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„Interactions between dissolved organic matter (DOM) and
bacteria in a river-floodplain system.“

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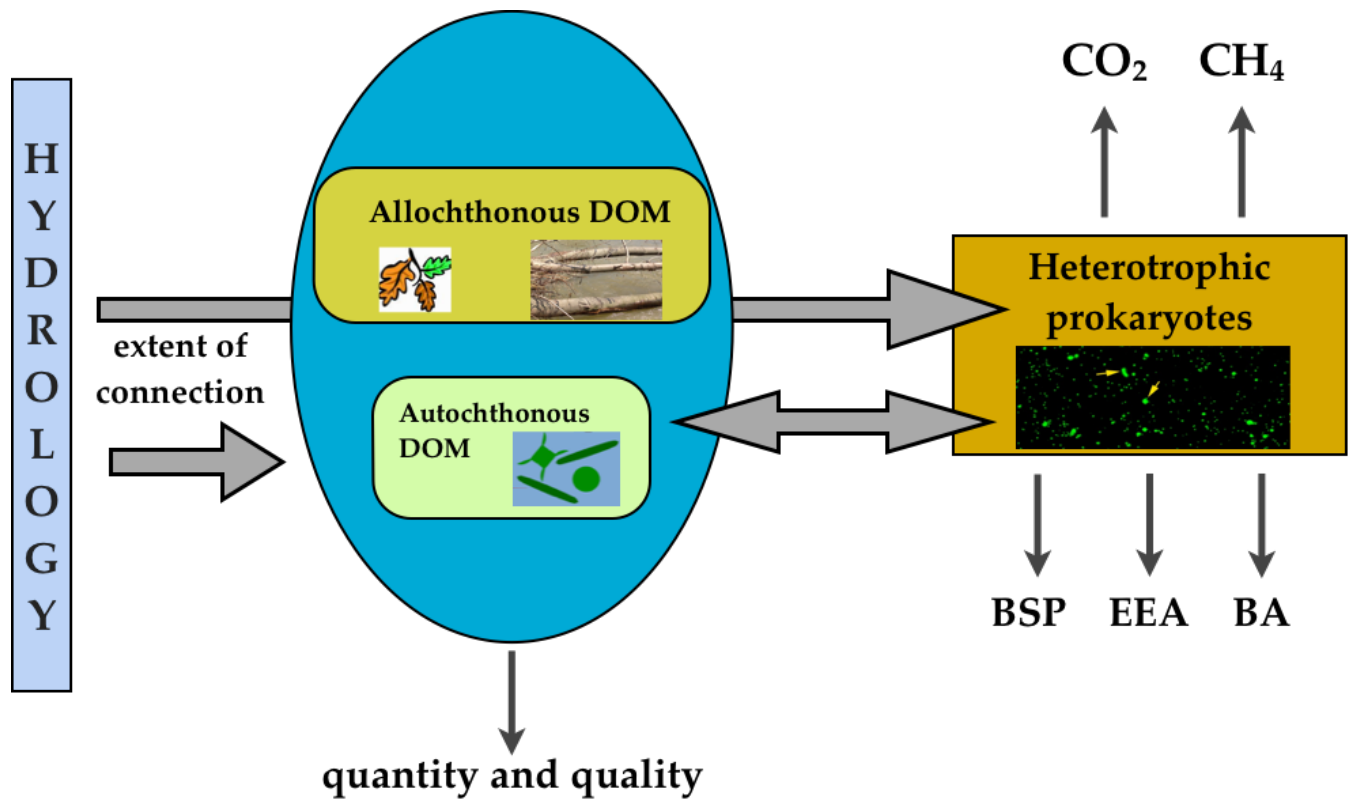


Fig. 1 Main pools, processes and interactions investigated in the Doctoral thesis: dissolved organic matter (DOM), bacterial secondary production (BSP), bacterial abundance (BA), extracellular enzymatic activity (EEA)

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1. Summary

River-floodplain systems are among the most diverse and productive ecosystems on earth. High hydrological stochasticity, which allows for recurrent changes from lentic to lotic habitats, is the main driving force in these systems. Hence, periodical floods have major consequences for functioning of river-floodplains. Due to prolonged residence time of water, river-floodplains also act as biogeochemical hot spots, what highlights their considerable role in the inland water carbon cycle. Despite their importance the majority of river-floodplains, especially in Europe, have been significantly altered by human activity, what carries consequences for DOM cycling. Hence studying the hydrological changes and the effect of human impact is essential to understand functioning of these systems.

In my Doctoral thesis I focused on the role of hydrology as a key aspect shaping composition of the DOM pool and microbial DOM processing in different subsystems of the river-floodplain system. My aim was also to highlight the role of river-floodplains in inland water C flux and their significance as a source of CH₄. I applied two different, but complementary approaches to study changes in DOM quality. With spectroscopic measurements, I identified that flooding altered DOM quality significantly and introduced allochthonous, refractory and aromatic DOM. However with the application of extracellular enzymatic activity (EEA) measurements, I discovered that flood-imported DOM was bioavailable and could be utilized relative quickly. I propose that EEA measurements can be used as a tool to track availability of non-chromophoric DOM during prompt hydrological changes, which otherwise would be overlooked when using only traditional spectroscopic measurements.

Another focus was on the post-flood period, which created opportunity to utilize less reactive, terrestrial DOM delivered to the floodplain. This highlights the significance of floodplain waters for processing refractory DOM (RDOM). Enhanced activity of enzymes degrading RDOM was enabled in the presence of fresh labile material derived from primary production. Thus, another aim was to investigate the EEA response to photosynthetic extracellular release (PER) in different subsystems. The results demonstrated that EEA measurement is a valuable tool to elucidate the link between non-chromophoric, algal-derived DOM and bacterial secondary production (BSP). I demonstrated that even in closely located waters, but with

different connectivity, BSP was fueled by DOM derived from distinct sources. I revealed that in a disconnected section with high allochthonous load, autochthonous sources supported BSP.

Furthermore I showed that the type and magnitude of hydrological connectivity carried major consequences for dynamics of CH₄. The results pointed to disconnected subsystems as the strongest sources of CH₄ in the river-floodplain. However during surface connection the river served as an “exhaust pipe” for the CH₄, produced in highly isolated habitats. Additionally, the magnitude of the flood was identified as factor, which affected CH₄ fluxes significantly.

My findings contribute to the current knowledge on DOM-microbial interactions in temperate river-floodplain systems and emphasize the crucial role of hydrology. They also suggest that ongoing disconnection of these systems will have consequences not only for the floodplain, but also for the river carbon cycle.

2. Introduction

2.1 River-floodplain systems in the inland water carbon cycle

River-floodplain systems cover from 0.8 to 2×10^6 km² globally (Gardner et al. 2015; Mitsch and Gosselink 2000), however, their entire and actual area is difficult to assess (Whigham et al. 1993). This uncertainty derives from the ability of “active” floodplains to largely expand their aquatic surface during periodic flooding events. An active floodplain is described as a terrestrial area, which is flooded at least once in 100 years (Tockner and Stanford 2002). Although globally the area covered by river-floodplains is relatively small, they are crucial in functioning of river systems. Due to their unique features, they are considered as model ecosystems to study the effects of environmental heterogeneity on ecosystem functioning (Tockner et al. 2010). The changing mosaic of terrestrial and aquatic habitats provides high productivity and species diversity, which shifts dynamically depending on hydrological stochasticity. Beside high species richness, river-floodplains are considered as biogeochemical hot spots and also “second chance” places for utilization of riverine organic matter (Battin et al. 2009), which makes these systems important in the inland water carbon cycle.

As described by the microbial loop model (Azam et al. 1983), heterotrophic microorganisms are essential in decomposition of organic matter and nutrient cycling. The role of extracellular enzymatic activity (EEA), which enables utilization of highly variable and composite dissolved organic matter (DOM), is a crucial process in remineralization of carbon. Aerobic and anaerobic microbial respiration is mainly responsible for supersaturation of floodplain waters with two major greenhouse gases (GHG): CO₂ and CH₄, which are then subsequently emitted to the atmosphere (Bastviken et al. 2008; Bianchini Jr et al. 2010). However, photosynthetic uptake of CO₂ or methanotrophy, which act as GHG sinks, are responsible for great fluctuations in the water column, leading to under-saturated conditions, especially regarding CO₂. This results in high inter-annual variability in CO₂ and CH₄ concentrations (Teodoru et al. 2015), when river-floodplains serve as either sinks or sources of GHG. The significance of inland waters in the global carbon cycle has been recognized (Cole et al. 2007) and studied in various aquatic ecosystems, which include not only rivers (Butman and Raymond 2011; Hotchkiss et al. 2015) and streams (Raymond et al. 2013) but also lakes (Cole et al. 1994), reservoirs (Barros et al. 2011), estuaries (Abril and Borges 2005) and some coastal waters (Bange 2006). The numerous

attempts to quantify global C evasion from inland waters resulted in a broad range, from 0.75 (Cole et al. 2007) to 3.3 Pg y⁻¹ (Aufdenkampe et al. 2011) with a recent emphasize on the role of small streams (2.1 Pg C yr⁻¹) (Raymond et al. 2013). The importance of river-floodplains has also been recognized. However, the recent estimate of global C evasion “focuses on the river network only and **ignores the contribution of floodplains** to the total water-air gas exchange” (Lauerwald et al. 2015). Nevertheless, the significance of large river-floodplains, such as the Amazon (Melack et al. 2004; Sawakuchi et al. 2014) or the Pantanal (Marani and Alvala 2007), as important spots of gas evasion has been raised (Richey et al. 2002). Although most of the research focused only on these large tropical systems, some attention was also placed on gas fluxes from arctic floodplains (Van Huissteden et al. 2005). However, the emission of GHG from European, temperate river-floodplain systems, has often been overlooked and their importance underestimated. Although some estimates included temperate floodplains, they focused mainly on Northern American areas (Aufdenkampe et al. 2011). Further, few research recognized the contribution of European wetlands and watercourses as sources of CH₄ (Saarnio et al. 2009) and CO₂ (Armentano and Menges 1986; O'Shea et al. 2014). However, these estimates carry large uncertainty (Kirschke et al. 2013) and they are poorly quantified due to incomplete temporal and spatial coverage.

Compared to tropical, pristine river-floodplains, European systems cover a relatively small area, however they possess distinct features that can affect gas fluxes significantly. A great part of European rivers (~75%) has been severely impacted by human activity, which resulted in modifications of their flow and disconnection of numerous floodplains from their adjacent rivers. Such anthropogenic alterations have inevitable consequences for the floodplain carbon cycle, affecting gaseous fluxes to the atmosphere (Perga et al. 2016). Hence, the numerous anthropogenic changes have altered the inputs of carbon from soils to inland waters and the strength of the air–water gas exchange (Regnier et al. 2013). In this light there is necessity to investigate and include temperate, human-impacted river-floodplains into global estimates of C emissions.

2.2 Importance of hydrology in river-floodplain systems

Hydrological connectivity is the main factor shaping numerous processes in river-floodplain systems. The variable riverine discharge regimes, characteristic for river-floodplains, are responsible for transformations of lentic floodplain waters into lotic systems during the flood events. Depending on the magnitude and duration of spates, they shift from biologically to mainly hydrologically controlled systems (Hein et al. 2003; Preiner et al. 2008). The flood pulse concept introduced by Junk et al. (1989) emphasized the importance of floods as a crucial force, which drives river-floodplain systems and maintains them in a dynamic equilibrium. However, the stochasticity and frequency of such events has considerable impact on various biotic and abiotic floodplain processes and interactions. The floodplain habitat heterogeneity depends on expansion-contraction events, induced by pulsing of the river discharge, which changes terrestrial habitats of an “active” floodplain into aquatic areas (Tockner et al. 2000).

2.2.1 Role of hydrology for DOM heterogeneity

The lateral exchange of the river with the adjacent floodplain is the main factor controlling the delivery and composition of DOM (Peduzzi et al. 2008). Hence, the heterogeneity and origin of DOM depends on the hydrological connectivity, when the floodplain serves either as a source or a sink for this material. The main river can be an important source of autochthonous material, which emphasizes the contribution of local primary production for riverine food webs (Thorp and Delong 2002). According to the recent River Wave Concept (Humphries et al. 2014), which describes functioning of the system as a wave, the spatial and temporal contribution of riverine or floodplain allochthonous and autochthonous material depends on the position of the wave. The floodplain serves as sources of allochthonous and autochthonous DOM for the main channel (Moreira-Turcq et al. 2013) during the crest of the wave. However, during the troughs of the waves, storage and transformation of the material prevails, when local autochthonous and allochthonous input is of higher importance. Accordingly, the allochthonous upstream input of DOM becomes most important during the rising and falling water level, hence ascending or descending limbs of river waves. Therefore, hydrological events affect the magnitude of DOM release and

retention within the system (Butturini et al. 2016). Such complexity and dynamic hydrological changes in river-floodplains generate a heterogeneous and composite mixture of DOM. Typically, DOM is comprised of organic compounds with various molecular weight and reactivity (Amon and Benner 1996). However, only 10 ~ 25% of DOM present in aquatic systems, including river-floodplains, consists of currently identified compounds of known structure (i. e. carbohydrates, proteins), while the rest of the DOM pool (75 – 90%) falls into a broad category of fulvic and humic acids (Thurman 1985). They are often environmentally altered (i. e. by UV radiation), have composite structure (i. e. contain aromatic rings), and derive from residue of plant, bacterial, fungal or other origin. However, depending on the hydrological phase and the strength of connectivity between river and its floodplain, the quality of DOM shifts substantially. Flooding events deliver high amounts of polymeric, high molecular weight organic matter (Fasching et al. 2015), which is typically considered as refractory, hence difficult to be processed by heterotrophs. Due to soil-derived DOM, brought by flood waters from upstream, river DOM consists of substantially higher share of humic substances compared to proteins (Nguyen et al. 2010). However, a significant increase in organic matter bioavailability during the floods has been reported as well (Stepanauskas et al. 2000), what implies that allochthonous DOM, delivered with inflow events, may be more available than previously thought (Hitchcock and Mitrovic 2015). During the post-flood period, when primary production is higher (Hein et al. 1999; Hein et al. 2004; Schagerl et al. 2009), the share of autochthonous, more labile DOM is larger. The productivity, which is stimulated by short pulses followed by lentic periods (Schiemer et al. 2006), is a substantial source of high quality DOM with greater protein content (Hu et al. 2016). Additionally, after the flooding, during more stagnant conditions, the DOM undergoes UV degradation, which changes its structure and availability. Such DOM complexity in river-floodplains carries great challenges to study its composition. However, recent advances in fluorometric spectroscopy (Murphy et al. 2013) allow for improved characterization of DOM. Fluorescence excitation/emission matrix (EEM) spectroscopy combined with multivariate data analysis (PARAFAC) has been applied to identify fluorescent components of DOM in river-floodplains (Hertkorn et al. 2015). With this method it is possible to characterize dynamic changes in floodplain allochthonous and autochthonous DOM. However, only a fraction of the DOM pool can be optically characterized, hence this technique possesses major drawbacks. Thus,

with the application of spectroscopic techniques a substantial part of the non-chromophoric DOM pool is entirely overlooked. This calls for searching for supplementary techniques to assess DOM quality, especially during sudden hydrological changes.

2.2.2 Microbial utilization of DOM and the role of hydrology

Most of the organic material (up to 96%) present in aquatic ecosystems, including floodplains, is in the form of large, high molecular weight and complex compounds (Perdue and Ritchie 2003), which cannot be transported easily through membranes into the bacterial cell. In order to make these substrates available, they first must be hydrolyzed outside the cell into smaller components by extracellular enzymes (EE). Hence, also in river-floodplain systems DOM composition is one of the keys that restricts microbial utilization of this material, and acts as an important control mechanism for bacterioplankton growth. The metabolic fate of DOM is shaped by its quantity and quality, when prokaryotes act either as link or sink for DOM, thus either transferring DOM to higher trophic levels or mineralizing organic C, what results in CO₂ and CH₄ production (Sanseverino et al. 2012). Typically, autochthonous, phytoplankton-derived DOM is labile and rapidly exhausted, whereas allochthonous, semi-labile and recalcitrant material requires enzymatic degradation. However, products of photosynthetic extracellular release (PER) often are of polymeric structure (Myklestad 2000) and also require enzymatic degradation. Hence, the synthesis of EE may be induced not only by high amounts of composite DOM (polysaccharides, proteins), which are released due to algae senescence (Chróst 1992b), but also during the earlier stages of a phytoplankton bloom (Riemann et al. 2000).

The process of EE expression is complex and controlled by various environmental factors (Cunha et al. 2010). Hence, increase in EEA can be governed by nutrient limitation, which prompts cells to produce enzymes to enable acquirement of the limiting nutrient (Hoppe 1983). The optimal resource allocation strategy describes the stoichiometry of EE, which is regulated by up and down enzyme expression in order to fulfil microbial growth requirements (Allison and Vitousek 2005; Sinsabaugh and Follstad Shah 2012). Except biochemical regulation (availability of limiting nutrients) (Hill et al. 2006) and temperature (Baltar et al. 2016), in rivers also hydrology is

reported to be an important factor, which influences EEA (Bodmer et al. 2016; Romani et al. 1998; Romani and Sabater 1999). Especially the flooding events may affect the hydrological-biogeochemical linkage, thus inhibiting or promoting enzymatic activity. However, depending on duration and magnitude of the flooding, the microbial EEA response to different DOM delivered to the floodplain, may be shifted. High amounts of terrestrial DOM transferred during such seasonal disturbance events may foster EEA, thus increasing microbial metabolism (Chróst and Riemann 1994). Because flood-introduced, allochthonous DOM possesses a high carbon content (Hein et al. 2003), it is likely that it serves as a better substrate for carbohydrate than protein degrading EE. Prompt enzymatic response of glucosidases, shortly after the flood, emphasizes the importance of short spates for DOM utilization (Burns and Ryder 2008). Also, high proteolytic activity has been associated with flooding event, however usually its peak is linked with phytoplankton blooms with the highest activity after/during the post-flood period (Chróst 1991; Karrasch et al. 2003). Therefore it is essential to track not only the variability of C and N-acquiring EE, but also account for dynamic changes in their activity, which carries the crucial information on DOM utilization patterns.

The examination of EEA patterns, especially in such stochastic systems like river-floodplains, delivers vital information on the dynamically changing composition of DOM. However, knowledge about EEA in temperate river-floodplains is still fragmentary, especially for periods during and after seasonal flooding. While the EEA dynamics have been investigated and described in many aquatic ecosystems (Chróst 1992a), their functioning under hydrological stress remains largely unclear.

2.2.3 Role of hydrology for greenhouse gases dynamics

River-floodplain systems are considered not only as sinks and hot-spots for DOM transformation, but also as sources of carbon to the atmosphere. Due to their high potential for aerobic or anaerobic processing of organic matter, they release substantial amounts of the two major greenhouse gases, CO₂ and CH₄. Recently, increasing concern has been placed on emissions of CH₄ from natural sources, because this greenhouse gas possesses 25 times higher warming potential than CO₂. Especially, the contribution of wetlands, including floodplains is substantial on a global scale; they are considered the largest natural sources of CH₄ (Wang et al.

1996). Moreover, the fluctuations in CH₄ outgassing from wetlands are responsible for global interannual variability of CH₄ emissions (Ciais et al. 2014). In this light it is crucial to define the main drivers for CH₄ evasion from these systems. Beside temperature (Dinsmore et al. 2013), which affects respiration and gas emission rates, hydrological variability is also a key factor, which controls dynamics of CH₄ in river-floodplains (Gatland et al. 2014). Hence, flooding events play a crucial role in the exchange of gases between water, land and atmosphere, thus affecting also CH₄ flux (Louis et al. 2000). Sudden and substantial emission of CH₄ may occur with the onset of the flood, temporarily altering gaseous dynamics in temperate (Boon et al. 1997) and subtropical (Gatland et al. 2014) wetlands. There is evidence that the emissions of CH₄ are highest during spates (Borges et al. 2015), however the effect of the flooding magnitude, especially during extreme incidents, is overlooked. In particular, this may be important in the light of a global change, such as predicted rise in discharge for major rivers (Van Vliet et al. 2013) and increasing frequency of flooding episodes (Lehner et al. 2006). This calls for studies on the consequences of increasing stochasticity of spates, because such alterations will inevitably have consequences for CH₄ flux on a global scale.

Understanding the magnitude and variability of CH₄ evasion from floodplains requires also recognizing the importance of temporarily flooded areas. Occasionally inundated zones may serve as a substantial source of gases for the river during the flooding (Abril et al. 2014), supporting evasion from the entire river-floodplain systems. Hence, river-floodplains can be responsible for large carbon fluxes from land to water and to atmosphere (Mayorga et al. 2005). Furthermore, the frequency of inundation of terrestrial systems may control rates of gaseous emissions (Altor and Mitsch 2006) and the emission may also depend on the duration of inter-flooding intervals (Sánchez-Andrés et al. 2010). Therefore, a critical step is to account for the effect of pulsing hydrology as a main driver for high temporal variability in CH₄ dynamics in temperate river-floodplain systems.

3. Objectives of the Thesis

3.1 Main aim and sampling approach of the Doctoral thesis

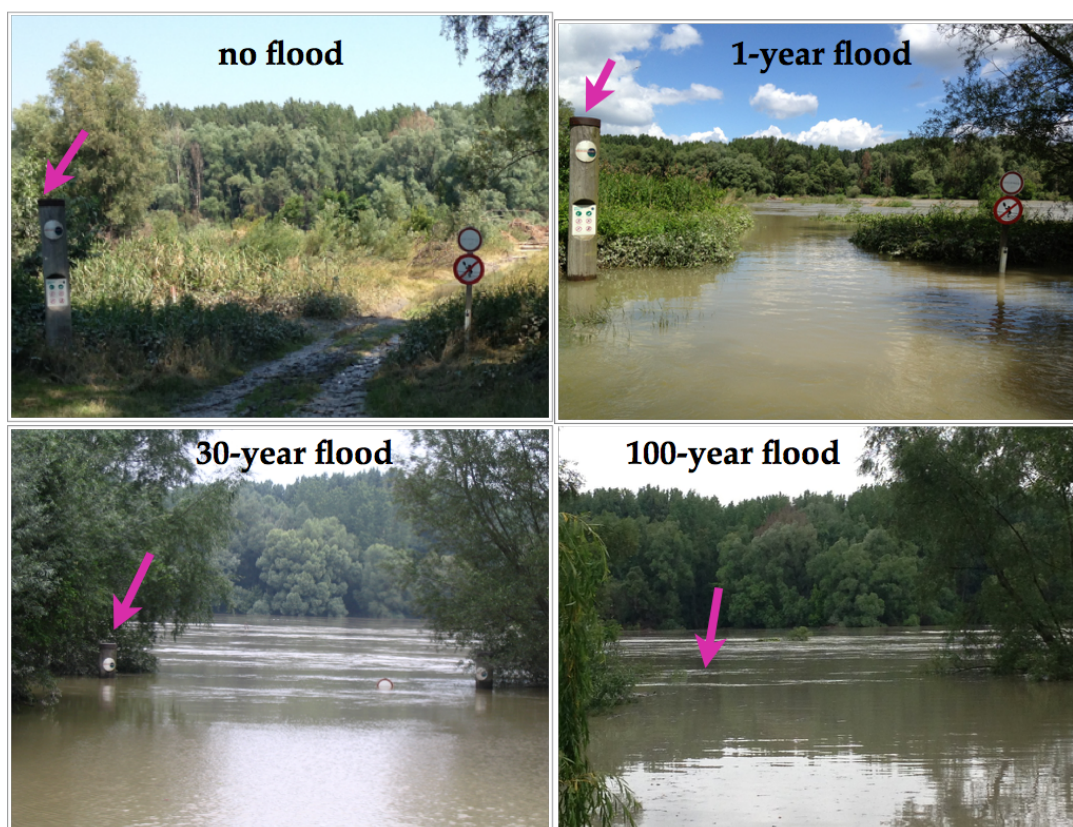


Fig. 2 Typical floodplain site during different hydrological situations.

The main objective of my Doctoral thesis was to understand the effect of variable hydrology on the microbial response to quantitative and qualitative changes in the DOM pool in a temperate river-floodplain system. An important goal was to investigate if floodplains and their hydrological connectivity with the main channel could contribute to local and global CH_4 emission and carbon flux. The aim was also to explain the consequences of severing the connection and isolating the river from its floodplain.

One of the goals was to investigate whether floodplain backwaters show highly variable DOM, due to mixing of long distance transported dissolved organic carbon (DOC) versus autochthonously produced DOM, and local input of terrestrial

DOM. I tested if such heterogeneity of carbon sources could significantly influence microbial metabolism in floodplain waters with different surface connectivity (**Paper I&II**). Another aim was to investigate whether freshly introduced terrigenous DOM during seasonal floods, although more refractory, could stimulate microbial processes when exposed to changed physical and biological settings. I tested if in “active” i.e. dynamically connected river-floodplain systems conditions allow for significant utilization also of allochthonous carbon introduced from flood pulses (**Paper I**). The third major focus was on the significance of connectivity and magnitude of flooding as important driving factors for C-remineralization. I investigated whether floods, differing in magnitude, have a distinct impact on concentration and evasion of CH₄. The objective was to emphasize the role of floodplains as CH₄ sources compared to the main channel (**Paper III**).

To study the above outlined scientific questions, an area within the Danube Floodplain National Park (DFNP) downstream of Vienna (Austria) was chosen. This area was selected due to its heterogeneity in surface connection to the main channel, reflecting the different levels of human impact. Parts of DFNP exhibit restored, semi-natural conditions, where frequent connection of floodplain waters exists, whereas other sections are strongly impacted by human activity and almost entirely disconnected from the river. Also, due to seasonal flooding, this area possesses an excellent opportunity to investigate effects of changing hydrology on various microbe-DOM interactions. In the restored section, two water bodies were selected, which are connected ~ 220 days per year to the main channel, hence they often experience direct through flow. In the regulated section, five water bodies were selected, which display a system of backwaters with a gradient of connectivity to the river, from ~180 days per year to a completely disconnected station. More detailed description of the investigated area is provided in the respective papers. The aim of the sampling program was to cover variable hydrological situations, which are representative for this river-floodplain system. Hence, the sampling schedule was established in accordance with the hydrological stochasticity. The selected locations were routinely sampled on a biweekly basis, however during characteristic hydrological situations such as flooding, retention periods with algal blooms, low water periods or leaf fall, the program was intensified (up to daily intervals). With this approach I was able to cover a broad range of diverse hydrological situations, which included typically occurring 1-year flood, but also 30- and 100-year flooding events.

3.2 Specific aims of Paper I

River-floodplain systems are susceptible to hydrological changes, which include rapidly occurring flooding events as well as long-lasting low water levels. Such incidents have major consequences for the functioning of river-floodplain system, however their effect on composition of DOM and related microbial processes is not clearly deciphered so far. Typically, changes in quality of DOM are investigated with spectroscopic techniques, which relate optical properties of DOM to its chemical composition. However this approach has its disadvantages such as overlooking the highly reactive and non-chromophoric pool of DOM. In Paper I, I investigated the effect of flooding on composition of DOM and related microbial processes. To explore changes in DOM quality, additionally to spectroscopic analyses, the extracellular enzymatic activity approach was applied. With this approach I investigated whether allochthonous DOM, introduced with the flood event, was reactive and susceptible to microbial metabolism.

3.3 Specific aims of Paper II

River-floodplains are hydrologically controlled during the flood, however they become biologically regulated systems after the spates. The phase after a flood is typically characterized by elevated primary production, which usually provides substrate preferential for bacterial metabolism. Hence in Paper II, I focused on the post-flood period and investigated the response of microbial extracellular enzymatic activity to phytoplankton productivity. The emphasis was on the importance of phytoplankton extracellular release in different subsystems of the river-floodplain. I investigated if in subsystems, usually dominated by allochthonous DOM, autochthonous DOM could be a main substrate for bacterial growth.

3.4 Specific aims of Paper III

Although flooding has also pronounced effect on concentration and evasion of greenhouse gases, its importance has been mainly studied in large river-floodplain systems such as the Amazon or Pantanal. However, the stochasticity of hydrology, as a major determinant of emissions of GHG, from human-modified European river-floodplains is largely unknown. In Paper III, I studied the effect of hydrological events with different magnitude on concentration and evasion of CH₄ from floodplain waters with different surface connectivity to the Danube. The focus was to compare CH₄ evasion from floodplain waters with the main channel. I also discussed how varying anthropogenic alterations of river-floodplain sections might affect emissions of CH₄.

4. Paper I

Sieczko A, Peduzzi P (2014) *Origin, enzymatic response and fate of dissolved organic matter during flood and non-flood conditions in a river-floodplain system of the Danube (Austria)*. Aquatic Sciences 76:115-129. doi: 10.1007/s00027-013-0318-3

My contribution to this study was designing the field campaign, performing the entire field sampling and processing the samples in the laboratory. Furthermore, I interpreted the results, completed statistical analysis and wrote the manuscript as a first author.

Origin, enzymatic response and fate of dissolved organic matter during flood and non-flood conditions in a river-floodplain system of the Danube (Austria)

Anna Sieczko · Peter Peduzzi

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Abstract Spectroscopic techniques and extracellular enzyme activity measurements were combined with assessments of bacterial secondary production (BSP) to elucidate flood-pulse-linked differences in carbon (C) sources and related microbial processes in a river-floodplain system near Vienna (Austria). Surface connection with the main channel significantly influenced the quantity and quality of dissolved organic matter (DOM) in floodplain backwaters. The highest values of dissolved organic carbon (DOC) and chromophoric DOM (CDOM) were observed during the peak of the flood, when DOC increased from 1.36 to 4.37 mg l⁻¹ and CDOM from 2.94 to 14.32 m⁻¹. The flood introduced DOC which consisted of more allochthonously-derived, aromatic compounds. Bacterial enzymatic activity, as a proxy to track the response to changes in DOM, indicated elevated utilization of imported allochthonous material. Based on the enzyme measurements, new parameters were calculated: metabolic effort and enzymatic indices (EEA 1 and EEA 2). During connection, bacterial glucosidase and protease activity were dominant, whereas during disconnected phases a switch to lignin degradation (phenol oxidase) occurred. The enzymatic activity analysis revealed that flooding mobilized reactive DOM, which then supported bacterial metabolism. No significant differences in overall BSP between the two phases were detected, indicating that heterogeneous sources of C sufficiently support BSP. The study demonstrates that floods are important for delivering

DOM, which, despite its allochthonous origin, is reactive and can be effectively utilized by aquatic bacteria in this river-floodplain systems. The presence of active floodplains, characterized by hydrological connectivity with the main channel, creates the opportunity to process allochthonous DOC. This has potential consequences for carbon flux, enhancing C sequestration and mineralization processes in this river-floodplain system.

Keywords Dissolved organic matter · Bacteria · Extracellular enzymatic activity · Floodplain

Introduction

River-floodplain systems often have a broad range of lotic, lentic and semi-aquatic habitats with high species richness (Ward and Stanford 2006). Such environmental diversity creates opportunities for numerous linkages between the aquatic and terrestrial ecosystem and promotes interactions between abiotic factors and biotic processes. These couplings have received insufficient attention, particularly the linkage between the hydrological cycle and the carbon cycle (Battin et al. 2008). In particular, floodplain backwaters are seldom included in calculations of global carbon flux; they play a significant role in organic matter cycling and carbon mineralization, sequestration and transport (Battin et al. 2008).

One of the dominant forms of organic material in aquatic environments, including floodplains, is dissolved organic matter (DOM). DOM is a heterogeneous mixture of molecules, some of which originate from the surrounding area (allochthonous DOM), while others are generated within the system (autochthonous DOM). Allochthonous input of organic material is considered to be the dominant

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substrate supply for microorganisms in inland waters (Tranvik and Jansson 2002). Most DOM of terrestrial origin is believed to enter streams (Royer and David 2005), tropical rivers (Wiegner et al. 2009) and floodplains (Peduzzi et al. 2008) during seasonal floods and storms.

Bacterial utilization of DOM can be highly variable, depending on the lability of the organic matter. In rivers (del Giorgio and Pace 2008) and floodplains (Mladenov et al. 2007), bacterial metabolism may be supported by two different pools: quickly cycling DOM together with more slowly utilized semi-labile DOM. The highly labile pool, typically linked to phytoplankton development, is often rapidly exhausted. In contrast, the semi-labile pool appears to be strongly coupled to terrestrial DOC, which increases after flood events (del Giorgio and Pace 2008). This implies that aquatic food webs are coupled to watershed inputs of organic carbon (Pace et al. 2004). Some lake carbon cycles are reported to be heavily subsidized by organic carbon from the surrounding landscape (Carpenter et al. 2005; Cole et al. 2011). The importance of terrestrial material for bacterial metabolism has been emphasized in boreal headwater streams (Berggren et al. 2009), temperate lakes (Cole et al. 2006; Carpenter et al. 2005; Pace et al. 2004), oligotrophic humic lakes (Daniel et al. 2005) and estuaries (Van den Meersche et al. 2009).

Aquatic heterotrophic bacteria may act as links that transfer organic carbon to higher trophic levels, and/or sinks that mineralize DOM, resulting in CO₂ production. Numerous studies have coupled bacterial respiration to allochthonous sources, underlining that little of the terrestrial C is passed to higher trophic levels (Cole et al. 2002; Kritzberg et al. 2005; Karlsson et al. 2007). Other evidence, however, suggests that autochthonous organic matter may be significantly respired as well (McCallister and Giorgio 2008). Among the many environmental factors (nutrient availability, temperature, photochemical impacts) (McCallister et al. 2005; Apple et al. 2006) that influence substrate partitioning between biomass generation and respiration, also seasonal disturbance events (floods, storms etc.) clearly affect bacterial processes. Flood pulses deliver substantial amounts of bioavailable DOM that may stimulate bacterial metabolism, for example in tropical rivers (Wiegner et al. 2009), streams (Ågren et al. 2008) and lakes (Lennon and Cottingham 2008). These sudden inputs of different carbon sources may increase microbial productivity (Docherty et al. 2006), respiration and rates of biogeochemical cycles (Kritzberg et al. 2005). Hydrological connectivity appears to be a crucial driving force in river-floodplain systems as well, influencing the quantity and quality of DOM and the related microbial processes (Schiemer et al. 2006; Mladenov et al. 2007; Peduzzi et al. 2008). In such highly variable water bodies, DOM bioavailability and the fate of utilized DOM, i.e. its partitioning into bacterial catabolic and anabolic processes,

remains largely unresolved. Therefore, the factors determining DOM bioavailability in river-floodplain systems need to be better understood, as does the balance between anabolic and catabolic processes.

Losses and changes of organic matter due to microbial degradation have been assessed in several ways. One approach applies spectroscopic techniques to characterize and trace DOM dynamics (McKnight et al. 2001; Weishaar et al. 2003). This method relates the optical properties of DOM to its chemical composition, thus inferring DOM bioavailability. Nonetheless, characterizing DOM based solely on absorbance and fluorescence techniques has drawbacks. These include overlooking the highly reactive and quickly cycling DOM pool rapidly metabolized by microbial activity and also the non-chromophoric DOM. When faced with diverse DOM sources, bacteria produce a broad array of enzymes (Chróst 1991). Therefore, techniques that measure extracellular enzymatic activity (EEA) link bacterial activity to DOM composition and allow carbon sources and utilization patterns to be examined more closely (Foreman et al. 1998; Romaní et al. 2006). This approach may be especially valuable to study the bacterial response to rapidly released DOM during floods (Burns and Ryder 2008). We combined spectroscopic and enzymatic approaches, together with bacterial secondary production (BSP), to elucidate flood-pulse-linked differences in C sources and the related microbial processes. First, we asked whether surface connection with the main Danube channel significantly influenced DOM quantity and quality in floodplain backwaters. We then explored whether flooding mobilized a significant fraction of the semi-labile DOM from the catchment, which could then promote bacterial metabolism. We also attempted to understand whether metabolized DOM resulted more in catabolic or more in anabolic processes in relation to DOM origin and hydrological changes in river-floodplain systems. The focus was on the importance and availability of allochthonous DOM for microbial utilization.

Methods

The Danube is one of the longest rivers in Europe. The river and its floodplain characteristics are described in more detail in Heiler et al. (1995) and Schiemer et al. (1999a). Our investigation was conducted in the main channel of the Danube (I) and two sections of the “Danube Floodplain National Park” near Vienna, Austria (Fig. 1). Although the entire river segment is strongly impacted by regulation, it represents one of the very few remaining semi-natural river-floodplain systems in central Europe. It offers a broad spectrum of river-floodplain connections from strongly dammed sites to areas exhibiting a high degree of hydrological connectivity. Within this floodplain

Fig. 1 Study site: main channel of the Danube and its floodplains downstream of Vienna (side-arm system of Lobau and Regelsbrunn). Circles and numbers indicate sampling stations

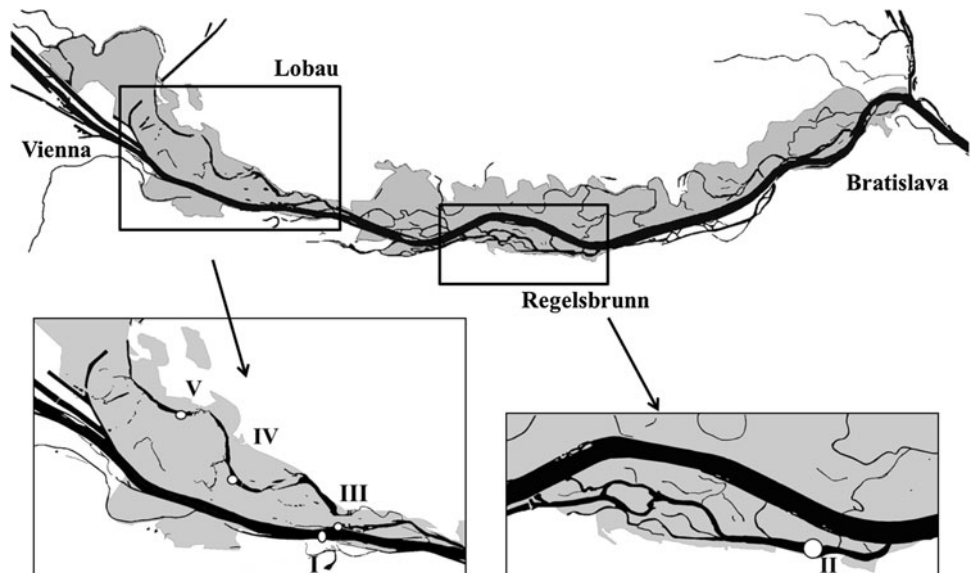


Table 1 Yearly average surface hydrological connectivity and distance from main stem for the study locations (II, III, IV, V)

Station	Location	Distance from the connection point with the main stem of the Danube (m)	Average yearly surface connectivity to the Danube (days)
II	Mannsdorfer Hagel	50	138
III	Regelsbrunn	300	220
IV	Kühwörther Wasser	820	18
V	Mittelwasser	1,680	18

area, four stations were selected. They display diverse hydrological characteristics representing a gradient of connectivity to the main channel (Table 1). Two dynamically connected side arms were chosen: one (II) located in section more isolated from the main channel (called Lobau), another (III) situated in recently reconnected section (Schiemer et al. 1999b). At a high water level in the river, both locations experience direct through-flow, changing the conditions from disconnected to connected. Stations designated as IV and V (Fig. 1) are located farther from the main channel (Table 1) in a semi-separated section of the floodplain; they are severed from direct through-flow by a levee and additional weirs. This results in connection to the Danube only at high flood (approx. 18 days per year, Federal Waterway Agency). During previous years, an average flood lasted for ~10 days and usually occurred in May–June (Tockner et al. 1999; Hein et al. 2003; Peduzzi et al. 2008); the years when no flood occurred were also recorded (Preiner et al. 2008).

Since the investigated backwaters are relatively shallow pools (0.63–1.5 m) and usually well mixed by wind and

currents (Hein et al. 1999), each water body was sampled at a selected point known to be representative for the respective area. Surface water samples were collected on 16 dates from May–October 2009, typically monthly but intensified during changing hydrological situations (e.g. flood events, receding water level) to 2–3-day intervals. Water level and temperature were measured in situ at each location. The water samples were taken with rinsed polyethylene bottles (10 l) and transported to the laboratory within 3 h in dark boxes at in situ temperature. Three nitrogen compounds—nitrate, ammonia and dissolved organic nitrogen (DON) and soluble reactive phosphorus (SRP)—were determined within 24 h of collection. The water samples were analyzed following German standard methods for the examination of water: nitrate: DIN 38405-29, ammonia: DIN 38406-5, SRP: DIN EN ISO 6678. DON was calculated by subtracting ammonia from soluble Kjeldahl-N (Peduzzi et al. 2008). The method DIN 38406-5 was applied to obtain soluble Kjeldahl-N.

Hydrology

The hydrological status was described by applying an “extent of connection” parameter (EC), defined as the deviation in meters from the level of connection [m] between the main channel and the respective floodplain section (Peduzzi et al. 2008). The degree of connectivity was expressed as the difference in height between the water level of the river at the time of sampling and a given side arm-specific topographic level at which inflow conditions are established. The amount of riverine water input into the floodplain section or the degree of isolation is a function of EC, which is related to the Danube water level. This value can be either positive (surface connection established to

some degree), zero or negative (no surface connection). The EC-values of four locations (II, III, IV, V) were tested for significant differences in water level between connected and disconnected phases ($p < 0.001$). Hence, the measured parameters were split into 3 data sets: (1) data collected from stations II, III, IV and V during surface connection with the main river and (2) data obtained from stations II, III, IV and V when no surface connection existed (henceforth termed connected and disconnected, respectively). The third data set contains only Danube data obtained from the whole sampling period.

Dissolved organic matter properties

Dissolved organic matter quantity and quality was analyzed by filtering water through pre-combusted (500 °C, GF/F, 0.7 µm, pore size) filters within 3 h of collection. DOC concentration [mg l^{-1}] was obtained by high-temperature combustion using a Shimadzu TOC 5000 analyzer (Benner and Strom 1993), and the ratio DOC/DON was determined. The optical properties of DOM were investigated to obtain proxies for DOM quantity and quality. The absorption coefficient at 350 nm (a_{350}) was chosen to describe changes in CDOM quantity (Kowalczyk et al. 2003; Para et al. 2010). The a_{350} [m^{-1}] was obtained using the relationship: $a_{350} = 2.303 A(350 \text{ nm})/L$, where L is path length (0.01 m). To obtain an aromaticity index of DOC, carbon specific UV absorbance (SUVA_{254}) was calculated. SUVA_{254} [$\text{l mg}^{-1} \text{m}^{-1}$] is the ratio of absorption at 254 nm to the DOC concentration and is positively correlated with DOC aromaticity (Weishaar et al. 2003). To classify the origin of DOM, the fluorescence index (FI), proposed by McKnight et al. (2001), was determined with a Shimadzu spectrofluorophotometer RF-5301 PC. FI is the ratio of the emission intensity at 450 to 500 nm under excitation at 370 nm. The index allows distinguishing DOM originating from microbial sources (including algal derived) from terrestrially derived DOM. An endpoint index value of ~ 1.3 indicates allochthonously produced DOM, whereas an index of ~ 2.0 implies autochthonously derived DOM. This method was already successfully applied in Danube waters (Peduzzi et al. 2008).

Bacterial parameters

All bacterial measurements were performed on unfiltered samples. Bacterial abundance (BA) was determined from the formaldehyde-fixed water sample (1–2 ml), which was stained with 4,6-diamidino-2-phenylindole-dihydrochloride (DAPI) ($10 \mu\text{g ml}^{-1}$); bacterial cells were enumerated by epifluorescence microscopy (Nikon E 800) in 20–30 randomly chosen fields according to (Porter and Feig 1980) with a minimum of 500–600 cells counted

(Noble and Fuhrman 1998). Bacterial secondary production (BSP) was measured based on the incorporation of [^3H] thymidine (20 µl, 0.1 mCi ml^{-1} final concentration) in three replicate samples; two formaldehyde-killed samples served as controls (Fuhrman and Azam 1982). BSP [$\mu\text{gC l}^{-1} \text{h}^{-1}$] was assessed using a Danube-specific conversion factor of 3.2×10^{18} cells produced per mol of thymidine incorporated (Berger et al. 1995).

The activity of three hydrolases: α -, β - glucosidases and leu-aminopeptidase (EEAa, EEAb, EEAlau, respectively) was calculated using fluorogenic substrates: 4-methylumbelliferyl (MUF)- α -D-glucoside, 4-MUF- β -D-glucoside and 4-methyl-7-coumarinylamide (MCA)-leucine; final concentration 0.5 mmol. Fluorescence (excitation at 360 nm, emission at 444 nm) was measured with a spectrofluorophotometer (Shimadzu RF-5301 PC) at (t_0) and after incubation in the dark (t_1) at in situ temperature and results were calculated as nmol substrate hydrolyzed per hour (Hoppe 1993). Phenol oxidase (PhOx) activity was determined using L-3,4-dihydroxyphenylalanine (L-DOPA) as a substrate (Pind et al. 1994). Two ml of unfiltered sample water and MilliQ water (control) were mixed with 2 ml of DOPA stock solution (5 mM DOPA in 2.5 mM NaHCO_3 buffer, pH 8.3). The absorbance was measured at 460 nm in a spectrophotometer (Hitachi U-2000) immediately after adding the substrate (t_0) and after incubation in dark and at in situ temperature for 180–220 min (t_1). Activity of PhOx [$\text{nmol product h}^{-1}$] was calculated using Beer's Law and the molar absorptance coefficient for the L-DOPA product 3-dihydroindole-5,6-quinone-2-carboxylate (diqc) (3.7×10^4) (Mason 1948).

Statistical data analyses

The data were processed using Microsoft Excel. Data were tested for normal distribution and homogeneity of variances with R 2.11.1 and SPSS 17.0 for Windows. Parametric tests were applied because data were, for the most part, normally distributed (Independent samples T test and one-way ANOVA). For non-normally distributed data, non-parametric tests (Wilcoxon- and Kruskal–Wallis tests) were used. Principal Component Analysis (PCA) was used to highlight relations among the composite variables measured in this study. PCA reduced our large data set to a few independent, uncorrelated variables (principal components) that explained much of the variance of the original data. In order to minimize the variation among the variables under each factor, the factor axes were subsequently varimax rotated. As recommended by Tabachnick and Fidell (1996), we interpreted variables with loading > 0.45 . The software package SPSS 17.0 for Windows was used to complete PCA. The abbreviations of parameters used in this study are listed in Table 2.

Table 2 Table of abbreviations used throughout the text

Parameter	Abbreviation
Bacterial abundance	BA
Bacterial secondary production	BSP
Carbon specific UV absorbance	SUVA ₂₅₄
CDOM absorption coefficient	a_{350}
Chlorophyll <i>a</i>	chl <i>a</i>
Chromophoric dissolved organic matter	CDOM
Dissolved inorganic nitrogen	DIN
Dissolved organic carbon	DOC
Dissolved organic matter	DOM
Dissolved organic nitrogen	DON
Extent of connection	EC
Extracellular enzymatic index 1	EEA1
Extracellular enzymatic index 2	EEA2
Fluorescence index	FI
Leucin-aminopeptidase	EEA _{leu}
Metabolic effort	MEF
Metabolic effort of leucin-aminopeptidase	MEF _{leu}
Metabolic effort of phenol oxidase	MEF _{phOx}
Metabolic effort of α -glucosidase	MEF _a
Metabolic effort of β -glucosidase	MEF _b
Phenol oxidase	PhOx
Soluble reactive phosphorus	SRP
Temperature	Temp
α -Glucosidase	EEA _a
β -Glucosidase	EEA _b

Results

Hydrology

The year 2009 was characterized by relatively high discharge of the Danube (average $2,222 \text{ m}^3 \text{ s}^{-1}$), with a 30-year flood event in June. From May until October, 59 sampling events were conducted on 16 dates. Particular focus was placed on the hydrologically most dynamic period (May–August). The repeatedly fluctuating Danube level altered the hydrological conditions in the floodplain backwaters and created a diverse range of surface connections with the main river. Starting in May and continuing until mid-August, the water level was typically above mean water ($1,930 \text{ m}^3 \text{ s}^{-1}$), with discharge values varying from $1,743$ to $8,190 \text{ m}^3 \text{ s}^{-1}$ (Fig. 2I). On 23 June, the first notably higher water level (discharge $4,332 \text{ m}^3 \text{ s}^{-1}$) occurred, and reached even the most distant locations at the Lobau (Fig. 2II, III, IV). The highest Danube discharge occurred on 25 June. It dropped 5 days after the peak, gradually disconnecting the more remote stations (IV and V) (Fig. 2IV, V). Two additional but

smaller rises were noted in July and in August, $4,409$ and $4,102 \text{ m}^3 \text{ s}^{-1}$, respectively. Both also flooded more distant locations (IV and V). Finally, in mid-August, even the frequently flooded section (II) in Lobau became disconnected (Fig. 2II, III).

Temporal and spatial patterns of DOM quantity and quality

During the study, DOM quantity and quality in backwaters was clearly affected by the introduction of the Danube water. During connected conditions, DOC in backwaters increased from 1.36 to 4.37 mg l^{-1} and did not differ significantly from the Danube DOC, which remained in the same range (1.38 – 3.27 mg l^{-1}) (Fig. 3a). During disconnected conditions, however, DOC was significantly lower compared with connected conditions ($p < 0.01$, $n = 58$) (Fig. 3a). The DOC/DON ratio in backwaters increased with the flood, from average 20:1 before the flood, up to 32:1 noted few days after the peak. However, the DOC/DON ratio in the main channel (I) remained lower and stayed in the range of 15:1–21:1. This resulted in significantly lower values of DOC/DON in the Danube compared to backwaters during connected and disconnected phases ($p < 0.05$, $n = 66$). The values of the quantity and quality proxies (a_{350} , FI, SUVA₂₅₄) of the backwaters did not differ significantly from the Danube during the connected phases (Fig. 3b–d). However, lack of surface connection with the main channel resulted in significant differences between connected and disconnected phases in the a_{350} , SUVA₂₅₄, and FI values (Fig. 3b–d). The CDOM quantity and DOM quality were related to EC, either positively (Fig. 4a, b) or negatively (Fig. 4c). When disconnected conditions were established, DOC concentration was not mirrored by its optical properties, whereas during surface connection with the main channel and in the main channel itself, DOC was significantly negatively correlated with the FI ratio, but positively with SUVA₂₅₄ (Table 3).

Temporal and spatial bacterial parameters

Bacterial abundance

BA varied over the course of the study with changes in river flow. At the onset of the flood, bacterial numbers decreased, followed by continuous changes until October in a range of 0.41 – $7.00 \times 10^6 \text{ cells ml}^{-1}$ (Fig. 5a). This resulted in significantly higher numbers of bacteria in backwaters during disconnected phases in comparison to the Danube ($p < 0.05$, $n = 32$) and connected phases ($p < 0.001$, $n = 58$).

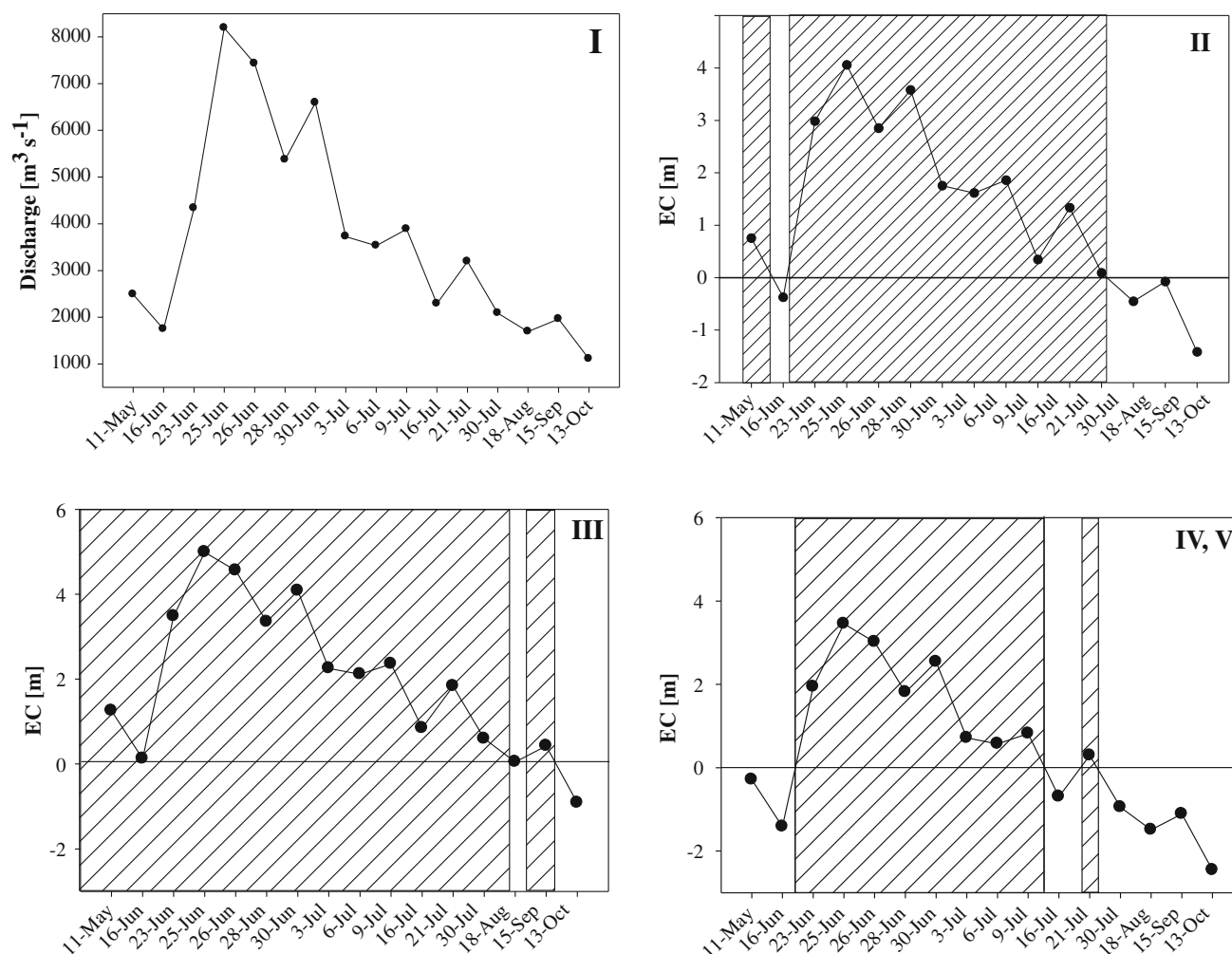


Fig. 2 Danube discharge (main channel, I) and extent of connection (EC) for each location (II, III, IV and V) over the sampling period. Two distinct hydrological phases are defined: connected (lined area) and disconnected (white area)

Bacterial production

Bacterial secondary production (BSP) fluctuated considerably within a range of $0.22\text{--}3.46 \mu\text{g C l}^{-1} \text{h}^{-1}$ (Fig. 5b). Due to strong fluctuations, there were no significant differences in BSP between connected and disconnected phases, however BSP was generally significantly higher in backwaters during disconnected phases than in the Danube, ($p < 0.01$, $n = 32$).

Enzyme activities

In general, an initial drop of EEA at flood onset was followed by increased hydrolytic enzyme activity 3–5 days after establishment of the surface connection at stations II, III, IV and V (Fig. 5c–f). Later in the season, hydrolytic enzyme patterns were less consistent at all the locations, with more pronounced fluctuations at rarely connected water bodies. PhOx activity was considerably higher when

water bodies were decoupled ($p < 0.001$, $n = 46$), whereas there were no significant differences in hydrolytic enzyme activities between the two different hydrological phases and the Danube main channel (EEAa, $p = 0.58$; EEAb, $p = 0.24$; EEALeu, $p = 0.88$, $n = 71$). Hydrolytic enzyme activity did not change significantly with extent of connection, while PhOx was positively related to EC ($r^2 = 0.2$, $p < 0.001$, $n = 57$). Activities of EEAa and EEAb were strongly coupled to DOC of the Danube ($r = 0.80$, $r = 0.86$, $p < 0.01$) and during connection ($r = 0.46$, $r = 0.46$, $p < 0.01$), (Table 3). No correlations were detected between oxidase and hydrolases throughout the season.

A metabolic effort (MEF) parameter [$\text{nm of enzyme produced} \times \mu\text{g C incorporated}^{-1}$] was calculated by dividing the activity of each enzyme by BSP in order to normalize bacterial enzymatic activity. Since extracellular hydrolysis of the substrate and uptake of the hydrolysis product are strongly related (Chróst and Siuda 2002), the

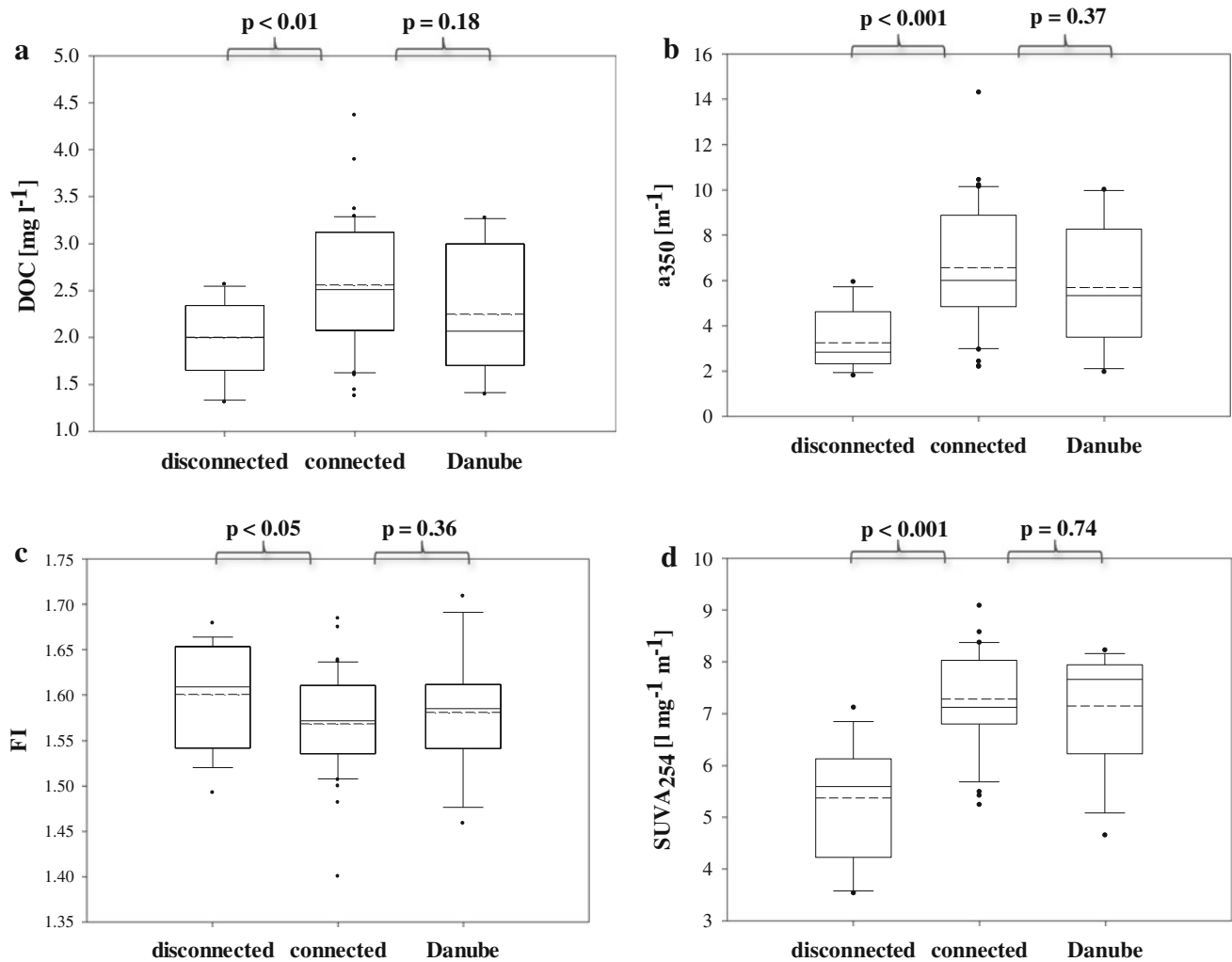


Fig. 3 Boxplots of DOM quantity (**a**) and quality (**b**, **c**, **d**) for connected, disconnected conditions and main channel of the Danube. The boundaries of the box plot indicate the 25th and 75th percentiles, points indicate outliers, the *solid line* in the box marks the median, *dashed line* marks the mean. Statistical differences are indicated on

the top of each panel. The *t* test was used to check for significant differences in the measured variables between the main channel of the Danube and connected conditions and between connected and disconnected conditions

MEF parameter was applied to evaluate the magnitude of enzyme production, which is inversely related to the bacterial carbon assimilation. MEF of all the enzymes fluctuated considerably throughout the season. There were no significant relations between hydrology (EC) and MEF: MEF α -glucosidase (MEFa): $p = 0.46$; MEF β -glucosidase (MEFb): $p = 0.25$; MEF leu-aminopeptidase (MEFleu): $p = 0.76$; MEF phenol oxidase (MEFphOx): $p = 0.24$; $n = 59$, hence also no significant differences between connected and disconnected situations (MEFa: $p = 0.66$; MEFb: $p = 0.88$; MEFleu: $p = 0.78$; $n = 45$). The only exception was the significantly higher MEFphOx during disconnection ($p < 0.05$).

Two enzymatic indices were calculated. Although an enzymatic index approach has already been applied in other

studies (Clinton et al. 2010; Sinsabaugh et al. 2010), no hydrolytic to oxidative activity has been assessed so far, especially for floodplain backwaters. The ratios of EEALeu to PhOx and EEAA + EEAB to PhOx (further termed extracellular enzymatic index: EEA 1 and EEA 2, respectively) were calculated (Sinsabaugh and Moorhead 1994). They served as proxies to indicate the relative importance of protein and carbohydrate utilization versus lignin degradation for microbial metabolism. EEA 1 and EEA 2 increased with flooding, reaching a maximum at the beginning of the flood. Afterwards, the decrease of indices resulted in significantly lower EEA 1 and EEA 2 ($p < 0.05$, $p < 0.01$, $n = 57$) during the disconnected phase. EEA 1 and EEA 2 were negatively correlated with FI ($r = -0.35$, $r = -0.39$, $p < 0.05$, $n = 39$), but only during connected phases.

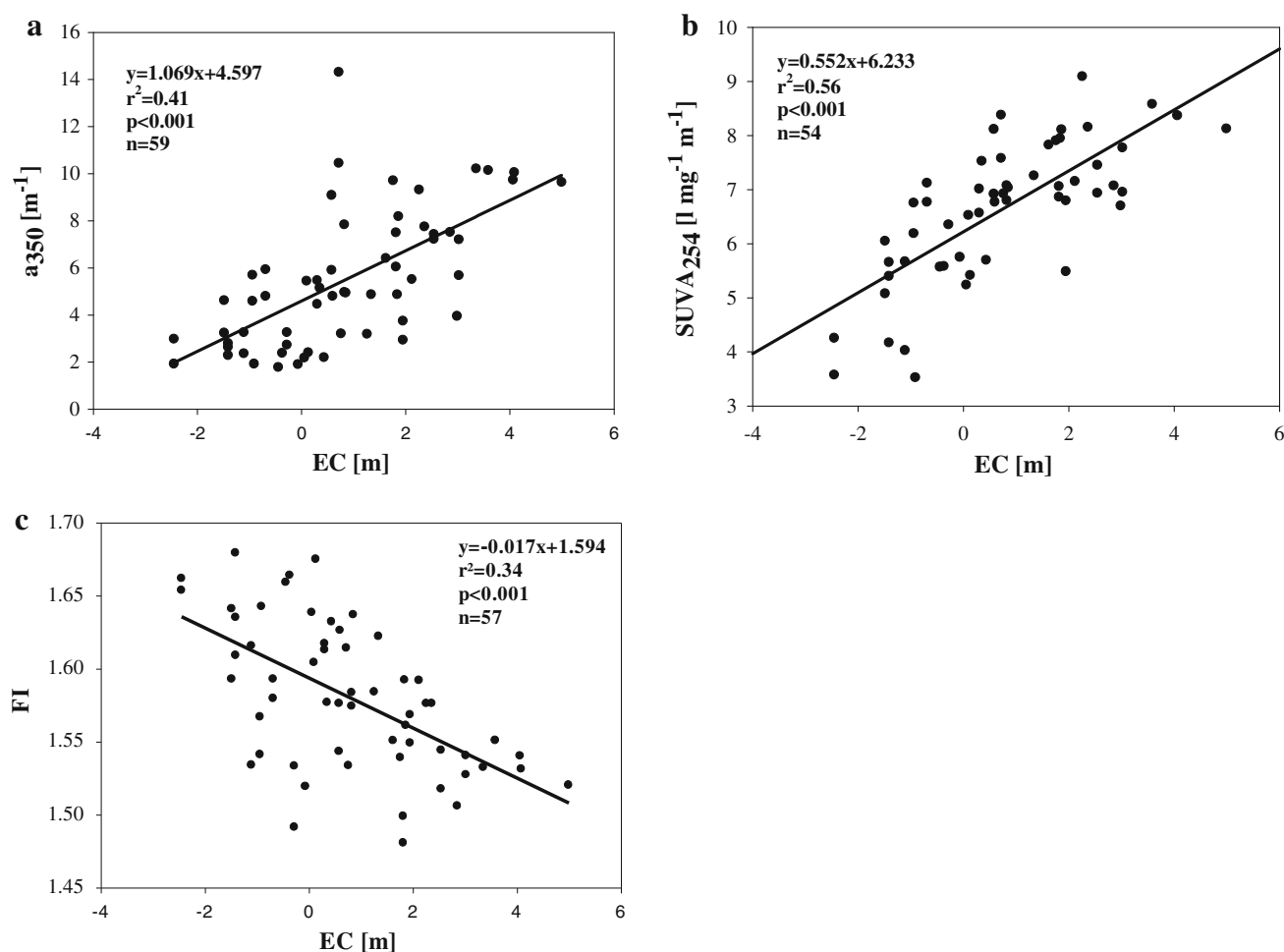


Fig. 4 Relationships between hydrology (EC), CDOM quantity (a) and DOM quality: SUVA₂₅₄ (b) and FI (c). For abbreviations see Table 2

Table 3 Correlation coefficients between DOM quantity (DOC) and qualitative parameters and selected enzymatic activities; connected (CON), disconnected phases (DISC) and main channel of the Danube

Parameter	DOC		
	DISC	CON	Danube
FI	ns	−0.57**	−0.71**
SUVA ₂₅₄	ns	0.65**	0.69*
EEAa	ns	0.46**	0.80**
EEAb	ns	0.46**	0.86**

For abbreviations see Table 2

* $p < 0.05$, ** $p < 0.01$

A PCA was performed to provide an overview of the patterns and relations within measured parameters during hydrological connection/disconnection phases. For the PCA input, we used the variables listed in Table 4. The variables were calculated for stations II, III, IV and V throughout the sampling season (May–October). Five components were distinguished that explained 81 % of

variability in the data. Component 1 accounted for 23.9 % of variability and represented hydrology (EC) and origin of dissolved organic matter (autochthonous vs. allochthonous). Component 1 was positively correlated with EC, a_{350} and SUVA₂₅₄ and negatively with FI. Component 2 explained 23.6 % (Table 4) and illustrated bacterial activity (described as MEF). It was positively correlated with enzymatic investment by heterotrophic bacteria. In Fig. 6 we present an overview of the observed patterns based on PCA by relating it to four distinct hydrological situations. The patterns during surface connection/disconnection phases are illustrated in Fig. 6 and are mainly grouped by component 1 (hydrology and DOM origin); component 2 appears to be less important regarding connection of backwaters to the main channel. The other components were less important, but worth mentioning is component 4, which explained only 12.2 % of variability. Component 4 determined phytoplankton biomass (chlorophyll *a* [chl *a*]) and inorganic nutrients (Table 4).

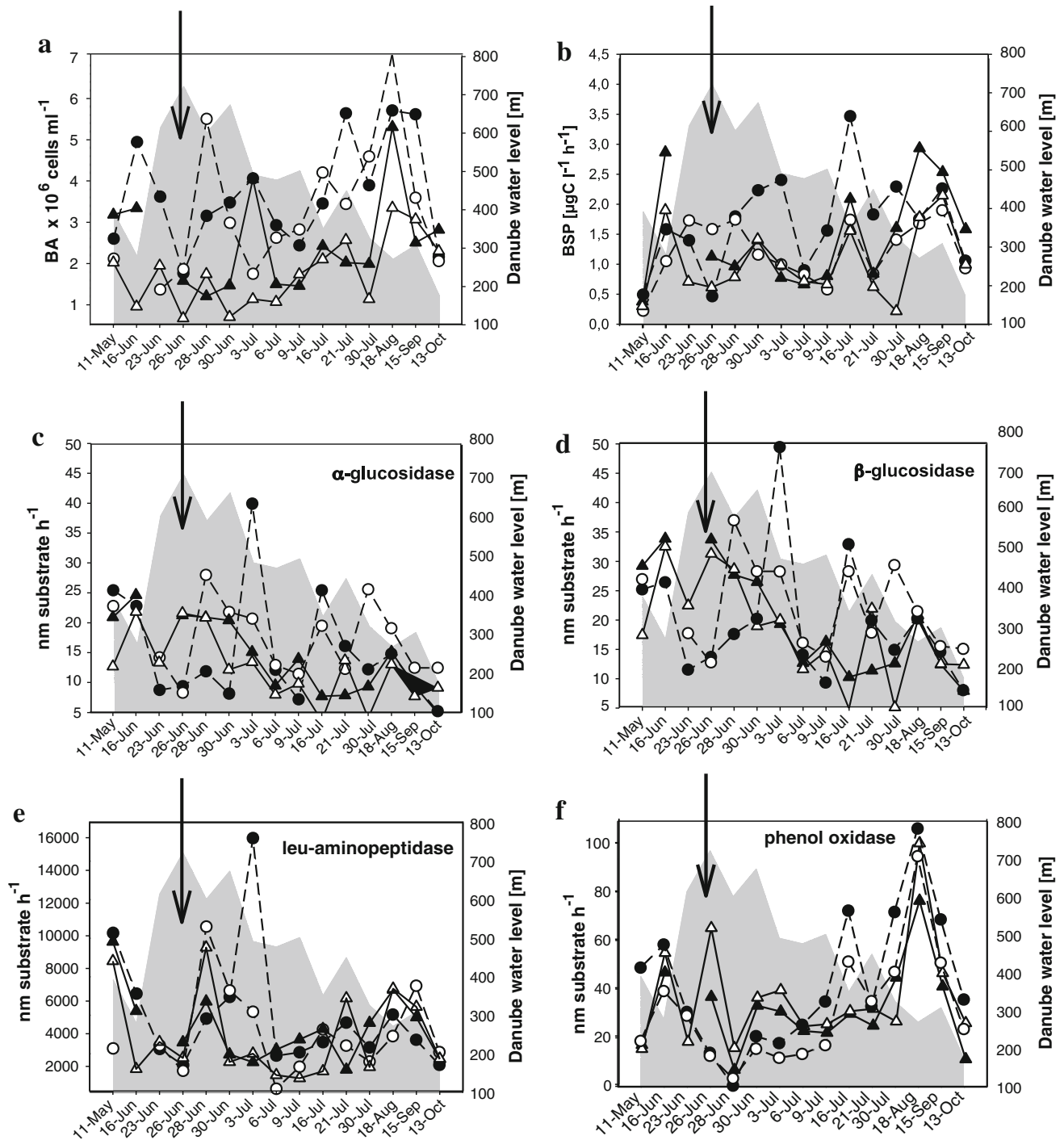


Fig. 5 Temporal patterns of bacterial abundance (a) and activity parameters: BSP (b), α -glucosidase (c), β -glucosidase (d), leu-aminopeptidase (e) and phenol oxidase (f) in stations II (filled triangle), III (open triangle), IV (filled circle), and V (open circle).

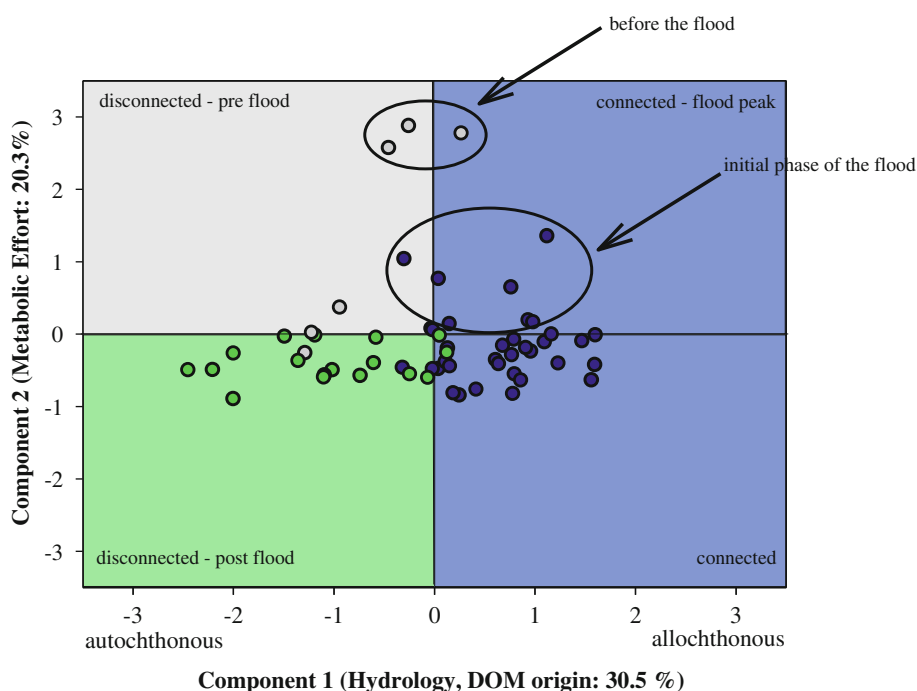
Arrows indicate peak of the flood in the main channel of the Danube. Grey area indicates water level in the main channel of the Danube. For analytical variations and replicates, see “Methods” (analytical variance <5 %, except BSP (10 %))

Table 4 PCA: Explained variation in percent and correlation coefficients of environmental variables with five components

Variable	Correlation coefficients				
	Component 1 (23.86 %)	Component 2 (23.66 %)	Component 3 (12.35 %)	Component 4 (12.28 %)	Component 5 (9.31 %)
EC	0.851	0.014	−0.309	0.014	−0.216
FI	− 0.689	−0.253	−0.028	−0.255	−0.319
SUVA ₂₅₄	0.916	0.102	0.077	−0.188	−0.067
a ₃₅₀	0.861	−0.109	−0.024	−0.222	0.199
DOC/DON	0.020	0.076	0.023	−0.109	0.931
DIN/SRP	−0.048	0.119	0.145	0.878	−0.014
Temp	0.126	−0.009	0.919	−0.051	0.012
Chl <i>a</i>	−0.363	−0.046	−0.013	0.573	−0.327
MEFa	0.110	0.937	−0.063	0.005	0.136
MEFb	0.137	0.949	−0.095	0.005	0.112
MEFleu	0.013	0.850	−0.055	0.183	−0.053
MEFphOx	−0.191	0.670	0.169	−0.502	−0.097
BA	−0.323	−0.128	0.769	0.218	0.017

Boldface: correlation coefficients >0.5. Bartlett's Test of Sphericity was *** $p < 0.001$. For abbreviations see Table 2. For more details see "Methods"

Fig. 6 Score plot for the first two principal components from PCA of variables listed in Table 4. The plot was generated from PCA after varimax rotation. Data from disconnected and connected conditions of stations II, III, IV and V were used as PCA input. Different colours of the figure quarters indicate distinct hydrological phases. Data points are separated according to the type of connectivity with the main channel. Grey circles indicate stations sampled before the flood, blue circles during surface connection, green circles after disconnection from the main channel. Stations are represented by all parameters listed in Table 4. For more details on PCA see methods and Table 4



Discussion

Importance of surface connectivity for DOM heterogeneity

Our results confirm that the flood introduced into the backwaters DOM that consisted of more allochthonously derived, aromatic compounds, as has also been found in other rivers (Guéguen et al. 2006) and wetlands (Hunsinger et al. 2010). During the floods, inputs of allochthonous

DOC in streams are under hydrological control (Royer and David 2005), and elevated discharge is often related to higher DOC concentrations (Guéguen et al. 2006; Mladenov et al. 2007; Peduzzi et al. 2008).

In our study, the flood also introduced significantly more DOC, but more interestingly, the optical properties, related to EC (Fig. 4a–c) suggest that hydrological events altered DOM composition in backwaters substantially. Positive relations between DOC and SUVA₂₅₄, and negative with FI (Table 3) in the Danube and during connected phases,

indicate that DOC in the floodplain backwaters was mostly derived from the main channel and contained a high proportion of chromophoric, allochthonous and aromatic material. This agrees with previous reports indicating hydrological control of the FI (Peduzzi et al. 2008; Besemer et al. 2009). However, the lower DOC/DON values in I, compared to DOC/DON during connected phases in backwaters, support the idea that the floodplain itself is a potential DOM source during flood conditions (Mladenov et al. 2005). The above findings suggest that the DOM was a mixture of river and floodplain-derived DOM. Nonetheless, we could not observe that the flood was a source of very bioavailable DOM, since its chemical properties pointed to a refractory nature of introduced material.

At disconnection, we recorded significantly lower SUVA₂₅₄, which denotes that the DOM pool during the disconnected phase is composed of less aromatic molecules. Significantly higher FI implies the introduction of other, most likely autochthonous material to the DOM pool of the floodplain system (Peduzzi et al. 2008; Besemer et al. 2009). Note that the increase in FI could also reflect the removal of allochthonous organic matter in conjunction with microbial/autochthonous production.

EEA as a tool to track alterations in DOM availability

Responses to changes in DOM sources can be traced by examining bacterial enzymatic activity (Clinton et al. 2010), by linking EEA to DOC composition (Burns and Ryder 2008). Within a bacterial community, the composition of extracellular enzymes may shift in relation to changing sources of DOM. Accordingly, enzyme activity patterns imply potential availability of different DOM components (Chróst 1991). EEAb activity is repressed by the presence of readily utilizable bacterial substrates (i.e. glucose), whereas the appearance of more complex saccharides strongly induces EEAb activity (Chróst 1991). Pronounced temporal peaks and significant correlation of DOC with EEAA and EEAb (Table 3) point to rapid utilization of available carbon sources (Burns and Ryder 2008). Simultaneous increase of EEAA and EEAb right after the flood peak (Fig. 5c, d) indicates introduction of more complex, storage and structural polysaccharides (Sinsabaugh and Foreman 2001). The quick response of glucosidases implies that even short pulses stimulate activity of bacterial glucosidases (Burns and Ryder 2008). The delayed, but pronounced, EEALeu reaction (5–11 days) (Fig. 5e) shows that longer floods promote hydrolysis of proteins and demonstrates that the introduction also of bioavailable DOM was linked to the flood (Stepanauskas et al. 2000). We suggest that, investigation of EEA during rapid environmental changes is a very useful tool to assess the reactivity of DOM. Although results of the optical

properties pointed to a more aromatic and refractory nature of DOM during connected phases, the EEA approach revealed that some introduced material was bioavailable and could be utilized by the bacterial community. The pronounced changes in EEA point to the presence of bioavailable polysaccharides introduced with the flood event. Some of the polysaccharides are not chromophoric and they would not be detected based on optical properties. Therefore, previous studies, which relied solely on DOM optical properties, might have missed the mobilization of non-chromophoric, but bioavailable and ecologically important polysaccharides and proteins.

Despite extensive studies on hydrolytic enzyme activity, less is known about PhOx in aquatic environments. This enzyme degrades recalcitrant, aromatic, phenolic compounds such as lignin or humic material into more readily available substrates (Sinsabaugh 2010). Fungi are responsible for initial degradation of native lignin, while bacteria are considered to play a main role in mineralization of lignin-derived low-molecular weight compounds in soils (Masai et al. 2007). However, in aquatic ecosystems, bacteria seem to play a leading role in decomposing lignin (Li et al. 2008). The significant increase of phenol oxidase associated with lignin degradation (DeAngelis et al. 2011) typically is a longer process compared to the quicker response of glucosidases (Smart and Jackson 2009). PhOx and glucosidases are usually decoupled, (as shown in our study) which suggests different controlling mechanisms on these two classes of enzymes (Sinsabaugh 2010).

Most PhOx studies focused on activity in soil and litter (Pind et al. 1994; Williams et al. 2000; Sinsabaugh 2010), whereas activity in inland waters is less explored (Münster and De Haan 1998). Factors that control PhOx activity in soil include pH, concentration of soluble phenolic compounds, lignin content of plant litter, along with oxygen and nitrogen availability (Sinsabaugh 2010). Our results showed that PhOx was influenced by hydrology, with significantly higher activity during disconnected phases. This suggests that hydrological changes such as flooding and subsequent lowering of the water table promoted activity of PhOx. Flooding can strongly stimulate loss rates of lignin in soils (Lockaby et al. 1996), whereas in ponds, activity of PhOx may be triggered after flooding (Alvarez and Guerrero 2000). Nonetheless, there is some discrepancy about the response of PhOx to water level changes. Freeman et al. (2001) reported a significant increase as the water table dropped in peatlands, whereas Williams et al. (2000) found no relation of PhOx to wetland hydrology. Additionally, our results show that during the disconnection, activity of phOx was promoted by aromatic content of DOM ($r = 0.48$, $p < 0.05$), which points to bacterial utilization of lignin-derived aromatic compounds (Masai et al. 2007) during disconnected phases. Significantly higher

PhOx after the flood also implies that degradation of aromatic material could be responsible for significant decrease of SUVA₂₅₄ after the flood.

Effect of surface connectivity on the fate of utilized DOM

Our results confirm that DOM quality is an important factor regulating bacterial production in floodplain areas (Peduzzi et al. 2008). Typically, DOM of autochthonous origin is higher-quality material that stimulates BSP (Cole et al. 2002), as opposed to allochthonously derived DOM, which is recalcitrant with much longer turnover times (del Giorgio and Pace 2008). At the same time, there is evidence that BSP can also be controlled by allochthonous organic carbon rather than by within-system primary production alone (Tranvik 1992; Kritzberg et al. 2004; Daniel et al. 2005). One suggestion is that allochthonous material supports almost exclusively respiration, whereas algae prevalently support carbon assimilation (Cole et al. 2002; Kritzberg et al. 2005; Karlsson et al. 2007). Nonetheless, abundant allochthonous DOM input may not only boost respiration but also increase bacterial production (Thottathil et al. 2008).

In order to describe DOM utilization patterns and quality during connected and disconnected phases, we applied new parameters: MEF, EEA 1 and EEA 2, (see “Results”). MEF serves as an assimilation efficiency parameter that indicates the proportional importance of catabolic versus anabolic processes. Lower values indicate that substrate, which undergoes enzymatic degradation, drives bacterial metabolism towards anabolism. We did not measure bacterial respiration, however with PCA analysis (Fig. 6), where we incorporated the MEF parameter, we attempted to explain the importance of catabolic versus anabolic processes. Our results of the PCA demonstrate (Table 4) that hydrological disturbances and DOM quality (Component 1) appear to be crucial in the functioning of this system. The MEF parameter loaded higher on Component 2 (Fig. 6; Table 4), and MEFa and MEFb were significantly higher in dynamic stations (II, III) ($p < 0.05$, $n = 19$) during the first 7 days of the flood. These results imply that the onset of the flood boosts catabolic processes (del Giorgio and Pace 2008). At the same time, the lack of significant differences of MEFa, MEFb and MEFleu between connected and disconnected phases indicates that prolonged connectivity with the main channel only minimally influences the assimilation efficiency of hydrolyzed products (Fig. 6). What is surprising is the pre-flood situation: despite the few measurements, the degraded substrates (even of autochthonous origin) generate less bacterial biomass per energy expenditure (Fig. 6). Significantly higher MEFphOx during disconnected conditions

supports the conclusion that longer water retention promotes this enzymatic activity per unit of production (Sinsabaugh 2010). This calls for studying the factors that determine elevated MEFphOx in floodplain waters during disconnected phases.

Although we did not conduct photodegradation experiments, photodegradation may be important during disconnected phases. The flood introduced significantly higher amounts of aromatic and chromophoric material. Such material is considered to be photo-labile DOM (Stubbins et al. 2010) and may be substantially photo-altered in the post-flood period (less turbid conditions). However, the issue of photodegradation in our system remains to be investigated.

Bacterial utilization of high vs. low reactive DOM

The EEA 1 and EEA 2 indicate which part of the DOM pool (high/low reactivity DOM) was a dominant component for bacterial metabolism. This enables assessing bioavailability and quality of material from different sources. The substantial increase of the EEA 1 and EEA 2 in all the backwaters, with a maximum at the 5th day of connection, indicates a switch to polysaccharide and protein metabolism. The negative relation of EEA 2 with the FI during connected phases suggests that microbial production of glucosidases is induced by allochthonous DOM. Therefore, we suggest that DOM introduced with this flood water is more bioavailable than previously thought and contains reactive organic matter that is processed relatively quickly. High aquatic BSP during and shortly after floods may be supported by the more bioavailable fraction of the small, readily available sub-pool of the terrestrial DOM that is flushed from the surrounding area (Ågren et al. 2008).

In floodplains, during seasonal floods, rapidly decaying plant-derived sources (terrestrial and aquatic) dominate the DOM pool. In contrast, non-flood periods are better described by the presence of a more slowly processed pool (Mladenov et al. 2007). In our study, a switch from protein and polysaccharide (highly reactive) to lignin (low reactive) degradation was indicated by significantly lower EEA 1 and EEA 2 during the disconnected phase. An increased importance of lignin material for heterotrophic metabolism is further supported by significant negative relations between BSP and EEA 1 or EEA 2 (Fig. 7) during disconnection. Microbial degradation of lignin material occurs constantly throughout the water column and is an important process in removing dissolved lignin phenols (Hernes and Benner 2003). Although this semi-labile pool is used up more slowly (days-months) (Benner and Kaiser 2011), it still supports some level of bacterial metabolism (del Giorgio and Pace 2008).

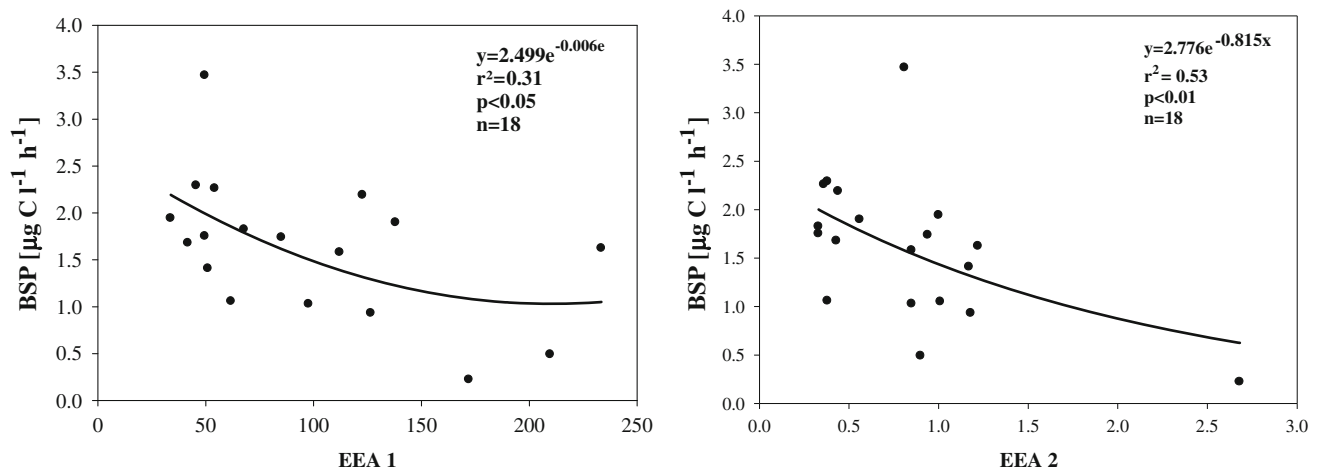


Fig. 7 BSP as a function of extracellular enzymatic indices (EEA 1 and EEA2) during disconnected phases. For abbreviations see Table 2

Although carbon flux in water-linked landscapes is still poorly understood, floodplains play a significant role in carbon sequestration and mineralization processes (Battin et al. 2008). Due to the reduced hydrologic connectivity in human-impacted, regulated rivers, numerous services and functions of floodplains are impaired (Tockner et al. 2006). This includes the reduced opportunity to process large amounts of organic carbon, severely altering the overall carbon flux. Our results stress the importance of seasonal hydrological events that connect a river channel with adjacent floodplains (Schiemer et al. 2006). Flooding mobilized a significant fraction of the reactive DOM from the catchment, which then subsidizes bacterial metabolism (Carpenter et al. 2005); sudden and increased amounts of different carbon sources can promote microbial productivity (Docherty et al. 2006). Thus, terrestrial material may be also processed quickly (Lam et al. 2007), and carbon flow through bacteria can in certain situations be dominated by allochthonously derived DOM (Carpenter et al. 2005). Therefore, allochthonous DOM imported during floods is important for BSP (Berggren et al. 2009). Interestingly, utilization of different C sources does not necessarily substantially affect the proportions of energy expenditure versus microbial biomass generation. The minor influence of prolonged connectivity on the assimilation efficiency of hydrolyzed products could be explained by a very adaptive metabolic capacity of the bacterial community (compare Fig. 6). Within the frame of physiological possibilities, the bacterial assemblage may respond with enzymatic and/or community composition changes.

Thus metabolic changes can be based on the metabolic flexibility of ecotypes of specific species, or be a result of changes in community structure. Nonetheless, the changed conditions for processing of allochthonous DOM, when residing in backwaters, provide different opportunities for

carbon processing in floodplains. The existence of such a river-floodplain connection should also be important for downstream carbon transport. Further studies are necessary to better understand the share of the DOM pool in bacterial C assimilation versus respiration during/after flood events.

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5. Paper II

Sieczko A, Maschek M and Peduzzi P (2015) *Algal extracellular release in river-floodplain dissolved organic matter: response of extracellular enzymatic activity during a post-flood period*. *Frontiers in Microbiology* 6. doi:10.3389/fmicb.2015.00080

I contributed to this study by designing and performing field sampling. I also processed most of the samples in the laboratory, carried out the calculations and statistical analysis, interpreted the results and wrote the manuscript as a first author. Also as a corresponding author I completed the submission process, including the revisions and replied to the comments of the reviewers.



Algal extracellular release in river-floodplain dissolved organic matter: response of extracellular enzymatic activity during a post-flood period

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River-floodplain systems are susceptible to rapid hydrological events. Changing hydrological connectivity of the floodplain generates a broad range of conditions, from lentic to lotic. This creates a mixture of allochthonously and autochthonously derived dissolved organic matter (DOM). Autochthonous DOM, including photosynthetic extracellular release (PER), is an important source supporting bacterial secondary production (BSP). Nonetheless, no details are available regarding microbial extracellular enzymatic activity (EEA) as a response to PER under variable hydrological settings in river-floodplain systems. To investigate the relationship between bacterial and phytoplankton components, we therefore used EEA as a tool to track the microbial response to non-chromophoric, but reactive and ecologically important DOM. The study was conducted in three floodplain subsystems with distinct hydrological regimes (Danube Floodplain National Park, Austria). The focus was on the post-flood period. Enhanced %PER (up to 48% of primary production) in a hydrologically isolated subsystem was strongly correlated with β -glucosidase, which was related to BSP. This shows that—in disconnected floodplain backwaters with high terrestrial input—BSP can also be driven by autochthonous carbon sources (PER). In a semi-isolated section, in the presence of fresh labile material from primary producers, enhanced activity of phenol oxidase was observed. In frequently flooded river-floodplain systems, BSP was mainly driven by enzymatic degradation of particulate primary production. Our research demonstrates that EEA measurements are an excellent tool to describe the coupling between bacteria and phytoplankton, which cannot be deciphered when focusing solely on chromophoric DOM.

Keywords: extracellular enzymatic activity, photosynthetic extracellular release, autochthonous DOM, non-chromophoric DOM, river-floodplain

INTRODUCTION

In river-floodplain systems seasonal floods or low water episodes create a range of lotic to lentic conditions in the backwaters. Depending on the duration and frequency of spates and on the distance of a side arm from the main channel, different types of side backwaters can be distinguished. These range from frequently connected to fragmented and very rarely flooded parts to side-arms that are completely disconnected from the main channel. Rapid hydrological events introduce substantial loads of terrestrial dissolved organic matter (DOM) into the floodplain (Hein et al., 2003). Moreover, disconnected and stable hydrological situations, observed after a flood, enhance phytoplankton productivity (Hein et al., 1999). This creates a complex DOM pool with a mixture of allochthonously and autochthonously derived material, potentially available for bacterial utilization.

Autochthonous autotrophic production is an important carbon source also for secondary production in rivers (Thorp and Delong, 2002; Bunn et al., 2003). Nonetheless, DOM generated *in situ* often is insufficient to exclusively support aquatic food

webs (Kritzberg et al., 2004). Particularly in aquatic systems where primary production is low compared with the terrestrial load of DOC (Kritzberg et al., 2005), allochthonous DOC can become essential for bacterial secondary production (BSP) (Jansson et al., 2000). BSP may be much higher when faced with pulse resource addition compared to the same quantity of DOC supplied continuously over a longer time period (Lennon and Cottingham, 2008). This points to the importance of seasonal disturbance events (such as floods) for BSP.

Even if most of carbon needed for bacterial growth may be supplied from other than autochthonous sources (Fouilland and Mostajir, 2010), the autochthonous material is preferentially utilized prior to terrestrial DOM. In pelagic zones, an important source of autochthonous carbon is photosynthetic extracellular release of phytoplankton (PER). The link between PER and bacterial heterotrophic metabolism has been studied in freshwater and marine systems. In floodplain studies, however, this is rarely measured routinely. The rate and quality of the exudated material depends on the phytoplankton species, cell size (López-Sandoval et al., 2013) and on the type of aquatic ecosystem (Fogg, 1983).

Nutrients (Meon and Kirchman, 2001; Wyatt et al., 2014), light (Hulatt et al., 2009), UV radiation (Pausz and Herndl, 1999) and other factors can also significantly impact the percentage, composition and microbial utilization of PER (Sarmiento and Gasol, 2012; Landa et al., 2013). Related phytoplankton species may produce similar suits of DOM, which has implications for shaping bacterial communities (Becker et al., 2014). However, the composition of PER may depend on the growth phase of phytoplankton (Barofsky et al., 2009). In general, most primary production exudates are high-quality substrates, comprised mainly of monomeric sugars, carboxylic acids and amino acids (Bertilsson and Jones, 2003), which can be directly assimilated by bacteria, stimulating the growth and abundance of microbial communities (Norrman et al., 1995). Numerous studies have revealed the importance of PER for bacterial metabolism and the direct utilization of PER by bacteria (Cole, 1982; Chrzanowski and Hubbard, 1989; Baines and Pace, 1991). Nonetheless, other studies could not demonstrate direct coupling between BSP and PER (Teira et al., 2003; López-Sandoval et al., 2010). Thus, beside monomeric compounds, phytoplankton exudates may be composed also of high molecular weight material, mostly polymeric sugars (Myklestad, 1995; Giroldo and Vieira, 2005), which are not directly available for bacteria. In such cases, an enzyme-mediated step is necessary to assimilate these polymeric exudates. The optical properties of PER provide information on PER composition (Stedmon and Markager, 2005; Romera-Castillo et al., 2011). Such investigations, however, do not cover the broad range of non-chromophoric compounds that are also exudated (Rochelle-Newall and Fisher, 2002). Measurements of extracellular enzymatic activity (EEA) is an alternative approach that enables tracking the microbial response to non-chromophoric, but quickly cycling DOM. The activity of extracellular enzymes enables detecting shifts in the microbial response to varying resources (Wagner et al., 2014), especially those that are relatively quickly exploited by the bacterial community. Production of extracellular enzymes by bacteria can be regulated by the DOM supply (Chróst, 1991) and it is directly linked to mechanisms of DOM processing (Sinsabaugh and Foreman, 2001). Hence EEA likely is regulated by the composition of phytoplankton exudates (Chróst and Siuda, 2006).

Model enzymes used to study bacterial degradation of carbohydrates and proteins are β -glucosidase and leu-aminopeptidase (Cunha et al., 2010), but the activity of other enzymes has also been measured frequently (Chróst and Siuda, 2002; Sinsabaugh, 2010). Although some studies report the extracellular enzymatic response to phytoplankton exudates in the water column (Obernosterer and Herndl, 1995; Fajon et al., 1999) and sediments (Goto et al., 2001), this topic remains understudied. Especially under variable hydrological settings in river-floodplain systems this process has been neglected.

Our study was designed to compare water column processes in three different floodplain sections located in a river-floodplain system of the Danube. The focus was on the post-flood period. We investigated relationships between natural phytoplankton productivity and microbial EEA in these distinct subsystems. The main goals of the study were:

- to characterize potential environmental differences between subsystems located in relatively close vicinity in the same floodplain area,
- to investigate how extracellular enzymes respond to primary production, emphasizing the effect of phytoplankton extracellular release in temporarily compared to permanently disconnected water bodies,
- to elucidate the importance of autochthonous, phytoplankton-derived DOM for microbial uptake in an environment typically dominated by allochthonous DOM,
- to elucidate whether the main DOM sources for bacterial growth are different in hydrological individual and diverse subsystems of a floodplain after a flood.

MATERIALS AND METHODS

STUDY SITE

The study was conducted in the area of the “Danube Floodplain National Park” (DFNP) downstream of Vienna, Austria. Some parts of this area are severely impacted by human activity (river regulation), whereas others were restored about 15 years ago (Schiemer et al., 1999). Water samples were collected after a 30-year flood from backwaters located in three different subsystems of the DFNP (**Figure 1**). These subsystems, although close to one another, exhibit distinct hydrological regimes, with different types of surface connectivity with the main Danube channel. Subsystem I (hereafter termed: I) is located in a semi-natural and restored part of the river-floodplain, where two side arms (A and B) were chosen (**Figure 1**). Although A and B are situated in different sections of the DFNP, both have very similar characteristics. They have been restored by lowering the riverbank, which results in frequent changes of lentic to lotic and flowing conditions (220 (A) and 180 (B) days per year). In subsystem II (hereafter: II) two temporarily disconnected backwaters (C) and (D) were selected (**Figure 1**). Section C and D are located in a semi-separated area of the floodplain, which is protected from direct flow-through by a levee and additional weirs. Here, a surface connection of backwaters with the main channel is established only rarely (average 18 days per year), during higher water level of the Danube (>1.30 m above mean water). Both backwaters are open water bodies, where DOM derives from external (fallen leaves, rare river water import) as well as from internal sources (phytoplankton, macrophytes). Subsystem III (hereafter: III) in former times was an integral part of the “active” floodplain area, but due to a major regulation of the river in the nineteenth century (embankment and additional weirs), subsystem III has been permanently separated from the main channel. Therefore, it is never flushed even during exceptional floods. Hence, surface connection with the Danube is never established; the only connection with the Danube is through infiltration from groundwater. Subsystem III is situated in a highly shaded area (average light intensity at midday: $195 \mu\text{mol photons PAR m}^{-2} \text{ s}^{-1}$) where the dominating DOM input is probably of allochthonous origin (leaves, debris).

Water samples from all stations were always collected between 10 and 12 a.m. with rinsed polyethylene bottles (10 l, single sample) and transported to the laboratory within 3 h in dark boxes at *in situ* temperature. Surface water sampling was performed

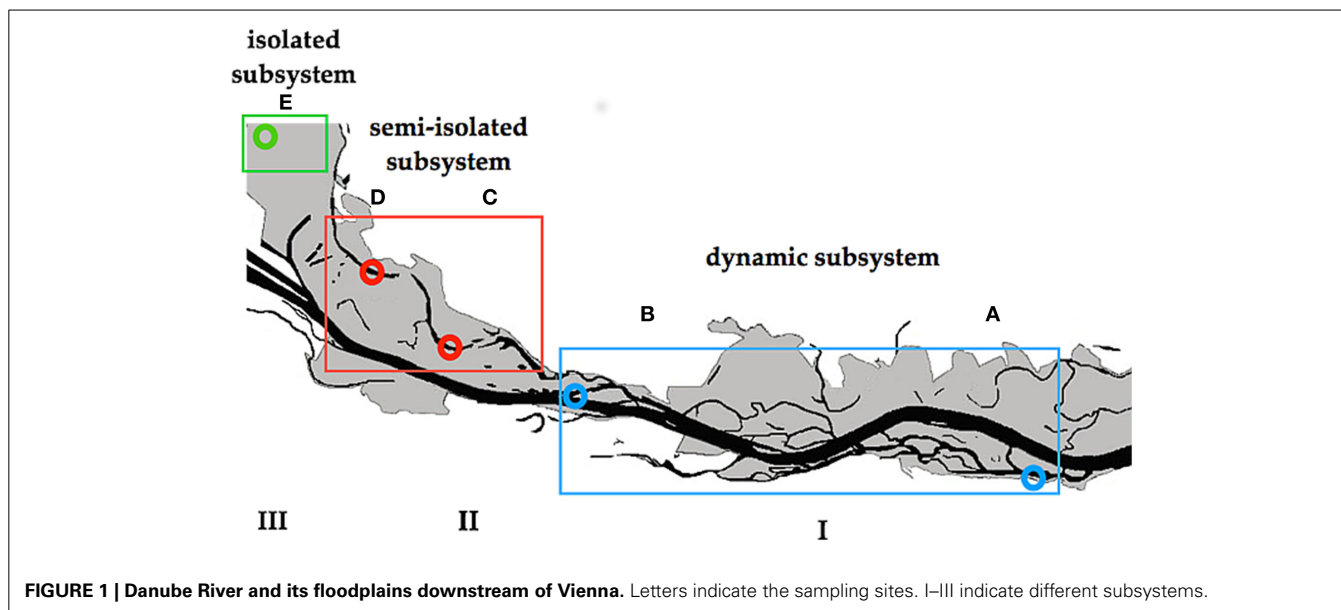


FIGURE 1 | Danube River and its floodplains downstream of Vienna. Letters indicate the sampling sites. I–III indicate different subsystems.

between July and October 2009, which covered the post-flood period. In subsystems II and III the sampling included a 2-weeks-longer period than in I. Because both locations in subsystem I still experienced flooding conditions in early July, we did not include these samples into our post-flood analyses. Water samples from subsystem I were collected on 5 dates, whereas in II and III, samples were taken on 9 dates. The intensity of sampling was more frequent at the onset of the post-flood phase (every few days) and decreased toward the end of the investigation period (every month). In subsystem I the first sample was taken 7 days after flood while in subsystem II, 3 days after the flooding.

ANALYSES OF ABIOTIC PARAMETERS

Water level, temperature, oxygen saturation (WTW, Oxi 315i) and vertical profiles of light intensity ($\mu\text{mol photons PAR m}^{-2} \text{ s}^{-1}$, every 10 cm, LICOR sensor) were measured *in situ* at each location. The key nutrients determined included: three nitrogen compounds—nitrate, ammonia, dissolved organic nitrogen (DON)—and soluble reactive phosphorus (SRP). These analyses were completed within 24 h using the German standard methods: nitrate: DIN 38405-29, ammonia: DIN 38406-5, SRP: DIN EN ISO 6678, soluble Kjeldahl-N: DIN 38406-5. DON was assessed by subtracting ammonia from soluble Kjeldahl-N (Peduzzi et al., 2008) and the ratio DOC/DON was determined. The ratio of dissolved inorganic nitrogen [(nitrate+ammonia) (DIN)] and SRP was calculated as DIN/SRP.

PRIMARY AND SECONDARY PRODUCERS

Phytoplankton productivity and biomass

To make our measurements comparable, the samples for primary production were taken at the same time of day during all sampling events right below the water surface (10–20 cm). Particulate primary production (pPP) and net photosynthetic extracellular release (PER) of phytoplankton were determined using a ^{14}C method, originally proposed by Steemann-Nielsen (1974), modified according to Preiner et al. (2008). We measured the rate of

carbon fixed in particulate material (cells; pPP), while PER was assessed to estimate dissolved organic carbon released by phytoplankton into the surrounding water. Total primary production (PPT) was calculated as the sum of pPP and PER. Assessing PPT was needed to calculate the relative fractions. For each station, 8 bottles with a defined volume ($\sim 117 \text{ ml}$) were incubated in a water basin at *in situ* temperature for 3.5–4 h after adding sodium bicarbonate ($\text{NaH}^{14}\text{CO}_3$) ^{14}C ($20 \mu\text{Ci/ml}$). This time period was selected according to Mykkestad (2000) and Preiner et al. (2008) assuming that, on one hand, it was not too short to obtain measurable results. On the other hand, avoiding that the bacterial community has not passed its latent period and not reached maximum uptake rates. With this it was attempted to minimize a bias due to potentially remaining bacterial uptake. Bottles were exposed in a light gradient covering the environmental conditions ($10\text{--}560 \mu\text{mol Photons m}^{-2} \text{ s}^{-1}$). The sample water was permanently mixed by placing the bottles on a rolling table. Two additional bottles were kept in dark to determine the unspecific adsorption of ^{14}C . After the incubation, filtration ($0.45 \mu\text{m}$; Millipore HAWP) separated the particulate from the dissolved fraction (Wetzel and Linkens, 1990) using low vacuum pressure ($<50 \text{ mBa}$). The filtrate from each sample (5 ml), including dark-incubated samples, was collected. The filters were placed in scintillation vials with 0.5 ml 0.5N HCl to remove the inorganic ^{14}C (Peterson, 1980). The filtrate was decontaminated by acidifying the samples to pH 4 and bubbling with air for 15 min (Peterson, 1980; Parsons et al., 1984). Filters were dissolved with ethyl acetate to minimize self-adsorption (Peterson, 1980) and scintillation cocktail was added. After 24 h radioactivity of each sample was measured in a scintillation counter (Parsons et al., 1984) to determine disintegrations per minute (DPMs). DPMs of dark-incubated samples were subtracted to calculate pPP and PER. For the characterization of the light climate, the light attenuation coefficient was determined from vertical light profiles and light curves (P/I curves) were created. Primary production [$\mu\text{g C l}^{-1} \text{ h}^{-1}$] was calculated according to Jassby and Platt (1976) based

on surface radiation, the attenuation coefficient and the depth where the samples were taken (Riedler and Schagerl, 1998). The analytical variability was always <10%. The rate of carbon fixed in particulate organic matter (cells; pPP) was compared to the rate of dissolved organic carbon released extracellularly by phytoplankton (PER). The relative extracellular release was defined as percentage of total primary productivity (PPt): $\%PER = PER \times 100 / (PP + PER)$. Additionally, for a biomass estimate, the concentrations of chlorophyll *a* (chl *a*) were determined using standard methodology (Lorenzen, 1967); the method is described in more details by Schagerl et al. (1996).

Bacterial secondary production

Bacterial secondary production (BSP) was assessed based on the method of [^3H] thymidine (20 μ l, 20 nM final concentration) incorporation into DNA (Fuhrman and Azam, 1982). Three replicate samples and two additional formaldehyde-killed (2% final concentration) samples (5 ml), which served as controls, were incubated in dark at ambient temperature for 0.45–1.5 h. After the incubation, samples and controls were filtered (0.45 μ m; Millipore HAWP). Filters were collected in scintillation vials and dissolved in ethyl acetate. Afterwards scintillation cocktail was added and samples were placed into the scintillation counter. The analytical variability was always <10%. BSP [μ g C l $^{-1}$ h $^{-1}$] was calculated using an average Danube-specific conversion factor of 3.2×10^{18} cells produced per mol of incorporated thymidine (Berger et al., 1995). The ratio of BSP:PPt was calculated, based on hourly rates as an instantaneous BSP:PPt ratio, the ratio right at the day time of sampling. This ratio was used to establish the relative share of primary production used by BSP, hence to characterize a potential coupling between the bacterial and phytoplankton compartments (Ducklow and Carlson, 1992).

Bacterial abundance

Sample water (1–2 ml) was fixed with formaldehyde (2% final concentration) and stained with 4,6-diamidino-2-phenylindole-dihydrochloride (DAPI) (10 μ g ml $^{-1}$). Bacterial abundance (BA) was determined by epifluorescence microscopy (Nikon E 800) in 20–30 randomly chosen fields according to Porter and Feig (1980).

DOM characterization

Water samples were filtered through pre-combusted (500°C) GF/F filters (0.7 μ m, pore size) within 3 h of collection. Samples for DOC concentration [mg l $^{-1}$] were acidified to pH 3 and analyzed by high-temperature combustion using a Shimadzu TOC 5000 analyzer (Benner and Strom, 1993). We applied optical DOM indices to obtain information about DOM quality in each subsystem. Absorbance at wavelengths of 364, 254, and 250 nm was measured in a 5-cm quartz cuvette with a spectrophotometer (Hitachi U-2000). Carbon-specific UV absorbance (SUVA₂₅₄) [l mg $^{-1}$ m $^{-1}$] was calculated as the ratio of absorbance at 254 nm to the DOC concentration. SUVA₂₅₄ is reported to be positively correlated with DOC aromaticity (Weishaar et al., 2003). SUVA₂₅₄ has also been used as an indicator of terrestrial sources of DOM (Jaffé et al., 2008). Additionally, the ratio of absorbance measured at 250 nm to absorbance at 364 nm (E2:E3 ratio), first proposed

by De Haan and Boer (1987), was calculated. The E2:E3 ratio was determined to track changes in the relative size of DOM molecules. E2:E3 is inversely related to average DOM molecular weight, hence higher E2:E3 ratios, indicate lower molecular weight of DOM. The E2:E3 ratio has been shown to be a good indicator to track alterations in molecular weight of DOM during and after rapid hydrological changes (Ågren et al., 2008). Furthermore, to distinguish DOM originating from microbial sources (including algal-derived) from terrestrially derived DOM, the fluorescence index (FI) (McKnight et al., 2001; Sieczko and Peduzzi, 2014) was determined with a Shimadzu spectrofluorophotometer RF-5301 PC. FI is the ratio of the emission intensity at 450–500 nm under excitation at 370 nm; FI end-values of ~1.3 indicate allochthonously produced DOM, whereas ~2.0 implies autochthonously derived material.

Extracellular enzymatic activity (EEA)

EEA was measured to infer available organic matter sources as suggested by Boschker and Capenberg (1998) for natural systems. Hence we used EEA as a tool to obtain information about availability of non-chromophoric but reactive and quickly cycling DOM (Sieczko and Peduzzi, 2014). Part of the rapidly metabolized DOM (including phytoplankton-derived DOM) is non-chromophoric, but it also includes bioavailable polysaccharides and proteins. Hence, this part of the DOM pool cannot be tracked with standard optical methods. Therefore, serving as a proxy, this ecologically important DOM was investigated by EEA measurements.

Fluorogenic substrate analogs were used to assess potential hydrolysis rates of α -, β - glucosidic and peptide bonds. To estimate the activity of α - (EEA α) and β - (EEA β) glucosidase, 4-methylumbelliferyl (MUF)- α -D-glucoside and 4-MUF- β -D-glucoside were used, respectively. To measure the activity of leucine aminopeptidase (EEA α leu), L-leucine 7-amido-4-methylcoumarin was used. For measuring the extracellular enzymatic activity, triplicates of 3 ml of sample water with 15 μ l fluorescently-labeled substrates (final, substrate saturating concentration: 2.5 μ M) were incubated in the dark at *in situ* temperature. Fluorescence of the replicates was measured immediately after adding the substrate (t_0) and after 45–90 min (t_1) at an excitation wavelength of 360 nm and an emission wavelength of 444 nm (Hoppe, 1983) with a spectrofluorophotometer (Shimadzu RF-5301 PC). Substrate degradation was calculated from the increase in fluorescence over time as nmol substrate hydrolyzed per liter per hour (Hoppe, 1993).

Phenol oxidase activity (PhOx) was measured spectrophotometrically following the method outlined by Pind et al. (1994). Incubations were completed by mixing 2 ml of unfiltered sample water with 2 ml of L-3,4-Dihydroxyphenylalanine (DOPA) stock solution (5 mM DOPA in 2.5 mM NaHCO₃ buffer, pH 8.3). Thus, the substrate saturating, final concentration for our samples was 2.5 mM DOPA. The absorbance was measured at 460 nm in a spectrophotometer (Hitachi U-2000) immediately after addition of DOPA (t_0) and after incubation in dark (t_1) at *in situ* temperature for 180–220 min. Results were calculated using Beer's Law and the molar absorptivity coefficient for the DOPA product 3-dihydroindole-5,6-quinone-2-carboxylate

(diqc) (3.7×10^4 ; Mason, 1948). Phenol oxidase activity was expressed in nmol of product (diqc) produced per liter per hour.

Statistical analyses

For the statistical tests we used R 2.15.0 and SPSS 17.0 for Windows. Most of the data were normally distributed and fulfilled the conditions to apply parametric tests (*T*-test and One-Way ANOVA), unless stated otherwise. In such cases, non-parametric tests such as Wilcoxon- and Kruskal-Wallis tests were applied. Only the data used for correlations and linear regressions were log-transformed and the residuals were tested for normal distribution and homogeneity of variances.

RESULTS

SELECTED ENVIRONMENTAL AND DOM CHARACTERISTICS

Most of the abiotic parameters of subsystem III were clearly distinct from parameters at subsystems I and II. Different nutrient conditions occurred: in subsystems I and II, SRP was significantly lower compared to III, ($p < 0.001$, $n = 42$), whereas the lowest DIN was noted in II. Hence the DIN/SRP-ratio diverged from the Redfield ratio and was significantly higher in I and II than in III ($p < 0.001$, $n = 42$) (Table 1). Average oxygen saturation [$O_2\%$] was significantly different between all subsystems ($p < 0.001$, $n = 42$), with the highest values at I (Table 1). The organic nutrient ratio (DOC/DON) ranged from 13:1 to 62:1 (Table 1), with significantly higher values at II and III compared to I ($p < 0.001$, $n = 42$). The DOC quantity and the qualitative proxies for DOM in the permanently disconnected subsystem (III) were distinct from I and II, with significantly higher DOC ($p < 0.001$), SUVA₂₅₄ ($p < 0.01$), higher E2:E3 ratio ($p < 0.05$) and lower FI ($p < 0.001$) in III (Table 1). The DOC quantity in I and II decreased by 50% during the investigation period, while in III it increased by 30%, (data not shown). Also SUVA₂₅₄ in I and II dropped (43, 57%, respectively) while E2:E3 increased by 20 and 35%, respectively, toward the end of the sampling season (early October) compared with the values measured at the onset of the post-flood period. DOM quantity and quality in subsystems I and II did not significantly differ from each other. In III SUVA₂₅₄ and E2:E3 were relatively constant; the only considerable increase was noted in the middle of the sampling season (data not shown).

PHYTOPLANKTON BIOMASS, PRIMARY PRODUCTION AND PHOTOSYNTHETIC EXTRACELLULAR RELEASE

Phytoplankton biomass and productivity varied distinctly along the connectivity gradient from dynamic to isolated sites. After the flood, in mid-August, the pPP increased markedly and reached the highest values in station A ($263.4 \mu\text{g C l}^{-1} \text{ h}^{-1}$) and B ($213.1 \mu\text{g C l}^{-1} \text{ h}^{-1}$) (Supplementary Figures 1A,B). In subsystem II, the pPP also increased after the flood, the highest pPP rate $181.9 \mu\text{g C l}^{-1} \text{ h}^{-1}$ occurring at the end of the sampling period (Supplementary Figures 1C,D). In subsystem III, pPP was much lower and fluctuated considerably, peaking in September ($41.9 \mu\text{g C l}^{-1} \text{ h}^{-1}$) (Supplementary Figure 1E).

On average, chl *a* and pPP rates were highest in I; pPP rates in III were even 7 times lower than in I (Figures 2A,B). Chl *a* and pPP were correlated significantly only in the semi-isolated subsystem (II) ($r = 0.82$, $p < 0.001$, $n = 14$).

Phytoplankton cells excreted variable amounts of photosynthesis products (PER) into the water. PER in I remained in the range of $1.54\text{--}7.46 \mu\text{g C l}^{-1} \text{ h}^{-1}$ (Supplementary Figures 2A,B). The highest PER coincided well with an increase in pPP rates in both locations in I. In II and III, PER fluctuated considerably ($0.68\text{--}11.3 \mu\text{g C l}^{-1} \text{ h}^{-1}$ and $2.02\text{--}17.0 \mu\text{g C l}^{-1} \text{ h}^{-1}$, respectively) with the highest rates in the end of the sampling period (Supplementary Figures 2C–E). Overall, subsystem III was characterized by generally higher rates of PER, but due to high variability, no significant differences in PER between the subsystems were noted (Figure 3A). Also the percentage of PER (% PER) was highly variable in each subsystem throughout the sampling season. Nonetheless, average % PER increased significantly at locations with lower connectivity with the main river channel (Figure 3B). The share of PER on total primary production (PPt) in I ranged from 0.9 to 3.4%, in II from 0.9 to 12.3% whereas in the isolated subsystem (III), the values were between 12.2 and 47.9% of PPt.

EXTRACELLULAR ENZYMATIC ACTIVITY IN DIFFERENT SUBSYSTEMS OF THE FLOODPLAIN

The EEA rates showed different patterns in the three subsystems (Supplementary Figure 2). In both stations of subsystem I, all the measured enzymes strongly coincided with each other (Supplementary Figures 2A,B); hence, hydrolases

Table 1 | Min-max values of: nutrients (DIN, SRP, DIN/SRP ratio), oxygen saturation and DOM quantity (DOC) and quality (DOC/DON, SUVA₂₅₄, FI ratio, E2:E3 ratio) in subsystems I–III; average in brackets.

Subsystem	DIN [$\mu\text{g l}^{-1}$]	SRP [$\mu\text{g l}^{-1}$]	Ratio DIN/SRP [g/g]	O_2 [%]	DOC [mg l^{-1}]	Ratio DOC/DON	SUVA [$\text{l mg}^{-1} \text{ m}^{-2}$]	FI	E2:E3 ratio
I	107.5–1478.9 (743.1)	0.88–25.7 (8.68)	37.3–485.1 (181.7)	74–147 (104)	1.30–2.61 (1.88)	12.9–22.9 (17.1)	3.53–7.53 (5.79)	1.52–1.68 (1.62)	4.66–6.79 (5.82)
II	86.9–349.7 (181.4)	0.78–3.24 (1.44)	60.7–332.5 (132.8)	50–100 (75)	1.98–4.37 (2.63)	13.3–41.5 (23.2)	3.58–8.38 (6.34)	1.40–1.66 (1.58)	4.34–6.69 (5.34)
III	368.7–1051.8 (771.8)	121.7–581.4 (364.9)	1.05–4.47 (2.41)	10–65 (38)	21.21–389.0 (270)	17.4–62.4 (25.6)	5.60–12.9 (7.94)	1.42–1.53 (1.51)	4.88–10.7 (6.26)

For abbreviations see text.

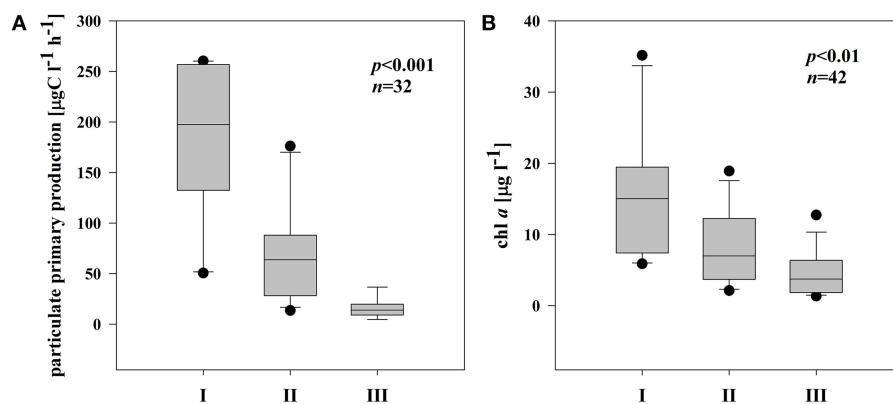


FIGURE 2 | Boxplots illustrating average chl *a* (A) and pPP rates (B) in three different subsystems (I–III) during the sampling period. The boundaries of the box plot indicate the 25th and 75th percentiles, points

indicate outliers, the solid line in the box marks the median. Statistical differences are indicated on the top of each panel (for applied statistical tests see Section Materials and Methods).

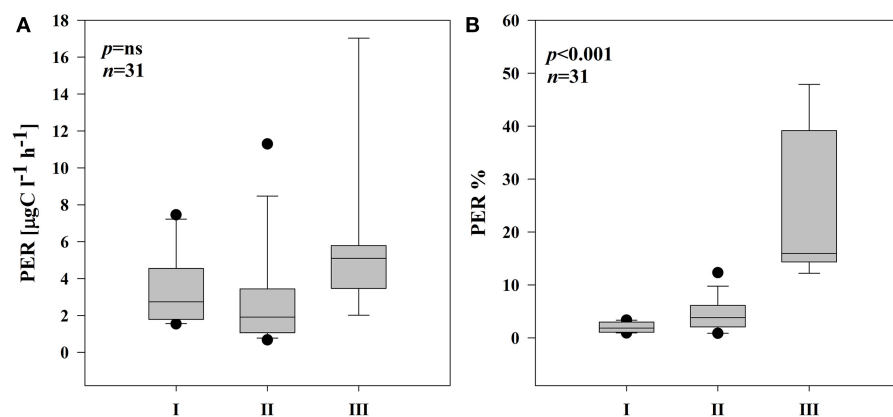


FIGURE 3 | Boxplots illustrating average PER rate (A) and % of PER release in Ppt (B) in three different subsystems (I–III) during the sampling period. The boundaries of the box plot indicate the 25th and 75th

percentiles, points indicate outliers, the solid line in the box marks the median. Statistical differences are indicated on the top of each panel (for applied statistical tests see Section Materials and Methods).

and oxidase were strongly and positively correlated (Table 2A). In subsystem II, EEA was more variable, but, the hydrolytic enzymes coincided quite well during the investigated post-flood period (Supplementary Figures 2C,D); correlations between hydrolytic enzymes (glucosidases with leu-aminopeptidase) were still observed (Table 2B). The hydrolase activity peaked in subsystem III, while PhOx showed the lowest rates here toward the end of the sampling period (Supplementary Material Figure 2E). Extracellular enzymes in III were more decoupled, but similarly to other subsystems the activities of the two glucosidases (EEAa and EEAb) coincided well throughout the investigated post-flood period (Table 2C). Activity of glucosidases (Figures 4A,B) and PhOx (Figure 4C) were on average highest in III, whereas EEAlau was highest in the dynamic subsystem (I) (Figure 4D). Among the hydrolytic enzymes, EEAlau displayed the highest activity, which was 3–4 orders of magnitude higher than EEAa and EEAb activity. In line with the gradient of connectivity between I, II, and III, decoupling of the different enzymatic activities occurred (Table 2).

BACTERIAL ABUNDANCE AND PRODUCTION

Total bacterial numbers (BA) in all the subsystems ranged from 1.1 to 7.0×10^6 cells ml^{-1} (Supplementary Figures 3A–E). They were on average higher in III but not significantly different between the three subsystems (Figure 5A).

BSP ranged from 0.24 – $3.91 \mu\text{g C l}^{-1} \text{h}^{-1}$ with occasionally highest rates in III (Supplementary Figures 3A–E). Due to high variability, however there were no significant differences between subsystems (Figure 5B). BSP followed a similar pattern as BA in subsystems I and II (Supplementary Figures 3A–D), hence BSP was significantly correlated with BA in I ($r = 0.82$, $p < 0.01$, $n = 10$) and in II ($r = 0.56$, $p < 0.05$, $n = 18$).

MICROBIAL RESPONSE ON DOM CHARACTERISTICS

BSP and BA were not related to DOM quality (based on optical properties) in any of the subsystems. No direct enzymatic response to any of the qualitative proxies was observed in subsystem I and III. In subsystem II, however, the synthesis of EEAa and EEAb was apparently related with SUVA_{254} (Figures 6A,B).

Table 2 | Correlation coefficients between extracellular enzymatic activity (EEAa, EEAb, EEAlau, phOx) in subsystem I (A), subsystem II (B), and subsystem III (C).

	EEAa	EEAb	EEAlau	phOx
I (A)				
EEAa	–	0.98***	0.86**	0.73*
EEAb		–	0.90***	0.70*
EEAlau			–	0.76*
phOx				–
II (B)				
EEAa	–	0.98***	0.50*	ns
EEAb		–	0.54*	ns
EEAlau			–	ns
phOx				–
III (C)				
EEAa	–	0.74*	ns	ns
EEAb		–	ns	–0.65*
EEAlau			–	ns
phOx				–

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. The data were log-transformed.

EEAa and EEAb were also negatively related to the E2:E3 ratio (Figures 6C,D).

RELATIONSHIPS BETWEEN EXTRACELLULAR ENZYMATIC ACTIVITY AND PRIMARY AND SECONDARY PRODUCTION

Depending on the subsystem, the extracellular enzymes exhibited distinct relationships with phytoplankton and bacterial parameters. In the dynamic subsystem (I), hydrolase activity was strongly promoted by the primary production rate and biomass (pPP and chl *a*) (Table 3). Beyond a positive relation between PhOx and chl *a* ($r = 0.46$, $p = 0.05$, $n = 14$) in II, no other direct enzymatic response to chl *a* occurred in subsystems II and III. There was, however, a clear enzymatic response to phytoplankton extracellular release. The synthesis of α - and β -glucosidase clearly coincided with PER in subsystem I (Supplementary Figures 2A,B); EEAa and EEAb were apparently promoted by PER (Figures 7A,B). However, in subsystem II, the opposite tendency was noted; the activity of EEAa and EEAb was negatively related to PER (Figures 7C,D). In subsystem III, EEAb corresponded to PER (Supplementary Material Figure 2E), hence it was positively related to PER (Figure 7E).

Since DOM properties, based on our optical assessment of DOM quality, were not related to BSP or BA, the enzymatic approach helped to elucidate which DOM was important for bacterial metabolism in each subsystem. Accordingly, we linked EEA to BSP to investigate which DOM pool was more important for bacterial metabolism in our three different subsystems. Our results imply that degradation of distinct material supported bacterial growth in each subsystem differently. More than one enzyme explained BSP variability; we therefore performed stepwise multiple regression analyses using a combination of EEAlau, EEAa, EEAb, and PhOx as independent variables. This revealed that in each subsystem different enzymes were the most significant predictors for BSP (Table 4). In I, only

EEAlau was directly related to bacterial production. In II, BSP was best described by the activity of EEAlau and PhOx, while in subsystem III, EEAb explained most of the variability in BSP (Table 4).

In I, pPP was directly related to BSP ($r = 0.67$, $p < 0.05$) and BA ($r = 0.64$, $p < 0.05$). Phytoplankton biomass (chl *a*) also positively related to BA in I ($r = 0.65$, $p < 0.05$) and BSP in II ($r = 0.49$, $p < 0.05$, $n = 17$). There was no direct response of bacterial production or abundance to photosynthetic extracellular release (PER) in any of the subsystems.

In subsystem I, where the highest pPP was observed (Figure 3), the BSP:PPt ratio was the lowest; bacterial production there ranged from 0.38 to 3.06% (average 1.07%) of the total primary production. On average the BSP:PPt ratio was more than 2 times higher in III than in II, where it remained in a range of 2.01–17.7% (8.65%) and 0.58–10.6% (3.66%), respectively.

DISCUSSION

ENZYMATIC ACTIVITY: RESPONSE TO DOM QUALITY

This study was designed to shed light on the microbial response to various allochthonous and autochthonous DOM sources in different sections of a floodplain. Our results show that DOM in all subsystems was a mixture of allochthonous and autochthonous material. Although no significant differences were noted between subsystem I and II after the flood, this study points to the more autochthonous, less aromatic DOM in subsystem I. However, in subsystem III DOM was clearly more allochthonous, higher molecular weight and more aromatic. This is very likely due to input of local terrestrial material (dense surrounding vegetation), because this subsystem is never connected to the main channel. In other subsystems (I and II) allochthonous material derives also from the main channel during occasional surface connection with the Danube (Sieczko and Peduzzi, 2014). Differences in the DOM composition and availability can be important in determining the functioning of microbial communities (Hoostal and Bouzat, 2008). Our results, however, do not generally support the idea that enzymatic activity is strongly linked to DOC quantity or quality based solely on optical measurements. Even though the quantitative and qualitative parameters of DOM were significantly different between the investigated subsystems, only in subsystem II did we find a significant relation between enzymatic activity and optical properties of DOM.

The positive relation between EEAa, EEAb activity and SUVA₂₅₄ or molecular size of DOM (Figures 6A–D) implies enhanced expression of glucosidases in the presence of terrestrially derived, higher molecular weight DOM. There is evidence that HMW substances can be even more reactive as long as they are diagenetically younger (Amon and Benner, 1996) compared to diagenetically older LMW residues of former bacterial metabolism (Ogawa et al., 2001). Nonetheless, molecular weight or origin are not the only factors regulating DOM bioavailability. For example, microbial communities are most productive when metabolizing their native DOM sources (Young et al., 2005). This could explain that, in other subsystems, EEA was not related to the molecular size of DOM and that other factors might have been important.

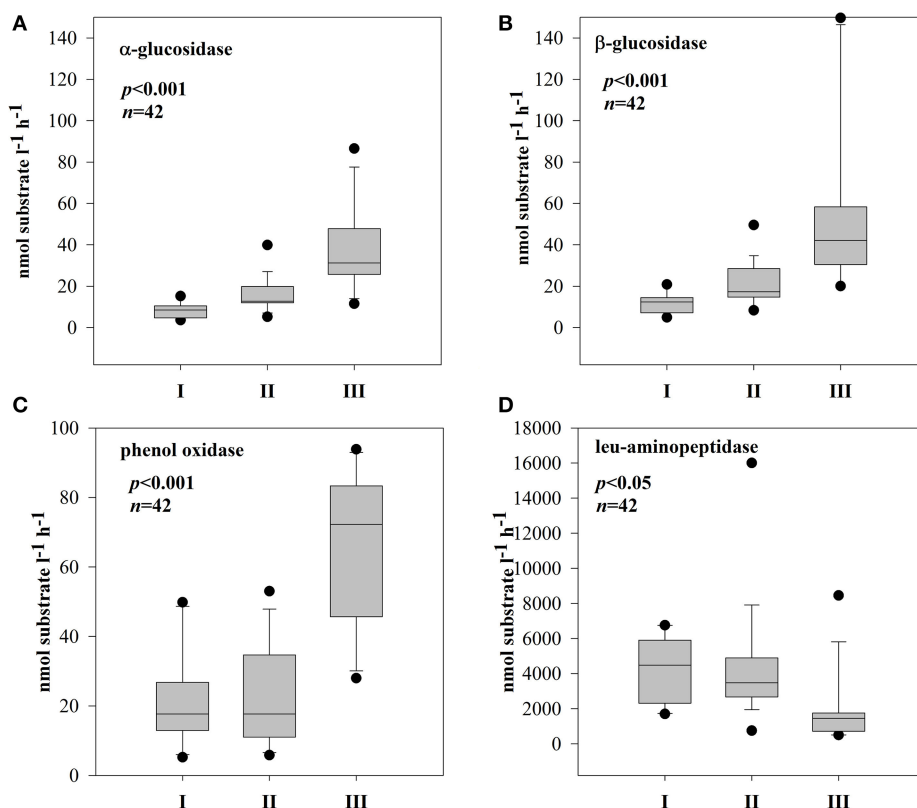


FIGURE 4 | Boxplots of extracellular enzymatic activity of α -glucosidase (A), β -glucosidase (B), phenol oxidase (C), and leu-aminopeptidase (D) in three different subsystems (I–III). The boundaries of the box plot indicate

the 25th and 75th percentiles, points indicate outliers, the solid line in the box marks the median. Statistical differences are indicated on the top of each panel (for applied statistical tests see Section Materials and Methods).

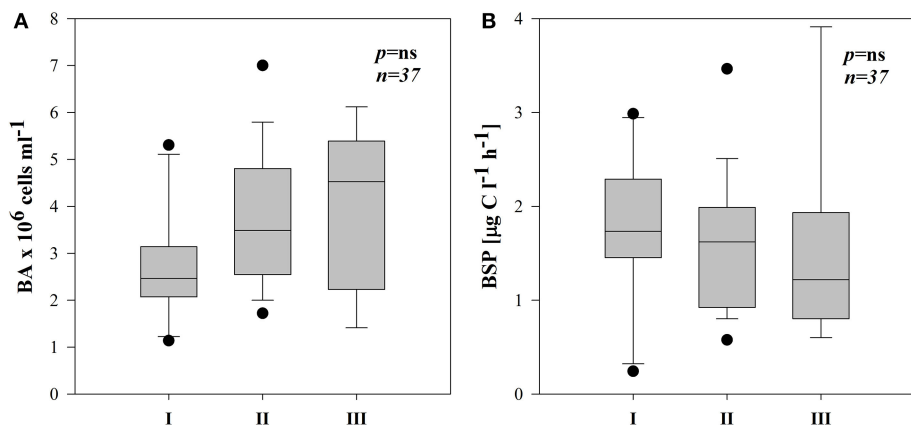


FIGURE 5 | Boxplots of average BA (A) and BSP (B) in three different subsystems (I–III). The boundaries of the box plot indicate the 25th and 75th percentiles, points indicate outliers, the solid line in

the box marks the median. Statistical differences are indicated on the top of each panel (for applied statistical tests see Materials and Methods).

RELATIONSHIP BETWEEN PHYTOPLANKTON AND MICROBIAL ENZYME ACTIVITIES

The mechanisms behind enzyme synthesis are regulated by a number of environmental factors which may induce or suppress production of extracellular enzymes (Sinsabaugh and Follstad

Shah, 2012; Arnosti et al., 2014). In our study, we attempted to evaluate the importance of autotrophic planktonic production for EEA. Our results show that in subsystem I, primary production (pPP and chl *a*) was strongly and positively related to EEAleu (Table 1), which indicates the importance of algal products here.

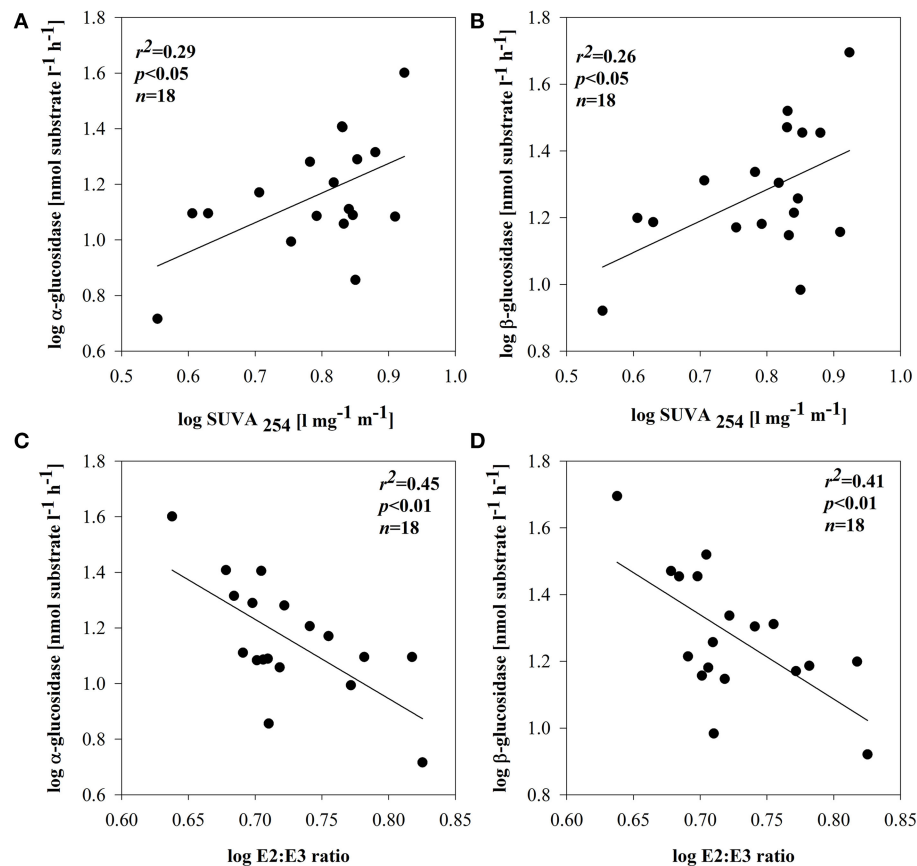


FIGURE 6 | Relationships between SUVA₂₅₄ and: α-glucosidase (A) or β-glucosidase (B) and E2:E3 ratio and: α-glucosidase (C) or β-glucosidase (D) in subsystem II. All data were log-transformed.

Table 3 | Correlation coefficients between extracellular enzymatic activity (EEAa, EEAb, EEAlu, PhOx) and phytoplankton parameters (pPP and chl *a*) in subsystem I.

	pPP	chl <i>a</i>
EEEa	0.82**	0.81**
EEAb	0.85**	0.87**
EEAlu	0.83**	0.77**
phOx	ns	ns

** $p < 0.01$. The data were log-transformed.

Studies in river environments showed that EEAlu was actively produced when autochthonous production of labile organic matter was high (Wilczek et al., 2005). One potential reason for quite high activity levels of EEAlu in our study, compared to the activity of other enzymes, is that aminopeptidases are not very specific in their cleaving (Chróst, 1992) and that they can hydrolyze many different peptides.

In I we also observed strong correlations between all the enzymes, including PhOx (Table 3). This indicates a tight coupling of metabolic pathways (Hoostal and Bouzat, 2008) and mutualism within the microbial community here. Further, in subsystem II, the strong and positive relation between PhOx

and chl *a* may indicate that input of fresh labile material from primary producers was able to augment synthesis of PhOx.

The phenomenon of enhanced production of extracellular enzymes degrading recalcitrant OM in the presence of labile OM is called priming effect (PE) (Guenet et al., 2010; Bianchi, 2012) and it has already been proven in laboratory assays (Shimp and Pfaender, 1985) and field studies (Treignier et al., 2006; Rier et al., 2007, 2014). Although our data may imply that in subsystems I and II phytoplankton-derived material possibly could influence the expression enzymes responsible for degrading recalcitrant DOM, more extensive studies on a potential priming effect here would be necessary. However, we suggest that river-floodplain systems are places where ample opportunities for this phenomenon are created. This calls for further investigations on the existence of a priming effect in hydrologically dynamic river-floodplain systems.

Carbon-rich exudates are often predominant products of phytoplankton growing under an imbalanced DIN/SRP ratio (Penna et al., 1999). Production of bacterial enzymes may be also related to the nutrient status. We propose that strong P-limitation (and low SRP) noted in subsystem II (Table 1) may have induced C-rich PER. Under P-depleted conditions, phytoplankton releases large amounts of carbohydrates (Obornosterer and Herndl,

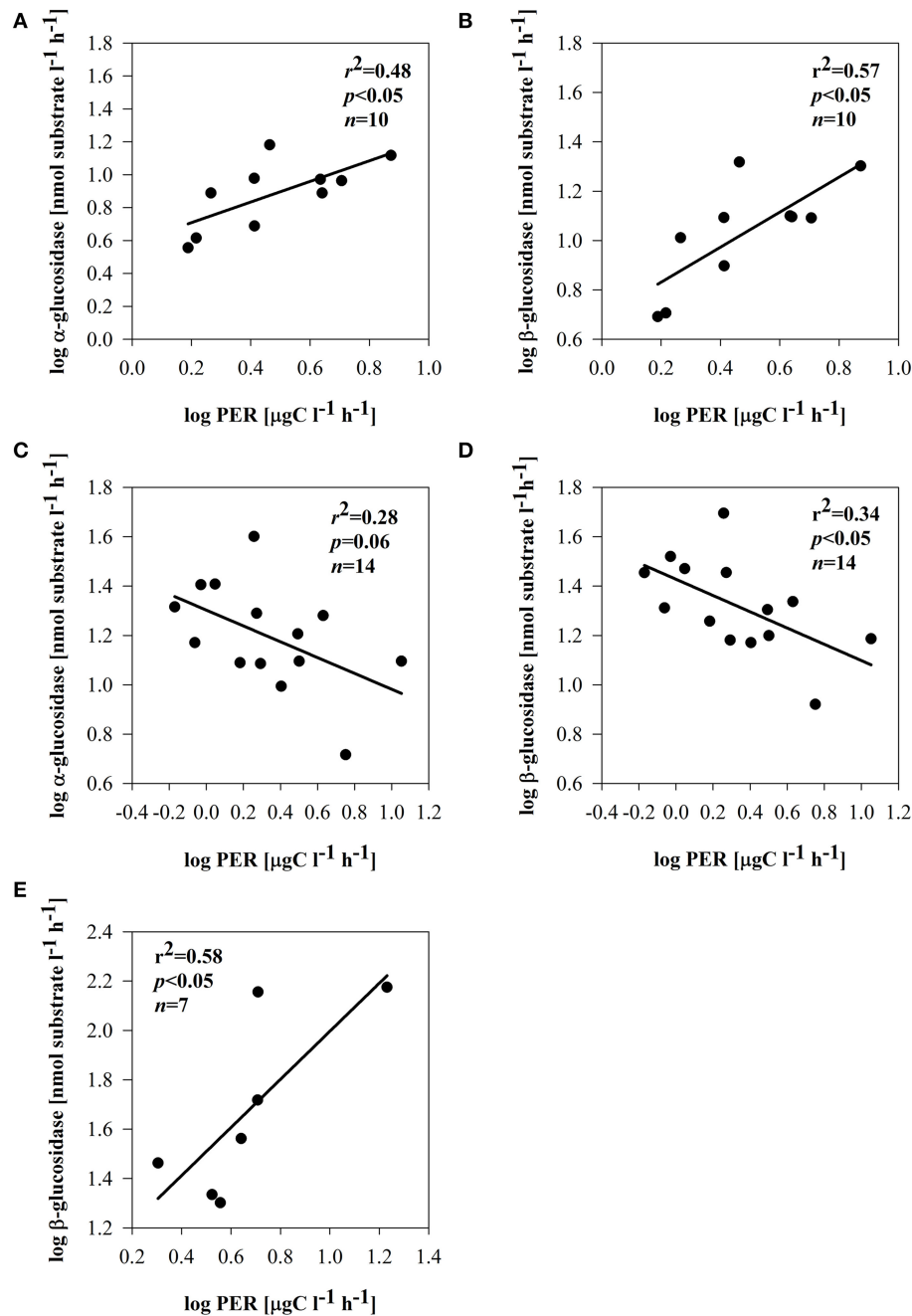


FIGURE 7 | Relationships between PER and α -glucosidase and PER and β -glucosidase in subsystem I (A,B), subsystem II (C,D), and subsystem III (E). All data were log-transformed.

1995), which may have induced a high utilization of PER by bacteria. Utilizable PER implies a reduction of EEAb activity, and this could be responsible for the negative relation of glucosidases with PER (**Figures 7C,D**). A decrease of β -glucosidase produced by aquatic bacteria has been described as being linked to the introduction of phytoplankton-derived DOM (Chróst and Siuda, 2002). Furthermore, also in lake water the catabolic repression of glucosidases in the presence of easily assimilable substrates is

described as an important mechanism controlling EEAb synthesis (Chróst, 1989).

However, in subsystem III nutrient limitation was unlikely, because DIN and SRP were high (**Table 1**). Other reasons (strong light limitation or viral lysis), potentially responsible for elevated PER here, need to be considered (compare Mykkestad, 2000). In subsystem III (**Table 1**), a large portion of total primary production was exudated, reaching up to 50% (**Figure 3B**). The

Table 4 | Multiple stepwise regressions between bacterial secondary production (BSP) and enzymatic activity (EEAa, EEAb, EEAlau, PhOx).

	Independent variables	Regression equation	Adjusted r^2
Subsystem I BSP	EEAlau(beta = 0.65; $p < 0.05$)	$\log \text{BSP} = 2.527 + 0.895 \log \text{EEAlau}$	0.35
Subsystem II BSP	PhOx(beta = 0.56; $p < 0.01$)	$\log \text{BSP} = 0.062 + 0.397 \log \text{PhOx}$	0.60
	EEAlau(beta = 0.45; $p < 0.05$)	$\log \text{BSP} = -1.080 + 0.342 \log \text{EEAlau}$	
Subsystem III BSP	EEAb(beta = 0.86; $p < 0.001$)	$\log \text{BSP} = -0.469 + 0.797 \log \text{EEAb}$	0.72

All data were log transformed. For abbreviations see text.

positive relation between PER and EEAb (**Figure 7E**) in subsystem III implies utilization of phytoplankton exudates that are mainly comprised of β -linked polysaccharides; these are often a predominant product of phytoplankton extracellular release (Biddanda and Benner, 1997) and from lysed cells (Weinbauer and Peduzzi, 1995). Although some of the primary production exudates contain quickly consumed compounds, actively growing phytoplankton also releases higher molecular weight organic matter, which is largely composed of polysaccharides (Lignell, 1990; Mykkestad, 2000). We suggest that the quality of exudates triggered a greater activity of EEAb.

WHAT SUPPORTS BACTERIAL PRODUCTION IN DISTINCT FLOODPLAIN SUBSYSTEMS?

Based on our study, EEA appears to be a valuable tool for elucidating the potential linkage between non-chromophoric, but rapidly cycling DOM and bacterial production. Our results imply that in each of the three subsystems, the degradation of distinct material supported bacterial growth. The similar average BA and BSP rates in all subsystems (**Figures 5A,B**)—but significantly different EEA (**Figures 4A–D**), phytoplankton biomass and pPP rates (**Figures 2A,B**)—suggest that in each of the three subsystems, bacterial growth was supported by DOM from different sources. Typically, DOM of autochthonous origin is higher-quality material that mainly controls BSP (Cole et al., 2002), as opposed to allochthonously derived DOM, which is recalcitrant with much longer turnover times. These pools are produced independently from each other but both are available for bacteria (Del Giorgio and Pace, 2008). Although correlation between BSP and pPP has been demonstrated both in culture (Gurung et al., 1999) and field studies (Cole et al., 1988), our field data show that only in subsystem I was primary production (pPP) directly coupled to BSP or BA (see Results). At increased primary productivity bacteria can assimilate more phytoplankton-derived DOM (Piontek et al., 2012). Hence, we suggest that in subsystem I (with highest pPP noted), pPP derived-material supported bacterial production directly or by an enzyme-mediated step. Other studies from this area (Preiner et al., 2008) report stimulated phytoplankton productivity after floods in frequently connected water bodies. Also, according to the River Wave Concept (Humphries et al., 2014), autochthonous production of DOM predominates after floods. Hence, strong relation of pPP with EEAlau (**Table 3**) and of BSP with EEAlau (**Table 4**) implies that pPP products, hydrolyzed by EEAlau, supported BSP in I. Our results suggest that in frequently flooded river-floodplain systems (such as subsystem I), elevated bacterial growth could be driven mainly by enzymatic degradation of particulate primary production.

In subsystem II, a more refractory pool appears to also support bacterial growth. PhOx and EEAlau were the only enzymes that could explain any variability in BSP in II (**Table 4**). Positive relations of these enzymes with BSP here imply that organic matter degraded by PhOx and EEAlau could have become relatively more important as a source and promoted bacterial growth and abundance in subsystem II. Other studies in different environments support our findings. Lignin material led to increased bacterial abundance in soils where the bacterial population assimilated lignin-derived carbon (Deangelis et al., 2011). Also in peatlands bacterial growth was linked to phenol oxidase activity (Fenner et al., 2005). In freshwater, PhOx activity is less explored (Münster and De Haan, 1998) but bacteria seem to play a leading role in decomposing lignin in aquatic ecosystems (Li et al., 2008). Lignin degradation is a relatively slow process (Benner and Kaiser, 2011) and significantly increases with higher temperature (Donnelly et al., 1990). Because lake water typically has a long residence time, such waters can be important places for microbial processing of carbon received from terrestrial sources. In river systems, occasional flood events are especially important in this process; they deliver significant amounts of low-age, terrestrial DOM (Berggren et al., 2009). We propose that semi-isolated floodplain side arms (such as subsystem II) provide opportunities for microbial processing of more refractory carbon. Sporadic inputs of fresh terrestrial material, reduced hydrodynamic stress due to rare flooding, high oxygen availability and the stimulating effect of phytoplankton on PhOx synthesis may create favorable conditions that could trigger the utilization of lignin-derived material.

Despite the elevated % PER in subsystem III, no direct link between BSP and pPP or PER was observed. This could be because bacteria in that subsystem did not take up algae-derived carbon directly. Our results, however, imply that BSP was driven by primary production here. This was found also in lakes with terrestrial input, where bacterial metabolism was driven by autochthonous primary production (Kritzberg et al., 2005). Even since mean pelagic primary production was significantly lower in III compared to the highly productive floodplain side arms of subsystem I, PER was still able to sustain bacterial metabolism also in subsystem III. In our study, close and positive relations of EEAb and BSP (**Table 4**) suggest that PER products, hydrolyzed by EEAb (**Figure 7E**), supported BSP. The activity of β -glucosidase is strongly stimulated by carbohydrates, which phytoplankton accumulates as a storage material (Mallet and Debroas, 2001; Børsheim et al., 2005). This may imply that β -linked polysaccharides derived from phytoplankton degradation could be beneficial sources sustaining bacterial growth in subsystem III. This allows

us to suggest that in isolated floodplain lakes, despite high terrestrial input, bacterial growth can be also driven by autochthonous carbon sources to a certain extent (Robertson et al., 1999).

EXTRACELLULAR ENZYMATIC ACTIVITY LINKING PRIMARY AND SECONDARY PRODUCTION

Our results stress the importance of primary production in sustaining BSP in backwaters with a connectivity gradient. Nonetheless, the only modest variation of BSP compared to more dynamic primary production (pPP, PER, and chl *a*) demonstrates that organic matter derived from sources other than phytoplankton also supported BSP in this river-floodplain system. The potential coupling between bacterial and phytoplankton components can be indicated by the BSP:PPT ratio (Van Wambeke et al., 2002). This ratio is a measure for the importance of heterotrophic bacteria in consuming material from primary production (Kirchman, 2010). A high ratio could reflect significant input of terrestrial OM (Kirchman, 2012). The highest value in subsystem III implies that allochthonous, non-planktonic sources were relatively more important than bacterial utilization of phytoplankton-derived C (compare Tranvik, 1989; Gao et al., 2007). EEA measurements, however, point to phytoplankton exudates as an important source supporting BSP in subsystem III. Our study demonstrates that, despite the lack of data for a direct relationship between bacterial and phytoplankton components, the EEA analyses are able to elucidate the patterns relating these two variables, especially in systems with high terrestrial input.

Bacteria can utilize an increasing proportion of primary production, when phytoplankton is less efficient (Conan et al., 1999). At increased primary productivity, when phytoplankton is most active, the BSP:PPT ratio is the lowest (Almeida et al., 2005; Morana et al., 2014). The lowest ratio has been sometimes interpreted as a low flux of labile OM to bacteria (Ducklow et al., 2012), hence indicating a weak coupling between the bacterial and phytoplankton component. In our study, however, the lowest BSP:PPT ratio in subsystem I was accompanied by a strong coupling of BSP and pPP. Moreover, EEA measurements emphasize the significance of primary production (pPP, PER, and chl *a*) for bacterial utilization. Hence, we suggest that in subsystem I a fast transfer of phytoplankton-derived carbon to bacteria occurred: at elevated primary production, more of phytoplankton-derived material was assimilated by bacteria (compare De Kluijver et al., 2014). Our conclusion is that EEA measurements are very useful in describing the coupling of bacterial and phytoplankton components. The BSP:PPT ratio alone does not fully describe the strength of this link (Morán et al., 2002). Drawing conclusions based solely on that ratio simplifies the mechanisms that relate these two components (Almeida et al., 2005) potentially overlooking important links between bacteria and phytoplankton.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <http://www.frontiersin.org/journal/10.3389/fmicb.2015.00080/abstract>

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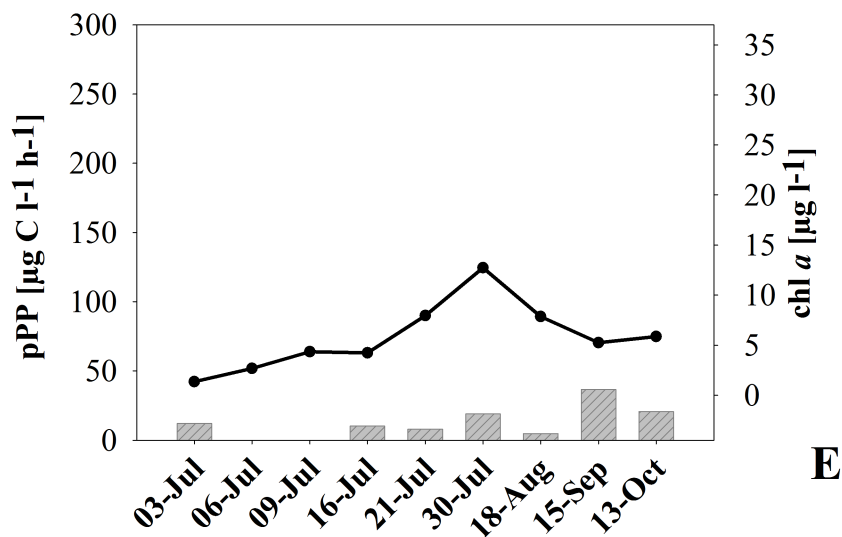
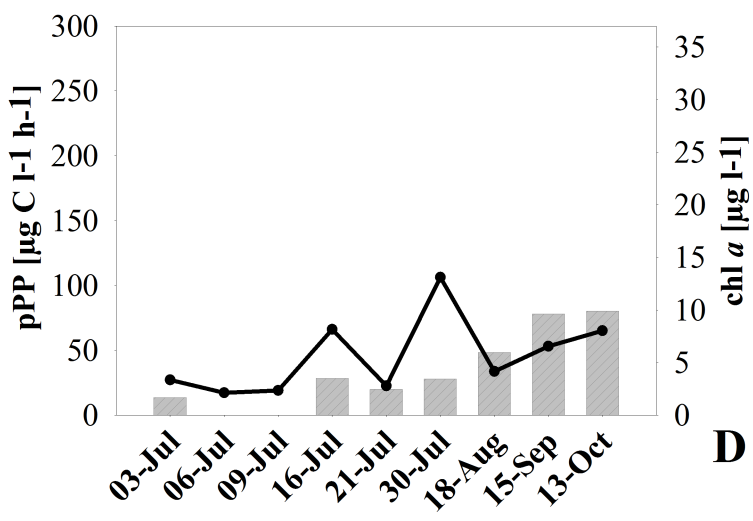
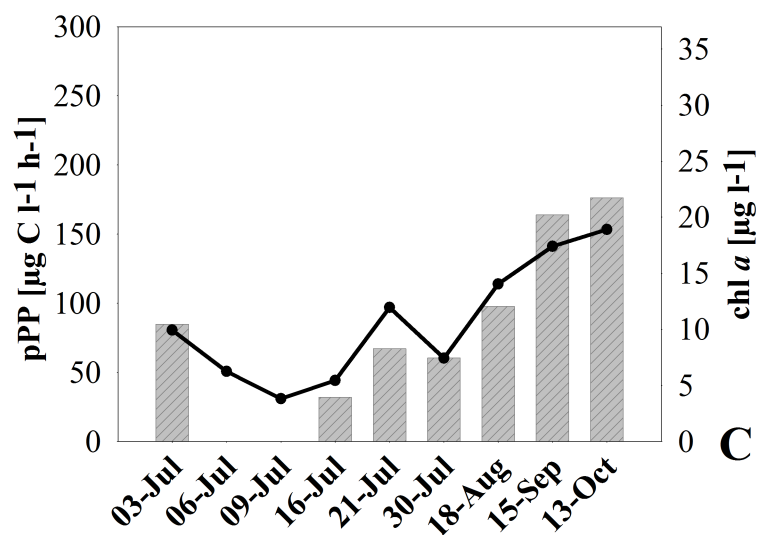
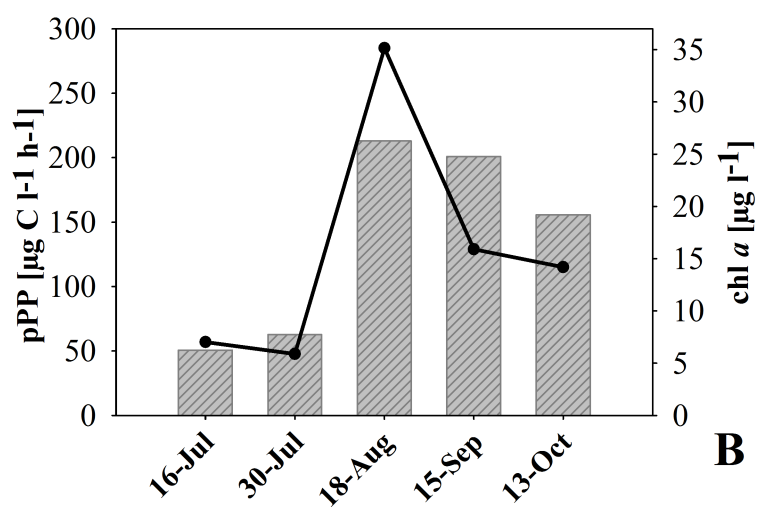
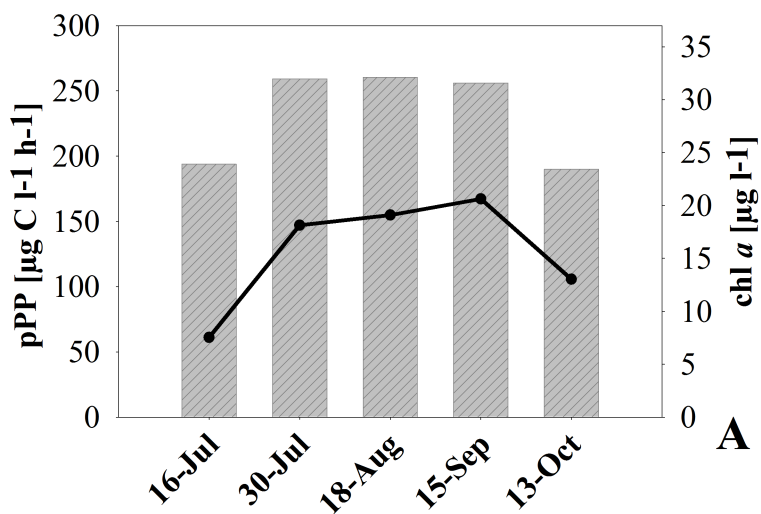
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Supplementary Figure 1

Temporal patterns of particulate primary production (grey bars) and chl *a* (●) in stations of subsystem I (A and B), subsystem II (C and D) and subsystem III (E).

For analytical variations and replicates, see material and methods.

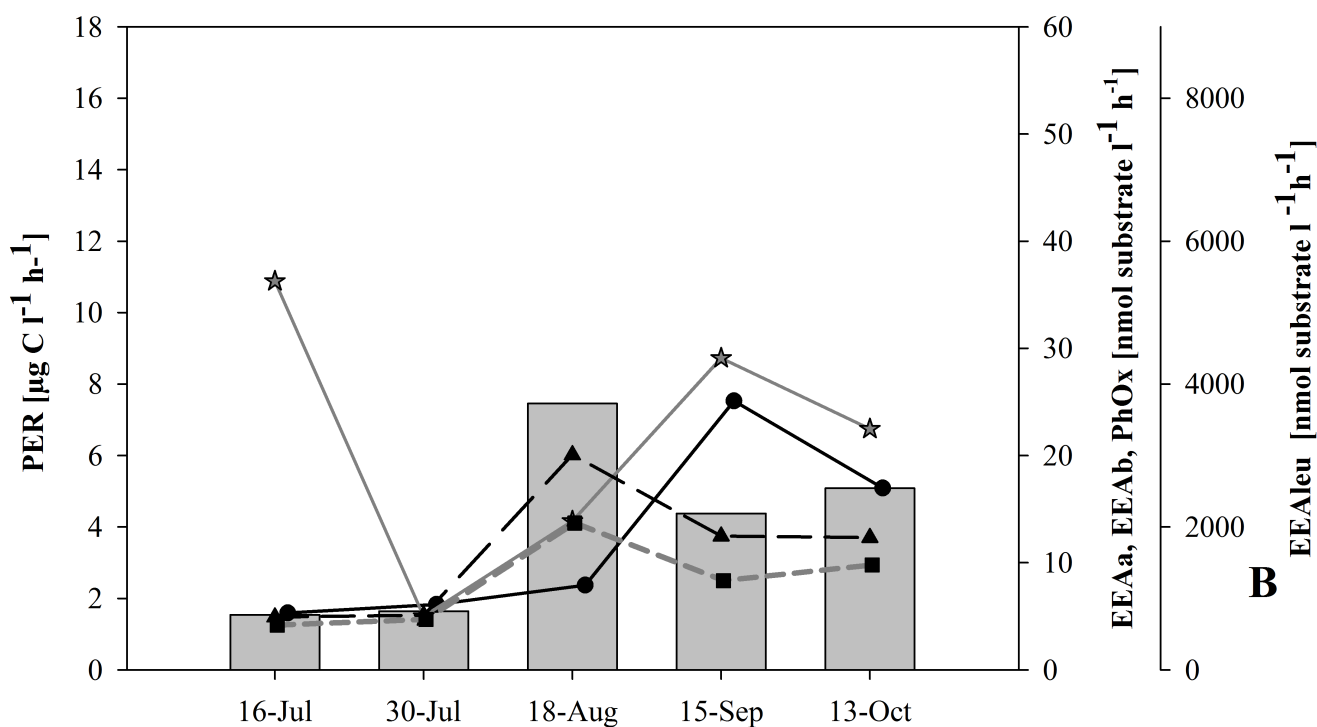
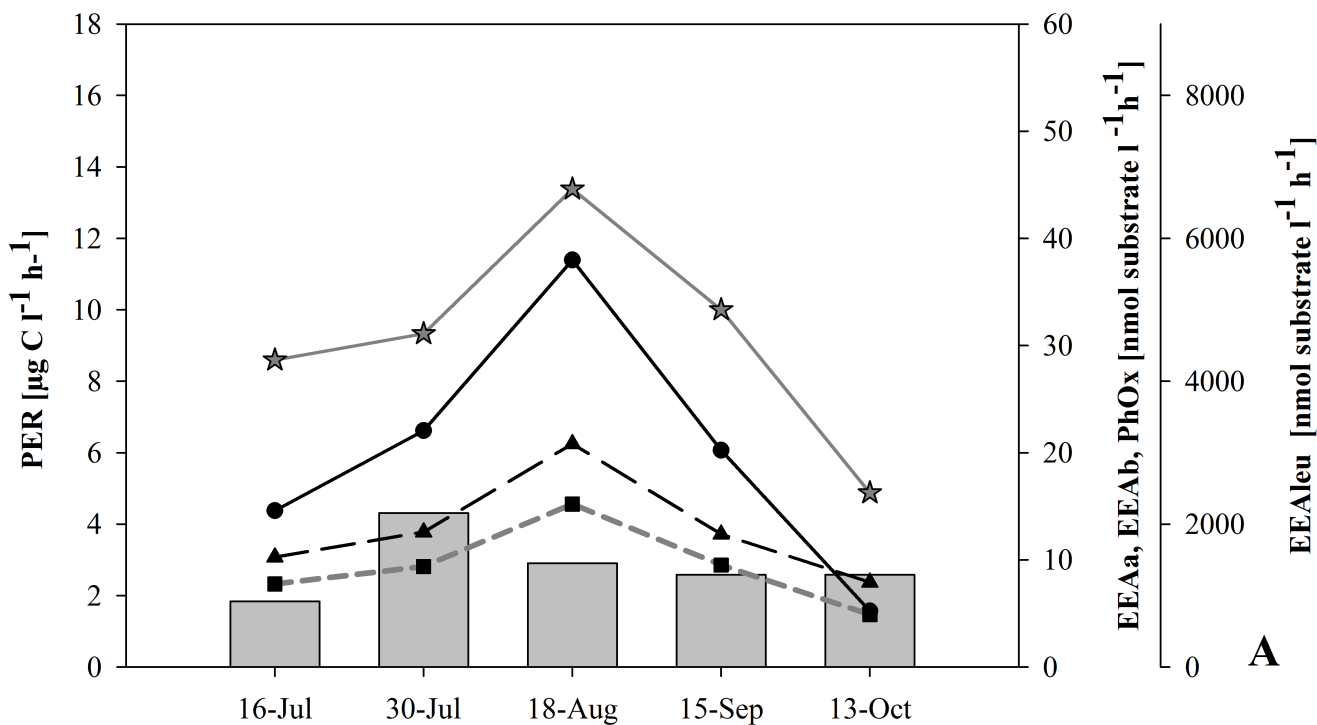


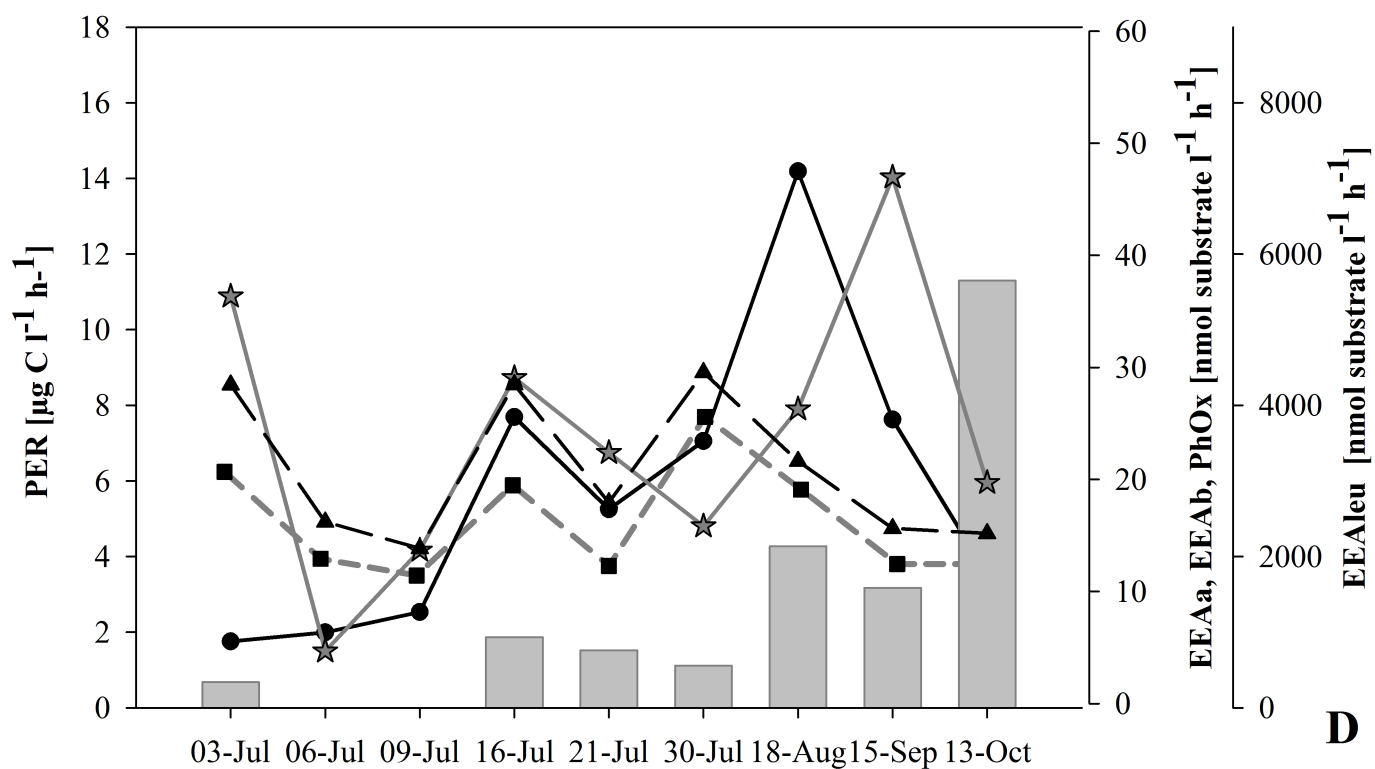
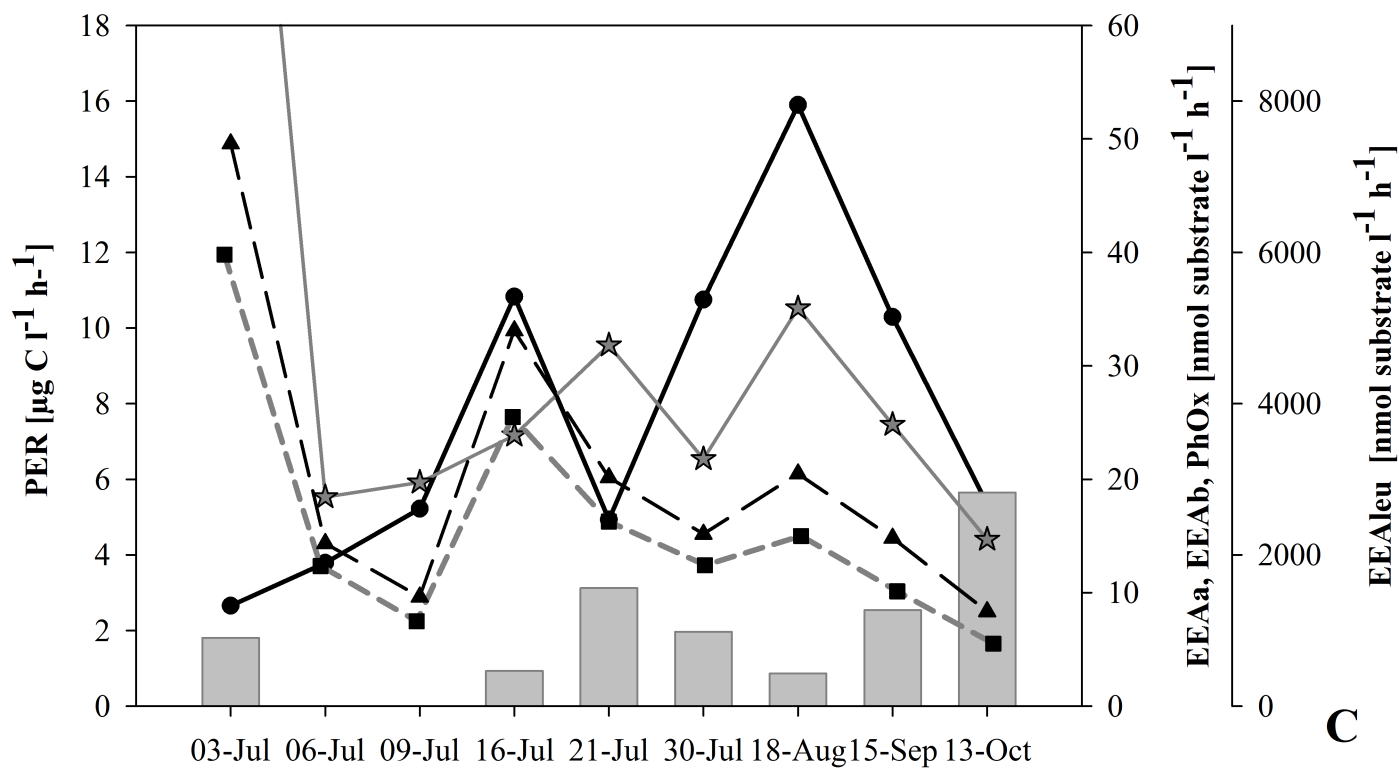
Supplementary Figure 2

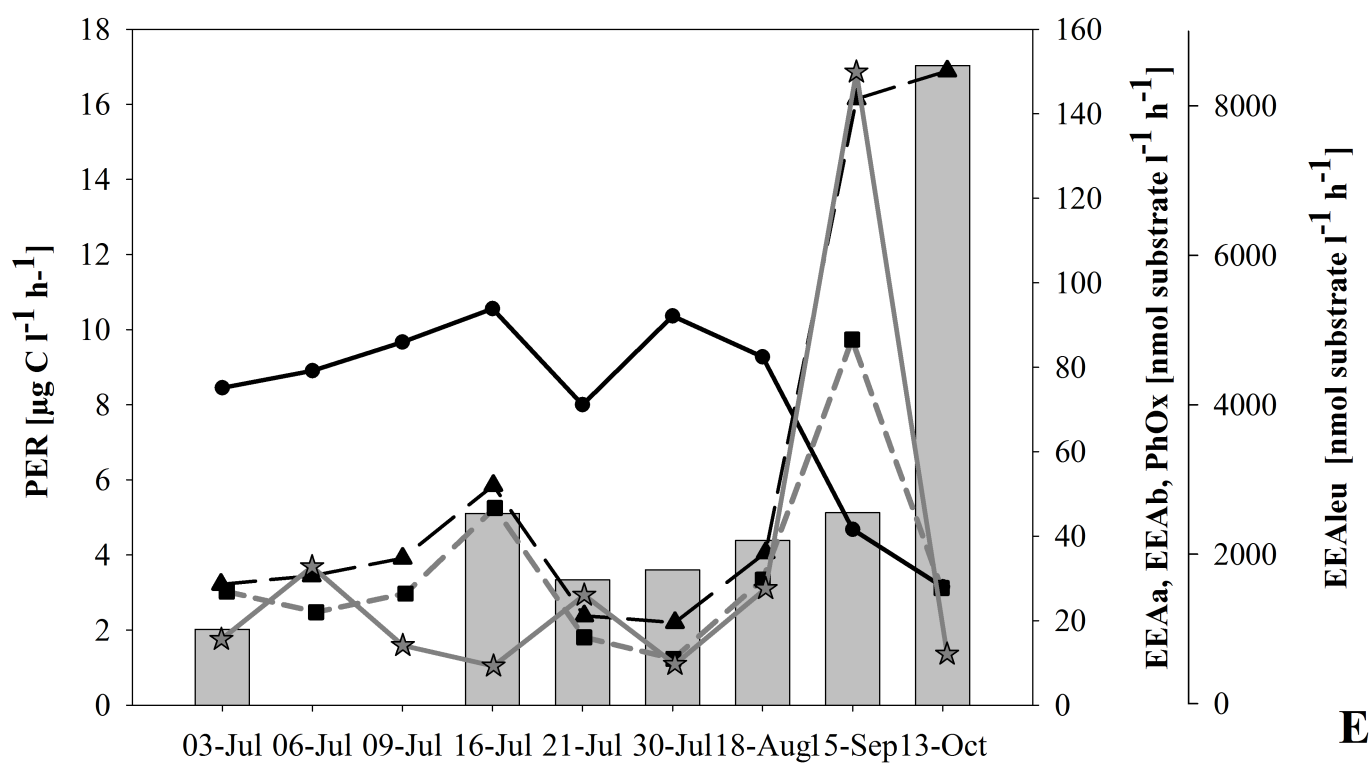
Temporal patterns of extracellular enzymatic activity and photosynthetic extracellular release in subsystem I (A, B), subsystem II (C, D) and subsystem III (E) during a post-flood period, between July and October. Grey bars indicate rate of PER, lines indicate EEA: α -glucosidase (■), β -glucosidase (▲), leu-aminopeptidase (★), and phenol oxidase (●).

For analytical variations and replicates, see material and methods (analytical variance < 5 %).

Note different scale for EEAA, EEAb and PhOx in E.

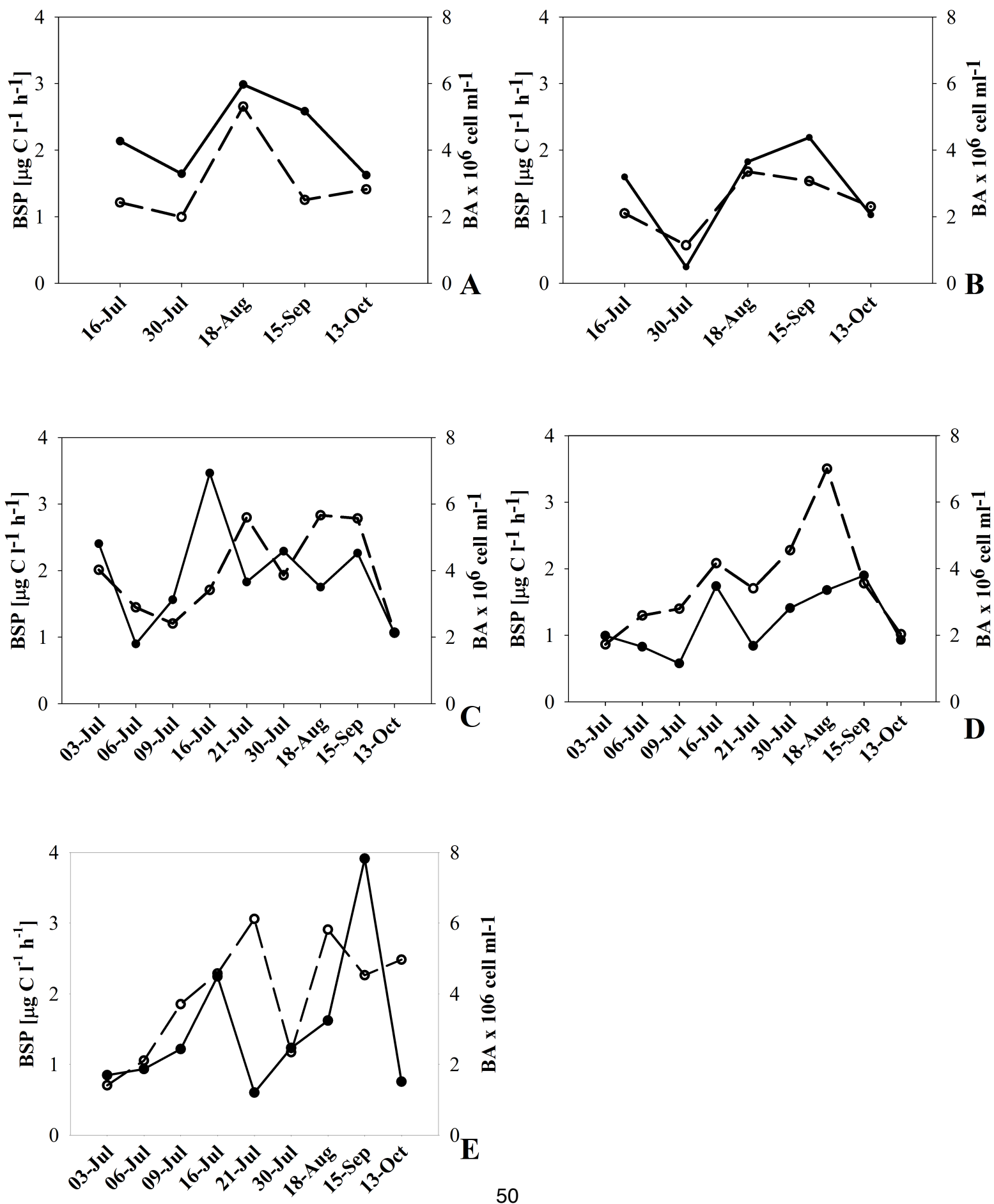






E

Supplementary Figure 3.
 Temporal patterns of bacterial secondary production (●) and abundance (○) in subsystem I (A, B), subsystem II (C, D) and subsystem III (E) during a post-flood period, between July and October. For analytical variations and replicates, see material and methods (analytical variance < 5 %).



6. Paper III

Sieczko A, Demeter K, Singer G, Tritthart M, Preiner S, Mayr M, Meisterl K and Peduzzi P (2016) *Aquatic methane dynamics in a human-impacted river-floodplain of the Danube*. Limnology&Oceanography: Special Issue: "Methane Emissions from Oceans, Wetlands, and Freshwater Habitats: New Perspectives and Feedbacks on Climate". doi: 10.1002/lno.10346.

My contribution to this study was designing and planning field campaigns. Due to rapid hydrological changes I also adapted the sampling schedule in order to cover all the hydrological phases, especially including a 100-year flood. I also performed most of the sampling and executed selected analyses in the laboratory. Moreover, I interpreted the results and made most of the statistical calculations. As a first author I wrote the first draft of the manuscript, which afterwards, with the cooperation of the co-authors, was finalized. Additionally, as a corresponding author I completed the submission process of Paper III.

Aquatic methane dynamics in a human-impacted river-floodplain of the Danube

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Abstract

River-floodplain systems are characterized by changing hydrological connectivity and variability of resources delivered to floodplain water bodies. Although the importance of hydrological events has been recognized, the effect of flooding on CH₄ concentrations and emissions from European, human-impacted river-floodplains is largely unknown. This study evaluates aquatic concentrations and emissions of CH₄ from a highly modified, yet partly restored river-floodplain system of the Danube near Vienna (Austria). We covered a broad range of hydrological conditions, including a 1-yr flood event in 2012 and a 100-yr flood in 2013. Our findings demonstrate that river-floodplain waters were supersaturated with CH₄, hence always serving as a source of CH₄ to the atmosphere. Hydrologically isolated habitats in general have higher concentrations and produce higher fluxes despite lower physically defined velocities. During surface connection, however, CH₄ is exported from the floodplain to the river, suggesting that the main channel serves as an “exhaust pipe” for the floodplain. This mechanism was especially important during the 100-yr flood, when a clear pulse of CH₄ was flushed from the floodplain with surface floodwaters. Our results emphasize the importance of floods differing in magnitude for methane evasion from river-floodplains; 34% more CH₄ was emitted from the entire system during the year with the 100-yr flood compared to a hydrologically “normal” year. Compared to the main river channel, semi-isolated floodplain waters were particularly strong sources of CH₄. Our findings also imply that the predicted increased frequency of extreme flooding events will have significant consequences for methane emission from river-floodplain systems.

Recent global estimates on the areal extent of inland waters are greater than previous appraisals (Downing et al. 2012; Verpoorter et al. 2014). This was also linked to higher global estimates of CO₂ evasion (Battin et al. 2008; Raymond et al. 2013). Methane (CH₄) is recognized as the second most important anthropogenic greenhouse gas (GHG), with a global warming potential 25 times that of CO₂, yet much less

is known about the emissions of this trace gas from inland waters (Bastviken et al. 2011). Regarding flowing waters, existing research has focused mostly on the emissions from large rivers and is associated with high uncertainty. Downing et al. (2012) emphasized that the contribution of rivers and streams may be +20 to +200% greater than previous estimates. The role of fringing floodplains and wetlands and their contribution to global methane emissions have received some attention (Aselmann and Crutzen 1989; Sha et al. 2011). Shallow lakes and wetlands are important for the Earth’s carbon balance, but the majority of existing research covers emissions from large river-floodplain systems such as the Amazon (Melack et al. 2004; Ringeval et al. 2014) or the Pantanal (Marani and Alvala 2007; Bastviken et al. 2010). More recently, some attention has been given to small wetlands because they possibly contribute more to global methane emissions than previously considered (Yavitt 2010).

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Due to variable hydrology and long-term retention between floods, river-floodplain systems possess ample opportunities to store and process carbon (Battin et al. 2008). Although some studies consider river-floodplains to be carbon neutral (Batson et al. 2015), most identify them as a source of CH_4 to the atmosphere (Denman et al. 2007; Sawakuchi et al. 2014). Saarnio et al. (2009), for example, evaluated total CH_4 emissions from European floodplains and wetlands in a meta-analysis, yet their estimates bear considerable uncertainties due to the small number of studies and high variations in CH_4 emission. One factor that potentially greatly affects CH_4 emissions is hydrological disturbance (Altor and Mitsch 2008; Gatland et al. 2014). Changing hydrology, including seasonal flooding events, results in large temporal changes in methane evasions from floodplains (Otter and Scholes 2000). Nonetheless, the importance of hydrology, especially the occurrence of extreme hydrological events, for CH_4 evasion from European river-floodplains has not received significant attention. An often overlooked, key aspect is the changing area of the inundated floodplain: it must be taken into account when estimating total evasion from such systems (Smith et al. 2000).

Due to river regulation, damming, construction of hydro-electric power stations, agriculture and other anthropogenic activities, the areas of many European floodplains have been reduced significantly (Klimo and Hager 2001), with substantial implications for floodplain functions. There is evidence that human impacts are responsible for increased methane fluxes from wetlands (Petrescu et al. 2015), but the study of Froelking et al. (2011) implies that anthropogenic disturbance may also result in reduced CH_4 emissions. This suggests that anthropogenic degradation of floodplain systems can influence their overall carbon flux. In this light, a potential human impact on CH_4 emissions from modified river-floodplains, including restored sections, remains unclear. Considering that much of Europe's floodplains have been degraded, the significance for CH_4 emissions must be considered.

In this study, we assess CH_4 emissions from a human-modified and from a partly restored river-floodplain section of the Danube near Vienna (Austria). We investigated whether hydrological events differing in magnitude affect the concentration and the evasion of CH_4 and hypothesized that floodplain waters are important sources of CH_4 in comparison to the main river channel. Finally, we discuss possible consequences of variously intense anthropogenic alterations of river-floodplain systems on CH_4 emissions.

Materials and methods

Study area and sampling design

Our study was conducted in a river-floodplain system located in the Danube Floodplain National Park downstream of Vienna (Austria, river km 1895–1918). Throughout the centuries, this area has been severely impacted by human alterations, considerably decreasing the active flood plain area (Hein et al. 2016).

Notably, levees built during the 19th century transformed lotic floodplain water bodies into lentic side-arms (Hohensinner et al. 2013). In recent decades, however, some floodplain sections have been successfully restored with the aim to re-establish conditions typical for “active,” natural floodplain systems. Thereby, near-natural-river-floodplain sections were recreated close to the town Regelsbrunn and in parts close to the area Lobau (Fig. 1) (Schiemer et al. 1999; Schiemer et al. 2006), where frequent surface connection with the main channel (~ 200 d per year) now occurs. Most parts of Lobau, however, are still heavily protected from direct through-flow (Fig. 1) because only a small downstream opening allows surface connection at higher water flow (Reckendorfer and Hein 2000). Lobau is thus characterized by dampened water level fluctuations (Schiemer et al. 1999) in a number of lentic backwaters, that are only occasionally connected by back-flowing floods (Reckendorfer et al. 2013). There are also completely disconnected parts of Lobau that formerly belonged to the active floodplain but now never experience surface connection to the Danube. Groundwater connection with the Danube, however, does exist (Griebler and Mösslacher 2003). However for this complex processes no detailed data are available so far.

For this study, we selected sites that represent a gradient of connectivity to the main channel. They ranged from isolated (station G, connected 0 d yr^{-1}), through semi-isolated (station F and E, connected 0–20 and 20–180 d yr^{-1} , respectively) to dynamic (station B, C, D, connected > 180 d yr^{-1}) (Fig. 1a). In dynamic water bodies (Fig. 1, stations B, C, D), lotic conditions can be frequently observed, whereas semi-isolated E and F are open-water backwaters mainly characterized by lentic conditions. The completely disconnected, isolated station G is comparably small, lake-like, highly sheltered and shaded. It was once part of the former, active floodplain, but because of the river regulation in the 19th century it is now never connected to the Danube, even during extreme flooding events.

We performed two sampling campaigns, one in 2012 (Apr–Dec) and one in 2013 (Apr–Aug), each covering a spring pre-flood, early summer flood and a post-flood hydrological phase. At the Austrian stretch the Danube receives large amounts of snowmelt and glacial runoff from its southern tributaries and its flow regime is therefore characterized by predictably high water levels in early summer (Heiler et al. 1995). Sampling frequency was adaptive, either bi-weekly or monthly in the pre- and post-flood phase, and increased to 2–3 times per week at the onset of a flood.

Hydrology and aquatic surface area

The Danube discharge and water level data were provided by the Hydrological Office of Lower Austria. They were obtained from station A located downstream of the floodplain. Mean water, 1-yr and 100-yr flooding events are defined by discharges of 1930 $\text{m}^3 \text{s}^{-1}$, 5300 $\text{m}^3 \text{s}^{-1}$, and 10,500 $\text{m}^3 \text{s}^{-1}$, respectively (www.noel.gv.at). Flow velocity in the main channel was calculated from the discharge time series and cross section data (“Via Donau”-company, unpublished data).

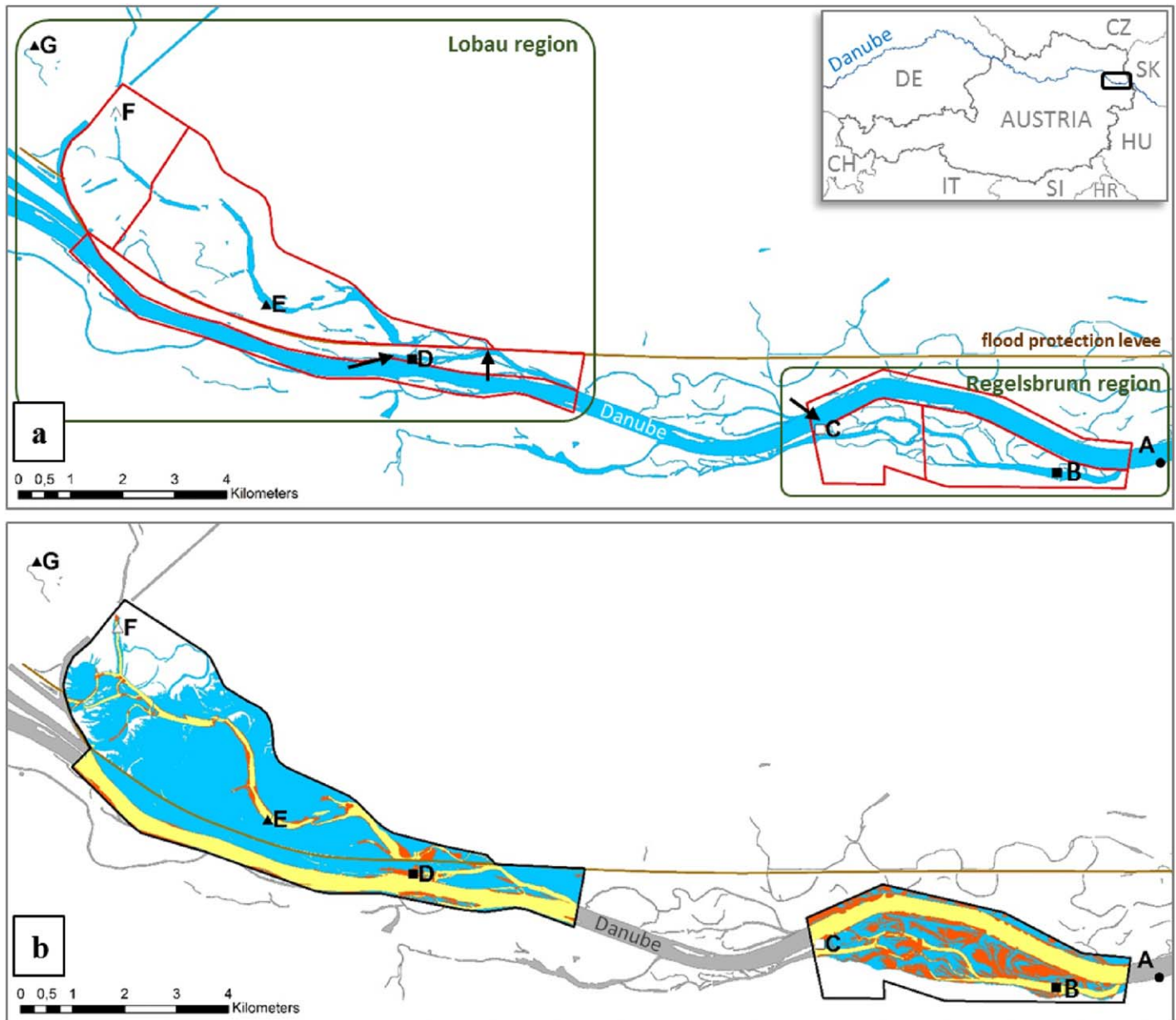


Fig. 1. The Danube River and its floodplains downstream of Vienna. The small map shows Austria. **(a)** The letters indicate the sampling stations. Regelsbrunn and Lobau regions indicate different subsystems of the river-floodplain and the arrows point at the inlet where the flooding water enters these floodplain areas. Floodplain sections used for upscaling are marked with the red line. **(b)** The aerial extent of mean water (yellow), a 1-yr flood (red) and a 100-yr flood (blue) in the two studied regions.

Stations B and C are located in a hydrologically different area than stations D through F. While the latter stations are subject to backwater flow only—as inflow and outflow take place through a single cross section—the first two are located in a river subsystem generally characterized by through-flow, hence characterized by separate boundary conditions for inflow and outflow. Therefore, different model approaches were selected for these two regions. For stations B and C, flow velocities were obtained from the model by Reckendorfer and Steel (2004), and water levels were computed from

gauge readings taken during each sampling campaign. For stations D, E and F, flow velocities were acquired from the hydrological model of Gabriel et al. (2015), and water levels were calculated for the discharges observed at sampling dates with the hydrodynamic model of Tritthart et al. (2011) developed for the Lobau area. The three referenced models are comparable in the quality of their output parameters because all of them were calibrated and validated successfully on measured hydrographs at several gauges located within the study site. As flow velocity follows directly from

an unsteady continuity condition in the river subsystems investigated, it is not expected to be associated with any larger error than the water surface, independent of the model used.

For each sampled water body and sampling time point, the volume and surface area of the flooded region were calculated based on the water level and the digital elevation model (DEM) (Via Donau, unpublished data, resolution: 2.5×2.5 m), using the “3D Analyst Tools” in ArcMap 10.1 (ESRI). The average depth of the water body was obtained by dividing the volume by the surface area of the water body. These values were used to calculate CH_4 flux.

Sampling and gas chromatography

Temperature, oxygen saturation (%), pH, and conductivity were determined *in situ* (WTW Oxi 330, WTW pH 330 and WTW Cond 330, respectively). Water and air samples from all stations were collected in triplicate in 50 mL serum bottles to measure CH_4 concentrations. Water samples were collected by filling bottles without headspace and closing them with crimp-seal gas-tight rubber stoppers under water. Bottles were prepared with a small amount of precipitated sodium azide (NaN_3 , final concentration 0.54 mol L^{-1}) for preservation prior to sampling. Samples were stored at 4°C pending analysis by gas chromatography (Agilent GC 6890N equipped with a flame ionization detector and an automatic gas-injection unit). The GC sampled a headspace that was created by replacing 15–20% of sample water with CH_4 -free N_2 and equilibrated with the water phase at a controlled temperature (30°C water bath placed on a rotary shaker); exact headspace volume was determined by mass difference. Equilibration pressure was recorded automatically by the gas-injection unit (Joint Analytical Systems) sampling the headspace. One CH_4 -standard (50 ppm) was analyzed prior to each batch of 15 samples. The CH_4 concentration in the original water sample (CH_4w [$\mu\text{mol L}^{-1}$]) was determined based on Henry's law using equilibration pressure and temperature, and water and headspace volume (Sander 2014). Field partial pressure of CH_4 was then computed based on Henry's law from concentration and using field water temperature and atmospheric pressure sourced from close-by meteorological stations (Austrian Central Institute for Meteorology and Geodynamics, ZAMG).

Water-air flux computations

The concentration gradient between water and atmosphere was computed as

$$d\text{CH}_4 = \text{CH}_4\text{w} - \text{CH}_4\text{eq} \quad (1)$$

where CH_4eq ($\mu\text{mol L}^{-1}$) is the concentration in water expected for atmospheric equilibrium. CH_4eq is computed from atmospheric pressure, CH_4 partial pressure in air ($p\text{CH}_4$), and field water temperature by Henry's law. CH_4 flux (F [$\mu\text{mol m}^{-2} \text{ h}^{-1}$]) was then calculated according to:

$$F = k_{\text{CH}_4} \times d\text{CH}_4 \quad (2)$$

where k_{CH_4} is the vertical gas transfer velocity of CH_4 (cm h^{-1}) at the respective water temperature.

k_{CH_4} was computed from:

$$k_{\text{CH}_4} = k_{600} \times (\text{Sc}_{\text{CH}_4} / 600)^{-n} \quad (3)$$

where k_{600} is the gas transfer velocity normalized to a Schmidt number of 600 and Sc_{CH_4} is the Schmidt number of CH_4 at the temperature measured in the field (Wanninkhof 2014). We used $n = 0.67$ for wind speeds in 10 m height $u_{10} < 3.7 \text{ m s}^{-1}$ and water velocities $v < 30 \text{ cm s}^{-1}$. Whenever either u_{10} or v exceeded the threshold, $n = 0.5$ was applied.

We predicted k_{600} (cm h^{-1}) based on an additive model developed from CO_2 data by (Borges et al. 2004):

$$k_{600} = 1.0 + 1.719v^{0.5}h^{-0.5} + 2.58u_{10} \quad (4)$$

from flow velocity v (cm s^{-1}), water depth h (m), and wind speed at 10 m height u_{10} (m s^{-1}). The wind speed was obtained from ZAMG measured at nearby meteorological stations. Notably, Eq. 4 takes into account both hydrological conditions and wind as controls on gas transfer velocity, which contrasts classical approaches (Cole and Caraco 1998; Crusius and Wanninkhof 2003; Juutinen et al. 2009), but is considered vital for dynamic floodplain water bodies (Alin et al. 2011). For the completely disconnected station (no flow, sheltered from wind) we used a k_{600} of $2.13 \text{ (cm h}^{-1}\text{)}$ proposed for small, temperate, wind-sheltered lakes by Cole et al. (2010).

To calculate the total CH_4 evasion from the whole area ($t\text{CH}_4$) (kmol h^{-1}), the floodplain section belonging to each sampled water body was delineated based on the average connection to the Danube (Reckendorfer and Steel 2004; Tritthart et al. 2011) (Fig. 1a). The total area covered with water during distinct hydrological phases was calculated (Fig. 1b). Then, based on daily mean discharge, the following time periods were established: “mean water,” “1-year flood,” and “100- year flood.” Afterwards, each CH_4 flux datapoint was assigned to one of these categories, in each year, respectively. Assuming that our investigation period covered most of the hydrological situations, $t\text{CH}_4$ for each section and for the whole river-floodplain was determined by multiplying the average CH_4 flux within one time period by the aquatic surface area of the respective section. We then scaled up the CH_4 flux to obtain the total, annual evasion of $t\text{CH}_4$ (kmol yr^{-1}) from the whole river-floodplain (Lobau and Regelsbrunn) for each year.

Statistical analyses

Statistical analyses were performed with R 3.1.2 (R Core Team 2014), using the package gam (Hastie 2015). Analytical replicates ($n = 1\text{--}3$) were averaged for each site and date prior to any statistical analysis to avoid pseudoreplication. To

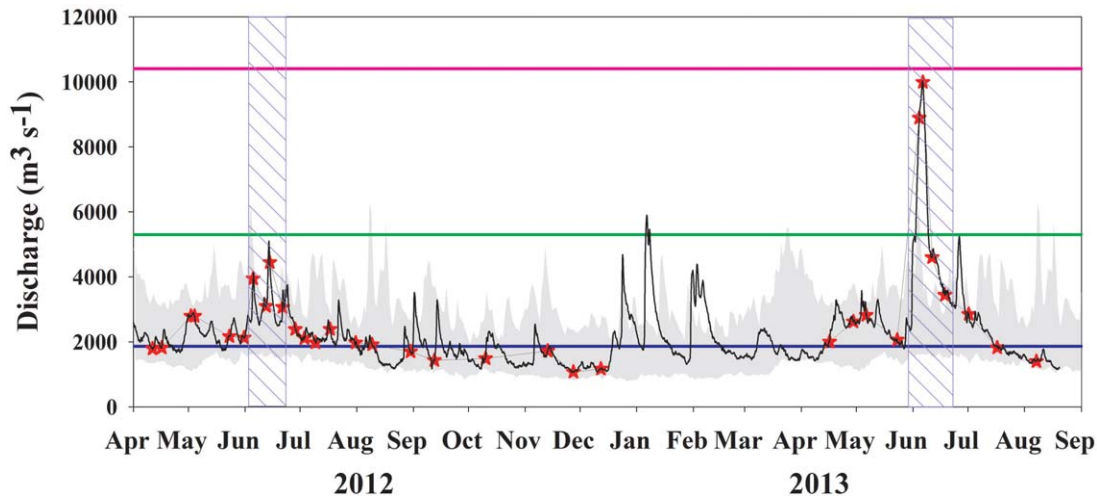


Fig. 2. Discharge of the main channel of the Danube during the investigation period. Grey area indicates the range between the 10th and the 90th percentile of the daily mean discharge (1996–2012). Stars indicate sampling events. Dashed area indicates flooding events during the investigation period. Blue line indicates Danube discharge for mean water, green line 1-yr flood and pink line 100-yr flood.

analyze the effects of the 1-yr and 100-yr floods on concentrations and fluxes, we computed bootstrap-confidence intervals (percentile method, [Manly 2006]) for the differences between pre-flood and flood phases at the various stations. To analyze effects of hydrological isolation on CH_4 data, we tested for differences among individual sites and accounted for temporal autocorrelation by a cubic spline smoother in general additive models (Hastie and Tibshirani 1990). To avoid variance heterogeneity, we used log-transformed CH_4 concentration or flux as individual responses. In these models, “site” is considered an ordered factor due to differing degrees of hydrological isolation (see above). We also included an interaction between the temporal smoothing term and site to investigate differences in temporal dynamics across sites.

Results

Hydrological conditions in the river-floodplain system

Hydrological conditions in the Danube (and in the floodplain) differed markedly between the two years. In 2012, the main channel discharge ranged from 1061 to 5101 $\text{m}^3 \text{s}^{-1}$ (Fig. 2), with a typical 1-yr flood in June. According to the gradient of connectivity, the dynamic stations were connected 82% (station C) and 65% (station D) of the sampling events. The semi-isolated stations (E and F) were connected during the flood only, yet without flowing water conditions in 27% and 5% of the sampling events, respectively. In 2013, the Danube discharge ranged between 1338 $\text{m}^3 \text{s}^{-1}$ and 10,041 $\text{m}^3 \text{s}^{-1}$ (Fig. 2). The discharge maximum in early June corresponded to a 100-yr flood and resulted in flowing water conditions at the dynamic and semi-isolated stations. Despite the large differences in peak flow, surface connectivity in 2013 was similar to 2012. The surface connection with

the Danube in dynamic stations occurred in 90% (B, C) and 65% (D), while in stations E and F it was established in 55% and 18% of the sampling events, respectively. A surface connection between the Danube and station G was never established.

Concentrations of CH_4 across the river-floodplain system

At all sampling events, river-floodplain waters were supersaturated with CH_4 . Atmospheric partial pressures of CH_4 were low (1.70–7.46 ppm) and concentrations of CH_4 in water were 29–22,281 times higher than expected theoretically for an equilibrium with the atmosphere. Notably, concentrations showed distinct spatial variation, with clear differences among stations depending on connectivity (Fig. 3). Overall, the mean CH_4 concentrations in water were lowest in the Danube main channel and increased with decreasing connectivity of floodplain waters (Table 1). The water in the completely isolated station (G, data for 2013 only) was exceptionally oversaturated; reaching extreme values 22,281 times in excess of the atmospheric equilibrium. There was clear temporal autocorrelation as shown by a significant smoothing term for CH_4 on time ($R^2_{\text{adj}}=0.59$, deviance explained=61.7%, $p<0.05$, $n=177$). Also, we identified a significant interaction between the temporal smoothing term and site, pointing to differences in CH_4 dynamics among sites. Notably, excluding the sites measured only in the second year, this interaction can be graphically recognized by converging trends of average CH_4 concentration in the second year, that is, less spatial variation across this subset of sites in the year with the 100-yr flood (Fig. 3). The temperature proved to be a driving force for high methane concentrations here, resulting in a significant correlation between CH_4 concentrations in station G and temperature ($r=0.62$, $p<0.05$). Also for dynamic stations, some

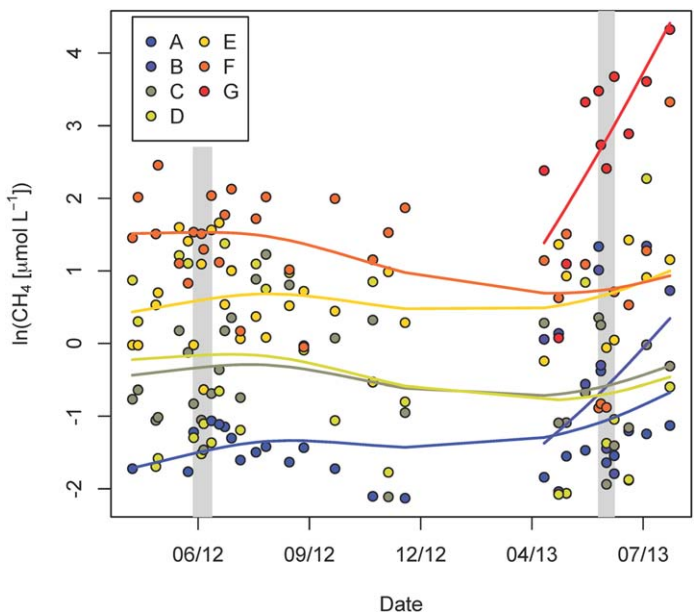


Fig. 3. CH₄ concentrations at the 7 investigated sites differing in hydrological isolation (surface connectivity with the Danube) across the whole study period. The color gradient indicates increasing hydrological isolation (from blue toward red). Lines are cubic spline smoothers of time resulting from a general additive model with an interaction term between (smoothed) time and site. Note converging trends of average CH₄ concentration in the second year, that is, less spatial variation across the continuously sampled subset of sites in the year with the 100-yr flood. For stations B and G data was only available for 2013. These two stations showed a strong increase in CH₄ concentrations over the short period of sampling in 2013.

Table 1. Average aquatic CH₄ concentration (μmol L⁻¹) in the main channel of the Danube, dynamic, semi-isolated and isolated stations during all sampling events.

Danube main channel	Dynamic stations	Semi-isolated stations	Isolated station
0.41 (0.12–3.80)	1.51 (0.12–9.70)	3.58 (0.41–27.85)	24.70 (1.08–75.53)

In parentheses: min–max

dependency on temperature was recorded ($r = 0.34$, $p < 0.01$); at other stations no such correlation was observed.

We calculated the differences in CH₄ concentrations (ΔCH_4) between floodplain sites and the main channel for all sampling dates during times of surface connection with the Danube. Both revealed significant inverse relationships with the Danube discharge (Fig. 4a,b). ΔCH_4 was much larger at low discharge and decreased markedly during times of higher flow (including the 1-yr flood). During the 100-yr flood, the differences between the floodplain and the main channel were either very small or showed negative values, indicating considerable increase in the CH₄ concentration in

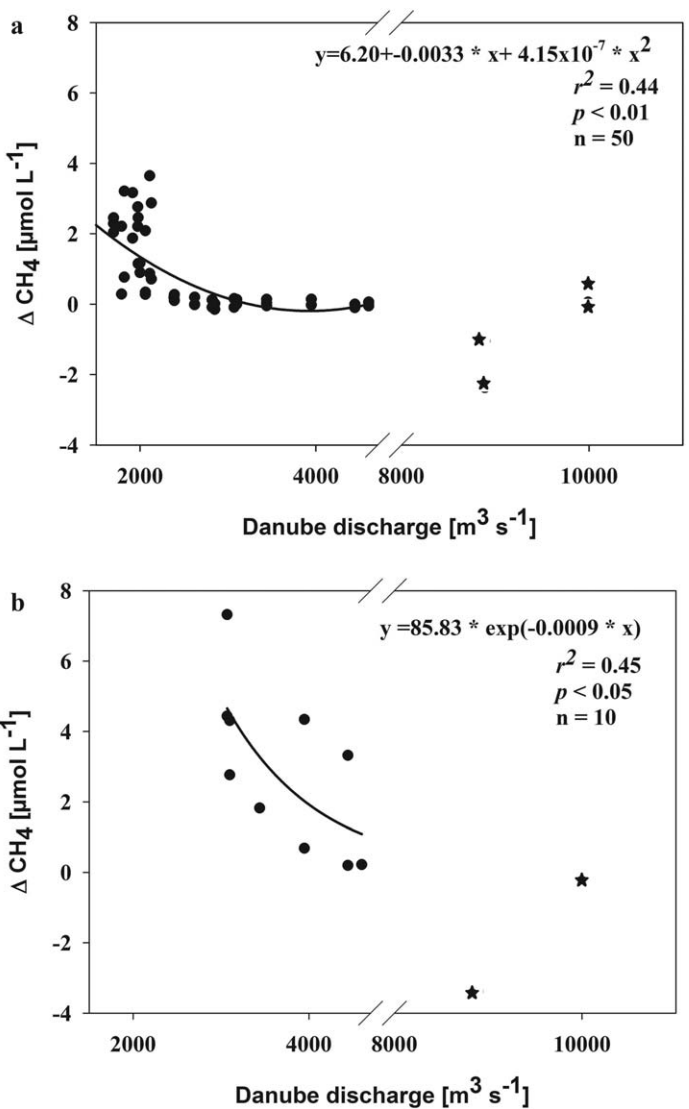


Fig. 4. Relationships between main channel discharge and river-to-floodplain differences in CH₄ concentrations in dynamic (a) and semi-isolated stations (b) during connection with the main channel. Stars indicate samples taken during the onset of the 100-yr flood event.

the main channel and concurrent decrease in the floodplain (Fig. 4a,b).

CH₄ fluxes across the river-floodplain system—effect of flooding

Overall, methane evasion differed markedly along the connectivity gradient from the main channel, through dynamic, semi-isolated to isolated stations and revealed distinct spatial variation with clear differences among stations (Fig. 5). The lowest average methane fluxes were observed in the Danube main channel (Table 2), and the highest average values always occurred in floodplain waters; the only exception was the 100-yr flood in the main channel of the Danube (Table 2). Furthermore, similar to CH₄ concentration

data, differences in the dynamics of CH₄ flux among the sites were indicated by a significant interaction between the temporal smoothing term and site ($R^2_{\text{adj}} = 0.17$, deviance explained = 22.5%, $p < 0.05$, $n = 181$).

Flooding had variable impact on CH₄ evasion from the various river-floodplain stations. The CH₄ flux from the Danube main channel and the dynamic stations was higher during the 100-yr flood (Table 2) but due to high variability the difference to pre-flood conditions was not significant (Fig. 6). The 1-yr flood resulted in lower fluxes in dynamic floodplain stations, yet also these could not be identified as signif-

icantly different to spring-pre flood conditions (Fig. 6). At semi-isolated stations a significant decrease of CH₄ evasion occurred during the 100-yr flood (Fig. 6). At the least connected station (G), the flood had no significant effect on CH₄ flux. No significant correlation between CH₄ fluxes and temperature was noted except semi-isolated ($r = 0.26$, $p < 0.05$) and isolated station ($r = 0.70$, $p < 0.05$).

Floodplain waters vs. main channel as important sources of methane

Based on the evasion rates and the flooded area of the two river-floodplain systems, we evaluated the share of floodplain evasion of CH₄ (mol h⁻¹) compared to the entire river-floodplain system (Regelsbrunn + Lobau + main

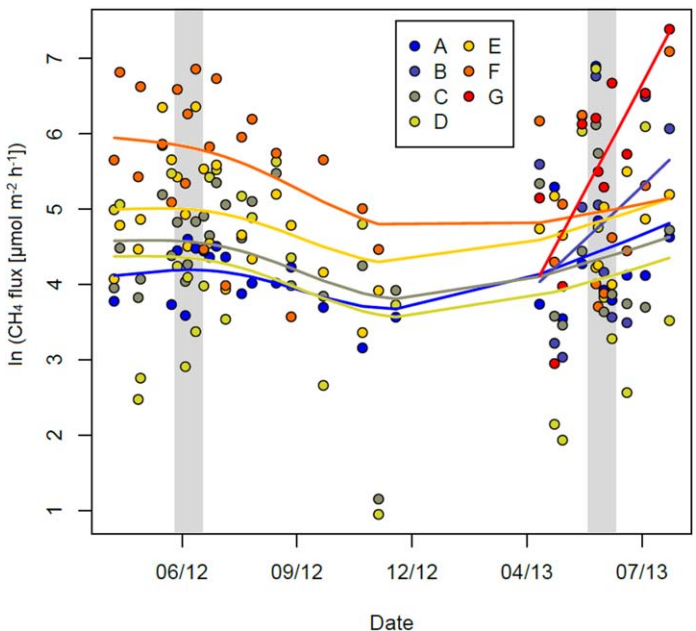


Fig. 5. CH₄ fluxes at the 7 investigated sites differing in hydrological isolation (surface connectivity with the Danube) across the whole study period. The color gradient indicates increasing hydrological isolation (from blue toward red). Lines are cubic spline smoothers of time resulting from a general additive model with an interaction term between (smoothed) time and site. Note converging trends of average CH₄ concentration in the second year, that is, less spatial variation across the continuously sampled subset of sites in the year with the 100-yr flood. For stations B and G data were only available for 2013. These two stations showed a strong increase in CH₄ fluxes over the short period of sampling in 2013.

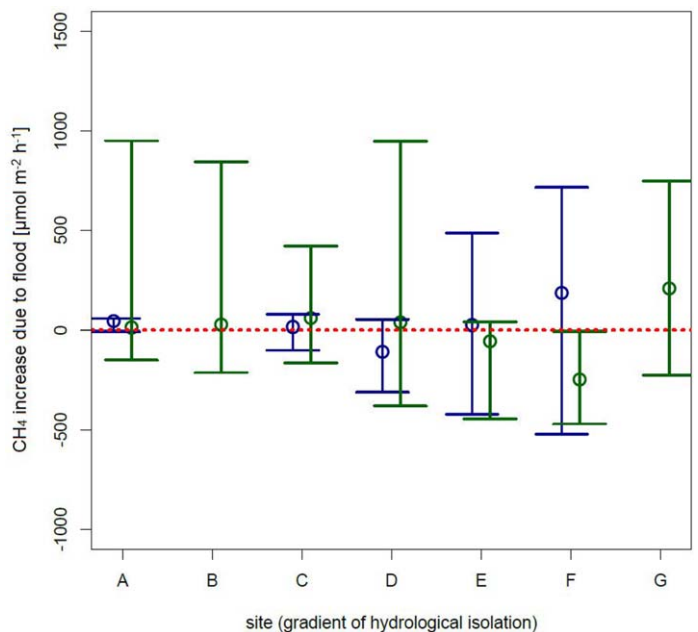


Fig. 6. 95% bootstrap confidence intervals (computed from percentiles) for the flood-driven CH₄ flux increase (differences flood minus pre-flood) at the various sites ordered along hydrological isolation (surface connectivity) in 2012 (blue) and 2013 (green). Non-overlap of confidence intervals with the zero line indicates a significant difference between hydrological phases. Similarly, non-overlap among pairs of confidence intervals indicates a significant difference between pairs of sites or years. The means of analytical replicates were used for bootstrapping.

Table 2. Average CH₄ flux (μmol m⁻² h⁻¹) from the main channel of the Danube, dynamic, semi-isolated and isolated stations during different hydrological phases.

	Danube	Dynamic stations	Semi-isolated stations	Isolated station
Spring pre- flood (both years)	72.2 (34.7–199.1)	116.7 (6.9–417.9)	304.1 (58.8–913.6)	175.9 (19.1–458.8)
1-yr flood	77.4 (36.2–99.7)	69.5 (18.4–125.8)	430.9 (90.5–952.5)	no data
100-yr flood	303.2 (44.3–990.2)	260.1 (26.6–955.6)	74.1 (40.7–152.8)	432.8 (198.7–791.1)
After flood (both years)	63.3 (23.5–102.5)	138.1 (2.6–661.2)	228.7 (28.8–1203.2)	871.9 (307.9–1616.2)

In parentheses: min-max.

channel). Overall, our results revealed that during both years, floodplain waters (dynamic and semi-isolated) accounted for 53% (in 2012 and in 2013) of the CH_4 evasion from the investigated river-floodplain system (Fig. 7). The contributions of the isolated station (G) were negligible due to its small surface area.

Most of the CH_4 (mol h^{-1}) was lost during the 100-yr flood (Table 3). The highest evasion was estimated from the water surface of the Danube stretch, but high losses were also recorded from the regulated floodplain of Lobau and restored section at Regelsbrunn (Table 3). Our results also revealed that 34% more CH_4 (kmol yr^{-1}) were emitted from the entire river-floodplain during the year with the 100-yr flood (Fig. 7).

Discussion

CH_4 concentration in river-floodplain sections

High spatial and temporal variability in aquatic CH_4 concentrations is characteristic for wetlands (Bloom et al. 2012), rivers (Shelley et al. 2014) as well as for floodplains (Crill et al. 1988). In this study, methane concentrations also cov-

ered a broad range (Table 1) (Fig. 3); the main channel had lower values (29–1206 times higher than atmospheric equilibrium), while semi-isolated and isolated floodplain waters were highly supersaturated with CH_4 (32–22,281 times higher than atmospheric equilibrium). Hence, floodplain waters, as well as the main channel of the Danube, were always sources of CH_4 to the atmosphere. Average aquatic methane concentrations in this study resembled the concentrations reported for the Brazilian Amazon (Belger et al. 2011) rather than the lower concentrations for temperate lowland rivers (Sanders et al. 2007; Trimmer et al. 2009). The elevated aquatic methane concentrations are likely caused by in-situ methanogenesis, which can be considerably high in riverbed sediments (Shelley et al. 2015). The activity of methanogens is affected by temperature (Fu et al. 2015; Tveit et al. 2015), and methane formation is apparently sensitive to temperature increase (Duc et al. 2010). In our study, temperature was the strongest controlling mechanism for CH_4 concentration at the completely disconnected station (G). However, the temperature-dependency was less pronounced in other stations, where methane concentrations could also be influenced by other factors such as availability of organic carbon (Christensen et al. 2003; Liu et al. 2011). The quantity and quality of organic substrates delivered to the river-floodplain are highly variable and fluctuate substantially, from mostly allochthonous material during the flood (Sieczko and Peduzzi 2014) to autochthonous material during the post-flood period (Sieczko et al. 2015). The elevated sedimentation of organic matter delivered with a spate could also be responsible for enhanced activity of methanogens during the post-flood period (Van Huissteden et al. 2005; Gatland et al. 2014), especially at semi-isolated stations. Hence, beside fluctuations in methanogen activity, spatio-temporal variation in aquatic CH_4 concentration across the river-floodplain system can be purely physically induced by hydrological fluctuations affecting gas exchange (Pulliam and Meyer 1992). This includes flooding events, which may alter CH_4 concentrations substantially by flushing otherwise hydrologically isolated habitats. In our study, the highest differences in concentrations between floodplain waters and the Danube were noted when the river flow was lower (Fig.

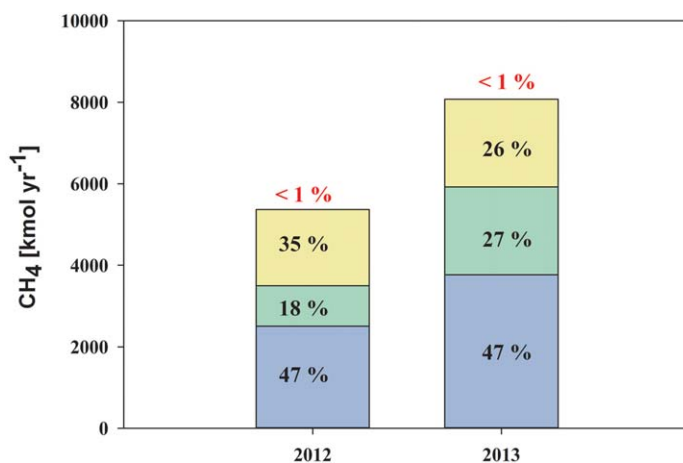


Fig. 7. Total evasion of CH_4 (kmol yr^{-1}) from the entire river-floodplain (Regelsbrunn and Lobau) in 2012 and 2013 and relative contribution (%) of different sections: Danube (blue), dynamic (green), semi-isolated (yellow), isolated (red).

Table 3. Average total fluxes of CH_4 (mol h^{-1}) from the Danube main channel stretch of the investigated area, Regelsbrunn (restored) and Lobau (regulated) floodplains during different hydrological phases.

	Spring pre-flood		1-yr flood		100-yr flood		After flood	
	Total flux [mol h^{-1}]	Area [km^2]	Total flux [mol h^{-1}]	Area [km^2]	Total flux [mol h^{-1}]	Area [km^2]	Total flux [mol h^{-1}]	Area [km^2]
Danube main channel	341.37	4.87	466.46	6.16	2196.20	7.24	296.43	4.87
Regelsbrunn	63.59	0.67	229.99	2.43	1039.91	4.35	121.36	0.67
Lobau	284.25	1.21	545.80	2.02	1935.67	14.96	201.74	1.21

4a,b). In agreement with Pulliam and Meyer (1992), our results indicate that dissolved CH_4 concentrations in floodplain waters are driven by changes of main channel discharge. This suggests that surface connection between the floodplain and the river may be an important factor controlling CH_4 concentrations in the main channel (Richey et al. 1988). In fact, due to its much higher gas exchange efficiency the river main channel can act as an “exhaust pipe” of the entire river-floodplain system, responsible for evasion of CH_4 produced in floodplain waters. This mechanism have been especially important during the 100-yr flood when floodplain CH_4 concentrations decreased due to dilution, while the main channel CH_4 concentration increased (Fig. 4a,b) at the same time. Hence, a pulse of methane flushed from the slow-flowing floodplain into the river could be responsible for elevated CH_4 in the main channel. This suggests that most of the river CH_4 has originated in the super-saturated waters adjacent to the main channel and suggests that river carbon delivery was driven by surface floodwater supply (Abril et al. 2014; Gatland et al. 2014). Additionally, the high CH_4 peak during the 100-yr flood could also be associated with run-off from upstream forest and agricultural soils (Angelis and Lilley 1987).

Importance of floods: Implications of increased flooding frequency for methane evasion

High interannual variations of CH_4 emissions, characteristic for river-floodplains, point to the importance of these systems as methane sources (Marani and Alvala 2007). Rapid hydrological changes, typical for river-floodplains, can substantially affect evasion of greenhouse gases from temporarily inundated systems (Altor and Mitsch 2008), and flooded environments are recognized as sources of methane to the atmosphere (Belger et al. 2011). Our study shows that flooding significantly altered methane evasion. It also emphasizes the importance of spates with different magnitude for CH_4 emission from stations with different connectivity (Fig. 6). During a high-water period, methane evasion can be significantly higher (Devol et al. 1990), with rapid emissions occurring shortly after the onset of the flood (Boon et al. 1997). Similar to a study of Gatland et al. (2014), our findings show that the highest CH_4 loss from the floodplain area occurred during the extreme 100-year flood (Table 3). This suggests that an improved, detailed characterization of hydrological processes is necessary to correctly assess the CH_4 dynamics in river-floodplain systems (Zhu et al. 2013).

Methane emissions from wetlands have been linked to climate change (Mitsch et al. 2010). Yvon-Durocher et al. (2014) suggested that global warming has a large effect on the relative contributions of CH_4 to total GHG from aquatic ecosystems. Also, impacts of global change, such as increasing frequency of droughts and flooding, summarized by Lehner et al. (2006), are expected to occur in Europe. However, the effect of a shift in hydrological variability—as possibly

caused by global climate change—on methane emissions still remains unknown both at tropical latitudes (Mitsch et al. 2010) and in temperate regions. Future scenarios for the Danube River predict an increase in discharge magnitude and in water temperature during spring floods (Zweimüller et al. 2008). In our study, tCH_4 evasion (Fig. 7) was 34% higher during the year of extreme flooding compared to the hydrologically “average” year, suggesting that increased flooding frequency, increase of the flooded area will have marked consequences on overall methane emissions (Sha et al. 2011). Hence, higher emissions from river-floodplains during extensive floods may be expected in the future. Note also that rivers can be important CH_4 sources to the coastal ocean (Scranton and Mcshane 1991). Pavel et al. (2009), for example, observed a significant input of riverine CH_4 into the Danube Delta during post-flood conditions. Thus, the predicted, intensified spate occurrence in the Danube may also have consequences for the carbon cycle in the Danube Delta and the coastal Black Sea.

Methane evasion from floodplains with different levels of human impact

The global emissions of methane from tropical wetlands (Smith et al. 2000) and from temperate regions are most likely underestimated and considerably uncertain (Kirschke et al. 2013). Typically, large tropical floodplains have been considered to be the most important sources of methane due to their extensive area (Bartlett and Harriss 1993). Our data (Table 2), however, show that the average diffusive fluxes from semi-isolated and isolated stations of a temperate floodplain are similar to average methane fluxes measured over flooded tropical Amazon forest (Bartlett and Harriss 1993). Our study also shows that isolated and semi-isolated stations, although exhibiting variability in their relative contribution (Fig. 7), were significantly stronger sources of CH_4 to the atmosphere than the dynamic sites and the respective river stretch (Table 2). Occasionally, the flux per unit water surface area reached 94% higher values than in the main channel (Table 2). Hence, our study points to the importance of semi-isolated and isolated floodplain waters as important sources of methane.

In general, human activities significantly affect the contribution of inland waters to the carbon cycle (Regnier et al. 2013). Due to river regulation, damming or agriculture, most of the European floodplains have been severely impacted. Today, spatially much reduced floodplain areas experience a markedly lowered frequency of connection with the main river (Buijse et al. 2002). The Danube River is regulated along 80% of its length, resulting in 81% reduction of floodplain areas in the entire river basin (Günther-Diringer 2001). Our findings emphasize that most of the CH_4 emissions originated from human-altered, semi-isolated floodplain waters. Approximately 47% of CH_4 emission occurred from the main river stretch (Fig. 7). The investigated isolated station,

due to its small area, did not contribute substantially to the total CH₄ loss from the whole river-floodplain. High CH₄ fluxes from this isolated station (Table 2, Fig. 5), however, suggest that ongoing, complete disconnection of floodplain sections due to river regulation will produce more areas with such water bodies, thus increasing their contribution to the overall CH₄ budget. Our findings indicate that human-induced disconnection of floodplain waters from the river may have significant consequences for the overall methane budget and emphasize the importance of floodplain waters as sources of greenhouse gases. River-floodplains—beside small, dynamic streams and rivers (Benstead and Leigh 2012)—also apparently play an important role in global greenhouse gas fluxes.

Here, we focused only on diffusive fluxes; the ebullition, plant-mediated flux or recently reported microbubble flux (Prairie and Del Giorgio 2013) have not been investigated. Especially ebullition could be an important component of the total methane flux in floodplain waters (Smith et al. 2000; Ringeval et al. 2014). In rivers, diffusive flux is usually the main component of the total CH₄ emission, but ebullition hotspots may contribute substantially (up to 50%) to the total flux (Sawakuchi et al. 2014). The ebullition rates are also temperature-dependent (Wilkinson et al. 2015) and may thus play an increasing role in the total CH₄ flux in the context of global warming. Because ebullition can be the dominant mechanism in floodplains (Devol et al. 1990) and may exceed diffusive fluxes several-fold or even by orders of magnitude in some systems, we consider this study's estimate as conservative for the contribution of floodplain waters to the overall river-floodplain methane emission. Nevertheless, in line with other studies, our work stresses the importance of CH₄ emissions from temperate river-floodplain systems with different levels of human impact. Temperate river-floodplain systems should be considered in global budgets of CH₄ emission.

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7. Discussion

7.1 Effect of variable hydrological connectivity for DOM heterogeneity

The stochasticity of hydrological events in river-floodplains generates multiple opportunities for numerous interactions between terrestrial and aquatic ecosystems (Tockner et al. 1999). This creates a heterogeneous mixture of allochthonous and autochthonous DOM, which changes its composition depending on the hydrological phase and duration of connectivity to the main channel (**Paper I&II**). Especially, flooding events are responsible for introducing high quantity of allochthonous DOM into the floodplain (Mladenov et al. 2005, **Paper I**), however bioavailability of this material is not fully understood so far. Although, the flood-associated shifts in DOM suggest either decrease (Leff and Meyer 1991), or no change in DOM quality (Buffam et al. 2001), some studies report an increase in bioavailability of the spate-originated material (Fellman et al. 2009). Typically, DOM quality when assessed with spectroscopic measurements, often resembles an aromatic and refractory character of flood introduced material with higher DOC concentration compared to non-flood conditions (**Paper I**, Hood et al. 2006). However, information about DOM quality, based solely on optical properties, can be misleading, because ecologically important, non-chromophoric compounds are entirely overlooked. To obtain knowledge also on the non-chromophoric DOM pool, I applied extracellular enzymatic activity measurements. They indicate potential availability patterns of different DOM components (Chróst 1991). With this method, I found that the flood-introduced pool of polysaccharides and proteins was bioavailable and susceptible to microbial metabolism. Therefore, the application of the “enzyme fingerprinting” method is very useful to track mobilization and changes in non-chromophoric DOM sources, especially during sudden hydrological events (Clinton et al. 2010, **Paper I**). With this technique I was able shed a light on bioavailability of this highly heterogeneous material, when introduced with flood pulses. In my Doctoral thesis, I propose that the EEA approach should be used as a proxy to track bioavailability of non-chromophoric DOM in addition to typically measured optical properties, particularly under variable hydrological conditions.

7.2 Microbial utilization of DOM under different hydrological settings

DOM quality is a crucial factor, which regulates bacterial growth and respiration in aquatic ecosystems (Apple and Del Giorgio 2007; Del Giorgio and Cole 1998). Bacterial secondary production (BSP) is generally supported by high quality, autochthonously-derived DOM (Kirchman 2003). However it may also be fueled by allochthonous material in some systems (Burrows et al. 2013; Figueroa et al. 2016). The presence of more labile allochthonous DOM compared to autochthonous DOM may affect bacterial productivity substantially (Catalán et al. 2013). River floodplains carry highly heterogeneous DOM, where mixing zones between different water bodies create hot spots for BSP (Farjalla 2014). Hence, investigating the linkage between DOM origin, quality and BSP is challenging. Similarly as in the Amazon floodplains (Vidal et al. 2015), I showed that hydrological changes and DOM quality were the key elements that affect functioning of this European temperate river-floodplain system (**Paper I, II**). Typically, lentic periods are associated with higher BSP (Peduzzi et al. 2008), whereas during periods of high water, river and floodplain BSP are lower and more homogenous (Castillo 2000). However, the effect of hydrology may not always be pronounced, when rather the quality of DOM plays the main role in maintaining BSP (Judd et al. 2006). This results in similar rates of bacterial productivity, when in even closely located water bodies of the same river-floodplain system, with different hydrological connectivity, BSP can be supported by degradation of material derived from different sources (**Paper I, II**). Thus, both biologically reactive allochthonous DOM and primary production are considered to play an important role in maintaining heterotrophic metabolism (Del Giorgio et al. 1999). However the enzymatic hydrolysis of allochthonous and autochthonous polymers, which mediates formation of assimilable products, is considered as rate-limiting process to BSP (Sinsabaugh et al. 1997). With the application of the EEA approach, I showed that primary production was an essential source, sustaining bacterial growth, however, an enzyme-mediated step was necessary to make this material available for bacteria (**Paper II**). Because up to date very scarce information is available on EEA response to photosynthetic extracellular release of phytoplankton (PER) (Romani and Sabater 2000) in river-floodplains, I particularly focused on this aspect. My results showed that in completely isolated water bodies, with high input of local, allochthonous, refractory DOM, BSP was driven by enzymatic degradation of

PER products (**Paper II**). Although studies point to the importance of algal in situ production (Chanton and Lewis 2002), also decoupling of BSP from phytoplankton production in some systems has been reported earlier (Bird and Karl 1999; Cheol et al. 1994; Van Wambeke et al. 2002). However, with my approach, by investigating the relationship between EEA and phytoplankton productivity, it was possible to reveal the linkage between these two components. I found that in frequently flooded water bodies, BSP was sustained by enzymatic degradation of particulate primary production, whereas in rarely connected parts, despite readily available autochthonous DOM, utilization of the more refractory pool also appeared to support BSP (**Paper II**). Therefore, because autochthonously-derived DOM is not always directly available for bacteria due to its polymeric structure (Meon and Kirchman 2001), I propose that the EEA approach is an excellent tool to decipher the linkage between bacteria and non-chromophoric, but ecologically important phytoplankton-derived DOM. My findings also show that backwater systems with variable hydrological connectivity are important places for processing carbon derived from allochthonous and autochthonous sources with different availability (**Paper I&II**).

One of the goals in DOM related studies is to decipher the role of microbes, which may act either as a link, transferring DOM to higher trophic levels, or/and as a sink, resulting in CO₂ and CH₄ respiration. Microbial resource partitioning depends mainly on the quantity (Eiler et al. 2003) and quality of DOM (Apple and Del Giorgio 2007), but also on temperature (Apple et al. 2006) and nutrient levels (Allison and Vitousek 2005). Consistent with another study (Welti et al. 2012), I also found that, beside DOM quality, the hydrological connectivity was the crucial factor controlling the fate of the utilized material (**Paper I**). To describe DOM utilization patterns during different hydrological phases, including the pre-flood, initial phase of the flood, the flood and after-flood conditions, I calculated a Metabolic Effort (MEF) parameter, which indicates proportional importance of catabolic versus anabolic processes. With the application of MEF, I was able to show that the onset of the flood was a phase that accelerated catabolic processes (**Paper I**). This finding is supported by other studies, which suggest that the highest respiration rate could be expected during the initial phase of the flood (Valett et al. 2005). Further, I also found that there was no difference between connected and disconnected phases, what implies that the prolonged connectivity to the main channel had no influence on assimilation efficiency of hydrolyzed products. Nevertheless, my results indicate that initial phases

of flooding events may have a great impact on microbial respiration in river-floodplain systems (**Paper I**). It also shows that, to properly evaluate ecosystem functioning in highly dynamic river-floodplain systems, coverage of all typical hydrological phases is necessary.

7.3 Importance of hydrology for CH₄ dynamics

One of the recent goals in ecology is to quantify the emissions of CH₄ from inland waters, including floodplains (Bastviken et al. 2011). Although the effect of temperature, water depth or aquatic area on concentrations and emissions of CH₄ has been recognized (Bastviken et al. 2008; Teodoru et al. 2015; Yvon-Durocher et al. 2014), the influence of hydrology in temperate river-floodplains has been largely overlooked so far. The modulating role of connectivity between floodplain and main stem appears to be an important factor in tropical systems, however predicting dynamics of dissolved CH₄ bears difficulties (Borges et al. 2015). In my Doctoral thesis I showed that hydrological connectivity of floodplain waters with the main channel had a significant influence on CH₄ concentrations. I found that along a decreasing connectivity gradient, the concentration of CH₄ increased (**Paper III**), in semi-isolated parts reaching or even exceeding values reported for lakes in tropical latitudes (Bastviken et al. 2010). Further, a clear dependence also of CH₄ flux on hydrological connectivity was observed, which points to hydrology as a crucial factor controlling emissions of CH₄ from river-floodplain systems (Gatland et al. 2014, **Paper III**). However, not only the type of connectivity, but also flooding and especially its magnitude may have a significant impact on CH₄ dynamics (Altor and Mitsch 2008; Boon et al. 1997; Nahlik and Mitsch 2011). I discovered that during the flooding, when even more isolated waters were involved, CH₄ was transported from the floodplain to the main channel. This pattern, which was especially pronounced during an extreme 100-year flood event, suggests that the main channel acted as an “exhaust pipe” for the whole system. Given that some studies in tropical floodplains found similar pattern for CO₂ (Abril et al. 2014), it implies that floodplains are important places that fuel main rivers with greenhouse gases (**Paper III**). The flood magnitude had also a pronounced effect on CH₄ flux. I observed that during the year when a 100-year flood occurred, 34 % more CH₄ was emitted from the entire river-floodplain system (**Paper III**). This finding may be especially important in the light of predicted increasing frequency of extreme flooding events in Europe (Alfieri et al.

2015; Feyen et al. 2012), which together, with the forecasted temperature raise (Betts et al. 2011), may lead to higher CH₄ emissions from temperate floodplains in the future. Therefore, I suggest that, although the area of the European river-floodplains is smaller compared to tropic, more frequently studied systems, the importance of temperate river-floodplains in the global carbon budget will increase due to climate change. Further from my study, it must be emphasized that only ~ 50 % of the total CH₄ flux occurred from the main studied river stretch (**Paper III**), what suggests that, to accurately estimate emissions from inland waters, considering adjacent floodplains is mandatory. Thus, my Doctoral thesis emphasizes that, to properly calculate emissions of CH₄ from European river-floodplains, the temporal and spatial aspects must be taken into account (**Paper III**, Bridgham et al. 2013). Hence, to understand the significance of river-floodplains in carbon budgets, it is necessary to cover all distinct hydrological phases. Future research should focus on the effect of hydrology, and include the consequences of biogeochemical disturbances, such as seasonal flooding, on emission of GHG from river-floodplain systems.

7.4 Implications of human impacts

Human-induced disturbances change flow significantly and carry major consequences, not only for biota, and the carrying capacity of the system, but also for the exchange of material and energy in riverine ecosystems (Hein et al. 2016; Rolls et al. 2012). Due to damming and river regulation (weirs, levees), the delivery of allochthonous DOM from river to the floodplain is highly disturbed. It leads to alterations in biogeochemical processes, affecting rates of DOM utilization and river metabolism (Young et al. 2008). This highlights the importance of the post-flood periods for utilization of refractory DOM, delivered to a floodplain during the spate. In agreement with a lake study (Ylla et al. 2013), my findings also emphasize that during longer retention time after a hydrological event, there is an increased opportunity to process refractory material, which was introduced into the floodplain (**Paper I & II**). This implies that human-induced reduction of surface connectivity between river and its floodplain and extended drying periods may have severe consequences for system metabolic activities (Ylla et al. 2010) and the carbon budget (**Paper III**, Hupp et al. 2009; Román et al. 2006). Therefore, the ongoing disconnection of floodplains from adjacent rivers will not only have local impact on carbon sequestration and mineralization, but also will have consequences for river-

related carbon flux. Additionally, the predicted rise in temperature of rivers (Van Vliet et al. 2011) may also increase organic matter utilization (Gudas et al. 2010), thus suggesting consequences for the river-floodplain carbon cycle (Yvon-Durocher et al. 2014). Beside the enhanced mineralization of C, the global rise in temperature has also an impact on river discharge patterns (Zweimüller et al. 2008). Thus, changes in flood regime due to human activity have also significant consequences on the CH₄ dynamics within river-floodplains (Pearson et al. 2016). This was particularly true in my study (**Paper III**); the strongest source of CH₄ was from the most anthropogenically modified, completely disconnected part of the river-floodplain. The CH₄ flux noted there was on average 6 times higher than in dynamic, frequently connected parts of the system. Consistent with my results, also another study stressed the implication of human perturbations, reporting significantly higher GHG emissions from artificially drained wetlands (Page and Dalal 2011). Therefore, my findings underline that also temperate, human-altered and disconnected floodplain waters can be important sources of CH₄ (**Paper III**), compared to undisturbed systems. Taking into account that many European rivers have been severely modified, which resulted in separation of numerous floodplains from the main channel, I suggest that any further modification and disconnection will have an impact on GHG budgets.

7.5 Conclusions

In conclusion, the variable degree of connectivity in river-floodplain systems appears to be an important driver for occurrence of “hot spots” and “hot moments” for microbial substrate utilization. Particularly, studies on the enzymatic response to flood-introduced DOM provide essential knowledge on functioning of river-floodplains and their ability to process DOM (Wilczek et al. 2005). Moreover, the changing hydrology, which alters the resource allocation, has consequences not only for the floodplain but also for functioning of riverine-food webs in general (Reid et al. 2012). My findings have important implications for further studies on temperate river-floodplains, and suggest that, to fully understand the complex issue of DOM-microbial interactions, detailed spatial and temporal investigations, including hydrological variability, are absolutely necessary. Therefore, this calls for further, comprehensive studies on the contribution of temperate river-floodplain systems to the inland water carbon budget. Further research should aim to consider not only hydrological variability, but also the human-induced effects.

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9. Zusammenfassung

Fluss-Auen-Systeme gehören zu den vielfältigsten und produktivsten Ökosystemen der Erde. Starke hydrologische Stochastizität, die wiederkehrende Änderungen in lentischen und lotischen Lebensräumen ermöglicht, ist die treibende Kraft in diesen Ökosystemen. Daher haben periodische Überschwemmungen erhebliche Folgen für das Funktionieren von Fluss-Auen. Aufgrund längerer Verweilzeit von Wasser in Fluss-Auen wirken diese auch als biogeochemische “Hot Spots”, was ihre wichtige Rolle im Kohlenstoffkreislauf hervorhebt. Trotz ihrer ökologischen Bedeutung wurde ein Grossteil der Fluss-Auen, vor allem in Europa, durch den Menschen erheblich verändert, was Folgen für den Kreislauf von organischem Material hat. Daher ist es wichtig die Auswirkungen von hydrologischen Veränderungen und den Einflusses des Menschen auf die Funktionalität von Fluss-Auen zu verstehen.

In meiner Doktorarbeit konzentrierte ich mich auf die Rolle der Hydrologie als wichtiger Einflussfaktor auf die Zusammensetzung des DOM-Pools (gelöstes organisches Material) sowie der mikrobiellen Verarbeitung von DOM in verschiedenen Subsystemen von Fluss-Auen. Mein Ziel war es auch, die Rolle von Fluss-Auen im Kohlenstoffkreislauf und ihre Bedeutung als CH₄-Quelle zu erforschen.

Ich habe zwei verschiedene, sich ergänzende Ansätze gewählt, um Veränderungen der DOM-Qualität in diesen Systemen zu studieren. Durch spektroskopische Messungen habe ich herausgefunden, dass Überschwemmungen die DOM-Qualität erheblich verändern und terrestrisches, aromatisches DOM zum C-Pool hinzugefügt wird. Durch die Messung von extrazellulären enzymatischen Aktivitäten habe ich entdeckt, dass durch Überschwemmungen auch bioverfügbares DOM, welches relativ schnell durch Bakterien abgebaut werden kann, in die Fluss-Auen importiert wird. Daher können Messungen der extrazellulären Enzymaktivitäten verwendet werden, um die Verfügbarkeit von nicht-chromophorem DOM während schneller hydrologischer Veränderungen zu verfolgen, die nicht erfassbar sind, wenn nur traditionelle spektroskopische Messungen eingesetzt werden.

Ein weiterer Forschungsschwerpunkt lag auf der Periode direkt nach einer Überschwemmung, in der potenziell weniger reaktives, terrestrisches DOM in den Fluss-Auen umgesetzt werden könnte. Dies unterstreicht die Bedeutung von

Überschwemmungswasser für die Verarbeitung von refraktärem, schwer abbaubaren, DOM (RDOM). Erhöhte Aktivität von RDOM-abbauenden Enzymen wurde in der Gegenwart von frischem, labilen DOM, welches unmittelbar von der Primärproduktion stammt, beobachtet. Daher war ein weiteres Forschungsziel die Enzym-Reaktionen auf phototrophe extrazelluläre Freisetzung von organischem Material in verschiedenen Subsystemen der Fluss-Auen zu untersuchen. Die Ergebnisse zeigten, dass Enzym-Messungen ein wertvolles Werkzeug sind, um die Interaktion zwischen nicht-chromophorem, von Primärproduzenten stammenden DOM und der bakteriellen Sekundärproduktion zu erfassen. Ich konnte zeigen, dass selbst in benachbarten Gewässern mit unterschiedlicher Konnektivität die bakterielle Sekundärproduktion durch DOM aus verschiedenen Quellen angetrieben wurde. Ich habe gezeigt, dass in einem abgetrennten Abschnitt der Fluss-Au, mit hoher Konzentration an terrestrischem DOM, eher frisches und labiles DOM die bakterielle Sekundärproduktion unterstützt hat.

Ich konnte des Weiteren zeigen, dass die Art und das Ausmaß der hydrologischen Vernetzung, erhebliche Konsequenzen für die Dynamik von CH_4 haben. Die Ergebnisse weisen darauf hin, dass abgetrennte Subsysteme der Fluss-Auen die stärksten Quellen von CH_4 sind. Hingegen, wenn Oberflächenverbindung zwischen den Subsystemen bestand, diente der Fluss als eine Art "Auspuff" für die Weiterleitung von CH_4 , welches in sehr isolierten Lebensräumen produziert wurde. Zusätzlich wurde die Intensität der Überschwemmung als wichtiger Faktor identifiziert, welcher die CH_4 -Dynamik signifikant beeinflusst.

Meine Ergebnisse tragen wesentlich zum derzeitigen Kenntnisstand bezüglich der Interaktionen zwischen DOM und Bakterien in Fluss-Auensystem bei und betonen die entscheidende Rolle der Hydrologie in diesen Ökosystemen. Sie deuten darauf hin, dass die laufende Abschottung von Subsystemen im Zuge von Regulierungsmaßnahmen nicht nur direkte Folgen für die Auen haben wird, sondern auch generell für den Kohlenstoffkreislauf in Flüssen.

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