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Import and decomposition of dissolved organic carbon in pre-dams of drinking water reservoirs

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Karoline Morling

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Reviewer:	PD Dr. Norbert Kamjunke
Reviewer:	Prof. Dr. Hans-Peter Grossart
Reviewer:	Prof. Dr. Stefan Bertilsson

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“Sometimes when I consider
what tremendous consequences
come from little things,
I am tempted to think
there are no little things.”

Bruce Barton

Preface

This work was conducted at the Helmholtz Centre for Environmental Research – UFZ, Department of Lake Research, Brückstr. 3a, 39114 Magdeburg (Germany) as part of the project “Load of drinking water reservoirs by dissolved organic carbon - Prediction, precaution, management options **(TALKO)” (BMBF grant number 02WT1290A)** under the main supervision of PD Dr. Norbert Kamjunke and Dr. Jörg Tittel.

List of abbreviations

β/α	freshness index
B.P.	before present (i.e. before 1950)
Chl. *a*	chlorophyll *a*
CRA	conventional radiocarbon age
Da	unified atomic mass unit (Dalton)
DBE	double bond equivalents
DBP	disinfection by-products
DI	degradation index
DIC	dissolved inorganic carbon
DOC	dissolved organic carbon
DOM	dissolved organic matter
EEM	excitation-emission matrix
EI	enrichment index
FT-ICR MS	Fourier transform ion cyclotron resonance mass spectrometry
GP	gross production
HIX	humification index
IC	inorganic carbon
m/z	mass to charge ratio
NP	net production
OC	organic carbon
OM	organic matter
PAR	photosynthetically active radiation
PE	priming effect
PLS	partial least square
POC	particulate organic carbon
POM	particulate organic matter
Q	discharge
Resp	respiration
RI	relative peak intensity
RSD	relative standard deviation
TIC	total inorganic carbon
Temp	temperature
TERENO	terrestrial environmental observatories
TOC	total organic carbon
TP	total phosphorus
UFZ	Helmholtz Centre for Environmental Research (Umweltforschungszentrum)
UV	ultra violet
WRT	water residence time

List of papers

I Morling, K., Kamjunke, N., Tittel, J., 2016. A simplified method of recovering CO_2 from bacterioplankton respiration for isotopic analysis. Journal of Microbiological Methods 121, 8-10. http://dx.doi.org/10.1016/j.mimet.2015.12.008

II Morling, K., Herzsprung, P., Kamjunke, N., 2017. Discharge determines production of, decomposition of and quality changes in dissolved organic carbon in pre-dams of drinking water reservoirs. Science of the Total Environment 577, 329-339. http://dx.doi.org/10.1016/j.scitotenv.2016.10.192

III Morling, K., Raeke, J., Kamjunke, N., Reemtsma, T., Tittel, J. (2017) Tracing aquatic priming effect during microbial decomposition of terrestrial dissolved organic carbon in chemostat experiments. Microbial Ecology. https://doi.org/10.1007/s00248-017-0976-0

Zusammenfassung

Gelöster organischer Kohlenstoff (dissolved organic carbon, DOC) bildet nicht nur eine zentrale Komponente des aquatischen Kohlenstoffkreislaufs, sondern auch für die Gewinnung von Trinkwasser aus Oberflächengewässern. In den letzten Jahrzehnten stiegen die DOC-Konzentrationen in Gewässern der nördlichen Hemisphäre an und führen sowohl zu ökologischen Konsequenzen als auch zu Wasserqualitätsproblemen. Die Zusammensetzung des DOC im Pelagial von Seen und Talsperren wird erheblich durch biologische Aktivität beeinflusst, da DOC-Verbindungen gleichzeitig produziert und abgebaut werden. Im Fokus meiner Dissertation standen ein konzeptionelles Verständnis des organischen Kohlenstoffkreislaufs und die damit verbundenen Änderungen in der DOC-Qualität unter verschiedenen hydrologischen und trophischen Bedingungen. Weiterhin wurde das Auftreten eines aquatischen Priming-Effektes untersucht, welcher den mikrobiellen Abbau von stabilem allochthonem DOC im Pelagial fördern könnte.

Quantitative und qualitative Untersuchungen wurden unter verschiedenen hydrologischen Bedingungen in drei Vorsperren von Trinkwassertalsperren durchgeführt, die einen Gradienten an DOC-Konzentrationen und Trophie aufwiesen. Alle Vorsperren waren im Epilimnion überwiegend autotroph. Abfluss und Temperatur wurden als Schlüsselfaktoren identifiziert, die Produktion und Respiration in den oberen Wasserschichten der Vorsperren regulieren. Eine vergleichsweise hohe autotrophe Produktion wurde während der Sommermonate bei hoher Trophie und Basisabfluss beobachtet. Bis zu 30% des gesamten eingetragenen organischen Kohlenstoffes wurde im Epilimnion produziert. Dies beeinflusste die DOC-Qualität in den Vorsperren erheblich und es traten vermehrt Charakteristiken von algenbürtigem DOC unter Basisabfluss in den Sommermonaten auf. Allochthoner DOC dominierte bei hohen Abflüssen und unter oligotrophen Bedingungen, wenn Produktion und Respiration gering waren. Diese Ergebnisse unterstreichen, dass auch kleine Speicherbecken mit typischerweise kurzen Wasseraufenthaltszeiten „Hotspots“ für Kohlenstoffumsetzung sind und die Wasserqualität signifikant in Abhängigkeit von Abflussbedingungen, Temperatur und Trophie verändern. Diese Faktoren sind auch für zukünftiges Wassermanagement bedeutsam, da steigende Temperaturen und veränderte Niederschläge im Zuge des Klimawandels prognostiziert werden.

Unter Basisabfluss verwerteten heterotrophe Bakterien vorrangig ältere DOC-Komponenten mit einem konventionellen Radiokarbonalter von 195-395 Jahren B.P. („before present“, d. h. vor 1950). Im Gegensatz dazu wurden jüngere DOC-Komponenten (modern, d. h. nach 1950 produziert) nach einem Regenwetterzufluss abgebaut. Daraus lässt sich schließen, dass Alter und mikrobielle Verwertbarkeit des DOC voneinander unabhängig sind. Um das Alter des genutzten DOC zu bestimmen, wurde eine vereinfachte Methode entwickelt, die das Auffangen des bakteriell respirierten CO_2 und eine anschließende Analyse der Kohlenstoffisotope (^{13}C, ^{14}C) ermöglicht. Die Vorteile der Methode liegen vor allem in der Verwendung von Replikaten, die bei in-situ Temperaturen inkubiert werden können und in der Nutzung von gängiger Laborausstattung. Dies ermöglicht eine Anwendung der Methode unter einer weiten Bandbreite von Bedingungen.

Der aquatische Priming-Effekt wurde in Laborexperimenten während des mikrobiellen Abbaus von zwei terrestrischen DOC-Substraten (Moorwasser und Bodeneluat) untersucht.

Phytoplankton diente als Quelle für labile organische Substanz und die DOC-Konzentrationen nahmen durch Exudation und Zelllysis des wachsenden Phytoplanktons während des Experimentes zu. Ein Priming-Effekt wurde für beide terrestrischen DOC-Substrate mittels Analyse von Kohlenstoffisotopen und Mischungsmodellen nachgewiesen, wobei der Priming-Effekt für das Moorwasser stärker ausgeprägt war als für das Bodeneluat. Analysen mittels hochauflösender Massenspektrometrie zeigten, dass verstärkt oxidierte und ungesättigte Verbindungen während des Primings (d. h. in Anwesenheit von Phytoplankton) abgebaut wurden. Aus den angestiegenen DOC-Konzentrationen während des Experimentes kann geschlussfolgert werden, dass ein aquatischer Priming-Effekt nicht allein über Konzentrationsänderungen nachweisbar ist und vielmehr als ein qualitativer Effekt betrachtet werden kann.

Diese Arbeit trägt zum Verständnis des aquatischen Kohlenstoffkreislaufs bei und zeigt wie DOC-Dynamiken in Süßgewässern mit hydrologischen, saisonalen und trophischen Bedingungen variieren. Weiterhin wurde gezeigt, dass der aquatische Priming-Effekt zu dem mikrobiellen Umsatz von organischem Kohlenstoff und dem beobachteten Abbau von terrestrischen DOC während des Transportes in Binnengewässern beiträgt.

Summary

Dissolved organic carbon (DOC) depicts a key component in the aquatic carbon cycle as well as for drinking water production from surface waters. DOC concentrations increased in water bodies of the northern hemisphere in the last decades, posing ecological consequences and water quality problems. Within the pelagic zone of lakes and reservoirs, the DOC pool is greatly affected by biological activity as DOC is simultaneously produced and decomposed. This thesis aimed for a conceptual understanding of organic carbon cycling and DOC quality changes under differing hydrological and trophic conditions. Further, the occurrence of aquatic priming was investigated, which has been proposed as a potential process facilitating the microbial decomposition of stable allochthonous DOC within the pelagic zone.

To study organic carbon cycling under different hydrological conditions, quantitative and qualitative investigations were carried out in three pre-dams of drinking water reservoirs exhibiting a gradient in DOC concentrations and trophic states. All pre-dams were mainly autotrophic in their epilimnia. Discharge and temperature were identified as the key factors regulating net production and respiration in the upper water layers of the pre-dams. Considerable high autochthonous production was observed during the summer season under higher trophic status and base flow conditions. Up to 30% of the total gained organic carbon was produced within the epilimnia. Consequently, this affected the DOC quality within the pre-dams over the year and enhanced characteristics of algae-derived DOC were observed during base flow in summer. Allochthonous derived DOC dominated at high discharges and oligotrophic conditions when production and respiration were low. These results underline that also small impoundments with typically low water residence times are hotspots of carbon cycling, significantly altering water quality in dependence of discharge conditions, temperature and trophic status. Further, it highlights that these factors need to be considered in future water management as increasing temperatures and altered precipitation patterns are predicted in the context of climate change.

Under base flow conditions, heterotrophic bacteria preferentially utilized older DOC components with a conventional radiocarbon age of 195-395 years before present (i.e. before 1950). In contrast, younger carbon components (modern, i.e. produced after 1950) were mineralized following a storm flow event. This highlights that age and recalcitrance of DOC are independent from each other. To assess the ages of the microbially consumed DOC, a simplified method was developed to recover the respired CO_2 from heterotrophic bacterioplankton for carbon isotope analyses (^{13}C, ^{14}C). The advantages of the method comprise the operation of replicate incubations at in-situ temperatures using standard laboratory equipment and thus enabling an application in a broad range of conditions.

Aquatic priming was investigated in laboratory experiments during the microbial decomposition of two terrestrial DOC substrates (peat water and soil leachate). Thereby, natural phytoplankton served as a source of labile organic matter and the total DOC pool increased throughout the experiments due to exudation and cell lysis of the growing phytoplankton. A priming effect for both terrestrial DOC substrates was revealed via carbon isotope analysis and mixing models. Thereby, priming was more pronounced for the peat water than for the soil leachate. This indicates that the DOC source and the amount of the added labile organic matter might influence the magnitude of a priming effect. Additional analysis via high-resolution mass spectrometry revealed that oxidized, unsaturated compounds were more strongly decomposed under priming (i.e. in phytoplankton presence). Given the observed increase in DOC concentrations during the experiments, it can be concluded that aquatic priming is not easily detectable via net concentration changes alone and could be considered as a qualitative effect.

The knowledge gained from this thesis contributes to the understanding of aquatic carbon cycling and demonstrated how DOC dynamics in freshwaters vary with hydrological, seasonal and trophic conditions. It further demonstrated that aquatic priming contributes to the microbial transformation of organic carbon and the observed decay of allochthonous DOC during transport in inland waters.

Contents

1. Introduction

1.1 Chemical nature of dissolved organic carbon

Carbon is considered as the building block of life and is exchanged between the atmosphere, the oceans, terrestrial systems and fossil reservoirs (Houghton 2003). Organic carbon (OC) is produced by living organisms and represents a class of very heterogeneous compounds. It is operationally divided into dissolved organic carbon (**DOC**) and particulate organic carbon (**POC**). For instance, DOC can represent all organic carbon compounds passing a medium grade glass fibre filter (GF/F) which retains fine particles down to 0.7 µm during filtration (Fig. 1-1, Aitkenhead-Peterson *et al.* 2003). As a result of this separation, bacteria partly belong to DOC as they occasionally pass these filters (Fig. 1-1, Verdugo *et al.* 2004). In contrast, the European Standard determines DOC as all organic carbon compounds passing a membrane filter with a given pore size of 0.45 µm (DIN EN 1484 1997).

DOC comprises a continuum of small organic molecules to highly polymeric humic substances with molecular weights ranging from 100 to 100,000 Da (Aitkenhead-Peterson *et al.* 2003). Molecular weight classes can be used for a simple characterization of DOC compounds. A biologically derived categorization is applied by grouping DOC into fractions of **labile** (i.e. easily decomposable) and **refractory** (i.e. not decomposed) DOC. These are obtained from biodegradation experiments with heterotrophic bacteria and are thus always bound to the employed incubation times and experimental conditions such as temperature, inoculum size and addition of inorganic nutrients (del Giorgio and Davis 2003).

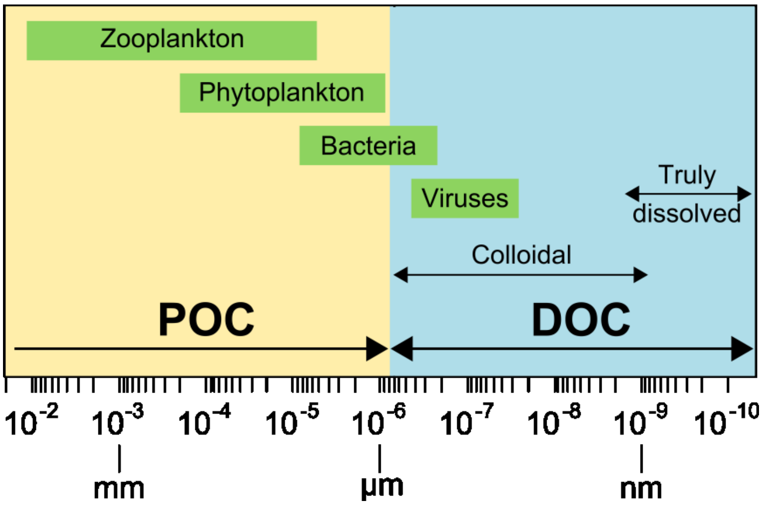


Fig. 1-1: Size continuum of organic carbon with separation between particulate (POC > 0.7 µm) and dissolved organic carbon (DOC < 0.7 µm). Adapted from Verdugo *et al.* (2004).

DOC compounds differ considerably in their chemical structure depending on their origin. DOC from terrestrial sources is usually nitrogen-poor, optically dense and rich in aromatic structures, whereas algae-derived DOC is nitrogen-rich and relatively transparent (Curtis 1998, Bertilsson and Jones 2003). **Humic substances** make up a large fraction (up to 40-60%; McKnight and Aiken 1998, Aitkenhead-Peterson *et al.* 2003) of DOC in freshwaters and are typically derived from terrestrial sources. They comprise a continuum of organic acids soluble under different pH ranges and are operationally defined as the fraction of DOC adsorbing onto XAD resins (Benner 2003). Humic substances are believed to be formed directly from lignified tissues of plant material or by polymerization of simple products generated in the degradation of plant material via both rapid and long-term processes (Choudhry 1984, McKnight and Aiken 1998). Humic substances contain **chromophoric groups**, absorbing ultra-violet (UV) and visible light (Lean 1998), making them responsible for the typically observed yellow to brown colour of DOC. The spectral absorption coefficient at the wavelength of 254 nm is widely used as a proxy for DOC concentration (Frimmel and Kumke 1999, Rinke *et al.* 2013) and was established as a controlling factor in water treatment of surface waters (ATT 2010). **Qualitative characterizations** of DOC compounds are frequently obtained by UV-visible light absorption spectra and excitation-emission matrix spectra (McKnight *et al.* 2001, Fellman *et al.* 2010, Dittmar and Stubbins 2014), allowing the differentiation of different DOC sources and different levels of aromaticity and biological activity (McKnight *et al.* 2001, Fellman *et al.* 2010, Hansen *et al.* 2016). Hypothetical molecular structures of humic substances have been proposed (Fig. 1-2; Choudhry 1984, McKnight and Aiken 1998, Maranger and Pullin 2003), demonstrating the diversity of functional groups allowing reactions and complexing with other compounds. Although advanced spectroscopic techniques and high resolution mass spectrometry revealed major structural characteristics, the chemical structures of DOC compounds are still largely unknown (Dittmar and Stubbins 2014). Further, a significant fraction of DOC (up to > 30%) is still lost from the analytical window due to its resistance to isolation or the exclusion during preparative procedures (Dittmar and Stubbins 2014).

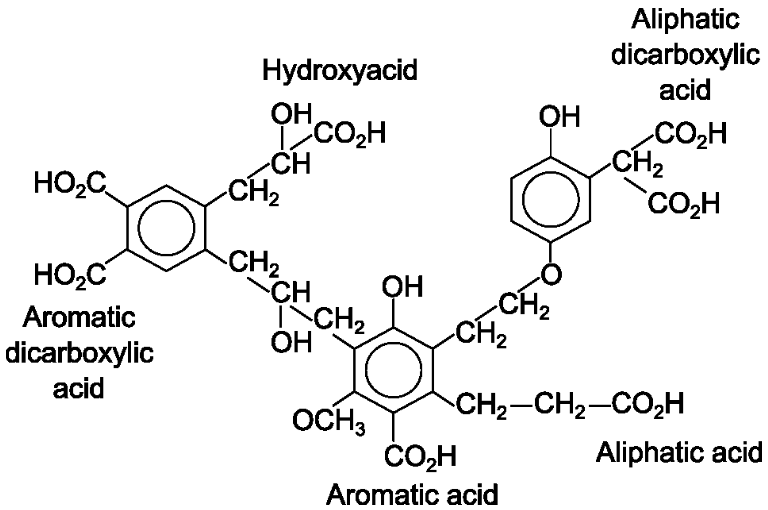


Fig. 1-2: Hypothetical molecular structure of humic acid. Important functional groups are illustrated. Adapted from Matilainen *et al.* (2010).

1.2 Dissolved organic carbon in freshwater ecosystems

DOC represents a key constituent in aquatic systems (Prairie 2008), connecting soil organic carbon stores to those of the ocean (Dittmar and Stubbins 2014). In aquatic systems, it can be derived from external (allochthonous; Aitkenhead-Peterson *et al.* 2003) or internal (autochthonous; Bertilsson and Jones 2003) sources. Carbon isotopes have been used in several studies to distinguish between allochthonous and autochthonous sources in freshwater systems (del Giorgio and France 1996, Raymond and Bauer 2001, Cole *et al.* 2006, Guillemette *et al.* 2013, Wilkinson *et al.* 2013) and whole lake experiments provided deeper insight into the cycling of both allochthonous and autochthonous carbon (Cole *et al.* 2002, Kritzberg *et al.* 2004, Pace *et al.* 2004, Kritzberg *et al.* 2006, Scharnweber *et al.* 2014).

Allochthonous material represents the predominant part of DOC found in surface waters and is originating from terrestrial organic matter (OM), which is either directly (e.g. in terms of leaf litter) or indirectly (e.g. via leaching from soils) introduced into inland waters. Up to 88% of the bulk soil solution DOC originates from **organic horizons** (Aitkenhead-Peterson *et al.* 2003). Its quality is largely determined by vegetation and land use and is controlled by various abiotic and biotic processes such as physical and chemical alterations, desorption and dissolution as well as microbial degradation of the soil OM (Kalbitz *et al.* 2000). The adsorption of DOC compounds (i.e. their retention in soils) is positively correlated to the amount of Fe and Al oxides/hydroxides in soils (Tipping 1981, Moore *et al.* 1992, Kalbitz and Knappe 1997). Soil pH was found to have no direct effect on the release of DOC (Kalbitz and Knappe 1997), although a higher mobilization of DOC was observed with increasing pH in forest floors (Michalzik *et al.* 2001). The chemical composition of soil OM is significantly affected by pH and nitrate: Agricultural soils hold more nitrogen-containing and aromatic compounds, while forest soils contain compounds of higher molecular weight and higher oxygen to carbon (O/C) ratios (Seifert *et al.* 2016).

The delivery of terrestrial derived DOC to inland waters (Fig. 1-3) depends on the balance between production and losses from soil solution and on opportunities for **hydrological transport** (Curtis and Schindler 1997, Curtis 1998, Kalbitz *et al.* 2000, Aitkenhead-Peterson *et al.* 2003, Laudon *et al.* 2011). Precipitation increases DOC fluxes from soils (Tipping *et al.* 1999) and higher DOC releases were observed during rain events following long drought periods (Kalbitz and Knappe 1997). Most of the terrestrial derived OC reaches freshwaters in short time periods during rainstorm events or snow melts (Brooks *et al.* 1999, Dalzell *et al.* 2007, Raymond and Saiers 2010, Graeber *et al.* 2015b). Such events enable surface runoff, flow through organic-rich near-surface layers (Easthouse *et al.* 1992, Boyer *et al.* 1996, Mulholland 2003) and the connection of upland domains (Laudon *et al.* 2011, Lambert *et al.* 2014). During base flow conditions, the dominating contributions derive from lower soil layers and groundwater, which exhibit typically lower DOC concentrations (Easthouse *et al.* 1992, Curtis 1998). Attributes of the **catchment areas** such as size, topography, soil types and land use determine the mobilization, quantity and quality of the allochthonous DOC delivered to freshwaters. DOC concentrations and export have been linked to soil C:N ratios (Aitkenhead and McDowell 2000), percentages of peat cover (Dillon and Molot 1997, Aitkenhead *et al.* 1999), watershed slopes (Xenopoulos *et al.* 2003), runoff (Dillon and Molot 2005) and the ratios of catchment area to lake area as well as water residence time (WRT; Curtis and Schindler 1997). In a comparison of watersheds from geographical regions worldwide, DOC concentrations in lakes were found to be significantly determined by elevation and percentage of wetlands (Xenopoulos *et al.* 2003). Drainage from organic rich **wetlands** tends to have high DOC concentrations (Dillon and Molot 1997, Lambert *et al.* 2014) and serve as a DOC source to rivers and lakes all-the-year (Laudon *et al.* 2011, Lambert *et al.* 2014).

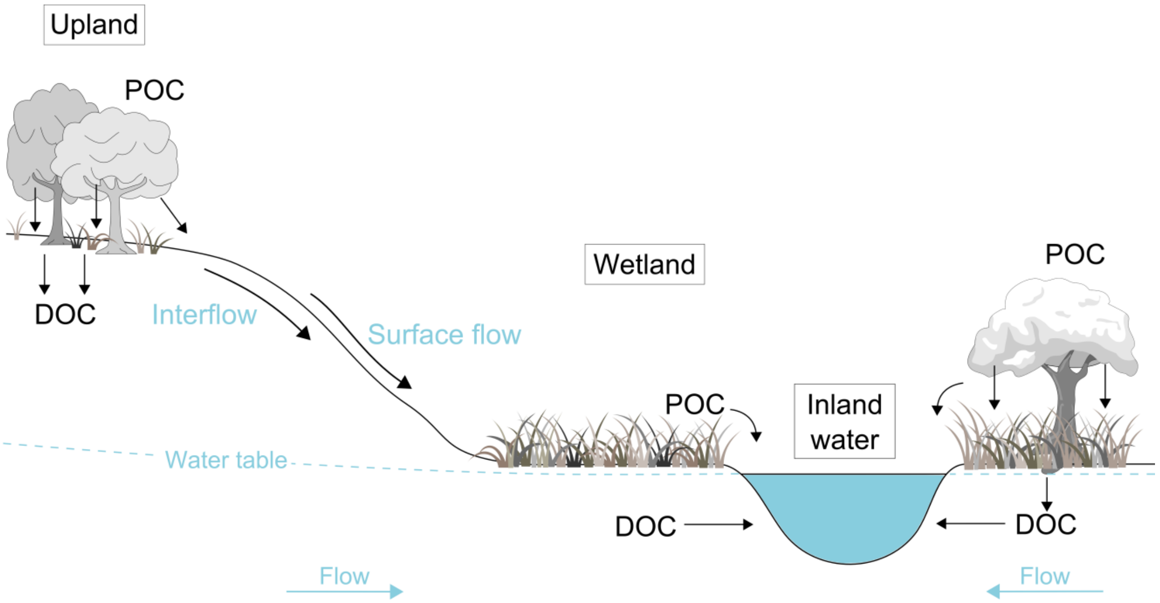


Fig. 1-3: Terrestrial-aquatic coupling of the DOC cycle illustrating pathways of DOC delivery to inland waters. Modified after Schwoerbel and Brendelberger (2013).

In the last decades, DOC concentrations increased in many freshwaters of the northern hemisphere (Evans *et al.* 2005, Monteith *et al.* 2007), leading to a “**browning**” of inland waters. Several possible causes have been suggested for this increase (reviewed by Porcal *et al.* 2009) such as increased temperatures and terrestrial primary production (Tipping *et al.* 1999, Freeman *et al.* 2004, Hagedorn *et al.* 2008, Larsen *et al.* 2011, Reichstein *et al.* 2013), decreased sulphate deposition (Driscoll *et al.* 2003, Monteith *et al.* 2007, Ekström *et al.* 2011, Löfgren and Zetterberg 2011) as well as continued nitrogen deposition (Pregitzer *et al.* 2004, Findlay 2005, Evans *et al.* 2008, Maaroufi *et al.* 2015). Further, changes in catchment hydrology (Schindler *et al.* 1997, Hejzlar *et al.* 2003, Evans *et al.* 2005, Worrall and Burt 2008, Fenner and Freeman 2011), land use management (Mattsson *et al.* 2005, Yallop and Clutterbuck 2009, Stergiadi *et al.* 2016) or increasing concentrations of iron in freshwaters caused by an increased reduction of soil Fe-oxyhydroxides (Kritzberg and Ekström 2012, Knorr 2013, Lambert *et al.* 2013, Weyhenmeyer *et al.* 2014, Musolff *et al.* 2016a) have been related to the increase in DOC concentration. Most likely, several of the above mentioned mechanisms are interacting and influencing the DOC dynamics, while at the same time being highly dependent on the local conditions of the catchments such as vegetation and soil type, bedrock, topography and climate.

Autochthonous DOC refers to carbon compounds produced within aquatic systems (Bertilsson and Jones 2003). Such dissolved organic compounds are directly released by active excretion from growing phytoplankton (Larsson and Hagström 1979, Zlotnik and Dubinsky 1989, Obernosterer and Herndl 1995, Børsheim *et al.* 2005), decay of senescent cells or by viral lysis as well as by sloppy feeding of higher trophic level organisms (Bertilsson and Jones 2003). Also, macrophytes and epiphytic algae growing on them represent an important source of autochthonous DOC in freshwaters (Bertilsson and Jones 2003) as they typically attain high production and high DOC release rates (Wetzel 1990, Vis *et al.* 2007, Demarty and Prairie 2009). Particulate detritus is representing a secondary source of autochthonous DOC due to decomposition processes taking place within aquatic systems (Bertilsson and Jones 2003).

1.3 Impacts and cycling of dissolved organic carbon in aquatic systems

Allochthonous DOC and especially the colored humic substances therein exert extensive impacts on freshwater systems on both abiotic and biotic level (reviewed by Solomon *et al.* 2015). High DOC concentrations lead to higher **attenuation and absorption** of visible and ultraviolet (UV) light (Kirk 1994, Scully and Lean 1994, Morris *et al.* 1995). While the attenuation of UV-B light protects aquatic organisms of all trophic levels from damaging effects of UV radiation (Little and Fabacher 1994, Moeller 1994, Zagarese *et al.* 1994, Morris *et al.* 1995, Williamson 1995), the heat balance and **stratification** of lakes is highly affected as well. The heat absorption of humic substances warm up the water quickly, leading to thin epilimnia during the summer periods (Lean 1998, Eloranta 1999, Zwart *et al.* 2016a). Photosynthetic organisms are directly inhibited by attenuated light penetration and indirectly affected by the changes in water column structure and stability (Jones 1998, Lean 1998). Phytoplankton species composition seems to be not influenced by the content of humic substances, although chrysophytes and cryptophyte flagellates are well presented in nutrient-poor humic lakes (Jones 1998, Arvola *et al.* 1999). Typically, primary production decreases and respiration increases with increasing DOC concentrations in oligotrophic lakes, resulting in **net heterotrophy** of these systems (Hanson *et al.* 2003, Ask *et al.* 2009, Ask *et al.* 2012, Seekell *et al.* 2015), which refers to the condition of respiration exceeding gross production. Thus, high DOC concentrations in freshwaters with low primary production are able to alter planktonic species composition towards more chemoorganotrophic food webs (Jansson *et al.* 2000). In accordance with this, biomass production of higher trophic level organisms such as fish can be suppressed under high DOC concentrations (Rask *et al.* 1999, Karlsson *et al.* 2015). Moreover, it was suggested that humic substances directly interact with aquatic organisms and modify individual fitness through hormone-like effects, irritations of biomembranes, altered ion regulation and inducing of stress defense reactions if present in high concentrations (reviewed by Steinberg *et al.* 2007).

The complex and diverse chemical structures of humic substances enable the **complexation** of trace metals such as Cu, Al and Fe (Driscoll *et al.* 1994, Perdue 1998, Maranger and Pullin 2003, Aiken *et al.* 2011). Both Al and Fe have long been known to be associated with high amounts of DOC (Perdue *et al.* 1976, Moore 1988). The complexation with humic substances prevents the precipitation of these metals. Thus, Fe is made available for enhanced uptake by phytoplankton after photochemical reduction, but humus-metal complexes were also found to suppress primary production (Jones 1998, Maranger and Pullin 2003).

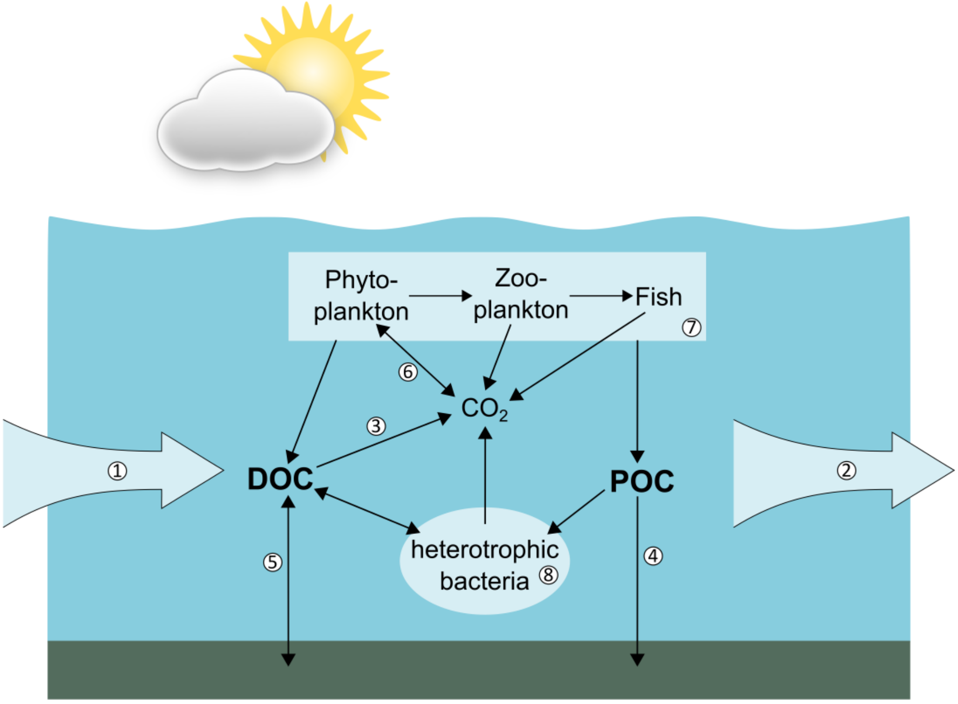


Fig. 1-4: Schematic illustration of the carbon cycling within the pelagic zone of freshwater systems. Abiotic processes comprise the (1) inflow and (2) outflow of both DOC and POC, the (3) conversion of DOC to CO_2 via photochemical reactions (photolysis), (4) sedimentation and (5) adsorption and desorption of DOC compounds to sediments under oxic and anoxic conditions. Biotic pathways include (6) photosynthesis as well as the uptake and release of DOC and POC via cell lysis, excretion or sloppy feeding and CO_2 via respiration by the organisms of the (7) traditional food chain and by (8) heterotrophic bacteria.

DOC depicts a highly dynamic component of the aquatic carbon cycle as it underlies several abiotic and biotic transformation processes (Fig. 1-4). Hydrology and precipitation patterns are important control factors not only for the delivery of DOC to inland waters (see section 1.2), but also for the **retention and transport** of both allochthonously and autochthonously derived DOC to downstream water bodies (Algesten *et al.* 2004). DOC retention in lakes is higher during dry periods and low discharges (Dillon and Molot 2005) as the WRT is longer.

Photochemical reactions lead to the direct formation of CO_2 and CO from DOC compounds (Miller and Zepp 1995, Granéli *et al.* 1996, Lean 1998, Miller 1998, Bertilsson and Tranvik 2000, Tranvik and Bertilsson 2001). The **photoreactivity** of DOC compounds depends on their molecular structure and hence also on their sources. Aromatic compounds were found to be more photoreactive than saturated ones (Gonsior *et al.* 2009) and both labile and refractory substances can be formed during light exposure of DOC (Tranvik and Kokalj 1998, Tranvik and Bertilsson 2001). Increased bacterial growth was found on light exposed terrestrial DOC material (which is usually of more aromatic character), while the bacterial growth on algal material (which is more aliphatic) was decreased (Tranvik and Bertilsson 2001). Definitely, photoreactions occur only in the upper water layers, where light can penetrate, and are damped with increasing content of humic substances in the water. Thus, photoreactions can play a substantial role in surface layers, but only a minor role on a depth-integrated basis (Moran and Covert 2003).

Another mechanism for DOC removal from the water column is the **aggregation** of compounds and subsequent sedimentation. Predominantly allochthonous DOC is removed via this pathway (von Wachenfeldt and Tranvik 2008). Further, DOC compounds are efficiently taken up by sediments and bound to ferric minerals under oxic conditions (Lalonde *et al.* 2012, Dadi *et al.* 2016), but might be released as well from anoxic sediments under reducing conditions (Dadi *et al.* 2016).

Biologically driven transformation processes of DOC within aquatic systems comprise the conversion of inorganic carbon to living biomass by **photosynthetic** organisms and the subsequent release of DOC via cell lysis, excretion or sloppy feeding by the organisms of the traditional food chain as well as the transformation and mineralization of DOC compounds through the **microbial loop** (Tranvik 1998, Bertilsson and Jones 2003, Jansson *et al.* 2007, Guillemette and del Giorgio 2012). Seasonal variations influence the production and release of allochthonous and autochthonous DOC on both abiotic and biotic level and DOC is processed at higher or lower efficiencies depending on its source and quality.

A substantial fraction (up to ≥ 50%) of terrestrial derived DOC is mineralized in freshwaters (Algesten *et al.* 2004, Caraco and Cole 2004, Weyhenmeyer *et al.* 2012) and the majority of lakes were found to be net-heterotrophic (Cole *et al.* 2000, Jansson *et al.* 2000, Prairie *et al.* 2002, Hanson *et al.* 2003), making them CO_2 sources to the atmosphere and pointing to the decomposition of allochthonously derived OC. Consequently, lakes and reservoirs were identified as **hotspots of carbon cycling** (Cole *et al.* 2007, Tranvik *et al.* 2009), despite making up only ~3% of the terrestrial land surface (Downing *et al.* 2006). Globally, the terrestrial carbon input into lakes and reservoirs accounts for ~2.9 Pg C y^{-1}, from which ~20% is buried in sediments and ~48% is released as CO_2 to the atmosphere (Tranvik *et al.* 2009).

1.4 The role of heterotrophic microorganisms in the decomposition of dissolved organic carbon

Up to 90% of the organic carbon in inland waters is in dissolved form (Wetzel 2001), favoring its uptake and decomposition by heterotrophic microorganisms via aerobic and anaerobic metabolic pathways (Tranvik 1998). Microbial DOC decomposition is important for higher trophic levels in both positive (nutrient recycling) and negative (O_2 consumption, production of fermentative metabolic end products) way (Wetzel 1995). Heterotrophic bacteria are maintaining the carbon cycle and directly influencing ecosystem dynamics. Bacterial biomass serves as a food source for heterotrophic nano-flagellates and ciliates, which are consumed by zooplankton. Bacteria are connected with the traditional food chain via the **microbial loop** and make energy available to higher trophic levels (Azam *et al.* 1983, Jansson *et al.* 2007). However, the majority of the carbon flux through pelagic bacteria is through respiration (Gaedke *et al.* 1996, Cotner and Biddanda 2002, Karlsson 2007), leading to a higher energy loss via the microbial loop in comparison to the traditional grazing food chain (Gaedke *et al.* 1996).

Microbial carbon turnover is highly influenced by various environmental factors such as temperature or availability of nutrients. Further, the composition of the DOC pool plays an important role for **microbial mineralization**. Autochthonous DOC is regarded as more labile and preferentially utilized by heterotrophic bacteria (Coveney 1982, Chen and Wangersky 1996, Kritzberg *et al.* 2004, Nguyen *et al.* 2005, Guillemette *et al.* 2013, Guillemette *et al.* 2015) and primary production and respiration are often coupled in lake systems (Cole 1982, Cole *et al.* 1988, Bertilsson and Jones 2003, Solomon *et al.* 2013). Allochthonous DOC is mineralized as well (Tranvik 1998, Karlsson *et al.* 2007) and bacterial biomass was found to be positively correlated with the amount of humic substances (Tranvik 1988, Jansson 1998, Eiler *et al.* 2003), whereby photochemical reactions might enhance the microbial decomposition of humic substances (Geller 1986, Lindell *et al.* 1995, Wetzel *et al.* 1995, Miller 1998, Tranvik 1998). Bacterial growth was found to be positively correlated with the H/C and N/C ratios of the DOC source, but was negatively correlated to O/C (Sun *et al.* 1997). This resulted in a selective removal of aliphatic compounds and the alteration of the DOC pool towards more aromatic characteristics during riverine transport (Sun *et al.* 1997). Thus, the chemical structure of the DOC pool in freshwaters results to a large extent from microbial activity and decomposition processes (Moran *et al.* 2000), whereby microbes fulfil a dual role in simultaneously mineralizing and producing DOC compounds (Guillemette and del Giorgio 2012). Thereby, such microbially produced DOC compounds could be of more refractory character (Geller 1985, Ogawa *et al.* 2001, Lechtenfeld *et al.* 2015).

Heterotrophic bacteria preferentially utilize mixtures of multiple substrates (Geller 1985, Tranvik 1998, Grover 2000, Farjalla *et al.* 2009, Fonte *et al.* 2013), giving rise to the question if priming is a possible mechanism in microbial DOC decomposition in aquatic systems. The **priming effect** often refers to an increased mineralization of a refractory OC pool after the addition of a labile OC source (Kuzyakov *et al.* 2000). Suggested mechanisms underlying the occurrence of the priming effect are co-metabolism, the increase in microbial biomass due to the supplied labile OC substrate or nutrient mining (Fontaine *et al.* 2003, Blagodatskaya and Kuzyakov 2008, Guenet *et al.* 2010). The priming concept is derived from soil sciences, where it has been widely observed and studied (Fontaine *et al.* 2007, Blagodatskaya and Kuzyakov 2008, Kuzyakov 2010). In contrast, research on the priming effect in aquatic systems started only recently and experimental results are contradictory. While some studies found an aquatic priming effect (Guenet *et al.* 2014, Kuehn *et al.* 2014, Bianchi *et al.* 2015), others did not (Bengtsson *et al.* 2014, Catalán *et al.* 2015, Dorado-García *et al.* 2016) or only under low-nutrient conditions (Danger *et al.* 2013). The contrasting results might be attributed to the great variety of different terrestrial DOC sources used as the refractory material (e.g. soil leachate, lake water, leaf litter, leaf leachate) and varying types of the added labile carbon substrates (e.g. sugar compounds, algae extracts, growing autotrophs).

1.5 Dissolved organic carbon in the context of reservoir management and drinking water production

As man-made constructions, reservoirs serve several purposes such as power generation, flood protection, water storage and drinking water production. The increasing DOC concentrations observed in surface waters in the last decades depict a serious issue for the water industry, intensifying several problems for **drinking water production**. Higher amounts of flocculants are needed to remove the DOC from raw waters, resulting in shorter filter operation times and making drinking water production from surface waters more costly (Matilainen and Sillanpää 2010, Ledesma *et al.* 2012). Further, DOC compounds act as precursors for the formation of potentially carcinogenic disinfection by-products (DBP) during the purification treatment (Singer 1994, Nguyen *et al.* 2002, Chow *et al.* 2003, Richardson *et al.* 2007, Matilainen *et al.* 2010, Jeong *et al.* 2012, Lavonen *et al.* 2013).

In Germany, drinking water reservoirs are mostly located in secondary mountain ranges and are usually constructed with pre-dams. **Pre-dams** are small reservoirs located upstream of the main drinking water reservoirs and possess typically low WRT of a few days or weeks and are usually constructed with a surface overflow similar to natural lakes. They serve mainly for sediment trapping and nutrient removal (Benndorf and Pütz 1987a, b, Pütz and Benndorf 1998) and represent an important tool for water quality management (Klapper *et al.* 1974, Paul 2003). Pre-dams exhibit a much higher ratio of catchment to surface area as most lakes and a strong coupling to their drainage basins. Thus, they can be expected to react much faster to changes in environmental conditions and anthropogenic impacts than their corresponding main reservoirs.

1.6 Study sites

Field surveys included in my thesis were carried out in three pre-dams, which all belong to drinking water reservoirs located in Germany and differed in their DOC concentrations and trophic states (Tab. 1-1). The oligotrophic and DOC-rich Rote Mulde pre-dam (Tittel *et al.* 2013) is located in the Western Ore Mountains and its small catchment is almost entirely dominated by forest (Tab. 1-1, Fig. 1-5A). The mesotrophic Rappbode pre-dam and the eutrophic Hassel pre-dam (Rinke *et al.* 2013, Friese *et al.* 2014) are part of the Rappbode reservoir system in the Harz Mountains and belong to the TERENO (terrestrial environmental observatories) network in Germany (Zacharias *et al.* 2011). They exhibit lower DOC concentrations and their catchment areas possess higher proportions of grasslands and agriculture, leading to higher P concentrations in the pre-dams (Tab. 1-1, Fig. 1-5B). Due to alternating construction works at the dams, sampling took place in the Hassel pre-dam in 2013 and in the Rappbode pre-dam in 2014. The Rote Mulde pre-dam was sampled in both years.

Tab. 1-1: Properties of the three studied pre-dams. Data provided by the reservoir management authority "Talsperrenbetrieb Sachsen-Anhalt" and the State Reservoir Administration Saxony. Chemical values represent ranges during sampling campaigns in 2013 and 2014 (Morling *et al.* 2017).

Pre-dam	Rote Mulde	Rappbode	Hassel
Catchment area [km²]	5.35	39.1	40.5
- Forest [%]	98	72	37
- Grasslands and acres [%]	1	24	58
- Urban areas [%]	1	3	5
Surface area [km²]	0.098	0.218	0.288
Volume [Mio m³]	0.06	1.25	1.45
Mean depth [m]	1.8	5.3	5.0
Max. depth [m]	4	17	14
Water residence time [days]	6	16	27
pH	5.2-6.6	7.1-7.8	6.8-8.9
Total P [µg L^{-1}]	9.5-33	14-40	22-51
DOC [mg L^{-1}]	5.1-21.1	2.4-5.4	4.2-6.9

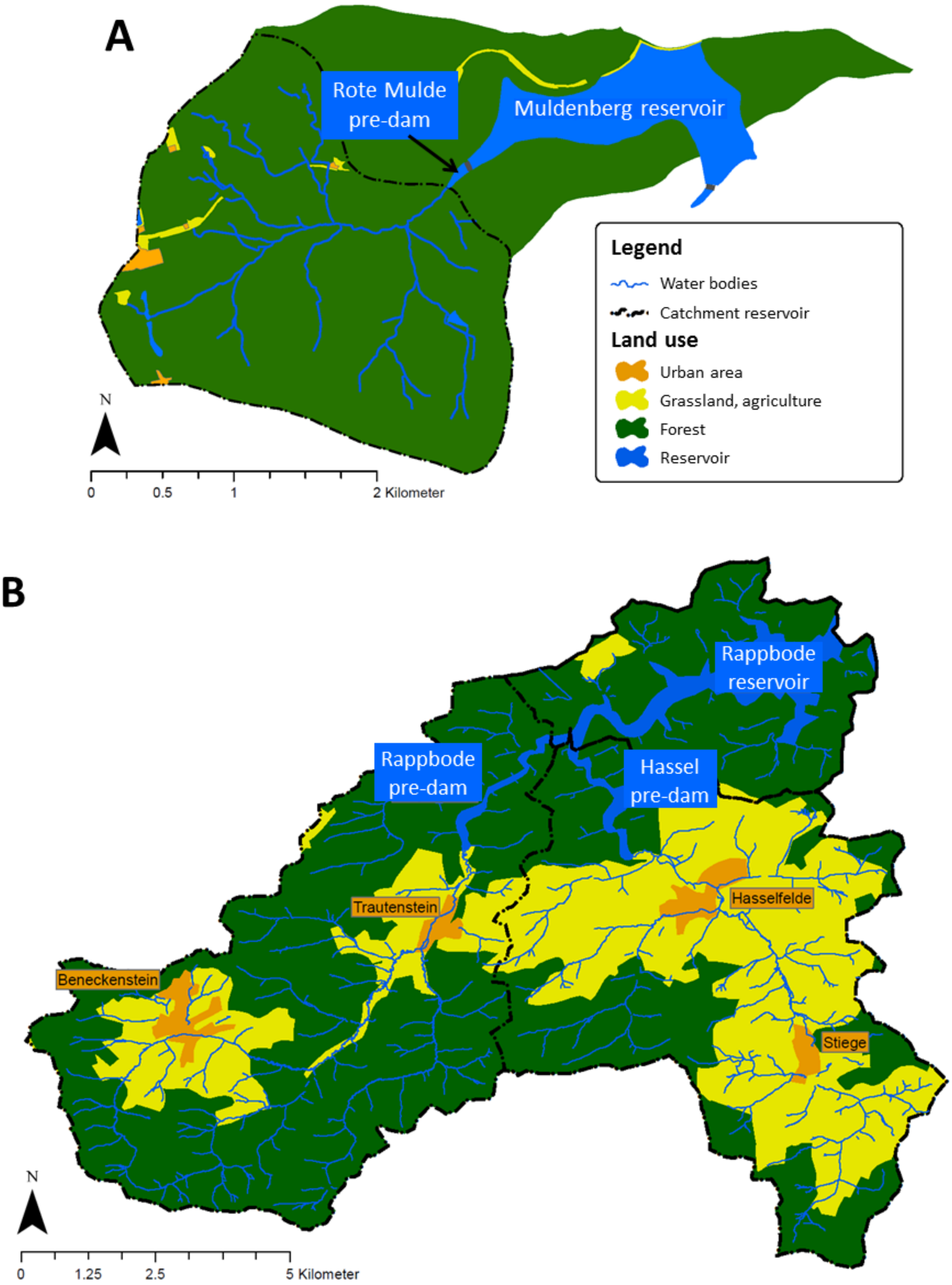


Fig. 1-5: Maps of the two study areas: (A) Muldenberg reservoir with the tributary and pre-dam Rote Mulde and (B) Rappbode reservoir with the tributaries Rappbode and Hassel and their corresponding pre-dams. Maps prepared by O. Büttner, UFZ.

2. Thesis outline and declaration of contributions

The major objective of my thesis comprised the study of DOC dynamics within the pelagic zone in pre-dams of drinking water reservoirs. I combined quantitative and qualitative investigations in lab experiments and field surveys in collaboration with other members of the UFZ and subprojects within the TALKO project (Musolff *et al.* 2016b, Tittel *et al.* 2016).

Two main aspects arose from this work: (1) studying the OC cycling and DOC quality changes in the pelagic zone of pre-dams under different hydrological and trophic conditions to gain a conceptual understanding and (2) investigating if aquatic priming is a potential process contributing to DOC dynamics and facilitating the microbial decomposition of terrestrial DOC within the pelagic zone. These two major aspects are addressed within three manuscripts.

Manuscript I concerns the development of a simplified method for recovering CO_2 respired by heterotrophic microorganisms for isotopic analyses (^{13}C, ^{14}C) in order to investigate sources and ages of utilized DOC. A previously established method was adapted for the use with standard laboratory equipment, avoiding the construction of a technically demanding harvest line and allowing replicate incubations at in-situ temperatures. The recovered respiratory CO_2 was more depleted in ^{14}C than the DOC source, suggesting a selective respiration of older carbon by heterotrophic bacteria and giving evidence for the independence of age and recalcitrance of DOC.

The concept of simplifying the previously established method was formed by J. Tittel. I evaluated the approach and conducted the lab experiments assisted by N. Kamjunke. I prepared the samples for carbon isotope analyses. I analyzed the results and wrote the manuscript for which I received comments by all co-authors.

Manuscript II aims for a conceptional understanding of production and decomposition of OC within the pelagic zone of pre-dams in relation to differing hydrological and trophic conditions. Combined results of field investigations and laboratory experiments on both quantitative and qualitative level are presented. The studied pre-dams were found to be mainly autotrophic in their upper water layers and on average 0-30% of the total gained OC was produced within the pre-dams. The method described in manuscript I was applied to analyze respired CO_2 and revealed that heterotrophic bacteria preferentially utilized older DOC components (195-395 years before present) under base flow conditions and younger DOC components (modern, i.e. OC produced after 1950) following a rain event. DOC quality within the pelagic zone changed significantly over the year as revealed by fluorescence measurements. High proportions of algae-derived DOC were observed during base flow conditions in summer, and the freshness index (β/α ratio) decreased significantly with increasing discharge.

The experimental design of the field survey was conceived by J. Tittel. I conducted in-situ measurements of net production and respiration as well as the accompanying lab experiments on microbial DOC decomposition. Fluorescent measurements were conducted by P. Herzsprung in the context of the UFZ monitoring program at the Rappbode reservoir. I analyzed the data supported by N. Kamjunke. I wrote the manuscript and received comments by all co-authors.

Manuscript III presents quantitative and qualitative results of laboratory experiments testing for the potential occurrence of aquatic priming effect during microbial decomposition of two terrestrial DOC sources in flow-through cultures (chemostats) in absence and presence of growing phytoplankton. Carbon isotope data and mixing models revealed a priming effect in the presence of phytoplankton, although the total DOC concentrations increased for both substrates due to exudation and cell lysis of the phytoplankton. Samples analyzed via ultra-high resolution mass spectrometry revealed that predominantly the same saturated, aliphatic molecules were decomposed in absence and in presence of phytoplankton. However, under priming, oxidized unsaturated compounds were more strongly decomposed. We conclude that aquatic priming effect is not easily detectable via net concentration changes alone and requires qualitative investigations as well.

J. Tittel and T. Reemtsma conceived the study. I conducted the chemostat experiments and prepared the samples for carbon isotope analyses. Extraction of the chemostat samples and measurements via high resolution mass spectrometry were performed by J. Raeke. I analyzed the experimental results in teamwork with N. Kamjunke, J. Raeke and J. Tittel. I wrote the manuscript and received comments by all co-authors.

The manuscripts are self-containing papers, which have been published or submitted to reviewed journals. Implications of the results and further perspectives are summarized in the comprehensive discussion.

3. Compendium of Manuscripts

Manuscript I

A simplified method of recovering CO_2 from bacterioplankton respiration for isotopic analysis

Morling, K., Kamjunke, N., Tittel, J., 2016. A simplified method of recovering CO_2 from bacterioplankton respiration for isotopic analysis. Journal of Microbiological Methods 121, 8-10. http://dx.doi.org/10.1016/j.mimet.2015.12.008

A simplified method of recovering CO_2 from bacterioplankton respiration for isotopic analysis

Karoline Morling[a]*, Norbert Kamjunke[a,b], Jörg Tittel[a]

[a]UFZ – Helmholtz Centre for Environmental Research, Department of Lake Research, Brückstr. 3a, 39114 Magdeburg, Germany
[b]UFZ – Helmholtz Centre for Environmental Research, Department of River Ecology, Brückstr. 3a, 39114 Magdeburg, Germany

*corresponding author: Tel.: +49 391 8109447, email: karoline.morling@ufz.de

Key words:
Dissolved organic carbon (DOC), inorganic carbon (IC), degradation, carbon isotopes, reservoir

Abstract

A method for recovering CO_2 respired by bacterioplankton for analysis of carbon isotopes was adapted for use with standard laboratory equipment without a technically demanding harvest line. The recovered CO_2 was more depleted in ^{14}C than the dissolved organic carbon (DOC) source, which suggests a selective respiration of older carbon.

Main text

Bacterial respiration makes up a large fraction of total respiration in aquatic systems (Cotner and Biddanda, 2002). McCallister et al. (2006) have developed a method of directly recover CO_2 from bacterioplankton respiration for analyses of carbon isotopes to determine the sources and ages of organic carbon (OC) consumed. However, their method requires the construction of a technically demanding harvest line for the recovery process. Thus, we developed a simplified method, which was tested in three experiments using water taken from two drinking water pre-reservoirs: Rappbode (Harz Mountains, Germany; Rinke et al. 2013) and Rote Mulde (Ore Mountains, Germany; Tittel et al. 2013) in June and July 2014. Samples were filtered through pre-rinsed filters (0.22 µm, MPGL06GH2, Millipore). Filtered subsamples (2 L) were used for isotope analyses of the source DOC (Tittel et al. 2013). Each experiment consisted of two treatments with filtered DOC and one control with low background DOC medium (basal salts medium; Sanders et al., 1990) of ambient nutrient concentrations. Each treatment and control was comprised of two 10 L glass vessels (demijohns).

To remove the ambient inorganic carbon, filtered DOC and control medium were acidified to pH ~2.5 (37% HCl) in 54 L demijohns. Aeration with CO_2-free air for 24 h was provided by scrubbers consisting of air pumps and two connected gas washing bottles filled with silica gel and solid NaOH. After re-adjusting pH to initial values (<15 mL 10 M NaOH), the TIC-free filtered DOC and control medium were distributed into 10 L vessels sealed with silicone rubber stoppers into which glass rods were inserted (Fig. 1). The longer glass rods enabled a mixture of the substrates during aeration, the shorter one was a port to the gas phase. Silicone tubing and luer-lock tube connectors were attached to the rods and were closed with tubing clips, applied close to the rods to minimize intrusion of atmospheric CO_2 through the tubing (Fig. 1).

As bacterial inoculum, 50 mL pre-filtered (2 µm) water from the pre-reservoirs was added. Start samples of TIC and DOC were taken while CO_2-free aeration was still provided. TIC and DOC concentrations were measured by infrared detection using a DIMATOC® 2000 (Dimatec, Germany). Start TIC was zero in all experiments. Vessels were placed in darkness close to ambient temperatures (15°C for the Rote Mulde experiment and 20°C for both Rappbode experiments).

TIC concentrations were monitored monthly during incubation using a 20 mL glass syringe with luer-lock connection. Prior to sampling, vessels were shaken vigorously and the same volume of CO_2-free air was injected. To ensure that sufficient carbon could be harvested a minimum TIC concentration of 0.5 mg L^{-1} was considered necessary. Incubation times resulted in 91 (A) and 125 days (B) for Rappbode, and 131 days for Rote Mulde experiment.

Final samples for TIC, DOC and bacterial biomass (fixed with 5% Lugol's solution) were taken before CO_2-recovery. Vessels were acidified to pH ~2.5 (37% HCl) and were gently aerated with UHP nitrogen for at least 4 h. The tubes of the shorter glass rods of the two vessels used for each treatment and each control were joined. Outstripped CO_2 was extracted by transferring the gas through saturated $Ba(OH)_2$ solution (pH 12.7) according to Tittel et al. (2013). Volumes were determined by weighing the full and empty vessels. Carbonate precipitates were washed with N_2-aerated, high-purity water under forming gas (5% H_2 in N_2) atmosphere before drying at 60°C under N_2 aeration.

About 0.5 mg C of the source DOC and dried carbonates were weighed and placed in tin capsules before being sent for $\delta^{13}C$ analysis via isotope-ratio mass spectrometry to the Stable Isotope Facility Davis (USA). Carbonates of the treatments were sent for $\Delta^{14}C$ analysis by accelerator mass spectrometry to the Poznan Radiocarbon Laboratory (Poland). Samples for $\delta^{13}C$ and $\Delta^{14}C$ were analyzed with respect to VPDB and oxalic acid II standard, respectively, and are reported according to standard notations (Stuiver and Polach, 1977). Reported isotope values are individual measurements. Bacterial biomass was determined as described in Kamjunke et al. (2005).

The measured $\Delta^{14}C$ values of the treatments were corrected for intruded atmospheric CO_2. It was assumed that an equal amount of TIC as measured in the controls intruded as CO_2 into the treatments. Using a simple, two-source mixing model, the isotopic signature of the respiratory CO_2 ($\Delta^{14}C_{respired}$) was calculated as:

$$\Delta^{14}C_{respired} = \frac{TIC_{treat} * \Delta^{14}C_{treat} - TIC_{con} * \Delta^{14}C_{atm}}{TIC_{treat} - TIC_{con}} \tag{1}$$

where $\Delta^{14}C_{treat}$ was the measured $\Delta^{14}C$ of the treatments. The $\Delta^{14}C_{atm}$ represented the $\Delta^{14}C$ of atmospheric CO_2 (21.5‰; Tittel et al., 2015). TIC_{treat} and TIC_{con} were the final TIC concentrations measured in treatments and controls, respectively. The correction resulted in ^{14}C-depleted respiratory CO_2 (Table 1).

Source DOC in both Rappbode experiments was modern (i.e. contained OC produced after 1950); while it had a conventional radiocarbon age (CRA) of 48 years before present (B.P., i.e. before 1950) in the Rote Mulde experiment. The recovered CO_2 was older than the source DOC in all experiments (CRA 58-166 years B.P., Table 1), demonstrating a selectively older fraction of the substrates being respired. A respiration of considerably older carbon was also found by McCallister and del Giorgio (2012). DOC degradation in the treatments was 13 to 20%. Final bacterial biomasses were higher in treatments than in controls (Table 1), indicating no substantial bacterial growth in controls.

The presented simplified method allows the assessment of respiratory CO_2 in replicate incubations at in-situ temperatures. A re-oxygenation step (McCallister et al., 2006) is omitted; avoiding potential effects of changes in redox conditions on source DOC. A small inoculum and long incubation time allow the bacterial community to adapt to the substrates in contrast to large inocula and short incubation times (McCallister et al., 2006). The Incubation time of 91-131 days is representative for residence times of many inland waters and was still shorter than in other degradation experiments (Bastviken et al., 2004, Kalbitz et al., 2003, Rossel et al., 2013). Bacterial growth efficiencies are difficult to interpret over this long period due to increasing importance of loss processes, but they ranged from 7 to 24%, indicating actively growing cells.

Table 1

Results from three incubation experiments using DOC from the Rappbode and Rote Mulde pre-reservoirs. Values of $\Delta^{14}C$ (analytical error 3‰) and $\delta^{13}C$ (analytical error 0.09‰) are individual measurements of the source DOC and recovered CO_2. Values of respiratory CO_2 ($\Delta^{14}C_{respired}$) were derived by correction for intruded atmospheric CO_2 (see text). n.d. = not determined.

		Start DOC [mg L^{-1}]	Final DOC [mg L^{-1}]	Bacterial biomass [µg C L^{-1}]	Final TIC [mg L^{-1}]	$\delta^{13}C$ [‰]	$\Delta^{14}C$ [‰]	$\Delta^{14}C_{respired}$ [‰]
Rappbode A	Source DOC					-28.7	4	
	Control	0.3	0.3	1.5	0.1	-20.1	n.d.	
	Treatment 1	4.9	4.1	4.9	0.6	-27.0	-26	-36
	Treatment 2	4.7	3.9	5.6	0.5	-26.6	-28	-40
Rappbode B	Source DOC					-28.9	13	
	Control	0.4	0.4	3.3	0.2	-19.8	n.d.	
	Treatment 1	5.2	4.3	3.2	0.8	-27.4	-15	-27
	Treatment 2	5.3	4.2	4.5	0.7	-26.7	-18	-34
Rote Mulde	Source DOC					-27.3	-14	
	Control	0.4	0.2	2.5	0.2	-17.2	n.d.	
	Treatment 1	9.3	8.0	13.4	0.8	-28.5	-27	-43
	Treatment 2	9.4	8.0	11.7	0.8	-25.4	-21	-35

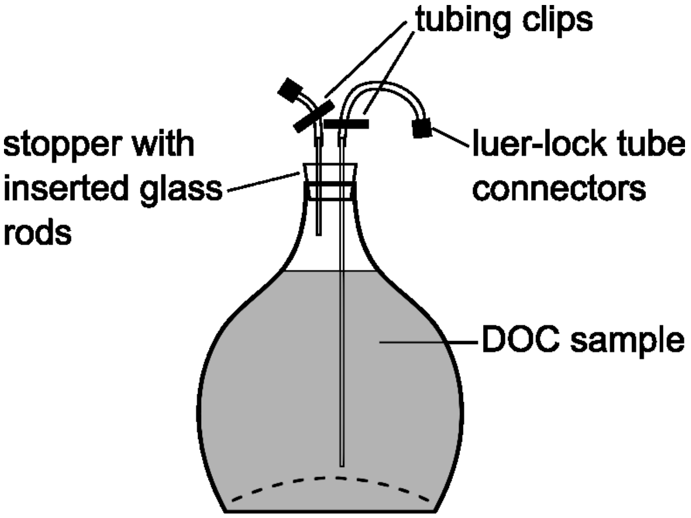
Figure legends

Fig. 1

Experimental setup consisting of commercially available 10 L glass demijohns, which contained the filtered DOC sample and the natural bacterial inoculum. See text for details.

Figures

Fig. 1



Conflict of interest

The authors declare no conflicts of interest.

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Manuscript II

Discharge determines production of, decomposition of and quality changes in dissolved organic carbon in pre-dams of drinking water reservoirs

Morling, K., Herzsprung, P., Kamjunke, N., 2017. Discharge determines production of, decomposition of and quality changes in dissolved organic carbon in pre-dams of drinking water reservoirs. Science of the Total Environment 577, 329-339. http://dx.doi.org/10.1016/j.scitotenv.2016.10.192

Discharge determines production of, decomposition of and quality changes in dissolved organic carbon in pre-dams of drinking water reservoirs

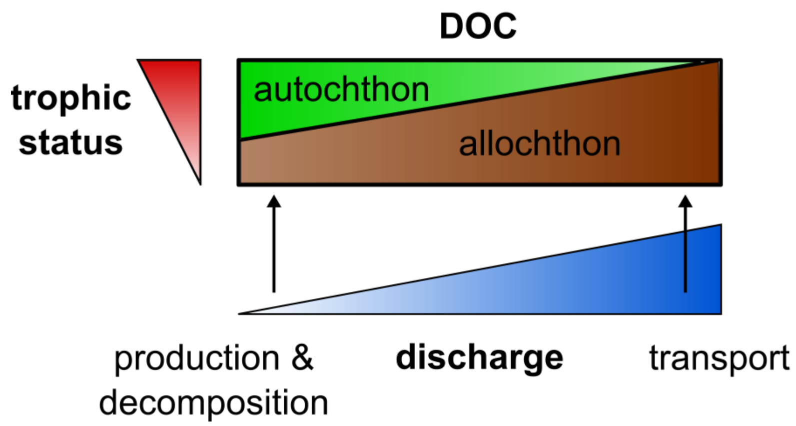
Karoline Morling[a]*, Peter Herzsprung[a], Norbert Kamjunke[a,b]

UFZ – Helmholtz Centre for Environmental Research, Brückstr. 3a, 39114 Magdeburg, Germany
[a]Department of Lake Research
[b]Department of River Ecology

*corresponding author: Tel.: +49 391 8109447, email: karoline.morling@ufz.de

Key words: Carbon isotopes, ^{14}C, Autotrophy, Microbial decomposition, Fluorescence, Net production

Graphical abstract



Highlights

- A proportion of 0-30% of the total gained OC was produced within the pre-dams
- Ratio of OC production to total OC gain was negatively related to discharge
- Proportion of algae-derived DOC (β/α index) decreased with higher discharges
- Heterotrophic bacteria utilized older DOC under base flow conditions

Abstract

Pre-dams are small reservoirs constructed upstream of the main drinking water reservoirs and are used for nutrient removal and sediment trapping. Little is known about the role of pre-dams regarding the production and decomposition of dissolved organic carbon (DOC) in relation to discharge and how this affects the quality of DOC in the water. We combined quantitative and qualitative investigations under different hydrological conditions at three pre-dams exhibiting a gradient from oligotrophic/high-DOC to eutrophic/low-DOC. All pre-dams were mainly autotrophic in their upper water layers. The ratio of OC production to total gained OC (i.e. OC import + OC production) decreased with increasing discharge. On average, 0-30% of the total gained OC was produced within the pre-dams. The amount of microbially decomposed DOC increased with the average water residence time (WRT) and with the trophic status of the pre-dams. Radiocarbon analyses of respired CO_2 revealed that heterotrophic bacteria preferentially utilized old DOC components (195-395 years before present) under base flow conditions, whereas younger components (modern, i.e. OC produced after 1950) were utilized at high discharge. DOC quality changed significantly over the year within the pre-dams: High proportions of algae-derived DOC were observed during base flow in summer, and the freshness index (β/α ratio) decreased significantly with higher discharges. DOC production and quality changes in response to hydrological conditions should be considered for future water quality management in reservoirs, as climate scenarios for temperate regions predict decreased runoffs leading to longer WRT and increased eutrophication and production of algae-derived OC.

1. Introduction

The majority of organic carbon (OC) in inland waters is in dissolved form (Wetzel, 2001) and originates from external (i.e. allochthonous; Aitkenhead-Peterson et al., 2003) and internal (i.e. autochthonous; Bertilsson and Jones, 2003) sources. Over the last few decades, concentrations of dissolved organic carbon (DOC) have been increasing in freshwaters in the northern hemisphere (Evans et al., 2005; Monteith et al., 2007), leading to a browning of inland waters. This increase has been attributed to several causes such as decreased acid deposition (Monteith et al., 2007), increased terrestrial primary production (Freeman et al., 2004; Hagedorn et al., 2008), changes in catchment hydrology (Evans et al., 2005; Schindler et al., 1997), land use changes (Mattsson et al., 2005), and increasing iron concentrations (Kritzberg and Ekström, 2012; Weyhenmeyer et al., 2014) due to the release of formerly adsorbed soil organic compounds by increased reduction of Fe-oxyhydroxides (Knorr, 2013; Lambert et al., 2013). Elevated DOC concentrations affect the lake ecosystem structure (reviewed by Solomon et al., 2015) at both the abiotic level (e.g. changes in light attenuation and heat balance; Lean, 1998; Zwart et al., 2016) and the biotic level (e.g. planktonic species composition; Jansson et al., 2000), as well as lake metabolism and its biomass production (Ask et al., 2009; Hanson et al., 2003; Karlsson et al., 2015). DOC cycles in freshwaters as it is simultaneously mineralized by heterotrophic bacteria (Eiler et al., 2003; Franke et al., 2013; Tranvik, 1998), produced and released by photosynthetic organisms (Børsheim et al., 2005; Fogg et al., 1965; Larsson and Hagström, 1979) or transformed by chemical pathways such as photo-reactions (Bertilsson and Tranvik, 2000; Granéli et al., 1996; Tranvik, 1996). DOC quality in freshwaters is affected by hydrological conditions as well. For instance, old carbon (100 to 1,000 of years) is introduced from soils into freshwaters during base flow conditions (Schiff et al., 1997; Trumbore, 2009), while OC derived from recent terrestrial vegetation is often transported during rain events or snow melts (Raymond et al., 2007; Schiff et al., 1997).

Reservoirs alter the DOC load and DOC quality. They can be both sinks and sources of DOC during different times of the year (Kraus et al., 2011). Likewise, reservoirs can be both sources and sinks of CO_2 depending on hydrological conditions, showing reduced CO_2 evasion under low-flow conditions during dry summer periods (Knoll et al., 2013). Parks and Baker (1997) observed that reservoirs in arid regions can be a significant source of autochthonously derived DOC. Nearly one-third of the exported DOC was produced within their studied reservoirs, although these accounted to < 1% of the watershed area. In contrast, Romero-Martínez et al. (2013) observed net heterotrophy in the mixed layers of two monomictic Mediterranean reservoirs. Such diverse results underline that lakes and reservoirs are hotspots of carbon cycling (Cole et al., 2007; Tranvik et al., 2009) and indicate the importance of hydrological conditions and lake turnover time for DOC dynamics in inland waters (Dalzell et al., 2007; Köhler et al., 2013; Lambert et al., 2013; Raymond and Saiers, 2010). High DOC concentrations cause problems in drinking water production (Ledesma et al., 2012; Matilainen and Sillanpää, 2010) by increasing the amount of precipitants to remove DOC, shortening filter operation times and forming potentially carcinogenic disinfection by-products (DBP) during the purification treatments (Chow et al., 2003; Jeong et al., 2012; Lavonen et al., 2013; Matilainen et al., 2010).

In Germany, drinking water reservoirs are predominantly constructed in low mountain ranges and are usually built with pre-dams. Pre-dams are small reservoirs constructed upstream of the main reservoirs and have typically low water residence times (WRT) of a few days or weeks. Pre-dams are constructed with a surface overflow similar to natural lakes and are used for sediment trapping and nutrient removal (Benndorf and Pütz, 1987a; Benndorf and Pütz, 1987b; Paul, 2003; Pütz and Benndorf, 1998). Thus, they represent an important tool for reservoir water quality management (Pütz and Benndorf, 1998; Zhang et al., 2011). A high carbon turnover might be expected within pre-dams, as the water is impounded after a short travel time of a few days from the headwaters. However, to our knowledge, a study investigating the role of pre-dams regarding the production and decomposition of OC is missing to date. Hydrological conditions greatly influence the WRT in these systems, and discharge is therefore expected to have a strong impact on OC decomposition and production within and the export from the pre-dams (into the main reservoirs). The upper 3 m of the water column (commonly corresponding to the euphotic zone) is the zone of phytoplankton growth, nutrient cycling and water transport during stratification. Therefore, we focused on the processes taking place within the upper 3 m of the water column of three pre-dams which differed in DOC concentrations and trophic status.

We addressed the following hypotheses: (1) higher trophy (higher P concentrations) and/or low discharge lead to considerably higher amounts of autochthonously produced OC, (2) higher P concentrations and longer WRT within the pre-dams increase the amount of DOC decomposed by heterotrophic microbes, and (3) the proportion of algae-derived DOC to total DOC is negatively related to discharge. Further, we hypothesized that (4) the age of microbially utilized DOC is dependent on discharge conditions, with older carbon preferentially decomposed at low discharge. To investigate these hypotheses, we conducted a combination of quantitative and qualitative investigations such as in-situ oxygen production and consumption measurements, lab incubation experiments, radiocarbon analyses and fluorescence measurements to characterize the production, decomposition and quality of the DOC within the pre-dams.

2. Methods

2.1 Study sites and sampling

All three study pre-dams belong to drinking water reservoirs located in Germany and exhibit a gradient in DOC and phosphorus concentrations (Table 1). The oligotrophic and DOC-rich Rote Mulde pre-dam (Tittel et al., 2013) is located in the Western Ore Mountains. The meso- and eutrophic Rappbode and Hassel pre-dams (Friese et al., 2014; Rinke et al., 2013) show considerably lower DOC concentrations (Table 1) and are part of the Rappbode reservoir in the Harz Mountains and the TERENO (terrestrial environmental observatories) network in Germany (Zacharias et al., 2011). The land use within the catchments of the three pre-dams range from almost entirely forestry-dominated landscape around the oligotrophic Rote Mulde pre-dam to agriculture-dominated landscape around the eutrophic Hassel pre-dam (Table 1).

A total of six sampling campaigns were conducted in each pre-dam along a gradient of hydrological and temperature conditions (Table S1). Campaigns took place in the Hassel pre-dam in 2013 (from April to November) and in the Rappbode pre-dam in 2014 (from March to October) due to alternating construction work at the dams. In the Rote Mulde pre-dam, campaigns took place in both years (three times each year, from April to September). Throughout the campaigns, net production and respiration were measured (see Section 2.2) and water samples were taken from the inflows and the water columns of the pre-dams for chemical analyses. Water samples were also taken for decomposition and respiration experiments (see Sections 2.4 and 2.5). Temperature profiles were recorded with multi-parameter probes (CTD 90 M, Sea & Sun Technology; Ocean Seven 316 CTD, Idronaut). Discharge was measured during the campaigns at the inflows of the pre-dams as described in Kamjunke et al. (2016) using table salt as a conservative tracer. Data on fluorescence (see Section 2.6) were available from biweekly monitoring of the two pre-dams located in the Harz Mountains. Fluorescence measurements were related to further discharge data which were provided by the reservoir management authority “Talsperrenbetrieb Sachsen-Anhalt”. For comparison, discharge data were normalized to the catchment areas and expressed in mm d^{-1}.

2.2 Measurements of net production and respiration

In-situ measurements of net production and community respiration were conducted during every sampling campaign. The Winkler method was used as described by Carignan et al. (1998). Bottles used for obtaining the oxygen production and consumption rates were exposed close to the dam walls in the upper 3 m of the water column. This depth corresponds to the euphotic zone in the Rappbode and Hassel pre-dams and to almost the total water column of the shallow Rote Mulde pre-dam (Table 1). Water samples were taken at depths of 0.5, 1.5 and 2.5 m using a 5 L Limnos water sampler (Turku, Finland) and filled into triplicate Karlsruhe BOD bottles made of glass via a tube. The bottles were kept in covered boxes to avoid exposing them to sunlight. Bottles for dark incubations were wrapped in aluminum foil prior to sampling and additionally around their top subsequently to placing the stoppers. Bottles were fastened at their necks in customized acrylic glass frames and placed at the three depths for an incubation duration of 3 to 4 h around noon. The Winkler reagents were immediately added to the triplicate bottles used for the start concentrations of oxygen and were placed in cooling boxes filled with reservoir water to avoid temperature changes. After incubation, both the clear and the dark bottles were collected from the depths and the Winkler reagents were added immediately. The bottles were placed in cooling boxes and transported to the lab within 2 h, where they were placed in a climate room at in-situ temperatures corresponding to the water at 0.5 m depth. Titrations were conducted in the climate room within 24 h over a 3 h interval using a Metrohm titrator (736 GP Titrino, Herisau, Switzerland) equipped with a 10 mL burette and a Pt electrode. All reagents were prepared and oxygen concentrations were calculated as described in Carignan et al. (1998). Net production (NP) was calculated as the difference between the averages of triplicate clear and start bottles. Dark respiration (Resp) was calculated analogously as the difference between the averages of triplicate start and dark bottles. For conversion of oxygen production and consumption rates into carbon equivalents, a photosynthetic quotient of 1.25 (Carignan et al., 2000) and a respiration quotient of 1 were used. The use of triplicate bottles allowed the calculation of standard errors which were applied to the following calculations using error propagation rules. Daily values of NP [mg C m^{-3} d^{-1}] were calculated by multiplication with a light factor (total daily irradiance divided by the irradiance during the incubation period) (Larsson and Hagström, 1982). Irradiance was measured with HOBO® Pendant Temperature/Light Data Loggers UA-002-64 (Onset Computer Corporation). For calculation of photosynthetically active radiation (PAR), total daily irradiance was multiplied with empirically derived conversion factors, obtained from subsurface measurements with an underwater quantum sensor (Li-Cor LI-1000-32). For calculating daily values of Resp [mg C m^{-3} d^{-1}], it was assumed that respiration rates were equal during day and night. Both NP and Resp were converted into depth-integrated (0-3 m) daily values [mg C m^{-2} d^{-1}], and gross production (GP) was calculated as the sum of NP and Resp.

2.3 Calculation of OC gained by the pre-dams and OC turnover

For calculating the phototrophic OC production within the upper 3 m [kg d^{-1}], GP and Resp were converted into volumetric values (GP_{vol} and $Resp_{vol}$ [kg d^{-1}]) using the volume-depth-relationships of the pre-dams. OC production was then calculated as:

$$OC\ production = GP_{vol} - Resp_{vol} \tag{1}$$

The allochthonous OC import to the pre-dams [kg d^{-1}] was calculated as the product of the total organic carbon concentration at the inflows of the pre-dams (TOC_{in} [mg L^{-1}]) and the discharge (Q [L d^{-1}]):

$$OC\ import = TOC_{in} \times Q \tag{2}$$

The OC gain [kg d^{-1}] represents the sum of OC import and OC production:

$$OC\ gain = OC\ import + OC\ production \tag{3}$$

It comprises the total amount of OC received by the pre-dams as allochthonous material derived from the catchments and autochthonously produced within the pre-dams. The ratio of OC production to total OC gain was related as a function of discharge.

The stock of OC [t] present in the pre-dams was calculated as the sum of the DOC and algae-derived carbon by using the volume-depth-relationships of the pre-dams. DOC and Chlorophyll *a* samples were taken from the water columns during the sampling campaigns. Algae-derived carbon was estimated from Chlorophyll *a* samples assuming an average C:Chl *a* ratio of 50:1 (Coveney and Wetzel, 1995; Yacobi and Zohary, 2010). OC turnover [yr^{-1}] was then derived by dividing the OC production by the OC stock.

2.4 Decomposition experiments

Lab incubations were performed in order to assess the fraction of DOC which is potentially decomposed by heterotrophic bacteria. Water taken from the pre-dams (0.5 m) during each sampling campaign was filtered through pre-combusted (4 h at 450 °C) glass fiber filters (GF/F, Whatman). Subsamples of 300 mL were given into triplicate Erlenmeyer flasks. For the inoculum, water taken from the pre-dams was filtered through polycarbonate filters (0.8 µm pore size, Nuclepore). Each flask was inoculated with 3 mL of the filtrate. Samples for start DOC concentrations were taken. Flasks were wrapped with aluminum foil to keep them dark and the cultures were incubated at ca. 20 °C in the lab for approximately 6 months (182-209 days). Then final DOC concentrations were analyzed and the DOC decomposition was calculated as a percentage of the difference between DOC concentrations at the beginning and end of the incubations. The percentage of microbially decomposed DOC was related to the average WRT of the pre-dams.

2.5 Respiration experiments

To obtain radiocarbon ages of microbially respired CO_2 during DOC decomposition, experiments were carried out with water taken from the pre-dams Rappbode and Rote Mulde from April to September 2014 as according to Morling et al. (2016). In brief, approximately 60 L of water was taken from the pre-dams (1.5 m depth) and transported to the lab where the water samples were sterilized through filtration (0.2 µm), and subsamples for carbon isotope analysis of the start DOC were taken and enriched as described in Tittel et al. (2013). The filtered water was acidified to pH ~ 2 and aerated overnight with CO_2-free air to remove ambient inorganic carbon (IC). The pH was then adjusted to initial values and inoculated with a natural bacterial assemblage taken from the pre-dams (filtered through 0.8 µm pore size polycarbonate filters). Incubations were conducted in duplicates at in-situ temperatures for 91 to 279 days, depending on the amount of produced IC. To harvest the microbial respired carbon, the treatments were acidified (pH ~ 2) and aerated with N_2; the released CO_2 was precipitated in saturated $Ba(OH)_2$-solution. The carbonate precipitates were washed with deionized water and dried (60 °C) before being sent away for ^{14}C analyses. Carbonates were analyzed by accelerator mass spectrometry at the Poznan Radiocarbon Laboratory (Poland). Samples were analyzed with respect to the standard oxalic acid II and are reported according to standard notations (Stuiver and Polach, 1977).

2.6 Sample analyses and fluorescence measurements

For determination of DOC concentrations, the samples were filtered through pre-combusted (4 h at 450 °C) glass fiber filters (GF/F, Whatman) prior to analysis. DOC samples were analyzed by infrared detection using a DIMATOC® 2000 (Dimatec, Germany). Samples taken from the inflows and the water columns of the pre-dams were also analyzed for

Chlorophyll *a*, total phosphorus and nitrate following standard methods as described in Friese et al. (2014).

Excitation-emission-matrix fluorescence spectroscopy (EEM) measurements were performed on water samples obtained during the biweekly monitoring samplings at the Hassel and Rappbode pre-dams in 2013 and 2014. Fluorescence EEMs of filtered samples (GF/F, Whatman) were obtained using a spectrofluorometer (AQUALOG®, HORIBA Jobin Yvon, USA) with the intensity measured during emission scans (240 nm-600 nm every 3.27 nm, 8 pixel) at excitation wavelengths in 3 nm increments from 240 nm to 600 nm. A 5 nm bandpass for excitation and emission wavelength and 1 s integration time were used. Fluorescence data were corrected (including blank subtraction) using HORIBA internal software. The EEMF spectra were Rayleigh-scattering masked and corrected for inner-filter effects using an AQUALOG® internal recorded UV-Vis absorbance spectrum in the same quartz cell fluorescent sample. Two fluorescence indices were used to characterize the DOC quality. The freshness index β/α, which represents an indicator of autochthonous inputs and of recently produced DOC (Fellman et al., 2010; Halbedel et al., 2013; Kamjunke et al., 2015; McKnight et al., 2001), was calculated as the ratio of the emission intensity at 380 nm (β) divided by the highest detected emission intensity between 420 and 435 nm (α), all obtained for an excitation at 310 nm. The humification index (HIX) is a measure of the complexity and the condensed (aromatic) nature of DOC compounds (Ohno et al., 2007). Spearman’s rank correlation showed that it is correlated with an abundance of oxygen-rich and relatively unsaturated components (polyphenol-like components) (Lavonen et al., 2015). The HIX was calculated by dividing the peak area under the emission spectra between 435 and 480 nm with the peak area under the emission spectra between 300 and 345 nm, both at 255 nm excitation wavelength (Kamjunke et al., 2015; Zsolnay, 2003).

2.7 Statistics

Discharge data [mm d^{-1}] were log-transformed to base 10 prior to statistical analyses. These included linear regressions and t-tests and were performed using the program R, version 2.15.2 (R Core Team, 2012). To identify which variable classes were determining net production and respiration, we performed partial least square (PLS) regression analyses for two components using the package “plsdepot” in R.

3. Results

3.1 Production and respiration in the upper water column

The comparison of GP and Resp revealed that all three pre-dams were predominantly autotrophic (Resp < GP) in the upper water columns (0-3 m) (Fig. 1A), indicating that more carbon was photosynthetically fixed than decomposed in this layer. The lowest values of GP and Resp were observed in the oligotrophic Rote Mulde pre-dam; the meso- to eutrophic Rappbode and Hassel pre-dams showed the highest values of GP and Resp (Fig. 1A). NP and Resp were not significantly correlated to total phosphorus and nitrate concentrations ($p > 0.22$, data not shown). Both NP and Resp significantly correlated to mean water temperature (calculated as depth-weighted average of the temperatures in the upper 3 m) and discharge ($p < 0.05$, Fig. 1B to 1E). NP was also controlled by PAR ($p < 0.004$, Fig. 1F). The PLS analysis of NP revealed that discharge, temperature and PAR (Fig. 1G) were associated with the first component (explaining 68% of the variance). Thereby, temperature and PAR were highly correlated, whereas discharge was oppositely related to them. Total phosphorus (TP) and nitrate were highly correlated with the second component, which explained only a minor fraction of variance (4%). Likewise, the PLS analysis of Resp identified that discharge and temperature were correlated with the first component (explaining 63% of variance) and were oppositely correlated as well (Fig. 1H). TP was strongly related to the second component (which explained only 2% of the variance), while nitrate was of minor importance.

3.2 OC gain and DOC balance

OC import was highest in the Hassel pre-dam in April (during snow melt) and autumn 2013, and during a rain event in the Rote Mulde pre-dam in September 2014 (Fig. 2A). OC production was highest in the Hassel and Rappbode pre-dams during summer, and exceeded the OC import in both pre-dams in July (Fig. 2A). The OC production was very low in the oligotrophic Rote Mulde pre-dam (not visible in Fig. 2A) and ranged from 0 to 14% of the total OC gain.

A considerable OC removal (i.e. negative OC production) was observed during the sampling in the Rappbode pre-dam in April 2014 (Fig. 2A). Negligible OC removal (not visible in Fig. 2A) was observed in the Hassel pre-dam in November 2013 and in the Rote Mulde pre-dam in April 2013 and September 2014. The ratio of OC production to OC gain was highest at low discharges and high temperatures in summer (Fig. 2B). Production ratios of up to 98% and 81% were observed at low discharges in the eutrophic Hassel pre-dam and in the mesotrophic Rappbode pre-dam, respectively. The ratio decreased with increasing discharge (Fig. 2B, linear regression with log-transformed discharge data, $R^2 = 0.54$, $p < 0.001$). The annual average of OC production (as well as OC import) was highest in the eutrophic Hassel pre-dam (Table 2). The lowest annual average OC production was obtained for the Rote Mulde pre-dam, while the OC import was lowest in Rappbode pre-dam (Table 2). About 0-30% of the total OC gain was produced within the three pre-dams. The average OC stock and OC turnover was lowest in the Rote Mulde pre-dam (Table 2) due to its smaller volume and low production. The OC stock was highest in the eutrophic Hassel pre-dam, which is the pre-dam with the largest volume. Though, OC turnover was highest in the Rappbode pre-dam (Table 2).

3.3 Decomposition experiments

The percentage of microbially decomposable DOC differed between the three pre-dams and their average WRT (Fig. 3). For the oligotrophic and DOC-rich Rote Mulde pre-dam, which also shows the lowest average WRT, a significantly lower DOC decomposition was observed in comparison to the other two pre-dams (Fig. 3, t-Test, $p < 0.027$). The highest decomposition was observed for the eutrophic Hassel pre-dam, which has the highest average WRT and which also showed the highest level of autotrophy (Fig. 1A). The overall mean of DOC decomposition (± standard deviation) for all pre-dams amounted to 18% ± 11%.

3.4 Respiration experiments

In two experiments, the start DOC was old (Fig. 4, negative $\Delta^{14}C$ signals), with conventional radiocarbon ages (CRA) of 111 and 128 years before present (B.P., i.e. before 1950). In the other experiments, the start DOC was of modern origin (Fig. 4, positive $\Delta^{14}C$ signals), i.e. it contained bomb-carbon derived from nuclear testing after 1950. The microbial respired CO_2 was considerably older than the start DOC in most cases ($\Delta^{14}C$ signals below the 1:1 line in Fig. 4), with CRA ranging from 195 to 395 years B.P. These experiments were conducted during low discharge conditions ($Q < 0.6$ mm d^{-1}). DOC of modern origin was utilized and respired in only one experiment ($\Delta^{14}C$ signals above the 1:1 line in Fig. 4), which was conducted after a rain event (Q: 3.3. mm d^{-1}) at the Rote Mulde pre-dam in September 2014. Similar to the results observed in the decomposition experiments (Fig. 3), the DOC decomposition in all conducted respiration experiments ranged from 7% to 21%.

3.5 DOC quality

DOC quality in the water columns in both the Hassel and the Rappbode pre-dam changed over during the course of the study years (Fig. 5). In the Hassel pre-dam, the β/α ratio increased and the HIX decreased within the upper water column under base flow conditions during summer 2013 (Fig. 5A, C), indicating that more autochthonous material was produced. This pattern was not pronounced to the same extent in the Rappbode pre-dam in 2014 (Fig. 5B, D). The β/α ratio decreased significantly with increasing discharge (Fig. 6A, B; linear regressions, $R^2 \geq 0.36$, $p < 0.001$). In contrast, the HIX index increased significantly with discharge only in the Rappbode pre-dam (Fig. 6D; linear regression, $R^2 = 0.24$, $p < 0.008$), but not in Hassel pre-dam (Fig. 6C; $p = 0.32$).

4. Discussion

The three studied pre-dams were found to be mainly autotrophic in their upper water columns. OC production was related to discharge conditions. The amounts of imported and produced OC varied between the three pre-dams and seasons. Although net production and respiration were not significantly related to P concentrations in our dataset, a higher degree of autotrophy and a higher amount of produced OC was observed in the mesotrophic Rappbode and the eutrophic Hassel pre-dams, confirming hypothesis (1).

The obtained rates for net production and community respiration in our three pre-dams matched previously published values for both eutrophic (Knoll et al., 2003; Romero-Martínez et al., 2013) and oligotrophic systems (Carignan et al., 2000; del Giorgio and Peters, 1994; Seekell et al., 2015). The autotrophic behavior of all three pre-dams is quite different from that of lakes, which tend towards net heterotrophy (Cole et al., 1994; Duarte and Prairie, 2005). Especially DOC-rich, nutrient-poor lakes were found to be net heterotrophic (Cole et al., 2000; Jansson et al., 2000), and a DOC threshold of 4-6 mg L^{-1} for lakes was proposed (Hanson et al., 2003; Prairie et al., 2002; Seekell et al., 2015) to indicate the boundary between autotrophic and heterotrophic systems. However, the autotrophic behavior of the oligotrophic and DOC-rich Rote Mulde pre-dam is in agreement with previous studies (Carignan et al., 2000; Forget et al., 2009) which demonstrated that also oligotrophic lakes can be autotrophic in their pelagic zones. NP and Resp significantly decreased with increasing discharge, a fact which can be attributed to the development of planktonic communities under conditions of base flow and higher trophy. High discharges lead to a wash-out of the planktonic community. Our results are in accordance to findings in Canadian lakes, where summer excess respiration was positively related to water retention time (del Giorgio and Peters, 1994). Similarly, previous studies found that higher amounts of OC in lakes were lost by mineralization and sedimentation with increasing WRT (Algesten et al., 2004, Köhler et al., 2013).

Microbial decomposition differed between the three study pre-dams in relation to their average WRT. Decomposition was lowest in the oligotrophic and DOC-rich Rote Mulde pre-dam, which is the impoundment with the shortest average WRT. In contrast, DOC decomposition was highest in the eutrophic Hassel pre-dam, which exhibits the longest average WRT. The pH was not significantly correlated with microbial decomposition ($p = 0.11$, data not shown). This indicates that the lower pH conditions in the Rote Mulde pre-dam were not affecting microbial activity. This is in agreement with studies in acidic mining lakes, showing that microbial production is not inhibited by low pH conditions (Kamjunke et al., 2005). A higher decomposition can be attributed to higher nutrient concentrations facilitating microbial mineralization and/or to higher amounts of autochthonously derived DOC. Both the Rappbode and the Hassel pre-dams are usually enriched in P concentrations compared to the Rote Mulde pre-dam and showed higher rates and higher amounts of OC production. These two pre-dams also have longer WRT, allowing the development of more stable plankton communities and higher amounts of released algae-DOC. Thus, hypothesis (2) was confirmed. Algae-derived carbon is considered to be more labile and preferentially utilized by heterotrophic microbes (Chen and Wangersky, 1996; Guillemette et al., 2013; Kritzberg et al., 2004). Further, the occurrence of aquatic priming might have promoted the decomposition in the incubations of the Hassel and Rappbode pre-dams. Aquatic priming refers to an increased microbial mineralization of refractory terrestrial carbon compounds in presence of easily decomposable labile carbon substrates (Guenet et al., 2010). However, aquatic priming was observed in some studies (Bianchi et al., 2015; Guenet et al., 2014; Kuehn et al., 2014) and not in others (Bengtsson et al., 2014; Catalán et al., 2015; Dorado-García et al., 2016), allowing no clear answer as to whether priming is a mechanism potentially responsible for increased microbial decomposition occurring in aquatic systems.

Mostly older DOC components were utilized and respired by heterotrophic microbes under base flow conditions in our experiments, giving further evidence that age and recalcitrance are decoupled (McCallister and del Giorgio, 2012; Petsch et al., 2001) and confirming hypothesis (4). OC of considerable age (100 to 1000 of years) in aquatic systems could be derived from hydrological connection of deeper soil layers during base flow or groundwater entries (Schiff et al., 1997; Trumbore, 2009; Trumbore et al., 1992). Another pathway is given by imports of old dissolved inorganic carbon (DIC) originating from carbonate weathering (Casas-Ruiz et al., 2015; Ishikawa et al., 2015b; Marcé et al., 2015) and which is taken up by primary producers and incorporated into cells (Ishikawa et al., 2015a; Ishikawa et al., 2016). Subsequently it can be released as old DOC through active excretion by the algae, via cell lysis or by herbivory (Ishikawa et al., 2016). A substantial accumulation of old DOC via this pathway could be expected only when autotrophic production is high, i.e. preferably under base flow (i.e. longer WRT) and high nutrient supply in summer. Both modern and old DIC and DOC were found in the tributaries of the Hassel and Rappbode pre-dams in a previous survey in 2012 (Tittel et al., 2015), confirming that old carbon cycles in these systems and that carbonate weathering is a likely source of the old carbon found in our experiments. In contrast, the tributary of the Rote Mulde pre-dam showed modern DIC entries, but old DOC in an earlier study (Tittel et al., 2013). This indicates that it is unlikely that old carbon originates from carbonate weathering in this system, but rather is derived from groundwater or aged soil carbon. However, modern carbon (most probably derived from recent terrestrial vegetation) was flushed into the Rote Mulde pre-dam during a rain event in September 2014 (Q: 3.3 mm d^{-1}), and was utilized and respired by heterotrophic bacteria during decomposition.

DOC quality changed considerably within the studied pre-dams, as indicated by higher β/α ratios which are associated with recently fixed organic matter (Fellman et al., 2010; Hansen et al., 2016). The β/α index decreased significantly with increasing discharge, indicating a higher production of autochthonously derived DOC during base flow conditions in summer and confirming hypothesis (3). This can be related to a decomposition of terrestrially derived OC, which is replaced by algae-derived OC (Catalán et al., 2014; Köhler et al, 2013; Kraus et al., 2011; Weyhenmeyer et al., 2012). Greater contributions of humic-like substances during rainy periods and increases in HIX with increasing flow rate (Inamdar et al., 2011; Yang et al., 2015; Zhou et al., 2016) can be attributed with usually high amounts of terrestrial DOC entering inland waters during storm flow events (Dalzell et al., 2007; Raymond and Saiers, 2010). The HIX increased significantly with discharge in the Rappbode pre-dam but not in the Hassel pre-dam. This difference could be related to the different land use within the catchments, influencing the DOC quality of the water flowing into these two pre-dams. The proportion of forests was found to be positively related to the content of humic substances within the Bode catchment to which both pre-dams belong (Kamjunke et al., 2013). The Hassel catchment consists of only 37% forest and 58% grasslands and fields, whereas the Rappbode catchment consists of 72% forest and 24% grasslands and fields (Table 1). Thus, land use can account for the observed differences in the relationships between HIX and discharge in these two pre-dams.

It has been acknowledged that characteristics of DOC cycling in inland waters are dependent on land use within the catchment areas, and that they change during their transport from the land to the ocean due to decay of terrestrially derived OC (Weyhenmeyer et al., 2012) and production of autochthonous OC. In the studied pre-dams, an average of 0-30% of the total gained OC was produced within the impoundments, altering the water quality significantly from allochthonous to autochthonous characteristics under base flow conditions. This highlights that small impoundments with typically low WRT are also hot spots of carbon cycling. However, we investigated only a short time period under current climate conditions. Future climate scenarios predict changes in precipitation, runoff and in the amount of extreme rain events, most likely resulting in altered hydrological conditions and changes in WRT of inland waters. Temperate regions will most likely experience drier weather conditions with decreased runoffs and an increase in extreme rain events (Easterling et al., 2000; Tranvik et al., 2009). Less runoff will lead to more droughts, longer WRT and might increase eutrophication (Jeppesen et al., 2009; Tranvik et al., 2009; Trolle et al., 2011), which might then promote the production and transformation of algae-derived OC. Possible management options include the reduction of nutrient inputs to reservoirs by adapting land use (Chantigny, 2003; Schelker et al., 2012; Stanley et al., 2012; Tranvik et al., 2009) and stocking of piscivorous fish to reduce phytoplankton biomass via the trophic cascade, and the improvement of the water quality (Cooke et al., 2005; Drenner and Hambright, 2002; Ha et al., 2013). However, impacts of land use management on DOC are highly uncertain, as most restoration projects have only a small spatial and temporal extent, ignoring a delay between restoration actions and biochemical responses. Therefore, only slight changes are instantly visible (Chantigny, 2003; Stanley et al., 2012). Such mismatches should be considered for future reservoir management under changing hydrological conditions.

5. Conclusion

Pre-dams were found to be important sites of aquatic carbon cycling, depending on discharge conditions and trophic status (Fig. 7). We observed considerable autochthonous OC production and microbial decomposition of DOC in the summer period under higher trophic status and/or base flow conditions. This led to changes in the DOC quality towards more autochthonous characteristics. Under high discharge levels and/or low trophy, allochthonously derived OC passed the pre-dams and was transported into the main reservoirs. Our results emphasize that hydrology and trophic status greatly influence the carbon cycling within pre-dams and highlight the urgency of considering future changes in runoff and nutrient concentrations for the management of water networks.

Conflict of interest

The authors declare no conflict of interest.

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Tables

Table 1

Properties of the three studied pre-dams. Chemical values represent ranges during sampling campaigns in 2013 and 2014.

Pre-dam	**Rote Mulde**	**Rappbode**	**Hassel**
Catchment area [km²]	5.35	39.1	40.5
- forest [%]	98	72	37
- grasslands and fields [%]	1	24	58
- urban areas [%]	1	3	5
Surface area [km²]	0.098	0.218	0.288
Volume [Mio m³]	0.06	1.25	1.45
Mean depth [m]	1.8	5.3	5.0
Maximum depth [m]	4	17	14
Water residence time [days]	6	16	27
pH	5.2-6.6	7.1-7.8	6.8-8.9
Total P [µg L^{-1}]	9.5-33	14-40	22-51
DOC [mg L^{-1}]	5.1-21.1	2.4-5.4	4.2-6.9

Table 2

Average values for OC production (surface normalized values are given in brackets), OC import as well as calculated OC stocks and OC turnover in the three study pre-dams.

Pre-dam	**Rote Mulde**	**Rappbode**	**Hassel**
OC production [kg d^{-1}] (kg d^{-1} km^{-2})	0.3 (3.1)	32.8 (150.5)	48.3 (167.7)
OC import [kg d^{-1}]	114.1	76.9	467.9
OC production/OC gain [%]	0.2	29.9	9.4
OC stock [t]	0.7	3.6	6.9
OC turnover [yr^{-1}]	0.14	3.31	2.56

Figure legends

Fig. 1

Measurements of production and respiration. Symbols are Hassel pre-dam (open circles), Rappbode pre-dam (filled squares) and Rote Mulde pre-dam (grey triangles). A: Comparison of gross production (GP) and community respiration (Resp) in the upper water columns (3 m) of the three pre-dams throughout the study years. Values are depth-integrated daily values ± standard errors. The 1:1 line separates heterotrophic (Resp > GP, above the 1:1 line) and autotrophic (Resp < GP, below the 1:1 line) systems. B-F: Net production and respiration depending on mean water temperature, discharge and photosynthetically active radiation (PAR). Lines represent linear regressions ($p < 0.005$, B: $R^2 = 0.48$, C: $R^2 = 0.50$, D: $R^2 = 0.44$, E: $R^2 = 0.43$, F: $R^2 = 0.39$). G-H: Correlation circles obtained from the PLS analyses of (G) net production and (H) respiration. The closer a variable appears on the perimeter of the circle, the better it is represented. If two variables are highly correlated, they will appear next to each other. If two variables are negatively correlated, they will appear in opposite directions. The percentage of explained variance of the components is given in brackets on each axis.

Fig. 2

Organic carbon gains in the three studied pre-dams. A: OC production within and OC import into the pre-dams. Note different scales for y axes. B: Ratio of OC production to OC gain in the three pre-dams as a function of discharge. The line represents the linear regression line of log transformed discharge data ($R^2 = 0.54$, $p < 0.001$). Symbols are Hassel pre-dam (open circles), Rappbode pre-dam (filled squares) and Rote Mulde pre-dam (grey triangles).

Fig. 3

Percentages of decomposed DOC obtained from 6-month dark incubations (conducted in triplicate) of water samples taken from the study pre-dams related to average WRT. The horizontal, thick black line shows the median, the bottom and top of the boxes represent the 25th and 75th percentile. Whiskers show either the maximum value or 1.5 times the interquartile range of the data, and extremes outside this range are plotted as dots. The sample size is given by n. Significant differences between groups (t-Test, $p < 0.03$) are marked with different letters.

Fig. 4

Comparison of the radiocarbon signals of the start DOC and the microbially respired CO_2 obtained from duplicate respiration experiments conducted with water samples taken from the Rappbode pre-dam (squares) and the Rote Mulde pre-dam (triangles) in 2014. Grey and white symbols refer to results previously published in Morling et al. (2016). Values are individual measurements. The skewed line marks the 1:1 line.

Fig. 5

Temporal and spatial distribution of the freshness index (β/α) and humification index (HIX) within the Hassel pre-dam in 2013 (A, C) and the Rappbode pre-dam in 2014 (B, D), respectively.

Fig. 6

Freshness index (β/α) and humification index (HIX) in the outflows of the Hassel pre-dam (A, C) and Rappbode pre-dam (B, D) depending on discharge. Lines represent linear regressions ($p < 0.008$, A: $R^2 = 0.36$, B: $R^2 = 0.45$, D: $R^2 = 0.24$). The unfilled symbols in B and D were regarded as outliers and removed from the regression analysis.

Fig. 7

Conceptual summary of DOC processing and transport within the upper water columns in the pre-dams of drinking water reservoirs depending on discharge conditions and trophic status. During low discharges and high trophy, release of autochthonous DOC by photosynthetic organisms and DOC decomposition by heterotrophic microbes dominates. Under high discharge conditions and low trophy, mainly allochthonous DOC passes the pre-dams and is transported into the main reservoirs.

Figures

Fig. 1

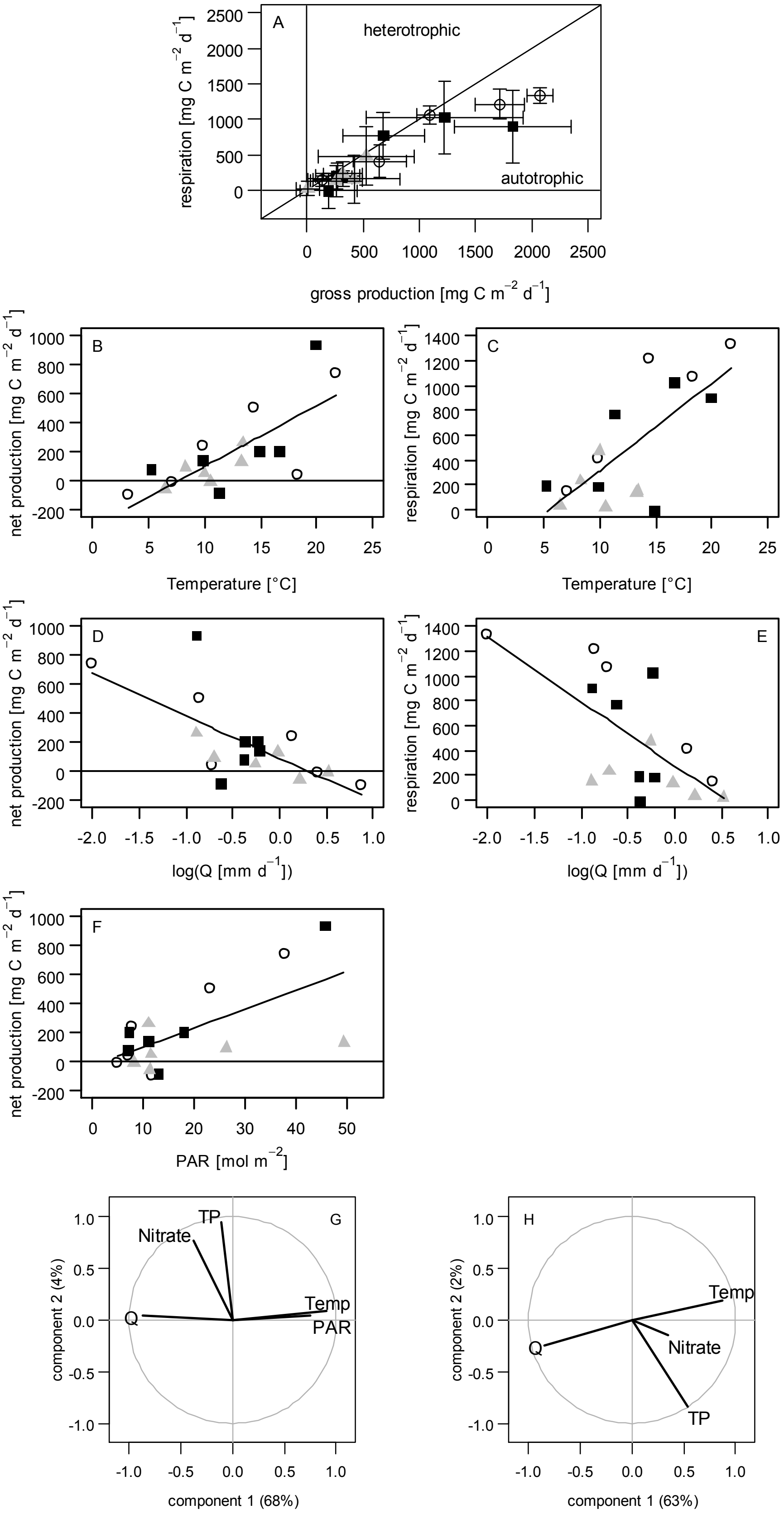


Fig. 2

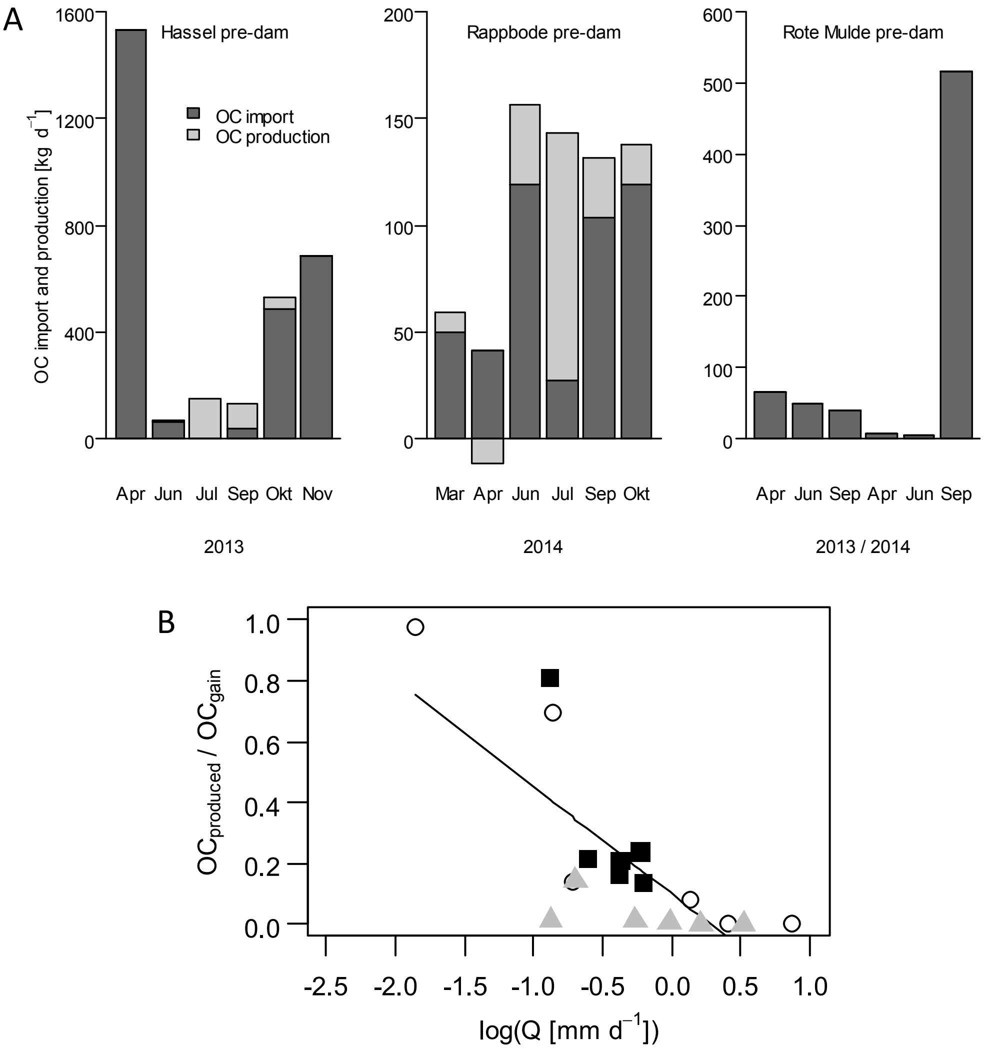

A
Hassel pre-dam
Rappbode pre-dam
Rote Mulde pre-dam
OC import and production [kg d^{-1}]
OC import
OC production
1600
1200
800
400
0
200
150
100
50
600
500
400
300
200
100
Apr Jun Jul Sep Okt Nov
Mar Apr Jun Jul Sep Okt
Apr Jun Sep Apr Jun Sep
2013
2014
2013 / 2014
B
OC$_{produced}$ / OC$_{gain}$
1.0
0.8
0.6
0.4
0.2
0.0
-2.5 -2.0 -1.5 -1.0 -0.5 0.0 0.5 1.0
log(Q [mm d^{-1}])

Fig. 3

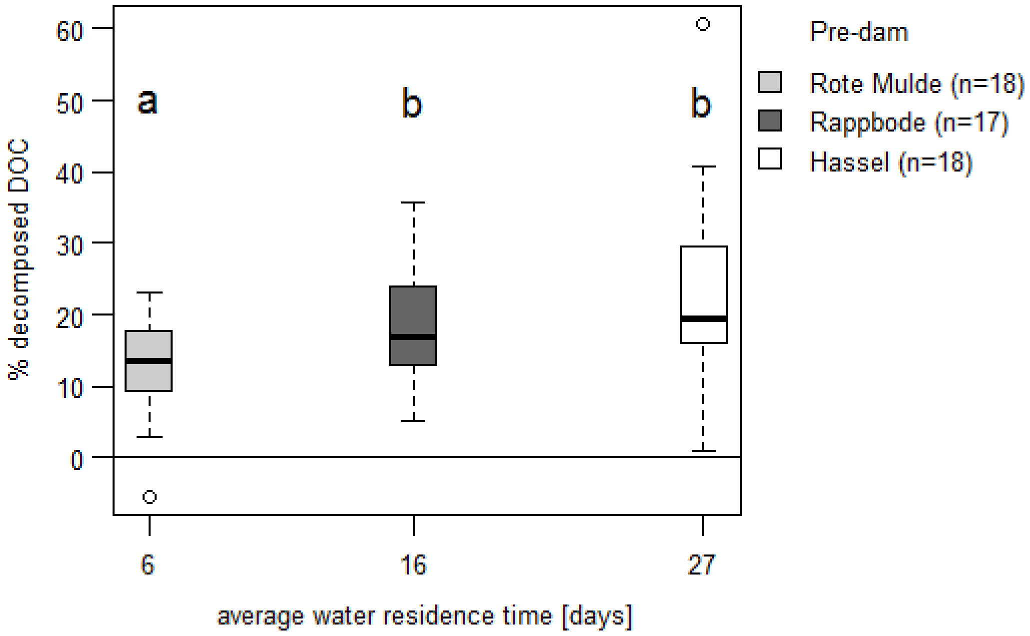

Pre-dam
Rote Mulde (n=18)
Rappbode (n=17)
Hassel (n=18)
a
b
b
% decomposed DOC
60
50
40
30
20
10
0
6
16
27
average water residence time [days]

Fig. 4

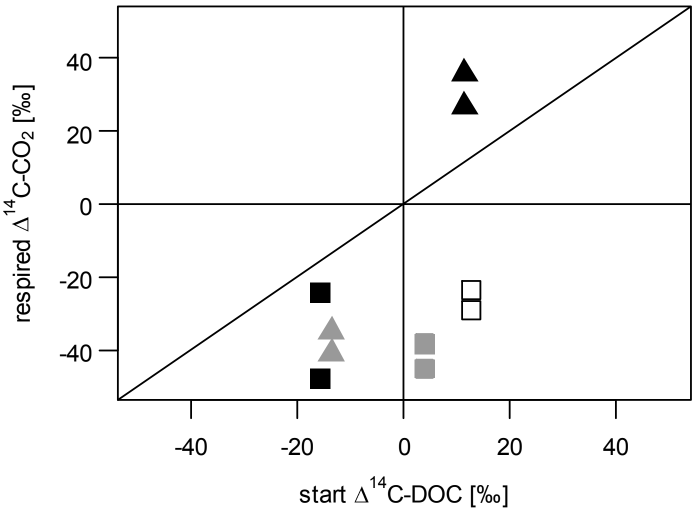

40
20
0
-20
-40
-40
-20
0
20
40
respired Δ^{14}C-CO$_2$ [‰]
start Δ^{14}C-DOC [‰]

Fig. 5

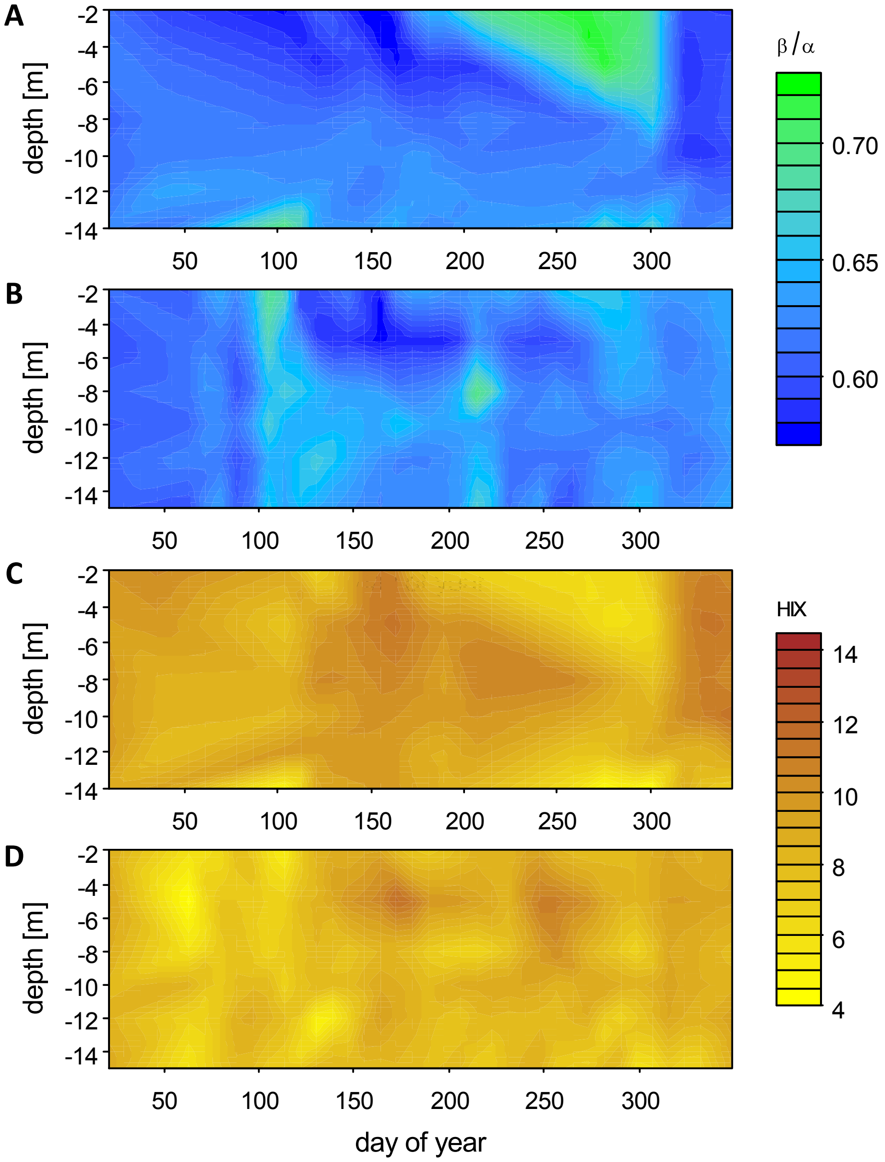

A
B
C
D
depth [m]
-2
-4
-6
-8
-10
-12
-14
50
100
150
200
250
300
day of year
β/α
0.70
0.65
0.60
HIX
14
12
10
8
6
4

Fig. 6

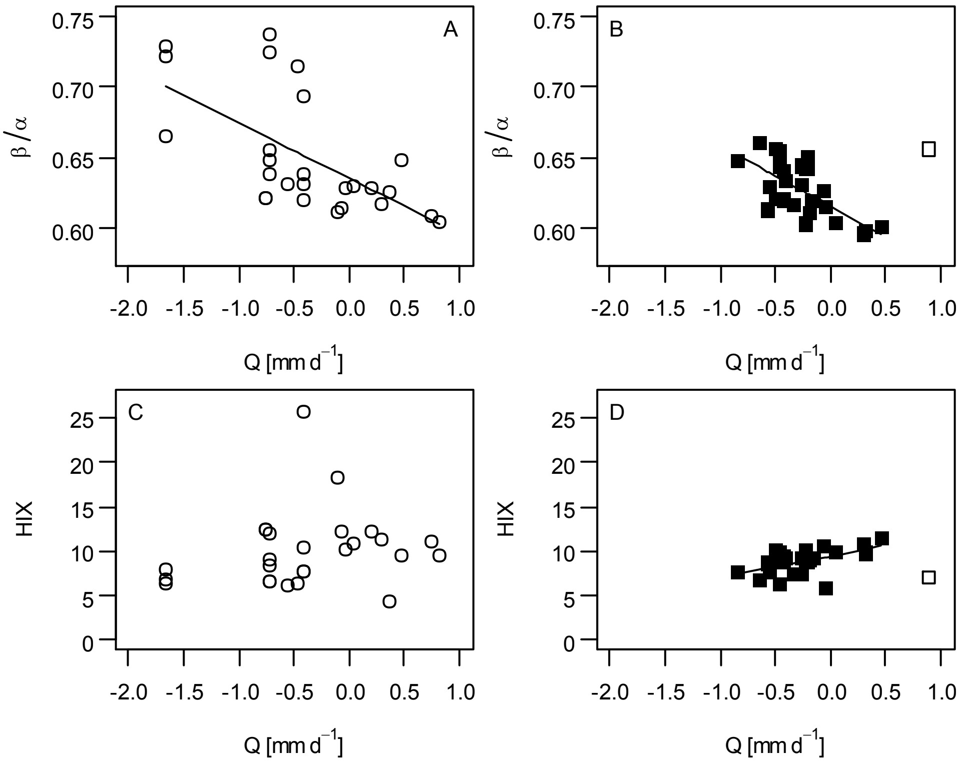
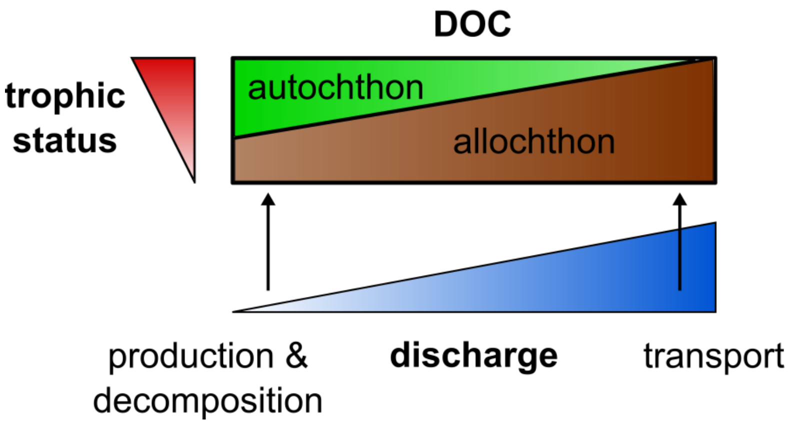

A
B
C
D
β/α
HIX
0.75
0.70
0.65
0.60
25
20
15
10
5
0
-2.0
-1.5
-1.0
-0.5
0.0
0.5
1.0
Q [mm d^{-1}]

Fig. 7


DOC
trophic status
autochthon
allochthon
production & decomposition
discharge
transport

Supporting information

Table S1

Overview of the conducted sampling campaigns in the three study pre-dams. Discharge was measured at the inflow of each pre-dam. The daily water residence time (WRT) was calculated by dividing the volumes of the dams by the daily inflow rates. Temperature is given as depth-weighted average of the temperatures in the upper 3 m. Respiration experiments were only conducted in 2014 during selected sampling campaigns. Measurements of net production and respiration as well as decomposition experiments were carried out for each sampling campaign.

Pre-dam	**Sampling dates**	**Discharge [mm d^{-1}]**	**daily WRT [days]**	**Temperature [°C]**	**Respiration experiments**
Hassel	15.04.2013	7.42	5	3.25	
	25.06.2013	0.19	140	18.35	
	23.07.2013	0.01	2382	21.69	
	17.09.2013	0.14	201	14.46	
	15.10.2013	1.38	19	9.82	
	12.11.2013	2.56	12	7.05	
Rappbode	18.03.2014	0.42	54	5.25	
	23.04.2014	0.24	90	11.30	X
	11.06.2014	0.59	40	16.69	X
	23.07.2014	0.13	164	19.89	X
	02.09.2014	0.43	56	14.90	
	28.10.2014	0.62	38	9.91	
Rote Mulde	23.04.2013	1.62	1	6.42	
	18.06.2013	0.97	1	13.23	
	24.09.2013	0.55	3	9.96	
	02.04.2014	0.20	27	8.29	
	25.06.2014	0.13	3	13.37	X
	23.09.2014	3.31	0.23	10.51	X

Manuscript III

Tracing aquatic priming effect during microbial decomposition of terrestrial dissolved organic carbon in chemostat experiments

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Tracing aquatic priming effect during microbial decomposition of terrestrial dissolved organic carbon in chemostat experiments

Karoline Morling[a]*, Julia Raeke[b], Norbert Kamjunke[a,c], Thorsten Reemtsma[b], Jörg Tittel[a]

Helmholtz Centre for Environmental Research – UFZ
[a] Department of Lake Research, Brückstr. 3a, 39114 Magdeburg, Germany
[b] Department of Analytical Chemistry, Permoserstr. 15, 04318 Leipzig, Germany
[c] Department of River Ecology, Brückstr. 3a, 39114 Magdeburg, Germany
*corresponding author: Tel.: +49 391 8109447, email: karoline.morling@ufz.de

Keywords: radiocarbon, ^{14}C, ultra-high resolution mass spectrometry, FT-ICR MS, DOM quality

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Abstract

Microbial decomposition of terrestrial carbon may be enhanced by the addition of easily decomposable compounds, a phenomenon referred to as priming effect. We investigated the microbial decomposition of terrestrial dissolved organic carbon (DOC) in one-stage and two-stage flow-through cultures (chemostats) in the absence and presence of growing phytoplankton as phytoplankton-derived organic matter might facilitate the mineralization of more refractory terrestrial compounds. Peat water and soil leachate were used as terrestrial substrates and only slight DOC decomposition was observed in the absence of phytoplankton for both substrates. A priming effect was revealed via ^{14}C data. Priming was more pronounced for the peat water substrate than for the soil leachate. The total DOC concentrations increased for both substrates in the presence of phytoplankton due to exudation and cell lysis. Samples from the soil leachate experiments were analyzed using ultra-high resolution mass spectrometry (FT-ICR MS). Predominantly the same saturated, aliphatic molecules with H/C ratios > 1.5 were completely decomposed in the absence and in the presence of phytoplankton. The decomposition of more stable molecules differed in their intensity. Oxidized and unsaturated molecules with H/C ratios < 1.0 and O/C ratios > 0.4 were more strongly decomposed in phytoplankton presence (i.e. under priming). We conclude that an aquatic priming effect is not easily detectable via net concentration changes alone, and that qualitative investigations of the DOC processed by bacterial decomposition are necessary to detect aquatic priming.

Introduction

Terrestrial organic matter is an important source of carbon in freshwater ecosystems [1-3]. Most of the organic carbon which inland waters receive from their catchments is in a dissolved form [4] favouring microbial decomposition. Respiration typically dominates over primary production in lakes [5-7] and a substantial fraction (up to ≥ 50%) of the terrestrially derived dissolved organic carbon (DOC) is mineralized [8-10]. The DOC found in inland waters consists of a large variety of different compounds originating not only from terrestrial (allochthonous, [11]) but also internal (autochthonous, [12]) sources, most likely influencing the efficiency of microbial mineralization. If organic carbon (OC) is easily decomposable it is referred to as being labile, while it is termed refractory or stable if decomposition is low within a given time frame. The addition of labile OC may increase the mineralization of the more refractory OC. This phenomenon is referred to as priming effect (PE) and has been often observed in soil science studies [13-15]. PE is commonly differentiated into positive and negative priming: A positive PE refers to increased decomposition of the refractory terrestrial organic matter (OM) compared to a control without added labile OC; while a PE is considered to be negative if OM decomposition decreases [15]. Suggested mechanisms underlying the occurrence of PE are co-metabolism, the increase in microbial biomass due to the supplied labile OC substance (primer), nutrient mining or the preferential use of the added primer instead of refractory OC [13, 16, 17].

Although widely studied in terrestrial systems, the occurrence of PE in aquatic systems has rarely been examined [16] and experimental results are contradictory. Guenet et al. [18] found a positive PE in degradation of soil OM in waterlogged microcosm experiments after the addition of glucose, and Kuehn et al. [19] observed a stimulation of microbial mineralization by periphytic algae during leaf litter decomposition. Priming of pine tree needle leachate was found in a controlled lab-based experiment by Bianchi et al. [20]. However, Danger et al. [21] only found a PE under low-nutrient conditions. No PE was found for the degradation of DOC in lake water [22, 23], and the PE was not significant in the hyporheic zone microcosms studied by Bengtsson et al. [24]. Most of these studies used sugar compounds, e.g. glucose, trehalose or cane sugar as a primer added to the refractory OC [18, 20, 22-24]. Only a few studies used more natural priming substances such as algal extracts [20, 24] or related PE to the presence of growing autotrophs during leaf litter decomposition [19, 21]. None of these studies investigated the composition of the terrestrial carbon sources, although it can be expected that they widely differ in terms of the complexity of molecules, their oxidation status and availability to microbial decomposition. OC from allochthonous sources is usually regarded as more refractory [25-27], whereas autochthonous OC is regarded as labile and expected to be preferentially utilized by aquatic microbes [28-31]. The majority of the extracellularly released OM from phytoplankton comprises bioavailable low-molecular weight compounds such as monomeric sugars, carboxylic acids, amino acids and alditols [12]. A major fraction of these released exudates is utilized by bacteria and therefore algae-derived OM might represent a suitable primer substance in natural freshwaters.

Priming was suggested to occur ubiquitously not only in terrestrial, but also in freshwater and marine ecosystems [16]. Given the observed selective decay of terrestrial OC during transport from land to sea [8, 10], we tested for the potential occurrence of an aquatic PE during the microbial decomposition of stable terrestrial derived DOC using algae-derived OM as primer substance. We combined quantitative and qualitative investigations in one-stage and two-stage flow-through cultures (chemostats). Chemostats were chosen, as they allow a continuous supply of the substrates, preventing starvation of the bacterial communities when substrates are exhausted as it might occur in batch cultures. A steady state is achieved when growth and losses of the community (which are determined by the dilution rate) are equal. DOC concentrations and DOC quality obtained from different chemostat experiments can be assigned to defined steady state conditions and are therefore comparable. Algae-derived OM served as labile substrate and was provided by actively growing phytoplankton. We define priming as an increased decomposition of the terrestrial DOC source in presence of the primer compared to the control without primer. Priming was determined by analyzing changes in radiocarbon (^{14}C) signatures of the microbially processed DOC using isotope mixing models. Further, qualitative changes were investigated via Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) to provide insight into which molecules are preferentially decomposed and whether or not these differ in the presence and in the absence of the primer.

Methods

Preparation of terrestrial DOC substrates

Two terrestrial substrates, peat water and soil leachate, were used as refractory carbon sources. Peat water was taken from a peat bog (N 51° 47’ 51.3’’, E 10° 40’ 10.8’’) in the Harz Mountains (Germany) at a depth of 40 to 60 cm under shady conditions. The water was filtered in the dark using pre-combusted (4 h at 450°C) GF/A and subsequently GF/F glass fiber filters

(Whatman). For the soil leachate, soil was taken from a beech forest in the same region (N 51° 44′ 27′′, E 10° 52′ 27′′) at a depth of 16 to 60 cm. Stones and larger root material were removed, and the homogenized soil was transported to the lab in plastic boxes lined with aluminum foil. The soil was transferred to an acid-rinsed plastic barrel, placed in a climate room at 4°C, and then doused and stirred with ~40 L of high-purity water (Milli-Q Synthesis A10). After allowing sedimentation for two days, the supernatant was centrifuged (10,000 rpm, 20 min, 15°C) to remove larger soil particles, and then filtered (pre-combusted GF/F). For carbon isotope analyses, subsamples (2 L) were taken from both substrates to obtain ^{14}C signatures of the start DOC (see sample analysis). Both terrestrial DOC substrates were stored frozen until preparation for the experiments.

To prepare the growth media for the experiments, the DOC substrates were thawed and diluted to a concentration of ~5 mg C L^{-1} with high-purity water. N and P were added in all experiments as KNO_3 and KH_2PO_4 and adjusted to an atomic ratio of C:N:P = 50:10:1 [32] to prevent bacterial inorganic nutrient limitation. Vitamins as well as the trace elements Co, Mo and Cu were added according to the concentrations of f/2 growth medium [33]. Previous analysis of the DOC substrates revealed concentrations below the detection limit for these trace elements. The media were sterile-filtered (Nalgene™ Rapid-Flow™, 0.2 µm, 600 mbar vacuum pressure) into 5 L pre-combusted (4 h at 550°C) glass bottles. Control measurements of the initial DOC, dissolved nitrogen and dissolved phosphorus were conducted. From both DOC media, 100 mL subsamples were derived and stored frozen in acid-rinsed and pre-combusted glass bottles for later extraction and analysis by Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS, see below). Samples for extraction had to be stored frozen as they could not be processed at once.

Inoculum preparation

A mixed natural bacterioplankton community was chosen as an inoculum, as synergism can be expected within an assemblage of different bacterial species for the decomposition of OM [34]. Water samples were collected from the inflows and outflows of two small reservoirs (Hassel and Rappbode) during monitoring sampling [35, 36]. Samples were stored at 8°C for one or two days until preparation. The samples were mixed and filtered through 5 µm pore size polycarbonate filters (Nuclepore, Whatman) at under 200 mbar vacuum pressure to remove grazers. Small phytoplankton were also part of the natural inocula, but were only able to grow in the light exposed stages of the two-stage setups (No. 2 and 4, Fig. 1). Aliquots of 5 mL L^{-1} were inoculated to each chemostat vessel.

Experimental setup

The prepared DOC media were filled into the autoclaved chemostat vessels (made of borosilicate glass) and bacterioplankton was subsequently inoculated to start the experiments. The setup of the chemostats has been previously described [37, 38]. The chemostats were run for at least 2 h prior to the first sampling. Chemostats were run in triplicates with a dilution rate of 0.05 day^{-1} (by way of a syringe pump: AL-6000, World Precision Instruments), and were placed in a climate room at 15 ± 1°C (water temperature). The cultures were continuously aerated with sterile, filtered air (0.2 µm). Chemostats had a volume of 1 L in the one-stage setups which were run as controls (Fig. 1). In the two-stage experiments testing for priming, two chemostat vessels were consecutively connected (No. 2 and 4, Fig. 1). The dark stages had half the volume of the light stages in order to maintain the dilution rate in the dark stages, as the supplies were reduced by the sample volumes derived from the light stages. In the peat two-stage experiment (No. 2, Fig. 1), the volume of 1 L in the light stages enabled only carbon isotope but not FT-ICR MS analyses. For the soil two-stage experiment (No. 4, Fig. 1), the volumes of the light and dark stages were adjusted to 1.5 and 0.75 L, respectively, to enable both analyses. The connected chemostats allowed the transport of the DOC and organisms including phytoplankton from the first stage into the second one, in which additional OM might be released due to algal cell lysis in darkness. In the second dark stage, we tested whether or not, priming can reduce the total DOC (terrestrial + algal DOC) in the medium below the start concentrations of the terrestrial DOC. In both two-stage experiments testing for priming, the first stages were continuously exposed to light (200 µmol photons m^{-2} s^{-1}, fluorescent tubes HO ES 20 W/840, OSRAM), while the second stages were kept in darkness by wrapping the chemostat vessels with aluminum foil. The used lamps emit predominantly light (> 90%) in the wavelength range of photosynthetically active radiation (PAR, 400-700 nm). PAR is known to be not effective in the photodegradation of DOC [39-42] as it does provide less energy for chemical reactions. Instead, ultra violet (UV) radiation is the most important and energy-rich part responsible for most of the photodegradation of DOC [43-46]. The lamps used in our experiments emit only a minor fraction of UV light (313 mW m^{-2}) and we found no significant effect of such a low intensity of UV light on both DOC quantity and quality (see supporting information). Hence, we concluded that photochemical decomposition was of minor importance in our experiments.

Experiments were run until steady states were achieved, i.e. until no further considerable changes in DOC concentrations could be observed. Both one-stage control experiments were run for two weeks as the bacterial abundances and DOC concentrations did not change considerably, indicating a steady state of cellular growth and dilution losses and constant DOC decomposition rates. Priming experiments were run for a longer period of time (36 and 50 days) due to the time needed for the phytoplankton communities to establish and due to more variable concentrations of DOC and total organic carbon (TOC).

Sampling

Samples for the analyses of DOC, TOC and abundances of bacteria and phytoplankton were drawn from the chemostat cultures after shaking the vessels and mixing the cultures. The one-stage controls were run in darkness and were sampled under red light. Plankton samples were preserved with 5% Lugol's solution. Samples of DOC and plankton were taken daily, except in the two-stage soil DOC experiment (No. 4, Fig. 1) from which samples were taken every second day. The last five samplings at steady state conditions were taken daily. TOC sampling in the two-stage setups started on days 13 (No. 2, Fig. 1) and 11 (No. 4, Fig. 1) when algal photosynthetic biomass production became significant. At the end of the soil priming experiment, phytoplankton material was collected from the triplicate light stages on pre-combusted GF/A filters for ^{14}C analyses in order to obtain the isotopic signature of the autochthonous source. Afterwards, the cultures were filtered (GF/F). Subsamples of 100 mL were removed and frozen separately for measurements via FT-ICR MS (see below) from the soil DOC experiments (No. 2 and 4, Fig. 1). The filtered DOC was prepared for carbon isotope analysis (see sample analysis).

Sample analysis

DOC samples were filtered using pre-combusted GF/F filters at < 200 mbar vacuum pressure. DOC and TOC were analyzed by non-dispersive infrared detection using a DIMATOC® 2000 (Dimatec, Germany). Bacteria were counted on every second day via epifluorescence microscopy (Axioskop2, Zeiss) after staining with Acridine-Orange according to Kamjunke et al. [47]. Phytoplankton was monitored by inverse microscopy (Axiovert100, Zeiss).

For ^{14}C analyses, DOC of the light stages from the two-stage experiments and subsamples of the DOC substrates (see above) were enriched under N_2 aeration at 60°C and acidified with H_3PO_4 (pH<2) according to Tittel et al. [48]. Enriched samples were stored frozen. The three GF/A filters used for obtaining a phytoplankton signal were dried and fumigated with HCl (37%) for 4 h to remove inorganic carbon [49] and then dried at 60°C. Pieces of equal size taken from each filter were combined and sent for radiocarbon analysis. All ^{14}C analyses were carried out at the Leibniz-Laboratory for Radiometric Dating and Isotope Research Kiel (Germany). DOC samples were freeze-dried and combusted at 900°C in the presence of CuO and a silver catalyst. Purified CO_2 was reduced to graphite for accelerator mass spectrometry with H_2 at 600°C. Concentrations were analyzed with respect to oxalic acid II standard and were corrected for fractionation. Notations are reported according to Stuiver and Polach [50]. One of the soil DOC replicate samples of the light stages did not contain enough carbon for the ^{14}C analysis.

Additionally, stable isotope ^{13}C analyses were carried out for aliquots of the peat DOC substrate and for the peat two-stage experiment (No. 2, Fig. 1) at the Colorado Plateau Stable Isotope Laboratory (CPSIL), Flagstaff (USA). Samples from the soil two-stage experiment (No. 4, Fig. 1) were analyzed at the Hydrogeology Stable Isotope Laboratory Erlangen (Germany) as ^{13}C analyses in liquids were no longer possible at the CPSIL. Samples were analyzed by conventional isotopic ratio mass spectrometry. The measured $\delta^{13}C$-values ranged from -26.6‰ to -23.2‰ with standard deviations of duplicate samples ranging from 0.0‰ to 0.1‰.

The DOC samples for FT-ICR MS measurements were acidified to pH 2 with hydrochloric acid (30%, Suprapur, Merck), and 50 mL of each sample were extracted using solid-phase extraction (SPE, Agilent, Bond Elut PPL, 500 mg, 6 mL volume) according to Raeke et al. [51]. The SPE extracts were stored at -20°C in the dark. Extraction efficiency ranged between 48% and 74% ($n = 6$). The extracts were diluted with methanol (ULC/MS grade, Biosolve, Valkenswaard) and high-purity water to a final DOC concentration of 12 mg L^{-1} before FT-ICR MS analysis. The samples were analyzed using a FT-ICR mass spectrometer, equipped with a dynamically harmonized analyzer cell (solariX XR, Bruker Daltonics Inc., Billerica, MA) and a 12 T refrigerated actively shielded superconducting magnet (Bruker Biospin, Wissembourg, France). An electrospray ionization (ESI) ion source was used in negative mode (capillary voltage +4.5 kV, infusion flow rate 120 µL h^{-1}). For each spectrum, 256 scans were co-added in the mass to charge ratio (m/z) range of 150-3000 m/z. Mass spectra were internally calibrated with a list of peaks (m/z 150-600, $n > 150$) commonly present in natural organic matter. The mass accuracy after internal calibration was better than 0.15 ppm. The mass resolution $m/\Delta m$ was around 800,000 at m/z 400. Peaks were considered if the signal/noise ratio was greater than three.

Calculations and statistics

Steady state comparisons. For start DOC concentrations in the chemostat experiments, the first 3 samplings were averaged. Steady states were assigned from day 10 on in both control experiments and from day 33 on in both two-stage setups as these were run for a longer period of time and due to more variable concentrations of DOC and TOC. DOC change (i.e. decomposition or gain of DOC) was calculated as the percentage changes between the start and the steady state concentrations. Wilcoxon rank sum tests were performed to test for differences in the DOC change between controls and both light and dark stages of the two-stage setups. Further, DOC and TOC concentrations at steady states were compared between the light and dark stages of the priming experiments by using Wilcoxon rank sum tests as well. The same test was also used to compare the bacterial abundances at steady states between the controls and the treatments. All tests were performed using the program R, version 2.15.2 [52].

Isotope mixing models. The fraction of decomposed terrestrial DOC (f_d) in the light stages of the chemostats was calculated using two isotopic-endmember mixing models for each of the triplicates. The calculated f_d were then compared to the observed decomposition in the controls. For the first isotope-mixing model it was assumed that the radiocarbon signal of the start DOC ($\Delta^{14}C_{start}$) can be differentiated into a remaining ($\Delta^{14}C_r$) and a decomposed part ($\Delta^{14}C_d$):

$$\Delta^{14}C_{start} = f_r \times \Delta^{14}C_r + f_d \times \Delta^{14}C_d \tag{1}$$

where f_r is the remaining fraction. The remaining fraction (f_r) and the decomposed fraction (f_d) are linked as follows:

$$1 = f_r + f_d \tag{2}$$

For the second isotope-mixing model it was assumed that the radiocarbon signals of the DOC at steady states in the light stages ($\Delta^{14}C_s$) were a mixture of the remaining terrestrial DOC ($\Delta^{14}C_r$) and the DOC derived from the growing algae ($\Delta^{14}C_a$):

$$DOC_s \times \Delta^{14}C_s = DOC_r \times \Delta^{14}C_r + DOC_a \times \Delta^{14}C_a \tag{3}$$

whereby DOC_s, DOC_r and DOC_a are the DOC concentrations at steady states, the remaining terrestrial DOC and the algal-derived DOC, respectively. DOC_a is the difference of DOC_s and DOC_r. Further DOC_r is the remaining fraction (f_r) of the terrestrial source DOC present at the start of the experiments (DOC_{start}):

$$DOC_s \times \Delta^{14}C_s = f_r \times DOC_{start} \times \Delta^{14}C_r + (DOC_s - f_r \times DOC_{start}) \times \Delta^{14}C_a \tag{4}$$

By solving equations (1) and (2) for $\Delta^{14}C_r$ and f_r, respectively, and inserting both parameters into equation (4), the fraction of decomposed terrestrial DOC (f_d) can be calculated for a given $\Delta^{14}C_d$ (see supporting information for derivation of equation (5)):

$$f_d = \frac{DOC_s \times (\Delta^{14}C_s - \Delta^{14}C_a) + DOC_{start} \times (\Delta^{14}C_a - \Delta^{14}C_{start})}{DOC_{start} \times (\Delta^{14}C_a - \Delta^{14}C_d)} \tag{5}$$

We calculated f_d for a range of $\Delta^{14}C_d$ values (see results). Note that equation (5) is valid only if $\Delta^{14}C_a \neq \Delta^{14}C_d$, i.e. these two endmembers must be distinct. If $f_d = 1$, then $\Delta^{14}C_s = \Delta^{14}C_a$ and $\Delta^{14}C_d = \Delta^{14}C_{start}$. If $f_d = 0$, then the DOC at steady states would reflect a simple mixture of the not decomposed terrestrial source DOC present from the start of the experiment and the additional algae-derived DOC:

$$DOC_s \times \Delta^{14}C_s = DOC_{start} \times \Delta^{14}C_{start} + (DOC_s - DOC_{start}) \times \Delta^{14}C_a \tag{6}$$

DOM quality assessment. For the samples measured by FT-ICR MS, elemental formulae were calculated considering the elements C, H, O, N and S ($N \leq 2$, $S \leq 1$; [53]). The molecules were singly negatively charged, therefore the mass-to-charge ratio (*m/z*) was equivalent to the mass of the $[M\text{-}H]^-$ anion. A molecular formula of a mass peak is referred to as 'molecule' in this article although one molecular formula can represent several different structural isomers. Double-bond equivalents (DBE) were calculated for $C_cH_hN_nO_oS_s$:

$$DBE = \frac{2 \times c - h + n + 2}{2} \tag{7}$$

The DBE represents the number of double bonds and ring systems and is a parameter for the degree of unsaturation [54]. Moreover, the oxygen to carbon (O/C) and the hydrogen to carbon (H/C) ratio were calculated for each molecule. Relative peak intensities (RI) were calculated in relation to the highest peak in the mass spectra of the chemostat samples. For the soil DOC samples this was the mass peak at *m/z* 341.0878 ($C_{15}H_{18}O_9$). Most likely, this mass peak is of terrestrial origin as it was already present at high intensity in the source soil DOC. The intensity of this peak was not significantly different in the mass spectra of the control and two-stage experiments. Therefore, we expected that the relative intensities calculated in relation to this peak were not biased by the addition of newly formed algae-derived DOC. Only peaks with a relative intensity above 2.5% and an *m/z* of 150-600 were considered for further analysis.

Intensity-weighted average parameters (e.g. O/C_{wa}) were calculated using the RI of each sum formula.

To evaluate the overall reproducibility of replicate mass spectra, the relative standard deviation (RSD) between the relative peak intensities of the replicate chemostat experiments was calculated as proposed by Sleighter et al. [55]. In the control samples 79% of the peaks common in all three replicates had a RSD ≤ 10%, and 98% of the peaks had a RSD ≤ 25%. In the two-stage experiment, 62% of all peaks common in the light and dark stages (n = 6) had a RSD ≤ 10%, while 98% of the peaks had a RSD ≤ 25%. This is within the limits proposed by Sleighter et al. [55] (60% and 90% of the peaks within 10% and 25% RSD respectively).

To compare the molecular formulae present in the two start DOC (peat and soil), an enrichment index (EI) was calculated:

$$EI = \frac{RI(start\ peat)}{RI(start\ peat) + RI(start\ soil)} \tag{8}$$

Molecules present only in the start peat DOC have an EI of 1, whereas molecules present only in the start soil DOC are indicated by an EI of 0; molecules occurring in both samples with the same RI have an EI of 0.5

In order to compare molecules which were decomposed and produced during the experiments, a degradation index (DI) was calculated between the start DOC and the samples taken at the end of the chemostat experiments:

$$DI = \frac{mean\ RI(end)}{RI(start) + mean\ RI(end)} \tag{9}$$

For calculations, the mean RI of the triplicates was used for the soil control samples. For the samples of the soil two-stage experiment, the mean RI of the light and dark stages (n = 6) was calculated. Molecules with a DI of 0 were referred to as completely decomposed, whereas molecules with a DI of 1 were referred to as newly produced molecules. A DI of 0.5 implies no net change in the relative peak intensities of the molecules.

The intensity of decomposition differed in the control and in the two-stage experiment as the molecules showed differently decreasing RI. Such differences in the decomposition were assessed by selecting molecules containing only C, H and O with a DI < 0.5. For these molecules, the difference between the DI values of the molecules present in the two-stage setup and the DI value of the molecules present in the control was calculated as:

$$\Delta DI = DI_{priming} - DI_{control} \tag{10}$$

A ΔDI of 0 indicates that the molecules were decomposed in equal measure in both the control and the two-stage experiment. A negative ΔDI indicates that the molecules were more strongly decomposed in the two-stage experiment than in the control, whereas a positive ΔDI indicates that the molecules were more strongly decomposed in the control than in the two-stage setup. When calculating the ΔDI between the replicate samples of the same chemostat experiments, more than 90% of all ΔDI values ranged between -0.2 and 0.2. We therefore considered only ΔDI values below -0.2 or above 0.2 as significant changes when comparing the intensity of decomposition in the control and two-stage experiments.

Results

Carbon concentrations and communities

The DOC concentrations in the controls decreased slightly by an average of 5% and 9% for the peat and soil substrates, respectively (Fig. 2, Tab. 1). In both two-stage experiments, DOC and TOC concentrations increased in the light as well as in the dark stages (Fig. 3, Fig. 4, Tab. 1). For the peat substrate, DOC concentrations increased by an average of 29% within the light stage and of 33% within the dark stage compared to the start of the experiments. A much higher average increase of 62% within the light and of 67% within the dark stage was observed for the soil substrate. In the peat two-stage experiment, the steady state DOC concentration in the dark stage was significantly higher than in the light stage (Wilcoxon rank sum test, $p < 0.001$). In contrast, in the soil two-stage experiment the DOC concentration in steady state was slightly higher in the light stage than in the dark stage, but this difference was not significant (Wilcoxon rank sum test, $p = 0.305$). In both two-stage experiments, the TOC concentrations in the dark stages were significantly lower by 8% (peat substrate) and 17% (soil substrate) than in the light stages at steady state conditions (Wilcoxon signed rank test, $p < 0.003$).

The bacterial abundances at steady states were significantly higher in both the light and dark stages of the two-stage experiments compared to the control setups for both DOC substrates (Wilcoxon rank sum test, $p < 0.001$). The phytoplankton communities in the peat light stages consisted predominantly of diatoms (mainly the genera *Asterionella*, *Nitzschia*, *Cyclotella*) at the beginning of growth. At steady states the cyanobacterium *Oscillatoria limnetica* dominated in two replicates, whereas green algae (mainly the genera *Chlamydomonas*, *Ankistrodesmus*, *Dictyosphaerium*) dominated in the third replicate. In the soil light stage, the phytoplankton consisted almost entirely of a flagellate similar to *Chlamydomonas* spec. in all replicates.

Carbon isotope data

The peat DOC substrate was enriched in ^{14}C, i.e. it contained modern carbon produced after 1950 (Tab. 2). Likewise, the DOC in the light stages at the end of the priming experiment was enriched in ^{14}C. The phytoplankton OC yielded a $\Delta^{14}C$ of 9‰ (Tab. 2), which resemble the radiocarbon signature of atmospheric CO_2 (~20‰) [56, 57]. The ^{14}C-DOC abundances of the three replicates were between those of the start peat DOC and the phytoplankton signal (Tab. 2), indicating that DOC with positive $\Delta^{14}C$-values (i.e. modern carbon) must have been decomposed during the experiment. According to equation (5), DOC with a $\Delta^{14}C$ ranging from 115‰ to 142‰ (100% decomposition of the start DOC) to 900‰ (12% to 15% decomposition) could have been decomposed during the bacterial mineralization in the three replicates (Fig. 5), explaining the measured $\Delta^{14}C$-DOC at the end of the experiments. Summer mean values of $\Delta^{14}C$-CO_2 higher than 900‰ were never observed in the atmosphere [58] and can therefore not occur in photosynthetic biomass or in derived OC. Hence, a decomposition of less than 12% can be ruled out, revealing a PE compared to the 5% decomposition in the control (Fig. 5). To our knowledge, the highest reported $\Delta^{14}C$ value of microbially respired CO_2 is close to 400‰ [59]. We consider this as the maximum possible $\Delta^{14}C$ value of decomposed DOC (dashed line in Fig. 5). Such a high $\Delta^{14}C_d$ value is rather unlikely to occur in organic matter as summer mean values of atmospheric $\Delta^{14}C$-CO_2 > 400‰ were only observed between 1963 and 1974 [58]. Therefore, it can be used to test if the hypothesis of the occurrence of a PE in our experiments can be rejected. For the maximum $\Delta^{14}C_d$ of 400‰, f_d ranged from 27% to 34% for the three peat replicates (Tab. 2). This is more than the observed average 5% decomposition in the control, confirming a PE.

The start soil DOC (Tab. 2) was older than the start peat DOC, with a conventional radiocarbon age (CRA) of 23 years before present (B.P.; i.e. before 1950). The DOC in the light stages was even more depleted in ^{14}C (CRA 84 and 139 years B.P.) than the start soil DOC. This suggests that the start soil DOC was partly decomposed, and older compounds remained despite an increase in DOC concentrations (Fig. 4) due to phytoplankton exudation and cell lysis. Just as for the peat substrate, DOC with positive $\Delta^{14}C$-values must have been decomposed to explain the observed results, and could have ranged from 27‰ to 60‰ (100% decomposition) to 900‰ (2% to 5% decomposition) in the two replicates (Fig. 5). For the assumed maximum $\Delta^{14}C_d$ of 400‰, f_d results in 5% and 13% decomposition (Tab. 2), indicating priming only for one of the two replicates. Further, the mean of both replicates resulted in a slightly lower f_d of 8% compared to the average 9% decomposition in the control (Tab. 2). Thus, for the soil leachate DOC, the PE was not as pronounced as for the peat DOC.

DOM quality data

Qualitative investigations of the molecular composition were carried out for the peat and soil substrates and for the soil DOC experiments (No. 3 to 4, Fig. 1). To investigate differences between the terrestrial substrates and between the experimental samples, we used van Krevelen diagrams. In such a diagram, each dot represents in general several molecular formulae. Molecules with different masses but the same O/C and H/C ratio are superimposed.

The peat substrate was less saturated (higher DBE_{wa}, lower H/C_{wa} ratio) and more oxidized (higher O/C_{wa} ratio, Tab. 3) and contained molecules with an H/C ratio < 1.0 at higher intensities than the soil substrate (Fig. 6). It also revealed a higher proportion of nitrogen-containing molecules, but a lower proportion of sulfur-containing molecules (Tab. 3).

Considerable changes were observed between the samples taken at the end of the chemostat experiments and the start soil DOC. On average, the molecules became more oxidized (higher O/C_{wa}), less saturated (lower H/C_{wa}) and increased in molecular weight (higher m/z_{wa} ratio, Tab. 3) during the experiments. This indicates that mainly aliphatic molecules with low oxygen content were decomposed. Compounds containing only C, H and O represented the main fraction of the molecules in all investigated samples, and ranged from 85% in the start soil DOC to 95% at the end of the chemostat experiments (Tab. 3). Predominantly, molecules with the same molecular composition were completely decomposed (they had a DI of 0, see eq. (9)) in both the control and the two-stage setup (light and dark stage), and were situated in the upper part of the van Krevelen diagrams with H/C ratios above 1.5 (Fig. 7a, c, e). Particularly for the $C_xH_yO_z$ molecules but also for the nitrogen-containing molecules, even more molecules were completely decomposed in the control than in the two-stage experiment (Tab. 3, Fig. 7a, c). Sulfur containing molecules were almost completely decomposed in both chemostat experiments (Fig. 7e). For incompletely decomposed molecules, the intensity of decomposition differed between the control and the two-stage setup (Fig. 8). In the control experiment, less oxidized (O/C < 0.4) molecules with intermediate H/C ratios (0.5 to 1.5) were usually more strongly decomposed (Fig. 8). In contrast, more-oxidized (O/C ratios 0.4 to 1.0) and less-saturated (H/C ratios < 1.0) molecules were more strongly decomposed in the two-stage experiment (Fig. 8).

Fewer molecules were newly produced (DI of 1 according to eq. (9)) than were completely decomposed. More of the newly produced molecules were found in the two-stage experiment than in the control (Fig. 7). These were less oxidized (lower O/C ratios) and more saturated (higher H/C ratios) than in the control (Fig. 7). A part of the newly formed, nitrogen-containing molecules were found in the upper left of the diagram, with an H/C ratio above 1.5 (Fig. 7d). Almost no sulfur-containing molecules were newly produced in the control or in the two-stage setup (Fig. 7f).

Discussion

We investigated aquatic priming during the microbial decomposition of two terrestrial DOC substrates in presence and in absence of fresh OM derived from growing phytoplankton. Priming was defined as an enhanced decomposition of the terrestrial derived DOC, which included the possibility that total DOC (terrestrial + algae-derived) increased.

We used peat water as one of the two tested terrestrial substrates as peatlands are areas with a close coupling of terrestrial and aquatic environments and are considered to be one of the main sources of terrestrial DOC to aquatic systems [60-62]. We used a soil leachate as a second substrate as it can be expected that such carbon material enters aquatic systems via surface or underground flows during rain events or erosion processes, therefore contributing to natural river and lake DOC. The two substrates differed in their molecular composition. The peat DOC contained a higher number of unsaturated molecules with an H/C ratio < 1.0 (Fig. 6). These are considered to be less decomposable than molecules with an H/C ratio > 1.5 [63-65], which predominated in the start soil DOC. Thus, the peat DOC was more stable than the soil DOC, which could explain the lower percentage of decomposition observed in the peat control compared to the soil control experiment (Tab. 2).

Although sugar compounds such as glucose are often used in terrestrial PE studies, they might not reflect naturally occurring, labile carbon substances which could act as primers. Algal extracts were used as primers in only a few of aquatic priming studies, leading to contradictory results on the occurrence of an aquatic PE [20, 24]. We used chemostat cultures as the steady state conditions prevent starvation of the microbial communities as it might occur in batch cultures. In contrast to previous studies, the algae-derived OM used as a primer in our study was freshly produced and continuously provided by actively growing phytoplankton during the experiments. Growing phytoplankton releases a substantial fraction of labile low-molecular weight compounds [12], making it a suitable source of primer substances. Growing phytoplankton might also release specific compounds which could serve as “growth factors”, stimulating the growth of bacteria. For instance, by providing algae with vitamin B_{12}, bacteria are supplied with carbohydrates released by algae [66]. Further, our setup allows direct interactions between the organisms such as attachment of bacteria on phytoplankton cells [67]. Our multi-species approach easily leads to a development of different communities in controls and treatments (different algae in triplicate chemostats, see results section), which resulted in a higher variability between replicates (Fig. 3 and 4). However, these communities are adapted to the respective DOC quality within the chemostat cultures. We chose these settings on purpose as we were interested in the decomposition of the DOC substrates by natural bacterial assemblages. Thus, we defined steady state conditions by constant DOC concentrations and used longer time intervals for the assessment of the steady states.

To assess the PE, we applied natural abundance ^{14}C analyses and isotope mixing models, which are often used in studies on carbon cycling. The use of the stable isotope ^{13}C is more complicated, as it typically varies in a narrow range and underlies several fractionation processes [68]. The variation of ^{14}C over a wider range and the corrections for fractionation during analyses makes it more suitable for distinguishing different OC sources. Although methods exist to determine the ^{14}C of the decomposed DOC [59, 69, 70], these were not applicable in our experimental setup. It would have been not possible to distinguish the contributions of terrestrial and algae-derived sources. Further, different components of the terrestrial DOC substrates may be decomposed with and without priming (Fig. 7 and 8). Thus, even if we would have measured the ^{14}C signature of the decomposed terrestrial DOC in the control experiments, it could not be expected that it has the same signature as in the priming setups. According to the isotope mixing models, modern carbon must have been decomposed in both two-stage experiments to explain the measured $\Delta^{14}C$ in the light stages at the end of the experiments. The source peat DOC was of modern origin and thus the decomposition of DOC components with positive $\Delta^{14}C_d$ values seems reasonable for this substrate. The source soil DOC showed a radiocarbon signature of -10‰ (Tab. 2) and most likely, constituents of different ages contributed to this bulk signal. For instance, radiocarbon signatures of respired CO_2 from soils represent a combination of younger (e.g. fine roots) and older constituents (e.g. humified material) [71]. Bacteria have been found to utilize distinct DOC pools of different ages, resulting in the respiration of both ancient and modern $^{14}CO_2$ [59, 72, 73]. Berggren & del Giorgio [59] reported a $\Delta^{14}C$ value of respired CO_2 close to 400‰ following the microbial decomposition of DOC from a headwater stream. To our knowledge, this is the highest reported value and we considered it as the upper maximum of possible $\Delta^{14}C_d$ values during microbial utilization in our experiments. Applying this threshold, we could prove aquatic PE in our experiments on the basis of the measured ^{14}C data (Tab. 2, Fig. 5) except for one of the soil DOC replicates. Published $\Delta^{14}C$ data of microbially respired CO_2 in freshwater systems are sparse [59, 72, 73]. However, if heterotrophic bacteria utilized modern organic carbon pools, these had almost exclusively ^{14}C signatures of less than 250‰ [59, 72, 73] and are thus more likely to occur than the only once observed maximum of 400‰ [59]. According to our isotope mixing model, the calculated decomposed fraction (f_d) increases with lower and more reasonable values of $\Delta^{14}C_d$ (Fig. 5). In the first soil DOC replicate, priming could have been assigned with an assumed $\Delta^{14}C_d < 209‰$ (Fig. 5b), which indicates that priming likely occurred as well in this replicate.

Priming was more pronounced for the peat DOC than for the soil leachate. This could be related to a longer running of the peat two-stage experiment, allowing for a more extensive microbial mineralization of the terrestrial DOC. In soil science, however, the magnitude of PE has been linked to the amount and quality of the added primer [74-77]. Rising concentrations of autochthonous DOC enhanced the microbial decomposition and led to a decrease of humic substances in the allochthonous DOC pool in a study by Attermeyer et al [78]. In our soil priming experiment, PE differed between the two replicates for which ^{14}C data were available (Tab. 2, Fig. 5). At steady states, DOC concentrations in the light stages were lower in replicate I (6.11 mg C L^{-1}) than in replicate II (9.08 mg C L^{-1}). Thus, there was a potentially higher amount of algae-derived DOC present in the second replicate, which could have enhanced the PE. However, this needs further research for validation.

Photochemical reactions have been known to contribute to the decomposition of and changes in molecular characteristics of DOC [43, 79-81]. The lamps used in our experiments for growing the phytoplankton emit predominantly PAR, which provides less energy for chemical reactions and is thus not effective in the photodegradation of DOC [39-42]. Most reactions are enabled by the energy-rich UV light [43-46]. For instance, photochemical DOC degradation ranged from 5% to 35% using UV light similar to natural sunlight (5.1 to 18.9 W m^{-2}) [43, 82, 83]. However, the lamps we used emit only a minor fraction of UV light (0.31 W m^{-2}), corresponding to less than 6.1% of the UV light used in experiments on DOC photodegradation [43, 82, 83]. In additional experiments (see supporting information), we found no significant effect of such a low intensity of UV light on both DOC quantity and quality. Likewise, Hansen et al. [84] found little (< 3%) or no change in DOC concentrations following irradiation with a similar low intensity of UV light (0.45 W m^{-2}). Thus, we concluded that photochemical reactions were of minor importance in our experiments. Flocculation is a further pathway which removes DOC from the water column [85, 86] and could be falsely interpreted as DOC decomposition, especially in the two-stage experiments which were run for a longer time period. However, we propose that DOC flocculation did not considerably account for the formation of the particulate organic carbon (POC) within the two-stage experiments. The ^{14}C-signature of the POC sample, which we used to obtain an isotopic signal of the phytoplankton, was close to that of atmospheric CO_2 (Tab. 2), giving no hint for a substantial contribution of a terrestrial signal.

The total DOC (i.e. terrestrial + algal DOC) concentrations increased in both two-stage experiments (Tab. 1, Fig. 3 and 4). The net increase in DOC concentrations was lower for the peat than for the soil substrate, a fact which can be associated to the different communities of phytoplankton species with different species-specific growth rates. Intuitively, one would expect a net decrease in the total OC pool during PE, as the added primer is expected to be decomposed as well. However, this is also not the case in studies of soil PE, where more carbon from the added primer could remain at the end of the incubation than was lost due to additional OM mineralization [74, 75] and an increased turnover due to priming does not necessarily lead to a decrease of the total C stock [15]. In our experiments, the concentration of TOC at the end of the experiments was higher than that of the DOC at the start. Algae-derived DOC accumulated, leading to a net increase in the total DOC at steady states. Phytoplankton not only produces labile and easily decomposable organic compounds, but also releases a fraction of OM stable to microbial decomposition [28, 87, 88]. The question arises as to whether or not such overcompensation of the PE is also likely to occur in natural freshwater systems as the concentration of naturally derived algae DOC might not usually be as high as under our experimental conditions. This also leads to the question if aquatic PE is of quantitative importance regarding the total DOC (i.e. terrestrial + algal DOC) concentrations observed in natural waters. Guillemette et al. [25] found a preferential removal of phytoplankton-derived OC from terrestrially dominated C pools by aquatic bacteria, and gave further evidence that the supply of labile, phytoplankton-derived OC could enhance the utilization of more stable terrestrial OC. In our experiments, priming took place as an increased decomposition of the terrestrial DOC, although the total OC pool was not reduced.

FT-ICR MS analyses revealed that predominantly molecules with the same molecular composition were completely decomposed in both the control and the two-stage experiments (Tab. 3, Fig. 7). A labile fraction with an O/C ratio < 0.4 and an H/C > 1.5 in DOC of different origins was also identified in other studies using FT-ICR MS analyses [63-65]. Completely decomposed molecules were generally of aliphatic character with low oxygen content (Tab. 3, Fig. 7). More molecules were completely decomposed in the control than in the two-stage experiment (Tab. 3, Fig. 7). In the presence of phytoplankton, molecules with higher O/C (more oxidized) and lower H/C ratios (less saturated) were more strongly decomposed (Fig. 8), giving evidence that the observed PE was acting on these compounds. Such differences were not observed for nitrogen- and sulfur-containing molecules (data not shown). A preferential removal of oxidized, aromatic compounds during decomposition was also observed by Kellerman et al. [89] in a survey of dissolved organic matter in Swedish lakes. As priming is referred to an increased decomposition of more stable compounds, the greater amount of more aromatic molecules present in the peat substrates (Fig. 6) provides a reason as to why the observed PE was more pronounced for the peat than for the soil substrate (Tab. 2). The start peat DOC also exhibited more nitrogen-containing molecules (Tab. 3) which might have additionally facilitated the higher intensity of the PE observed in the peat two-stage setup (Tab. 2).

Produced molecules differed between the control and the two-stage setup (Fig. 7): Newly formed molecules in the control derive from either microbial or chemical transformation processes of DOC. These molecules were mostly below an H/C ratio of 1.5, and are thus regarded as more stable molecules [63, 65]. This provides evidence that bacterial activity may be responsible for the production of more stable compounds, as has also been found in earlier studies [90-93]. In the two-stage experiment, the produced molecules were more saturated and less oxidized than the ones in the control (Fig. 7b, d, f). A part of the newly formed, nitrogen-containing molecules in the two-stage experiment was situated in the upper left part of the van Krevelen diagram (Fig. 7d), where typically labile and easily decomposable molecules can be found [63, 65, 94]. These labile molecules are most likely quickly decomposed as well. They appear in the diagram due to the calculation of the DI (see methods) as they were not present in the start soil DOC. Hence, they derive from either the exudates of growing phytoplankton or from the decomposition and transformation of algae-derived substances. A reactive pool of dissolved organic nitrogen in the same region of the van Krevelen diagram was also found in a bioassay study by Osborne et al. [95], suggesting a rapid cycling of these molecules in aquatic systems. We also observed a pool of newly formed nitrogen-containing molecules in the center of the van Krevelen diagram in our experiments (Fig. 7d). Osborne et al. observed a pool of refractory nitrogen-containing molecules in the same region which they termed “lignin-like”. A similar conclusion of the existence of two pools of nitrogen-containing compounds in aquatic systems, a fast cycling and a resistant one, was also made by Kellerman et al. [89].

In conclusion, the net increase in DOC concentrations during the two-stage experiments does not point to priming at first sight. However, carbon isotope results and mixing models showed that more terrestrial DOC was decomposed in presence of fresh photosynthetic substrates. Qualitative changes in the molecular composition of the soil DOC were investigated via FT-ICR MS, and substantial changes were observed during the experiments. In general, the same labile molecules were completely decomposed in control and two-stage setup. However, the intensity of the decomposition differed, showing that owing to PE, oxidized, aromatic compounds were more strongly decomposed than without PE. Given the observed net increase in DOC concentrations in the presence of phytoplankton growth in our experiments, it might be that PE in aquatic environments is not easily detectable. The question arises how important PE is in natural ecosystems, highlighting the need for further studies which combine quantitative and qualitative investigations on the bacterial decomposition of OC in aquatic systems.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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Figure legends

Fig. 1 Overview of conducted chemostat experiments for both terrestrial DOC substrates (peat and soil). Control experiments were run in one-stage setups in darkness. Priming was tested in two-stage setups, whereby the first stages were exposed to light to allow phytoplankton growth and the second stages were kept in darkness. No. indicates the number of the experiment. See methods for detailed descriptions

Fig. 2 DOC concentrations and bacterial abundances in the peat (a, b) and soil (c, d) control experiments (Mean ± standard error of the mean, n=3)

Fig. 3 DOC and TOC concentrations in the light (a) and dark stages (b) as well as bacterial abundances in both stages (c) of the peat two-stage experiment (No. 2, Fig. 1) (Mean ± standard error of the mean, n=3)

Fig. 4 DOC and TOC concentrations in the light (a) and dark stages (b) as well as bacterial abundances in both stages (c) of the soil two-stage experiment (No. 4, Fig. 1) (Mean ± standard error of the mean, n=3)

Fig. 5 The calculated fraction of decomposed terrestrial DOC (f_d) in dependence of its assumed radiocarbon signature ($\Delta^{14}C_d$). f_d was calculated according to equation (5) for the (a) peat water and (b) soil leachate DOC. Replicates of both two-stage experiments (No. 2 and 4, Fig. 1) are indicated with Roman numerals (Repl. I to III). Note that the third soil DOC replicate was lost during ^{14}C analysis. Horizontal lines mark the average decomposition observed in the control experiments (see Tab. 1). Dashed vertical lines indicate the assumed maximum possible value of $\Delta^{14}C_d$ (see results).

Fig. 6 Van Krevelen diagram showing the differences in the composition of $C_xH_yO_z$ molecules of the peat and the soil substrates. The color code indicates the enrichment index (EI, see methods) calculated for the two substrates. An EI of 0 indicates molecules present only in the soil substrate, whereas an EI of 1 indicates molecules present only in the peat substrate

Fig. 7 Van Krevelen diagrams of the soil DOC molecules, which were (1) completely decomposed (a) $C_xH_yO_z$, (c) $C_xH_yN_{1-2}O_z$, (e) $C_xH_yO_zS_1$ and (2) newly formed (b) $C_xH_yO_z$, (d) $C_xH_y N_{1-2}O_z$,

(f) $C_xH_yO_zS_1$ in the soil control and in the soil two-stage setup (light and dark stages), and the ones common in both experiments. Grey background dots represent the molecular formulae of the start soil DOC

Fig. 8 Van Krevelen diagram of $C_xH_yO_z$ molecules, which were only partly decomposed in the soil control and the two-stage experiment. The color code indicates the intensity of decomposition (ΔDI, see methods). Positive ΔDI values represent molecules which were more strongly decomposed in the control than in the two-stage setup, while negative ΔDI values represent molecules which were more strongly decomposed in the two-stage setup than in the control. A ΔDI between -0.2 and 0.2 indicate molecules which were decomposed in equal measure in both the control and the two-stage setup

Tables

Tab. 1 DOC concentrations and bacteria abundances at the end of the experiments. No. indicates the number of the experiment (see Fig. 1). Steady state DOC concentrations, DOC changes and bacterial abundances are given as means (± standard errors of the mean, n=3). DOC change describes the decomposed (negative values) and gained (positive values) DOC expressed as percentage change from the start values. Significant differences in DOC change between the controls and the light and dark stages are marked.

DOC Substrate	**No.**	**Treatment**	**Steady state DOC [mg C L^{-1}]**	**DOC change [%]**	**Bacterial abundance [10^9 cells L^{-1}]**
Peat	1	Control	6.29 ± 0.05	-5 ± 1	2.41 ± 0.06
Peat	2	Light stage	6.51 ± 0.07	+29 ± 2*	5.75 ± 0.67
Peat	2	Dark stage	7.80 ± 0.11	+33 ± 2*	6.21 ± 0.78
Soil	3	Control	5.20 ± 0.03	-9 ± 1	1.61 ± 0.14
Soil	4	Light stage	7.69 ± 0.33	+62 ± 6*	4.55 ± 0.34
Soil	4	Dark stage	7.19 ± 0.34	+67 ± 8*	4.67 ± 0.63

* – significant at the 0.001 probability level

Tab. 2 Measured Δ^{14}C-DOC, results of the isotope mixing model and derived conclusions about a priming effect. Data are given for the two terrestrial substrates, the replicate values (I to III) in the light stages of the two-stage chemostats (No. 2, No. 4, Fig. 1) and their means. Reported Δ^{14}C values of the replicates are single measurements (the analytical error ranged from 2‰ to 4‰). The Δ^{14}C signal of the phytoplankton represented algae OC contributions in the isotope mixing model (see methods). For both terrestrial substrates, the fraction of decomposed terrestrial DOC (f_d) was derived from the mean decomposition (± standard errors of the mean) observed in the triplicate control experiments (see Tab. 1). For the replicates and their means, f_d was calculated assuming a decomposition of ^{14}C-DOC ($\Delta^{14}C_d$) with a maximum possible value of 400‰ (see results). Priming was assigned as proved (P) when f_d was higher than the decomposition in the control or as not proved (NP) if it was not.

		$\Delta^{14}C_d$ = 400‰	
	Δ^{14}C [‰]	**f_d [%]**	**Priming**
Peat substrate	185	5 ± 1	
Peat replicate I	54	32	P
Peat replicate II	68	27	P
Peat replicate III	45	34	P
Peat mean (I to III)	56	31	P
Soil substrate	-10	9 ± 1	
Soil replicate I	-18	5	NP
Soil replicate II	-25	13	P
Soil replicate III	–[a]		
Soil mean (I to II)	-22	8	NP
Phytoplankton	9		

[a] – Sample was lost during ^{14}C analysis.

Tab. 3 FT-ICR MS parameters for the samples of the two start substrates (peat and soil) and the samples of the soil DOC chemostat experiments. No. indicates the number of the experiment (see Fig. 1). Parameters were calculated as intensity-weighted averages (see methods) and percentages of $C_xH_yO_z$, $C_xH_yN_{1\text{-}2}O_z$ and $C_xH_yO_zS_1$ molecules (numbers of assigned sum formulae are given in parentheses) are provided.

sample	**No.**	***m/z*$_{wa}$**	**DBE$_{wa}$**	**O/C$_{wa}$**	**H/C$_{wa}$**	**$C_xH_yO_z$ [%]**	**$C_xH_yN_{1\text{-}2}O_z$ [%]**	**$C_xH_yO_zS_1$ [%]**
Peat substrate		360	8.7	0.54	1.09	84.1 (2004)	12.5 (963)	3.4 (324)
Soil substrate		365	6.5	0.49	1.38	85.4 (1770)	5.3 (369)	9.1 (323)
Soil control	3	380 ± 6	8.6 ± 0.1	0.55 ± 0.01	1.13 ± 0.01	95.6 ± 0.2 (1330)	3.7 ± 0.1 (190)	0.7 ± 0.1 (33)
Soil light stage	4	388 ± 2	8.3 ± 0.1	0.52 ± 0.01	1.18 ± 0.01	94.7 ± 0.3 (1532)	4.8 ± 0.4 (259)	0.5 ± 0.1 (33)
Soil dark stage	4	382 ± 2	8.4 ± 0.1	0.52 ± 0.01	1.18 ± 0.01	94.7 ± 0.3 (1517)	4.8 ± 0.6 (255)	0.6 ± 0.1 (35)

Figures

Fig. 1

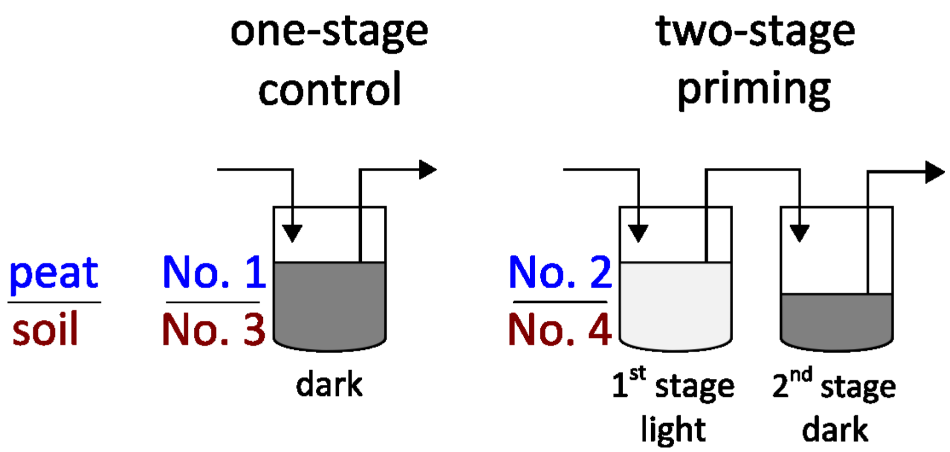


Fig. 2

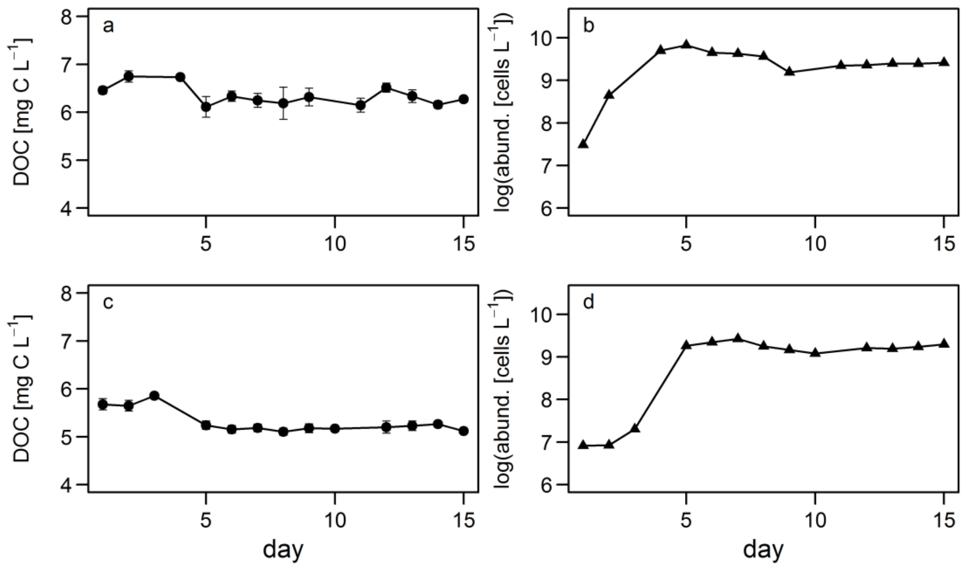


Fig. 3

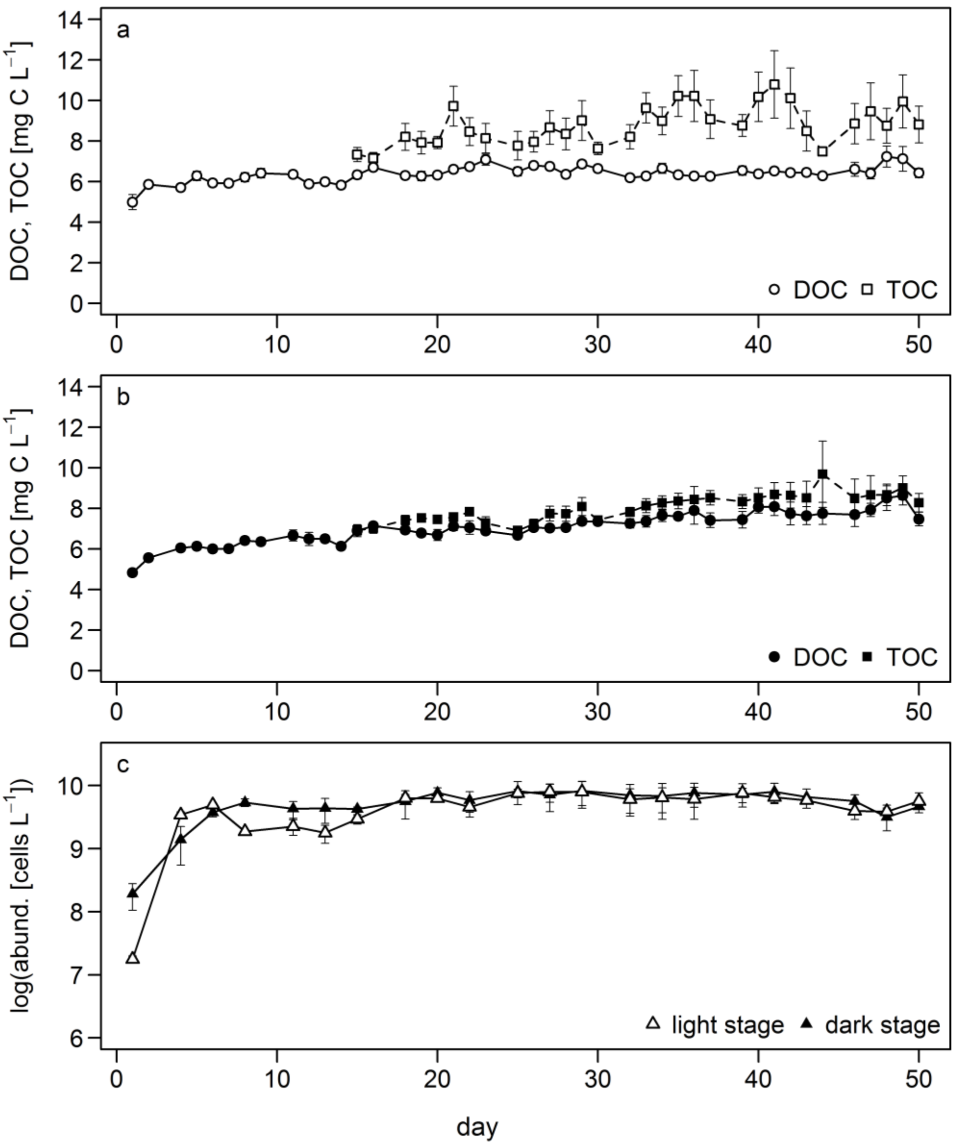
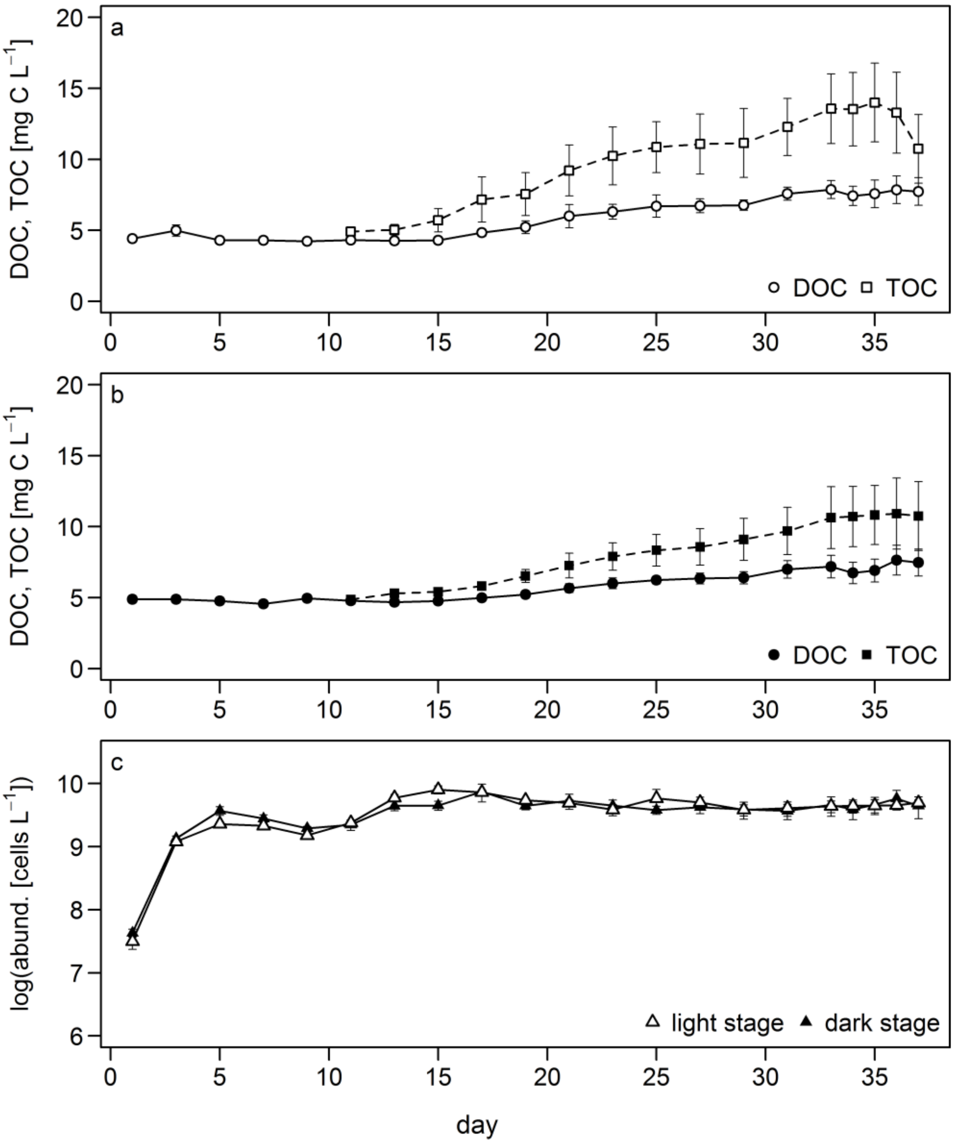

a
DOC, TOC [mg C L^{-1}]
○ DOC □ TOC
b
DOC, TOC [mg C L^{-1}]
● DOC ■ TOC
c
log(abund. [cells L^{-1}])
△ light stage ▲ dark stage
0
10
20
30
40
50
day
0
2
4
6
8
10
12
14
6
7
8
9
10

Fig. 4


a
DOC, TOC [mg C L^{-1}]
0
5
10
15
20
○ DOC □ TOC
b
DOC, TOC [mg C L^{-1}]
● DOC ■ TOC
c
log(abund. [cells L^{-1}])
6
7
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△ light stage ▲ dark stage
0
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25
30
35
day

Fig. 5

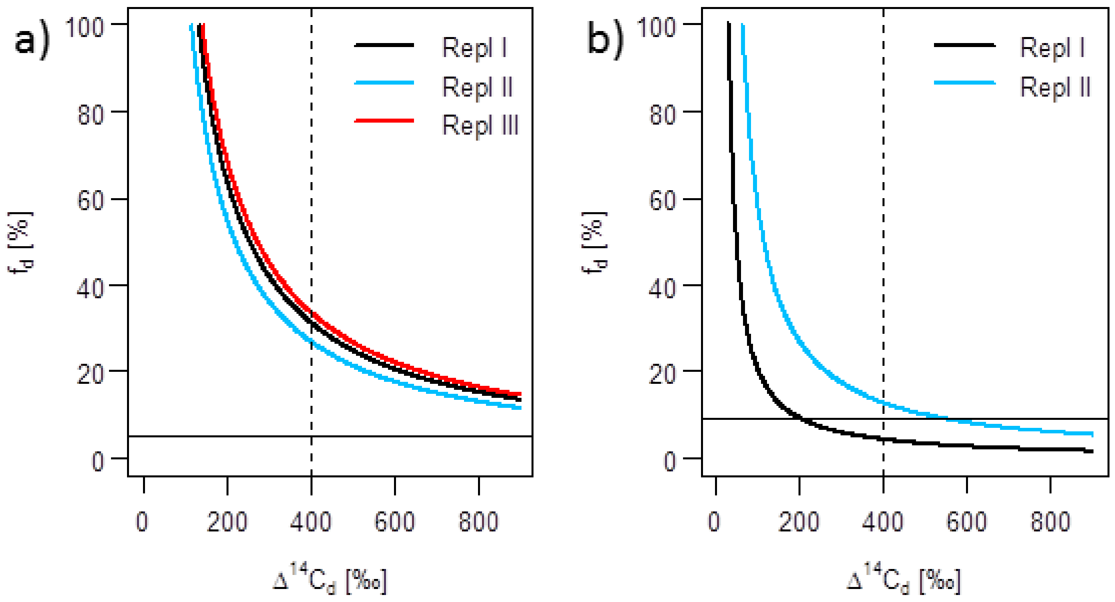

a)
b)
100
80
60
40
20
0
f_d [%]
0
200
400
600
800
$\Delta^{14}C_d$ [‰]
Repl I
Repl II
Repl III

Fig. 6

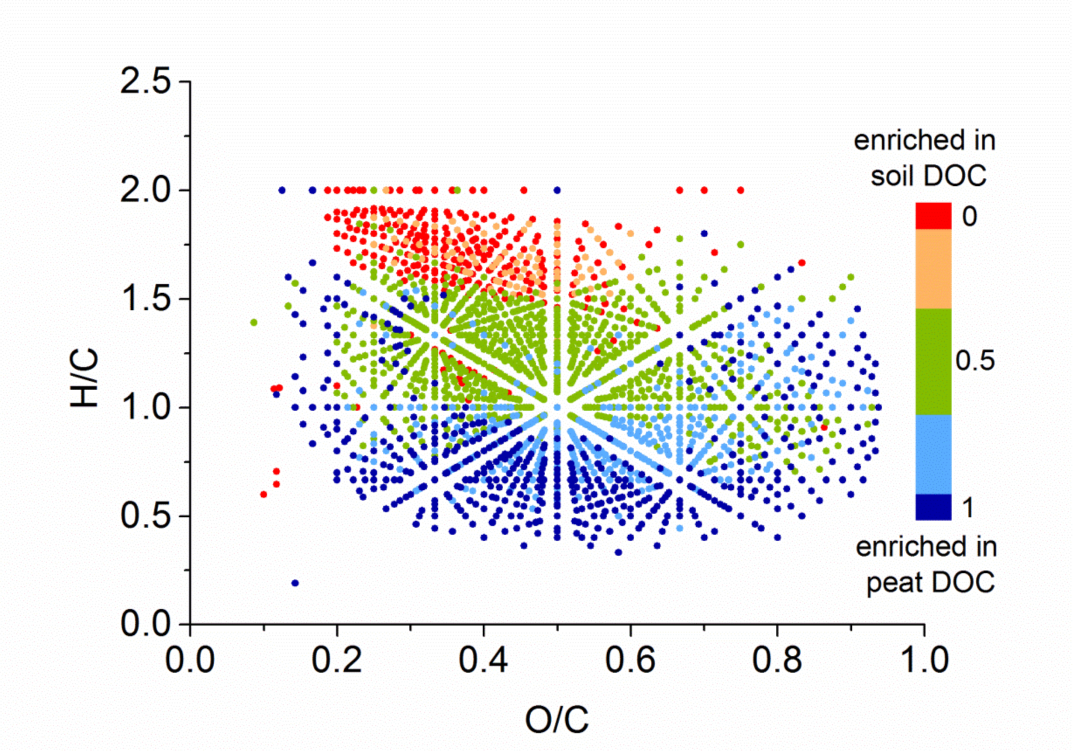

2.5
2.0
1.5
1.0
0.5
0.0
H/C
0.0
0.2
0.4
0.6
0.8
1.0
O/C
enriched in soil DOC
0
0.5
1
enriched in peat DOC

Fig. 7

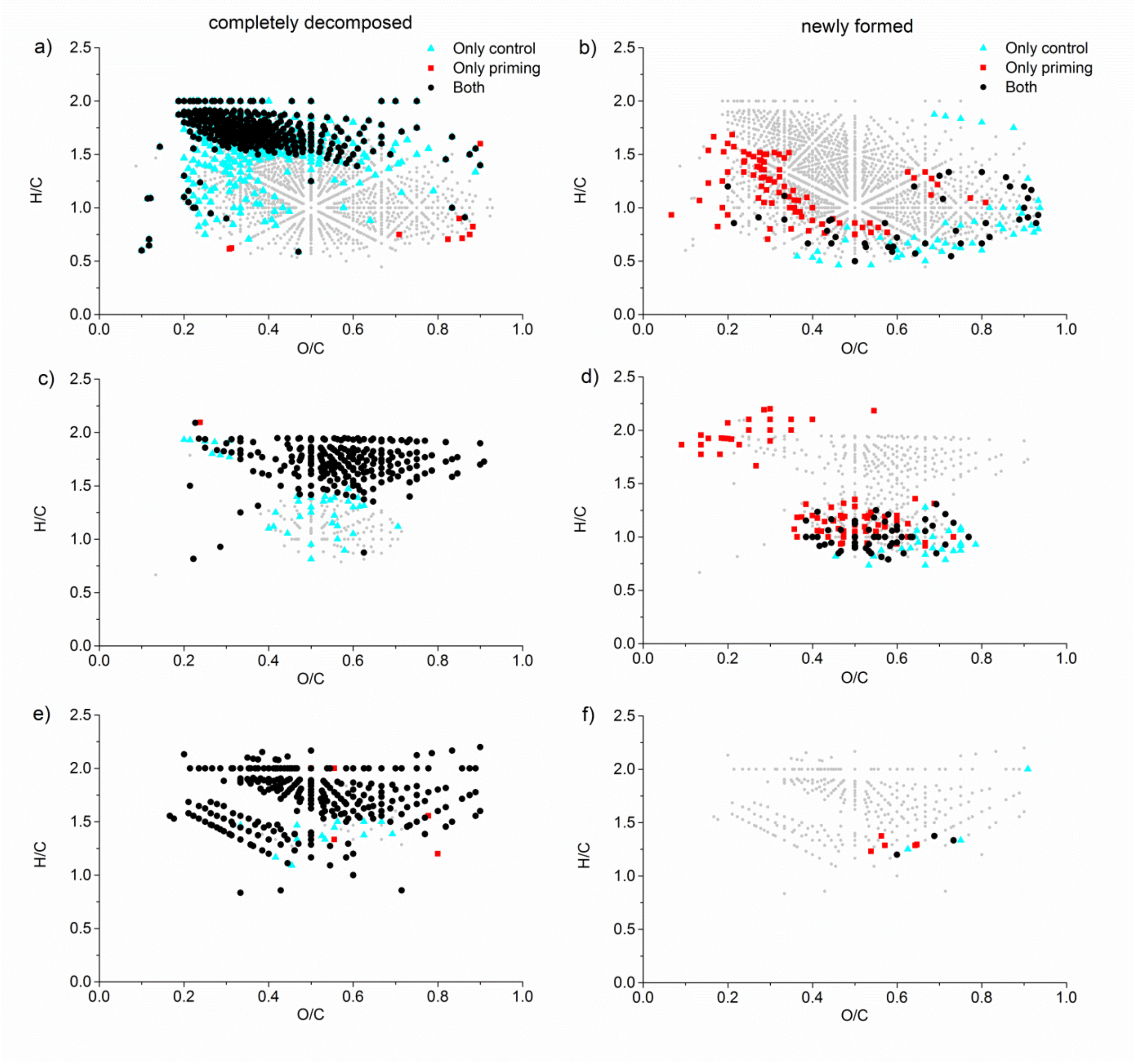
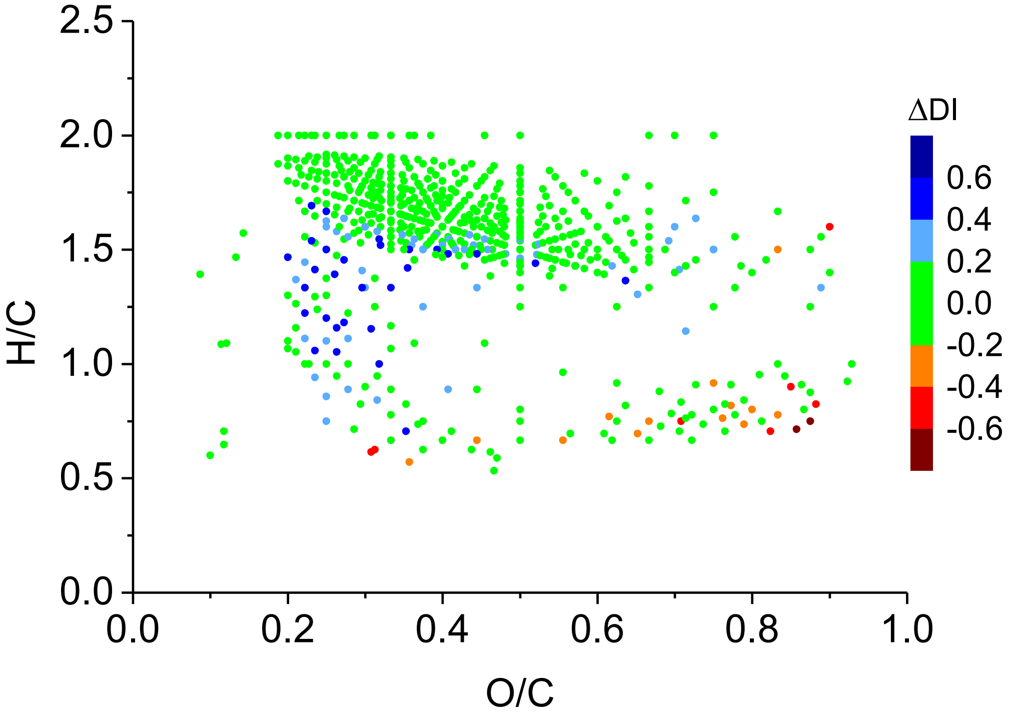

completely decomposed
newly formed
a)
b)
c)
d)
e)
f)
Only control
Only priming
Both
H/C
O/C
0.0
0.5
1.0
1.5
2.0
2.5
0.0
0.2
0.4
0.6
0.8
1.0

Fig. 8



Supporting information

Microbial Ecology

Tracing aquatic priming effect during microbial decomposition of terrestrial dissolved organic carbon in chemostat experiments

Karoline Morling[a]*, Julia Raeke[b], Norbert Kamjunke[a,c], Thorsten Reemtsma[b], Jörg Tittel[a]

Helmholtz Centre for Environmental Research – UFZ
[a] Department of Lake Research, Brückstr. 3a, 39114 Magdeburg, Germany
[b] Department of Analytical Chemistry, Permoserstr. 15, 04318 Leipzig, Germany
[c] Department of River Ecology, Brückstr. 3a, 39114 Magdeburg, Germany
*corresponding author: Tel.: +49 391 8109447, email: karoline.morling@ufz.de

Description and results of pre-irradiation experiments

The influence of photolytic effects was tested by exposing both DOC substrates (peat water and soil leachate) to UV light before running one-stage chemostats in the dark. DOC media were prepared as described in the method section, filled in glass bottles (transmitting 80% of the UV radiation) and exposed to UV light (fluorescent lamp PL-S 9W BLB/2P 1CT, Philips) for 24 h before starting the experiments. The UV light intensity (316 mW m^{-2}) was similar to that of the emitted UV light in the conducted two-stage setups (313 mW m^{-2}). No DOC was decomposed during the 24 h irradiation. With the pre-irradiated DOC media, we performed triplicate one-stage chemostat experiments in the dark. The settings, sampling procedure and analyses as well as calculations were conducted as described in the method section.

Only slight changes in DOC concentrations were observed in the chemostat experiments with pre-irradiated media (Fig. S1): an increase of 7% was observed for the peat substrate, but a decrease of 6% for the soil substrate (compare also with Tab. 2). The DOC change in the peat pre-irradiation experiment was significantly higher than in the peat control (Wilcoxon sign rank test, $p < 0.001$). For the soil pre-irradiation experiment the DOC change was not significantly different from the soil control (Wilcoxon sign rank test, $p > 0.05$). Bacterial abundances differed not significantly between the pre-irradiation and the control experiments for both substrates.

Extraction of the samples from the soil DOC pre-irradiation experiments and subsequent measurement via FT-ICR MS were performed as described in the method section. FT-ICR MS parameters of the soil pre-irradiation experiments are given in Tab. S1. To evaluate the influence of the light on the molecular composition, the degradation index (DI) was calculated first (see methods). In a second step, the ΔDI was calculated to compare the soil pre-irradiation and soil control experiments ($\Delta DI = DI_{pre-irradiation} - DI_{control}$), but only marginal differences were observed between these samples. The vast majority (95%) of all ΔDI values ranged from -0.2 to 0.2 (Fig. S2, see also methods), which indicates molecules decomposed in equal measure in both the control and the pre-irradiation setup. We therefore concluded that the light had no substantial effect on the chemical composition of the soil DOC substrate.

Tab. S1 FT-ICR MS parameters for the samples of the soil pre-irradiation experiment. Parameters were calculated as intensity-weighted averages (see methods). Percentages of $C_xH_yO_z$, $C_xH_yO_zN_{1\text{-}2}$ and $C_xH_yO_zS_1$ molecules (number of assigned sum formulae are given in parentheses) are provided.

	Soil pre-irradiation experiment
m/z_{wa}	388 ± 1
DBE_{wa}	8.7 ± 0.1
O/C_{wa}	0.55 ± 0.01
H/C_{wa}	1.13 ± 0.01
$C_xH_yO_z$ [%]	95.1 ± 0.1 (1429)
$C_xH_yO_zN_{1\text{-}2}$ [%]	4.1 ± 0.1 (184)
$C_xH_yO_zS_1$ [%]	0.9 ± 0.1 (35)

Fig. S1

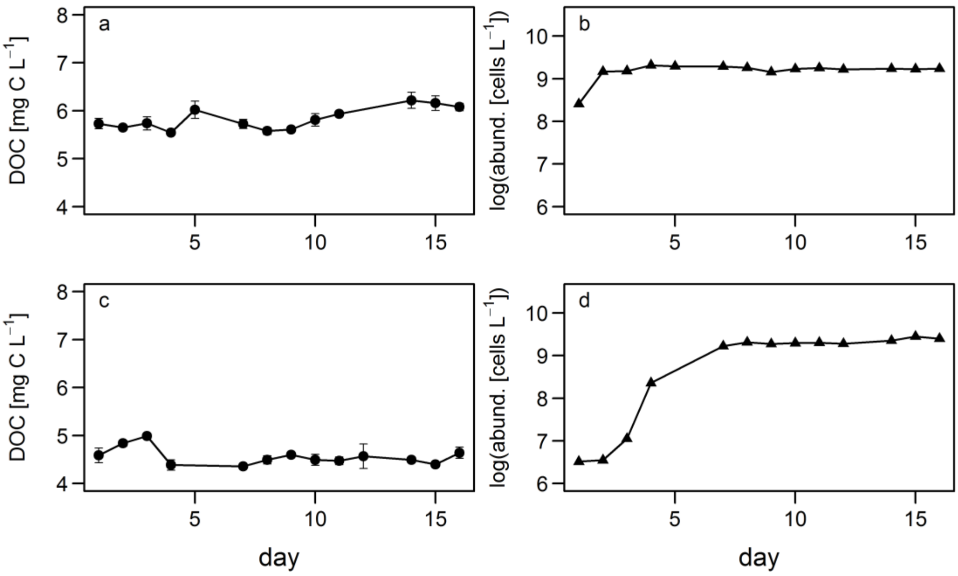


Fig. S1 DOC concentrations and bacterial abundances in the peat (a, b) and soil (c, d) pre-irradiation experiments (Mean ± standard error of the mean, n=3)

Fig. S2

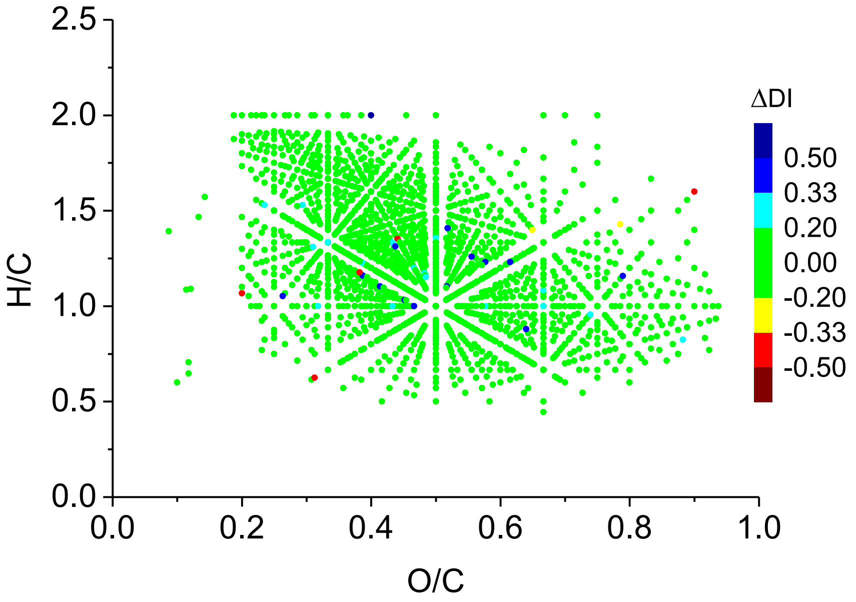


Fig. S2 Van Krevelen diagram of the $C_xH_yO_z$ molecules, which were partly decomposed in the soil control and the pre-irradiation experiment. The color code indicates the intensity of decomposition (ΔDI), calculated as $\Delta DI = DI_{pre-irradiation} - DI_{control}$. Positive ΔDI values represent molecules which were more strongly decomposed in the control than in the pre-irradiation setup, while negative ΔDI values represent molecules which were more strongly decomposed in the pre-irradiation setup than in the control. A ΔDI between -0.2 and 0.2 indicates molecules which were decomposed in equal measure in both the control and the pre-irradiation setup (see also methods)

Derivation of equation (5)

To obtain the fraction of decomposed terrestrial DOC (f_d), equations (1), (2) and (4) from the methods sections are used.

Equation (2) is solved for f_r:

$$f_r = 1 - f_d \tag{S1}$$

Equation (1) is solved for $\Delta^{14}C_r$:

$$\Delta^{14}C_r = \frac{\Delta^{14}C_{start} - f_d \Delta^{14}C_d}{f_r} \tag{S2}$$

Replacing f_r by inserting (S1) in (S2), thus:

$$\Delta^{14}C_r = \frac{\Delta^{14}C_{start} - f_d \Delta^{14}C_d}{1 - f_d} \tag{S3}$$

Replacing f_r in equation (4) by inserting (S1), which gives:

$$DOC_s \times \Delta^{14}C_s = (1 - f_d) \times DOC_{start} \times \Delta^{14}C_r + (DOC_s - (1 - f_d) \times DOC_{start}) \times \Delta^{14}C_a \tag{S4}$$

Then, $\Delta^{14}C_r$ is replaced by inserting (S3) in (S4):

$$DOC_s \times \Delta^{14}C_s \;= (1 - f_d) \times DOC_{start} \times \frac{\Delta^{14}C_{start} - f_d \Delta^{14}C_d}{1 - f_d} + (DOC_s - (1 - f_d) \times DOC_{start}) \times \Delta^{14}C_a \tag{S5}$$

(S5) can be cancelled down to:

$$DOC_s \times \Delta^{14}C_s \;= DOC_{start} \times \Delta^{14}C_{start} - f_d \times DOC_{start} \times \Delta^{14}C_d + (DOC_s - DOC_{start} + \; f_d \times DOC_{start}) \times \Delta^{14}C_a \tag{S6}$$

(S6) can be expanded to:

$$DOC_s \times \Delta^{14}C_s \;= DOC_{start} \times \Delta^{14}C_{start} - f_d \times DOC_{start} \times \Delta^{14}C_d + (DOC_s - DOC_{start}) \times \Delta^{14}C_a \; + \; f_d \times DOC_{start} \times \Delta^{14}C_a \tag{S7}$$

All terms not containing f_d are shifted to the other side of the equation:

$$DOC_s \times \Delta^{14}C_s \; - DOC_{start} \times \Delta^{14}C_{start} - (DOC_s - DOC_{start}) \times \Delta^{14}C_a \; = f_d \times DOC_{start} \times \Delta^{14}C_a \; - f_d \times DOC_{start} \times \Delta^{14}C_d \tag{S8}$$

Then, f_d can be factored out:

$$DOC_s \times \Delta^{14}C_s \; - DOC_{start} \times \Delta^{14}C_{start} - DOC_s \times \Delta^{14}C_a \; + DOC_{start} \times \Delta^{14}C_a \; = f_d \times (DOC_{start} \times \Delta^{14}C_a \; - DOC_{start} \times \Delta^{14}C_d) \tag{S9}$$

For simplification, DOC concentrations are factored out on the left side of equation (S9):

$$DOC_s \times (\Delta^{14}C_s - \Delta^{14}C_a) + DOC_{start} \times (\Delta^{14}C_a - \Delta^{14}C_{start}) = f_d \times (DOC_{start} \times \Delta^{14}C_a - DOC_{start} \times \Delta^{14}C_d) \tag{S10}$$

Now, f_d can be separated by dividing the bracket term:

$$\frac{DOC_s \times (\Delta^{14}C_s - \Delta^{14}C_a) + DOC_{start} \times (\Delta^{14}C_a - \Delta^{14}C_{start})}{(DOC_{start} \times \Delta^{14}C_a - DOC_{start} \times \Delta^{14}C_d)} = f_d \tag{S11}$$

Finally, equation (5) is obtained by factoring out DOC$_{start}$ concentrations in the denominator:

$$f_d = \frac{DOC_s \times (\Delta^{14}C_s - \Delta^{14}C_a) + DOC_{start} \times (\Delta^{14}C_a - \Delta^{14}C_{start})}{DOC_{start} \times (\Delta^{14}C_a - \Delta^{14}C_d)}$$

4. Discussion

DOC represents not only a key element in the aquatic carbon cycle (Prairie 2008), but also for water quality and drinking water production (Delpla *et al.* 2009, Matilainen and Sillanpää 2010, Ledesma *et al.* 2012, Lavonen *et al.* 2013). In my thesis, I focused on quantitative and qualitative aspects of OC cycling in freshwater systems. In the first part, I investigated the utilization of DOC by heterotrophic microbes and the OC cycling in pre-dams of drinking water reservoirs under different hydrological conditions and over a gradient of trophic states. In the second part, I investigated the potential occurrence of aquatic priming effect as a possible process contributing to the DOC transformation in freshwaters. First, I will discuss the main findings and give an overview on implications in the context of climate change and subsequently address future research questions.

4.1 Organic carbon cycling and DOC transformation in the pelagic zone of pre-dams

Within my thesis, several key components regulating the OC cycling and DOC quality changes within the pelagic zone of pre-dams were identified (Fig. 4-1). NP and Resp in the upper water layers were predominantly regulated by **discharge and temperature**. It should be noted that discharge and temperature are partly negatively correlated as discharge is typically lower throughout the summer season in Germany. Hence, higher NP and Resp were observed in summer under base-flow conditions. The same relationship was observed in the tributaries of the pre-dams, where bacterial DOM degradation was significantly influenced by discharge and temperature as well, and higher degradation was observed in summer during base-flow conditions (Kamjunke *et al.* 2016). Both discharge and temperature (season) have been also identified as key factors explaining the intra-annual DOC variability in other running waters (Raymond and Saiers 2010, Winterdahl *et al.* 2014, Wallin *et al.* 2015). Further, **trophic status** was of importance for OC production and decomposition, although no significant relationship was found between P concentrations and both NP and Resp. This can be explained by higher P concentrations and higher P loads when discharge is high and NP and Resp are negligible due to the flushing effect. For instance, this was observed during the snow melt event at Hassel pre-dam in 2013. However, generally higher rates of NP and Resp were measured in the meso- and eutrophic pre-dams.

The studied pre-dams were mainly **autotrophic** in their epilimnia. It should be noted, that the in-situ measurements for NP and Resp were conducted only in the upper 3 m, which comprises the whole water column in the oligotrophic Rote Mulde pre-dam, but not in both pre-dams located in the Harz Mountains. Thus, these two pre-dams most probably could have shown a more heterotrophic behavior when the whole water column had been included in the measurements. However, as pre-dams are usually constructed with an overflow similar to natural lakes, processes in the upper water layers are most important for OC cycling, water quality changes and the delivery of these waters to the main reservoirs.

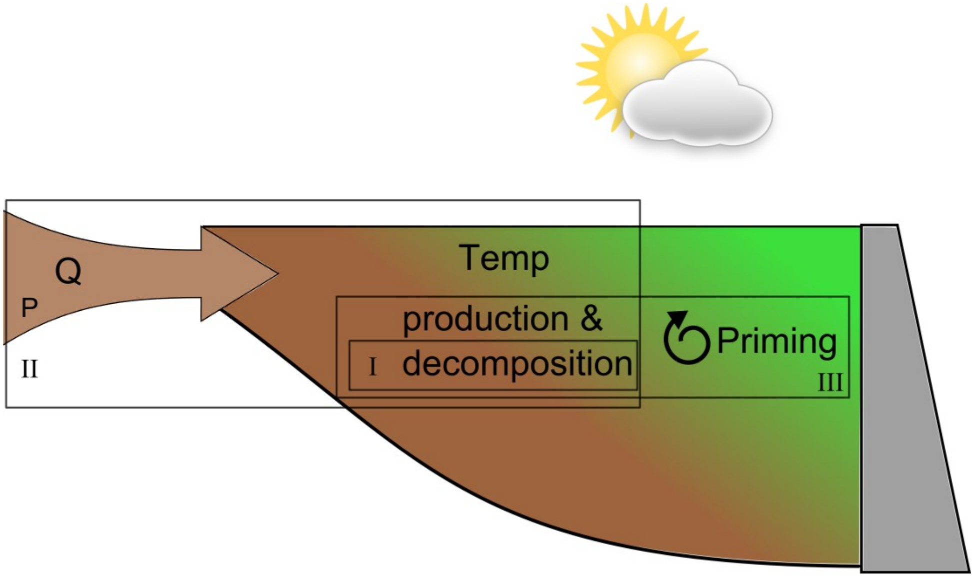


Fig. 4-1: Conceptual overview of identified key components influencing OC cycling and DOC quality changes in the pelagic zone of pre-dams. Boxes and Roman numerals refer to the numbering of the corresponding manuscripts within my thesis.

On average 0-30% of the total gained OC was produced within the upper water layers of the pre-dams. Interestingly, the highest percentage was estimated for the mesotrophic Rappbode pre-dam. Although the eutrophic Hassel pre-dam exhibited the highest average OC production, it also received the highest OC import. This was driven mainly by a long winter and an intense snow melt event taking place in mid-April in 2013. In contrast, 2014 was a year with an exceptionally warm winter. Thus, comparisons between consecutive years are hampered by weather-related variability and differences in the magnitude of runoff delivered from the catchments to the pre-dams. This indicates the importance of **hydrological extremes** such as snow melts or heavy rain events, which are often responsible for the delivery of large fractions of the annual DOC load within short time periods (Brooks *et al.* 1999, Nguyen *et al.* 2002, Dalzell *et al.* 2007, Raymond and Saiers 2010, Graeber *et al.* 2015b, Zwart *et al.* 2016b). Such extreme events affect not only the loadings of OC and nutrients and change lake water chemistry (Weyhenmeyer *et al.* 2004, Li *et al.* 2016), but also lead to shifts in plankton communities (Weyhenmeyer *et al.* 2004) and are thus able to change lake metabolism. For instance, Zwart *et al.* (2016b) observed predominantly shifts towards lake net heterotrophy but also autotrophic responses following extreme precipitation events. The magnitude and direction of these changes most likely depend on the quality of the material delivered during the event and its subsequent utilization within the lake. Increasing exports of bioavailable DOM with increasing discharges were found within streams (Fellman *et al.* 2009, McLaughlin and Kaplan 2013, Wilson *et al.* 2013, Wilson *et al.* 2016). In line with this, we observed the highest amount of microbially decomposed DOC (40-61%) following the snow melt event at Hassel pre-dam in April 2013.

The importance of hydrological conditions was also reflected in the microbial utilization of the DOC pool. Heterotrophic bacteria preferentially utilized older DOC under base-flow conditions, whereas younger carbon components were respired following a storm flow event at Rote Mulde pre-dam in September 2014. This confirms previous results, showing that **^{14}C age** and **recalcitrance** are **independent** (Petsch *et al.* 2001, Fontaine *et al.* 2007, McCallister and del Giorgio 2012). To analyze the ages of the microbially utilized DOC, I adapted and simplified a method for recovering CO_2 from respiration of heterotrophic bacterioplankton for isotopic analysis. In comparison to the previously established method by McCallister *et al.* (2006), investigations can be carried out in replicate incubations at in-situ temperatures under standard laboratory conditions, enabling its application in a broad range of conditions. An additional re-oxygenation step prior to the incubation is omitted and the microbially respired CO_2 can be recovered without the construction of a technically demanding harvest line by precipitation in barium hydroxide solution. Currently, the method relies on running a control with TIC-free medium to estimate the potential entry of atmospheric CO_2 during the incubation. However, this can be improved in future applications by choosing air-tight combinations of vessels and lids. The developed method holds manifold potential for future research. For instance, replicate incubations allow investigations on the variability of microbially utilized and respired CO_2 originating from the same DOC source. Further, it can be easily combined with qualitative investigations of the remaining DOC pool using methods such as fluorescence spectroscopy or high resolution mass spectrometry. Another possibility would be the investigation of bacterial community compositions.

A removal of older DOC during base-flow could be enabled by two pathways. Under base-flow conditions, entries of groundwater DOC are dominating and groundwater-derived DOC was found to be usually depleted in ^{14}C, showing radiocarbon ages of 100 to 1000 of years (Trumbore *et al.* 1992, Schiff *et al.* 1997, Trumbore 2009). A second pathway was identified in riverine food web studies applying extensive carbon isotope analyses. Carbonate weathering contribute to ^{14}C-depleted dissolved inorganic carbon (DIC), which is then taken up by photosynthetic organisms (Ishikawa *et al.* 2015a). Subsequently, this carbon can be released by algae and circulating in the food web in form of DOC and POC with high radiocarbon ages (Ishikawa *et al.* 2015a, Ishikawa *et al.* 2015b). In the catchments of both pre-dams located in the Harz Mountains, isotope studies revealed that old DIC is circulating in the tributaries (Tittel *et al.* 2015). Thus, the second pathway via algae-derived old DOC gives a very likely explanation for the observed utilization of older DOC compounds during the incubations. In contrast, in the Rote Mulde catchment such ^{14}C depleted DIC was not observed in a previous study (Tittel *et al.* 2013), giving evidence that groundwater entries delivering old DOC make a more likely explanation in this catchment under base flow conditions. These results point to the consideration of local geological properties and hydrological connections within catchments when interpreting results of carbon isotope studies in freshwater systems.

Naturally, production and decomposition of OC are taking place simultaneously, which lead to **qualitative changes** of the DOC pool. We observed such changes in the two pre-dams located in the Harz Mountains throughout the study years. The freshness index (β/α), an indicator of recently produced DOM, was negatively correlated with discharge. Complementary to this, the humification index (HIX), an indicator of humic substance content, was positively related with discharge in the Rappbode pre-dam. This is in agreement with the observed dependence of NP and Resp on discharge. Greater contributions of aromatic and humic-like substances and increases in HIX are related to rainy periods and increasing flow rate (Inamdar *et al.* 2011, Wallin *et al.* 2015, Yang *et al.* 2015, Zhou *et al.* 2016), as usually higher amounts of terrestrial DOC enter inland waters during storm flow events (Weyhenmeyer *et al.* 2004, Dalzell *et al.* 2007, Raymond and Saiers 2010, Graeber *et al.* 2015b). Likewise, Kellerman *et al*. (2014) identified hydrological conditions (precipitation and WRT) and temperature as the main factors shaping the molecular composition of DOM in Swedish lakes. However, land use and vegetation patterns significantly influence the DOC quality of the water flowing into lakes and reservoirs. Within the Bode catchment, to which Hassel and Rappbode pre-dams belong, the proportion of forests was positively related to the content of humic substances (Kamjunke *et al.* 2013). A recent study within the three studied catchments revealed that the riverine DOM in forested areas was more oxygenated, more aromatic and of higher molecular weight compared to the DOM in areas with higher contributions of grasslands and agriculture (Raeke *et al.* 2017). In contrast, riverine DOM from agricultural catchments was found to be of more microbial character, showing a less plant-derived composition with higher proportions of sulfur- and nitrogen-containing molecules (Graeber *et al.* 2012, Graeber *et al.* 2015a, Raeke *et al.* 2017).

To conclude, we identified pre-dams as **hot spots of OC cycling**, significantly altering the DOC quality despite being much smaller in size and having much shorter WRT than their corresponding main reservoirs and most natural lakes. The magnitude and direction of OC cycling and DOC quality changes depend mainly on discharge conditions, temperature (season) and trophic status.

4.2 Occurrence of aquatic priming effect during microbial DOC decomposition

Within the second part of my thesis, I investigated if aquatic priming effect depicts a potential process facilitating the decomposition of allochthonous derived DOC in freshwater systems. We combined quantitative and qualitative investigations to trace aquatic PE and to follow changes in DOC composition. While previous studies used predominantly sugar compounds and algal extracts as primer (Bengtsson *et al.* 2014, Guenet *et al.* 2014, Bianchi *et al.* 2015, Catalán *et al.* 2015), we established **growing phytoplankton** as a source of labile OM within the two-stage chemostat cultures to mimic natural conditions. Autochthonous produced DOC is regarded as labile and preferentially utilized by heterotrophic microbes (Coveney 1982, Chen and Wangersky 1996, Bertilsson and Jones 2003, Kritzberg *et al.* 2004, Nguyen *et al.* 2005, Guillemette *et al.* 2013), making it a suitable primer substance.

Priming was revealed via radiocarbon analyses and isotope mixing models for both tested terrestrial DOC substances, although the priming was more pronounced for the peat water DOC than for the soil leachate DOC. Predominantly younger DOC components of both terrestrial substrates were decomposed by the microbial assemblages during priming as indicated by the applied isotope mixing models.

Although we observed priming in our experiments, the **total DOC concentrations** even increased. That appears counterintuitive at first sight as one would expect that priming leads to a depletion of the total DOC pool. However, even in studies of soil PE more carbon from the added primer remained at the end of the incubation than was lost due to additional OM mineralization (Hamer 2004) and an increased turnover due to priming does not necessarily lead to a decrease of the total C stock (Neff *et al.* 2002, Kuzyakov 2010). In our experiment, the phytoplankton growth within the chemostat cultures was not restricted by herbivores as it would be the case in natural systems. Thus, the high algae biomass most likely promoted the observed accumulation of autochthonous DOC due to extracellular release, which can reach considerably high rates (Anderson and Zeutschel 1970, Berman and Holm-Hansen 1974, Larsson and Hagström 1979, Obernosterer and Herndl 1995, Kamjunke and Tittel 2009). Interestingly, Attermeyer *et al.* (2014) observed in a laboratory experiment that rising concentrations of added phytoplankton lysate increased the bacterial consumption and lead to a decrease of humic substances within the allochthonous DOC pool. Thus, the high amounts of algae-derived DOC in our experiments might have even increased the observed PE. Further, it suggests that priming mediated by phytoplankton-derived labile OM might be most important in eutrophic systems where considerable autochthonous production exists.

Samples from the soil leachate experiments were analyzed with ultra-high resolution mass spectrometry (**FT-ICR MS**). Predominantly the same saturated molecules with H/C ratios > 1.5 were completely decomposed in both control and priming experiment. However, the decomposition of more stable molecules differed and oxidized, unsaturated molecules with H/C ratios < 1.0 were more strongly decomposed under priming (i.e. in presence of phytoplankton). Although such molecules are considered as less decomposable than molecules with an H/C ratio > 1.5 (Sleighter *et al.* 2014, D'Andrilli *et al.* 2015, Lu *et al.* 2015), a preferential removal of oxidized, aromatic compounds during decomposition was also observed by Kellerman *et al.* (2015) in a survey of DOM in Swedish lakes.

In soil science, the occurrence and magnitude of priming has been linked to the type and concentration of the added primer as well as to the soil properties (Hamer 2004, Rasmussen *et al.* 2007, Zimmerman *et al.* 2011, Pascault *et al.* 2013). Further, the **availability of N** plays a crucial role in soil OM decomposition and priming (Kuzyakov *et al.* 2000, Neff *et al.* 2002, Hamer 2004, Conde *et al.* 2005, Blagodatskaya and Kuzyakov 2008, Kuzyakov 2010, Gärdenäs *et al.* 2011). In aquatic systems, bacterial growth on amino acids has been known to be more efficient than on monosaccharides (Kirchman 2003) and the C/N ratio of the substrate was identified as a major determinant of bacterial growth (Kroer 1993, Sun *et al.* 1997). We observed two pools of newly produced N-containing molecules (a labile and a stable one) in the presence of phytoplankton which were not found in the control experiment. Both a reactive and a refractory pool of dissolved organic nitrogen were also identified in a bioassay study by Osborne *et al.* (2013). Similarly, Kellerman *et al.* (2015) concluded that N-containing compounds are either resistant to degradation or tightly cycled within freshwaters. In a recent study, Hannides and Aller (2016) observed an increased mineralization of sedimentary OM after addition of mucus from a benthic gastropod and related the priming effect to an accelerated remineralization of sedimentary N. Thus, priming in aquatic systems might be coupled to the dynamics of N-containing compounds and their availability for bacterial growth.

From our results, we can conclude that the supply of labile phytoplankton derived OM facilitated the microbial decomposition of stable terrestrial derived DOC. Although, the total DOC pool even increased, priming was revealed via carbon isotope analysis and oxidized, unsaturated compounds were more strongly decomposed in phytoplankton presence. Thus, aquatic priming could be mainly considered as a **qualitative effect**.

4.3 Implications for water quality in relation to changing climate

Climate change is predicted to globally further increase surface temperatures and altering precipitation patterns (IPCC 2014), thus affecting the global water cycle and leading to tremendous changes in (1) the leaching of carbon and nutrients from catchments and their transported loads, (2) abiotic and biotic responses within inland waters as well as (3) water quality. Thus, climate change will also have tremendous impacts on OC cycling and DOC quality within freshwaters.

Hydrological changes significantly affect DOC exports and might lead to either increased DOC inputs to lakes (Freeman *et al.* 2001, Tranvik *et al.* 2009) or even decreased DOC concentrations (Schindler *et al.* 1997) depending on local topographic and climatic conditions within the catchments. Likewise, the leaching and transport of nutrients are affected and increases in N and P loadings to freshwaters are usually obtained in modelling studies for future scenarios (Arheimer *et al.* 2005, Andersen *et al.* 2006, Kaste *et al.* 2006, Jeppesen *et al.* 2009, George 2010, Trolle *et al.* 2011). Simultaneously, in-lake processes are affected by increasing temperatures in a complex manner. A prolonged stratification period might lead to an increased O_2 demand in the hypolimnion, resulting in hypoxia and P release from sediments (Komatsu *et al.* 2007, Nixdorf *et al.* 2009, George 2010, Shatwell *et al.* 2013). Thus, the internal P loading could enhance algae growth and promote the formation of algal blooms (Komatsu *et al.* 2007, Nixdorf *et al.* 2009, George 2010, Shatwell *et al.* 2013).

In Germany, **climate scenarios** predict in general increased precipitation in the winter season and decreased precipitation during summer, which might lead to higher discharges in winter and lower ones in summer (DWA 2014, UBA 2015). Higher precipitation during winter potentially lead to a higher soil erosion as the plant cover is reduced. In turn, this might also increase diffuse inputs from agricultural lands into water bodies. Soil erosion is predicted to be most pronounced in alpine uplands and secondary mountain ranges (UBA 2015), where most of the German drinking water reservoirs are located. In contrast, less water will be available during summer, which will increase WRT and affect water quality (DWA 2014). Warmer water, reduced inflow and enhanced evaporation in summer are expected to increase the trophic status of inland waters and might promote algae growth (Nixdorf *et al.* 2009). Higher bioactivity and prolonged thermal stratification during summer (Shatwell *et al.* 2013) could lead to increased oxygen consumption and increase internal P loadings in German lakes and reservoirs (Nixdorf *et al.* 2009, Shatwell *et al.* 2013). Hence, the impacts of climate change could counteract the reduction of trophic states by load reductions (Nixdorf *et al.* 2009, Shatwell *et al.* 2013, Horn *et al.* 2015).

As a consequence, two contrasting extremes can be identified in terms of the expected **DOC quality** in pre-dams and reservoirs: (A) drier summers with lower discharges will lead to higher contributions of autochthonous produced DOC, while (B) wetter winters with higher discharges will result in more allochthonous derived DOC (Fig. 4-3). Both extremes can be expected to be much more pronounced in future years. Simultaneously, an expected increase in heavy rain events (Easterling *et al.* 2000, UBA 2015, Fischer and Knutti 2016) could cause abrupt shifts from situation A to situation B and increasing the variability in DOC quality during the summer season. Such shifts will be of particular importance in pre-dams due to their small sizes and their location in headwater regions and will affect the DOC load and quality of the water delivered from the pre-dams to the main reservoirs.

In future, priming of stable allochthonous DOC in freshwaters could potentially increase during the summer season as bioactivity can be expected to be higher and more autochthonous DOC as labile primer substance might be available during the prolonged stratification periods. However, at the same time less of the stable terrestrial DOC might be imported into the water bodies during the summer season due to decreased precipitation. Instead, large amounts of terrestrial DOC might be transported during winter and/or during heavy rain events, thereby passing the pre-dams and reaching the main reservoirs or regions located even further downstream. Thus, priming of such transported allochthonous DOC might likely take place with a time lag in downstream regions.

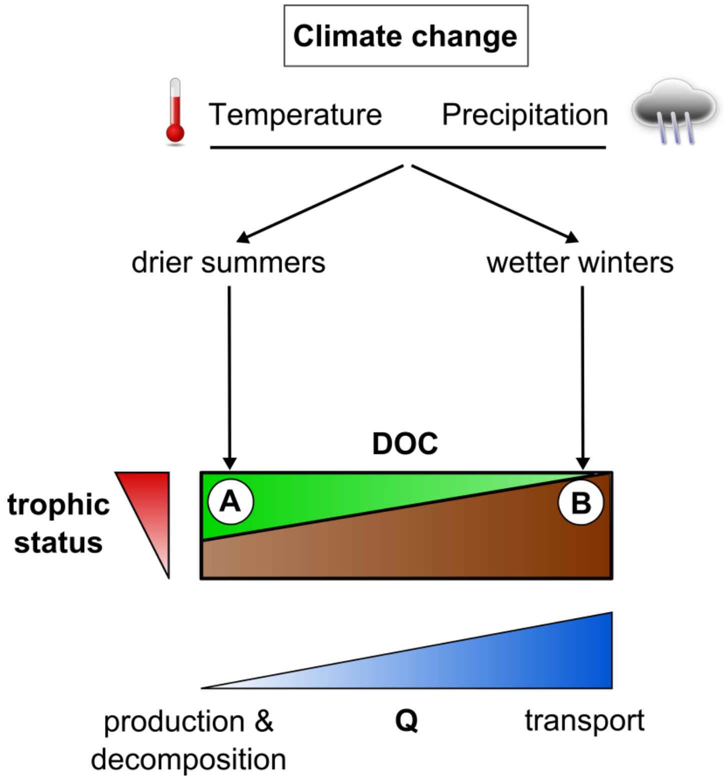


Fig. 4-3: Overview of climate change effects on DOC quality in freshwaters systems. Climate change is characterized by higher temperature and changes in precipitation patterns, which might lead to a stronger pronounciation of two seasonal contrasts: (A) autochthonous produced DOC might dominate during drier summers with lower discharges; while (B) allochthonous derived DOC might be delivered in higher quantities during wetter winters with higher discharges.

4.4 Concluding remarks and future research questions

The cycling of DOC plays an important role in freshwater systems both on the small scale (e.g. bacterial decomposition) and for whole ecosystems (e.g. transport within catchments) as well as for water quality and drinking water production. An understanding of OC cycling and DOC quality changes in inland waters is essential to assess potential effects of anthropogenic alterations of aquatic systems and their catchments and to understand impacts of climate change. The major outcomes of this thesis are:

- Simplification of a method for the recovery of CO_2 respired by heterotrophic bacterioplankton to study sources and ages of utilized DOC

- Identification of discharge, temperature (season) and trophic state as the key factors regulating the production and decomposition of OC in pre-dams of drinking water reservoirs

- Demonstration of pre-dams as hot spots of OC cycling, where DOC quality significantly alters in dependence of hydrological and trophic conditions

- Identifying aquatic priming as a potential process facilitating the microbial decomposition of terrestrially derived DOC

- Presentation of aquatic priming as a qualitative effect contributing to the DOC transformation within freshwater systems

Within the thesis, pre-dams were identified as hotspots of OC cycling, tremendously altering the DOC quality due to internal production and decomposition. Further research is needed to evaluate the role of pre-dams as **net sources or sinks** of DOC and how this varies on temporal scales. For instance, the Horseshoe-Bartlett reservoir system (USA) was estimated to internally produce 41% of the inflow DOC loading in a survey conducted from 1994-1995 (Parks and Baker 1997), but was found to reduce the DOC loading by 21-38% in a survey conducted from 1997-1998 (Nguyen *et al.* 2002). The role of reservoirs as net sources or sinks of DOC also changes during different times of the year. High phytoplankton activity during summer is able to turn a reservoir into a DOC source, while degradative processes during winter turn it into a DOC sink (Kraus *et al.* 2011). Of particular interest is the question how extreme events such as heavy rain or snow melt affect the DOC budgets of pre-dams as hydrological conditions are expected to change in future. Lake metabolism changes in response of heavy precipitation events and these shifts even occur with a certain delay in time (Zwart *et al.* 2016b). This can be attributed to the flush-out and re-establishment of aquatic organisms and will greatly influence DOC loads and quality within reservoirs. Measurements over several years and with appropriate temporal resolution will be needed to study the long-term behavior and to capture the influence of contrasting weather periods and abrupt changes in hydrological conditions on DOC budgets in pre-dams. Such analyses might give valuable results for reservoir and water quality management, especially in the context of climate change and predicted changes in precipitation and discharge conditions. High DOC concentrations during flood events can be expected to further challenge drinking water treatment, making optimizations and investments in treatment technology necessary to efficiently remove DOC from raw waters.

Aquatic priming was identified as a process facilitating the bacterial mineralization of allochthonous DOC. Future studies should aim for capturing DOC quality changes and studying if priming is acting generally on the same kind of DOC components or if there might be differences depending on the DOC source. Priming in soils has been linked to changes in the **bacterial community composition** and certain microbial groups, which are stimulated by substrate additions (Nottingham *et al.* 2009, Pascault *et al.* 2013). In aquatic systems, the use of mixed substrates is known to enhance microbial diversity, resulting in a more efficient carbon use (Geller 1985, Tranvik 1998, Grover 2000, Farjalla *et al.* 2009, Fonte *et al.* 2013, Attermeyer *et al.* 2014) and might thus enable co-metabolism and priming in aquatic environments. Hence, the identification of certain bacterial key players and metabolic pathways enabling an aquatic priming effect is a question for future research and can be carried out by examining enzyme activities during incubation studies and by tracking changes in the functional diversity of microbial communities.

The quantification of aquatic priming in natural systems and how much it contributes to the observed loss of allochthonous DOC along the continuum of inland waters would greatly improve the understanding of the aquatic carbon cycle. To understand the occurrence and magnitude of aquatic priming, it is essential to investigate which **factors** might influence it. Nutrient availability and DOC quality play a crucial role for bacterial decomposition rates (Reche *et al.* 1998, Olsen *et al.* 2002, Findlay 2003). Results from FT-ICR MS analysis in our study revealed that priming might be coupled to the dynamics of N-containing compounds. To elucidate this, further studies could examine the influence of (organic) N additions on the occurrence and magnitude of aquatic PE. The OC cycling in the pre-dams was mainly influenced by discharge and temperature. Consequently, aquatic priming in the pelagic zone of freshwaters could be influenced by these factors as well. A higher production of autochthonous OM as a suitable primer substance can be expected at base-flow in summer and could promote aquatic priming under natural conditions. Further, bioactivity is higher at warmer temperatures and might increase the microbial mineralization and the magnitude of aquatic priming. However, the decomposed allochthonous DOC might be compensated for by newly produced autochthonous DOC, especially under eutrophic conditions. In that case, aquatic priming might be of low relevance for water quality management.

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Curriculum Vitae

Personal

Name	Karoline Morling
Birth	14.04.1987 in Cottbus, Germany
Citizenship	German

Education

12/2012 – present	PhD student Helmholtz Centre for Environmental Research (UFZ), Department of Lake Research
10/2009 – 09/2012	M. Sc. Ecology, Evolution and Nature Conservation University of Potsdam
10/2006 – 09/2009	B. Sc. Life Sciences (specialization in Organismic Biology) University of Potsdam

Participation in Conferences

06/2016	Oral presentation at the Association of the Sciences of Limnology and Oceanography (ASLO) Summer Meeting, Santa Fe, United States
02/2015	Poster presentation at the Association of the Sciences of Limnology and Oceanography (ASLO) Aquatic Sciences Meeting, Granada, Spain
10/2014	Oral presentation at the Annual Meeting of the German Society of Limnology (DGL), Magdeburg, Germany
08/2014	Poster presentation at Radiocarbon in the environment, Belfast, United Kingdom
09/2013	Poster presentation at the Annual Meeting of the German Society of Limnology (DGL), Potsdam, Germany

Teaching and Advising

03/2015	Helping in supervision of students in lab course Plankton Ecology at University of Potsdam (2 weeks)
04/2013 – 03/2014	Instruction of two student assistants in lab work at the Helmholtz Centre for Environmental Research in Magdeburg

Volunteering

07/2012	Volunteer for Turtle Foundation, Boa Vista, Cape Verde (4 weeks)

Magdeburg, 09.03.2017 Karoline Morling

Declaration of originality

This work was carried out from 12/2012 until 03/2017 at the Helmholtz Centre for Environmental Research in the Department of Lake Research. I hereby declare that this thesis or parts of it have not been submitted for a degree at any university. Where other persons work has been used, this has been acknowledged and referenced.

Die vorliegende Dissertation wurde von 12/2012 bis 03/2017 am Helmholtz-Zentrum für Umweltforschung im Department Seenforschung erstellt. Hiermit erkläre ich, dass die vorliegende Arbeit selbständig und nur unter der Verwendung der angegebenen Quellen und Hilfsmittel erstellt wurde. Die Arbeit wurde an keiner weiteren Hochschule vorgelegt.

Magdeburg, 09.03.2017 Karoline Morling