

Investigation of the use of biochar to enhance nitrate removal in Danish woodchip bioreactors and reduce nitrous oxide emissions

Raphaël Offermann

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Investigation of the use of biochar to enhance nitrate removal in Danish woodchip bioreactors and reduce nitrous oxide emissions

Par: Raphaël OFFERMANN





Soutenu à Rennes, le 21/10/2021

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Les analyses et les conclusions de ce travail d'étudiant n'engagent que la responsabilité de son auteur et non celle d'AGROCAMPUS OUEST

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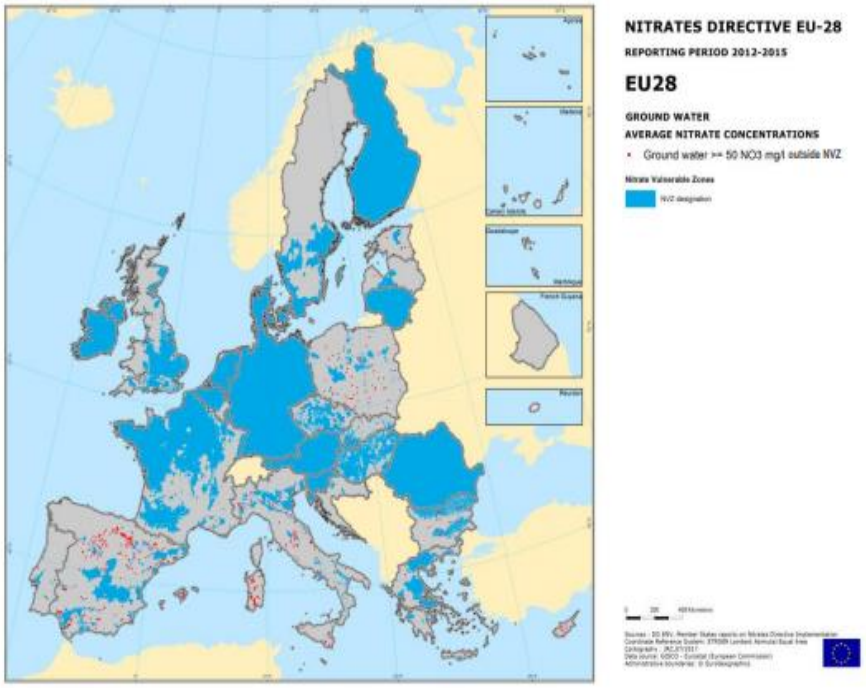
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Figure 1: Nitrates Vulnerable Zones, European Commission, 2018

1.Introduction

1.1 Agricultural nitrogen use and regulation in the European Union

Agricultural nitrogen is used in crops to maintain or restore soil nutrients and improve yields (Hirel et al., 2011). In intensive agricultural systems, the N used as fertilizer often exceeds the nutrients demands of the crops, which means some of the nitrogen applied will eventually be lost, through mineralization processes and leaching (Daalgard et al., 2011). The lost nitrogen then pollutes the aquatic environment, including shallow groundwater. High nitrate rates in drinking water may threaten human health, with diseases such as methemoglobinemia in infants and cancers in adults (Chae et al.,2009; Schullehner et al, 2018).

To reduce the threats and damages caused by nitrate, the European Nitrate Directive of 1991 ((ND91/676/EEC) EU Commission 1991) is the main European reference. This directive aims to "protect water quality across Europe by preventing nitrates from agricultural sources polluting ground and surface waters and by promoting the use of good farming practices" (Musacchio et al., 2020). The main outcomes of this directive were to designate Nitrate Vulnerable Zones (NVZ), where the groundwater pollution is likely to be more than or equal 50 mg/L of nitrate, and to limit the use of agricultural nitrogen in these zones to 170 kg $N^{-1}ha^{-1}yr^{-1}$. For farmer in NVZs, failure to comply to these regulations would mean loss of European subsidies (ND91/676/EEC).

1.2 Nitrogen pollution in Northern European countries

In Northern Europe, including Denmark, a major part of cultivated areas is in NVZ (Figure 1). Moreover, due to climate change, an increase of the average yearly precipitations and flow in rivers and undergrounded water has been predicted in northern lowlands. These regions are especially at risk of water quality degradation, due to higher precipitation in winter and a higher frequency in extreme events such as drought (Navarro et al, 2018). This could increase the risk of crop nutrients leaching. Anthropogenic stressors, such as high-density populations, intensive agriculture and flood protection amplify the pollution and eutrophication of freshwater streams, lakes, estuaries, and coastal waters (Hering et al., 2015 *in* Navarro et al., 2018). Artificial drainage networks used in agricultural fields can transport contaminants and affect the water quality downstream and exacerbate NO_3–N transport by providing a direct conduit from field to surface and subsurface water bodies (Dragon et al., 2016, Hoover et al., 2016). Drainpipes may gather contaminants from dispersed sources and aggregate these contaminants at cumulative concentrations downstream (Spaling & Smit, 1995).

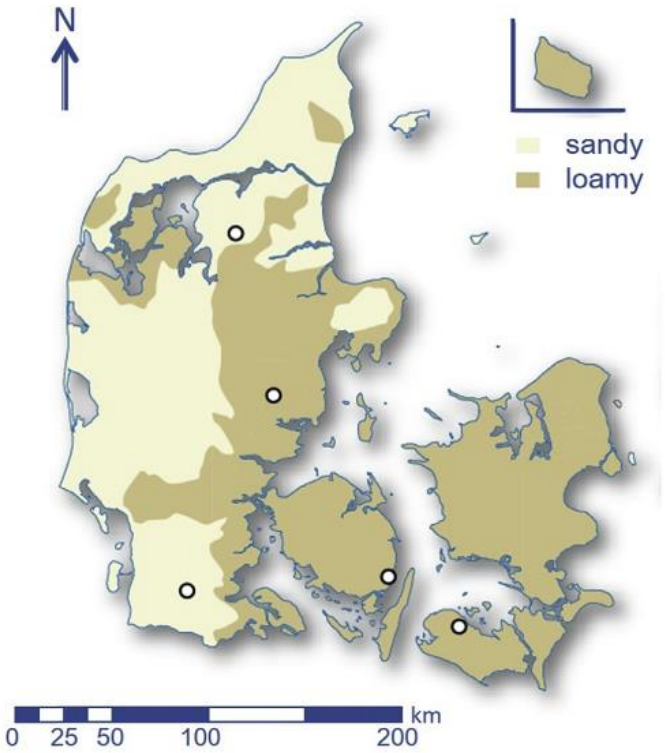


Fig. 1. Location of the five small agricultural catchments (the LOOP areas) in Denmark in sandy and loamy catchments included the Danish National Environmental Monitoring Program.

Figure 2: Sand and loam soils in Denmark, Hansen et al., 2019

1.3 Danish agricultural nitrogen pollution and mitigation strategies

Agriculture in Denmark is among the most intensive in the world, with 59% of the land area under cultivation (FAO,2019) and with an average use of 175 kg $N^{-1}ha^{-1}yr^{-1}$ and 25 kg P ha^{-1} y^{-1} (Blicher-Mathiesen et al., 2019), causing important crop nutrient pollution of groundwater (Schullehner et al., 2018). Diffuse losses of nutrients, especially nitrogen, generate concern over the health of the population because in Denmark groundwater is the only source of drinking water (Kaspersen et al., 2016; Schullehner et al., 2018).

Over the last 30 years, efforts have been made to regulate the anthropogenic N use and minimize nitrogen pollution of the environment. In 1998, a restriction on fertilization was introduced which lowered the use of crop nutrients for all farmers lowered to 10% below the economic optimum of crop nutrients (Kronvang et al., 2008). The restriction on fertilization was implemented with the 2nd National Environmental Action Plan for the Aquatic Environment Between 1989 and 2002, N discharges were reduced by 74%, N surplus by 30% and nitrogen leaching was reduced by 33%.

However, in many regions, in particular ones with sandy soil, nitrate was present in higher concentrations than the groundwater standard of 50 mg/L (Figure 2). In Danish ecosystems such as the Odense fjord, eutrophication led to the disappearance of seabed vegetation and fauna (Conley et al, 2007).

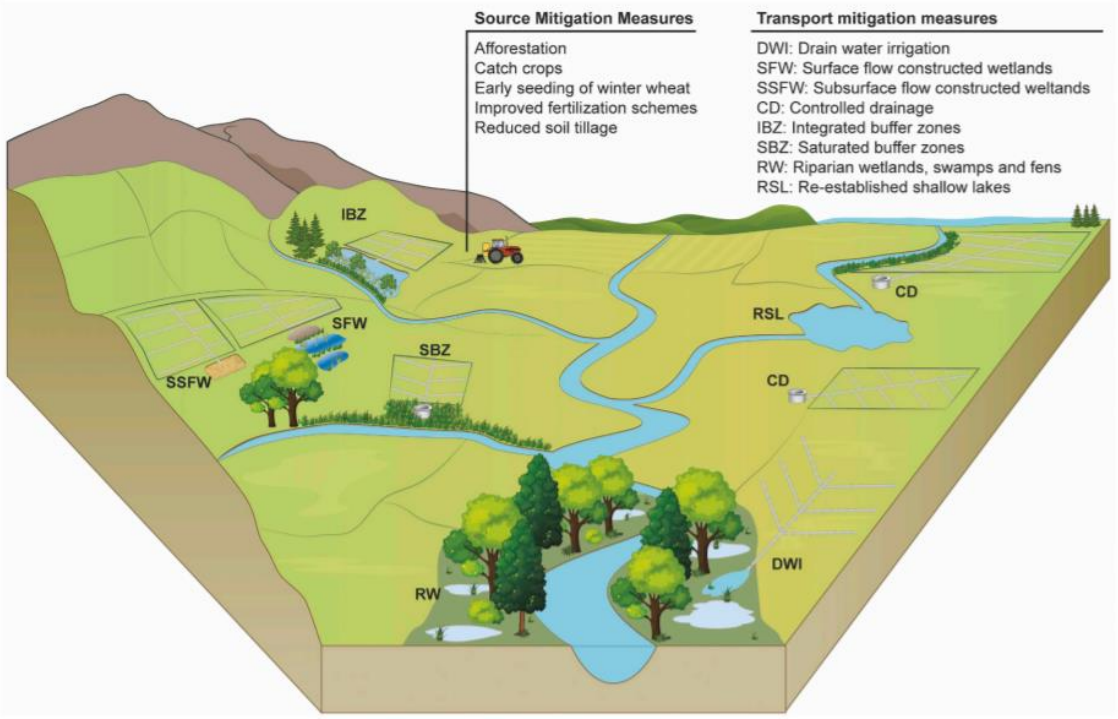


Fig. 1. Nutrient reduction efforts in Denmark since 1985. Modified after Carstensen et al., 2020.

Figure 3: Nitrate mitigation measures from Hoffmann et al.,2020

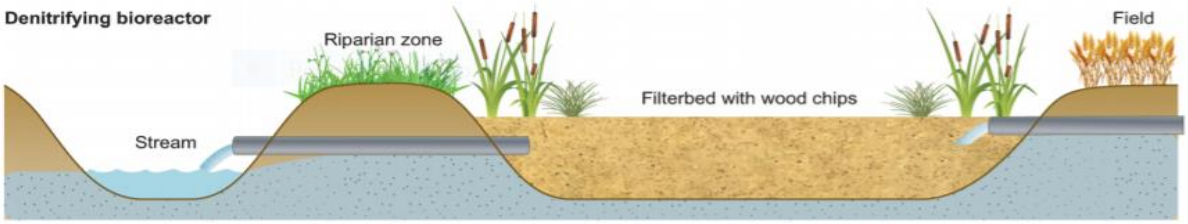


Figure 4: Woodchip bioreactors from Hoffmann et al.,2020

In Denmark, a new set of laws, the Agricultural Package of 2016, allowed an increased application rate of N fertilizers to allow farmers to reach an economic optimal. However, in return the implementation of farmers of nutrient mitigation measures is mandatory. Indeed, the Agricultural Package does not comply with the EU Nitrates Groundwater and Water Framework Directives without these environmental protection plans, as the application of nitrogen fertilizer often exceed 170 kg $N^{-1}ha^{-1}yr^{-1}$

Denmark has requested many derogations and Danish farmers gained the right to use up to 230 kg in 2002 $N^{-1}ha^{-1}yr^{-1}$ from Commission implementing Decisions of the European Union Decision 2002/915/EC. Other derogations were granted in 2005, 2008, 2012 and 2018 to further allow a higher fertilization rate (Official Journal of the European Union, Commission implementing decision 2020/1074, 21.7,2020). The last derogation issued was in 2020 and was agreed on the condition that Denmark will pursue its Nutrient-Reducing measures, or nutrient mitigation measures (EU Commission, 2020).

Mitigation measures are a set of techniques that reduce nitrate pollution of aquatic ecosystems that are a pillar of the Agricultural Package of 2016 (Figure 3). Source mitigation measures reduce nitrate at the source by limiting its propagation with good agricultural practices (low tillage, improved fertilization schemes), or capturing it (catch crops, afforestation). Transport mitigation measures consist of nitrate denitrification (constructed wetlands) and careful anticipation and the transport of contaminants (controlled drainage), (Hoffmann et al., 2020, Carstensen et al., 2020). Here we will focus on one transport mitigation measure, subsurface constructed wetlands, or bioreactors (Figure 4) having denitrifying properties thus reducing the pollution downstream.

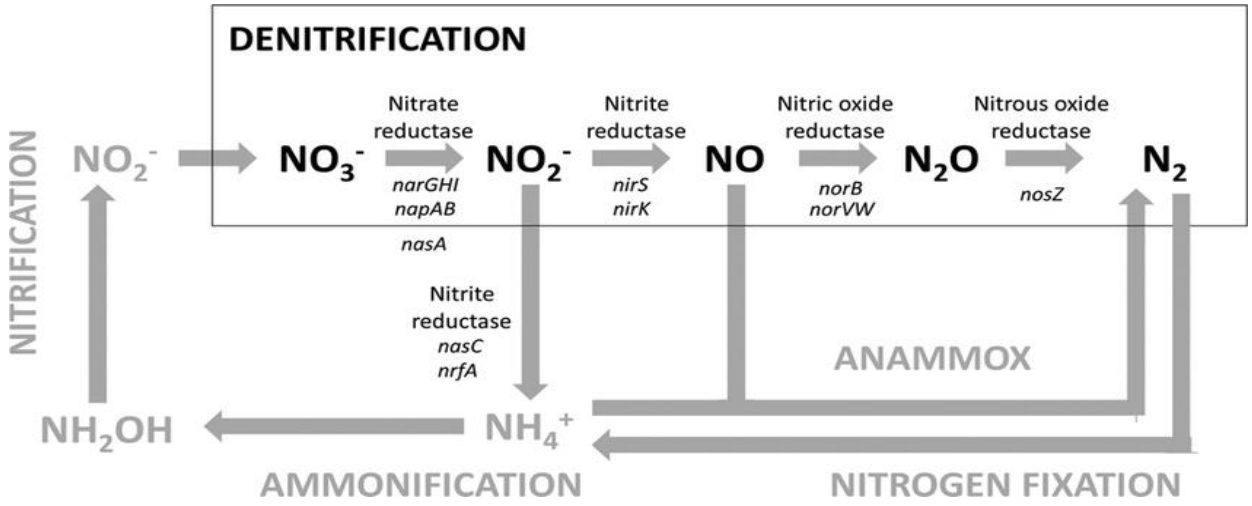


Figure 5: Nitrogen Cycle, Alvarez et al., 2014

1.4 Woodchip bioreactors

Denitrification is a key process in the biochemical nitrogen cycle, led mostly by heterotrophic, facultative anaerobic bacteria (Ye et al.,1994). For microbiologists, “denitrification is the process of accepting electrons generated from the carbon source metabolism and then consuming them in sequential bio-reductions: nitrate NO_3- reductase, nitrite NO_2- reductase, nitric oxide NO reductase, nitrous oxide N_2O reductase” (Berks et al., 1995 *in* Wu et al., 2019; Wan et al., 2016 *in* Wu et al.,2019), (Figure 5)

Facultative anaerobic denitrifying bacteria prefer to use O2 as their electron accepter due to energetic advantages, but when O2 is limited, a reduction of nitrogen oxides is possible. Thus, the combination of available carbon from woodchips and nitrate from contaminated drainage water creates a suitable habitat for denitrification. Woodchip bioreactors should be designed to have low O2 concentrations from the start, because they rely on the anaerobic bacterial respiration to denitrify the water (Giles et al., 2012).

Woodchip bioreactors are a relatively new technology for edge-of-field treatment of subsurface agricultural drainage water and have not been extensively evaluated yet (Hoover et al., 2015). However constructed wetlands have been used in Denmark since 1983 for wastewater treatment, mainly for small villages domestic sewage, and have averaged a 30-50% N and P removal over a 20-year period without nitrifying ammonium (Brix et al., 2007)
That said, many factors influence the performance of woodchip bioreactors in removing nitrate.

Experimental designs are either columns in controlled areas or pilot fields bioreactors either in beds or walls. Walls had significantly lower results, but the studies on walls very few and had heterogenous results. (Addy et al., 2016).

Variations of hydraulic retention time (HRT) showed a consistent impact on nitrate mass removal (Hoover et al, 2016). Longer HRTs will in fact allow the denitrification cycle to be completed, and can be obtained by larger bioreactors, a higher density of woodchips or a reduction of the influent flow (Addy et al., 2016).

Biological rates increase with higher temperatures, in fact in their meta-analysis, Addy et al (2016) have found that bioreactors with water temperatures below 6°C had a slower denitrification rate than those between intermediate temperatures (6°C to 16,9°C). Yet, in Denmark, most of the nutrient discharge in water occurs during the winter, the metabolism of denitrifying microorganisms will be slowed down by low temperatures.

It is also important to consider the pH in the woodchip bioreactor ecosystem, as an optimal pH for denitrifying bacteria would be rather neutral, ranging pH 6 to pH 8 (Reddy & DeLaune, 2008).

1.5 Nitrous oxide

The denitrification process inside woodchip bioreactors emits a greenhouse gas, nitrous oxide, which contributes to global warming. In fact, nitrous oxide is a potent greenhouse gas rated as approximatively 300 times stronger than CO2 (Larsen et al., 2009) This is because the denitrification cycle is not completed and that microorganisms have not fully reduced N20 to N2 (Raes et al., 2016) or dissimilatory NO3− reduction to ammonium through DNRA (Aalto et al., 2020) this linked to *nrfA*, resulting in nitrite reductase to NH4+ (APPENDIX 1)

In Denmark, agriculture account for about 16% of the total national green-house gases emissions (Nielsen et al, 2013). To comply with the “good ecological status” (GES) of the European Water Framework Directive appropriate measures are needed, that consider the potential impacts of climate change as well as its mitigation through the reduction of emission of greenhouse gases (Kaspersen et al., 2015).

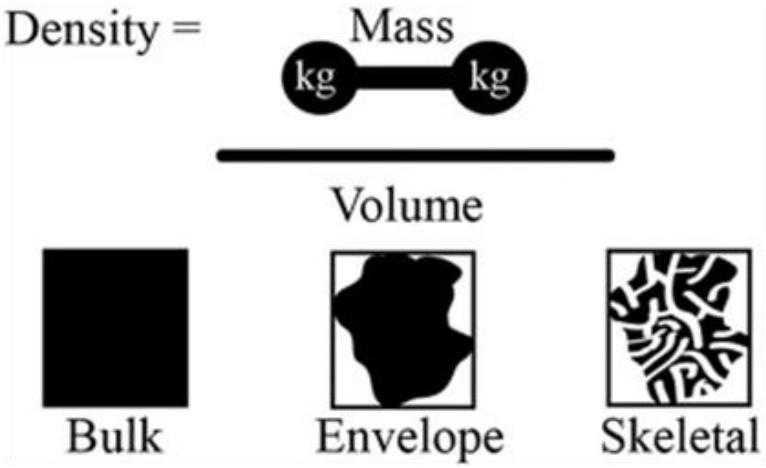


Figure 6: Bulk, envelope and skeletal densities, Brewer et al. 2014

1.6 Biochar

A way to increase the denitrifying rate is the increase of the total organic carbon release (TOC). Bioreactors with fresh woodchips have high TOC (> 20 mg/L) with a peak directly at the start, that can be as high as 200 mg/L (Hoover et al., 2016). Consequently, they need less time to denitrify 50% of the N than 2-7 years weathered woodchips (Robertson, 2010).

However, a high TOC from the start will mean a faster decrease of the organic carbon available in the woodchips, thus compromising the sustainability of the denitrification system. Most importantly, a high TOC would lead to high dissolved organic carbon (DOC) rates that may endanger aquatic ecosystems, and lead to a depletion of O_2 (Hoover et al., 2016).

Another aspect to consider in woodchips is their essence, softwoods, allow oxygen into dead wood, which make them more subject to decay and depletion of C supply more quickly than denser woods (Cornwell et al., 2009).

The pyrolysis method will, with the biochar´s raw material, determine its envelope density i.e. the density considering the pores as opposed to the skeletal density only considering the carbonaceous structures (excluding the pores, Figure 6). Considering the density of biochar particle is important for practical reasons, if their density is below 1g/cm3 they will float and play a role within the soil or water environments (Brewer et al., 2014).

Wood biochar have a skeletal density higher than 1g/cm3, meaning that in time the pores may be filled with water and the particles will sink. The mobility of biochar particles inside the woodchip reactors will be an aspect to consider in *Materials and Methods.*

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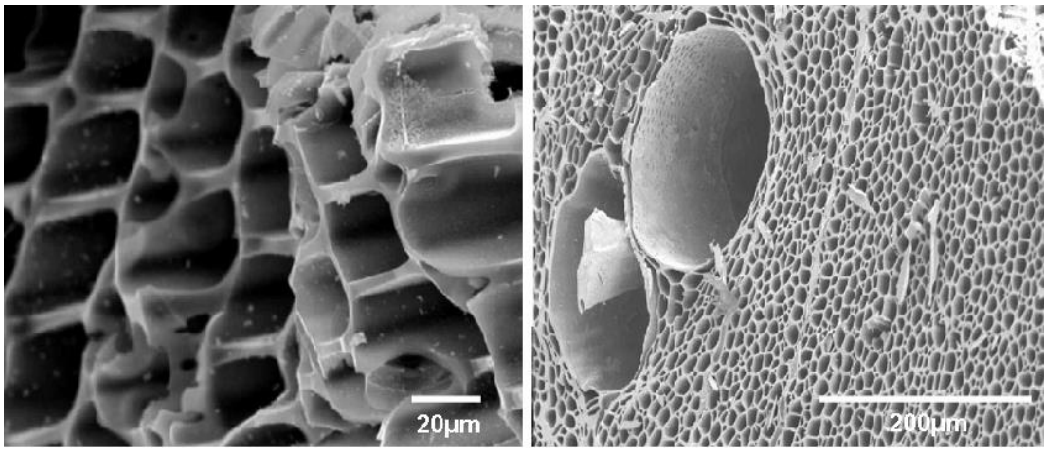


Figure 7: Porous structure of biochar from Thies & Rillig 2009

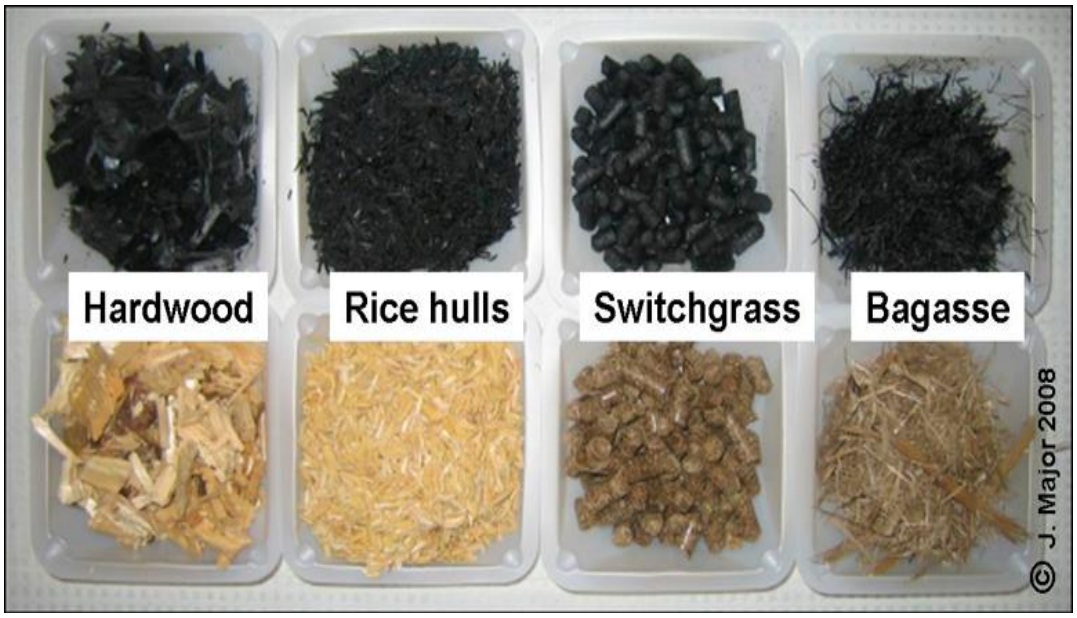


Figure 8: Example of plant raw materials for biochar, International Biochar Intitiative (IBI)

1.7 Biochar use in woodchip bioreactors

One potential way to decrease nitrous oxide emissions of woodchip bioreactors is the addition of biochar. On average there is a 38% N_2O emissions reduction with biochar on arable soils and a 40% N_2O reduction paddy fields (Borchard et al., 2018). However, theses nitrous oxide reduction rates greatly vary from study to study. Agriculture accounts for approximately 60 % of N_2O emissions, (Borchard et al., 2018).

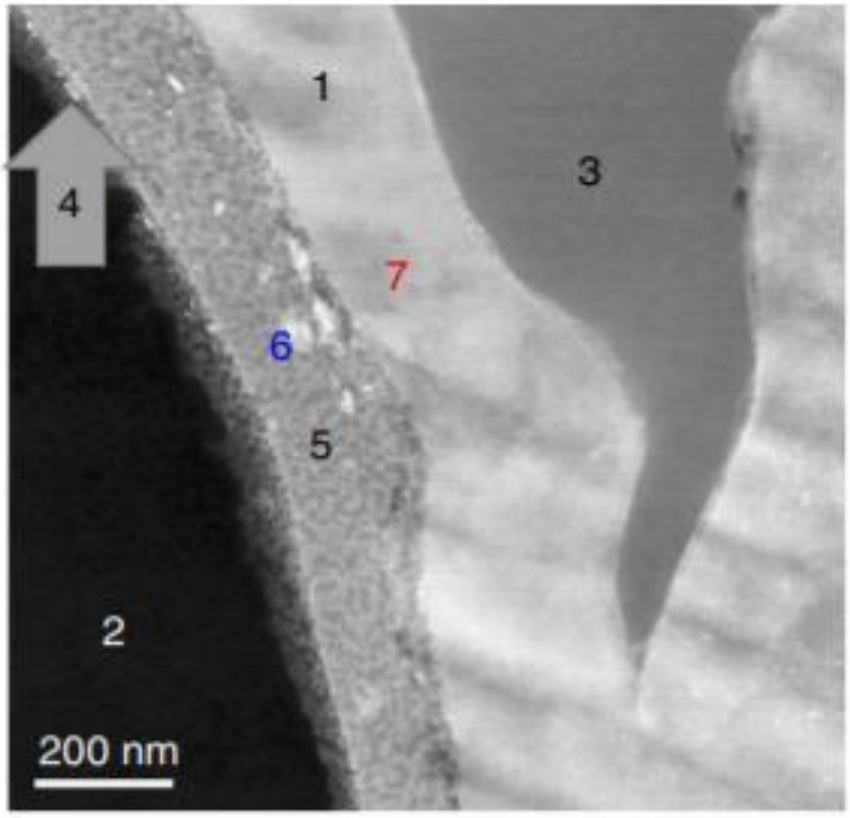
In addition, biochar has been shown to reduce mineral leaching. It is also efficient against other pollutants for example pesticides such as atrazine, 24D, benzotriazole, etc. (Ashoori et al., 2019).

Biochar is a carbonaceous porous material Figure 7) obtained by pyrolysis of biomasses an oxygen limited environment (Kammann et al, 2015) It can be obtained from various carbon sources (wood, straw, animal waste, etc.) (Figure 8) for various uses: removal of fertilizers, chemicals, composted and used with compost, soil enrichment Biochar, as defined by the International Biochar Institute (IBI), is “a solid material obtained from thermochemical conversion of biomass in an oxygen limited environment,” (Bock et al, 2015).

It could prove very useful for water treatment, especially, with continuous flows during winter or storm events in the summer (Ashoori et al. 2019). As a further advantage, none or at least non-significant nitrification processes were reported to occur in biochar (Bock et al 2015, Borchard, 2018).

Porosity in biochars can be ranged in three size categories: micropores (<2 nm), mesopores (2-50 nm), and macropores (>50 nm), (Tan et al., 2018). The source of the biochar as well as the pyrolysis method and highest temperature treatment (HTT) will strongly influence the pore size distribution, the macropores are the most present for biochar shaped under 600 °C. Mesopores are the predominant pores for treatments between 300°C and 800 °C. Treatment over 600°C will increase the proportion of micropores, thus having a greater capacity of capturing phosphorous but be less ideal for nitrate removal as microbial communities tend to colonize macropores (Tan et al., 2018).

Biochar retains pollutants through its ion sorption properties (Fidel et al, 2018). Four major classes of mechanisms for describing ion sorption onto the surface of biochar particles: surface precipitation (salt precipitation), chemical adsorption (chemical reaction with surface functional groups), entrapping of the solution present in interior pores (physical absorption) and electrostatically absorption (cation or anion exchange to charged functional groups on biochar surfaces). Pollutant’s absorption will predominantly occur in micro and mesopores (Brewer et al., 2014).



Ultra-thin section of co-composted biochar

- **6 Organic coating**
- **7 Biochar**

Figure 9: Organic coating, Hagemann et al., 2017

The presence of microorganisms, especially those who can produce biofilm enhances the retention of crop nutrients. This phenomenon is described as organic coating (Figure 9) and allows an addition of hydrophilicity, of redox interactions and leads to more meso-porosity (Hagemann et al, 2017), on top of the denitrification of nitrate due to denitrifiers metabolism. Microorganisms' colonization is due to the attractive properties of biochar as a habitat as well as a source of carbon. The geometry of the particle determines accessibility of the pores from the exterior and their connectivity, which conditions an autochthonous microbial colonization process, (Brewer et al., 2014; Schnee et al., 2016). In addition, biochar particles can protect microbial denitrifiers against their predators (Palansooriya et al., 2019).

Biochar has been reported to have a screening effect, decreasing the bacterial diversity and increase denitrifying communities from the phylum *Proteobacteria,* especially from the genus *Pseudomonas* (Wu et al., 2019).

2. Hypotheses

Could biochar enhance the nitrate removal woodchip bioreactors and its good ecological status? The aim of this study is to access the effect of biochar introduction on the woodchip bioreactor´s nitrate removal efficiency in the pilot plant, by evaluating nitrate and nitrous oxide fluctuations in the pilot plant and studying the bacterial communities within biochar particles. This was done by testing the following hypotheses:

1. Biochar may remove nitrate through sorption
2. Biochar may act as a hot spot for microbial denitrifiers, hosting bacterial communities and being a source of carbon
3. Biochar may reduce N_2O emissions

Analyses of nitrate removal and nitrous oxide emissions were conducted on set HRT and temperature. pH, DOC, and ammonium were tested to verify that our system did not create unfavorable conditions for the aquatic ecosystem downstream.



Figure 10: Region Midtjylland, rm.dk

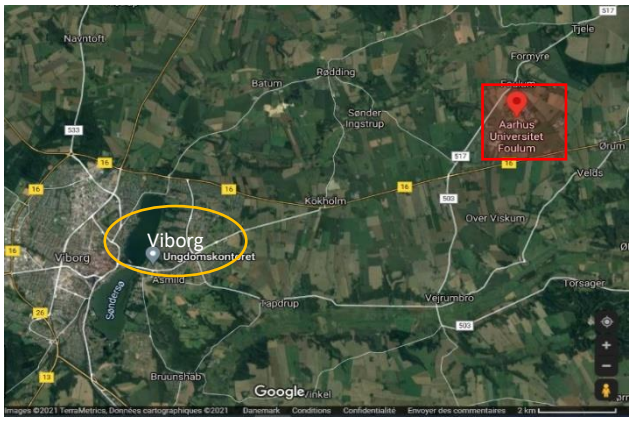


Figure 11: Satellite view of the university and its nearby city, Google Maps, 2021

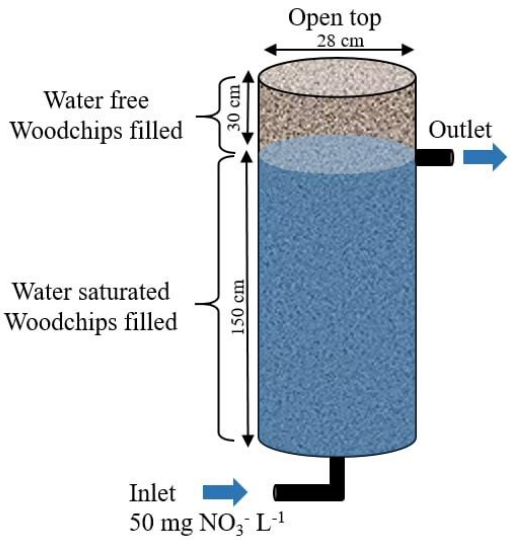


Figure 12: Functionning of a bioreactor, with the courtesy of Arnaud Jéglot



Figure 13: Willow woodchips within a bioreactor



Figure 14: Entry pumps

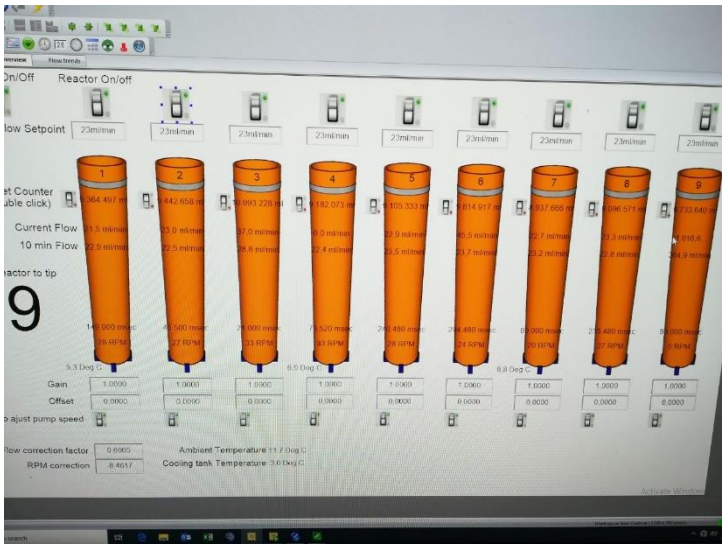


Figure 15: Computer monitoring

3.Materials and methods

3.1 Pilot plant design of the woodchip bioreactors

The pilot plant facility is in Tjele, on the Jutland peninsula, in the Region Midtjylland (Figure 10 & Figure 11). Agricultural production is dominated by livestock farming (Madsen et al., 2010). In this region agricultural fields and infrastructures accounted for 788 961 ha in 2020 and approximatively 60 % its total area (Danmarks Statistik, 2020). Danish towns are municipalities with at least 200 habitants and are considered urban area. 17,5% of Midtjylland reside in rural areas. When agricultural production in Midtjylland accounts for 4% of all jobs, in rural areas 20% of the people are employed in the primary sector (Madsen et al., 2010). A pilot field woodchip bioreactor was also implemented near the university, benefiting from being surrounded by many agricultural fields. Though it is not our focus here, pictures of this facility and a brief description on how it is operating is available in APPENDIX 2.

The material used for the trial was in the basement of a storehouse at Aarhus University Foulum to have cooler ambient temperature, and the possibility to install a cool tank set on 5 °C -to create the similar temperatures than a Danish agricultural outflows during winter. A water tank of the capacity of 12 000 L is used to fill the columns with tap water, in which KNO3- was added at a 50mg NO3- L^{-1} concentration, the European Union maximal concentration allowed for drink water and often found in NVZ (ND91/676/EEC).

The pilot plant is considered as semi-field design, woodchip bioreactors used are columns with an ascending flow: they are between laboratory scale and pilot field design. The flow inside the reactors is vertical and ascendant. The aim is to get closer to the pilot field design than at a smaller scale but be able to better monitor the entries and so test woodchip bioreactors in winter flow simulated situations the hole year-round. It was designed in the context of a PhD thesis and already on site at the beginning of the experiment and preliminary experiments.

Nine columns, or bioreactors, of a volume of 92,6 litres are filled with hardwood willow woodchips (Figure 12 & Figure 13) from the company Ny Vraa Bioenergi based north of Jutland. A computer program (allows to parameter the flow, temperature, rotation per minute, and indirectly the HRT as it is linked to the flow by the equation HRT= V/Q and is connected to pumps for each reactor (Figure 14 & Figure 15)



Figure 16: Water sampling at the INLET



Figure 17: Tipping bucket flowmeter and water sampling of reactor (picture from Lars Elsgaard)

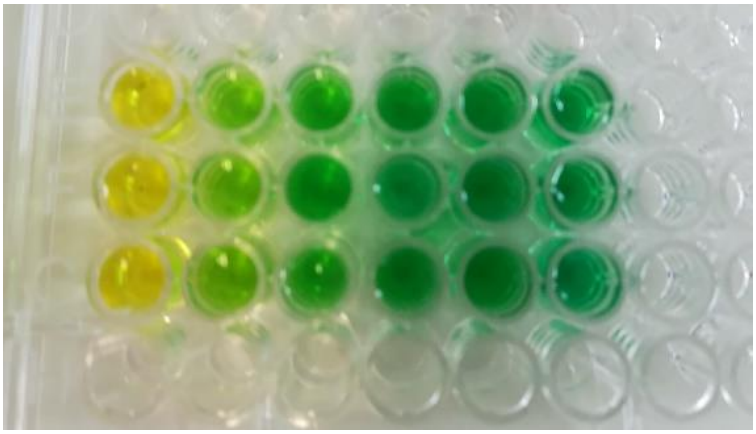


Figure 18: Prepared ammonium samples for the spectrophotometer

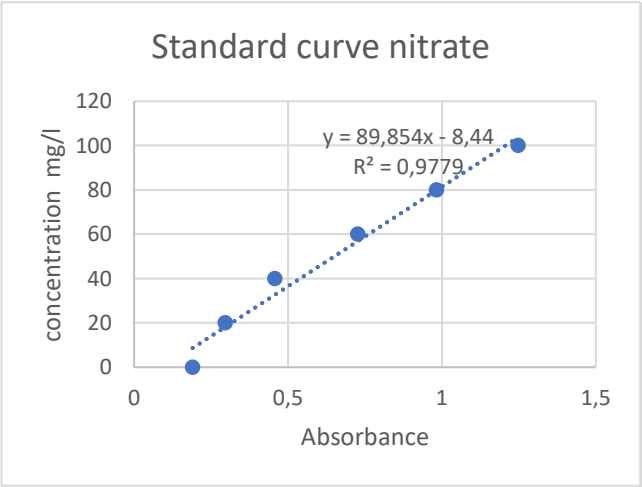


Figure 19: Standard curve for nitrate

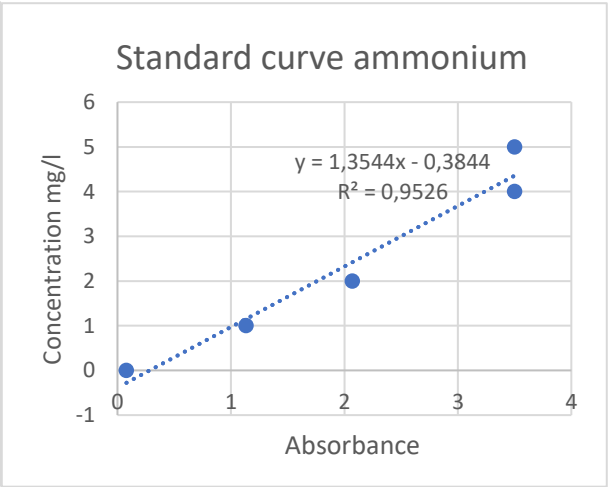


Figure 20: Standard curve for ammonium

3.2 Pilot plant measures of the woodchip bioreactor

The system was not designed nor set up by the author but were already present as an ongoing PhD project, as well as the measuring methods.

3.2.1 Flow

The outlet flow is measured by a flowmeter for each reactor using the tipping bucket technology (Figure 16). A flowmeter was also installed at the entry of the cool tank to be able to know how much water was injected in the system.

3.2.2 Nitrate

Nitrate being the focus, it was the most frequently measured factor, every day or every two weekdays. 10 ml nitrate samples are taken at the exit of the bioreactors and at the entry (Figure 16 & Figure 17). Measurements of absorbance in triplicate were made to determine the concentration using Spectroquant ® Nitrate test kit from the firm Supelco®.

3.2.3 Ammonium

Ammonium was also measured using Spectroquant ® Ammonium test from the firm Supelco®. In our system nitrification of ammonium should not happen, and it has been reported in a 20 years study by Brix et al, 2007 on Danish constructed wetlands. Monitoring ammonium is important to ensure that our system does not release additional nitrite or nitrate in aquatic ecosystems. As we added KNO3- in our system the concentrations of ammonium should remain around 0 mg/L and be equal in inlets and outlets.

3.2.4 Standard curves

Standard curves were obtained using the Spectroquant ® Nitrate test and Ammonium respectively for concentrations ranging from 0 to 100 mg/L and 0 to 5 mg/L and at pace of 20 mg/L and 1 mg/L. Figure 18 shows the variation of colours predetermined concentration. The spectrometer is set on a wavelength of 690 (yellow) where the absorbance of the sample would be close to zero, thus its concentration. Triplicates were always used to minimize the error. + From there, it was possible to establish a relation between absorbance and concentration specific to the experiment.

As in both cases, the R^2 was over 0.95, the equation of the linear curve allows the calculations of concentration for the measured absorbances. (Figure 19 & Figure 20)



Figure 21: Nitrous oxide sampling



Figure 22: Thermic isolation system

3.2.5 Nitrous oxide

Nitrous oxide measurements are crucial to access the greenhouse gases emissions of our woodchip bioreactors. Before sampling hermetic conditions were created by putting a hermetic lid tightly closed with rubber band (Figure 21). Sampling was made with a syringe and the samples were then took to a gas chromameter to measure concentrations in N_2O by the lab technicians of the research centre.

3.2.6 Dissolved Organic carbon (DOC)

DOC is another pollutant resulting from the carbon source and the bacteria metabolism. Therefore, measurements allow to access the pollution caused by the woodchip bioreactors. 50 ml samples are taken at the entry and exit of the bioreactors using syringe filters to prevent the entering of bacteria colonies and debris. It was then handed over to lab technicians.

3.2.7 pH

pH is an important to consider to for two mains reasons: it needs to allow microbial growth and not endanger the aquatic ecosystems downstream. 15 ml samples were taken both for the inlet and outlet and analysed with probe apparatus

3.2.8 Temperatures

Temperatures at the cool tank were kept under 5°C, however the temperatures within the bioreactors were not measured during the experiment, but in the pilot plant facility room, within the cool tank and three thermometers were placed. Isolation was installed in winter, as part of the PhD thesis project, to avoid the water to freeze, the isolation also allowed, in combination with being in a cellar to avoid drastic temperature changes in the summer (Figure 22)



Figure 23: Biochar applied inside the bioreactors

3.3 Introduction of biochar

3.3.1 Characteristics of the biochar

The biochar was originally planned to be produced out of fresh willow woodchip that were available at the pilot plant facility. This would have had the advantage of been able to precisely trace the raw material as well as define the optimal size and pyrolysis for the biochar particles. Moreover, Biochar derived from wood contains more carbon than those obtained from herbaceous sources (Tan et al., 2018) and a stabler structure.

Unfortunately, due to time constraints, and the context of a global pandemic, an alternative had to be found. Instead, we bought 10 kilograms of spruce biochar produced by the Finnish company Carbofex. This biochar has the EBC (European Biochar Certificate) quality label, elaborated by the European Biochar Industry Consortium (EBI). This means the producer must send samples to an EBC accredited laboratory to be analysed and be below thresholds for water soluble phosphorous, total potassium, total nitrogen, heavy metal, etc. The production needs to meets those criteria:

- the incoming goods must be plant biomasses inspected and documented, the risk associated with the use of non-certified biochar could also come from sludge, livestock, municipal waste, certain biogas digestates or bones, and contain dangerous pollutants such as paint residue, medication residues… (EBC guidelines, 2021). The spruce was produced in an 80 kilometres perimeter from the biochar facility and is approved for organic production.

- The technology for pyrolysis must be specified. Carbofex biochar´s HTT is 600 °C. There is no consensus at to what the best HTT for biochar is, but if the aim is to allow bacterial colonization, lower HTT are more efficient. The HTT of the biochar used for the trial is not optimal for bacterial colonization but still is in a range that would allow it. It will however be richer in mesopores which will allow more nitrate sorption (Tan et al., 2018). The biochar amount used in this trial is shown on Figure 23.

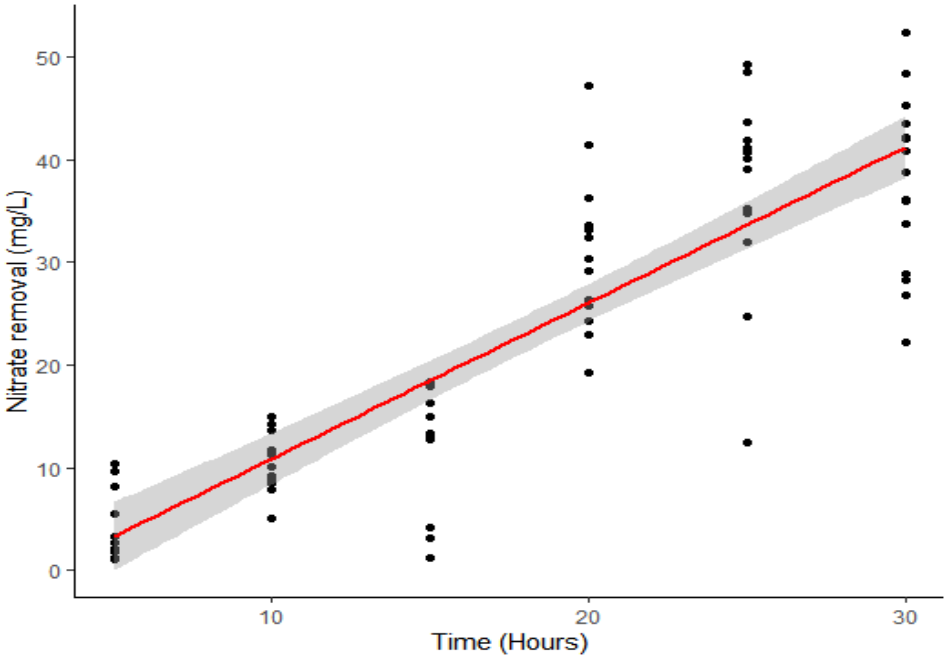


Figure 24: Impact of HRT on nitrate removal

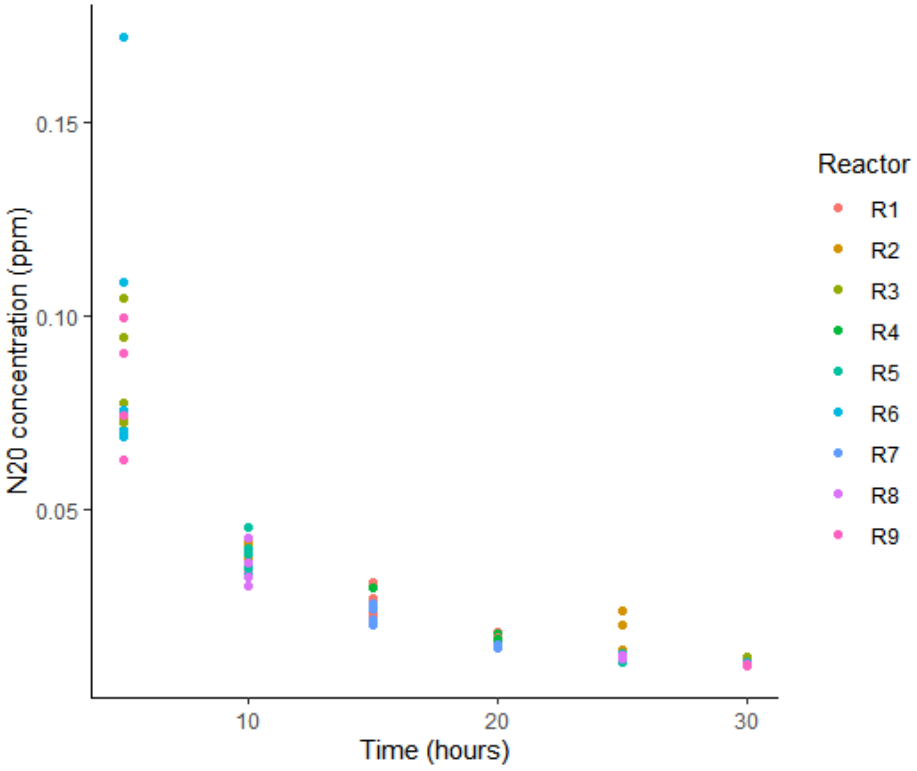


Figure 25: Impact of HRT on N_2O emissions

3.3.2 HRT settings

As a preliminary trial and part of an ongoing study, HRT settings were assessed between the 15th and the 25th of March 2021. This allowed to confirm that the relationship between HRT and Nitrate Removal was linear, and that HRT is likely to be a key factor in denitrification in our system (Figure 24).

It was also noticed that an HRT of 20 hours has good rates and would be a nearly optimal HRT for the system regarding N_2O emissions. However, in winter flow can be important and though HRT much shorter than 20 hours (Figure 25). A 15-hour HRT was chosen as a compromise between the optimal conditions and too stressful ones which would make N_2O emissions too difficult to see and necessitate more water to be filled in the tank.

3.3.3 Preliminary flow characterization with bromide

As part of an ongoing thesis project and to help have a better understanding of the flow BrNA was used as a tracer between the 21st and 25th of June 2021. The HRT was set on 15 hours. The aim of this tracing experiment is to compare the set HRT with the actual HRT in the bioreactors.

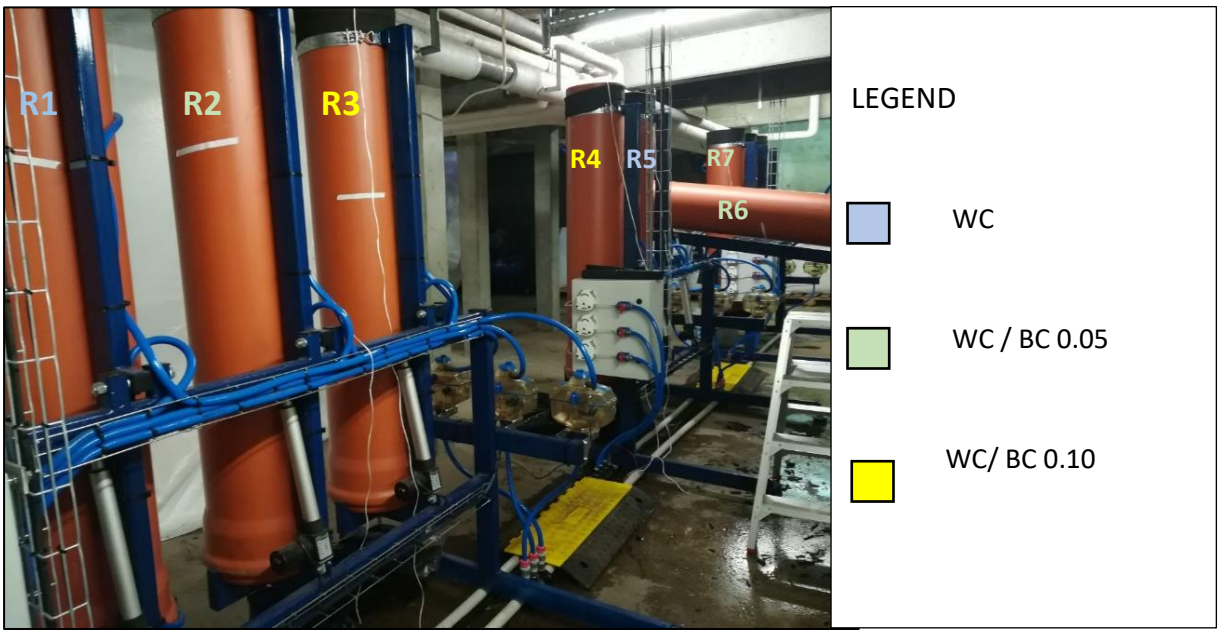
BrNa was injected into the inlet then samples were taken every hour or every 2 hours from each reactor outlet for during the first two days were the peak or the breakthrough curve was to be expected, then less and less frequently. Unfortunately, the results were not available upon the submission of this report. Such a curve can be found in literature and provided in APPENDIX 3. Even though no water was running in inlet and the reactors were supposed to be empty if only looking at the preset conditions on the computer, there was still water when the reactors were emptied for the installation of the woodchip/ woodchip+ biochar experiment.

3.3.4 Installation

The woodchips used were present within the bioreactors since December 2020 or January 2021 for previous trials. They can be considered as weathered woodchips as their peak of carbon release has already passed. This is because eliminating a flush of DOC that would drastically increase bacterial activity was seen as more reliable in the interpretation of the results. (Rivas et al, 2019).

What is more, the aim was the improvement of a pre-existing system with pre-existing microbial communities.

The total mass of Carbofex's Biochar used for this experiment was 6, 4 kg. We measured the volumic mass of woodchip bioreactors in our system, 153 g/l and from the information delivered by Carbofex we deduced the quantity needed for 5% and 10% of biochar in mass as its volumic mass is different (330 g/l)



WC =Woodchips; BC= Biochar 0.05 and 0.10= mass proportions

Figure 26: Trial pseudo-randomized repartition of the treatments

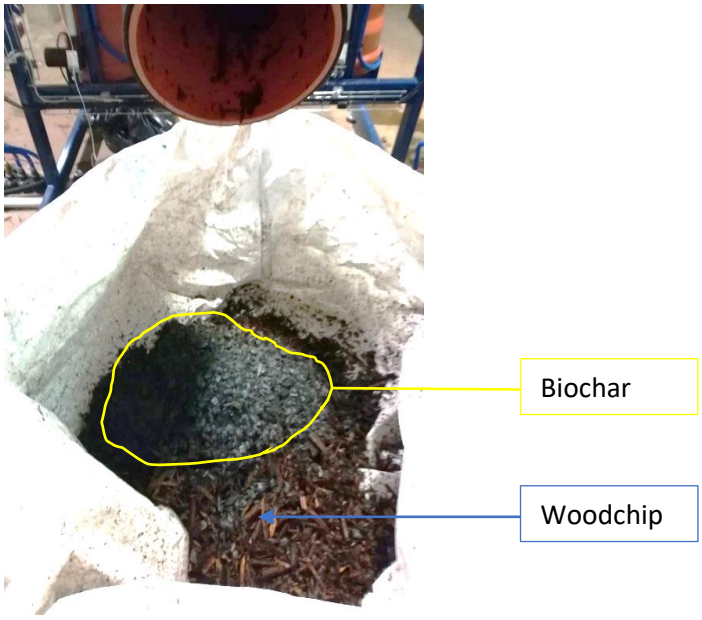


Figure 27: Emptying a bioreactor and addition of biochar



Figure 28: Permeable sampling bag

The trial of the 28th of June 2021 run at 5 pm and is considered day 1 of the experiment. It was originally planned to start 3 weeks earlier but delayed due to shipments related issues of the biochar.

It lasted 6 weeks until the 13 of August. Installation was done by the internship supervisor, his PhD Student, and the author. Each reactor was emptied into a big bag and then mixed to create perturbations among the bacterial communities, not just in the bioreactors receiving biochar but also the control to homogenize the method. We added biochar in two triplicates: 5% and 10% of the volume of the woodchip bioreactors (Figure 27).

As visible on Figure 26, the 9 bioreactors are divided into three compartments. The modalities were pseudo-randomized:

- Reactors R1, R5 and R9 were filled with only woodchips (WC)
- Reactors R2, R6 and R7 were filled with woodchips and 5% biochar (WC / 0.05)
- Reactors R3, R4 and R8 were filled with woodchips and 10% biochar (WC / 0.10)

It was also originally planned to incubate samples of each reactor at four weeks after the beginning of the trial, thus inserting a permeable bag (Figure 28). This project was later abandoned due to lack of time.

3.3.5 Measurements during the biochar addition trial PART 1

All samples were taken by the author, both at the inlet and the outlet from the 29th of June to the 9 of July.

Being the focus of our study, nitrate samples are taken every weekday, at the same hour (9 am). Unfortunately, there was not enough of the Spectroquant ® Nitrate test kit from the firm Supelco® to test all taken samples on the day they were taken, due to shipment delays and change of regulations.

Improvement of nitrate removal is also linked to oxygen depletion. As we have emptied the reactors to mix the woodchip a lot of oxygen has be introduced in the system, we have decreased the nitrate removal rate, as denitrifying bacteria are mostly facultative aerobes. Having sufficient data on the dynamics of the first days is important better understand future results.

N_2O samples of every bioreactor were taken 3 times a week at t0=9 am and t1=10 am. Sampling was done by putting 12 ml of gas within the syringe and compressing it into 10 ml vials. We will analyse the results using the same statistical methods than for nitrate.

Ammonium samples were first taken the first of July, both at the entry and at the exit of the bioreactors. We will not make a full statistical analysis on ammonium, because it is not the prime subject of this study, however we will verify that our system does not produce NH_{4+} that would be detrimental to the aquatic ecosystem downstream.

Because the pH of biochar is high (10) it is important to ensure that it won´t cause a drastic increase in pH of our system as it becomes less alkaline and therefore more hospitable for bacterial colonies to grow.

DOC samples of the outlet of each reactor were taken weekly in 50 ml tubes to ensure is not detrimental in regards of organic carbon being released into the outflow.

3.3.6 Measurements during the biochar addition trial PART 2

Due to the unexpected leave of the author, this experiment is divided into 2 parts, Part 2 covering results from the 14th of July 2021 to the 13th of August.

Water samples were taken every weekday and stored in a -18°C refrigerating room. Nitrate and ammonium analysis was made through an autoanalyzer.

N_2O analysis was made less frequently but in the same conditions as in part one and will therefore not be divided into two parts in the result section.

3.3.7 Statistical analysis

Nitrate removal was calculated this way :

Nitrate removal= *NO_3^-inletDay(x) - NO_3^-outletDay(x)Reactor(n)*

where *NO_3^-inletDay(x)* was the nitrate concentration of the water entering inside the reactors, with one measurement per *Day(x).* This is because the inlet could only be measured at one area (Figure 16). *NO_3^-outletDay(x)Reactor(n)* was the nitrate concentration of the water coming outside of the reactors and was measured for the 9 bioreactors of the trial.

Samples were taken 7 times during , measuring the impact of 3 treatments, and measured in triplicates in the spectrophotometer. We have therefore 62 observations which enables us to make statistical analyses such as ANOVAS in this time frame. We will also verify the normality (Kolmogorov Smirnov) of the observations and the residuals, outliers, as well as the homoscedasticity (Q-Q plot; see APPENDIX 4 & 5), Part 1 and Part 2.

For nitrous oxide we were not able to use ANOVAs because the hypothesis of normality of the residuals was not verified, in fact the Kolmogorov Smirnov was test resulted in a p-value of 0.03478, even after the removal of the values of day 46, which were aberrant. Kruskal-Wallis (see APPENDIX 6) was used as an alternative to a One-Way ANOVA.

All calculations were made using the software R (see code in APPENDIX 7)

4.Results

4.1 Nitrate Removal

4.1.1 Nitrate Part 1

Between the 29th of June to the 9 of July, no observation of significant differences was made regarding nitrate removal. In fact, the p-value of both unifactorial and bifactorial ANOVAS (Table 1 & Table 2, Figure 30 & Figure 31) was close to 1. Interestingly, though non-significant, the effect of time on the bioreactors is greater as the p-value is much smaller. This can partly be explained by a problem of mixing within the cool tank leading to fluctuations in the nitrate concentration. Still, the bioreactors are less fluctuations and well below the 50 mg/l of NO3- as demanded by the EU commission. Based on these results, even in a short period of time we can say that our system is resilient and working (Figure 32).For half of the observations, the nitrate removal was equal or superior to 55% (Figure 29).

4.1.2 Nitrate Part 2

Between 14th of July 2021 to the 13th of August, results showed no significant difference between treatments, however there is a very significant impact of time within the system (p-value 4.027e-06 ***, Table 3)

The reasons as to why the results vary so much from day to day are unclear, so we can only make hypotheses from there. A changing schedule or method of testing could be a way of explaining those differences as temperatures may vary throughout the day, so does the denitrification rate. In fact, due to the author's leave several members of the university took samples at various times of day. Outside temperatures were higher in Part 2 than in Part 1, similar nitrate removal concentrations were observed, however proportionally there was an increase of the median value to 0.65 (Figure 33). What is more, there was a flooding incidence on the reactor 7.

Table 1: One way ANOVA on Nitrate removal (mg/l) Part 1

	Df	Sum of Sq	RSS	AIC	F value	Pr(>F)
residuals			2804.9	242.34		
nitrate.removal$Treatment	2	4.3273	2809.2	238.44	0.0455	0.9555

Table 2: Two-way ANOVA on Nitrate (mg/l) Part 1

	Df	Sum of Sq	RSS	AIC	F value	Pr(>F)
residuals			2752.7	243.18		
nitrate.removal$Treatment	2	3.700	2756.4	239.26	0.0390	0.9618
nitrate.removal$Day	1	52.237	2804.9	242.34	1.1007	0.2985

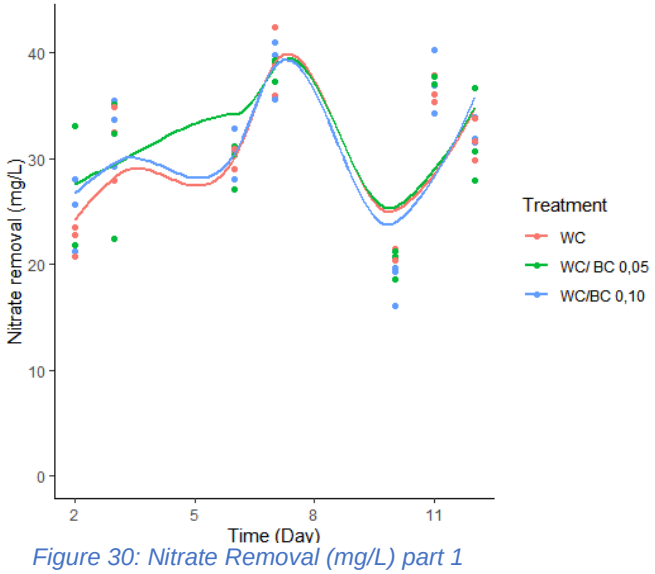


Figure 30: Nitrate Removal (mg/L) part 1

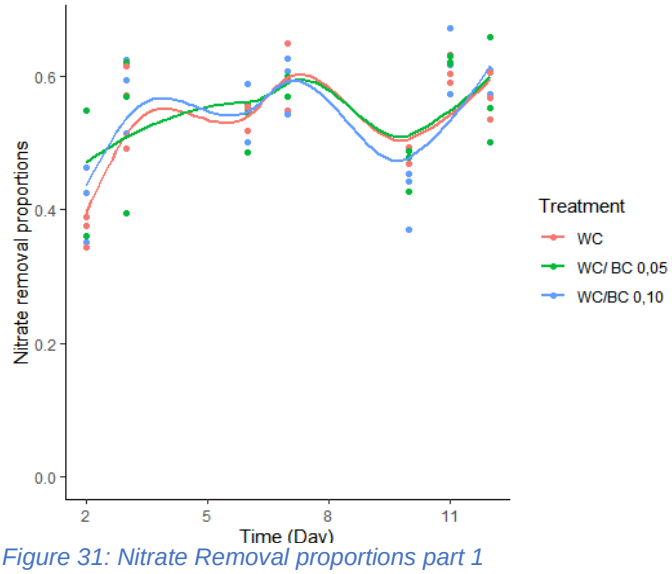


Figure 31: Nitrate Removal proportions part 1

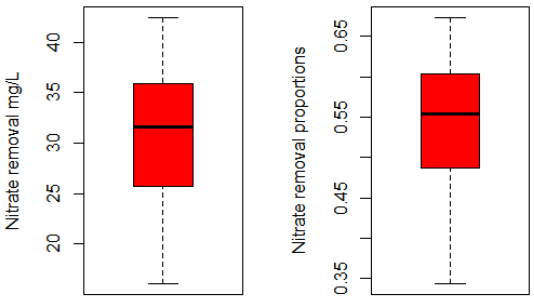


Figure 29: Nitrate Removal Boxplot

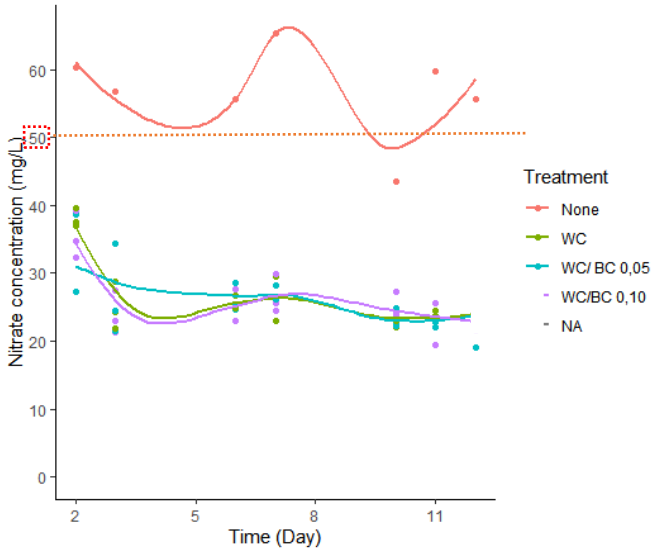


Figure 32: Nitrate concentrations (mg/lL) part1

Table 3: Two-way ANOVA on Nitrate (mg/l) Part 2

	Df	Sum of Sq	RSS	AIC	F value	Pr(>F)
residuals			3635.6	511.97		
NitratePart2$Treatment	2	51.84	3687.4	510.26	1.1264	0.3268
NitratePart2$Day	1	525.17	4160.7	531.83	22.8237	4.027e-06 ***

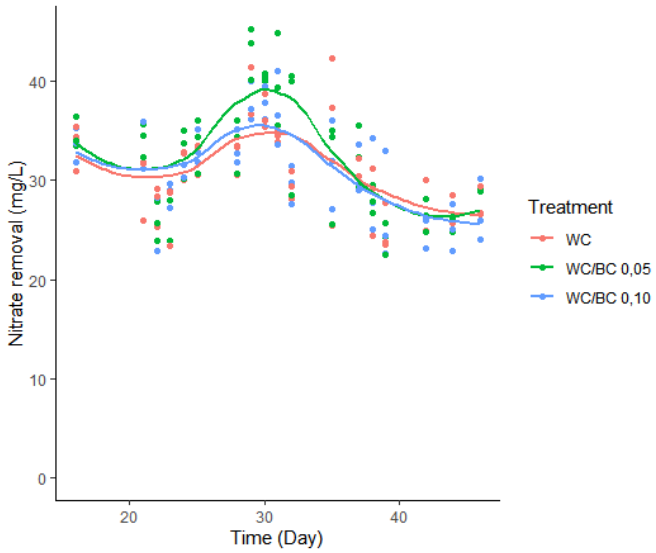


Figure 34: Nitrate removal (mg/L) Part 2

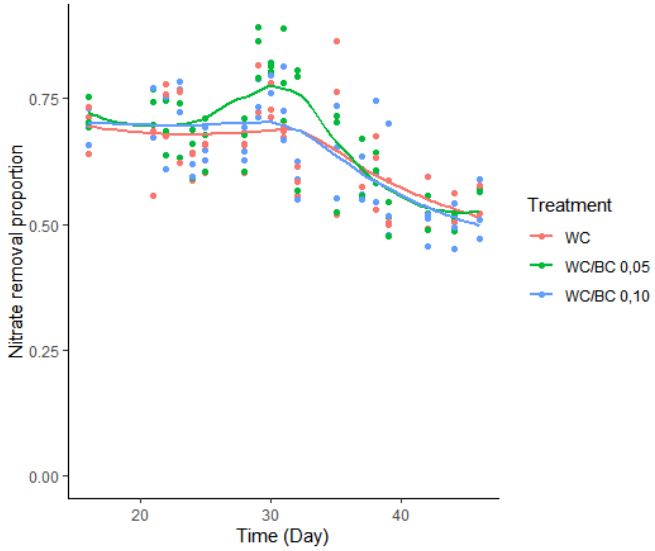


Figure 35: Nitrate removal proportions Part 2

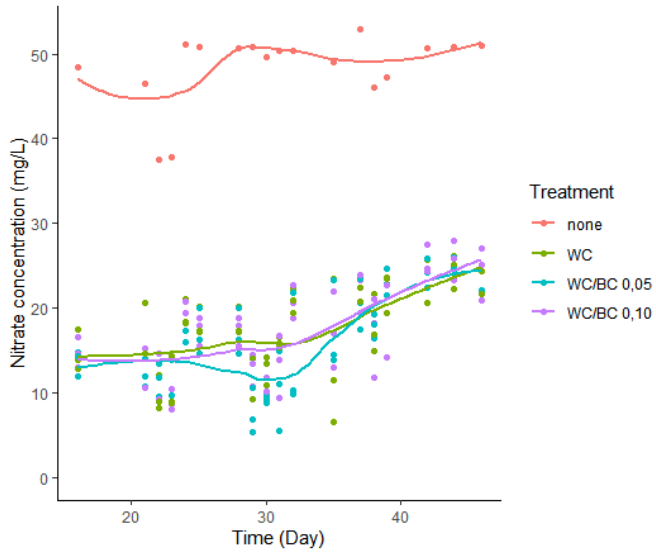


Figure 36: Nitrate concentrations (mg/L) Part 2

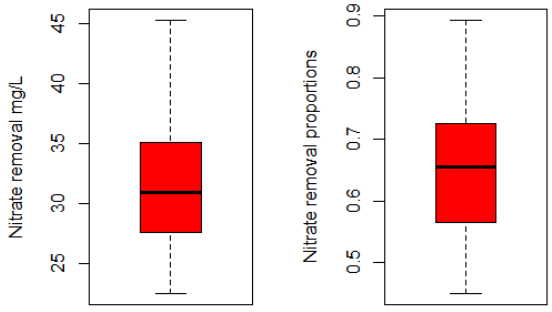


Figure 33: Nitrate concentrations Part 2

4.2 Ammonium

4.2.1 Ammonium Part 1

Between the 29th of June to the 9 of July, ammonium was scarce or not present. The first of July, there was one occurrence of high concentration in the reactor 9, with a clear shift in coloration, following sampling of that reactor showed no significant difference with the other replicates, and the inlet having very low to no ammonium concentrations (Figure 37 & Figure 38).

4.2.2 Ammonium Part 2

Between the 14th of July and the 13th of August, ammonium was partly not detectable by the autoanalyzer because it was in very low concentrations. Occurrences of higher ammonium concentrations were scarce and with a median value of 0.03 mg/l and a mean value of 0.0315 mg/l (Figure 39).

4.3 Nitrous oxide

Three tests were conducted on the effect on treatment, reactor, and day on the nitrous oxide concentrations (Table 4). The effect of treatment was found to be significant, with a p-value of 0.01601. Dunn-test (Table 5) serve a similar purpose than a Tukey test and we were therefore able to determine that there was a significant difference between WC treatments and WC/BC 0,05 treatments.

However, the differences of concentrations between reactors were way more significant than the influence of treatment with a p-value of 0.007995. This heterogeneity as represented on Figure 40 is to consider for further analyses. Figure 41 shows us that although most values are slightly below 0.5 ppm, there are several of aberrant values reaching up to 2.5 ppm.

The variation of N_2O concentrations with was not significant but still, the p-value was found to be low (0.06523). It was therefore interesting to test our dataset including the aberrant values of day 46. It was found that in that case the impact of time was very significant on N_2O concentrations with a p-value of 0.003366.

With no normal distribution of the dataset, the huge heterogeneity of the reactors, as well as a likely effect of time, we cannot conclude for sure that there is an impact of biochar on nitrous oxide, even if the treatment p-value is significant.

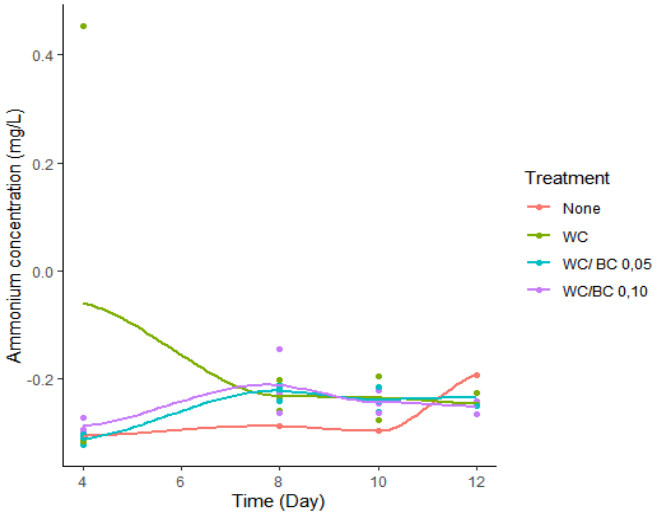


Figure 37: Ammonium concentrations (mg/L) calculated with the standard curve (Figure 20) Part 1

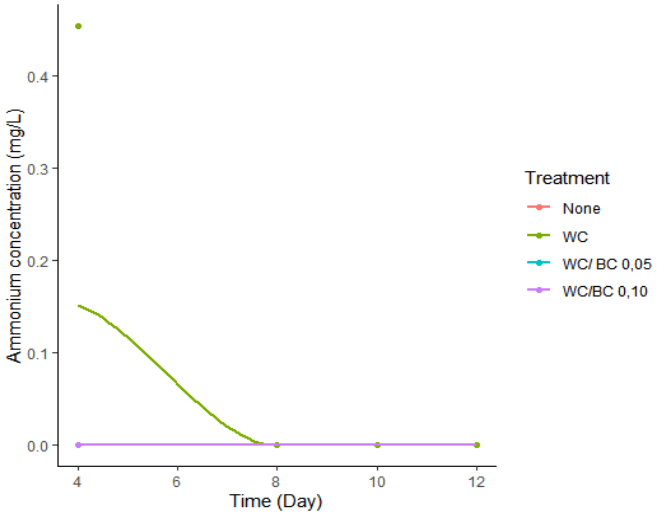


Figure 38: Ammonium adjusted Part 1

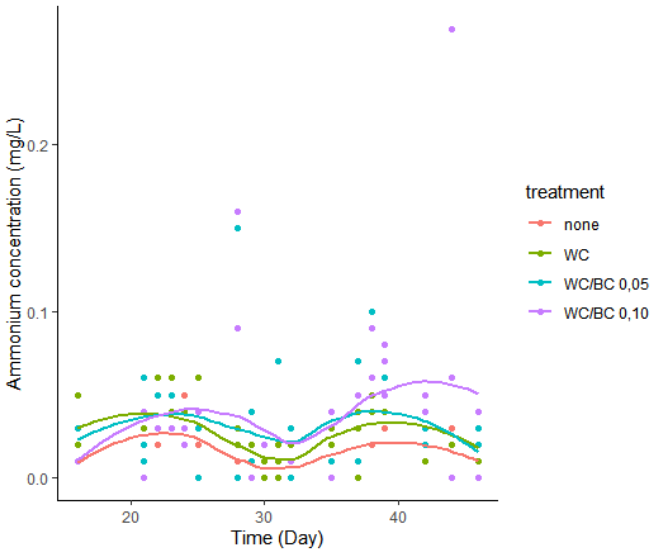


Figure 39: Ammonium concentrations (mg/L) Part 2

Table 4: Kruskal-Wallis tests: influence of treatment, reactor, and day on N_2O concentrations

	Chi-squared	Df	p-value
Treatment	8.2688	2	**0.01601**
Reactor	20.698	8	**0.007995**
Time	11.857	6	**0.06523**

Table 5: Dunn-test of the impact of treatments on N_2O concentrations

Comparison	Z	P.unadj	P.adj
WC - WC/BC 0,05	2.874795	0.004042902	**0.01212871**
WC - WC/BC 0,1	-1.380575	0.167409691	0.50222907
WC/BC 0,05 - WC/BC 0,1	-1.494220	0.135118170	0.40535451

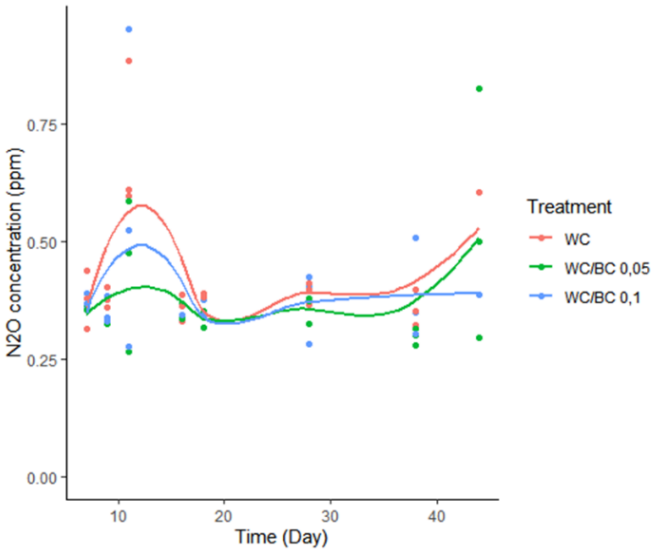


Figure 40: N_2O concentrations (ppm)

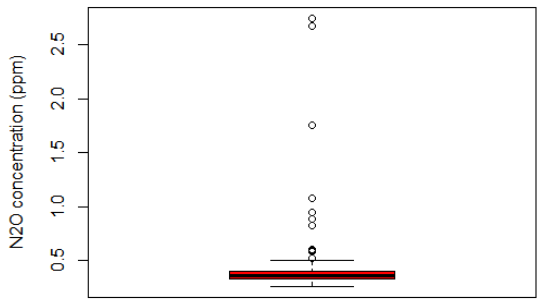


Figure 41: Boxplot N_2O concentrations (ppm)

Table 6: DOC concentrations (mg/l) on the 8th of July

R1 WC	R2 WC/BC 0.05	R3 WC/BC 0.10	R4 WC/BC 0.10	R5 WC	R6 WC/BC 0.05	R7 WC/BC 0.05	R8 WC/BC 0.10	R9 WC
2,4	2,63	3,53	2,61	3,01	3,36	3,28	4,01	3,07

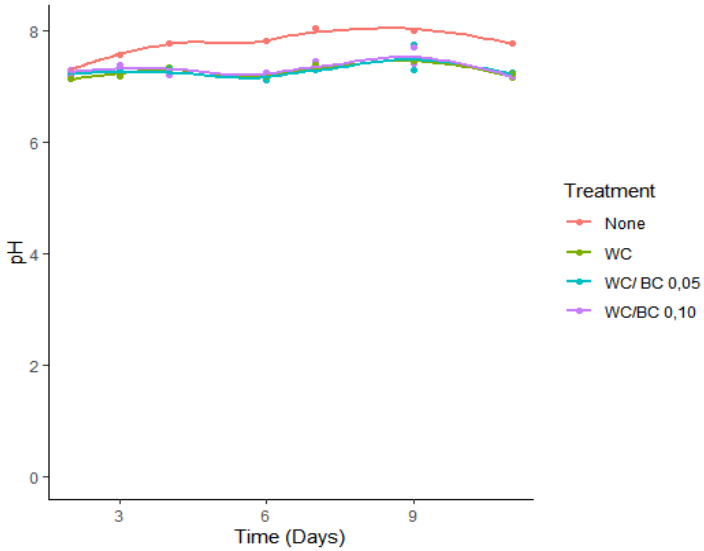


Figure 42: pH values according to treatment

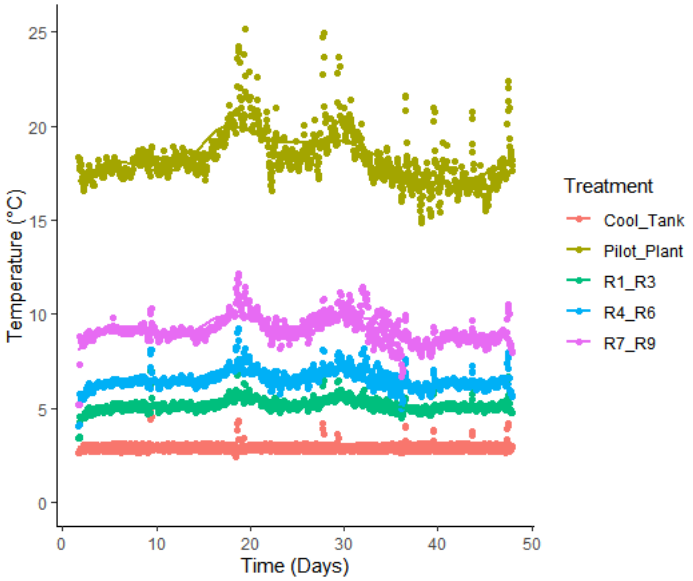


Figure 43: Temperature overview during the trial: cool tank, pilot plant, and reactors

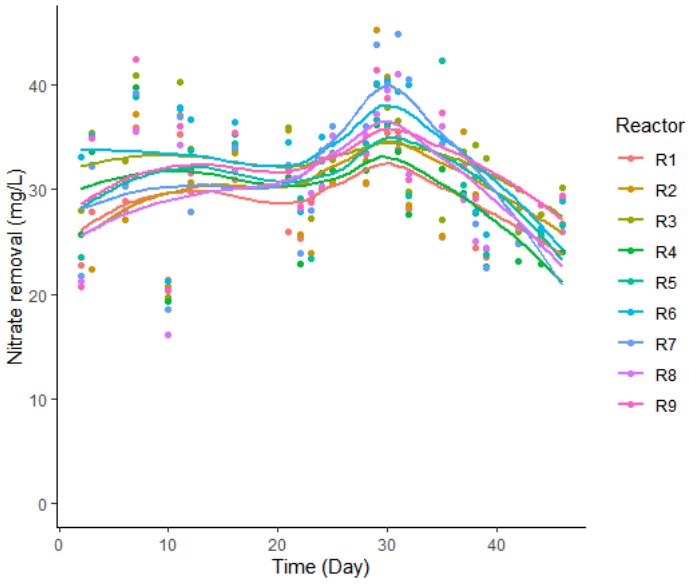


Figure 44: Nitrate removal overview during the trial: reactors

4.4 DOC

DOC results were provided by the laboratory only for one sampling day: the 8th of July 2021 (Table 6), with an average of 3,01 mg/L at the reactors outlet. Organic matter levels in fresh waters typically range from 0.1 mg/L to10–20 mg (Volk et al., 2002), and are close to studies conducted in Britany, another European NVZ (Dupas et al., 2021) and a below 5mg/l so this value reflects a good water quality regarding the DOC (Syndicat Mixte Oise Ardennes, Agence de l'eau, Eau Seine Normandie 2017) From this single measure we cannot conclude if our system is polluting the downstream aquatic environment with DOC, but we have at least some insights

4.5 pH

Data on pH is only available at the beginning of the trial from the 29th of June to the 7th of July. An unexplained slight water alkalinization was noticed within the cool tank. pH at the outlet rose at the same rate than the inlet (Figure 42). However, the bioreactor's woodchips lowered it by being more acidic than water. On the ten days that pH was measured, its values were favorable for denitrification within the bioreactor as well as for aquatic ecosystems downstream. The pH of the biochar in our system is 10. A rising pH at the outlet could have been noticed over time, as a sign that the biochar's pH is getting lower,+ but it was not the case in the system.

4.6 Temperature

Temperatures fluctuated a lot during the trial, with much higher temperatures than those that could be expected of a drainage season in Denmark (Figure 43). Air temperatures 17 and 25°C, and differences have been observed between the repetitions, R1-R3, R4- R6, R7-R9. The pipe supplying the reactors was not thermally insulated and was therefore subjected to the temperatures of the facility. Reactors 7,8 and 9 being the furthest from the source their temperature in entry was much higher.

Temperatures have been known to greatly impact bacterial communities (Addy et al, 2016).

However, there is no clear correlation between higher temperatures and higher nitrate removal rates, as shown on Figure 44 where the nitrate removal function Part 1 and Part 2 is not like the temperature fluctuations over the timeframe of the study. Further statistical aspects between temperature and nitrate removal were not explored as they were not central to the study.

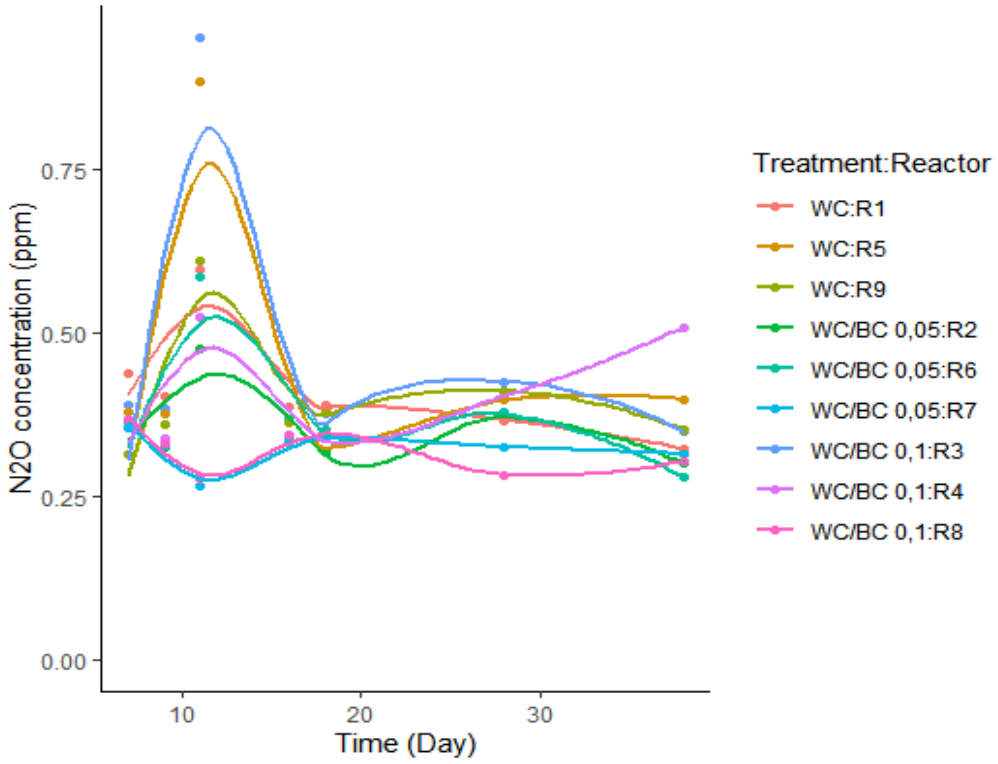


Figure 45: Nitrous oxide concentrations (ppm) per reactor

5. Discussion

5.1 Results in the context of a “semi field pilot plant”

The columns used for this experiment were much bigger than those used in labs and were meant for longer and larger trials but less monitored to be a transition between the lab scale and the field scale. However, no example of such designs was found in literature. Though being innovative, and setting a precedent, it also complicates the exploitation and potential application of the results.

Therefore, we initially planned to also run a lab scale incubation experiment to better understand the behavior of each bioreactor regarding nitrous oxide. The permeable sampling bags (Figure 28) would have been put in an anoxic controlled environment, for example airtight bottles for a week. We could have then more precisely measure the quantities of N_2O produced for each reactor and by extension of the treatment. Measures of cumulated emissions during the incubation trial might have given more insight on the efficiency of the treatments (Pacholski et al., 2010).

The impact of treatment on nitrous oxide was found to be significant with a p-value of 0.01601. However, it is hard to make any conclusion on its efficiency when the differences between the bioreactors with a p-value of 0.007995 (Figure 45). As seen in *Materials and Methods,* three bioreactors form a triplicate for each treatment, to have more reliable results. But, when bioreactors of the same treatment greatly differ it is necessary to be cautious.

Many factors could have played a role in the heterogeneity of the reactors. Error could have occurred in sampling, especially as there were different experimenters who sampled at different times of day, and different frequencies (the last measures were taken every 10 days). Temperature in inlet differ within each triplicate and could also be a source of variation. There is also a big unknown as to which bacteria were present in each column.

The Dunn-test has shown that only WC and WC/BC 0.05 were significatively different, and that it has lower nitrous oxide concentrations than woodchip alone. All that can be said is that the biochar probably had some lowering effect in nitrous oxide concentrations.

The impact of biochar on nitrate removal in our system was clearly non-significant. In Part 1 (from the 29th of June to the 9 of July) the One-way ANOVA on nitrate removal, testing treatment, had a p-value of 0.9555 and the Two-way ANOVA on nitrate removal, testing treatment and time, had a p-value of 0.9618 for the impact of treatment.

In Part 2 (from the 14th of July 2021 to the 13th of August) the p-value of the Two-Way ANOVA on nitrate removal testing treatment and time, found a p-value of 0.3268 and a very significant effect of time with a p-value of 4.027e-06. The impact of time could again be a combination of varying temperatures, errors in sampling and experimental methods as well as unknown bacterial communities and activities.

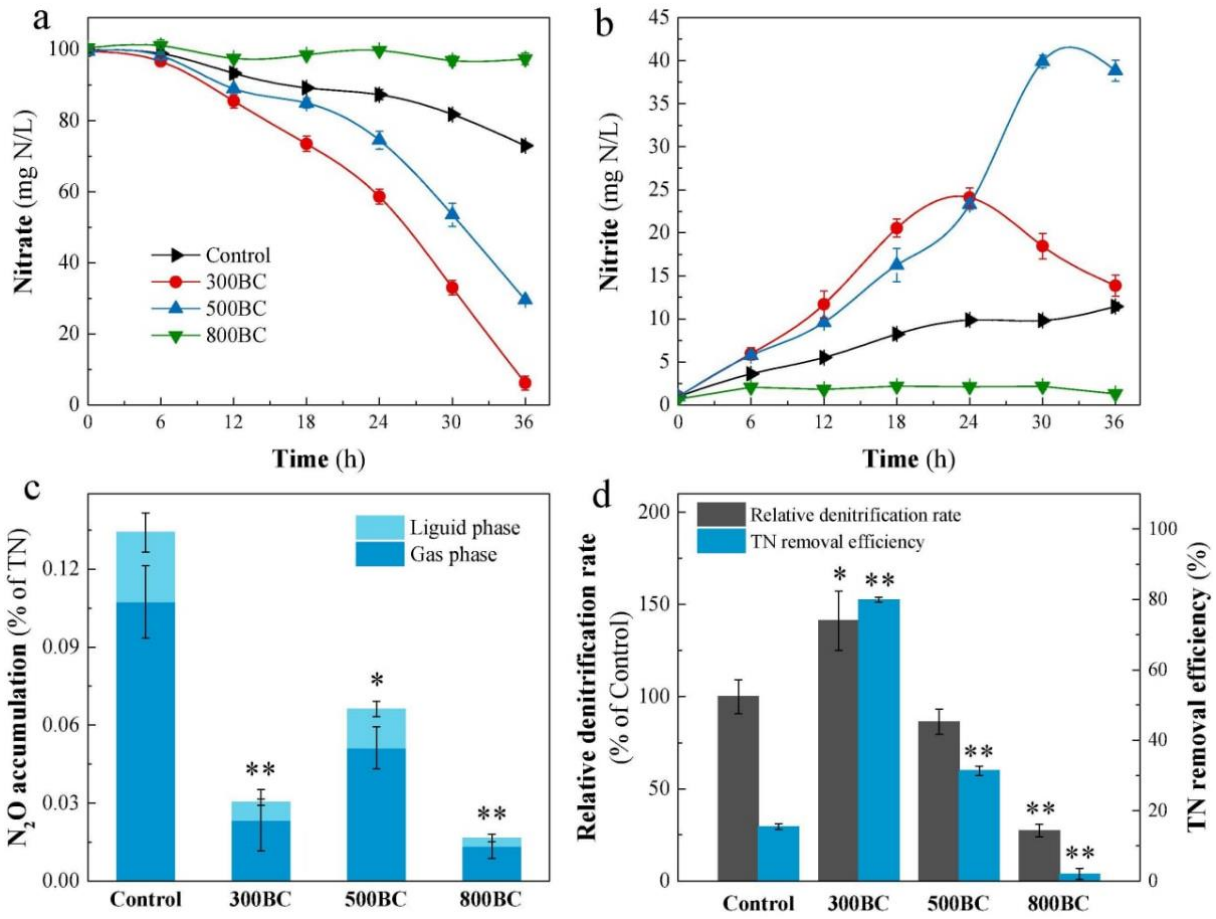


Fig. 1. Effects of biochars on NO_3^--N reduction (a), NO_2^- accumulation (b), N_2O accumulation (c), relative denitrification rate and total nitrogen removal efficiency (d) during denitrification process in 36 h experiment. Error bars represent the standard deviations of triplicate tests. Asterisks indicate significant differences with control (one-way ANOVA, $^{*}p<0.05$, $^{**}p<0.01$).

Figure 46: Main results Wu et al., 2019

5.2 Comparison with published studies

In Wu et al, 2019, biochar had a significant impact on nitrate removal. The aim of this study was to determine the influence of the biochar's HTT on nitrate removal. The trial was lab scaled and lasted 36 hours. Biochar was added in an anaerobic environment, with sludge and added glucose. The study showed promising results for both nitrate removal and nitrous oxide emissions for biochar made of cattail with an HTT of 300°C and 500°C. Nitrate removal even increased by 416% with the biochar with an HTT of 300°C. One of the reasons is a screening effect the biochars with lower HTT being more favorable for genus such as *Pseudomonas*, well known denitrifiers. The biochar with an HTT of 800°C, had the opposite effect, with nitrate removal results lower than the control. All the modalities with biochar very significatively reduced N_2O emissions (Figure 46).

Although the time frame and settings are very different than our trial, a way of improving nitrate removal could be to test the impact of biochar with low HTT temperatures.

On the other hand, a study conducted by Coleman et al., in 2019 observed that biochar failed to enhance nutrient agricultural excess following saturation. They designed laboratory scaled horizontal columns with varying HRT and nutrients loading rates. Their conclusion was that woodchips were able to successfully at removing nitrate and the impact of biochar was non-significant in their study.

In their meta-analysis on arable soil, Borchard et al., 2018 showed that biochar reduced N_2O emissions by 38% for the first year but then tended to be negligible the next year. This could question the sustainability of adding biochar both economically and environmentally speaking as pyrolysis requires a great amount of energy. Regarding nitrate removal, the results were heterogeneous but found that while nitrate concentrations in the soil remained unaffected, leaching was reduced by 13% with biochar applied on the fields. Unfortunately, no similar meta-analysis was found for biochar's properties in agricultural downstream during the time of this study.

There is a great heterogeneity of results within studies regarding the efficiency of biochar regarding nitrate removal, there is no consensus among the scientific community (Coleman et al., in 2019). In our study we found no significant improvement of denitrification when adding biochar.

Biochar's ability of reducing N_2O emissions is still not clear, though more studies described a significant decrease of N_2O emissions when implementing biochar into their systems, which is also coherent with our results.

5.3 Full scale Danish woodchip bioreactors and policies

In full scale woodchip bioreactors using willow, nitrate removal means of 50 % have been recorded (Hoffmann et al., 2020). Even if woodchip bioreactors are relatively low cost and low tech, they are an investment in term of price of the woodchips and surface area needed to implement those projects. What is more, they do not increase macro-biodiversity, contrary to integrated buffer zones (IBZ) or restored riparian wetlands, nitrate mitigation measures focusing more on the ecosystem. In a nutshell, woodchip bioreactors are not the one and only nitrate mitigation measure to go for.

To be in accordance with the EU Commission, nitrate mitigation systems must be planned in a precise legal framework. However, it has been concluded that the Danish sector laws contain a series of flaws, for instance limits of trivial matters regarding regulation (Maalgaard et al., 2016, Jensen et al., 2004) and is still a controversial topic as of today.

6. Conclusion

Woodchip bioreactors have been found to be an efficient nitrate mitigation measure in literature, empirically in the fields, and in this study. Nitrate removal in this study was on average 61,76% with a median value of 61,02%, during the six weeks of the trial.

The improvement of nitrate removal by adding biochar has yet to be seen in a similar system because it was clearly non-significant, with a p-value of 0.9618 between the 29th of June to the 7th of July 2021 and a p-value of 0.3268 between the 14th of July and the 13th of July 2021. In the six weeks of the trial, no significant difference was witnessed. With limited time and quantities of biochar, there is no definitive answer to the biochar enhancing the system. Would the trial have lasted longer; it is unknown if biochar would eventually have been a host to denitrifiers. Not knowing the microbial communities and how and if they interact with biochar is one of the biggest limits of this study. In fact, we could not explore our hypotheses about biochar being a hot spot for microbial denitrifiers and being a source of carbon nor if they remove nitrate through sorption in our system.

A significant effect of biochar has been found in reducing N_2O, however with heterogeneity of results between the reactors, as well as aberrant values over time it is impossible to reach any conclusion as to the real impact biochar had on N_2O emissions, only that it might have had a reducing effect, which is coherent with our hypothesis that biochar may reduce N_2O emissions.

Further trial and studies will be necessary to assess if the combination of woodchip and biochar is more efficient in bioreactors than woodchip on its own regarding nitrate removal. It is also essential to have an estimate of the N_2O emission reducing impact, to enable decision makers and farmers to weigh the costs and benefits of implementing biochar in woodchip denitrifying systems.

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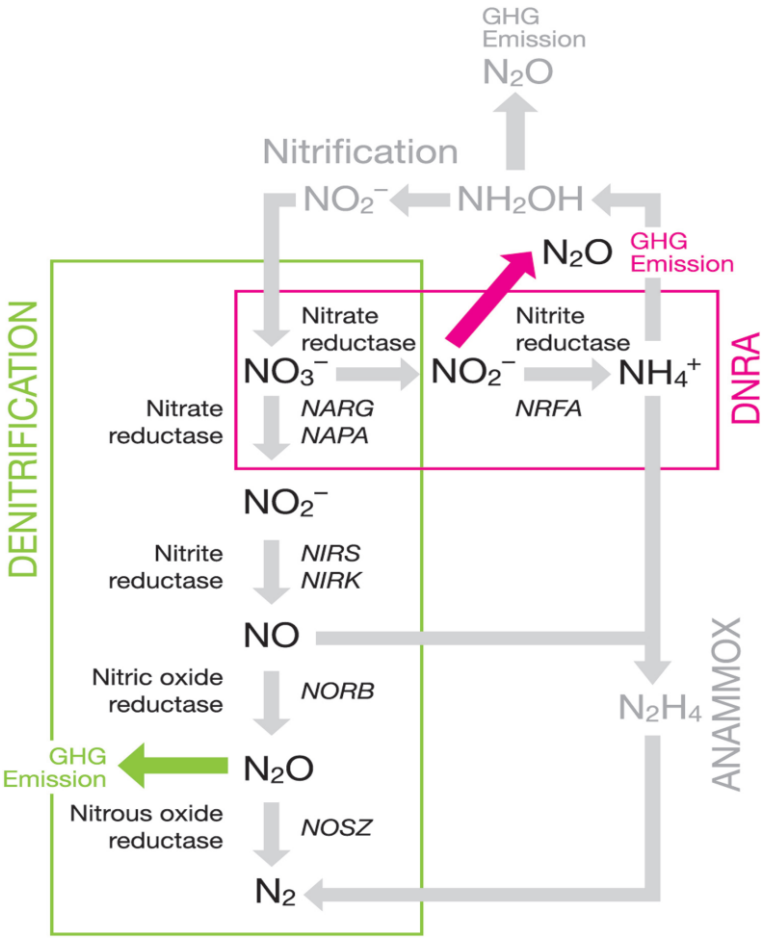
Volk, C., Wood, L., Johnson, B., Robinson, J., Zhu, H. W., & Kaplan, L. (2002). Monitoring dissolved organic carbon in surface and drinking waters. *Journal of Environmental Monitoring*, *4*(1), 43–47. https://doi.org/10.1039/b107768f

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APPENDIX 1



Nitrogen cycle, Giles et al., 2012

APPENDIX 2



Pilot scale woodchip bioreactor or sub-surface constructed wetland



Subsurface draining system leading to the woodchip bioreactor

APPENDIX 3

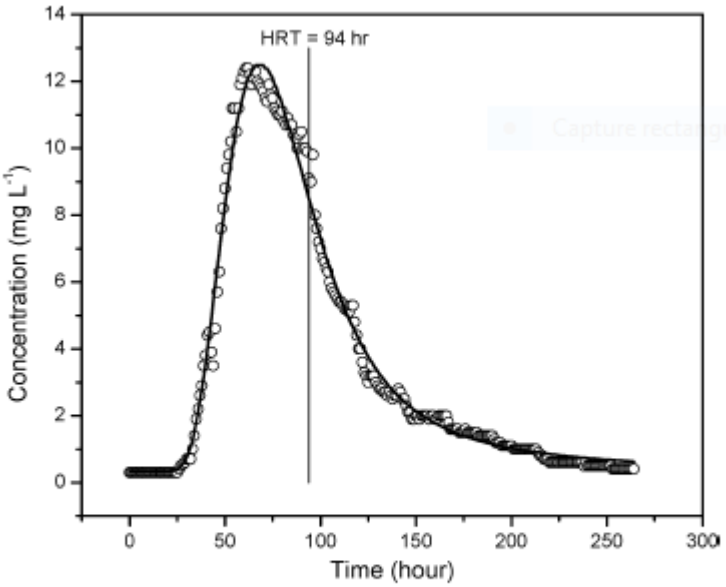
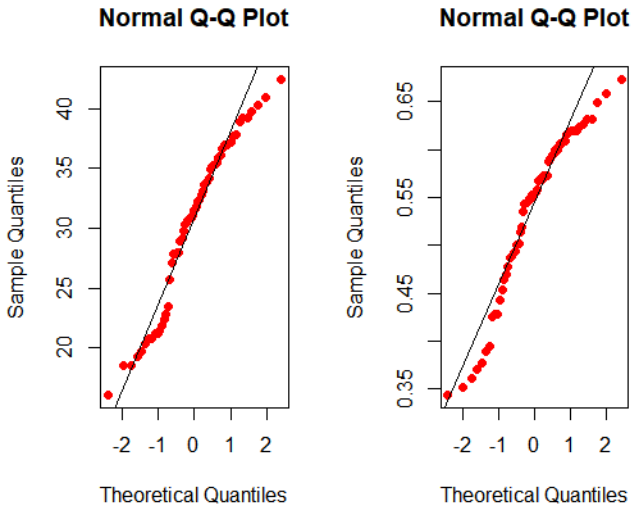


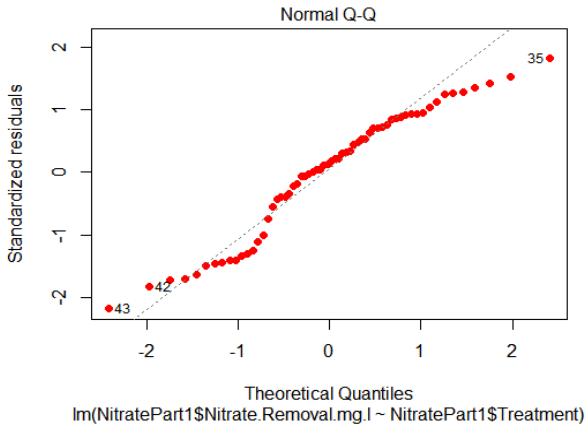
FIGURE 2. Bromide tracer test breakthrough curve and OTIS model simulation for the Hayfield 1 wetland, June 24–July 6, 1999 (*27*) (HRT = hydraulic retention time)

Bromide breakthrough curve from Keefe et al., 2004

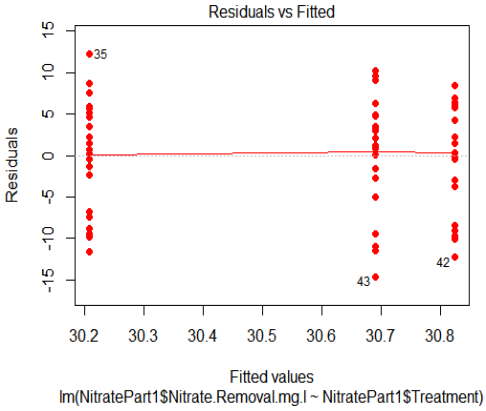
APPENDIX 4



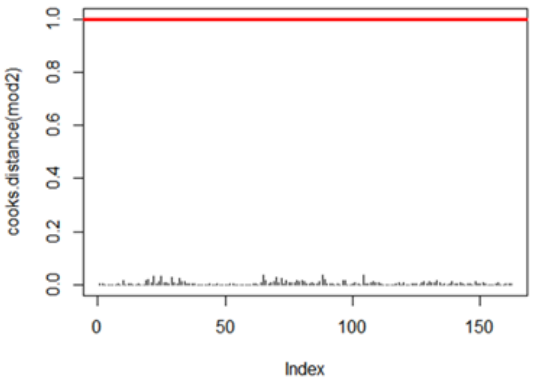
Normality of the nitrate removal measurements part 1



Normality of the residuals part 1

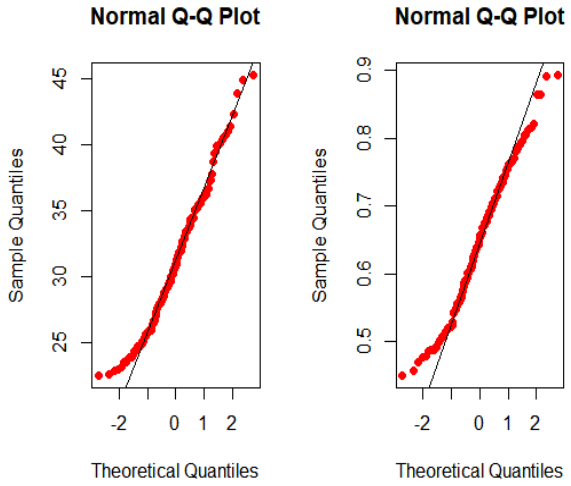


Homoscedasticity of the residuals part 1

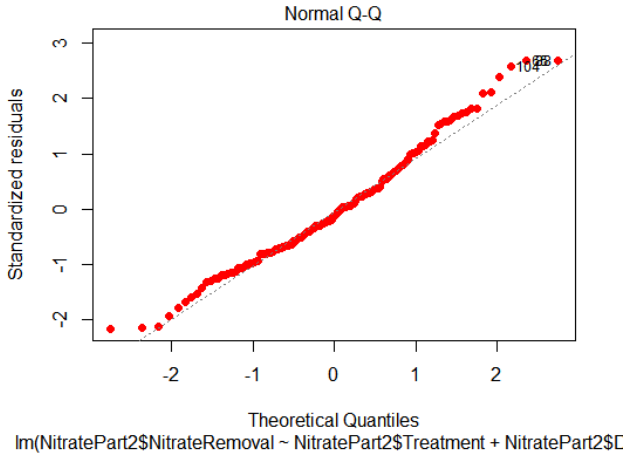


Cook's distance: impact of single and potential aberrant observations part 1

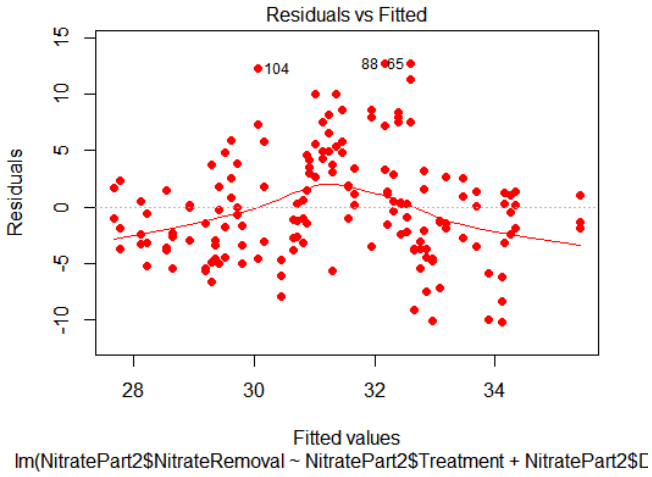
APPENDIX 5



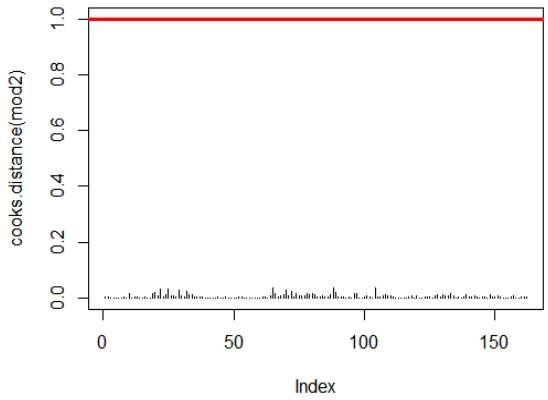
Normality of the nitrate removal measurements part 2



Normality of the residuals part 2



Homoscedasticity of the residuals part 2



Cook's distance: impact of single and potential aberrant observations part 2

APPENDIX 6

One-sample Kolmogorov-Smirnov test, data: residuals(mod2)

D = 0.17638, p-value = 0.03478

alternative hypothesis: two-sided

Kruskal-Wallis rank sum test data: N2OW46$N2O.concentration.ppm.mean and N2OW46$Treatment

Kruskal-Wallis chi-squared = 8.2688, df = 2, p-value = **0.01601**

Kruskal-Wallis rank sum test data: N2OW46$N20.concentration.ppm.mean and N2OW46$Reactor

Kruskal-Wallis chi-squared = 20.698, df = 8, p-value = **0.007995**

Kruskal-Wallis rank sum test data: N2OW46$N2O.concentration.ppm.mean and N2OW46$Day

Kruskal-Wallis chi-squared = 11.857, df = 6, **p-value = 0.06523**

APPENDIX 7

```
library(ggplot2)

library(ggthemes)

library(ggpmisc)

library(ggrepel)

library(FSA)

median(NitrateGlobal$Nitrate.Removal.per.cent)

mean(NitrateGlobal$Nitrate.Removal.per.cent)

########### Preliminary graphs ######################################################

ggplot(NErcor,aes(x=HRT,y=ERcor.N2O,color=Reactor))+geom_point()+labs(x="Time
(Hours)",y="N20 concentration (ppm)")+theme_classic()
ggplot(NErcor,aes(x=Day,y=ERcor.N2O))+geom_point()+geom_smooth(method='lm',color="Red")+lab
s(x="Time (Days)",y="N20 concentration
(ppm)")+theme_classic()+scale_x_continuous(breaks=c(0,3,6,9))
ggplot(`Nitrate.R.(2)`,aes(x=HRT,y=NRemoval))+geom_point()+geom_smooth(method=lm,color="red"
)+labs(x="Time (Hours)",y="Nitrate removal (mg/L)")+theme_classic()
mean(NErcor$ERcor.N2O,)

###################### Results Nitrate Global graphs ###################################

ggplot(`NitrateGlobalInlet`,aes(x=Day,y=`Concentration mg/L`,
color=Treatment))+geom_point()+labs(x="Time (Day)",y="Nitrate concentration
(mg/L)")+geom_smooth(se=FALSE)+ylim(0,max(NitrateGlobalInlet$`Concentration
mg/L`))+theme_classic()
ggplot(NitrateGlobal,aes(x=Day,y= Nitrate.Removal.mg.l,
color=Treatment))+geom_point()+labs(x="Time (Day)",y="Nitrate removal
(mg/L)")+geom_smooth(se=FALSE)+ylim(0,max(NitrateGlobal$Nitrate.Removal.mg.l))+theme_classic
()
ggplot(`NitrateGlobal`,aes(x=Day,y= Nitrate.Removal.per.cent,
color=Treatment))+geom_point()+labs(x="Time (Day)",y="Nitrate removal
proportions")+geom_smooth(se=FALSE)+ylim(0,max(NitrateGlobal$Nitrate.Removal.per.cent))+theme
_classic()

###################### Results Part 1 graphs ###########################################

#Nitrate

ggplot(`NitratePart1Inlet`,aes(x=Day,y=`Concentration mg/L`,
color=Treatment))+geom_point()+geom_smooth(se=FALSE)+labs(x="Time (Day)",y="Nitrate
concentration
(mg/L)")+scale_x_continuous(breaks=c(0,2,5,8,11))+scale_y_continuous(breaks=c(0,10,20,30,40,50,6
0),limits=c(0,max(NitratePart1Inlet$`Concentration mg/L`)))+theme_classic()
ggplot(NitratePart1,aes(x=Day,y= Nitrate.Removal.mg.l,
color=Treatment))+geom_point()+geom_smooth(se=FALSE)+labs(x="Time (Day)",y="Nitrate removal
(mg/L)")+scale_x_continuous(breaks=c(0,2,5,8,11))+ylim(0,max(NitratePart1$Nitrate.Removal.mg.l))+t
heme_classic()
ggplot(`NitratePart1`,aes(x=Day,y= Nitrate.Removal..,
color=Treatment))+geom_point()+geom_smooth(se=FALSE)+labs(x="Time (Day)",y="Nitrate removal
proportions")+scale_x_continuous(breaks=c(0,2,5,8,11))+ylim(0,max(NitratePart1$Nitrate.Removal..))
+theme_classic()
```

#Ammonium

```
ggplot(AmmoniumPart1Zero,aes(x=Day,y=Concentrations.mg.L,
color=Treatment))+geom_point()+geom_smooth(se=FALSE)+labs(x="Time (Day)",y="Ammonium
concentration
(mg/L)")+ylim(min(AmmoniumPart1Zero$Concentrations.mg.L),max(AmmoniumPart1Zero$Concentrat
ions.mg.L))+theme_classic()
ggplot(AmmoniumPart1Zero,aes(x=Day,y=X,
color=Treatment))+geom_point()+geom_smooth(se=FALSE)+labs(x="Time (Day)",y="Ammonium
concentration (mg/L)")+ylim(0,max(AmmoniumPart1Zero$Concentrations.mg.L))+theme_classic()
```

###################### Results Part 2 graphs ################################

#Nitrate

```
ggplot(`NitratePart2Inlet`,aes(x=Day,y=Concentration,
color=Treatment))+geom_point()+geom_smooth(se=FALSE)+labs(x="Time (Day)",y="Nitrate
concentration
(mg/L)")+scale_y_continuous(breaks=c(0,10,20,30,40,50,60),limits=c(0,max(NitratePart2Inlet$Concen
tration)))+theme_classic()
ggplot(NitratePart2,aes(x=Day,y= NitrateRemoval,
color=Treatment))+geom_point()+geom_smooth(se=FALSE)+labs(x="Time (Day)",y="Nitrate removal
(mg/L)")+ylim(0,max(NitratePart2$NitrateRemoval))+theme_classic()
ggplot(NitratePart2,aes(x=Day,y= NitrateRemoval.percent,
color=Treatment))+geom_point()+geom_smooth(se=FALSE)+labs(x="Time (Day)",y="Nitrate removal
proportion")+ylim(0,max(NitratePart2$NitrateRemoval.percent))+theme_classic()
```

#Ammonium

```
ggplot(AmmoniumPart2,aes(x=Day,y=Concentration.mg.l,
color=treatment))+geom_point()+geom_smooth(se=FALSE)+labs(x="Time (Day)",y="Ammonium
concentration (mg/L)")+ylim(0,max(AmmoniumPart2$Concentration.mg.l))+theme_classic()
```

###################### N2O graphs ###################################

```
ggplot(N2O,aes(x=Day,y=N20.concentration.ppm.mean,
color=Treatment))+geom_point()+geom_smooth(se=FALSE)+labs(x="Time (Day)",y="N2O
concentration (ppm)")+ylim(0,max(N2OW46$N20.concentration.ppm.mean))+theme_classic()
ggplot(N2OW46,aes(x=Day,y=N20.concentration.ppm.mean,
color=Treatment:Reactor))+geom_point()+geom_smooth(se=FALSE)+labs(x="Time (Day)",y="N2O
concentration (ppm)")+ylim(0,max(N2OW46$N20.concentration.ppm.mean))+theme_classic()
```

###################### Stats analysis ################################

######################### Preliminary hypothesis ###################################

Outliers in X and Y

```
par(mfrow=c(1,2))
```

Boxplot

```
boxplot(NitratePart2$NitrateRemoval,col='red',ylab="Nitrate removal (mg/L)")
```

Cleveland plot - outliers

```
dotchart(NitratePart2$NitrateRemoval,pch=16,col='red')
# Quantile-Quantile Plot
par(mfrow=c(1,1))
qqnorm(NitratePart2$NitrateRemoval, col='red', pch=16)
qqline(NitratePart2$NitrateRemoval)
```

```
#Kolmogorov smirnov test

ks.test(NitratePart2$NitrateRemoval,"pnorm",mean=mean(NitratePart2$NitrateRemoval),sd=sd(Nitrat
ePart2$NitrateRemoval))
#One-sample Kolmogorov-Smirnov test
#data: NitratePart2$NitrateRemoval
#D = 0.053954, p-value = 0.7332
#alternative hypothesis: two-sided

# X and Y relations

par(mfrow=c(2,2))
boxplot( NitratePart2$NitrateRemoval~ NitratePart2$Treatment,
    varwidth = TRUE,
    ylab = "N2o ppm",
    xlab = "Treatment",
    main = "")
boxplot( NitratePart2$NitrateRemoval~ NitratePart2$Day,
    varwidth = TRUE,
    ylab = "N2O ppm",
    xlab = "Day",
    main = "")
boxplot( NitratePart2$NitrateRemoval~ NitratePart2$Reactors,
    varwidth = TRUE,
    ylab = "N2O ppm",
    xlab = "Reactors",
    main = "")

# Interactions

boxplot(NitratePart2$NitrateRemoval~NitratePart2$Treatment*NitratePart2$Reactors,
   varwidth = TRUE,
   ylab = "Nitrate removal mg.l",
   main = "")

###################### Stat modelisation ################################

mod1<-lm(NitratePart2$NitrateRemoval~NitratePart2$Treatment)
drop1(mod1,test='F')

###################### Hypothesis validation on residuals ###################

par(mfrow=c(1,1))
hist (residuals(mod1)
  , col='red'
  , xlab='Valeurs des résidus'
  , ylab='Effectifs')
plot(mod1,
  pch=16,
  col="red")
plot(cooks.distance(mod1), type = "h", ylim = c(0, 1))
abline(h = 1, col = 2,lwd = 3)
ks.test(residuals(mod1),"pnorm",mean=mean(residuals(mod1)),sd=sd(residuals(mod1)))
#                    One-sample Kolmogorov-Smirnov test
#data: residuals(mod1)
#D = 0.051606, p-value = 0.7813
#alternative hypothesis: two-sided
```

```
###################### N2O stats analysis###################################

###################### N2O preliminary tests ###################################

par(mfrow=c(1,2))

# boxplot

boxplot(N2O$N20.concentration.ppm.mean,col='red',ylab="N2O concentration (ppm)")

# Cleveland plot - outliers

dotchart(N2O$N20.concentration.ppm.mean,pch=16,col='red')

#QQ plot

par(mfrow=c(1,1))
qqnorm(N2O$N20.concentration.ppm.mean, col='red', pch=16)
qqline(N2O$N20.concentration.ppm.mean)
ks.test(N2O$N20.concentration.ppm.mean,"pnorm",mean=mean(N2O$N20.concentration.ppm.mean),
sd=sd(N2O$N20.concentration.ppm.mean))

#One-sample Kolmogorov-Smirnov test

#data: N2O$N20.concentration.ppm.mean
#D = 0.34053, p-value = 1.12e-07
#alternative hypothesis: two-sided
#Histogram
hist(N2O$N20.concentration.ppm.mean
   ,breaks=10
   , col='red'
   , xlab='N2O concentration (ppm)'
   , ylab='Number')

####Log TRANSFORMATION

LogCN2O<-log(N2O$N20.concentration.ppm.mean)
par(mfrow=c(1,2))

# boxplot

boxplot(LogCN2O,col='red',ylab="N2O concentration (ppm)")

# Cleveland plot - outliers

dotchart(LogCN2O,pch=16,col='red')
#Histogram
hist(LogCN2O
   ,breaks=10
   , col='red'
   , xlab='N2O concentration (ppm)'
   , ylab='Number')

#QQ plot

par(mfrow=c(1,1))
qqnorm(LogCN2O, col='red', pch=16)
qqline(LogCN2O)
ks.test(LogCN2O,"pnorm",mean=mean(LogCN2O),sd=sd(LogCN2O))
#One-sample Kolmogorov-Smirnov test
#data: LogCN2O
#D = 0.28386, p-value = 1.827e-05
#alternative hypothesis: two-sided
```

```
####Inverse transformation

InvCN2O<-1/N2O$N20.concentration.ppm.mean

# boxplot

boxplot(InvCN2O,col='red',ylab="N2O concentration (ppm)")

# Cleveland plot - outliers

dotchart(InvCN2O,pch=16,col='red')

#Histogram

hist(InvCN2O
   ,breaks=10
   , col='red'
   , xlab='N2O concentration (ppm)'
   , ylab='Number')

#QQ plot

par(mfrow=c(1,1))
qqnorm(InvCN2O, col='red', pch=16)
qqline(InvCN2O)
ks.test(InvCN2O,"pnorm",mean=mean(InvCN2O),sd=sd(InvCN2O))
#One-sample Kolmogorov-Smirnov test
#data: InvCN2O
#D = 0.20237, p-value = 0.005493
#alternative hypothesis: two-sided

##### N2O not normally distributed --> Non parametric test Kruskal Wallis

##Inverse transformation without day 46

INVN2O<-1/N2OW46$N20.concentration.ppm.mean
# Outliers in X and Y
par(mfrow=c(1,2))
# Boxplot
boxplot(INVN2O,col='red',ylab="Inverse de N2O")
# Cleveland plot - outliers
dotchart(INVN2O,pch=16,col='red')
# Quantile-Quantile Plot
par(mfrow=c(1,1))
qqnorm(INVN2O, col='red', pch=16)
qqline(INVN2O)

#Kolmogorov smirnov test

ks.test(INVN2O,"pnorm",mean=mean(INVN2O),sd=sd(INVN2O))
#One-sample Kolmogorov-Smirnov test
#data: INVN2O
#D = 0.14409, p-value = 0.1461
#alternative hypothesis: two-sided
```

```
# X and Y relations

par(mfrow=c(2,2))
boxplot( INVN2O~ N2OW46$Treatment,
    varwidth = TRUE,
    ylab = "N2o ppm",
    xlab = "Treatment",
    main = "")
boxplot( INVN2O~ N2OW46$Day,
    varwidth = TRUE,
    ylab = "N2O ppm",
    xlab = "Day",
    main = "")
boxplot( INVN2O~ N2OW46$Reactor,
    varwidth = TRUE,
    ylab = "N2O ppm",
    xlab = "Reactors",
    main = "")

# Interactions

boxplot(INVN2O~N2OW46$Treatment*N2OW46$Reactor,
   varwidth = TRUE,
   ylab = "Nitrate removal mg.l",
   main = "")

#Stats analysis

mod2<-lm(INVN2O~N2OW46$Treatment+N2OW46$Day)
drop1(mod2,test='F')

## Hypothesis validation on residuals

par(mfrow=c(1,1))
hist (residuals(mod2)
  , col='red'
  , xlab='Valeurs des résidus'
  , ylab='Effectifs')
plot(mod2,
  pch=16,
  col="red")
plot(cooks.distance(mod2), type = "h", ylim = c(0, 1))
abline(h = 1, col = 2,lwd = 3)
ks.test(residuals(mod2),"pnorm",mean=mean(residuals(mod2)),sd=sd(residuals(mod2)))
#One-sample Kolmogorov-Smirnov test
#data: residuals(mod2)
#D = 0.17638, p-value = 0.03478
#alternative hypothesis: two-sided

#Without day 46 the preliminary hypothesis are verified but the residuals are not normally distribued --
> Non parametric test Kruskal Wallis

###################### N2O Kruskall-Wallis ###################################

kruskal.test(N2O$N20.concentration.ppm.mean, N2O$Treatment, data=N2O)
kruskal.test(N2O$N20.concentration.ppm.mean, N2O$Day, data=N2O)
kruskal.test(N2O$N20.concentration.ppm.mean, N2O$Reactor, data=N2O)
dunnTest(N2O$N20.concentration.ppm.mean,N2O$Treatment,method="bonferroni")
dunnTest(N2O$N20.concentration.ppm.mean,N2O$Reactor,method="bonferroni")
```

```
###################### Graphs Temperature ###################################

ggplot(TemperatureExperienceBiochar,aes(x=Day,y=Temperature,color=Treatment))+geom_point()+geom_smooth(se=FALSE)+labs(x="Time (Days)",y="Temperature (°C)")+ylim(0,max(TemperatureExperienceBiochar$Temperature))+theme_classic()

###################### Graphs pH ###################################

ggplot(pH,aes(x=Day,y=pH,color=Treatment))+geom_point()+geom_smooth(se=FALSE)+labs(x="Time (Days)", y="pH")+ylim(0, max(pH$pH))+theme_classic()
```

l'institut Agro agriculture • alimentation • environnement	Diplôme : Ingénieur Spécialité : Horticulture Spécialisation / option : Agroecology Enseignant référent : Safya Menasseri

Auteur(s) : **Raphaël Offermann** Date de naissance* : 06/01/1997	Organisme d'accueil : Aarhus University Blichers Allee, 20 8830 Tjele DANMARK Maître de stage : Lars Elsgaard

Nb pages : 28 Annexes :7

Année de soutenance :2021

Titre français : Recherche sur l'usage du biochar pour améliorer l'élimination de nitrate dans les bio-réacteurs à copeaux de bois au Danemark et réduire les émissions de peroxyde d'azote

Titre anglais : Investigation of the use of biochar to enhance nitrate removal in Danish woodchip bioreactors and reduce nitrous oxide emissions

Résumé (1600 caractères maximum) :

Au Danemark, les méthodes de mitigation du transport du nitrate sont des outils cruciaux pour lutter contre la pollution de l'eau liée aux excédents de nitrate provenant des effluents agricoles. En effet ; l'ensemble de la surface agricole du pays est classée ZVN (Zone vulnérable aux nitrates), c'est-à-dire qu'en l'absence de mesures visant à réduire la concentration de nitrate dans l'eau, le seuil réglementaire de 50mg/l de nitrate de la Commission Européenne serait atteint ou dépassé. Les bioréacteurs à copeaux de bois permettent la dénitrification des affluents agricoles par l'action enzymatique des communautés bactériennes. Il a cependant été observé que ces réacteurs rejetaient du peroxyde d'azote dû à ce processus de dénitrification. L'objet de cette étude était d'évaluer si l'introduction de biochar augmentait la dénitrification et permettait de réduire les émissions de N_2O. Un essai en système semi ouvert de 6 semaines a été mené avec des triplicats suivant 3 modalités : copeaux de bois seuls, copeaux de bois plus de 5% de charbon actif, copeaux de bois plus 10% de charbon actif. Aucune différence significative entre les modalités n'a été observée pour la dénitrification. Concernant la réduction d'émissions de N_2O, bien qu'un effet significatif ait été noté, d'autres facteurs, comme l'hétérogénéité des triplicats et l'effet jour étaient nettement plus significatifs. On ne peut donc conclure qu'en une probable réduction des émissions de N_2O en présence de biochar. Ces résultats sont proches études et métanalyses lors d'études similaires. Seules de futures études permettront de statuer sur l'efficacité du biochar pour la dénitrification et la réduction d'émission de N_2O.

Abstract (1600 caractères maximum) :

In Denmark, transport mitigation measures are crucial tools to limit water pollution linked to exceeding nitrate in agricultural outflows. In fact, the entire arable surface is described as NVZ (Nitrate vulnerable zone) by the European Commission and would have nitrate concentrations equal or above the legal threshold of 50mg/l without preventing measures. Woodchip bioreactors allow the denitrification of agricultural outflows by the enzymatic activity of microbial communities. However, it has been reported that those reactors emit nitrous oxide due to the denitrification process. The aim of this study was to assess the efficiency of the introduction of biochar to enhance denitrification and reduce N_2O emissions. A semi-field trial lasting 6 weeks was conducted with 3 modalities: woodchips, woodchips with 5% biochar and woodchips with 10% biochar. For denitrification, no significant difference was found between the modalities. Regarding N_2O emission reduction, a significant effect was found in presence of biochar. However other factors were more significant such as the heterogeneity of the triplicates and the impact of time. It can only be concluded that biochar probably has a reducing effect on, N_2O emissions. These results are close to similar studies and metanalyses. Only further research will enable a full understanding of the efficiency of biochar to enhance denitrification and reduce N_2O emissions.

Mots-clés : dénitrification, bioréacteurs à copeaux de bois, charbon actif, émissions de protoxyde d'azote, Danemark, mitigation du lessivage

Key Words: denitrification, woodchip bioreactors, biochar, nitrous oxide emissions, Denmark, leaching mitigation

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