Denitrification rates in the Jiulong river in South East China
Measured by Membrane Inlet Mass Spectrometry

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Photo: Caroline Fredriksson, 2010

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Abstract

The Jiulong River is located in the Fujian province in southeast China. It has high inputs of anthropogenic nitrogen. The high load of nutrients is further transported out to the Xiamen Sea. Harmful algal blooms are severely affecting the marine and riverine ecosystems and the drinking water quality. To improve the nutrient management in the region, denitrification is studied by an N₂/Ar method, using a MIMS (HPR-40) from HIDEN Analytical Corporation. Samples will be collected monthly 2010-2011. Analysis of the measurements in June 2010 shows the existence of denitrification in the Jiulong River with the net increase of dissolved N₂ concentration ranging from 6.99 μM to 49.16 μM. The denitrification rate shows great variation within the river reach. The denitrification rates are in general higher in the West Jiulong River (on average 16.4 mmol m⁻² d⁻¹) compared to the North River (on average 7.5 mmol m⁻² d⁻¹). In the West Jiulong River also the nitrate concentration is higher but there is no significant relationship between the denitrification rate and the nitrate concentration. The present study suggests a good prospect of N₂:Ar method in the future researches concerning denitrification process and mechanism in aquatic ecosystem.
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Introduction
Eutrophication in rivers, lakes and seas is a worldwide problem. Nitrogen is one of the main contributors to the excess growth of toxic algae in the sea. The nitrogen is transported from agricultural fields and outlets within the drainage basin to the estuarine waters via streams and rivers. Excessive use of fertilizers and deficient waste water treatment are examples of human activities contributing to the problem.

Nitrogen is entering the rivers in the form of nitrogen bound to organic matter (org-N), ammonium (NH₄⁺), nitrite (NO₂⁻) or nitrate (NO₃⁻). During aerobic conditions the ammonium is oxidized to nitrite which is further oxidized to nitrate. This process is called nitrification. During anoxic conditions, when there is low concentration of free oxygen, denitrifying bacteria use the nitrate as an electron acceptor and thus reduce the nitrate to nitrogen gas (N₂). Also nitric oxide (NO) and nitrous oxide (N₂O) which are potent greenhouse gases are formed (Groffman et al., 2006).

Inorganic nitrogen is also removed from aquatic systems by sorption or conversion to organic matter. Nitrogen is one of the building bricks in proteins and thus consumed during biomass growth. The denitrification is preferable for removal since the nitrogen compounds are transformed into the inert N₂-gas and removed from the system. Denitrification has been very difficult to measure given its high background concentration in the environment. Quantification of denitrification is also hindered by high spatial and temporal variation in the process in aquatic systems. Some common ways of estimating the denitrification rate are acetylene-based methods and mass balance calculations. But most of the methods used are indirect methods. The plentiful measurements result in a large risk of accumulating measurement errors (Pribyl et al., 2004).

In order to study the denitrification process, in-situ accurate measurement methods are crucial. This report gives an overview of an N₂/Ar-method for measuring denitrification directly. We also applied this method to the Jiulong River in the Fujian province in Southeast China. This report is part of a larger project where the denitrification and water quality in the watershed will be further investigated.

Background

The Jiulong River
The Jiulong River is located in the Fujian province in Southeast China. The drainage basin is about 14,700 km². The annual precipitation is between 1400 and 1800 mm of which 75 % fall from April to November. The annual mean temperature is 18-21 °C. The watershed is mainly forestland (67%), followed by arable land (17.5 %), water body (7.5 %), bare land (5%) and residential land (3%). The watershed is densely populated and more than 5 million people are taking their drinking water from the Jiulong River. Deteriorating water quality is an ecological, economical and human threat. (Chen et al., 2008)

The Jiulong River has two main tributaries, the North River and the West River. Every year the Jiulong River discharges 14 billion m³ of water into the Xiamen Sea. The estuary has have problems with poor water quality. Harmful algal blooms are common phenomena mainly due to high concentrations of nutrients in the water. The estuary receives N input both from riverine water and from adjacent
urban sewage. The main sources of the high nutrient load in the estuary has been identified as agricultural contributions from Jiulong River catchment, urban wastewater from the cities within the watershed and urban sewage effluent from Xiamen city. The contribution from the agriculture and anthropogenic activities in the watershed is the major N source in the estuary and coastal waters outside Xiamen (Cao et al., 2004).

Chen et al. (2008) have made a nitrogen budget of the Jiulong River watershed based on data from 2004. The budget suggests that the inputs was about two times higher than the outputs, about 80 000 t N year⁻¹ is lost to the environment every year. The main contributions to the N inputs where fertilizers (67.1 %) and animal feed (16.5 %). The main outputs where ammonia volatilization (32.6 % of the input) and runoff (13.8 % of the input). The denitrification in the watershed was estimated to account for removal of 11,258 t N year⁻¹ (5.9 % of the input). The runoff (13.8 % of the input) makes up the contribution to the estuary and corresponds to 26,138 t N year⁻¹.

Eutrophication is not the only threat to the ecosystem in the Jiulong River. The River has more than 130 hydropower stations and the loss of ecosystem services in the river has not been correctly accounted and compensated for during the development (Wang et al., 2009). There is also an important bank erosion and sediment transport.

The river is very important for the people who live in the region. It is e.g. used as a drinking water source, for transport and as a food source. Figure 1 illustrates fishermen living by the river.

![Figure 1 Fishermen living on boats in a tributary to the Jiulong River. Photo: Caroline Fredriksson](image-url)
Denitrification

Ammonium (NH₄⁺), nitrite (NO₂⁻) and nitrate (NO₃⁻) contain bioavailable nitrogen. In natural systems nitrogen enters the system mainly by plant fixation. Anthropogenic activities have increased the concentration of nitrogen in aquatic environments causing excess growth of algae and toxic levels of nitrite. Anthropogenic sources are e.g. fertilizers, increased N₂ fixation by cultivated leguminous crops, sewage water and combustion of fossil fuels. The anthropogenic sources contribute with more than the double amount of N than from natural fixation (Seitzinger et al., 2006).

In figure 2, a schematic picture of the N-cycle is presented.

![Figure 2 Schematic picture of the N-cycle. Source: www.stockton.edu (modified)](image)

Denitrification is carried out by denitrifying bacteria e.g. species of *pseudomonas*, *bacillus* and *micrococcus*. In this paper they are all referred to as denitrifiers. Denitrifiers are facultatively anaerobic bacteria so if oxygen is present, they use oxygen and at low oxygen levels they use nitrite or nitrate to conduct respiration. The respiration is a heterotrophic process where organic matter is degraded to produce energy. In the case of low oxygen levels (< 0.5 mg L⁻¹), nitrate or nitrite is used as the terminal electron acceptor. Denitrifiers are present in terrestrial, aquatic and marine ecosystems. For denitrification to occur, the conditions of nitrate or nitrite availability, low oxygen
Concentrations and sufficient amounts of organic matter need to be fulfilled. The optimal temperature for denitrification is 24 °C. (Seitzinger et al., 2006)

During aerobic conditions ammonia is oxidized into nitrite and nitrate by nitrifying bacteria. Nitrifying bacteria are autotrophic and use the energy they gain from the nitrification to assimilate CO₂. *Nitrosomonas* oxidize ammonia to nitrite. This reaction is the most time consuming. When the nitrite is formed, *nitrobacter* quickly oxidize the nitrite further into nitrate. Therefore the nitrate concentrations are normally much higher than nitrite concentrations in soil and water. The nitrification and denitrification are described by equation 1-3. Please note that the reactions in equation 3 are not balanced. (Beckman, 2005)

\[
\begin{align*}
\text{NH}_4^+ + 1.5 \text{O}_2 & \rightarrow \text{NO}_2^- + 2\text{H}^+ + \text{H}_2\text{O} + \text{energy} \\
\text{NO}_2^- + 0.5 \text{O}_2 & \rightarrow \text{NO}_3^- + \text{energy} \\
2\text{NO}_3^- & \rightarrow 2\text{NO}_2^- \rightarrow \text{NO} \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2
\end{align*}
\]

Equation 3 is simplified and only shows the molecules that contain nitrogen. If the pH is low (< 5) or the oxygen concentration is high, the reduction of nitrogen will not be complete and the production of NO and N₂O will be larger. (Beckman, 2005)

High concentration of nitrate will increase the denitrification rate. Therefore increased levels of nitrogen are mitigated by increased denitrification. Denitrification affects the ecosystem on a local, regional and global scale. In the local and regional scale, the denitrification removes dissolved nitrogen and thus decreases the N/P ratio. Denitrification can also protect downstream ecosystems by preventing further transport of nitrate. An example of this is that denitrification in rivers and lakes may prevent harmful algal blooms in the ocean. On the global scale denitrification is linked to the C-cycle. Increased concentrations of nitrogen in aquatic systems may increase primary production and affect the dissolved CO₂ level in the oceans and in the atmosphere. Denitrification affects the global climate indirect by controlling primary production. Denitrification also has a direct effect on the global climate through the production of nitric oxide (NO) and nitrous oxide (N₂O) which are both greenhouse gases. Nitric oxide may also contribute to formation of the toxic tropospheric ozone. (Seitzinger et al., 2006)

Accurate measurement methods and monitoring the concentrations of nitrous oxide in water is important. It is both a 300 times more efficient greenhouse gas than carbon dioxide and negatively affecting the ozonosphere. The atmospheric concentration is increasing due to biomass burning, nitrification, and denitrification. It has been shown that the levels of dissolved nitrous oxide in the seawater often is supersaturated, indicating that the oceans are a net source of N₂O. (Careri et al., 1999)

Altering the N-cycle affects the C-cycle and in a global perspective also the climate. The climate change will also affect the N-cycle by enhancing the denitrification. This effect may be more significant in marine systems. In lentic systems, this change is considered small in comparison to changes in land-use within the watershed. (Harrison et al., 2008)

On a global scale, considering denitrification of both natural and anthropogenic nitrate, the continental shelf sediments accounts for 44 % of total global denitrification, terrestrial soils 22 %,
oceanic oxygen minimum zones 14%, groundwater, lakes and rivers together 20% and estuaries 1%. The distribution is illustrated in figure 3. (Seitzinger et al., 2006)

![Figure 3 Distribution of total denitrification](image)

**Figure 3** Distribution of total denitrification according to Seitzinger et al., 2006. The lentic contribution is about 20%.

At the watershed scale, terrestrial soil is most important followed by groundwater, rivers, lakes, reservoirs and estuaries in the given order. Although the amount of removed N is largest from soils, the denitrification rate per unit area is about 10 times faster in lakes, rivers, reservoirs and estuaries. (Seitzinger et al., 2006)

Harrison et al (2008) made a similar study about the distribution of the global N removal with focus on small reservoirs and lakes (< 50 km²). They found that 19.7 Tg N is removed per year from watersheds and that small lakes and reservoirs retain almost half that amount. They also emphasize that although reservoirs only make up 6% of the lentic surface area, they remove 33% of the N removed within lentic systems. The high efficiency of reservoirs is explained by the low flow causing high settling velocities and larger catchment sizes in proportion to the surface area contributing to a higher load. In general N-removal in lentic systems correlate positively with N-loading and water residence time and negatively with the water depth. (Harrison et al., 2008)

Although freshwater systems play an important role in the nitrogen cycle, the denitrification rates have been poorly investigated. This is due to the difficulties to measure the denitrification but also the complexity and heterogeneity of the aquatic systems.

The denitrification does not account for the entire amount nitrate removed. There are several other pathways for the nitrate to leave the system. Although respiratory denitrification (described above) is the most important and common pathway for nitrate removal, the following alternatives are important to be aware of (Burgin et al., 2007):
✓ Assimilation Nitrate is incorporated into biomass either by plant or microbial uptake.

✓ Dissimilatory nitrate reduction to ammonium (DNRA) Nitrate is reduced to ammonia that can either be assimilated into biomass or oxidized back to nitrate. The reduction is either carried out by fermentative bacteria or linked to sulfur oxidation. The DNRA occurs during the same conditions as denitrification but is favored by nitrate-limited environments with access to easy degradable carbon.

✓ Nitrate reduction coupled to iron oxidation The reaction is either abiotic or biotic. The abiotic reaction includes reduction of ferrous iron or manganese that converts nitrate into nitrite which is rapidly transformed to N₂ or binds to organic substances and produce dissolved organic nitrogen, DON. The biotic reaction is mediated by microbes and similar to the abiotic one. Characteristic for the biotic reaction is that it normally occurs at relatively low temperatures and at pH 5.5 – 7.2.

✓ Anaerobic ammonium oxidation (anammox) Ammonium and nitrite is combined during anaerobic conditions and produce N₂. The process seems to be most important in deep marine environments. The optimal temperature is lower than for denitrification, 12 °C compared to 24 °C. Studies have showed that anammox also occurs in freshwater systems. It is assumed to be more important in deep lakes than in more shallow systems.

Methods for measurements of denitrification
Denitrification is difficult to measure. The major problem is that the measurement process may change the substrate concentration, disturb the physical settings (e.g. temperature, water velocity), lack sensitivity or be too expensive and time consuming. In small, well known systems e.g. wastewater treatment plants, the denitrification rate can be satisfactory determined by mass balance methods. A mass balance is an indirect way of measuring denitrification. When measuring the denitrification direct, the high background concentration of N₂ calls for a very precise method that can detect small changes in concentration of the major end product. Technical development and affordable apparatus has increased the use of direct measurement methods were the denitrification rate is measured in situ. (Groffman et al., 2006)

The measurement methods used to measure denitrification in aquatic systems are more precise and developed than for measurements in terrestrial environments. Groffman et al. (2006) have made a review of the available techniques used to measure denitrification. They are listed below with short descriptions of the method and its advantages and disadvantages with focus on aquatic systems:

✓ The Acetylene Inhibition Method Acetylene (C₂H₂) inhibits the reduction of N₂O to N₂. Thus N₂O becomes the terminal product of the denitrification. The N₂O is easier to measure since the background concentration is much lower. The measurements are easy to carry out and therefore a large number of samples can be run. The main disadvantage of the method is that the C₂H₂ also inhibits nitrification. If there is limited amounts of NO₃⁻ present in the system, denitrification rates may be underestimated.

✓ ¹⁵N Tracer Methods There are several applications of using the isotope ¹⁵N as a tracer. The most common method is to add ¹⁵NO₃⁻ and ¹⁵NH₄⁺ to the system and measure the production of ¹⁵N gases. The method is based on an assumption of homogenous isotope mixing and linearity
between denitrification rates and bulk water \( \text{NO}_3^- \) concentrations. The conditions are rarely fulfilled in aquatic systems with several different types of organisms and solid substrates. Therefore the method is not suitable for streams, wetlands and littoral zones.

- **Direct \( \text{N}_2 \) and \( \text{N}_2/\text{Ar} \) Measurements** In aquatic systems the background concentration of \( \text{N}_2 \) is lower than in terrestrial systems with free gas exchange with the atmosphere. Therefore the increased concentration of \( \text{N}_2 \) due to denitrification in aquatic systems can be measured by the use of mass spectrometry and gas chromatography. The method has recently increased in popularity due to availability of low-cost mass spectrometers. A disadvantage of the method is that it only measures net denitrification which is the difference between total denitrification and \( \text{N}_2 \)-fixation. The method is described in detail in the section *Membrane Inlet Mass Spectrometer (MIMS) to determine \( \text{N}_2 \)*.

- **Mass Balance Methods** Aquatic systems are complex and to facilitate calculations steady state is often assumed. Only inputs and outputs are quantified and change of storage is not accounted for. Still there are many parameters to keep track of and estimation and measurement errors may accumulate. To calculate the denitrification, two different approaches may be taken; either the denitrification is calculated as the difference between inflow and outflow, or the denitrification is estimated from literature values and incorporated into the mass balance. In agricultural watersheds the assumption of steady state may be a large source of error since the N pools are very big and even small changes may greatly affect the flux. Groffman et al. consider the mass balance method useful to control the accuracy of other methods, e.g. by checking so that denitrification rates are not larger than the N input to the system.

- **Stoichiometric Approaches** The method is based on mass balance and investigations of the elemental ratio of organic matter. It is assumed that when the organic matter is decomposed using an electron acceptor (e.g. \( \text{O}_2 \) or \( \text{NO}_3^- \)), there will be a set of products corresponding to the known elemental ratio. The consumption of electron acceptor is calculated and an expected concentration of inorganic N is derived. The difference between expected and observed dissolved inorganic N is used to calculate denitrification. The stoichiometric approach has the same problems with error accumulation as other mass balance methods and also the problem with measuring the net denitrification as the difference between fixation and actual denitrification. The method is best suited for marine environments since marine organisms have a more uniform elemental ratio.

- **Methods Based on Stable Isotope Abundances** The general idea is that denitrification alters concentrations and isotopic compositions of many different aquatic solids and species. For example the fraction of isotope \( ^{15}\text{N} \) and \( ^{18}\text{O} \) is often increasing in the water during \( \text{NO}_3^- \) reduction. These changes and many more isotopic alterations of other species (e.g. \( \text{H}, \text{S}, \text{C} \)) can be studied to determine the denitrification rate but also the origin of the water, sources of reactants and products of denitrification. The method is a useful indicator of denitrification but is difficult to use in physically heterogeneous systems.

Also use of environmental tracers are discussed in the review by Groffman et al. but since it is mainly used for historical estimates it is not further discussed here. The molecular approaches have also been excluded since they do not aim to quantify the denitrification rate.
**Membrane Inlet Mass Spectrometer (MIMS) to determine \([N_2]\)**

Mass spectrometry is an analysis method where molecules are specified and quantified by measuring their mass. The molecules are first ionized and then accelerated through vacuum so that they are not destroyed in collision with other particles. An electromagnetic field causes the ions to decline from their trajectories. The declination depends on the molecule’s mass and charge. A detector registers the ions that have the mass/charge ratio required to reach the detector. (Ellervik and Sterner, 2004)

The ionization can be done by several techniques but is normally accomplished by “electron impact”, i.e. shooting electrons at the sample, creating positive ions. The ions are unstable and immediately fragmented. The fragments are detected and create a fragment pattern that is characteristic for each molecule. This technique is typically used when analyzing organic compounds. (Ellervik and Sterner, 2004)

A schematic picture of a mass spectrometer is presented in figure 4.

![Schematic picture of a mass spectrometer. Source: www.wpcontent.answers.com](image)

The method used in this experiment was developed by Kana et al (1994). The method is based on measuring gases dissolved in water. \(N_2\), \(O_2\), and \(Ar\) are the most concentrated gases in water. \(N_2\) and \(O_2\) are affected by biological and physical processes but \(Ar\) is only affected by physical processes. When \(N_2\) and \(O_2\) are deviating from the equilibrium concentrations, corrected for physical changes, biological activities are indicated. The deviation may be small (<1%) and therefore very precise measurements are required. (Kana et al., 1994)

A MIMS apparatus (see figure 5 and 6) constitutes of a probe that is connected to mass spectrometers via a liquid \(N_2\) trap. The liquid \(N_2\) trap removes water vapor and carbon dioxide, \(CO_2\) so that they do not interfere with measurements of the studied gases. The probe that is submerged into the water or sediment sample consists of steel capillary tubing with a silicone membrane allowing for gas diffusion into the tube where it is vacuum. (Hartnett et al., 2003)
Membrane Inlet Mass Spectrometry, MIMS, has been used for measurements of denitrification rates in several different aquatic environments such as wetlands (Poe et al., 2003), rivers (Pribyl et al., 2004; Laursen and Seitzinger, 2002) and estuarine sediment pore waters (Kana et al., 1998; Hartnett et al., 2003).

Normally the ratio N₂/Ar is measured instead of the N₂ concentration, because mass spectrometer data is more precise when measuring ratios. The precision is <0.5% for N₂, O₂, and Ar and <0.05% for N₂/Ar and O₂/Ar ratios. The N₂/Ar ratio has also the advantage of not being affected by O₂ concentration. (Kana et al., 1994)

When using the MIMS method the temperature needs to be stable and it is important that no gas bubbles are present in the samples because they will disturb the uniform gas flow across the membrane. (Kana et al., 1994)
Pribyl et al. made a study where MIMS was compared to the mass balance method determining the denitrification in a river. They concluded that:

- the modern MIMS equipment has overcome a previous problem with separating the N₂ dissolved during denitrification from the atmospheric N₂ flux,
- denitrification rates that are two low to be analyzed with the mass balance method can be determined by MIMS,
- the two methods showed similar seasonal patterns but the mass balance estimates were in general slightly lower,
- the MIMS method is best suited for well-mixed streams with evenly distributed concentrations of N₂,
- potential errors for the MIMS method is losses of denitrified N₂ through bubbles, nitrogen fixation and N₂O.

**Purge-Trap-Gas Chromatography to determine [N₂O]**

Gas chromatography is an analysis method used to separate and specify molecules. The sample is moving through a column mixed with an inert gas, e.g. nitrogen (N₂) or helium (He). The walls of the column are covered with either a liquid or a polymer. The molecule’s affinity for the substrate on the column walls will determine the transport time through the column. In this way the sample will be separated and can also be quantified. Gas chromatography is often used together with mass spectrometry (Ellervik and Sterner, 2004).

The purge and trap method is used to isolate volatile compounds, see figure 7. An inert gas is bubbled through the sample. The molecules that are extracted from the sample are then trapped on an absorbent material. When the extraction is complete the absorbent material is heated so that the molecules are desorpted and returned to gas phase. (Research Group Weimar, 2003)

![Figure 7 Schematic of a purge-and-trap system. Source: Research Group Weimar, 2003](image)
Gas chromatographic methods are most frequently used when analyzing nitrous oxide in biological and environmental samples. Careri et al. (1999) made a study where the purge-and-trap method was compared to a dynamic head method using synthetic saltwater samples with known nitrous oxide concentrations. In the dynamic head method, the gases are separated from the sample by stripping. It was concluded that the purge-and-trap method was more sensitive and had a lower detection limit. (Careri et al, 1999)

**Methods to estimate denitrification rates**

The method used in this project is based on direct measurements of the increase of dissolved N₂ due to denitrification. The measured N₂ concentration is corrected for the atmospheric exchange. The dissolved N₂ concentration in water is a function of the production and the atmospheric exchange (Laursen et al, 2002):

\[ [N_2] = f(N_2 \text{ production, atmospheric exchange}) \]  \hspace{1cm} (4)

The total N₂ concentration is determined from the N₂/Ar ratio determined by MIMS. The solubility of argon determined by Weiss (1970) is used. Since argon concentration is only affected by physical factors, a tabled value can be found for saturated conditions. The solubility is depending on temperature and salinity. The N₂ concentration is calculated as:

\[ [N_2] = \frac{N_2}{Ar} \times [Ar]_{\text{Weiss}} \]  \hspace{1cm} (5)

The surplus N₂, \([N_2]_{\text{denitrification}}\), that is due to the denitrification is determined as the difference between the measured concentration of N₂, \([N_2]\), and the theoretical saturated concentration of N₂, \([N_2]^*\), which can be determined by Weiss (1970):

\[ [N_2]_{\text{denitrification}} = [N_2] - [N_2]^* \]  \hspace{1cm} (6)

The denitrification rate is the flux of nitrogen, F, determined by:

\[ F = k \times [N_2]_{\text{denitrification}} \]  \hspace{1cm} (7)

where k is the diffusion constant determined by (Wanninkhof, 1992):

\[ k = 0.31 \ u_{10}^2 (S_c/600)^{-1/2} \]  \hspace{1cm} (8)

where S_c is the Schmidt number (Wanninkhof, 1992),

\[ S_c = 2301.1 - 151.1 \times T + 4.7364 \times T^2 - 0.0059431 \times T^3 \]  \hspace{1cm} (9)

where T is the water temperature (0-30 °C).

N₂O is also measured as an end product from denitrification, although it accounts for a fairly small fraction. The contribution is calculated by the same method as for the denitrification with N₂ as end product. The total denitrification rate is the sum of the rate calculated from N₂ measurements and the rate calculated from N₂O measurements:

\[ \text{Total denitrification} = F_{N_2} + F_{N_2O} \]  \hspace{1cm} (10)
A more complicated whole-reach approach developed by Laursen and Seitzinger (2002) can be used for more detailed calculations. The method is presented here but not used in the project at this state.

The atmospheric exchange of a gas across the air-water interface (F) is determined from (Laursen et al, 2002):

\[ F = hK(C_{\text{mean}} - C_{\text{equil}}) \]  

(11)

where \( h \) is the mean water depth, \( K \) is the first order gas transfer rate, \( C_{\text{mean}} \) is the mean gas concentration in the water column and \( C_{\text{equil}} \) is the atmospheric equilibrium concentration. (Laursen et al, 2002)

The transfer rate, \( K \), can be determined by the use of non-reactive tracers. Laursen et al (2002) determined \( K_{N_2} \) and \( K_{Ar} \) based on experiments with propane gas and isobutene gas. The transfer rate is temperature dependent and \( K \) at new temperatures can be determined by:

\[ \frac{K_{N_2}}{K_{N_2}} = \left( \frac{S_{C_{N_2}}}{S_{C_{N_2}}} \right)^n \]  

(12)

where \( K_{N_2} \) is the unknown first order transfer rate at some new water temperature, \( K_{N_2} \) is first order transfer rate at the previous water temperature and \( S_{C_{N_2}} \) and \( S_{C_{N_2}} \) is the Schmidt number for the new respectively previous water temperature. The \( K_{Ar} \) is calculated in the same way. (Laursen et al, 2002)

The denitrification rate is modeled as the difference between the measured \([N_2]\) and the expected change in \([N_2]\) if no denitrification occurs. The expected \([N_2]\) is modeled in a parcel of water moving downstream, given \( K_{N_2} \). \( K_{N_2} \) is recalculated at each time step to allow for temperature changes according to equation 12. The surplus of \([N_2]\) is assumed to be due to denitrification. The model requires meteorological and physical parameters. The equations and necessary model input is taken from *Mesurement of denitrification in rivers: an integrated, whole reach approach* by Laursen and Seitzinger, 2002.

**Table 1 Model Inputs to Laursen and Seitzingers (2002) denitrification model.**

<table>
<thead>
<tr>
<th>Model input</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Channel depth</td>
<td>M</td>
</tr>
<tr>
<td>Channel width</td>
<td>M</td>
</tr>
<tr>
<td>Velocity</td>
<td>m min(^{-1})</td>
</tr>
<tr>
<td>( \Delta t )</td>
<td>H</td>
</tr>
<tr>
<td>Initial ( N_2 ) concentration</td>
<td>mmol ( N_2 ) m(^{-3})</td>
</tr>
<tr>
<td>Initial equilibrium ( N_2 ) concentration</td>
<td>mmol ( N_2 ) m(^{-3})</td>
</tr>
<tr>
<td>Initial temperature (upstream)</td>
<td>°C</td>
</tr>
<tr>
<td>Final temperature (downstream)</td>
<td>°C</td>
</tr>
<tr>
<td>Initial ( K_{N_2} )</td>
<td>h(^{-1})</td>
</tr>
<tr>
<td>Denitrification rate</td>
<td>mmol ( N_2 ) m(^{-3}) min(^{-1})</td>
</tr>
</tbody>
</table>

Model calculations, (Laursen et al, 2002) units according to table 1:

\[ \text{Tot} \ N_2 \ (\text{mmol} \ N_2) = \text{\( N_2 \) concentration} \times \text{width} \times \text{distance travelled in 1 min} \]  

(13)
At each time step, total N2 is recalculated as

\[ N_2 = (N'_2 + N_2 \text{ prod}) - N_2 \text{ loss} \]  

(14)

where

\[ N'_2 = \text{tot} N \text{ at previous time step} \]  

(15)

\[ N_2 \text{ prod} = \text{denitrification rate} \times 1 \text{ min} \times \text{width} \times \text{distance traveled in 1 min} \]  

(16)

\[ N_2 \text{ loss} = N_2 \text{ flux} \times 1 \text{ min} \times \text{width} \times \text{distance traveled in 1 min} \]  

(17)

\[ N_2 \text{ flux} = \text{depth} \times \frac{1}{60} \text{ min} \times K_{N_2'} \times [N_2 \text{ measured} - N_2 \text{ equilibrium'}] \]  

(18)

\[ K_{N_2'} (h^{-1}) = K_{N_2} \text{ for the current temperature and } N_2 \text{ equilibrium'} = \text{equilibrium concentration at the current time step} \]

Dissolved N2 (mmol N2 m\(^{-3}\)) is then recalculated from total N2 after each 1 min time step.

The N2O concentration should also be accounted for so the N2O flux will be calculated and added to the nitrogen production. It is also important to be aware of the potential error due to gas (N2 and N2O) lost by ebullition (i.e. through bubbling). (Pribyl et al, 2005)

When using a method based on measurements of dissolved N2 in water, knowledge about how the N2 concentration varies depending on external effects is crucial. Laursen et al. (2005) has made a model based investigation of how channel geometry, wind velocity, sampling interval and temperature inputs of N2-enriched groundwater affect the N2 concentration. They have come up with guidelines regarding when it is possible to calculate the denitrification based on measurements of N2 concentrations. They found that N2 could accumulate easy in shallow rivers at low wind speeds. During such conditions it is possible to detect denitrification rates as low as 30-100 \(\mu\)mole N m\(^{-2}\) h\(^{-1}\). They also found that varying diurnal temperature leads to lag effects that alter the sensitivity of the method, because the diffusion is temperature dependent. In deep rivers the method is less sensitive. This is due to the relatively larger water volume to the sediment surface area, therefore a higher rate of N2 flux is needed to detect changes. They point out the importance of knowledge of the groundwater system since input of N2 enriched groundwater otherwise could be misinterpreted as denitrification. (Laursen et al, 2005)

Materials and Methods

Sampling

There are 5 sampling points in the West Jiulong River and 4 sampling points in the North Jiulong River. There are also 3 seawater sampling points in the estuarine waters. The sampling points are indicated on the map in figure 8. The samples are collected 0.5 m below the water surface at central stream channel under well mixed conditions. At Station D5 (downriver of West River), water is also collected from depths of 2, 3, and 3.5 m (close to sediment). The samples were assigned number Z1, Z2, Z3, respectively.
Figure 8 The green dots indicate the position of the sampling points. Source: JRW Group

From each sampling point two groups of triplicate samples of 40 mL are collected for analysis of dissolved N\textsubscript{2} and N\textsubscript{2}O. To avoid mixing with air, samples are taken from the bucket to the vial via a siphon, see figure 9. They are allowed to overflow several times and are screw capped with no head space. These samples for N\textsubscript{2} and N\textsubscript{2}O are preserved by adding 200 µL of HgCl\textsubscript{2} (to a final concentration of 0.5 %) to stop continued denitrification by killing the bacteria. Samples for DOC (dissolved organic carbon) are filtered by a 0.7 µm filter and preserved with H\textsubscript{3}PO\textsubscript{4} (to a final concentration of 0.1 %). Samples for nutrients were also collected. Samples for N\textsubscript{2} are kept in a temperature of 1-2 °C below the lowest water temperature. Samples for N\textsubscript{2}O and DOC are kept in a temperature of 4 °C. Samples for nutrients are filtered in lab and are frozen to -20 °C before analysis.
Figure 9 Samples are collected from the bucket to the brown glass vials via a siphon.

At every sampling point, pH and dissolved oxygen is measured by an Orion 4 pH.DO portable from Thermo Scientific. Also water and air temperature is measured and the time is noted.

**Analysis of \( N_2 \)**

**Instrumentation**

A Membrane Inlet Mass Spectrometer of the model HPR40 from Hiden co. is used, see figure 10.

Figure 10 The MIMS apparatus used in the experiment. Photo: Caroline Fredriksson, 2010
Standards

Standards consist of 5 samples with different salinity. The samples are prepared from deionized water and NaCl. The concentrations (salinities) are 0%, 10‰, 20‰, 30‰ and 40‰. The samples have been air equilibrated for at least 52 hours and the temperature is kept constant closed to the sample water temperature (e.g., 24°C for June samples). Before the standards are measured, the instrument is stabilized for about one hour with a solution of 5‰ salinity. The analyzed samples need to be stabilized for at least 6-8 minutes before reach the stable signal. An average value of N₂/Ar is determined from 2 minutes measurements.

A standard curve is created from the 5 measurement points. The measured ratio of N₂/Ar is plotted against the theoretical, tabulated [N₂]/[Ar] ratios. (Weiss, 1970).

Analysis of N₂O

Instrumentation

The instrument used is a 6890N Network GC System from Agilent Technologies.

Standards

The standard curve is created by the use of standard gases with N₂O/ N₂ ratios of 0.506•10⁻⁶ and 0.995•10⁻⁶. The 6 standard samples have N₂O concentrations of 0.87*0.506 ppm, 0.87*0.995 ppm, 1.65*0.506 ppm, 1.65*0.995 ppm, 4.65*0.506 ppm and 4.65*0.995 ppm.

Analysis of nutrients

The methods used to analyze the nutrients are summarized in table 2.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method</th>
<th>Instrument</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dissolved total nitrogen</td>
<td>Alkaline potassium persulfate digestion-UV spectrophotometric method</td>
<td>UV-Visible Spectrophotometer Cary 100 BiD from Varian</td>
</tr>
<tr>
<td>Nitrate</td>
<td>Ultraviolet spectrophotometry</td>
<td>UV-Visible Spectrophotometer Cary 100 BiD from Varian</td>
</tr>
<tr>
<td>Nitrite</td>
<td>Griess reaction method</td>
<td>UV-Visible Spectrophotometer Cary 100 BiD from Varian</td>
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<tr>
<td>Ammonium</td>
<td>Indophenol blue method</td>
<td>UV-Visible Spectrophotometer Cary 100 BiD from Varian</td>
</tr>
<tr>
<td>Dissolved reactive phosphorus</td>
<td>Ammonium molybdate spectrophotometric method</td>
<td>UV-Visible Spectrophotometer Cary 100 BiD from Varian</td>
</tr>
</tbody>
</table>

Table 2. Methods and instruments used for analysis of nutrients.
**External Data**

Wind speed data was collected from the Zhangzhou weather station. An average wind speed for the month of June from the period 1954 – 2009 was used for the denitrification rate estimation. The average wind speed was 1.55 m/s.

**Results and discussion**

There is no data of N₂O in June because the instrument was out of function. The results for the N₂ concentration and denitrification rate are presented in Appendix 1. The result from the nutrient analysis and the pH is presented in Appendix 2.

Since there are no measurements of N₂O in June, the denitrification rates may be underestimated. The denitrification resulting in N₂O as end product is not accounted for.

The result shows that the average increase of [N₂] due to denitrification is 26.5 µM for all stations. The increased concentration is varying from 6.99 to 49.16 µM. The highest concentration is found in sampling point D3 (49.16 µM) located in the West River. The lowest concentration is found in sampling point D7 (6.99 µM) located in the North River (Fig. 11).

The [N₂]$_{\text{denitrification}}$ is higher in the West River with an average of 35.49 µM. In the North River, the [N₂]$_{\text{denitrification}}$ is 15.26 µM. The higher concentration in the West River is probably due to the higher concentration of nitrate. In the West River, the average [NO$_3^-$] is 3.06 ± 1.32 mg L$^{-1}$ and in the North River the average [NO$_3^-$] is 1.47 ± 0.67 mg L$^{-1}$.

![Figure 11. The net increase of N₂ due to denitrification.](image)

In figure 12, the denitrification rate and the nitrate concentrations are plotted together for each sampling point. The result shows that the average denitrification rate is 12.4 mmol m$^{-2}$ d$^{-1}$. The denitrification rate is varying from 3.2 to 22.7 mmol m$^{-2}$ d$^{-1}$. The highest rate is found in sampling point D3 and the lowest rate is found in sampling point D7. As the [N₂]$_{\text{denitrification}}$ indicated, the
denitrification rate is higher in the West River with an average of 16.4 mmol m⁻² d⁻¹. In the North River, the average denitrification is 7.5 mmol m⁻² d⁻¹.

![Figure 12. The denitrification rate and nitrate concentration at the different sampling points.](image)

To evaluate the relationship between the denitrification rate and the nitrate concentration, the denitrification was plotted to the nitrate concentration, figure 13. A straight line was fitted to the scatterpoints. The result shows no significant linear relation, $R^2=0.075$.

![Figure 13. The denitrification rate as a function of nitrate concentration shows no significant relation.](image)

The vertical samples were collected in sampling point D5. The vertical distribution of denitrification is shown in figure 14. The in situ $[N_2]$ is higher close to the bottom sediment. This is normal because the denitrification rate is higher there. The high concentration close to surface may depend on turbulence when sampling or origin of water. The $[N_2]$ in the surface water may have been transported from an upstream site with higher denitrification rate. Or the $[N_2]$ close to the surface my have been affected by the heavy rainfall on the sampling day.
Conclusions
The denitrification rate shows great variation within the river reach. The denitrification rates are in general higher in the West Jiulong River where also the nitrate concentration is higher. There is no significant relationship between the denitrification rate and the nitrate concentration. But this report is based on data from only one month, and maybe further studies will validate a relation. It is also difficult to isolate the relationship, because of the complexity of denitrification. Denitrification is also affected by e.g. pH, oxygen concentration, water temperature, hydrological conditions etc.

When finished, this project will probably contribute with valuable information about the capacity of the river to remove nitrogen. The results from the MIMS are showing a satisfactory coherence, but since the results so far only have been studied for June 2010 it is difficult to make any conclusions or suggestions regarding management. The water treatment within the watershed needs to be improved in order to reduce the nutrient transport and to ensure a good drinking water quality downstream. Also input of pig manure and fertilizers to the river needs to be decreased.

It is also important to continue sampling the production of N₂O to determine the effect of denitrification on the global climate.

Figure 14 Vertical distribution of in situ N₂ concentration in Zhengdian Station (D5).
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**Webpages**


http://www.ipc.uni-tuebingen.de/weimar/research/maintopics/supporting/hp7695.htm

**Figures**

Figure 2, accessed [07/05/2010] at

http://www.stockton.edu/~cromartw/ecology/images/N%20cycle.jpg

Figure 3, accessed [07/05/2010] at


Figure 6, accessed [07/05/2010] at

http://www.ipc.uni-tuebingen.de/weimar/research/maintopics/supporting/hp7695.htm

Figure 8

JRW group
## Appendix 1

Results for the N$_2$ analysis.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Water T ('C)</th>
<th>N$_2$(μM)</th>
<th>N$_2$(water) - N$_2$(eq) (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1  2  3 average</td>
<td>1  2  3 average</td>
</tr>
<tr>
<td>D1</td>
<td>26.5</td>
<td>NULL</td>
<td>515.72 509.43 512.58</td>
</tr>
<tr>
<td>D2</td>
<td>25.1</td>
<td>511.46</td>
<td>514.52 506.89 510.96</td>
</tr>
<tr>
<td>D3</td>
<td>27.0</td>
<td>NULL</td>
<td>516.40 523.76 520.08</td>
</tr>
<tr>
<td>D4</td>
<td>28.0</td>
<td>500.79</td>
<td>500.31 497.71 499.60</td>
</tr>
<tr>
<td>D5</td>
<td>28.0</td>
<td>496.19</td>
<td>494.27 NULL 495.23</td>
</tr>
<tr>
<td>D6</td>
<td>24.3</td>
<td>508.45</td>
<td>505.77 505.65 506.62</td>
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<tr>
<td>D7</td>
<td>27.0</td>
<td>479.16</td>
<td>476.98 477.60 477.91</td>
</tr>
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Note: null indicates missing sample
### Appendix 2

Results for the estimation of denitrification rate

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<tr>
<th>Samples</th>
<th>T (°C)</th>
<th>Sc</th>
<th>k</th>
<th>$\text{N}_2$ (water) - $\text{N}_2$ (eq)</th>
<th>exchange flux of air-water (mmol m$^{-2}$ d$^{-1}$)</th>
<th>flux average</th>
<th>standard deviation</th>
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<td>D1-1</td>
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<td>19.21</td>
<td>17.74</td>
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</tr>
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<td>13.98</td>
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<td>11.00</td>
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</table>
### Appendix 3

Results from the nutrients analysis, the pH.

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH</th>
<th>DTN (mg/L)</th>
<th>NO₃-N (mg/L)</th>
<th>NO₂-N (mg/L)</th>
<th>NH₃-N (mg/L)</th>
<th>DRP (mg/L)</th>
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<tbody>
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