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Spatial Variability of Denitrification Rates in the Riparian Zone of the Selke River

Masterarbeit unter der Leitung von PD Dr. Christine Stumpp

Freiburg i. Br., April 1, 2016

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Referent: PD Dr. Christine Stumpp
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Abstract

During the past decades, an increased application of nitrogen-rich fertilizer led to alarming nitrate related problems. In fact, excess nitrate has substantial negative impacts on groundwater quality and has been related to cause eutrophication and human diseases. Nitrate can be removed from the ecosystems by denitrification where nitrate (NO_3^-) is reduced to dinitrogen (N_2) which is subsequently released to the atmosphere. Riparian buffer zones are considered as "hot spots" of denitrification in ecosystems. However, their specific nitrate removal capacity often remains unknown as denitrification rates are highly heterogeneous in time and space.

This study investigates factors limiting denitrification and determines the spatial variability of denitrification rates in riparian zone sediments. To examine the effect of DOC and nitrate concentrations, temperature and soil depth on denitrification rates batch experiments were conducted. Sediments from the saturated zone and the mixing zone of a riparian corridor at the Selke river were amended with different quantities of DOC and labeled nitrate ($^{15}NO_3^-$). Over the course of a week, the increase of labeled ^{15}N was measured in the headspace, while the decrease of NO_3^- was simultaneously measured in the liquid phase.

The results of this study reveal significant impacts of both, temperature and soil depth on denitrification rates. With 0.015 µmol N g^{-1} soil d^{-1}, denitrification rates from samples stored at average groundwater temperature (12°C) were significantly smaller (p-value < 0.01) compared to rates of samples stored at 20°C with 0.019 µmol N g^{-1} soil d^{-1}. Furthermore, rates from the saturated zone were significantly smaller (p-value < 0.01) than rates from the mixing zone with 0.009 µmol N g^{-1} soil d^{-1} and 0.017 µmol N g^{-1} soil d^{-1}, respectively. No significant influence of nitrate and DOC content on denitrification rates was found. Most importantly, findings from this study were comparable with literature values and especially with results from prior studies at the field site which revealed denitrification rates of 0.013 µmol N g^{-1} soil d^{-1}.

The study demonstrated the applicability of batch experiments to determine factors limiting denitrification. Further research is demanded to validate these findings and provide more data for coupled hydrological denitrification models.

KEYWORDS: Riparian Zone, Denitrification, Batch-Experiments, Hot-Spots, $^{15}NO_3$

Abstract

Durch den Einsatz von stickstoffhaltigen Düngern zur Nahrungsmittelproduktion kam es in den vergangenen Jahrzehnten zu erhöhten Nitratbelastungen in Böden und Gewässern. Gelangt überschüssiges Nitrat in das Grundwasser, so kann es die Eutrophierung von Gewässern begünstigen und gesundheitliche Folgen für den Menschen hervorrufen. Eine Möglichkeit Nitrat aus dem Ökosystem zu entfernen ist die Denitrifizierung, bei der Nitrat (NO_3^-) zu Stickstoff (N_2) reduziert wird. Dieser wird als Hauptbestandteil der Atmosphäre zurückgeführt und damit endgültig aus dem Ökosystem entfernt. Eine besondere Rolle bei der Denitrifizierung spielen Gewässerrandstreifen oder Flussauen. Durch hohe Nitratumsatzraten werden sie in der Literatur als "Hot Spots" bezeichnet. Jedoch gibt es große Unterschiede zwischen diesen Gebieten und ihre Denitrifizierungs Raten sind oftmals unbekannt.

Im Verlauf dieser Studie wurden verschiedene Faktoren und ihr Einfluss auf Denitrifizierungsraten untersucht. Hierfür wurden die Veränderungen der Raten durch den Einfluss von verschiedenen DOC und Nitratkonzentrationen, sowie Abhängigkeiten von Temperatur und Bodenschicht in Batchexperimenten verglichen. Durch die Zugabe von markiertem Nitrat ($^{15}NO_3^-$) konnte der durch Denitrifizierung bedingte Anstieg von markiertem Stickstoff in der Atmosphäre der Batches über die Zeit gemessen werden. Gleichzeitig wurde die Abnahme von gelöstem Nitrat in der flüssigen Phase gemessen.

Die Ergebnisse der Studie zeigen einen signifikanten Einfluss (p-Wert < 0.01) von Temperatur und Bodenschicht auf Denitrifizierungsraten. Durch die Erhöhung der Temperatur von 12°C auf 20°C , stiegen die Raten von 0.015 µmol N g^{-1} soil d^{-1} auf 0.019 µmol N g^{-1} soil d^{-1}. Zudem konnten signifikant höhere Raten im Kapillarsaum gemessen werden, als in der gesättigten Zone. Hier wurden Werte von 0.017 µmol N g^{-1} soil d^{-1} bzw. 0.009 µmol N g^{-1} soil d^{-1} gemessen. Die gemessenen Raten sind vergleichbar mit Werten aus anderen Studien (0.013 µmol N g^{-1} soil d^{-1}).

Somit zeigt diese Studie, dass Batchversuche ein geeigneter Ansatz zur Überprüfung von limitierenden Faktoren der Denitrifizierung sind. Zukünftige Forschung sollte die hier erhobenen Daten durch weitere Versuche validieren. Durch zusätzliche Datenerhebungen kann die Grundlage für die Modellierung von Denitrifizierungsraten geschaffen und somit zukünftige Modelle verbessert werden.

KEYWORDS: Riparian Zone, Denitrification, Batch-Experiments, Hot-Spots, $^{15}NO_3$

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List of Abbreviations

ANOVA	**AN**alysis **O**f **VA**riance
AUC	**A**rea **U**nder the **C**urve
BNF	**B**iological **N**itrogen **F**ixation
DNRA	**D**issimilatory **N**itrate **R**eduction to **A**mmonium
DOC	**D**issolved **O**rganic **C**arbon
GCMS	**G**as **C**hromatography **M**ass **S**pectrometry
IC	**I**on **C**hromatography
ID	**I**nside **D**iameter
MQ	**M**illi- **Q** water
NDIR	**N**on **D**ispersive **I**nfra**R**ed gas analyzer
NPOC	**N**on **P**urgeable **O**rganic **C**arbon
PCB	**P**rinted **C**ircuit **B**oard
PVC	**P**oly **V**enyl **C**hloride
STD	**ST**andar**D** gas
TOC	**T**otal **O**rganic **C**arbon
WHO	**W**orld **H**ealth **O**rganization

Chapter 1

Introduction

1.1 Overview

Public water supplier rely on high quality surface- and groundwater to deliver clean drinking water to the customer. If the quality of drinking water is inadequate by nature or cannot be assured, expensive treatments have to meliorate the water quality with incremental costs for the customer. Avoiding water treatment and high costs, the protection of water bodies is of particular importance (Bannick et al., 2008; Rivett et al., 2008).

Recently, high concentrations of nitrate stress surface- and groundwater quality in many European countries. Due to intensification of agriculture and the involving application of fertilizers to the fields, an increase of agricultural nitrogen compounds can be observed in many aquifers (Groenigen et al., 2015).

Consequences of increased nitrate concentrations were shown by Sutton et al. (2011). If nitrate concentration in fresh water exceed the threshold of 6.7 mg/L it might start to affect the biodiversity in fresh waters and pose threats to the consumers (Sutton et al., 2011). High nitrate concentrations in water might cause algal blooms which will subsequently deplete the oxygen levels, thus indirectly killing fish. Moreover, if humans consume drinking water with increased nitrate concentrations, the risk of bowel cancer and methaemoglobinaemia increases (WHO, 2011; Sutton et al., 2011; Galloway et al., 2008). Thus, international policies aim to minimize the effects of nitrate on ecosystems and foremost the effects on human health. For this reason, the world health organization (WHO) set up guideline values for maximum nitrate concentrations in drinking-water of 50 mg/L.

One natural removal process of nitrate from ecosystems is denitrification during which nitrate is reduced by bacteria to dinitrogen. Dinitrogen is subsequently released to the atmosphere and permanently removed from the ecosystem. The reduction of nitrate, however, requires anoxic conditions and organic carbon as electron donor (Groffman et al., 1999). These conditions can typically be found in transition zones between

surface and groundwater, and especially in riparian zones. Here, the interplay of fluctuating groundwater tables and high DOC contents can facilitate high denitrification rates (Woodward et al., 2009; McClain et al., 2003). Therefore, riparian zones are regarded as an important aspect in the nitrogen cycle (Mayer et al., 2005). However, denitrification rates are highly variable in time and space and problematic to quantify under field conditions (Groffman et al., 2006). Thus, batch experiments were conducted in this study to determine denitrification rates under controlled conditions.

1.2 State of the Art

1.2.1 Nitrate in Groundwater

Rivett et al. (2008) documented that many European countries are currently facing serious issues with surface- and groundwater quality. Especially, contamination with nitrate causes many complications. Although there has been a minimal decrease in overall nitrate concentrations in groundwater over the last decade, 14.4 % of all European groundwater monitoring sites exceed the WHO guideline value of 50 mg/L of nitrate. Another 5.9 % range between 40 and 50 mg/L (EU-Commission, 2013; WHO, 2011).

In Germany, groundwater contributes with about 74 % to the public water supply (Bannick et al., 2008). Likewise to the EU, 14 % of the groundwater monitoring sites exceeded the guideline value in 2005. Another 34 % ranged between 10 and 50 mg/L and in 52 % measured values were below 10 mg/L. With more and more aquifers failing to meet the drinking water standards, the stress on aquifers with low-nitrate water for blending will eventually increase (Stoewer et al., 2015; Rivett et al., 2008).

Bannick et al. (2008) indicated, that elevated nitrate concentrations closely correlate with growing agricultural production and the vicinity of agricultural areas and drinking water sources. A European study by Sutton et al. (2011) illustrates this situation. About 50 % of 11 million tonnes of nitrogen fertilizer added to European fields is lost to the environment. This underlines the low nitrogen-use efficiency in agricultural processes and explains why agriculture is regarded as one main source for nitrate inputs (Erisman et al., 2008).

Different approaches can eventually improve nitrate management and help the preservation of ecosystems while using the benefits of nitrate for food production. Therefore, Stoewer et al. (2015) state the necessity of tracing and understanding the sources of nitrate into the ecosystem. With this knowledge, a decrease of nitrate inputs into the ecosystem and the improve of fertilizer efficiency can be achieved (Erisman et al., 2008).

1.2.2 Riparian Zones

Riparian zones have attracted widespread attraction as they facilitate a multitude of nitrogen turnover processes. (Saunders and Kalff, 2001; Ranalli and Macalady, 2010; Cey et al., 1999; Maître et al., 2003; Vidon and Hill, 2004; Hill et al., 2000; Gold et al., 1998).

Generally, riparian zones are defined by a non-cultivated, permanent vegetation cover with a high root density which subsequently results in high contents of organic matter (Maître et al., 2003; Woodward et al., 2009). Furthermore, their vicinity to streams often results in shallow groundwater tables and low flow gradients under riparian zones (Rassam et al., 2006; Kellogg et al., 2005). The interaction between high organic carbon contents, induced anoxic conditions and low groundwater flow rates consequently favors high denitrification rates.

This position was further characterized by McClain et al. (2003). They described riparian zones as an ecotone, an area where two ecosystems meet. Here the confluence of waters with different chemical properties leads to the occurrence of "hot spots" and "hot moments". "Hot spots" are defined as isolated zones with increased biogeochemical activity. Compared to the surrounding area, they show disproportional high reaction rates. "Hot moments" are defined as periods with comparable high reaction rates.

In this study, riparian zones are "hot spots" as interface between terrestrial and aquatic compartments. At this interface, waters with different chemical properties mix and facilitate the confluence of reactants which are required for denitrification. This spatial entity is illustrated in figure 1.1. The situation in the riparian zone at the field site is best exemplified by scenario (b). Excess nitrate from agriculture (Reactant B) leaches into the riparian zone. Here, high DOC concentrations and anoxic conditions (Reactant A) allow denitrifying bacteria to process nitrate to N_2 (Product C). "Hot moments" at the field site can be exemplified as pulses of nitrate from fertilizer applications or as fluctuating groundwater tables. They can subsequently induce "hot spots" (McClain et al., 2003).

Riparian zones function of regulating the transport of nitrate into the groundwater is of certain significance (Lowrance et al., 1997). In addition to high nitrate removal rates by denitrification, assimilation of nitrate by plants and microbes contributes to this regulatory function. By minimizing nitrate leaching into deeper aquifers or surface waters, riparian zones eventually serve as a protective buffer zone (Lowrance et al., 1997; Woodward et al., 2009; Mayer et al., 2005).

Furthermore, this natural protection by riparian zones reduces costs for drinking water treatment as contaminants can be retained or removed before entering the drinking

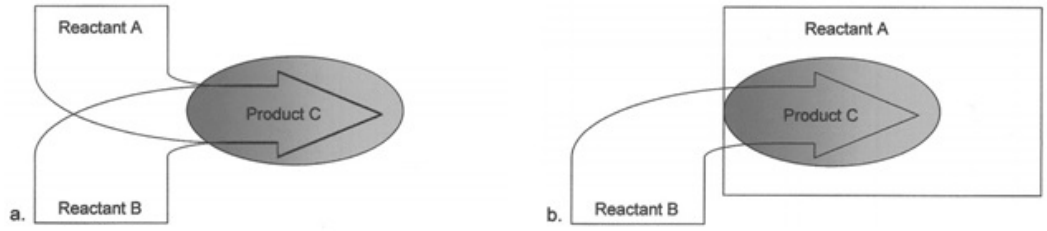

FIGURE 1.1: Schematic illustration of "hot spot" creation (a) confluence of two hydrological flowpaths with different chemical properties (b) infiltration of hydrological flowpath into a zones with different chemical properties (McClain et al., 2003).

water (Rivett et al., 2008). Therefore, riparian buffer zones are included in the German water law. It sets a minimum width of five meter for riparian zones with the purpose of improving ecosystem services, increasing water storage and water retention and reduce leaching of diffuse contaminants into river systems (BfJ, 2009).

Though mentioned natural protection functions of riparian zones, Saunders and Kalff (2001) state the concern about declining retention and removal of contaminants by riparian zones. They criticize that further river regulations will decrease the exchange of stream water with other ecosystems like riparian zones. Thus, preventing major denitrification sites from effectively removing or retaining contaminants. Furthermore, Mayer et al. (2007) report that under adverse conditions, riparian zones might even function as a source of nitrate caused by nitrification.

1.2.3 Methods for Measuring Denitrification Rates

Many different approaches have been carried out to quantify denitrification rates under varying conditions. Usually, denitrification rates are quantified by the emissions of the gaseous end products N_2 or N_2O. Some studies used ^{15}N as a tracer and analyzed its concentrations in the headspace. Due to the natural abundance of the nitrogen isotope ^{14}N of 99.64% compared to a contribution of only 0.36% ^{15}N (Steingruber et al., 2001; Holtappels et al., 2010), the atmospheric contamination with ^{15}N is relatively small. Based on this fact, Hauck and Melsted (1955) developed a method where labeled $^{15}NO_3^-$ was added to sediment samples and afterwards they measured the increase of ^{15}N in the headspace of a batch via GC-MS. Thus, they minimized the effect of ^{14}N contamination. Over the years, this method was used and developed in many studies (Hauck et al., 1958; Mariotti, 1983; Boast et al., 1988; Evsbech and Sørensen, 1990; Hart and Myrold, 1996). Protocols are provided by Dalsgaard et al. (2000) and Groffman et al. (1999).

Results from ^{15}N measurements can be interpreted and improved with different mathematical approaches. They aim to differentiate between the reactions responsible

for the ^{15}N concentrations in the headspace and can further increase the accuracy and precision of headspace measurements (Russow et al., 1996; Spott and Stange, 2007). Spott and Stange (2007) for instance, developed a method to distinguish between ^{15}N from denitrification or from anammox under consideration of atmospheric contamination. However, all ^{15}N methods require considerable amendments of labeled substances like nitrate to increase the detection limit of ^{15}N, which eventually alters the experimental conditions. Therefore, these methods are rather inappropriate for systems with low nitrate background concentrations (Groffman et al., 2006).

A different approach to solve the problem of measuring gaseous end products of denitrification is the acetylene method. The application of acetylene inhibits the formation of N_2 from N_2O by bacteria. As a consequence, N_2O prevails as intermediate denitrification product which can be directly measured due to negligible background concentrations in the atmosphere (Ryden, 1983; Dodds and Jones, 1987; Christensen et al., 1990; Schipper et al., 1993; Pfenning and McMahon, 1996). Since this method quantifies the amount of N_2O and assumes that it represents denitrification rates, an even distribution of acetylene is necessary to inhibit the activity of all bacteria. This, however, is not given in many studies as the diffusion of acetylene into "hot spots" can be restricted (Evsbech and Sørensen, 1990). This consequently leads to the underestimation of denitrification which can be 5 to 10 times smaller compared to the ^{15}N method (Evsbech and Sørensen, 1990; Lindau et al., 2011; Butterbach-Bahl and Willibald, 2002). However, the acetylene method is comparably inexpensive and simple, therefore a large number of samples can be analyzed. All in all, the disadvantages of the acetylene method outbalance positive effects which is why it is not recommended to apply this method (Groffman et al., 2006).

Measuring the increase of N_2 from denitrification in the headspace without further amendments of ^{15}N or acetylene would require totally gas tight systems as high contamination with atmospheric air (78% N_2) would results in major uncertainties in the analysis. This idea was approached by Butterbach-Bahl and Willibald (2002) who quantified denitrification rates without major intrusions in a gas-flow soil core system. They installed intact soil cores into an incubation vessel, replaced the initial atmosphere with He and continuously measured emerging N_2 and N_2O in a flow-through system. This method avoids the limitations from the ^{15}N and the acetylene inhibition technique, however, it requires substantial effort to seal the whole system from atmospheric contamination. Furthermore, this method is not suitable for measuring a multitude of parameters or a large number of samples.

Groffman et al. (2006) stated the difficulty of determining denitrification rates in the field. Large uncertainties arise with regard to dynamic cycling processes of nitrate and DOC, complex microbial interactions in the soil (Groenigen et al., 2015) and fluctuation of hydrological conditions (McClain et al., 2003).

One field method was established by Addy et al. (2000) who developed an in-situ method with ^{15}N as a tracer. Their approach is described as a push-pull method during which they inject labeled nitrate and conservative tracers (bromide) into a well and, after a certain incubation period, they collected water samples at periodic intervals. They finally analyzed the samples for dissolved ^{15}N, and calculated the denitrification rates based on the tracer recovery of bromide. This method was adapted by Kellogg et al. (2005) who investigated vertical variability of denitrification rates in three depths. They reported that denitrification rates generally follow DOC concentrations in the soil and do not necessarily decrease with depth. In addition to that, the found higher rates within 10 m of the stream channel. A similar approach was conducted by Woodward et al. (2009), however, they added acetylene to 50 % of the wells instead of labeled nitrate and measured N_2O concentrations in the water samples.

However, most measurement from batch or tracer tests only provide information about punctual denitrification rates but due to the occurrence of "hot spots" and "hot moments" upscaling of denitrification rates is still challenging (Groffman et al., 2009). Pinay et al. (2015) proposed upscaling of denitrification rates based on catchment topography and landscape pattern. These factors stand as a proxy for exposure time and different denitrification capacities of particular landscape units. Though, this approach is currently restricted to small scale catchments. Schuetz et al. (2015) and Seitzinger et al. (2002) modeled potential denitrification rates or nitrate removal capacity of small catchments based on in stream nitrate concentrations. In particular, they investigated nitrate concentrations along the stream and integrated the data into a in-stream mixing and removal model. They stated, that especially small headwater catchments can remove disproportionately large amounts of nitrate. Denitrification rates on a global scale are presented by Seitzinger et al. (2006). They report that large scale nitrogen models are most commonly based on nitrogen budgets and nitrogen mass balances.

Chapter 2

Research Gap and Objectives

Riparian zones are regarded as important reactive zone in the nitrogen cycle, mainly because of their denitrification potential (Mayer et al., 2005). However, the importance and dynamics of denitrification rates in ecosystems still remains uncertain (Martin et al., 1999). In fact, the response of bacterial communities to a multitude of environmental factors (Groenigen et al., 2015), unknown fluxes of compounds and the spatial and temporal variability (McClain et al., 2003) are not yet understood and especially challenging to determine at the field site. In addition to that, quantifying denitrification rates, especially the product N_2, is a major challenge (Groffman et al., 2006). Developing denitrification models that incorporate heterogeneities of denitrification rates in time and space to predict and manage future nitrogen scenarios are of certain importance. However, to create robust and comprehensive denitrification models more high resolution denitrification measurements are required (Groffman et al., 2009).

My hypothesis is that highest denitrification rates occur in transition zones between aquatic and terrestrial ecosystems, i.e hyporheic- and riparian zones or the mixing zone between the saturated and the unsaturated zone. These areas are especially suitable for the denitrification process as they facilitate the confluence of waters with different chemical properties. Riparian zones for example, provide an DOC enriched rhizosphere and most commonly saturated conditions with low flow rates induced by fluctuating water levels. Leaching of nitrate into this zone would consequently lead to denitrification. In addition to that, I assume that conditions in the mixing zone are even more appropriate for denitrification in comparison to the unsaturated and saturated zone. The unsaturated zone features a DOC enriched rhizosphere but lacks anaerobic conditions which are required for denitrification. In contrast, the saturated zones DOC contents might not be sufficient to facilitate high denitrification rates. Fluctuating water tables in the mixing zone favour the creation of denitrification "hot spots" with anaerobic conditions in soil aggregates and furthermore, the mixing zone receives high DOC contents from the rhizosphere. Therefore, I assume that highest denitrification rates will eventually emerge

from the contact zone between unsaturated and saturated conditions, the mixing zone.

The main objective of this study was to estimate potential denitrification rates in a riparian zone. Hence the multitude of unknown parameters at the field site, a method was developed to measure denitrification rates under controlled conditions in batch experiments. Further, the setup allowed variations of factors limiting denitrification, i.e. low nitrate and DOC concentrations and low temperature.

Based on prior studies, a spatial variability of denitrification potential with soil depth was expected. This was investigated with different sediments from the saturated zone and sediments from a mixing zone with high interactions between surface- and groundwater. The method was eventually evaluated with other studies and comparable literature values.

The results of this study finally contribute to a better understanding of denitrification rates and its variability in riparian zones. Denitrification rates obtained in this study can be incorporated in catchment models and help to improve the description of catchment processes. Key objectives of this study are:

1. Measure denitrification rates with lab incubation experiments
2. Compare the results to literature values
3. Evaluate the limitations of the used method in comparison to other available methods

Chapter 3

Nitrogen Cycle

Nitrogen is a component of every protein in living organisms, but the majority of nitrogen in the global N-cycle is present as inactive dinitrogen (N_2, 78 %) in the atmosphere (Stumm and Morgan, 1996; Galloway et al., 2008). A schematic approach to the N-cycle is presented in figure 3.1 and depicts possible physical and chemical processes of nitrogen related compounds.

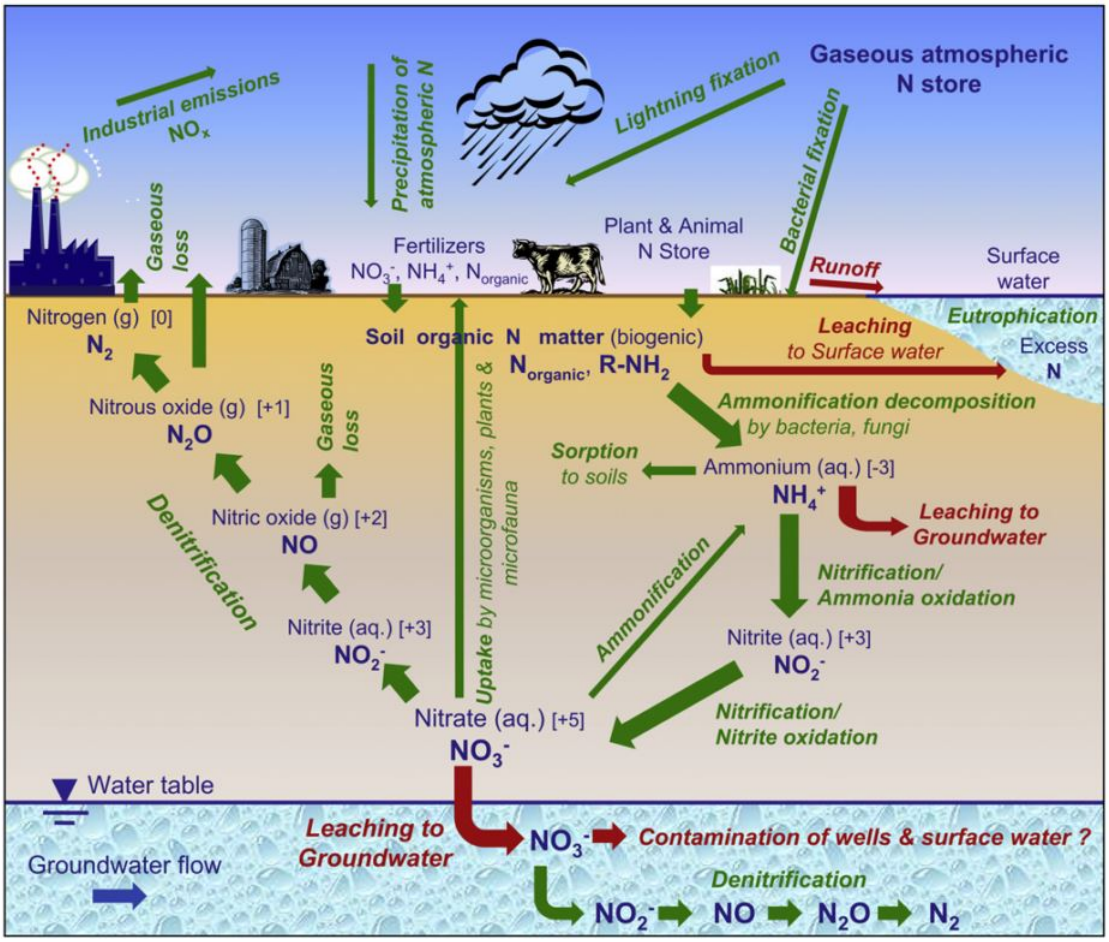

FIGURE 3.1: The nitrogen cycle with focus on environmental impacts (Rivett et al., 2008).

Due to its particularly stable triple bond, the assimilation of dinitrogen is not feasible for most plants and bacteria (Mortimer, 2001). However, some species managed to fix atmospheric N_2 and process inactive nitrogen into active nitrogen. These processes of biological N fixation (BFN) can be facilitated by free living bacteria and lichens or a symbiotic association of plant roots and bacteria (Groenigen et al., 2015). Reed et al. (2011) document, that BFN might even be the dominant N source in many terrestrial ecosystems. Other pathways for nitrogen to enter the terrestrial system are atmospheric deposition and to a small extend lightning fixation (Rivett et al., 2008).

Once nitrogen enters the terrestrial ecosystems in a reactive form, there is a multitude of pathways combined with chemical and biological transformation processes. Nitrogen sources are organic (manure) and inorganic fertilizer, biological and lightning N-fixation or atmospheric deposition. Inorganic nitrogen mostly occurs as nitrate (NO_3^-) or as ammonium (NH_4^+) which is the prioritized product from decomposition of biomass (organic carbon). This process is called mineralization. Under oxic and temperate conditions, ammonium is subsequently nitrified by bacteria to nitrate which is called nitrification. During this process aerobic microbes such as *Nitrosomas* and *Nitrobacter* metabolize ammonium (NH_4^+) to nitrate (see equations 3.1 and 3.2). Thereby, providing fresh supply for denitrification. The exothermic reaction can be divided into two steps (Schachtschabel et al., 1998).

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 2H_2O + 4H^+ \tag{3.1}$$

$$2NO_2^- + O_2 \rightarrow 2NO_3^- \tag{3.2}$$

Nitrogen sinks are the assimilation of nitrogen by bacteria and plants (Woodward et al., 2009), leaching and erosion (Schachtschabel et al., 1998) and gaseous losses of nitrous oxide (N_2O), nitrogen oxide (NO_x), ammonia (NH_3) and dinitrogen (N_2).

During the last years the understanding of processes contributing to gaseous losses of nitrogen like anammox (Holtappels et al., 2010), Feammox (Groenigen et al., 2015), nitrifier denitrification (Wrage et al., 2001) and co-denitrification (Spott and Stange, 2011) emerged. However, denitrification is still considered the most important nitrate sink (Galloway et al., 2008). Denitrification is the successive reduction of nitrate to elementary nitrogen by bacteria under the consumption of DOC (see equation 3.3).

$$4NO_3^- + 5CH_2O \rightarrow 2N_2 + 5HCO_3^- + H^+ + 2H_2O \tag{3.3}$$

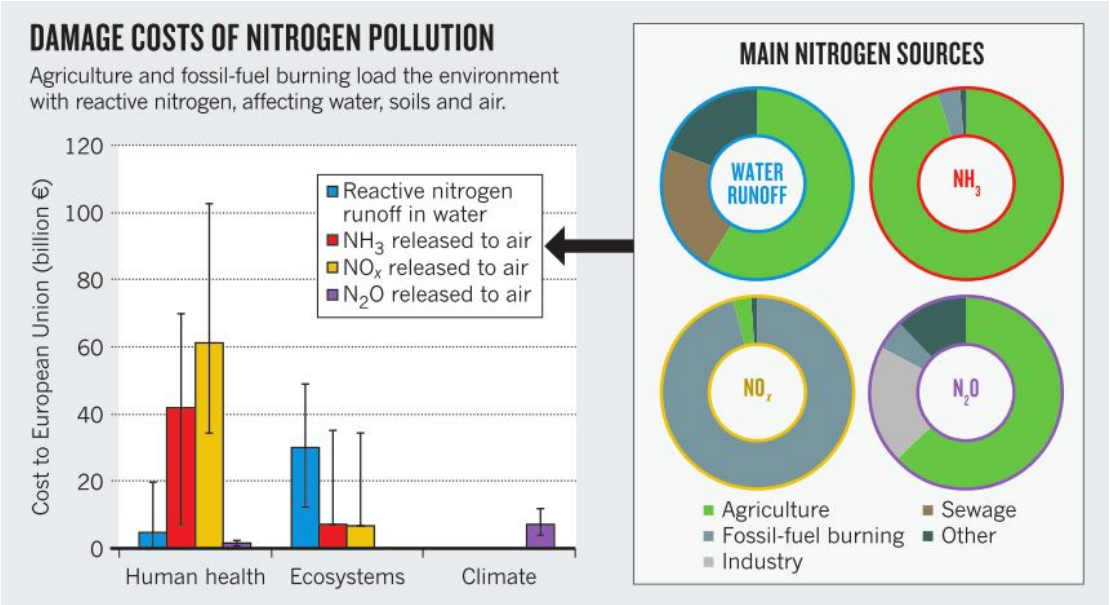

FIGURE 3.2: Damage costs of nitrogen pollution (Sutton et al., 2011).

This process is subdivided into multiple steps which are shown in equation 3.4. Interrupted denitrification can also lead to the emission of any intermediate product presented in the following formula (Martin et al., 1999).

$$NO_3^- \rightarrow NO_2^{2-} \rightarrow NO \rightarrow N_2O \rightarrow N_2 \tag{3.4}$$

Given the presence of nitrate, electron donors (DOC) and anaerobic conditions, denitrification is most commonly conducted by the heterotrophic bacterium *Pseudomonas denitrificans*, or the chemolithoautotrophic *Thiobacillus denitrificans*, (Rivett et al., 2008; Clark and Fritz, 1997; Kendall and Mcdonnell, 1998). Hence the need for an electron donor like dissolved organic carbon (DOC), highest denitrification rates have been measured in top soils or transition zones. Likewise dependent on anaerobic conditions, denitrification mostly occurs when soil saturation is above 70-80 % (Schachtschabel et al., 1998).

However, the N-cycle is not in equilibrium and the formation of reactive nitrogen and thus the input of nitrogen into the ecosystems accelerates (Galloway et al., 2008). Historically, nitrogen was a limiting factor in many ecosystems. This changed in 1910 with the synthetic production of nitrogen fertilizer by the Haber-Bosch synthesis. Due to additional reactive nitrogen, food production and food security increased over the last 100 years (Galloway et al., 2004). While in 1908 one hectare of arable land was suitable to provide food for 1.9 persons, this area feeds 4.3 persons in 2008. (Erisman et al., 2008).

The disadvantages of releasing additional nitrogen into the ecosystems are environmental damages like water- and air-quality degradation and a loss in biodiversity due to eutrophication caused by excess nitrate (Rivett et al., 2008; Kulkarni et al., 2014; James et al., 2005; Kendall and Mcdonnell, 1998; Volterra et al., 2002; Galloway et al., 2008; Erisman et al., 2008). Furthermore, ammonia (NH_3) and nitrogen oxides (NO_x) can increase ground-level ozone, and lead to respiratory and cardiovascular diseases. Nitrous oxide (N_2O) on the other hand is considered a greenhouse gas and contributes to climate change (Sutton et al., 2011; Erisman et al., 2008).

With regard to the EU, the financial consequences of excess reactive nitrate, such as nature restoration and health care, add up to 70-320 billion € per year (see figure 3.2), which is about 200% of the income generated by fertilizer (Sutton et al., 2011). Moreover, Galloway et al. (2008) established, that the N-cycle will further alter as the imbalance between regions producing N-containing products and regions consuming these products will grow. Producing regions will eventually pay the costs for environmental damage caused by reactive nitrogen.

Chapter 4

Field Site

4.1 Site Description

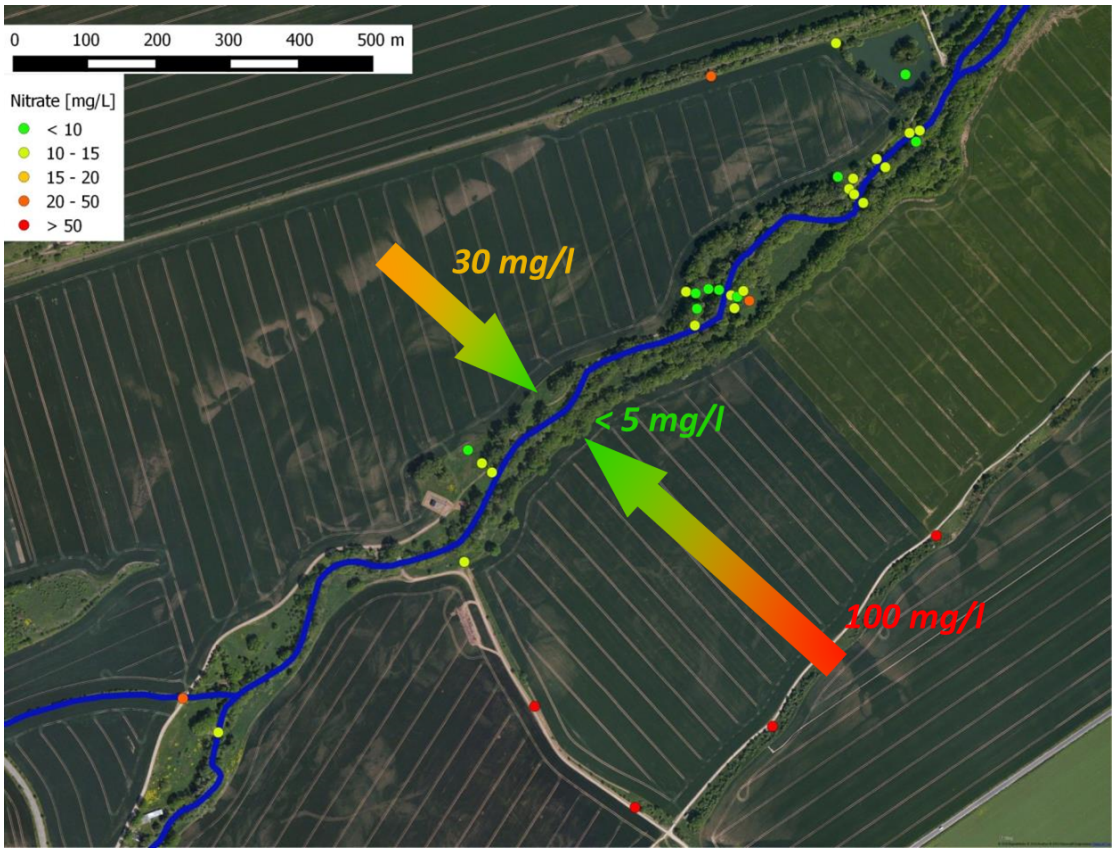

FIGURE 4.1: Overview of the field site at the Selke River (Trauth et al., 2016)

The field site at the Selke river is part of the TERENO observatories for studying hydrological processes. The Selke catchment covers 483 km^2 and drains into the Bode river. 35 % of the Selke catchment can be described as forested area which is the prevalent landuse in upper regions. Another 53 % are dedicated to agricultural use (LHW, 2008). A riparian forest borders the Selke river on both sides. The study site stretches over

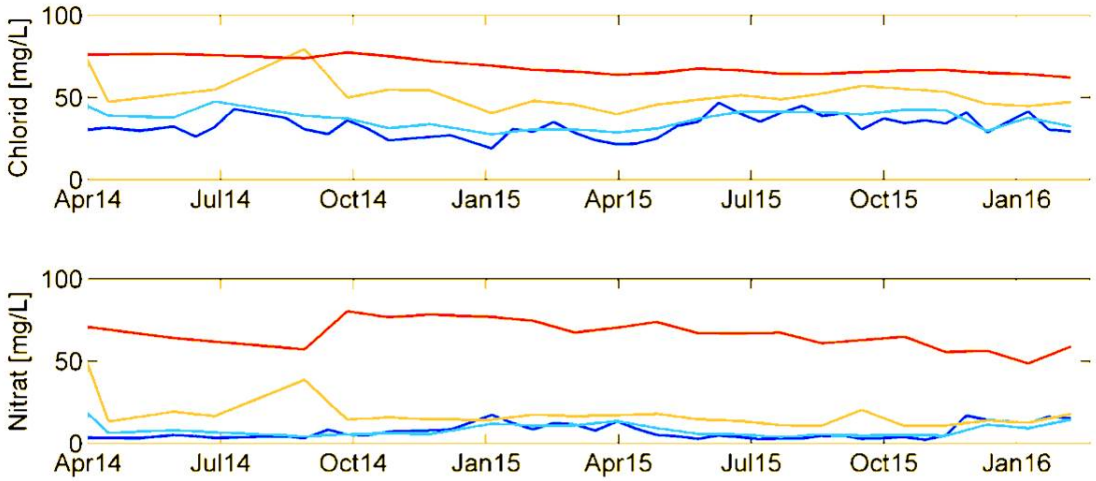

FIGURE 4.2: Nitrate and chloride concentrations from the field site. In red: groundwater > 100 m distance to stream, in yellow: groundwater 25 < 100 m distance to stream, in light blue: groundwater < 25 m distance to stream and in blue concentrations from the stream Trauth et al. (2016).

1 km along the river and is equipped with several observation wells measuring important groundwater parameters like groundwater level, electrical conductivity, temperature and oxygen concentrations. Figure 4.1 shows the locations of observation wells at the field site and nitrate concentrations measured by Trauth et al. (2016). A horizontal gradient with decreasing nitrate concentration towards the stream can be observed.

Trauth et al. (2016) reported data from wells at the Selke site which is illustrated in figure 4.2. The data shows nitrate and chloride concentrations in dependence to stream distance. The first graph shows a time series of chloride concentrations in observation wells from April 2014 until January 2016. The second graph describes the evolution of nitrate. The different colors identify different distances to the stream. A horizontal gradient with decreased concentrations towards the stream can be observed for nitrate and chloride. The NO_3^-/Cl^- ratio, however, indicates non-conservative behavior. Therefore, the horizontal gradient of nitrate cannot be explained by dilution which finally indicates additional nitrate removal processes in the riparian zone. Consequently, the riparian zone can be considered a "hot spot" for nitrate removal.

Furthermore, three multi-level wells were installed in order to resolve vertical gradients in high resolution. In each multi-level well, 22 ports ranging from 40 cm to 375 cm facilitate water sampling from the unsaturated zone, the capillary fringe and the saturated zone (Gassen et al., 2016). Further information can be obtained from figure 4.3 which shows vertical depth profiles from a multi-level well (Gassen et al., 2016). The left graph shows that nitrate concentrations between 1 and 2 m are reduced compared to lower regions. This implies a second "hot spot" in the mixing zone between

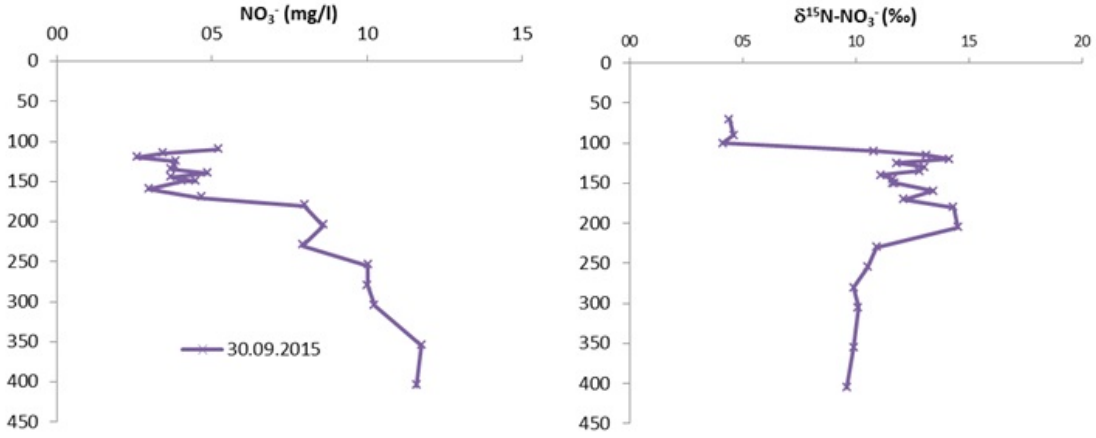

FIGURE 4.3: Depth profile of nitrate concentrations and nitrogen isotopes from the field site (Gassen et al., 2016).

groundwater from unsaturated zone and saturated zone. The second graph illustrates the gradient of ^{15}N isotopes in nitrate. As lighter isotopes are preferred during denitrification, the enrichment of heavy isotopes between 1 and 2 m indicates that decreased nitrate concentrations can be explained with denitrification (Kendall and Mcdonnell, 1998).

So far, investigation of in stream reactions and hyporheic zones have been conducted at the field site (Trauth et al., 2013; Trauth et al., 2014; Trauth et al., 2015). They indicated that the hyporheic zone is a significant sink for nitrate. Trauth et al. (2015) reported nitrate concentration in the groundwater of up to 100 mg/l, compared to 10 mg/L of nitrate in the stream (see figure 4.1). In addition to that, groundwater O_2 concentrations ranged from 0 to 5.5 ml/L thus favourable conditions for denitrification. The DOC content was lower than 2.0 mg/L. They modeled in-stream denitrification and nitrate removal. Their results indicated that denitrification accounts for in-stream nitrate removal of up to 8 % over a 1 km stream. Nixdorf and Trauth (2016) investigated groundwater travel times in the riparian zone under different hydraulic conditions. Further information about transit times will provide useful information for denitrification models because the longer the groundwater stays in contact with the riparian interface, the more nitrate can be processed.

4.2 Field Work

Two field campaigns were conducted during the course of this study. During the first campaign, samples were taken at the riparian corridor and from the nearby river bed (see figure 4.1). Samples from the riparian corridor were obtained with a *Sonic Drill*. Fresh samples from the streambed were taken with a spade from a bank of gravel inside the

FIGURE 4.4: Metal tube filled with sediments from the field site after 5 month exposure to groundwater. Considerable orange discoloration from 0-80 cm in contrast to the top 20 cm without discoloration.

FIGURE 4.5: Driving core with loose sediments from second field campaign. Sediments from the mixing zone on the left and from the saturated zone on the right.

Selke river. All sediments were sieved to fractions from 0.18 mm to 2.5 mm, representing medium sand to small gravel. The homogenized sediments were then embedded into 6 meshed metal tubes. In order to expose the sediments to its natural conditions, the tubes were installed into shielded 1 inch monitoring wells from the 15^{th} of July until the 7^{th} of December 2015. Hereby, it was intended to obtain the establishment of natural microbial communities on the sediments (Zhou et al., 2012).

To minimize the contact with the atmosphere and thus a contamination with oxygen during sampling in December, the cores were emptied into 1 L *Schott* bottles which were prior filled with groundwater. Here, it was assured that the bottles were filled from the bottom part under flooded conditions. Each tube was filled into a separate bottle, allowing measurements of pH and dissolved oxygen in the laboratory. In addition to that, extra groundwater samples were taken for the geochemical analysis. All samples were stored at 12°C which approximately represents the average groundwater temperature at the field site. However, a considerable orange color of the metal tubes was observed, caused by unexpected iron oxidation (see picture 4.4). This coloring was also visible in the sampling bottles.

Sediments from the second field campaign, which took place on the 14^{th} of January 2016, were taken from driving cores. A 1-inch core was pile driven into the soil filling the core with sediments. Unfortunately, the core got clogged and the bore hole partly collapsed into the core. This led to unsorted and loose sediments (see picture 4.5). Based on soil moisture content and approximate position, we divided the sediments

into a saturated fraction (> 200 cm) and a fraction from the mixing zone (100-200 cm). Afterwards, sediments were packed into two gas tight plastic bags and stored at 12°C.

Chapter 5

Methods

The following chapter contains information about how the data was gathered and processed. First, procedures in the field will be explained. Further, methods from the laboratory experiments will be illustrated as well as instruments and their respective mode of operation. Chemicals and materials used in this study can be found in table A.2 and table A.3, respectively.

5.1 Experimental Setup

Batch experiments were conducted as they allowed precise variations of parameters such as nitrate or DOC concentrations under controlled conditions. Particularly, the possibility of manipulating parameters in a closed system allows to represent a huge variety of scenarios which might not (yet) be represented by the field site. This is an advantage compared to field experiments, where concentrations can be measured over time but no precise rates can be measured as too many parameters remain unknown. To obtain representative denitrification rates for the Selke field site, all sediments were sampled on site and processed within 8 days, minimizing the influence on established microbial communities for the lab experiments.

5.1.1 Batch Preparation

This study comprises two different experiments from which the first test (test-1) was used to investigate limiting factors of denitrification and potential denitrification rates and the second test (test-2) was used to determine the spatial variability of actual denitrification rates. Despite different sediment sampling (see section 4.2) the procedure in the lab and the experimental setup were equal. The experimental setup is illustrated in figure 5.1. 20 g of sediment was filled into 100 ml headspace vials and a solution containing labeled nitrate (KNO_3) and DOC (acetate) in different concentrations was added. Groundwater and artificial groundwater were used as a medium for the solution.

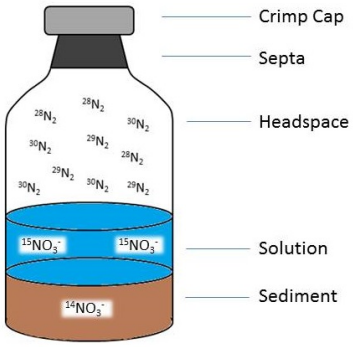

FIGURE 5.1: Sketch of the experimental setup. Batches filled with sediments containing residue unlabeled nitrate and a solution containing DOC and labeled nitrate. End products of denitrification measured in headspace as dinitrogen.

After the amendment with nitrate and DOC, batches were crimp sealed with gas tight rubber septa and subsequently flushed with a $Ar - CO_2$ mixture for 10 minutes to remove ambient air, containing O_2 and $^{28}N_2$. Here, one canula was inserted and adjusted at the very bottom edge of the tilted batch to achieve maximum flow cross-section. A second canula was injected to release the overpressure from the batches, thus allowing a constant flow rate (see picture 5.2). This canula was not in contact with the liquid phase. After flushing the batches for 10 minutes, the second needle was removed and 1.5 bar overpressure was subsequently established to compensate the constant withdrawal of solution and gas from the batches. Afterwards, batches were placed on a shaker for best mixing of the solutes.

5.1.2 Procedure during Headspace Measurements

Mixing of the headspace was achieved by rotating the batches in different directions, shaking was avoided. Samples were taken by injecting the needle with closed valve through the septa into the headspace, opening the valve and flushing the total volume of the syringe fivefold. Thereby, mixing the atmosphere inside the batches and removing any residues from the syringe. Hereafter, the required amount of gas was adjusted by a slow uptake of gas to the maximum, followed by a slow release of gas to the required amount. The valve was then closed and the needle was slowly drawn out from the septa, allowing the septa to consolidate before the needle was removed.

To release the overpressure from the syringe, which was obtained by the overpressure inside the batches, the valve was quickly opened and closed inside a water quench. As

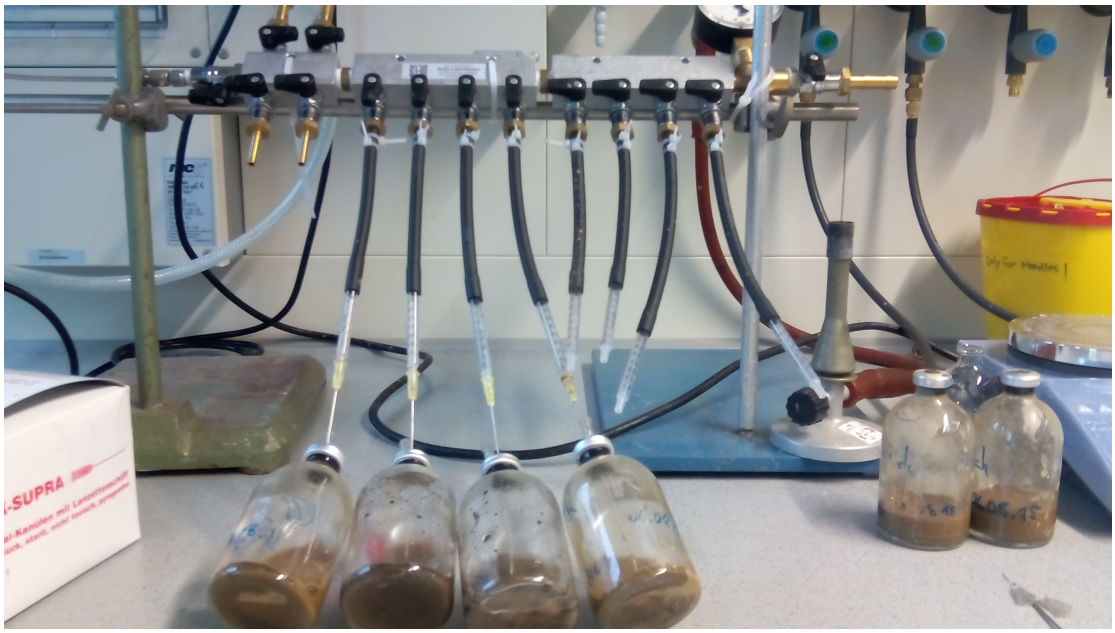

FIGURE 5.2: Instrumental design for simultaneous flushing. Device equipped with pressure valve allowing flushing of up to 8 batches under controlled gas flow rate.

the gas emission (water bubbles) was clearly visible, this method has the advantage of an indication of overpressure compared to a pressure release without a water quench. Additionally, a possible clogging of the needle can be identified. After releasing the pressure, the syringe was quickly inserted into the injection port. The valve was opened and the sample was injected by evenly and quickly depressing the plunger.

5.1.3 Procedure during Liquid Phase Measurements

For water sampling, a sterile canula was injected through the septa of the tilted batch. As the septa was now overlaid by water, air leaking was prevented. Three milliliter of well mixed sample were collected in a 3 ml syringe, thus successively reducing the liquid phase in each measurement. The canula was removed and replaced by a sterile 45 µl filter. The first milliliter was subsequently discarded to flush the residue from the filter. Thereafter, each milliliter was filled into IC-vials and prepared falcon tubes, respectively. IC-vials were directly installed into allocated trays and analyzed for cations and anions. As for the DOC samples, 1 ml of sample was mixed with 5 ml of MQ in specially designed glass tubes for DOC analysis.

The tightness of the batches was checked via syringe plunger. Due to the overpressure inside the batches the plungers were consequently pushed hereby filling the syringe. As long as there was overpressure inside the batches, they were considered as tight since

no ambient air could leak into the batches. Sampled water was not replaced by artificial groundwater to prevent contamination with oxygen. In the case of the abiotic control batches, additional contamination with bacteria was avoided.

5.2 Experiments

The first test was used to identify limiting factors for denitrification. Therefore, the influence of different quantities of DOC, nitrate and different temperature were compared. In test-2, the spatial variability of actual denitrification rates was investigated using sediments from different depths, which received DOC and nitrate amendments comparable to field conditions. Detailed information about pre-experiments and the preparation of stock solutions for labeled nitrate and DOC can be found in chapter A.1.

5.2.1 Potential Rates and Limitations

Preparation

In order to investigate limiting factors for denitrification rates, batches with varying amounts of nitrate, DOC and different temperatures were set up. Table 5.1 illustrates the 5 different treatments. By comparing treatment 1 and 2, the effect of DOC can be observed. Treatment 2 and 3 differ in terms of temperature. Finally, treatment 3 and 4 differ in nitrate concentration. Treatment 5 was autoclaved, serves as a control and thus provides information about abiotic dinitrogen production or contamination.

TABLE 5.1: Composition of batches from first experiments, each treatment consists of three batches which were measured in duplicates. Treatment 5 was autoclaved and serves as abiotic control.

Treatment	**15 KNO3**		**DOC**		**Solution**		**Sediment**	**Temp**
	µL	mg/L	µL	mg/L	ml	Type		°C
1	133,0	27.9	46,9	3,2	30	AGW	Mix	20
2	133,0	27.9	11,7	0,8	30	AGW	Mix	20
3	133,0	27.9	11,7	0,8	30	AGW	Mix	12
4	66,5	13.9	11,7	0,8	30	AGW	Mix	12
5	133,0	27.9	46,9	3,2	30	AGW	Mix	20

The pH of sediment suspension was measured and compared with initial groundwater composition to check for processes during the transport. The pH ranged around 7.5 both in samples and initial groundwater. All batches were filled with about 20 g of moist, homogenized soil. According to literature values 10 µg N g^{-1} soil was added which is from now on referred to as value for 100%. In order to imitate present groundwater values for the Selke, nitrate amendment for treatment 4 was set to 25%. The amount

of added DOC (100 %) was chosen as it, according to the redox formula (see equation 5.1), represents the amount of DOC to facilitate the total reduction of nitrate to N_2.

$$4NO_3^- + 5CH_2O \rightarrow 2N_2 + 5HCO_3^- + H^+ + 2H_2O \tag{5.1}$$

To investigate dependencies of denitrification rates and DOC content, treatment 2-4 received a reduced amount of DOC of 25%. Further, this value of 0.8 mg/L approximates DOC concentration in the field. Temperature was chosen as further parameter and varied from 12 to 20°C. Batches from treatment 5 were autoclaved according to the procedure presented by Pfenning and McMahon (1996) and served as abiotic control. They received 100% Nitrate and 100% DOC and were crimp sealed and autoclaved for 20 minutes at 120°C.

Headspace Measurements

The concentration of labeled nitrogen in the headspace was measured with the *Agilent GC-MS*. The first series comprises four measurements days from the 9^{th} of December until the 14^{th} of December (day 1, 2, 3 and day 5) with duplicate measurements of each batch. Batches stored at 12°C were collected briefly before the measurement to avoid unnecessary heating. Results were obtained from *Agilent MSD ChemStation* containing information about retention times, peak heights and peak areas (AUC) for each isotope. Data was exported and further processed in *OpenOffice 4.0*.

Liquid Phase Measurements

Measurements in the liquid phase were carried out between the 9^{th} of December until the 14^{th} of December (day 0, 2, 5, 7 and 9). In each case 3 ml of liquid sample was withdrawn and prepared for IC and DOC measurements.

Ferrozine Assay

The Ferrozine assay was conducted to analyze samples for possible formation of ferrous iron over the course of this series of measurements. Thus, samples from the day 0 were compared to such from the day 9. For preparation, 0.1 ml sample was dissolved in 0.9 ml HCl (1 mol) and stored in *Falcon tubes*. Standards for Fe^{2+} were prepared using different MQ water and FE-solution ratios. Standards and samples were shaken for 1 hour before further treatment. Triplicates of each 0.02 ml sample (or standard) were pipetted into microtiter plates and mixed with 0.18 ml FERR-solution. After an incubation time of 15 minutes in the dark (covered in tin foil), the absorbance was measured.

5.2.2 Actual Rates and Spatial Heterogeneity

Preparation

Based on the results of the first series of measurements, where different factors were analyzed for their impact on denitrification rates, a comparison of sediments from the saturated and the mixed zone was intended for the second series of measurements. The respective batch setup can be found in table 5.2.

TABLE 5.2: Composition of batches from second experiments, each treatment consists of three batches which were measured in duplicates. Treatment 6 was autoclaved and serves as abiotic control.

Treatment	**15 KNO3**		**DOC**		**Solution**		**Sediment**	**Temp**
	µL	mg/L	µL	mg/L	ml	Type		°C
1	133,0	27.9	2,1	1,4	30	AGW	Mix	12
2	133,0	27.9	-	-	30	GW	Mix	12
3	133,0	27.9	2,1	1,4	30	AGW	Sat	12
4	133,0	27.9	-	-	30	GW	Sat	12
5	133,0	27.9	8,3	5,7	30	GW	Sat	20
6	133,0	27.9	-	-	30	GW	Sat	20

Data from Gassen et al. (2016) indicated a zone with high DOC and depleted nitrate concentrations in the mixing zone between saturated and unsaturated zone. Groundwater from this division was collected and artificial groundwater was reproduced accordingly (see table A.1). Nitrate amendments from the previous test were adopted. Demoling et al. (2007) demonstrated that the availability of carbon and nitrate has substantial impact on bacterial growth. However, due to residue nitrate and high DOC concentrations in the groundwater it was assumed that the addition of nitrate and DOC into the AGW solution would not result in bacterial growth. As high DOC background concentrations were suspected in the groundwater, no acetate was added to the groundwater-nitrate solution. Finally, serum bottles were filled with 20 g homogenized sediments from either saturated or mixing zone. AGW or GW solution was added and batches were crimp sealed and flushed for 10 minutes. Abiotic controls were autoclaved prior to flushing. Between the measurements, all samples were placed on shakers at 12°C and 20°C, respectively .

Headspace Measurement

GC-MS measurements were performed between the 18^{th} and 25^{th} of January 2016 (day 0, 1, 2, 3, 4, 5, 7). Each batch was measured in duplicates with a headspace withdrawal of 20µL, respectively. These headspace samples were subsequently measured in the *Agilent GC-MS*.

Liquid Phase Measurements

Samples for IC and DOC analysis were taken at day 0, 2, 4 and day 7. DOC was immediately measured after day 7, IC samples were stored at 4°C for delayed measurements at the central analytic laboratory.

5.3 Sample Analysis

The following section comprehends information about the instruments used and theoretical background information on their respective mode of operation.

5.3.1 Headspace Measurements

Gas Chromatography Mass Spectrometry (GC-MS) was used to obtain data about isotopic gas concentrations in the batch headspaces.

Gas Chromatography Mass Spectrometry

The combined approach of Gas Chromatography (GC) and Mass Spectrometry (MS) in one measurement facilitates the quantification and qualification of different N_2, CO_2 and N_2O species. Figure 5.3 illustrates the instrument setup of the *Agilent GC-MS*. The GC measurement separates between molecules with different physical and chemical properties (for example N_2, CO_2 and N_2O) but does not differentiate between lighter or heavier isotopes ($^{28}N_2$, $^{29}N_2$). In contrast to the GC, a MS measurement separates the molecules based on their mass/charge equivalent (m/z). However, there are many different molecules that exhibit the same mass/charge ratio like N_2 and CO (m/z: 28), or N_2O and CO_2 (m/z: 44) that could not be divided by a MS. The combination of GC and MS however facilitates this comprehensive approach.

The GC basically consists of three parts: the injector, the column and the detector which are connected and seeped through by the carrier gas (high purified Argon or Helium). The samples get injected through a rubber septa into the heated injection port. Here the volatile sample mixes with the carrier gas and is transported into the column. The column used in this study was a 60 m *Rt-QPlot* column made of fused silica and coated from the inside with a stationary phase to separate the mixture. Based on boiling point and solubility of the compounds, they gradually transfer from the column into the detector. This information gets converted into a chromatogram which represents sample elution and response.

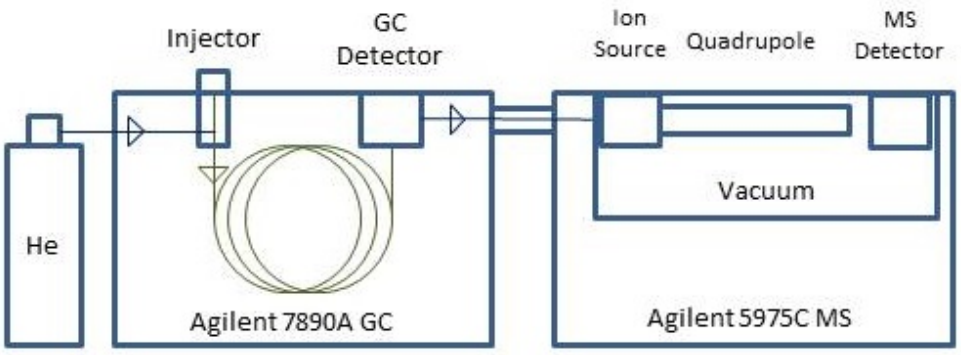

FIGURE 5.3: Schematic representation of combined Agilent 7890A GC and 5975C MS.

Hereinafter, the compounds enter the ion source of the MS via the transfer line from the GC. Here, an electrical current in the filament ionizes the molecules. These are subsequently focused into the electrical field of the quadrupole mass filter. The quadrupole filters non ionized ions which are not affected by the electrical field and thus in random motion. While ionized molecules follow the electrical field and enter the mass detector, non ionized molecules are removed from the vacuum by a vacuum pump. Analytes continue to the detector and are split up by their mass/charge characteristics. The results can be visualized in a mass spectra.

Figure 5.4 depicts a the final output from the GC-MS. Each graph describes the chromatogram of one specific mass/charge ratio. The first graph (Ion 28) for instance, shows the abundance of molecules with different retention times of molecules with m/z: 28. The AUC of the first peak represents the amount of $^{14}N^{14}N$ whereas the AUC of the second peak represents the amount of $^{12}C^{16}O$. The difference in isotopic composition of one species, i.e. N_2 can be interpreted by comparing different graphs, which result from MS measurements. For instance, the ratio of different N_2 species can be obtained by comparing the AUC of the first peak from graphs with m/z: 28 ($^{14}N^{14}N$), m/z: 29 ($^{14}N^{15}N$) and m/z: 30 ($^{15}N^{15}N$).

First, the *Thermo Finnigan* GC-MS with a 60 m *Rt-QPlot* Column (0.25 mm ID by *Restek*) was used for pre-experiments. However, it was not possible to receive reproducible results. Therefore, several maintenance operations like cleaning of the ion-source and exchange of the filament, cleaning of quadrupole and prefilter, the replacement of the PCB, replacement of a broken cable harness between prefilter and

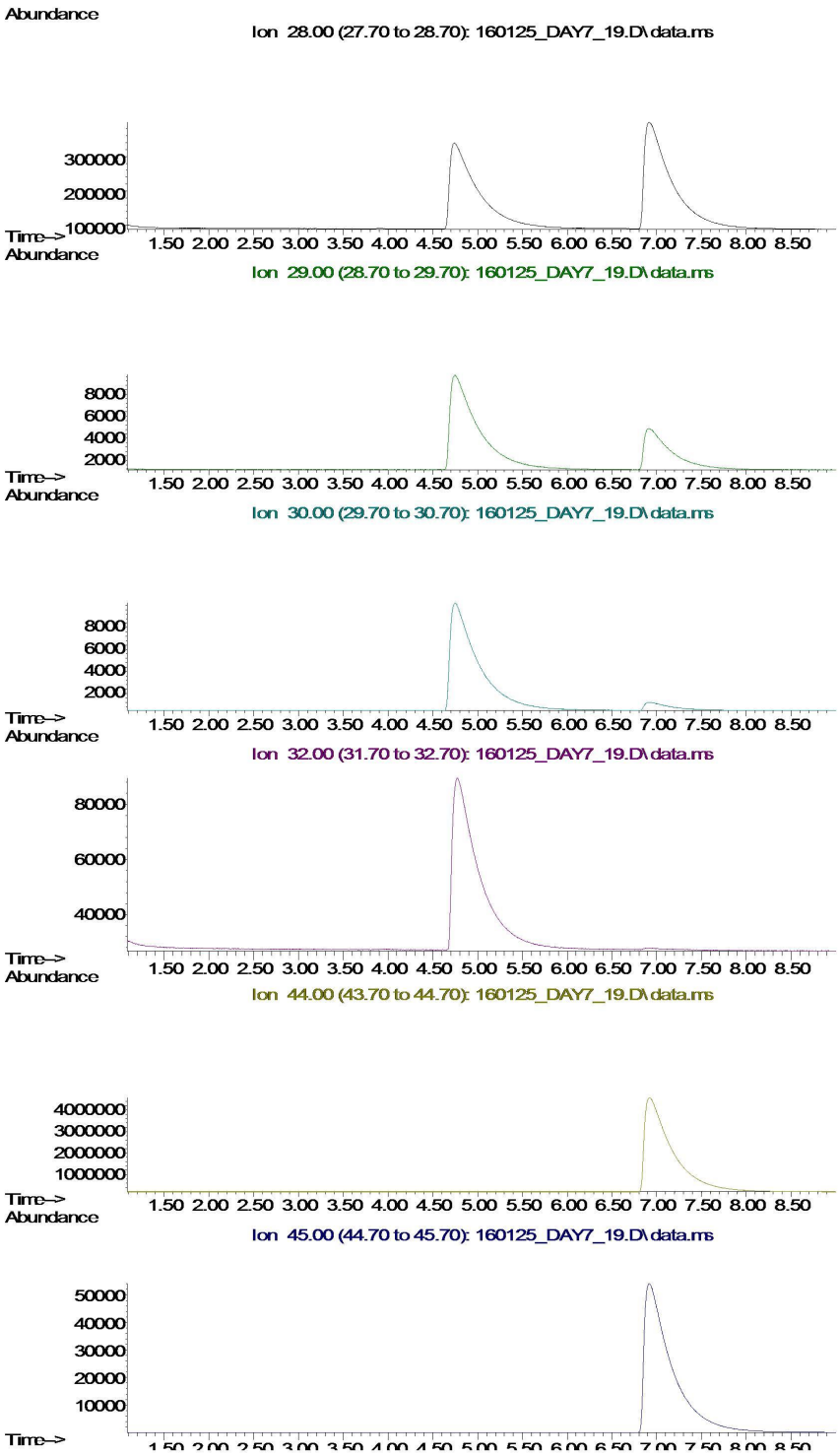

FIGURE 5.4: Chromatogram of GC-MS Analysis. Retention time of molecules on the x-axis, abundance on the y-axis. Each graph represents a specific m/z ratio. m/z: 28 - N_2 and CO, m/z: 29 - N_2 and CO, m/z: 30 - N_2 and NO, m/z: 32 - O_2, m/z: 44 - CO_2, m/z: 45 - CO_2.

quadrupole and the exchange of the column had to be conducted. However the improvements were only temporary.

The final series of measurements were performed by a an *Agilent 7890A* GC connected to an *Agilent 5975C* quadrupole mass selective detector (see figure 5.3). The GC was equipped with the identical *Rt-Qplot* column (60 m in length, 0.25 mm ID) used by the *Thermo Finnigan GC-MS*. Highly purified helium was used as carrier gas. As columns had to be installed before each series of measurements, we were obliged to bake out the column and remove any residue compounds and perform an autotune to check for system stability.

As no prior N_2 measurements were performed with the *Agilent GC-MS*, a new method had to be developed. Basic settings were adjusted referring to and orientated at literature values. However, there was no study with identical column setup. To develop the most suitable method for detection of N_2 a standard mixture (STD) for N_2, N_2O and CO_2, as well as representative batches were tested. By successively changing the flow-through rate of the carrier gas and the temperature at different components (injector, transfer line or oven temperature) it was possible to break the measurement duration down to 9 minutes. In this case it was crucial to get clear and sharp peaks in the GC chromatograph as some nitrogen and carbon species (CO and N_2, CO_2 and N_2O) interfere in the MS. Finally, the samples were manually injected into a 250°C inlet in splitless mode, with a septum purge flow of 3 ml/min. The oven temperature of the GC was set to 30°C with a constant flow rate of 1.3 ml/min at a pressure of 23.2 psi.

Calibration

GC-MS measurements were conducted to determine the concentration of different isotopic species of labeled dinitrogen in the batch headspace. For this calculation, the partial pressure of each gas species was a crucial parameter. Hence the unknown pressure gradient in the batches over time, two pressure scenarios were calculated. The first scenario assumes a constant pressure in the batch headspace over time. Here, bacterial gas production compensates the sample withdrawal. Scenario 2 presumes a linear decrease of the pressure with increasing headspace volume according to the ideal gas law (see equation 5.2). This scenario is based on the assumption of negligible bacterial gas production rates. The following section will describe the calibration method to obtain results from either scenario.

Hence the relative measurement, it was necessary to calibrate the instrument with samples of known concentrations of N_2. Thus creating a linear relation between amount of substance and detected area. Different volumes of a pre-mixed standard gas (STD)

were measured as duplicates to calibrate the instrument. The range of the STD was selected to cover all values of the batch measurements and was therefore individually adjusted for each series of measurements. The number of moles for the three standard volumes was calculated using equation 5.2.

$$p_x V = nRT \tag{5.2}$$

with $\boldsymbol{p_x}$*, partial pressure [*Pa*],* $\boldsymbol{V}$*, volume injected [L],* $\boldsymbol{n}$*, amount of substance [mole],* $\boldsymbol{R}$*, universal gas constant [*$\mathrm{J\,K^{-1}\,mol^{-1}}$*] and* $\boldsymbol{T}$*, room temperature [*K*].*

Where the partial pressure (p_x) for fractions of $^{28}N_2$, $^{29}N_2$ and $^{30}N_2$ in the standard gas was calculated as a function of partial pressure in the standard gas (1‰) multiplied by a specific factor representing the ratio between the isotope abundances as described in equation 5.3.

$$p_x = 101.325 \cdot 0.01 \cdot \frac{N_x}{N_{28} + N_{29} + N_{30}} \tag{5.3}$$

with $\boldsymbol{p_x}$ *partial pressure [*Pa*],* $\boldsymbol{N_x}$ *AUC of respective dinitrogen isotope [-].*

As a drift by the instrument was observed over time, series of standard measurements were performed twice a day, before and after each series, to get a linear relation between detected area and time. Accordingly, individual standard peak areas for the three molecules ($^{28}N_2$, $^{29}N_2$, $^{30}N_2$) were calculated for each time step. Hereafter, slope and intersect were calculated individually for each sample and isotope using the specific standard peak areas on the x-axis and number of moles on the y-axis.

Finally, the amount of substances was calculated for each injection following equation (5.4). This amount represents the number of moles at standard pressure for the injection volume of 20 µL.

$$n_s = ma + b \tag{5.4}$$

with $\boldsymbol{n_s}$*, amount of substance in the syringe [mole],* $\boldsymbol{m}$*, individual slope [-],* $\boldsymbol{a}$*, sample peak area [-],* $\boldsymbol{b}$*, individual intercept [-].*

5.3.2 Liquid Phase Measurements

Ion Chromatography and DOC measurements were performed to validate GC-MS results and monitor compounds in the liquid phase. Finally, the Ferrozine Assay was conducted to exclude potential side effects from test-1.

Ion Chromatography

The analysis of anions and cations of liquid samples from test-1 was performed by a *RFIC Dionex ICS-1100*. IC analysis for test-2 was outsourced to the central analytic. The fundamental principle of IC is the separation of ions by their net charge. The system consists of two separation columns and a detector. The separation columns are packed with charged beads, one column positive, one negative. A liquid sample is flushed through the system by salt solutions called eluents. Again, one solution is positively charged while the other is negatively charged. The separation is facilitated by the fact that, depending on their net charge, substances are differently attracted or repelled by the column. As the absorption is reversible, they are subsequently eluted from the column in relation to their interaction with the column. The travel time of a substance through the columns is specific for each molecule and therefore it leads to a qualitative separation. Finally, substances enter the detector where the quantity of each substance is analyzed by electrical conductivity. Hence the relative measurement, it is necessary to calibrate the instrument with samples of known TOC concentrations. Thus creating a linear relation between amount of substance and detected area.

DOC Analysis

For the preparation of DOC measurements, 2 ml falcon tubes were acidified with 50 µl HCl (1 mol) and filled with 1.5 ml sample. The quantification of DOC was performed using a *Shimadzu TOC-5000A*. The instrument applies a combustion catalytic oxidation method to obtain total combustion of TOC into measurable CO_2. This is subsequently quantified via a non-dispersive infrared gas analyzer (NDIR). After the injection of the liquid samples into the 680°C combustion furnace, equipped with platinum catalyst and purged with purified oxygen as carrier gas, the TOC converts into CO_2, H_2O and residual substances, R (see equation 5.5). Following the combustion, the gases undergo cooling processes and pass through a moisture trap. Thereafter, the dehumidified gas is detected by a NDIR hereby quantifying duration and intensity of the CO_2 peak. Finally, measurement results were obtained as relative values and had to be calibrated for quantification.

$$R - C + O_2 \rightarrow CO_2 + H_2O + R \tag{5.5}$$

To differentiate between TOC and DOC, water samples were filtrated with a 0.45µm syringe filter as no differentiation by the instrument was possible. By definition, DOC particles are smaller than 0.45µm and pass through the filter (Thurman, 1985; Zsolnay, 2003).

Ferrozine Assay

Due to the substantial orange coloring of the batches during the first measurement series and the assumption of iron reduction by bacteria, samples were analyzed for reactive, ferrous iron (Fe^{2+}). Samples from day 0 and day 7 were compared to detect a possible increase in ferrous iron. The method is based on the formation of stable iron complexes by ferrozine and ferrous iron. However, ferrozine does not react with ferric iron (Fe^{3+}). The produced complex is highly soluble and intensely colored. By measuring the absorbance at 560 nm with a *Wallac Victor* plate reader, the amount of Fe^{2+} can be qualified. To quantify this amount for each sample, a linear regression between standard solutions of known concentrations and absorbance was implemented (Stookey, 1970; Riemer et al., 2004).

5.4 Data Analysis

5.4.1 Nitrogen Interpolation

The production of either $^{29}N_2$ or $^{30}N_2$ can be referred to biological activity and the denitrification of labeled nitrate to dinitrogen. However, due to inevitable contamination of samples with atmospheric $^{28}N_2$, the determination of unlabeled dinitrogen production by denitrification was impossible to measure and had to be interpolated.

For a total sum of denitrification produced dinitrogen, the amount of labeled and unlabeled dinitrogen in the headspace had to be added to the respective amounts dissolved in the liquid phase. Therefore, dissolved labeled dinitrogen was calculated using Henry's law with variable solubility constants for 12 and 20°C. The concentration of unlabeled dinitrogen in either phase was interpolated based on the enrichment of labeled nitrate in the nitrate source pool.

First of all, the number of moles calculated for each injection had to be calculated from injection volume to the batch headspace volume. To obtain the number of moles per batch for each molecule ($^{28}N_2$, $^{29}N_2$, $^{30}N_2$), the results from section 5.3.1 were converted with regard to specific headspace volumes and pressure inside the batches

(see equation 5.6). The headspace volume was calculated as difference between the batch weight after the final measurements and the weight after filling the batch with MQ to the maximum. For this purpose, batches were filled up to the top and were sealed with a septa equipped with a canula. By pushing the septa into the batches, excess water was displaced through the canula and the exact headspace volume could be determined. Finally, headspace volumes were calculated for each day by adding the volume extracted for IC and DOC measurements to the endpoint headspace volume.

$$n_b = \frac{n_s}{V_s} \cdot V_b \cdot p_b \tag{5.6}$$

with $\boldsymbol{n}_b$, amount of substance in the headspace [mole], $\boldsymbol{n}_s$, amount of substance in the syringe [mole], $\boldsymbol{V}_s$, syringe volume [L], $\boldsymbol{V}_b$, headspace volume [L], $\boldsymbol{p}_b$ batch overpressure [atm].

The amount of labeled nitrogen in the headspace was calculated using equation 5.7.

$$n_{^{15}N} = 2 \cdot n_{30} + n_{29} \tag{5.7}$$

with $\boldsymbol{n}_{^{15}N}$, amount of labeled nitrogen [mole], $\boldsymbol{n}_{30}$, amount of $^{30}N_2$ [mole], $\boldsymbol{n}_{29}$, amount of $^{29}N_2$ [mole].

The ^{15}N content of the nitrate source pool was assessed in two different approaches: (i) the concentration of labeled nitrate solution was compared to values of total nitrate from IC measurements. Equation 5.8 depicts this approach. (ii) since no potassium was added to AGW, total potassium in the solution derives from labeled potassium nitrate. The deviation from initial K/NO_3^- ratio can be interpreted as dilution by unlabeled nitrate.

$$e = \frac{c(^{15}NO_3^-)}{c(NO_3^-)} \tag{5.8}$$

with $\boldsymbol{e}$, ^{15}N enrichment in the nitrate source pool, $\boldsymbol{c}_{^{15}NO_3^-}$, labeled nitrate concentration in solution on day 0 [mg/L], $\boldsymbol{c}_{NO_3^-}$, total nitrate concentration in solution on day 0 [mg/L].

Further steps were performed to receive the amount of dinitrogen molecules dissolved in the liquid phase. Henry's Law relates the concentration of dissolved gases with the partial pressure of the atmosphere. It was applied to calculate the amount of dinitrogen in the liquid phase (Mortimer, 2001; Sander, 2015; Domenico and Schwartz, 1990;

Stumm and Morgan, 1996). The solubility constant for the respective temperature was calculated using the van't Hoff equation 5.9 modified from Sander (2015).

$$H_{12}^{cp} = H_{25}^{cp} \cdot exp\left(C \cdot \left(\frac{1}{T_{12}} - \frac{1}{T_{25}}\right)\right) \tag{5.9}$$

with $\boldsymbol{H^{cp}}$, Henry's solubility constant for N_2 at different temperatures, $\boldsymbol{C}$, constant = 1300, $\boldsymbol{T}$, temperature of liquid phase [K].

Hereinafter, the concentration of labeled dinitrogen molecules in the liquid phase was obtained by applying equation 5.10.

$$C_{^{15}N} = H^{cp} \cdot P \tag{5.10}$$

with $\boldsymbol{C}$ liquid phase concentration for molecule A [moles/L], $\boldsymbol{H^{cp}}$ Henry's law constant [-], $\boldsymbol{P}$, partial pressure in the headspace for molecule A [atm].

According to Kulkarni et al. (2014), total dinitrogen production in the headspace or in the liquid phase n_N was calculated by dividing the amount of labeled nitrogen in either phase by the ^{15}N enrichment (see equation 5.11). Under the assumption of random paring of nitrogen atoms (Thamdrup and Dalsgaard, 2002; Holtappels et al., 2010; Groffman et al., 2006) the total dinitrogen production, including the production of $^{28}N_2$ was calculated.

$$n_N = \frac{n_{^{15}N}}{e} \tag{5.11}$$

with $\boldsymbol{n}_N$ total dinitrogen-N production [mole], $\boldsymbol{n}_{15N}$ amount of labeled nitrogen [mole] and $\boldsymbol{e}$ ^{15}N enrichment in the nitrate source pool [-].

However, fractionation effects during denitrification (Kendall and Mcdonnell, 1998) were not integrated which might result in the underestimation of denitrification rates.

5.4.2 Statistics

The data was checked for normal distribution with Komogorov-Smirnov and Shapiro-Wilk-test. Bartlett-test was used to check for equal variances which is required for ANOVA (Analysis of Variance). If the data was not parametric, it was necessarily transformed into normal distribution. For parametric distribution with a sample size > 2, one-way ANOVA and Welch-test for one-way analysis of means were used (independent

data groups). Students and Welch-test were used to compare two mean values. If distribution was non-parametric and sample size was > 2, Kruskal & Wallis (independent) was applied. Mann & Whitney (independent) and Wilcoxon-test (dependent) were used two compare two values (Singer and Karwautz, 2014).

Microsoft Excel 2010 was used for data compilation from the peak detection software. Advanced statistical analysis and descriptive statistics were conducted in R-Studio (0.98.1091).

Chapter 6

Results

6.1 Potential Rates and Limitations

The following section comprises results from the first series of measurements. Sediments were embedded in meshed metal tubes and exposed to groundwater. Five different treatments were prepared to investigate the influence of nitrate, DOC and temperature limitation on denitrification rates.

6.1.1 Isotope Approach

The growth of total ^{15}N (headspace and dissolved) from scenario 1 can be seen in figure 6.1. Each box contains values from 6 measurements per day (n = 6). The top and the bottom of the boxes represent the first and third quantile, respectively. The median or second quantile is expressed as horizontal band.

The results for treatment 1 show significant differences between ^{15}N production and days in a one-way ANOVA (F = 35.62, d.f1 = 1, d.f2 = 22, Pr(>F) < 0.001). Pairwise comparison of means by t-test revealed significant differences between day 1 to 3 (p-value < 0.01) but no significant changes between day 3 and 5 (p-value = 1). The overall shape of ^{15}N increase over time shows a logarithmic trend with maximum rates between day 1 and 2 and successively decreasing rates between day 2 and 5. Labeled nitrogen values reached a constant level on day 3 without further increase on day 5. In contrast, treatment 2 shows a linear increase over time with significant ^{15}N increase over time (F = 135.1, d.f1 = 1, d.f2 = 22, Pr(>F) < 0.001). Significant differences between each day were identified (p-value < 0.001). Despite lower DOC content, treatment 2 produced comparable ^{15}N concentrations to treatment 1 (p-value = 0.15). Labeled nitrogen production in treatment 3 was generally slower than in treatment 1 and 2, resulting in less ^{15}N production over the course of 5 days compared to treatment 2 (20°C). Concentrations differed significantly from treatment 2 (p-value < 0.05). Further, the increase of ^{15}N over time was significant (F = 125.3, d.f1 = 1, d.f2 = 22, Pr(>F) < 0.001) with significant differences between each day (p-value < 0.001). Test results from

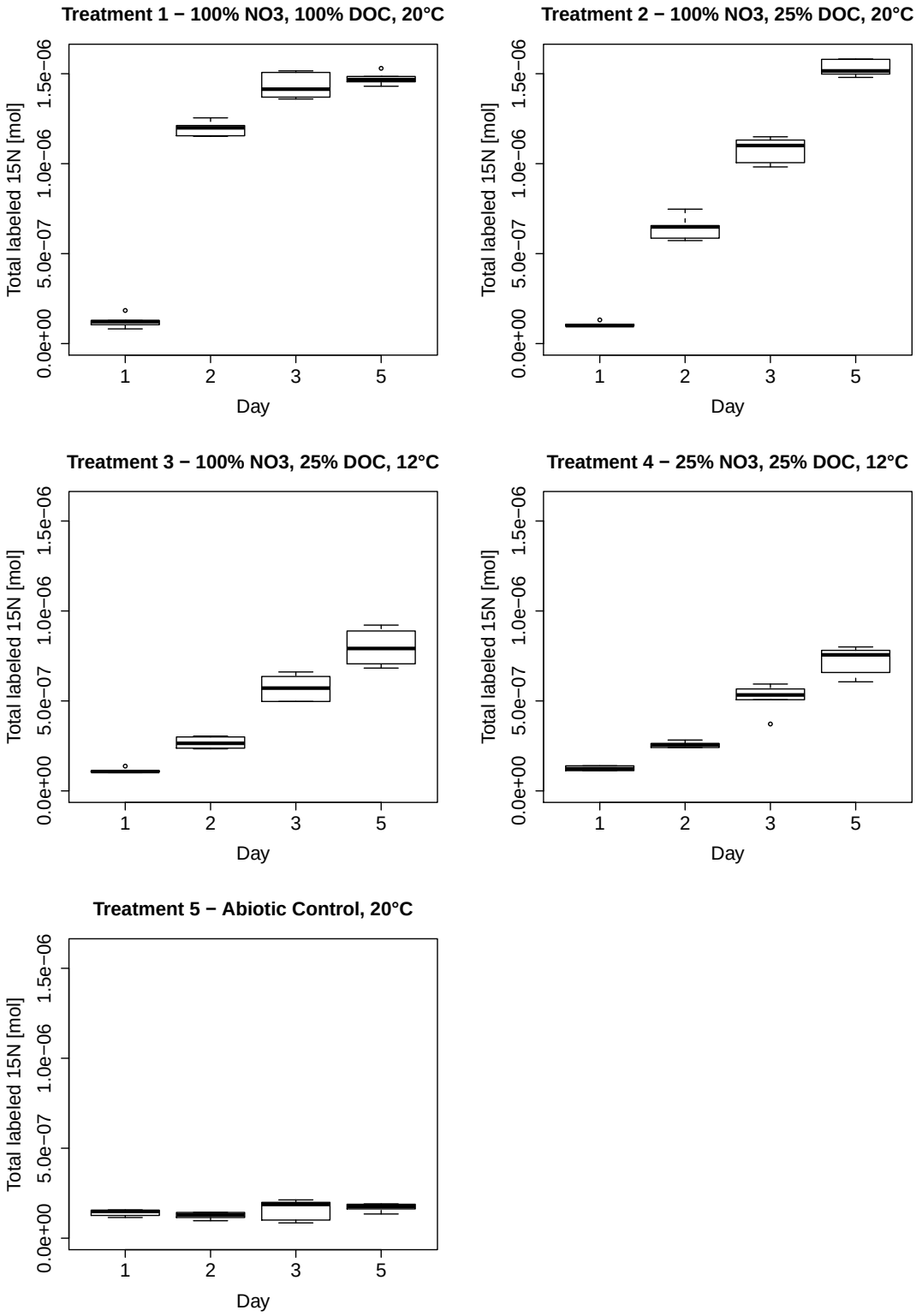

FIGURE 6.1: Comparison of labeled dinitrogen evolution between different treatments from the first series of measurements.

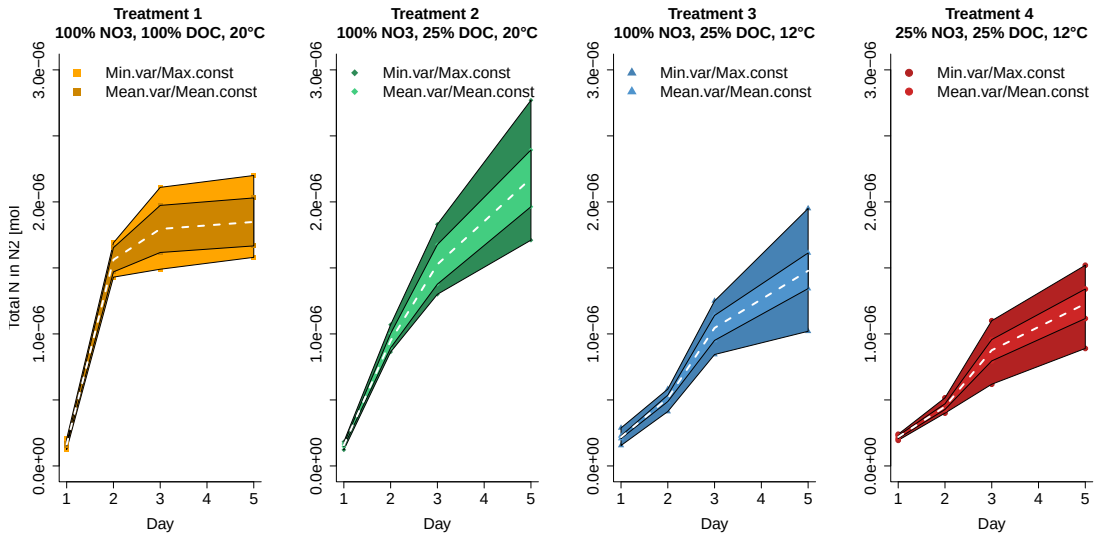

FIGURE 6.2: Evolution of denitrification nitrogen in different treatments from the first series of measurement. Mean evolution of nitrogen expressed as white, dashed line.

ANOVA indicated significant differences of ^{15}N production between days in treatment 4 (F = 146.4, d.f1 = 1, d.f2 = 22, Pr(>F) < 0.001) with significant differences between each day (p-value < 0.001). However, there was no significant difference to treatment 3 (p-value = 0.30). No significant differences were found with a Wilcoxon rank sum test in treatment 5, the abiotic control (p-value > 0.1). Labeled nitrogen concentrations stayed leveled over the time.

The results may be summarized as follows: All treatments differed from the abiotic control. Variation of DOC (compare treatment 1 and 2) and nitrate (compare treatment 3 and 4) showed no significant impact on ^{15}N production. Whereas a drop in temperature from 20 to 12°C caused significantly lower denitrification rates (compare treatment 2 and 3).

As explained in section 5.3.1, results from GC-MS measurements were calculated for two pressure scenarios. Figure 6.2 depicts the evolution of total dinitrogen-N (^{14}N and ^{15}N) from denitrification in different treatments. Each treatment comprehends three batches with identical conditions.

The colored areas represent possible ranges of total nitrogen for treatment 1 to 4. Treatment 5, the abiotic control was not displayed as no significant increase of nitrogen was observed. The larger area stands for a maximum range which consists of minimum values from scenario 2, assuming pressure changes in the headspace and maximum values from scenario 1 assuming constant pressure in the headspace over time. In contrast, the overlying area represents a range of values between the means

of scenario 1 and 2. The white dashed line stands for the mean values of scenario 1 and 2.

Denitrification rates were calculated as nitrogen difference between day 1 and day 5. They can be found in table 6.1. Rates were calculated in µmol nitrogen-N per day for constant and variable pressure scenarios. Furthermore, the amount of labeled nitrogen-N can be compared to the amount of total nitrogen-N. Recovery of labeled nitrogen is displayed as percentage over the course of five days. Statistical analysis was performed using data from the main population (batch 1-12) and not the mean values displayed in this table.

TABLE 6.1: Denitrification rates from the first series of measurements. Mean rates per day for labeled nitrogen and total nitrogen calculated for both pressure scenarios. Further, percentage of processed total labeled ^{15}N.

	Scenario 1: Constant pressure			Scenario 2: Variable pressure		
Treatment	µmol ^{15}N d^{-1}	µmol N d^{-1}	% ^{15}N	µmol ^{15}N d^{-1}	µmol N $^{-1}$	% ^{15}N
1	0.2739	0.3718	10.27	0.2229	0.3025	8.36
2	0.2887	0.4454	10.83	0.2355	0.3114	8.83
3	0.1256	0.2771	4.71	0.1125	0.3327	4.22
4	0.1202	0.2220	4.51	0.0983	0.3634	3.69
5	-	-	-	-	-	-

No significant influence of DOC content on nitrogen production was observed between treatment 1 and 2 (t = -2.4818, df = 3.91, p-value = 0.06952). Recovery of labeled nitrogen ranged between 8 and 10 % over five days in both treatments. Apart from a different storage temperature, treatment 2 and 3 were set up identically. However, denitrification rates differed significantly (t = -12.318, df = 2.436, p-value < 0.01). Recovery rates in treatment 3 are within the range of 4 %. The effect of reduced nitrate amendments can be asses by comparing treatment 3 (100 % $^{15}NO3\_$) with treatment 4 (25 % $^{15}NO3\_$). Nevertheless, there was no significant difference between denitrification rates of treatment 4 compared to treatment 3 (t = 1.1004, df = 3.878, p-value = 0.3347). Due to low nitrogen changes in the abiotic control, which did not exceed the measurement accuracy, no denitrification rates were calculated for treatment 5.

6.1.2 Mass Balance

IC and DOC measurements were carried out to validate denitrification rates. Assuming no fractionation effects during denitrification, optimal behavior would be a linear relation with a slope of -1 between nitrogen increase and nitrate decrease. As DOC was considered a crucial parameter for limitation of denitrification rates, this parameter allows further understanding of denitrification processes in the batches.

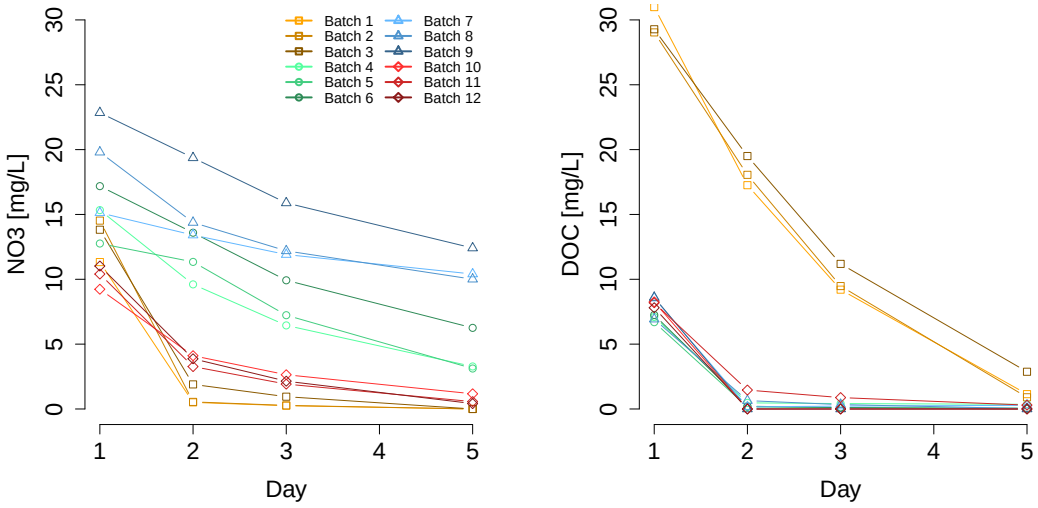

FIGURE 6.3: Comparison of nitrate and DOC decrease from the first series of measurements over time. Batches from treatment 1 (1-3, orange), treatment 2 (4-6, green), treatment 3 (7-9, blue) and treatment 4 (10-12, red).

Figure 6.3 illustrates the decrease of nitrate and DOC over time. Measurements from batch 1 to 12 are presented and colors label the respective treatments. Batches from treatment 1 and 4 showed similar nitrate reduction curves. Both treatments received DOC and nitrate amendments in equal ratios, but different quantities (treatment 1: 100 %, treatment 2: 25 %). However, they were stored at different temperatures (treatment 1: 20°C, treatment 4: 12°C). Their curves are defined by a steep decrease between day 1 and 2 and a further slight decline until day 5. In treatment 1, a mean reduction of 13.2 ± 1.7 mg/L nitrate was measured. Whereas nitrate concentrations in treatment 4 declined by 9.5 ± 1.3 mg/L over the five day period. On day 5, no nitrate was detected in treatment 1 and values below 1 mg/L were measured in treatment 4. Nitrate decrease in treatment 2 and 3 can be described as gradual with a mean of 10.9 ± 1.3 and 8.2 ± 3.1 mg/L over five days, respectively. Measured mean concentrations on day 5 for treatment 2 were 4.1 ± 1.8 mg/L and 10.9 ± 1.2 mg/L for treatment 3.

Treatment 2 to 4 received DOC amendments of 25 % compared to treatment 1. This is validated by DOC concentrations of 7.1 ± 0.2, 8.0 ± 0.9 and 8.1 ± 0.3 mg/L in treatments 2 to 4, compared to 29.8 ± 1.1 mg/L in treatment 1. From day 2, the amount of DOC in treatment 2 to 4 was below 1 mg/L, in contrast to treatment 1 where the amount declines until day 5. Over the course of five days, DOC concentrations in treatment 1 decreased 28 ± 1.9 mg/L compared to 6.9 ± 0.4 in treatment 2, 7.9 ± 0.9 in treatment 3 and 8.0 ± 0.3 mg/L in treatment 4.

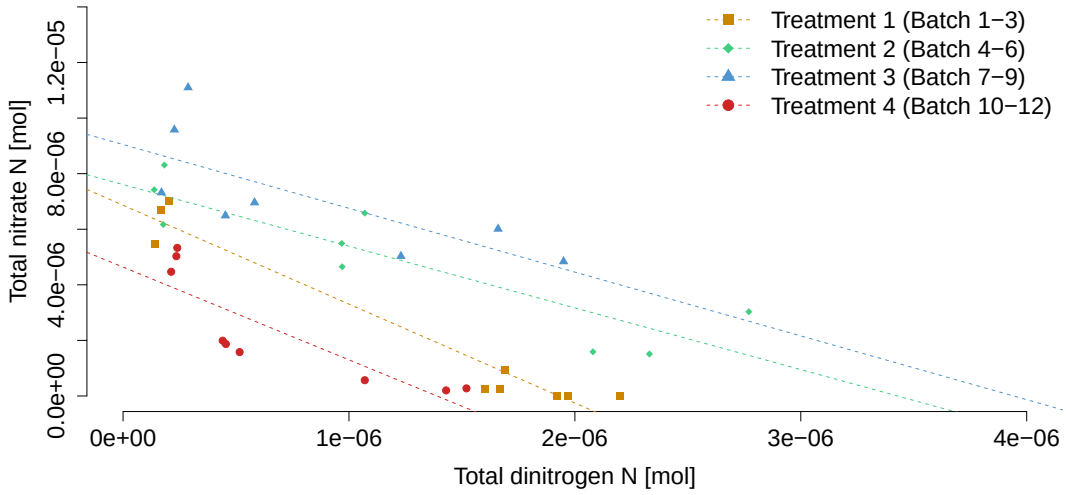

FIGURE 6.4: Relation between dinitrogen increase and nitrate decrease. Linear relation of each treatment illustrated by dashed lines.

To summarize, a decrease in nitrate and DOC was observed in in treatment 1 to 4. Despite treatment 1, all batches were under limiting DOC circumstances from day 2. However, nitrate reduction continued unchanged in treatment 2 and 3.

It was assumed, that the reduction of 1 mole nitrate-N during denitrification would consequently emerge 1 mole of dinitrogen-N in the headspace. This would imply a slope of -1 comparing N in the headspace and residual nitrate in the liquid phase. However, fractionation effects were neglected, thus leading to underestimation of headspace nitrogen. The relation of nitrate-N decrease and dinitrogen-N increase can be seen in figure 6.4. Calculation of the adjusted r^2 for treatment 1 resulted in 0.94. However, only two cluster on either side of the trendline dominate the relation. Data points for treatment 2 and 3 scatter around the trendline, whereas data points from treatment 4 show a rather exponential decline and cannot properly be described by the linear regression. The coefficients from the linear regression are illustrated in table 6.2.

A slope smaller than -1 indicates a faster decrease of nitrate-N in contrast to nitrogen-N. In this case, nitrate-N declines 2.2 to 3.6 times faster than nitrogen-N increases.

TABLE 6.2: Coefficients from linear regression between nitrogen on the x-axis and nitrate on the y-axis (see figure 6.4).

Treatment	Slope	Intercept	r^2
1	-3.56	6.86E-06	0.94
2	-2.22	7.61E-06	0.76
3	-2.29	9.05E-06	0.46
4	-3.34	4.64E-06	0.68

6.1.3 Ferrozine Assay

The Ferrozine assay was conducted to gain information about the reliability of test-1. As an considerable discoloration was observed, it was assumed that bacteria could produce ferrous iron under the consumption of DOC. If this was the case, denitrification results would be error-prone due to the competitive consumption of DOC. Therefore, samples from day 1 were compared with samples from day 5 and results indicated the absence of ferrous iron on both days. No values differed significantly from the zero stock check. This led to the assumption that denitrification processes during this test were not influenced by iron reduction and the results obtained were comparable to the second series of measurements.

6.2 Actual Rates and Spatial Heteogeneity

Results from the second series of measurements will be presented in the following section. Fresh sediments from the mixing zone between unsaturated and saturated zones, and sediments from the saturated zone were collected. Hereafter, six treatments were prepared to compare denitrification rates from the mixing zone (treatment 1 and 2) and the saturated zone (treatment 3 and 4) with potential denitrification rates (treatment 5). Treatment 6 is the abiotic control.

6.2.1 Isotope Approach

The growth of total ^{15}N (headspace and dissolved) from scenario 1 over time can be seen in figure 6.5. Each box contains values from 6 measurements per day (n = 6).

A significant difference in nitrogen concentration was found for treatment 1 (F = 382.7, d.f1 = 1, d.f2 = 40, Pr(>F) < 0.001) with significant differences between day 1 and 2 (p-value < 0.01) and between day 4 and 7 (p-value < 0.001). No significant increase in nitrogen could be found between day 2 and 4. Similar pattern was found for treatment 2 (F = 355, d.f1 = 1, d.f2 = 40, Pr(>F) < 0.001), however with a higher significance between day 1 and 2 (p-value < 0.001). Nitrogen concentration in treatment 3 show a more linear increase until day 4 without significant differences between the days. Test results from ANOVA revealed significant differences though (F = 129.7, d.f1 = 1, d.f2 = 40, Pr(>F) < 0.001) which could be identified between day 4 and 7 (p-value < 0.001). Treatment 4 showed significant nitrogen differences (F = 380, d.f1 = 1, d.f2 = 40, Pr(>F) < 0.001) which were found between day 1 and 2, 4 and 5 (p-value < 0.001) and 5 and 7 (p-value < 0.05), respectively. Treatment 5 received DOC amendments and significant nitrogen differences were found between the days (F = 107.6, d.f1 = 1, d.f2 = 34, Pr(>F)

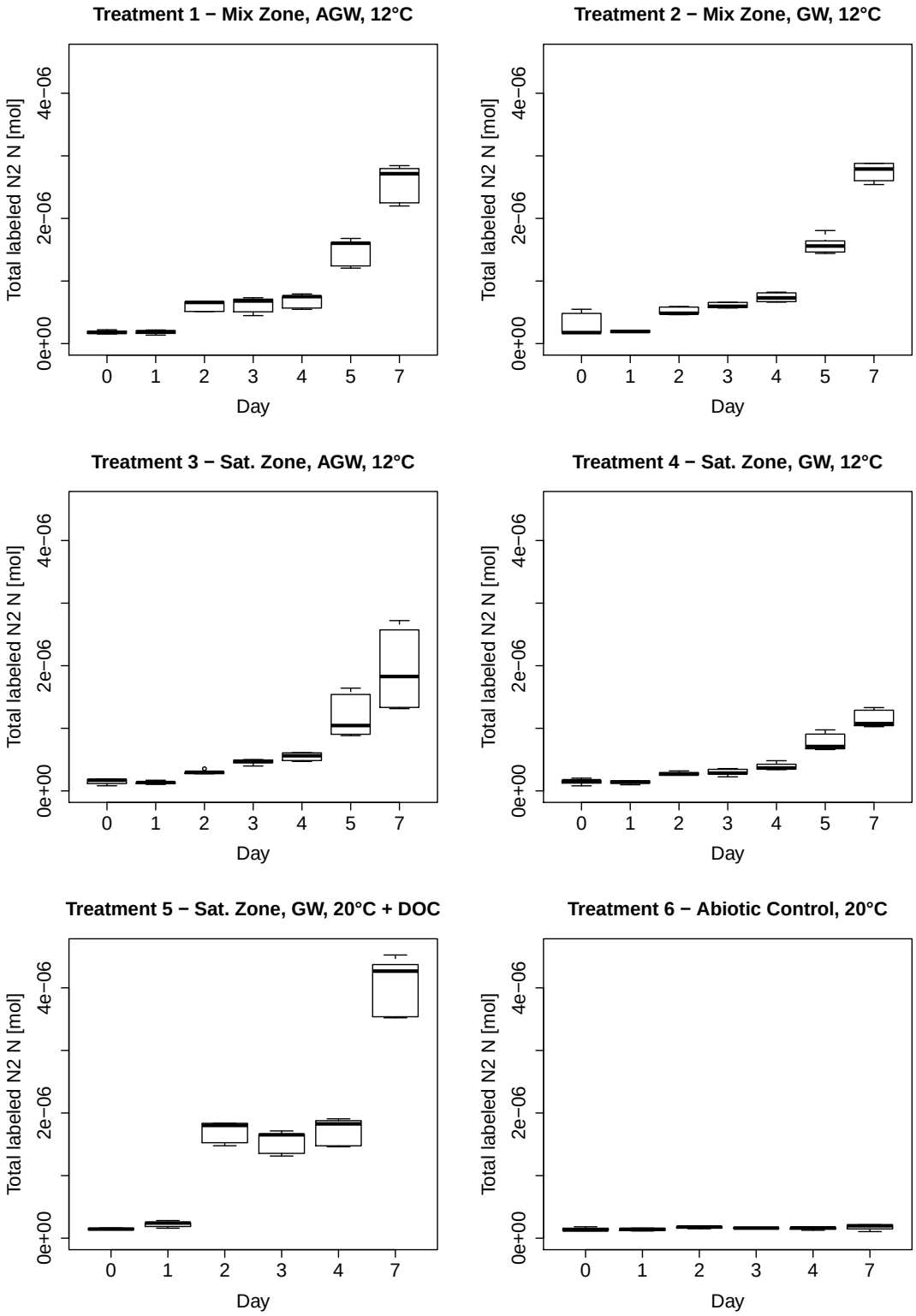

FIGURE 6.5: Comparison of labeled dinitrogen evolution on different days from the second series of measurements.

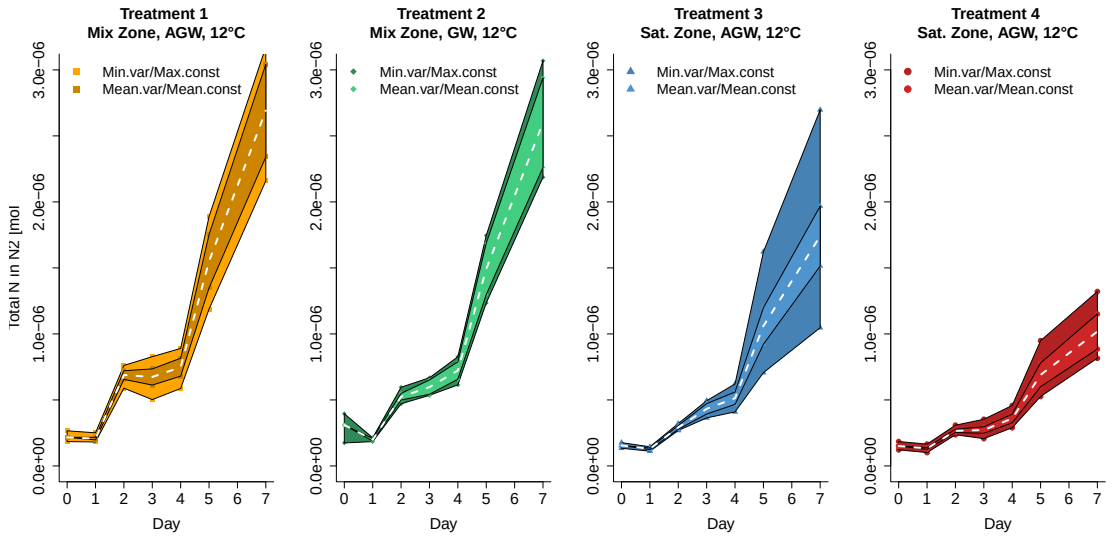

FIGURE 6.6: Evolution of denitrification nitrogen in different treatments from the second series of measurement. Mean evolution of nitrogen expressed as white, dashed line.

< 0.001). To specify, significant differences were identified between day 0 and 1, day 2 and 3 and day 4 to 7 (p-value < 0.001). Finally, no significant differences were found in the abiotic control ((F = 3.33, d.f1 = 1, d.f2 = 34, Pr(>F) = 0.08).

The development range of nitrogen derived from denitrification can be seen in figure 6.6. Based on uncertain pressure changes in the batch headspaces, results for scenario 1 were calculated for constant pressure, whereas results for scenario 2 were calculated for a linear decreasing pressure.

Background areas represent the maximum range of total denitrification derived nitrogen. The boundaries are defined by minimum values from scenario 2 and maximum values from scenario 1. The overlying area is defined by mean values from both scenarios 1 and 2. The white dashed line finally stands for the mean nitrogen value. In contrast to test-1, first measurements were conducted on day 0, the same day the batches were prepared. The evolution of nitrogen proceeds comparable in all treatments. Between day 0 and 1, no considerable amount of nitrogen was produced. In contrast, nitrogen stays constant or depending on the scenario and treatment, even decreases. Between day 1 and 2, a steep increase can be observed which subsequently levels or attenuates until day 4. Until the end of the measurements on day 7, a steep linear increase can be observed in treatment 1 and 2 (mixing zone). Nitrogen concentrations in treatment 3 and 4 are less steep with a more logarithmic shape curve between day 5 and 7 compared to treatment 1 and 2.

Table 6.3 illustrates denitrification rates from the second series of measurements. Furthermore, tracer recovery is expressed as percentage of measured ^{15}N compared to the labeled nitrate amendments.

TABLE 6.3: Denitrification rates from the second series of measurements. Mean rates per day for labeled nitrogen and total nitrogen calculated for both pressure scenarios. Further, percentage of processed total labeled ^{15}N.

	Scenario 1: Constant			Scenario 2: Variable		
Treatment	µmol ^{15}N d^{-1}	µmol N d^{-1}	% ^{15}N	µmol ^{15}N d^{-1}	µmol N d^{-1}	% ^{15}N
1	0.3434	0.4037	18.03	0.2581	0.3034	13.55
2	0.3516	0.3756	18.46	0.2610	0.2787	13.70
3	0.2544	0.2590	13.35	0.1906	0.1941	10.01
4	0.1415	0.1428	7.43	0.1039	0.1049	5.45
5	0.5620	0.5951	29.51	0.4275	0.4526	22.44
6	0.0055	0.0059	0.29	0.0012	0.0013	0.06

No significant difference was detected between either treatments with sediments from the mixing zone (treatment 1 and 2, t = 1.158, df = 3.408, p-value = 0.3215) or treatments with sediments from the saturated zone (treatments 3 and 4, t = 1.9902, df = 2.204, p-value = 0.1729). This fact indicates no influence of the medium (GW or AGW) on denitrification rates. In contrast to that, a significant difference was found between mixing zone and saturated zone (t = 4.855, df = 6.144, p-value < 0.01). Treatment 5 was set up with sediments from the saturated zone and received additional DOC amendments. Therefore it was not compared with treatment 3 and 4 but regarded separately. Significant differences were found in comparison with denitrification rates from the mixing zone (t = -4.2322, df = 2.291, p-value < 0.05) and the saturated zone (t = -6.6128, df = 4.535, p-value < 0.01). All treatments differed from the abiotic control (p < 0.001).

6.2.2 Mass Balance

Figure 6.7 shows the evolution of nitrate and DOC over the course of 7 days. Batch 1 to 15 are color coded and represent treatment 1 (orange), treatment 2 (green), treatment 3 (blue), treatment 4 (red) and treatment 5 (purple). Treatment 1 and 2 were set up with sediments from the mixing zone and treatments 3 and 4 were set up with sediments from the saturated zone. As it can be seen, treatments 1 to 4 show a gradual decline in nitrate concentrations over time. In contrast to treatment 1 to 4, treatment 5 represents potential denitrification rates and received supplementary DOC amendments.

The different batch setup is reflected by the difference between nitrate concentration on day 0 with higher concentrations in batches from the mixing zone (t = 4.1352, df =

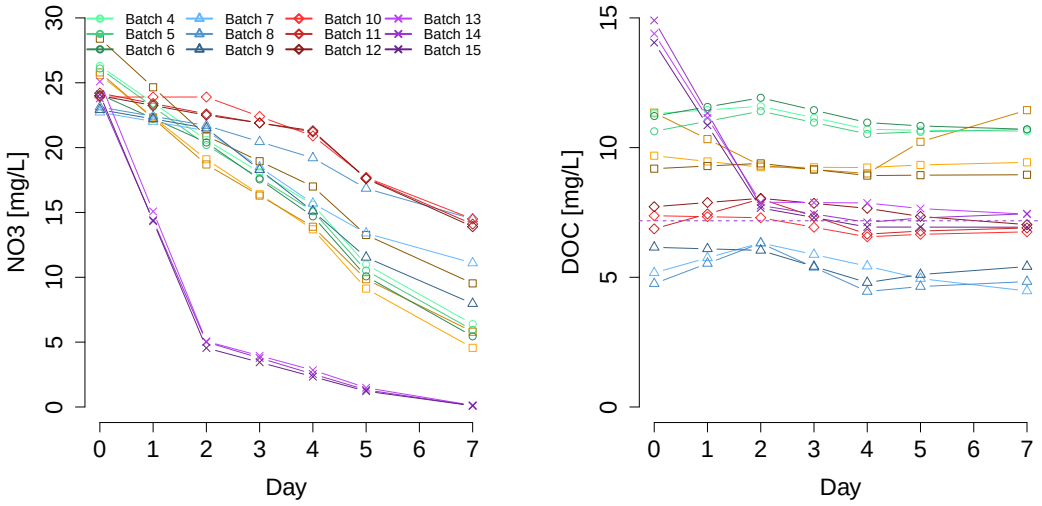

FIGURE 6.7: Comparison of nitrate and DOC decrease from the second series of measurements over time. Batches from treatment 1 (1-3, orange), treatment 2 (4-6, green), treatment 3 (7-9, blue), treatment 4 (10-12, red) and treatment 5 (13-15, purple).

7.035, p-value < 0.01). Mean nitrate reduction in treatment 1 was 19.9 ± 1.1 mg/L with residue nitrate on day 7 of 6.6 ± 2.6 mg/L, compared to treatment 2 with 19.6 ± 0.8 mg/L reduced and 5.9 ± 0.5 mg/L remaining nitrate. However, no significant difference was found between rates in batches of treatment 1 and 2 (t = 0.5075, df = 3.699, p-value = 0.6406). Nitrate reduction rates from treatment 1 and 2 however, were significantly different from treatment 3 and 4 with sediments from the saturated zone (t = 9.0507, df = 6.49, p-value < 0.001). Nitrate in treatment 3 was reduced by 11.7 ± 3.2 mg/L to 11.2 ± 3.3 mg/L, whereas nitrate reduction in treatment 4 was 9.9 ± 0.4 mg/L with remaining 14.2 ± 0.3 mg/L. Again, nitrate reduction rates did not significantly differ between batches of treatment 3 and 4 (t = 1.011, df = 2.065, p-value = 0.4155). Finally, all treatments significantly differed from treatment 5 (p-value < 0.01) with nitrate reduction rates of 24.2 ± 0.6 mg/L and final nitrate concentrations of 0.1 ± 0.02 mg/L. In contrast to the other treatments, a steep drop in concentration between day 0 and 2 followed by a gradual decline could be observed in this treatment.

With regard to DOC, no significant decline could be observed in treatment 1 to 4. Mean concentrations dropped 0.16 ± 0.13, 0.40 ± 0.35, 0.48 ± 0.42 and 0.43 ± 0.38 mg/L over seven days. Once again, treatment 5 differs from the rest and shows a steep drop between day 0 and 2, followed by a comparable gradient with other treatments between day 2 and 7. DOC concentrations declined by 7.16 ± 0.27 mg/L with 7.27 ± 0.29 mg/L remaining. No significant difference was found between treatment 1 and 2 (t = -1.0782,

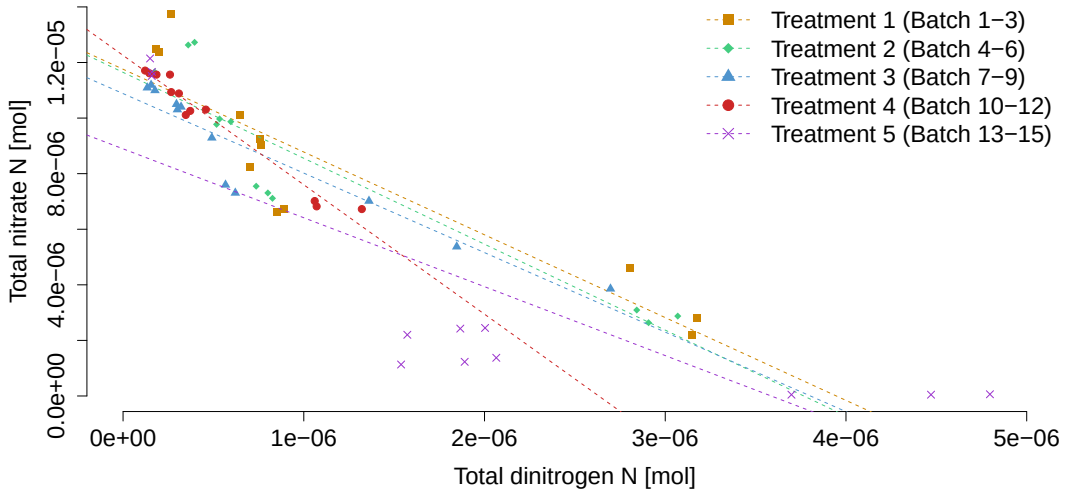

FIGURE 6.8: Relation between dinitrogen increase and nitrate decrease. Linear regression of each treatment illustrated as dashed lines.

df = 2.602, p-value = 0.3706) or treatment 3 and 4 (t = 0.144, df = 3.96, p-value = 0.8925). However, all treatments differed from treatment 5 (p-value < 0.001).

All in all, a considerable decline in nitrate concentrations could be observed in each treatment. However, this decline did not coincide with the development of DOC concentrations which remained stable over time. Finally, rates differed significantly from the remaining treatments in treatment 5 which received additional DOC. In fact, a high decrease in nitrate and DOC concentrations was observed until day 2, followed by a gradual evolution comparable to treatment 1 to 4.

Figure 6.8 depicts the relation between nitrate-N decrease on the y-axis and dinitrogen-N increase on the x-axis. The coefficients from the calculated linear regression can be found in table 6.4. Based on the assumption of a linear relation between these two parameters, the deviation from a potential slope of -1 and the coefficient of determination r^2, can provide information about the processes.

TABLE 6.4: Coefficients from linear regression between nitrogen on the x-axis and nitrate on the y-axis (see figure 6.8).

Treatment	Slope	Intercept	r^2
1	-2.98	1.18E-05	0.81
2	-3.09	1.17E-05	0.84
3	-2.86	1.09E-05	0.86
4	-4.66	1.23E-05	0.96
5	-2.48	8.89E-06	0.62

It was assumed that the consumption of 1 mole nitrate-N would consequently result in the enrichment of 1 mole dinitrogen-N in the headspace. This would be described with a slope of -1. However, the deviation of all slopes differed significantly from -1 ($t = -11.2356$, $df = 4$, p-value < 0.001). Treatments 1 to 4 show a similar relation with gradually declining values at high nitrate and low nitrogen concentrations. However outlier at low nitrate and high nitrogen concentrations reduce the coefficient of determination r^2 in treatment 1, 2, 3 and 5. These points origin from day 7 measurements where a strong increase of dinitrogen could be observed in figure 6.5. Without obvious outliers, the linear regression for treatment 4 reveals the highest accuracy with $r^2 = 0.96$.

Meanwhile, it seems that nitrate decreases about 2 to 5 times faster than nitrogen emerges. Furthermore, no relations between the slope and denitrification rates or batch setup could be drawn.

Chapter 7

Discussion

This research examined the limiting factors of denitrification in batch experiments and further, limitation of batch experiments itself. In particular, two series of measurements were conducted (i) to test the influence of nitrate, DOC and temperature on denitrification rates and (ii) to compare the spatial variability of denitrification rates with sediments from different depths.

7.1 Factors Limiting Denitrification Rates

Whether denitrification rates are limited by nitrate and DOC concentrations or temperature was examined in the first test described in section 6.1. Four different treatments were set up with 3 identical batches each (see section 5.2.1). Treatment 1 received 100 % of either DOC and nitrate. The specific amounts were calculated as they should represent non limiting conditions, thus potential denitrification rates. To compare the effect of DOC limitation, the DOC amendment was reduced to 25 % in treatment 2. Influence of temperature was observed by comparing treatment 2 (20°C) with treatment 3 (12°C). Treatment 4 mimics field conditions with 25 % of nitrate and DOC and a storage temperature comparable to the average groundwater temperature of 12°C. In direct comparison to treatment 3, the influence of reduced nitrate concentrations in treatment 4 can be observed.

As shown in section 6.1, a significant influence of temperature on denitrification rates was found. In particular, denitrification rates in treatment 2 (20°C, 0.019 µmol N g^{-1} soil d^{-1}) were significantly higher (p-value < 0.01) than in treatment 3 (12°C, 0.010 µmol N g^{-1} soil d^{-1}). This was confirmed by nitrate isotope data from the field site which indicated higher denitrification during summer months compared to winter (K. Knöller, personal communication). The finding was further supported by Lu et al. (2009), Pfenning and McMahon (1996) and Sirivedhin and Gray (2006) who found significantly higher denitrification rates at higher temperatures. Pfenning and McMahon (1996) for example, reported a decrease in denitrification rates of 77% resulting from a

temperature decrease from 22 to 4°C.

Surprisingly, no impact of tested nitrate or DOC contents on mean denitrification rates was observed. In fact, denitrification rates of treatment 1 and 2, and of treatment 3 and 4 were all in the same range and did not differ significantly.

Previous research by Lu et al. (2009), Addy et al. (2000) and Hoffmann et al. (2000) however, indicated the influence of DOC on denitrification rates. They reported that the addition of highly degradable DOC as acetate or glucose can significantly increase the denitrification potential in batch experiments.

The fact that no significant difference between treatments with variable DOC concentrations was found, can generally be explained by a nitrate limitation in treatment 1 from day 2. This can be seen in figure 6.3 which illustrates the evolution of nitrate and DOC in test-1. However, a faster increase of nitrogen production in treatment 1 compared to treatment 2 can be seen at the beginning of the experiment where both treatments had sufficient nitrate supply (see figure 6.1). Here, a significant difference between nitrogen production from treatment 1 and 2 was found on day 2 (t = 18.5509, df = 8.312, p-value < 0.001).

This leads to the assumption that DOC and nitrate might have an influence on denitrification rates which could not be explained in this study. Longer time series with comparable conditions in both treatments are required to provide more informative values about these limitation.

7.2 Spatial Variability of Denitrification Rates

Previous research has demonstrated that highest denitrification rates occur in transition zones, or "hot spots" (McClain et al., 2003). Therefore, samples from the saturated and the mixing zone were taken to examine the spatial variability of denitrification rates in the riparian zone.

The hypothesis of this study was that highest denitrification rates would occur in the prior mentioned mixing zones. This was confirmed (see section 6.2) and significantly higher (p-value < 0.01) denitrification rates were found in the mixing zone (0.0177 ± 0.001 µmol N g^{-1} soil d^{-1}) compared to the saturated zone (0.0088 ± 0.004 µmol N g^{-1} soil d^{-1}). These findings are in accordance with data from Gassen et al. (2016), indicating lower nitrate concentrations and enriched nitrogen isotopes in the mixing zone (see figure 4.3).

A decrease in denitrification rates with depth was found by Hoffmann et al. (2000) and Woodward et al. (2009). However, they linked decreased denitrification rates with

lower DOC availability with depth and measurements were performed with a much lower vertical resolution. Thus, they compared unsaturated and saturated sediments. In contrast to test-1, no limitations in nitrate or DOC were detected. Both treatments from the mixing and saturated zone show a gradual decrease of nitrate and almost no changes in DOC concentrations (see figure 6.7).

7.3 Comparison with Literature Values

Table 7.1 compares denitrification rates from literature with values from this study. For comparison, all literature values were transformed into the unit $\mu mol\,N\,g^{-1}\,soil\,d^{-1}$. First of all, this table demonstrates that all measured denitrification rates can be confirmed with literature values. Even if no comparable studies were found, all measured rates lie within the range of published studies. Furthermore, the relation between denitrification rates and spatial distribution was approved by significantly lower rates in the saturated zone. Though quantitative differences between calculated rates from the ^{15}N and the NO_3^- method, both methods can be compared on a qualitative level. Qualitative comparison between saturated zone and mixed zone show around 50 % lower denitrification rates in the saturated zone. Unfortunately, a clear trend between denitrification rates and method or field site was not detected. Studies of Woodward et al. (2009) and Pinay et al. (1993) examined denitrification rates of riparian forests at the field site and in batch experiments using the acetylene inhibition method (AIM). However, they measured mean denitrification rates of 0.239 and 0.002 $\mu mol\,N\,g^{-1}\,soil\,d^{-1}$, respectively which is one of the highest rates compared to the second smallest rate. Mass balance approaches revealed highest rates, however, they rather represent nitrate removal rates than denitrification rates.

Further, the number of studies is limited due to missing values about the amount of used soil in many other studies. Often, denitrification rates are given in N per area over time, for example $g\,N\,m^{-2}\,d^{-1}$. This value can be transformed into the unit used in this study, if information about the surface of the experimental setup and either volume of sediments or sediment mass is provided. Unfortunately, many studies lack this information. In addition to that, the comparison of denitrification rates is always challenging. Taking all important parameters into account, there are very few studies using the same method, in the same ecosystem, in the same climate, during the same vegetation period and so forth.

All in all, this table illustrates the heterogeneity of denitrification measurements. Hence, the interpolation of rates to a method or a spatial unit is error-prone and not precise. Therefore, denitrification rates should be investigated at each specific field site,

ideally in temporal and spatial extend to obtain detailed and precise information about the quantity of denitrification rates.

TABLE 7.1: Comparison of denitrification rates expresses as $\mu mol\,N\,g^{-1}\,soil\,d^{-1}$. Values sorted top-down by the mean rate. Methods from different studies like ^{15}N, mass balance approaches (MB) and acetylene inhibition methods (AIM).

Study	Method	Min rate	Max rate	Mean rate	Comment
Yeomans et al., 1992		0.0262	2.4810	1.2536	Arable field
Cooper, 1990	MB	0.0007	2.3143	1.1575	Riparian forest
Schipper et al., 1993	MB	0.7543	0.8914	0.8229	Riparian forest
Woodward et al., 2009	In-situ AIM	0.0643	0.4143	0.2393	Riparian forest
Warneke et al., 2011	AIM	0.1731	0.1841	0.1786	Denitrification bed
Pfenning et al., 1996	AIM	0.0700	0.2000	0.1350	Nitrate rich riverbed
Dodds and Jones, 1987	AIM	0.0065	0.2178	0.1122	Oligotrophic freshwater
Test-2, Treat. 5	MB	-	-	0.0837	Potential rate
Test-2, Treat. 1	**MB**	-	-	**0.0690**	**Mixing zone, AGW**
Test-1,Treat. 2	MB	-	-	0.0687	DOC limited
Test-2,Treat. 2	**MB**	-	-	**0.0676**	**Mixing zone, GW**
Test-1,Treat. 1	MB	-	-	0.0611	Potential rate
Test-1,Treat. 3	MB	-	-	0.0555	DOC, Temp limited
Test-1,Treat. 4	MB	-	-	0.0478	DOC, Temp, NO3 limited
Test-2, Treat. 3	**MB**	-	-	**0.0406**	**Saturated zone, AGW**
Clague et al., 2015	15N	0.0005	0.0686	0.0346	Dairying catchment
Test-2,Treat. 4	**MB**	-	-	**0.0341**	**Saturated zone , GW**
Test-2,Treat. 5	15N	0.0226	0.0298	0.0262	Potential rate
Mathieu et al., 2006	15N	0.0006	0.0423	0.0214	Arable field
Test-1,Treat. 2	15N	0.0156	0.0223	0.0189	DOC limited
Test-2,Treat. 1	**15N**	**0.0152**	**0.0202**	**0.0177**	**Mixing zone, AGW**
Test-1,Treat. 1	15N	0.0151	0.0186	0.0169	Potential rate
Test-2,Treat. 2	**15N**	**0.0139**	**0.0188**	**0.0164**	**Mixing zone, GW**
Test-1,Treat. 3	15N	0.0139	0.0166	0.0153	DOC, Temp limited
Lindau et al., 2011	15N	0.0068	0.0218	0.0143	River swamp
Silver et al., 2010	15N	0.0071	0.0214	0.0143	Tropical forest
Trauth et al., 2016	MB	-	-	0.0130	Riparian forest
Test-2,Treat. 3	**15N**	**0.0097**	**0.0130**	**0.0113**	**Saturated zone, AGW**
Test-1,Treat. 4	15N	0.0111	0.0090	0.0101	DOC, Temp, NO3 limited
Bateman and Baggs, 2005	AIM	0.0033	0.0143	0.0088	Arable field
Addy et al., 2000	In-situ 15N	0.0067	0.0088	0.0079	Riparian forest
Test-2,Treat. 4	**15N**	**0.0052**	**0.0071**	**0.0062**	**Saturated zone, GW**
Kellogg et al., 2005	In-situ 15N	0.0007	0.0084	0.0046	Riparian wetland
Pinay et al., 1993	AIM	0.0002	0.0039	0.0020	Riparian forest
Yang et al., 2014	15N	0.0011	0.0022	0.0017	Tropical forest

7.4 Limitations of Denitrification Experiments

7.4.1 Batch Experiments

Batch experiments were conducted to determine denitrification rates under controlled conditions and investigate potential limiting factors. A major uncertainty of these findings is related to the inherent nature of batch experiments, initial conditions had to be changed. Even though batch conditions might represent in-situ conditions in chemical and physical matters, many parameters were altered with unknown consequences.

In particular, sediments were incubated with a groundwater medium which might not reflect the actual groundwater conditions. Furthermore, the water-sediment ratio was altered. Albeit, taking sediments from zones which were not limited in nitrate, it could not be excluded that amendments of labeled nitrate did not stimulate bacterial growth in the batches. The same accounts for DOC amendments. Bacterial growth would consequently result in higher denitrification rates and would not represent the processes in the field site. Furthermore, unlike natural conditions, strictly anoxic conditions were created in the batches. This would consequently enforce bacteria to denitrify which would therefore lead to overestimation of denitrification.

On the contrary, this study exclusively measured denitrification rates, excluding other nitrogen related processes like nitrification. To be precise, the calculation of total denitrification rates depends on the consumption of labeled nitrate, thereby neglecting additional nitrate sources. As a result, the labeled nitrate source depletes over time, thus leading to nitrate limited systems. Therefore, these batch experiments might underestimate denitrification rates in the riparian zone by excluding potential nitrate inputs.

7.4.2 Differences between Methods

With regard to the the methods used in this study, differences in denitrification rates were found between the two different methods. Specifically, denitrification rates obtained from the decrease of nitrate in the liquid phase were around 3 - 4 times higher than results obtained from dinitrogen increase (see table 7.1). One assumption was that nitrate might diffuse into soil aggregates or bind with, or adsorb on some other substances thereby concealing from liquid sampling. However, this theory could be rejected as nitrate concentrations in the abiotic control did not decline over time. This led to the assumption of bacterial related processes causing the discrepancy.

Dissimilatory reduction of nitrate to ammonium (DNRA) was identified by Silver et al. (2010) to cause major losses of nitrate in denitrification experiments, accounting for

75 % of consumed nitrate. Rivett et al. (2008) emphasizes the role of DNRA in nitrate limited systems. Besides excess ammonium and limited nitrate concentrations, DNRA requires highly reducing conditions. These conditions were created by increasing the soil water content in the experiments. Thereby, representing a rather flooded riparian zone. As low nitrate contents were observed in treatment 1 and 4 from test-1, DNRA might explain some deviation.

Different approaches to describe the discrepancy between nitrate decrease and nitrogen increase could be bacterial assimilation of nitrate for the conversion into biomass (Woodward et al., 2009), the underestimation of denitrification rates due to missing NO and N_2O measurements (Rivett et al., 2008), or inaccuracy during the GC-MS calibration. In fact, the uncertainty of the headspace pressure evolution might play a major role in the underestimation of denitrification rates. Furthermore, the interpolation of $^{28}N_2$ was based on the enrichment of labeled nitrate in the nitrate source pool after preparation and. Uncertainties derived from this measurements propagated and affected all future calculations. Furthermore, disregarding the fractionation effects would subsequently lead to the underestimation of produced $^{28}N_2$ and thus underestimation of denitrification rates (Kendall and Mcdonnell, 1998).

7.4.3 DOC Amendments

DOC was added to some treatments to achieve conditions which were not limited in DOC and thus, would represent potential denitrification rates. Nevertheless, it was expected that DOC contents in the sediments would be high enough to facilitate denitrification under limited conditions. However, this was not the case in test-1. Here, around 11.7 mg/L of DOC was added as acetate to treatment 2-4 (see table 5.1) but DOC measurements shortly after the preparation showed concentrations below the amendment value (see batch 4-12 in table A.4). This implies that sediments did not contain considerable and measurable DOC and that DOC was consequently diluted by the residue groundwater from sediment pores. In contrast to test-1, the natural DOC concentrations in sediments from test-2 ranged between 5 and 10 mg/L. Thus, representing field conditions (see batch 1-12 in table A.4).

This difference can be caused by the different sediments sampling technique. Sediments from test-1 were collected from an in-stream gravel bank and from soil cores at the riparian zone. In contrast to test-2, where fresh sediments were used without further processing, sediments from test-1 were sieved and incubated in the groundwater. Furthermore, high concentration of iron oxides were present in the soil-groundwater continuum of test-1. These two factors might explain why natural DOC concentrations in test-1 were substantially smaller compared to test-2. First, DOC can be adsorbed on

soil particles and by sieving the sediments, smaller soil fractions with comparatively high surface areas were removed. Thus, the available area for adsorption and eventually the amount of DOC in the sediments was reduced (Jardine et al., 1989; Davis, 1982). Secondly, Gu et al. (1995) reported that natural organic matter can adsorb on iron oxide surfaces where they are strongly bound. This can be applied to test-1 as the metal soil tubes were extremely covered with oxidized iron. As a result, organic carbon was not reversely adsorbed to the soil and available during the experiments, but bound to the iron oxides at the metal tube or as inactive complexes in the groundwater. To conclude, the approach of incubating sediments in groundwater might have influenced the conditions in test-1 because fine particles were removed from the sediments and further effects from iron oxidation might have influenced DOC availability.

The second uncertainty is related to the steep decline of concentration that can be observed in all treatments from test-1 (see figure 6.3) and in treatment 5 from test-2 (see figure 6.7). These were treatments substantially amended with DOC (acetate). Obvious differences in DOC variability over time in the different treatments were observed in test-2. Treatments 1 to 4 received only minor DOC additions in comparison the treatment 5. Treatments 1 to 4 show no significant decrease over the course of 7 days (see treatment 1-4 in figure 6.7) but varying concentrations were observed with minor increase and decrease. In treatment 5, however, a sharp decline was observed until day 2. Later, the concentrations remained constant for the rest of the experiment. The steep decline in treatment 5 cannot be found in other treatments from test-2, but remarkably coincides with curves from test-1. While in test-1 acetate was the sole source for DOC, treatments in test-2 contained natural organic carbon and only treatment 5 received considerable additional DOC amendments of around 5.7 mg/L (see table 5.2). It appears that all treatments with DOC amendments show a fast consumption of the added acetate. In fact, treatment 5 received 5.7 mg/L of acetate and 6.7 mg/L of DOC were consumed during the first two days. After the consumption of this additional DOC source, bacteria might switch back to natural DOC, resulting in much smaller denitrification rates (see treatment 5 in figure 6.7).

The consumption of artificial DOC as acetate seems to facilitate much higher nitrate removal rates than organic carbon from the field site. This assumption is supported by Sirivedhin and Gray (2006) and Pfenning and McMahon (1996). They state that the addition of highly degradable acetate supports significantly higher denitrification rates than amendments of DOC as phenol or humic acid. Furthermore, Pfenning and McMahon (1996) reported that organic carbon derived from surface water might be

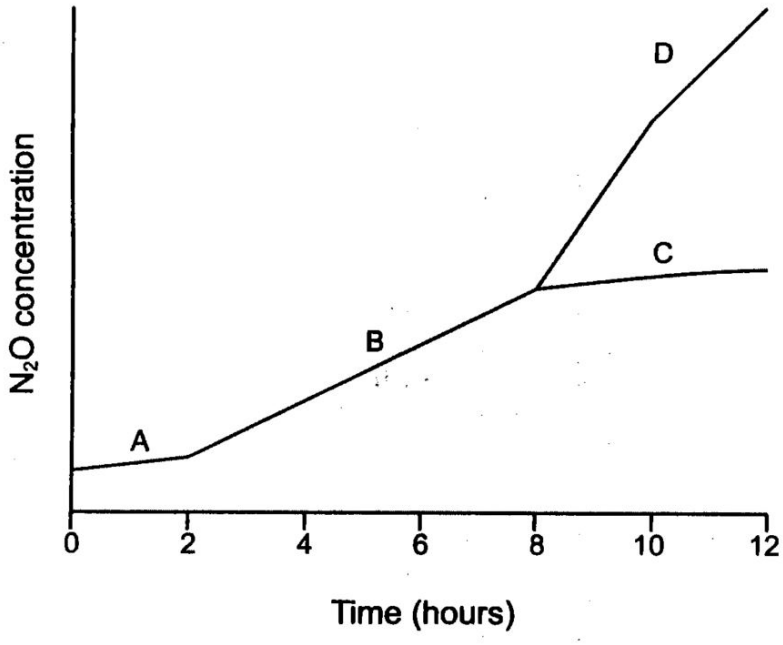

FIGURE 7.1: Phases of denitrification in batch experiments by Groffman et al. (1999).

more labile compared to sedimentary carbon. The addition of DOC as acetate in batch experiments will consequently lead to overestimation of denitrification rates.

7.4.4 Interpretation

One main objective in this study was the quantification of denitrification rates in batch experiments. Therefore, the increase of N_2 in the headspace was measured and compared to the decrease of nitrate. The decrease of nitrate, however, might not represent denitrification rates as it integrates nitrate removal from the assimilation of N into biomass and respiration of N during denitrification.

Intentionally, it was planned to measure rates representing the initial bacterial communities, thus respiration without significant assimilation. A schematic approach by Groffman et al. (1999) can be seen in figure 7.1 and shows interpretations for different phases during denitrification in batch experiments. Compared to the results from figure 6.1 and 6.5, this schematic approach helps to identify the processes during denitrification in test-1 and test-2.

The phases shown in this figure could be adopted for this study as follows: Phase A, a lag phase between day 0 and 1. During this phase the bacterial community adopts to new conditions without significant production of dinitrogen. Phase B, a linear phase of denitrification between day 1 and depending on the treatment day 2 (treatment 1 and 2) or 4 (treatment 3 and 4). The decreased rate between day 2 and 4, in treatment 1 and

2, would represent phase C, the nitrate depletion phase, during which denitrification is limited by nitrate concentrations. In fact, this phase describes the depleting phase in treatment 1 from test-1. However, this theory cannot be adopted for test-2 as nitrate levels range still between 25 and 15 mg/L (see figure 6.7).

As an alternative for phase C, they present phase D, the oxygen depletion phase with increased rates in contrast to phase B. This would require residue oxygen in the batches after preparation which was consumed between day 0 and 4 in test-2. Therefore, this phase would imply insufficient outgassing with the Ar-CO_2 mixture during batch preparation and high residue O_2 concentrations at day 0. This scenario would imply that residue oxygen was consumed until day 4 which would consequently lead to limited denitrification rates. In this scenario, actual denitrification rates would have been measured from day 4 and phase D would represent these denitrification rates.

On the other hand, the increased dinitrogen production from day 4 could also be the result of bacterial growth, stimulated by nitrate and DOC amendments. If this was the case, rates between day 4 and 7 would falsify actual denitrification rates, with consequential overestimation of denitrification rates.

As no evidence for either process C or D could be found in the current study, it was assumed that rates between day 0 and 7 would generally describe denitrification rates the best. Thus, factors such as bacterial growth and inadequate aerobic conditions were neglected due to missing evidence. However, oxygen saturation and bacterial activity should be investigated in future studies.

7.5 Implications

Denitrification rates obtained in this study revealed comparable denitrification rates to field experiments by Trauth et al. (2016) at the field site. Nixdorf and Trauth (2016) investigated the hydraulic conductivity at the field site. With this information about flow rate and travel time and conceptualized denitrification kinetics from different studies, a first model approach approximated the nitrate removal function of this riparian zone.

Furthermore, higher denitrification rates were measured in the mixing zone which is supported by results from Gassen et al. (2016). In fact, this implies the overall possibility and reliability of batch experiments to determine representative denitrification rates. Significant influence of temperature on denitrification rates was detected which is in accordance with nitrate isotope data from the field site. The data indicated higher denitrification rates during summer months compared to winter (K. Knöller, personal communication). The influence of temperature should be considered in future experiments to classify results with regard to seasonality and temperature gradients in the soil.

Moreover, significantly higher denitrification rates were found in the mixing zone, indicating a vertical gradient of denitrification potential in the soil-groundwater continuum. Incorporating these findings into hydrological denitrification models could therefore approve the accuracy of denitrification rates in specific zones.

Results from this study, from observation wells (Trauth et al., 2016) and from multi-level wells (Gassen et al., 2016) suggest high nitrate turnover rates for the field site. Therefore, this riparian zone should be maintained and protected to conserve the inherent ecosystem services. However, new studies should be performed to validate this study and especially because spatial variability on a horizontal level remains unknown.

7.6 Outlook

Future research should attempt to clarify the potential for denitrification at the field site. The use of multi-level wells and a large amount of observation wells facilitate high resolution tracer tests at the field site (Addy et al., 2000). More batch experiments should be conducted to obtain high resolution data about vertical and horizontal variability of denitrification rates which can be incorporated into heterogeneity models.

To improve future batch experiments, batches should be sacrificed after each day. This would imply to create one batch per day and treatment assuming that conditions in each batch would be the same. However, this would require a lot of sediment from each spatial unit and the amendment of more tracer in total. Even though it would not be possible to measure the increase of nitrogen in one batch over time, sacrificing batches would not interfere with the batch ecosystems and could produce undisturbed results. Furthermore, as batches would be sacrificed after each measurement, this method would allow detailed sampling of sediment and solution each day. Hence, it would facilitate to measure oxygen saturation, nitrate isotopes, headspace pressure or bacterial activity. Oxygen saturation would provide information if favourable conditions for denitrification would be present. Sampling of nitrate isotopes would allow to calculate denitrification rates via isotope pairing method. Headspace pressure measurements would help to improve the accuracy of GC-MS calibrations. Finally, bacterial activity would state whether the bacterial community would represent in-situ conditions. Due to the limited amount of sediment and solution in this study and the large impact on the batches, we were unable to measure these important parameters.

Furthermore, batches should be stored and transported at their respective temperature, especially during GC-MS measurements. Thus, avoiding unnecessarily increasing the batch temperature which evidently influences denitrification rates. Here, the use of a cooler is suggested to keep the batches at constant temperature between duplicate

measurements. If possible, IC measurements should be performed on a daily basis to analyze residue nitrate concentrations in the batches. GC-MS measurements should subsequently continue until the nitrate pool is depleted. Unfortunately, this was impossible for this study and GC-MS measurements had to stop at a point where dinitrogen concentrations increased steeply.

Chapter 8

Conclusions

Overall, this study demonstrated the feasibility of batch experiments for the determination of denitrification rates in a riparian zone. Two experiments were conducted to determine (i) potential denitrification rates and limitations and (ii) actual denitrification rates and spatial heterogeneity. A $^{15}NO_3^-$ tracer was applied to sediments and ^{15}N was subsequently quantified in the headspace. This method was further validated with nitrate and DOC measurements from the liquid phase.

Temperature was identified as a limiting factor for denitrification and substantially higher rates were found at 20°C compared to 12°C, with 0.019 and 0.015 µmol N g^{-1} soil d^{-1}, respectively. No limiting influences of nitrate and DOC concentrations were found in this study. However, this was caused by the experimental setup and results indicated that both, nitrate and DOC might limit denitrification rates under different conditions. A vertical heterogeneity of denitrification rates was found in the second test where sediments from the mixing zone and from the saturated zone were compared. With 0.017 µmol N g^{-1} soil d^{-1}, denitrification rates in the mixing zone were significantly higher than rates from the saturated zone with 0.009 µmol N g^{-1} soil d^{-1}.

Measured rates were in the same order of magnitude with literature values for comparable study sites. Moreover, the results from this study were comparable to prior studies from the field site which identified the mixing zone as a "hot spot" for nitrate removal, higher denitrification rates during summer months and particularly, measured similar denitrification rates with a mass balance approach. Nevertheless, a difference between the headspace and mass balance approach was found with 3-4 times larger denitrification rates in the mass balance approach. In this case, however, the mass balance approach represents a multitude of different nitrate removal processes and not only denitrification which eventually results in higher nitrate removal.

Two problems concerning the quality and quantity of DOC were identified in this study. First, sediments from the first test were sieved and small fractions were removed.

This can explain why sediments did not contain substantial DOC concentrations. Secondly, DOC was amended as acetate which is more labile compared to natural organic carbon led to the overestimation of denitrification rates in certain treatments.

However, if validated with in-situ measurements, batch experiments from this study can provide considerable information as they measured high resolution denitrification rates under controlled conditions. According to Groffman et al. (2009), a deficit in denitrification data restricts the implementation of heterogeneity models for upscaling of denitrification rates. Therefore, the urge for more data is unambiguous. Future studies should consequently focus on the understanding of spatial variability on different scales. Horizontal transects from field to stream, and high resolution vertical transects from unsaturated to saturated zone could provide useful information

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Appendix A

Appendix - Additional Information

A.1 Pre-Experiments and Stock Solution Preparation

Pre-experiments were conducted to get information about the optimal amount of sediment as well as optimal DOC and nitrate concentrations. Thus, pre-experiments consisted of several measurement series with varying amounts of sediment, DOC and nitrate. Furthermore, containers, syringes and septa were compared for best feasibility during measurements.

A.1.1 Influence of Headspace Volume

The effects of different headspace volumes were tested using different sized serum bottles (see figure 5.1), filled with identical volumes of sediment and liquid, resulting in different headspace volumes. Preliminary results of multiple replicate measurements show evidence to suggest a higher accuracy as well as a higher detection limit at smaller headspace volumes. Due to the limited amount of sediment, 100 ml serum bottles were used for further experiments resulting in a headspace volume between 30 and 41 ml.

A.1.2 Syringes and Injection Volume

To minimize contamination with ambient air during the injection, different syringes as well as different injection techniques were tested. The *VICI Precision Sampling A-2* syringe was chosen for further measurements. This syringe was especially designed for high pressure gas sampling and is equipped with a push-button valve to lock in the sample. Furthermore, all parts were replaceable and easy to repair, allowing constant maintenance to keep the quality of the measurements high over time. To avoid the puncture of the septa, side-port needles with a cone tip were used. Furthermore, the injection of 20 µL gas sample produced convincing results in the GC-MS. The amount was large enough to detect small changes in concentrations. Furthermore, the readability of the syringe was convincing for 20 µL and allowed precise adjustment of this volume.

A.1.3 Septa

The air tightness of the experimental setup and precision of the measurements were improved by changing the different septa on a regular basis. For instance, the septa at the injector was changed before each series of measurements and the septa of the pressure reducer (STD) was changed as soon as a drop in chamber pressure was observed between two measurements. The batches were sealed using gas tight rubber septa and aluminum seal crimps. To yield a higher durability of the septa, each injection was performed on a different spot of the septa and the use of side-port needles with cone tips was preferred to bevel tips. Additionally, the septa was compacted after each measurement by pressing and rolling to a solid surface like the lab bench. Afterwards, tightness was checked visually by controlling the bulge of the septa which indicated overpressure inside the batches. Furthermore, all instrumental interfaces were checked for air leaks with leak detection spray.

A.1.4 Stock Solution Preparation

Stock solutions of sodium acetate and labeled potassium nitrate were prepared and stored for future measurements. Basic calculations for the preparation of stock solutions will be explained by the following equations in this section. A comparison of cation and anion concentrations in groundwater and artificial groundwater can be found in table A.1.

Following equation A.1, 1 gram of labeled potassium nitrate was dissolved in 9.7943 ml MQ to obtain a 1 mol stock solution.

$$m = McV \tag{A.1}$$

with ***m****, mass [g],* ***M****, molar mass [g/mol],* ***c****, concentration [mol/L],* **V**, volume [L]

For further application the solution was diluted 1:10 to receive 10 ml of a 0.1 mol solution by using equation A.3. Hereafter, the solution was stored in *Eppendorf* tubes at -20°C for future application. The production of the sodium acetate stock solution was conducted likewise. However, this solution was filtered prior to storage using 0.45 µm filters, attached to sterile syringes, and was stored in sterile *Falcon* tubes.

Studies by Clague et al. (2015) and Cannavo et al. (2004) suggested the application of 1 to 200 µg $^{15}N\ g^{-1}$ soil. The amount of 10 µg $^{15}N\ g^{-1}$ soil used by Clague et al. (2015) was finally chosen as it further represents the current groundwater conditions in the study area. The required volume of the nitrate solution and the amount of moles were calculated using equation A.1 and equation A.2.

$$n = \frac{m}{M} \tag{A.2}$$

with ***n****, number of moles,* ***m****, mass [g],* ***M****, molar mass [g/mol]*

The total required amount of sodium acetate to facilitate a complete transformation from nitrate to dinitrogen was calculated in mole. The calculation from required moles to the respective volume of the sodium acetate solution was performed using equation A.2 and equation A.1.

TABLE A.1: Composition of groundwater from the field site and stock solution of artificial groundwater. Comparison of main cations and anions.

	Mg^{2+}	Ca^{2+}	Na^{+}	SO_4^{2-}	Cl^{-}	HCO_3^{-}
GW [mmol/L]	0.617	3.000	1.304	0.521	0.423	3.000
AGW [mmol/L]	0.120	0.185	1.141	0.475	0.329	3.026

Based on prior measured groundwater parameters of cations (Na^{+}, Mg^{2+}, Ca^{2+}) and anions (SO_4^{2-}, HCO_3^{-}) artificial groundwater (AGW) was prepared. The required amount of each element was calculated (see equation A.2 and A.1) and the respective amounts of $MgSO_4$ x $7*H_2O$, Na_2SO_4, $CaCl_2$ x $2*H_2O$ and $NaHCO_3$ were diluted in MQ. Table A.1 shows groundwater concentrations from prior measurements compared to AGW.

The final solutions were prepared by mixing the nitrate stock solution with the sodium acetate stock solution and either artificial groundwater or groundwater. The final concentrations were calculated with equation A.3 and further validated with IC and DOC measurements. Concentrations of nitrate and DOC for the final solution from test-1 are documented in table 5.1 and for test-2 ind table 5.2.

$$c_1 V_1 = c_2 V_2 \tag{A.3}$$

with c_1*, concentration of solution 1 [g/L],* V_1*, volume of solution 1 [L],* c_2*, concentration of solution 2 [g/L],* V_2*, volume of solution 2 [L]*

A.2 Chemicals and Materials

The following chapter will comprise chemicals and materials used in this study. Basic laboratory equipment is not listed.

A.2.1 Chemicals

Table A.2 lists the most important chemicals used in this study.

TABLE A.2: Chemicals used in this study

Abb. or formula	Name	Company	Comments
HSG	20% CO_2, rest Ar	Linde AG	
STD	1% N_2, 1% CO_2, 0.5% N_2O, rest Ar	Linde AG	
$MgSO_4$ x 7 $*$ H_2O	Magnesium sulfate heptahydrate	Sigma-Aldrich	Cat.: 63138
Na_2SO_4	Sodium sulfate	Sigma-Aldrich	Cat.: 71962
$CaCl_2$ x 2 $*$ H_2O	Calcium chloride dihydrate	Sigma-Aldrich	Cat.: C5080
$NaHCO_3$	Sodium hydrogencarbonate	Sigma-Aldrich	Cat.: 401676
$K^{15}NO_3$	Potassium nitrate-^{15}N	Sigma-Aldrich	Cat.: 335134
$C_2H_3NaO_2$	Sodium acetate	Sigma-Aldrich	Cat.: S2889
FERR	Ferrozine	Sigma-Aldrich	Cat.: 82950
HAHCL	Hydroxylamin-HCl	Sigma-Aldrich	Cat.: 238074
FE	Fe(II)sulphate heptahydrate	Sigma-Aldrich	Cat.: 215422

A.2.2 Materials

Table A.3 lists the most important materials used in this study. Focus is set on consumable supplies and considerable equipment. Basic laboratory supplies like volumetric flasks, Eppendorf pipettes etc. are considered as basic requirements and not listed.

TABLE A.3: Materials used in this study

Name	Details	Company	Comments
Syringe 50µL	Vici high precision A-2	Sigma-Aldrich	Cat.: 22269-U
Syringe 100µL	Vici high precision A-2	Sigma-Aldrich	Cat.: 22270-U
Serum vials	N20 crimp neck	Ochs Laborbedarf	Cat.: 102046
Crimp caps	N20, center hole	Macherey-Nagel	Cat.: 702804
Crimp septa	Butyl septa, black	Ochs Laborbedarf	Cat.: 102049
Syringe filter	45 µm, PVDF filter	Sigma-Adrich	Cat.: Z355518
Eppendorf tubes		Sigma-Aldrich	Cat.:
Falcon tubes		Sigma-Aldrich	Cat.:
Ferrozine plate	Nunc Microwell 96K	Thermo Scientific	Cat.: 269620

A.3 Results

TABLE A.4: Evolution of Nitrate and DOC concentrations from the first series of measurements

	Nitrate Concentrations [mg/L]				DOC concentrations [mg/L]			
Batch	Day 1	Day 2	Day 5	Rate	Day 1	Day 2	Day 5	Rate
1	11.33	0.51	0.00	11.33	30.99	17.26	1.14	29.84
2	14.52	0.55	0.00	14.52	29.04	18.05	0.90	28.14
3	13.82	1.90	0.00	13.82	29.28	19.50	2.87	26.41
4	15.34	9.61	3.28	12.06	7.09	0.47	0.36	6.73
5	12.76	11.34	3.12	9.64	6.69	0.16	0.07	6.62
6	17.18	13.59	6.26	10.93	7.25	0.21	0.00	7.25
7	15.13	13.41	10.41	4.72	8.55	0.16	0.28	8.27
8	19.80	14.38	10.01	9.79	6.93	0.64	0.03	6.90
9	22.84	1.74	12.41	10.43	8.60	0.00	0.00	8.60
10	9.23	4.11	1.17	8.06	8.26	0.00	0.00	8.26
11	10.40	3.27	0.57	9.84	8.19	1.46	0.30	7.89
12	11.02	3.87	0.42	10.61	7.81	0.00	0.00	7.81
13	22.77	9.23	9.58	13.19	27.35	30.60	29.39	-2.04
14	22.83	8.79	8.02	14.81	33.89	27.19	26.47	7.42
15	22.90	7.43	5.85	17.05	38.30	27.22	25.05	13.25

TABLE A.5: Evolution of Nitrate and DOC concentrations from the second series of measurements

	Nitrate Concentrations [mg/L]					DOC concentrations [mg/L]				
Batch	Day 0	Day 1	Day 2	Day 5	Rate	Day 0	Day 1	Day 2	Day 5	Rate
1	25.60	19.10	13.70	4.56	21.04	9.69	9.25	9.23	9.43	0.26
2	25.80	18.70	13.90	5.80	20.00	11.35	9.31	9.00	11.44	-0.10
3	28.40	20.90	17.00	9.53	18.87	9.19	9.39	8.92	8.95	0.23
4	26.30	20.60	15.60	6.39	19.91	11.31	11.59	10.71	10.63	0.68
5	26.10	20.20	15.10	5.94	20.16	10.63	11.41	10.53	10.70	-0.07
6	24.10	20.40	14.70	5.45	18.65	11.22	11.92	10.97	10.71	0.51
7	22.73	21.30	15.70	11.10	11.63	5.17	6.33	5.43	4.47	0.70
8	23.13	21.70	19.20	14.50	8.63	4.75	6.31	4.45	4.83	-0.08
9	22.93	21.50	15.10	7.97	14.96	6.16	6.04	4.80	5.41	0.74
10	23.90	23.90	20.90	14.50	9.40	7.37	7.30	6.56	6.75	0.62
11	24.20	22.60	21.20	14.10	10.10	6.87	8.02	6.66	6.90	-0.03
12	24.00	22.50	21.30	13.90	10.10	7.72	8.05	7.66	7.03	0.69
13	25.10	5.06	2.84	0.13	24.97	14.39	7.90	7.87	7.43	6.96
14	23.80	5.01	2.54	0.10	23.70	14.90	7.77	7.12	7.45	7.45
15	24.10	4.55	2.34	0.10	24.00	14.05	7.67	6.94	6.93	7.12
16	25.00	25.50	25.90	24.50	0.50	36.08	35.63	33.45	33.39	2.69
17	24.70	26.00	25.00	21.70	3.00	32.20	31.57	31.00	28.37	3.83

Appendix B

Appendix - Figures

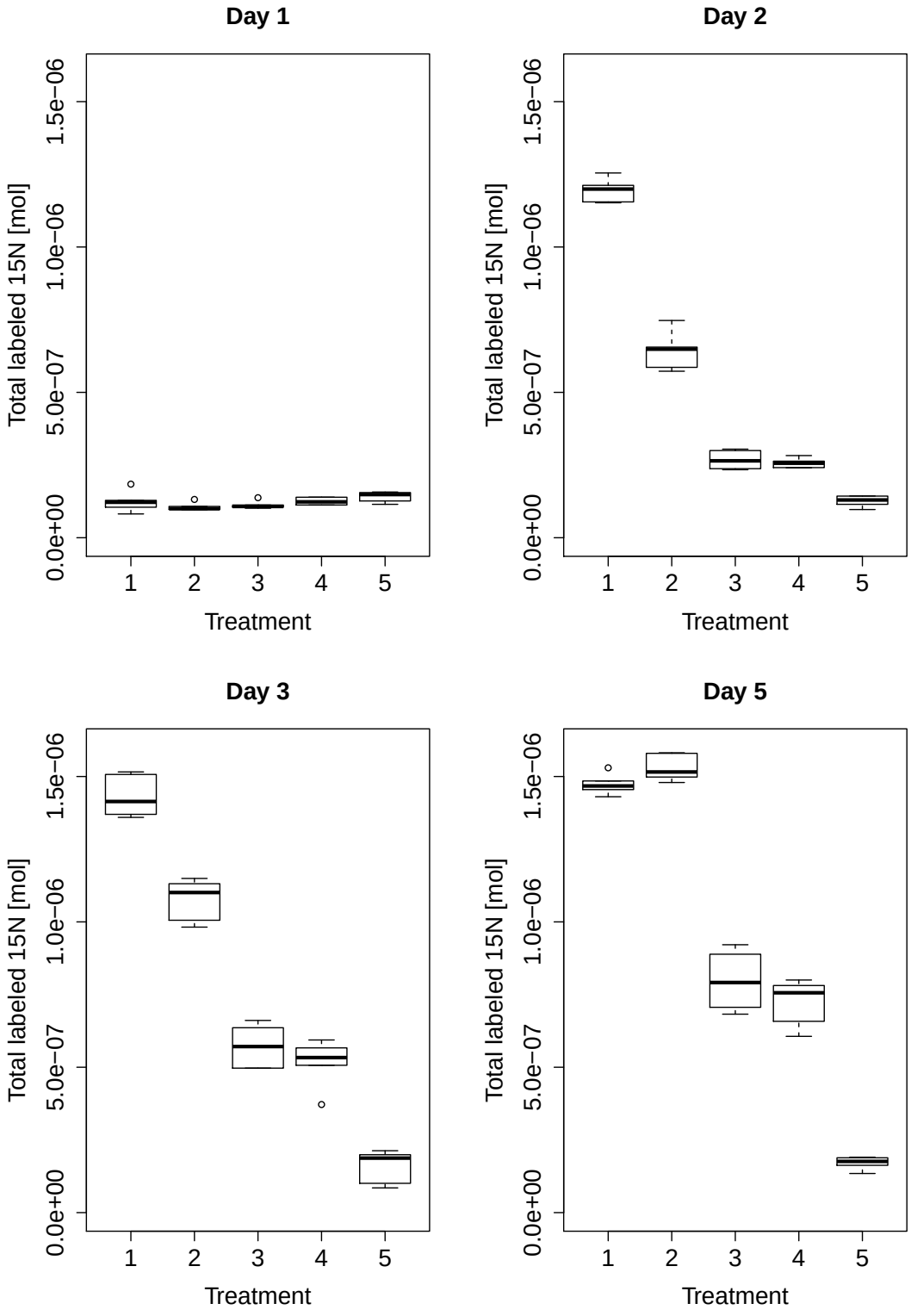

FIGURE B.1: Comparison of dinitrogen evolution between different treatments on different days from the first series of measurements. Treatment 1: 100% DOC, 100% NO_3^-, 20°C, Treatment 2: 25% DOC, 100% NO_3^-, 20°C, Treatment 3: 25% DOC, 100% NO_3^-, 12°C, Treatment 4: 25% DOC, 25% NO_3^-, 12°C, Treatment 5: Abiotic control, 20°C.

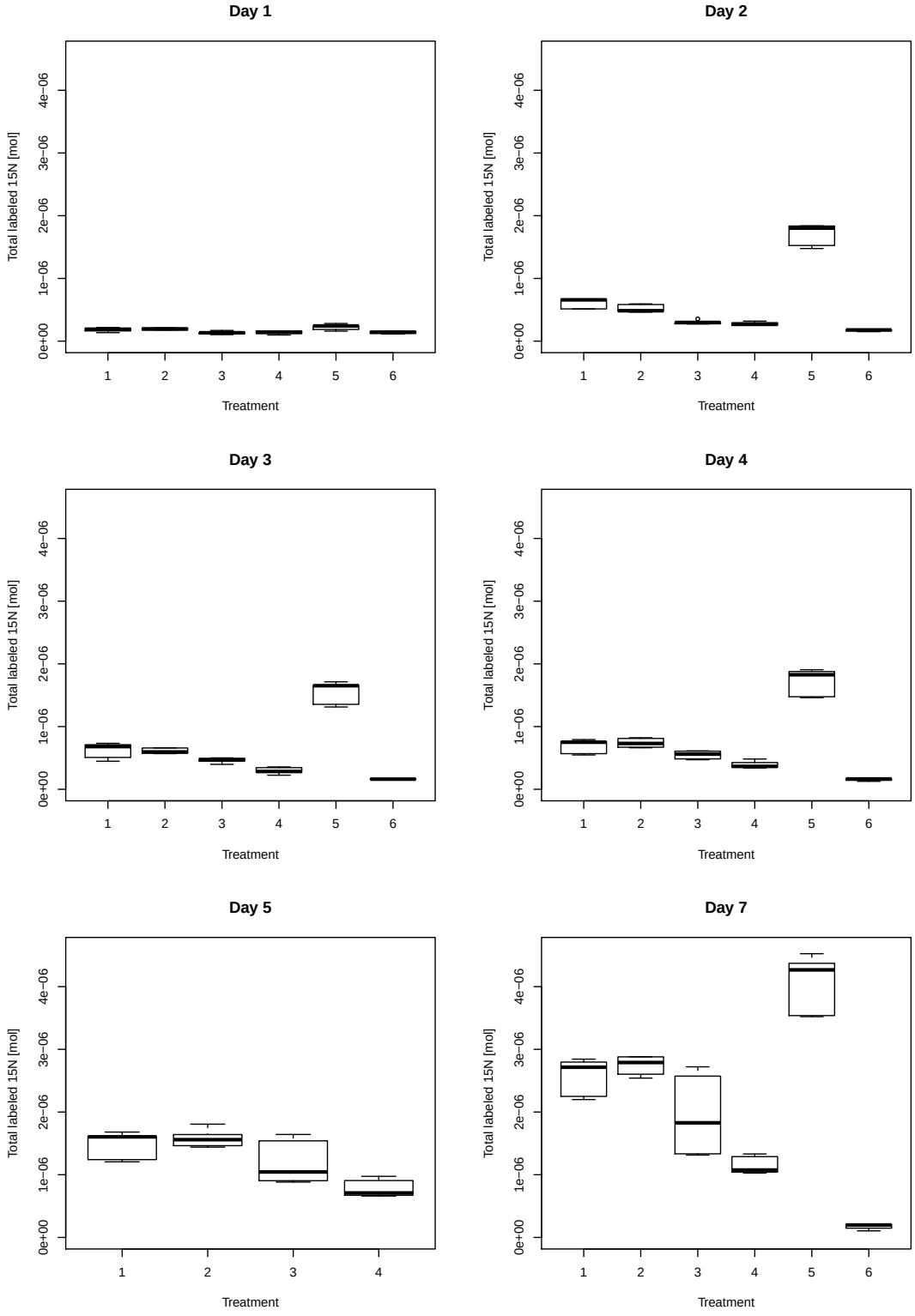

FIGURE B.2: Comparison of labeled dinitrogen evolution between different treatments on different days from the second series of measurements. Treatment 1: Mix zone, AGW, 12°C, Treatment 2: Mix zone, GW, 12°C, Treatment 3: Sat. zone, AGW, 12°C, Treatment 4: Sat. zone, GW, 12°C, Treatment 5: Sat. zone, GW, 20°C+ DOC, Treatment 6: Abiotic control, 20°C.

Declaration of Authorship

Hiermit erkläre ich, André Böker, dass die Arbeit selbständig und nur unter Verwendung der angegebenen Hilfsmittel angefertigt wurde.

Signed:

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Date:

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