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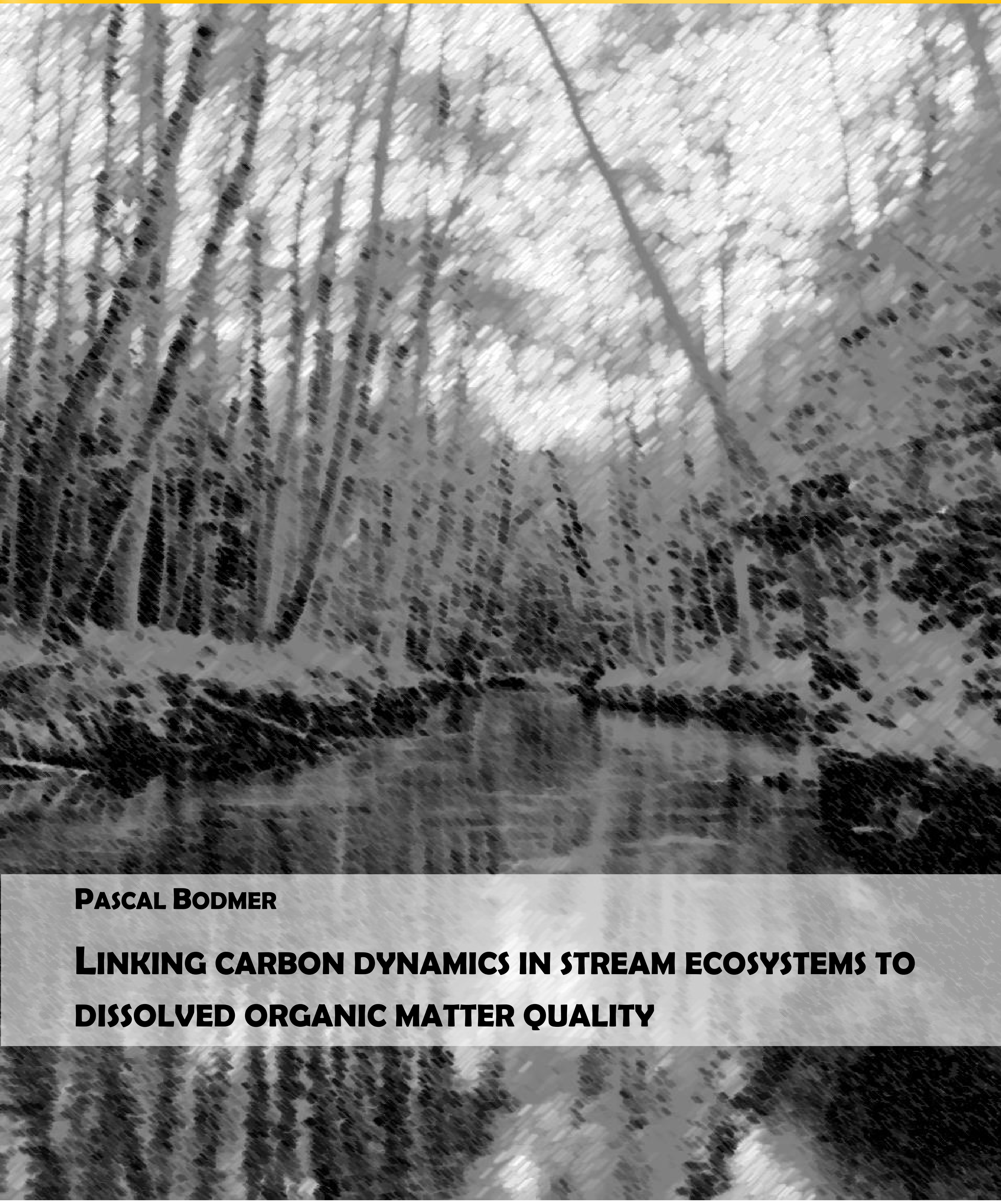

PASCAL BODMER

LINKING CARBON DYNAMICS IN STREAM ECOSYSTEMS TO DISSOLVED ORGANIC MATTER QUALITY

Linking Carbon Dynamics in Stream Ecosystems to Dissolved Organic Matter Quality

Inaugural-Dissertation
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SUMMARY

Stream ecosystems form an active component of the carbon (C) cycle, and are identified as “hotspots” for carbon dioxide (CO_2) emissions. However, the mechanisms driving CO_2 emissions from streams are not completely understood. Beside the input of C in the form of CO_2 from groundwater, streams receive organic matter from aquatic and terrestrial origins which is partly mineralized to inorganic nutrients and CO_2. Future predictions suggest enhanced input of terrestrial organic matter into streams. As such, surrounding land use may highly influence dissolved organic matter (DOM) composition and turnover in streams. The quality, i.e. bioavailability or lability, of aquatic and terrestrial organic matter, as well as which quality feature provides which bioavailability, is controversially discussed and the research is still in its infancy. Thus, the main goal of my thesis is to enhance the understanding of the role of organic matter quality as a potential driver for organic matter turnover in stream ecosystems. A further goal is to shed light on C dynamics with main focus on CO_2 of streams surrounded by different land use.

The presented work is based on an experimental approach in the laboratory, supported by seasonal field studies and a developed model in order to explore C dynamics and the corresponding drivers in stream ecosystems. The underlying mechanisms and the importance of DOM quality as a main driver was assessed on the small scale in laboratory experiments. The C emissions from streams were quantified and the influence of DOM quality was examined on a stream reach scale by investigating two stream types with different organic matter quality inputs. By developing a process-based model, the understanding of the daily and seasonal scale of C turnover in stream ecosystems was amplified.

The results from the experiment under controlled conditions demonstrate that DOM quality governs microbial metabolism (i.e. respiration and bacterial protein production). Moreover, I revealed significant quality differences between two terrestrial DOM sources, while respiration and bacterial protein production increased with the available proportion of the labile DOM source. The molecular weight of DOM was the strongest predictor of bacterial protein production and respiration, while among others, the concentration of low molecular weight substances was another highly influential predictor. The importance of molecular size/weight and DOM quality for microbial metabolism was further confirmed on the stream reach scale where we demonstrated among others a significant linkage between molecular size of DOM and pCO_2 across agricultural and forest streams. Moreover, agricultural streams contained significantly higher pCO_2 compared to forest streams during all seasons. However, CO_2 emissions measured with the powerful drifting chamber method were not significantly different between the stream types. Modeled dissolved oxygen (O_2) and CO_2 dynamics calibrated with field data resulted in respiratory quotients (RQ = mole of CO_2 produced per mole of O_2 consumed), which are

intimately linked to the elemental composition of the respired compounds across four seasons and two stream types. RQ values were not related to adjacent land use or season. Nevertheless, I found significant relationships between RQ values and DOM quality indicators, such as fluorescing component characteristic for higher plant material and molecule size of DOM in agricultural streams.

In conclusion, this thesis demonstrates that DOM quality is an important driver for organic matter turnover in streams. Consequently, my results indicate that ongoing and future land use change and enhanced terrestrial DOM input into streams may influence CO_2 emissions, and underline the status of streams as C turnover “hotspots”. Thus, my thesis contributes to the mechanistic understanding of organic matter cycling in stream ecosystems and their role in the regional and global C cycle. Therefore, organic matter quality should be considered in future models and studies with respect to C cycling.

ZUSAMMENFASSUNG

Bäche und Flüsse stellen eine aktive Komponente des Kohlenstoffkreislaufes dar und wurden als „Hotspots“ für Kohlendioxid (CO_2) Emissionen identifiziert. Die Mechanismen, welche die CO_2 Emissionen aus Bächen steuern, sind bisher aber noch nicht vollständig verstanden. Zusätzlich zum Grundwassereintrag von Kohlenstoff in Form von CO_2, wird in Bäche organisches Material aquatischen und terrestrischen Ursprungs eingetragen, welches teilweise zu anorganischen Nährstoffen und CO_2 mineralisiert wird. Studien weisen auf einen derzeitig erhöhten Eintrag von terrestrischem organischem Material in Bächen hin, der in naher Zukunft weiter ansteigen soll. Es ist daher zu vermuten, dass die Landnutzung in der unmittelbaren Umgebung der Bäche, die Zusammensetzung und Umsetzung des gelösten organischen Materials in den Bächen stark beeinflusst. Die Qualität des organischen Materials aquatischer und terrestrischer Herkunft, also die Bioverfügbarkeit oder Labilität, sowie welches Qualitätsmerkmal welchen Grad an Bioverfügbarkeit darstellt, wird bisher noch kontrovers diskutiert. Die Forschung zu diesen Aspekten befindet sich noch am Anfang. Daher ist das Hauptziel meiner Doktorarbeit, das Verständnis zur Rolle der Qualität des organischen Materials als potentiell kontrollierenden Faktor für die Umsetzung von organischem Material in Bachökosystemen, zu erhöhen. Ein weiteres Ziel ist es, die Kohlenstoffdynamik, hauptsächlich CO_2 Dynamiken, in verschiedenen Bächen mit unterschiedlichen Einflüssen durch die umgebende Landnutzung näher zu untersuchen.

Die vorliegende Arbeit basiert dabei auf einem experimentellen Ansatz im Labor, ergänzt durch saisonale Feldstudien und der Entwicklung eines Models, um Kohlenstoffdynamiken sowie deren entsprechende kontrollierenden Mechanismen in Bachökosystemen zu untersuchen. Die Mechanismen und die Bedeutung der Qualität des gelösten organischen Materials als Einflussfaktor wurden im kleinen Maßstab untersucht. In einem Vergleich zweier Bachtypen mit unterschiedlichen Einträgen von organischem Material auf Bach-Abschnitts Ebene, wurden Kohlenstoffemissionen quantifiziert sowie der Einfluss der Qualität des organischen Materials untersucht. Die Entwicklung eines prozessbasierten Modells, ermöglichte ein besseres Verständnis der Kohlenstoffumsätze in Bachökosystemen auf täglicher und jahreszeitlicher Ebene.

Die Ergebnisse aus dem Experiment mit kontrollierten Bedingungen zeigen, dass die Qualität des gelösten organischen Materials den mikrobiellen Metabolismus, also Atmung und bakterielle Produktion, reguliert. Darüber hinaus konnte ich erhebliche Qualitätsunterschiede zwischen zwei gelösten organischen Kohlenstoffquellen terrestrischer Herkunft aufzeigen. Die Intensität der Respiration und bakteriellen Produktion nahm dabei mit dem verfügbaren Anteil an labilem gelöstem organischen Material zu. Das Molekulargewicht des gelösten organischen

Materials war der stärkste Einflussfaktor für die Respiration und bakterielle Produktion. Die Konzentration der niedermolekularen Substanzen stellte einen weiteren sehr einflussreichen Faktor dar. Die Bedeutung von Molekülgrösse/-gewicht des gelösten organischen Materials und dessen Qualität für den mikrobiellen Metabolismus wurde in der Feldstudie bestätigt. Dort habe ich unter anderem auch einen signifikanten Zusammenhang zwischen der Molekülgrösse des gelösten organischen Materials und dem Partialdruck von CO_2 gefunden, der sich unabhängig von der umgebenden Landnutzung (Landwirtschaft oder Wald) gezeigt hat. Des Weiteren wiesen landwirtschaftliche Bäche im Vergleich zu Waldbächen signifikant höhere CO_2 Partialdrücke zu allen Jahreszeiten auf. Im Gegensatz dazu zeigten die CO_2 Emissionen, gemessen mit der zuverlässigen Methode der driftenden Kammer, keine signifikanten Unterschiede zwischen den beiden Bachtypen. Modellierte Dynamiken von gelöstem Sauerstoff und CO_2, die mit Felddaten kalibriert wurden, resultierten in Respirationsquotienten (RQ = Verhältnis von Mol CO_2 produziert per Mol Sauerstoff konsumiert), welche eng mit der Elementarzusammensetzung der respirierten Komponenten zusammenhängen, in zwei Bachtypen (Landwirtschaft und Wald) zu allen vier Jahreszeiten. Die RQ-Werte zeigten jedoch keinen Zusammenhang mit angrenzender Landnutzung oder der Jahreszeit. Ich konnte dennoch signifikante Zusammenhänge zwischen RQ-Werten und einigen Qualitätsindikatoren von gelöstem organischen Material, wie zum Beispiel einer Fluoreszenz-Komponente, welche für höhere Pflanzen charakteristisch ist oder der Molekülgrösse vom gelösten organischen Material in landwirtschaftlichen Bächen, nachweisen.

Abschließend lässt sich sagen, dass meine Arbeiten die Rolle der Qualität des gelösten organischen Materials als wichtigen Einflussfaktor für die Kohlenstoffumsätze innerhalb von Bächen hervorheben. Meine Arbeiten zeigen, dass die derzeitigen und zukünftigen Landnutzungsänderungen und die erhöhten Einträge terrestrischen Materials einen großen Einfluss auf die CO_2 Emissionen darstellen können. Die Rolle der Bäche als „Hotspots“ von Kohlenstoffumsätzen konnte in meinen Untersuchungen bestätigt werden. Meine Dissertation trägt daher zu einem besseren mechanistischen Verständnis dieser Umsätze und der Rolle der Bäche in regionalen und globalen Kohlenstoffkreisläufen bei. Folglich sollte die Qualität des eingetragenen Materials in zukünftigen Modellen und Untersuchungen zum Kohlenstoffkreislauf berücksichtigt werden.

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THESIS OUTLINE

This thesis is composed of four manuscripts that are either published (manuscript II), submitted (manuscript III), or in preparation stage to be submitted to peer-reviewed journals (manuscript I & IV). Each manuscript contains an introduction, methodology, results and discussion section. In the general introduction section, I provide the overall context of the thesis, the main aims of this thesis, as well as the individual aims of each manuscript. In the general discussion section I discuss the main findings of this thesis, connect them to previous studies and provide an outlook for potential future studies.

Manuscript I

P. Bodmer, J. Fabian, N. Kamjunke, O. J. Lechtenfeld, J. Raeke, D. Zak, and K. Premke. Dissolved organic matter quality – not source – drives microbial metabolism. (to be submitted)
Author contributions (abbreviations of author names above are used):
PB, KP, and JF designed the experiment. **PB**, NK, and JF performed sampling/data collection. NK measured bacterial protein production, OJL and JR measured FT-ICR-MS data, **PB** measured and analyzed gas data. **PB** and KP conceptualized the manuscript. **PB** performed statistical analyses and drafted the manuscript. All authors revised the manuscript.

Manuscript II

A. Lorke, **P. Bodmer**, C. Noss, Z. Alshboul, M. Koschorreck, C. Somlai-Haase, D. Bastviken, S. Flury, D. F. McGinnis, A. Maeck, D. Müller, and K. Premke (2015) Technical note: drifting versus anchored flux chambers for measuring greenhouse gas emissions from running waters. Biogeosciences, 12, 7013–7024, doi:10.5194/bg-12-7013-2015
Author contributions (abbreviations of author names above are used):
KP and AL initiated project. **PB**, ZA, MK, and CSH mesured and analysed field data. CN and ZA performed additional experiment. **PB,** AL, and KP conceptualized the manuscript. AL performed statistical analyses and drafted the manuscript. All authors revised the paper.

Manuscript III

P. Bodmer, M. Heinz, M. Pusch, G. Singer, and K. Premke. Carbon dynamics and their link to DOM quality across contrasting stream ecosystems. (submitted to Science of the Total Environment)
Author contributions (abbreviations of author names above are used):

PB and KP designed the field study. **PB** performed sampling/data collection. **PB** and GS performed statistical analyses. MH performed PARAFAC modeling. **PB**, and KP conceptualized the manuscript**.** **PB** drafted the manuscript. All authors revised the manuscript.

Manuscript IV

P. Bodmer, A. Marzadri, G. Singer, K. Premke, and A. Bellin. Simultaneous diel trends of dissolved oxygen and carbon dioxide resolve the chemistry of stream ecosystem metabolism. (to be submitted)
Author contributions (abbreviations of author names above are used):
PB, AB and KP designed the study. **PB**, AM, GS, and AB conceptualized and developed the model. **PB** and AM wrote the model code. **PB** performed statistical analyses and drafted the manuscript. All authors revised the manuscript.

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KEY TO ABBREVIATIONS

BPP	Bacterial protein production
C	Carbon
CH_4	Methane
CO_2	Carbon dioxide
DIC	Dissolved inorganic carbon
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
DOM_{leaf}	Dissolved organic matter leached from leaves
DOM_{peat}	Dissolved organic matter leached from peat
ER	Ecosystem respiration
FI	Fluorescence index
GHG	Greenhouse gases
GPP	Gross primary production
HIX	Humification index
HMWS	High molecular weight substances
HS	Humic-like substances
LMWS	Low molecular weight substances
O_2	Oxygen
OC	Organic carbon
OM	Organic matter
PARAFAC	Parallel Factor Analysis
pCO_2	Partial pressure of CO_2
PP	Primary production
R	Respiration
RQ	Respiratory quotient
SRP	Soluble reactive phosphorus
SUVA	Specific UltraViolet absorption

GENERAL INTRODUCTION

Role of running waters in the carbon cycle

Running waters are estimated to cover 0.30 – 0.56 % of the land surface (Downing et al., 2012). Although running waters only cover a small fraction of the land surface, they are key sentinels and integrators of environmental change in the surrounding terrestrial landscape (Williamson et al., 2008). Moreover, running waters are tightly connected to their surrounding landscape and thus important agents in the coupling of biogeochemical cycles among terrestrial environments, freshwaters, and atmosphere (Aufdenkampe et al., 2011). Running waters form an active component of the carbon (C) cycle, (Cole et al., 2007, Battin et al., 2009, Aufdenkampe et al., 2011) and are identified as “hotspots” for carbon dioxide (CO_2) and methane (CH_4) emissions (Striegl et al., 2012, Raymond et al., 2013, Campeau and Del Giorgio, 2014, Borges et al., 2015). However, the mechanisms that define running waters as C-hot-spots are not fully understood.

Recently, many investigations revealed that stream size is an important aspect regarding CO_2 emissions (Teodoru et al., 2009, Hotchkiss et al., 2015). For instance, Butman and Raymond (2011) were able to show that small streams contributed most to the total CO_2 emission of running waters in the United States. This can be explained, for example, by a high number of small streams and consequently high surface area (Butman and Raymond, 2011, Downing et al., 2012), tight connection to their terrestrial environment, and high CO_2 input through groundwater (Hotchkiss et al., 2015), compared to larger running waters. However, the mechanisms that drive CO_2 and CH_4 emissions from streams are not completely understood (Raymond et al., 2013), underlining the need for further research on this subject.

Organic matter turnover in stream ecosystems

Streams receive organic matter (OM) from different origins (Fig. 1). Allochthonous (terrestrial derived material) OM comprises matter from production that occurs outside the stream channel, i.e. terrestrial matter such as leaves, branches or soil (Richardson et al., 2010) and enters the stream either through groundwater, lateral inflow, or during storm events (Mulholland, 1997, Buffam et al., 2001). Autochthonous (in-stream produced material) OM derives from aquatic primary producers, such phytoplankton and macrophytes (Allan and Castillo, 2007) and enter streams via extracellular release or die back of these aquatic plants (Bertilsson and Jones Jr, 2003). Both sources reveal different qualities in respect to their bioavailability, i.e. resistances to natural degradation. Traditionally, autochthonous OM is thought to be more bioavailable than allochthonous OM (Chen and Wangersky, 1996, McKnight and Aiken, 1998) due to the absence of e.g. lignin structures. Nevertheless, recent studies showed that allochthonous OM can contain a highly bioavailable organic C fraction (Berggren et al., 2010, Guillemette et al., 2013). The OM has three major fates in streams: transportation downstream, biological degradation and/or storage. However, storage of OM in running waters is generally small and can thus be neglected (Cole et al., 2007).

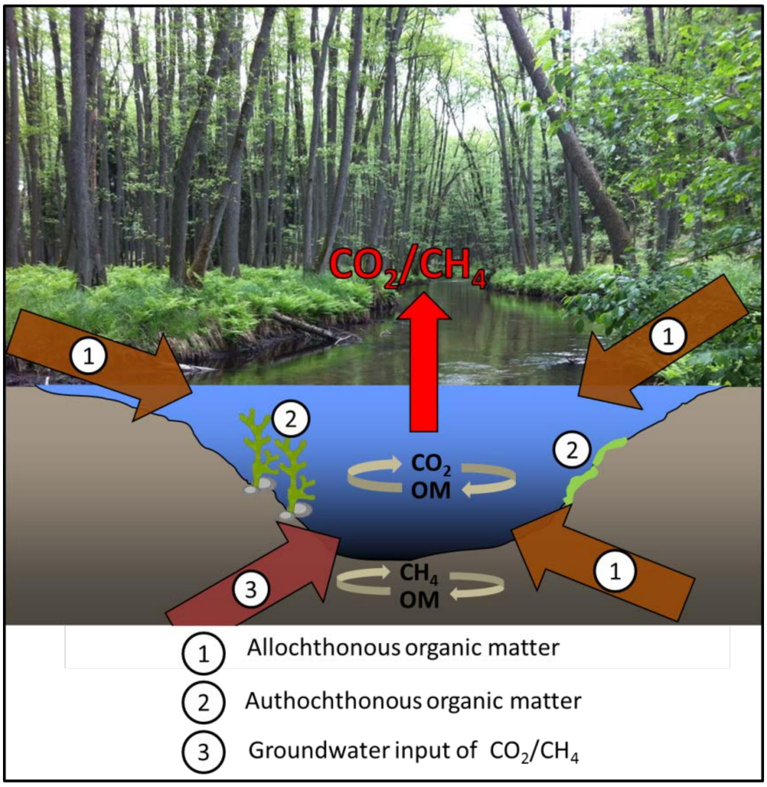

Figure 1. Simplified conceptual figure of different sources of organic matter, its potential turnover and groundwater input of CO_2/CH_4 in running waters.

With respect to biological degradation of OM, the particle size is a crucial attribute. Usually, OM is divided into three size classes: coarse particulate OM (> 1 mm), fine particulate OM (FPOM, < 1 mm and > 0.5 µm), and dissolved OM (DOM, < 0.5 µm) (Fisher and Likens, 1973, Allan and Castillo, 2007). The size division between FPOM and DOM is usually determined by what passes a 0.45 µm filter. In running waters, DOM usually contributes the largest fraction (about 70 %) to the organic C pool in running waters (Fisher and Likens, 1973), and contains a variety of organic compounds that are potential C sources for microorganisms (Allan and Castillo, 2007). In literature, DOM and DOC are often used interchangeable due to the high C content of DOM (45-50 % by mass) (Allan and Castillo, 2007).

DOM in streams can be processed either abiotically or biotically. Abiotic processes encompass physical-chemical adsorption to components of the stream sediment or water (Dahm, 1981) and photodegradation (Moran and Covert, 2003). During biotic degradation, DOM can be directly taken up by microorganisms (Battin et al., 2008) and is either incorporation into biomass (assimilation) and/or respired to CO_2 and/or methane (CH_4) (del Giorgio et al., 1997), thus underlining the importance of DOM in the C cycle (Battin et al., 2008). Microorganisms have been recognized to occupy an important role with respect to the transformation and mineralization of DOM in aquatic environments, including streams (Kominkova et al., 2000, Kawasaki and Benner, 2006, Allan and Castillo, 2007). Hereby, the sediment-water interface represents a site of intense microbial activity. The high microbial density, compared to the running water column, creates a highly active environment (Capone and Kiene, 1988) and therefore, the sediments often contribute to a higher proportion of CO_2 production than in the running water column (Fischer and Pusch, 2001). While CO_2 is produced under aerobic and anaerobic conditions, CH_4 is mainly produced anaerobically during organic matter degradation (Schulz and Zabel, 2006). Both gases are strong greenhouse gases (GHG) and can potentially be emitted to the atmosphere. However, whether a system is a GHG sink or source strongly depends on the interplay of primary production, organic matter input, and respiration.

Streams vary strongly with respect to density and biodiversity of microorganisms, primary production, and allochthonous OM input. Consequently, the transformation of organic matter may significantly differ among different streams, leading to either C sources or sinks of these systems. For instance, if primary production (PP) exceeds ecosystem respiration (ER), the stream system is characterized as autotrophic (i.e. C sink); and when ER is greater than the PP, the aquatic system is characterized as heterotrophic (i.e. C source) (Odum, 1956, Fisher and Likens, 1973). In heterotrophic systems, the partial pressure of CO_2 (pCO_2) in the water is larger than in the atmosphere and thus supersaturated with $CO_{2,}$ which consequently leads to CO_2 emissions to the atmosphere (Jones et al., 2003). Beside the above described in-stream processes, groundwater is highly supersaturated in CO_2 (Cole et al., 2007), contributing to stream CO_2 supersaturation (Crawford et al., 2014) (Fig. 1).

Main drivers for organic matter transformation in streams

OM turnover in streams is very complex. It is influenced by multiple factors such as nutrient availability (Farjalla et al., 2009, Teodoru et al., 2009, Bergfur and Friberg, 2012, Guillemette et al., 2013), photochemical reactions (Wetzel et al., 1995, Tranvik and Bertilsson, 2001, Moran and Covert, 2003) and temperature, among others. Since biological rates are temperature dependent, ecosystem processes such as OM degradation and biological metabolism are affected as well (Uehlinger et al., 2000, Allan and Castillo, 2007, Yvon-Durocher et al., 2010). For example, Sinsabaugh (1997) showed that benthic respiration in streams was directly proportional to temperature.

Besides temperature, mechanical parameters also play a major role in the dynamics of OM transformations in streams. Discharge regulates the retention time of the water on the sediment; i.e. how long OM is in contact with biologically active surfaces (Battin et al., 2008). For instance, Acuña et al. (2004) observed in a headwater Mediterranean stream a negative effect of discharge on organic matter accumulation and ER. Precipitation increases discharge and consequently may reduce ER; however, precipitation also increases the input of allochthonous OM trough surface run off (Lynch et al., 2010, Delpla et al., 2011), which can boost the OM turnover (Roberts et al., 2007). Roberts et al. (2007) observed that in a forest headwater stream, ER rates revealed a sharp decline when discharge increased after a storm event. However, after the initial decline, ER rates increased again for several days to levels exceeding those prior the storm, presumably due to the input and turnover of terrestrial OM.

Another important factor is the OM quantity (Teodoru et al., 2009, Ellis et al., 2012). Ellis et al. (2012), for example, found in streams of varying sizes a strong positive correlation between respiration and fine particulate organic carbon concentration. Moreover, an investigation of seventy boreal streams of different sizes revealed that pCO_2, was strongly positively correlated with DOC concentration (Teodoru et al., 2009). This positive relation between pCO_2 and DOC concentrations was also found by Lapierre et al. (2013). In addition to respiration or pCO_2, bacterial production also correlates positively with DOC concentration (Cole et al., 1988, Attermeyer et al., 2013).

Beside the OM quantity, the quality of OM is also likely an important factor influencing carbon turnover in streams, yet at present, mostly neglected as a controlling factor. DOM generally represents a chemically highly heterogeneous and complex pool of different compounds. Whereas, stream DOM composition is mainly influenced by surrounding land cover (Graeber et al., 2012, Kothawala et al., 2015). About 10% to 25% of the DOM consist of carbohydrates, amino, hydroxy and fatty acids (Allan and Castillo, 2007). The remaining 50% - 75 % consists of humic, fulvic and hydrophilic acids. The latter part can contribute up to 90% in colored water (Allan and Castillo, 2007).

Alongside the chemical heterogeneity, DOM can exhibit different levels of bioavailability. The state of bioavailability can be traced back to the intrinsic molecular properties, such as size, molecular weight, aromaticity, or molecular complexity (del Giorgio and Davis, 2003, Bastviken et al., 2004, Docherty et al., 2006). The origin of DOM mainly determines its composition (Hudson et al., 2007). Autochthonous DOM consists of more low molecular weight carbon compound characteristics (Chen and Wangersky, 1996), while allochthonous DOM contains more complex and aromatic compound characteristics (McKnight and Aiken, 1998). However, a reasonable part of allochthonous DOM also exhibits low molecular weight compounds, such as shown for e.g. beech leaf leachates (Attermeyer et al., 2014).

The effect of OM quality on degradation processes was shown for instance by Del Giorgio and Pace (2008). They suggest that the Hudson River functions as a reactor; degrading intensively autochthonous OM (Del Giorgio and Pace, 2008) while the majority of the allochthonous OM is transported to sea. In contrast, a study by Berggren et al. (2010) found an efficient microbial utilization of allochthonous DOM. Kaiser and Sulzberger (2004) and Amon and Benner (1996) show two examples where the size of DOM is a main factor that controls degradation processes. In the Tagliamento River, bacteria preferentially used low molecular weight substances rather than high molecular weight substances for biomass production (Kaiser and Sulzberger, 2004). In contrast, the size-continuum model proposed by Amon and Benner (1996), suggests that bioavailability decreases with decreasing molecular weight/size. They argue that the high molecular weight DOM is diagenetically less altered than the low molecular weight DOM, leading to differences in bioavailability of these two size fractions (Amon and Benner, 1996, Benner and Amon, 2015).

Such contradictory findings continue also with respect to CO_2 emissions. Del Giorgio and Pace (2008) found that CO_2 emissions of the Hudson River are fueled by highly bioavailable autochthonous OC. On the other hand, Ward et al. (2013) showed that lignin and other allochthonous derived macromolecules contribute significantly to CO_2 emissions in the Amazon River. Additinally, the effect of DOM age is a controversially discussed issue. For example Mayorga et al. (2005) revealed that the dominant source of the emitted CO_2 in Amazonian rivers is the respiration of young organic matter (less than five years old). On the other hand, McCallister and del Giorgio (2012) state that a significant fraction of the respired allochthonous OM (here specifically organic carbon) in streams is old (in the range of 1,000–3,000 y B.P.). Such contradictory results demonstrate that the linkages between carbon fluxes, sources of OM and quality aspects are very complex and are not yet fully understood. Eventually, which features of DOM lead to what level of bioavailability depends strongly on its origin, transition across the landscape and the transformation process by microorganisms.

Measuring carbon turnover in streams

Streams are dynamic systems and highly variable in space and time, which makes measuring carbon turnover in such systems complex. There exists multiple approaches, of which a few are presented in this thesis. A straight forward approach is to measure the CO_2 concentration. Measuring CO_2 concentrations in stream-water is an integrative measure, since the origin of the CO_2 can be in-stream processes, groundwater or lateral inflow. CO_2 measurements can be done e.g. with distinct sampling, indirectly calculated with pH or by deploying autonomous sensors.

With discrete sampling, the CO_2 concentration is generally determined via a headspace equilibration technique (Kling et al., 1991, Hope et al., 2001, Teodoru et al., 2009, Halbedel and Koschorreck, 2013). Briefly, a water sample is taken and the sampling vessel sealed gas tight and without headspace. Afterwards, a headspace is created with an inert gas and the sampling vessel shaken until the dissolved gas components (here CO_2) diffuse into the gas phase until the headspace reaches quilibrium (Goldenfum, 2010). The headspace gas concentration is then measured with a gas chormatograph and dissolved concentrations are calculated based on Henry's law (Goldenfum, 2010).

CO_2 concentrations can also be calculated using the carbonate equilibrium. The pH dependent carbonate systems consist of four measurable parameters: total alkalinity, total dissolved inorganic carbon (i.e. sum of dissolved CO_2, carbonates, and the bicarbonates), pH and partial pressure of CO_2 (Lewis et al., 1998, Dickson et al., 2007). With two parameters out of these four, it is possible to calculate the other two. In the studies of Butman and Raymond (2011) and Raymond et al. (2013) for example, dissolved CO_2 concentrations were calculated from measurements of pH, alkalinity and water temperature. In one of the presented studies in this thesis (M IV), total dissolved inorganic carbon was estimated from measurements of pCO_2, water temperature, and pH.

Another way to measure CO_2 concentrations directly in streams is by deploying autonomous sensors, which allow long-term and continuous measurements (Lynch et al., 2010). Since a few years ago, more sensors based on membrane equilibration and infrared spectrometry are used (Johnson et al., 2010, Fietzek et al., 2014). In thisthesis (M III), I used an autonomous carbon dioxide sensor developed by CONTROS (HydroC™ CO_2; Kongsberg Maritime Contros GmbH, Germany) to continuously monitor pCO_2 in the stream water. In short, the pCO_2 sensor is equipped with a water pump and a flow head, in which the pumped water is directed in a planar manner (Fietzek et al., 2014). Subsequently, the partial pressure equilibration occurs at a semipermeable membrane separating the water from the internal head space, while a pump continuously circulates the gas between the membrane equilibrator, a heater, and the nondispersive infrared detector (Fietzek et al., 2014).

In summary, all the before-mentioned methods to measure or calculate pCO_2 have advantages and disadvantages. Calculating CO_2 concentrations is an elegant way, since data of pH, alkalinity and temperature are often available for large areas and long periods of time (Butman and Raymond, 2011, Raymond et al., 2013). However, since all the variables needed for the calculation show a distinct diurnal (Fig. 2 in “General discussion”) and seasonal dynamic, one must be careful about the timing with respect to sampling as well as the meaningfullness of the calculated data. Taking water samples and measuring CO_2 concentrations via headspace equilibration technique (Kling et al., 1991) is a time-efficient method. However, CO_2 concentrations may reveal strong diurnal dynamics (Fig. 2 in “General discussion”); therefore, the timing of sampling is crucial. Furtermore, biological activity between the time of sampling and analysis should be prevented, which is technically not trivial without changing the CO_2 concentration in the water sample (Goldenfum, 2010). Although some autonomous CO_2 sensors need to be completely under water, which may limit their application to a certain water depth, they provide, in my opinion, the most promising approach to resolving CO_2 concentrations in streams. The provided continuous and long-term data give a representative and meaningful insight into the CO_2 dynamics in streams.

To assess the role of streams in the carbon cycle, it is crucial to measure the CO_2 fluxes between the water and the atmosphere. The flux (F) can be described by the following equation: $F = k\ (C_{water} - C_{air})$, where k is the gas exchange coefficient, and C is the CO_2 concentration in the water and air, respectively (MacIntyre et al., 1995). While C_{water} and C_{air} are directly measured, the gas exchange coefficient is either estimated based on several physical factors or empirically determined with tracer experiments. The gas exchange coefficient is mainly controlled by turbulence at the water-side of the air-water interface (MacIntyre et al., 2010), while turbulence in running waters is driven by stream velocity, depth and bottom roughness (Marion et al., 2014). Hence the gas exchange coefficient in running waters is often parametrized with e.g. stream order, slope, discharge, width and depth (Alin et al., 2011, Wallin et al., 2011, Raymond et al., 2012). However, the gas exchange coefficient can be derived by gas tracer experiments, where a volatile tracer (e.g., sulfur hexafluoride or propane) is injected upstream and the longitudinal decrease of its dissolved concentration is measured (Raymond et al., 2012). An alternative method - independent of the often ‘unknown’ *k* - to directly determine CO_2 fluxes is by deploying floating chambers (Alin et al., 2011, Beaulieu et al., 2012). Briefly, air is enclosed in a chamber that floats at the surface of the water (Goldenfum, 2010). The fluxes are then calculated according to the concentration change of CO_2 over time (McGinnis et al., 2015, M III). In running waters, floating chambers have been deployed anchored at one spot (Sand-Jensen and Staehr, 2012, Crawford et al., 2013), or freely drifting with the water (Alin et al., 2011, Beaulieu et al., 2012). I discuss and compare these two approaches in detail in M II and conclude that anchored chambers enhance turbulence under the chambers and thus artificially elevate fluxes, while drifting chambers have a very small impact on the water turbulence under the chamber and thus generate more reliable fluxes.

A more holistic apporach is the measurement of ecosystem metabolism, which provides an integrative measure of stream structure and function. Ecosytem metabolism comprises two major processes: Gross primary production (GPP) and ecosystem respiration (ER) (Izagirre et al., 2008, Bernot et al., 2010), which are key processes for production, transformation, and retention of organic carbon (Hotchkiss and Hall, 2014). Ecosystem metabolism can be determined using either diurnal oxygen (O_2) or CO_2 dynamics (Odum, 1956, Hanson et al., 2003). O_2 dynamics is much more often used since the sensors are more cost-effective and robust (Hanson et al., 2003, Staehr et al., 2010) than CO_2 sensors. In addition to the direct measurements to calculate metabolism, there are equations describing the processes of GPP and ER as a function of external forcing factors, such as solar radiation and temperature (Uehlinger et al., 2000, Marcarelli et al., 2010). Such equations can be used to model O_2 and CO_2 dynamics in aquatic systems. In stream ecosystems, O_2 dynamics are often modeled (Uehlinger et al., 2000, Birkel et al., 2013, Riley and Dodds, 2013), whereas CO_2 models are rare (Dinsmore et al., 2013). A combined model for O_2 and CO_2 dynamics in streams does not exist to my knowledge. Such a combined model however would provide new mechanistic insights into carbon turnover in stream ecosystems - I discussed this issue in M IV.

Measuring dissolved organic matter quality

Beside classical approaches like stoichiometry (Sun et al., 1997, Sterner and Elser, 2002) and bioassays (Wiegner et al., 2006, Del Giorgio and Pace, 2008), a common approach to assess DOM quality is the identification of the proportions of different size fractions. This can be done in multiple ways, for example, by centrifuge ultrafiltration (Burdige and Gardner, 1998, Ellis et al., 2012), high-pressure size exclusion chromatography (Young et al., 2004, Docherty et al., 2006), or liquid chromatography – organic carbon detection (LC-OCD; Huber et al., 2011, Attermeyer et al., 2014).

This method was applied in M I, M III, and M IV. LC-OCD can detect the following size classes: biopolymers, humic-like substances, building blocks, low molecular weight acids and humic-like substances, and low molecular neutrals (Fig 2) (Huber et al., 2011) of which, high molecular weight substances (biopolymers), humic-like substances (humic-like substances + building blocks), and low molecular weight substances (low molecular weight acids and humic-like substances + low molecular neutrials) are used in this thesis (similar to Attermeyer et al., 2014). The 'bypass' is not chromatographically separated and provides the total DOC concentration of the sample (Huber et al., 2011).

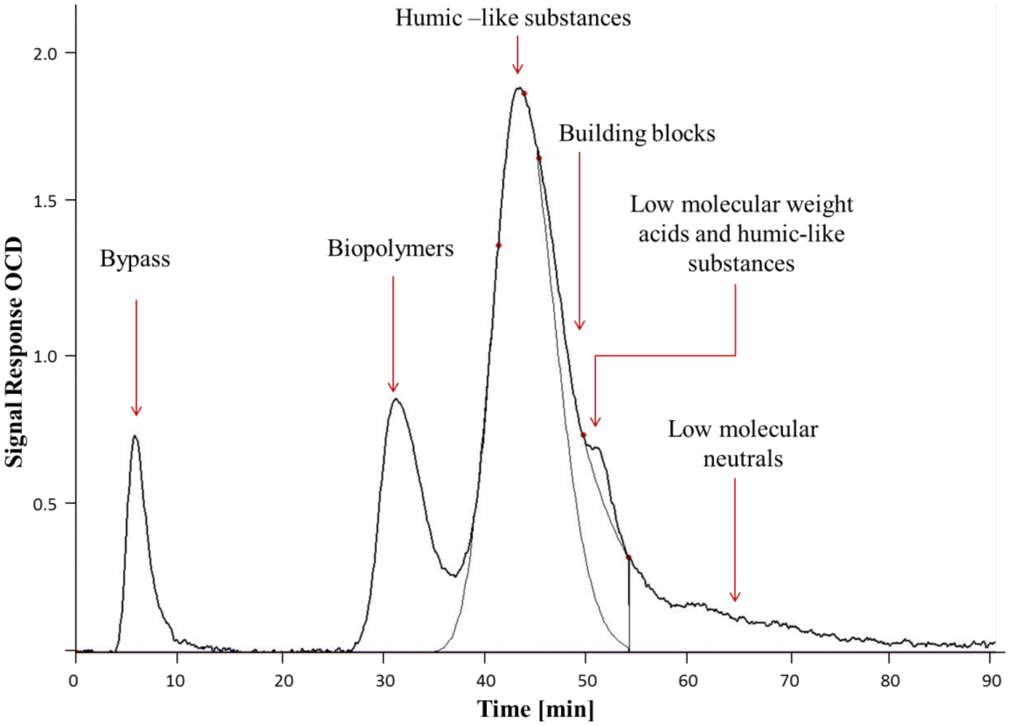

Figure 2. Example chromatogram of liquid chromatography – organic carbon detection measurements showing the different size classes of substances that can be identified. The thin lines represent the integrals for the calculation of individual size classes.

Furthermore, optical properties of DOM can provide information about its chemical characteristics (Mueller et al., 2012). Compositional changes of DOM can be inferred from fluorescence and light absorption properties of the optical fraction (chromophoric dissolved organic matter, CDOM) (Jaffé et al., 2008). Generally, fluorescence DOM measurements are collected as three-dimensional excitation-emission matrices (EEMs, McKnight et al., 2001), which provide proxies for the source, the redox state, and the biological availability of CDOM (Jaffé et al., 2008, Fellman et al., 2010). Parallel factor analysis (PARAFAC), a multivariate modeling technique, can be used to analyze EEMs (Cory and McKnight, 2005). Briefly, PARAFAC is a method that decomposes the fluorescence signature of DOM into individual components while estimating the relative contribution of each component to total DOM fluorescence (Stedmon and Bro, 2008, Fellman et al., 2010). Consequently, PARAFAC components provide information about the origin, chemical composition, and biogeochemical role of aquatic DOM (Fellman et al., 2010). As an illustration example, figure 3 shows three components (C1, C2, C3) identified via PARAFAC modeling in M III.

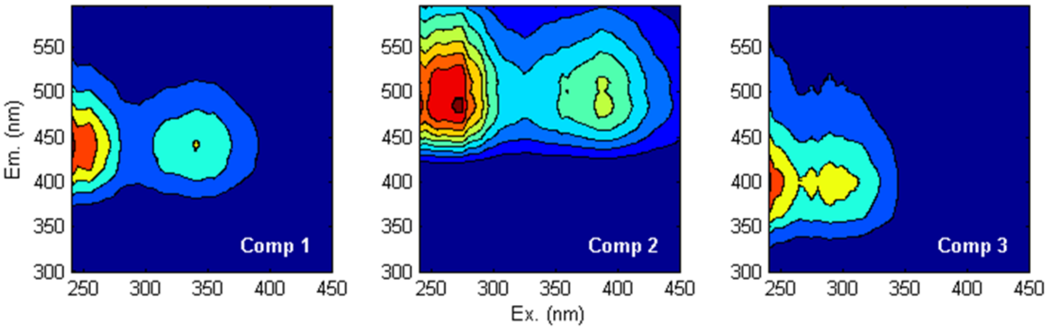

Figure 3. Three components identified via Parallel factor analysis modeling (M III + M IV); Comp = component, Em = emission, Ex = excitation.

Briefly, C1 is considered a humic-like, terrestrially derived, high molecular weight compound (Stedmon and Markager, 2005, Fellman et al., 2010). In this example, C2 is similar to published PARAFAC components resembling high molecular weight, humic-like, terrestrial material (Fellman et al., 2010) with increased aromatic carbon content, indicating higher plant material as a likely source (Cory and McKnight, 2005). However, C2 may also be the product of biological production and degradation (Ishii and Boyer, 2012). C3 resembles a humic-like, terrestrial component associated with agriculture and wastewater (Cory and McKnight, 2005, Fellman et al., 2010). Beside the PARAFAC components, it is also possible to calculate DOM quality indices from fluorescence data. For instance, the freshness index indicative of fresh microbially produced DOM (Parlanti et al., 2000), the humification index indicating the extent of humification (Ohno, 2002), and the fluorescence index as a proxy for DOM source (i.e., terrestrial versus microbially derived DOM; McKnight et al., 2001). In addition to the DOM characterization by fluorescence measurements, the measurement of ultraviolet-visible (UV) absorption spectra is another tool to characterize DOM optically (Helms et al., 2008). From UV absorption spectra one can calculate different indicators related to DOM molecular weight or DOM aromaticity for example (Weishaar et al., 2003, Helms et al., 2008).

Research gaps, aims and structure of this thesis

The mechanisms driving CO_2 emissions from streams are not completely understood. Streams receive organic matter from aquatic and terrestrial origins which are partly respired to CO_2. Moreover, future predictions suggest enhanced input of terrestrial organic matter into streams (Porcal et al., 2009). Consequently, surrounding land use may highly influence dissolved organic matter (DOM) composition and turnover in streams. As highlighted in the introduction above, the quality, i.e. bioavailability, of aquatic and terrestrial organic matter, as well as which quality feature provides which bioavailability, is controversially discussed. Thus, the main goal of this thesis was to enhance the understanding of the role of organic matter quality as a potential driver for organic matter turnover in stream ecosystems. A further goal was to shed light on carbon dynamics including CO_2 fluxes of streams surrounded by different land use. To reach these goals, I combined laboratory, field, and modeling approaches, ranging from small to stream-reach scale (Fig. 4).

In manuscript I, I aimed at understanding how different mixtures of two specific terrestrial DOM sources with distinct qualities influence the microbial usage of DOM (i.e. used for respiration or assimilation), and to explore the driving factors (nutrients, DOM quality characteristics, stoichiometry) of bacterial production and respiration. Hence, I performed a short term incubation experiment in which I mixed a more bioavailable DOM source (beech leaf leachate) with a less bioavailable DOM source (peat leachate) in different proportions and with a natural microbial community inoculum.

In manuscript II, I assesed an appropriate method to measure carbon fluxes (i.e. CO_2 and CH_4) in the streams and applied it in the field study described in manuscript III. In manuscript two, I evaluated and improved together with my colleges the flux chamber method, a powerful approach to measure carbon fluxes in running waters. We compared measurements of anchored and freely drifting chambers on various streams in order to compare the reliability of the respective results.

In manuscript III, I compared seasonal dynamics of CO_2/CH_4 concentrations and the respective emissions to the atmosphere of different stream types, as bordered by contrasting types of land use (agriculture and forest). Additionally, these two land use types should provide contrasting DOM quality inputs to the streams. I furthermore explored linkages between DOM quality and CO_2 concentrations across all investigated streams. I used autonomous sensors to measure CO_2 and methane concentrations continuously in the stream surface water, and applied the drifting chamber method evaluated in manuscript two to measure CO_2/CH_4 fluxes between the water and the atmosphere. Moreover, I took water samples in order to derive multiple DOM quality characteristics.

In manuscript IV, I modeled the measured diurnal CO_2 and O_2 dynamics of the contrasting stream ecosystems of manuscript three. Therefore, I developed a model based on ecosystem metabolism components, i.e. ecosystem respiration and gross primary production. I linked the underlying differential equations with the respiratory quotient which describes the mole of CO_2 produced per mole of O_2 consumed. Consequently, the respiratory quotient can be related to the quality of respired substrates. I furthermore explored the respiratory quotient spatially and temporally as well as the relationship between respiratory quotient and DOM quality characteristics.

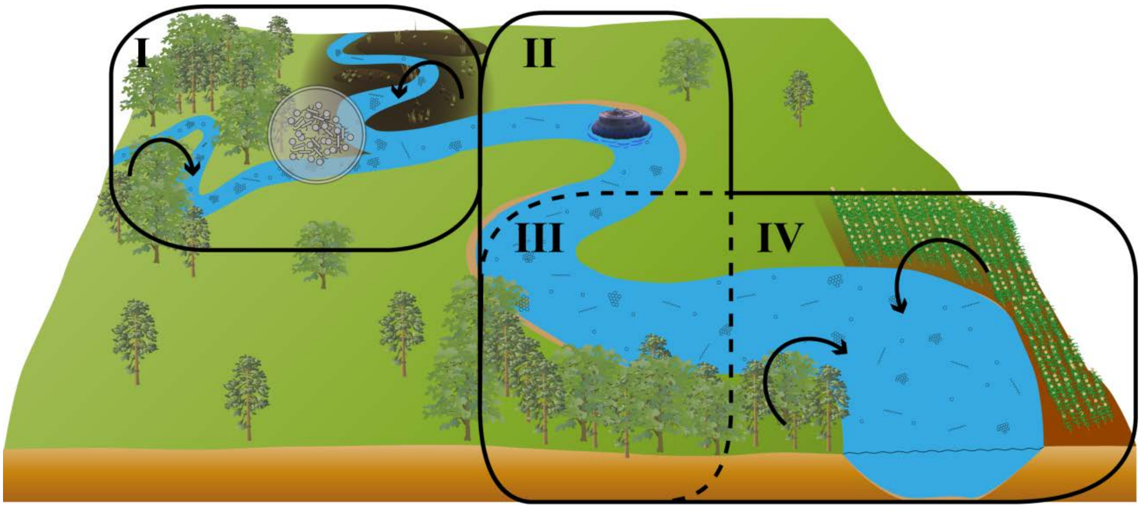

Figure 4. The conceptual overview of the four manuscripts presented in this thesis. Each black box encloses the content of one manuscript investigating different carbon dynamic aspects in stream ecosystems. The Roman numerals indicate the respective manuscript within the thesis. As illustrated in the overview, manuscript I deals with the effect of two distinct allochthonous dissolved organic matter (DOM) sources on microbial DOM utilization; manuscript II deals with drifting chambers as powerful tool to measure CO_2/CH_4 fluxes from running waters; manuscript III deals with comparing concentration and emission dynamics of CO_2/CH_4 in forest and agricultural streams as well as the role of DOM quality in carbon turnover across these stream ecosystems; and manuscript IV deals with modeling dissolved oxygen and CO_2 dynamics in forest and agricultural streams, while exploring the respiratory quotient spatially and temporally as well as the relationship between respiratory quotient and DOM quality characteristics. The arrows indicate input of different organic matter; and the molecular structures in the stream symbolize a mix of different DOM qualities.

MANUSCRIPT I

DISSOLVED ORGANIC MATTER QUALITY – NOT SOURCE – DRIVES MICROBIAL METABOLISM

DISSOLVED ORGANIC MATTER QUALITY – NOT SOURCE – DRIVES MICROBIAL METABOLISM

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Abstract

Future hydrological changes will likely lead to changes in both quantity and quality of DOM exported from the terrestrial sources to the freshwater systems. Currently there exists a controversy on the importance of DOM quality on the microbial mediated C cycle in freshwater systems. Thus, this study aims (i) to understand how two specific terrestrial DOM sources with distinct qualities and different mixes of them influence the microbial usage of DOM (i.e. used for respiration or bacterial production), and (ii) to explore the driving factors of bacterial production and respiration. We performed a short term incubation experiment, where we mixed a labile DOM source (isotopically labelled beech leaf leachate; DOM_{leaf}) and a less labile DOM source (peat leachate; DOM_{peat}) in different proportions to a natural microbial community inoculum. We measured the respiration (R) intensity and isotopic signature, and bacterial protein production (BPP), nutrients as well as DOM quality characteristics. We observed a strong treatment effect on BPP and R, while BPP and R intensity was higher with increasing proportion of labile DOM (DOM_{leaf}) in the treatment. Moreover, the results of the stable isotope approach show that respiration in the different treatments was dominated by mainly DOM_{leaf} with changes over time. Finally, slope ratio (Sr), indicating apparent DOM molecular weight was the strongest predictor of BPP and R, while among others, the concentration of low molecular weight substances, C:N ratio and the specific UV absorption as a proxy for DOM aromaticity were further highly influential predictors. The results of this study suggest large interaction between microbial metabolism and DOM quality, indicating that labile terrestrial DOM likely supports high levels of BPP and respiration in freshwater ecosystems.

1 Introduction

Dissolved organic matter (DOM) is ubiquitous in aquatic ecosystems and represents a crucial source of carbon and nutrients for the metabolism of aquatic microorganisms (Volk et al., 1997, Young et al., 2004, Docherty et al., 2006). Inland waters receive large quantities of terrestrial DOM from which a substantial fraction is metabolized during passage towards the ocean (Koehler et al., 2012). It has long been recognized that microorganisms or more specific heterotrophic bacteria and fungi occupy a critical role in the transformation, metabolization and mineralization of organic matter in aquatic environments (Baldy et al., 1995, Kominkova et al., 2000, Kawasaki and Benner, 2006). The ability of microorganisms to degrade the inherently heterogeneous and complex pool of DOM is a critical constraint on its mineralization (Amon and Benner, 1996, Hedges, 2002). Consequently, beside the major drivers temperature (Gudasz et al., 2012) and DOM quantity (Attermeyer et al., 2014), the microbial turnover of DOM is determined by the quality (i.e. bioavailability) of its different constituents (del Giorgio and Davis, 2003). Thus, DOM reveals a large gradient of resistance to natural degradation, consisting of better bioavailable, i.e. more labile constituents and less bioavailable constituents for microorganisms (del Giorgio and Davis, 2003, Guenet et al., 2010).

DOM quality is a very broad term that has been the subject of increasing attention in recent years. Due to the DOM complexity and not yet fully understood factors that determine DOM degradability (del Giorgio and Davis, 2003), it is crucial to combine several measures to determine DOM quality. For instance, DOM quality can be defined according to molecular weight/size; however, the reactivity of small or large molecules is yet a subject to controversy (e.g. Amon and Benner, 1996, Docherty et al., 2006). A potential method to determine different size classes of DOM is liquid size-exclusion chromatography in combination with UV- and IR- organic carbon detection (LC-OCD; Huber et al., 2011, Attermeyer et al., 2014). This method provides information about absolute concentrations and relative amounts of different size classes, such as high molecular weight substances, humic-like substances and low molecular weight substances of specifically DOC (Huber et al., 2011). Furthermore, optical properties can be related to chemical characteristics of DOM (Mueller et al., 2012). DOM quality indicators can be, for example, parameterized by ultraviolet-visible (UV) absorption spectra (Helms et al., 2008) or fluorescence characterization (Fellman et al., 2010). From UV absorption spectra it is possible to calculate for example indicators related to DOM molecular weight or DOM aromaticity, i.e. structural complexity (Weishaar et al., 2003, Helms et al., 2008). Meanwhile, from fluorescence data it is possible to calculate e.g. the freshness index, indicating the contribution of recently produced DOM, or the humification index, indicating the extent of humification of the DOM (Fellman et al., 2010). Moreover, extensive new insights into the molecular composition of DOM can be derived by the application of ultrahigh-resolution mass spectrometry via Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS; e.g. Stenson et al., 2002, Stenson et al., 2003).

Another aspect will be the decomposition of DOM concerning the effects that could be expected due to a global change, resulting in warmer temperatures and higher precipitation (Porcal et al., 2009). The concentrations of DOM increased over the last decade in many streams and rivers of central and northern Europe as well as North America, primarily driven by the input of humic compounds of terrestrial origin (Hansson et al., 2013, Weyhenmeyer et al., 2015). Beside DOM quantity, Porcal et al. (2009) state that future hydrological changes are likely leading to changes in the quality of DOM exported from terrestrial sources to freshwater systems. In addition to the fact that for example bacterial production correlates positively with DOM (or more specifically DOC) concentrations (Cole et al., 1988, Attermeyer et al., 2013), it is crucial to understand how the expected change in quality of the exported DOM will affect the microbial carbon cycle in freshwaters.

Conventionally, it has been assumed that algal DOM should be more readily consumed by bacteria than terrestrial DOM (Guillemette et al., 2013). However, a recent study of Berggren et al. (2010a) revealed an efficient microbial utilization of terrestrial derived DOM. Additionally, decomposition experiments have been performed to investigate the degradation dynamics including its effect on microbial metabolism of algal and terrestrial DOM (Guillemette et al., 2013) or macrophyte leachates and humic lagoon DOM separately and equally mixed (Farjalla et al., 2009). These studies indicated that DOM quality interacts with microbial metabolism.

In order to enhance the understanding how DOM quality, specifically from different terrestrial sources, is influencing the microbial metabolism, we set up a two factorial laboratory experiment. Therefore, we incubated a natural bacterial stream community with two distinct natural dissolved organic matter sources, more precisely peat and ^{13}C labeled beech leaves (DOM_{peat} and DOM_{leaf}). Preliminary investigations indicated that DOM of leaves, at least of wetland plants, consists of substantial amounts of labile biopolymers like polypeptides and low molecular substances. This is in contrast to DOM_{peat} which was dominated by less labile humic substances (Zak et al., 2015). As such, DOM_{peat} was supposed to consist of less bioavailable organic substances, while DOM_{leaf} represents a more labile DOM source. Furthermore, pre-tests have shown that these two DOM sources reveal large differences regarding the amount of low and high molecular weight substances and C:N ratios (unpublished data, P. Bodmer). The two DOM sources were mixed in different proportions resulting in five treatments with different fractions of labile and less labile DOM-sources. To assess the DOM quality, we combined multiple methods such as LC-OCD, optical measurements (i.e. absorbance and fluorescence), FT-ICR-MS and stable isotopes.

Our presented study aims at understanding how DOM quality with respect to more labile and less labile compositions affects the turnover of two different terrestrial DOM sources. Consequently, we aim at investigating terrestrial-aquatic DOM cycling in a mechanistic approach. In particular, we aim at (i) understanding how two specific terrestrial DOM sources with distinct qualities and different mixes of them influence the microbial utilization of DOM (i.e. used for respiration or assimilation) along an incubation period, and (ii) exploring the driving factors of bacterial protein production and respiration.

2 Material and methods

2.1 *Experimental design*

We run the incubation experiment for 12 days in dark under controlled temperature conditions (15 °C). Therefore, we inoculated artificial lake water (ALW; Lehman, 1980) with a natural microbial community ($0.2*10^6$ Bacteria ml^{-1}) at an initial nutrient level of 15 mg L^{-1} $MgSO_4$ $7H_2O$, 20 mg L^{-1} $NaHCO_3^-$,19.9 mg L^{-1} $CaCl_2$ $2H_2O$, 3.8 mg L^{-1} NH_4Cl, 0.6 mg L^{-1} Na_2HPO_4, and 114 mg L^{-1} K_2HPO_4 3 H_2O (Bastviken et al., 2004, modified) in order to avoid nutrient limitation. We further added 9 mg L^{-1} DOC (mean ± standard deviation over all treatments and replicates: 9.2 ± 0.52 mg L^{-1} DOC; n = 4) in form of leaf and/or peat leachates. We mixed both DOC sources in different proportions to get a gradient in lability resulting in five treatments: 100% DOM_{peat}, 75% DOM_{peat} and 25% DOM_{leaf}, 50% DOM_{peat} and 50% DOM_{leaf}, 15% DOM_{peat} and 85% DOM_{leaf}, and 100% DOM_{leaf} (Fig. 1). We split mixtures into five replicates by filling 1.2 L of each into five acrylic glass incubation chambers/cylinders (11 x 20 cm; diameter x height) resulting in an overlying headspace of 0.7 L at starting conditions. The water was recirculated downwards to keep mixture homogenous and to ensure high gas exchange between water and overlying headspace (see *inset* Fig.1). Concentration and isotopy of the emitted CO_2 were measured automatically (Valco 10 mpos dead-end path, Vici, USA) every 12 hours. We sampled each treatment at the start of the experiment, after two, seven, and twelve days in order to analyze dissolved organic carbon DOC concentrations, nutrients, and DOM quality characteristics as described below.

2.2 Preparation of inoculum and leachates

Five days prior to start of the experiment, we sampled a natural microbial community from the Rheinsberger Rhin (52°34'25"N 14°6'12"E; Germany), a forest stream bordered by coniferous forest, while its stream banks are lined with alders. We filtered the stream water through pre-combusted (450°C for 3 hours) GF/C filters (Whatman, Dassel, Germany; mesh size: 1.2µm), in order to remove grazers and particles. The filtrate was centrifuged (Sorvall Evolution RC, Thermo Fisher Scientific, Waltham, MA, USA) for 10 minutes at 5000 g, resulting in a microbial pellet at the bottom of the centrifuge tubes. The supernatant was discarded gently and the microbial pellet was resuspended through shaking in 12.5 ml of ALW for five minutes. The final concentrate was transferred to a pre-autoclaved glass bottle and stored at 4°C until start of experiment. A subsample of the concentrate was fixed in glutardialdehyde (Carl Roth GmbH, Germany; 0.9 % final concentration) and counted for cell numbers according to Attermeyer et al. (2013).

We prepared leachates from natural particulate organic matter; therefore, we sampled degraded peat in the rewetted polder Zarnekow. A detailed description of this calcareous minerotrophic peatland located in the River Peene valley in NE Germany as well as the sampling site can be found elsewhere (Zak and Gelbrecht, 2007) and in the supplementary materials. The newly formed mud layer including fresh plant tissue at the top the inundated peat was removed carefully before single monoliths of about 10 dm^3 were collected and homogenized with hands in a 90 L box. The box was stored in a climate room at 8 °C in the dark until we prepared the peat leachate (DOM_{peat}). To obtain leachate, we combined 500 g of peat with 500 ml ALW and incubated it for six days at 10°C in darkness. For the production of leaf leachate (DOM_{leaf}) we used beech leaves (*Fagus sylvatica*, L.) that were stored dry at room temperature after they were sampled from trees grown under a $^{13}CO_2$ enriched atmosphere in greenhouses in Nancy, France. We leached 11.5 g of the ^{13}C-labelled beech leaves to 3.2 L ALW for 48 hours at 10 °C in the dark. Both substrates (peat and leaf) were shaken from time to time to foster the leaching process. Two days prior to start of the experiment, we filtered both leachates through a cascade of pre-rinsed filters, starting from 8.0 µm (cellulose acetate, Sartorius), 0.45 µm (cellulose acetate, Sartorius), and finally 0.2 µm (Durapore Membrane PVDF Filter, Merck Millipore, Molsheim, France) to get two microbial-free DOM sources.

2.3 Measurements

2.3.1 Dissolved organic and inorganic carbon and nutrients

The collected water samples were filtered through a pre-rinsed 0.45 µm membrane filter (cellulose acetate, Sartorius) for the analysis of DOC, dissolved inorganic carbon (DIC), soluble reactive phosphorus (SRP), nitrate (NO_3^-), and ammonium (NH_4^+). To remove all inorganic carbon and for conservation, samples for DOC, NO_3^-, and NH_4^+ were acidified with 2 N hydrochloric acid (HCl) to pH 2. For details of the specific analyses, see supplementary materials. For the data analysis we summed NO_3^- and NH_4^+ to dissolved inorganic nitrogen (DIN).

2.3.2 DOM quality parameters

The composition of organic fractions in the water samples of each treatment and replicate were determined for each sampling using liquid size-exclusion chromatography in combination with UV- and IR- organic carbon detection and UV-organic nitrogen detection (LC-OCD-OND method, see Huber et al., 2011). This size exclusion chromatography (SEC) allows separate determination of at least three DOC size class fractions: high molecular weight substances (HMWS) of hydrophilic character (polysaccharides, proteins, amino sugars), humic-like substances (HS), and low molecular weight substances (LMWS) which refer to neutral, hydrophilic and to amphiphilic substances (alcoholes, aldehydes, ketones, sugars, amino acids; Huber et al., 2011). However, it must be noted that those substance were analyzed as DOC (a sum parameter) but in the following we will refer to them as DOM quality characteristics for reasons of terminological consistency. Additionally, the instrument is equipped with a nitrogen detector so we were able to determine dissolved organic nitrogen (DON) concentrations.

Additionally, we determined DOM absorbance and fluorescence using an Aqualog (Horiba, USA). From absorption coefficients we derived the specific UV absorption ($SUVA_{254}$) as a proxy for DOM aromaticity (Weishaar et al., 2003, modified) and the brown color of DOM (a440) (Cuthbert and Del Giorgio, 1992, modified) as a proxy for amount of humic substances in the DOM. Furthermore, we calculated the ratio of absorption coefficients a254/a365 (Dehaan, 1993, Dahlen et al., 1996, modified) and the slope ratio (Sr) (Helms et al., 2008, modified), which are both related to apparent DOM molecular weight. From fluorescence data we calculated the humification index (HIX) indicating the extent of humification (Ohno, 2002, modified), the b/a index (freshness index) indicative of fresh microbially produced DOM (Parlanti et al., 2000, modified), and the fluorescence index (FI) as a proxy for DOM source (i.e., terrestrial versus microbially derived DOM) (McKnight et al., 2001, modified). The fluorescence intensities at excitation wavelengths were measured with an increment of 3 nm; therefore, we had to slightly modify the calculations of the optical indices. Since we use the optical indices to compare DOM quality characteristics among our treatments, these modifications do not have further consequences. For details of the calculations and modifications please see the supplementary materials.

Furthermore, we assessed DOM quality of three replicates of 0 % DOM_{leaf}, 50 % DOM_{leaf} and 100 % DOM_{leaf}, respectively, by Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS). The samples were filtered through a pre-rinsed 0.45 µm membrane filter (cellulose acetate, Sartorius) and acidified with 300 µl HCl (0.95 molar) to about pH 2 and stored at 5 °C until extraction. 20 ml of each sample were extracted using solid phase extraction (Bond Elut PPL, 50 mg), according to (Dittmar et al., 2008). Briefly, the SPE cartridges were rinsed with 6 ml of methanol (Biosolve, ULC/MS) and conditioned 6 ml of Millipore® water pH 2. After the samples passed through the cartridges, the cartridges were rinsed with 6 ml of Millipore® water pH 2, dried under nitrogen gas for 10 minutes and eluted with 1.5 ml of methanol. The average carbon extraction efficiency was 64 %.

An aliquot of each sample was diluted with Millipore® Water and methanol (Biosolve, ULC/MS; 50/50 v/v) to a final concentration of 10 mg DOC/L. The samples were analyzed using a FT-ICR mass spectrometer (Bruker solariX XR, 12 T). An ESI ion source was used in negative mode (capillary voltage +4500 V, infusion flow rate 120 µL h^{-1}). For each spectrum, 300 scans were co-added in the mass range 147 - 3000 m/z. The acquired spectra were externally calibrated with arginine cluster, and on-line calibration was performed with m/z 311.11363. Spectra were internally recalibrated with a list of masses (n = 190) commonly present in natural organic matter. The average root mean square mass error of the calibration mass peaks was 62 ± 8 ppb (n = 39).

Only peaks with a signal to noise ratio above 5 were considered. Elemental formulas were calculated considering the elements C (1-60), H (1-122), O (0-40), N (0-2) and S (0-1) for the mass range 150 - 700 Da (Lechtenfeld et al., 2014). Relative peak intensities were calculated by dividing the peak magnitude by the magnitude of the highest peak in each mass spectrum (excluding contaminant peaks). Intensity weighted average (wa) molecular masses and elemental ratios were calculated from the relative peak magnitudes.

2.3.3 Bacterial protein production (BPP)

We measured Production of heterotrophic bacteria using the leucine technique (Simon and Azam, 1989) as described by Kamjunke et al. (2015). Briefly, 5 ml aliquots from the four replicates and one formalin-treated control (3.7%, final concentration) were spiked with ^{14}C leucine (12.2 MBq μmol^{-1}, Sigma, 50 nM final concentration). Samples were incubated in the laboratory at 15°C for 1 h in the dark on a shaker. Incorporation was stopped with formalin, and 0.6 ml 50 % trichloracetic acid (TCA) was added. We extracted proteins for 15 min and filtered onto 0.2 µm Nuclepore membranes. Filters were rinsed twice with 1 ml 5% TCA and once with 80% ethanol. After dissolving the filters in 0.5 ml Soluene (Packard) and adding 2.5 ml Hionic Fluor (Packard) to each scintillation vial, radioactivity was measured using a Liquid Scintillation Analyzer (2300 TR, Packard). The external standard ratio method was used for quenching. Carbon production was calculated using the equations of Simon and Azam (1989).

2.3.4 Respiration measurements and calculation for respired carbon source elucidation

Concentration and isotopy of headspace CO_2 was measured every 12 hours in an automatic sampling system that connected the incubation chambers to an Off-Axis Integrated-Cavity Output Spectroscopy Stable Isotope Carbon Dioxide analyzer (Off-Axis ICOS CCIA, Los Gatos Research, CA, USA). During gas measurements the chambers head space was flushed with outside air. For every incubation interval (12h), the increase in headspace CO_2 was calculated by subtracting the base value before the closing of the chamber from the peak value after the following opening (hereafter referred as respiration). Measurements of outside air in every 12 hours were used to check for isotope drifting, yielding a precision of 2 ‰ for $\delta^{13}C$ and 1 ppm for CO_2 concentrations. The data was later on referred to a standard containing 70% N_2, 30% O_2 and 0.15% CO_2.

In order to distinguish which carbon source was respired (DOC_{peat} and DOC_{leaf}) in the mixed treatments, we applied a two source mixing model approach according to the following equation (Karlsson et al., 2007):

$$F_{DOC_{leaf}} = (n - 0.5‰ - \delta^{13}C_{DOC_{peat}}) / (\delta^{13}C_{DOC_{leaf}} - \delta^{13}C_{DOC_{peat}}) \,,$$

(1)

where $F_{DOCleaf}$ is the fraction of DOC_{leaf} that contributed to the respiration, n is the intercept of keeling plots of each chamber over 24 hours (i.e. the mixing $\delta^{13}C$ signal of both respired DOM sources; (Keeling, 1958, Pataki et al., 2003), *0.5 ‰* accounts for a fractionation during respiration (Hullar et al., 1996), $\delta^{13}C_{DOMleaf}$ is 48.9 ‰, and $\delta^{13}C_{DOMpeat}$ is -29 ‰. The cutoff for the R^2 of the keeling plots per replicate over 24 hours was set to 0.5.

$\delta^{13}C_{DOM}$ were determined using an TOC Analyzer (OI Analytical, College Station, TX , Analytical Model 1030) interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) utilizing a GD-100 Gas Trap Interface (Graden Instruments, UC Davis, California). The analytical precision for ^{13}C of DOC was 0.4‰. For further details of the measurement please see the supplementary materials.

2.5 Statistics

We applied non-parametric Kruskal-Wallis tests to test treatment effects (n=4 per treatment) regarding consumption of DOC, and total relative changes of different size fractions of DOM (HMWS, HS, LMWS) during the duration of the experiment. Consequently, post hoc Dunn´s tests with Bonferroni correction were applied, to detect specific differences among treatments (R-package dunn.test).

To test treatment effects over the course of the experiment regarding weighted averages of m/z, O:C, and O:H, as well as BPP and cumulative respiration, we applied a linear mixed-effects model (LME). We used the 'lme' command of the R-package 'nmle' with Restricted Maximum Likelihood estimation, whereby the fixed structure was set as interaction for treatment (3 - 5 levels) and sampling time (3 - 22 levels) and for the random structure we allowed different intercepts for each replicate. Each LME was followed by a model validation, checking the residuals for normal distribution and homogeneity of variances. Statistical significance of the interaction was tested using a likelihood-ratio test by comparing the model with and without the interaction. When the interaction was found significant, we analyzed each sampling individually. The LMEs were followed by the conservative Turkey's post hoc test (R-package multcomp) to test significances among treatments. All the above-mentioned analyses were tested at a significance level of $P \leq 0.05$ performed with the program R (R-Development-Core-Team, 2010).

We applied Partial Least Squares Regression (PLS; e.g. Eriksson et al., 2006, Abdi, 2010) in order to explore how different treatment describing parameters perform as predictors of BPP and respiration (response variables). For PLS, data need not to be normally distributed and its underlying algorithm is tolerant to missing values. Analyses was performed after Sobek et al. (2003) and detailed analyses could be found there. Briefly, in order to increase the model performance, variables with a skewness > 2 or min/max-ratio < 0.1 were log-transformed beforehand. Moreover, all input variables were scaled prior modeling. The model performance of the PLS is expressed by R^2Y and Q^2, while R^2Y is comparable to R^2 in a linear regression. Furthermore, the closer Q^2 values are to R^2Y, the higher is the predictive power of the model. The spatial distribution of the variables in the plot area provides information about the correlation structure of the dataset. Positively correlated variables are situated close to each other; negatively correlated variables are situated oppositely. We used the variable influence on projection (VIP) to express the influence of every predictor variable on the response variables. The VIP scores are weighted according to the amount of explained variance of the response variables in each component (Eriksson et al., 2006). According to Eriksson et al. (2001), the VIPs can be classified in highly influential (VIP < 1), moderately influential (0.8 < VIP < 1), and less influential (VIP < 0.8). All PLS modeling was done on Statistica 12 software.

3 Results

3.1 Quantity and quality dynamics of DOM and carbon during the experiment

In general we observed in all treatments (0 % DOM_{leaf}, 25 % DOM_{leaf}, 50 % DOM_{leaf}, 85 % DOM_{leaf}, 100 % DOM_{leaf}; Fig. 1) a consumption of DOC, a decrease of the relative amount of LMWS, as well as an increase of the relative amounts of HMWS and HS in the remaining DOM.

The DOC consumption over the time of the experiment was significantly different among treatments (Kruskal-Wallis, $\chi^2_{(4)} = 18.299$, p = 0.001; Fig. 2). The DOC consumption in treatment 85

% DOM_{leaf} (Dunns's test, p = 0.021) and treatment 100 % DOM_{leaf} (Dunns's test, p = 0.001) were significantly higher than in treatment 0 % DOM_{leaf}, while the DOC consumption in treatment 100 % DOM_{leaf} was significantly higher than in treatment 25 % DOM_{leaf} (Dunns's test, p = 0.021).

Also the decrease in the relative amount of LMWS was significantly different among treatments over the time of the experiment (Kruskal-Wallis, $\chi^2_{(4)}$ = 16.104, p = 0.003; Fig. 3). Relative amount of LMWS decrease in treatment 85 % DOM_{leaf} (Dunns's test, p = 0.042) and treatment 100 % DOM_{leaf} (Dunns's test, p = 0.003) were significantly higher than in treatment 0 % DOM_{leaf}, while the LMWS decrease in treatment 100 % DOM_{leaf} was significantly higher than in treatment 25 % DOM_{leaf} (Dunns's test, p = 0.027).

Additionally, there was a significant treatment effect in the relative amount of HMWS over the time of the experiment (Kruskal-Wallis, $\chi^2_{(4)}$ = 16.643, p = 0.002; Fig. 3). Relative amount of HMWS increase in treatment 85 % DOM_{leaf} (Dunns's test, p = 0.008) and 100 % DOM_{leaf} (Dunns's test, p = 0.002) was significantly higher than in treatment 0 % DOM_{leaf}.

Finally, we also found a significant treatment effect in respect to the increase of the relative amount of HS over the time of the experiments (Kruskal-Wallis, $\chi^2_{(4)}$ = 15.420, p = 0.004; Fig. 3). Relative amount of HS increase in treatment 100 % DOM_{leaf} was significantly higher than in treatment 0 % DOM_{leaf} (Dunns's test, p = 0.012) and treatment 25 % DOM_{leaf} (Dunns's test, p = 0.015).

A high-resolution mass spectrometry view in the DOM revealed that the weighted average of mass-to-charge ratio (m/z) decreased during the experiment in treatment 0 % DOM_{leaf}, 50 % DOM_{leaf} and 100 % DOM_{leaf} (Fig. 4a). There was no interaction effect among the treatments (LME, LLR = -142.525, p = 0.257) and the posthoc test showed that m/z was significantly higher in treatment 0 % DOM_{leaf} compared to treatment 50 % DOM_{leaf} (Turkey's test, p = 0.015) and treatment 100 % DOM_{leaf} (Turkey's test, p < 0.001).

Furthermore, the weighted average of oxygen to carbon (O:C) decreased during the experiment in treatment 0 % DOM_{leaf}, 50 % DOM_{leaf} and 100 % DOM_{leaf} (Fig. 4b). There was no interaction effect among the treatments (LME, LLR = 90.726, p = 0.872) and the posthoc test showed that O:C was significantly higher in treatment 0 % DOM_{leaf} compared to treatment 100 % DOM_{leaf} (Turkey's test, p = 0.032).

Finally, the weighted average of hydrogen to carbon (H:C) remained the same during the experiment in treatment 50 % DOM_{leaf} and 100 % DOM_{leaf}, and increased slightly in treatment 0 % DOM_{leaf} after 2 days (Fig. 4c). Consequently, we detected an interaction effect among the treatments (LME, LLR = 68.920, p = 0.020). Therefore, we analyzed the treatment effect on H:C at each sampling (at the start and after 48, 168, and 288 hours) and H:C was always significantly different among treatments (p values ranging from < 0.0001 to 0.017).

3.2 BPP and respiration dynamics during the experiment

The BPP showed different dynamics over the time of the experiment among the different treatments, whereby all treatments started at a similar level (Fig. 5). Treatment 0 % DOM_{leaf} revealed a constant increase up to seven days after the experiment start and then stayed similar until the end of the experiment. Treatments 25 % DOM_{leaf} and 50 % DOM_{leaf} revealed a peak after two days and decreased afterwards, while BPP increased again in the end of the experiment in treatment 25 % DOM_{leaf}, whereas BPP in treatment 50 % DOM_{leaf} remained stable. BPP in treatment 85 % DOM_{leaf} and 100 % DOM_{leaf} showed a BPP peak after two days of the experiment and decreased afterwards. Consequently, we detected an interaction effect of BPP among the treatments (LME, LLR = -354.170, $p < 0.0001$). Therefore, we analyzed the treatment effect on BPP at each sampling (after 48, 168, and 288 hours) and BPP was always significantly different among treatments (p values ranging from < 0.0001 to 0.0001).

Beside BPP, also the total respiration (R) over the time of the experiment was significantly different among treatments (Kruskal-Wallis, $\chi^2_{(4)} = 16.689$, $p = 0.002$; Fig. S1). R in treatment 85 % DOM_{leaf} (Dunns's test, $p = 0.037$) and treatment 100 % DOM_{leaf} (Dunns's test, $p = 0.001$) were significantly higher than in treatment 0 % DOM_{leaf}; while R in treatment 100 % DOM_{leaf} was significantly higher than in treatment 25 % DOM_{leaf} (Dunns's test, $p = 0.050$). The cumulative R for each incubation interval allows a closer look at the temporal dynamics over the course of the experiment among treatments (Fig. 6). It shows a consistent more intense R with increasing amount of DOM_{leaf} in the treatments (treatment 100 % > 85 % > 50 % > 25 % > 0 % DOM_{leaf}) during the entire experiment. Furthermore, it is noticeable that treatment 50 % DOM_{leaf} reveals the highest standardizations, indicating more heterogeneity of the replicates within the same treatment. We detected an interaction effect of R among the treatments (LME, LLR = 448.656, $p < 0.0001$), confirming the different dynamics over the course of the experiment among treatments.

3.3 Driving factors of BPP and respiration (R)

In order to identify the main drivers related to BPP and R, we performed PLS analysis with the parameters collected after the start of the experiment related to DOM quality characteristics, nutrients and DOC concentrations (Table 1). The PLS regression model describing the response BPP and R (Fig. 7) extracted three significant components that explained, in total, 63 % of the variance (R^2Y_{cum} 0.63). The model predictability power was moderate (Q^2_{cum} 0.54). The slope ratio (Sr), indicating apparent DOM molecular weight is the most important predictor of BPP and R. Based on VIP scores, other important predictors include LMWS and C:N (positively correlated), DOC concentration, and fluorescence index (FI) and $SUVA_{254}$ (positively correlated). Furthermore, the PLS model also describes the correlation structure of the X-variables. Variables reflecting humic-like substances (a440, HS and HIX) were well-correlated with each other (Fig. 7). Moreover, indicators of more complex DOM (HMWS and $SUVA_{254}$) were well correlated and HMWS showed an inverse relationship with LMWS. BPP and R revealed a strong inverse relationship with HMWS and parameters indicating humic-like substances (HS, a440 and HIX). Moderately influential predictors include the freshness index (b/a) and SRP (positively correlated), as well as HIX, HS, DON, HMWS, a440, and a254/a365 (all positively correlated).

3.4 *Carbon source used for respiration*

The continuous measurements of the isotopy of R combined with the isotopically labeled DOM_{leaf} enabled us to distinguish between the two added DOM sources and to track, over time, which was used for R. By applying the keeling plot method we were able to trace the mixing ^{13}C signal of the emitted CO_2, coming from the respired dissolved inorganic carbon, over 24 hours, during the entire course of the experiment in each of the mixed treatments. Due to the set cutoff of R^2 for the keeling plots at 0.5, we had to accept some missing values (Fig. 8). The two-source mixing model revealed a clear dominance of labile DOM sources in R. The higher the proportion of labile DOM (DOM_{leaf}) in the treatment, the more DOM_{leaf} was used for respiration. Although the respired fraction of DOM_{leaf} was at some points in time over 60 %, a reasonable amount of DOM_{peat} must have been used for microbial respiration.

4 Discussion

Since pre-industrial times, anthropogenic perturbation has been increasing the input of carbon to inland waters mainly due to enhanced organic matter export from soils (Regnier et al., 2013). This is while, future hydrological changes (specially runoff) are likely leading to changes not only in the quantity but also in the quality of DOM exported from terrestrial sources to freshwater systems (Porcal et al., 2009). In this study we addressed the interaction of two natural terrestrial DOM sources with distinct differences in quality to spot the importance of DOM quality on its microbial utilization. Moreover, we combined multiple DOM quality assessment approaches (LC-OCD, optical properties, FT-ICR-MS) with multiple microbial DOM turnover assessments (BPP, respiration, stable isotopes) in order to investigate how microbial DOM turnover and thereby terrestrial-aquatic coupling is related to the composition of the DOM in respect to quality.

The results of this study suggest large interactive effects between microbial metabolism and the quality of the two investigated terrestrial DOM sources. Consequently, these findings enhance the understanding of terrestrial-aquatic coupling and the relevance of DOM quality as a driver of DOM turnover in freshwater ecosystems.

4.1 *DOM quality drives microbial metabolism*

The data concerning the size fractions of the consumed DOM reveal that LMWS were consumed more intense, and the relative amount of HMWS as well as humic-like substances (HS) increased (Fig. 3). Consequently, the remaining DOM in the treatments may got more complex and relatively heavier. This underlines the fact that the microbial community selectively consumed the more labile LMWS first (Middelburg, 1989). Hereby DOM can be directly taken up by the microbes via assimilation without mediation by an external process (Findlay and Sinsabaugh, 1999). Consequently, the mostly likely materials to flow along this pathway are small monomeric substrates such as short peptides, fatty acids or saccharides (Findlay and Sinsabaugh, 1999). Beside our study, this mechanism was underlined from Meyer et al. (1987) who showed that microbial metabolism on blackwater river was greatest in LMWS DOM-enriched treatments (Meyer et al., 1987). In contrast, other studies demonstrated an utilization of HMWS during the experiment, with a consequently decreased of the molecular weight of the DOM (Docherty et al., 2006, Kritzberg et al., 2006). This can be explained by the size-reactivity continuum model proposed by Amon and Benner (1996), which is originally based on seawater bioassays. It suggests that the bulk of HMW DOM is more bioreactive than the bulk of LMWS DOM. The fact that molecular weight of DOM is an important quality characteristic is underlined by the result of the PLS modeling, revealing slope ratio as an indicator of apparent DOM weight as the most influential predictor for BPP and R (Fig. 7). However, concentration of LMWS was a further highly influential predictor of BPP and R. Since this topic is scientifically discussed in such a contradictive manner, we conclude that DOM quality cannot be derived from molecular size of DOM alone but must be supported by further DOM quality indicators such as optical properties.

Furthermore, with our experimental setup, we could clearly show that DOM quality influences microbial metabolism, i.e. BPP and R (Fig. 7). BPP was highest in the treatments with the highest proportions of labile DOM (DOM_{leaf}), especially after two and seven days after the star of the experiment (Fig. 5). These results indicate that the labile DOM boosted BPP, but had also a quite short turnover time. In comparison, the treatment with the highest proportion of less labile DOM (DOM_{peat}) revealed a lower but steady level of BPP. This finding goes along with the concept of "baseline metabolism" suggested by Guillemette et al. (2013). In their study, the labile DOM (algal DOM) is quickly degraded on short time scales, whereas the less labile DOM pool (terrestrial DOM) is mainly degraded on long term. Likewise, previous similar studies showed that BPP was highest in most labile DOM treatments (Kritzberg et al., 2006, Farjalla et al., 2009) compared to treatments with less labile DOM. Furthermore, Berggren et al. (2010b) showed by a modeling approach that terrestrial exported low molecular weight DOM supported about 50–100% of BPP in a boreal lake in northern Sweden. Finally, we found an interaction between BPP and the relative amount of LMWS in the remaining DOM: when BPP increased, the relative amount of LMWS decreased, indicating that they were used for BPP. However, when BPP decreased, the relative amount of LMWS increased, indicating that maybe bacterial cell lysis took place, which we consequently measured in the LMWS fraction (Fig. 5 b, c, d, and e). Also R was highest in the treatments with the highest proportions of labile DOM (DOM_{leaf}), but compared to BPP throughout the whole duration of the experiment (Fig. 6). These results are in line with previous studies, which showed as well that R was highest in more labile and fresh DOM treatments compared to more refractory and aged DOM (Kritzberg et al., 2006, Farjalla et al., 2009). Since we performed a short term experiment (12 days), we cannot predict how the DOM source utilization will develop on a long term scale. Koehler et al. (2012) showed in a long term experiment over 3.7 years that the decay rate of DOM of clear-water lakes and brownwater lakes converged within five months. However, with the result that R intensity follows the gradient of increasing proportions of labile DOM in the treatment, we can clearly say that labile DOM boosts R in the short term. Additionally, the before mentioned statement can be underlined by a strong positive relationship between total R and total relative consumption of LMWS during the experiment (Fig. S1).

The mixtures of labile and less labile DOM did neither led to a higher BPP nor R compared to a single carbon source, as could be expected by the aquatic priming concept (Guenet et al., 2010, Bianchi, 2011). Briefly, the initially terrestrial derived concept describes that the supply of labile organic matter can stimulate the mineralization of the less labile organic matter (Kuzyakov et al., 2000). For example, a study of Farjalla et al. (2009) showed that BPP and R were higher in the mixture of DOM leached from aquatic macrophytes and DOM accumulated from a tropical humic lagoon than expected in single substrate cultures. However, another study by Catalan et al. (2015) used a multifactorial microcosm experiment to test the conditions under which priming may be observed in freshwater ecosystems. Despite the extensive range of tested conditions, they found no clear evidence of a priming effect on DOM degradation (Catalan et al., 2015). Hence, it may be concluded that priming in freshwater systems may be of limited importance (Bengtsson et al., 2014, Catalan et al., 2015).

Traditionally, it has been assumed that DOM originated from aquatic plants should be better bioavailable for bacteria than terrestrial DOM, due to simple, low molecular weight carbon compounds characteristics of the former (Chen and Wangersky, 1996) and more complex and aromatic compound characteristics of the latter (McKnight and Aiken, 1998). Recent studies, however, call this assumption into question (Berggren et al., 2010a, Attermeyer et al., 2014) by indicating that DOM quality -rather than the source of DOM itself- determines DOM turnover. This new insight is supported by an early study of Tranvik and Höfle (1987), which revealed that bacterial biomass produced in cultures, which were based on water from a humic lake, was doubled compared to a clear-water lake. The results of this study further confirm that DOM quality is too complex to be simplified to its origin. We showed that two terrestrial DOM sources show strong quality differences leading to significant different microbial metabolism.

4.2 DOM source for microbial respiration

We determined the proportion of DOM_{leaf} vs. DOM_{peat} respired by a natural microbial community over time by using a novel method to continuously measure the isotopic signature ($\delta^{13}C$) of respiratory CO_2. In a similar study, Guillemette et al. (2013) tracked the isotopic signature of bacterial respiratory CO_2 in lake water incubations and reconstructed the bacterial consumption dynamics of algal and terrestrial DOM. Compared to the study of Guillemette et al. (2013), where the CO_2 production and its isotopic signature was measured three times within 20 days of experiment, we were able to reach a much higher temporal resolution (Fig. 8). As such, our results suggest that the labile labeled DOM source (DOM_{leaf}) was used, for respiration by the microbial community to a higher extend compared to the more refractory DOM source (DOM_{peat}) for specific time periods; for example, in treatment 85 % DOM_{leaf}, between 84 and 132 hours of the experiment DOM_{leaf} was used for respiration by 61 %. However, it seems that a reasonable proportion (e.g. in treatment 25 % DOM_{leaf} 67 % between 36 and 60 hours of the experiment) of the microbial respiration have been fueled by DOM_{peat}. Our results are lower, but yet in line with the findings of Guillemette et al. (2013), which revealed that the labile algal DOM pool was respired in proportions and at rates twice and 10 times as high as the terrestrial DOM pool, respectively. Additionally, in all their experiments, the proportion of labile respired algal DOM decreased systematically over time (Guillemette et al., 2013), which was also observed in our study. Furthermore, McCallister and Del Giorgio (2008) determined the proportion of terrigenous vs. algal-derived organic carbon respired by bacteria in eight lakes in southern Québec (Canada). They found a negative relationship between percentage of respiratory CO_2 from terrigenous OC and chlorophyll *a* concentration in the investigated lakes (McCallister and Del Giorgio, 2008). However, they also found a positive relationship between total planktonic respiration and chlorophyll *a* concentration (McCallister and Del Giorgio, 2008). These results may indicate that if more labile DOM is available, it is also mainly used for respiration.

4.3 Conclusions

Our results underline the importance of DOM quality in carbon turnover, suggesting that there is a significant labile proportion in the terrestrial DOM, which is likely to boost microbial metabolism. Furthermore, our results go along with a study of Berggren et al. (2010a) showing a rapid utilization of low molecular weight compounds of terrestrial origin in aquatic ecosystems. One step further goes a study by Lapierre et al. (2013) which shows a strong causal link between DOM concentrations and aquatic CO_2 fluxes, mediated by the degradation of terrestrial organic matter in aquatic ecosystems. Finally, our study provides a further puzzle piece for the mechanistic understanding of the microbial degradation processes of terrestrial derived DOM, which may lead to an even stronger contribution of inland waters to the regional and global carbon budgets in the future.

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Table 1. Variables used in PLS modeling

Variable in PLS model and abbreviation	**Category**
Bacterial protein production (BPP)	Response (Y)
***Respiration (R)**	Response (Y)
DOC concentration	Predictor (X)
SRP concentration	Predictor (X)
***High molecular weight substances (HMWS, mg L^{-1})**	Predictor (X)
Humic-like substances (HS, mg L^{-1})	Predictor (X)
Low molecular weight substances (LMWS, mg L^{-1})	Predictor (X)
Dissolved organic nitrogen (DON)	Predictor (X)
***C:N ratio**	Predictor (X)
DOM color (a440)	Predictor (X)
Apparent molecular size (a254/a365)	Predictor (X)
Slope ratio (Sr)	Predictor (X)
Specific ultraviolet absorbance ($SUVA_{254}$)	Predictor (X)
***Fluorescence index (FI)**	Predictor (X)
Humification index (HIX)	Predictor (X)
Freshness index (b/a)	Predictor (X)

* log transformed prior PLS modeling

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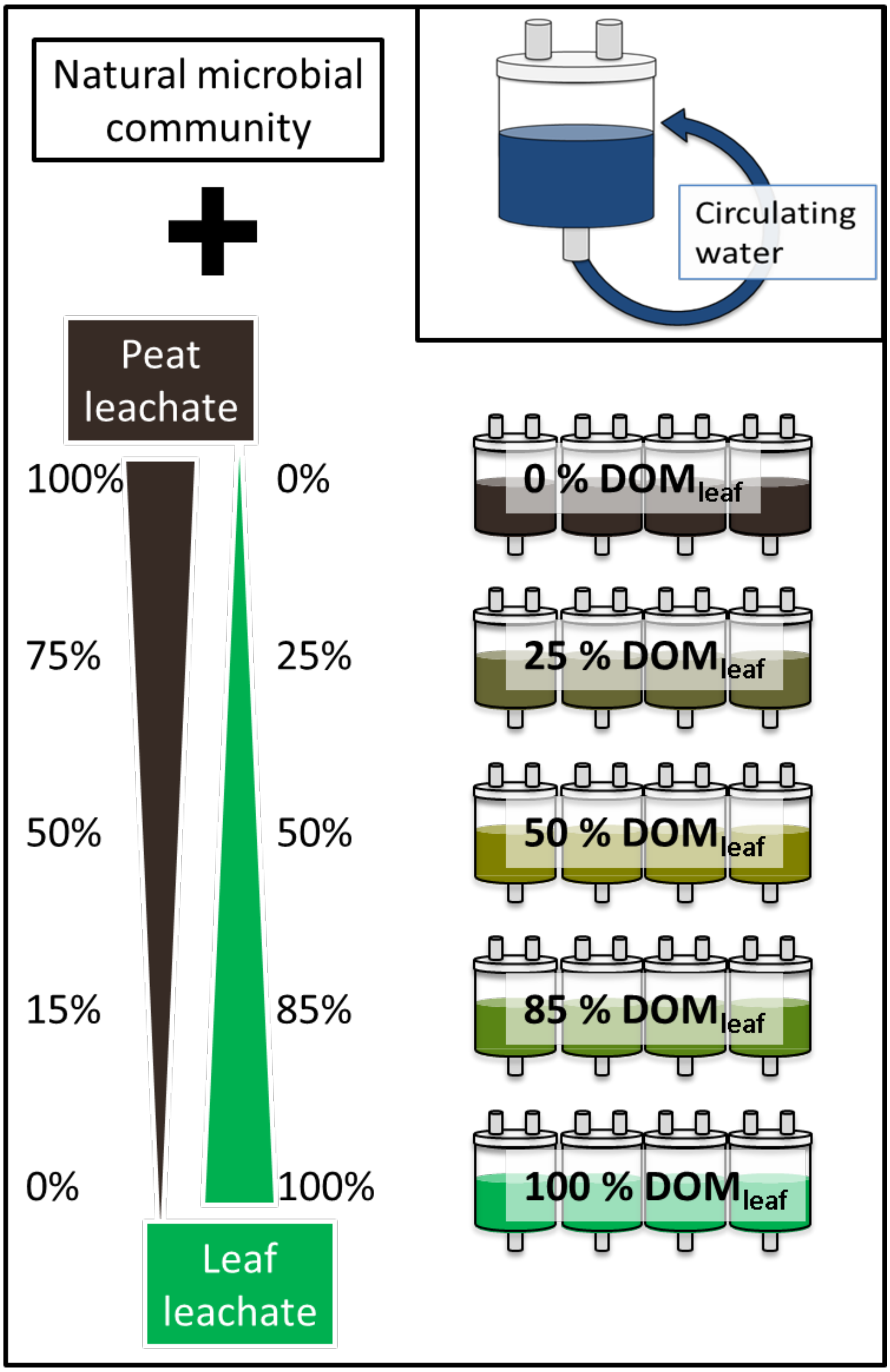

Figure 1. Overview of the experimental design: a natural microbial community was mixed with artificial lake water and different proportions of two distinct dissolved organic matter sources (DOM_{peat} and DOM_{leaf}). *Inset graph*: shows an exemplary chamber which was continuously pumped through.

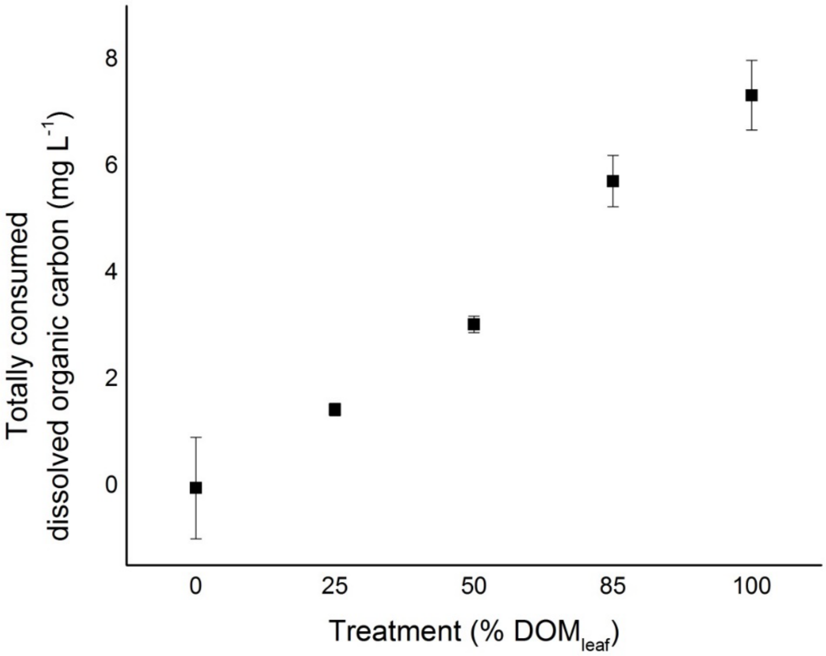

Figure 2. Totally consumed dissolved organic carbon during experiment per treatment. Squares = mean, vertical lines = standard deviation; n = 4 per treatment.

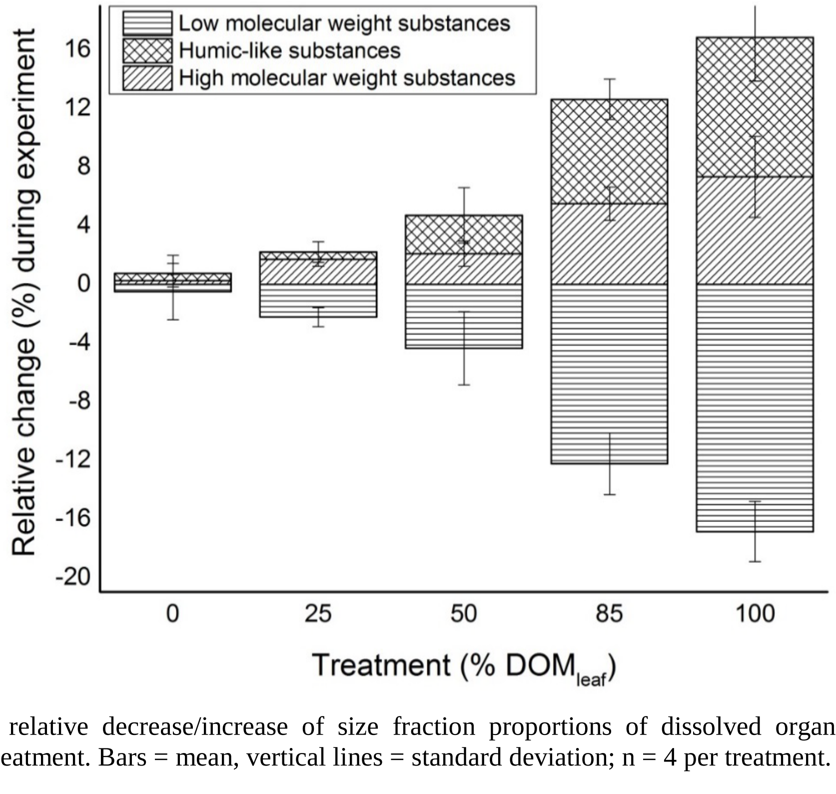

Figure 3. Total relative decrease/increase of size fraction proportions of dissolved organic matter during experiment per treatment. Bars = mean, vertical lines = standard deviation; n = 4 per treatment.

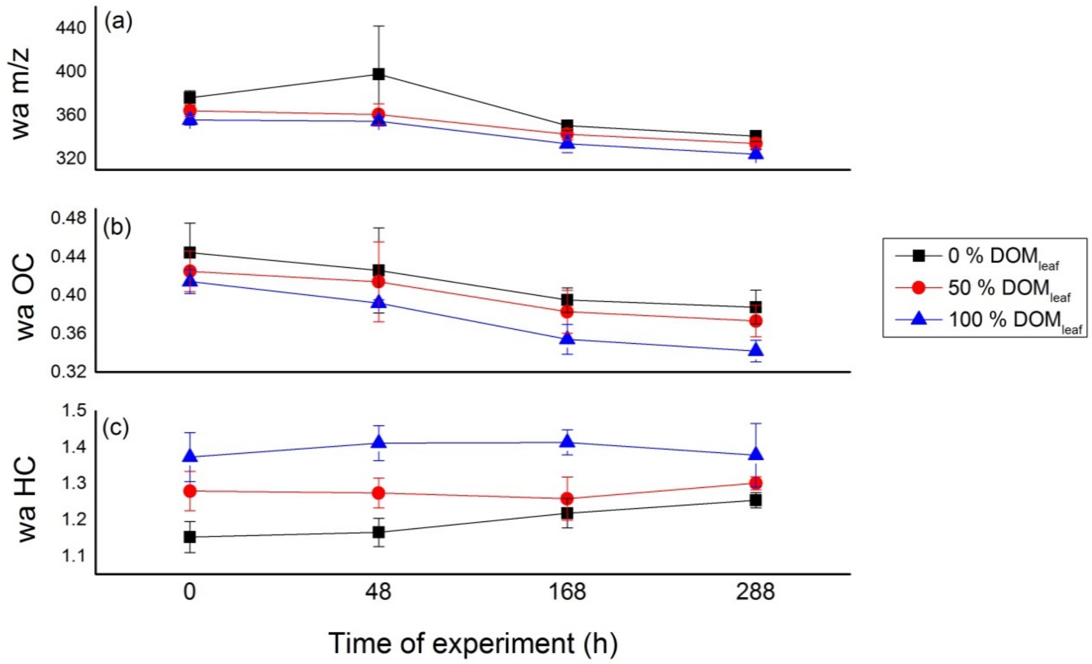

Figure 4. Change of weighted averages (wa) of (a) m/z, (b) O:C, and (c) H:C of treatment 0 % DOMleaf, 50 % DOMleaf and 100 % DOMleaf over the duration of the experiment. Filled symbols = mean, vertical lines = standard deviation, n = 3 per treatment.

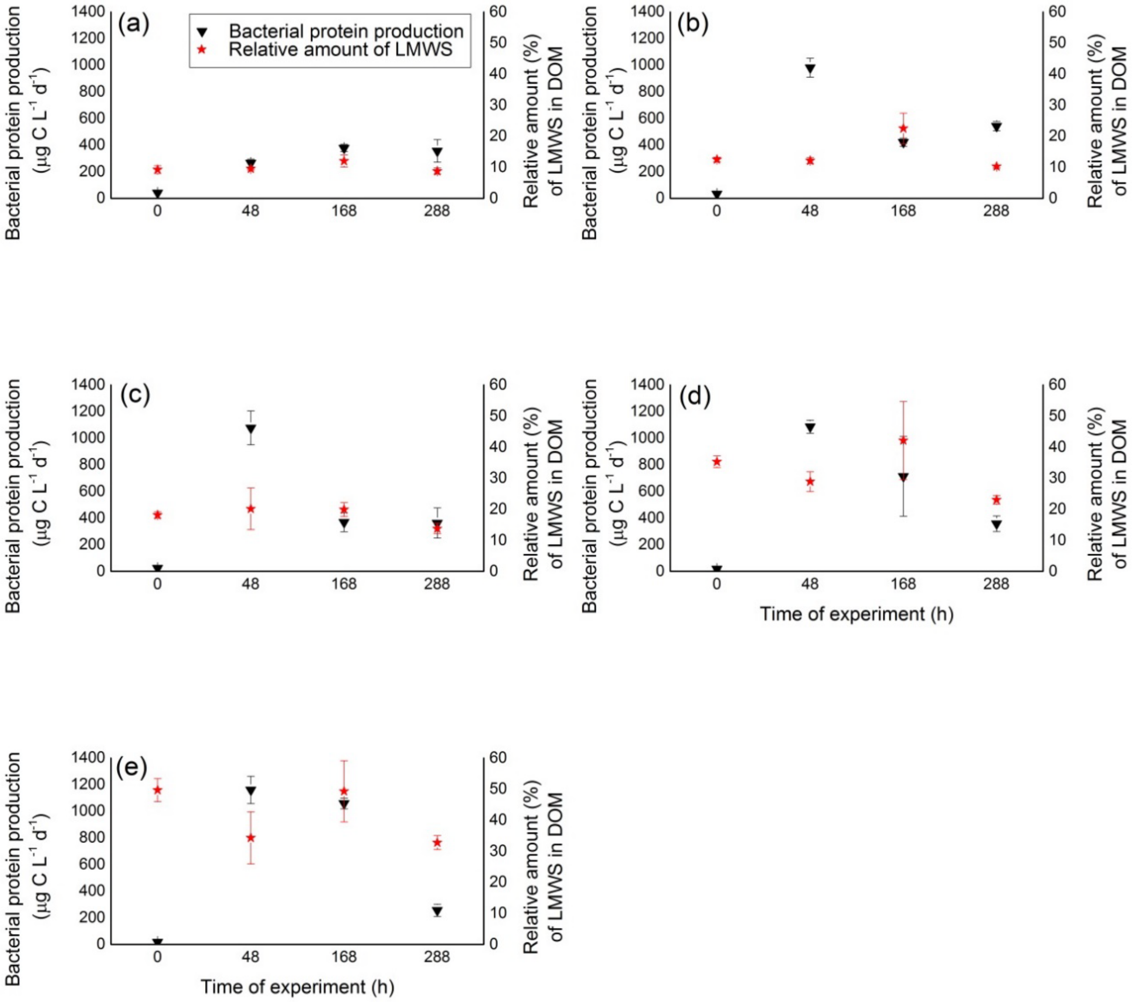

Figure 5. Bacterial protein production and relative amount of low molecular size substances in treatment 0 % DOM$_{leaf}$ (a), 25 % DOM$_{leaf}$ (b), 50 % DOM$_{leaf}$ (c), 85 % DOM$_{leaf}$ (d), and 100 % DOM$_{leaf}$ (e), over the duration of the experiment. Filled symbols = mean, vertical lines = standard deviation, n = 4 per treatment.

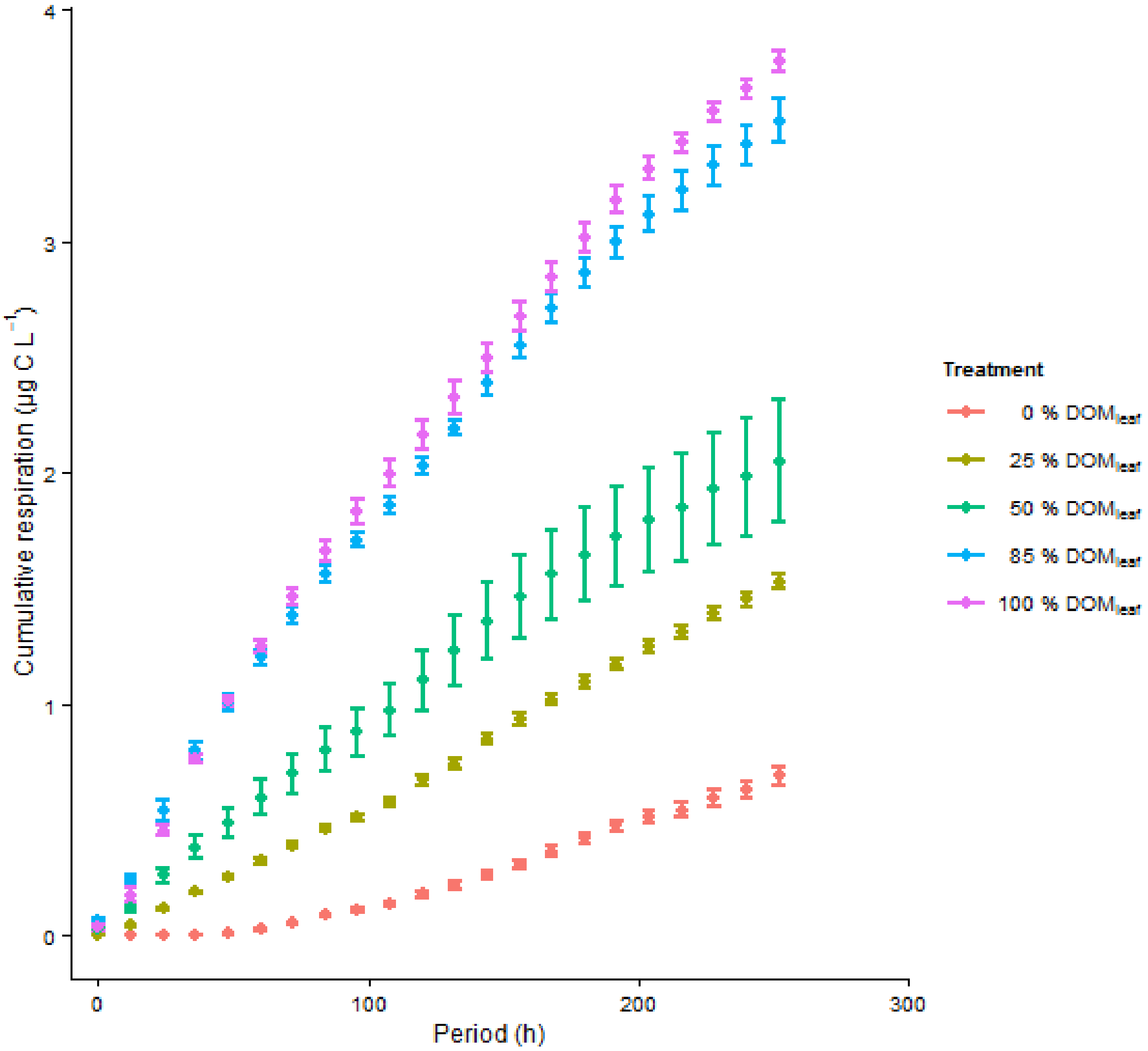

Figure 6. Cumulative respiration over the duration of the experiment of different treatments. Dots = mean; vertical lines = standard deviation; n = 4 for treatment 0 % DOM$_{\text{leaf}}$, 25 % DOM$_{\text{leaf}}$, 50 % DOM$_{\text{leaf}}$ and 0 % DOM$_{\text{leaf}}$, n = 3 for treatment 85 % DOM$_{\text{leaf}}$.

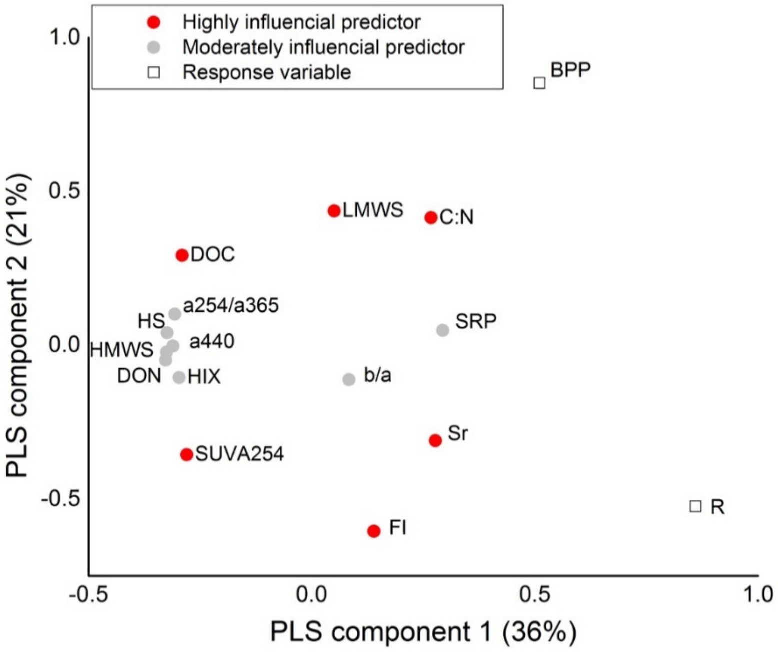

Figure 7. Loading plot of partial least squares (PLS) model for 3 samplings during the experiment. The graph depicts the correlation structures between the predictor variables and the response variables (bacterial protein production and respiration). Variables situated along the same directional axis correlate with each other. For explanation of abbreviations, see methods section and table 1.

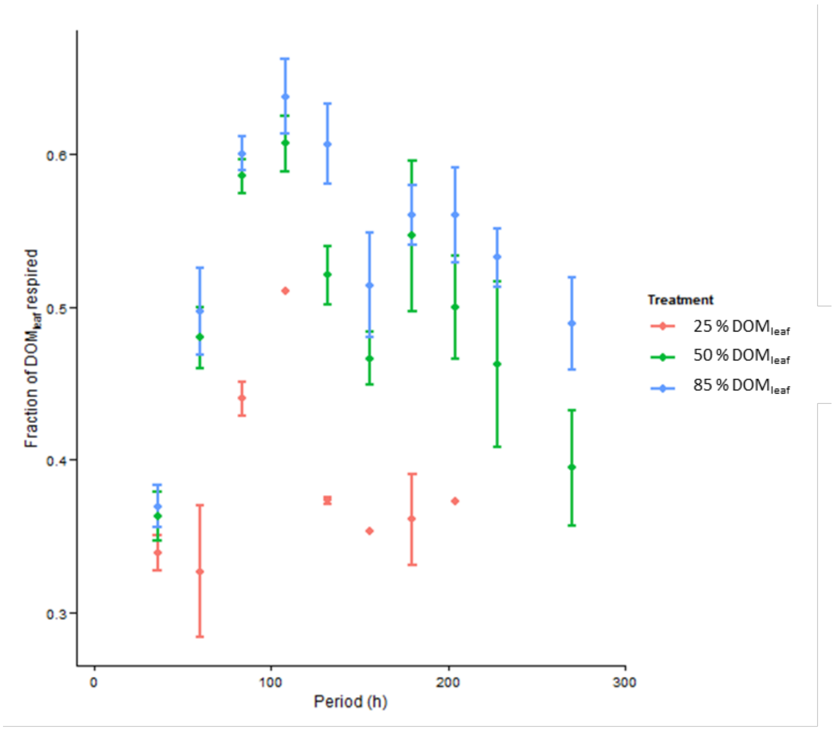

Figure 8. Fraction of DOM_{leaf} respired over the duration of the experiment of different treatments. The fraction of DOM_{peat} is consequently 1-fraction DOM_{leaf}. Dots = mean; vertical lines = standard deviation; n = between 1 and 4.

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SUPPLEMENTARY MATERIALS OF MANUSCRIPT I

Supplemental methods

Brief description of the peatland site

Briefly the peatland site experienced intensive drainage activities and agricultural use, over about three decades, provoking a proceeded degradation of the surface peat layer. The upper about 0.3 dm became heavily decomposed, i.e. the originally less decomposed sedge and reed-derived peat changed to so called muck soils (Okruszko, 1995). A previous incubation of this substrate have shown an elevated mobilization of DOM but comparatively low greenhouse gas fluxes, implying that this material was less accessible for microbial degradation (Hahn-Schofl et al., 2011).

Dissolved organic and inorganic carbon and nutrients

The samples were analyzed in duplicates on a multi N/C 3100 Analyzer (Jena Analytics, Germany) by applying infralyt detection after combustion and according to DIN EN 1484 for DOC and DIC. Water samples for SRP were analyzed photospectrometrically on a UV/VIS-Photometer CARY 1E (VARIAN, Germany). The protocol for SRP (DIN EN 1189) was slightly modified, whereby the molybdate solution was changed according to Murphy and Riley (1962). NH_4^+ was measured according to EN ISO 11732, using automated segmented flow analysis (SCAN++ - System, Skalar), while NO_3^- was analyzed by an ion chromatography system with suppressor (Shimadzu).

Optical indices

Samples for optical analysis were measured within 2 days of sampling. We used an Aqualog (Horiba Scientific) to generate absorbance scans and excitation emission matrices EEMs simultaneously. Fluorescence intensities were measured at excitation wavelengths ranging from 250 to 600 nm (3-nm increments) and emission wavelengths from 212.14 to 621.78 nm (1.64-nm increments). Since the fluorescence intensities at excitation wavelengths were measured with an increment of 3 nm, the optical indices were adapted slightly. The water Raman peak of Milli-Q water served as reference, and EEMs were corrected for blanks (MilliQ) and absorbance.

We computed the following optical indices from DOM absorbance and fluorescence.

The specific UV absorption ($SUVA_{254}$) was calculated as the absorption coefficient at 255 nm (m^{-1}) (instead of 254 nm (m^{-1})) relative standardized to the DOC concentration (mg l^{-1}) (Weishaar et al., 2003, modified). The absorption coefficient at 441 nm (m^{-1}) (instead of 440 nm (m^{-1})) served as an indicator for DOM color/color intensity (Cuthbert and Del Giorgio, 1992, modified). The ratio of absorbance at 254 and 365nm (a254/a365) was calculated as ratio of absorbance at 255 and 366 nm instead (Dehaan, 1993, Dahlen et al., 1996, modified). Furthermore, the slope ratio (S_R) was calculated as the ratio of $S_{276-294}$ to $S_{349-399}$ instead of $S_{275-295}$ to $S_{350-400}$ (Helms et al., 2008, modified).

The humification index (HIX) was calculated as the area under the emission spectra 434 (instead of 435) - 480 nm divided by the peak area 300-345 nm plus 434 (instead of 435) - 480 nm, at an excitation wavelength of 255 nm (instead of 254 nm) (Ohno, 2002, modified). The b/a (freshness) index was computed as the ratio of emission intensity at 381 nm (instead of 380 nm) (b) to the maximum emission intensity between 421 and 434 nm (instead of 420 and 435 nm) (a) at an excitation wavelength of 309 nm (instead of 310 nm) (Parlanti et al., 2000, modified). Finally, the fluorescence index (FI) was calculated as the ratio of emission intensity at 450 nm to 500 nm at an excitation wavelength of 369 nm (instead of 370 nm) (McKnight et al., 2001, modified).

Isotopic ^{13}C signature DOC_{peat} and DOC_{leaf}

To remove all dissolved inorganic carbon (DIC), samples were acidified and purged with helium off-line. Aliquots of 1-9 mL of sample (depending on sample concentration) were transferred into a heated digestion vessel and reacted with sodium persulfate to convert DOC into a pulse of CO_2. The CO_2 was carried in a helium flow to the isotope ratio mass spectrometer where the $^{13}C/^{12}C$ ratios were measured. Samples were corrected based on included laboratory standards, and calibrated against NIST Standard Reference Materials (UC Davis, California).

Supplemental figures

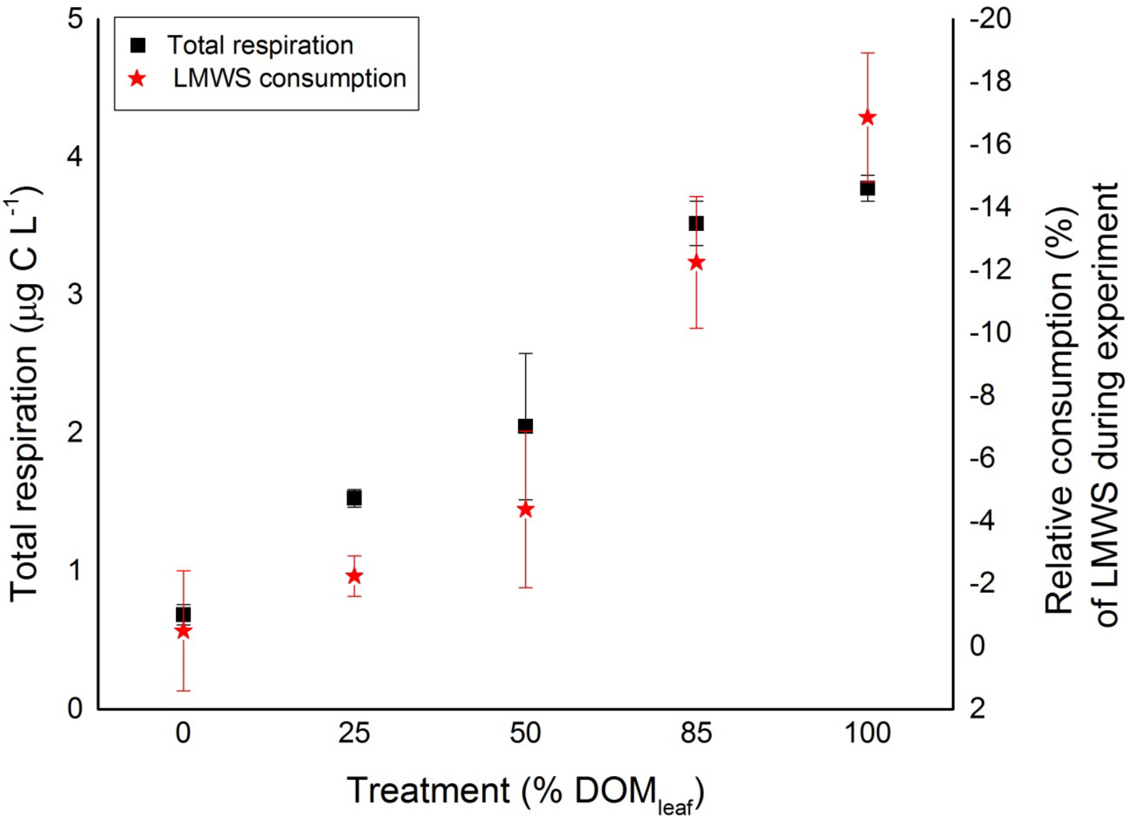

Figure S1. Total respiration of different treatments. Squares = mean; vertical lines = standard deviations; n = 4 for treatment 0 % DOM$_{leaf}$, 25 % DOM$_{leaf}$, 50 % DOM$_{leaf}$ and 0 % DOM$_{leaf}$, n = 3 for treatment 85 % DOM$_{leaf}$

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MANUSCRIPT II

TECHNICAL NOTE: DRIFTING VERSUS ANCHORED FLUX CHAMBERS FOR MEASURING GREENHOUSE GAS EMISSIONS FROM RUNNING WATERS

Published in Biogeosciences:

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Technical note: drifting versus anchored flux chambers for measuring greenhouse gas emissions from running waters

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Abstract. Stream networks have recently been discovered to be major but poorly constrained natural greenhouse gas (GHG) sources. A fundamental problem is that several measurement approaches have been used without cross-comparisons. Flux chambers represent a potentially powerful methodological approach if robust and reliable ways to use chambers on running water can be defined. Here we compare the use of anchored and freely drifting chambers on various streams with different flow velocities. The study clearly shows that (1) anchored chambers enhance turbulence under the chambers and thus elevate fluxes, (2) drifting chambers have a very small impact on the water turbulence under the chamber and thus generate more reliable fluxes, (3) the bias of the anchored chambers greatly depends on chamber design and sampling conditions, and (4) there is a promising method to reduce the bias from anchored chambers by using a flexible plastic foil collar to seal the chambers to the water surface, rather than having rigid chamber walls penetrating into the water. Altogether, these results provide novel guidance on how to apply flux chambers in running water, which will have important consequences for measurements to constrain the global GHG balances.

1 Introduction

Rivers and streams have been identified as important links in the global carbon cycle. They receive and transport terrestrial carbon from the land to the ocean and are also shown to be a net source of greenhouse gases (GHG), i.e., carbon dioxide (CO_2) and methane (CH_4) (Aufdenkampe et al., 2011; Battin et al., 2008; Cole et al., 2007; Tranvik et al., 2009). In a recent study, the global CO_2 emissions from rivers and streams were estimated to be 1.8 ± 0.25 Gt C year^{-1} (Raymond et al., 2013), which corresponds to 70 % of the global ocean carbon sink (Le Quéré et al., 2014). Due to the lack of knowledge of surface area and gas exchange velocity, the smallest streams are considered to be a major unknown component of regional- to global-scale GHG emission estimates (Bastviken et al., 2011; Cole et al., 2007). Despite these knowledge gaps,

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there are strong indications that small streams have the highest gas exchange velocities (Aufdenkampe et al., 2011), highest CO_2 partial pressures (Koprivnjak et al., 2010) and cover the largest fractional surface area within fluvial networks (Butman and Raymond, 2011). A continental-scale analysis of CO_2 efflux from streams and rivers revealed a continuous decline of the fluxes with increasing size and discharge of the aquatic systems (Hotchkiss et al., 2015).

Ecosystem-scale fluxes of CO_2 and CH_4 from running waters are often derived indirectly using measured gas partial pressure in the surface water in combination with estimates of a gas exchange velocity. For sparingly soluble gases, the exchange velocity is mainly controlled by turbulence at the water-side of the air–water interface. In smaller rivers and streams, turbulence is driven by stream velocity, depth, and bottom roughness (Marion et al., 2014), and the resulting gas exchange velocities are often parameterized with one or more of the following terms: stream order, slope, flow velocity, discharge, width, and depth (Alin et al., 2011; Raymond et al., 2012; Wallin et al., 2011). In small streams, reach-scale estimates of the gas exchange velocity can also be derived from gas tracer experiments, whereby a volatile tracer (e.g., propane or sulfur hexafluoride) is injected upstream and the longitudinal decrease of its dissolved concentration is measured (Halbedel and Koschorreck, 2013; Raymond et al., 2012). For practical reasons, tracer gas injections are limited to application in small streams and alternative methods suitable for a greater range of stream sizes are needed. Moreover, recent studies have suggested that the gas exchange velocity of CH_4 can be enhanced by microbubbles (Beaulieu et al., 2012) and can therefore differ from that of the volatile tracer. To better constrain ecosystem-scale estimates of GHG emissions and to improve the understanding of the flux drivers in small running waters, reliable methods are required that allow direct measurements.

As eddy-covariance (Baldocchi, 2014) measurements are not suitable for small streams, gas flux chambers that float on the water surface are a straightforward and inexpensive method for direct measurements of gas fluxes, and can easily be replicated over time and space (Bastviken et al., 2015). The gas flux is determined from the change of the gas concentration in the chamber headspace over time. Floating chambers have been frequently applied for measuring gas fluxes in large rivers, reservoirs and lakes (e.g., Beaulieu et al., 2014; DelSontro et al., 2011; Eugster et al., 2011).

Chamber measurements have been criticized because submerged chamber edges are thought to disrupt the aquatic boundary layer, thereby affecting the gas exchange (Kremer et al., 2003). Comparisons of floating chambers with other flux measurement techniques were performed in lakes, rivers, and estuaries. While some studies have reported a tendency of floating chambers to yield higher fluxes than other methods (Raymond and Cole, 2001; Teodoru et al., 2015), others found reasonable agreement (Gålfalk et al., 2013; Cole et al., 2010).

In streams and rivers, floating chambers have been deployed anchored at one spot (anchored chambers; Sand-Jensen and Staehr, 2012; Crawford et al., 2013), or freely drifting with the water (drifting chambers; Alin et al., 2011; Beaulieu et al., 2012). Although based on the same principle, the two deployment modes have fundamental differences. Because of the higher velocity difference between the chamber and the surface water, anchored chambers in running waters may create additional turbulence around the chamber edges (Kremer et al., 2003). If the effect of this turbulence on fluxes is minor, anchored chambers would be advantageous as the area covered by the chamber can be controlled and because practical work with anchored chambers is relatively simple. Drifting chambers will likely induce less turbulence in the surface water; however it is difficult to control their coverage, potentially resulting in spatially biased measurements. Drifting chambers are also complicated for several reasons, e.g., the presence of obstacles in the streams or in terms of logistics, as the chambers may travel far during measurement periods.

While the establishment of efficient methods for running water gas emissions is needed to improve the global GHG budget, progress in chamber-based methods is prevented by the lack of comparative assessments of anchored versus drifting chambers. In this study, we compared measurements of GHG fluxes and the gas exchange velocity using drifting and anchored chambers in various streams and rivers. Because chamber performance is expected to depend strongly on chamber design, the field experiments were conducted using three different chamber types. In laboratory experiments, we analyzed the flow field and the turbulence under both anchored and drifting chambers at different flow velocities. The primary objective of this study was to answer the following question: do anchored chambers produce reliable measurements of localized GHG fluxes in running waters?

2 Methods

2.1 Chamber measurements in the field

Field measurements were conducted in nine different rivers and streams in Germany and Poland using three different chambers (Table 1). All three data sets included anchored measurements, where the chambers were tethered to stay at a fixed position as well as drifting measurements, where the chambers freely moved with the current. In two of the data sets (A and B), the temporal change of CO_2 and CH_4 concentration in the chamber headspace was measured on a boat using infrared gas analyzers (A: off-axis integrated cavity output spectroscopy (OA-ICOS) gas analyzer, UGGA, Los Gatos Research Inc. USA; B: Fourier transform infrared (FTIR) analyzer, Gasmet 4010, Gasmet, Finland). In the third data set (C), the gas concentration was measured using a built-in and low-cost CO_2 sensor (ELG, SenseAir, Swe-

Table 1. Summary of the three data sets obtained in field measurements. Pictures show the three different chambers used for the anchored and drifting approach. Additional information about the sampling procedures is provided in the Supplement.

Data set	A	B	C
			
Site	Five different streams, north-central European Plain in Germany and Poland	Bode river, Harz Mountains, central Germany	Three different streams, Upper Rhine Valley, southwest Germany
Chamber volume (m^3)	0.0168	0.0147	0.0068
Chamber area (m^2) (shape)	0.126 (circular)	0.098 (rectangular)	0.066 (circular)
Chamber height (m)	0.175	0.15	0.13
Penetration depth (m)	0.018	0.023	0.025
Chamber gas measurement	LosGatos, CO_2, CH_4 on boat	FTIR analyzer (GASMET, Finland) on boat	Built-in low-cost CO_2 logger (ELG by SenseAir, Sweden)
Dissolved gas measurement	Contros CO_2 and CH_4	Contros CO_2, CH_4 with GC	UGGA with membrane contactor
Drifting measurements	Following boat or vice versa	Freely drifting while followed with boat	Freely drifting
Anchored measurements	Tethered to a rack in the middle of the stream	Tethered to anchored boat	Tethered with rope from above
Number of measurements	At five sites: two–five pairs of anchored chamber measurements (upstream) and subsequent floating chamber runs	For two different discharge situations: 10–13 pairs of subsequent drifting and anchored chamber measurements down the river using a single chamber	At three sites: two–three subsequent floating chamber runs and five parallel anchored chambers distributed along the trajectory of the floating chamber

den). The chamber used in C is described in detail elsewhere (Bastviken et al., 2015), the chamber used in A is described in McGinnis et al. (2015).

The chamber flux measurements were supplemented by measurements of dissolved gas concentrations (CO_2 and in data set A and B also CH_4) in the stream water and in the atmosphere (Table 1). Additional measurements include water temperature and near-surface current velocity, which was measured at selected sites within the study reaches using acoustic or electromagnetic current meters. More details on sampling and instrumentation are provided in Appendix A.

The flux F ($\mathrm{mmol\,m^{-2}\,d^{-1}}$) of CO_2 (all data sets) and CH_4 (parts of data set A and B), was calculated from the observed rate of change of the mole fraction S ($\mathrm{ppm\,s^{-1}}$) of the respective gas in the chamber using (Campeau and Del Giorgio, 2014)

$$F = (S \cdot V/A) \cdot t_1 \cdot t_2, \tag{1}$$

where V is the chamber gas volume (m^3), A is the chamber area (m^2), $t_1 = 8.64 \times 10^4\,\mathrm{s\,d^{-1}}$ is the conversion factor from seconds to days, and t_2 is a conversion factor from mole fraction (ppm) to concentration ($\mathrm{mmol\,m^{-3}}$) at in situ temperature (T in K) and atmospheric pressure (p in Pa), according to the ideal gas law:

$$t_2 = p/(8.31\,\mathrm{J\,K^{-1}\,mole^{-1}} \cdot T) \cdot 1000. \tag{2}$$

The gas exchange velocity of the respective gas at in situ temperature k ($\mathrm{m\,d^{-1}}$) was estimated from measured fluxes as

$$k = F/(K_H \cdot (p^{\text{water}} - p^{\text{air}})), \tag{3}$$

using the partial pressure of CO_2 and CH_4 in the stream water (p^{water}) and in the atmosphere (p^{air}). The partial pressures were obtained by multiplication of the measured mole fraction by atmospheric pressure. K_H is the temperature-dependent Henry constant (mmol m^{-3} Pa^{-1}; Goldenfum, 2011). The in situ gas exchange velocities were converted to a standardized (independent of temperature and gas diffusivity) exchange velocity k_{600} using the Schmidt number dependence:

$$k_{600} = k \cdot (600/\text{Sc})^{-n}, \tag{4}$$

where the temperature-dependent Schmidt numbers (Sc) of both gases were estimated according to Goldenfum (2011). The Schmidt number exponent n describes the dependence of the gas exchange velocity of a particular gas on the diffusion coefficient of this gas in water. We used $n = 0.5$, which showed best agreement with measurements for wave-covered and turbulent water surfaces (Jähne and Haußecker, 1998).

2.2 Turbulence measurements in the lab

The flow fields under freely drifting and anchored chambers were measured using particle image velocimetry (PIV) in a 3 m long laboratory flume. The chamber type and geometry was identical to the chamber in data set C (Table 1). The flow field under the drifting chamber was measured for 50 repeated chamber runs (58 s cumulative velocity observations under the chamber) at a mean flow velocity of 0.10 $m\,s^{-1}$, the highest flow velocity that could be realized in the flume. Measurements under anchored chambers were performed for 90 s at a mean flow velocity of 0.10 $m\,s^{-1}$. Additional measurements were performed at reduced mean flow velocities of 0.08 and 0.06 $m\,s^{-1}$. As a reference, the undisturbed flow field without chambers was measured for 90 s. Due to the limited length of the laboratory flume it was not possible to measure gas fluxes or estimate the gas exchange velocities.

The flow fields were analyzed by illuminating neutrally buoyant seeding particles (diameter of 20 µm, polyethylene) within a thin light sheet produced by a double-pulse laser (DualPower 200-15, DantecDynamics) with 5 ms between pulses. The sampling frequency was 7.5 Hz. Images were recorded in a 145×145 mm^2 field of view with a charge-coupled device (CCD) camera (FlowSense 4M MKII, 2048×2048 pixels, DantecDynamics). The camera was inclined by 30° to the horizontal, which allowed flow velocities below the chamber to be observed.

The two-dimensional (longitudinal and vertical) flow velocities within the field of view were estimated using an adaptive correlation algorithm (Dynamic Studio, DantecDynamics) with a final spatial resolution of 2.6×2.6 mm^2. The longitudinal extent of the observed flow fields (433 mm for anchored and 395 mm for drifting chambers) covered the complete chamber diameter and velocities are reported as a function of distance from the leading chamber edge in both the anchored and the drifting deployment.

The turbulent kinetic energy (TKE) was estimated by assuming isotropy in the unresolved velocity component to be

$$\text{TKE} = \frac{3}{4}\overline{u'^2 + w'^2}, \tag{5}$$

where u' and w' denote the temporal fluctuations of the longitudinal and vertical velocity component, respectively, and the overbar denotes temporal averaging.

2.3 Statistics

The mean fluxes measured with anchored and drifting chambers in the respective field data sets were compared using paired t tests, comparisons between the data sets were performed using two-sample t tests. Spearman rank correlation coefficients (r_S) were estimated when testing for correlations between gas exchange velocities from anchored and drifting chambers for each data set. All analyses were performed at a significance level $p < 0.05$, unless stated otherwise.

3 Results

3.1 Drifting vs. anchored chamber measurements in the field

In all measurements, the CO_2 and CH_4 fluxes were positive, i.e., the streams were sources of both gases to the atmosphere. While the mean CO_2 fluxes measured by drifting chambers did not differ significantly among the data sets B and C, they were about 7-fold higher in data set A (Table 2). In all data sets, anchored chamber fluxes were significantly higher than the corresponding drifting chamber fluxes.

Gas exchange velocities k_{600} estimated from CO_2 measurements in the drifting chamber deployments ($k_{600\_CO_2\_d}$) ranged between 0.2 and 8.1 $m\,d^{-1}$. They varied widely within each data set (Table 2), but in contrast to the current velocities mean values of $k_{600\_CO_2\_d}$ did not significantly differ among the data sets. In all data sets, however, k_{600} from anchored chambers ($k_{600\_CO_2\_a}$) differed significantly from that of drifting chambers (Fig. 1a). Except for data set A, both were weakly correlated to each other ($r_S = 0.49$, $p = 0.01$, $r_S = 0.76$, and $p < 0.001$ for data set B and C, respectively) (Fig. 1b). With only a few exceptions, the gas exchange velocities under anchored chambers were higher than those under drifting chambers with individual measurements, $k_{600\_CO_2\_a}$ being up to 20 times higher than $k_{600\_CO_2\_d}$. The average ratio of both velocities was 2.2, 6.2, and 4.0 for data set A, B, and C, respectively (Table 2).

When both gases were measured, the gas exchange velocities estimated from CO_2 fluxes were strongly correlated to

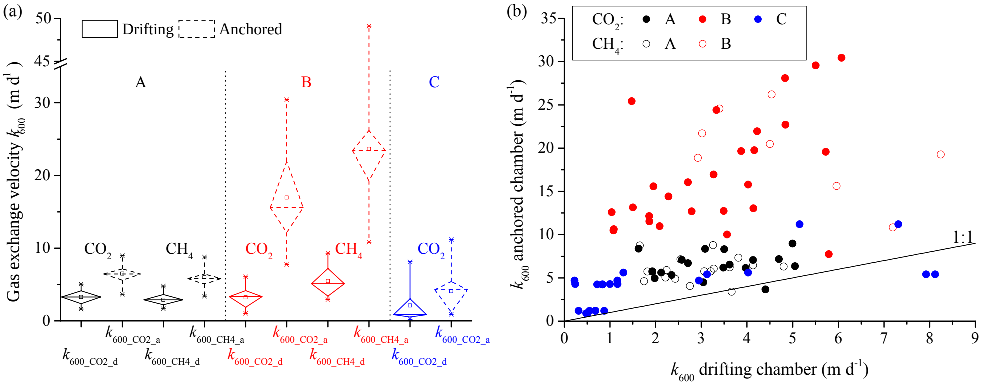

Figure 1. (a) Box plots of the standardized gas exchange (k_{600}) velocity measured using drifting (solid lines) and anchored (dashed lines) flux chambers in data set A (black), B (red), and C (blue). The diamond-shaped boxes encompass the 25–75 percentile range, whiskers show minimum and maximum, and open squares and horizontal lines mark mean and median values, respectively. **(b)** k_{600} estimated from anchored chamber deployments versus that from drifting chambers for the data sets A–C (see color code in the inset.). Filled symbols show k_{600} estimated from CO_2 fluxes; open symbols are based on CH_4 fluxes. The solid line shows a 1 : 1 relationship.

Table 2. Discharge rate, flow velocities, gas fluxes (F_{CO_2}, F_{CH_4}), and gas exchange velocities ($k_{600\_CO_2}$, $k_{600\_CH_4}$) estimated from drifting chambers (subscript d) and from anchored (subscript a) chambers during the three field campaigns (A–C, cf. Table 1). Except for discharge, all values are given as mean ± standard deviation.

Data set	A	B	C
No. of samples n	$n_{CO_2} = 18$ $n_{CH_4} = 18$	$n_{CO_2} = 27$ $n_{CH_4} = 9$	$n_{CO_2} = 24$ $n_{CH_4} = 0$
Discharge (m^3 s^{-1})	0.6–1.4	7.7–12.8	0.1–7.6
Flow velocity (m s^{-1})	0.21 ± 0.07	0.60 ± 0.12	0.30 ± 0.07
$F_{CO_2\_a}$ (mmol m^{-2} day^{-1})	742 ± 282	302 ± 148	103 ± 47
$F_{CO_2\_d}$ (mmol m^{-2} day^{-1})	363 ± 139	55 ± 30	49 ± 36
$k_{600\_CO_2\_a}$ (m day^{-1})	6.5 ± 1.4	17 ± 6.4	4.1 ± 2.8
$k_{600\_CO_2\_d}$ (m day^{-1})	3.3 ± 1.1	3.2 ± 1.5	2.1 ± 2.5
$k_{600\_CO_2\_a}/k_{600\_CO_2\_d}$	2.2 ± 0.9	6.2 ± 3.2	4.0 ± 5.0
$F_{CH_4\_a}$ (mmol m^{-2} day^{-1})	4.31 ± 1.35	1.55 ± 0.71	–
$F_{CH_4\_d}$ (mmol m^{-2} day^{-1})	2.12 ± 0.86	0.37 ± 0.16	–
$k_{600\_CH_4\_a}$ (m day^{-1})	6.0 ± 1.4	23.0 ± 10.8	–
$k_{600\_CH_4\_d}$ (m day^{-1})	2.9 ± 0.9	5.5 ± 2.4	–
$k_{600\_CH_4\_a}/k_{600\_CH_4\_d}$	2.3 ± 1.0	4.8 ± 2.1	–

those estimated from CH_4 measurements for both deployment types. Small but significant differences were observed between $k_{600\_CO_2\_d}$ and $k_{600\_CH_4\_d}$, whereas the CO_2-based estimates were on average slightly higher in data set A and lower in data set B (Fig. 1a). In accordance with the CO_2-based estimates, k_{600} estimated from CH_4 was higher under anchored than under drifting chambers (Table 2), and the ratio k_{600_a}/k_{600_d} did not differ significantly between both gases.

When combining all data sets, there was no correlation between gas exchange velocities and the measured current velocity for drifting chambers for either CO_2 or CH_4 (Fig. 2a). However, for anchored chamber deployments, k_{600_a} was positively correlated to current speed in data set A ($r_S = 0.54$, $p = 0.02$) and B ($r_S = 0.7$, $p < 0.001$). The ratio of the gas exchange velocities estimated from both deployment types was positively correlated to current speed when all three data sets were combined ($r_S = 0.66$, $p < 0.001$), but no significant correlations were observed within the individual data sets (Fig. 2b).

3.2 Flow field and turbulence under chambers

The laboratory measurements revealed pronounced differences in the flow fields and turbulence under the anchored and drifting chambers. The mean longitudinal flow velocity was strongly reduced within the submerged part of the anchored chamber and increased below the submerged chamber edge. Recirculating eddies were formed under the leading (upstream) edge of the chamber (vector graphs of the mean velocity distributions are provided in Appendix B). These eddies detached and injected turbulence below the chamber (Fig. 3). The turbulent kinetic energy which was produced by the submerged edge of the anchored chambers increased with increasing current speed (Appendix B). Under the drifting chambers, the flow velocities were slightly enhanced below the submerged chamber edge, but no recirculating eddies were formed.

The penetration depth of the chamber edges varied with time as the chamber moved vertically on the rough water surface (see Appendix B for snapshots of instantaneous velocity distributions and chamber penetration). However, at the same flow velocity the average penetration depth of the anchored chamber was higher than that of the drifting chamber (Fig. 3).

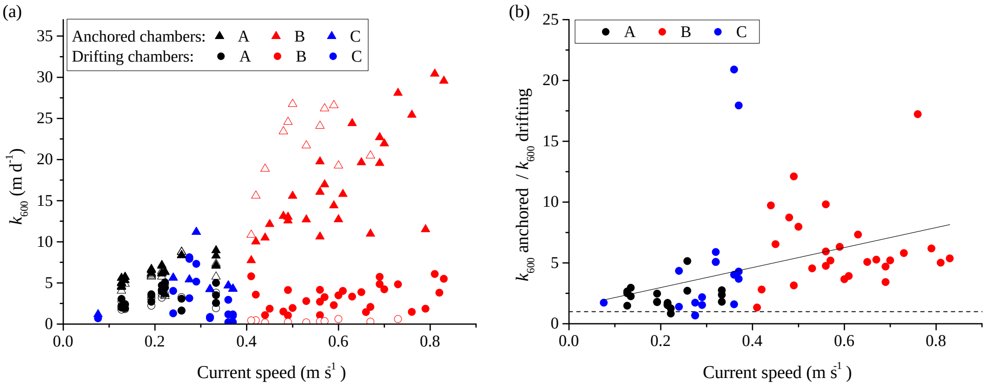

Figure 2. (a) Gas exchange velocity k_{600} from anchored (triangles) and drifting (circles) chambers versus current velocity for the three field data sets (A–C, colors). Filled symbols show data obtained from CO_2, open symbols are based on CH_4 fluxes. **(b)** Ratio of the gas exchange velocities from anchored and drifting chambers versus current speed (filled symbols: CO_2; open symbols: CH_4; colors correspond to the different data sets). The dashed line indicates a constant ratio of 1 and the solid line shows a linear regression of the combined data sets ($r_S = 0.66$, $p < 0.001$).

4 Discussion

4.1 Chamber bias in anchored deployments

Our field observations showed consistently higher gas exchange velocities and gas fluxes measured with anchored in comparison to freely drifting chambers in a variety of small streams with flow velocities between 0.08 and 0.8 m s^{-1}. Detailed observations of the flow field and turbulence under both types of chambers in the laboratory revealed a reduction of mean flow velocity and the generation of chamber-induced turbulence due to the shedding of eddies at the upstream part of the submerged edge of the anchored chamber. Under identical hydraulic conditions, anchored chambers penetrated deeper into the water, which we attribute to a partial diversion of the strong horizontal drag force imposed by the flow into the vertical direction. In combination, horizontal current shear and deeper penetration caused an increase in magnitude of chamber-induced turbulence with increasing difference in velocity between the water flow and the chamber (Fig. B1). This mechanism has been suggested in previous studies of floating chamber performance in water bodies, although there are mixed results regarding its importance (Cole et al., 2010; Gålfalk et al., 2013; Vachon et al., 2010).

The laboratory observation agrees with our field measurements, where the ratio of the fluxes measured with anchored and with drifting chambers was comparably small at flow velocities < 0.2 m s^{-1}. However, even at low flow velocities, the gas exchange velocity was enhanced by more than a factor of 2 in the anchored deployment. At higher flow velocities (> 0.2 m s^{-1}) typical for rivers and streams, chamber-induced turbulence obviously dominated the gas flux into the anchored chambers.

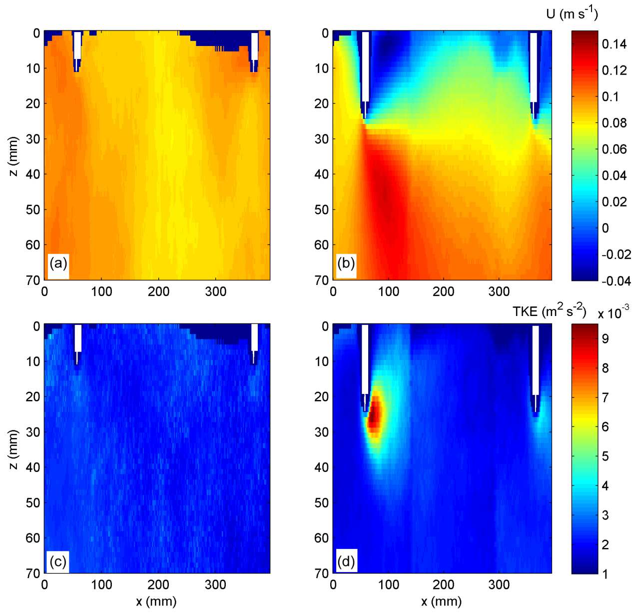

Figure 3. Laboratory measurements of the mean longitudinal flow velocities (U) **(a)** below a drifting chamber and **(b)** below an anchored chamber. Mean turbulent kinetic energy (TKE) of the flow fields below **(c)** the drifting chamber and **(d)** the anchored chamber. z and x refer to depth and longitudinal distance respectively. Chamber edges are blocked out (white) and regions without sufficient observations for temporal averaging are marked by a dark blue color. The flow direction is from left to right and the mean flow velocity was 0.1 m s^{-1}.

The large (several-fold) potential overestimation of fluxes measured with anchored chambers calls into question its suitability for application in running waters, particularly at high flow rates. This agrees with the observations of Teodoru et al. (2015) who reported a linear dependency of the gas ex-

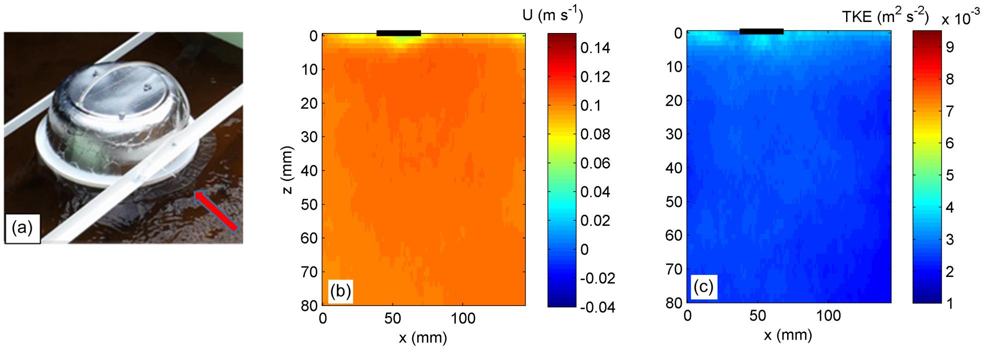

Figure 4. (a) Flying chamber design without penetration of the water surface by the chamber edges but using a plastic foil collar (marked by the red arrow) for sealing. The chamber is fixed above the water surface by a supporting frame. **(b)** Distribution of mean longitudinal flow velocities (U) and **(c)** turbulent kinetic energy (TKE) of the flow field below the front edge of a piece of static foil (marked by the black bar) at the water surface. The direction of flow was from left to right; x and y refer to longitudinal distance and depth, respectively. The mean flow velocity was $0.10\,\mathrm{m\,s^{-1}}$. Color scales are identical to that of Fig. 3.

change velocity under anchored chambers on the water velocity relative to the chamber in a large river.

4.2 Correction methods and chamber optimization

The correlation of the anchored chamber gas exchange velocity with flow velocity observed in our study could provide a potential means for correcting the artificial chamber flux, if the corresponding drifting chamber gas exchange velocity were also a function of flow velocity. However, no such correlation was present in our field observations, indicating that near-surface flow velocity is a poor predictor for the gas exchange velocities in streams. Therefore, it can be expected that river depth and bed roughness affect the near-surface turbulence more than flow velocity (Moog and Jirka, 1999; Raymond et al., 2012).

As the correction of the effects of chamber-induced turbulence on measured fluxes seems unlikely, it would be more reasonable to optimize the chamber design to completely avoid or to at least reduce this effect. The rectangular chamber B produced the largest error, although it remained unclear from our measurements whether this was caused by the geometry of the chamber or by the high flow velocity in data set B. On this basis, we recommend the use of more streamlined circular chambers to minimize the error under drifting conditions. Crawford et al. (2013) and McMahon and Dennehy (1999) used streamlined (canoe-shaped) instead of cylindrical or rectangular chambers to minimize the generation of chamber-induced turbulence at the upstream chamber edge during anchored chamber deployments. However, they did not provide evidence that this goal was reached.

Another approach to minimize the bias of anchored chambers would be to design chambers without submerged rigid walls. Submergence of the chamber edges can be avoided completely by using a piece of thin plastic foil which adheres to the water surface to seal the chamber headspace (Fig. 4a). Laboratory (PIV) measurements of the flow field were performed under a piece of foil, mimicking a chamber deployed in anchored mode. The measurements revealed a strong reduction of flow disturbances and chamber-induced turbulence (Fig. 4) in comparison to both anchored and drifting chambers. Such “flying” chambers require a frame to keep the chamber above the water surface, which can be supported by floats at a larger lateral distance to the chamber or, in small streams, also by a fixation at the river bank.

4.3 Implications for chamber-based flux measurements

Our study clearly shows that anchored chambers strongly overestimate the gas flux in running water and are not suited to quantify greenhouse gas fluxes in streams and rivers. One possible way forward to reduce this bias while still maintaining the practical advantages of the anchored chambers could be the use of “flying” (anchored) chambers with flexible foil sealing at the water surface. Drifting chambers provide a practical and reliable solution, although they are not free of potential spatial bias. Because their measurement locations are difficult to control, their trajectories may not be representative of the areal mean flux from the study reach. Regions with locally enhanced turbulence, e.g., stream reaches with large emerging roughness of the river bed, cannot be surveyed with drifting chambers; however the gas exchange velocity is highest at these sites (Moog and Jirka, 1999). Similarly, mean flow trajectories may bypass backwaters and regions of reduced flow velocity along the stream banks. Observations in reservoirs and river impoundments revealed that the enhanced sedimentation of particulate organic matter can make these zones emission hot spots (Maeck et al., 2013; DelSontro et al., 2011). Anchored chamber deployments may provide a useful extension of drifting chamber measurements at such sites, if the flow velocity is sufficiently small. To truly validate a reliable chamber method for small streams, a multi-method comparison study, including tracer additions, should be performed.

This study shows that flux chamber approaches to measure GHG fluxes from running waters have a high potential, given sufficient knowledge about appropriate chamber design and deployment approaches. Thus, flux chambers are emerging as an important method to constrain greenhouse gas fluxes from stream networks.

Appendix A: Additional information on the field data sets

A1 Data set A

Field measurements of five streams in the north-central European Plain in Germany and Poland were conducted during October 2014. Gaseous CO_2 and CH_4 emissions were measured at the water–air interface with a drifting chamber attached to an Ultraportable Greenhouse Gas Analyzer (UGGA; Los Gatos Research, Inc., USA). The chamber was connected to the UGGA placed in a boat via two gas-tight tubes (Tygon 2375), creating a circulation of air being sucked in and pumped out. For the anchored measurements, we tethered the chamber to a rack in the middle of the respective stream, in which we placed the sensors for continuously dissolved CO_2 and CH_4 measurements (HydroC™; CONTROS Systems & Solutions GmbH, Germany). Subsequently, we floated the same chamber down a predefined stream section following the boat freely at the speed of the current. During the chamber measurements, the UGGA continuously measured the gaseous CO_2 and CH_4 accumulation in the chamber (frequency 1 s). Flow velocity was measured with an Acoustic Digital Current meter (OTT, Germany).

A2 Data set B

Measurements were performed on the Bode River between Egeln-Nord and Staßfurt on 7 April 2014 (summer base flow $7.7\,m^3\,s^{-1}$) and 12 March 2015 (winter high flow $12.8\,m^3\,s^{-1}$).

The flux of CO_2 and CH_4 between water and the atmosphere was measured by a rectangular floating chamber, which was connected to an FTIR analyzer (GASMET 4010, Finland). Measurements were performed from a boat while it was drifting down the river. For a single measurement, the chamber was placed at the water surface for up to 5 min and CO_2 and CH_4 change inside the chamber was measured every 30 s. To compare drifting and fixed chamber measurements, the boat was then stopped by an anchor and measurements continued for another 3–5 min. During this stationary measurement, current velocity was measured with an electromagnetic current meter (MF-Pro, Ott, Germany) and water temperature were measured by handheld probes (ProfiLine Multi,WTW, Germany).

The concentration of CO_2 in the water was continuously measured by a submersible probe (HydroC™; CONTROS Systems & Solutions GmbH, Germany). Additionally, samples for CH_4 analysis were taken in plastic syringes and later analyzed by headspace gas chromatography.

Water temperature was continuously measured by temperature loggers (Tidbit, Onset, USA). The barometric pressure was recorded by the FTIR analyzer.

Under drifting conditions the CH_4 flux was often below the detection limit; while there was always a positive CH_4 flux in anchored chamber deployments.

A3 Data set C

Chambers with a cross-sectional area of $0.066\,m^2$ and volume of 6.8 L were covered by aluminum foil to reduce the internal heating and equipped with a Styrofoam material to keep the chamber body floating on water surface. The chambers were equipped with an internal CO_2 logger system that is positioned inside the headspace of the chamber (Bastviken et al., 2015). The non-dispersive infrared (NDIR) CO_2 logger (ELG, SenseAir, Sweden; www.senseair.se) measures CO_2 in the range of 0–5000 ppm. The logger measures simultaneously CO_2, temperature, and relative humidity, and operates at temperature and humidity of 0–50 °C and 0–99 % (non-condensing conditions) respectively. The loggers were calibrated by the manufacturer and operated with 9 V batteries. The measurement interval was adjusted to be 30 s; more information of technical specifications are provided elsewhere (Bastviken et al., 2015).

Chambers were deployed fixed at a certain position (anchored) and freely drifting. Triplicate measurements were conducted during each drifting run, and three runs were conducted at each site. The anchored chambers were then used for measuring the flux of CO_2 at different locations along the pathways of the drifting chambers. The chamber flux measurements were supplemented by measurements of dissolved gas CO_2 and CH_4 concentrations in the stream waters at each anchored stations for each run. Continuous measurements of CO_2 and methane in the middle of the stream were conducted using a membrane equilibrator (Liqui-Cel MiniModule, Membrana, USA) connected with an Ultraportable Greenhouse Gas Analyzer (UGGA; Los Gatos Research, Inc., USA). The water samples were pumped through the membrane contactor using a peristaltic pump at a constant flow rate.

Appendix B: Mean flow and turbulence under anchored chambers at different current speeds

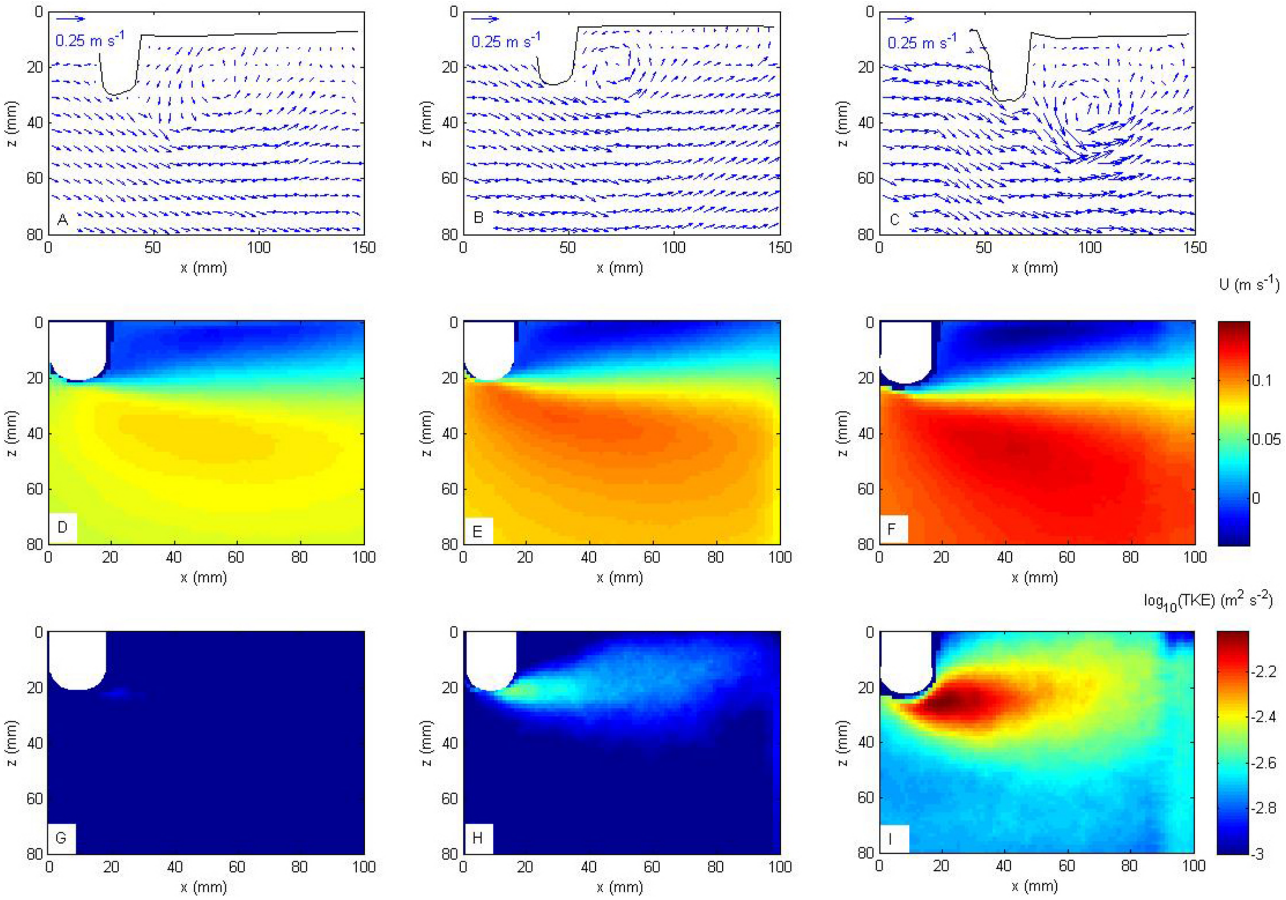

Figure B1. Laboratory measurements of flow velocity and turbulence under anchored chambers at different mean current speeds (left: 0.06 $\mathrm{m\,s^{-1}}$, middle: 0.08 $\mathrm{m\,s^{-1}}$, right: 0.10 $\mathrm{m\,s^{-1}}$. Panels **(a–c)** show examples of instantaneous velocities around the leading edge of the chambers. The water surface and the leading chamber edge are marked by solid black lines. **(d–f)** Temporal mean longitudinal flow velocity (U). **(g–i)** Mean turbulent kinetic energy (TKE). The chamber edges are masked out (white) and regions without sufficient observations ($<$ 90 s for the anchored cases) are displayed in dark blue. The direction of flow was from left to right; x and z refer to longitudinal distance and depth, respectively.

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MANUSCRIPT III

CARBON DYNAMICS AND THEIR LINK TO DISSOLVED ORGANIC MATTER QUALITY ACROSS CONTRASTING STREAM ECOSYSTEMS

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CARBON DYNAMICS AND THEIR LINK TO DISSOLVED ORGANIC MATTER QUALITY ACROSS CONTRASTING STREAM ECOSYSTEMS

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Abstract

Streams represent active components of the carbon cycle, with net global emissions of carbon dioxide (CO_2) and methane to the atmosphere. However, the mechanisms and governing factors of these emissions are still largely unknown, especially concerning the effect of land use. Thus, we compared dissolved and gaseous carbon dynamics in streams bordered by contrasting types of land use, specifically agriculture and forest. Carbon dioxide and methane partial pressures (pCO_2 and pCH_4, respectively) in the water body and carbon emissions were studied for 24 hours during four field expeditions. pCH_4 did not differ between the two systems. pCO_2 was constantly oversaturated and was significantly higher in agricultural streams (annual mean 4282 ppm) compared to forest streams (annual mean 2189 ppm) during all seasons. However, emissions of CO_2 were not significantly different between the stream types. This can be related to the higher hydraulic turbulence in forest streams, as the standardized gas transfer velocity was significantly higher in forest compared to agricultural streams. pCO_2 was significantly positively correlated to the concentrations of dissolved organic carbon, dissolved nitrogen and soluble reactive phosphorus in the water. Furthermore, pCO_2 was correlated to optical parameters of dissolved organic matter (DOM) quality, e.g., it increased with indicators of molecular size and the allochthonous component C2 identified by Parallel Factor Analysis (PARAFAC). This study demonstrates that different forms of land use may trigger a cascade of effects on the carbon production and emission of the investigated streams, involving changes in DOM quality.

Keywords: carbon dioxide, methane, drifting chamber, PARAFAC modeling, liquid chromatography with organic carbon detection (LC-OCD), land use

1 Introduction

Running waters, including rivers and streams, cover only 0.30–0.56% of the Earth’s land surface (Downing et al., 2012). However, streams form an important active component in the global carbon (C) cycle (Cole et al., 2007, Aufdenkampe et al., 2011, Bastviken et al., 2011) and have been identified as ‘hot spots’ for carbon dioxide (CO_2) and methane (CH_4) emissions (Striegl et al., 2012). Due to their length and small ratio of surface area to adjacent area, streams are tightly connected to their terrestrial environment. While receiving organic and inorganic carbon (OC and IC, respectively) from the terrestrial landscapes and passively transporting them to the oceans, streams are also actively processing organic carbon from allochthonous (i.e., originating from surrounding terrestrial areas) and autochthonous (i.e., released by plants within the water) sources (Pusch et al., 1998, Wanner et al., 2002, Cole et al., 2007, Battin et al., 2008, Butman and Raymond, 2011). During the aerobic respiration of OC, carbon dioxide (CO_2) is produced, which, together with the CO_2 entering streams with groundwater, leads to oversaturation of CO_2 in rivers and streams. The resulting CO_2 emissions into the atmosphere are globally estimated at 1.8 petagrams of carbon (Pg C) per year, which represents approximately 70% of the global CO_2 emissions from all inland waters (Raymond et al., 2013). In addition, streams and rivers often constitute substantial sources of methane (CH_4), an even stronger greenhouse gas (Bastviken et al., 2011). CH_4 emissions are not as well constrained as those of CO_2, but recent estimates show that rivers emit 1.5 Tg CH_4 per year (Bastviken et al., 2011). Despite the knowledge of these globally relevant numbers, investigations of the ecological mechanisms governing the emissions of these two gases across various stream and river ecosystems are rare (e.g. Shelley et al., 2015).

Organic carbon may enter aquatic ecosystems as dissolved organic carbon (DOC) or as particulate organic carbon (POC). Both components are metabolized microbially, but POC only becomes accessible to microbes after enzymatic cleavage into dissolved compounds (Pusch et al., 1998). Therefore, DOC represents the most important intermediate in carbon cycling (Battin et al., 2008) and is often positively correlated to CO_2 (Teodoru et al., 2009). DOC represents the main component of dissolved organic matter (DOM), which also includes other components, such as dissolved organic nitrogen or dissolved organic phosphorous.

It has been shown that the quantity and composition of DOM (hereafter referred to as DOM quality) in streams may be influenced by land use (Williams et al., 2010).With an increasing ratio of croplands to wetlands, the DOM is less humic and has lower molecular weight (Wilson and Xenopoulos, 2009). These effects of land use on the characteristics of riverine DOM should have important implications for carbon cycling in agricultural stream systems. That is because the rates of microbial carbon processing (and ensuing outgassing) depend, beside nutrient availability (Bernot et al., 2010) which is high in agricultural streams, on DOM quality (Wilson and Xenopoulos, 2009). Moreover, land use may influence areal carbon-budgets and especially gas emissions (Huotari et al., 2013), as streams are often supersaturated with carbon dioxide (Wallin et al., 2014) and methane (Campeau and Del Giorgio, 2014) with respect to the atmosphere. Investigations of the mechanisms that regulate the outgassing of carbon in streams such as Halbedel and Koschorreck (2013) are rare. Considering the quality of DOM and linking it to carbon emissions could contribute considerably to the understanding of carbon metabolism in aquatic systems.

The most promising approach to assessing the quality of an extensive set of DOM samples consists of the analysis of its optical properties, which can then be related to its chemical characteristics (Stedmon et al., 2003). Fluorescence measurements can thus provide information about the source, redox state and biological reactivity of DOM (Miller et al., 2009).

There are several indirect (Koprivnjak et al., 2010) and direct (Kling et al., 1991, Hope et al., 2001, Teodoru et al., 2009) approaches to measuring partial pressures of carbon dioxide (pCO_2) and methane (pCH_4) in the water. In recent years, direct and continuous measurements of pCO_2 (Johnson et al., 2010, Fietzek et al., 2014) and pCH_4 (Maeck et al., 2013) have also been used, but to our knowledge, the combination of direct and continuous measurements of pCO_2 and pCH_4 used in this study has never been applied to streams before.

To measure actual emissions fluxes, floating chambers measurements which allow a direct estimation of CO_2 and CH_4 emissions provide a powerful method. Floating chamber measurements have been criticized (Kremer et al., 2003), but comparisons with other flux measurement techniques revealed reasonable agreement (Cole et al., 2010, Gålfalk et al., 2013). We applied the floating chamber method in a drifting manner, since a recent study by Lorke et al. (2015) revealed that anchored floating chambers tend to provide overestimated fluxes.

The main objectives of this study were (1) to compare the seasonal dynamics of pCO_2 and pCH_4 and CO_2 and CH_4 emissions between streams characterized by different adjacent land use as forest and arable land (agriculture) and (2) to test for relationships between DOM quality and pCO_2 and pCH_4, respectively.

We hypothesize that land use triggers a cascade of effects on DOM quality, nutrient availability, organic carbon quantity and stream hydromorphology, resulting in clear differences in C partial pressures and C emissions.

2 Material and Methods

2.1 Study sites

During the course of the year 2013/2014, sections of six streams located in the North Central European Plains in Germany and Poland were investigated (Fig. 1). Three of them are bordered by coniferous forest, while the stream banks are lined with alders (hereafter referred to as forest stream type; F1-F3; Fig. 1b; Table 1; CORINE Land Cover (CLC2006); Federal Environment Agency, DLR-DFD 2009), the other three are bordered by non-irrigated arable land or pastures (hereafter referred to as agricultural stream type; A1-A3; Fig. 1a; Table 1; CORINE Land Cover (CLC2006); Federal Environment Agency, DLR-DFD 2009). All six streams have sandy sediments and similar hydro-geomorphological characteristics (Table 1). However, the two stream types differ with respect to their physico-chemical water characteristics (Table 1). In each stream, we chose a 65- to 220-m-long section that featured similar environmental characteristics without such disturbing factors as in- or outflow from or to other streams, beaver dams, or fallen trees. The influence of groundwater is comparable in the investigated systems, as indicated by measurements of the vertical hydraulic gradient (VHG), which is a dimensionless metric for which positive values indicating upwelling and negative values down welling conditions. The measurements showed a stable and continuous slight upwelling in all investigated systems over the year of investigation, with vertical hydraulic gradient values ranging from 0.009 to 0.274.

2.2. Sampling design

To study seasonal dynamics, we conducted four two-day field expeditions at each stream over an annual cycle in May (spring), July/August (summer), and October (autumn) 2013 and January/February (winter) 2014. Weather and hydrological conditions in each field expedition were stable and representative for the respective seasons. During each field expedition, we measured the following parameters in the water column continuously over 24 hours: partial pressure of carbon dioxide (pCO_2), partial pressure of methane (pCH_4), water temperature, pH, and dissolved oxygen. Additionally, we measured chamber-based carbon dioxide and methane emission (E_{CO2} and E_{CH4}) several times during the daylight. Furthermore, we took water samples (n = 4) at the downstream end of our pre-defined stream section approximately 10 cm below the water surface for the analysis of total phosphorous (TP), soluble reactive phosphorus (SRP), ammonium (NH_4^+), nitrate (NO_3^-), dissolved nitrogen (DN), dissolved inorganic carbon (DIC), total organic carbon (TOC), dissolved organic carbon (DOC), absorbance and fluorescence of dissolved organic matter (DOM), and size classes of DOC (high molecular weight and low molecular weight). During transport, we stored the samples in a cooling box filled with ice and transported them to the laboratory for further analyses. In the field, we filtered 500 - 1000 ml of stream water using pre-combusted (4 h, 500 °C) glass fiber filters (Whatman GF/F 0.7 µm; n = 4), for particulate organic matter (POM) determination. After the 24-hour measurement cycle, we took nine sediment cores along the stream section (inner diameter = 59 mm; Uwitec - Niederreiter Richard, Austria). We took the uppermost 2 cm of each core and pooled these in groups of three to produce three representative sediment samples for determination of organic matter content and carbon to nitrogen ratios. Sediment samples were cooled during transportation to the lab and stored at -20 °C before analysis.

2.3. Measurements and calculations

Hydrological parameters

In order to detect potential changes in discharge during the two-day field expeditions, discharge measurements were performed always at the beginning and the end of the expedition. During the spring and summer expeditions, discharge measurements were performed with a mini-propeller-based apparatus (Schiltknecht, Switzerland), and during autumn and winter, measurements were performed with an Acoustic Digital Current Meter (OTT, Germany). The two measuring methods are comparable, as a comparison revealed a deviation of approximately 10% maximum. Furthermore, we installed two piezometers (total 2 m length, 0.051 m diameter, 1 m in the sediment) equipped with pressure data loggers (AquiLite Beaver ATP10, AquiTronic, Germany) in close proximity in each investigated stream section, one to monitor the water level of the surface water and another one to monitor the groundwater level. With these two continuously measured parameters (frequency 1 h), we were able to calculate the vertical hydraulic gradient (VHG) over time, which indicates the direction and intensity of groundwater–stream water exchange. The VHG was calculated according to equation 1 (Baxter et al., 2003):

$$VHG = \frac{\Delta h}{\Delta l}, \tag{1}$$

where Δh is the difference in hydraulic head between the water level in the piezometer and the level of the stream surface (m) and Δl is the depth from the surface of the streambed to the first opening in the piezometer sidewall.

Carbon dioxide (pCO_2) and methane (pCH_4) partial pressure

We measured pCO_2 and pCH_4 using a carbon dioxide sensor (HydroC™) and a methane sensor (CH_4 HydroC™), respectively (Kongsberg Maritime Contros GmbH, Germany). The pCO_2 and pCH_4 sensors were factory calibrated before (pCO_2: December 2012; pCH_4: October 2012) and in the middle (pCO_2: July 2013) of the four measuring expedition over the annual cycle. Furthermore, the pCH_4 sensor was controlled in the middle (July 2013) of the four measuring expeditions over the annual cycle. All pCO_2 data were corrected for drift post-expedition by Kongsberg Maritime Contros GmbH. All pCH_4 data were corrected for linear offset and a discrete water sample post-expedition by Kongsberg Maritime Contros GmbH. For further details, measuring procedures, and drift correction, see Fietzek et al. (2014). The sensors were mounted on a frame to ensure a stable position and avoid direct contact with the sediment. They were placed in the main current at the downstream end of our pre-defined stream section and received their power supply from two car batteries per sensor (12 V, 70 Ah). We measured for 24 hours at a maximal measuring frequency of 10 s^{-1}.

Oxygen, pH and temperature measurements

In addition, we recorded water temperature, pH, and dissolved oxygen over 24 hours at one-minute intervals using a Yellow Springs Instruments monitoring probe (YSI; 6600 V2, Xylem Inc., Yellow Springs, USA).

Emission of carbon dioxide and methane

We measured gaseous CO_2 and CH_4 emissions (mmol m^{-2} d^{-1}) at the water-air interface with a drifting chamber attached to an Ultraportable Greenhouse Gas Analyzer (UGGA; Los Gatos Research, Inc., USA). The drifting chamber consisted of an inverted rain barrel (bottom area: 0.13 m^2; volume: 16.8 L) with attached foam elements to ensure flotation. The position of the edge of the drifting chamber was adjusted to 2 cm below the water level in order to separate the chamber from ambient air, while creating a minimum of artificial turbulence (Gålfalk et al., 2013). The chamber was painted white to prevent its heating up in sunshine.

The drifting chamber was connected to the UGGA placed in a boat via two 5-meter-long gas-tight tubes (Tygon 2375), creating a circulating gas loop between the chamber and the UGGA. As the boat floated down the stream section (3 to 10 minutes depending on the length of the stream section) with the drifting chamber following the boat freely at the speed of the current, the UGGA continuously measured the gaseous CO_2 and CH_4 accumulation in the chamber (frequency 1 s^{-1}). During the measurements, we avoided any disturbance that could create additional turbulence and consequently increase emissions of CO_2 and CH_4. Emissions were obtained from the slopes of the measured CO_2 and CH_4 curves, which were linear throughout all measurements, as expected for measurements performed at conditions far from equilibrium (McGinnis et al., 2015).

Calculations

We calculated the emission flux *E* (mol m^{-2} d^{-1}) of CO_2 and CH_4 according to equation 2 (Duc et al., 2013):

$$E = S * 10^{-6} \frac{PV}{RTA} * 60 * 60 * 24 , \tag{2}$$

where *S* is the slope (ppm s^{-1}), *P* is the atmospheric pressure (atm), *V* is the volume (mL) of the drifting chamber, *R* is the gas constant (82.0562 mL atm K^{-1} mol $^{-1}$), *T* is the temperature (K), *A* is the bottom area of the drifting chamber (m^2), and the last term is the conversion from seconds to days.

Subsequently, we used *E* to calculate a strictly diffusive gas transfer velocity (k) by inverting the equation for Fick's law of gas diffusion, as follows (equation 3):

$$k = \frac{E}{kH(\Delta pC)} , \tag{3}$$

where *k* is the gas transfer velocity in m d^{-1} specific for CO_2 or CH_4, *E* is taken from eq. (1), *kH* is Henry's constant (in mol L^{-1} atm^{-1}) adjusted for temperature (Goldenfum, 2010) and *ΔpC* is the difference between the partial pressure of CO_2 or CH_4 in the surface waters and in the atmosphere (µatm). To compare *k* among different streams, we standardized *k* of CO_2 and CH_4 measured at in-site temperature to k_{600} (equivalent to k of CO_2 at 20 °C) computed according to Jähne et al. (1987):

$$k_{600} = k \left(\frac{600}{S_c} \right)^{-0.5} , \tag{4}$$

where *k* is the calculated *k* (from eq. 2) at the in situ temperature (T) for CO_2 and CH_4, respectively, *Sc* is the Schmidt number for in situ temperature T for CO_2 or CH_4, and the Schmidt number for 20°C in freshwater is 600 (Jähne et al., 1987). The exponent of –0.5 should be appropriate for low-wind conditions (Jähne et al., 1987) like those encountered during our field expeditions. The Schmidt numbers for freshwater were calculated as a function of temperature (Wanninkhof, 1992).

Water analyses

A portion of the collected water samples was filtered through a pre-washed 0.45 µm membrane filter (cellulose acetate, Sartorius) for the analysis of SRP, DN, NO_3^-, NH_4^+, DIC and DOC. From the unfiltered homogenized fraction we measured TOC, TN and TP. To remove all inorganic carbon and prevent biological activity, samples for NO_3^-, NH_4^+, DOC and TOC were acidified with 2 N hydrochloric acid (HCl) to pH 2. For details of the specific analyses, see supplementary materials.

Size classes of DOC and dissolved organic nitrogen (DON)

We characterized DOC using liquid chromatography and organic carbon detection according to Huber et al. (2011). This size exclusion chromatography allows separate determination of three DOC size class fractions: humic substances (HS), high-molecular weight non humic substances (HMWS) and low molecular weight substances (LMWS). As the instrument is equipped with a nitrogen detector, these size class fractions are also available for DON.

Absorbance and fluorescence

We analyzed chemical characteristics of the optically active DOM fraction by absorbance and fluorescence analyses, which provide proxies for DOM source or biological availability, among others (Jaffé et al., 2008, Fellman et al., 2010). Absorbance was measured at room temperature with a 1 cm cuvette (Shimadzu UV-2401 UV/VIS spectrometer; Duisburg, Germany). We calculated the specific absorption at 254 nm (SUVA), which is an indicator for aromaticity (Weishaar et al., 2003), and the ratio E2:E3, which declines with increasing molecular size (Helms et al., 2008) and thus indicates DOM quality. Absorbance data were corrected for instrument baseline offset (Green and Blough, 1994). Fluorescence measurements (Perkin Elmer LS-50B fluorescence spectrometer, Rodgau, Germany) produced 92 excitation (ex) - emission (em) matrices, which were subjected to Parallel Factor Analysis (PARAFAC). PARAFAC is a multivariate three-way modeling technique that decomposes the fluorescence signal into individual components and provides estimates of the relative contribution of each component to the additively formed total signal (Bro, 1997, Stedmon and Bro, 2008). See supporting information for details of fluorometry and PARAFAC. The resulting three-component PARAFAC model consisted of components C1, C2, and C3. C1 (fluorescence maximum at ex: <240, 330-360, em: 440) is a previously reported humic-like, terrestrially derived, high molecular weight fluorophore (Stedmon and Markager, 2005, Fellman et al., 2010). C2 (ex: 255-275, (385-395) em: 480 – 515) was similar to published PARAFAC components resembling high molecular weight, humic-like, terrestrial material (Fellman et al., 2010) with increased aromatic carbon content, indicating higher plant material as a likely source (Cory and McKnight, 2005). However, C2 may also be the product of biological production and degradation (Ishii and Boyer, 2012). While C1 and C2 represent terrestrial components widespread in all environments, C3 (ex: <240, 280-310, em: 390 – 410) resembles a humic-like, terrestrial component associated with agriculture and wastewater (Cory and McKnight, 2005, Fellman et al., 2010). The PARAFAC components were expressed as relative fluorescence intensities contributing to the total fluorescence of the sample for all subsequent data analyses. In addition to PARAFAC, fluorescence measurement were also used to compute the humification index (HIX) following Ohno and Bro (2006), the fluorescence index (FI) following McKnight et al. (2001), which indicates more microbial (FI~1.9) or terrestrial and higher plant (FI~1.4) origin of DOM, and the β:α ratio (freshness index) following Parlanti et al. (2000), indicating the freshness of the material (>1: freshly produced and released to water, 0.6-0.8: more terrestrial input).

Particulate organic matter (POM) and sediment properties

We determined suspended POM concentrations (g L^{-1}) by freeze drying the filters with the adhered POM and subtracting the filter weight. Sampled sediment (ca. 50 ml) was freeze-dried, weighed (dry weight, DW), ashed at 550°C for 5 hours, and reweighed. The difference in sediment weight was recorded as organic matter (g OM g DW^{-1}).

2.4. Statistical analysis

In the case of univariate responses (pCO_2, pCH_4, CO_2 emission, CH_4 emission, $k_{600,CO2}$, $k_{600,CH4}$, discharge) we tested for effects of land use and season by a non-parametric ANOVA (F1-LD-F1 design), which is robust to outliers and exhibits competitive performance for small sample sizes (Brunner et al., 2002). Further testing of controls on pCO_2 and pCH_4 were performed by bivariate linear regression analyses using selected nutrient concentrations, OC quantity and DOM quality descriptors as individual predictors. Residuals of regression models were tested for deviation from normal distribution by the Shapiro-Wilk test. Relationships between $k_{600,CO2}$ and discharge were explored using simple bivariate correlation analysis.

Principal component analysis (PCA) was used to condense multivariate information on nutrient concentrations, quantitative and qualitative organic carbon data to a few principal components. PCA was run separately for (i) nutrients (based on the variables TN, DN, TP, SRP, NO_3^-, DON, and N content of the sediment), (ii) organic carbon (OC) quantity (DOC, TOC, organic matter content, particulate organic matter, and C content of the sediment), and (iii) DOM quality (PARAFAC components C1, C2, and C3, HMWS (in %), HS (in%), LMWS (in %), SUVA, HIX, FI, freshness index, E2:E3, and C to N ratio in the water). All concentration data were log-transformed prior to PCA, i.e., all variables describing nutrient concentrations and OC quantity. The PCAs served two purposes: a) to represent the variation across stream types and seasons in figures and b) to produce "meta-variables", i.e., principal components, may carry the information of several measurement variables and are suitable for further use in multiple linear regression analyses due to little collinearity within each PCA. We complement PCA biplots with a permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001, McArdle and Anderson, 2001) based on Euclidean distances among sites computed from standardized variables that were transformed identically to the PCAs. Three PERMANOVAs were set up as two-way designs with an interaction term (dropped from the model if insignificant) to test for effects of land use and season on (i) nutrients, (ii) OC quantity and (iii) DOM quality. PERMANOVAs were accompanied by tests of homogeneity of dispersion by permutation (Anderson, 2006).

Controls on pCO_2 were also explored in a multivariate context by using the most important principal components from the three PCAs on nutrients, OC quantity, and DOM quality in a multiple linear regression context. Important principal components were chosen based on the Kaiser-Guttmann criterion with eigenvalues > 1 (Quinn and Keough, 2002); these were the first 2, 3 and 4 from the PCAs on nutrients, OC quantity and DOM quality, respectively. Further, we included the gas exchange velocity k as a proxy for physical controls, i.e., outgassing in the various streams that may happen upstream to the sampling location in the stream and that may perturb the relationships of pCO_2 to biological/biogeochemical controls. Using hierarchical partitioning (Chevan and Sutherland, 1991), we considered a maximum of 9 predictors as controls on log-transformed pCO_2 in all possible purely additive multiple linear regression models. This exhaustive model building procedure partitions R^2 to determine the proportion of variance explained independently and jointly by each variable. It is designed to overcome problems of collinearity and non-identification of important predictors, and it identifies predictors strongly and independently correlating with the response, in contrast to those suffering from collinearity and thus sharing explanatory power with other predictors. Finally, predictors with high independent explanatory power were analyzed in bivariate regressions with pCO_2.

All statistical analyses were performed with R version 3.1.1 (R-Development-Core-Team, 2010) using the packages *MASS* (Venables and Ripley, 2002), *nparLD* (Noguchi et al., 2012), *vegan* (Oksanen et al., 2011) and *hier.part* (Walsh and Mac Nally, 2008). Figures were created in ArcGIS (version 10.0), MSOffice (2010) and R version 3.1.1 (R-Development-Core-Team, 2010).

3 Results

3.1 Nutrients, DOM quantity and quality

The PCA based on nutrients (Fig. 2a) summarizes nutrient conditions across stream types (agricultural and forest) and seasons; the first two PCA axes cover 82% of the total dataset variance. PCA axis 1, clearly the axis of main variation in the dataset with 62% of the total variance, strongly separates forested from agricultural streams; it was best defined by TN, DN, and DON as well as TP and SRP. Axis 2, in terms of variance explained (21%) a clearly minor PCA axis, separates seasons – especially winter and summer, which occupied opposite ends along this gradient – and was best defined by NO_3^- and the nitrogen content in the sediment. Subsequent PERMANOVA revealed nutrient conditions to be significantly affected by stream type (pseudo-$F_{1,19}$=16.4, P<0.001) and season (pseudo-$F_{3,19}$=2.4, P<0.05). An insignificant interaction term was dropped from the model.

A PCA based on OC quantity resulted in the identification of similar gradients as those from the nutrient data, albeit with slightly more variation within stream types and seasons and thus more overlap of the various groups in the PCA plot (Fig. 2b). PCA axis 1 covered 46% of the total variation and, again, separated stream types mainly based upon TOC and DOC concentration. Axis 2, in contrast, covered 32% of the total variance, separated seasons and was mostly driven by organic matter and carbon in the sediment and particulate organic matter in the water column. Subsequent PERMANOVA revealed significant effects of both stream type (pseudo-$F_{1,19}$=5.1, P<0.01) and season (pseudo-$F_{3,19}$=2.4, P<0.05); the interaction term was insignificant. In comparison to the multivariate analysis of nutrient data, more balanced variation covered by the first two axes and more similar F-values in the PERMANOVA point to roughly equal contribution of stream type and season as sources of variance of OC quantity.

In contrast to the data on nutrients and OC quantity, the DOM quality data set turned out to be more complex, and the PCA needed 4 dimensions to adequately represent the information (Fig. 2c + supplementary figure S1). PCA axis 1 and axis 2 explained 45% and 24% of the total variance, respectively. Axis 1 could not be obviously related to either stream type or season; it was best defined by the PARAFAC components C1 and C2, SUVA, HIX and freshness index. Axis 2 again separated seasons, and it was best defined by the PARAFAC component C2, the concentration of low molecular weight substances in the water column and the molecular size. A notable result from the first two PCA axes is the suggested higher variation of DOM quality among forested streams; these cover a larger area in the PCA plot compared to agricultural streams (Fig. 2c). PCA axes 3 and 4 (supplementary figure S1) further achieved some seasonal separation mainly based on measurements achieved by size exclusion chromatography (i.e., small vs. larger and humic-like molecules), yet stream types were not consistently different in this space of minor variation either. In agreement with the PCA results, PERMANOVA revealed a significant effect of season on DOM quality (pseudo-$F_{3,19}$=2.1, P<0.05), but no effect of stream type nor an interaction. However, stream types had significantly different dispersions (pseudo-$F_{1,18}$=7.5, P<0.05), confirming the increased variation of DOM quality among forest streams – or the higher homogeneity of DOM quality across agricultural streams – visible in the PCA plot (Fig. 2c).

3.2. Carbon dioxide (pCO_2) and methane (pCH_4) partial pressures in water and emissions

We found that pCO_2 was significantly higher in agricultural streams (nparLD, $F_{1,6}$ = 10.9, P = 0.001; Fig. 3a) and differed among seasons (nparLD, $F_{3,6}$ = 7.8, P < 0.001). pCH_4, however, was not significantly different between stream types (nparLD, $F_{1,6}$ < 0, P = 1.00; Fig. 3d) or among seasons (nparLD, $F_{3,6}$ = 1.7, P = 0.18).

In contrast to concentrations, neither emissions of CO_2 nor emissions of CH_4 differed significantly between stream types (P= 0.30, 3b; and P = 0.40, for E_{CO2} and E_{CH4}, respectively; Fig. 3b,3e) nor among seasons (P= 0.11 and P= 0.36 for E_{CO2} and E_{CH4}, respectively). This result indicates higher gas exchange efficiency in forest streams and also differences among seasons. Indeed, the standardized gas transfer velocity (k_{600}) computed from CO_2 data differed significantly between stream types (nparLD, $F_{1,6}$ = 14.9, P < 0.001; Fig. 3c) and among seasons (nparLD, $F_{3,6}$ = 3.8, P

=0.03). Gas transfer velocity is known to correlate with discharge, as a measure of stream size, when assessed over large ranges (Melching and Flores, 1999, Raymond et al., 2012), and we therefore checked for confounding of these results by discharge. k_{600} correlated only weakly and insignificantly with stream size (as log(discharge), Pearson´s (R=0.40, P=0.08) in our dataset. However, agricultural streams turned out to have on average lower discharge than the forest streams (nparLD, $F_{1,6} = 4.8$, $P = 0.03$), suggesting some possibility for confounding of stream type with stream size (and gas exchange efficiency) and limiting the meaning of the identified differences in CO_2 concentrations among the two stream types. A similar, although weaker, covariance of discharge was found with seasons (nparLD, $F_{3,6} = 9.4$, $P < 0.01$; data not shown), and spring especially had relatively high discharge across all streams. We thus have to conclude that – when assessed using two-way ANOVAs and targeting effects of stream type and season – effects on pCO_2 by stream type and season may be partly mediated by discharge differences. This result also points towards the necessity to include a proxy for gas exchange when further exploring controls on pCO_2. For k_{600} computed from CH_4 data we could only detect an effect of stream type ($P < 0.001$; Fig. 3f) and not of season ($P = 0.24$).

3.3. Potential influencing factors on pCO_2

To further explore the influence of nutrients, OC quantity and DOM quality on pCO_2, we conducted hierarchical partitioning (HP) using all important axes from the three PCAs. Based on the Kaiser-Guttman criterion (eigenvalues >1), we included the first 2, 3 and 4 principal components from the PCAs based on nutrients, OC quantity and DOM quality, respectively. Additionally and as a consequence of the previous analyses of pCO_2, we added k_{CO2} as an indicator for physical processes, i.e., as a proxy for gas exchange occurring in the upstream reach and perturbing the imprint of metabolic processes on pCO_2. HP identified PC1 of nutrients, PC1 of OC quantity, and k_{CO2} and PC2 of DOM quality as the most important predictors for CO_2 (Fig. 4). These 4 variables had the highest independent contributions, but all variables also had high joint contributions, indicating a high degree of colinearity.

As a last step, we performed single linear regressions with seasonal means of the highest structural coefficients (i.e., highest correlation of scores with original data) of the 3 most important PC axes (see above). We found a positive linear relationship between DOC and pCO_2 (ANOVA, $F_{1,20} = 27.6$, $r^2 = 0.58$, $P < 0.001$, Fig. 5a). Furthermore, we found positive linear relationships between pCO_2 and the nutrient parameters TP (ANOVA, $F_{1,20} = 27.5$, $r^2 = 0.58$, $P < 0.001$, Fig. 5b) and DN (ANOVA, $F_{1,20} = 22.4$, $r^2 = 0.53$, $P < 0.001$, Fig. 5c). Finally, we found a positive linear relationship between pCO_2 and the PARAFAC component C2 (ANOVA, $F_{1,20} = 13.5$, $r^2 = 0.40$, $P = 0.002$, Fig. 5d) and a negative linear relationship between pCO_2 and molecular size (ANOVA, $F_{1,20} = 15.5$, $r^2 = 0.43$, $P = 0.001$, Fig. 5e), both representative parameters for DOM quality. However, there was no significant relationship between pCO_2 and water temperature on a seasonal basis (ANOVA, $F_{1,20}$ = 1.6, r^2 = 0.03, *P*=0.22).

4. Discussion

In this study, we link aquatic pCO_2 to the large-scale controls of land use and season, but we only achieve this by relating pCO_2 to nutrient and carbon data used to indicate, or causally connected to, in-stream metabolic processes. Our study contributes to the ongoing discussion on streams as pipes for soil-derived CO_2 (e.g. Johnson et al., 2008) vs. streams as landscape bioreactors metabolizing terrestrially derived organic matter (e.g. Fasching et al., 2014). We argue that a careful selection and analysis of causally interpretable controls on pCO_2 may greatly contribute to an increased understanding of the complex interplay of factors influencing pCO_2 in stream ecosystems. The links between $pCO_{2,}$ DOM quality, OC quantity and dissolved nutrients discovered in the streams in this study point towards the importance of in-stream metabolic processes driving CO_2 concentrations, especially respiration as a source of CO_2. We cannot rule out, however, that some of the discovered relationships, e.g., between pCO_2 and DOM quality indicators, are driven by covariance of several variables on the basis of a common hydrological source in soils or intruding groundwater.

4.1. Land use effect and seasonality on carbon dynamics

Previous studies examining patterns of pCO_2 and CO_2-emissions (E_{CO2}) have mostly been performed in forest catchments (Teodoru et al., 2009, Campeau and Del Giorgio, 2014), despite the fact that approximately 40% of the Earth's ice-free terrestrial surface is covered by cropland or pasture (Foley et al., 2005). We are not aware of studies explicitly investigating CO_2 levels or dynamics in agricultural streams. Although some studies report seasonal or diurnal dynamics of CO_2 concentrations (Johnson et al., 2010, Dinsmore et al., 2013, Peter et al., 2014), most scaled-up emission estimates are based on CO_2 samples taken at unknown times or during the day, thereby biasing emission estimated for larger spatial scales (Raymond et al., 2013). Most studies on CO_2 emissions are biogeochemically motivated and focus on emission flux descriptions and physical controls rather than ecological mechanisms. CO_2 emissions are often reported as positively correlated to pCO_2, which, however, is partly a consequence of computing emission fluxes by simply multiplying oversaturation by an estimate for gas transfer velocity (Halbedel and Koschorreck, 2013). Across longer gradients of stream size, CO_2 concentrations and gas transfer velocities are in general positively correlated. Small streams receive spring and groundwater commonly supersaturated with CO_2, i.e., carrying a CO_2 legacy from respiration in underground and soil environments, as such shallow streams with high turbulence result in high gas exchange efficiency. Other studies showed that turbulence strongly influences the transport coefficient *k*, which in turn influences emission rates (Jahne and Haussecker, 1998, Hope et al., 2001).

In this study, we independently estimated CO_2 concentration and emissions. We found higher CO_2 concentrations in the agricultural streams, but not higher emissions due to concomitantly decreasing gas transfer velocities (Fig. 3c). Forest streams in our study were characterized by an increased abundance of coarse woody debris (author's observation), which generated rapids with higher levels of turbulence. This result underlines to the complexity of controls on CO_2 emissions, as higher CO_2 concentrations do not necessarily lead to higher emission fluxes. Further, this result points to difficulties in using either pCO_2 or CO_2 emissions as proxies for in-stream metabolic performance, unless differences in gas transfer velocities are simultaneously accounted for.

Previously, land use gradients have often been exploited for studies of aquatic metabolism based on diurnal oxygen dynamics (Bernot et al., 2010). Changes in catchment land use and riparian vegetation alter light availability and organic matter supply, which are fundamental factors controlling organic matter production and respiration in streams (Young and Huryn, 1999). Furthermore, Bernot et al. (2010) shows a direct effect of land use on GPP as well as SRP and DIN, whereas the organic matter affects ER. In our study, the agricultural streams showed a stronger diurnal dynamic of pCO_2 compared to forest streams, probably related to the changed light availability (supplementary figure S2). This finding, combined with the fact that the emission measurements were taken in a diurnal schedule, leads to a high standard deviation of the measured emissions (Fig. 3b). This fact may also lead to emission rates being not significantly different between forest and agricultural streams.

However, for instance, Halbedel et al. (2013) studied the temporal and spatial variability of DOM in concert with whole stream metabolism in forest streams (almost exclusively surrounded by trees) and non-forest streams (surrounded by open space with little or no vegetation). Using the two-station oxygen change technique and focusing on light regime effects, these authors identified forest streams as more heterotrophic than non-forest streams based on ratios of primary production to respiration . However, in the catchments of the investigated non-forest streams, only 6% and 14% of the area was used by agriculture; therefore, their results are consequently not directly comparable to our study.

Consistent with previous findings that agricultural land use usually increases the delivery of nutrients such as nitrogen or phosphorus to fluvial ecosystems (Carpenter et al., 1998), our results revealed a higher amount of dissolved nutrients in agricultural streams compared to forest streams. The high availability of nutrients in agricultural characterized stream ecosystems may facilitates the in-stream degradation of organic matter, resulting in a high amount of dissolved CO_2 in the investigated agricultural streams. In contrast aquatic photosynthesis can also be stimulated in streams dominated by agriculture and nitrate input, which consequently reduces pCO_2 levels (Wang et al., 2007). For instance, Wang et al. (2007) showed that dissolved CO_2 in the Changjiang River revealed a declining trend since the 1960s, attributed to an increase in the trophic status of its drainage basin. These findings could be explained by the dimension of the River Changjiang compared to our investigated streams. It is the largest river on the Euro-Asian continent, while we investigated small streams with much higher terrestrial connectivity, where the potential effect of autochthonous primary production on the overall stream metabolism remains much lower.

In a global context across countries and climate zones, the annual mean of pCO_2 of the forest streams studied here lies within the range of the temperate climate zone (Table 2). The annual pCO_2 mean of the agricultural streams lies higher, and corresponds to the range of streams within the subtropical/tropical climate zone (Richey et al., 2002, Alin et al., 2011, Wang et al., 2011). This is a remarkable outcome because all streams investigated in this study are located in a radius less than 200 km. Consequently, this finding may underlines the influence of bordering land use on the variability of C turnover in streams.

4.2. Carbon quality, quantity and nutrient effects on C dynamics

In order to increase the understanding of the above discussed adjacent land use and season, we investigated small-scale mechanisms such as the influence of temperature, nutrients, OC quantity and DOM quality on C dynamics.

Our study shows that neither pCO_2 nor pCH_4 are influenced by seasonal water temperature. However, an influence of water temperature alone on pCO_2 has previously been a matter of controversy (e.g. Demars et al., 2011, Campeau and Del Giorgio, 2014). The differing findings in the available studies indicate that carbon dynamics in running water are governed by several major factors that may not yet have been fully recognized. Additionally, the variation in results may have been increased by the use of different approaches such as day and night measurements, a higher number of investigated streams, or the interaction of temperature variation with other seasonal changes. In contradiction to our results, a strong temperature dependence of pCH_4 has been previously suggested for 46 streams and rivers in two distinct boreal landscapes of Northern Québec (Canada), where the winter conditions are much harsher compared to Northern Germany (Campeau and Del Giorgio, 2014). In general, we could not find correlating abiotic factors in our investigated streams that substantially control stream methane emission or concentration. As such, we will not discuss this part in more detail.

As shown previously, pCO_2 in streams is influenced by several factors. A review of Guenet et al. (2010) discussed the differentiation between labile and recalcitrant organic matter (i.e., different qualities in respect of bioavailability) and the consequences of their interaction with each other regarding mineralization intensities in aquatic systems. They state that inputs of labile organic matter frequently tend to increase the mineralization of the more recalcitrant organic matter, an effect called the priming effect. Furthermore, they suggest that recalcitrant organic matter may contribute substantially to the CO_2 emissions of aquatic ecosystems through the priming effect. These new insights into the influence of DOM quality on OC mineralization underline its importance in the C cycle. The quality of OM is highly variable in running waters, as it can be seen, for example, by a study of Holmes et al. (2008), who showed that there is a substantial seasonal variability in the lability of DOC (which is the main constituent of DOM in Alaskan rivers) transported by Alaskan rivers to the Arctic Ocean. Moreover, Williams et al. (2010) indicated a more labile and accessible DOM for the microbial community in agricultural streams than in wetland streams (Williams et al., 2010). In accordance with most other studies (Teodoru et al., 2009, Lapierre et al., 2013), we found a significant positive relationship between pCO_2 and DOC concentration. This can be explained by the high microbial bioavailability of DOC (Battin et al., 2008). Thus, DOC gets easily respired by microbes and results in enhanced pCO_2.

In spite of the hypothesized effect of bioavailability and priming, up to now, no study has shown a direct relationship between pCO_2 and DOM quality. In field-based studies like that described here, it is difficult to completely disentangle the effects of DOM quality from those of OC quantity. We can demonstrate the linkage between DOM quality and pCO_2 by showing the positive correlations between pCO_2 and molecular size of DOM and PARAFAC component C2, respectively (Fig. 5d, e). Our findings with respect to molecular size go along with the ones of Amon and Benner (1996), where aquatic respiration measurements indicated that high molecular weight DOC (i.e., large molecules) was utilized to a greater extent than low molecular weight DOC (i.e., small molecules). Consequently, they suggest a new size-reactivity continuum model where the bulk of HMW DOM is more bioreactive and less diagenetically altered than the bulk of LMW DOM (Amon and Benner, 1996). The component C2 has been previously associated with material derived from terrestrial plants (Cory and McKnight, 2005) but also with biological products and degradation of terrestrial precursor material (Ishii and Boyer, 2012). This finding is supported by the study of Ward et al. (2013) using gas chromatography time-of-flight mass spectrometry, showing that lignin and other terrestrially derived macromolecules contribute significantly to CO_2 outgassing from rivers. Furthermore, the fact that nutrients, DOM quality and OC quantity contribute to similar extents to the predictability of pCO_2 (Fig. 4) points clearly to a tight and complex interplay among these aspects. However, the importance of looking not only at quantity but also at quality is still in its infancy.

5 Conclusion

To our knowledge, this is the first study that directly compares measured dynamics of pCO_2 and pCH_4 and respective C emission in different agricultural and forest streams. Moreover, we demonstrate that continuous pCO_2 measurements coupled with multiple CO_2 emission measurements via drifting chambers provide a powerful combination to enhance the understanding of carbon dynamics in stream ecosystems (supplementary figure S3). We show that pCO_2 in streams is strongly influenced by the two investigated adjacent land uses and season. Furthermore, in addition to carbon quantity, we demonstrate that parameters of carbon quality are significantly linked to pCO_2. As such, our study sheds more light into the small-scale mechanistic links of carbon turnover in stream ecosystems and thus underlines the need of such knowledge to recognize and understand large-scale patterns of carbon dynamics in streams.

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Tables of Manuscript III

Table 1. (Continiueing to the next page) General, hydromorphological and physico-chemical information on the six investigated streams. Seasonal range of mean and standard deviation (SD) are given. Replicates: n = 4; F1-F3 = forest streams; A1-A3 = agricultural streams; stream width was measured at the downstream end of the investigated stream section; T = water temperature, DO = dissolved oxygen, TP = total phosphorus, SRP = soluble reactive phosphorus, NH_4^+ = ammonium, NO_3^- = nitrate, TOC = total organic carbon, DN = dissolved nitrogen, DIC = dissolved inorganic carbon, and DOC = dissolved organic carbon.

		F1	**F2**	**F3**	**A1**	**A2**	**A3**
General	**Coordinates**	52°34'25"N 14°6'12"E	53°1'7"N 12°54'23"E	52°16'27.4"N 14°43'51.9"E	53°6'55"N 14°2'33"E	53°20'32"N 13°42'56"E	52°10'38"N 14°18'14"E
	Catchment size above measured section (km^2)	134	244	423	414	95	64
	Stream order	2	4	2	3	2	2
Hydromorphology	**Stream width (m)**	7.7	8.8	13.0	6.5	3.3	3.9
	Mean stream depth (m)	0.268 ± 0.072 (0.163 - 0.316)	0.454 ± 0.034 (0.422 - 0.494)	0.519 ± 0.082 (0.418 - 0.607)	0.366 ± 0.072 (0.298 - 0.467)	0.346 ± 0.019 (0.324 - 0.360)	0.329 ± 0.063 (0.279 - 0.411)
	Discharge (m^{3}s^{-1})	0.530 ± 0.305 (0.217 - 0.942)	1.658 ± 0.439 (1.394 - 2.31)	2.048 ± 1.043 (1.359 - 3.601)	0.627 ± 0.108 (0.565 - 0.788)	0.184 ± 0.124 (0.059 - 0.309)	0.180 ± 0.056 (0.143 - 0.262)
	Flow velocity (m s^{-1})	0.258 ± 0.123 (0.173 - 0.44)	0.428 ± 0.121 (0.32 - 0.6)	0.289 ± 0.095 (0.219 - 0.430)	0.275 ± 0.113 (0.188 - 0.44)	0.126 ± 0.101 (0.055 - 0.242)	0.147 ± 0.050 (0.09 - 0.21)
Water parameters	**T (°C)**	13.1 ± 8.7 (1.9 - 22.3)	11.7 ± 7.6 (2.7 - 21.0)	11.5 ± 7.3 (1.9 - 19.4)	11.2 ± 7.8 (1.3 - 17.5)	9.4 ± 5.7 (1.1 - 13.6)	11.3 ± 8.5 (-0.6 - 19.7)
	pH	7.8 ± 0.1 (7.6 - 7.9)	7.7 ± 0.2 (7.5 - 8.0)	7.5 ± 0.2 (7.4 - 7.8)	7.7 ± 0.2 (7.5 - 7.9)	7.5 ± 0.3 (7.1 - 7.8)	7.2 ± 0.2 (7.0 - 7.3)

(continiueing from the previous page, table 1)

		F1	**F2**	**F3**	**A1**	**A2**	**A3**
Water parameters	**DO (mg L^{-1})**	8.89 ± 2.47 (6.56 - 12.38)	9.52 ± 2.05 (7.38 - 12.30)	8.54 ± 2.63 (5.52 - 11.90)	9.60 ± 1.90 (7.98 - 12.28)	7.90 ± 2.33 (5.70 - 11.15)	8.48 ± 1.59 (7.01 - 10.40)
	TP (µg L^{-1})	56 ± 19 (33 - 78)	49 ± 9 (40 - 62)	184 ± 93 (107 - 311)	184 ± 26 (152 - 212)	77 ± 17 (61 - 100)	253 ± 101 (174 - 387)
	SRP (µg L^{-1})	25 ± 9 (14 - 34)	13 ± 10 (4 - 25)	61 ± 25 (28 - 85)	35 ± 24 (10 - 67)	20 ± 13 (11 - 39)	165 ± 69 (104 - 242)
	NH4+ (mg L^{-1})	0.05 ± 0.01 (0.04 - 0.05)	0.04 ± 0.02 (0.01 - 0.06)	0.12 ± 0.15 (0.05 - 0.34)	0.23 ± 0.21 (0.03 - 0.52)	0.09 ± 0.08 (0.00 - 0.19)	1.00 ± 0.49 (0.35 - 1.49)
	NO3- (mg L^{-1})	0.14 ± 0.14 (0.03 - 0.35)	0.13 ± 0.05 (0.07 - 0.19)	0.40 ± 0.18 (0.14 - 0.50)	0.85 ± 0.35 (0.52 - 1.31)	1.81 ± 1.12 (0.69 - 2.95)	0.18 ± 0.03 (0.15 - 0.23)
	TOC (mg L^{-1})	6.2 ± 0.6 (5.7 - 7.0)	7.0 ± 0.2 (6.7 - 7.3)	8.6 ± 2.9 (5.9 - 12.4)	10.5 ± 1.3 (8.8 - 11.9)	9.9 ± 4.3 (5.4 - 15.5)	9.7 ± 2.0 (7.4 - 12.3)
	DN (mg L^{-1})	0.5 ± 0.1 (0.4 - 0.7)	0.6 ± 0.0 (0.5 - 0.6)	0.8 ± 0.2 (0.5 - 0.925)	1.4 ± 0.3 (1.0 - 1.7)	1.9 ± 1.0 (1.0 - 3.0)	1.9 ± 0.5 (1.1 - 2.3)
	DIC (mg L^{-1})	51 ± 3 (47 - 53)	32 ± 2 (29 - 35)	44 ± 4 (39 - 47)	59 ± 8 (50 - 67)	69 ± 7 (63 - 75)	29 ± 5 (23 - 35)
	DOC (mg L^{-1})	5.6 ± 0.4 (5.3 - 6.0)	5.8 ± 0.4 (5.4 - 6.3)	7.0 ± 2.2 (4.3 - 9.2)	8.3 ± 1.4 (6.5 - 9.8)	9.6 ± 4.5 (4.8 - 15.3)	9.0 ± 1.3 (7.2 - 10.0)

Table 2. Compilation of mean water pCO_2 values across different countries and climate zones.

Country	Region	Climate	Water pCO_2 Mean (µatm)	Reference
Northern Germany	North German Plain; forest area	Temperate	2189	This study
Northern Québec, Canada	Eastmain River region	Temperate	1858	Teodoru et al. 2009
Northern Québec, Canada	Abitibi, James Bay	Temperate	2959	Campeau and Del Giorgio 2014
Northern Germany	North German Plain; agricultural area	Temperate	4282	This study
Brazil	Amazon	Tropic	4350	Richey et al. 2002
China	Upper stream of Maotiao	Subtropic	3740	Wang et al. 2011
Brazil	Caixiuana	Tropic	4283	Alin et al. 2011
Brazil	Badeira Branca	Tropic	3918	Alin et al. 2011
Brazil	São José	Tropic	4451	Alin et al. 2011

Figures of Manuscript III

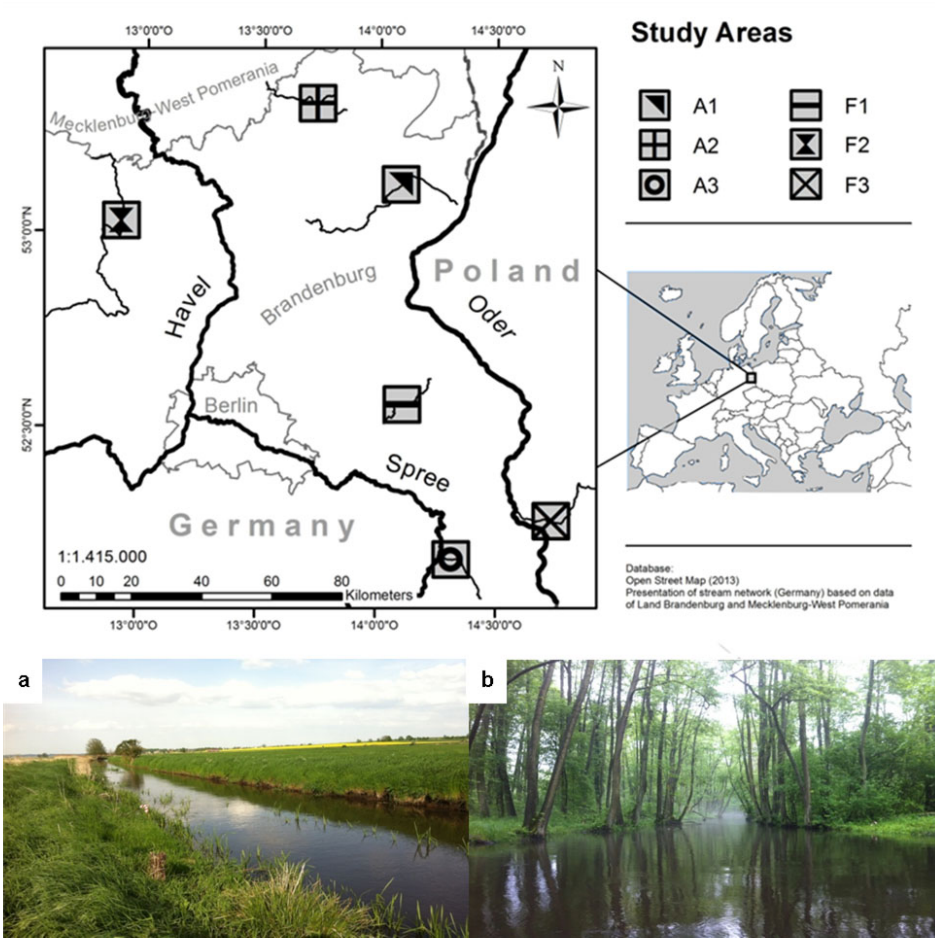

Figure 1. Up: Overview of investigated stream sections in North Germany and adjacent Poland; Forest streams (F1-F3: Rhin, Stöbber, Ilanka) and agricultural streams (A1-A3: Welse, Quillow, Oelse). Down: Examples of two investigated stream types (May 2013); a) agricultural stream (A1), b) forest stream (F3).

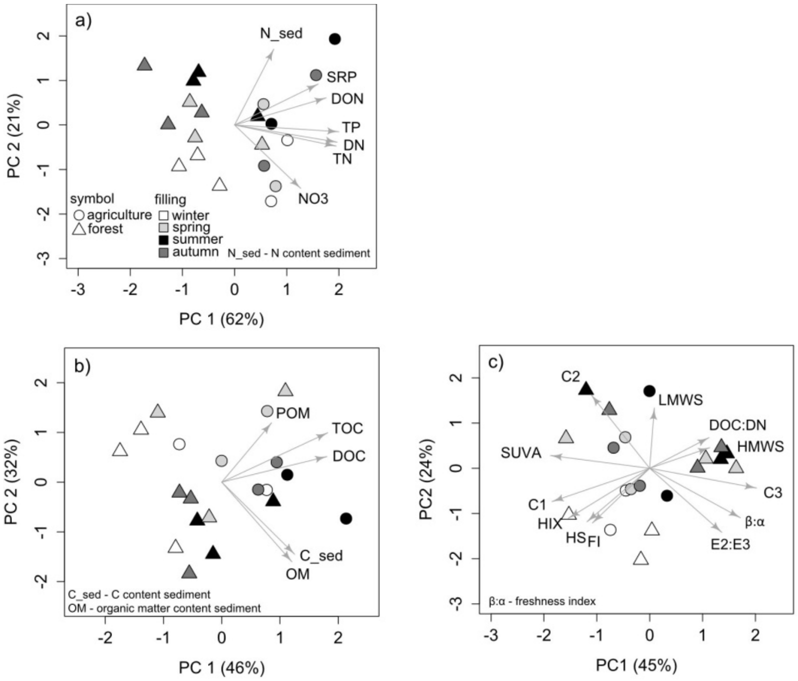

Figure 2. PCA biplots summarize conditions of (a) nutrient concentrations, (b) carbon quantity, and (c) DOM quality across all sites and seasons. PCAs are based on the variables (a) total nitrogen (TN), dissolved nitrogen (DN), soluble reactive phosphorous (SRP), nitrate (NO_3^-), dissolved organic nitrogen (DON) nitrogen content of the sediment; (b) dissolved organic carbon (DOC), total organic carbon (TOC), organic content of the sediment (OM), particulate organic matter (POM), carbon content of the sediment; and (c) PARAFAC components C1, C2 and C3, high molecular weight substances (HMWS), low molecular weight substances (LMWS), humic like substances (HS), specific absorption at 254 nm (SUVA), humification index (HI), fluorescence index (FI), freshness index β:α, and molecular size (E2:E3).

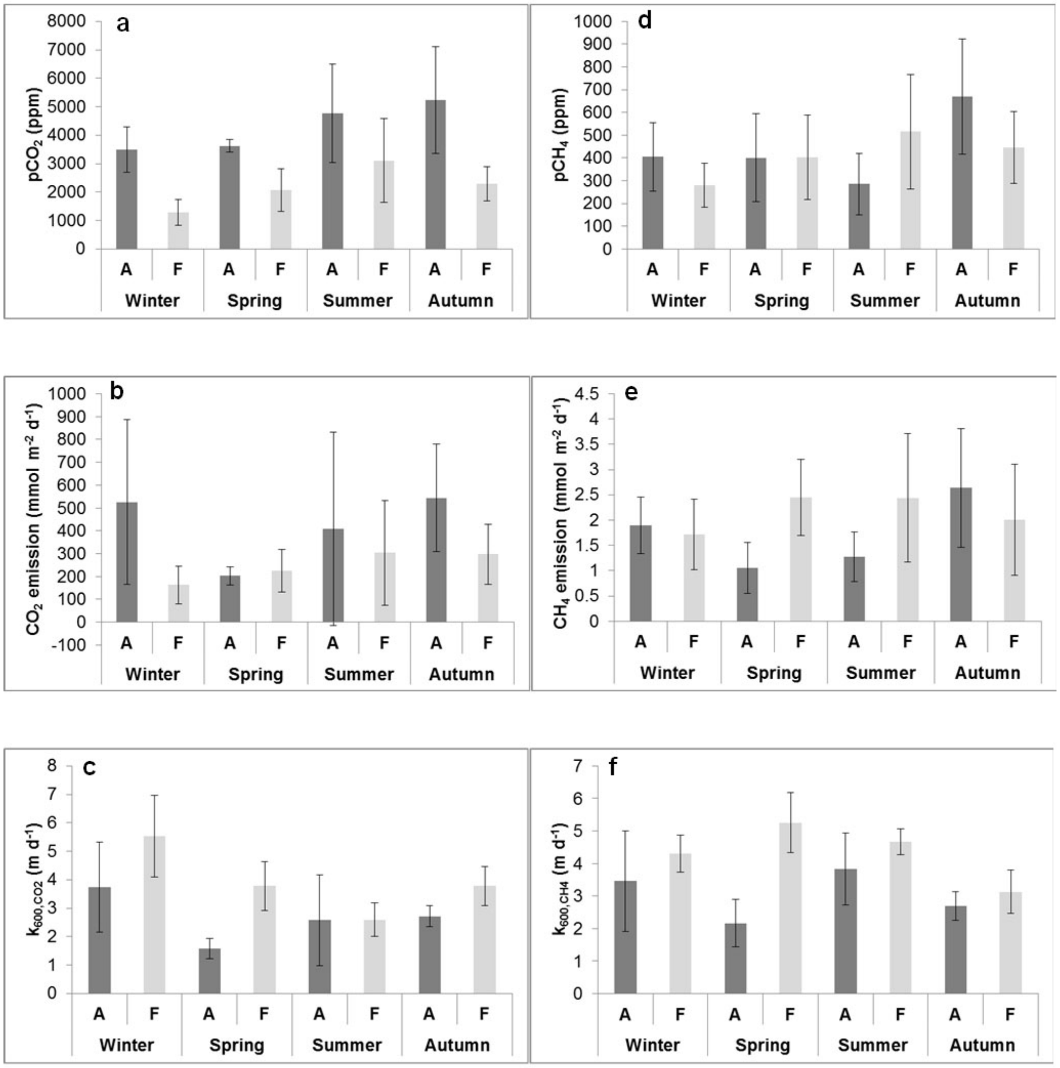

Figure 3. Mean ± 1SD of pCO$_2$ (a), CO$_2$ emissions (b), the standardized gas transfer velocity (k$_{600}$) of CO$_2$ (c), pCH$_4$ (d), CH$_4$ emissions (e), and the standardized gas transfer velocity (k$_{600}$) of CH$_4$ (f) over four seasons (winter, spring, summer and autumn) for agricultural (A) and forest (F) stream type (n = 3 per season and stream type).

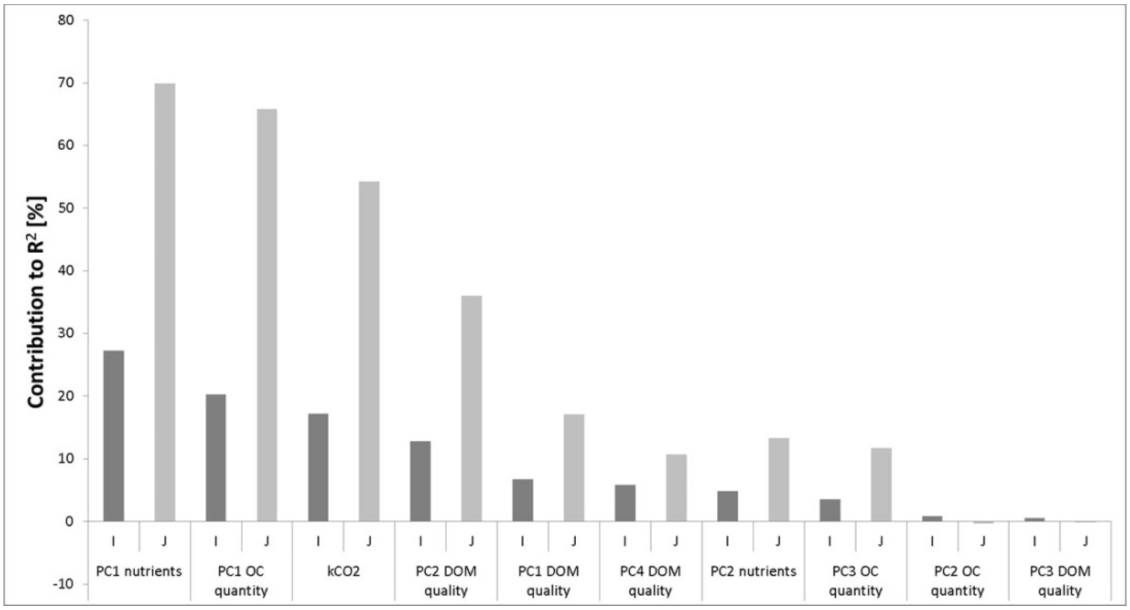

Figure 4. Independent (I) and joint contributions (J) of the 10 considered predictors for pCO_2 in exhaustive model building by hierarchical partitioning; predictors were ranked by their independent contributions. Joint contributions are contributions to R^2 shared with collinear predictors.

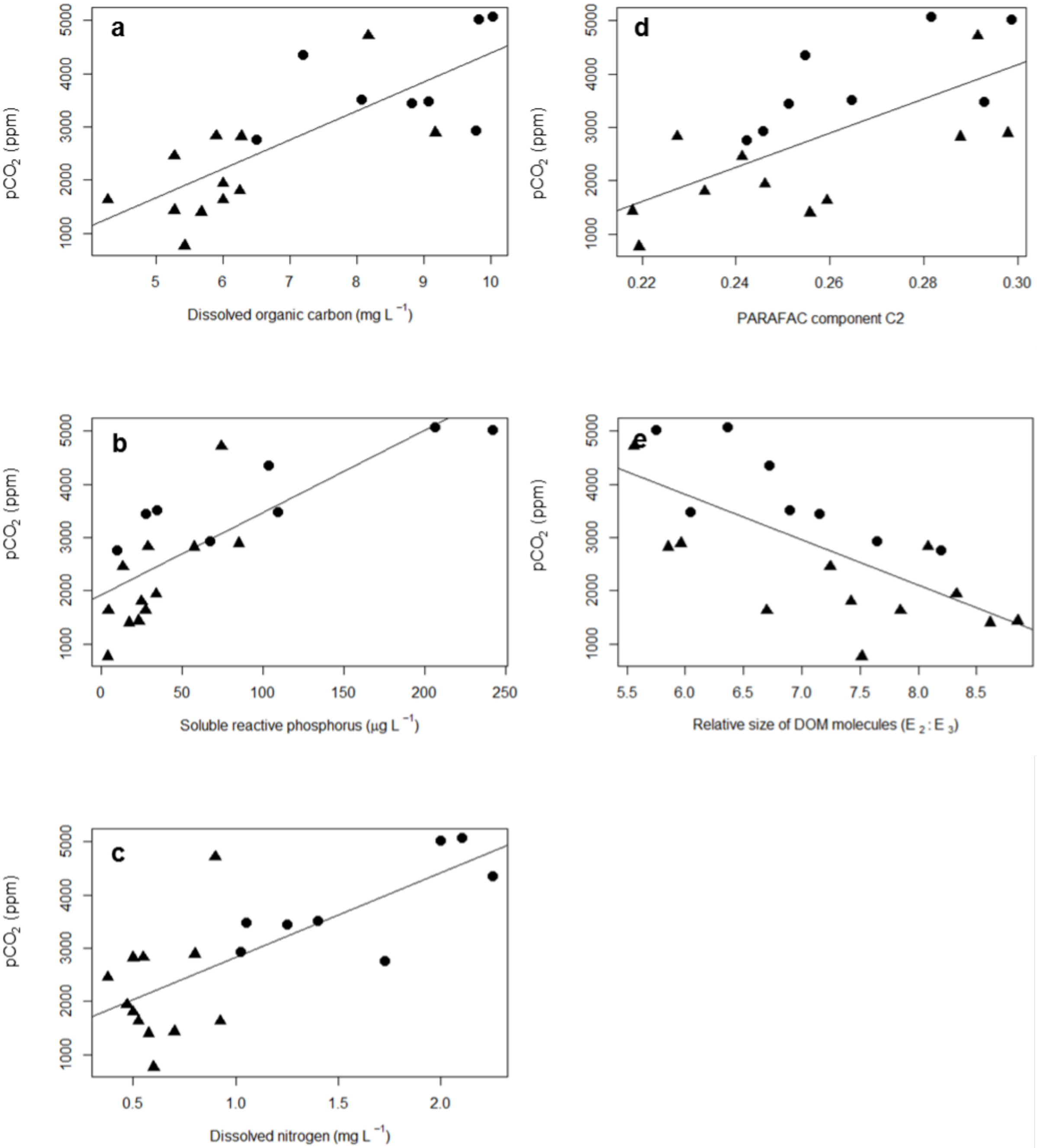

Figure 5. Single linear regressions of pCO$_2$ (ppm) against (a) dissolved organic carbon (mg L^{-1}), (b) total phosphorous (µg L^{-1}), (c) dissolved nitrogen (mg L^{-1}), (d) PARAFAC component C2 and (e) the relative size of DOM molecules (E2:E3); circles: agricultural streams, triangles: forest streams.

SUPPLEMENTARY MATERIALS OF MANUSCRIPT III

Supplementary Methods

Water analyses: The samples were analyzed in duplicates on a multi N/C 3100 Analyzer (Jena Analytics, Germany) by applying infralyt detection after combustion according to DIN EN 1484 for TOC/DOC/DIC and EN 12260 for TN/DN, respectively. Water samples for TP, SRP and were analyzed photospectrometrically on a UV/VIS-Photometer CARY 1E (VARIAN, Germany). The protocol for TP and SRP (DIN EN 1189) was slightly modified whereby the molybdate solution was changed according to Murphy and Riley (1962). NH_4^+ was measured according to EN ISO 11732 using automated segmented flow analysis (SCAN++ - System, Skalar), while NO_3^- was analyzed by an ion chromatography system with suppressor (Shimadzu).

Fluorescence and Parallel Factor Analysis (PARAFAC): To produce excitation-emission-matrices (EEMs), we measured the excitation (240 - 450 nm, 2 nm steps) and the emission (300 - 596 nm, 5 nm steps), using a Perkin Elmer LS-50B fluorescence spectrometer (Rodgau, Germany) with a slit width of 5 nm for both excitation and emission. The fluorescence data were corrected for daily variations of instrument stability Raman correction; (Lawaetz and Stedmon, 2009) and primary and secondary inner-filter effects (Lakowicz, 2006). The emission spectra was corrected using the BAM fluorescence calibration kit (Pfeifer et al., 2006), and normalized by the area under the Raman peak at 350 nm excitation wavelength (Lawaetz and Stedmon, 2009). All corrections and the subsequent decomposition of the EEMs into their underlying chemical components (PARAFAC analysis) were performed using Matlab (R2011, MathWorks, Isamning, Germany), the drEEM (version 0.1.0, August 2013; Murphy et al., 2013,and the N-way version 3.20, July 2012) toolboxes following the tutorial of Murphy et al. (2013). For model evaluation split half analysis was performed and replicate samples were assigned to the same splidts (Murphy et al., 2013).

Supplementary Figures

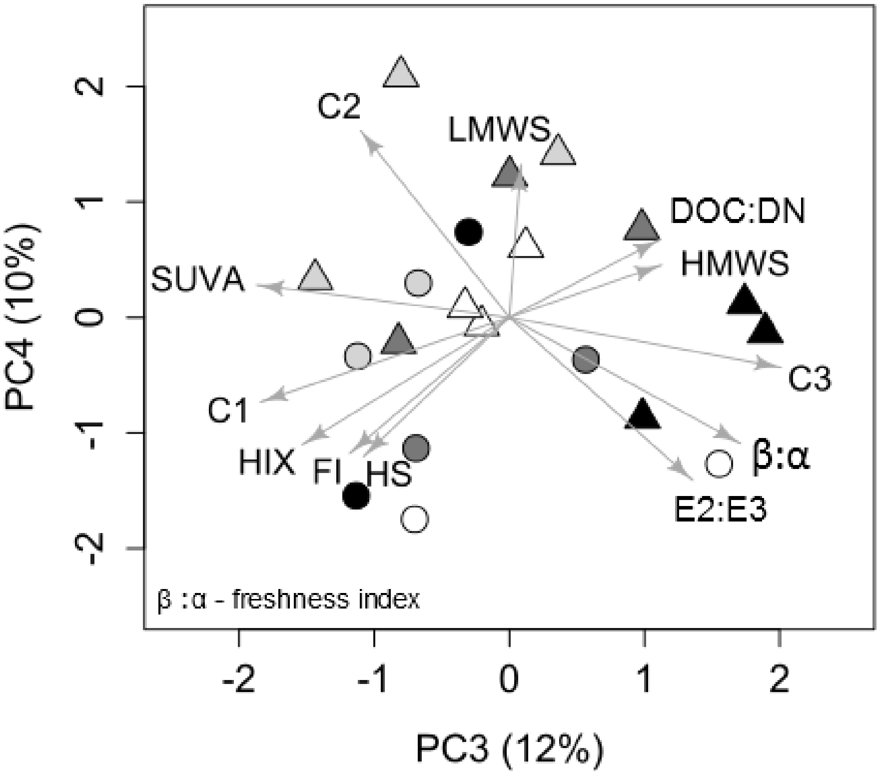

Figure S1 PCA biplot summarizes conditions of DOM quality across all sites and seasons. PCAs are based on the variables PARAFAC components C1, C2 and C3, high molecular weight substances (HMWS), low molecular weight substances (LMWS), humic like substances (HS), specific absorption at 254 nm (SUVA), humification index (HI), fluorescence index (FI), freshness index β:α, molecular size (E2:E3).

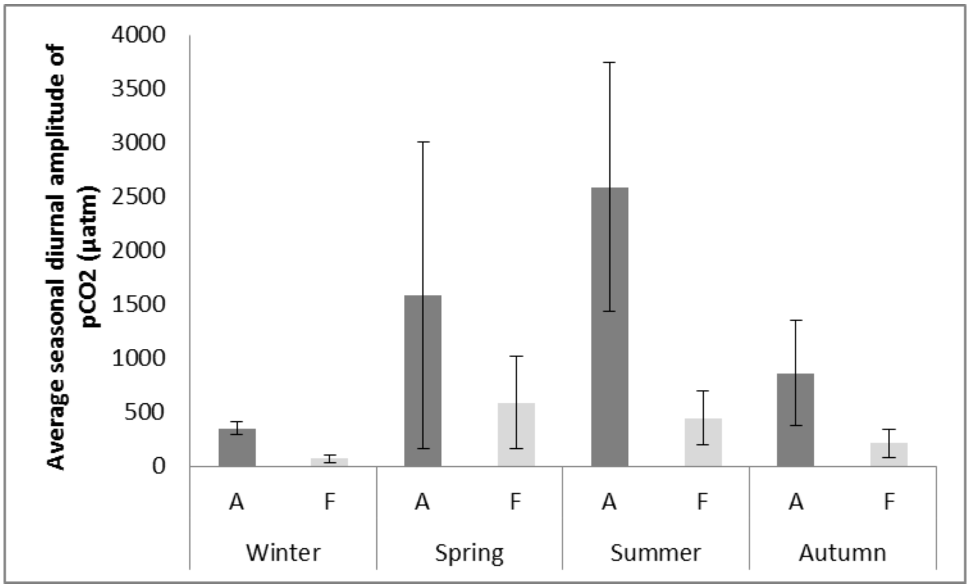

Figure S2 Mean ± 1SD of average seasonal diurnal amplitudes of pCO_2 over four seasons (winter, spring, summer and autumn) for agricultural (A) and forest (F) stream type (n = 3 per season and stream type).

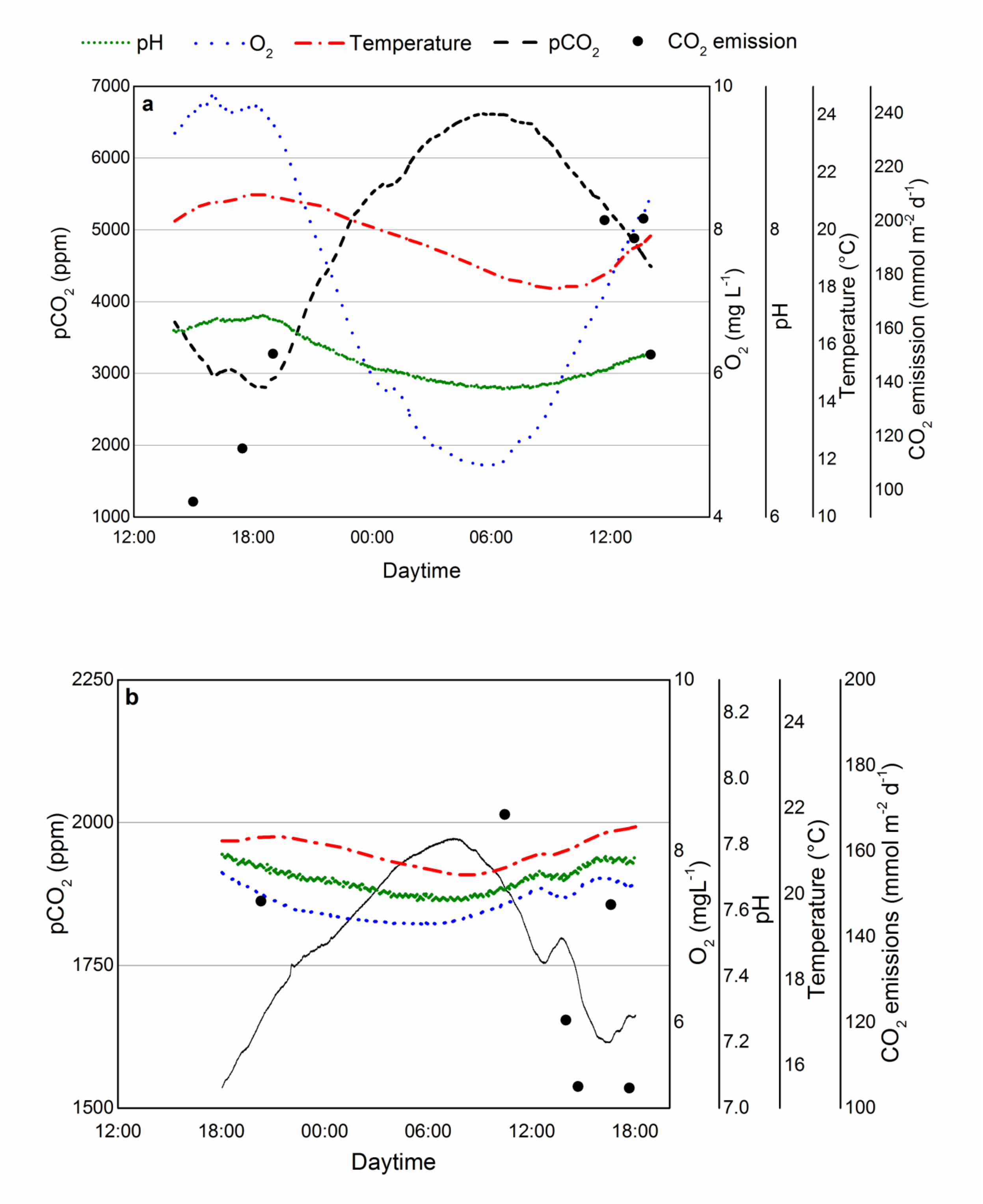

Figure S3 Representative diurnal dynamics (24 h) of key parameters measured in the agricultural stream A3 (a) and F2 (b) during the summer sampling. Black circles = simultaneously drifting chamber measurements; Short dots = pH; dots = O_2; dash dots = temperature; dash = pCO_2.

Supplementary References

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MANUSCRIPT IV

SIMULTANEOUS DIEL TRENDS OF DISSOLVED OXYGEN AND CARBON DIOXIDE RESOLVE THE CHEMISTRY OF STREAM ECOSYSTEM METABOLISM

SIMULTANEOUS DIEL TRENDS OF DISSOLVED OXYGEN AND CARBON DIOXIDE RESOLVE THE CHEMISTRY OF STREAM ECOSYSTEM METABOLISM

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Abstract

Stream metabolism describes the production and transformation of various pools of organic carbon (OC) at the level of the whole ecosystem. Its component processes, namely gross primary production (GPP) and ecosystem respiration (ER), are often estimated from diurnal dynamics of dissolved oxygen (O_2), although ideally they should be measured and expressed in terms of carbon (C). Here, we present a process-based model creating diurnally dynamic conditions of dissolved O_2 and Dissolved Inorganic Carbon (DIC) based on light and water temperature as external forces. The model includes saturation of primary production at high light. It models all relevant DIC-fractions depending on pH as an additional constraint on the carbonate equilibria. The underlying differential equations for O_2 and DIC as state variables are linked by photosynthethic (PQ) and respiratory (RQ) quotients, which describe the mole of CO_2 used or produced per mole of O_2 consumed or produced, respectively. Specifically, RQ is related to the quality of respired substrates, which are likely chemically more diverse than those produced and affecting PQ. The model is then used to interpret time series of CO_2 and $O_{2,}$ collected in four streams, differing in adjacent land use across four seasons. The model is able to reproduce, with varying accuracy, the main features of diurnal O_2 and CO_2 dynamics over 24 hours in 15 out of 16 stream scenarios. The resulting RQs ranged between 0.82 and 3.44 but were not related to adjacent land use or season. However, we found significant relationships between RQ values and proximate descriptors of organic carbon chemistry: a fluorescing component characteristic for higher plant material and an indicator for dominant molecule size of dissolved OC in agricultural streams. These results suggest agreement between chemical descriptions of available OC by water chemistry and a process-based ecosystem-scale assessment of the quality of OC used in ecosystem respiration. Our modeling approach deepens understanding of carbon turnover in stream ecosystems, leading towards an improved assessment of the role of streams in the regional and global carbon cycle.

1 Introduction

Stream ecosystem metabolism provides an integrative measure of stream structure and function, and comprises two major processes: Gross primary production (GPP) and ecosystem respiration (ER) (Izagirre et al., 2008, Bernot et al., 2010). They are key processes in production, transformation, and retention of organic carbon (Hotchkiss and Hall, 2014). Consequently, understanding the factors that control stream metabolism dynamics is fundamental to assess stream ecosystem functioning (Williamson et al., 2008).

Ecosystem metabolism can be quantified as a function of O_2 and CO_2 dynamics (Odum, 1956, Hanson et al., 2003). O_2 is usually the measure of choice since dissolved oxygen sensors are the more robust, cost-effective tools in widespread use compared to dissolved carbon dioxide sensors (Hanson et al., 2003, Staehr et al., 2010). Traditionally, metabolism was directly calculated from diel oxygen data alone, based on the (probably oversimplified) assumption that ER remains constant over night and day, while GPP must be limited to periods of daylight (Odum, 1956, Bott, 1996). However, relationships exist providing GPP and ER as a function of external forcing factors, such as solar radiation and temperature (Uehlinger et al., 2000, Birkel et al., 2013). These can be used to model O_2 or CO_2 dynamics in aquatic systems and – in an inverse model fitting approach – estimate GPP and ER. This has been done based on O_2 (e.g. Uehlinger et al., 2000, Birkel et al., 2013, Riley and Dodds, 2013), but, surprisingly, modeling of CO_2 dynamics are rare (Dinsmore et al., 2013) and a combination of O_2 and CO_2 in stream ecosystems has not been attempted so far.

The big advantage of measuring or modeling both gases (O_2 and CO_2) (e.g. Hanson et al., 2003) is the additional information gain about the photosynthetic quotient (PQ) and respiratory quotient (RQ). PQ, providing the moles of O_2 produced per mole of CO_2 consumed and vice versa for RQ (Bott, 1996). The ranges of variation of these two quotients differ widely. Williams and Robertson (1991) state PQ values for a 'typical' algal cell (40% proteins, 40% carbohydrates, 15% lipids, 5% nucleic acids) to range between 1.0 and 1.36. In contrast, RQ can vary more, even though it is often assumed to be one, for instance, to compute C production in the whole lake metabolism studies based on O_2 (e.g. Brothers et al., 2014). A study conducted by Berggren et al. (2012) showed that for bacterioplankton RQ varied across 52 streams, with the average RQ of this dataset converging to around 1.2. Apparently, RQ must be intimately linked to the elemental composition of the respired pools of OC, which are known to vary across freshwater gradients (Wilson and Xenopoulos, 2009). Contemporary studies of the chemical composition of available OC (Fellman et al., 2010) have meanwhile greatly transcended traditional, ecologically motivated operational pool definitions; yet it is the actual respiration of sub-pools of highly diverse OC and parameters like RQ providing information about actual aquatic ecosystem functioning (Berggren et al., 2012).

The main objective of this study is to exploit differences in the dynamics of O_2 and CO_2 in metabolism modeling to explore qualities of substrate respiration at ecosystem scale. Consequently, we (i) develop a process-based model describing O_2 and DIC dynamics, (i) confront it against field data, and (ii) explore relationships of resulting estimates RQ with chemical descriptors of available OC.

2 Methods

2.1 Modeling approach

The dynamics of dissolved oxygen (O_2) and dissolved inorganic carbon (DIC) are controlled by in-stream metabolic processes and gas exchange with the atmosphere, according to the following two coupled differential equations (Fig. 1):

$$\frac{d}{dt}O_2(t) = (GPP(t) - ER(t) + F_{O_2})\left(\frac{1}{z}\right), \tag{1}$$

$$\frac{d}{dt}DIC(t) = (-\frac{GPP(t)}{PQ} + ER(t)RQ + F_{CO_2})\left(\frac{1}{z}\right) \tag{2}$$

Here, *GPP* is gross primary production, *ER* is ecosystem respiration, *F* is the flux of gas (with subscript O_2 and CO_2 for oxygen and carbon dioxide, respectively) to or from the atmosphere, *z* is stream depth, *RQ* is the respiratory quotient, and *PQ* is the photosynthetic quotient. Modeling O_2 and DIC over time based on equations (1) and (2) requires the definition of additional expressions for GPP and ER, which are described subsequently.

Gross primary production (GPP) was represented by:

$$GPP(t) = \frac{I(t)}{p1 + p2I(t)}, \tag{3}$$

where *I* is the incoming short wave radiation, while *p1* and *p2* are suitable parameters (Uehlinger et al., 2000). At *p2* = 0 GPP is linearly related to *I* through coefficient *p1*, while for large radiation, GPP tends to a constant saturation value controlled by *p2*. It is convention in ecology to report GPP on a daily basis; for this Equation (3) is numerically integrated over 24 hours:

$$GPP_{24} = \sum_{i=1}^{144} GPP_i \ \Delta t \tag{4}$$

where Δt is the time step (1/6 hours).

Ecosystem respiration (ER) was represented as proposed by Parkhill and Gulliver (1998):

$$ER(t) = (R_{20})\Theta^{T(t)-20}, \tag{5}$$

where R_{20} is the respiration rate at 20°C; *T* is the temperature; and Θ was set to 1.0241 (Elmore and West, 1961). Similar to GPP, ER is aggregated on a daily basis by convention in ecological studies:

The integration of modeled ER fluxes results in daily values:

$$ER_{24} = \sum_{i=1}^{144} ER_i \ \Delta t \tag{6}$$

where Δt is the time step (1/6 hours).

Exchange of gases between stream and atmosphere was modeled according to the following equation:

$$F_i = k_i \left([C_i]_{sat} - [C_i]_{obs} \right), \tag{7}$$

in which, $[C_i]_{sat}$ and $[C_i]_{obs}$ are gas concentrations (O_2 or CO_2) at saturation (i.e. in equilibrium with the atmosphere) and observed in the stream water, respectively. $[C_i]_{obs}$ for CO_2 is the quantity $[CO_2*]$ linked to carbonate equilibria and calculated according to equation (13). CO_2 concentration at saturation (CO_{2sat}) was calculated using Henry's law (Goldenfum, 2010) and assuming an atmospheric molar gas fraction of 400 ppm for CO_2. For this, barometric pressure was calculated from altitude according to (National-Oceanic-and-Atmospheric-Administration, 1976). Oxygen concentration at saturation (O_{2sat}) was calculated according to Benson and Krause Jr (1984) from temperature and barometric pressure. k_i is the piston velocity modeled according to the formulation proposed by Jähne et al. (1987). We computed k_{O2} and k_{CO2} from the standardized k_{600}:

$$k_{O2} = \frac{k_{600}}{(\frac{600}{Sc_{O2}})^{-n}}, \tag{8}$$

$$k_{CO2} = \frac{k_{600}}{(\frac{600}{Sc_{CO2}})^{-n}}, \tag{9}$$

with *Sc* being the Schmidt number for in-situ temperature of the respective gas and 600 being the Schmidt number for 20°C in freshwater (Jähne et al., 1987). Similar to Guerin et al. (2007) and Prairie and del Giorgio (2013), the exponent n is taken as $n = 2/3$ for low wind speed, since the investigated streams were more or less sheltered.

The Schmidt numbers (Sc) for O_2 (eq. 10) and CO_2 (eq. 11) were calculated according to Raymond et al. (2012):

$$Sc_{O2} = 1568.0 - 86.04 T_{mean} + 2.142 T_{mean}{}^2 - 0.0216 T_{mean}{}^3, \tag{10}$$

$$Sc_{CO2} = 1911.1 - 118.11 T_{mean} + 3.4527 T_{mean}{}^2 - 0.04132 T_{mean}{}^3, \tag{11}$$

where the mean temperature over 24 hours (T_{mean}) was expressed as °C.

Carbonate equilibria: In aqueous solution gaseous CO_2 is only one fraction of the DIC pool; it readily forms carbonic acid and exchanges with ionic carbonate fractions in dependence of pH (Stumm and Morgan, 1996, Dickson et al., 2007). The total DIC pool is given by:

$$DIC = [CO_2^*] + [HCO_3^-] + [CO_3^{2-}], \tag{12}$$

where $[CO_2*]$ denotes the operationally defined sum of dissolved gas and hydrated carbonic acid, and the species HCO_3^- and CO_3^{2-} represent carbonate anions counterbalanced by H^+ or alternative cations. Notably, variable amounts of carbonates may be paired with alternative cations, e.g., the alkaline cations Ca and Mg, which can markedly increase the total DIC pool; this DIC-fraction is chemically defined and measurable as ‘alkalinity’. The various chemical concentration-dependent dissociation equilibria governing DIC species fractionation are well described (Stumm and Morgan, 1996, Dickson et al., 2007). Here, with regard to our model, four aspects need to be taken into account: first, chemical equilibration happens fast at timescales below biological and physical processes and thus have no effect in the time domain of the model. Second, while biological processes access various fractions of the DIC pool (Allan and Castillo, 2007), gas exchange across the water-atmosphere interface is limited to $[CO_2*]$. Third, our study ‘measures’ DIC dynamics based on dynamics of pCO_2, which is directly translatable to $[CO_2*]$, but may react weakly to even intense biological processes due to buffering by carbonates at high alkalinity. Thus, fourth, if model calibration to pCO_2 measurements is an aim, a full description of all DIC fractions is needed. For this, the measurement of one additional variable is necessary: either a single value of alkalinity, which can be assumed as constant, or continuous records of DIC or pH. The first and second of these options are prone to error or not feasible, respectively. However, continuous measurements of pH are easily and accurately achievable with sondes. We opted for the last and solved the carbonate equilibria for $[CO_2*]$ (Stumm and Morgan, 1996, Dickson et al., 2007):

$$DIC = [CO_2^*]\left(1 + \frac{K_1}{[H^+]} + \frac{K_1 K_2}{[H^+]^2}\right), \tag{13}$$

which then – as a function of DIC (a model state variable) and pH (as an external force) – was introduced to the gas flux equation eq. (7). Rearranging eq. (13) to solve for DIC is the basis for translating empirical measurements of pCO_2 and pH into dynamic DIC. In eq. (13) K_1 and K_2 are the dissociation constant of carbonic acide and H^+ is the hydrogen ion concentration computed from measured pH as 10^{-pH}. The constants were temperature-adjusted following Stumm and Morgan (1996):

$$K_1 = 10^{-356.3094 - 0.06091964 \cdot \mathrm{T} + 21834.37/\mathrm{T} + 126.8339 \cdot \mathrm{LOG(T)} - 1684915/T^2}, \tag{14}$$

$$K_2 = 10^{-107.8871 - 0.03252849 \cdot \mathrm{T} + 5151.79/\mathrm{T} + 38.92561 \cdot \mathrm{LOG(T)} - 563713.9/\mathrm{T}^2}, \tag{15}$$

where temperature T is expressed in Kelvin (K).

Respiratory quotient (RQ) and photosynthetic quotient (PQ) – Since we modeled oxygen and CO_2 dynamics simultaneously we implemented PQ and RQ in our DIC equation (eq. 2):

$$Respiratory\ quotient\ (RQ) = \frac{mole\ CO_2\ produced}{mole\ O_2\ consumed}, \tag{16}$$

$$Photosynthetic\ quotient\ (PQ) = \frac{mole\ O_2\ produced}{mole\ CO_2 consumed}, \tag{17}$$

where PQ was set to one.

To model dynamics of O_2 and DIC (and subsequently compute dynamics of its various fractions), equations (1) and (2) were solved analytically using the average concentration of the two species ($\bar{O}_2$ and $\overline{DIC}$) over the 24 hours as initial conditions:

$$O_2(t) = \left[\frac{1}{z}\int_0^t e^{(k_{O2}\tau/z)}\left(GPP(\tau) - ER(\tau) + k_{O2}O_{2_{sat}}(\tau)\right)d\tau + \overline{O_2}\right]e^{(-k_{O2}t/z)}, \tag{18}$$

$$DIC(t) = \left[\frac{1}{z}\int_0^t e^{(k_{CO2}/z\int f_{DIC}(\tau)d\tau)}\left(-\frac{GPP(\tau)}{PQ} + ER(\tau)RQ + k_{CO2}CO_{2_{sat}}(\tau)\right)d\tau + \overline{DIC}\right]e^{(-k_{CO2}/z\int f_{DIC}(t)dt)}, \tag{19}$$

where:

$$f_{DIC}(t) = \frac{DIC(t)}{1+\frac{K1(t)*K2(t)}{H^+(t)^2}+\frac{K1(t)}{H^+(t)}}, \tag{20}$$

The analytical solutions (18) and (19) were implemented into a code written in *Fortran* 95. For every time step, we additionally calculated the CO_2 concentration from the modeled DIC concentration according to equation (20).

Model calibration

The model was calibrated to diurnal O_2 and CO_2 curves measured during the field campaigns (see below). The model uses time series of stream water temperature (°C), pH and incoming short-wave radiation (W m^{-2}) as external forcing factors determining light-saturated GPP, temperature-dependent ER and temperature - and pH - dependent carbonate equilibria. Further, mean stream depth (m) is required to turn areal fluxes into changes of volumetric concentrations.

We calibrated iteratively five model parameters ($p1$, $p2$, k_{600}, RQ, R_{20}). Associated climate variables (stream temperature and solar radiation) drove the model and the parameters were calibrated by running through the complete oxygen and DIC 24-hour time series.

A multi-objective framework has been applied to calibrate optimal parameters. The index of agreement (IA; Willmott and Wicks, 1980, Willmott, 1981) has been used to define two distinct metrics of model performance: one providing a measure of model capabilities in simulating observed oxygen dynamics (IA_{O2}), and the other in simulating observed CO_2 dynamics (IA_{CO2}). These two metrics have been aggregated into a single objective function IA_{tot} according to the following equation:

$$IA_{tot} = (1.0 - a)IA_{O_2} + aIA_{CO_2} \tag{21}$$

Here, the weighting factor *a* can range between 0 (only O_2 data considered) and 1 (only CO_2 data considered), yet we used $a = 0.5$ as the best default choice in the absence of prior information. *IA* is defined as follows:

$$IA = 1 - \frac{\sum_{i=1}^{n}(P_i - O_i)^2}{\sum_{i=1}^{n}(\left|P_i - \overline{O}\right| + \left|O_i - \overline{O}\right|)^2}, \tag{22}$$

where *P* stands for the quantity predicted by the model to be compared with the observed (O) quantity. *IA* varies between $-\infty$ and 1.0, with the latter implying perfect agreement with the data.

The optimization of IA_{tot} has been performed by implementing the Particle Swarm Optimization (PSO) algorithm, an evolutionary and self-adaptive search optimization technique (Kennedy, 2010). In short, in PSO the parameter space is searched by a swarm of particles that each move by a combination of a random jump with two attraction components at each iteration. A local optimum is identified as the location with the best fit (the largest value of *IA*) among those experienced by a single particle; the global optimum is the absolute best found by all particles. We used 500 particles and 1000 iterations to exhaustively explore the parameter space defined by plausible parameter ranges (Table 1). Calibrated parameters were obtained for all 16 scenarios under the assumption that they remain constant during the entire dial cycle but may vary among streams and seasons. With these limits we executed the final model for O_2 and DIC for all 16 scenarios.

Consequently, our modeled RQ values represent averages across a day and across various processes in a stream.

2.2 Field data

2.2.1 Study sites and measurements

Empirical measurements were done in four streams located in the North Central European Plain in Germany (Fig. 2): Two ‘forest’ streams (F1 & F2) are surrounded by coniferous forest with banks lined with alders (CORINE Land Cover; Federal Environment Agency, DLR-DFD 2009); two ‘agricultural‘ streams (A1 & A3) are surrounded by non-irrigated arable land or pastures. Each stream was investigated during four two-day field campaigns covering all four seasons (May, July/August, October and January/February in 2013-2014). Weather and hydrological conditions in each field campaign were stable and representative for the respective seasons.

During each field campaign we continuously measured partial pressure of CO_2 at a maximal measuring frequency of 10 s^{-1} over 24 hours using a carbon dioxide sensor (HydroC™ CO_2; Kongsberg

Maritime Contros GmbH, Germany), which was factory calibrated before (December 2012) and in the middle (July 2013) of the measuring campaigns. All pCO_2 data were corrected for signal drift post-hoc by the manufacturer. For further details on measuring procedures and drift correction see Fietzek et al. (2014). The battery-powered (12V, 70 Ah) sensor was stably mounted in the main current avoiding direct contact with the sediments. In addition, we continuously recorded water temperature, pH and dissolved oxygen over 24 hours at one-minute intervals using a Yellow Springs Instruments monitoring probe (YSI; 6600 V2, Xylem Inc., Yellow Springs, USA) mounted next to the CO_2 sensor. Calibration of the dissolved oxygen sensor was achieved in water-saturated air, that of pH by two-point calibration at pH 7 and 10.

Solar radiation data was sourced from the AWEKAS network (Automatic Weather Map System; http://www.awekas.at/de/index.php), which pools weather data of scattered private weather stations including necessary quality checks. We chose the weather stations closest to our study sites, at distances of 43 km, 11 km, 20 km and 21 km from sampling sites A1, A2, F1 and F3, respectively. The data had observation frequencies from min^{-1} to h^{-1} and were pre-processed to provide values every 10 min over 24 hours.

We further collected 4 replicate water samples at the position of the sensor to analyze nutrient concentrations (dissolved nitrogen and soluble reactive phosphorus), concentrations and size classes of two dissolved organic carbon fractions (high molecular weight and low molecular weight), and proxies of dissolved organic matter quality by fluorescence and absorbance measurements.

2.2.2 Analyses and calculations

Prior to chemical analyses, the collected water samples were filtered through a pre-washed 0.45 μm membrane filter (cellulose acetate, Sartorius) in the laboratory. Samples for dissolved organic carbon (DOC) and total dissolved nitrogen (DN) were acidified with 2 N hydrochloric acid (HCl) to pH 2 for preservation and measured in duplicate on a multi N/C 3100 Analyzer (Jena Analytics, Germany) by IR-absorption after combustion according to DIN EN 1484 for DOC and EN 12260 for DN, respectively. Soluble reactive phosphorous (SRP) was analyzed photospectrometrically on a UV/VIS-Photometer CARY 1E (VARIAN, Germany) following a modified (Murphy and Riley 1962) protocol based on DIN EN 1189. We further characterized DOC using liquid chromatography and organic carbon detection (LC-OCD) (Huber et al., 2011). This size-exclusion chromatography allows separate concentration determination (in mg L^{-1}) of three DOC size class fractions: humic-like substances (HS), high-molecular weight non-humic substances (HMWS) and low-molecular weight substances (LMWS). Absorbance was measured with a 1 cm cuvette on a Shimadzu UV-2401 UV/VIS spectrometer (Duisburg, Germany) at room temperature. Absorbance data were corrected for instrument baseline offset (Green and Blough,

1994) and used to compute specific UV absorption at 254 nm ($SUVA_{254}$) as an indicator for aromaticity (Weishaar et al., 2003) and the ratio E2:E3 that is related to apparent DOM molecular size (Helms et al., 2008). Further, measurements of fluorescence (Perkin Elmer LS-50B fluorescence spectrometer, Rodgau, Germany) produced excitation-emission (ex and em) matrices that were subjected to Parallel Factor Analysis (PARAFAC). Briefly, PARAFAC is a multivariate three-way modeling technique which decomposes the fluorescence signal into individual components and provides relative contributions of each component to the additively formed total signal (Bro, 1997, Stedmon and Bro, 2008). For details of fluorometry and PARAFAC see Manuscript III of this thesis. The resulting PARAFAC model consisted of three components: C1, C2, and C3. C1 (fluorescence maximum at ex: <240, 330-360, em: 440) was a previously reported humic-like, terrestrially derived, high molecular weight fluorophore (Stedmon and Markager, 2005, Fellman et al., 2010). C2 (ex: 255-275, (385-395) em: 480 – 515) was similar to published PARAFAC components resembling high molecular weight, humic-like, terrestrial material (Fellman et al., 2010) with increased aromatic carbon content, indicating higher plant material as a likely source (Cory and McKnight, 2005). C3 (ex: <240, 280-310, em: 390 – 410) resembled a humic-like, terrestrial component associated with agriculture and wastewater (Fellman et al., 2010; C6, Cory and McKnight, 2005). For all subsequent data analyses, the PARAFAC components were expressed as relative fluorescence intensities contributing to the total fluorescence of the sample. Beside for PARAFAC modeling, fluorescence measurement were also used to compute the β:α ratio (freshness index) (Parlanti et al., 2000) indicating the freshness of DOC (>1: freshly produced, 0.6-0.8: more terrestrial input), the humification index (HIX) (Ohno and Bro, 2006), and the fluorescence index (FI) indicating more microbial (FI~1.9) or terrestrial higher plant (FI~1.4) origin of DOC (McKnight et al., 2001). We used the suite of water-chemical measures to test for relationships with calibrated RQ values by Pearson correlations computed in R (R-Development-Core-Team, 2010).

3 Results

3.1 *Calibrated results: Stream diurnal O_2 and CO_2 dynamics and RQ*

After calibration the model was able to capture the diurnal O_2 and CO_2 dynamics (Fig. 3) over 24 hours in 15 out of 16 stream scenarios (i.e. combinations of 4 streams in 4 seasons). Fitting quality, expressed by the index of agreement (IA_{tot}; eq.21), ranged 0.80 – 0.99. For one scenario, we were not able to model the CO_2 dynamics. In general we could reasonably capture the dynamics of both measured gases, though, notably, model performance was often better for CO_2 than for O_2. As GPP was formulated as a function of solar radiation, O_2 and CO_2 dynamics tended to reflect the spiky behavior of the light data. In general the model worked best in spring and summer and worst in winter, probably because of low metabolism at colder temperatures and short light periods.

Representative O_2 and CO_2 dynamics in spring in the four investigated streams are shown in Figure 3. The behavior of a too fast drop and too fast rise of O_2 (Fig. 3B.1 and D.1) can be explained by a suboptimally represented gas exchange model term, i.e. the interaction of O_2 with the atmosphere could not be perfectly modeled, or by a process which is not properly represented in the model.

Calibration of the model to data from the 16 scenarios worked successfully and produced reasonable best parameters for the five parameters ($p1$, $p2$, R_{20}, k_{600} and RQ) in the range of the prior set limits (Table 1). When the calibration results for the key parameters $p1$, $p2$ and R_{20} (Table 1) were combined with the data on light (eq. 3) and stream temperature (eq. 5), this resulted in comparably high stream ecosystem metabolism estimates (Fig. 4): ER ranged from $1.26*10^{-6}$ to 79.59 g O_2 m^{-2} d^{-1} and GPP from 1.36 to 82.60 g O_2 m^{-2} d^{-1}. In general, there was higher metabolism (both for GPP as for ER) in summer than in winter and in the two agricultural streams compared to the forest streams. For the parameter RQ, four scenarios suggested RQ values outside the admissible range of variation and were excluded from further analyses. The RQs inside the admissible range were between 0.82 and 3.44 (Table 1).

3.2 Nutrients, dissolved organic matter quality characteristics and their link to modeled RQ values

Nutrient concentrations, size classes of DOC and several optical DOM quality characteristics varied across land uses and seasons (Table 2). DOC concentrations ranged between 6.50 ± 0.22 mg L^{-1} (mean ± 1 SD) and 10.03 ± 0.13 in agricultural streams and between 5.28 ± 0.13 and 6.25 ± 0.06 in forest streams. LMWS concentrations ranged between 0.81 ± 0.1 and 1.77 ± 0.36 in agricultural streams, while in forest streams LMWS concentrations ranged between 0.50 ± 0.06 and 2.76 ± 0.16. Moreover, E2:E3 (indicating the relative size of DOM) ranged from 5.75 ± 0.05 to 8.20 ± 0.13 in agricultural streams, whereas forest streams showed a smaller range between 7.24 ± 0.25 and 8.85 ± 0.26. Furthermore, the data revealed seasonal effects, e.g., HMWS concentration were generally lower in winter than in summer in both stream types.

When agricultural and forest stream data were pooled, modeled RQ values did not result in any significant correlation (Pearson, $p > 0.05$) with nutrient concentrations, size classes of dissolved organic carbon or optical proxies of dissolved organic matter quality. However, when the two stream types were analyzed separately significant linkages were identified in agricultural streams but not in forest streams (Table 3). In agricultural streams RQ was positively correlated to the PARAFAC component C2 (Pearson, $r = 0.92$, $p = 0.001$), which indicates higher plant material as a likely source of respiration, and negatively correlated to the size ratio of DOM (Pearson, $r = -0.84$, $p = 0.04$), indicating higher RQ values when larger DOM molecules are present in the system. Moreover, we detected a borderline in-significant trend indicating correlation between RQ and the freshness index (Pearson, *r* = -0.77, *p* = 0.07), denoting higher RQ values with more terrestrial input and lower RQ values with more freshly produced DOM.

4 Discussion

With the process-based model discussed in Section 2, for which underlying differential equations are linked by the respiratory and photosynthetic quotients, we were able to model reasonable diurnal O_2 and CO_2 dynamics in 15 out of 16 stream scenarios (i.e. four streams in four seasons). The novelty of our approach is that we are able to model O_2 and CO_2 dynamics simultaneously, which allows calibration of a daily-averaged ecosystem-scale respiratory quotient parameter (RQ). Finally, validity of this parameter was confirmed by successful identification of linkages between RQ and DOM quality features, underlining the indicatory potential of RQ derived from O_2/CO_2 records for assessments of stream ecosystem functioning.

4.1 Model performance in capturing simultaneous diurnal O_2 and CO_2 dynamics

The essential, novel element of our model is the exploitation of a mismatch in the O_2 vs. CO_2/DIC dynamics for estimating RQ. This mismatch is driven by three conditions (i) differences in the diffusion constants that translate to differences in gas exchange efficiency (parameters k_{O2} and k_{CO2}), (ii) alkalinity that defines carbonate equilibria and buffering of diurnal CO_2 dynamics relative to O_2 dynamics, and (iii) PQ and RQ that depend on chemical features of organic matter produced and respired and thus potentially both differ from the commonly assumed standard value of 1 (e.g. McCallister and Del Giorgio, 2008). In the following we discuss, among others, which circumstances regarding (i) and (ii) influence the model performance.

The transition phase, in which – due to just recent light-driven production – O_2 still deviates enough from its quite constant dynamic equilibrium between respiration and gas exchange, is the period most critical for fitting the reaeration flux term (parameter k_{O2}) in a standard modeling approach based on oxygen alone. When low GPP co-occurs with high k_{O2}, diurnal variations are dampened, transition phases are short, and the model's parameters for the reaeration flux and GPP can hardly be inferred; consequentially also ER cannot be estimated as the reaeration term remains unknown (Reichert et al., 2009). Clearly, such conditions would also prevent successful CO_2-based modeling of GPP. Ideally, both gases show some limited diurnal variability that can be aligned to a well-resolved record of light.

Using CO_2 data for metabolism estimation faces an additional challenge: alkalinity that is positively related to pH and thus negatively to free gaseous CO_2 concentration (Rebsdorf et al., 1991, Stumm and

Morgan, 1996). High concentrations of ionic DIC-fractions (carbonates) represent a buffer exchanging readily with free gaseous CO_2 and thereby dampening any ER-or GPP-driven CO_2 dynamics. This decreases the signal exploitable for model calibration and thus adds uncertainty to the model, i.e., it causes unreliable parameter estimations. A possible way to tackle this problem is to adjust the weighting factor *a* in the equation of the fitting quality for multi-objective optimization during the calibration (eq. 21). We so far used equal weights for both gases (O_2 and CO_2). However, usage of unequal weights, one for O_2 and the other one for CO_2 measurements, may allow making the best possible use of all the available information, taking into account both model deficiencies and errors in the measurements. For example, different sensor sensitivities or sensor noise levels, alkalinity dampening the CO_2 but not the O_2 signal, or responsiveness of CO_2 and O_2 to changing functions may differently affect the time series of the two gasses. The analysis of the Pareto front is an effective way to deal with cases in which more than one metric can be defined, with the shape of the Pareto front highlighting model limitations and structural problems (Gupta et al., 1998). We computed the Pareto front for every stream and season, by performing the optimization separately eleven times by changing the weighting factor *a*, starting at 0 (only O_2 data considered) until 1 (only CO_2 data considered) with steps of 0.1 (Madsen, 2000, Piccolroaz et al., 2015). The resulting Pareto fronts revealed that the Index of agreement (IA) for CO_2 is generally higher than the IA for O_2. This suggests more noise for the O_2 sensor and/or dampening of the CO_2 signal, most likely by alkalinity. In some cases, the choice of the weighting factor *a* also influenced modeled RQ values.

Overall, the model performed well in 15 out of 16 stream scenarios, with relatively high indices of agreement and optimal parameters ($p1$, $p2$, k_{600}, R_{20}) within their range of variability that was identified by prior information. The parameter k_{600} was in general rather low, yet this is not surprising given the slow-flow lowland character of our streams. Visual inspections (Fig. 3 and 4) confirm that the model is able to capture the daily dynamics of both O_2 and CO_2. However, the goodness of fit for O_2 and CO_2 dynamics varied considerably among seasons and streams. The model performance –especially for O_2 – was clearly best in spring and summer, and worst in winter. An explanation for the not-optimal model performance in winter months could be the absence of sinusoidal patterns of O_2 and CO_2, including specific trends and peaks. This kind of patterns were labeled "non-ideal" by Birkel et al. (2013) and the model was rejected in their case. A further issue in winter could be the low rates of GPP and ER due to low and short light intensities and low stream water temperatures. In these circumstances neglected effects, such as external lateral fluxes and groundwater contributions carrying CO_2 (see e.g. Crawford et al., 2014) may become dominant (or not longer negligible), thereby driving the system into a situation that the model is not able to represent.

With respect to modeled O_2 dynamics, we faced some difficulties. Similar to Riley and Dodds (2013), we got a saw-blade pattern in the modeled curves, which means that modeled O_2 and CO_2 respond quicker to changes in light compared to the measured CO_2 and O_2. One of the reasons may be the fact that we used a formulation of GPP which is strongly dependent on the parameters *p1* and *p2*, which in turn are a function of light (eq. 3). Furthermore, the model produces a flat behavior of O_2 during the nighttime, where the light input is zero. These patterns were observed also on other studies (Riley and Dodds, 2013, Hotchkiss and Hall, 2014) and calibration of *p1* and *p2* did not solve this problem. Consequently, a more sophisticated model of GPP may be necessary to finally solve these problems.

With respect to aquatic CO_2 dynamics and consequently CO_2 fluxes, the chemical enhancement of gas exchange is yet another important issue. The exchange of CO_2 between the water and atmosphere phases is primarily influenced by reactions diffusion (Emerson, 1975), but for CO_2 chemical reactions with OH^- at high pH conditions can substantially increase the mass transfer of CO_2 between air and water (Wanninkhof and Knox, 1996, Bade and Cole, 2006). Since the pH of our investigated streams was typically much less than nine, chemically enhanced CO_2 gas exchange was assumed to be negligible (Emerson, 1975, Bade and Cole, 2006).

Furthermore, in our model we did not account for possible oxygen consumption by nitrification and the activity of anoxic and anaerobic mineralization processes, which additionally would be able to influence the observed O_2 and CO_2 dynamics (Reichert et al., 2009). Since we are not aware of any important ammonia sources or large anoxic sections of the water body in our investigated stream sites we considered these processes as negligible (Reichert et al., 2009).

4.2 Exploration of calibration results with a focus on RQ

In five scenarios (i.e. combination of streams and seasons), our model was not able to produce reliable RQs, i.e. the values obtained calibrating the model on the measured O_2 and CO_2 concentrations were unrealistically high from an ecological point of view. This failure can be explained in two ways: (1) these extreme RQ values could point towards groundwater input. Groundwater may have high DIC and low O_2 at some very biased ratio, like low amount of O_2 and a high amount of DIC. This additional input makes the RQ calibration impossible without the inclusion of a groundwater component in the budget described by equations (1) and (2). (2) In our model formulation, RQ is strongly coupled to ER (eq. 2). Therefore, if ER is very small compared to GPP, the value of RQ becomes unconstrained by observations and can therefore assume unrealistically large or small values during the calibration process.

Furthermore, modeled GPP_{24} and ER_{24} values are partly surprisingly high compared to existing literature (e.g. Acuña et al., 2004, and the compiled values therein). Possible explanations could be the high macrophyte cover observed in the field or the effect of O_2-bubles.

Generally, RQ (eq. 17) is used to transform O_2 consumption into C production (Bott, 1996). These kinds of conversions are applied in a variety of studies ranging from calculations of bacterial growth efficiency (e.g. del Giorgio and Cole, 1998, Amado et al., 2013, Dinasquet et al., 2013) to determination of freshwater DOC lability by bacterial respiration measurements (Guillemette and del Giorgio, 2011) to whole lake metabolism (Brothers et al., 2014). In most of these studies, RQ is fixed to 1 (e.g. McCallister and Del Giorgio, 2008, Amado et al., 2013) without a clear justification other than the assumption that glucose is the very first product of photosynthesis. An extensive study of Berggren et al. (2012) revealed significant correlations of RQ with ecosystem-level, substrate-level and bacterial community-level characteristics. Our modeled RQs range between 0.82 and 3.44, similar to the range observed by Berggren et al. (2012), revealing a wide range of reduced substrates (i.e. low RQ) to highly oxidized substrates (i.e. high RQ) used for respiration. Transferred to our stream systems high RQs occur in combination with high CO_2 and low O_2 concentrations.

Since we modeled stream ecosystem RQ's, which describe a mixture of DOC pools for respiration, it is not possible to ascribe them to a single substrate. By analyzing relationships of the modeled RQs separately for each stream system with environmental parameters (Table 3) we were able to find a significant relationship of RQ with molecular size of DOM as well as with a fluorescing component characteristic for higher plant material in agricultural streams. This result indicates that there is a relationship between higher RQ values and larger DOM in agricultural stream system. Berggren et al. (2012) found a similar relationship between high RQs and high molecular weight DOM across lakes, shallow ponds and tarns systems. However, Berggren et al. (2012) found additionally a relationship between RQ and SUVA, which we neither found in agricultural nor forest streams. Nevertheless, we could show that RQ - an indicator of stream ecosystem functioning- is linked to DOM quality indicators in agricultural streams. Several studies revealed that DOM quality in streams differs in respect to land use (Wilson and Xenopoulos, 2009, Williams et al., 2010, Graeber et al., 2012). By revealing a link between RQ and DOM quality we conclude, that these DOM quality differences may have actual consequences on stream ecosystem functioning.

Nevertheless, the presented RQ's in this study are average daily values; therefore, they are averaged across autotrophic respiration and respiration based on terrestrial subsidies and constant over time, i.e. averaged across time. An option for further development of the model could be to introduce two different TOC pools in the model: the autochthonous TOC pool, i.e. GPP derived, and the allochthonous TOC pool, i.e. terrestrial input derived. These two pools are definitely respired at different RQ's. Further assuming these two different TOC pools to have different dynamics - one depends on GPP, while the other does not - would turn the RQ for autochthonous material into a dynamic variable, in contrast to our so far constant average RQ. This further development would deepen the understanding of carbon turnover in stream ecosystems even more, leading towards an improved assessment of the role of streams in the regional and global carbon cycle.

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Notation

O_2	Dissolved oxygen concentration (mg L^{-1})
DIC	Dissolved inorganic carbon concentration (mg L^{-1})
CO_2	Dissolved carbon dioxide concentration (mg L^{-1})
ER	Ecosystem respiration rate (g O_2 m^{-2} h^{-1})
ER_{24}	Daily ecosystem respiration (g O_2 m^{-2} d^{-1})
GPP	Gross primary production rate (g O_2 m^{-2} h^{-1})
GPP_{24}	Daily gross primary production (g O_2 m^{-2} d^{-1})
z	Stream depth (m)
k_{O2}	Piston velocity for oxygen (m h^{-1})
k_{CO2}	Piston velocity for carbon dioxide (m h^{-1})
O_{2sat}	Saturation concentration of oxygen (mg L^{-1})
CO_{2sat}	Saturation concentration of carbon dioxide (mg L^{-1})
PQ	Photosynthetic quotient (-)
RQ	Respiratory quotient (-)
K_1	Dissociation constant 1 (mol L^{-1})
K_2	Dissociation constant 2 (mol L^{-1})
H^+	Hydrogen ion concentration (mol L^{-1})
I	Incoming short-wave radiation (W m^{-2})
p1	Linear photosynthesis parameter (W h g O_2^{-1})
p2	Light saturation parameter (m^2 h g O_2^{-1})
R_{20}	Standard total dark respiration rate parameter at 20 °C (g O_2 m^{-2} h^{-1})
Ө	Arrhenius coefficient (-)
k_{600}	Standardized gas transfer velocity (m h^{-1})
Sc	Schmidt number (-)
$[CO_2^*]$	Dissolved CO_2 concentration (mol L^{-1})
P	Predicted value (mg L^{-1})
O	Observed value (mg L^{-1})
$\bar{O}$	Observed mean value (mg L^{-1})

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Tables of Manuscript IV

Table 1. Parameters put in calibration, initial parameter ranges and range of best fit; Notes: Literature reference for initial parameter range

Parameter	Unit	Initial parameter range	Range of best fit	Notes
p1	(W h g O_2^{-1})	0 - 5000	$1.20*10^{-5}$ - 1058.98	Birkel et al., 2013
p2	(m^2 h g O_2^{-1})	0 - 50	0.04- 2.54	Birkel et al., 2013
k_{600}	m h^{-1}	$3.00*10^{-5}$ – 0.28	$3.00*10^{-5}$ - 0.01	Campeau and Del Giorgio, 2014
Respiratory quotient	-	0 – 5	0.82 - 3.44	Berggren et al., 2012
R_{20}	(g O_2 m^{-2} h^{-1})	0 - 4	$8.00*10^{-8}$ - 3.79	Birkel et al., 2013

Table 2. (continiueing to the next page) Mean ± 1 SD of nutrients (DOC, DN, SRP), optical proxies of dissolved organic matter quality (C1-C3, HIX, FI, Freshness index, E2:E3, SUVA254) and size classes of dissolved organic carbon (HMWS, HS, LMWS) of the two investigated agricultural streams (A) and forest streams (F) in four seasons. Win. = winter, Spr. = spring, Sum. = summer, Aut. = autumn; n = 4 per stream and season.

	A1				**A3**				**F1**				**F2**			
Parameter	**Win.**	**Spr.**	**Sum.**	**Aut.**	**Win.**	**Spr.**	**Sum.**	**Aut.**	**Win.**	**Spr.**	**Sum.**	**Aut.**	**Win.**	**Spr.**	**Sum.**	**Aut.**
DOC	6.50 ± 0.22	8.08 ± 0.13	9.78 ± 0.21	8.83 ± 0.26	7.20 ± 0.00	9.08 ± 0.43	9.83 ± 0.19	10.03 ± 0.13	5.28 ± 0.13	6.00 ± 0.10	5.90 ± 0.24	5.28 ± 0.13	5.43 ± 0.15	5.68 ± 0.26	6.25 ± 0.06	6.00 ± 0.22
DN	1.73 ± 0.05	1.40 ± 0.00	1.03 ± 0.05	1.25 ± 0.06	2.25 ± 0.06	1.05 ± 0.06	2.00 ± 0.00	2.10 ± 0.00	0.70 ± 0.08	0.47 ± 0.06	0.55 ± 0.06	0.38 ± 0.05	0.60 ± 0.00	0.58 ± 0.05	0.50 ± 0.00	0.53 ± 0.05
SRP	9.75 ± 0.50	34.50 ± 0.58	67.25 ± 1.71	27.50 ± 1.73	103.75 ± 1.26	109.50 ± 0.58	241.75 ± 1.26	206.5 ± 2.38	23.25 ± 0.50	34.00 ± 1.00	29.00 ± 0.82	13.50 ± 0.58	4.00 ± 0.00	17.25 ± 1.26	25.00 ± 0.82	4.50 ± 0.58
C1	0.47 ± 0.00	0.42 ± 0.00	0.41 ± 0.00	0.43 ± 0.00	0.45 ± 0.01	0.42 ± 0.00	0.39 ± 0.01	0.44 ± 0.01	0.45 ± 0.00	0.38 ± 0.00	0.39 ± 0.02	0.39 ± 0.00	0.45 ± 0.01	0.36 ± 0.01	0.39 ± 0.00	0.39 ± 0.01
C2	0.24 ± 0.00	0.26 ± 0.00	0.25 ± 0.00	0.25 ± 0.00	0.25 ± 0.00	0.29 ± 0.00	0.30 ± 0.00	0.28 ± 0.01	0.22 ± 0.00	0.25 ± 0.00	0.23 ± 0.00	0.24 ± 0.00	0.22 ± 0.00	0.26 ± 0.00	0.23 ± 0.00	0.26 ± 0.00
C3	0.29 ± 0.00	0.31 ± 0.00	0.35 ± 0.01	0.32 ± 0.00	0.30 ± 0.00	0.29 ± 0.00	0.31 ± 0.00	0.28 ± 0.00	0.33 ± 0.00	0.37 ± 0.00	0.38 ± 0.02	0.37 ± 0.00	0.33 ± 0.01	0.38 ± 0.01	0.37 ± 0.00	0.35 ± 0.00
HIX	0.98 ± 0.00	0.95 ± 0.01	0.93 ± 0.01	0.93 ± 0.00	0.96 ± 0.01	0.95 ± 0.00	0.92 ± 0.01	0.95 ± 0.01	0.98 ± 0.00	0.94 ± 0.00	0.94 ± 0.01	0.91 ± 0.00	0.98 ± 0.00	0.92 ± 0.01	0.91 ± 0.00	0.89 ± 0.00
FI	1.61 ± 0.01	1.50 ± 0.02	1.5 ± 0.05	1.55 ± 0.01	1.52 ± 0.01	1.50 ± 0.01	1.45 ± 0.03	1.50 ± 0.03	1.54 ± 0.06	1.42 ± 0.01	1.46 ± 0.02	1.49 ± 0.04	1.52 ± 0.04	1.40 ± 0.04	1.51 ± 0.04	1.46 ± 0.02
Freshness index	0.67 ± 0.01	0.63 ± 0.01	0.68 ± 0.02	0.68 ± 0.00	0.68 ± 0.01	0.61 ± 0.01	0.61 ± 0.01	0.62 ± 0.02	0.67 ± 0.01	0.68 ± 0.01	0.71 ± 0.05	0.69 ± 0.01	0.70 ± 0.01	0.70 ± 0.01	0.71 ± 0.01	0.69 ± 0.02

(continueing of previous page, table 2)

	A1				**A3**				**F1**				**F2**			
Parameter	**Win.**	**Spr.**	**Sum.**	**Aut.**	**Win.**	**Spr.**	**Sum.**	**Aut.**	**Win.**	**Spr.**	**Sum.**	**Aut.**	**Win.**	**Spr.**	**Sum.**	**Aut.**
HMWS	0.30	0.47	0.73	0.58	1.07	1.17	1.70	1.32	0.39	0.51	0.58	0.46	0.60	0.93	1.00	1.02
	± 0.02	± 0.01	± 0.01	± 0.00	± 0.02	± 0.03	± 0.02	± 0.14	± 0.31	± 0.02	± 0.02	± 0.01	± 0.03	± 0.04	± 0.02	± 0.03
HS	5.25	7.16	7.99	6.80	5.09	6.28	6.19	7.10	5.11	4.57	4.44	3.94	3.96	4.22	4.01	4.23
	± 0.08	± 0.13	± 0.04	± 0.03	± 0.09	± 0.17	± 0.10	± 0.13	± 2.47	± 0.19	± 0.20	± 0.02	± 0.04	± 0.14	± 0.01	± 0.18
LMWS	1.73	0.81	0.97	1.58	0.90	0.97	1.77	1.37	0.50	1.63	2.76	1.00	0.54	0.69	1.98	0.99
	± 0.61	± 0.1	± 0.03	± 0.05	± 0.01	± 0.14	± 0.36	± 0.18	± 0.06	± 0.30	± 0.16	± 0.38	± 0.07	± 0.04	± 0.14	± 0.1
E2:E3	8.20	6.89	7.65	7.15	6.72	6.05	5.75	6.36	8.85	8.33	8.08	7.24	7.51	8.62	7.42	7.85
	± 0.13	± 0.05	± 0.16	± 0.10	± 0.32	± 0.05	± 0.05	± 0.07	± 0.26	± 0.41	± 0.28	± 0.25	± 0.03	± 0.65	± 0.19	± 0.47
SUVA$_{254}$	2.63	2.90	2.58	2.63	2.57	2.66	2.58	2.82	2.59	2.73	2.62	2.49	2.26	2.02	1.91	2.12
	± 0.10	± 0.03	± 0.04	± 0.08	± 0.03	± 0.12	± 0.06	± 0.04	± 0.06	± 0.06	± 0.11	± 0.08	± 0.06	± 0.09	± 0.02	± 0.08

DOC = Dissolved organic carbon (mg L^{-1}); DN = Dissolved nitrogen (mg L^{-1}); SRP = Soluble ractive phosphorus (µg L^{-1}); C1 = PARAFAC component C1; C2 = PARAFAC component C2; C3 = PARAFAC component C3; HIX = Humification index; FI = Fluorescence index; HMWS = High molecular weight substances (mg L^{-1}); HS = Humic-like substances (mg L^{-1}); LMWS = Low molecular weight substances (mg L^{-1}); E2:E3 = Relative size of DOM molecules; SUVA$_{254}$ = Specifiv ultraviolet absorption (L $(mg*m)^{-1}$)

Table 3. Pearson correlations (*r* values) of modeled respiratory quotients (RQs) and nutrients and dissolved organic matter quality characteristics (pooled data of four seasons and two streams per stream type). Four modeled RQ values outside the admissible range of variation were excluded. Significant correlations ($p < 0.05$) are highlighted in bold.

	Agricultural streams (n = 6)		**Forest streams (n = 5)**	
Independent variable	***r***	***p***	***r***	***p***
Dissolved organic carbon (mg L^{-1})	0.41	0.42	0.21	0.74
Dissolved nitrogen (mg L^{-1})	-0.06	0.91	0.34	0.58
Soluble ractive phosphorus (µg L^{-1})	0.62	0.19	0.66	0.22
PARAFAC component C1	-0.07	0.89	0.43	0.47
PARAFAC component C2	**0.92**	**0.01**	-0.21	0.74
PARAFAC component C3	-0.71	0.11	-0.53	0.36
Humification index	0.26	0.62	0.67	0.22
Fluorescence index	-0.36	0.48	0.1	0.87
Freshness index	-0.77	0.07	-0.81	0.10
High molecular weight substances (mg L^{-1})	0.74	0.09	-0.41	0.49
Humic-like substances (mg L^{-1})	-0.17	0.75	0.76	0.14
Low molecular weight substances (mg L^{-1})	0.16	0.76	-0.18	0.78
Relative size of DOM molecules	**-0.84**	**0.04**	0.57	0.31
Specifiv ultraviolet absorption (L $(mg*m)^{-1}$)	0.09	0.86	0.52	0.37

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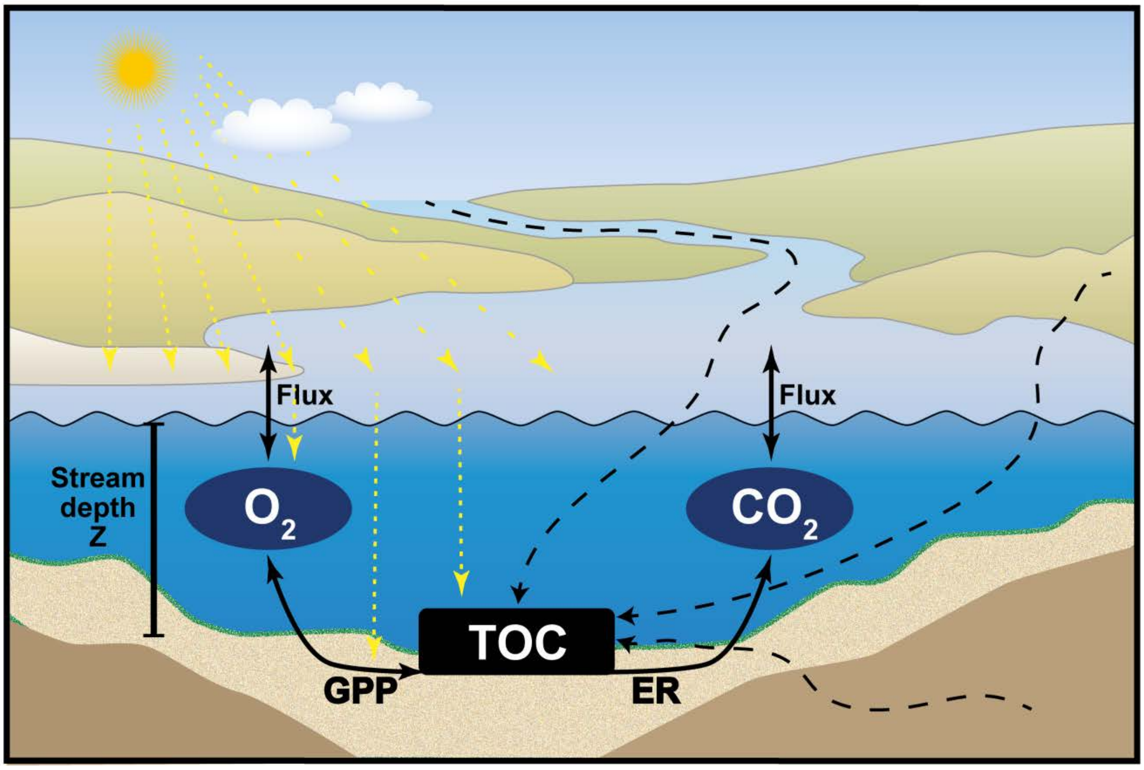
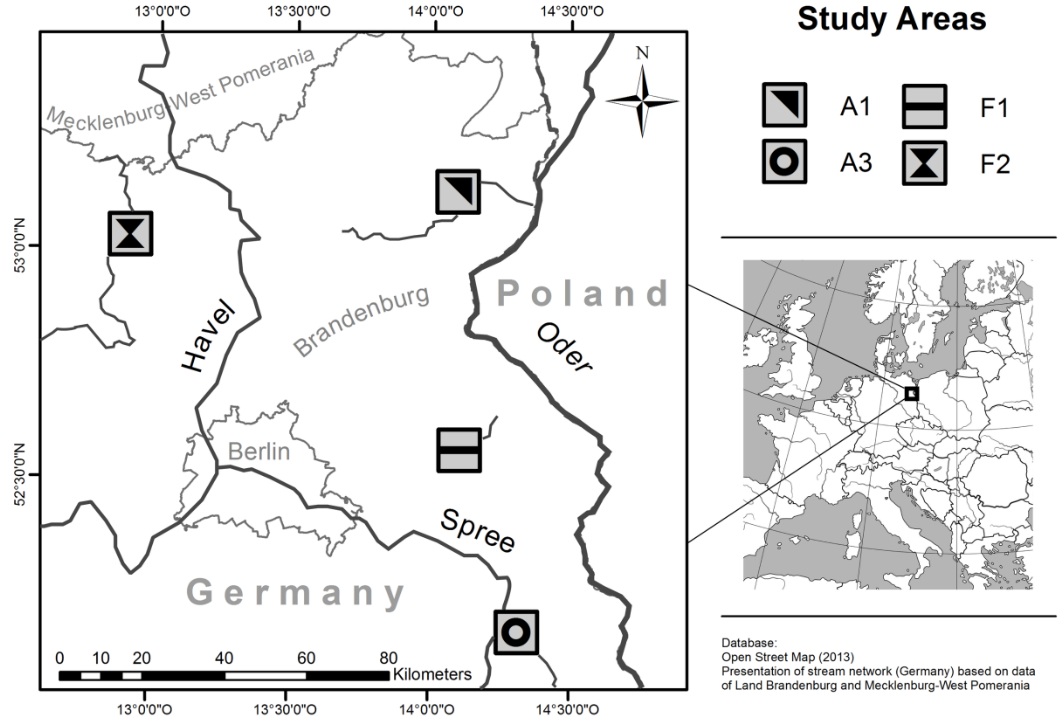


Figure 1. Conceptual model of in-stream metabolism processes implemented in the model equations. GPP = gross primary production, ER = ecosystem respiration, TOC = total organic carbon, Flux = interaction with the atmosphere of the respective gas.



Database:
Open Street Map (2013)
Presentation of stream network (Germany) based on data of Land Brandenburg and Mecklenburg-West Pomerania

Figure 2. Overview of investigated stream sections in North Germany; Forest streams (F1 & F2: Stöbber, Rhin) and agricultural streams (A1 & A3: Welse, Oelse)

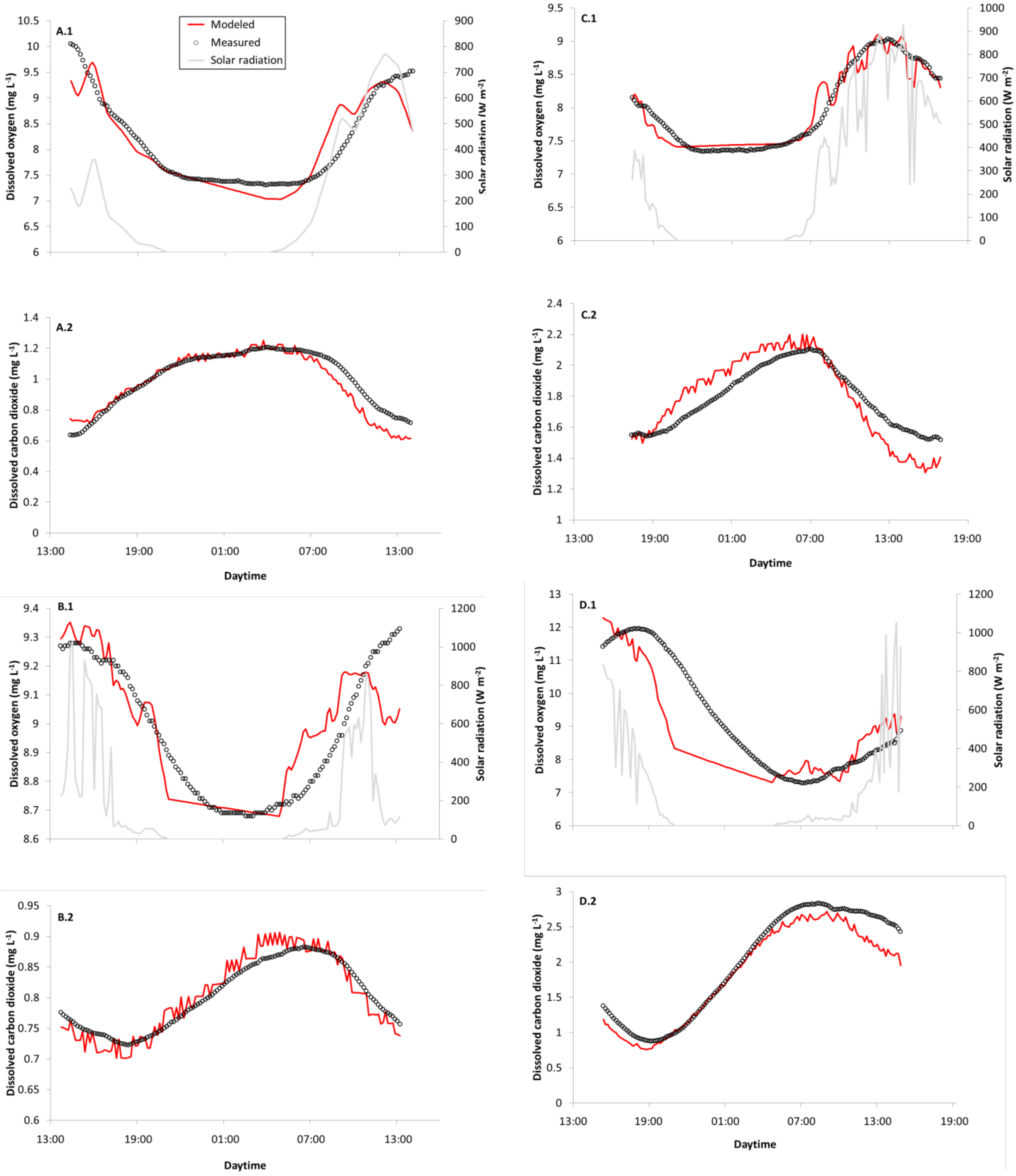


Figure 3. Modeled and measured dissolved oxygen (A.1 – D.1) and CO_2 dynamics (A.2 – D.2) in four stream sites for a selected 24h period in spring (time step 1/6 hours). A) and B) are forest streams (F2 and F1, respectively), B) and C) are agricultural streams (A1 and A2, respectively)

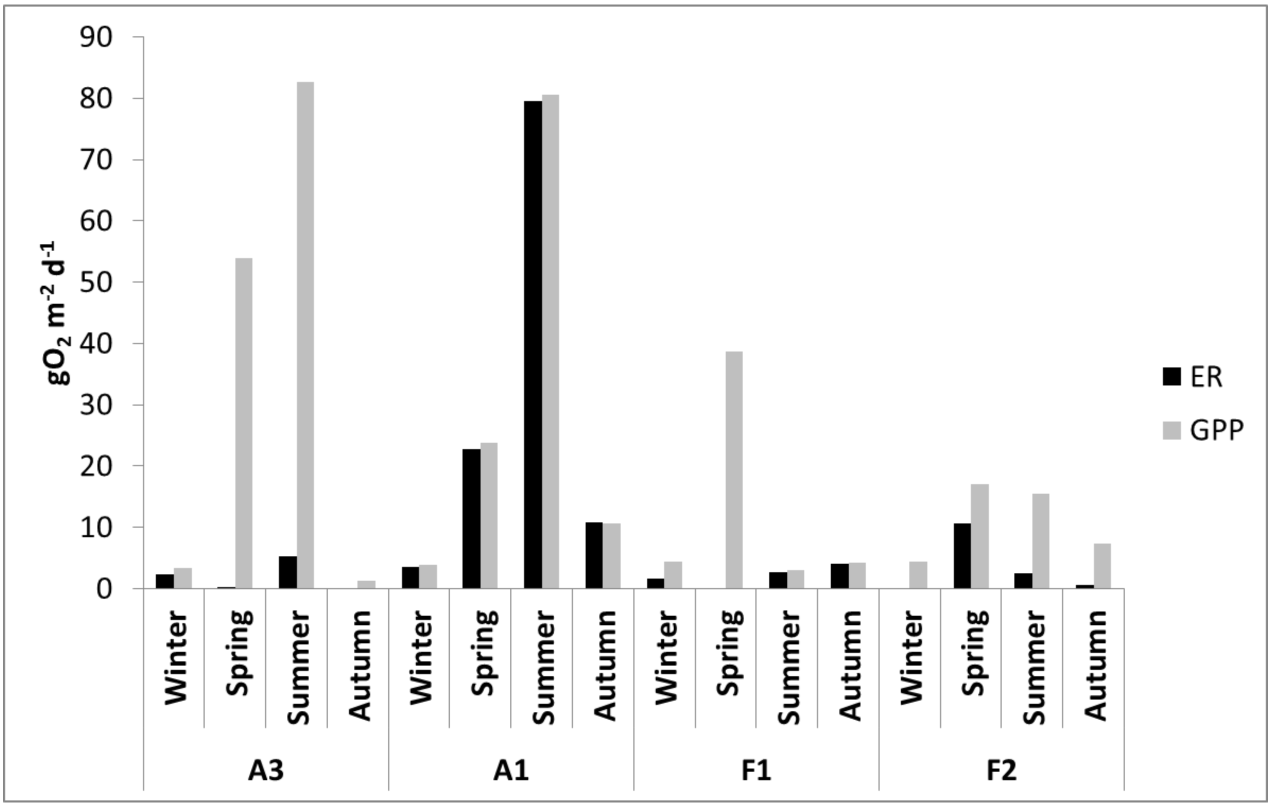


Figure 4. Calculated ecosystem respiration (ER) and gross primary production (GPP) for the four stream sites (A1 & A3, F1 & F2) in different seasons

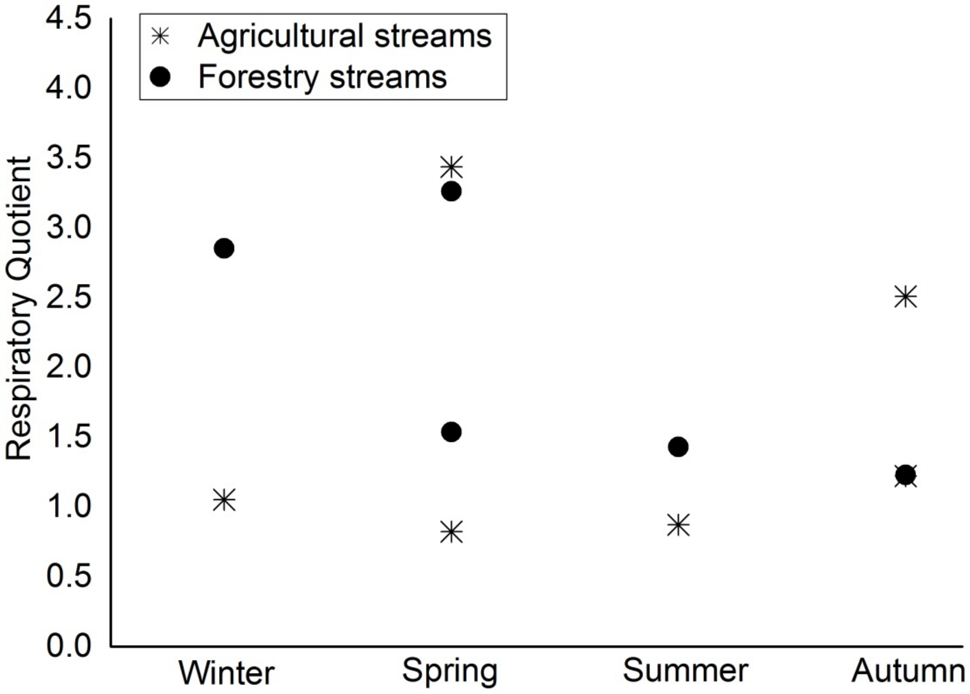


Figure 5. Modeled daily ecosystem respiratory quotients in different seasons and stream ecosystems

GENERAL DISCUSSION

Rationale

The underlying mechanisms with respect to organic matter (OM) turnover in streams, contributing to the often heterotrophic state of these systems, are not yet fully understood. Nevertheless, this understanding is crucial in order to manage and predict the role of streams in the carbon cycle on regional and global scales. There exist many studies about CO_2 dynamics in forest streams (Teodoru et al., 2009, Koprivnjak et al., 2010, Campeau and Del Giorgio, 2014), however similar studies in agricultural streams are rare. Furthermore, several studies focused on the microbial utilization (comprising either assimilation or respiration) of allochthonous and autochthonous OM (Cole and Caraco, 2001, Del Giorgio and Pace, 2008), which reveal different OM qualities (i.e. levels of bioavailability). Beside the traditional view that autochthonous dissolved organic matter (DOM) is better bioavailable for microorganisms (Del Giorgio and Pace, 2008), other studies show that allochthonous DOM can also support significant amounts of microbial respiration and assimilation (Berggren et al., 2010, Ward et al., 2013). These findings suggest that at least a part of the allochthonous DOM is highly bioavailable (Guillemette et al., 2013). In turn, this may have significant implications for the organic matter turnover for stream ecosystems which are dominated by allochthonous inputs (e.g. Fisher and Likens, 1973, Smock, 1997). Consequently, further investigations on different allochthonous DOM sources as well as DOM quality as potential drivers of OM turnover in stream ecosystems are needed.

Small scale: microbial usage of terrestrial DOM

Microorganisms are the major consumers of the DOM (Cole, 1999, Battin et al., 2008) and thus studying their OM degradation activities is key to the understanding of CO_2 dynamics in streams. Within an experimental approach, I compared the microbial utilization of two distinct allochthonous sources with distinct DOM quality characteristics (DOM_{leaf}: more labile and DOM_{peat}: less labile) mixed in different proportions (M I). Pre-tests have shown that DOM_{leaf} reveals a higher proportion of low molecular weight substances(LMWS) and lower proportion of high molecular weight substances, respectively, compared to DOM_{peat} (unpublished data, P. Bodmer). The results were striking: bacterial protein production (i.e. bacterial assimilation) and respiration intensity were higher with increasing proportion of labile allochthonous DOM (DOM_{leaf}), i.e. with increasing proportion of LMWS in the treatment. These results reveal the importance of DOM quality in carbon turnover as the DOM quantity was the same in all treatments. Furthermore, my results underline that there is a significant labile proportion in the terrestrial DOM which is likely to boost bacterial protein production and microbial respiration in stream ecosystems. The boosted microbial respiration by allochthonous DOM may contribute significantly to the heterotrophic state of most streams, leading to CO_2 emissions to the atmosphere (Cole and Caraco, 2001, Ward et al., 2013, M III). Thus, the small-scale mechanisms can be very important for large scale processes.

Furthermore, Guillemette et al. (2013) proposed a concept which illustrates the degradation dynamics of autochthonous (specified as algal) and allochthonous DOC in lakes (Fig. 1a). The autochthonous DOC is more rapidly degraded compared to the allochthonous DOC. However, at least a fraction of allochthonous DOC is highly reactive and processed on short time scales, while the rest is processed on longer time scales (Guillemette et al., 2013).

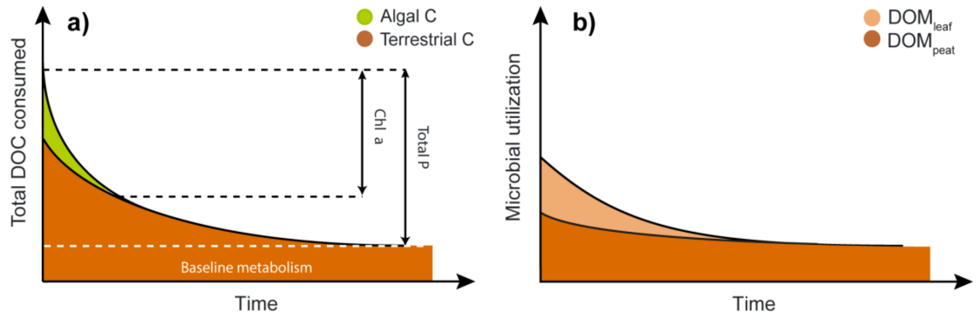


Figure 1. Schematic illustration according to Guillemette et al. (2013) (a) showing the degradation dynamics of authochthonous (specified as algal) and allochthonous DOC, and a modified illustration (b) showing microbial utilization dynamics of the two allochthonous DOM sources (leaf DOM and peat DOM) investigated in M I. There, I demonstrated that allochthonous DOM sources may reveal significant quality differences, indicated by the time and intensity in which they are microbially utilized.

Despite the lack of an autochthonous DOM pool in my experiment, my results underpin the proposed aspect of the allochthonous DOM in respect to its microbial utilization dynamics (Fig. 1b). This is demonstrated by the fact that bacterial protein production as well as the fraction of DOM$_{leaf}$ respired over time show initially high utilization rates that decline after a few days (M I). In contrast, the less labile DOM$_{peat}$ is microbially utilized much slower, making it a rather long-term DOM source. These results confirm that DOM quality is not exclusively controlled by its origin (Attermeyer et al., 2014). Consequently, the traditional division between autochthonous and allochthonous sources is too generic to predict the bioavailability of DOM. Instead, bioavailability may be the result of its molecular composition, i.e. DOM quality. From a chemical perspective, DOM can be understood as a supramolecular association of heterogeneous and relatively small molecules (Piccolo, 2001, Sutton and Sposito, 2005), including carbohydrates, amino acids and fatty acids (Allan and Castillo, 2007, Huber et al., 2011). Furthermore, polyphenols are ubiquitous in DOM from various environments (Cory and McKnight, 2005). These structural units are significantly affecting DOM quality since they play an important role in the stability of DOM in the environment by e.g. inhibiting microbial enzyme activity (Freeman et al., 2004). Aeschbacher et al. (2012) quantified phenolic properties of allochthonous and autochthonous DOM sources, revealing rather small differences. Consequently, the two sources primarily differ with respect to their amount of labile compounds. The depletion of labile fractions of DOM may be rationalized by DOM age (Amon and Benner, 1996) rather than by its source. Autochthonous DOM is freshly produced while allochthonous DOM is generally older and already more biogeochemically processed when it reaches the stream. However, DOM degradation depends always on the time scale. Microorganisms may generally consume what they get, the molecular size and other factors (such as nutirents or temperature) determine the process time. For instance, Koehler et al. (2012) investigated the first-order decay coefficient, as an indicator of microbial decomposition of DOC over 3.7 years in brown and clear water lakes. After an initial fivefold larger initial decay coefficient in clearwater lakes compared to brownwater lakes, indicating a better DOC quality of the former, the decay coefficients converged within five months (Koehler et al., 2012).

Mixtures of labile and less labile DOM sources, as performed in M I, allow the examination of the priming effect (PE). Briefly, the initially soil-derived concept describes that the supply of labile OM can stimulate the mineralization of the less labile OM (Kuzyakov et al., 2000). This concept has been increasingly discussed as a potential mechanism in aquatic environments (Guenet et al., 2010, Bianchi, 2011). However, the evidence for an aquatic priming effect is currently under debate. While some studies report a positive priming effect (Kuehn et al., 2014, Steen et al., 2015, Stutter and Cains, 2015), others do not find significant evidence (Bengtsson et al., 2014, Catalan et al., 2015). My results support the latter ones, none of the mixtures of labile (DOM_{leaf}) and less labile DOM (DOM_{peat}) led to an increase in bacterial protein production, respiration or bulk DOC consumption (M I). Hence, I come to the same conclusion as Catalan et al. (2015) and Bengtsson et al. (2014), that priming in freshwater systems may be of limited importance. However, I cannot exclude that the applied methods were not sensitive enough to capture the PE. For instance, the specific isotopic labelling of low and high molecular weight substances of different DOM sources may provide deeper insights into their microbial utilization.

Increasing complexity: from small-scale to stream-reach scale

Next to the experimental approach, the measurements of OM quality and its impact on carbon dynamics in running waters in situ are crucial to understanding their role in the carbon cycle. However, reliably measuring CO_2 fluxes (M III) from the water to the atmosphere with minimal measuring bias in the field is a huge challenge. Common approaches such as measuring or approximating the transport coefficient by tracer injections and hydromorphological parameters, respectively, provide often only one or few general transport coefficients in time (Alin et al., 2011, Wallin et al., 2011). Additionally, the required CO_2 concentrations in the water and in the atmosphere are often measured only one to two times per day (Teodoru et al., 2009, Halbedel and Koschorreck, 2013), but thereafter used to calculate daily CO_2 emissions and upscale to regional or even global estimates. Thus, it is important to develop and test new methods that are efficient, reliable and easily applied in the field. Flux chambers, for example, provide a powerful tool to measure CO_2 emissions multiple times per day at different locations and consequently get a more realistic value with respect to CO_2 emissions. However, an appropriate deployment approach of the flux chambers is crucial. The results of M II clearly showed that anchored chambers enhance turbulence under the chambers and thus elevate fluxes, while drifting chambers have a very small impact on the water turbulence under the chamber and thus generate more realistic fluxes. Therefore, the results from this study will pave the way for more realistic CO_2 emission estimates from running waters which are known to contribute to a large proportion to total freshwater CO_2 gas emissions (Raymond et al., 2013).

The combination of drifting chamber measurements with continuously measuring autonomous CO_2 sensors applied in M III provides an extremely powerful measuring combination, which has not been applied in streams so far. Both, the transport coefficient (unpublished, P. Bodmer) and the CO_2 concentration in the water change during the day, therefore CO_2 fluxes also change during the day (Fig. 2; from M III). These diurnal variations cannot be caught with single measurements and one can thus assume that most up-scaled daily and annual estimates for CO_2 emissions from running waters to date are biased and should be re-evaluated on a broader scale. Thus, the provided daily means of CO_2 fluxes in M III calculated from multiple measurements during the day are much more representative compared to studies which apply methods that cannot be replicated so often over time.

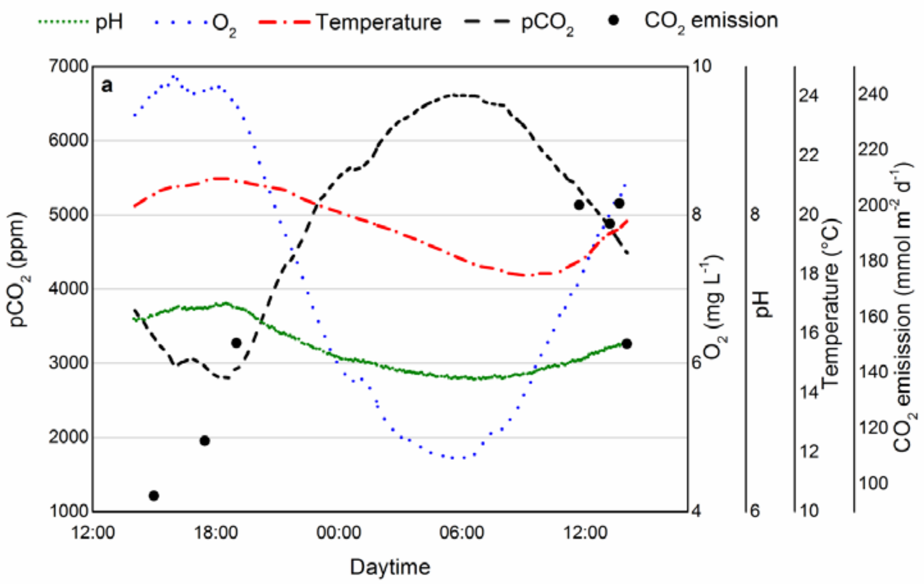


Figure 2. Diurnal dynamics of key parameters measured in an agricultural stream during the summer sampling (M III). The combination of different methods and sensors provide a sound picture of the interplay among pH, oxygen (O_2), water temperature, partial pressure of CO_2 and CO_2 emission to the atmosphere. Such combined continuous/repeated measurements provide a powerful tool to enhance the understanding of CO_2 dynamics in stream ecosystems.

Another challenge moving from the laboratory to field studies is certainly the increasing complexity. As described in the introduction, there are multiple factors influencing OM turnover in running waters that also interact with each other. In the field, they all come together and consequently, it is difficult to disentangle the effect of single influencing factors. In the performed field study, I found a linkage between different DOM quality indicators and measured pCO_2, which was however superimposed by nutrients and DOC concentrations (M III). Nevertheless, I found a linkage between apparent molecular size and pCO_2 across agricultural and forest streams, indicating higher pCO_2 while larger DOM molecules were present (M III). Despite the much more complex environment compared to the laboratory experiment (M I), I found in both studies that the DOM quality characteristic molecular size/weight may be a potential influencing factor with respect to OM turnover. However, agricultural streams were generally characterized by higher nutrient concentrations such as dissolved nitrogen as well as higher DOC concentration compared to forest streams. My analysis revealed that these parameters (nutrients and OC quantity) were, in addition to DOM quality, the main drivers of the significantly higher pCO_2 in agricultural streams compared to forest streams (M III).

The findings of M III and M I with respect to molecular size can be put into context of the size-reactivity continuum model proposed by Amon and Benner (1996) (Fig.3). Briefly, the model which is originally based on seawater bioassays, describes the major pathway of OM degradation as a continuous change from bioreactive (i.e. labile) macromolecules to small less labile (i.e. “stable”) organic molecules (Amon and Benner, 1996).

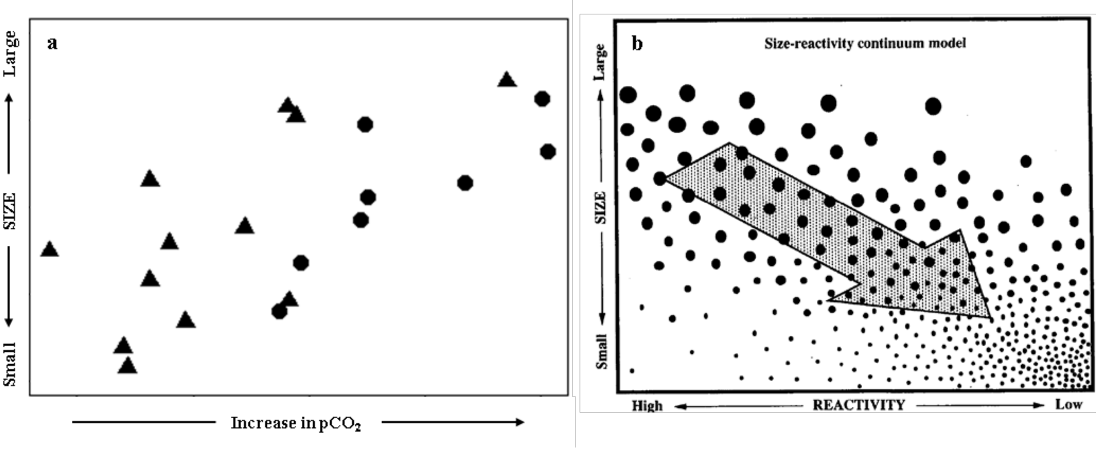


Figure 3. My findings of M III compared to the findings of Amon and Benner (1996) revealing implications of molecular size of DOM as quality indicator. I found a linkage between pCO_2 and relative size of DOM across agricultural (dots) and forest (triangles) streams (a), while Amon and Benner (1996) proposed a the size-reactivity continuum model, linking molecular size of DOM to biological reactivity (b). My findings underline the importance of molecular size DOM as quality indicator, although my results can be interpreted in a contradictive manner compared to the size-reactivity continuum model proposed by Amon and Benner (1996).

My results of M III in relation to the size-reactivity continuum model can be interpreted in two different ways. As the first interpretation, the high pCO_2 in the presence of large DOM may indicate that the large DOM is more bioreactive compared to the small DOM which consequently results in higher respiration products (i.e. pCO_2). However, since I measured only a snapshot of the molecule size and pCO_2, a second interpretation may be that the large DOM molecules remained and the small DOM molecules were used for respiration. The results of that laboratory experiment (M II) support the latter interpretation, showing that mainly low molecular weight substances (i.e. small molecules) were utilized by microbes, which is in line with Kaiser and Sulzberger (2004). In general, molecular size/weight of DOM played a role in all applied approaches in my thesis (M I, III, IV), underlining the importance of this DOM quality characteristic. However, besides molecular size, also the diagenetic state (i.e. age) of DOM is an important quality characteristic which is also included in the size-reactivity continuum concept (Amon and Benner, 1996, Benner and Amon, 2015) but not addressed in my thesis. Amon and Benner (1996) state that DOM becomes less bioavailable with increasing age. Consequently, the assessment of DOM age could extend my so far applied DOM quality assessments, e.g. by measurements of the natural radiocarbon ($\Delta^{14}C$) (McCallister et al., 2004, McCallister and del Giorgio, 2012).

From reality to modeling: enhanced understanding of stream metabolism mechanisms

In order to enhancing the understanding of basic mechanisms with respect to measured diurnal CO_2 and O_2 dynamics in streams (M III), I developed a process-based model (M IV). Short-term diurnal dynamics of CO_2 have been studied in streams (Dawson et al., 2001, Johnson et al., 2010, Dinsmore et al., 2013), while long-term O_2 measurements in streams are an established method to calculate ecosystem metabolism (Uehlinger and Naegeli, 1998, Uehlinger, 2000, Acuña et al., 2004). However, continuous measurements of CO_2 and O_2 in running waters are rare (Johnson et al., 2010) and to my knowledge not yet extensively performed in agricultural streams. Consequently, the performed continuous measurements of diurnal O_2 and CO_2 dynamics in several agricultural and forest streams in different seasons are scientifically very valuable (M III). Furthermore, O_2 dynamics in streams have been modeled (Uehlinger et al., 2000, Birkel et al., 2013, Riley and Dodds, 2013), whereas CO_2 modeling approaches are rare (Dinsmore et al., 2013). Thus, the presented process-based model, simulating diurnal O_2 and CO_2 dynamics in two agricultural and forest streams in four seasons is innovative and enhances the mechanistic understanding of carbon processes in stream ecosystems (M IV). Although models similar to that presented in M IV are simple representations of complex phenomena, they provide powerful tools for testing and enhancing our understanding of carbon dynamics in stream ecosystems. Especially if coupled with field measurements, a well-conceptualized process-based model is ecologically very valuable. For instance, it can replace or complement long-term or permanent in situ measurements which are time, money and labor intensive. Moreover, the model parameters can be manipulated in order to predict certain future scenarios. A further advantage of such models is the possibility to simulate processes which cannot be directly measured, such as the contribution of microbubbles to methane emissions from the water to the atmosphere (McGinnis et al., 2015).

From parallel O_2 and CO_2 measurements and modeling, respectively, respiratory quotients (RQ) can be calculated. Since RQ describes the mole of CO_2 produced per mole of O_2 consumed, it can be related to the quality of respired substrates (Berggren et al., 2012). In the presented model approach in

M IV, I linked the RQ in the underlying differential equations. Thus I was able to calculate daily ecosystem RQs of agricultural and forest streams in different seasons. These calculations are extremely valuable to accurately express ecosystem metabolism parameters (ER and GPP) in terms of carbon for whole aquatic ecosystems, which is so far often done with an RQ of one (McCallister and del Giorgio, 2012, Brothers et al., 2014). Again, I was able to show that RQ can be linked to DOM quality, such as the molecular size of DOM or a fluorescing component characteristic for plant material in agricultural streams. As already shown in M I and II, this finding underlines again the importance of DOM quality for stream ecosystem functioning and deepens the understanding of carbon turnover in stream ecosystems.

Conclusions

To summarize, the results presented in this dissertation indicate that DOM quality, especially molecular size/weight is an important driving factor in respect to carbon turnover in stream ecosystems (M I, III, IV). It is linked to microbial metabolism at the small scale (M I), to pCO_2 in agricultural and forest streams (M III), as well as the modeled RQ in agricultural streams at stream-reach scale (M IV) (Fig. 4). In addition, the assessment and application of the powerful drifting chamber method provides a technical improvement in order to constrain CO_2 fluxes of running waters (M II). Those results pave the way for future studies on greenhouse gas emissions and carbon quality parameters to better disentangle and project carbon dynamics in streams. Finally, this thesis leads towards an improved mechanistic understanding of carbon turnover in stream ecosystems and indicates DOM quality as its potential driver (Fig. 4).

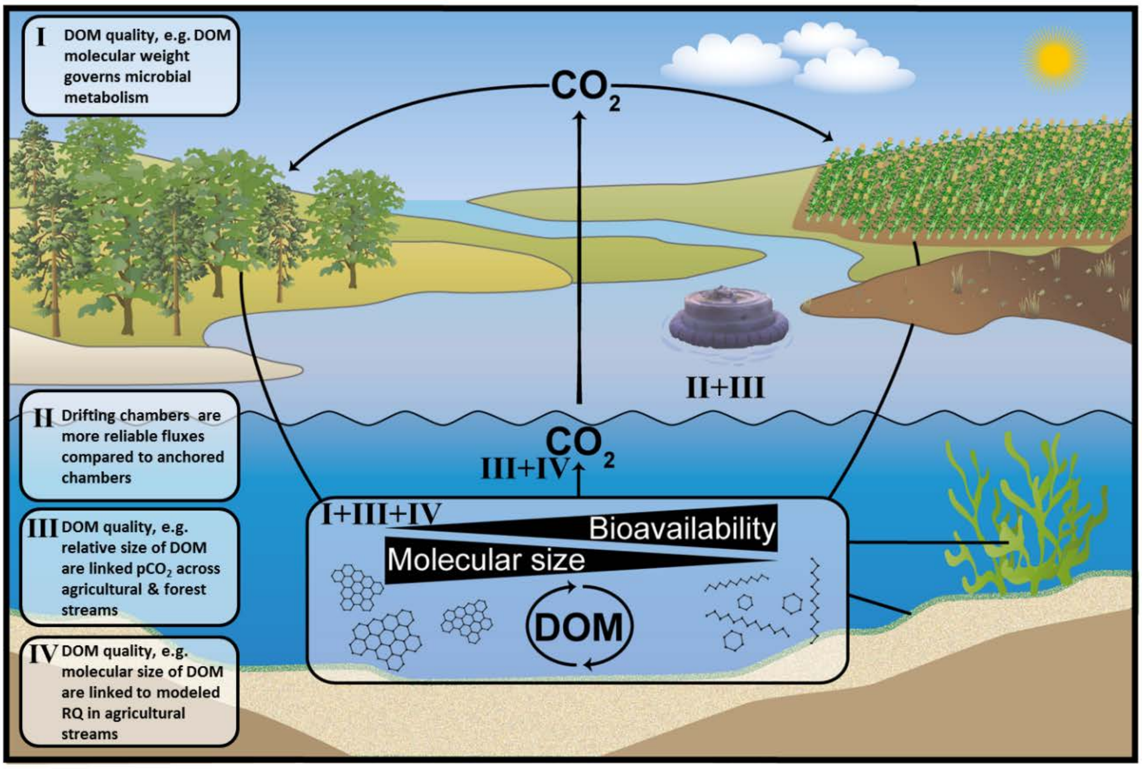


Figure 4. Summary scheme of the thesis and selected main messages from the four manuscripts (roman numbers) presented in this thesis. Abbreviations: DOM = dissolved organic matter, RQ = Respiratory quotient, pCO_2 = partial pressure of CO_2.

Future perspectives

Continuous measurements of multiple parameters such as shown in figure 2 demonstrate the possibilities of the latest autonomous sensor developments. For instance, a recent review of Meinson et al. (2015) revealed the broadening of the applications of continuous and high-frequency measurements in lakes at the spatial scale as a major future challenge, which can easily be transferred to stream ecosystems. A long-term instrumentation of a stream system with e.g. autonomous CO_2/O_2/temperature sensors may allow capturing spatial and temporal dynamics of theses parameters up to the catchment scale. Additionally, these measurements could be coupled with high-frequency measurements of entire UV absorption spectra (e.g. spectro::lyser sensor, scan Messtechnik GmbH, Austria), allowing the investigation of the DOM quality influence on CO_2 dynamics by optical proxies on the landscape scale. Furthermore, such a powerful setup may enable to capture “hot moments” (referring to McClain et al., 2003) such as episodic storm events, which may be missed by regular samplings but are crucial in order to understand carbon dynamics in stream ecosystems. Referring to a recent study of Krause et al. (2015) which discusses conceptual, technological and methodological challenges for real-time ecohydrological research, the approach above is an example of real-time biogeochemistry in a stream system. Such research approaches are especially relevant in the context of climate change. The assumptions and present observations reveal for example an increase in heavy rain events, storms and floods (IPCC, 2013) which will dramatically affect organic matter turnover and cycling in e.g. streams on the global scale. Thus, a better understanding of carbon dynamics in streams and more reliable and robust models will help to improve predictions of future climate change.

However, in order to understand large scale patterns of carbon dynamics in streams within the landscape, it is crucial to identify small-scale mechanisms. In my thesis, I investigated DOM quality as a potential driver of carbon dynamics in streams. Besides the presented DOM quality assessments, there are further possibilities to analyze the DOM quality in greater detail. So far, I mainly investigated the influence of DOM structure on microbial processes. Optical indicators (i.e. absorbance and fluorescence) as well as the liquid size-exclusion chromatography in combination with UV- and IR- organic carbon detection applied in this thesis (M I, III and IV) provide already powerful tools to characterize DOM in this sense. However, there exists novel high-resolution geochemical tools, such as ultrahigh-resolution mass spectrometry via Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS; e.g. Stenson et al., 2002, Stenson et al., 2003), which allows to determine elemental compositions of thousands of DOM compounds directly out of mixtures. Consequently, this method may bring extensive new insights into the molecular composition of DOM (Herzsprung et al., 2012). Moreover, it is also possible to combine the fluorescence measurement derived excitation emission matrices with FT-ICR-MS, providing a new tool to assess CDOM molecular properties (Herzsprung et al., 2012). Such approaches may provide the opportunity to investigate how chemodiversity, i.e. molecular diversity of DOM (Kellerman et al., 2014) influence smicrobial metabolism or microbial community composition. In conclusion, it is crucial to examine DOM turnover from different perspectives and to combine different methods, to achieve the full picture of DOM turnover in stream ecosystems.

STATEMENT OF ACADEMIC INTEGRITY

I hereby certify that the submitted thesis “Linking carbon dynamics in stream ecosystems to dissolved organic matter quality” is my own work, and that all published or other sources of material consulted in its preparation have been indicated. I have clearly pointed out any collaboration that has taken place with other researchers, and stated my own personal share in the investigations in the Thesis Outline. I confirm that this work has not been submitted to any other university or examining body for a comparable academic award.

Berlin, 22.12.2015

Pascal Bodmer

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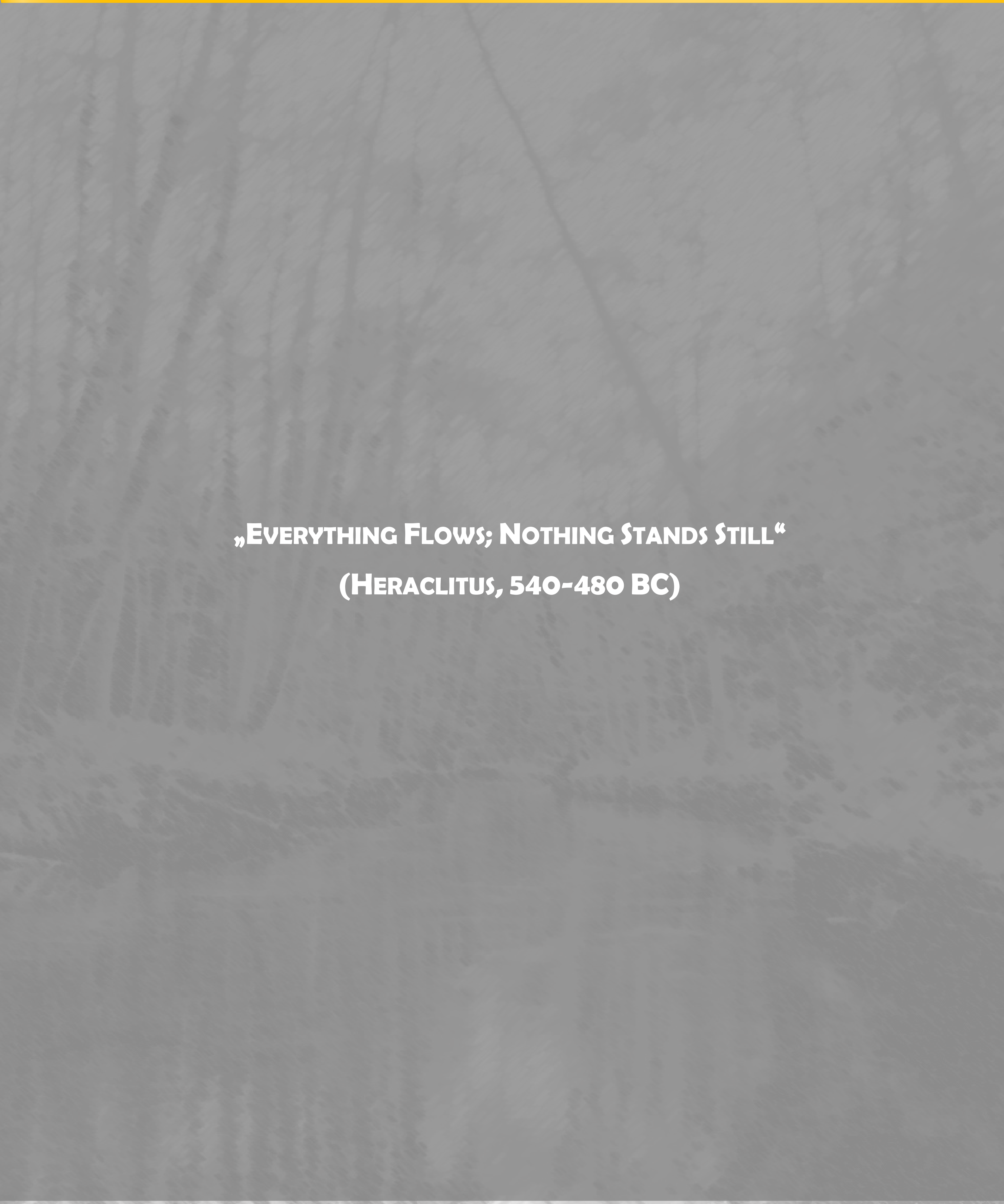

„Everything Flows; Nothing Stands Still“

(Heraclitus, 540-480 BC)