UNIVERSITY OF WARSAW

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NITROGEN REMOVAL PROCESSES BY WETLAND BUFFER ZONES IN NAREW TRIBUTARY

by

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This thesis is submitted for obtaining the Interdisciplinary Master's Degree in Environmental Management. By submitting the thesis, the author certifies that the text is from his own hand, does not include the work of someone else unless clearly indicated, and that the thesis has been produced in accordance with proper academic practices.

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1. Introduction

1.1. Introduction

Agricultural activities, production and usage of fertilisers, fossil fuels combustion and other activities related to increase of human population have significantly altered geochemical cycles of elements crucial for primary production. Moreover, increased food demands and fertiliser use are the cause of growing rate of nonpoint pollution sources (NPS). Additionally available nutrients accelerate the eutrophication process. Moreover, NPS might be the source of nutrients in the groundwater, which in turn can cause significant health-related issues. In the management of point pollution sources, treatment and water purification can be applied to majority, if not all, of discharges. In the case of NPS, however, this solution is simply undoable. With water treatment solutions being inadequate, the quality of water resources is worsening. The solution for this problem should be found quickly. One of the proposed solutions might be using riparian buffer zones in water management. Due to their high effectivity in nitrogen removal processes they can be considered to be means of ecological infrastructure (Mander 2017a), which is connecting natural and semi-natural ecosystems, as well as supporting them in providing the ecosystem services. For implementing this solution for water treatment, a thorough examination of its effectiveness should be carried out. In this paper, we compare nutrient removal efficiency by riparian buffer zones in several chosen transects, varying in terms of usage, hydrology and vegetation types. As an outcome, we aim at demonstrating the usefulness of riparian buffer zones in environmental management practices.

1.2. Nutrient sources

Anthropogenic pressure have significantly altered every major biogeochemical cycle (Falkowski 2000), also of those elements crucial from the anthropogenic perspective. In this paper we will focus on nitrogen, considering also the role of phosphorus and carbon, since those elements are considered limiting in aquatic ecosystems (Rabalais 2002, Paerl 1982). In recent years, the proportion of those nutrients available in aquatic inputs has changed, with significant increase of nitrogen availability. The increase of N availability might only increase: FAO estimates that due to growth of human population and consumption of meat, we will observe further steady increase of usage of nitrogen-based fertilisers (FAO 2015; similar estimations are cited by Mosier (2008)). Due to those changes, the elevation of nitrogen to phosphorus ratio has been observed, e.g. in the north western Pacific Ocean (Kim et al. 2011). Additionally, research suggest that while the inorganic N input increases, the ability of streams to remove this nutrient from the biogeochemical cycle decreases (Mulholland et al. 2008).

Increase in the availability of nitrogen can be linked mostly to food production (agriculture is assumed to be the main source of nitrogen in European streams), transportation, and energy consumption, generally increasing both the availability and the mobility of N on global scale (Klapproth and Johnson 2009, Kim et al. 2011). As stated by Sutton and Bleeker (2013), the changes are massive and modifications to the N cycle can be described as pan-dimensional. In case of aquatic ecosystems, there is a historically unprecedented increase of reactive nitrogen observed due to anthropopressure: namely, increased biological fixation of atmospheric N (dinitrogen) resulting from crop production, combustion of fossil fuels, and production of synthetic fertilisers (Mosier 2008).

This leads to decrease of the quality of groundwater and surface water bodies. High nitrate concentration in water can also potentially cause health-related issues in people, such as methaemoglobinaemia (blue baby syndrome, connected to elevated amount of methemoglobin in the newborns' blood) and cancer (Manassaram et al. 2010). Permissible levels for ammonium in drinking water is even lower than for nitrate (Maitre at al. 2003). While already known results stemming from elevated N inputs are alarming, overall results of this massive shift in nitrogen availability on a global scale have yet to be fully explored. Due to those causes World Health Organisation have set a standard for nitrate pollution in drinking water (Fewtrell 2004), and nitrate pollution in surface water and groundwater has attracted global attention (Jalali 2011, Jalali et al. 2018).

Recent changes can also be observed in the P cycle. Estimated amount of dissolved phosphorus in river streams has doubled compared to the amount from the era before significant human impact. The availability of P is on the rise due to the agriculture and other human activities (Mainstone and Parr 2002). One of human activities leading to altering the P cycle is fertilisation. The use of fertilisers contributes substantially to the dissolved P cycle. Another human activity significantly modifying the P cycle is deforestation, especially when it is based on harvesting selectively chosen tree species and burning the rest of them. Additionally, deforestation changes the bioavailability of phosphorus (Turrión et al. 2000, Lawrence et al. 2007). The soil, deprived of roots due to deforestation, is faster leaching the stored P (Filippelli 2008). Described alterations in the global P and N cycle lead to faster eutrophication of riverine and lake ecosystems, and are likely to cause enhanced biological production in the whole ocean (Harper 1992).

Increase of eutrophication is influencing the carbon cycle, and will continue further in the future. Carbon cycle is another biogeochemical cycle strongly effected by human presence. Scholars notice alterations in global C cycle connected to the anthropopressure for around 200 years. Modern atmosphere contains higher atmospheric carbon concentration than it had used to at any point in the past 800 000 years (Lüthi et al. 2008). Moreover, atmospheric carbon exchanges rapidly with aquatic and terrestrial ecosystems, influencing the concentration of C in them.

1.3. Need for purification

Health related issues, mentioned in previous chapter, as well as increase of human population lead to increase demand for drinking water. Public polls also suggest that water pollution is one of the most feared environmental issue among Europeans (EORG 2002). Public concern about the quality of waters was a major push in creating the Water Framework Directive (WFD) 2000/60/EC. The directive aims at achieving "good ecological and chemical status" of all European waters (surface and groundwaters) by 2015. The status is defined by the WFD as determined by low levels of chemical pollution and low level of other factors harming ecological well-being of rivers, such as morphological changes and water extraction for industrial purposes. The second aspect of waters status was groundbreaking for international legislations. To ensure this goal WFD requires the long-term sustainable use of both surface and groundwater and introduces certain monitoring measures that have to be fulfilled.

Another legislative mean to ensure access to clean waters in Europe was the so-called Nitrates Directive 91/676/EEC. The directive aims to protect water quality across Europe by focusing on the source of nitrates pollution. Identifying agriculture as main source of nitrates pollution, directive focuses at promotion of good farming practices. However the groundwater pollution still can be found exceeding quality limit of 50 mg NO3/L, with some data suggesting that national monitoring programme might underestimate nitrate levels in several local studies (Højberg et al., 2017), Nitrates Directive implementation has also introduced measurable improvement. In 2010 vast majority (70%) of monitoring sites located in EU-15 countries reported unchanged or decreasing nitrate concentration in surface water compared to the period 2000-2003 (Implementation of Nitrates Directive Factsheet, 2010).

Even with mentioned legislative actions being taken, waters are still a very important matter for European citizens. Survey published in 2012 shows that a majority of respondents (68% of surveyed citizens of EU25) considers water related issues as serious (Flash Eurobarometer 344 2012). Even bigger percentage (75%) thought that the EU need additional standards regulating further water problems in Europe. According to this group of surveyed citizens, the main focus of additional measures on water pollution should be pollution from industry and agriculture. Nitrates Directive is an example of the leading trend among legislations aimed at elevating water quality. It focuses mostly on the source of the pollutants and promotes actions that can diminish the amount of nutrients introduced to the environment. Another example of such legislations can be French EcoPhyto plan, introduced in 2008 (Stokstad, 2018). According to Stokstad (2018), one of the aims of legislation was to reduce the usage of fertilisers (and pesticides) by 50% in ten years, until 2018. As we now know, the plan did not succeed. However, a new project, based on revised assumptions, is to be undertaken

However, non-removed nutrients at some point are transferred to the waters, contributing to the pollution of aquatic ecosystems. It is difficult to estimate the exact percentage of NPS within all sources of pollution, especially that it can vary highly among countries. Research conducted in Ebro (Spain) proved that NPS are responsible for 64% of NO3 load (Torrecilla et al. 2005). The necessity to find a systemic solution for this problem has been noticed by both researchers and legislators. Riparian buffer zones, known for highly effective nutrient removal, could be a solution for this problem, also helping to achieve "good ecological status" of rivers.

First European legislative actions focused at using buffer strips in water treatment have already been implemented. One of them is a Buffer Strip Act, adopted by the Danish Parliament in June 2012 (Kronvang et al. 2015). According to Kronvang (205), this legislation required a mandatory buffer strip of minimal width of 10 meters to be established along chosen rivers and lakes. Waterbodies subjected to this form of protection have to have surface larger than 100 m^2. Buffer Strip Act came to life on 1st of September 2012, and first assessments of its efficiency have already been prepared (Zak et al., 2019).

1.4. Buffer zones in water purification

Often defined as the zone of vegetation adjacent to streams, rivers, or wetlands (Mayer et al. 2007), buffer zones are vital for the ecosystem functioning due to their role in sediment trapping, forming habitats crucial for semi-aquatic species, creating ecological corridors, and nutrients removal (Teiter, Mander 2005). Riparian buffer zones are considered to be highly heterogeneous, varying by factors such as hydrology, soil characteristics and biological processes. In this paper following terms - "buffer zones", "buffers" and "riparian buffer zones" - will be uses interchangeably to describe the same ecosystem.

High efficiency of riparian buffer zones in nitrogen removal has been acknowledged for thirty years (Teiter, Mander 2005). Nitrogen removal conducted by riparian buffers can help protect aquatic ecosystems from additional nitrogen inputs, mostly caused by anthropopressure. Nitrogen can be temporally removed from nitrogen cycle by various processes. The most potent processes include plant uptake and microbial removal (Mander 2017a). While there are many different types of microbial nitrate reduction processes, such as autotrophic denitrification, chemodenitrification, or dissimilatory nitrate reduction to ammonium, heterotrophic denitrification is found to be generally the dominant process in riparian zones (Hefting 2006). This process demands anoxic conditions and presence of nitrate as electron acceptor, and involves facultative anaerobic organisms. They use oxygen for respiration in aerobic conditions, and switch to nitrate in anaerobic conditions. The process consists of reduction of nitrate through nitrite nitrogen oxide and nitrous oxide. The end product of this process is nitrogen in gaseous form (dinitrogen). The rate of purification processes is thought to be controlled by factors both hydrological (such as water residence time (Kjellin et al. 2007)) and biological (plant species composition and microbial biodiversity (Liu 2017). Another processes causing N removal by riparian buffers are storage in the soils, groundwater mixing (Pinay et al. 1998) and microbial immobilisation (through assimilation into microbial biomass) (Mander 2017a, Rivett et al. 2008, Hefting et al. 2005). The latter is a temporal process, followed by the release of ammonium after bacterial die-off (Rivett et al. 2008). Despite its relatively small scale, research points to the importance of this process for buffer zones' plants communities. Plants that have mutualistic relationship with microorganisms living in rhizosphere can use excess nitrogen that is temporarily stored in microbial biomass. Therefore by rapid accommodation to increase in N input, microorganisms can trap inorganic nitrogen and prevent it from leaching from the ecosystem (Kuzyakov and Xu 2013).

Riparian buffer zones are also highly efficient in phosphorus sequestration. Storage of phosphorus in wetland and riparian buffer zones depends on processes such as plant and microbial uptake. In case of wetland buffer zones which consist of mires (ecosystems actively accumulating peat), part of organic phosphorus can be also incorporated into peat. Similarly to nitrate sequestration, effectivity of different processes temporarily removing P from the cycle varies greatly, depending on further characteristics of given ecosystem and other factors. Data quoted by Mander (2005, 2017b) suggest buffer strips more diverse in terms of vegetation types are generally more efficient in purification processes they support.

1.5. Role of bank vegetation

Recognition of the role of bank vegetation dates back to the mid-twentieth century. However, only recently scientists begun to apply complementary field, flume and theoretical/modelling investigations which contributed greatly to understanding the influence of plants on fluvial systems. Modelling investigations allowed scientists to examine in detail the role of bank vegetation taking into consideration a range of vegetation-related factors including canopy flow resistance, root-reinforcement of sediment, and growth of the plants that rely on highly wet habitats (including anaerobic conditions). The extant research shows that bank vegetation can influence stream dynamics, pattern and size both on micro and macro scale (Gurnell et al. 2012).

Beside being river system engineers influencing the geomorphology of the fluvial systems, bank vegetation influences greatly the water purification processes, impacting the amount and ratio of N cycling in aquatic ecosystems. Contrary to denitrification, this process is only temporary: plants immobilise N which is released after the mineralisation of the plant. Immobilisation period differs based on the rate of litter decomposition. There are many factors that may possibly influence the rate of litter decomposition, such as microclimate, soil nitrate availability (Hefting et al. 2005), sunlight penetration (which could potentially explain the difference in rates of leaf litter decomposition in forested and herbaceous areas) and litter quality (such as presence of resistant substrates), or oxygen accessibility. The only ecosystems, in which a part of N immobilised by plant uptake leaves the N cycle for a longer period of time are mires (wetlands with active peat-forming process) in which the slowed decomposition of organic matter keeps nutrients sequestrated in soil organic matter. Still, plants significantly increase the residence time of nutrients, and plant uptake is a significant mean of N mitigation. A study examining forested and herbaceous riparian buffers in six European countries (France, Switzerland, the Netherlands, Romania, Spain and Poland) demonstrated that the annual N retention in vegetation and litter accounts for from 13 up to 99% of the total N mitigation(Hefting et al. 2005). As Mander (1997) argues, the importance of plant uptake of N can increase with biomass harvesting.

Having that in mind, different types of buffers vary in terms of their N retention time. Results of research about the influence of vegetation type on efficiency of N retention shows inconsistent data. Besides the influence on water purification processes through direct N uptake and N incorporation in litter, bank vegetation can also stimulate denitrification activity through the supply of organic matter, impacting the rates on N removal indirectly (Hefting et al. 2005).

1.6. Buffer zones in Poland

Significant majority of Poland (75%) is lowland, with an average elevation of 113 m above sea level (FAO, 2001). Nearly 50% of area of the country consists of agriculture land, which is an important part of Polish economy - around 12.6% of Polish population is employed in agriculture (FAO Statistical Yearbook, 2014). When compared to the rest of European Union, relatively big percentage of farms is based on a small areas (with average of 9 ha). At the same time, the number of big market-oriented agricultural holdings is quickly raising, changing the landscape of polish agricultural market. We can see the reflection of those changes in the amount of used fertilisers, which, according to FAO estimations, is rising. The biggest increase of used fertilisers was visible especially for nitrogen-based fertilisers: starting from 895 500 tonnes of N in the year 2005 the amount of used nitrogen increased by almost 30 % (Olszanska et al. 2019).

With the potential increase of N input to the rivers trough the NPS pollution, the issue of rivers' quality seem to be especially pressing. Unfortunately for the water quality river management in Poland seem not to fulfil parts of WFD (SWD Working Document 53 2019). Buffer zones frequently fall victim to intensive and intrusive river regulation practices, which drastically change the ecosystem morphology, and trigger changes in the functioning of the ecosystem and species composition (Abril et al. 2015). Projects heavily disrupting hydromorphology of rivers are still undertaken by the National Water Management Authority (*Państwowe Gospodarstwo Wodne Wody Polskie*). Under the general term of 'maintenance works', a lot of small rivers is being a subject to process called *desluging* (in polish: *odmulanie*). The process consists of bottom sediment removal (sediment layer of 10 to 50 cm width). It is also frequently connected to re-shaping of river bed into a trapezoidal shape. According to estimations prepared by WWF Poland, even 16 000 kilometres (around 25%) of natural have been subjected to the described 'maintenance works' during years 2009 - 2013. Described practices have significantly harmful impact on the environment of the aquatic ecosystems, also potentially accelerating nitrogen load to the waterbodies (Jabłońska et al. 2013). According to estimations provided by the WWF Poland, the total number of rivers subjected to this practice during years 2010-2015 vary from 11 700 to even 20 000 kilometres (out of estimated 140 000 kilometres) (Nawrocki et al. 2014).

At the same time, also big rivers are potentially subjected to highly impactful management practices. European Agreement on Main Inland Waterways of International Importance (AGN convention), signed by Polish president in 2017, along with the "Strategy for the inland waterways development for years 2016-2020 with the perspective on 2030" present the plans of further management of main Polish rivers, with the main goal of achieving the international class of navigability for three main waterways (E-70, E-30 and E-40). Proposed project would also disrupt the connectivity between the river and riparian buffer zones and floodplains, further inhibiting water purification processes (Ministry of Marine Economy and Inland Navigation, 2017.).

Past and current management practices are reflected by the amount of nutrients carried with rivers to the sea. Vast majority of Poland lays within the Baltic Sea Basin, with only a very small area of the country being within the Black Sea Basin. The country is also the most significant polluter of the Baltic Sea, when it comes to the nutrients' loading. According to the HELCOM (Baltic Marine Environment Protection Commission - Helsinki Commission), the total input of nitrogen to the Baltic Sea in 2010 was 977,000 tonnes and the total input of phosphorus was equal to 38,300 tonnes. Poland was a leading contributor for both of those indicators, adding 30% and 37% of nutrients' total load (for N and P, respectively) in 2010 (Svendsen et al. 2013).

1.7 Research framework: CLEARANCE

The present study was a part of a much wider research project titled CLEARANCE (CircuLar Economy Approach to River pollution by Agricultural Nutrients with use of Carbon-storing Ecosystems) run collectively by scientists from Warsaw University (UW), Warsaw University of Life Sciences (SGGW), University of Greifswald (UG) and University of Århus (UA) . With applicability in mind, scientists working in CLEARANCE aim to implement the concept of paludiculture, understood as agriculture that uses a productive wetland in cultivating adapted crops (such as Common Reed or Cattail). Paludiculture would allow to maintain the land productive (and therefore useful from the owners' perspective) while mitigating environmental impact of the drained wetlands (such as greenhouse gasses emission and loss of nutrient removal ability) by rewetting them. Such reconstructed wetlands, serving as productive wetland buffer zones, could provide ecosystem services including water purification, and limit greenhouse gasses emission while still being profitable for their owners. Clearance focus on various aspects of wetland buffer zones created from restored wetlands, such as the relation between nutrient removal and biomass utilisation.

In order to assess feasibility of combining wetland restoration, buffer zone application and paludiculture, Clearance aims to address the existing gaps in knowledge regarding ecological, biochemical, social and economical aspects of wetland buffer zones existence. One of these gaps regard the role of wetland buffer zones in nutrient retention and removal. Since Clearance combines research teams from different countries, it became important to focus on case studies from each of them, looking into nutrient removal potential in various conditions.

1.8 Aim of the research

This study aimed at answering the questions regarding the potential of chosen study sites to retain inorganic nitrogen (nitrate and ammonium), and the role of both environmental variables and management practices in this process.

2. Methods and site description

2.1 Study site

Study sites are wetland buffer zones bordering agricultural field and a natural river stream. Two riparian buffers along unregulated stream were selected in the central Poland (around 52°6' N, 21°0' W). According to Köppen-Geiger classification, this area is characterised by continental warm-summer humid continental climate (Rubel and Kottek 2010). Mean annual temperature equals to 7.5 °C, with mean of warmest month (July) 18.6 °C and mean of coldest month (January) -4.3 °C. Mean annual precipitation is around 506 mm, with mean of driest month (March) 23 mm and mean of wettest month (July) 71 mm (Merkel 2018).

Both of the selected wetland buffer zones were in part unmown (or grazed by cattle) and in part left without human-induced disturbances. They both bordered Pokrzywnica, a Narew tributary of around 16 kilometres. The selected buffer zones were less than one kilometre apart on a straight line, yet located in different villages: Obrębek and Mory. The buffer zone in Obrębek was located upstream. It has bordered two arable fields, one covered with triticale, and the other with oats. According to local farmers, fields were fertilised with fertilisers based on nitrogen (Saletra, with chemical formula $2NH_4NO_3 + CaMg(CO_3)_2$) and potassium (Polifoska, fertiliser containing 24% of potassium (K_2O)). Unmown part was crossed by a network of small streams, with soil highly saturated with water. Later in this thesis, the described study site is referred to as research site 1.

The buffer zone in Mory was located downstream and consisted of riparian buffer zones, rewetted around 10 years ago probably due to a beaver dam construction (timespan given by local farmers). It bordered with rarely fertilised meadows and fertilised fields. Fields on top of the hill were fertilised either with manure or with Saletra and Polifoska. However, fertilised fields do not neighbour with research site directly - there is a strip of unfertilised fields between them and chosen transects. Above one side of the research area a small forest was situated. Later in this thesis, the described study site is referred to as research site 2. Both study sites are visualised in Fig. 1.

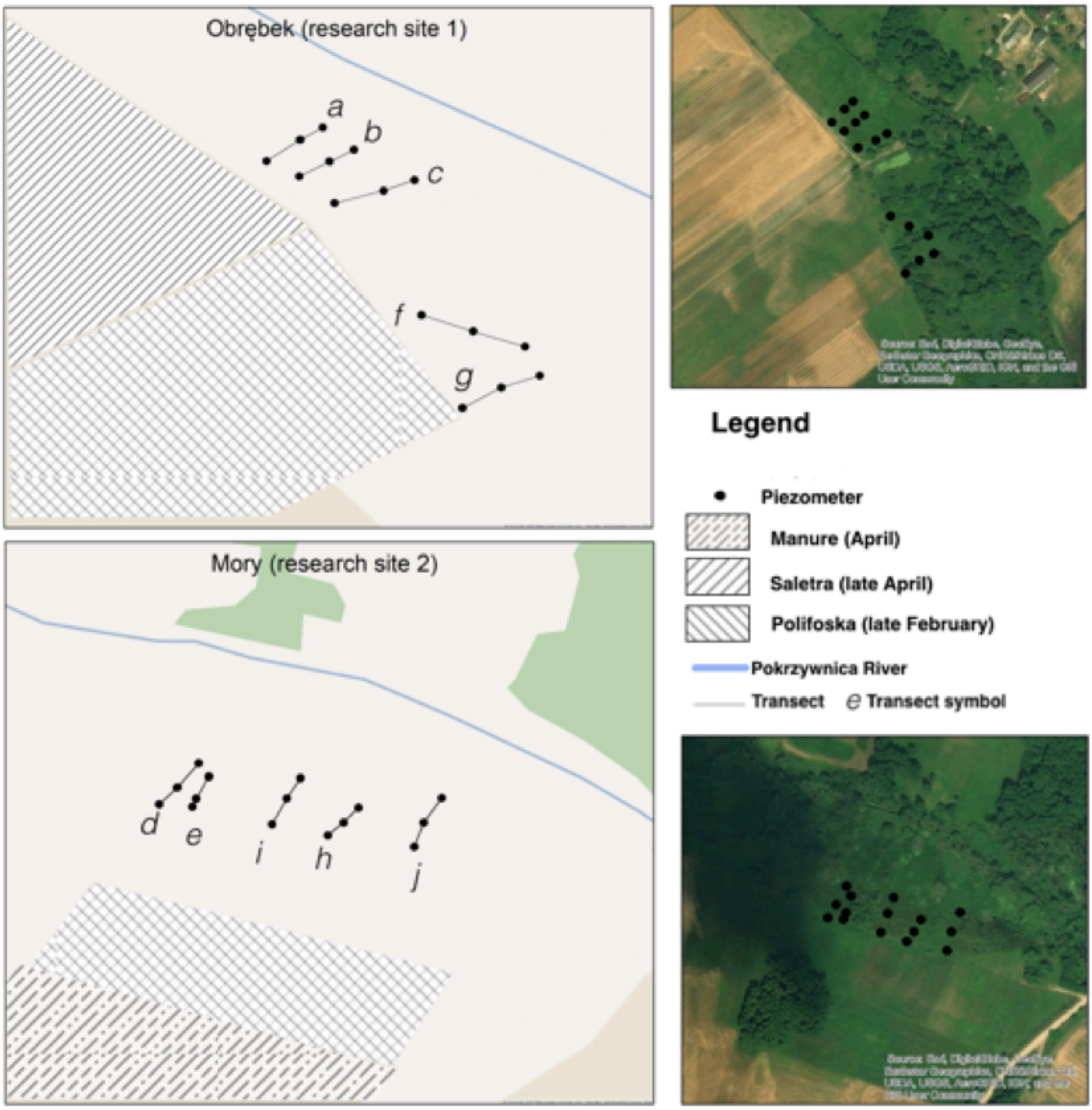


Figure 1. Fertilisation of the fields surrounding our study site. In the key to the map the type of fertiliser is given, with the estimated date of fertilisation given in the brackets.

2.2 Groundwater monitoring

A grid of piezometers (Fig. 1) was established on elevation gradient from agricultural fields to the stream for measurement of water table elevation and to facilitate measurements of water chemistry. Piezometers were grouped in 5 transects (between 23 and 44 meters long) in both study sites. Both study sites consisted of transects established on moved and transects established on unmoved areas.

In the research site 1 (Obrebek) there were 3 moved transects and 2 unmoved, while in research site 2 (Mory) 3 transects were unmoved while 2 were moved. Each transect consisted of 3 measuring points. In every point two piezometers were installed, one short (equal to 75 cm, or shorter, depending on the depth of clay layer) and one long (between 75 and 125 cm, depending on the depth of the impermeable clay layer). Piezometers were perforated along the bottom and covered with material filtering potential impurities, such as sand or organic matter. Piezometers were spatially divided into three groups: (1) those located on the border of wetland buffer zone and bordering with agricultural field, referred to as located at "field border", (2) those located on closest to the river stream, referred to as located at "stream border", and (3) piezometers located in-between ("intermediate"). Groundwater level was checked seasonally thought the year: in August 2017, November 2017, April 2018 and June 2018. Based on the water table elevation, we were able to establish groundwater level.

2.3 Groundwater physico-chemical parameters

Dissolved forms of N in groundwater, as well as related chemical parameters were studied for a year. Beside dissolved forms of nitrogen (nitrate and ammonia), phosphorus (phosphates) and total inorganic carbon were measured, along with other selected ions.

I obtained groundwater for analysis seasonally, in August, November, April, and June. I pumped out water standing in piezometers. After tubes were refilled, I sampled 100 ml of water using mechanical pump. All samples were stored in a portable cooler box during sampling and transportation. After arrival at the lab, I measured pH and electronic conductivity (EC) using HACH HQ40D multimeter.

Then, the samples were filtered (within 36 hours of sampling) and separated into three sets: 20 ml was analysed for total organic carbon (TIC), 10 ml of filtered water was preserved with 0,1 ml of HNO_3 and then analysed for ion concentration using ICP, and 20 ml was frozen and analysed for NO_3^-, NH_4^+ and PO_4^{3-}. The analyses (beside TIC measurement) were performed in laboratory of Department of Aquatic Ecology & Environmental Biology at Radboud University Nijmegen. Total organic carbon was measured in Laboratory of Biogeochemistry and Environmental Protection (Laboratorium Biogeochemii i Ochrony Środowiska) at CNBCH (Centrum Nauk Biologiczno-Chemicznych) at Warsaw University.

2.4 Nitrogen removal

Nitrate removal rates were calculated using relation between nitrate loading and output in relation to the length of the flow path of the analysed strip of buffer zone. To calculate the removal rate, measurements from the first and last piezometers were used. The following equation was used (adapted from Sabater et al. 2003):

$$nNO_3 = (N_{input} - N_{output})\ N_{input}^{-1}\ 100$$

where: N_{input} is N concentration from piezometers located at "field border" of a transect, N_{output} is N concentration from piezometers located at "stream border" of the transect. Value of nNO_3 shows the efficiency of a nitrate removal process by presenting a percent nitrate removed from the groundwater. If it is greater than zero, the removal process is effective. If nNO_3 is below zero, removal process was also negative and greater amount of nitrate was released than removed. In order not to disregard ammonium production we also obtained estimates regarding net inorganic nitrogen (Maitre et al. 2003). Following Sabater et al. (2003), similar approach was used for calculating the ability of chosen riparian strips to retain inorganic nitrogen (NO_{3-} -N and NH_{4+-} -N).

2.5 Vegetation analysis

Description of vegetation cover was performed for all measurement point during the establishment of transects (August 2017). Relevés were conducted near piezometers, on area of 2 m border square. I made 30 relevés and analysed them using TurboVeg and Canoco5 software. To analyse the relationship between plant species composition and the explanatory environmental variables, detrended correspondence analysis (DCA) and constrained correspondence analysis (CCA) analysis have been performed.

2.6 Statistical analysis

All of the analysis were performed using Excel for Windows and R Studio (ver 1.1.383). Significant part of concentration measures were either very small or equal to zero (for nitrate total of 98 observations showed nitrate concentrations close to zero, out of which 28 were taken in the strip bordering the field). Measurements from two points, both known to be contaminated, were excluded from the analysis. Collected data was checked for normality both graphically and with Shapiro-Wilk test. Unpaired t-test was used to compare N removal efficiency for study sites 1 and 2. Pearson correlation coefficient was used to detect possible relationships between N removal effectiveness to data related to N input, TIC concentration, and groundwater properties. For data that did not meet the assumptions of parametric statistical testing, the analysis was conducted based on non-parametric tests. Non-parametric tests (non-parametric ANOVA and signed rank tests) were used for checking the seasonal and spatial variation in removal rates and nutrients' concentration. For comparison of effectivity in N removal between the two sites and their mown and unmown parts in each, two-factor ANOVA was conducted. Test statistic for Kruskal-Wallis test (H), Wilcoxon test (W), Bonferroni-Dunn test (z) and the significance (p) are reported each time the test was used for calculations.

2.7 Gathering additional data

Context needed for understanding of research results has been gathered through 1) interviews with fields' owners (stakeholders) and 2) literature review. In order to interview fields' owners we needed to first identify them. Information about fields' owners of the fields we were interested in was gathered through village leaders. After having identifying fields' owners, I conducted a series of interviews. It allowed me to collected data on frequency and type of fertilisation on fields we have conducted our research on, as well as on neighbouring fields. When conversation with the owner was impossible (they have moved or lived too far to be reached), the knowledge on amount and frequency of fertilisation was gained through estimation made by neighbours or village leaders. In order to properly identify the fields, maps with parcels' numbers have been printed. Informants were asked to use printed maps when describing localisation of the field and the amount of fertilisers they use.

I also prepared a literature review with data regarding nutrient input and removal. If necessary, data was recalculated so it could be compared with our results. Additional information (on type of vegetation, hydrological data etc.) was also gathered. Obtained information allowed me to compare results from installed experimental setup with other measurement and therefore discuss it more thoroughly.

3. Results

3.1 Nitrogen inflow and fertilisation

A Kruskal-Wallis test on the collected data indicated significant seasonal variation in the median nitrate concentration measured at the field border (nitrate input) ($H = 8.68$, $p < .05$), with the highest concentration measured in June (mean 454 µg/l and median 30.5 µg/l), and the lowest, due to a large number of measurements equal to zero, in August (mean 1083 µg/l and median 0 µg/l) (Fig 2a). A significant difference between seasonal concentration of ammonium was also detected ($H = 45.19$, $p < .0001$), with the highest concentration measured in June (mean 775 µg/l and median 286 µg/l), and the lowest in November (mean 181 µg/l and median 66.7 µg/l) (Fig. 2b).

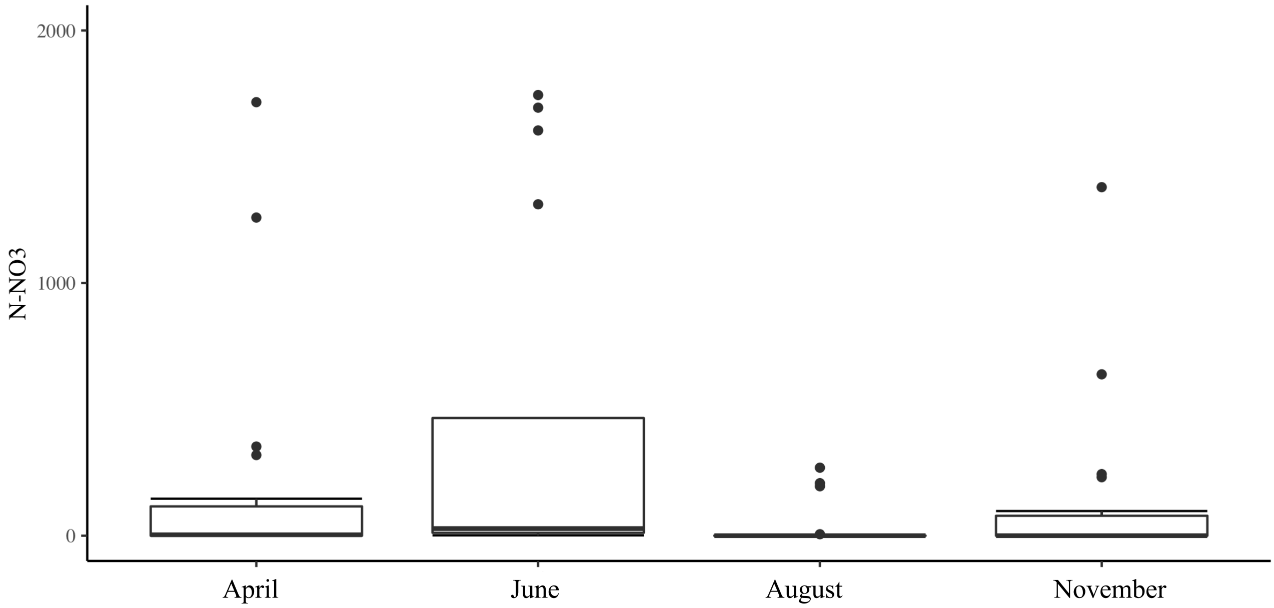


Figure 2a. Nitrate ($N\text{-}NO_3$ [µg/l]) concentration measured at the field border by seasons. For legibility of the graph, following outliers were removed: 13115.4 in transect b, field border, (August), 6790.4 in transect g, field border, (August), 7791.3 in transect b, field border (November), 2818.1 in transect g, field border (November), 2130.4 in transect g, field border (April) and 10484.3 in transect g, field border (April).

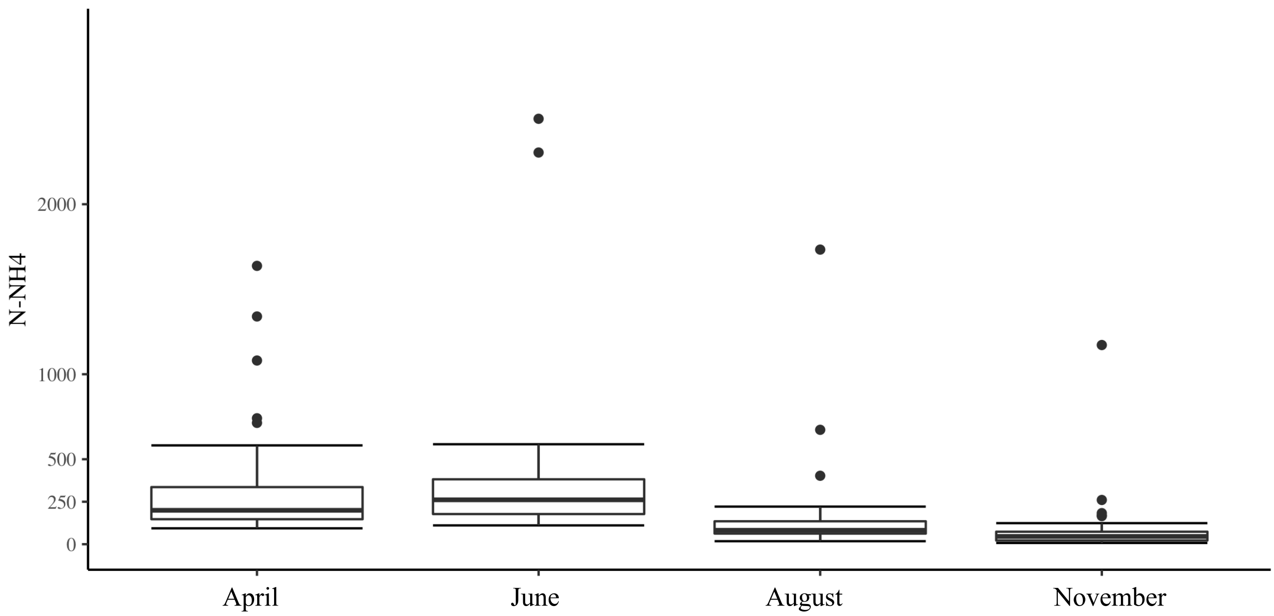


Figure 2b. Ammonium (N-NH_4 [µg/l]) concentration measured at the field border by seasons. For legibility of the graph, following outliers were removed: 5762.859 in transect f, field border (April), 6040.9 in transect d, field border (June), 3532.4 in transect f, field border (June), and 2304.1 in transect h, field border (June).

A Wilcoxon test on collected data did not indicate clear patterns connected to the influence of agricultural practices (mowing) on nitrate input, whereas concentration of ammonium differed significantly regarding the treatment of research sites ($W = 87$, $p < .05$). At the same time, there were significant differences in nitrate concentration between geographical locations of research sites ($W = 436$, $p < .01$) (being either 'upstream' or 'downstream' towards one another, namely: whether they were located in Mory [research site 2] or Obrębek [research site 1]) (Fig. 3), with the mean concentration measured in Obrębek being higher, although medians for both locations were below 100 µg/l. There was no such pattern observed for ammonium. Nitrate concentrations at the field border were also significantly different between the measured transects ($H = 35.5$, $p < 0.0001$) (Fig. 4).

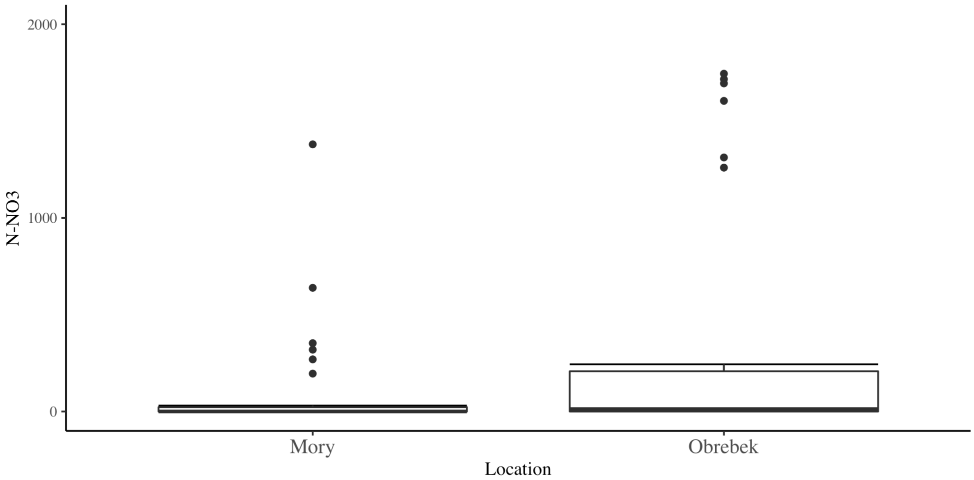


Figure 3. N-NO_3 [µg/l] input concentration measured in Mory versus N-NO_3 [µg/l] concentration measured in Obrębek. For legibility of the graph, following outliers were removed: 13115.4 in transect b, field border, (August), 6790.4 in transect g, field border, (August), 7791.3 in transect b, field border (November), 2818.1 in transect g, field border (November), 2130.4 in transect g, field border (April) and 10484.3 in transect g, field border (April).

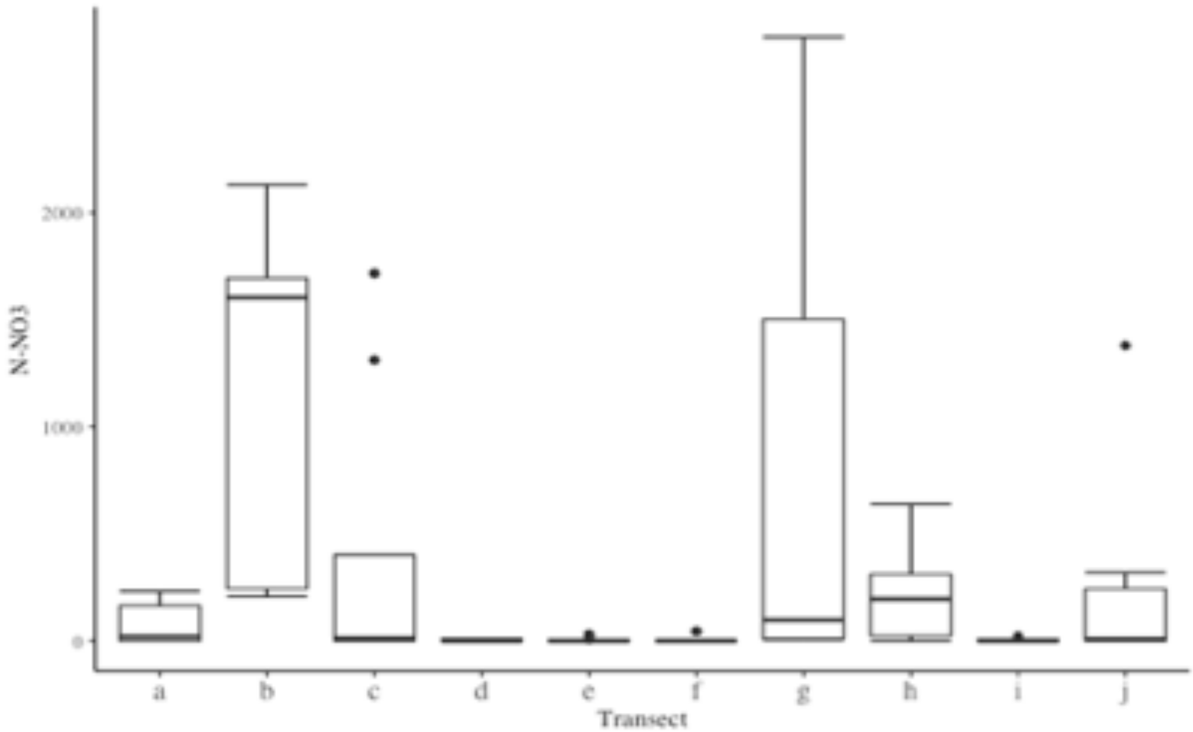


Figure 4. N-NO_3 [µg/l] concentrations at the field border for each transect. For legibility of the graph, following outliers were removed: 5762.859 in transect f, field border (April), 6040.9 in transect d, field border (June), 3532.4 in transect f, field border (June), and 2304.1 in transect h, field border (June).

Informal conversations with local farmers confirmed that the amount of used fertilisers was relatively small, and consisted mostly of industrially available fertilisers and manure, as presented on Fig.1. Similar practices were conducted in the recent years.

3.2 Nitrogen concentrations along groundwater flow

There was a significant difference between the concentration of nitrates in each measurement strip (field-border, intermediate and river-border) (H = 7.95, p < .05). A post-hoc Mann-Whitney test indicated that concentration of nitrates at the field border was significantly higher than in the intermediate strip (W = 3391.5, p < .05), with median concentration for both strips below 10 mg/m^3 Table 1). However, there were no significant differences between concentration measured in the intermediate and river border strips (W = 2570.5, p = .58). The overall difference between input and output concentration is on the verge of statistical significance (W = 3118, p = .066), as the main difference occurred between field border and intermediate strips (Table 1, Fig. 5a, Fig. 6).

Table 1. Median concentrations of nitrogen, EC and pH of the groundwater in strips parallel to the stream. n = number of observations.

	Field border	**Intermediate**	**River border**	**Deep groundwater**	**Shallow groundwater**
N-NO_3 [µg/l]	7.81	1.24	2.72	8.46	1.08
N-NH_4 [µg/l]	108.51	152.48	165.41	140.11	151.1
EC [µSm/cm]	592	662	805	647.5	700
pH	6.6	6.9	6.9	6.7	6.8
n	78	80	76	114	120

At the same time, median ammonium concentration increased significantly along the flow path (H = 6.0, p < .05) (Table 1, Fig. 5b, Fig 6). A post-hoc analysis (Mann-Whitney test) indicated that concentration of ammonium in the field border was lower than in the intermediate strip (W = 2103, p < .05), and in the river border (W = 1854, p < .01). The difference between intermediate and river border strips was not statistically significant (W = 2405, p = .551).

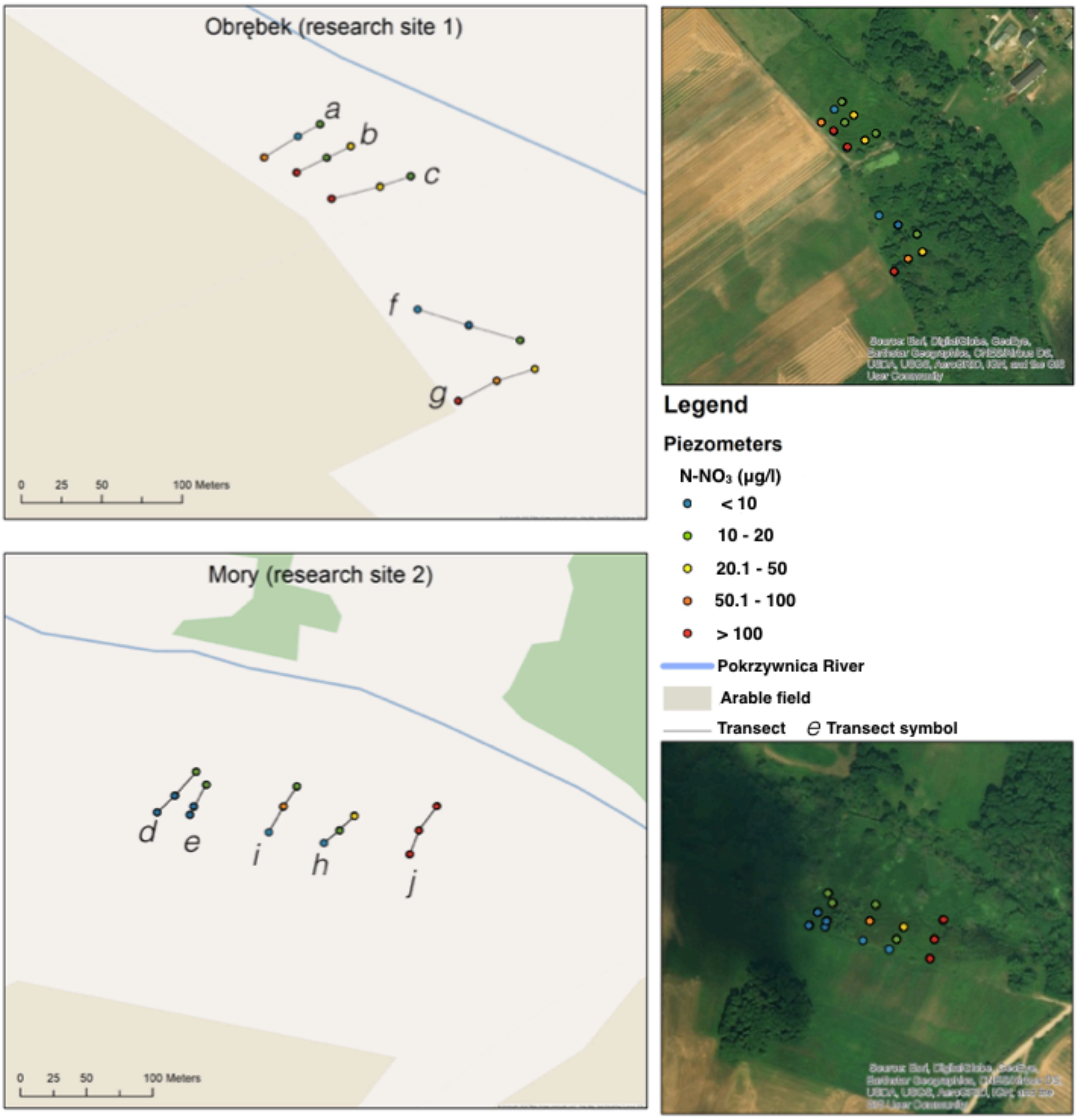


Figure 5a. Mean N-NO_3 concentration, computed for each piezometer from both depths (shallow and deep) and all measured seasons.

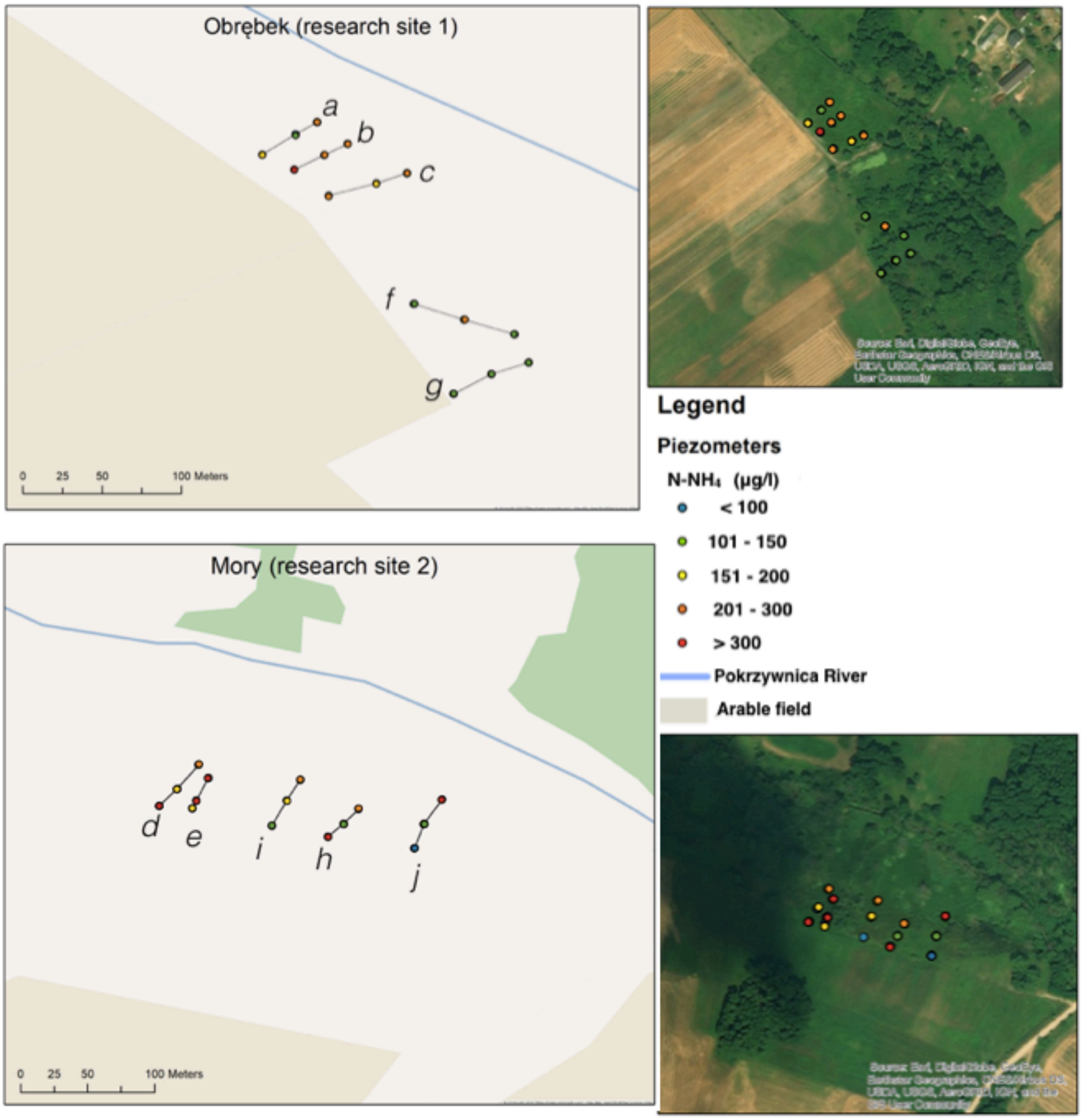
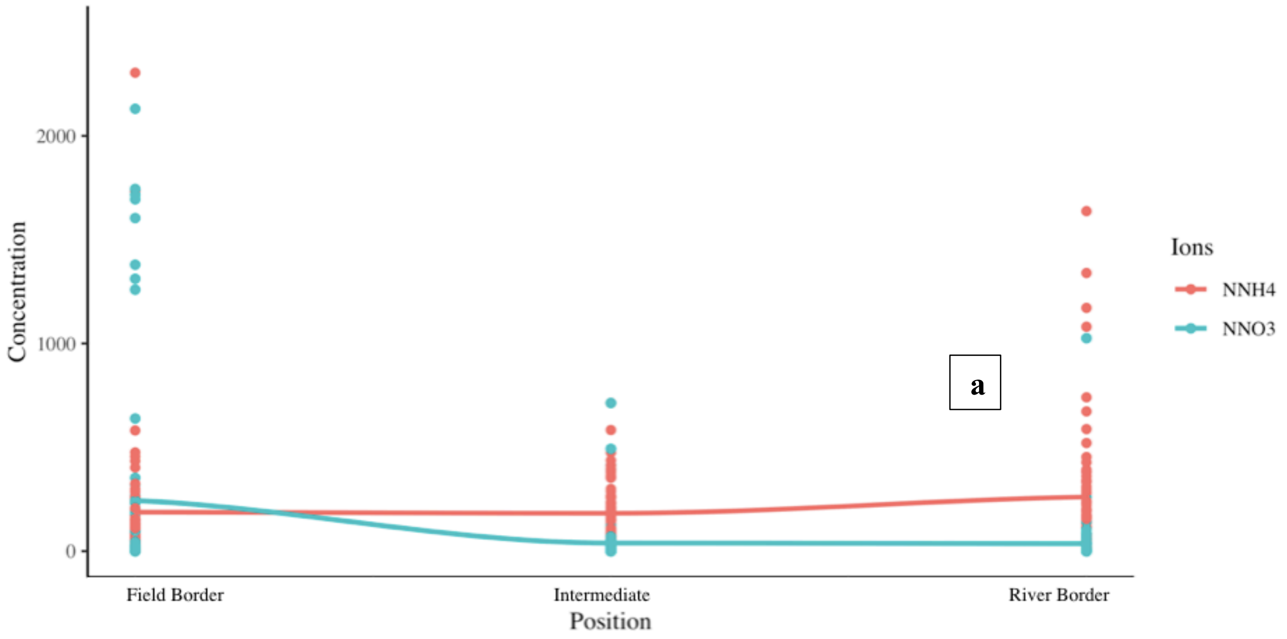


Figure 5b. Mean N-NH4 concentration, computed for each piezometer from both depths (shallow and deep) and all measured seasons.



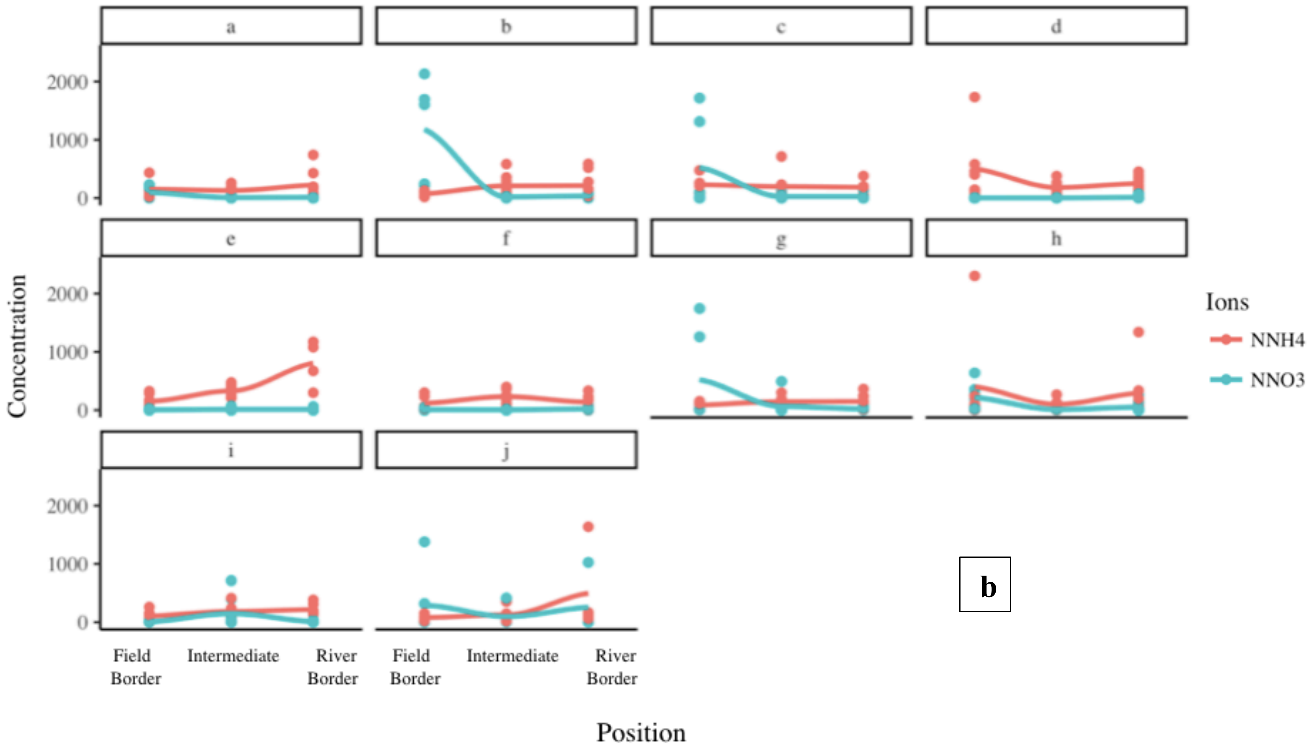


Figure 6. Changes in N-NO_3 and N-NH_4 concentration [µg/l] along the flow paths (**a** – together; **b** – for each transect). For legibility of the graph 5 outliers has been removed: N-NO_3 = 13115.44, 6790.41, 7791.29, 10484.26 and N-NH_4 = 6040.86, all measured in field border.

3.3 Groundwater chemistry

The concentration of majority of measured elements has significantly increased along the groundwater flow. At the same time sulphur decreased significantly. For Cl, K, and PO_4^{3-} the differences were not statistically significant. However, the differences in PO_4^{3-} concentration along the groundwater flow were significant when measured for Obrebek (research site 1). The highest median concentration of S was measured in transects "*b*", "*c*", and "*g*" (respectively 721.27, 679.92 and 530.38 µg/l). At the same time, concentration of S in deep groundwater (median value 644.22) was significantly higher than in shallow groundwater (median value 530.56) ($W = 6641.5$, $p < .05$).

Table 2. Median concentration of chosen elements in measured groundwater, given separately for both research sites (upstream and downstream). Asterisk (*) is used to identify elements for which the difference between input and output of selected element was statistically significant.

	Mory (downstream)			**Obrębek (upstream)**		
	Field border	Intermediate	River border	Field border	Intermediate	River border
Mn* [µg/l]	40.35	63.08	92.78	15.62	20.47	30.96
Zn* [µg/l]	101.54	148.49	175.22	110.17	188.53	240.13
Fe* [µg/l]	0.89	0.28	7.79	0.36	0.4	0.8
Ca* [µg/l]	1261.1	2670.9	3708.83	2780.7	2670.28	3370.76
K [µg/l]	7.01	9.54	9.28	36.25	36.53	20.2
Cl [µg/l]	117.02	253.4	188.33	486.13	355.45	355.83
Si* [µg/l]	165.49	189.52	267.3	209.43	309.74	443.57
Mg* [µg/l]	247.73	447.02	529.63	362.1	480.35	455.14
S* [µg/l]	539.76	388.9	166.88	811.82	441.14	388.45
TIC* [mg/l]	41.58	70.57	111.0	61.16	61.95	87.72
PO_4^{3-} [µg/l]	0.03	0.02	0	0.06	0.02	0
EC [µSm/cm]	352.5	653.25	869	621.5	633.75	750.25

Concentration of TIC [mg/l] shows statistically significant differences between different strips ($H = 63.529$, $p < .000001$), with the median concentration increasing along the groundwater flow. TIC concentration was weakly negatively correlated to N-NO_3 concentration ($p < 0.05$, r = -0.15). Pearson's correlation coefficient for N-NO_3 concentration and pH values was of negligible value ($r = 0.08$).

3.4 Removal rate

Nitrogen removal rate is expressed as the difference between the input and output total nitrogen loading in relation to the lengths of the flow paths. Nitrate removal rates calculated for the whole transect (from field border to river border) were mostly positive, ranging from 27,6% to 100% of removed nitrate. However, there were also several high negative values (up to -913,49%). Similarly, inorganic nitrogen removal rates were also mostly positive, ranging from 23% to 100% of removed nitrogen. However, there were also several high negative values (up to -1332,60%) (table 3).

Table 3. Concentration of nitrate and inorganic nitrogen (NO_3^- -N and NH_4^+ -N) in measured groundwater measured in input and output, as well as removal rate, given separately for each measurement.

transect	season	depth	N-NO_3 input [µg/l]	N-NO_3 output [µg/l]	N-NH_4 input [µg/l]	N-NH_4 output [µg/l]	Nitrate Removal Rate (%)	Inorganic N Removal Rate (%)
a	4	d	146,44	0,27	198,57	165,01	99,82	52,09
		k	20,80	44,94	127,97	740,93	-116,11	-428,27
	6	d	183,47	6,85	434,39	196,61	96,27	67,07
		k	NA	10,58	NA	428,02	NA	NA
	8	d	0,00	0,00	66,70	83,83	NA	-25,68
		k	0,00	0,00	172,85	74,47	NA	56,92
	11	d	231,58	39,12	24,86	74,26	83,11	55,79
		k	0,00	1,67	44,45	18,02	NA	55,69
b	4	d	10484,26	2,73	93,56	520,90	99,97	95,05
		k	2130,43	15,50	99,14	276,89	99,27	86,89
	6	d	1694,49	104,36	110,70	588,12	93,84	61,64
		k	1604,23	86,14	131,46	156,07	94,63	86,05
	8	d	13115,44	8,74	45,93	36,67	99,93	99,65
		k	207,93	0,00	67,77	30,71	100,00	88,86
	11	d	7791,29	22,15	22,38	61,04	99,72	98,94
		k	243,81	39,85	20,59	36,09	83,65	71,28
c	4	d	1716,26	0,00	259,68	380,61	100,00	80,74
		k	25,28	11,32	252,11	135,79	55,21	46,97
	6	d	1311,95	143,89	207,30	202,76	89,03	77,18
		k	97,92	0,00	475,70	189,11	100,00	67,03
	8	d	0,00	0,00	100,79	154,15	NA	-52,94
		k	0,00	0,00	77,29	55,62	NA	28,04
	11	d	0,00	0,00	NA	NA	NA	NA
		k	0,00	0,00	NA	NA	NA	NA
d	4	d	0,00	0,00	581,45	342,74	NA	41,05

		k	8,07	11,70	145,07	392,65	-44,97	-164,04
	6	d	8,17	13,35	6040,86	286,37	-63,46	95,05
		k	7,27	73,71	456,33	453,37	-913,49	-13,69
	8	d	0,00	0,00	402,77	116,92	NA	70,97
		k	0,00	0,00	1733,10	221,33	NA	87,23
	11	d	0,00	5,18	66,34	26,13	NA	52,80
		k	0,00	0,00	124,03	169,84	NA	-36,93
e	4	k	0,00	0,00	154,35	1080,64	NA	-600,14
	6	k	7,55	59,81	285,67	298,59	-691,84	-22,23
	8	k	0,00	0,00	169,79	672,99	NA	-296,36
	11	k	0,00	0,52	81,83	1171,73	NA	-1332,60
f	4	d	NA	16,44	NA	333,73	NA	NA
		k	0,00	0,00	235,12	175,85	NA	25,21
	6	d	NA	20,02	NA	173,70	NA	NA
		k	44,84	32,48	300,05	231,18	27,56	23,55
	8	d	0,00	0,00	62,98	65,29	NA	-3,67
		k	0,06	0,00	17,80	84,57	100,00	-373,63
	11	d	0,00	1,78	28,24	10,47	NA	56,63
		k	0,00	85,79	62,50	51,82	NA	-120,18
g	4	d	1259,47	19,45	147,44	360,92	98,46	72,96
		k	4,93	2,34	113,70	236,36	52,56	-101,21
	6	d	1744,51	57,99	114,88	241,78	96,68	83,88
		k	12,86	37,61	152,32	155,83	-192,37	-17,11
	8	d	6790,41	4,02	46,93	114,68	99,94	98,26
		k	5,90	0,00	89,94	50,40	100,00	47,41
	11	d	2818,13	47,29	9,48	18,53	98,32	97,67
		k	97,74	6,82	23,86	18,10	93,02	79,51
h	4	d	353,03	0,00	106,31	1339,75	100,00	-191,66
		k	0,00	0,00	240,74	143,13	NA	40,54
	6	d	25,99	145,01	2304,13	335,13	-457,84	79,39
		k	NA	31,14	NA	198,54	NA	NA
	8	d	195,75	0,00	59,58	73,57	100,00	71,19
		k	269,26	0,00	59,99	62,48	100,00	81,02
	11	d	638,87	259,26	7,61	113,60	59,42	42,33
		k	22,53	0,00	18,94	73,08	100,00	-76,23
i	4	d	2,41	0,00	134,85	158,89	100,00	-15,76
		k	0,00	3,43	260,10	310,69	NA	-20,77
	6	d	23,11	8,84	126,33	200,31	61,73	-39,96
		k	15,01	34,95	155,74	382,81	-132,87	-144,67
	8	d	0,00	0,00	65,79	162,67	NA	-147,25
		k	0,00	0,00	61,41	134,96	NA	-119,79
	11	d	0,25	0,00	12,38	0,00	100,00	100,00
		k	0,00	3,52	21,21	165,41	NA	-696,56
j	4	d	319,27	1025,63	110,71	1637,43	-221,24	-519,33
		k	0,00	2,70	152,09	166,37	NA	-11,17
	6	d	NA	NA	NA	NA	NA	NA
		k	NA	NA	NA	NA	NA	NA
	8	d	0,00	NA	78,28	NA	NA	NA
		k	0,38	0,00	82,09	117,58	100,00	-42,57
	11	k	15,53	0,00	45,90	61,49	100,00	-0,10

There were clear spatial differences observed in the inorganic nitrogen removal rates. There was a statistically significant difference between removal rates in the upstream and downstream sites ($W = 306$, $p < .001$), with median removal rates in Obrębek (upstream) being 56.9, and median in

Mory (downstream) being 0. Statistically significant difference has also been detected between removal rates in shallow and deep groundwater (Fig. 7).

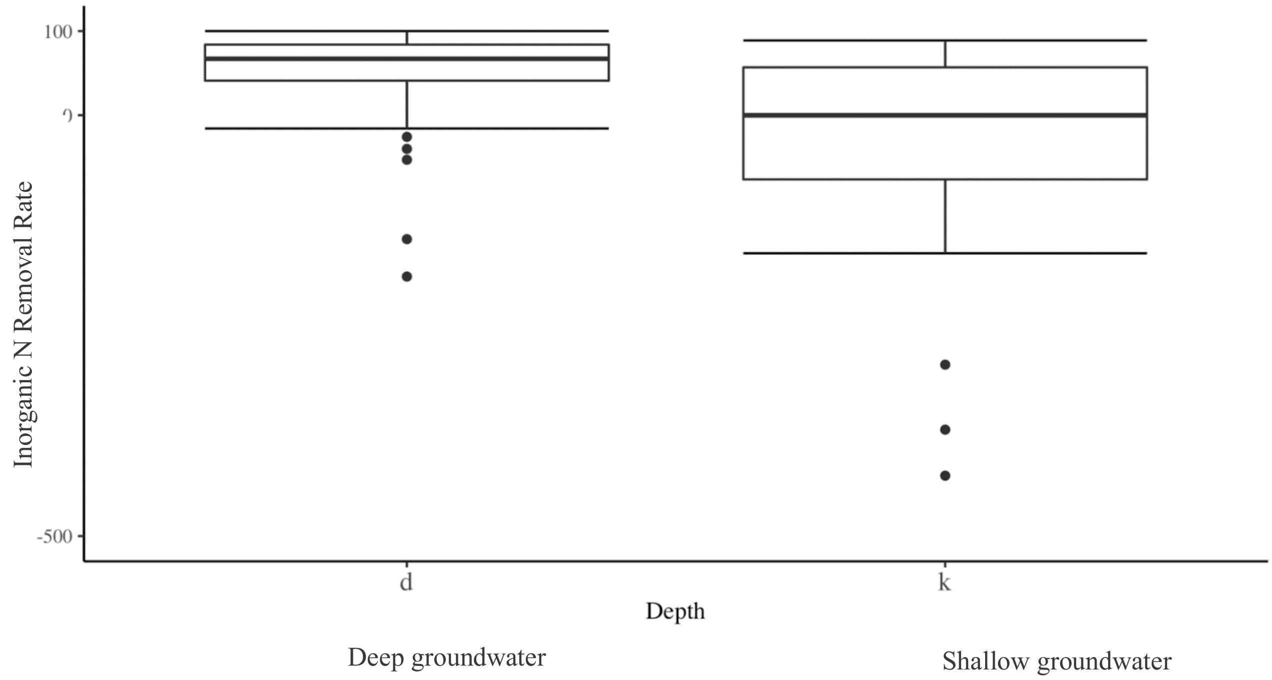


Figure 7. Nitrogen removal ra tage) at different depths of grou ing outliers were deleted for legibility of the graph: -600,14 and -1332,60 (both measured in transect "e"), -696,56 (transect "i") and -519,33 (transect "j") (Mann-Whitney test, $p < .005$, W = 765).

Nitrogen removal rates differed significantly also between each transect (H = 29.009, $p < 0.001$) (Fig. 8). Multiple comparisons following an analysis of variance were performed using the Bonferroni–Dunn test, which showed that removal rates calculated from measurement from transect "b" were significantly bigger from transects "b" ($z = 4.13$, $p < .05$), "f" ($z = 3.13$, $p < 0.05$), "i" ($z = 3.74$, $p < .001$), and "j" ($z = 3.22$, $p < 0.05$) with no significant differences for other transects. When filtered for positive results only, removal rates did not differ significantly between transects.

There was also observed a weak negative correlation between total inorganic carbon concentration (TIC) and nitrogen removal rate ($r(66) = -0.32$, $p < 001$). This correlation was much stronger for shallow water when compared to deeper water.

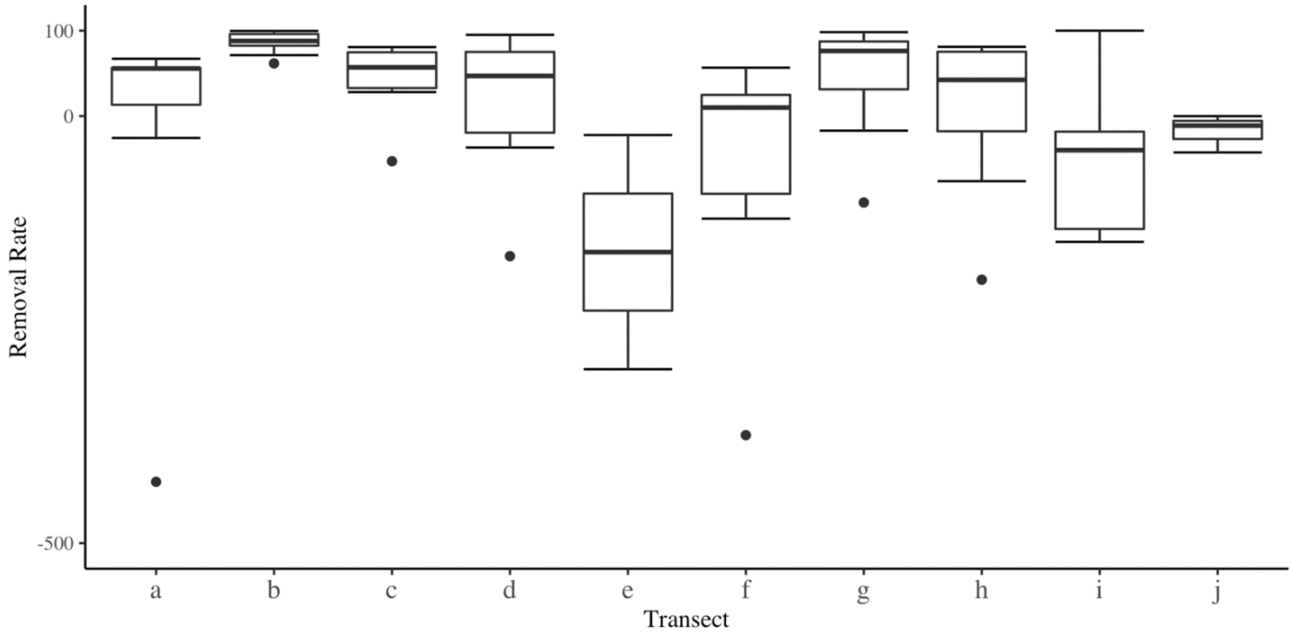


Figure 8. Nitrogen removal rates from all seasons for each transect. (H = 36.628, p < .0001). Following outliers were deleted for legibility of the graph: -600,14 and -1332,60 (both measured in transect "e"), -696,56 (transect "i") and -519,33 (transect "j") (Mann-Whitney test, p < .005, W = 765).

No clear spatial differences in nitrate removal rates were detected, neither between study sites (up- and downstream (W = 211, $p >$ 0.05)) nor between differently treated transects (mown and unmown) (W = 180.5, $p >$ 0.05).

There were statistically significant differences between nitrate removal rate by different seasons (H = 16.057, p < 0.005) (Fig. 9). Multiple comparisons following an analysis of variance were performed using the Bonferroni–Dunn test, which showed that removal rates for July differed significantly from those calculated for August (z = -3.900865, p < 0.001). There was also no significant correlation between TIC concentration and removal rates.

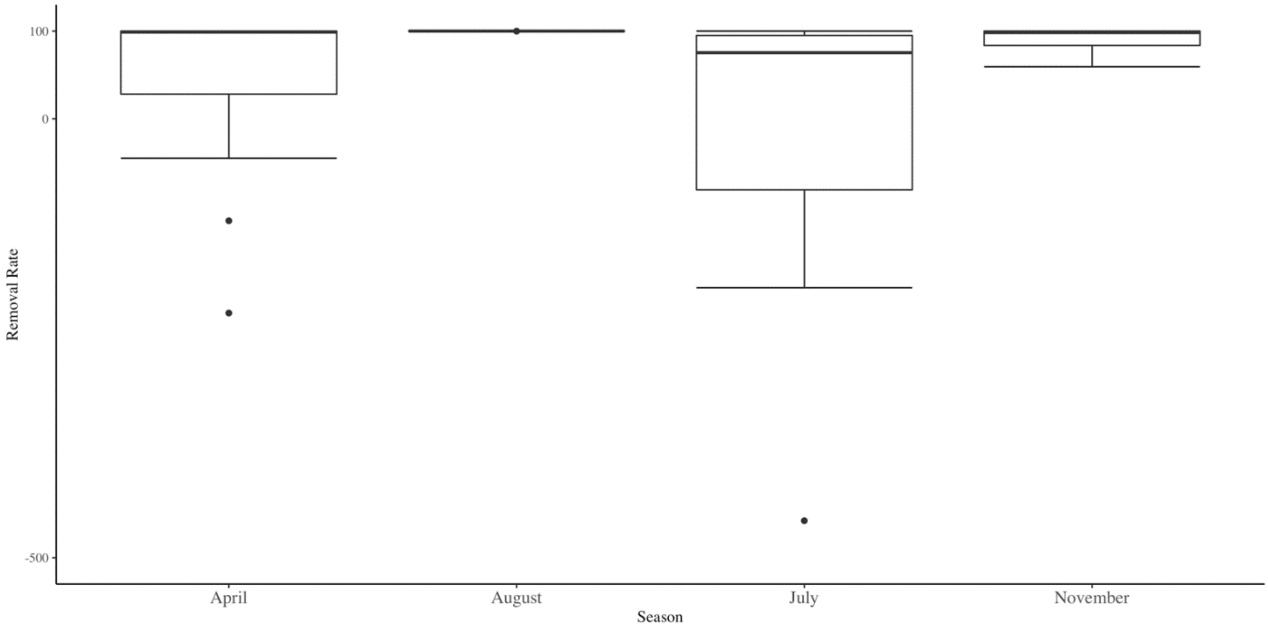


Figure 9. Nitrate removal rates, showed in percentages, differentiated by seasons.

3.5 Plant species and effective environmental factors

104 plant species were accounted for within chosen study sites. The length of both axes of DCA is sufficiently long (SD > 4) to suggest the unimodality of gathered data. Following species were noted as the most abundant (total number of relevés with the dominant, defined here as the most abundant, specie given in brackets): *Carex acuta* (18), *Scirpus sylvaticus* (2), *Carex acutiformis* (2), *Potentilla anserina* (2), *Juncus effusus* (1), *Carex riparia* (3), *Salix cinerea* (1) (if two species were equally abundant in chosen plot, both are quoted as dominant).

Relationship between plant species and environmental variables (pH, NO_3^-, NH_4^+, PO_4^{3-}, water table level, whether it was mowed or not, TIC and EC) was analysed in CCA (Fig. 10). Water table level and NO_3^- seem to be related to the first axis. At the same time, TIC, EC and PO_4^{3-} are related to the second axis. The arrow length shows the importance of the environmental variable in differentiating the dataset, which suggests strong impact of management practices (moving) as well as pH and water table level at vegetation variability. Configuration of arrows suggest that the environmental variables best differentiating vegetation (the pH and management practices) were not correlated.

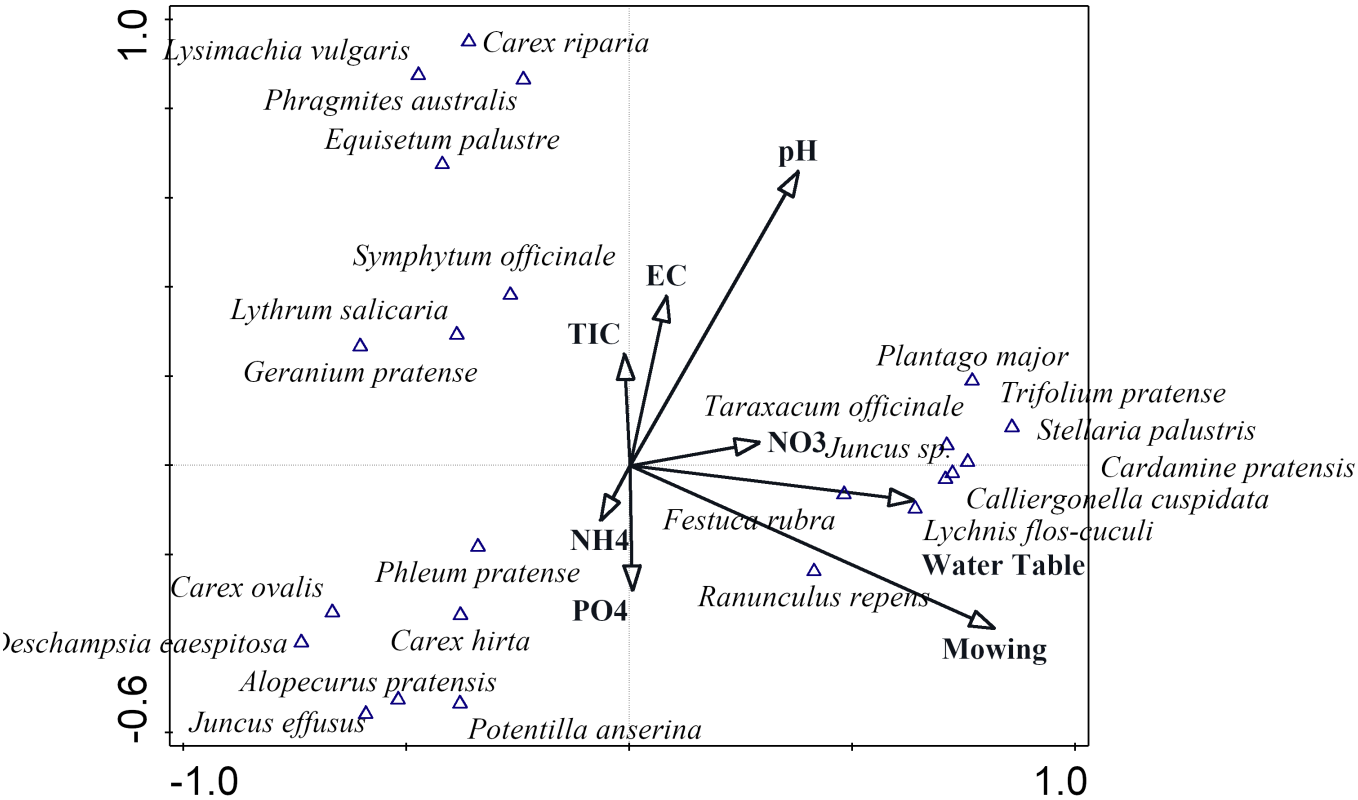


Figure 10. CCA ordination diagram of 1st and 2nd axes obtained from 6 environmental properties and 28 relevés. 104 species of the 28 relevés were analysed, with display limited to 25 best fitting of them.

3.6 Literature review

Data on varied riparian buffer zones, with information regarding vegetation types, soil type, N form and measured amount (input), as well removal rates, and buffer width were gathered from literature review (table 3) for comparison with data obtained from research sites.

Table 4. Literature review. Entries marked with asterisk (*) are quoted after Mayer 2005.

Data source	Vegetation (overview)	Soil types	N form	N-NO_3 [µg/l] amount	total N removal	Buffer width
Léonard Bernard-Jannin et al, 2017	Riparian forest closely to the river - mostly Salix alba and Fraxinus excelsior. Between the forest and the field there is a plantation of Populus alba.	Alluvial deposits covered with a silty soil layer 1–2m deep (based on literature)	nitrate	10603,92	no specific data given	not detailed (based on graphs > 300 meters, but its very imprecise)
Sergi Sabater et al, 2003	Riparian forest, dominant species: Urtica dioica, Alnus glutinosa.	sandy clay/peat	N-NO3 (NH4 also given)	20	~0%	14 m.
Sergi Sabater et al, 2003	Riparian forest, dominant species: Urtica dioica, Alnus glutinosa.	sandy clay/peat	N-NO3 (NH4 also given)	20	~0%	30 m.
Sergi Sabater et al, 2003	Acer pseudoplatanus, Fagus sylvatica	brown soil	N-NO3 (NH4 also given)	120	~0%	50m.
Sergi Sabater et al, 2003	Lolium, Poa, Trifolium	brown soil	N-NO3 (NH4 also given)	160	~100%	40m.
Sergi Sabater et al, 2003	Glyceria, Myosotis, Nasturtium	brown soil	N-NO3 (NH4 also given)	510	~100%	60 m.
Sergi Sabater et al, 2003	Alnus glutinosa	sand/peat	N-NO3 (NH4 also given)	350	~0%	10-20 m.
Sergi Sabater et al, 2003	Glyceria maxima, Urtica dioica	sand/peat	N-NO3 (NH4 also given)	1140	~100%	20 m.
Sergi Sabater et al, 2003	Alnus glutenosa, Salix sp.	reductisols	N-NO3 (NH4 also given)	1690	~100%	20 m.
Sergi Sabater et al, 2003	Cornus x sanguinea, Populus nigra	chromic luvisols	N-NO3 (NH4 also given)	3350	~0%	20 m.
Sergi Sabater et al, 2003	Lolium, Trifolium, Taraxacum	chromic luvisols	N-NO3 (NH4 also given)	8540	~100%	10.5 m.
Sergi Sabater et al, 2003	Salix sp., Quercus sp.	organic/clay	N-NO3 (NH4	10700	~75%	15 m.

			also given)			
Sergi Sabater et al, 2003	Salix sp., Rubus fruticosus	organic/clay	N-NO3 (NH4 also given)	11600	~0%	15 m.
Sergi Sabater et al, 2003	Holcus lanais, Dactylis glomerata	organic/clay	N-NO3 (NH4 also given)	12350	~100%	15 m.
Sergi Sabater et al, 2003	Alnus glutinosa, Platanus hybrida	sandy	N-NO3 (NH4 also given)	35000	~100%	20 m.
Magette, W. L. et al, 1988	grass	sandy loam soils	total N	non applicable (N and P were applied)	-15%	4.6 m.
Magette, W. L. et al, 1989	grass	sandy loam soils	total N	non applicable (N and P were applied)	35%	9.2 m.
Yates, Sheridan, 1983	cypress, gums, magnolia, red maple, tulip popla, in more open sites: Juncus and Carex.	sandy	NO3 (and N-NO2)	<1000	81%*	no data
Yates, Sheridan, 1983	winter cover of oats, followed by a summer crop of soybeans	sandy	NO3 (and N-NO2)	no data, only load given (Monthly average load of 210 g ha -1)	no data	no data
Hanson et al 1994	Oak and maple	derived from glaciofluvial deposits, with variable amount of alluvial material	nitrate	14170,2	59%*	31 m.
Hanson et al 1994	Red maple	derived from glaciofluvial deposits, with variable amount of alluvial material	nitrate	no data	59%	31 m.
Vellidis et al, 2003	Forested riparian wetland	loamy sand	nitrate	6915,6	78%	
Brüsch et al, 1993	Sweet grass (Glyceria declinata), with <15% of vegetation as jointed rush (Juncus articulatus), sedge (Carex sp.), and lotus (Lotus pedunculatis).	peat	nitrate	12882	12%*	20 m.

Brüsch et al, 1993	Sweet grass (Glyceria declinata), with <15% of vegetation as jointed rush (Juncus articulatus), sedge (Carex sp.), and lotus (Lotus pedunculatis).	peat	nitrate	12882	74%*	20 m.
Fustec et al, 1991	Alder (Alnus glutinosa) and reed (Phragmites communis) community	clay/ silt with alluvial deposits	nitrate	non applicable (N and P were applied)	95%*	200 m.
Puckett et al, 2002	Oak, ash, and poplar species (Quercus, Fraxinus, and Populus) with scattered spruce and fir (Picea and Abies)	sand / gravel / silty clay	total N	17400	~100%	40 m.
Faafeng and Roseth, 1993	Scirpus silvaticus and Glyceria fluitans	no data	total N	non applicable (N and P were applied)	~100%	non applicable
Faafeng and Roseth, 1994	Calamagrostis canescens and Carex acuta	no data	total N	non applicable (N and P were applied)	~100%	non applicable
Pomogyi, 1993	No 'real wetland vegetation' in the first part - mostly belonged to the Caricetum acutiformisripariae, Deschampsieetum caespitosae, Rubo-Solidaginetum, Alopecuretum and Agrostietum communities. The second part is covered mostly by different Phragmition, Magnocericion and an associateion of Lemno-Potamea.	peat	N-NO3	2000-3500	62% (mean for 5 years and a half, varies from -4% to 79%)	no data
Bratli et al, 1999	Emerged vegetation is dominated with Phragmites australis and Schoenoplectus lacustris. The submerged species are dominated with Ceratophyllum demersum, Potamogeton crispus, Myriophyllum spicatum, and Potamogeton perfoliatus.	no data	total N	no data	39%-51% (mean value 44%, calculated from 3 consecutive years)	250 m.
Jordan et al, 2003	Eleocharis obtusa, Ludwigia palustris and Schoenoplectus americanus	silty loam soils (more detailed description given)	total N and total Kjeldahl nitrogen	no data	38% in the first year of the study and no significant N removal in the second year of the study	no data

4. Discussion and Conclusion

4.1 Nitrogen inflow and groundwater chemistry

As expected, nitrate input and concentration along flow paths differed significantly between seasons, which is in line with the declared agricultural practices (fertilization). The mean values obtained in our study were relatively small (Table 1), compared to the existing literature from similar area (Table 3). The difference in nitrate concentration in the strip near field border between the two sites (in Mory and Obrebek) could have been caused by the differences in fertilisation of the nearby fields and the distance from the fields to the studied buffer zones.

Surprisingly, median nitrate concentration was higher in deeper groundwater (Table 1). Previous research suggest that nitrate concentration tends to be higher near water surface due to higher concentration of dissolved oxygen. Dissolved oxygen is preferred over nitrate by microbes as electron acceptors since it yields most energy to bacteria mediating reaction (Korom 1992). However, shallow groundwater from studied area might be also low in dissolved oxygen, since it was not collected near the surface. Stratification of N removal processes in groundwater may be also related to carbon distribution and its bioavailability (Kellogg et al. 2005). We might also assume that nitrate concentration stratification could be caused by plant uptake, influencing nitrate concentration in shallower groundwater. However, nitrogen removal rates are not higher for shallow groundwater, which does not support mentioned hypotheses.

Concentration of nitrate differed significantly between measurements taken at the field border and at the intermediate strips ($W = 3391.5$, $p < .05$). We describe processes responsible for removal of nitrogen in further parts of the paper. There were no significant differences between measurements taken in the intermediate and bordering river strips. We attribute it mostly to the low values of nitrate, which was totally removed in the first part of riparian buffers. Such interpretation is in accordance with previous studies (Haycock and Pinay 1993). Measurements from the river border strip with increase in nitrate concentration might also be linked to the river water infiltrating closest piezometers.

Increase in TIC along the flow path (Table 1) suggests active microbial production (Cole et al. 2013). While microbial activity can decrease pH (Hefting 2006), we have observed small increase in pH along water path flow (Table 1). It might be caused by ratio of production of OH^- to CO_2 in the process (Rivett et al. 2008).

Concentration of dissolved manganese (Mn) increased significantly from field border to intermediate strip in both upstream and downstream sites (Table 2). It also slightly decreased from intermediate to river border strip, while still being higher than in field border strip. Increase in concentration was also accounted for Fe (Table 2), which increased for both sites from the intermediate to the river border strip, with minimal or no increase in the first part of the transects (flow path between strips of field border and intermediate). Dissolved manganese, similarly to iron (Fe^{3+}), mobilises when reduced, but precipitates under oxidised conditions. Therefore, increase of dissolved manganese and iron suggests prevailing low (negative) redox potential at described study site (Søndergaard 2009, Naiman and Décamps 1997, Pinay et al. 1993). As argues Palmucci (Palmucci et al. 2016), the co-existence of reduction processes of NO_3^- and Fe^{3+} as well as NO_3^- and Mn can suggest mixed (anoxic) conditions, according to the USCG classification (USCG 2009, Palmucci et al. 2016, McMahon and Chapelle 2008).

Groundwater chloride measurements suggest, that while dilution of nitrate might play a role for some of studied transects, it cannot account for a major portion of the nitrate decrease observed in most of the transects. Over one third of transects with removal rate over 50% accounted for < 30% of difference, while 5 of them showed difference in chloride concentration being <10%. Since Cl in groundwater is recognised as conservative tracer (Macioszczyk 2007), changes in Cl concentration along established groundwater flow path can mean, in our study sites, mixing of chloride-poor groundwater with deeper chloride-rich groundwater, or infiltration of measured groundwater with river water contaminated with chloride (Vidon and Hill 2004).

4.2 Processes responsible for nitrogen removal

Plant uptake and microbially mitigated nitrogen removal processes are thought to be the main causes for N groundwater retention. The lack of statistically significant differences between removal rate during growing and non-growing seasons imply that plant uptake was not the main process responsible for nitrogen removal in chosen study sites (Figure 9). However, this does not mean that plant uptake was not an important process in the spring and summer. Research shows that microorganisms and plants uptake of inorganic N can be perceived as more of a cooperation than competition, since microorganisms immobilisation can prevent inorganic N from leaching from riparian ecosystems in winter, when plant uptake is arrested (Kuzyakov and Xu 2013).

As has been stated in the introduction, beside denitrification, nitrate can be depleted also by two other processes mitigated by microbes. Dissimilatory nitrate reduction to ammonium (DNRA) occurs in similar conditions as denitrification. It can be represented by the following equation (after Robertson et al. 1996):

$$2H^+ + NO_{3-} + 2CH_2O \rightarrow NH_{4+} + 2CO_2 + H_2O$$

DNRA is estimated to be less common than denitrification. Moreover, bacteria performing DNRA are obligatory anaerobes, unlike denitrifying bacteria. Therefore, they cannot occupy the same variety of niches.

The above processes can play a different role in retaining the nitrate depending on the specifics of the site. In some of our research transects (*b, c, g, h,* and *j*, Fig. 6b) decrease of nitrate is followed by the increase of ammonium, suggesting the process of DNRA. Ammonium generated by this process can be later converted to diatomic nitrogen during anaerobic ammonium oxidation (annamox), a process that was found responsible for up to 37% in terrestrial ecosystems (Hu et al. 2011). It can be a process responsible for ammonium decrease in some transects from selected study sites (eg. *e, d*, and *h,* Fig. 6b). High concentration of nitrate in water can inhibit ammonium oxidation, which can in turn lead to production of N_2O (Rivett et al. 2008). Nonetheless, the concentration of nitrate in the described transects was very low (Table 1), significantly discounting the possibility of N_2O emission.

4.3 Denitrification potential of selected sites

For some transects, nitrogen removal was a significant process, removing almost all of the nitrate. However, for some transects nitrate removal was not accounted for at all, and for some transects negative values of nitrogen removal were found (Table 3).

Dissimilatory nitrate reduction to ammonium is speculated to be the most important process for some of the transects. Research suggest that answer for the question regarding which microbial process is mostly responsible for nitrate retention lays in what limits the process (Pinay et al. 1993). Usually nitrate is retained by denitrifying bacteria when the rate of the process is limited by the amount of electron donors. For denitrifying bacteria, the most common electron donor is inorganic carbon. In C limited ecosystems reduced manganese (Mn^{2+}), ferrous iron (Fe^{2+}) and sulfides can also play the role of alternative electron donors. Moreover, several studies suggest that multiple electron donors can be present in a given aquifer (Korom 1992, Rivett et al. 2008). DNRA seems to be the main process for microbial nitrate depletion when the limiting factor is the amount of electron acceptors (in this case: nitrate), which is the line with amount of nitrate available in studied sites (Table 1) in comparison with data available from literature review (Table 3) (Jahangir et al. 2017).

Steady decrease of total sulphur in our study site (Table 2) also suggests that the process of mineralization of organic carbon with sulphur compounds as electron acceptors might have taken place, resulting in observed groundwater depletion of total S. Total sulphur decrease indicated that microbiological demand for electron acceptors was higher than that provided by nitrate and dissolved oxygen. Due to nitrate shortage SO_4^{2-} was used as electron acceptor and converted to gaseous H_2S. This indicates that nitrate amount was the factor limiting N removal processes, and therefore confirms the assumption about the denitrifying potential of a given site being greater than received nitrate load. The issue of denitrifying potential also raises the question of environmental conditions suitable for denitrifying bacteria and their acclimation time to changes in nitrate amount. However, research suggest that riparian wetlands can support a population of denitrifying bacteria with no additional (anthropogenic) nitrate input, and denitrifying bacteria can swiftly adapt to increase in nitrate amount (Rivett et al. 2008, Inselsbacher et al. 2010).

It is, however, important to keep in mind that between field border, intermediate, and river border piezometers, groundwater might have been subjected to several factors influencing nitrate and ammonium concentrations. During this research, we accounted for certain changes (such as mixing shallow groundwater with deeper groundwater) by tracking the changes in chloride concentration. At the same time, chosen study method (singular measurements repeated seasonally) made it impossible to closely follow temporal variations of water composition and exclude whether it was caused by external factors. Additionally, chemical composition of groundwater in river border could have been altered by the influence of riverine water flooding the floodplains. While a lot of researchers focusing on groundwater processes in riparian buffers use methods similar to the technique we used (point-in-time measurements, repeated seasonally, see: Sabater et al. 2003, Hefting et al. 2005, Hefting et al. 2006), some reach for techniques allowing more precision in identifying additional factors changing dependent and independent variables, such as stable isotopes analysis (Komor 1997, Bassett et al. 1995, Konohira et al. 2001, Clement et al. 2003, Nisi et al. 2011). Additionally, it has to be remembered while analysing the data, that high negative removal values (up to -1332,60%) were recorder in points that had almost no measured inorganic nitrogen concentration. Therefore, increase in N, while seemingly significant when presented in percentage, was still relatively small.

4.4 Spatial differences in nitrogen removal

Absolute nitrogen removal was statistically more significant for deeper groundwater than for shallow groundwater (Fig. 7). It has been thought for some time, that the potential for microbially mitigated N removal decreases with depth. Nitrate removal (namely: heterotrophic denitrification) is though to be far more efficient for shallow subsurface groundwater (Haycock and Pinay 1993, Vidon and Hill 2004). The main cause for this pattern is linked to electron donor (organic carbon derived from the organic matter) bioavailability. However, significant increase in nitrate removal with depth has also been described, and linked to stratified availability of different electron donors (Kolbe et al. 2019). For chosen study site the difference in nitrogen retention processes for shallower and deeper groundwater might alternatively be caused by observed increase in nitrate concentration with groundwater depth (Table 1).

While the anthropogenic influence on nitrate removal rates is still generally unknown, recent research suggests that agricultural practices, such as mowing, may affect microbially mitigated nitrogen retention. Potential cause of this phenomenon may lay in the soil compaction resulting from mowing. While seldom research was conducted regarding the impact of anthropogenic soil compaction on DNRA process, denitrification was reported to be more efficient in compacted soil when compared to uncompacted soil. It is thought to be the result of the increase of water-filled pore space (WFPS) amount in compacted soil. WFPS arrests supply of oxygen, which in turn stimulates denitrification activity (Li et al. 2014). However, since there was no significant difference in N removal capacity between differently treated transects, our data do not support this hypothesis. Since the structure of functional microbial groups and environmental factors influencing them are still not very well known (Braker and Conrad 2011, Li et al. 2014), more research is needed to support further hypothesis.

4.5 Relationship between plants species and environmental variables

Data obtained from CCA analysis (Fig. 10) shows that the species differentiation was correlated mostly to water table level and NO_3^- (axis one), and to TIC, EC and PO_4^{3-} (axis 2). Water table level as a primary factor for driving differentiation of plant species in riparian environment has been found in previous research and is widely supported by data (Jean and Bouchard 1993, Xu et al. 2015). *Plantago major, Tripholium pratense, Stellaria palustris* and *Cardamine pratensis* were among the species related to higher water level and mowing, while *Phragmites australis, Typha latifolia* and several *Carex* species were more frequently found in the sites characterised by lower water levels and lack of agricultural practices (mowing).

Since this first axis was correlated to, among others, nitrate levels, we might speculate about lower levels of nitrate in groundwater flowing through plots dominated by *Phragmites australis, Typha latifolia* and *Carex* species. One of the potential causes to this relationship could be higher uptake of nitrate by this group of species, which is in line with previously conducted research, proving that it is mostly highly productive plants that take up the nitrogen (Miller and Hawkins 2006).

However, there might be also alternative causes to this correlation, stemming from the spatial distribution of described species. *Phragmites australis, Typha latifolia* and *Carex* species were more frequently found in the river border strip, which was also characterised by significantly smaller amount of nitrate (Table 1). Therefore, we might also assume that the relation between those groups of species and smaller amount of nitrate was caused by a small amount of nitrate cycling in this ecosystem, being mostly taken out of biogeochemical cycle by plant and microbial uptake earlier in the groundwater path flow.

4.6 Conclusions

Nitrogen retaining processes have been described in scientific literature for decades. However, some of their aspects, as well as applicability to certain conditions, still remain underdeveloped. At the same time research shows that nitrogen retention potential might be decreasing in certain ecosystems due to the increase in anthropogenic nitrogen loading.

The undertaken measurements of the groundwater chemistry, its physical properties, analysis of plant species and conducted literature review led us to believe that detected NO_3^- concentrations were relatively low in chosen study sites, which might have impacted N retaining processes. At the same time, chloride concentration changes show the importance of groundwater dilution process and its possible influence on denitrification potential estimations. However, in some transects N removal (argued to be mostly caused by the DNRA) was still a significant process. Together with evident decrease in S concentration along the flow path suggest denitrification potential of chosen sites exceeding its nitrate load for studied period of time.

The effectivity of N retaining processes was not dependant on the type of treatment which chosen study site was subjected to. While recent research suggests that there are potential links between agricultural practices and nitrate removal rates, our findings do not support this hypothesis.

Water table level was found to be one of the most important variables influencing the structure of plant communities from chosen study sites. High impact of water table on microbially mitigated processes removing nitrogen from the groundwater has also been documented. Therefore, water table level seems to be one of the most important factors shaping nitrogen removal processes in chosen wetland riparian buffer.

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