

Introduction and fundamentals **Universität für Bodenkultur Wien**
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Master thesis

AQUATIC GREENHOUSE GAS PRODUCTION AND DISSOLVED ORGANIC MATTER QUALITY IN STREAMS OF DIFFERENT POLLUTION LEVELS

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Abstract

Anthropogenic impacts, such as increased nutrient loading of inland freshwater streams, stream straightening, channelization and other effects contribute - as indicated by new scientific research - to the production and increasing concentrations of greenhouse gases. The three superficial greenhouse gases emitted by inland freshwater streams are carbon dioxide (CO₂), nitrous oxide (N₂O) and methane (CH₄).

The main goal of this thesis was to investigate the production of in-stream greenhouse gases, depending on the ambient nitrogen and dissolved organic matter pollution. This was done by selecting 9 stream reaches comparable in size and hydromorphology, showing varying influence through agriculture. The streams were then grouped according to their pollution - resulting from the agricultural influence - which allowed a comparison between pristine or only slightly impacted streams and moderately / heavily polluted streams. The main influencing factors and parameters of stream greenhouse gas production were determined, as well as seasonal variations. For this purpose, for each stream, water, gas and sediment samples were taken and analysed in spring and summer of 2015. Gas samples were analysed for CO₂, N₂O and CH₄ concentration, water samples for ammonium (NH₄-N), nitrate (NO₃-N), nitrite (NO₂-N), dissolved organic carbon (DOC), dissolved organic matter (DOM) concentrations, and sediment samples for organic matter and nitrogen concentrations.

In specific, the quality and quantity of dissolved organic matter (DOM) in the study streams as potential main influencing factor for greenhouse gas production, was investigated. In addition, the denitrification potential of the stream sediments was determined via the acetylene inhibition technique.

The analysis of the gained data showed that the concentration of CO₂ and N₂O was highest in the highly polluted, and lowest in the lowly polluted streams. CH₄ and N₂O-concentrations were significantly higher in summer, while CO₂ concentrations were higher in spring. The dissolved CH₄ and CO₂ concentrations were positively correlated with DOC concentration as well as with most DOM parameters, water NO₂-N and NO₃-N concentration, while N₂O concentration only showed a negative correlation with sedimentary NO₃-N concentration. Optical properties of DOM analyses showed a dominance of humic-like (C) and tyrocine-like (B2) substances for all streams and the amount of all DOM fractions increased with the pollution. FIX values indicated a terrestrial source for most streams, especially in summer, and HIX values were quite low in spring and summer for all streams pointing towards plant biomass and animal manure as pollution source. β/α-ratio showed no clear tendency towards autochthonous or allochthonous material for most streams in spring and summer.

A global warming estimate, based on the greenhouse gas concentrations in the stream water and their specific global warming potential, was introduced in this thesis. It was highest in

streams of high and moderate pollution and lowest in streams of low pollution, underlining the set of problems possibly caused by anthropogenic influence.

The additional experiments showed that the denitrification potential was highest for sediments of streams showing high pollution levels and lowest for sediments of lowly polluted streams. The addition of organic carbon led to an increase of the denitrification potential.

No significant differences in greenhouse gas production could be found, neither concerning sampling in pools and riffles nor at different times of the day.

All in all, the thesis attests a direct connection between the in-stream nitrogen / DOC pollution (most likely caused by agriculture) and the production of greenhouse gases in the stream water. The sediment experiments, showing a linkage between increased pollution and denitrification potential, further support the findings. Global warming, driven by anthropogenically induced greenhouse gas production, and its effects on the planet is nowadays becoming more and more relevant. The findings of this thesis could be taken as an occasion to think about measures leading to a mitigation of the increasing stream water pollution through agriculture and therefore also to a possible decrease in the production of greenhouse gases.

Zusammenfassung

Neueste Forschungen belegen, dass anthropogene Einflüsse - wie zum Beispiel erhöhte Nährstoffbelastung der Fließgewässer infolge landwirtschaftlicher Nutzung nahegelegener Felder, Flussbegradigung oder Kanalisierung - entscheidend zur Produktion von Treibhausgasen in Fließgewässern beitragen können. Die drei wichtigsten Treibhausgase, die von Fließgewässern emittiert werden, sind Kohlendioxid (CO_2), Distickstoffmonoxid (N_2O) und Methan (CH_4).

Das Hauptziel dieser Arbeit war es, die Produktion von Treibhausgasen in Abhängigkeit der Belastung des Flusses mit Nährstoffen anthropogenen Ursprungs zu untersuchen. Zu diesem Zweck wurden 9 Flüsse (vergleichbar in Größe und Hydromorphologie) ausgesucht, die eine unterschiedliche Belastung vermutlich durch landwirtschaftliche Nutzung aufwiesen. Die Flüsse wurden anschließend gemäß ihrer Belastung – resultierend durch den landwirtschaftlichen Einfluss – gruppiert, was einen Vergleich von gar nicht oder nur sehr wenig belasteten Flüssen mit moderat oder stark belasteten Flüssen ermöglichte. Die Haupteinflussfaktoren für die Treibhausgasproduktion wurden ermittelt, ebenso wie saisonale Unterschiede. Zu diesem Zweck wurden in jedem der Flüsse im Frühling und Sommer des Jahres 2015 Wasser-, Gas- und Sedimentproben genommen. Die Gasproben wurden auf CO_2 -, N_2O - und CH_4 -Konzentrationen untersucht, während in den Wasserproben die Ammonium-, Nitrit- und Nitratkonzentrationen bestimmt wurden, ebenso wie DOC (dissolved organic carbon) und DOM (dissolved organic matter) Konzentrationen. In den Sedimentproben wurden die organische Materie sowie ebenfalls die Stickstoffkonzentrationen gemessen.

Ein Hauptaugenmerk der Arbeit liegt auf der Rolle der Qualität und Quantität von DOM als potentieller Haupteinflussfaktor der Treibhausgasproduktion in Flüssen. Des Weiteren wurde das Denitrifikationspotential der Flusssedimente mittels der Acetylen-Block Methode ermittelt, um mögliche Zusammenhänge mit der Gewässerbelastung zu erkennen.

Die Analyse zeigte, dass die Konzentration der im Wasser gelösten Treibhausgase am höchsten in Flüssen mit hoher, und am niedrigsten in Flüssen mit niedriger Verschmutzung war. CH_4 und N_2O -Konzentrationen waren im Sommer signifikant höher als im Frühling. CH_4 und CO_2 - Konzentrationen waren positiv mit DOC, den meisten DOM- Parametern sowie den NO_2 und $\text{NO}_3\text{-N}$ -Konzentrationen im Wasser korreliert, während N_2O lediglich eine negative Korrelation mit der sedimentären $\text{NO}_3\text{-N}$ -Konzentration aufwies. Die Analysen der optischen Eigenschaften ergab eine Dominanz von humic-artigen (C) und tyrocine-artigen (B2) Substanzen, und eine klare Steigerung aller DOM Fraktionen mit zunehmender Verschmutzung. Der berechnete FIX-Wert ergab eine Tendenz in Richtung terrestrischer Quellen, vor allem im Sommer. Der HIX-Wert war relativ niedrig für alle untersuchten Flüsse, was eine pflanzliche, beziehungsweise tierische Düngung als Verschmutzungsquelle

vermuten lässt. Die β/α -Rate ergab kein klares Bild in Bezug auf allochthones oder autochthones Material.

Ein „Global warming estimate“, das auf der Treibhausgaskonzentration im Flusswasser sowie dem jeweiligen „Global warming potential“ basiert, wurde in dieser Arbeit eingesetzt. Es war am höchsten in Flüssen mit hoher und mittlerer Verschmutzung und am niedrigsten in Flüssen mit niedriger Verschmutzung, was die in diesem Zusammenhang durch menschliche Einflüsse verursachten Probleme weiter verdeutlicht. Die zusätzlichen Experimente zeigten, dass das Denitrifikationspotential ebenfalls in hochverschmutzten Flüssen am höchsten, und in weniger verschmutzten Flüssen am niedrigsten war; die Zugabe von Kohlenstoff führte zu einer weiteren Steigerung. In Bezug auf Proben aus „Pool“ bzw. „Riffle“- Sektionen des Flusses, konnten keine signifikanten Unterschiede in der Treibhausgasproduktion festgestellt werden, ebenso für unterschiedliche Tageszeiten.

Zusammenfassend zeigt diese Arbeit, dass Landnutzung und die vermutlich daraus resultierende Nährstoff- und DOM- Belastung die Treibhausgasproduktion in Oberlauf Flüssen beeinflusst. Die Sedimentexperimente, die ebenfalls einen Zusammenhang zwischen steigendem Verschmutzungsgrad und Denitrifikationspotential belegen, unterstützen die Ergebnisse zusätzlich.

Klimawandel, begünstigt durch menschlich bedingte Treibhausgasproduktion, und seine Auswirkungen auf den Planeten werden heutzutage immer relevanter. Die Ergebnisse und Erkenntnisse dieser Thesis sollten zum Anlass genommen werden, um über Maßnahmen zur Reduktion der Flusswasserbelastung durch Landwirtschaft - und somit auch Maßnahmen zur Reduktion der Treibhausgasproduktion – nachzudenken.

Key words and abbreviations

Key words:

Aquatic greenhouse gas production, DOM quality, DOM quantity, seasonal patterns, denitrification potential, greenhouse gas production parameters.

Abbreviations:

| | |
|----------------------------------|---|
| DOC | dissolved organic carbon |
| DOM | dissolved organic matter |
| OM | organic matter |
| CO ₂ | carbon dioxide |
| N ₂ O | nitrous oxide |
| CH ₄ | methane |
| GWP | global warming potential |
| EEM | excitation emission matrix |
| DEA | denitrification enzyme activity |
| DEA _{cont} | denitrification enzyme activity- control with acetylene inhibition |
| DEA _{pot} | denitrification enzyme activity- potential with acetylene inhibition |
| N ₂ O _{cont} | denitrification enzyme activity- control without acetylene inhibition |
| N ₂ O _{pot} | denitrification enzyme activity- potential without acetylene inhibition |
| std. dev. | standard deviation |

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1. Introduction and fundamentals

1.1 Introduction

In the last centuries, climate change became a subject of intense discussion and scientific research. Today the effects of global warming become noticeable for everybody, and greenhouse gases have been identified as one of the strongest influencing factors in these processes.

The role of soil, wetland and lake greenhouse gas emissions was topic of intense research over the last years, but the role of stream water in context with greenhouse gas emissions still shows many lacks of clarity. The decisive greenhouse gases emitted by inland waters are carbon dioxide (CO₂), nitrous oxide (N₂O) and methane (CH₄). The total amount of emitted greenhouse gases may be smaller, due to the much smaller surface area of inland water bodies compared to agriculturally used land, but the composition of the emitted gases can be of high importance. According to the Intergovernmental Panel on Climate Change (IPCC) the global warming potential (GWP) of N₂O equals 289 and therefore it is a highly potent greenhouse gas with an additional negative effect on the stratospheric ozone (Khalil and Rasmussen 1983). CH₄, with a GWP of 21, is a quite violent greenhouse gas as well (IPCC).

The conventional carbon cycle does not clearly differentiate between the outgassing from inland waters and fluxes of terrestrial ecosystem respiration (Battin et al. 2009). The outgassing is therefore not well recorded and could be higher in number than thought. Especially the potential outgassing of the - highly relevant - greenhouse gas N₂O from streams could contribute to up to 20% of the global N₂O emission (Seitzinger and Kroeze 1998; Laursen and Seitzinger 2004). This underestimation could also apply to stream CH₄ emissions and their share of the global budget (Bastviken et al. 2011; Tranvik et al. 2009).

Integrating the role of inland waters in terms of greenhouse gas emission into the traditional approach will be a necessary step in the attempt of mitigating global warming.

The influencing factors of processes leading to outgassing of greenhouse gases are complex and often difficult to assign, these key factors in turn are influenced by anthropogenic impacts, as indicated by recent studies (Weigelhofer, Welti, and Hein 2013; Trimmer et al. 2012; Jake J. Beaulieu et al. 2008; Royer, Tank, and David 2004; Battin et al. 2009). Streams function as longitudinal transport and buffer system for nutrients; nutrient enrichment in streams, (caused by anthropogenic influences), however, can negatively affect these processes. Anthropogenic impacts can result in higher loads of organic matter and nutrients in streams, due to a variety of reasons, such as clear cuts of riparian zones, stream straightening, incisions, erosion and especially agricultural land use as well as the use of fertilizer. Not only do increased nutrient concentrations in streams hinder the transport and buffer functions, but they also are important

parameters of N₂O production, for N₂O is produced during denitrification and nitrification via microbes (Bianchi, Feliatra, and Lefevre 1999; E A Strauss, Mitchell, and Lamberti 2002), as well as of methanogenesis. The nutrients (especially nitrogen, which for the reason mentioned above is one of the most important factors for greenhouse gas production) can enter the stream water body through overland flow, erosion processes, a connection between groundwater body and the stream, but also through direct input. Sources include point sources - such as wastewater treatment plants - and non-point sources (Seitzinger and Kroeze 1998), especially agricultural land use (Wilson and Xenopoulos 2009). Apart from nitrogen, dissolved organic matter forms the second big influencing factor for greenhouse gas production. Stedmon, Markager, and Bro 2003 were able to show the influence of land use on quantity and quality of DOM exported to streams. Increased inorganic and organic nutrients in streams were also shown to exhibit linkages in particular to agricultural land use in the catchment (Wilson and Xenopoulos 2009; Bruesewitz, Hamilton, and Schipper 2011)

As mentioned, streams are not only longitudinal systems, but they also are influenced by lateral connections to the surrounding area. They therefore interact with groundwater, soil, atmosphere and the in-stream or terrestrial ecosystem, which leads to a high complexity of ongoing processes and interactions of these systems.

Single aspects have been examined, and detailed research has been done in the past in order to understand the role and the production of greenhouse gases within streams, but there are still parts of the puzzle missing. Recent studies (e.g. Fu et al. 2010; J. J. Beaulieu, Arango, and Tank 2009; Jonathan J Cole and Caraco 2001) for example solely concentrated on the role of N₂O emissions in context with agriculturally induced pollution, or other single aspects of this complex topic. In this thesis, nine small to medium sized streams were chosen to address the question of stream pollution and its effects on DOM quality and quantity, as well as the controlling parameters of the relevant greenhouse gas production of inland freshwater streams. The nine streams followed a gradient in terms of nutrient and DOM pollution, most likely induced by agricultural land use and other anthropogenic impacts.

1.2 Fundamentals

1.2.1 Relevant greenhouse gases in headwater streams

In the past centuries, intensification of agricultural land use in the catchment and other anthropogenic sources contributed to growing nitrogen and organic matter loads in small to medium sized streams. These increased loads can lead to augmented greenhouse-gas production and are therefore relevant for climate change. This chapter summarizes the three most important greenhouse gases and gives a short description of their relevance in terms of climate change, their origin and major influencing factors.

Carbon dioxide and methane:

Inland waters play a major role in mineralization of organic carbon (Battin et al. 2009); an increase in organic matter load fuels mineralization and therefore greenhouse gas production within headwater streams. Mineralization, respiration and in-stream metabolism are central processes in decomposition of organic matter as well as nutrient cycling (Mooney, Vitousek, and Matson 1987; Jr. and Mulholland 1998). During these processes, if the stream water is supersaturated with CO₂ (which, according to J J Cole et al. 1994; Panneer Selvam et al. 2014 is quite common), it can be released to the atmosphere. Organic matter (OM) and in specific dissolved organic carbon (DOC) form the basis for mineralization within streams and therefore are sources and major influencing factors of carbon dioxide release to the atmosphere (Battin et al. 2008; Trimmer et al. 2012; M. A. Baker, Dahm, and Valett 1999). Furthermore, CO₂ release and concentration in the stream water are also influenced by upland-, riparian- and groundwater processes (Mulholland 1998).

The sources of methane in lotic systems often include fluxes from groundwater and riparian zone, where methanogenesis, due to dominating anoxic conditions, is common (Jr. and Mulholland 1998; Jones and Mulholland 1998). But methanogenesis is also possible within anaerobic stream sediments if other electron acceptors, such as O₂, NO₃-N, sulphate or trivalent iron are depleted (Conrad 2007). Supersaturation with methane is typical for streams (Jones and Mulholland 1998), therefore emission of gas to the atmosphere occurs regularly. The major influencing factors for methane release include gas concentration, temperature and turbulence (Fortescue & Pearson, 1967; Jähne, Heinz, & Dietrich, 1987), but also the rate of methanogenesis and its influencing factors, especially O₂ dynamics. Methanogenesis is performed by microbes using carbon as the terminal electron acceptor; therefore anaerobic conditions are necessary for the process. For these reasons, organic carbon concentration – along the others – is also one of the controlling factors for methanogenesis. In case of occurrence of macrophytes in the stream, they can contribute in releasing methane to the atmosphere as well. Trimmer et al. (2012) indicates that dominant amounts of methane release could happen via the stems (aerenchyma) of macrophytes.

Nitrous oxide:

If nitrogen from fertilizer sources is not used by crops, depleted or processed by microorganisms, it can be transported into the stream water bodies via surface runoff, base flow or groundwater stream flow (Dodds et al. 2009; Keeler et al. 2012; Guentzel et al. 2014). Within streams, nitrogen can be transformed via denitrification, nitrification and other processes. If it is not transformed or retained otherwise, it can reach downstream ecosystems, where it can have strong negative impacts on the potentially sensitive ecosystem.

Nitrous oxide (N_2O) production refers to several different processes within inland waters. N_2O is produced during microbial processes via nitrification, an aerobic process where ammonium (NH_4^+) is processed to nitrate ($\text{NO}_3\text{-N}$) and N_2O is outgassed as a by-product (Bianchi, Feliatra, and Lefevre 1999; Eric A Strauss et al. 2004). It can also be produced via denitrification, an anaerobic process where $\text{NO}_3\text{-N}$ is reduced to NO_2 and furthermore into N_2 and small amounts of N_2O (Jake J. Beaulieu et al. 2008). This process is performed via free-living, facultative aerobic bacteria, which can be grouped into true and partial denitrifiers (Sciences and Gardner 2008). The most important factors influencing denitrification are the availability of organic carbon and nitrate. The processes of bacterial denitrification, as well as the single steps on the pathway of nitrate transformation, are controlled by reductase enzymes (Sciences and Gardner 2008) (see chapter 1.2.2). These enzymes are linked to the presence of oxygen, which can deactivate the enzymes (Murray and Knowles 2004).

The production of N_2O also depends on the rate of cycled N. Baulch (et al. 2012) highlight also other processes influencing the N cycle, such as anaerobic ammonium oxidation (Anammox), dissimilatory reduction of $\text{NO}_3\text{-N}$ to ammonium ($\text{NH}_4\text{-N}$) (DNRA), iron driven denitrification and sulphur driven nitrate reduction. The different processes potentially leading to the production of N_2O are summarized in Figure 1. As indicated by recent studies (Baulch et al., 2012; Beaulieu et al., 2008; Royer et al., 2004), the N_2O production in stream sediments, as well as the emission to the atmosphere, can be positively related to the $\text{NO}_3\text{-N}$ and the NO_2 concentrations. Beaulieu et al. 2008 in addition exhibit, that the production of N_2O takes place in the upper layer of stream sediments and is related to the sedimentary organic matter and organic carbon content, which are furthermore related to the agricultural use in the drainage basin. As other studies found out, further controlling factors for nitrification include $\text{NH}_4\text{-N}$, organic carbon concentration, temperature and the pH value (Bianchi, Feliatra, and Lefevre 1999; Eric A Strauss et al. 2004; Laursen and Seitzinger 2004). An aspect Laursen and Seitzinger (2004) pointed out in terms of denitrification, is the relation between oxygen dynamics and photosynthesis by microalgae.

The microbial consumption of O_2 also contributes to anaerobic conditions, which are needed for denitrification and methane production (Sciences and Gardner 2008). Increased OM and nutrient concentrations increase mineralization and, thus, oxygen consumption in streams, thereby promoting anoxic processes, such as denitrification and methanogenesis.

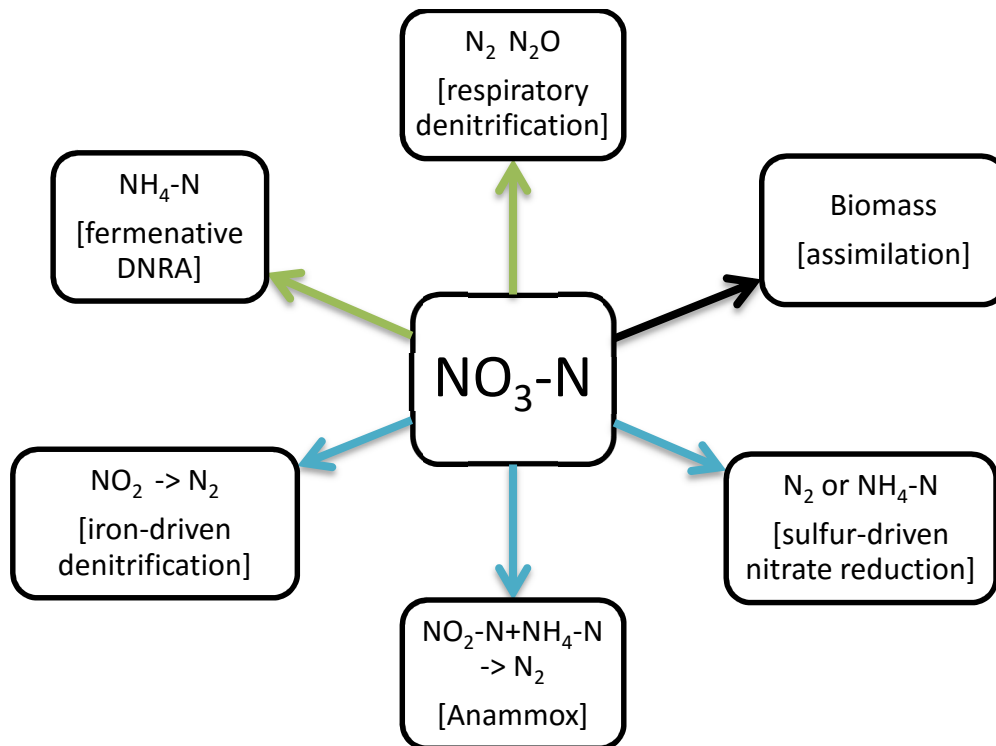


Figure 1 after Burgin and Hamilton (2007): Scheme of the „fate” of $\text{NO}_3\text{-N}$. Blue arrows indicate autotrophic pathways, green arrows indicate heterotrophic pathways.

1.2.2 Denitrification enzyme activities

The denitrification rates *in situ* usually show a highly spatial and temporal variation, therefore laboratory analyses can help to determine the potential denitrification enzyme activity (DEA). To assess the DEA, the acetylene inhibition technique after Smith and Tiedje (1979) is a commonly used approach. Denitrification can be defined as “the dissimilatory reduction of one or both of the ionic nitrogen oxides (nitrates $\text{NO}_3\text{-N}$, nitrites $\text{NO}_2\text{-N}$) to the gaseous oxides (nitric oxide NO , nitrous oxide N_2O), which may themselves be further reduced to dinitrogen N_2 by nitrous oxide reductase” (Sánchez-Pérez et al. 2003). In the presence of acetylene (C_2H_2), the N_2O reductase is disabled, and therefore the potential DEA for different preconditions can be determined.

It has to be mentioned, that the acetylene inhibition technique after Smith and Tiedje (1979) is seen as controversial. Acetylene inhibits the production of $\text{NO}_3\text{-N}$ via nitrification (Groffman et al. 1999). Knowles (1982) and Seitzinger et al. (1993) criticised the method because of the missing inhibition of N_2O reductase, but it still provides a good basis for comparison of stream denitrification rates among each other, as needed for this thesis.

1.2.3 Dissolved organic matter (DOM)

Dissolved organic matter (DOM) is a collective term for different organic substances which pass filters of a pore size of $0.45\mu\text{m}$ (Á. Zsolnay 2003). The quantity and composition of DOM is strongly influenced by the land use in the watershed of a stream, as it influences not only the terrestrial input of DOM but also the autochthonous production of DOM (A. Baker and Spencer 2004; Wiegner and Seitzinger 2004; Wilson and Xenopoulos 2009; Williams et al. 2010). DOM quality has a strong influence on the mineralization process and on the production of greenhouse gases, as DOM is providing the basis for carbon cycling in freshwater ecosystems. Williams et al. (2010) showed that agricultural land use in the catchment leads to higher DOM loads, higher rates of more labile DOM and higher DOM processing rates. This could result in higher gas production because of enhanced bacterial production. Findlay et al. (2001) again underline the influence of land use on the bioavailability of DOC and also on organic matter cycling. DOM can be divided into readily degradable and persistent components, which can originate from different sources. Major fractions of DOM are humic-acid-like fluorophores, tyrosine-like fluorophores and tryptophan-like fluorophores (A. Baker and Inverarity 2004). Because between 40-60 % of organic matter are fluorescent (A. Baker 2001), DOM-spectrophotometry analysis is a common approach to determine possible origins of DOM pollution and composition.

Excitation Emission Matrix (EEM) is a method used in the examination of dissolved organic matter (DOM), as it is fluorescent, has a strong absorption in the ultraviolet band and in the aggregate has explicit spectrophotometric properties (A. Baker and Inverarity 2004). Different fluorescent peaks, determined by scanning the sample simultaneously within a wide range of wavelength (Excitation 200nm till 450nm, 5nm slit and emission starting at 250nm till 600nm, 5 nm slit), result in an EEM matrix. Groups of functional fluorescents can be linked to DOM from natural and non-natural sources (A. Baker and Inverarity 2004). For this thesis the fractions of Humic-acids-like fluorophores, tyrosine-like fluorophores, tryptophan-like fluorophores and the Index of fluorescence (FIX), humification index (HIX) as well as the β/α -ratio, were analysed. The fractions are described below:

Major fractions:

Tryptophan, a protein-like fluorescent, occurs at a wavelength of 220nm excitation and 350nm emission (T220) and also at 280nm excitation and 350nm emission (T280) (A. Baker and Inverarity 2004; A. Baker 2002; Stedmon, Markager, and Bro 2003). It is indicated, that high values of T220 and T280 could be related to anthropogenic influence on the stream. Tyrosine (B1 & B2) are protein-like fluorescents as well, and can be found at the wavelength pair of 275nm excitation and 305nm emission. It has to be mentioned that tyrosine peaks are located very near to Raman line of water, which sometimes causes problems. Humic-like fluorescents

can be detected at a wavelength of 220-350nm excitation and 400-460nm emission (M. a. Baker and Vervier 2004; A. Baker 2002; Stedmon, Markager, and Bro 2003).

Fluorescence index (FIX):

Fulvic-acid represents a major fraction of DOM. Based on the difference in fluorophore emission, peaks of microbially derived fulvic acid can be compared to terrestrially derived fulvic acid. The FIX provides a method for distinguishing the origins of aquatic fulvic-acid, and therefore also distinguishing the sources of DOM in stream water. FIX-values higher than 1.9 indicate a microbial fulvic-acid source, values smaller than 1.4 indicate DOM from a terrestrial source (McKnight et al. 2001; Sazawa et al. 2011; Johnson et al. 2011). The lignin content of DOM is inversely related to FIX (Williams et al. 2010).

Humification index (HIX):

The humification index is a ratio which describes the degree of humification, originally developed by A. Zsolnay et al. (1999). During the humification-process the organic compounds become more condensed; this fact can be used for distinguishing the origin of the material (Chen et al. 2011). The higher the degree of humification, the higher the degree of aromaticity becomes, this in turn shifts fluorescence emission to longer wavelengths (Ohno 2002). To calculate the HIX, the fluorescence emission intensity starting at the wavelength of 435nm up to 480nm is divided by the emission intensity of 300nm to 345nm at an excitation of 254nm (Å. Zsolnay 2003; A. Zsolnay et al. 1999; Ohno 2002; Chen et al. 2011). Sazawa et al. (2011) show that low HIX values are linked to plant biomass and animal manure, while high HIX values indicate a high degree of humification. Williams et al. (2010) assign the HIX values from 1-2 to non-humified plant materials and state that HIX values are direct proportional to the humic content of DOM.

β/α -ratio:

The fluorescence intensity in the α -region, with an excitation of 310nm and an emission of 400nm, depends on the strain of highly degraded humic-like compounds, while the β -region (with also an excitation of 310nm but an emission of 350nm) is associated with the rapid degradation or transformation of microbially derived, protein-like DOM (Wilson and Xenopoulos 2009; Huguet et al. 2009; Williams et al. 2010). The ratio of β -region fluorescence-intensity, divided by the α -region fluorescence-intensity, provides a ratio which describes the proportion of recently produced to highly degraded DOM (Wilson and Xenopoulos 2009). In streams, values >1 are typically assigned with autochthonous DOM, while values <0.6 are usually linked to allochthonous DOM.

2. Objectives, hypotheses and rationale

2.1 Objectives

This master thesis is part of the research project “PowerStreams”, which is funded by the Austrian Federal Ministry of Science, Research and Economics. The overall aim of this research-education-cooperation is the investigation of the effects of in-stream nutrient loading on the efficiency and sustainability of their self-purification capacity. Different approaches are spanned by the project, including the measurement of nitrogen and phosphorous uptake along a gradient of nutrient loading, as well as a long-term DOC (dissolved organic carbon) addition experiment and the investigation of its effects on the stream metabolism and water quality.

The aim followed in this thesis is the investigation of greenhouse gas production in headwater streams of different pollution levels, and its interrelations with DOM quantity and quality. In addition, the effects water and sediment chemistry, as well as hydromorphology on the production of the three most relevant greenhouse gases (CO_2 , CH_4 , N_2O) were investigated. Furthermore, also the context between gas production and microbial and chlorophyll-a abundance was examined. These investigations were performed to identify the parameters possibly controlling the production of greenhouse gases in stream ecosystems. For this purpose, gas, water and sediment samples were taken at nine different streams in spring and summer 2015. To reveal seasonal patterns, the sampling was done in two similar blocks over the year; the first sampling block was in spring (between March and April) and the second in summer (from July to August). The streams were comparable in size and discharge, with different intensities of agricultural land use in the catchment. In order to determine the potential denitrification, additional experiments with stream sediments were performed, using the acetylene inhibition technique.

The gained parameters and data were analysed statistically, including a comparison between spring and summer, and the findings then were discussed in detail. An equation called the “global warming estimate by Bauer” (B_f) was introduced. This parameter produces a single parameter for each stream, summarizing all relevant gas productions of headwater streams, referring to the global warming potential (GWP). With the combined knowledge gained from all analyses and experiments, it was aimed to produce a holistic picture of the processes interconnected with the production of greenhouse gases in streams. This could help to identify options for a future sustainable management of stream ecosystems and to provide a basis for future research of greenhouse gas production within headwater streams.

2.2 Hypotheses

2.2.1 Hypothesis A

Increased agricultural land use in the catchment leads to higher organic matter and nutrient pollution in stream water, resulting in higher production of greenhouse gases.

Streams with higher pollution will show higher amounts of DOM, especially labile DOM fractions. This, in turn, due to stimulated microbial growth and activity (Williams et al. 2010), leads to increased production of CO₂ and CH₄.

A higher amount of nitrogen in the water and sediment leads to intensified denitrification processes and therefore increased N₂O production. Increased NO₃-N water pollution leads to an increased N₂O concentration in the stream water (Jake J. Beaulieu et al. 2008).

2.2.2 Hypothesis B

The amount of greenhouse gas produced in streams is higher in summer than in spring.

Higher water temperature leads to intensified biotic processes (autotrophic and heterotrophic) in the stream water, and therefore the greenhouse gas concentrations rise in summer. Apart from that, the lower discharge leads to higher concentrations of nitrogen and DOM in the water, which also triggers the gas production.

2.2.3 Hypothesis C

If tested under laboratory conditions, sediments from streams with intensified agricultural land use in the catchment and high nutrient pollution exhibit higher denitrification potential than streams of low pollution and extensive agricultural land use in the catchment.

This higher denitrification potential is a result of a bigger denitrifier community in these streams.

2.3 Rationale

There are already some studies focusing on the impacts of eutrophication caused by agricultural land use or morphological alterations on the production of greenhouse gases in a stream ecosystem. In the past, however, aquatic research mainly concentrated on single aspects of greenhouse gas production in streams (e.g. Fu et al. 2010; J. J. Beaulieu, Arango, and Tank 2009; Jonathan J Cole and Caraco 2001) or tried to fill the gaps concerning inland freshwater-induced greenhouse gas production. Recent studies, for example, concentrated solely on the role of N₂O emissions in context with agriculturally induced pollution.

Because of the great amount of influencing parameters and processes contributing to greenhouse gas production, it is quite difficult to get an overall picture. The role of soil, wetland and lake greenhouse gas emissions was topic of intense research over the last years (Hlaváčová et al. 2006; Panneer Selvam et al. 2014; Audet et al. 2013; Bastviken et al. 2011), but the role of stream water in context with greenhouse gas emissions still shows many lacks of clarity. Additionally these past studies were performed in climate settings differing to those of this study.

Agricultural land- use in the catchment area could be the strongest areal influencing factor in terms of pollution with nutrients and organic matter for the most small to medium sized inland streams (beside point sources of pollution) (Audet et al. 2014; Heinz et al. 2015; Wilson and Xenopoulos 2009; Teufl et al. 2013; Weigelhofer et al. 2012). The new approach in this thesis is to focus on all three important gases instead of only a single one and also the investigation of different seasons. Therefore, the most important factors - such as morphology, water chemistry and sediment parameters - that may be connected to agricultural land use and especially DOM quality and quantity, were investigated. As several other studies (Heinz et al. 2015; A. Baker and Spencer 2004; Williams et al. 2010) pointed out a change in DOM composition for streams of increased pollution, special focus in this thesis lies on DOM composition and DOM fraction properties.

As Lower Austria is a highly diverse state in terms of agricultural land use, from very intensive agricultural land use down to regions of pretty pristine conditions, it was of high interest to compare streams among different pollution conditions. This thesis covers a broad range of influencing factors, as it does not only consider physical, but also chemical and biological factors. It therefore represents a multilateral approach, illuminating the many different mechanisms potentially influencing and promoting the production of greenhouse gases in streams.

3. Methods

3.1 Study area and site description

3.1.1 Selected streams and criteria

For this master thesis, nine streams in the federal state of Lower Austria were chosen (see Figure 2). Lower Austria is a highly diverse state in terms of landscape, land coverage, land use and precipitation (see Table 1). The average annual precipitation in the catchment of the southern part of lower Austria, where two of the investigated streams flow, is 1686 mm a^{-1} (BMLFUW Abteilung IV/4 2012). The average annual precipitation in the northern part of lower Austria (where also 2 investigated streams lie) in contrast is only 551 mm/a , although the two locations are only $\sim 140 \text{ km}$ apart. Furthermore, the northern part is influenced by strong agricultural land use (especially the Weinviertel, where high pollution rates in terms of DOC/DOM and nitrate occur). With rising altitude (Figure 2) the agricultural land use becomes less intensive because of climatic, soil and site conditions.

All the chosen streams had to fulfil standard criteria to enable comparability. First the discharge had to be similar in all streams; variations in discharge however are very common for headwater streams of this size because they can strongly respond to rainfall events and during spring the snow melt increases discharge significantly. Sometimes streams can fall dry during hot summer periods, this fact had to be considered too. The riparian zone of the rivers had to be forested, ideally with a closed canopy. The stream course, especially in the investigated stream reach, had to be without strong anthropogenic influence, such as channelling or other artificial alterations, no big point sources of pollution (such as drainage pipes discharging into the stream) should be present, as they could have falsified the results. For this number of criteria, it sometimes was challenging to find suitable streams, especially in the Weinviertel region. Therefore, not all criteria could be fulfilled for the strongly influenced *Gmoosbach*, as it is channelized.

In order to determine a preselection of streams with increasing pollution levels, the following land use categories (Table 1 & Figure 2) were defined and the streams chosen according to them:

Intensively cultivated cropland with high soil erosion / high pollution level:

The *Rußbach* [1] and the *Gmoosbach* [2] were chosen as “high pollution” streams (red hexagons in Figure 2 & Table 1). They are located in the Weinviertel, a quite flat region with intense agricultural land use and usage of high amounts of fertilizer. In this region the most intensive agricultural land use in Austria has taken place across decades and still takes place until today.

Intensively cultivated grass- and cropland / moderate pollution level:

Sierning [3], *Zettelbach* [4] and *Schweinzbach* [5] are located near Mank in the south-western part of lower Austria (yellow hexagons in Figure 2 & Table 1). They are at the border of the Alps, where the land starts to form a valley. A lot of maize is grown here, but the agricultural land use is rather moderate and less intensive.

Intermediate (between moderate and low) pollution:

Feichsen [6] and *Grestenbach* [7] (yellow hexagons in Figure 2 & Table 1) flow only 20 km west of the *Schweinzbach*, *Zettelbach* and *Sierning*. The land is hillier, and land use changes towards meadows and stock farming. The *Feichsen* was only sampled in spring; it had to be excluded from summer sampling because of changed framework conditions.

Forested catchment / low pollution level:

Kothbergbach [8] and *Gamingbach* [9] lie within mountain valleys (blue hexagons in Figure 2), but only 10 km south of *Feichsen* and *Grestenbach*. There is almost no agricultural land use in their catchment and only few human settlements are present. They are pretty much in pristine conditions and therefore represent the general orientation for comparison.

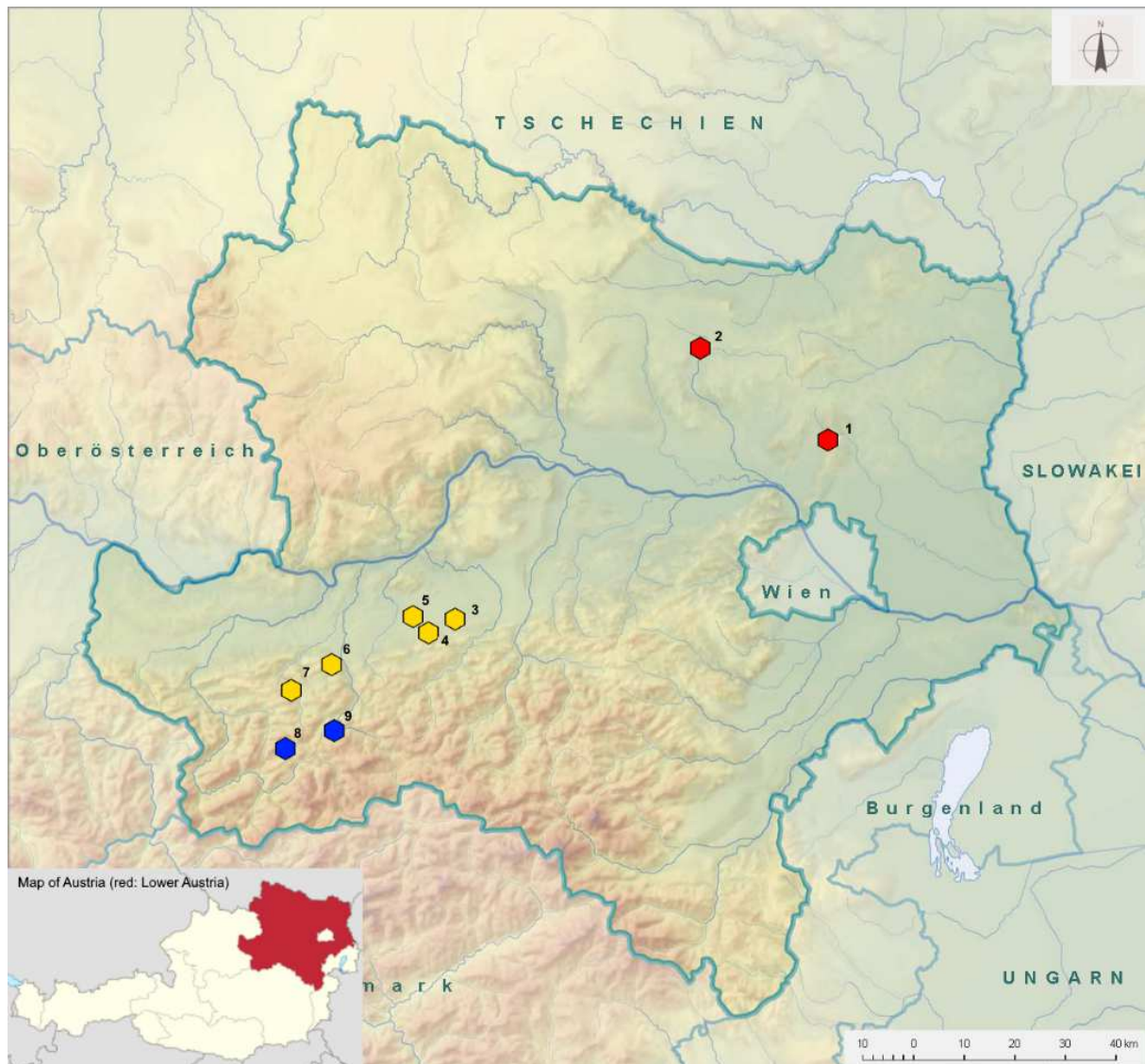


Figure 2: Overview map of the federal state of Lower Austria with numbered stream positions. Red marks represent highly polluted streams, yellow marks moderately & moderately to lowly, and blue lowly polluted streams. [Sources: Amt der Niederösterreichischen Landesregierung and Wikipedia]

Methods

Table 1: Summary of stream and catchment properties, such as land use and forested area in the catchment, size of catchment area, presence of riparian wood, average annual precipitation, erosion and orthometric height ([m. ü. A] meters above Adriatic sea level) of the study streams. [Source: BMLFUW Abteilung IV/4 2012]

| pollution | stream name | catchment | forest in catchment | land use in catchment | riparian wood | ground erosion | average annual precipitation in the catchment | orthometric height |
|-----------|--------------|--------------------|---------------------|---|---------------------------|------------------|---|--------------------|
| | | [km ²] | [%] | | | | [mm] | [m. ü. A] |
| high | Rußbach | 46.5 | 10.3 | cropland/settlements/ wood in Kreuttal | rarely | high | 588 | ~214 |
| | Gmoosbach | 125.5 | 3.4 | cropland/settlements/ wood only at the spring | rarely | high | 551 | ~241 |
| moderate | Schweinzbach | - | <1% | pastures/cropland/ settlements /wood only partially | bilateral wood stripes | medium | 764 | ~279 |
| | Sierning | 21.4 | 20.5 | pastures/cropland/ settlements/ wood at the spring | bilateral wood stripes | medium | 811 | ~279 |
| | Zettelbach | 19.5 | 58 | pastures/cropland/ settlements /wood at the spring | bilateral wood stripes | low to medium | 811 | ~279 |
| | Feichsen | 23 | 54 | pastures in the valley/ wood at the source | bilateral wood stripes | low | 1057 | ~295 |
| | Grestenbach | 7 | 54 | pastures in the valley/ wood at the source | wood | low | 1251 | ~399 |
| low | Gamingbach | 8.44 | 99.9 | single pastures (2) / only wood | wood | low | 1619 | ~402 |
| | Kothbergbach | - | 100 | only wood | wood | low | 1621 | ~450 |

3.2 Sampling design

In order to provide sufficient data for the later analyses, the following sampling design was used: Each of the chosen streams was sampled twice within one year, the first sampling block was in spring in the months of March and April and the second sampling block was in July. All samples were taken on one specific day for each stream and sample block (see Table 2).

In spring, 5 gas samples were taken at one pool section in each stream at around 10:00 o'clock, in order to measure the ambient greenhouse gas concentration in the water. To gain further information about potential changes in gas emissions of streams across differing daytimes and stream sections, the procedure for the summer time sampling was slightly changed: 4 samples were now taken at 10:00, 12:00 and 14:00 o'clock, respectively, to check for temperature and time variations of gas emission within stream water. Additionally, at each time slot the location of sample taking now included a pool and a riffle section of the stream, to explore possible outgassing variations due to turbulences. 2 samples per site (pool or riffle) were taken.

In addition to the gas samples, for each stream and sampling block 5 water samples (compare Table 2) were taken evenly distributed along a reach of approximately 200m to 300m length. Additionally, 10 sediment samples were taken at each stream site to test them for N concentrations, microbial abundance and organic matter concentration. The sediment samples were taken within a stream section visually assessed to be representative for the studied stream. In summer, an additional set of ten sediment samples was gathered (under the same conditions) to test them for their denitrification potential. For the analysis of the chlorophyll-a concentration in the stream reach, ten biofilm samples were taken as well.

Table 2: Summary of the total numbers of samples taken, split into type of sample

| Type of sample | Number of samples taken | |
|----------------|---|-------------------------|
| | Number of samples per stream and sample block | Total number of samples |
| Sediment | 10 | 170 |
| Water | 5 | 85 |
| Chlorophyll-a | 10 | 170 |
| Gas | 5 spring / 12 summer | 141 |

3.3 Sampling procedures

3.3.1 Channel morphology, transects and flow velocity

For each stream, 3 cross section transects were measured choosing representative locations. Water depth, wetted width as well as flow velocity were measured. With a foot rule the depth of the stream was measured every 30 cm. Flow velocity was measured in stopping the time a floating object needed to travel 4 m in the stream, this procedure was repeated 3 times for each transect.

3.3.2 Oxygen and light characteristics

After the sampling procedure, an oxygen probe (YSI 600 OMS V2) was installed submerged in the stream. The place was chosen under the aspect of warranted submerging of the probe and solid stability. A light-sensor (HOBO) was attached to the oxygen probe. The probe and sensor were then left in the stream for at least 48 hours to record diurnal and night patterns in oxygen, temperature and light, but also to calculate the whole-reach in-stream metabolism.

3.3.3 Water samples

5 water samples were collected using 250ml HDPE bottles (Lactan). The pre-cleaned bottles were rinsed with stream water 3 times and then filled with the same. In order to provide undisturbed conditions, the samples were taken with the help of a telescope rod, standing at the stream bank. Using a pre-cleaned 50 ml syringe (rinsed with Mill-Q water), the sample was filtered through a GF/F filter (Whatman Glass Microfibre Filter, 0.7 μm) and filled into a 50ml plastic tube.

Additionally, in summer, one water sample per stream was collected into a 250ml HDPE bottle (without filtering) for the use of denitrification experiments. All water samples were stored in the fridge and the analyses of $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$ and $\text{NO}_2\text{-N}$ were performed within 24 hours of sampling.

3.3.4 Dissolved organic matter (DOM) and dissolved organic carbon (DOC) samples

Using a 50ml syringe, a water sample of each stream was filtered through a GF/F filter (Whatman Glass Microfibre Filter, 0.7 μm) directly into a 40ml muffled DOC carbon free glass vial (Supelco 40ml Clear Via/Hole Cap with PTFE/Silicon Septa, Sigma Aldrich). The analysis of DOM and DOC was performed within 12 hours.

3.3.5 Sediment samples

Ten sediment samples per stream site were taken. For this purpose, the upper 3 cm of sediment were sieved through a 4 mm sieve, using the frame of the sieve with a diameter of

25 cm as a reference area. Excess water was removed, the sample poured into a synthetic bag and the bag then was closed. Weight varied due to different grain size composition, but in average about 200-250g of sediment sample were gained. Samples were stored in a cooling box until the transport to the laboratory. The amounts needed for analysis of dry weight, organic matter content, microbial abundances as well as for denitrification experiments were removed and used immediately in laboratory. For the analysis of microbial abundances, 1,5g of the fresh sediments were weighed into a tube and the microbes fixed with a 2,5% formaldehyde fixing solution. The rest of the sample was frozen at -20° and stored in the freezer for further use. In case of very fine sediments, direct samples with special areal reference were taken.

3.3.6 Gas samples

Gas samples were collected according to the protocol of Beaulieu et al. 2008, although slightly modified. A pre-washed 50 ml syringe (Omnifix 50ml steril, Lactan) was flushed with stream water three times - fully submerged - in order to avoid contamination. Afterwards the syringe was (still submerged) filled with 20 ml of stream water, approximately 5 cm below the water surface. A second syringe with an attached reservoir balloon filled with Ultra High Purity Helium (He) was connected to the valve of the water sample syringe. The next step was to suck 20 ml of the helium into the water sample syringe and close the valve. The syringe with the mixed sample of stream water and helium was shaken for 5 minutes. To assure complete outgassing of the water sample, the syringe was then set to rest for another 5 minutes. The headspace gas of the water sample syringe was then transferred into a pre-evacuated <10mTorr 10ml crimp gas vial (ND20/ Headspace gas vials, septum VWR). The vials were stored submerged in the stream.

For laboratory storage, the gas vials were stored in the refrigerator turned upside down, submerged in Mill-Q Water, in order to avoid any contamination or leakage until the analysis.

3.3.7 Chlorophyll-a samples

The epilithic biomass was scraped off the surfaces of ten stones per stream and the samples then were filled into 15 ml tubes. The scraped area was measured and noted, in case of big algae blooms, samples were gathered directly from the stone. Samples were cooled in the stream and afterwards kept frozen at -20°C until analysis.

3.4 Laboratory analysis

3.4.1 Gas sample analysis

The gas samples were analysed for methane (CH_4), carbon dioxide (CO_2) and nitrous oxide (N_2O) via gas-chromatography with a gas chromatograph Agilent 6890A (Santa Clara, USA) connected to an automatic sample-injection system DANI HSS 86.50 headspace-sampler (Cologno Monzese, Italy).

For the acetylene flushed gas samples of the denitrification experiments, additionally an Agilent 7697A Headspace Sampler (Santa Clara, USA) at the Institute for Soil Research of the University of Natural Resources and Life Sciences was used.

3.4.2 Water sample analysis

Nitrate ($\text{NO}_2\text{-N}$)

The water samples were analysed for $\text{NO}_2\text{-N}$ concentration using the continuous flow analysis (CFA) according to (ISO 13395 1996), at a detection limit of $1\mu\text{g l}^{-1}$.

Nitrite ($\text{NO}_3\text{-N}$)

The water samples were analysed for $\text{NO}_3\text{-N}$ concentration using the CFA method according to (ISO 13395 1996), at a detection limit of $100\mu\text{g l}^{-1}$.

Ammonium ($\text{NH}_4\text{-N}$)

The water samples were analysed for $\text{NH}_4\text{-N}$ concentration using colorimetric methods after APHA (1998). Two reagents (dichlorisocyanuric acid and a mixed solution containing sodium nitroprusside, NaOH and water) were produced. Mixed with the water sample, the mixture was analysed using a Hach DR2800 portable spectrophotometer with a detection limit of $4\mu\text{g l}^{-1}$.

3.4.3 Dissolved organic matter and dissolved organic carbon analysis

DOC:

The water samples, stored in carbon free vials, were analysed for dissolved organic carbon via Sievers*900 portable Total Organic Carbon Analyzer at a detection limit of 0.030 ppb (General Electric Company 2005).

DOM:

The water samples, stored in carbon free vials, were analysed using a Hitachi F-7000 FL Spectrophotometer. Instrument parameters for scanning are as follows:

| | |
|-------------------------------|--------------|
| Measurement type: | 3-D Scan |
| Data mode: | Fluorescence |
| Excitation start wavelength: | 200.0 nm |
| Excitation end wavelength: | 450.0 nm |
| Excitation sampling Interval: | 5.0 nm |
| Emission start wavelength: | 250.0 nm |
| Emission end wavelength: | 600.0 nm |
| Emission sampling Interval: | 2.0 nm |
| Scan speed: | 12000 nm/min |
| Excitation slit: | 5.0 nm |
| Emission Slit: | 5.0 nm |
| PMT Voltage: | 700 V |
| Response: | 0.01 s |

The resulting excitation-emission matrix was then analysed via peak picking (sensu Hudson and Reynolds 2007). Peaks were determined for corrected data within following spectrums (Table 3):

Table 3: Scanning bandwidths for tryptophan-like, tyrosine-like and humic-like fluorescence DOM scans

| description | Excitation bandwidth [nm] | Emission bandwidth [nm] | Parameter abbreviation |
|-----------------|------------------------------|----------------------------|---------------------------|
| tryptophan-like | 225-237 | 340-381 | T220 |
| | 275 | 340 | T280 |
| tyrosine-like | 225-237 | 309-321 | B1 |
| | 275 | 310 | B2 |
| humic-like | 300-370 | 400-500 | C |
| | 237-260 | 400-500 | A |

Indices:

For calculation of the used indices, scan parameter as described in Table 4 were used. Afterwards the indices were calculated using the equations 1, 2 and 3

Table 4: Scanning bandwidths for the indices FIX (fluorescence index), HIX (humification index) and β/α -ratio

| Index | Excitation bandwidth [nm] | Emission bandwidth [nm] | Parameter abbreviation |
|-----------------------|------------------------------|----------------------------|---------------------------|
| FIX | 370 | 420 | FL450 |
| | 370 | 470 | FL500 |
| HIX | 255 | 280 | HIX300 |
| | 255 | 420 | HIX434 |
| β/α -ratio | 310 | 350 | β 380 |
| | 310 | 400 | α 420 |

Fluorescence Index:

For the calculation of the fluorescence index (FIX), the intensity of 370nm excitation and 450nm emission was divided by the intensity of 370 nm excitation and 500nm emission (*sensu* McKnight et al. 2001, see Equation 1).

Equation 1: Fluorescence Index (FIX)

$$FIX = \frac{I_{370:450}}{I_{370:500}}$$

Humification Index:

For the calculation of the humification index (HIX), the sum of the intensity at each wavelength following from 435nm to 480nm was divided by the sum of the intensity at each wavelength from 300nm up to 345nm (*sensu* Ohno 2002, see Equation 2).

Equation 2: Humification Index (HIX)

$$HIX = \frac{\sum I_{435 \rightarrow 480}}{\sum I_{300 \rightarrow 345}}$$

 β/α -ratio:

For the β/α -ratio, the intensity of 310nm excitation and 350nm emission (β -region) was divided by the intensity of 310 nm excitation and 400nm emission (α -region) (see Equation 3).

Equation 3: β/α -ratio

$$\frac{\beta}{\alpha} = \frac{I_{310:350}}{I_{310:400}}$$

3.4.4 Sediment sample analysis

Water content: The water content of each sediment sample was determined following the protocol of ÖNORM B 4410 (2009). About 20g of sediments (wet weight) were dried in the oven at 80°C until constant weight (dry weight) and then the mass difference between the wet and the dry weight was calculated. This mass difference represented the absolute water content, which then was transformed into the water content relative to the wet weight (relative water content).

NH₄-N: All sediments were analysed for NH₄-N after extraction with potassium chloride (KCl) according to Mulvaney (1996). For this purpose, about 1,5g of sediments were put in a beaker, enriched with 25ml potassium chloride and the closed beakers then left on the shaker for 16h. The extract then was poured into glass tubes, put in the centrifuge and the supernatant then was analysed using the standard colorimetric methods after APHA (1998). For this purpose, a Hach DR2800 portable spectrophotometer was used. The exact weight of the sediment was noted to correct for the dry weight and calculate the concentration of nutrient per gram sediment.

NO₃-N: All sediments were analysed for NO₃-N after extraction with H₂O according to Mulvaney (1996) using the CFA method (ISO 13395 1996). The approach of sediment treatment is the same as described before for NH₄-N but with the difference that distilled water was used instead of KCl. The detection limit for this analysis lies at 20µg l⁻¹.

NO₂-N: All sediments were analysed for NO₂-N after extraction with H₂O (treatment procedure see point above) according to Mulvaney (1996) using the CFA method (ISO 13395 1996). The detection limit for this analysis lies at 1µg l⁻¹.

3.4.5 Microbial abundance sample analysis:

1.5 g of each sediment sample were weighted into a 15 ml tube and mixed with 3ml water and 0.75 ml formaldehyde (2,5% fixing solution). All sediments were then analysed for microbial abundance after the protocol of Duhamel & Jacquet (2006) via Flow cytometer (Beckman-Coulter) using a Cytoflex flow cytometer from Beckman Coulter.

3.4.6 Chlorophyll-a samples

The biofilm samples were unfrozen and 10ml of 90% acetone were added. The samples were homogenized using an ultrasonic device; afterwards the samples were shaken and stored in the dark for 24h at 4°C. After centrifugation, the supernatants were analysed fluorometrically for chlorophyll- a content following the directions of Steinman, Lamberti & Leavitt (2007).

3.5 Design and analysis of the denitrification experiments

For the measurement of the denitrification enzyme activity (DEA), the acetylene inhibition technique (after Smith & Tiedje 1979), as described in chapter 1.2.2, was performed using 10 subsets of 10 g (fresh weight) sediment samples. All the experiments were performed in July. For this purpose, the samples gathered at each stream in summer were used.

Using 10 sediment samples per stream and producing replicates with 5 different solutions (see Table 4 below), resulted in 50 septic flasks per treatment and stream. Thus, 100 septic flasks per stream (because of the two different treatments, one with and one without acetylene) were used, resulting in a total of 700 flasks.

Solutions:

There were 5 different solutions added to the ~10 g fresh weight sediment samples. 1mg N per g sediment according to Weigelhofer, Welti, & Hein (2013) is sufficient for achieving a clear increase in DEA_{pot} , while a higher N amount does not affect the DEA_{pot} rates further.

Table 5: Description of the concentrations of the solutions used for the denitrification experiments

| Solution name | abbreviation | Concentration of solution | comment |
|------------------------------|--------------|---------------------------|--|
| Carbon C-Glucose solution | +C | 2g C per litre | =5g glucose $C_6H_{12}O_6$ L^{-1} |
| Nitrogen solution | +N | 2g N per litre | =12,14 g $NaNO_3$ L^{-1} |
| Carbon and Nitrogen solution | +C&N | 2g N and 2g C per litre | =12,4g $NaNO_3$ + 5g $C_6H_{12}O_6$ |
| Stream water | +RW | 5ml of stream water | unfiltered |
| Bi-distilled water | +Mill-Q | 5 ml of Mill-Q | bi-distilled |

Methods

First 10 g of fresh-weight sediment sample were weighted into a pre-cleaned 100 ml Schott flask with septa. To get the potential-denitrification enzyme activity (DEA_{pot}), the nutrient solutions described in Table 5 were added to the sediment slurry. Then the flask was closed tightly and made anoxic by flushing the headspace with N_2 gas for 5 minutes, afterwards the flasks were brought to atmospheric pressure. In the next step the sediment samples were flushed with 10% (v/v) acetylene to block the N_2O reductase. Then the flasks were shaken and stored in the dark at $25^\circ C$ for 4 hours. After the incubation, a clean and gas-tight syringe was used to extract 10ml gas from the headspace of the flasks and inject it into pre-evacuated 10ml gas vials. The gas vials then were stored headfirst in the darkness at $5^\circ C$ until the analysis.

To get a reference value for DEA_{pot} , two additional treatments - one with the addition of bi-distilled water (called "Mill-Q") and one with the addition of stream water (called "+RW") - were performed, which provided the non-amended denitrification rate DEA_{cont} (see Table 5) (Bruesewitz, Hamilton, and Schipper 2011).

For the determination of the N proportion denitrified as N_2O during the analysis, a second set of samples was measured under the same conditions as described before and with the same added solutions (Table 5), but this time without the flushing of acetylene. Here, as well to get a reference (for this time N_2O_{pot}) two additional treatments were performed again, one with the addition of bi-distilled water ("Mill-Q") and one with the addition of stream water ("RW"). These samples provided the non-amended denitrification rate N_2O_{cont} (Table 5) (Bruesewitz, Hamilton, and Schipper 2011).

To express the denitrification efficiency, a ratio of $N_2O:DEA$ was used (Yoshinari and Knowles 1976; Weigelhofer, Welti, and Hein 2013).

3.6 Calculation

3.6.1 The global warming estimate by Bauer(B_f)

One main aim of this thesis was to reveal the influence of pollution, caused by agricultural land use, on greenhouse gas emissions. Therefore, I intended to create and test a value that sums up the negative effects of all greenhouse gas emissions of streams on the climate. As it can be seen in the results section, in terms of gas-emissions there is no general correlation between the different kinds of greenhouse gases; high CO₂ values don't necessarily equal high N₂O emissions. Therefore, the global warming estimate gained from a newly developed equation (Equation 4) was used to calculate a single value of greenhouse gas emission equivalent. This was achieved by summing up the greenhouse gas emissions multiplied by their individual global warming potential. This approach allowed ranking the streams in order of climate effective greenhouse gas emissions. The Global Warming Potential (GWP) for CO₂ equals 1, for CH₄ 21 and for N₂O 289 (referred to Kyoto protocol/ based on 100 years).

Equation 4: Global warming estimate by Bauer (B_f)

$$B_f = CO_2 [ppm] * GWP_{CO_2} + CH_4 [ppm] * GWP_{CH_4} + N_2O [ppm] * GWP_{N_2O}$$

$$GWP_{CO_2}=1$$

$$GWP_{CH_4}=21$$

$$GWP_{N_2O}=289$$

The streams were ranked after increasing CH₄ emission [in ppm] in order to create a reference and then each stream was consecutively numbered by its position from lowest to highest. Afterwards the streams were sorted after their CO₂ [ppm] emissions, N₂O [ppm] emissions and then after the calculated global warming estimate to check for differences in the classification.

3.6.2 Denitrification enzyme activity, DEA-ratio

First the conversion from N₂O [ppm] to N₂O [μg l⁻¹] was done:

Equation 5: Conversion of N₂O from [ppm] to N₂O [μg l⁻¹]

$$N_2O[\mu g/l] = \frac{N_2O[ppm] * molecular\ weight}{molecular\ volume}$$

Molecular weight of N₂O = 44

Molecular volume of N₂O= 24.5l (at standard air pressure and room temperature)

As a second step, the conversion from N₂O [μg l⁻¹] to N₂O [ng g⁻¹ h⁻¹] was done:

Equation 6: conversion from N₂O [μg l⁻¹] to N₂O [ng g⁻¹ h⁻¹]

$$N_2O[ng/g] = \frac{N_2O[\mu g/l] * [flask\ volume[ml] - added\ liquid\ solution[ml] - sample\ weight\ [g]]}{dry\ weight\ of\ sample\ [g] * incubation\ time\ [h]}$$

Then the conversion for the N₂O [ppm] results of the non-acetylene treatment was calculated, again using equation 5 and 6, to get N₂O_{pot}. Afterwards the N₂O [ppm] results of the acetylene treatment were converted, using the same equations, to get DEA_{pot}.

N₂O_{pot}...nitrous dioxide-potential

DEA_{pot}...denitrification enzyme activity-potential

The last step was to calculate the DEA-ratio by dividing N₂O_{pot} by DEA_{pot}:

Equation 7: DEA-ratio

$$DEA_{ratio} = \frac{N_2O_{pot}}{DEA_{pot}}$$

3.7 Statistical analysis

All the data were recorded digitally and organised with the program MS Excel. The statistical analyses were performed using the program IBM SPSS Statistics Version 21 for Windows (Armonk, NY: IBM Corp. Released 2012).

All data were tested for normality using the Kolmogorov-Smirnov test.

To examine the relationships between ambient nitrogen concentrations, DOC, DOM and greenhouse gas production, as well as possible correlations among hydromorphology, nutrient and sedimentary characteristics, Spearman's rank correlation was used. Spearman's rank correlation was useful due to low sample size, lack of normality and possible outliers.

Differences between the pollution groups in terms of in-stream and sedimentary nitrogen concentrations, gas concentration, as well as DOC and OM, were analysed using the Mann-Whitney-U-Test. For diurnal changes in stream water gas concentrations, the Kruskal-Wallis-Test was used. In addition, all the parameters were tested across spring and summer to detect significant differences between the two seasons. For this purpose, the Mann-Whitney-U-Test was used as well.

An alpha level of 0.05 was applied throughout the analyses.

4. Results

4.1 Morphological and hydrological parameters and water temperature

The mean discharge described a strong decline for all streams from spring to summer, resulting in a decrease of the parameters flow velocity, mean depth and wetted perimeter (Table 6). The mean water temperature showed a strong increase from spring to summer time, as it nearly doubled for all streams. Also the variability of temperature increased in summer time. The highest mean temperatures could be observed for the streams Rußbach, Gmoosbach and Schweinzbach and the lowest for Kothbergbach and Gamingbach.

Table 6: Hydromorphological parameters, discharge [Ls^{-1}], flow velocity (ms^{-1}) and water temperature [$^{\circ}\text{C}$] in the study streams during the investigations (mean, $n=1-4$ for spring and summer)

| Hydromorphological parameters and water temperature | | | | | | | | | | |
|---|--------------------------------|--------|------------------------------------|--------|----------------|--------|----------------------|--------|--|--------|
| Stream name | discharge [Ls^{-1}] | | flow velocity [ms^{-1}] | | mean depth [m] | | wetted perimeter [m] | | water temperature [$^{\circ}\text{C}$] | |
| | Spring | Summer | Spring | Summer | Spring | Summer | Spring | Summer | Spring | Summer |
| Rußbach | 181.0 | 80.30 | 0.29 | 0.20 | 0.18 | 0.12 | 3.85 | 3.73 | 8.8 | 21.2 |
| Gmoosbach | 260.3 | 92.90 | 0.31 | 0.11 | 0.33 | 0.37 | 3.17 | 3.03 | 12.6 | 23.9 |
| Sierning | 133.3 | 54.20 | 0.13 | 0.08 | 0.37 | 0.26 | 3.54 | 3.11 | 12 | 18.76 |
| Grestenbach | 193.0 | 49.70 | 0.19 | 0.09 | 0.33 | 0.22 | 3.72 | 2.97 | 9 | 19.84 |
| Zettelbach | 84.3 | 43.00 | 0.12 | 0.04 | 0.17 | 0.23 | 4.18 | 3.36 | 12.2 | 17.88 |
| Schweinzbach | 133.4 | 43.51 | 0.11 | 0.04 | 0.33 | 0.53 | 4.3 | 3.17 | 13.3 | 20.55 |
| Kothbergbach | 391.4 | 267.30 | 0.33 | 0.19 | 0.23 | 0.28 | 5.57 | 5.6 | 10.8 | 13.89 |
| Gamingbach | 873.3 | 70.40 | 0.26 | 0.10 | 0.98 | 0.18 | 5.34 | 4.07 | 8.8 | 15.22 |
| Feichsen | 85.6 | - | 0.15 | - | 0.46 | - | 5.09 | - | 10.2 | - |

4.2 Water chemistry

4.2.1 Dissolved inorganic nitrogen concentrations in the water

In terms of water nitrogen values, the composition was quite heterogenic (see Table 7). The Rußbach was the stream with the highest values in spring and summer for all three measured parameters (NO₃-N, NO₂-N and NH₄-N); it therefore suited the group of high pollution. Gmoosbach indicated still rather high values, but Sierning and Schweinzbach were higher, especially in the terms of NO₃-N in the stream water. Differences between summer and spring values of NO₃-N were not significant (Mann-Whitney-U; p=0.510; n=85).

NO₂-water concentrations were significantly different in summer compared to spring values (Mann-Whitney-U; p=0.010; n=85), the values of NO₂-N followed a distinct increase for all streams, and highly polluted streams showed much higher NO₂-N values. In total there was no significant difference between summer and spring in the NH₄-N concentrations (Mann-Whitney-U; p=0.929; n=84). There was also no clear pattern in increases and decrease of NH₄-N values among the pollution graduation.

With the exception of some variations in nitrogen values, a clear classification of pollution-level turns out to be resilient, in general, water chemistry parameters reflected the initial order of streams along a land use and pollution gradient. For all streams, NO₃-N showed the highest nitrogen values by far.

Table 7: Nitrate (NO₃-N), nitrite (NO₂-N) and ammonium (NH₄-N) concentrations [µg l⁻¹] in the study streams during the investigations (means and standard deviations, n =5)

| water parameters of nitrate, nitrite and ammonium | | | | | | | |
|---|----------|---|--------|---|--------|---|--------|
| stream name | | water NO ₃ -N [µg l ⁻¹] | | water NO ₂ -N [µg l ⁻¹] | | water NH ₄ -N [µg l ⁻¹] | |
| | | Spring | Summer | Spring | Summer | Spring | Summer |
| Rußbach | mean | 6857.2 | 4059.2 | 52.8 | 197.2 | 192.7 | 175.6 |
| | std. dev | 138.4 | 81.7 | 0.5 | 4.9 | 10.4 | 29.3 |
| Gmoosbach | mean | 2306.9 | 3497.3 | 28.1 | 58 | 21.9 | 30.6 |
| | std. dev | 196.6 | 91.5 | 0.3 | 1.4 | 2.4 | 9 |
| Sierning | mean | 4151.6 | 5604.6 | 45.8 | 142 | 94.9 | 163.7 |
| | std. dev | 50.1 | 34.6 | 0.2 | 3.9 | 2.6 | 9.9 |
| Grestenbach | mean | 976 | 1274.6 | 3.1 | 6 | 5.5 | 3.6 |
| | std. dev | 10.1 | 256.7 | 0.2 | 0.5 | 2.9 | 1.9 |
| Zettelbach | mean | 1492.2 | 1609.6 | 6 | 4.9 | 11.5 | 6.4 |
| | std. dev | 430.2 | 19 | 0.2 | 0.2 | 1.4 | 3.2 |
| Schweinzbach | mean | 3308.2 | 3020.7 | 19.5 | 22.5 | 5.9 | 14.4 |
| | std. dev | 130.2 | 99 | 1.2 | 1.5 | 1.3 | 4.7 |
| Kothbergbach | mean | 1017.4 | 961.3 | 1.3 | 2.5 | 6.3 | 4 |
| | std. dev | 26.9 | 19.4 | 0.2 | 1.1 | 1.4 | 0 |
| Gamingbach | mean | 1056.8 | 837.4 | 1.3 | 2.3 | 12.4 | 33 |
| | std. dev | 0.3 | 9 | 0.3 | 0.3 | 9.4 | 21.7 |
| Feichsen | mean | 1156.2 | | 4 | | 4.4 | |
| | std. dev | 15.4 | | 0.1 | | 10 | |

4.3 Dissolved organic matter

4.3.1 Dissolved organic carbon

The results of the DOC analysis showed a clear pattern, which correlated with the classification of streams of different pollution levels as described in chapter 3.1.1 (Figure 3). The streams flowing through areas of high areal and intense agricultural land use showed much higher DOC values than streams in areas of extensive land use.

Therefore, the chosen categorization of chapter 3.1.1 was kept throughout all further analyses, with Rußbach and Gmoosbach forming the group of “high pollution”, Sierning, Zettelbach, Schweinzbach, Grestenbach and Feichsen the group of “moderate”, and Gamingbach and Kothbergbach forming the group of “low pollution”.

In total, spring and summer values varied significantly (Mann-Whitney-U; $p=0.016$; $n=93$), with summer DOC values being higher for all streams. DOC in “high pollution” group was significantly higher than in the group “moderate pollution” for both, spring and summer values (spring; Mann-Whitney-U; $p=0.000$; $n=37$) (summer; Mann-Whitney-U; $p=0.000$; $n=32$). It was also significantly higher than the values of the “low pollution” group.

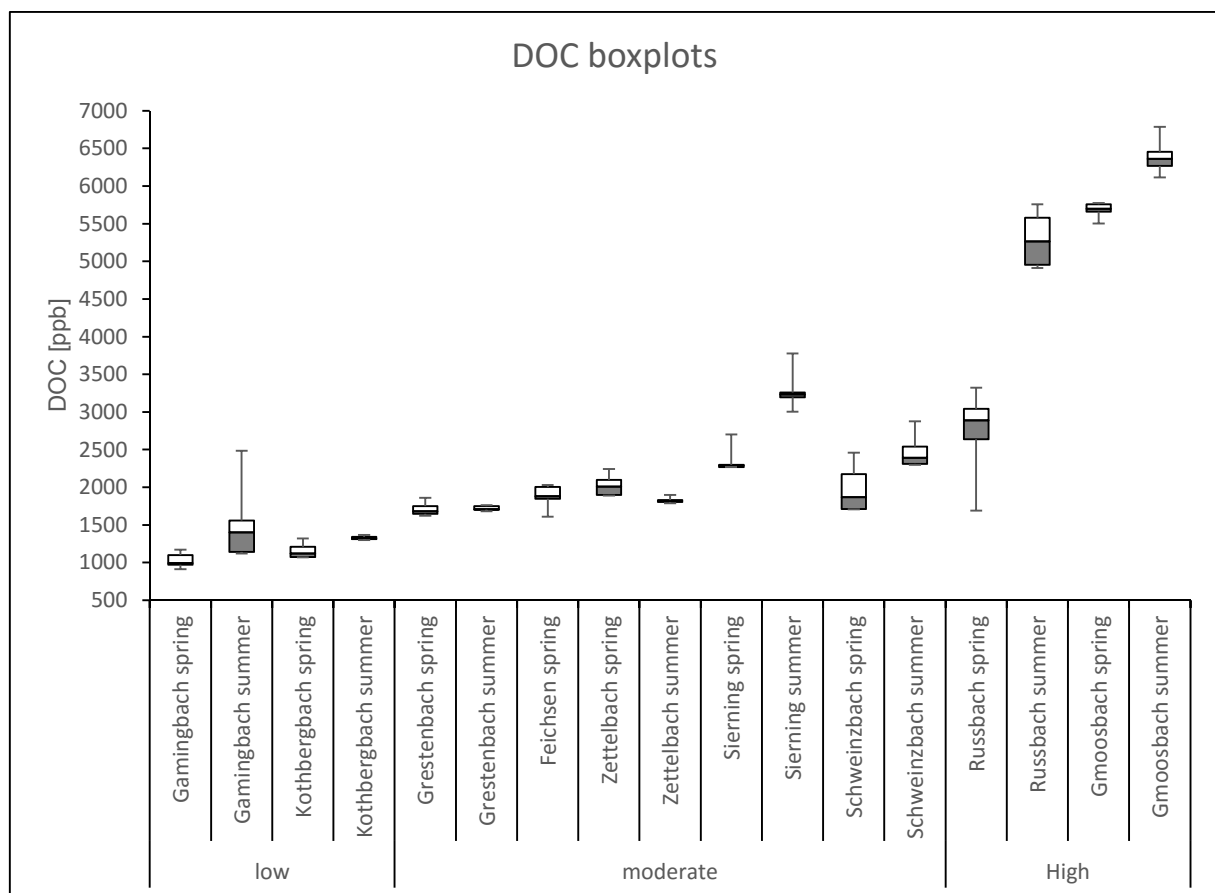


Figure 3: Dissolved organic carbon (DOC) content in the study streams during the investigations. Shown are median and 10, 25, 75 and 90% percentiles ($n=5-7$)

4.3.2 Optical properties of dissolved organic matter

Dissolved organic matter analysis produced a pattern reflecting the selected pollution groups of streams. The Gmoosbach showed distinctly different compositions than all the others, with extremely high values for all measured DOM parameters in both, spring and summer. For detailed values and standard deviations, check Table 26 and Table 27 in the appendix.

The stream with the second highest pollution, the Rußbach, also showed much higher values - especially in summer. In summer, the gradient between the different pollution groups generally was very clear. All DOM parameters were distinctly higher in the highly polluted streams, being in average 10 times higher for the Gmoosbach than for the Gamingbach (see Table 9). Moderately to highly polluted streams - including Sierning and Schweinzbach - showed distinctly lower values (T220=±330; T280=±340; B1=±110; B2=±800; A=±600; C=±5000). The rest of the DOM values ranged between those of Sierning, Schweinzbach and the lowly polluted stream Gamingbach (T220=140; T280=289; B1=78; B2=602; A=147; C=2978).

In spring the picture was more heterogeneous. Lowly polluted streams, like the Gamingbach and the Kothbergbach, showed the lowest values, fitting their assignment, with exception of the humic-like parameter C for the Kothbergbach, which lied in the moderate range. For the rest of the streams the parameters varied, but still the Rußbach showed high to moderate values. In general, the graduation was not as distinct as for summer DOM values. Especially the Schweinzbach varied very strongly in each DOM- parameter, for it showed the second highest value for tyrocine-like parameter B1, a moderate value for parameter T220 and the second lowest for humic-like C parameter.

Differences between spring and summer DOM values were quite distinct. All streams exhibited a strong decrease in summer for all parameters. The extent of decrease was more or less proportional to the pollution level, with rising total numbers for rising pollution gradient. Only the Rußbach showed an increase in all parameters of DOM, the Gamingbach increased in terms of T280, B2, A and C parameter. The humic-like C parameter of the Schweinzbach rose as well.

Table 9 and Table 10 show a summary of measured DOM parameters in spring and summer, respectively. A colour was assigned to each stream (compare Table 8) and the values were then ranked from highest to lowest.

Table 8: Colour code of the study streams for DOM parameter interpretation in Table 9 and Table 10

| color code | | | | | | | |
|-------------|---------|------------|-------------|----------|--------------|------------|--------------|
| stream name | | | | | | | |
| Gmoosbach | Rußbach | Zettelbach | Grestenbach | Sierning | Schweinzbach | Gamingbach | Kothbergbach |

Table 9: Dissolved Organic Matter (DOM) parameter values; colour coded and ranked from highest to lowest. Comparison among the study streams for mean spring fluorescence intensity values (tryptophan-like T220 and T280; tyrocine-like B1 and B2; humic-like A and C) (n=5-8)

| spring | | | | | |
|--------|------|-----|-------|------|-------|
| T220 | T280 | B1 | B2 | A | C |
| 4285 | 4042 | 655 | 10240 | 2872 | 33923 |
| 654 | 677 | 179 | 2140 | 1046 | 9937 |
| 496 | 573 | 163 | 1646 | 807 | 9806 |
| 478 | 556 | 152 | 1340 | 790 | 9032 |
| 463 | 497 | 139 | 1285 | 660 | 8782 |
| 384 | 472 | 118 | 1283 | 526 | 6671 |
| 272 | 379 | 86 | 1001 | 316 | 4290 |
| 160 | 255 | 76 | 439 | 143 | 2053 |

Table 10: Dissolved Organic Matter (DOM) parameter values; colour coded and ranked from highest to lowest. Comparison among the study streams for mean summer fluorescence intensity values (tryptophan-like T220 and T280; tyrocine-like B1 and B2; humic-like A and C) (n=5-8)

| summer | | | | | |
|--------|------|-----|------|------|-------|
| T220 | T280 | B1 | B2 | A | C |
| 1778 | 1361 | 875 | 2203 | 1938 | 11067 |
| 1774 | 1327 | 806 | 2194 | 1532 | 10190 |
| 353 | 364 | 114 | 842 | 694 | 6482 |
| 317 | 324 | 108 | 800 | 535 | 4874 |
| 264 | 304 | 98 | 743 | 427 | 4733 |
| 245 | 295 | 89 | 700 | 386 | 4221 |
| 226 | 289 | 83 | 657 | 274 | 3796 |
| 140 | 286 | 78 | 602 | 147 | 2978 |

4.3.3 Fluorescence Index (FIX) values

As mentioned before in the introduction, fulvic-acid represents a major fraction of DOM; and with the help of the FIX the sources of DOM in stream water can be distinguished. FIX-values higher than 1.9 point to a microbial fulvic-acid source, and therefore DOM from microbial sources, while values smaller than 1.4 indicate terrestrial fulvic-acid and therefore DOM from terrestrial source.

FIX values for nearly all streams, except Gamingbach and Gmoosbach, indicated a terrestrial source of fulvic-acid (see Table 11 and Table 12). Only the Gamingbach showed a microbial source in spring and summer time. The Gmoosbach seemed to run through a shift of source; while during spring a terrestrial source was detected, during summer a microbial source was predominant. In general, there was no significant difference between summer and spring in the FIX values, except for the Gmoosbach (Mann-Whitney-U; $p=0.161$; $n=16$). Independent of pollution gradient, FIX values for all categories had a tendency towards terrestrial sources.

Table 11: Mean fluorescence indices (FIX) and standard deviations in the study streams for spring values ($n=5-8$)

| stream name | | FIX spring | | |
|--------------|-----------|--------------------------------|--------------|------------------------------|
| | | terrestrial source FIX <1.4 | intermediate | microbial source FIX >1.9 |
| Rußbach | mean | 1.20 | | |
| | Std. dev. | 0.02 | | |
| Gmoosbach | mean | | | 2.35 |
| | Std. dev. | | | 0.13 |
| Zettelbach | mean | | 1.48 | |
| | Std. dev. | | 0.06 | |
| Grestenbach | mean | | 1.43 | |
| | Std. dev. | | 0.02 | |
| Sierning | mean | 1.30 | | |
| | Std. dev. | 0.02 | | |
| Gaming | mean | | | 2.15 |
| | Std. dev. | | | 0.09 |
| Schweinzbach | mean | 1.38 | | |
| | Std. dev. | 0.02 | | |
| Kothbergbach | mean | | 1.65 | |
| | Std. dev. | | 0.04 | |
| Feichsen | mean | | 1.44 | |
| | Std. dev. | | 0.02 | |

Table 12: Mean fluorescence indices (FIX) in the study streams for summer values (n=5-8)

| stream name | | FIX summer | | |
|--------------|-----------|--------------------------------|--------------|------------------------------|
| | | terrestrial source FIX <1.4 | intermediate | microbial source FIX >1.9 |
| Rußbach | mean | | 1.41 | |
| | Std. dev. | | 0.03 | |
| Gmoosbach | mean | 1.34 | | |
| | Std. dev. | 0.03 | | |
| Zettelbach | mean | 1.24 | | |
| | Std. dev. | 0.03 | | |
| Grestenbach | mean | 1.19 | | |
| | Std. dev. | 0.03 | | |
| Sierning | mean | 1.13 | | |
| | Std. dev. | 0.02 | | |
| Gaming | mean | | | 2.30 |
| | Std. dev. | | | 0.04 |
| Schweinzbach | mean | 1.14 | | |
| | Std. dev. | 0.02 | | |
| Kothbergbach | mean | | 1.49 | |
| | Std. dev. | | 0.03 | |
| Feichsen | mean | - | - | - |
| | Std. dev. | - | - | - |

4.3.4 Humification index (HIX) values:

The HIX describes the degree of humification. As mentioned in the introduction, high HIX values indicate aromatics of high molecular weight, while lower HIX values $\sim <2$ can be assigned to plant biomass and animal manure.

In total, the HIX values (Table 13) showed no significant difference between summer and spring values (Mann-Whitney-U; $p=1$; $n=16$), but for the Rußbach and the Gmoosbach, the HIX values changed between spring and summer. The HIX value of the Rußbach significantly decreased from 2.09 to 0.77, while for the Gmoosbach the HIX value increased from 0.45 to 1.02 from spring to summer. The other streams indicated no significant shifts in HIX values. Lowest HIX values could be assigned to the Gamingbach and the Kothbergbach, with values ranging from ~ 0.3 - 0.6 , while the highest HIX value (2.09) was found in spring at the Rußbach.

Table 13: Mean humification indices (HIX) and standard deviation in the study streams, summer and spring values (n=5-8)

| stream name | | HIX | |
|--------------|-----------|--------|--------|
| | | spring | summer |
| | | HIX | HIX |
| Rußbach | mean | 2.09 | 0.77 |
| | Std. dev. | 0.11 | 0.02 |
| Gmoosbach | mean | 0.45 | 1.02 |
| | Std. dev. | 0.02 | 0.03 |
| Zettelbach | mean | 0.87 | 0.89 |
| | Std. dev. | 0.04 | 0.03 |
| Grestenbach | mean | 0.79 | 0.83 |
| | Std. dev. | 0.05 | 0.01 |
| Sierning | mean | 1.36 | 1.61 |
| | Std. dev. | 0.04 | 0.05 |
| Gamingbach | mean | 0.29 | 0.27 |
| | Std. dev. | 0.01 | 0.01 |
| Schweinzbach | mean | 1.13 | 1.28 |
| | Std. dev. | 0.01 | 0.04 |
| Kothbergbach | mean | 0.61 | 0.50 |
| | Std. dev. | 0.02 | 0.01 |
| Feichsen | mean | 0.90 | |
| | Std. dev. | 0.01 | |

4.3.5 β/α -ratio

As described in chapter 1.2.2, β/α -ratio represents the relative portion of recently derived protein-like DOM in the β -region to the highly degraded humic-like compounds in the α -region. Values >1 are typically identified with autochthonous DOM, while values $<0,6$ are usually linked to allochthonous DOM.

For most streams, β/α -ratios showed a mixed result with a tendency towards autochthonous DOM (compare Table 14 and Table 15). There was no significant difference between spring and summer values (Mann-Whitney-U; $p=1$; $n=2$). The β/α -ratio of the Rußbach experienced a shift from allochthonous towards autochthonous material (from 0.6 to 1.09). Zettelbach, Sierning, Grestenbach, Schweinzbach and Gmoosbach showed a small decrease in β/α -ratio, with Schweinzbach revealing the strongest decrease from 0.96 to 0.66. The Kothbergbach and Gamingbach exhibited increases of the β/α -ratio with a difference of ~ 0.2 .

Table 14: Spring β/α -ratios of the study streams (n=5-8, mean and standard deviations)

| | | spring | | |
|--------------------|-----------|----------------------------------|---------------------|----------------------|
| stream name | | β/α | | |
| | | allochthonous | | autochthonous |
| | | <0.6 | intermediate | >1 |
| Rußbach | mean | 0.60 | | |
| | Std. dev. | 0.02 | | |
| Gmoosbach | mean | | | 1.17 |
| | Std. dev. | | | 0.03 |
| Zettelbach | mean | | 0.83 | |
| | Std. dev. | | 0.03 | |
| Grestenbach | mean | | 0.99 | |
| | Std. dev. | | 0.08 | |
| Sierning | mean | | 0.68 | |
| | Std. dev. | | 0.01 | |
| Gamingbach | mean | | | 1.71 |
| | Std. dev. | | | 0.29 |
| Schweinzbach | mean | | 0.96 | |
| | Std. dev. | | 0.36 | |
| Kothbergbach | mean | | | 1.07 |
| | Std. dev. | | | 0.05 |
| Feichsen | mean | | | 1.05 |
| | Std. dev. | | | 0.13 |

Table 15: Summer β/α -ratios of the study streams (n=5-8, mean and standard deviation)

| | | summer | | |
|--------------------|-----------|----------------------------------|---------------------|----------------------|
| stream name | | β/α | | |
| | | allochthonous | | autochthonous |
| | | <0.6 | intermediate | >1 |
| Rußbach | mean | | | 1.09 |
| | Std. dev. | | | 0.03 |
| Gmoosbach | mean | | 0.95 | |
| | Std. dev. | | 0.06 | |
| Zettelbach | mean | | 0.85 | |
| | Std. dev. | | 0.03 | |
| Grestenbach | mean | | 0.82 | |
| | Std. dev. | | 0.03 | |
| Sierning | mean | | 0.69 | |
| | Std. dev. | | 0.09 | |
| Gamingbach | mean | | | 1.91 |
| | Std. dev. | | | 0.13 |
| Schweinzbach | mean | | 0.66 | |
| | Std. dev. | | 0.01 | |
| Kothbergbach | mean | | | 1.27 |
| | Std. dev. | | | 0.08 |
| Feichsen | mean | - | - | - |
| | Std. dev. | - | - | - |

4.3.6 Correlations

The analysis showed that all DOM fractions were highly intercorrelated (see Table 16). The DOM ratios HIX and FIX were negatively correlated with each other, so were FIX and β/α -ratio. HIX and β/α -ratio, in turn, were positively correlated. FIX was also positively correlated to humic-like parameter A, also a positive correlation of HIX and mean flow velocity V was found. Water $\text{NO}_2\text{-N}$ concentrations were positively correlated with all DOM parameters; most of the DOM parameters were correlated to $\text{NO}_3\text{-N}$ water concentrations as well. $\text{NH}_4\text{-N}$ was positively correlated to the DOM parameter C. Organic matter was highly positive correlated to tryptophan-like (T220) and also to T280 and tyrosine-like B1, as well as to humic-like A. For temperature and chlorophyll-a no correlations with any of the tested parameters could be found.

Results

Table 16: Spearman's rank correlation matrix, including DOM parameter, water nitrogen values, mean flow velocity (V), mean temperature, organic matter (OM) and benthic chlorophyll-a (chl-a) of the study streams (n=17)

| | correlation matrix | | | | | | | | | | | | | | |
|----------------------------------|--------------------|----------------|----------------|----------------|----------------|-----------------|-----------------|-----------------|--------------------|--------------------|--------------------|-------------------------------|--------------------|----------------|-------|
| | DOM parameter | | | | | water parameter | | | | | | | sediment parameter | | |
| | T280 | B1 | B2 | A | C | HIX | FIX | β/α | NO ₂ -N | NO ₃ -N | NH ₄ -N | V mean [ms ⁻¹] | Temp mean [°C] | OM | chl-a |
| T220 | 0.956** | 0.914** | 0.919** | 0.890** | 0.733** | n.s | n.s | n.s | 0.620** | 0.507* | n.s | n.s | n.s | 0.642** | n.s |
| T280 | | 0.843** | 0.939** | 0.897** | 0.777** | n.s | n.s | n.s | 0.627** | 0.539* | n.s | n.s | n.s | 0.518* | n.s |
| B1 | | | 0.860** | 0.900** | 0.623** | n.s | n.s | n.s | 0.711** | 0.588* | n.s | n.s | n.s | 0.604* | n.s |
| B2 | | | | 0.836** | 0.853** | n.s | n.s | n.s | 0.549* | n.s | n.s | n.s | n.s | n.s | n.s |
| A | | | | | 0.689** | n.s | 0.512* | n.s | 0.863** | 0.792** | 0.512* | n.s | n.s | 0.513* | n.s |
| C | | | | | | n.s | n.s | n.s | 0.532* | n.s | n.s | n.s | n.s | n.s | n.s |
| HIX | | | | | | | -0.836** | 0.868** | -0.603* | 0.654** | n.s | 0.608** | n.s | n.s | n.s |
| FIX | | | | | | | | -0.897** | 0.637** | 0.777** | n.s | n.s | n.s | n.s | n.s |
| β/α | | | | | | | | | -0.564* | 0.681** | n.s | n.s | n.s | n.s | n.s |
| NO₂-N | | | | | | | | | | 0.909** | 0.625** | n.s | n.s | n.s | n.s |
| NO₃-N | | | | | | | | | | | 0.667** | n.s | n.s | n.s | n.s |
| NH₄-N | | | | | | | | | | | | n.s | n.s | n.s | n.s |
| V | | | | | | | | | | | | | n.s | n.s | n.s |
| temp | | | | | | | | | | | | | | n.s | n.s |
| OM | | | | | | | | | | | | | | | n.s |

4.4 Sediment characteristics

Sedimentary nitrogen values showed a clear gradient in terms of pollution (compare Table 17). In comparison to water nitrogen values, it is remarkable that sedimentary NO₂-N concentrations decreased in summer time, while water NO₂-N concentrations were higher in summer than in spring. For highly polluted streams (Rußbach, Gmoosbach and Schweinzbach as well) sedimentary NH₄-N values were the highest, in difference to water-nitrogen values where NO₃-N was the dominant nitrogen species. For moderately polluted streams in contrast, sedimentary NO₃-N was the predominant nitrogen value.

Table 17: Nitrate (NO₃-N), nitrite (NO₂-N), and ammonium (NH₄-N) concentrations [$\mu\text{g l}^{-1}$] in the study stream sediments during the investigations (means and standard deviations, n =5)

| Nitrate, nitrite and ammonium concentrations in the sediments | | | | | | | |
|---|----------|--|--------|--|--------|--|--------|
| stream name | | sediment NO ₃ -N [$\mu\text{g g}^{-1}$] | | sediment NO ₂ -N [$\mu\text{g g}^{-1}$] | | sediment NH ₄ -N [$\mu\text{g g}^{-1}$] | |
| | | Spring | Summer | Spring | Summer | Spring | Summer |
| Rußbach | mean | 3.5 | 3.4 | 1.5 | 0.2 | 27.1 | 3.7 |
| | std. dev | 1.6 | 0.8 | 0.5 | 0 | 11.8 | 0.5 |
| Gmoosbach | mean | 9.9 | 0.5 | 1.7 | 0.4 | 11.6 | 9.3 |
| | std. dev | 11.3 | 0.2 | 0.5 | 0.1 | 3.2 | 0.4 |
| Sierning | mean | 4.1 | 1.6 | 0.1 | 0.1 | 0.6 | 0.4 |
| | std. dev | 1.2 | 0.7 | 0 | 0.1 | 0.2 | 0.2 |
| Grestenbach | mean | 6.8 | 2.3 | 0.9 | 0.13 | 0.8 | 1.7 |
| | std. dev | 1.7 | 1.5 | 0.3 | 0.1 | 0.2 | 0.9 |
| Zettelbach | mean | 2.4 | 2.2 | 0.1 | 0.2 | 0.7 | 0.7 |
| | std. dev | 0.7 | 0.4 | 0 | 0 | 0.2 | 0.5 |
| Schweinzbach | mean | 1.1 | 2.1 | 0.3 | 0.05 | 2.8 | 0.5 |
| | std. dev | 1 | 0.2 | 0.2 | 0 | 0.9 | 0.1 |
| Kothbergbach | mean | 1.7 | 1.23 | 0.1 | 0.03 | 0.5 | 1.7 |
| | std. dev | 0.4 | 0 | 0 | 0 | 0.1 | 0.3 |
| Gamingbach | mean | 2.9 | 1.5 | 0.1 | 0.03 | 0.5 | 1.9 |
| | std. dev | 0.9 | 0.2 | 0 | 0 | 0.3 | 0.5 |
| Feichsen | mean | 1.6 | - | 0.5 | - | 0.7 | - |
| | std. dev | 0.6 | | 0.1 | | 0.6 | |

Organic matter content of the sediments decreased or stayed more or less constant for all streams during summer (Table 18). Highly polluted streams showed higher organic matter content - except spring values of the Gamingbach - but in general all streams showed low OM values. Microbial abundances in the sediments showed a heterogeneous pattern, with highest values around 25 billion per gram dry weight in the Rußbach, and lowest in the Grestenbach with around 1.7 billion per gram dry weight.

Benthic chlorophyll-a was as well rather different for each stream, but with a tendency of smaller concentrations for lowly polluted streams (Table 18). During summer, chlorophyll-a values dropped for most streams.

Table 18: Summary of organic matter, microbial abundance in billions [bn/g dry weight] and benthic chlorophyll-a concentration in microgram per square centimetre [μgcm^{-2}] in the study stream sediments during the investigations (means and standard deviations, n = 5-10)

| sediment characteristics | | | | | | | |
|--------------------------|----------|--------------------|--------|--|--------|--|--------|
| stream name | | organic matter [%] | | microbial abundances [bn/g dry weight] | | benthic chl-a [μgcm^{-2}] | |
| | | Spring | Summer | Spring | Summer | Spring | Summer |
| Rußbach | mean | 4.87 | 1.33 | - | 24.93 | 507.85 | 49.99 |
| | std. dev | 0.7 | 0.22 | | 11 | 445.36 | 24.61 |
| Gmoosbach | mean | 6.68 | 6.66 | - | 9.81 | 15.15 | 5.65 |
| | std. dev | 1.5 | 1.04 | | 2.4 | 14.84 | 2.17 |
| Sierning | mean | 1.02 | 1.53 | - | 3.58 | 32.14 | 2.83 |
| | std. dev | 0.2 | 0.26 | | 2.2 | 15.62 | 2.09 |
| Grestenbach | mean | 1.9 | 1.26 | - | 1.74 | 168.1 | 22.62 |
| | std. dev | 0.1 | 0.08 | | 1.4 | 13.54 | 24.09 |
| Zettelbach | mean | 1.5 | 1.04 | - | 2.11 | 63.2 | 7.18 |
| | std. dev | 0 | 0.13 | | 1.1 | 39.49 | 3.19 |
| Schweinzbach | mean | 1.1 | 1.33 | - | 4.49 | 74.74 | 100.45 |
| | std. dev | 0.1 | 0.13 | | 2.2 | 31.5 | 113.33 |
| Kothbergbach | mean | 0.42 | 0.58 | - | 8.96 | 13.57 | 5.87 |
| | std. dev | 0.1 | 0.13 | | 3.5 | 6.72 | 2.19 |
| Gamingbach | mean | 2.99 | 0.38 | - | 3.8 | 18.87 | 16.53 |
| | std. dev | 0.2 | 0.1 | | 1.9 | 7.62 | 2.34 |
| Feichsen | mean | 2.1 | - | - | - | 37.14 | - |
| | std. dev | 0.2 | | | - | 23.33 | |

4.5 Concentrations of dissolved greenhouse gases in the water

Gas concentrations showed highly significant differences for CH_4 and CO_2 between highly to moderately polluted streams and lowly polluted streams {(high to moderate CH_4 ; Mann-Whitney-U; $p=0.001$; $n=72$; Figure 4, Figure 5, Figure 6) (high to moderate CO_2 ; Mann-Whitney-U; $p=0.000$; $n=72$)}. Differences in N_2O concentrations were not significant among stream groups (Figure 6). For detailed gas values (in ppm) and standard deviations, check Table 28 in the appendix.

Figure 4 shows, that the CO_2 concentrations were highest in streams of high pollution as the Gmoosbach with ~ 2040 ppm in spring and ~ 1560 ppm in summer, while they were lowest in streams of low pollution (Feichsen with ~ 600 ppm in spring and in the Kothbergbach in the summer with ~ 180 ppm).

Figure 5 shows, that the CH_4 concentrations were highest in the group of high pollution (Gmoosbach ~ 11 ppm in spring; highest in the Schweinzbach ~ 16 ppm in summer). The lowest concentrations were observed in the streams of low pollution (Kothbergbach with ~ 1.3 ppm in spring and ~ 2 ppm in summer).

Figure 6 shows, that the N_2O concentrations were quite similar for all streams (values around 0.5 ppm). In summer values were higher for all pollution groups (values around 1 ppm).

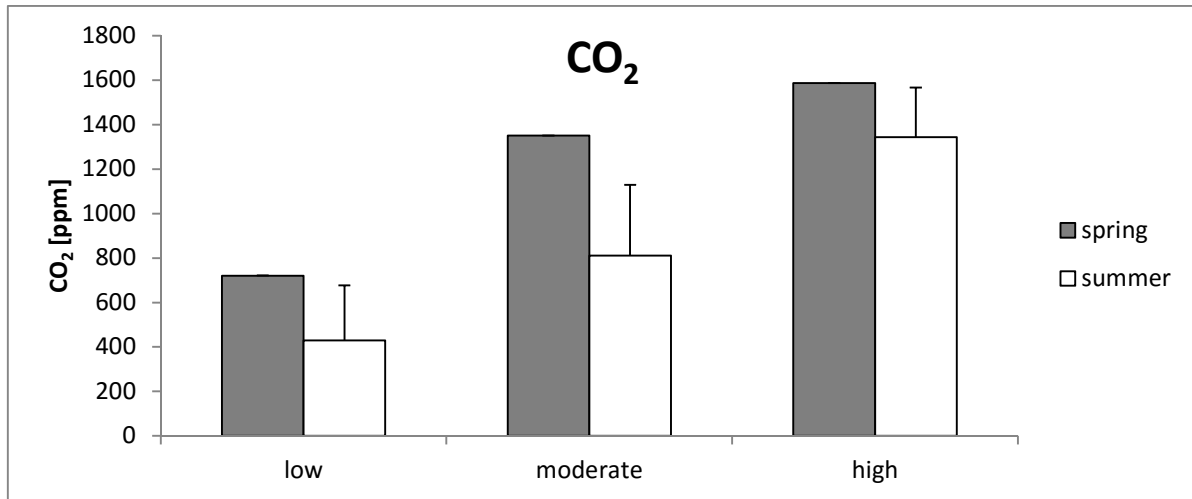


Figure 4: CO₂ concentrations of investigated streams [in ppm] for spring and summer (n=5-12) (mean and standard deviation)

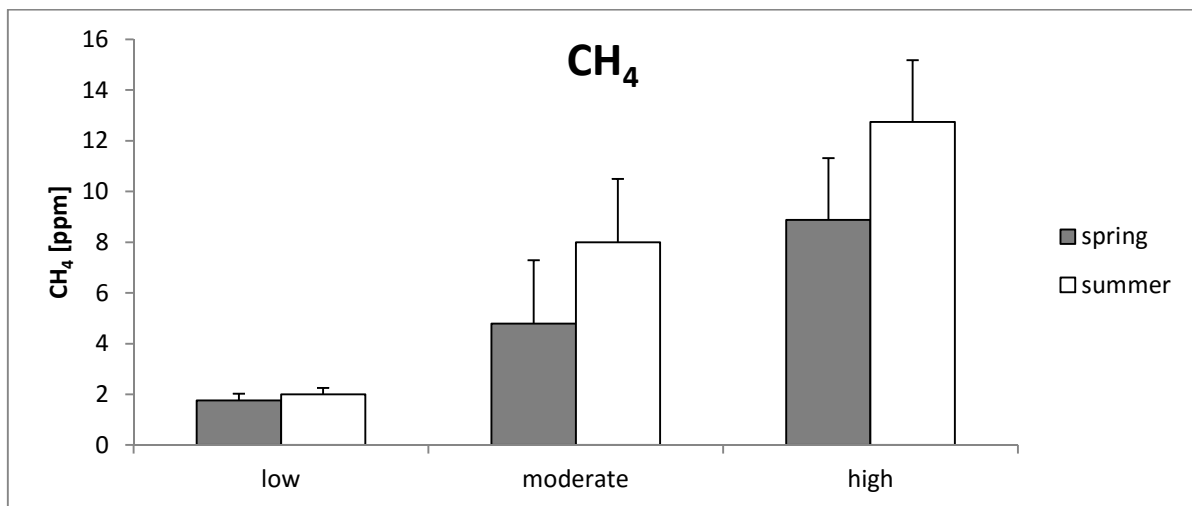


Figure 5: CH₄ concentrations of investigated streams [in ppm] for spring and summer (n=5-12) (mean and standard deviation)

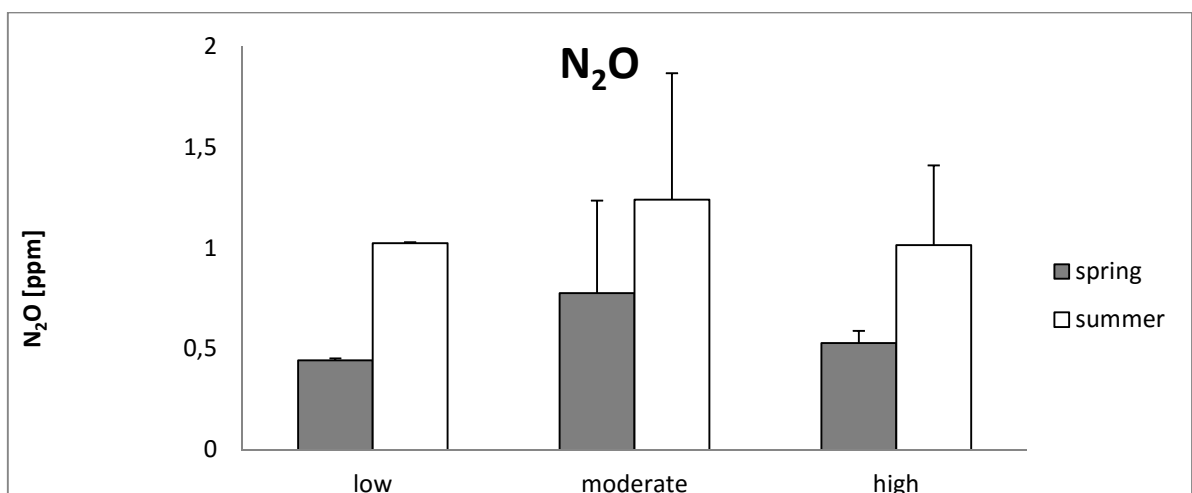


Figure 6: N₂O concentrations of investigated streams [in ppm] for spring and summer (n=5-12) (mean and standard deviation)

4.5.1 Seasonal differences in the gas concentrations

General comparison of all streams showed significant changes between summer and spring gas concentration (Figure 5; Figure 6; Figure 4). Methane (CH₄) concentrations were higher in summer (Mann-Whitney-U; p=0.000), so were the N₂O values (Mann-Whitney-U; p=0.000). CO₂ concentrations, on the other hand, were lower in summer (Mann-Whitney-U; p=0.000). The Rußbach showed high changes in CH₄ concentrations (increasing to the 2-fold of the spring value), and also N₂O values increased significantly. Only CO₂ values stayed quite stable across the seasons. Gmoosbach stayed almost constant in its CH₄ concentrations, but exhibited a decrease in CO₂ concentrations, while N₂O concentrations even triplicated.

4.5.2 The global warming estimate by Bauer (B_f)

The numbering, described in chapter 3.6.1, shows the differences of stream position depending on the greenhouse gas used for ranking. The introduced Bauer-Global Warming estimate (B_f) provides a clear ranking referenced to the global warming potential (Table 19 and Table 20).

In spring, the B_f more or less followed the ranking of DOM, DOC and nutrient loading for streams. Gamingbach and Kothbergbach showed the lowest global warming potential and Gmoosbach the highest. It shows that, if considering the GWP of all 3 gas species at the same time, even a highly polluted stream (like the Rußbach) can range in the middle field concerning its relevance for the atmosphere. This observation even strengthened for the summer values. In summer, as mentioned before, ranking in terms of DOM, DOC and nutrients was even clearer, but concentrations of greenhouse gases showed a more distinct picture. The lowly polluted stream Gamingbach was shown to produce more greenhouse gases relevant in terms of global warming than a moderately polluted stream like the Grestenbach.

Table 19: Ranking after greenhouse gas concentrations according to the dissolved greenhouse gas concentrations of CH₄, CO₂, and N₂O and the Bauer-Global Warming potential for the study streams in spring. The numbers in brackets indicate the position in the ranking compared to the initial CH₄ rank for interpretation

| spring | | | | | | | |
|-----------------------|-------|-----------------------|---------|------------------------|------|------------------|------|
| CH ₄ [ppm] | | CO ₂ [ppm] | | N ₂ O [ppm] | | BF | |
| [1] Kothbergbach | 1.39 | [3] Feichsen | 598.23 | [2] Gamingbach | 0.43 | [3] Feichsen | 770 |
| [2] Gamingbach | 1.91 | [2] Gamingbach | 738.41 | [3] Feichsen | 0.45 | [2] Gamingbach | 904 |
| [3] Feichsen | 1.98 | [1] Kothbergbach | 824.53 | [1] Kothbergbach | 0.45 | [1] Kothbergbach | 984 |
| [4] Grestenbach | 2.12 | [5] Zettelbach | 1099.21 | [4] Grestenbach | 0.47 | [5] Zettelbach | 1306 |
| [5] Zettelbach | 2.53 | [6] Rußbach | 1132.51 | [6] Rußbach | 0.47 | [6] Rußbach | 1405 |
| [6] Rußbach | 6.44 | [7] Schweinzbach | 1145.71 | [5] Zettelbach | 0.53 | [7] Schweinzbach | 1740 |
| [7] Schweinzbach | 6.70 | [4] Grestenbach | 1575.83 | [8] Sierning | 0.54 | [4] Grestenbach | 1756 |
| [8] Sierning | 7.81 | [8] Sierning | 1580.63 | [9] Gmoosbach | 0.59 | [8] Sierning | 1899 |
| [9] Gmoosbach | 11.31 | [9] Gmoosbach | 2039.81 | [7] Schweinzbach | 1.57 | [9] Gmoosbach | 2447 |

Table 20: Ranking after greenhouse gas concentrations according to Bauer-Global Warming potential for the study streams in summer. The numbers in brackets indicate the position in the ranking compared to the initial CH₄ rank for interpretation

| summer | | | | | | | |
|-----------------------|-------|-----------------------|---------|------------------------|------|------------------|------|
| CH ₄ [ppm] | | CO ₂ [ppm] | | N ₂ O [ppm] | | BF | |
| [1] Kothbergbach | 1.99 | [1] Kothbergbach | 182.16 | [6] Rußbach | 0.62 | [1] Kothbergbach | 519 |
| [2] Gamingbach | 2.00 | [4] Grestenbach | 449.97 | [4] Grestenbach | 0.73 | [4] Grestenbach | 721 |
| [4] Grestenbach | 2.83 | [5] Zettelbach | 626.02 | [5] Zettelbach | 0.89 | [5] Zettelbach | 958 |
| [5] Zettelbach | 3.55 | [2] Gamingbach | 677.42 | [1] Kothbergbach | 1.02 | [2] Gamingbach | 1018 |
| [8] Sierning | 9.66 | [8] Sierning | 866.66 | [8] Sierning | 1.03 | [8] Sierning | 1367 |
| [9] Gmoosbach | 12.74 | [6] Rußbach | 1120.55 | [2] Gamingbach | 1.03 | [6] Rußbach | 1567 |
| [6] Rußbach | 12.76 | [7] Schweinzbach | 1300.21 | [9] Gmoosbach | 1.41 | [9] Gmoosbach | 2240 |
| [7] Schweinzbach | 15.96 | [9] Gmoosbach | 1565.99 | [7] Schweinzbach | 2.31 | [7] Schweinzbach | 2302 |

4.5.3 Diurnal patterns and influence of pool and riffle sections on gas concentration in stream water during summer sampling

The data were interpreted statistically as well as visually (Figure 7; Figure 8; Figure 9; Figure 10; Figure 11; Figure 12). However, there was no general significant trend in gas concentrations concerning diurnal changes {(CH₄; Kruskal-Wallis-Test; p=0.826, n=48) (CO₂; Kruskal-Wallis-Test; p=0.436; n=48) (N₂O; Kruskal-Wallis-Test; p=1.294; n=48)}. Most variations were within the range of the standard deviation of the gas concentrations.

For CH₄ concentrations (Figure 7; Figure 8), Schweinzbach and Gmoosbach showed a slight diurnal pattern with a maximum at noon for the pool section. For the riffle section, they both showed a slight decrease in concentrations. The other streams did not reveal diurnal patterns. CO₂ concentrations (Figure 9; Figure 10) showed a decline for the Gmoosbach at pool and

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riffle sections, this pattern was also found at the Gamingbach. The rest of the study streams did not show any patterns for CO₂ concentrations.

N₂O concentrations (Figure 11; Figure 12) indicated a decline for water concentrations for the Sierning in the riffle section and an increase in the riffle sections of Kothbergbach and Gmoosbach.

In general, there was no significant difference between greenhouse gas water concentrations of “pool” sections and “riffle” sections of the study streams {(CH₄; Mann-Whitney-U; p=0.910; n=96) (CO₂; Mann-Whitney-U; p=0.786; n=96) (N₂O; Mann-Whitney-U; p=0.679; n=96)}

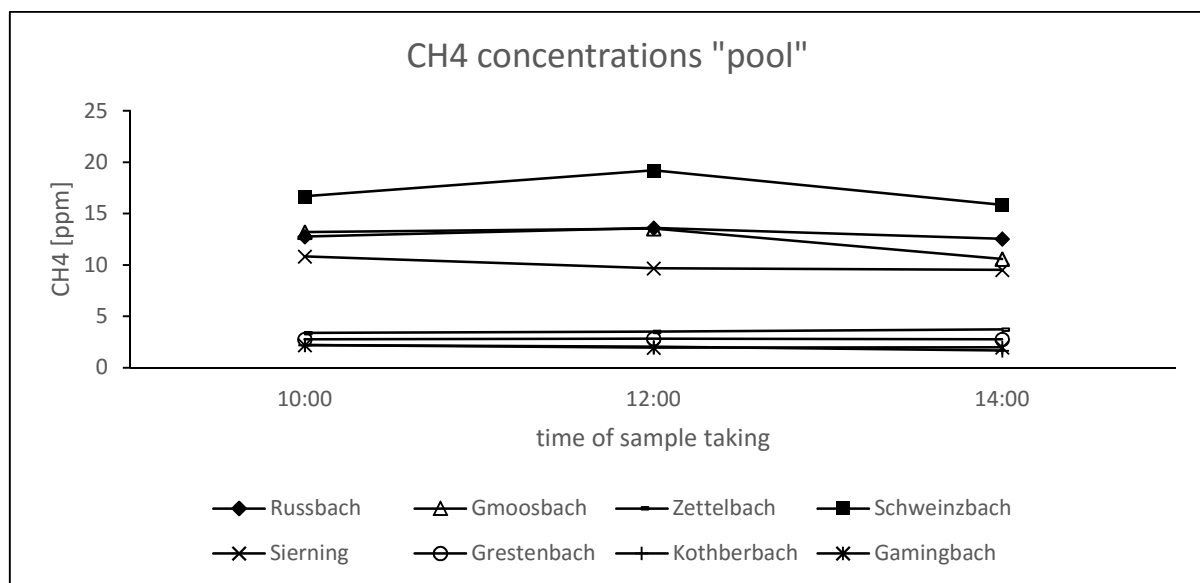


Figure 7: Comparison of CH₄ water concentrations in “pool” sections of the investigated streams

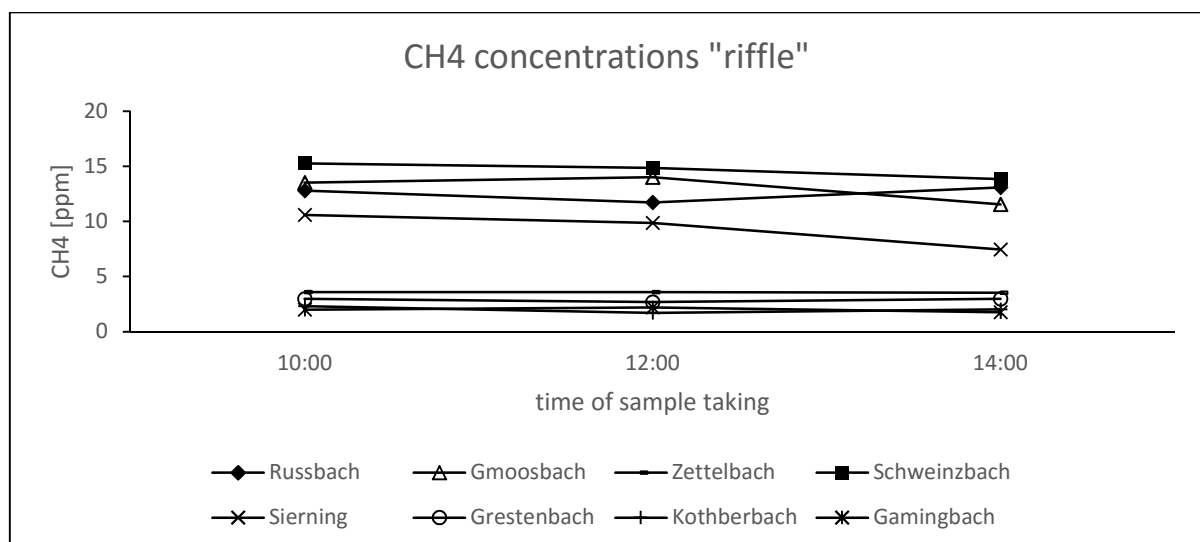


Figure 8: Comparison of CH₄ water concentrations in “riffle” sections of the investigated streams

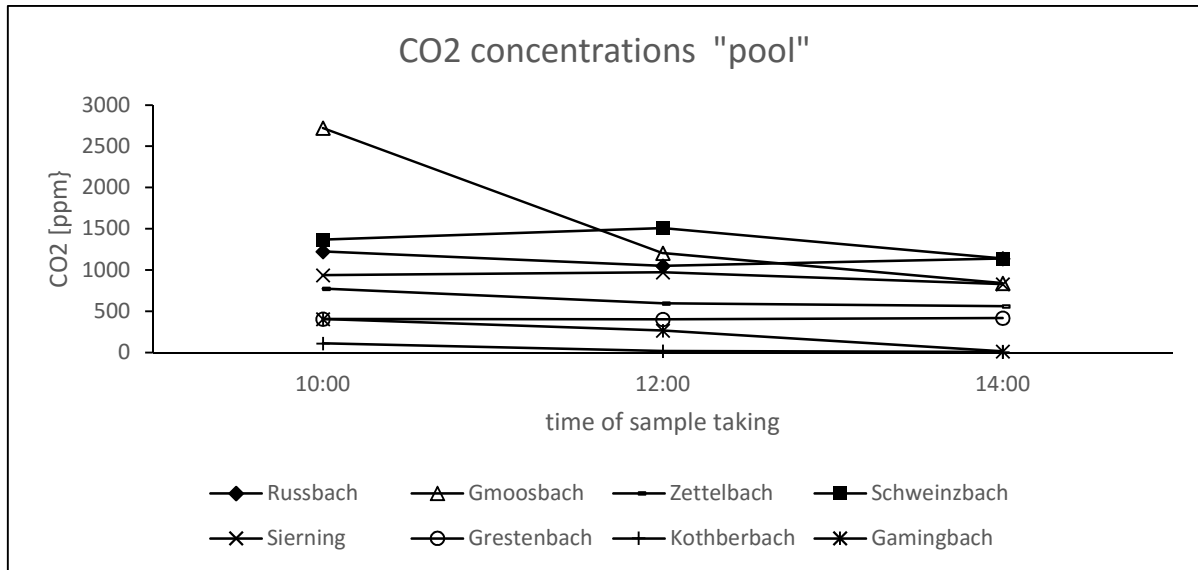


Figure 9: Comparison of CO₂ water concentrations in "pool" sections of the investigated streams

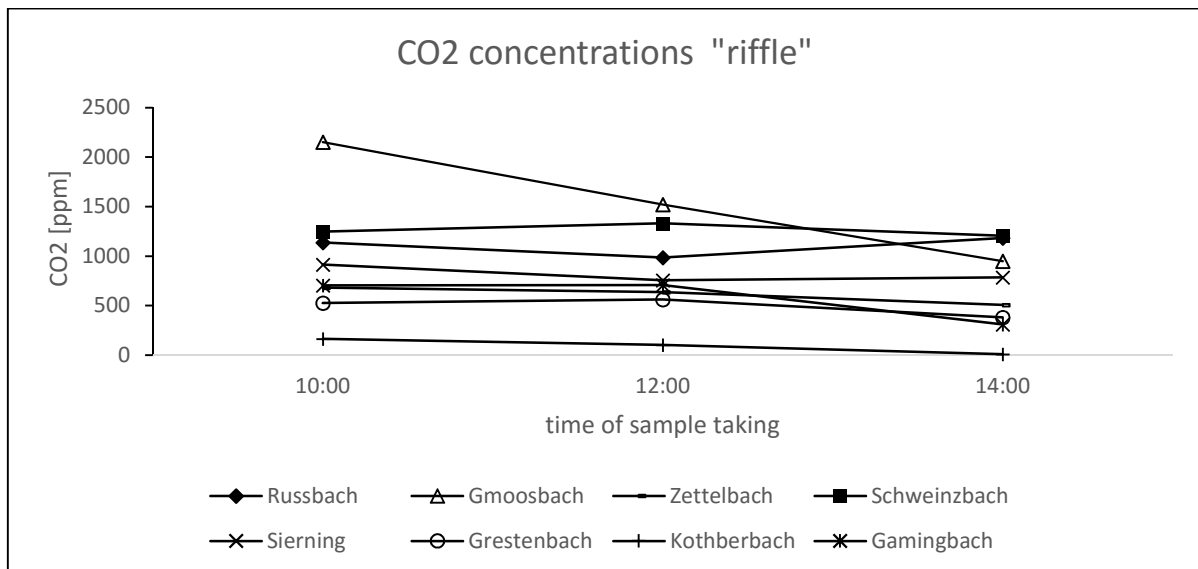


Figure 10: Comparison of CO₂ water concentrations in "riffle" sections of the investigated streams

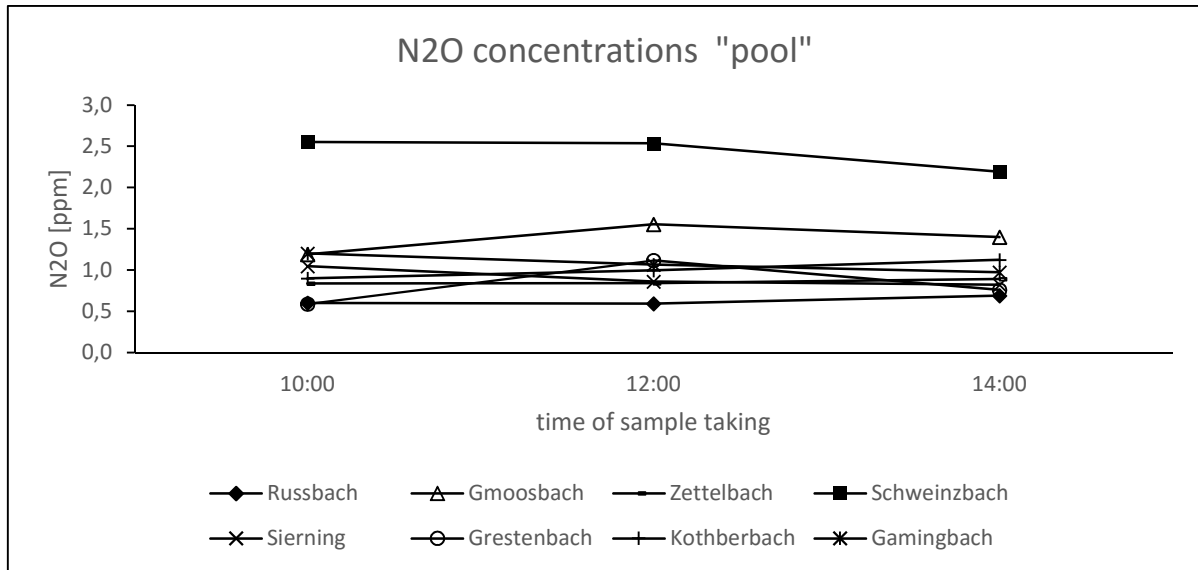


Figure 11: Comparison of N₂O water concentrations in "pool" sections of the investigated streams

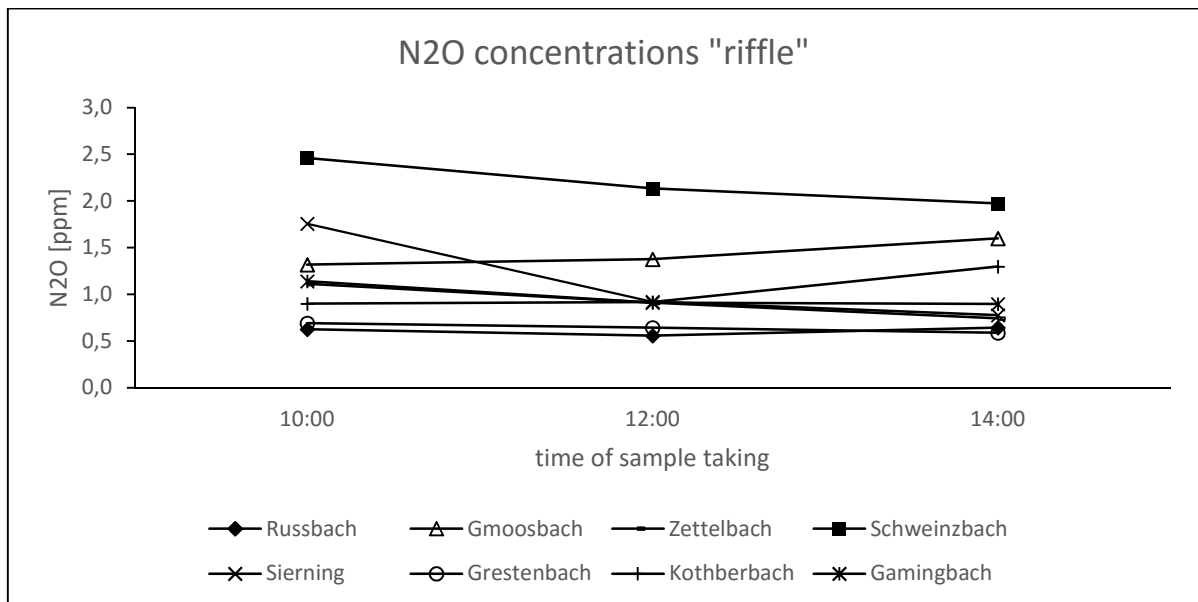


Figure 12: Comparison of N₂O water concentrations in "riffle" sections of the investigated streams

4.6 Factors influencing the concentrations of greenhouse gases

A strong correlation between CO₂ and CH₄ concentrations could be determined, while N₂O concentrations only correlated with CH₄ concentrations, but not with CO₂ (see Table 21).

Table 21: Spearman's rank correlation of the greenhouse gas concentrations with each other, with significance level

| correlations between gas concentrations | | | |
|--|---------------------|---------------------|----------------------|
| | gas CH ₄ | gas CO ₂ | gas N ₂ O |
| gas CH ₄ correlation coefficient | | 0.63** | 0.61** |
| gas CO ₂ correlation coefficient | | | n.s |
| gas N ₂ O correlation coefficient | | | |

** level of significance p<0.01
 * level of significance p<0.05

As shown in Table 22 and Figure 13, the DOC amount in the stream water showed positive correlations with CH₄ and CO₂ concentrations. N₂O concentrations however were not correlated to the amount of DOC, while B_f revealed a positive correlation with DOC concentration.

Table 22: Spearman's rank correlation of dissolved organic carbon (DOC) with greenhouse gas concentrations and global warming estimate by Bauer potential for the study streams, with significance level

| correlation DOC and gas concentrations | | | | |
|--|-----------------|-----------------|------------------|----------------|
| | CH ₄ | CO ₂ | N ₂ O | B _f |
| DOC correlation coefficient | 0.88** | 0.64** | n.s | 0.71** |

** level of significance p<0.01
 * level of significance p<0.05

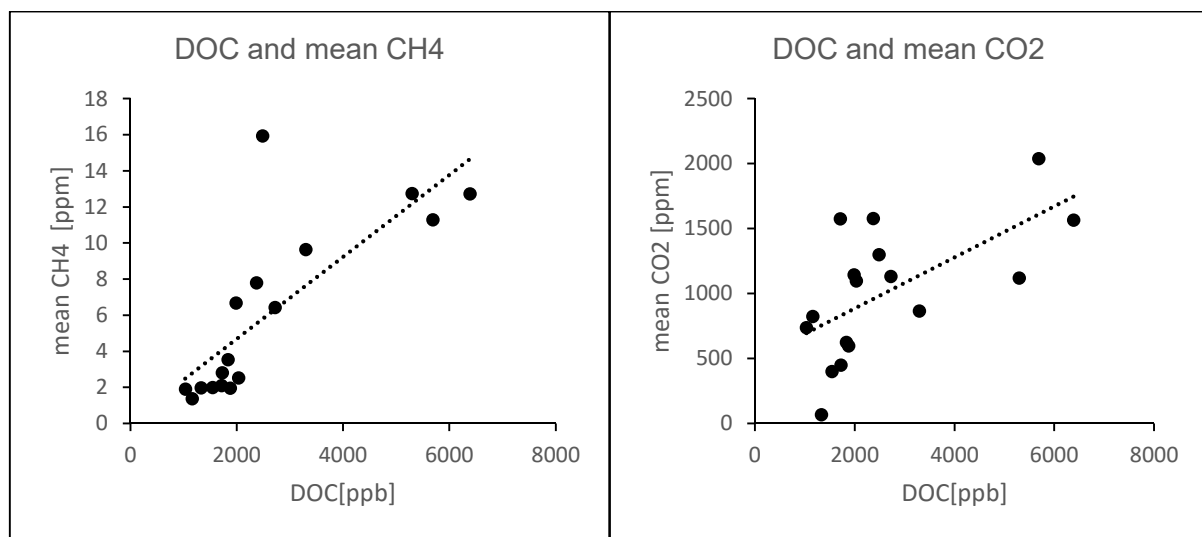


Figure 13: Correlations between dissolved organic carbon (DOC) and CH₄, N₂O and CO₂ concentrations of the study streams

No significant correlation between the different DOM parameters and the N₂O concentrations in the streams could be found (see Table 23).

Different results could be revealed in terms of CO₂, for they showed high positive correlations with tryptophan- and tyrocine-, as well as with humic-like parameters. CH₄ showed a quite diverse picture, with the tryptophan- and humic-like parameters showing strong correlation, unlike tyrosine-like B2. The B_f showed the same patterns as CH₄ and CO₂ correlation, with tendency towards humic-like substances. DOM quality therefore seemed to be decisive for CO₂ and CH₄, but not for N₂O production.

Table 23: Spearman's rank correlation between greenhouse gas concentrations and parameters of dissolved organic matter (DOM): tryptophan-like (T220; T280), tyrocine-like (B1; B2), humic-like (A; C) and indices (HIX; FIX; β/α) for study streams, with significance level

| | | | correlation DOM and GAS concentrations | | | |
|-----------------|----------------|-------------------------|---|-----------------------|-----------------------|----------------------|
| | | | CH₄ | CO₂ | N₂O | B_f |
| Tryptophan-like | T220 | correlation coefficient | 0.49* | 0.65** | n.s | 0.59* |
| | T280 | correlation coefficient | 0.50* | 0.66** | n.s | 0.62** |
| Tyrocine-like | B1 | correlation coefficient | 0.59* | 0.64** | n.s | 0.58* |
| | B2 | correlation coefficient | n.s | 0.66** | n.s | 0.59* |
| Humic-like | A | correlation coefficient | 0.75** | 0.73** | n.s | 0.71** |
| | C | correlation coefficient | 0.49* | 0.67** | n.s | 0.62** |
| Index | HIX | correlation coefficient | n.s | n.s | n.s | n.s |
| | FIX | correlation coefficient | 0.50* | n.s | n.s | n.s |
| | β/α | correlation coefficient | n.s | n.s | n.s | n.s |

** level of significance $p < 0.01$

* level of significance $p < 0.05$

Ambient water nitrogen parameters turned out to represent controlling factors for CH₄ and CO₂ concentrations (Table 24). Especially in-stream NO₂-N and NO₃-N concentration strongly positively correlated with both, CH₄ and CO₂, while NH₄-N positively correlated with CH₄.

Concerning the sediments, NO₂-N and NO₃-N, as well as chlorophyll-a values were not correlated to greenhouse gas production, with the exception of NO₃-N concentration in the sediment showing a significantly negative correlation with N₂O production. Sedimentary nitrogen concentrations therefore seemed to be of no high importance for greenhouse gas production in streams. The Bauer-factor only showed significance in terms of in-stream N-values. No correlation could be found between microbial abundance and any of the greenhouse gas concentrations, nor for organic matter. Temperature data indicated a strong correlation between maximum temperature and greenhouse gas concentrations (Table 24 and Figure 14 and 15), especially for CH₄ and N₂O production, but not for CO₂. For the mean flow velocity, a negative correlation with water N₂O concentration could be found.

Table 24: Spearman’s rank correlation between greenhouse gas concentrations and sedimentary and water nitrogen values (NO₂-N, NO₃-N, NH₄-N), benthic chlorophyll-a (chl-a) concentration, microbial abundance, maximum water temperature (temp_{max}), mean flow velocity (V_{mean}) and organic matter, with significance level

| | | gas concentration and sediment and water chemistry | | | |
|--------------------------|-------------------------|--|-----------------|------------------|----------------|
| | | CH ₄ | CO ₂ | N ₂ O | B _f |
| NO ₂ sediment | correlation coefficient | n.s | n.s | n.s | n.s |
| NO ₃ sediment | correlation coefficient | n.s | n.s | -0.55* | n.s |
| NH ₄ sediment | correlation coefficient | n.s | n.s | n.s | n.s |
| NO ₂ water | correlation coefficient | 0.89** | 0.55* | n.s | 0.59* |
| NO ₃ water | correlation coefficient | 0.77** | 0.55* | n.s | 0.54* |
| NH ₄ water | correlation coefficient | 0.56* | n.s | n.s | 0.53* |
| benthic chl-a | correlation coefficient | n.s | n.s | n.s | n.s |
| microbial abundance | correlation coefficient | n.s | n.s | n.s | n.s |
| organic matter | correlation coefficient | n.s | n.s | n.s | n.s |
| temp _{max} | correlation coefficient | 0.64** | n.s | 0.64** | n.s |
| V _{mean} | correlation coefficient | n.s | n.s | -0.72** | n.s |

** level of significance p<0.01

* level of significance p<0.05

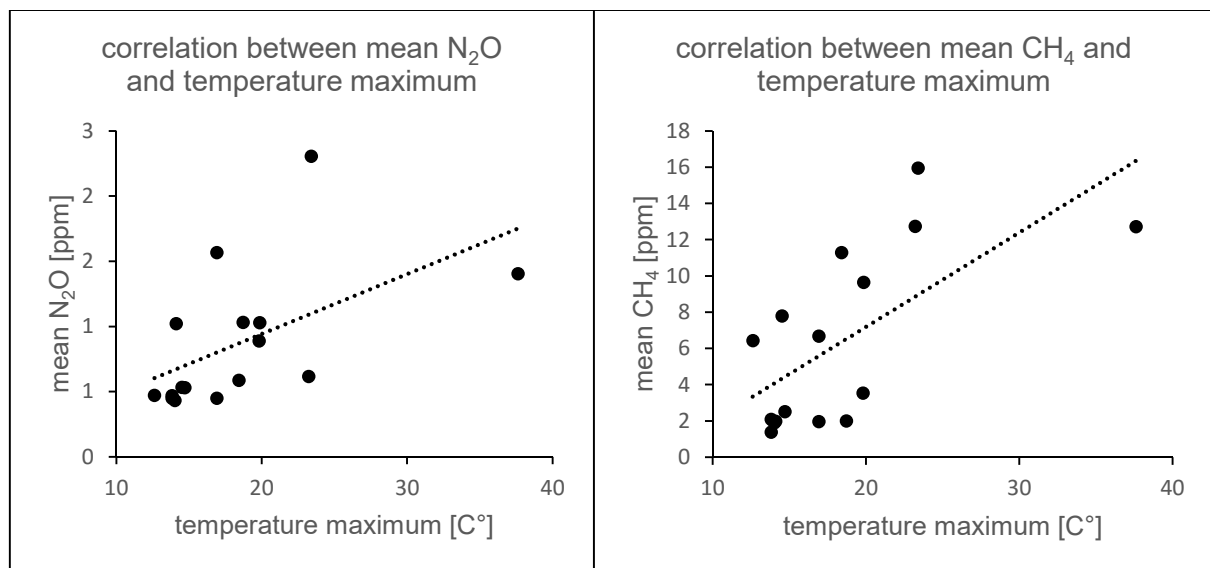


Figure 14: Correlation between mean N₂O and CH₄ concentrations [ppm] and temperature maximum [C°] of the study streams

4.7 Denitrification enzyme activity (DEA)

In the results sheets the different added solutions will be referred to as “+C” for carbon solution, “+N” for nitrate solution, “+C&N” for carbon and nitrate solution, “+Mill-Q” for the addition of bi-distilled water and “+RW” for the addition of the respective river water. All values of DEA_{pot} and N_2O_{pot} were checked for significant differences compared to $DEA_{cont.}$, respectively $N_2O_{cont.}$.

4.7.1 DEA_{pot} and N_2O_{pot}

The groups determined by N_2O_{pot} and DEA_{pot} (see chapter 3.5) represented more or less the land use gradient as described in chapter 3.1.1. The “high pollution” group was formed by the streams Rußbach and Gmoosbach, group “moderate pollution” by Schweinzbach and Grestenbach and the “low pollution” group consisted of Kothbergbach and Gamingbach. In the Sierning, variability was high and therefore no clear pattern was recognizable.

DEA_{pot}

In streams with high pollution, carbon amendment led to highly significant increases in DEA_{pot} compared to DEA_{cont} (Mann-Whitney-U; $p=0.000$; $n=16$) (see Figure 15). Moderately polluted streams also showed highly significant differences between DEA_{pot} and DEA_{cont} (Mann-Whitney-U; $p=0.001$; $n=16$) (Figure 16). Lowly polluted or pristine stream sediments in contrast showed no significant differences in the denitrification rates between carbon amended and non-amended treatments (Mann-Whitney-U; $p=0.222$; $n=18$) (Figure 17).

Nitrogen addition to stream sediments of high pollution caused marginally significant increases in DEA_{pot} (Mann-Whitney-U; $p=0.05$; $n=16$) (Figure 15). For moderately polluted streams, sediments showed no significant reaction to N amendment (Mann-Whitney-U; $p=0.505$; $n=16$) (Figure 16), neither did lowly polluted streams (Mann-Whitney-U; $p=0.931$; $n=18$) (Figure 17).

Addition of combined carbon and nitrogen solution caused a significantly high increase in DEA_{pot} (Mann-Whitney-U; $p=0,000$; $n=16$), likewise did the moderately polluted stream sediments (Mann-Whitney-U; $p=0.021$; $n=16$) (Figure 16). DEA_{pot} for lowly polluted streams in contrast showed no significant change (Mann-Whitney-U; $p=0.666$; $n=18$) (Figure 17).

N_2O_{pot}

In terms of carbon addition, N_2O_{pot} for highly polluted streams showed no significant differences compared to N_2O_{cont} (Mann-Whitney-U; $p=0.870$; $n=30$) (Figure 15), neither did moderately polluted streams (Mann-Whitney-U; $p=0.972$; $n=20$) (Figure 16). Only lowly polluted streams showed a significant increase of N_2O_{pot} because of carbon addition (Mann-Whitney-U; $p=0.009$; $n=20$) (Figure 17).

Nitrogen addition led to highly significant changes of N_2O_{pot} for highly polluted streams (Mann-Whitney-U; $p=0.000$; $n=30$), moderately polluted streams (Mann-Whitney-U; $p=0.000$; $n=20$)

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(Figure 16) and lowly polluted streams (Mann-Whitney-U; $p=0.000$; $n=20$) (Figure 17). Carbon and nitrogen addition showed the same pattern as nitrogen addition alone, namely significant changes for highly, (Mann-Whitney-U; $p=0.000$; $n=30$), moderately (Mann-Whitney-U; $p=0.000$; $n=20$) (Figure 16) and lowly polluted streams (Mann-Whitney-U; $p=0.000$; $n=20$) (Figure 17).

In general, the results of nitrogen-solution addition and combined carbon and nitrogen amendment showed no significant difference. A good differentiation of streams of different pollution levels could be seen for N_2O_{pot} and DEA_{pot} values.

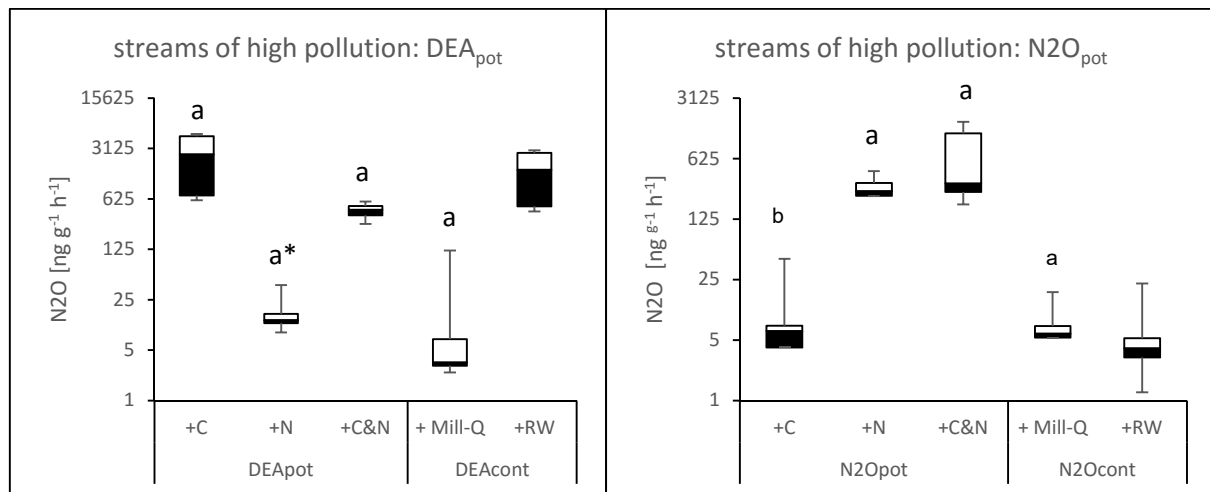


Figure 15: Graphical representation of N_2O_{pot} , DEA_{pot} , N_2O_{cont} and DEA_{cont} [$ng\ g^{-1}\ h^{-1}$] of stream sediments with high pollution (Rußbach and Gmoosbach) with different treatments: carbon solution (+C), nitrogen solution (+N), carbon and nitrogen solution (+C&N), distilled water (Mill-Q) and stream water (+RW) ($n=5$; per stream); a...significant deviation from Mill-Q treatment, a*...marginally significant deviation from Mill-Q treatment, b... no significant deviation from Mill-Q treatment

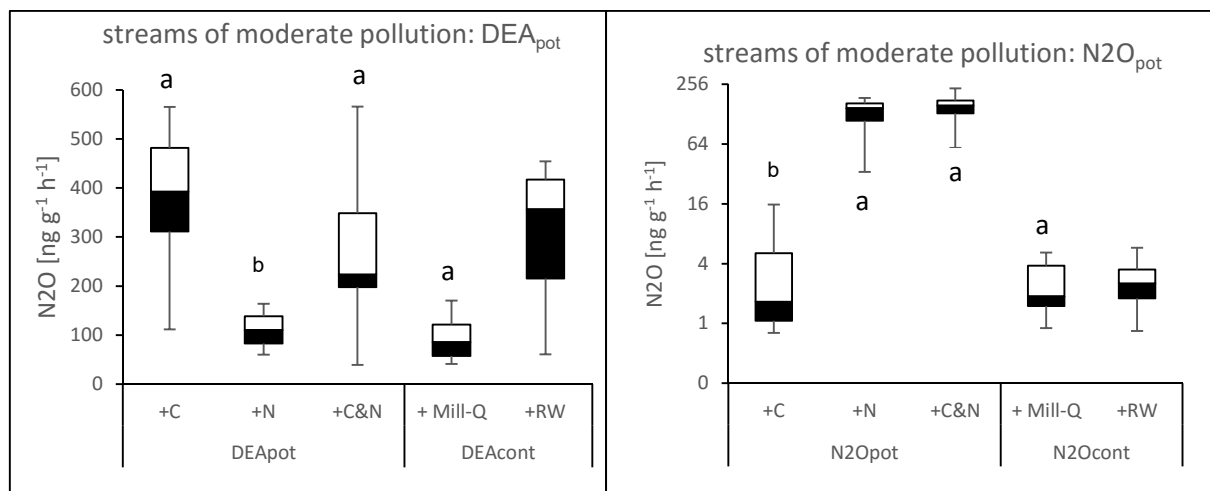


Figure 16: Graphical representation of N_2O_{pot} , DEA_{pot} , N_2O_{cont} and DEA_{cont} [$ng\ g^{-1}\ h^{-1}$] of stream sediments with moderate pollution (Grestenbach and Schweinzbach) with different treatments: carbon solution (+C), nitrogen solution (+N), carbon and nitrogen solution (+C&N), distilled water (Mill-Q) and river water (+RW) ($n=5$; per stream); a...significant deviation from Mill-Q treatment, b... no significant deviation from Mill-Q treatment

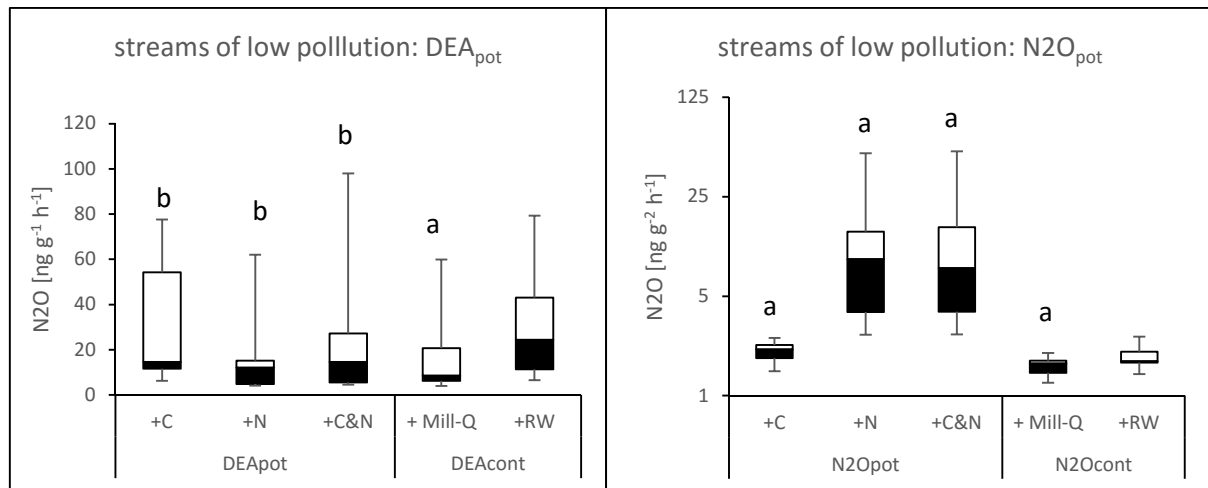


Figure 17: Graphical representation of N_2O_{pot} , DEA_{pot} , N_2O_{cont} and DEA_{cont} [$ng\ g^{-1}\ h^{-1}$] of stream sediments with low pollution (Gamingbach and Kothbergbach) with different treatments: carbon solution (+C), nitrogen solution (+N), carbon and nitrogen solution (+C&N), distilled water (Mill-Q) and river water (+RW) ($n=5$; per stream); a...significant deviation from Mill-Q treatment, b... no significant deviation from Mill-Q treatment

4.7.2 DEA-ratio

The efficiency of denitrification enzyme activity is expressed as $N_2O_{pot}:DEA_{pot}$, called DEA-ratio. For non-amended denitrification rate, DEA_{cont} were lowest for sediments of moderately polluted streams (Figure 19), such as the Schweinzbach and Grestenbach with values of ~ 0.03 . Efficiency in streams of low pollution (Gamingbach and Kothbergbach) varied from 0.15 to 0.24 (Figure 20). The highest efficiency of denitrification enzyme activity was found in streams of high pollution (Rußbach and Gmoosbach), with values from 0.98 to 2.10 (Figure 18). The Sierning lay between the moderate and highly polluted streams, with a DEA-ratio of 0.33.

For the highly polluted Rußbach, Gmoosbach and Sierning, the DEA-ratio showed significant difference to $DEA_{ratio_{cont}}$ in terms of carbon amendment (Mann-Whitney-U; $p=0.000$; $n=30$) (Figure 18). For moderately polluted streams there was no significant increase of DEA-ratio (Mann-Whitney-U; $p=0.165$; $n=20$) (Figure 19), the same applied for lowly polluted streams (Mann-Whitney-U; $p=0.529$; $n=20$) (Figure 20).

For nitrogen amendment, highly polluted streams showed significant differences to $DEA_{ratio_{cont}}$ (Mann-Whitney-U; $p=0.026$; $n=30$) (Figure 18) as well as moderately polluted streams (Mann-Whitney-U; $p=0.000$; $n=20$) (Figure 19) and lowly polluted ones did (Mann-Whitney-U; $p=0.000$; $n=20$) (Figure 20). Carbon and nitrogen amendment showed no significant impact for highly polluted streams (Mann-Whitney-U; $p=0.744$; $n=30$) (Figure 18) in contrast to strong significance for the moderately (Mann-Whitney-U; $p=0.000$; $n=20$) (Figure 19) and lowly polluted ones (Mann-Whitney-U; $p=0.005$; $n=20$) (Figure 20).

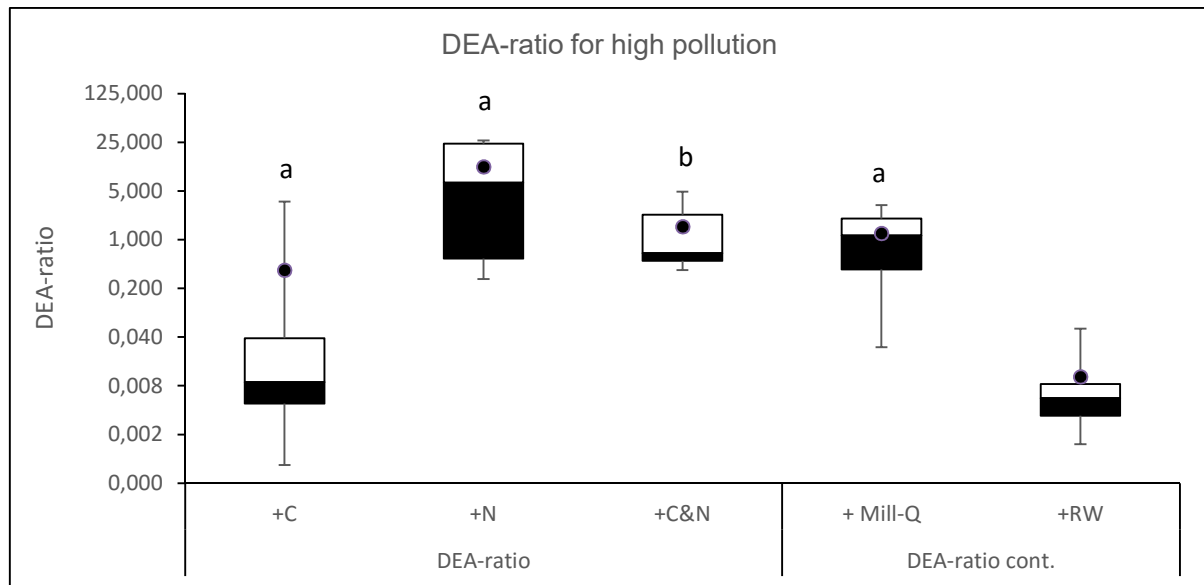


Figure 18: Denitrification Enzyme Activity (DEA) - ratio box-plots in sediments of high pollution with different treatments: carbon solution (+C), nitrogen solution (+N), carbon and nitrogen solution (+C&N), distilled water (Mill-Q) and river water (+RW) for 7 study streams; points indicate the mean value; a...significant deviation from Mill-Q treatment, b... no significant deviation from Mill-Q treatment

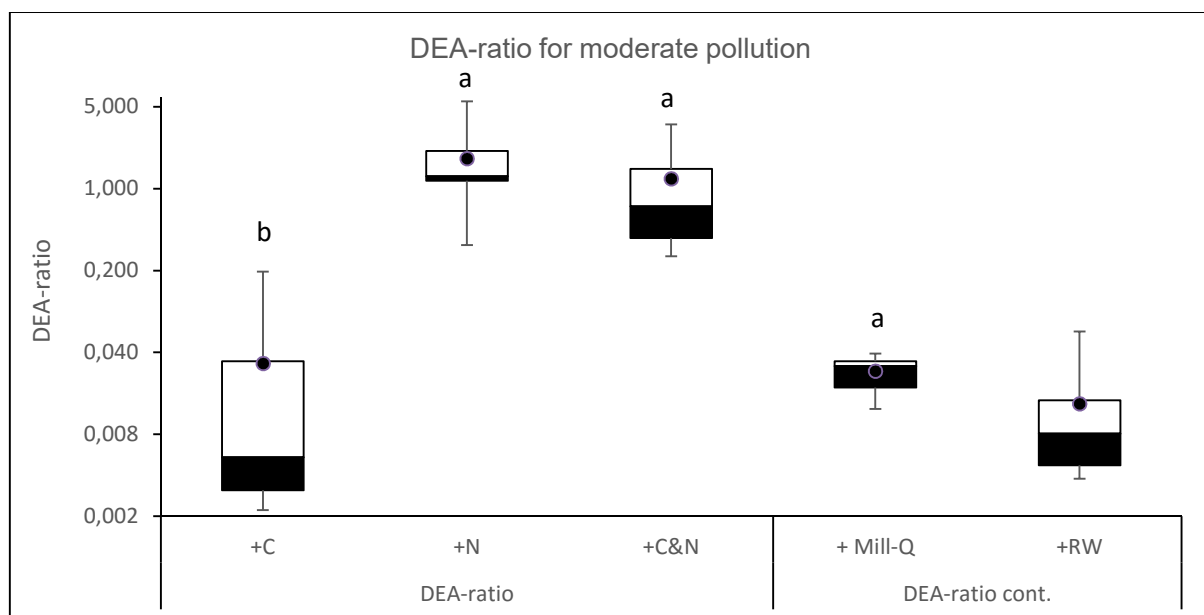


Figure 19: Denitrification Enzyme Activity (DEA) - ratio box-plots in sediments of medium pollution with different treatments: carbon-solution (+C), nitrogen solution (+N), carbon and nitrogen solution (+C&N), distilled water (Mill-Q) and river water (+RW) for 7 study streams; points indicate the mean value; a...significant deviation from Mill-Q treatment, b... no significant deviation from Mill-Q treatment

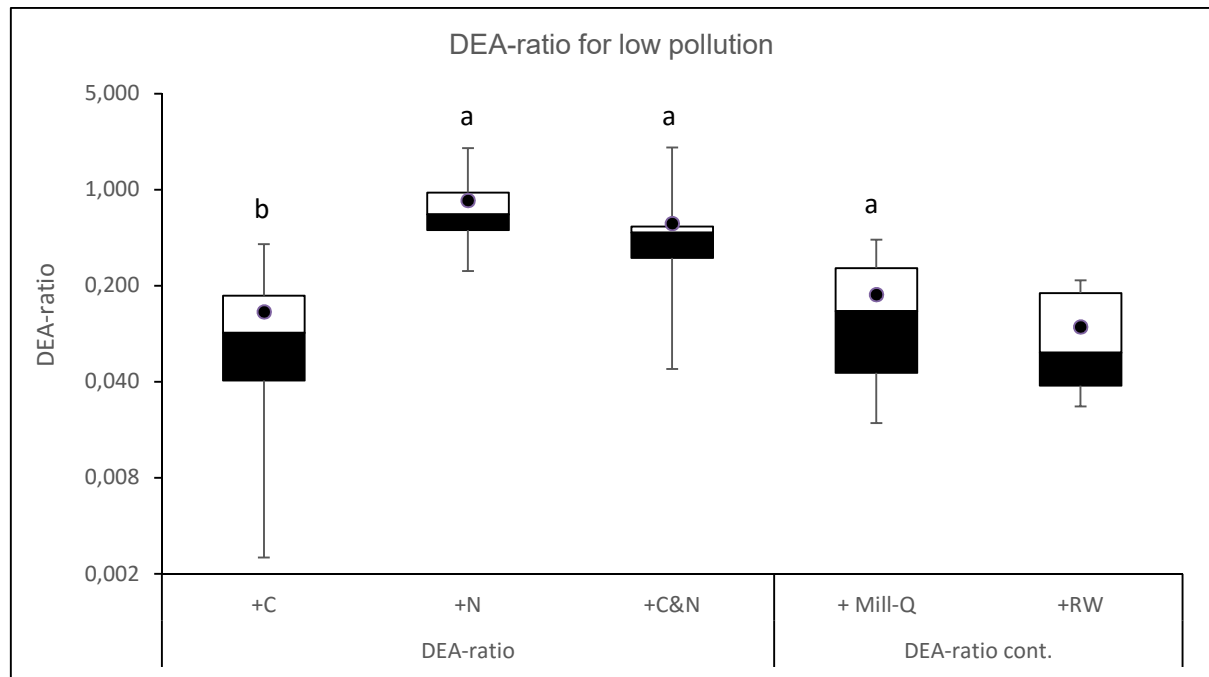


Figure 20: Denitrification Enzyme Activity (DEA) - ratio box-plots in sediments of low pollution with different treatments: Carbon-solution (+C), Nitrogen Solution (+N), Carbon and Nitrogen solution (+C&N), distilled water (Mill-Q) and river water (+RW) for 7 study stream; points indicate the mean value; a...significant deviation from Mill-Q treatment, b... no significant deviation from Mill-Q treatment

4.7.3 Correlations

DEA values of carbon and nitrate amended samples correlated positively with sedimentary organic matter, as well as with combined carbon and nitrogen addition (Table 25). The strongest correlation was observed for the addition of stream water. DOC was positively correlated to carbon amendment (+C) and stream water (RW). Sediment nitrogen values exhibited a correlation of nitrite ($\text{NO}_2\text{-N}$) for carbon and stream water amendment, for ammonium a negative correlation with nitrogen amendment could be recognized (Table 25). For water nitrogen values, positive correlations for combined carbon and nitrogen (+C&N) and stream water amendment (*RW) with water $\text{NO}_2\text{-N}$ and $\text{NO}_3\text{-N}$ could be detected. Microbial abundance only showed a negative correlation concerning distilled water (Mill-Q) amendment (Table 25).

Results

Table 25: Spearman's rank correlations between sedimentary organic matter content (OM), water and sediment nitrogen values, microbial abundance, benthic chlorophyll-a, dissolved organic carbon (DOC) and denitrification enzyme activity (DEA) values of different treatments (+C; +N; +C&N; +RW), with significance level

| correlation matrix | | | | | | | | | | | | | | |
|--------------------|--------------------------|------|--------|----------------|----------------|----------------|-----------------------|--------------------|--------------------|--------------------|--------------------|--------------------|---------------------|-----------------------|
| | +N | *C&N | Mill-Q | RW | OM | DOC | NO ₂ -N | NO ₃ -N | NH ₄ -N | NO ₂ -N | NO ₃ -N | NH ₄ -N | microbial abundance | benthic chlorophyll-a |
| | sediment nitrogen values | | | | | | water nitrogen values | | | | | | | |
| +C | n.s | n.s | n.s | 0.893** | 0.786* | 0.857* | 0.893** | n.s | n.s | n.s | n.s | n.s | n.s | n.s |
| +N | | n.s | n.s | n.s | n.s | n.s | n.s | n.s | -0.991** | n.s | n.s | n.s | n.s | n.s |
| *C&N | | | n.s | n.s | 0.786* | n.s | n.s | n.s | n.s | 0.929** | 0.964** | n.s | n.s | n.s |
| Mill-Q | | | | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | -0.929** | n.s |
| RW | | | | | 0.964** | 0.964** | 0.893** | n.s | n.s | 0.893** | 0.857* | n.s | n.s | n.s |

** level of significance p<0.01

* level of significance p<0.05

5. Discussion

5.1 Hypothesis A

Higher organic matter and nutrient pollution in stream water results in higher production of greenhouse gases.

“Streams with higher pollution will show higher amounts of DOM, especially labile DOM fractions. This, in turn, due to stimulated microbial growth and activity (Williams et al. 2010), leads to increased production of CO₂ and CH₄.”

Hypothesis A can be accepted in wide parts. DOM quantity showed distinct differences between groups of different pollution levels, linked to different intensities of land use in the catchment. Streams of high pollution therefore exhibited significantly higher values for all DOM fractions, than streams of low pollution. This result matches the findings of other recent studies concerning the influence of land use on DOM (Williams et al. 2010; Wilson and Xenopoulos 2009). The more heterogeneous picture in spring in terms of DOM quantity could be a result of higher inlet and precipitation, and therefore intensified erosion processes and DOM input (Kronvang et al. 2008). This was supported by the higher discharge levels of the study streams in spring.

DOM composition had similar tendencies for all streams, especially in summer, with humic-like A showing the highest values, followed by tyrocine-like B2. In spring the composition was more heterogeneous. Streams of high pollution exhibited a DOM quantity up to 10 times higher for every measured parameter than streams of low pollution. The DOC concentration measurements support this finding, as they exhibited a clear positive quantitative ranking for the study streams. The main difference in different pollution levels of the study streams. therefore, was not in DOM composition, but in DOM quantity.

As protein-like DOM is more labile, tryptophan and tyrocine-like DOM parameter were of special interest (Williams et al. 2010). Baker (2002) found in his study that high protein-like fluorescence could point towards farm waste as pollution source. Study streams with intense agricultural land use in the catchment showed higher values for protein-like DOM, ranging from 2 up to 10 times the values of pristine streams. These findings allow the conclusion of land use having an influence on DOM quantity and land use being a source of stream pollution. The results of the β/α -ratio support the thesis of labile DOM causing intensified production of CO₂ and CH₄, with a tendency for all study streams of ratios towards 1, meaning more autochthonous material. All streams indicated low HIX values, ranging from ~0.5 to ~2.1, meaning a low degree of aromaticity, and possible biological or aquatic bacterial origin of humic-like DOM (Huguet et al. 2009). FIX values ranged from ~1.2 to ~2.35 but predominantly were low, which indicates a terrestrial source for fulvic-like DOM. As DOC was correlated with

FIX ($r_s=0.54$), this could indicate an origin of DOC from fulvic-like terrestrial sources. FIX was also negatively correlated to HIX and β/α -ratio, which could indicate a terrestrial Fulvic-acid-like DOM source and autochthone humic-like DOM source. The exceptionally high values of tryptophan like parameters T280 (~4000) and T220 (~10000) for the Gmoosbach could point towards a sewage pollution source, as Baker, Inverarity, Charlton, & Richmond (2003) link high tryptophan-like values and high ammonium water concentrations to 'foul' water pollution.

Findlay, Quinn, Hickey, Burrell, & Downes (2001) showed the influence of differing land use on the quantity and the composition of DOC delivered to streams, while Tiquia (2010) mentions the positive correlation between stream pollution and DOC concentrations. Especially DOC revealed highly positive correlations with both, CH_4 and CO_2 concentrations, for the study streams, therefore this thesis underlines the positive effect of increased DOC concentrations on the production of greenhouse gases CH_4 ($r_s=0.88$) and CO_2 ($r_s=0.64$) in headwater streams. Especially CH_4 concentrations were significantly higher for streams with higher DOC concentration and nutrient load. I found that the water-nitrogen concentrations of $\text{NO}_3\text{-N}$ ($r_s=0.77$) and $\text{NO}_2\text{-N}$ ($r_s=0.89$) were significantly positively correlated to both, CH_4 and CO_2 concentrations, and CH_4 production was positively correlated to ambient $\text{NH}_4\text{-N}$ concentrations ($r_s=0.56$). The study indicates that DOC concentration and increased water nitrogen values can induce higher CO_2 and CH_4 production due to possibly intensified mineralization processes, and could emphasize the role of streams as sources and producers of greenhouse gases.

The study shows the positive correlation between organic matter load and greenhouse gas production. All measured DOM parameters were significantly positively correlated with CO_2 and CH_4 concentration, except Tyrosine-like B2. This indicates that one major driving force for mineralization could be the bioavailable DOM with resulting production of CO_2 and CH_4 in headwater streams. Protein-like and autochthonous DOM is more labile and therefore can stimulate microbial growth and activity (Williams et al. 2010). As other researches pointed out, microbial abundance and activity are linked to the denitrification process (Harrison et al. 2012), and therefore contribute to greenhouse gas production. Even though higher greenhouse gas production of streams with enriched nutrient and organic matter load would lead to the expectation of higher microbial abundance in those streams, microbial abundance could not be confirmed as significant influencing factor for this study and was not found to be higher in highly polluted streams. This contradiction could result from a too low sample size and a high spatial variability of microbes, and should be investigated in more detail.

The N_2O concentration showed no correlation towards any measured DOM parameter, suggesting that organic matter load has no direct influence on the N_2O concentration in stream water. This, however, does not automatically exclude the influence of organic matter load on N_2O producing processes.

“A higher amount of nitrogen in the water and sediment leads to intensified denitrification processes and therefore increased N₂O production. Increased NO₃-N water pollution leads to an increased N₂O concentration in the stream water (Jake J. Beaulieu et al. 2008).”

This part of **Hypothesis A** must be rejected. The water and sedimentary nitrogen values NH₄-N and NO₂-N had no statistical influence on the N₂O water concentration. Also no indication could be found that increased NO₃-N water concentrations lead to higher N₂O concentrations in the stream water. Only sedimentary NO₃-N concentrations were negatively correlated ($r_s = -0.55$) with N₂O water concentrations. In contrast to my findings, Beaulieu, Arango, Hamilton, & Tank (2008) and Beaulieu, Arango, and Tank (2009) reported significant indirect relation of water NO₃-N concentrations and N₂O production rates for their study of headwater streams located in the Midwestern United States of America, and suggest NO₃-N water concentrations as predictor for N₂O emissions. In these studies, however, NO₃-N values ranged from ~30 µg l⁻¹ up to ~2500 µg l⁻¹. Findlay et al. (2011) as well reported opposite results compared to my study, as positive correlations between NO₃-N water concentration and N₂O production could be found in their study of headwater streams in the United States. This contrast could be a result of lower sample size and different NO₃-N concentrations, or further reduction of produced N₂O during denitrification process, which is indicated by the DEA experiments in this study (see chapter 4.7.1). The negative correlation with sedimentary NO₃-N needs further investigation, but one explanation of the negative correlation could be, if sedimentary NO₃ gets depleted, then produced N₂O could be used and transformed in the denitrification process. Sedimentary NO₃ values were lower for most streams in summer, especially for the highly polluted stream Gmoosbach with a drop from ~10 µg g⁻¹ down to 0.5 µg g⁻¹, supporting this theory. Also DEA experiments showed, that the denitrification potential of highly polluted streams is much higher than for streams of low pollution, suggesting, that N₂O becomes further reduced. However, water nitrogen values were comparable to findings of similar studies in this region (Weigelhofer, Welti, and Hein 2013). In comparison they were quite high, with a range from ~5 µg l⁻¹ up to ~7000 µg l⁻¹ for the measured nitrogen parameters.

In general, N₂O concentrations did not vary significantly among the study streams. So streams of high pollution did exhibit quite the same range in N₂O concentrations as streams of low pollution. Deviating, higher N₂O concentrations of the Schweinzbach in summer could be a result of beaver activities in the summer time, which caused the impoundment of the stream section and therefore changed the whole hydrologic conditions. The negative correlation of flow velocity and N₂O concentration in stream water supports this explanation, for the impoundment contributed to a distinct decrease in flow velocity. So no significant influence of water nitrogen concentrations on N₂O concentrations on streams, following a pollution gradient, could be found in my study.

Even if no correlation in terms of N_2O could be found, concentrations of dissolved CO_2 and CH_4 in the water did increase significantly with $\text{NO}_3\text{-N}$, $\text{NO}_2\text{-N}$ and, in terms of CH_4 , also $\text{NH}_4\text{-N}$ concentrations in the water. Similar results of the positive effect of increased $\text{NO}_3\text{-N}$ concentrations on CH_4 and CO_2 production were found by other studies (Tortosa et al. 2011; Liu and Greaver 2009). Sediment nitrogen-parameters, however, turned out not to be correlated to production of CH_4 and CO_2 . A reason for the strong correlation of water nitrogen parameters with CH_4 and CO_2 production and the lack of correlation with sediment parameters could be that microbial communities – which are supposed to take the main part in mineralization - are located in the top layer or directly at the surface of the sediment, and could use the nitrogen directly from the water column or pore water. A detailed investigation should be done to reveal the influence of different sediment types, their grain size distribution and their capability of mineralization, to reveal more details.

As mentioned before, besides the positive effect of denitrification on the greenhouse gas production, the correlation between microbial abundance and greenhouse gas production turned out not to be significant. The missing N_2O trend contrasts the clear positive trend of CO_2 and CH_4 concentrations following the pollution gradient. One explanation for the missing N_2O trend could be the too small amount of evaluated samples ($n=5-10$) to get meaningful data because of the highly spatial variability of microbes within streams.

5.2 Hypothesis B

The amount of greenhouse gas produced in streams is higher in summer than in spring.

“Higher water temperature leads to intensified biotic processes (autotrophic and heterotrophic) in the stream water, and therefore the greenhouse gas concentrations rise in summer. Apart from that, the lower discharge leads to higher concentrations of nitrogen and DOM in the water, which also triggers the gas production.”

Hypothesis B can only be accepted in terms of CH_4 and N_2O concentration in the stream water. As the analysis showed, CH_4 and N_2O concentration indeed increased in summer, while CO_2 concentrations stayed on the same level or even decreased. For streams of high pollution, the increase was more distinct. Artigas, Romani, Gaudes, Muñoz, & Sabater, (2009) state, that microbial biomass and enzymatic activities (which is also an important factor for greenhouse-gas production) come to a maximum in late-summer. This supports the assumption of higher greenhouse gas production in summer. This study showed the distinct increase in stream water temperature in summer and its strong positive correlation with CH_4 and N_2O production in headwater streams. Campeau and del Giorgio (2014), reported the same pattern for their study, as CH_4 was positive correlated to water temperature while CO_2 showed no correlation, highlighting the role of water temperatures for metabolism.

Discussion

Neither chlorophyll-a nor microbial abundance concentration in the sediments were correlated to gas production in any form. Both, CH₄ and N₂O concentration, however, were significantly positively related to maximum water temperature ($r_s=0.62$). Dong, Thornton, Nedwell, & Underwood (2000) report that O₂ consumption, and thereby O₂ depletion in the sediments, was at maximum in their study stream in summer. This could be a likely explanation for increased N₂O and CH₄ concentrations in summer, as increased anoxic conditions in the sediments foster methanogenesis and denitrification. Also low flow velocity could favor N₂O concentration in water as pointed out in this study. The missing trend of increasing water CO₂ concentration in summer might be the result of higher outgassing rates due to supersaturation. With rising water temperature, the solubility decreases which could contribute to the decrease. Additionally, increased N₂O and CH₄ concentrations could point towards enhanced anaerobic processes in the study streams. CH₄ concentration increase could also be biased by groundwater inflow and riparian fluxes.

Hydromorphological parameters showed strong changes from spring to summer. All of the streams show a (pluvio-) nival regime, which explains the decline in discharge and the linked parameter decrease in summertime. Discharges were dropping and therefore water depth, mean flow velocity and - in consequence of that - also wetted perimeter, declined. Higher air temperature might have led to increased evaporation but the closed canopy and shadowing probably buffered this effect. Especially the changes in temperature were quite distinct, with all water temperatures being higher in summer than in spring. Only the more pristine streams (like the Kothbergbach and Gamingbach) showed less increase in water temperature because of the higher altitude they are located at and the far steeper hill flanks bordering the streams. This steep hill flanks shadowed the stream and prevented the streams from heating up. Also input of cold wells and the presence of riparian vegetation could have led to additional cooling.

The water chemistry analyses showed a general tendency for all streams. The water nitrogen parameters nitrate (NO₃-N), nitrite (NO₂-N) and ammonium (NH₄-N) increased in summer, but only NO₂-N significantly. This could be a result of less discharge and therefore an accumulation of stream nitrogen in water, again resulting in increases in CH₄ and CO₂. Additionally, due to lower discharge, the impact of pollution source could be stronger because of the decreased dilution.

5.3 Hypothesis C

If tested under laboratory conditions, sediments from streams with intensified agricultural land use in the catchment and high nutrient pollution exhibit higher denitrification potential than streams of low pollution and extensive agricultural land use in the catchment.

“This higher denitrification potential is a result of a bigger denitrifier community in these streams.”

The influence of agricultural land use in the catchment on the potential denitrification potential, as formulated in **Hypothesis C**, can be accepted. With rising agricultural land use, the pollution in terms of DOM /DOC concentration and nutrient load rose as shown. Potential denitrification showed distinct trends for different pollution levels. Streams of high pollution were carbon-adapted, as the highest N₂O production could be observed for C amendment with a range of $DEA_{pot} \approx 600$ to ~ 5000 ng N g⁻¹ h⁻¹. Streams of moderate pollution exhibited the same trend, but distinctly lower, with a range of $DEA_{pot} \approx 110$ to ~ 560 ng N g⁻¹ h⁻¹. Pristine or lowly polluted streams showed no trend for all added solutions, resulting in the more or less the same average amount of $DEA_{pot} \approx 25$ ng N g⁻¹ h⁻¹. These results are quite similar to results of recent studies concerning headwater streams in the Weinviertel, showing indeed a predominant nitrogen limitation, but also a significant increase of potential denitrification for carbon addition (Weigelhofer, Welti, and Hein 2013). The addition of readily bioavailable carbon seems to push potential denitrification for streams with moderate and high pollution. It is remarkable that the addition of stream water seems to result in quite the same rates as for potential denitrification measurements. This could be an indication for the dependency of potential denitrification on stream water carbon load and the adaption of the stream community. Other studies pointed out that quality and bioavailability of organic matter is also of importance for denitrification (Welti et al. 2012; DeForest 2009; Dodla et al. 2008) and is influenced by agricultural land use. Sedimentary organic matter content additionally showed the positive correlation with C amendment, which underlines the importance of carbon for the denitrification process.

Results of DEA-ratio, expressing the efficiency of denitrification, suggest, that if sufficient amounts of readily available carbon is present, the produced N₂O gets further reduced in the denitrification process. All streams indicated lowest DEA-ratios for C amendment and therefore N₂O produced during denitrification must have been further reduced. DEA-ratios were highest for N amendment, indicating that carbon deficit leads to higher production of N₂O. This could be one explanation for the relatively low N₂O concentrations found in all study streams, even highly polluted streams, which had clear positive correlation to CO₂ and CH₄ concentrations with pollution, but not to N₂O concentrations.

5.4 Summary

The pollution groups of streams, based on the different intensities of agricultural land-use in the catchment, turned out to be adequate in representing different stream pollution levels. As assumed, streams flowing through areas of high agricultural land use, showed much higher water-nitrogen concentrations than streams with extensive land use in the catchment. The reasons probably are fertilization in the immediate catchment, together with increased erosion processes, infiltration and groundwater interplay (Gordon, Finlayson, and Falkenmark 2010; Allan 2004). Organic matter as well reflected the different pollution levels, which can clearly be seen in the increasing DOC results and in the clear quantitative ranking of DOM in general. The composition of DOM was quite homogeneous, especially for summer, with humic-like A and tyrocine-like B2 forming the dominant fractions. For spring, the DOM data were more heterogeneous among the study streams. Chlorophyll-a and microbial abundance concentrations turned out to be no good indicator for describing greenhouse gas production within headwater streams in this study. The correlations of water nitrogen values and DOM fractions could reveal their interconnection. Further, more detailed investigation is needed to specify these connections. Organic matter in the sediment exhibited a correlation with tryptophan-like T220 and T280, tyrocine-like B2 and humic-like A, suggesting a connection of these parameters. Sedimentary nitrogen parameters were similar in their ranking as water nitrogen values, with a decrease in summer, while water nitrogen values increased (non-significantly). The gas concentrations also followed the ranking, with highly polluted streams showing the highest gas concentrations for CH₄ and CO₂, while N₂O concentrations were similar for all pollution levels. The newly introduced global warming estimate revealed that the global warming potential for streams of moderate to high pollution (with 1300 units up to 2500 units) was distinct higher than those of streams with low pollution (with 500 units up to 980 units) or more or less pristine conditions. This clearly shows the impact of anthropogenic influence on the global warming potential, respectively the greenhouse gas production of headwater streams. Worth mentioning is also the relatively strong summarized global warming potential of streams of moderate pollution, highlighting their role in global warming. The DEA experiments showed the carbon adaption of the denitrification process of the study streams, and the high potential of highly polluted streams in terms of N₂O production.

The main controlling factors of greenhouse gas production in headwater streams, according to this study are DOC concentration, DOM fractions of tryptophan-like, tyrocine-like and humic-like fluorescents, as well as ambient nitrate (NO₃-N) and nitrite (NO₂-N) concentrations. Additional, water temperature seems to be an important controlling factor for CH₄ and N₂O production, pointing towards temperature influence on anaerobic processes.

6. Conclusion and outlook

This thesis points out the importance of anthropogenic influence in the catchment of headwater streams on the DOM quantity and quality and the nutrient load in the water and sediment, leading to increased greenhouse gas production. Streams with high or moderate, but at least significant anthropogenic influence showed significantly higher greenhouse gas concentrations than streams of low pollution or pretty pristine conditions. As the probably strongest anthropogenic influence for the study streams, as indicated by the findings in this study, was the agricultural land use in the catchment the land-use in the surroundings of the streams should be paid high attention. Via direct input, groundwater inflow and erosion processes, nutrients enter the stream ecosystem, altering the self-cleaning potential of streams and causing problems in the local ecosystem and later on in bigger scale. Due to this increased input into streams, the greenhouse gas producing mechanisms in streams seem to be fuelled, as this study indicates. The newly introduced global warming estimate by Bauer (B_f) showed this increased greenhouse gas production and provides a good parameter for estimation of climate-relevant gas concentrations of streams. It therefore in future may be helpful to assess the part of global greenhouse gas concentrations produced by inland freshwater headstreams. As reference for future investigations, this study emphasizes sampling during summer, for values turned out to be more homogenous during this season and bias effects, especially for this type of headwater streams in the alpine-region, were lower. This thesis may form a basis for future assessment of greenhouse gas emissions of streams, as I tried to test a broad base of parameters in order to identify the main controlling parameters among them. These identified parameters should be investigated in more detail and also their interconnection to possible other relevant stream ecosystems processes. Future research has to address the revealed uncertainties and the detailed processes of greenhouse gas emissions in headwater streams, as well as the detailed origin of DOM and its exact pathways into the stream.

So, in order to improve the situation and to mitigate the effects of global warming caused by production of greenhouse gases in the long term, the implementation of best management practices will become necessary. These practices should aim to decrease the amount of nutrients reaching the stream ecosystem in the first place. The application of high loads of animal manure on the cropland in the stream catchment should be avoided, and in general a conservative strategy for the application of manure should be applied in order to reduce nutrient input into streams. Additional, cropland drainages should not be extended in number but rather be reduced, for they can transport huge amounts of nutrients into stream ecosystems within short time. Further, the interspace between cropland and stream should be as wide as possible, to lower the risk of stream pollution and counteract erosion processes.

Conclusion and outlook

Especially easily bioavailable, protein-like DOM, as it seems to fuel the greenhouse gas production processes in the stream ecosystem.

The increasing number of population on earth leads to a rising pressure on agriculture to increase and fasten the food production. The resulting effects can be seen in the whole ecosystem; inland streams are part of this system and should be taken into account. Like a blood vein in a body, headwater streams seem to be only a cog in the machine, but like for the body, headwater streams can be the source of pollution, too, and the starting point and transport system of bigger problems. In future, headwater streams should therefore be treated more carefully as they are today. The creation of more lateral space for streams could not only help mitigating flood water events, but also creating more buffer space for headwater streams. This created buffer space could help reducing nutrient input into streams, as indicated by recent studies (Verhoeven et al. 2006; Audet et al. 2013; Weigelhofer et al. 2012). It should be considered, if investing some “space” and some “thoughtfulness” in terms of stream pollution and land-use practice wouldn't be a good investment, and even would be a benefit for society in many ways.

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9. Appendix

Table 26: Dissolved Organic Matter (DOM) results of the tryptophan-like T220 and T280 and tyrocine-like fluorophores B1 and B2

| Dissolved Organic Matter (DOM) results of tryptophan-like and tyrocine-like fluorophores | | | | | | | | | | | | | | |
|--|--------|-----------|--------------------|------------------|-----------|--------------------|------------------|-----------|--------------------|------------------|-----------|--------------------|------------------|-----------|
| | | | Tryptophan-like | | | | | | Tyrocine-like | | | | | |
| | | | T220 | | | T280 | | | B1 | | | B2 | | |
| | | | Excitation [nm] | Emission [nm] | Intensity | Excitation [nm] | Emission [nm] | Intensity | Excitation [nm] | Emission [nm] | Intensity | Excitation [nm] | Emission [nm] | Intensity |
| Rußbach | spring | mean | 230 | 328 | 653.76 | 280 | 310 | 573.46 | 227 | 292 | 163.46 | 275 | 280 | 1285.40 |
| | | std. dev. | 0 | 1 | 205.16 | 0 | 0 | 87.03 | 3 | 0 | 38.40 | 0 | 0 | 165.94 |
| | summer | mean | 225 | 330 | 1774.07 | 280 | 310 | 1326.53 | 225 | 291 | 805.87 | 275 | 280 | 2194.00 |
| Gmoosbach | spring | mean | 226 | 329 | 4285.44 | 280 | 310 | 4041.72 | 225 | 292 | 654.84 | 275 | 280 | 10239.60 |
| | | std. dev. | 2 | 1 | 1266.21 | 0 | 0 | 109.44 | 0 | 1 | 294.51 | 0 | 0 | 348.30 |
| | summer | mean | 225 | 330 | 1778.20 | 280 | 310 | 1361.20 | 225 | 291 | 875.20 | 275 | 280 | 2202.87 |
| Zettelbach | spring | mean | 221 | 328 | 496.40 | 280 | 310 | 676.96 | 225 | 291 | 118.47 | 275 | 280 | 2140.00 |
| | | std. dev. | 2 | 2 | 65.09 | 0 | 0 | 12.74 | 0 | 2 | 23.92 | 0 | 0 | 62.16 |
| | summer | mean | 225 | 328 | 244.52 | 280 | 310 | 303.76 | 225 | 290 | 82.71 | 275 | 280 | 657.24 |
| Grestenbach | spring | mean | 223 | 329 | 477.98 | 280 | 310 | 471.66 | 225 | 291 | 139.36 | 275 | 280 | 1283.40 |
| | | std. dev. | 3 | 2 | 198.18 | 0 | 0 | 28.55 | 0 | 1 | 23.80 | 0 | 0 | 85.87 |
| | summer | mean | 223 | 329 | 225.74 | 280 | 310 | 294.70 | 225 | 290 | 89.44 | 275 | 280 | 742.58 |
| Sierning | spring | mean | 228 | 328 | 383.80 | 280 | 310 | 496.82 | 225 | 292 | 179.00 | 275 | 280 | 1646.20 |
| | | std. dev. | 3 | 2 | 55.00 | 0 | 0 | 15.93 | 0 | 1 | 18.81 | 0 | 0 | 205.44 |
| | summer | mean | 230 | 330 | 352.74 | 280 | 310 | 364.42 | 229 | 292 | 113.66 | 275 | 280 | 700.06 |
| Gaming | spring | mean | 222 | 330 | 160.12 | 280 | 310 | 255.42 | 225 | 291 | 85.86 | 275 | 280 | 439.18 |
| | | std. dev. | 3 | 1 | 53.77 | 0 | 0 | 11.69 | 0 | 2 | 35.76 | 0 | 0 | 33.09 |
| | summer | mean | 221 | 324 | 139.76 | 280 | 310 | 289.20 | 225 | 290 | 77.86 | 275 | 280 | 602.04 |
| Schweinzbach | spring | mean | 226 | 329 | 462.90 | 280 | 310 | 555.66 | 225 | 291 | 151.72 | 275 | 280 | 1339.80 |
| | | std. dev. | 2 | 2 | 121.12 | 0 | 0 | 20.70 | 0 | 1 | 34.93 | 0 | 0 | 83.09 |
| | summer | mean | 226 | 326 | 316.66 | 280 | 310 | 323.98 | 225 | 292 | 107.92 | 275 | 280 | 842.18 |
| Kothbergbach | spring | mean | 223 | 328 | 271.70 | 280 | 310 | 379.20 | 225 | 291 | 75.71 | 275 | 280 | 1001.20 |
| | | std. dev. | 3 | 3 | 110.04 | 0 | 0 | 20.63 | 0 | 1 | 37.09 | 0 | 0 | 211.16 |
| | summer | mean | 224 | 327 | 264.36 | 280 | 310 | 285.80 | 225 | 289 | 97.58 | 275 | 280 | 799.80 |
| Feichsen | spring | mean | 225 | 329 | 656.12 | 280 | 310 | 558.74 | 225 | 291 | 200.92 | 275 | 280 | 1407.60 |
| | | std. dev. | 0 | 2 | 79.26 | 0 | 0 | 15.62 | 0 | 2 | 20.74 | 0 | 0 | 55.94 |

Table 27: Dissolved Organic Matter (DOM) results of humic-like A and C and calculated indices Fluorescence Index (FIX). Humification Index (HIX) and β/α -ratio

| Dissolved Organic Matter (DOM) results of humic-like fluorophores and calculated Indices | | | | | | | | | | | |
|--|--------|-----------------|---------------|-----------|-----------------|---------------|-----------|----------------|------------------------|------------------------|------|
| | | Humic-like | | | | | | Index | | | |
| | | A | | | C | | | | | | |
| | | Excitation [nm] | Emission [nm] | Intensity | Excitation [nm] | Emission [nm] | Intensity | β/α | FIX Fluorescence Index | HIX Humification Index | |
| Rußbach | spring | mean | 239 | 416 | 1046.06 | 370 | 370 | 8781.54 | 0.60 | 1.21 | 2.09 |
| | | std. dev. | 2 | 4 | 55.20 | 0 | 0 | 1673.10 | 0.02 | 0.03 | 0.11 |
| | summer | mean | 235 | 414 | 1532.13 | 370 | 370 | 10190.00 | 1.09 | 1.41 | 0.77 |
| Gmoosbach | spring | mean | 235 | 416 | 2871.96 | 370 | 370 | 33922.80 | 1.17 | 2.35 | 0.45 |
| | | std. dev. | 0 | 6 | 454.83 | 0 | 0 | 4914.91 | 0.03 | 0.13 | 0.02 |
| | summer | mean | 235 | 411 | 1938.07 | 370 | 370 | 11067.33 | 0.95 | 1.34 | 1.02 |
| Zettelbach | spring | mean | 242 | 417 | 659.88 | 370 | 370 | 9937.32 | 0.82 | 1.48 | 0.87 |
| | | std. dev. | 8 | 6 | 37.24 | 0 | 0 | 139.93 | 0.03 | 0.06 | 0.04 |
| | summer | mean | 235 | 422 | 427.36 | 370 | 370 | 3796.00 | 0.84 | 1.24 | 0.89 |
| Grestenbach | spring | mean | 235 | 414 | 526.22 | 370 | 370 | 6671.00 | 0.99 | 1.43 | 0.79 |
| | | std. dev. | 0 | 8 | 38.96 | 0 | 0 | 1072.64 | 0.08 | 0.02 | 0.05 |
| | summer | mean | 235 | 420 | 386.12 | 370 | 370 | 4733.20 | 0.82 | 1.19 | 0.83 |
| Sierning | spring | mean | 236 | 418 | 806.70 | 370 | 370 | 9805.94 | 0.68 | 1.30 | 1.36 |
| | | std. dev. | 2 | 7 | 19.50 | 0 | 0 | 327.50 | 0.01 | 0.02 | 0.04 |
| | summer | mean | 236 | 411 | 693.60 | 370 | 370 | 4220.60 | 0.69 | 1.13 | 1.61 |
| Gaming | spring | mean | 235 | 408 | 142.94 | 370 | 370 | 2052.80 | 1.70 | 2.15 | 0.29 |
| | | std. dev. | 0 | 15 | 14.17 | 0 | 0 | 346.10 | 0.29 | 0.09 | 0.01 |
| | summer | mean | 235 | 413 | 146.94 | 370 | 370 | 2978.40 | 1.91 | 2.31 | 0.27 |
| Schweinzbach | spring | mean | 235 | 414 | 790.44 | 370 | 370 | 4289.80 | 0.95 | 1.38 | 1.12 |
| | | std. dev. | 0 | 11 | 6.39 | 0 | 0 | 759.81 | 0.13 | 0.04 | 0.01 |
| | summer | mean | 235 | 418 | 535.12 | 370 | 370 | 6481.80 | 0.66 | 1.14 | 1.28 |
| Kothbergbach | spring | mean | 235 | 417 | 315.94 | 370 | 370 | 1200.33 | 0.01 | 0.02 | 0.04 |
| | | std. dev. | 0 | 6 | 19.06 | 0 | 0 | 2007.75 | 0.05 | 0.04 | 0.02 |
| | summer | mean | 235 | 408 | 273.62 | 370 | 370 | 4874.40 | 1.27 | 1.49 | 0.50 |
| Feichsen | spring | mean | 235 | 410 | 688.30 | 370 | 370 | 1815.70 | 0.08 | 0.03 | 0.01 |
| | | std. dev. | 0 | 22 | 56.54 | 0 | 0 | 4123.20 | 1.05 | 1.44 | 0.90 |
| | summer | mean | 235 | 410 | 688.30 | 370 | 370 | 4123.20 | 1.05 | 1.44 | 0.90 |
| | | std. dev. | 0 | 14 | 20.36 | 0 | 0 | 452.75 | 0.13 | 0.02 | 0.01 |

Table 28: Gas-emission results in parts per million [ppm]

| gas results | | | | | | | |
|--------------------|-----------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| stream name | | CH₄ [ppm] | CO₂ [ppm] | N₂O [ppm] | CH₄ [ppm] | CO₂ [ppm] | N₂O [ppm] |
| | | spring | | | summer | | |
| Rußbach | mean | 6.44 | 1132.51 | 0.47 | 12.75 | 1120.55 | 0.62 |
| | std. dev. | 0.15 | 88.07 | 0.06 | 0.65 | 99.08 | 0.06 |
| Gmoosbach | mean | 11.31 | 2039.81 | 0.59 | 12.74 | 1565.99 | 1.41 |
| | std. dev. | 0.13 | 109.04 | 0.07 | 1.41 | 688.83 | 0.23 |
| Zettelbach | mean | 2.53 | 1099.21 | 0.53 | 3.55 | 626.02 | 0.89 |
| | std. dev. | 0.13 | 260.71 | 0.01 | 0.17 | 109.13 | 0.14 |
| Schweinzbach | mean | 6.70 | 1145.71 | 1.57 | 15.96 | 1300.21 | 2.31 |
| | std. dev. | 0.40 | 218.50 | 0.09 | 1.87 | 156.25 | 0.26 |
| Sierning | mean | 7.81 | 1580.63 | 0.54 | 9.65 | 866.66 | 1.03 |
| | std. dev. | 0.25 | 420.93 | 0.06 | 1.30 | 100.98 | 0.45 |
| Grestenbach | mean | 2.11 | 1575.83 | 0.47 | 2.83 | 449.97 | 0.73 |
| | std. dev. | 0.04 | 1223.77 | 0.03 | 0.16 | 75.22 | 0.21 |
| Kothbergbach | mean | 1.39 | 824.53 | 0.45 | 1.99 | 182.16 | 1.02 |
| | std. dev. | 0.08 | 125.18 | 0.04 | 0.28 | 40.20 | 0.19 |
| Gamingbach | mean | 1.91 | 738.41 | 0.43 | 2.00 | 677.42 | 1.03 |
| | std. dev. | 0.16 | 158.26 | 0.06 | 0.18 | 80.17 | 0.16 |
| Feichsen | mean | 1.98 | 598.23 | 0.45 | | | |
| | std. dev. | 0.10 | 67.57 | 0.05 | | | |

10. Affirmation

I certify that this master thesis was written by me, not using sources and tools other than quoted and without use of any other illegitimate support.

Furthermore, I confirm that I have not submitted this master thesis either nationally or internationally in any form.

Salzburg, 27.05.2016

Felix Bauer