Biogeosciences Discuss., 9, 4133–4176, 2012
www.biogeosciences-discuss.net/9/4133/2012/
doi:10.5194/bgd-9-4133-2012

Biogeosciences Discussions

Mimicking floodplain reconnection and disconnection using ^{15}N mesocosm incubations

N. Welti[1,2,*], E. Bondar-Kunze[1,2], M. Mair[1,2], P. Bonin[4], W. Wanek[3], G. Pinay[5], and T. Hein[1,2]

[1]University of Natural Resources and Life Science, Institute of Hydrobiology and Aquatic Ecosystem Management, Vienna, Austria
[2]WasserCluster Lunz Biologische Station GmbH, Lunz am See, Austria
[3]University of Vienna, Department of Terrestrial Ecosystem Research, Vienna, Austria
[4]LMGEM-UMR 6117, Centre d'Océanologie de Marseille, Marseille Cedex 9, France
[5]ECOBIO-OSUR-CNRS, Avenue du General Leclerc, Rennes Cedex, France
[*]University of Queensland and National Centre for Groundwater Research and Training, Brisbane, Australia

Received: 13 March 2012 – Accepted: 20 March 2012 – Published: 2 April 2012

Correspondence to: N. Welti (n.welti@uq.edu.au)

Published by Copernicus Publications on behalf of the European Geosciences Union.

Abstract

Floodplain restoration changes the nitrate delivery pattern and dissolved organic matter pool in backwaters but other effects are not yet well known. We performed two mesocosm experiments to quantify the nitrate metabolism in two types of floodplains. Rates of denitrification, dissimilatory nitrate reduction to ammonium (DNRA) and anammox were measured using ^{15}N tracer additions in mesocosms containing undisturbed floodplain sediments originating from (1) restored and (2) disconnected sites in the Alluvial Zone National Park on the Danube River downstream of Vienna, Austria. DNRA rates were an order of magnitude lower than denitrification and neither rate was affected by changes in nitrate delivery pattern or organic matter quality. Anammox was not detected at any of the sites. Denitrification was out-competed by assimilation which was estimated to use up to 70 % of the available nitrate. Overall, denitrification was higher in the restored sites, with mean rates of $5.7 \pm 2.8\,\mathrm{mmol\,N\,m^{-2}\,h^{-1}}$ compared to the disconnected site ($0.6 \pm 0.5\,\mathrm{mmol\,N\,m^{-1}\,h^{-1}}$). In addition, ratios of $N_2O : N_2$ were lower in the restored site indicating a more complete denitrification. Nitrate addition did not have any effect on denitrification, nor on the $N_2O : N_2$ ratio. However, DOM quality significantly changed the $N_2O : N_2$ ratio in both sites. Addition of riverine derived organic matter lowered the $N_2O : N_2$ ratio in the disconnected site, whereas addition of floodplain derived organic matter increased the $N_2O : N_2$ ratio in the restored site. These results demonstrate that increasing floodplains hydrological connection to the main river channel increases nitrogen retention and decreases nitrous oxide emissions.

1 Introduction

Floodplains are biogeochemical hot spots for carbon and nitrogen cycling and storage (McClain et al., 2003). Depending on the local morphology and hydrology (i.e. vegetation, mean water depth, redox conditions, sediment type, and discharge pattern), floodplains can act either as carbon and nitrogen sinks via microbial respiration and

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denitrification or as sources via organic matter production or nutrient export (Pinay et al., 2007). Flood pulses control organic carbon transformations and processes in floodplains and can trigger an increase of bacterial enzyme activity (Burns and Ryder, 2001; Wantzen et al., 2008). Surface water derived carbon and benthic organic carbon are adequate sources of energy for denitrification when these areas are receiving high nitrate inputs during floods (Arango et al., 2007; Pfenning and McMahon, 1996). Yet, several results suggest that none of these factors alone control denitrification (Dodla et al., 2008; Sutton-Grier et al., 2009; Wall et al., 2005).

Denitrification, a particular form of microbial respiration, is a process controlled by O_2, NO_3, and C availability (Knowles, 1982) which reduces nitrate (NO_3^-) to nitrite (NO_2^-), nitric oxide (NO), nitrous oxide (N_2O), and ultimately to dinitrogen (N_2) (Zumft, 1997). Incomplete denitrification results in the production of N_2O, a greenhouse gas with 300-times the warming potential of CO_2 and a precursor molecule for ozone-depleting NO radicals in the stratosphere (Bates et al., 2008; Dickinson and Cicerone, 1986). With rates ranging from 0 to 345 $\mu mol N m^{-2} h^{-1}$, rivers systems are estimated to contribute approx. 1 $Tg N y^{-1}$ to the global N_2O emissions (Seitzinger, 1988). Up to 80 % of denitrification is estimated to occur in soils and freshwater systems (Galloway et al., 2008).

Denitrification efficiency has been shown to decline with rising concentrations, particularly in larger streams (Bernot and Dodds, 2005; Mulholland et al., 2008). In the Upper Mississippi, denitrification in the floodplains was nitrate limited throughout the growing season, but the backwaters were capable of reacting quickly to a pulse of nitrate (Richardson et al., 2004). Increasing nitrate loads has also been shown to increase the N_2O emissions in both field and laboratory experiments (Barnard et al., 2005; Verhoeven et al., 2006).

Dissimilatory nitrate reduction to ammonium (DNRA) and anammox, two other anoxic nitrate removal processes, are also of interest in floodplains (Burgin and Hamilton, 2007). DNRA has the same environmental requirements as denitrification (anoxia, high nitrate and carbon substrate availability), but rather than a removal pathway, biore-

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active nitrogen is conserved and nitrate transformed into a more bio-available form (ammonium). Although DNRA has been reported as a significant pathway in marine and terrestrial systems accounting for 15–75 % of nitrate removal (An and Gardner, 2002; Morley and Baggs, 2010), it may be a minor route of nitrate removal in wetland systems (Matheson et al., 2002; Scott et al., 2008). While there are few studies that explicitly measured DNRA rates in floodplains, DNRA bacteria have been shown to survive in frequently flooded areas (Sgouridis et al., 2011). With restoration, the ratio of denitrification: DNRA may change along with the changing morphology and substrate availability, thus altering the nitrogen balance (Fazzolari et al., 1998). Anammox, the anaerobic oxidation of ammonium coupled to nitrite reduction with N_2 as the end product, is present throughout the marine system, but its presence in floodplains is not well documented (Jetten, 2001). Few studies have measured this pathway of nitrate removal in freshwater systems, let alone in riverine floodplains (Zhu et al., 2010). However, autotrophic NO_3 assimilation can be a dominating pathway in freshwater ecosystems and perhaps even out-compete denitrification, DNRA and anammox for substrates (Hall et al., 2009; James, 2010).

When water column nitrate was the main nitrate source Christensen et al. (1990) reported that denitrification was inversely proportional to the thickness of the oxic surface layer, as nitrate has to diffuse through this layer, and proportional to the nitrate concentration in the overlying water. Carbon supply stimulates denitrification activity directly by supplying the necessary substrate for growth and indirectly as the oxygen consumption is increased by the supply of carbon, thereby decreasing the thickness of the oxic zone (Chalamet, 1986; Seitzinger, 1988). Moreover, Kana et al. (1998) indicated that in situ denitrifying bacteria respond rapidly to increases in nitrate concentration in the overlying water. Gross primary production, rather than community respiration, has been shown to control NO_3^- uptake in streams (Hall and Tank, 2003). More primary producers (autotrophs) on the sediment surface would change the size of the oxic layer, which would not only drop the rate of denitrification but also disrupt the conversion of N_2O to N_2 because N_2O reductase is sensitive to changes in oxygen concentrations.

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In experiments conducted with soil-extracted bacteria, an oxic phase following anoxia decreased denitrification rates and resulted in more N_2O production (Morley et al., 2008).

Historically in Europe, river floodplains have been decoupled from their rivers, altering the natural nutrient spiraling. As a result, nutrients are transported downstream without being incorporated into floodplain biogeochemical processes (Hein et al., 2004; Tockner et al., 1999). Recent floodplain restoration efforts involve reconnecting the floodplain to the river, reestablishing the flow regime and altering the nutrient load in the floodplain (Buijse et al., 2002). Restoration of large floodplains via surface water reconnection provides an opportunity to observe the effects of changing nitrogen and carbon pools on denitrification, DNRA and anammox activity. Indeed, it is necessary to understand how these restoration efforts affect floodplain nitrogen removal and N_2O emissions in riverine landscapes (Welti et al., 2012).

We hypothesized that the restoring the hydrological exchange between a river and its floodplain would enhance denitrification rates by increasing nitrate and easily mineralizable organic carbon availability. We used mesocosm experiments to separate the effects of the riverine nitrate input and changes in DOM composition on the rate of anammox, DNRA, denitrification and the proportion of N_2O produced. These experiments were done on two types of sediments (1) disconnected and (2) reconnected (restored) floodplains of the Danube River, downstream Vienna, Austria.

We hypothesized that disconnected sites, naturally nitrate-depleted, would have lower denitrification rates than connected sites under high nitrate input, and would present higher N_2O to N_2 ratio. We tested this hypothesis by measuring the response of the denitrifying community of both disconnected and restored sites to pulsed or constant (over a 5 days period) inputs of ^{15}N labeled nitrate. We also hypothesized that adding Danube River water would increase the denitrification rate in the disconnected site due to a more heterogeneous carbon pool present in the Danube River. The resulting $N_2O : N_2$ ratio would decrease due to an increase of carbon substrate available.

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2 Methods

2.1 Study site description

Two sites were chosen within the boundaries of the Alluvial Zone National Park, located downstream of the city of Vienna, Austria. In this area, the Danube River is a 9th order river with a drainage basin of 104 000 km^2. The flow regime has an alpine character with variable and stochastic patterns (regulated low discharge = 915 m^3 s^{-1}, mean discharge = 1930 m^3 s^{-1}, annual flood discharge = 5300 m^3 s^{-1}, 30 y max. flood discharge = 9340 m^3 s^{-1}).

The two chosen sites represent (1) a typical disconnected, backwater pool, located in the Lower Lobau floodplain, and (2) a reconnected channel site (restored) located in the restored floodplain Orth. The restored site is connected via surface water exchange to the Danube main channel more often and for longer periods than the disconnected site (Table 1). The difference in hydrological conditions of the two sites also affects their water chemistry, with the restored site receiving frequent inputs of NO_3^- from the Danube (Table 1). These sites were chosen because they represent two distinct floodplain morphologies (channel vs. pool) and were predicted by a model to react differently with increasing hydrological connection in terms of sediment respiration (Tritthart et al., 2011) and potential denitrification (Welti et al., 2011).

The Lower Lobau floodplain, downstream of Vienna, covers approximately 23 km^2. Except for groundwater-surface water exchange and a controlled small water intake, the primary water exchange is through an artificial small breach in the flood levee located at the downstream end at 1908 river km. This artificial opening in the flood protection dam allows limited connection to the main river at discharges above 1500 m^3 s^{-1} (approx. 235 days y^{-1}). Three major retention structures with culverts prevent the side arms to fall completely dry during low flow periods, resulting in shallow lake-like conditions throughout the floodplain. The selected disconnected sampling site is typical of this floodplain as it is a shallow pool, dominated by groundwater flow and rarely con-

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nected to the Danube River via surface water. High macrophyte coverage and stands of *Phragmites sp.* are present at this site.

The floodplain Orth is directly downstream of the Lower Lobau, covering approximately 5.5 km^2 (Fig. 1), and featuring very diverse flow characteristics. Generally characterized as a through-flow system above a river discharge of 2230 $m^3 s^{-1}$, some sites are only connected during higher discharges. Most of the historical retention structures present in the Orth floodplain have been removed in recent years, increasing the side-arm discharge significantly as well as the connection duration (Tritthart et al., 2009). The three openings (at river km 1906.5 and two at river km 1905) and one outlet (river km 1902) connect parts of this side-arm system to the main river at discharges of 4400 $m^3 s^{-1}$ (approx. 7 days y^{-1}), 1500 $m^3 s^{-1}$ (approx. 235 days y^{-1}), and less than 900 $m^3 s^{-1}$ (approx. 365 days y^{-1}), respectively. The selected restored sampling site is a flow-through channel site, bounded on one side by a gravel bend and the other by fine silt and sand. No macrophytes or other floating vegetation are present at this site.

2.2 Mesocosm study preparation

Plexiglas mesocosms were used for core incubations. These mesocosms were 50 cm tall with a diameter of 24 cm and total volume of 22.6 l. The bottom of the mesocosm was sealed tightly with a plate bolted to the mesocosm (Fig. 2).

Three sets of triplicate sediment cores (depth = 10 cm) and the overlying water (15–17 l) ($n = 9$) were taken from each site. In the field, the mesocosms were emptied except for the last 2 l of water in order to maintain sediment saturation and anoxic conditions during transport. Black plastic sheeting was wrapped around the bottom of mesocosms in order to prevent light penetration into the sediment layer. Upon returning to the lab, the mesocosms were re-filled with in situ water, which had been collected and filtered on site (10 µm) to remove large phytoplankton assemblages, macrophytes, and coarse sediments. Triplicate cores were connected to a reservoir containing 40 l of filtered site water. Water was pumped via a peristaltic pump from the reservoir to the individual cores at a rate of 5 l h^{-1}, creating a residence time of approximately 2 h

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in each mesocosm. Mixing tests prior to the start of the experiments showed complete mixing within the mesocosms. The mesocosms were completely closed to the atmosphere, but the reservoirs were open. Between the mesocosm triplets and the reservoir, a filter (10 µm) prevented large phytoplankton assemblages from occurring and removed coarse suspended sediments. In order to prevent 29/30 N_2 accumulation in the mesocosm cores, water in the reservoirs was bubbled with air before returning to the mesocosms. All corers were kept at constant in situ water temperature at the time of sampling (range 19–22 °C) for the entirety of the incubation with 12 h dark/light cycles.

2.3 Tracer addition experiments

In order to mimic the chemistry of a flood, two incubation setups were used – one to follow the effect of nitrate input (Experiment 1), the other to follow the effect of changing DOM composition (Experiment 2). Mesocosms were stabilized for 48 h until N-NO_3 and N-NH_4 reached constant concentrations. Following the 48 h stabilization period, labeled nitrate ($K^{15}NO_3$) was added to each of the treatments to quantify nitrate transformations throughout the experiments.

The purpose of Experiment 1 was to simulate the nitrate input of either flooding or long-term surface water reconnection. Following the stabilization phase, the mesocosm either received (1) a spike addition (PEAK) of ^{15}N-NO_3 (target concentration: 130 µmol ± 10 %) or (2) a constant addition of ^{15}N-NO_3 to maintain a concentration of 75 µmol ± 10 % (PLATEAU). The control treatment (CONTROL N) received no increase in absolute nitrate concentration, but labeled ^{15}N-NO_3 was added to increase ^{15}N to circa 20 at%.

In Experiment 2, in situ water was replaced with water from (1) an open backwater pool in the Lobau (POOL) or (2) the Danube main channel (RIVER) in order to assess the role of the available organic matter source on denitrification rate and the $N_2O : N_2$ ratio. For control treatment (CONTROL C), no water was exchanged and water orig-

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inating from the sampling site was used. Once the chambers were re-filled, $^{15}N\text{-}NO_3$ was added to a concentration of 130 µmol ± 10 % to all treatments and kept constant for the five day incubation in order to prevent N limitation for denitrification.

2.4 N measurements

Water column sampling occurred through a tube extending into the water column, ending 1 cm above the sediment surface, separated from the atmosphere with a three-way stopcock. Water samples for nutrients, dissolved gases, and isotope analysis were collected using the protocols established in the NICE handbook (Dalsgaard et al., 2000). Water samples were taken at times 0, 2, 4, 8, 10, 24, 36, 48, 72, 96, and 108 h through the tube with a 60 ml syringe. Water samples (50 ml) for $N\text{-}NH_4$, $N\text{-}NO_3$, and $N\text{-}NO_2$ were filtered through a Whatman GF/F filter (pore size 0.7 µm) and analyzed using a continuous flow analyzer (CFA, Systea Analytical Technology).

Before the incubations, 50 g sediment subsamples were taken from the field site and again upon completion of the incubation from the incubated sediments. Nitrogen concentrations in the sediment were analyzed for $N\text{-}NH_4^+$ (KCl extraction), $N\text{-}NO_3^-$, and $N\text{-}NO_2^-$ (H_2O extraction) using standard colorimetric methods for a continuous flow analyzer (APHA, 1998; CFA, Systea Analytical Technology). Organic matter content of the sediment fractions was determined as weight loss by ignition (LOI %) of dry sediment at 450 °C for 4 h. Dry weight of the sediment samples was determined by oven-drying sediments at 70 °C to constant mass.

2.5 N species isotope composition

To measure the isotopic composition of N_2 and N_2O, water samples (50 ml) were collected by 60 ml plastic syringes equipped with a 10 cm long Nalgene® tube at sampling times 0, 2, 4, 8, 10, 24, 36, 48, 72, 96, and 108 h. The syringe was flushed with sample water prior to the transfer of the actual sample and no bubbles were present during sampling. The water was transferred to a gas tight vial (12 ml Exetainer, Labco, High

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Wycombe, UK) which was filled without air bubbles and preserved with 250 µl $ZnCl_2$ (50 % *m*/*v*).

N_2 and N_2O were extracted from the water in the Exetainers by introducing a helium headspace to remove 6 ml of water which was simultaneously replaced with an equivalent volume of He. Vials were shaken vigorously for 5 min so that more than 98 % of the N_2 and N_2O would be in the headspace (Weiss, 1970). All vials were frozen and shipped to LMGEM (CNRS Marseilles, France) for analysis. Corrected against air, samples were measured for $^{28}N_2$, $^{29}N_2$, $^{30}N_2$, $^{44}N_2O$, $^{45}N_2O$, $^{46}N_2O$, Ar, and O_2, with a mass spectrometer (Quadruple mass spectrometer Anagaz 100, MKS, England) in the headspace.

Signals at different m/z values were collected every 0.5 s intervals and were stored by a desktop computer for later analysis. N_2 was measured at $m/z = 28$, 29 and 30 corresponding to $^{28}N_2$, $^{29}N_2$ and $^{30}N_2$, respectively, and O_2 and Ar were measured at $m/z = 32$ and $m/z = 40$, respectively. Ar was used as an internal standard. The raw value collected at $m/z = 30$ was corrected according to Minjeaud et al. (2008) in order to take into account interference due to NO_x ions formation from N_2 and O^+ inside the MS. As $m/z = 44$, 45, and 46 can either originate from N_2O or CO_2, these were summed and corrected using the calculated ratio of $N_2O : CO_2$ from potential denitrification assays prior to the mesocosm incubation.

The isotopic composition of N-NO_3^-, N-NO_2^- and N-NH_4^+ in the overlying water column and in the sediments was determined according to Lachouani et al. (2010) and measured on a 96-slot autosampler with a double-hole needle (GC-PAL, CTC Analytics, Zwingen, Switzerland) connected via a Gasbench II headspace analyzer (Thermo Fisher, Bremen, Germany) to an IRMS (Delta V Advantage, SILVER lab, University of Vienna). Organic N and C concentration and isotope abundances from sediment samples were measured with an elemental analyzer (EA 1110, CE Instruments, Milan, Italy) connected to an isotope ratio mass spectrometry IRMS (DeltaPLUS, Finnigan MAT, Bremen, Germany) in Vienna (SILVER Lab, University of Vienna).

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2.6 Denitrification, anammox, and dissimilatory nitrate reduction to ammonium (DNRA)

At each time step, denitrification and anammox rates were calculated using the comprehensive method as outlined by Spott and Stange (2007). This approach allows for a precise calculation of the contribution of denitrification and anammox to an N_2 mixture, while taking into consideration the contamination by atmospheric N_2. The following calculations (Eqs. 1–3) were used to determine the portion of atmosphere (A), denitrification (B), and anammox (C) contributing to the N_2 mixture.

$$A = \frac{2b(\alpha_{30} - cd) + (c+d)(b^2 - \alpha_{30}) - (b^2 - cd)(\alpha_{29} + 2\alpha_{30})}{(a-b)[2(ab+cd) - (a+b)(c+d)]} \quad (1)$$

$$B = \frac{2a(cd - \alpha_{30}) + (c+d)(\alpha_{30} - a^2) + (ba^2 - cd)(\alpha_{29} + 2\alpha_{30})}{(a-b)[2(ab+cd) - (a+b)(c+d)]} \quad (2)$$

$$C = \frac{2ab - \alpha_{29}(a+b) + 2\alpha_{30}(1-b-a)}{2(ab+cd) - (a+b)(c+d)} \quad (3)$$

Where α_{28}, α_{29}, and α_{30} are the mole fractions of masses 28, 29, and 30 within the N_2 mixture and a, b, c, d are the ^{15}N atom fraction of N_2 (a), NO_3^- (b), NO_2^- (c), and NH_4^+(d).

The rate of dissimilatory nitrate reduction to ammonia (DNRA) was determined in the sediment after completion of the five day incubation. Rates were calculated using Gilbert et al. (1997) (Eq. 4).

$$\text{DNRA} = \frac{(\text{at\%}\,NH_4^+)([NH_4^+])}{(\text{at\% enrichment}\,NO_3^-)(\text{incubation duration})} \quad (4)$$

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Where at% NH_4^+ is the mole fraction of $^{15}N\text{-}NH_4^+$ determined at the end of incubation. All rates were calculated per square meter for the upper 10 cm of the sediment layer.

The sum of masses 28, 29, and 30 (m_{28}, m_{29}, and m_{30}), and the corrected sum of masses 44, 45, and 46 (m_{44}, m_{45}, and m_{46}), were used to calculate ratios of N_2 produced as an end product versus the amount of N_2O produced (Eq. 5). The closer the ratio is to zero, the larger the percentage of N_2 is produced relative to N_2O.

$$\frac{N_2O}{N_2} = \frac{m_{44} + m_{45} + m_{46}}{m_{28} + m_{29} + m_{30}} \quad (5)$$

The percentage of used nitrogen was estimated for denitrification and DNRA; pelagic and bacterioplankton production (BP); and biomass assimilation for each treatment. A C : N ratio of 5 : 1 was used to estimate the N-requirement for BP (Gruber and Galloway, 2008, references therein). All unaccounted nitrogen loss was attributed to biomass assimilation.

2.7 Bacterioplankton and benthic bacterial production

Bacterioplankton production (BP) was measured at times 0 h, 72 h, and 108 h according to Kirchman et al. (1986), while benthic bacterial production (BBP) was measured at time 0 and 108 h with a modified method of the Leu incorporation technique according to Wieltsching et al. (1999) and Fischer and Pusch (1999). Three replicate sub-samples taken from the sediment and two blanks (0.2 g) were weighted into 1.7 ml screw-cap microcentrifuge tubes. The samples were then incubated at in situ temperatures for 1 h. The incubation was terminated by the addition of formaldehyde (final concentration = 3.2 %).

The fixed samples were vortexed, sonicated (10 min, 60 % power) in a sonication bath (Elma T 710 DH) and vortexed again. After this step, trichloroacetic acid (TCA) was added to a final concentration of 5 %. In order to dissolve the non-protein fraction of the cells, the samples were then incubated at 95 °C for 30 min. After cooling on ice,

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the remaining precipitate was filtered onto 0.2-mm-pore-size membrane filters (polycarbonate filters [Nuclepore]). Filters were thoroughly rinsed with deionized water to eliminate unincorporated leucine. Filters were then put into 7-ml scintillation vials and completely dissolved in 5 ml scintillation cocktail (Ultima Gold; Canberra Packard). Radioactivity was measured in a Beckman 6500 scintillation counter. Controls were fixed with formaldehyde (final concentration, 3.2 %) immediately at the start of the incubation and generally contributed less than 10 % of the total leucine incorporation.

2.8 Dissolved organic matter (DOM) and dissolved organic carbon (DOC) measurements

Dissolved organic matter (DOM) and dissolved organic carbon concentration (DOC) were measured from the water column at each time step for all mesocosms during the carbon exchange experiment. Fluorescence excitation-emission matrices (EEMs) – three-dimensional contour plots which display fluorescence intensities as a function of a range of both excitation and emission wavelengths – were used to characterize DOM (dissolved organic matter) composition (Baker and Spencer, 2004).

The water samples were filtered through a prepared Whatman GF/F filter (2.5 h at 490 °C; diameter 0.7 µm) and stored in purged glass tubes (24 h in 10 % HCl, 4 h combusted at 490 °C) at 4 °C and analyzed within 24 h. DOC was measured using a TOC analyzer (Sievers 900).

The fluorescence measurements were undertaken using a Hitachi Fluorescence Spectrophotometer F-7000 and all samples were scanned in the following wavelength regions: excitation 200–400 nm at 5 nm steps and emission 280–500 nm at 2 nm steps. Blank water scans were run before and after every sample run using Milli-Q water to measure the Raman signal at excitation 350 nm (emitted at 397 nm) and all results are standardized to a mean Raman peak of 150 intensity units.

For characterization of DOM, we used three fluorescence peaks (B, T and C) according to Coble (1996), fluorescence index (FI), beta to alpha ($\beta : \alpha$) ratio, and the humification index (HIX). Peaks B and T were recorded at excitation wavelengths of

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225–275 nm and emission at wavelengths of 300–325 nm and 340–385 nm, respectively, and have been related to protein-like substances (peak B = Tyrosine-like, peak T = Tryptophan-like) (Baker, 2001). Peak C is a fluorophore at 300–370 nm excitation and 400–500 nm emission and is attributed to humic-like substances. Ratios between the fluorescence peaks (C, T, and B) and DOC were calculated to allow the partitioning of humic and protein-like DOM. The ratios T : DOC and B : DOC were summed to create a total protein-like pool of DOM in the overlying water column.

FI was calculated from excitation 370 nm as the ratio of intensities at 450 nm and 500 nm (McKnight et al., 2001). FI is inversely related to the lignin content of DOM, where values around 1.3 suggest a dominant terrestrial DOM and values around 1.8 suggest a dominant microbial DOM source. The $\beta : \alpha$ ratio was calculated at excitation 310 nm from the emission intensity at 380 nm divided by the emission intensity maximum observed between 420 and 435 nm (Wilson and Xenopoulos, 2009). The $\beta : \alpha$ indicates the relative contribution of microbially-derived autochthonous DOM (Huguet et al., 2009). Finally, HIX was calculated from excitation 255 nm as the ratio of the peak area under each curve at emissions 434–480 nm and 300–346 nm (Zsolnay et al., 1999). HIX values around 1–2 are associated with non-humified plant material and values > 10 are commonly reported for fulvic acid extracts (Ohno, 2002).

2.9 Statistics

Mann-Whitney *U* tests were used to test differences between the sites and treatments. General linear models were used to assess the change of N species over time for the individual treatments. One-way independent ANOVA was used to test the change of N-species over time between the sites and treatments. Stepwise multiple linear regression models between water chemistry ($N\text{-}NO_3^-$, $N\text{-}NO_2^-$, $N\text{-}NH_4^+$), carbon quality (Peaks C, T, and B and DOC concentration) were used to elucidate their overall influence on the denitrification rate and ratio of $N_2O : N_2$. Significance for all tests was set at $p < 0.05$. All tests were performed using the SPSS software package.

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3 Results

3.1 Measured denitrification rates

Denitrification rates varied between sites but not among treatments or over time. In the restored site N_2 fluxes were higher and had a larger range of rates with an of average 5.7 mmol N_2 m^{-2} h^{-1} whereas in the disconnected sites fluxes were on significantly lower with an average of 0.6 mmol N_2 m^{-2} h^{-1} ($U = 2520$, $p < 0.05$).

More incomplete denitrification was measured at the disconnected site than at the restored site with higher average calculated $N_2O : N_2$ ratios in the control treatments (Control N and Control C) (Isolated mean 0.07 ± 0.02; Restored mean 0.02 ± 0.01) ($U = 5610$, $p < 0.001$).

Since denitrification rates and $N_2O : N_2$ ratios did not change significantly over time at either site during any treatment (linear regression; $R^2 < 0.5$, $p > 0.05$), all subsequent analyses use five-day averages. As well, no significant differences were observed between the control replicates at either site for denitrification or the $N_2O : N_2$ ratio (disconnected; $p = 0.98$ and $p = 1.0$; Restored $p = 0.86$ and $p = 0.88$, respectively).

We measured higher denitrification rates in the restored site compared to the disconnected site ($U = 2520$, $p < 0.001$) in treatments that did not receive nitrogen additions (Fig. 3). In addition, in these same treatments higher $N_2O : N_2$ ratios (e.g., greater incomplete denitrification) were measured at the disconnected site than at the restored site ($U = 5610$, $p < 0.001$) (Fig. 4).

No anammox was detected during the five day incubation at either site. DNRA rates were calculated after 5 days and were higher and more variable at the backwater site than in the restored site ($U = 31.0$, $p < 0.001$) (Table 2).

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3.2 Measured BP and BBP

BP and BBP were higher in the disconnected site ($2.45 \pm 1.26\,\mu g\,C\,l^{-1}\,h^{-1}$; $2063 \pm 1040\,\mu g\,C\,kg^{-1}\,h^{-1}$, respectively) than in the restored site ($1.94 \pm 1.2\,\mu g\,C\,l^{-1}\,h^{-1}$; $1294 \pm 331\,\mu g\,C\,kg^{-1}\,h^{-1}$, respectively) ($U = 873$, $p < 0.05$; $U = 370$, $p < 0.001$, respectively).

3.3 Experiment 1: effect of NO_3^- addition on denitrification rates

3.3.1 Disconnected site

The mean in situ N-NO_3^- and N-NH_4^+ concentrations in the overlying water column measured prior to the incubation were 3.84 µM N-NO_3 and 16.4 µM N-NH_4. The ^{15}N-NO_3 additions increased the N-NO_3 concentration and ^{15}N enrichment (at%) in all core treatments (34.7 µM, 22 at% Control N; 106 µM, 88 at% Plateau; 189 µM, 90 at% Peak). Within two hours, once mixing within the cores and the reservoir was complete, the Plateau and Peak treatments reached the target concentrations of 75 µM N-NO_3 and 130 µM N-NO_3, respectively. The control treatment decreased from the increased addition to 25 µM N-NO_3^- after two hours.

Denitrification rates or the $N_2O : N_2$ ratio were not significantly different between the three N treatments (Control N, Peak and Plateau) ($F = 4.6$, $p = 0.06$) (Figs. 3, 4). DNRA rates were an order of magnitude lower than denitrification rates (Table 2) and were also not significantly different between N treatments.

DOC increased significantly over the five days for all treatments ($R^2 = 0.80$, $p < 0.01$) during the experiment and was not correlated with denitrification. In addition, NO_3^- concentration was not correlated to denitrification rates any treatment. But, changing the nitrate delivery regime (i.e. Peak vs. Plateau) resulted in a significant (one-way independent ANOVA) decrease in the percentage of N consumed by BP between the treatments from 66 % (Control N) to 17 % (Peak) and 26 % (Plateau) ($F = 35.5$, $p < 0.01$) and increase in biomass assimilation from 27 % (Control N) to 77 % (Peak) and 71 % (Plateau) ($F = 35.6$, $p < 0.01$) (Fig. 5). However, the estimated percentage of N up-

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take via denitrification did not change significantly and remained low (< 10 %) ($F = 1.8$, $p = 0.2$).

3.3.2 Restored site

The mean in situ N-NO_3^- and N-NH_4^+ concentrations in the overlying water column were 74 µM N-NO_3^- and 24.7 µM N-NH_4^+. Because the in situ concentration of N-NO_3^- was higher than the goal concentration for the plateau treatment, water from another adjacent restored site was used. This site was pre-selected because of its similar DOM characteristics and average hydrology. The in situ N-NO_3^- concentration at this site was 4.20 µM N-NO_3^- at the sampling time. Tracer additions increased the N-NO_3 concentrations in all mesocosm treatments (140.1 µM N-NO_3^-, 43 at% Control N; 111.0 µM N-NO_3^-, 93 at% PLATEAU; 171.9 µM N-NO_3^-, 46 at% PEAK). Within two hours, once mixing within the mesocosms and the reservoir was complete, the Plateau and Peak treatments reached the target concentrations of 75 µM N-NO_3^- and 130 µM N-NO_3^-, respectively. The control treatment decreased from the initial increase to 123 µM N-NO_3^- after 2 h.

Denitrification rates ranged from 0.6–8.6 mmol N m^{-2} h^{-1} (mean = 1.1 ± 1.9 mmol N m^{-2} h^{-1}) (Fig. 3). Denitrification rates and the $N_2O : N_2$ ratios were not significantly different between the nitrogen treatments ($p = 0.32$, $p = 0.91$, respectively) (Fig. 4). DNRA rates were low in all treatments (Table 2).

Changing the nitrate delivery regime significantly decreased (one-way independent ANOVA) the estimated nitrogen use by BP from 40 % (Control N) to 20 % (Peak) and 30 % (Plateau) ($F = 9.4$, $p < 0.05$) (Fig. 5). Although assimilation was estimated to be < 1 % in the control treatment, due to the high standard deviation in the treatments, the increase of biomass assimilation was not significant ($F = 2.3$, $p = 0.2$). No significant changes were estimated for denitrification ($F = 1.5$, $p = 0.3$).

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3.4 Experiment 2: effect of carbon quality on denitrification rate and on $N_2O : N_2$ ratio

3.4.1 Disconnected site

Prior to the water exchange, the in situ concentrations of NO_3 and NH_4 were 6.0 µM $N\text{-}NO_3^-$ and 5.8 µM $N\text{-}NH_4^+$ respectively Tracer additions increased the $N\text{-}NO_3^-$ concentrations in all treatments to 225.4 µM, 93 at% (Control C), 183.7 µM, 86 at% (POOL) and 189.9 µM, 45 at% (RIVER). Within two hours, all treatments reached the target concentrations of 130 µM (140.7 µM CONTROL C; 131.2 µM POOL; 149 µM RIVER). Once the target concentration was reached, $N\text{-}NO_3^-$ was kept constant by an addition of $N\text{-}NO_3^-$ after each sampling time.

In situ DOC varied from 2.6 $mg\,l^{-1}$ in the Danube River and 13.2 $mg\,l^{-1}$ in the open pool site (13.2 $mg\,l^{-1}$). In situ DOC concentrations in the disconnected site were in between these two extremes with an average of 6.9 $mg\,l^{-1}$ (Table 3). In two of the three treatments the DOC increased significantly over the five day incubation (Control $R^2 = 0.35$ $p < 0.05$; RIVER $R^2 = 0.82$ $p < 0.01$; POOL $R^2 = 0.01$ $p = 0.97$). Although the DOC was lowest in the Danube River water, the ratios of T : DOC and B : DOC were highest at this site (Table 3) (one-way ANOVA $p < 0.01$ and $p < 0.01$) indicating a high content of protein-like DOC.

Denitrification rates ranged from 0.02–10.7 $mmol\,N\,m^{-2}\,h^{-1}$, and were not significantly different between treatments ($F = 2.9$, $p = 0.06$) (Fig. 3). The $N_2O : N_2$ ratios were different (Fig. 4) between the Control C and the two treatments ($p < 0.001$) with a further decrease between RIVER and POOL (one-way ANOVA $p < 0.001$).

No significant differences in DNRA rates were observed between any of the treatments ($p = 0.13$) and ranged from 15–22 $\mu mol\,N\,m^{-2}\,h^{-1}$ (Table 2). The percentage of N used did not change significantly between treatments, with denitrification accounting for < 20 %, BP < 20 % and assimilation > 50 % in all treatments (one-way independent ANOVA) ($F = 0.6$, $p = 0.6$; $F = 4.3$, $p = 0.07$; $F = 1.2$, $p = 0.4$, respectively).

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3.4.2 Restored site

Prior to the water exchange, the in situ concentrations in the restored site were 89.3 µM $N\text{-}NO_3^-$ and 1.9 µM $N\text{-}NH_4^+$. Tracer additions increased the $N\text{-}NO_3^-$ concentration in all treatments to 159.5 µM, 51 at% (CONTROL C), 188 µM, 96 at% (POOL) and 152 µM, 33 at% (RIVER). Within two hours, two treatments reached the target concentrations of 130 µM (137 µM POOL; 135 µM RIVER). The control treatment reached the target concentration (144 µM) within 4 h.

At the restored site DOM was higher in overall protein-like carbon relative to the dissolved organic carbon pool (T + B : DOC) than the disconnected site in the water column ($U = 521$, $p < 0.001$) (Table 3). Yet, the DOC at the disconnected site was significantly higher ($U = 957$, $p < 0.01$) than at the restored site. Using the calculated ratios for FI, HIX, and $\beta : \alpha$ to distinguish the DOM characteristics in the water column, small, but significant differences were only observed for HIX and $\beta : \alpha$ between the sites ($U = 36$, $p < 0.01$; $U = 0.5$, $p < 0.01$, respectively) with the disconnected site having higher humic content in the DOM pool than the restored site (Table 3).

The in situ DOC was very similar to the Danube River. The water from the Danube River was highest in protein-like DOM. DOC increased slightly over the five day incubation in the POOL treatment (Control $R^2 = 0.14$ $p = 0.23$; RIVER $R^2 = 0.03$ $p = 0.59$; POOL $R^2 = 0.38$ $p < 0.05$). No significant differences in the measured denitrification rates were observed between the two control treatments (Control N & Control C) for either site ($p = 1.0$). No significant differences in DNRA were observed between the POOL and RIVER treatments ($F = 1.29$, $p = 0.329$).

A significant increase of the $N_2O : N_2$ ratio was observed between the CONTROL C and POOL treatments ($p < 0.05$), but not between the CONTROL C and RIVER treatments. DNRA rates remained low in all treatments (Table 2). No change was measured in the estimated percentage N uptake for denitrification (< 10 %), BP (9–30 %) and assimilation (66–90 %) (One-way independent ANOVA) ($F = 1.1$, $p = 0.4$; $F = 2.9$, $p = 0.1$; $F = 3.1$, $p = 0.1$, respectively).

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3.5 Factors influencing $N_2O : N_2$ ratio

All water chemistry and DOM quality data were combined by sites and treatments in Experiment 2 to investigate their effect on denitrification rates and $N_2O : N_2$ ratios in the sites. A stepwise regression was used for the $N_2O : N_2$ ratio ($R^2 = 0.67$). In the final regression model three variables entered significantly. $N\text{-}NO_2^-$ concentration in the water column was positive/negative, whereas the proportion of humic-like carbon relative to the total DOC pool (C : DOC) was positively related to the $N_2O : N_2$ ratio and the total protein-like carbon pool relative to the total DOC pool (T : DOC + B : DOC) was negatively related (Table 4).

3.6 Mass balance

Based on our mass balance estimate, biomass assimilation by algae and bacteria was estimated to be the main biological mechanism of N retention in the disconnected site (Fig. 5). The portioning of N retention was not affected by the experimental treatments (NO_3 or DOM quality). However, the low replicates ($n = 3$) and high standard deviation did not allow us to calculate significance (Mann-Whitney U) (Fig. 5).

4 Discussion

We found high denitrification rates at both restored and disconnected sites within the floodplain of the Danube River thus indicating the importance of microbial nitrate removal. Our rates are on the high side of global estimates for rivers (up to 700 µmol N m^{-2} h^{-1}) (Pina-Ochoa and Alvarez-Cobelas, 2006) and those for headwater streams (100–1000 µg N m^{-2} h^{-1}) (Mulholland et al., 2008).

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4.1 Importance of connected floodplains

Higher denitrification rates were measured in the restored site compared to the disconnected site. A similar trend in higher denitrification rates has also been documented for wetlands connected to the Po River (Racchetti et al., 2010). Higher denitrification rates ($94\,mg\,N\,m^{-2}\,h^{-1}$, ca. $279\,\mu mol\,m^{-2}\,h^{-1}$) were estimated by James (2010) for a backwater system of the Mississippi River, which receives similar $N\text{-}NO_3^-$ inputs from the river, accounting for 57 % of nitrate removal. This highlights the buffer capacity of floodplains and the potential for nitrate removal therein, which can be up to 100 % of a river's nitrate load (Fennessy and Cronk, 1997). Accordingly, nitrate concentrations in the floodplain lakes of the Wisconsin River declined below detection level after being disconnected for 6 days from the river channel (Forshay and Stanley, 2005). Flooding tends to increase NO_3^- concentrations and has a similar effect on denitrification rates even in disconnected channels (Hein et al., 1999).

The importance of floodplain connection is even more significant when we estimate denitrification rates on an annual basis. Indeed, using our measured denitrification rates and the restored Orth floodplain average yearly discharge of approx. $2000\,m^3\,h^{-1}$, we calculate that the restored floodplain would reduce $130\,\mu mol\,l^{-1}$ $N\text{-}NO_3^-$ within 24 h, compared to the estimated 6 days that it would be required in the Lobau floodplain. Previous models have estimated that denitrification rates are highly variable in these floodplains (Welti et al., 2011, 2012). As such, sites within the Lobau floodplain that receive more frequent inputs from the Danube River can have denitrification rates similar to those measured in the Orth floodplain.

Higher, but variable, denitrification rates were consistently measured at the restored site compared to the disconnected site, demonstrating higher denitrification capacity when floodplains are linked to the river (Fig. 3). As well, restored site undergo a more complete denitrification as $N_2O : N_2$ ratios are lower compared to disconnected sites. This increased incomplete denitrification in the disconnected sites could be due to

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differences in the microbial community structure (Philippot, 2002) or a decoupling between the water column and the anoxic sediment layer (Dodds et al., 2000).

We found an increase of $^{15}N\text{-}NH_4^+$ in the water column which suggests algal assimilation of NO_3^-. The communities in the water column may out-compete denitrification for both nitrogen and organic carbon via nitrification (Sloth et al., 1995). Despite being short term, biomass assimilation can provide a rapid sink for nitrate (Hefting et al., 2005). Although autotrophs are generally assumed to prefer ammonium than nitrate, biological assimilation and BP and BBP seem be of more importance than denitrification, accounting for the majority of DIN uptake in both systems (Fig. 5).

In many constructed wetlands, up to 36 % of nitrate loss can be accounted for via anammox (Scott et al., 2008), suggesting that this pathway could complement the nitrate removal process. However, we did not find anammox to be an important pathway in this floodplain system. Another pathway, DNRA, was detected to occur in our sites but was especially low in these floodplains. DNRA tends to increase in sites with high carbon and vegetation (Matheson et al., 2002; Fazzolari et al., 1998). In our study, the disconnected site was higher in overall C and terrestrially-rooted vegetation than the restored site, which might explain the observed differences between the DNRA rates in our two study sites (in the disconnected site, DNRA was 3 % of denitrification while it was 0.1 % in the restored one). Despite these differences, DNRA does not appear to be a quantitatively important pathway, as these rates were an order of magnitude lower than denitrification in both sites.

4.2 NO_3^- as a regulator of denitrification

Denitrification rates and denitrifier efficiency (as shown by $N_2O : N_2$) in stream sediments varies with nitrate concentration and discharge (Alexander et al., 2009). We predicted that, due to the high supply of carbon and anoxic conditions in the disconnected sediments, the disconnected site would be nitrate-limited, as has been demonstrated in different riverine sediments (Forshay and Stanley, 2005; Hill et al., 2000; Silvennoinen et al., 2008), streams (Smith et al., 2006), constructed wetlands (Scott et al., 2008)

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and estuaries (Teixeira et al., 2010). Therefore, by increasing the nitrate concentration in the water column of the disconnected site, which is high in organic carbon, we expected to measure a corresponding increase in denitrification. However, the addition of nitrate did not increase denitrification rates implying that denitrification at both sites was not nitrate limited (Table 2). After 5 days of constantly elevated NO_3^- concentrations, no adaptation effects were observed in the denitrification rate for either site or any treatment. While the percentage of N used by denitrification did not change with the treatments, the estimated amounts for assimilation and BP did, suggesting that these processes can react quickly with changes in nitrate concentration (Fig. 5). The opposite trends observed (increasing assimilation, decreasing BP) in the disconnected site suggest that assimilation can out-compete the heterotrophs for the available nitrogen.

Although we did not measure bacterial density or microbial community structure in this study, previous studies have shown that the bacterial community is conditioned to respond quickly and efficiently to flooding events (Sanchez-Perez, 2003). Nitrogen saturation can occur when bacterial communities are overloaded with constantly elevated nitrate concentrations. However, communities in pulsed systems with short-term increases (ex floodplains) the bacterial communities may not experience this overloading and can be stimulated by such pulses (Bernot and Dodds, 2005; Burns and Ryder, 2001). In this study, the restored floodplain typically receives frequent pulsing from the river which could be a reason for the described differences in denitrification rates.

4.3 Effects of DOM quality on denitrification

Dynamic floodplains are affected by the complete exchange of their water mass with that of the river water. This changes the available organic carbon substrate pool, originating in the riverine water column, to the bacterial community, located in the sediment of the floodplain. By changing the overlying water column in the mesocosms, we altered the available organic carbon pool quantity and quality. Our hypothesis that increasing the DOC and NO_3 would stimulate denitrification was not supported (Fig. 3).

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However we found that the additional DOM changed the ratio of $N_2O:N_2$ between the sites and treatments. Generally, the ratio of $N_2O:N_2$ was lower in the restored site (Fig. 3), implying that denitrification was more efficient. N_2O production has been related to high organic sediment content and eutrophic environments (Kenny et al., 2004; Sloth et al., 1995; Teixeira et al., 2010), which are the conditions found at both sites. However, when riverine water was added to the disconnected site (i.e. mimicking a reconnection event), the $N_2O:N_2$ ratio decreased, increasing the fraction of denitrification resulting in N_2. The reverse was true when water from a backwater site was added to the restored site (i.e. mimicking a disconnection event), increasing N_2O over N_2 production.

It was not the purpose of this study to investigate the different available substrates in each of the source waters, but rather the effect of the mixture of substrates on denitrification. In soils with changing oxygen conditions, it has been demonstrated that the carbon source becomes important for N_2O production (Morley and Baggs, 2010). Using the three DOM indices, we observed minor, but significant, differences in the carbon pools of the Danube River and the floodplains (mostly a dominant terrestrial DOM source and non-humified plant material). The HIX and FI of both sites were in the range associated with humic material and suggest DOM of primarily allochthonous origin. Nevertheless, the $\beta:\alpha$ ratio was higher in the restored site, indicating a higher contribution of recently derived autochthonous microbial DOM. As well, as shown by the $T+B:DOC$ ratios, the DOM pools between the source waters were significantly different: the Danube River had lower DOC compared to waters originating from the backwaters, but this carbon pool was more protein-dominated and therefore more bioavailable (labile) to the sediment microbe community (Table 3). This difference could be the reason for the different responses in the $N_2O:N_2$ ratio we observed. In the disconnected system, where the oxic layer may be changing diurnally due to a higher number of autotrophs, the same co-regulation between oxygen and carbon source may be occurring (Christensen et al., 1990; Laursen, 2004).

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The DOM pool is indeed an important predictor variable in the regression model produced for the ratio of $N_2O:N_2$. The negative relationship between the relative proportion of protein-like carbon and the $N_2O:N_2$ suggests that protein-like DOC reduces the $N_2O:N_2$ ratio, resulting in more N_2 production. The appearance of NO_2^- in the multiple regressions can be interpreted as a proxy for nitrogen cycling. Nitrate was kept in constant abundance in the experiment, but NO_2^- increased throughout the incubation period, a result of NO_3^- reduction. DOM originating from the Danube River has been shown to be a mixture of terrestrial and microbial derived sources, depending on the discharge and season (Besemer et al., 2009; Peduzzi et al., 2008; Preiner et al., 2008). Previous studies have suggested that OC is primarily derived from terrestrial sources (dominated by protein-like signatures) during average flow conditions (Hein et al., 2004), however during high discharge, more humic carbon may be transported into the floodplains.

4.4 Importance of restoration

In this study, the restored site experienced an increase in denitrification rates compared to the disconnected site, suggesting that increasing the continuous and long lasting surface water connection periods will increase the overall denitrification rate as well as its efficiency (Kjellin et al., 2007; Klocker et al., 2009; Racchetti et al., 2010). Yet, as shown in Experiment 1, increasing nitrate concentration does not lead to higher denitrification rates in short time. Rather, changing water sources led to changes in the $N_2O:N_2$ ratio. Therefore, prolonged connection to the river may increase the denitrification efficiency; however, surface water connections solely during floods will not increase the overall, long term denitrification efficiency as these sites do not respond quickly to an increase of NO_3. Previous work modeled the response of the $N_2O:N_2$ ratio in the similar floodplains at the floodplain scale, which predicted similar responses of DEA to flooding (Welti et al., 2011).

In the case of the studied restored floodplain, opening the embankments and allowing the Danube water to pulse into the floodplain changed the flow pattern and physical

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characteristics of the site. The restored site was in a channel dominated by a gravel bed, whereas the disconnected site was a shallow pool higher in organic material and fine sediments. In the restored floodplain (Orth), the depth of sediment as well as the sediment organic substrates decreased with the level of connection to the Danube (Reckendorfer, 2006). Compared to the disconnected floodplain (Lobau), which covers more surface area with finer and more organic rich sediments, the absolute area available for denitrification was lower. Reconnection of the Lobau floodplain would increase the rate of denitrification and lower overall N_2O production, resulting in a net gain of ecosystem services. Along with the changes in denitrification, increasing the surface water connection could prime the benthic and pelagic algal communities, thus increasing the nutrient retention capacity of the floodplain (Ahearn et al., 2006; Scott et al., 2009).

In this study, we demonstrated that while denitrification rates were not directly influenced by NO_3^- or DOC in the overlying water, the end product of denitrification was controlled by changes of carbon quality in the overlying water column. By increasing the frequency of flooding into the backwaters, N_2O production could be mitigated and the NO_3^- removal capacity of the floodplain could be increased. Creating regular surface water connection to the Danube River would reduce N_2O emissions by 50 % in the disconnected site. Hydrologic pulsing has been shown to decrease greenhouse gas emissions, organic matter accumulation and increase nutrient retention (Mitsch et al., 2008). In terms of ecosystem management and restoration, it is apparent that frequent, longer lasting, pulsing creates ideal conditions for efficient denitrification, resulting in lower N_2O production.

Acknowledgements. This project was funded by the Austrian Science Fund (FWF) project CANFLOOD (P19907-B17) and the Austrian Committee Danube Research of the International Association of Danube Research. We would like to acknowledge C. Hinterleitner for sediment and water nutrient analysis, S. Preiner and M. Felkl for their help in the field, P. Lachouni and B. Hood-Nowotny for their help with the isotope analyses, E. Lautsch for statistical guidance, and G. Weigelhofer and M. Xenopoulos for their helpful comments on the manuscript.

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Table 1. Site descriptions of hydrology, of the sediment carbon pools, and in situ water chemistry prior to mesocosm incubations ($N = 6$) (mean ± stdev). The two water ages refer to the two separate sampling days.

	Restored		Disconnected	
Days Connected (days y^{-1})	71.5		3.5	
Duration of Disconnection (30 y average)	30.6		426	
Duration of Connection (30 y average)	7.8		3.0	
C : N (Sediment)	8.99	±1.3	8.97	±1.6
$\delta^{13}C$ (Sediment)	−29.41	±3.5	−25.55	±2.4
N-NH_4^+ (µmol l^{-1})	8.2	±2.1	11.3	±6.1
N-NO_2^- (µmol l^{-1})	0.4	±0.1	0.7	±0.5
N-NO_3^- (µmol l^{-1})	80.6	±7.7	5.0	±1.3
DOC (µmol l^{-1})	4.1	±2.5	6.8	±3.8
Water Age (days)	7; 8		294; 24	

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Table 2. Mean DNRA rates ($N = 3$) measured and standard deviation in the sediment after 5 day incubation.

Site	Treatment	DNRA $\mu mol\,N\,m^{-2}\,h^{-1}$	
Disconnected	Control N	1.7	±0.1
	PEAK	1.5	±0.3
	PLATEAU	1.5	±0.7
Restored	Control N	2.7	±3.1
	PEAK	2.8	±3.5
	PLATEAU	0.9	±0.6
Disconnected	Control C	15.1	±12.3
	POOL	15.7	±12.9
	RIVER	22.7	±19.8
Restored	Control C	0.1	±0.1
	POOL	0.0	±0.0
	RIVER	0.3	±0.3

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Table 3. Dissolved organic matter quality and dissolved organic carbon (DOC) over five days and standard deviation in italics ($N = 33$). Peaks B and T occur at excitation wavelengths of 225–275 nm and emission at wavelengths of 300–325 nm and 340–385 nm, respectively, and have been related to protein-like substances (peak B = Tyrosine-like, peak T = Tryptophan-like). Peak C is a fluorophore at 300–370 nm excitation and 400–500 nm emission and is attributed to humic-like fluorescence. Fluorescence index (FI) $\beta : \alpha$ ratio, and the humification index (HIX) were calculated using the fluorescence peaks as described in the methods.

		DOC ($mg\,C\,l^{-1}$)		B : C		T : C		B : DOC		T : DOC		(B + T) : DOC		C : DOC		FI		HIX		$\beta : \alpha$	
Disconnected	Control	6.9	*0.7*	0.8	*0.2*	0.9	*0.1*	99.2	*13.2*	119.2	*9.4*	218.4	*20.8*	127.3	*13.4*	1.2	*0.02*	5.0	*0.2*	0.6	*0.0*
	RIVER	2.6	*0.8*	1.7	*0.4*	1.8	*0.3*	196.6	*50.3*	212.8	*42.5*	409.4	*91.1*	115.4	*11.1*	1.1	*0.01*	3.5	*0.3*	0.6	*0.1*
	POOL	3.2	*0.6*	0.4	*0.06*	0.7	*0.02*	41.3	*5.8*	62.8	*3.9*	104.5	*8.5*	93.8	*4.9*	1.0	*0.01*	5.9	*0.2*	0.6	*0.0*
Restored	Control	2.9	*0.2*	2.2	*0.2*	0.4	*0.02*	286.4	*24.5*	305.7	*27.7*	592.1	*47.4*	129.9	*14.4*	1.1	*0.02*	3.3	*0.2*	0.7	*0.01*
	RIVER	2.6	*0.2*	2.3	*0.1*	0.4	*0.02*	329.9	*37.3*	397.4	*54.2*	727.3	*90.3*	142.4	*13.3*	1.1	*0.03*	2.9	*0.3*	0.8	*0.07*
	POOL	10.7	*1.3*	0.7	*0.02*	0.9	*0.01*	104.3	*14.9*	161.6	*21.9*	265.9	*36.6*	138.8	*16.4*	1.1	*0.02*	3.8	*0.8*	0.7	*0.01*

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Table 4. Predictor variables and coefficients (β) influencing the calculated 5-day $N_2O : N_2$ ratio.

	β
Constant	
$N\text{-}NO_2^-$ µmol	0.44 *
C : DOC	0.62 *
T : DOC + B : DOC	−0.83 *

Dependent variable: $N_2O : N_2$
$R^2 = 0.67$
*$p < 0.001$

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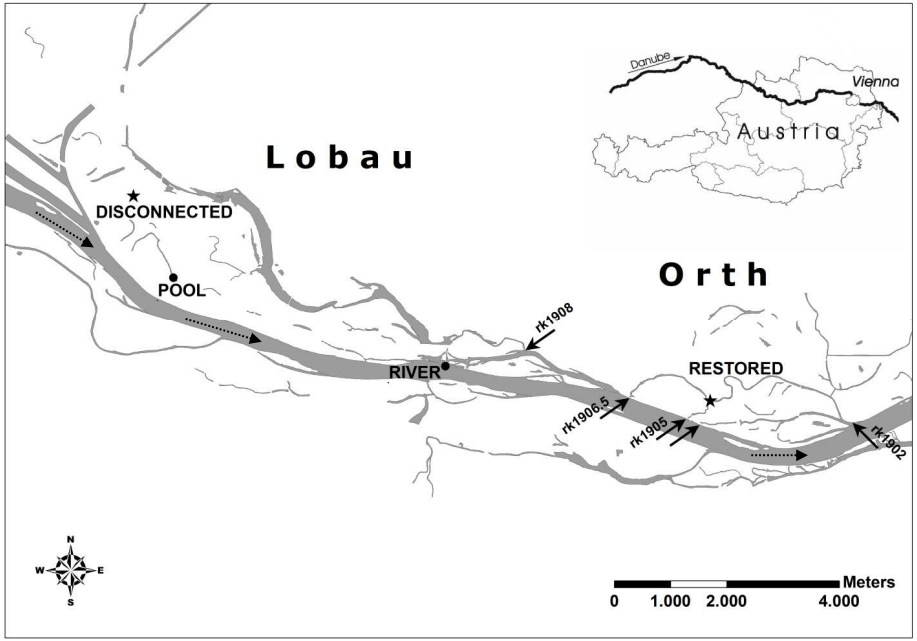

Fig. 1. Sampling sites are marked with stars; water exchange sites for Experiment 2 are marked with circles. Dashed arrows show the flow direction; solid arrows mark the openings to the Danube River.

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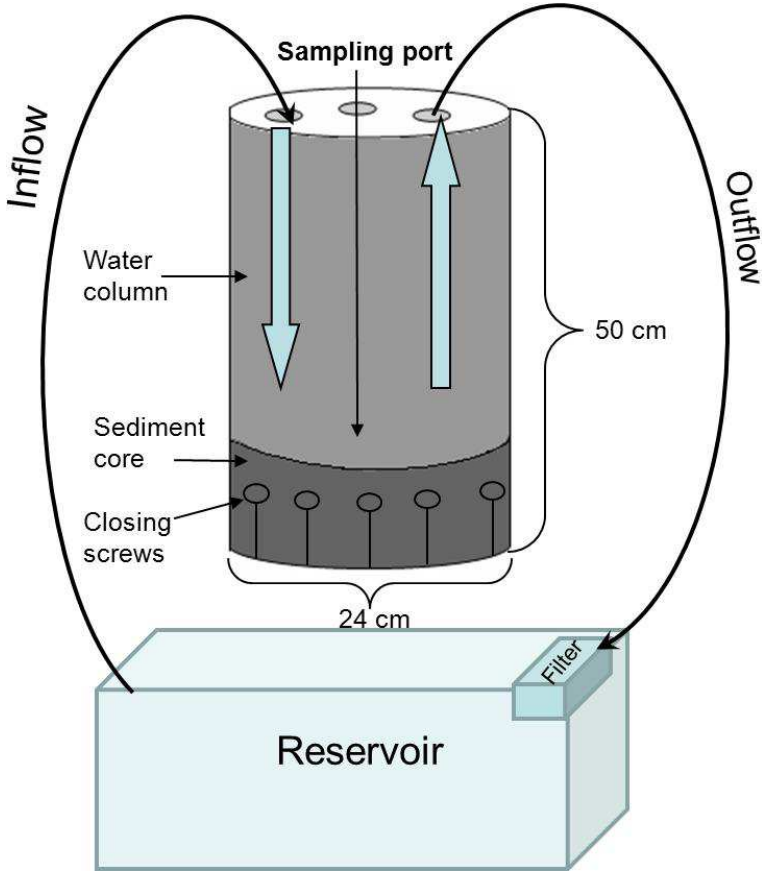

Fig. 2. Schematic of one mesocosm chamber setup. Each reservoir was connected to three mesocosm chambers.

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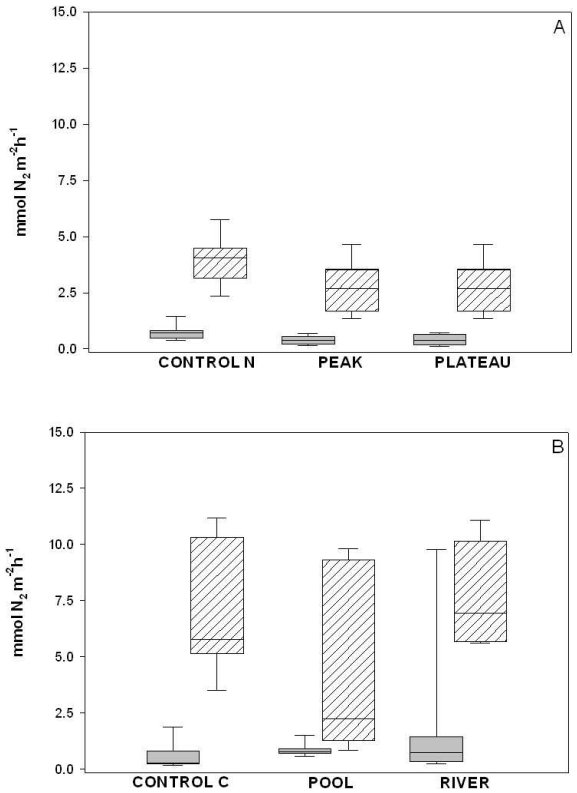

Fig. 3. Denitrification rates as measured over 5 days in **(A)** Experiment 1: NO_3 and **(B)** Experiment 2: DOM changes. Grey boxes are measurements from the disconnected site, hatched boxes from the restored site. Whiskers extend to the 95th and 5th percentiles. $N = 33$.

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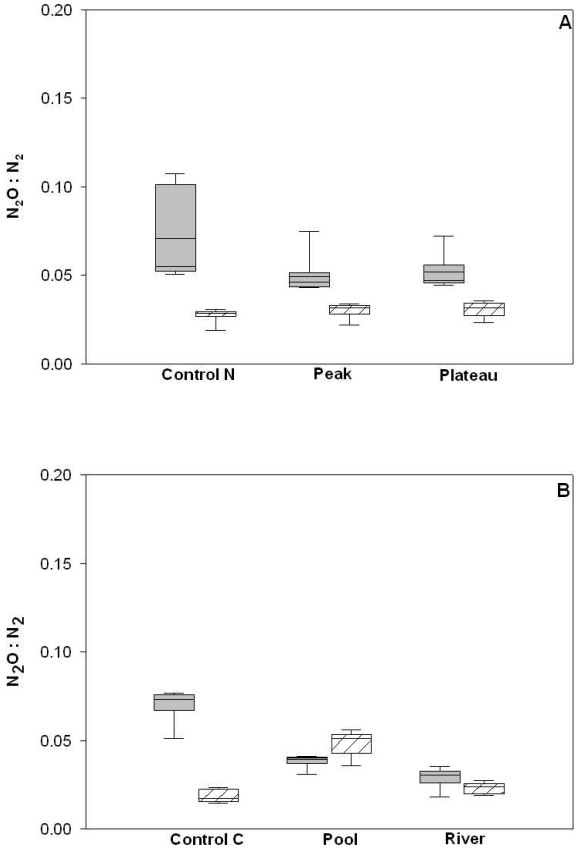

Fig. 4. Calculated $N_2O : N_2$ ratios over the 5 day incubation from **(A)** Experiment 1: NO_3 and **(B)** Experiment 2: DOM changes. Grey boxes are measurements from the disconnected site, hatched boxes from the restored site. Whiskers extend to the 95th and 5th percentiles. $N = 33$.

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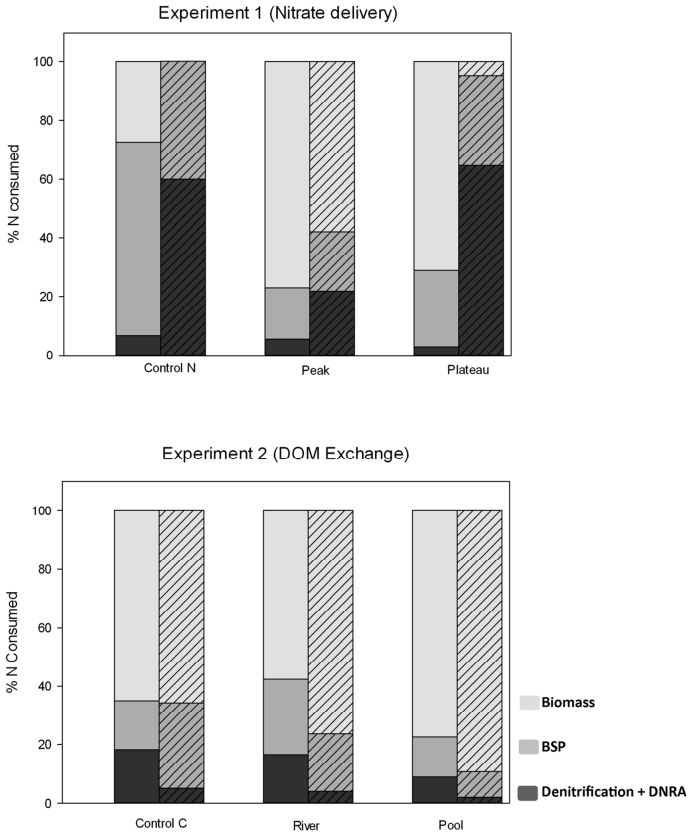

Fig. 5. Estimated percentage of total N pool used by denitrification and DNRA (dark gray), bacterial secondary production (middle gray) and biomass assimilation (light gray) using the 5 days averages. Solid bars are estimates from the disconnected site, hashed bars from the restored site. $N = 3$.

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