CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Environmental Sciences

BACHELOR THESIS

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Environmental Engineering
Department of Applied Ecology
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CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

FACULTY OF ENVIRONMENTAL SCIENCES
DEPARTMENT OF APPLIED ECOLOGY

Effects of C: N:P ratios on wastewater treatment in arbuscular mycorrhizal fungi assistant constructed wetlands

Bachelor Thesis

Author: Nina Ninovska
Supervisor: Doc. -Ing. Zhongbing Chen

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Objectives of thesis

Arbuscular mycorrhizal fungi (AMF) have been widely reported to play important roles in terrestrial plant resistance to abiotic stresses (e.g., heavy metals, drought, emerging pollutants, and nutrients). However, regarding the different nutrients (e.g., carbon (C), nitrogen (N), phosphorus (P) in wastewater), the effects of nutrient concentrations on wastewater purification in AMF assistant CWs under submerged water levels are poorly studied. Furthermore, it is unclear whether different nutrient ratios influence AMF colonization in CWs. Therefore, the objective of this thesis is to evaluate how nutrient concentrations affect wastewater purification in CWs, and whether AMF colonization can be affected by nutrient ratios in CWs.

Methodology

This study will use eight vertical subsurface flow CWs at the Czech University of Life Sciences Prague. The eight PVC pipes will be established to simulate the subsurface flow CWs with the dimensions of each system being 15× 55 cm (diameter ×Height). Each CW will be filled with 15 cm gravel (4-5 cm), and 25 cm sand will be used as substrates. For the AMF system, the substrates from the bottom to top are: 15 cm gravel, 10 cm sand, 10 cm sand mixed with 50 g AMF, then planted Iris pseudacorus, afterward add 5 cm sand. AMF inoculum will be Rhizophagus irregularis. The influencing factors of this study are N concentrations (low, high), P concentration (low, high) and AMF (with and without). Therefore, eight different treatments will be set. Inlet water of CWs will be simulated as municipal sewage. In order to keep the CWs under the submerged water condition, 3L of simulated municipal sewage with different pollutants concentrations will be fed into each CW, and the hydraulic retention time is seven days. The CWs will be protected from rain throughout the experiment.

The proposed extent of the thesis

50

Keywords

C: N:P ratios; wastewater treatment; arbuscular mycorrhizal fungi; constructed wetlands

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Recommended information sources

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Expected date of thesis defence

2022/23 SS – FES

The Bachelor Thesis Supervisor

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Supervising department

Department of Applied Ecology

Electronic approval: 7. 3. 2023

prof. Ing. Jan Vymazal, CSc.

Head of department

Electronic approval: 7. 3. 2023

prof. RNDr. Vladimír Bejček, CSc.

Dean

Prague on 29. 03. 2023

Statement:

I hereby declare that I have independently elaborated the bachelor/final thesis with the topic of: ''Effects of C: N:P ratios on wastewater treatment in arbuscular mycorrhizal fungi assistant constructed wetlands'' and that I have cited all the information sources that I used in the thesis as listed at the end of the thesis in the list of used information sources. I am aware that my bachelor/final thesis is subject to Act No. 121/2000 Coll., on copyright, on rights related to copyright and on amendments of certain acts, as amended by later regulations, particularly the provisions of Section 35(3) of the act on the use of the thesis. I am aware that by submitting the bachelor/final thesis I agree with its publication under Act No. 111/1998 Coll., on universities and on the change and amendments of certain acts, as amended, regardless of the result of its defense. With my own signature, I also declare that the electronic version is identical to the printed version and the data stated in the thesis has been processed in relation to the GDPR.

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Abstract:

Arbuscular mycorrhizal fungi (AMF) have been widely reported to play an essential role in enhancing resistance to abiotic stresses for terrestrial plants. However, it is unclear whether different nutrient concentrations of carbon (C), nitrogen (N), and phosphorus (P) in wastewater affect the purification process in constructed wetlands (CWs) under submerged water levels. Moreover, the effect of AMF in CWs with different nutrient concentrations is poorly studied. Therefore, this thesis investigates how different nutrient concentrations affect wastewater purification in CWs, and whether the presence of AMF influence wastewater purification in CWs under different nutrient concentrations.

The methodology consisted of vertical substrate flow CWs filled with a substrate and planted with *Iris Pseudacorus* as wetland vegetation and *Rhizophagus irregularis* as AMF inoculum. The influencing factors of this thesis are N concentrations (low, high), P concentrations (low, high), and AMF (with, without). Furthermore, water samples were taken for analysis which included the measurement of C (Total Organic Carbon, Total Carbon (TC), Inorganic Carbon), N (Ammonia, Total Nitrogen (TN), Nitrates, Nitrites) and P (Phosphates (PO_4^{3-}-P)).

The results demonstrated that the application of different concentrations of N:P influenced the pollutant removal efficiency, where TC removal was higher with low N and high P concentrations at 80%, TN removal was higher when concentrations of N were initially high at 96%, and PO_4^{3-}-P removal rate was higher when N and P concentrations were both low with 98%. Meanwhile, AMF enhanced removal efficiency for TN and PO_4^{3-}-P, specifically by 2% and 5%. However, the removal of TC was more effective without AMF presence, by 4%. Therefore, these results indicated that different nutrient concentration ratios, as well as AMF presence, could influence pollutant removal effectiveness in CW wastewater purification. This research could present useful information for potentially applying AMF in CWs for removing pollutants, as well as monitoring different nutrient concentrations for more successful future treatments.

Key Words: C: N:P ratios; wastewater treatment; arbuscular mycorrhizal fungi; constructed wetlands

Abbreviations:

CWs – Constructed Wetlands
AMF – Arbuscular Mycorrhizal Fungi
ORP – Oxidation/Reduction Potential
TOC – Total Organic Carbon
TC – Total Carbon
IC – Inorganic Carbon
NH_4 - N – Ammonia Nitrogen
TN – Total Nitrogen
NO_2 - N – Nitrite Nitrogen
NO_3 - N– Nitrate Nitrogen
PO_4^{3-} - P – Phosphate Phosphorus

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1. Introduction

With the recent increase in population growth, industrial and agricultural developments, climate change and global warming effects, wastewater treatment and management are an essential global concern that needs to be properly addressed (Chauhan and Prajapati, 2022). Challenges such as increased resource consumption, changes in land use, and air, water and soil pollution require the implementation of new sustainable strategies and practices which would enable more resilience to different types of stressors for habitats (Stefanakis et. 2019). Therefore, ecologically friendly technologies such as constructed wetlands (CWs) are being increasingly used in wastewater treatment, especially in rural areas (Saeed et al. 2019). Multiple studies have confirmed the positive effects on pollutant removals and wastewater purification of CWs, especially subsurface flow CWs (Ennabili and Radoux et. 2020; Kumar and Singh et. 2017; Hu et al. 2022; Tee et al. 2011).

Additionally, arbuscular mycorrhizal fungi (AMF) have repeatedly demonstrated having an important role in enhancing plants' resistance to abiotic stresses, promoting plant growth and soil fertility (Jeffries et al. 2003), as well as supporting nutrient acquisition (Hu et al. 2022). Moreover, AMF contribute to the maintenance of ionic homeostasis, osmoregulation, photosynthesis efficiency, cell membrane protection and enhancing salinity tolerance in plants (Evelin et al. 2019). As these mycorrhizal fungi are known to occur naturally in saline wetland environments, they show equal importance to wetland plant species as they do for terrestrial plant species (Ramirez-Viga et al. 2018).

However, different nutrient ratios of carbon (C), nitrogen (N) and phosphorus (P) in wastewater purification under submerged water levels in CWs with the assistance of AMF are poorly studied. Direct absorption of nutrients and removal of excessive concentrations is an essential aspect of wetland vegetation in wastewater treatment processes (Ennabili and Radoux, 2020). And so, the objective of this thesis is to research, experiment and evaluate how different nutrient concentrations, specifically nitrogen and phosphorus ratios, affect wastewater purification in CWs, as well as the effect of AMF on wastewater purification, and the relationships it forms with the wetland vegetation.

2. Objectives

- Evaluating the effects of different N:P nutrient concentration ratios on wastewater purification in constructed wetlands
- Assessing the effects of arbuscular mycorrhizal fungi on wastewater purification in constructed wetlands under different N:P nutrient concentrations

3. Literature review

3.1 Constructed Wetlands

CWs are defined as engineered systems intended for utilizing natural processes which contain the wetland vegetation, soil, and different microorganisms that contribute to wastewater purification (Vymazal et. 2010). These wetlands play an essential role in maintaining the climate and hydrological cycle globally, as well as protecting and enhancing the ecosystem's biodiversity (Saquib et al. 2022). They act as natural biofilters, removing pollutants, absorbing nutrients and eliminating toxic chemicals (Saquib et al. 2022).

CWs have recently become a popular environmentally friendly approach for urban wastewater treatment due to their affordability, reliability and simple design and construction (Wu et al. 2014a). These artificial wetlands enhance the naturally occurring functions in the purification of water by using natural resources such as aquatic vegetation, microorganisms and the filter bed (substrate) which often consists of sand, gravel or soil (Hota et al. 2022).

Furthermore, according to a cost-benefit analysis, CWs indicate advantages regarding the system construction and the operational costs when compared to other frequently used wastewater treatment plants (Zhang et al. 2012). Concepts which are taken into consideration include land usage, investment and operating costs, energy efficiency, and sustainability benefits, which are in favour of artificial CWs. However, a limiting factor includes land usage requirements, especially in regions where the land resources are limited, and the land is densely populated (Wu et al. 2014a). Additionally, the lifecycle cost of CWs is reported to be increasing due to the technology needed to achieve higher removal efficiency (Wu et al. 2014a).

Nevertheless, CWs remain a promising alternative to wastewater treatment, especially in developing countries (Chen et al. 2011), where they're frequently used in domestic and municipal wastewater purification, as well as agricultural and industrial runoff, mine drainages and more (Wu et al. 2014b).

3.1.1 Types of Constructed Wetlands

Several CW systems have been introduced in recent years in order to improve the removal of various contaminants and pollutants from wastewater (Jiang et al. 2016). There are different ways to categorize CWs according to an article by Mahmood et al. 2013, which can be based on two factors: type of macrophytic growth and water flow regime. Furthermore, according to Vymazal et. al 2010, considering the water flow regime, CWs can be divided into surface and subsurface flow. Surface flow wetlands can be further separated into free water surface and subsurface flow CWs. In addition, subsurface flow CWs can be further divided into horizontal and vertical subsurface flow **(See Figure 1)**.

On account of these different types of CWs, the performance and efficiency of pollutant removal varies according to the different structures of each CW (Zhang et al. 2012) and on the type of vegetation, the applied hydraulic load and the structure of the substrate (Parde et al. 2020).

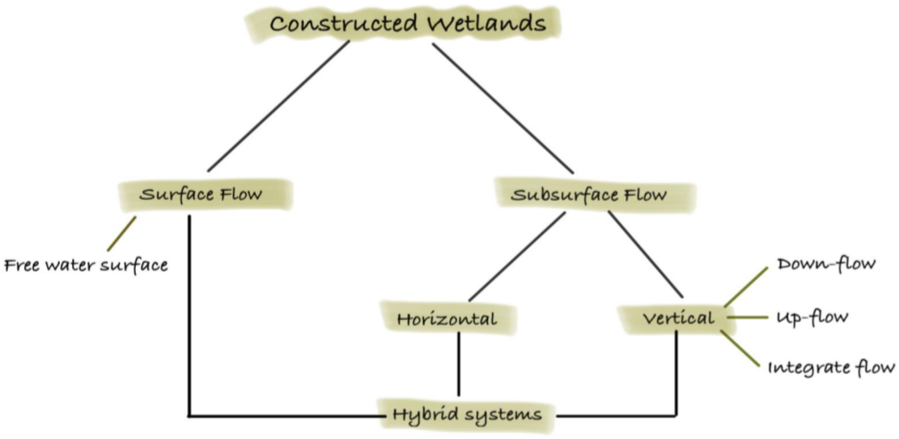

Figure 1*: Types of constructed wetlands.*

Source: Author

3.1.1.1 Free Water Surface Constructed Wetlands

Free water surface CWs is a natural type of wetland where the wastewater flows over the surface (Parde et al. 2020), preventing flood damages and promoting shoreline erosion control while improving the wastewater purification quality (Farooqi et al. 2008). These types of wetlands have a similar appearance to natural marshes (Kadlec and Wallace, 2008) and are especially effective in the removal of organic material through microbial degradation.

Additionally, suspended solids are efficiently removed by filtration from the applied dense wetland vegetation (Vymazal et. 2010). N is removed mostly through the process of nitrification, denitrification and ammonia volatilization, while the P retention is low due to the limited connection between water and soil particles (Vymazal et. 2010).

These types of CWs are often used in North America (Kadlec and Wallace, 2008) and Australia (Greenway et. 2005), and only recently became popular in parts of Europe, for the purpose of nitrogen elimination from diffuse pollution (Vymazal et al. 2006).

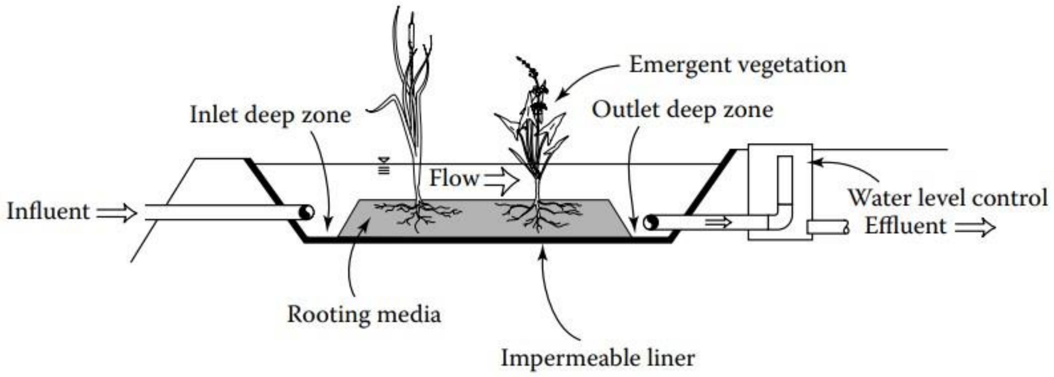

***Figure 2**: Basic structure of free water surface constructed wetland.*

Source: (Kadlec and Wallace et. 2008) Treatment Wetlands: Second Edition

3.1.1.2 Horizontal Subsurface Flow Constructed Wetlands

Horizontal subsurface flow CWs, also known as reed bed systems (Parde et al. 2020), are typically used in treating primary effluent before the water discharge and soil distribution (Kadlec and Wallace, 2008). These CWs are typically implemented with a substrate consisting of gravel or similar materials which are then sealed by a permeable layer and wetland vegetation (Vymazal et. 2010), where wastewater is kept below the surface of this substrate, while also horizontally flowing from the inlet to the outlet (Kadlec and Wallace, 2008).

Furthermore, the removal of N in the wastewater is primarily by the process of denitrification, while P is removed by ligand exchange reactions (Vymazal et. 2010), but horizontal flow CWs are often considered as having low removal of P, unless special treatment is used (Vymazal et. 2006). Moreover, suspended solids are usually retained in the process of filtration and sedimentation, and the removal efficiency is reportedly high in these types of CWs (Vymazal and Kropfelova, 2008). Additionally, ammonia removal is often limited due to the lack of oxygen availability in the substrate bed (Vymazal et. 2006).

These CWs have been widely used for domestic and municipal wastewater treatment, as well as industrial, agricultural, landfill leachate and water runoff nowadays (Vymazal et. 2010).

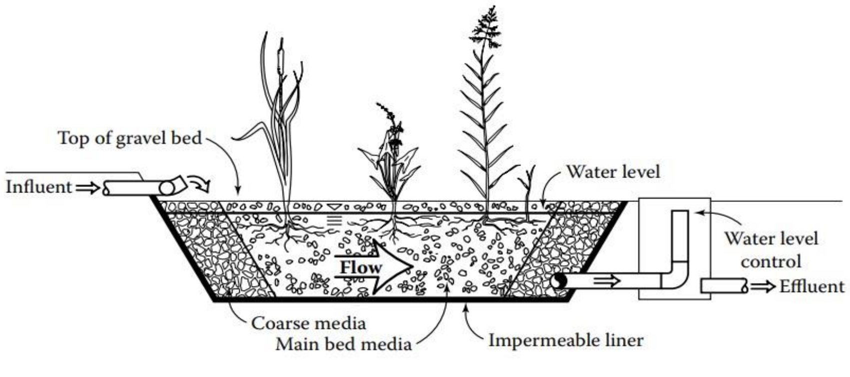

***Figure 3**: Basic structure of horizontal flow constructed wetland.*

Source: (Kadlec and Wallace et. 2008) Treatment Wetlands: Second Edition

3.1.1.3 Vertical Subsurface Flow Constructed Wetlands

Vertical flow CWs are a specific type of wetland consisting of substrate built from sand or gravel planted with wetland vegetation, where wastewater is submerged at the top of the CW and drained at the end, offering aerobic conditions that proceed to achieve high nitrification, COD and BOD in the pollutant removal process (Parde et al. 2020).

Additionally, vertical flow CWs enhance the oxygen supply from the atmosphere to the wastewater flow which further oxidizes ammonia (Abdelhay and Abunaser, 2020), in comparison to horizontal flow CWs that are limited in oxygen transfer (Kadlec and Wallace, 2008).

Therefore, these types of CWs are widely used in municipal and domestic wastewater treatment due to their capacity to nitrify successfully (Burgoon et al. 1999). Moreover, vertical flow CWs are efficient in the removal of organic and suspended solids, while the removal of P appears to be low unless technology with high sorption capacity is used (Vymazal et. 2010).

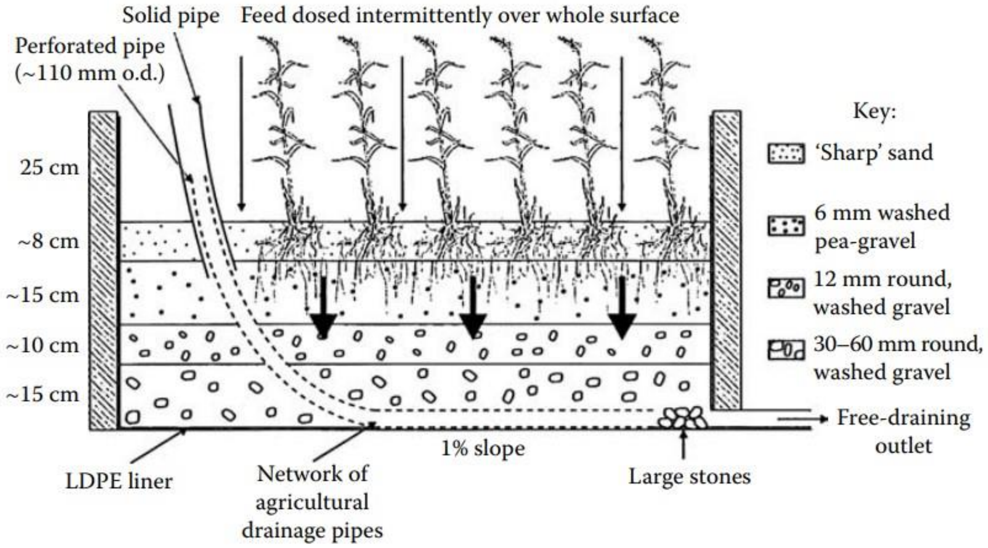

***Figure 4**: Basic structure of vertical constructed wetland.*

Source: (Kadlec and Wallace et. 2008) Treatment Wetlands: Second Edition

3.2 Wetland Vegetation and Arbuscular Mycorrhizal Fungi

Using vegetation restoration in CWs is an environmentally friendly, efficient and economically viable method for wastewater purification techniques (Ma et al. 2021). However, the growth and development of these wetland plants heavily depend on the environmental conditions, and very frequently, abiotic factors such as salinity concentration can exceed the plant's tolerance threshold (Evelin et al. 2009). This causes nutrient loss, growth problems and overall plant weakness (Evelin et al. 2009).

However, microorganisms have been shown to have a significantly positive effect on improving plants' performance and health in these extreme conditions (Evelin et al. 2009). Therefore, having microbial assistance in CW environments joined with wetland vegetation is an essential part of maintaining plant health and development in extreme conditions, such as municipal wastewater purification in CWs.

3.2.1 Arbuscular Mycorrhizal Fungi in Constructed Wetlands

AMF are an essential group of soil microorganisms that form symbiotic relationships with vascular plant species, where mutual exchange of nutrients and other beneficiary substances occurs (Smith and Read, 2008). They have reportedly shown their importance in establishing the health and stability of plant communities (Xu et al. 2016) while enhancing the resistance to abiotic stresses such as heavy metal exposures (Chen et al. 2022), salinity (Silva et al. 2022) and water stress such as drought (Chitarra et al. 2016). Additionally, AMF assist plants in nutrient uptake by occupying more space in the soil around them (Johnson and Gehring, 2007).

These mycorrhizal fungi are present in a lot of natural environments, especially aquatic habitats, and they play a significant role in promoting the biodegradation of pollutants in wastewater (Xu et al. 2016), as well as enhancing the translocation of N and P through the hyphae of the fungi successfully to the plant itself (Solaiman and Hirata, 1998).

Therefore, the presence of AMF in an ecologically based technology for wastewater treatment such as CWs has received increased attention in the past three decades (Xu et al. 2016).

3.3 Nutrients in Constructed Wetlands

CWs perform various essential biogeochemical functions in wetland ecosystems, including the transformation of different types of nutrients, the removal and retention of them as well as storage space. Therefore, the proper treatment and management of N and P concentrations play an important role in the functioning of CWs.

Excessive concentrations of nutrients such as ammonia N and total P in CWs can lead to a variety of problems related to the security of water sources (Zhu et al. 2022). This can include a lack of water purification capacity, diseases and pests in plants, a decline in the systems' stability, leakage of the CW and even overgrowth of vegetation (Zhu et al. 2022). This presents a challenge of maintaining the water quality and operating CWs efficiently in order to ensure water supply security.

After operating for a long time, CWs are becoming increasingly saturated which results in weakening the processes of adsorption, a transformation of pollutants and a decline in purification quality (Qualls and Heyvaert, 2017). Sediments are an important component of CWs where nutrients are stored, and under some circumstances, pollutants can be accumulated and can cause negative effects on the water quality in these wetlands (Zhu et al. 2022). In addition, the prediction and control of long-term effects on the accumulation of pollutants can be difficult because of different construction methods and management, the hydraulic systems, and the plant conditions of CWs (Zhu et al. 2022). Proper management and finding suitable materials for the substrate in CWs are crucial in enhancing the removal of pollutants in the process of water purification (Wu et al. 2022).

3.3.1 Nitrogen and Phosphorus Removal in Constructed Wetlands

The compounds of N include a variety of forms which are essential for all biological life; the most important forms include inorganic compounds such as ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-) (Vymazal et al. 2006). These forms are constantly transforming from inorganic to organic and back allowing for the N cycle to take place. While some compounds need energy, others release it, which is then used by biological organisms for growth and development, allowing for proper wetland ecosystem functioning (Vymazal et al. 2006).

P occurs as inorganic or organic compounds of phosphates in CWs (Vymazal et al. 2006). Orthophosphate has shown to be the only free organic form of P which is used directly by wetland vegetation and algae, representing a significant cycle between organic and inorganic P in wetland environments (Vymazal et al. 2006).

The transformation or removal of N and P depends on various conditions including physical, chemical and biological processes, and different environmental factors (Jakubaszek et. 2020).

The removal and retention of N during wastewater treatment purification in CWs include processes such as nitrification, denitrification, NH_3 volatilization, uptake by plants or microorganisms, ammonification, etc. (Vymazal et. 2006). On the other hand, processes that involve the removal of P include sorption, precipitation, plant uptake and soil accretion, however, the removal rates of this nutrient are low unless high sorption capacity is used in specific CW technologies (Vymazal et. 2006).

The removal and retention rates differ according to the type of CW: vertical flow CWs contribute to successful ammonia removal, however, denitrification occurs less and horizontal flow CWs increase the denitrification process, however, nitrification occurs in limited amounts (Vymazal et. 2006).

4. Methodology

4.1 Experimental Setup

The experimental setup consists of eight vertical flow subsurface flow CWs at the Czech University of Life Sciences, Prague. It comprises PVC pipes filled with a substrate mixture containing the wetland vegetation and mycorrhizal inoculum which are then connected to water outlets, enabling samples to be collected. Vertical subsurface flow CWs were chosen to be used because this type of wetlands has achieved higher removal efficiency than surface flow CWs, specifically 40% in contrast to 80% in subsurface vertical flow CWs (Yi et al. 2017).

Moreover, the eight vertical subsurface flow CWs are each with a diameter of 15 cm and height of 55 cm, filled with substrate consisting of 15 cm of gravel and 30 cm of sand **(See Figure 5)**. Additionally, *Iris Pseudacorus* is planted in the substrate as wetland vegetation since this species can withstand very damp habitats where it tolerates extreme conditions (Yousefi and Mohseni-Bandpei, 2010). 50 g of *Rhizophagus irregularis* was used as the AMF inoculum as it has reportedly been observed in wetland ecosystems (Xu et al. 2016).

Furthermore, in order to keep the CWs under submerged water conditions, 2.5 L of simulated wastewater with different pollutants is fed to each CW with 7 days being the hydraulic retention time. The bottom of each CW has an airtight cap which allows for the water samples to be collected every week, simultaneously the top of the CW is open in order to allow for plant growth. Due to this exposure on the top, the CWs were protected from rainfall and other potential influencing parameters by a rooftop situated in a greenhouse throughout the experiment.

Since the main goal of this research experiment is to determine whether AMF influence wastewater purification under different nutrient ratios, the influencing factors are as follows: **(See Figure 6)**

1) N concentrations (low, high)
2) P concentrations (low, high)
3) the presence of AMF (with AMF, without AMF)

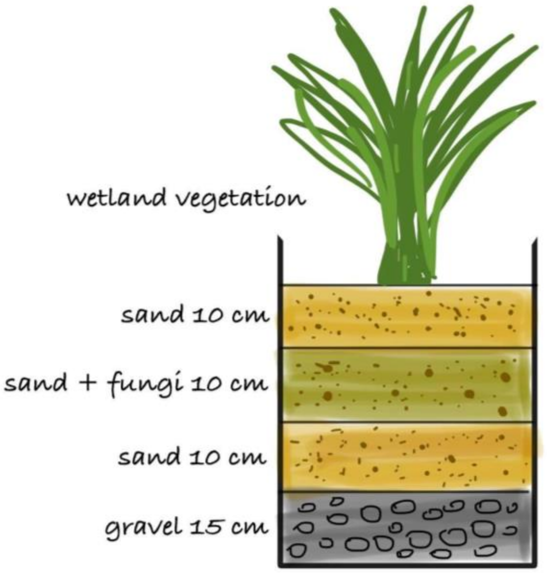

***Figure 5**: Composition of vertical subsurface constructed wetland.*

Source: Author

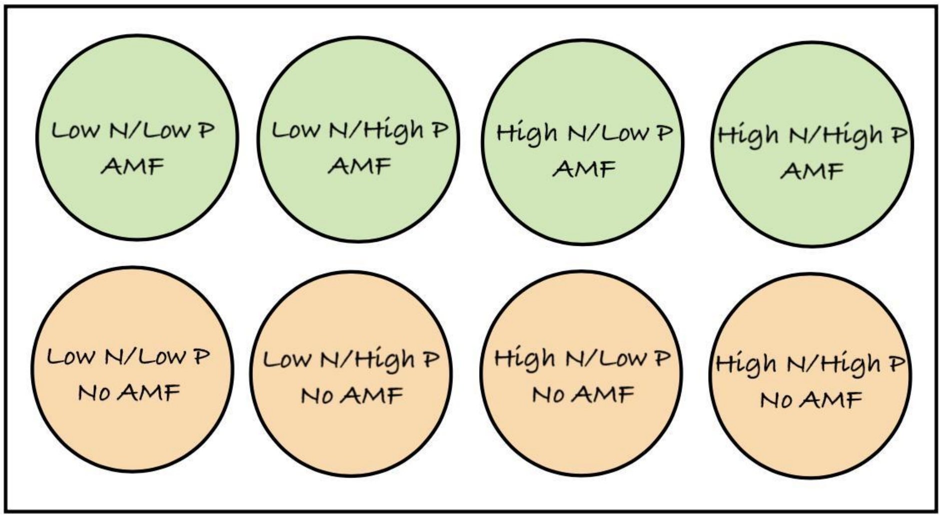

***Figure 6**: Composition of experimental setup. 8 vertical flow constructed wetlands with different N:P concentration ratios and AMF presence.*

Source: Author

4.2 Water Sample Analysis

After *Iris Pseudacorus* successfully started to grow and demonstrated signs of adaptation to the constructed wetland environment, while forming a symbiotic relationship with *Rhizophagus irregularis*, 2.5 L of pre-prepared simulated wastewater was fed into each CW system **(See Figure 7)**.

Subsequently, water samples were collected every week from each CW into 8 labelled 50 mL falcon tubes for further analysis (outflow measurements). At the same time, 4 samples were collected from the simulated wastewater (inflow measurements).

Afterwards, these samples were taken to the laboratory where different water parameters were analyzed. This includes:

- physicochemical parameters
    - pH
    - oxidation/reduction potential (ORP)
- wastewater parameters
    - TOC (Total Organic Carbon)
    - TC (Total Carbon)
    - IC (Inorganic Carbon)
    - NH_4 (Ammonia)
    - TN (Total Nitrogen)
    - NO_2 (Nitrites)
    - NO_3 (Nitrates)
    - PO_4^{3-} (Phosphates)

Reagents	Low N/Low P (g)	Low N/High P (g)	High N/Low P (g)	High N/High P (g)
Urea	0.52	0.52	0.52	0.52
NH_4Cl Ammonium Chloride	0.04	0.04	0.4	0.4
$CH_3COONa \bullet 3H_2O$ Sodium Acetate	0.65	0.65	0.65	0.65
Peptone	0.1	0.1	0.1	0.1
Yeast extract	0.66	0.66	0.66	0.66
Skim milk	0.295	0.295	0.295	0.295
$NaHCO_3$ Sodium Bicarbonate	0.125	0.125	0.125	0.125
$MgCl_2 \bullet 6H_2O$ Mineral bischofite	0.17	0.17	0.17	1.17
KH_2PO_4 Monopotassium phosphate	0.1	0.41	0.1	0.41
Microelements	Low N/Low P (mL)	Low N/High P (mL)	High N/Low P (mL)	High N/High P (mL)
$CuSO_4 \bullet 5H_2O$ Copper (II) sulfate	0.25	0.25	0.25	0.25
$FeSO_4 \bullet 7H_2O$ Iron (II) sulfate	0.25	0.25	0.25	0.25
H_3BO_3 Boric acid	0.25	0.25	0.25	0.25
$Na_2MoO_4 \bullet 2H_2O$ Sodium molybdate	0.25	0.25	0.25	0.25
$KCr(SO_4)_2 \bullet 12H_2O$ Chromium (III) potassium sulfate dodecahydrate	0.25	0.25	0.25	0.25

Figure 7*: Composition of simulated wastewater (inflow).*
Source: Author

4.2.1 pH and Oxidation/Reduction Potential

Firstly, the pH and ORP were measured using the device Multi 3430 (WTW) on each sample. After the device was calibrated, the probe was rinsed between each sample measurement to reduce the risk of systematic errors. Results are recorded.

4.2.2 Ammonia Measurement

Secondly, the NH_4 was measured by performing the indophenol method using the Agilent Technologies Cary 60 UV-Vis spectrophotometer with a wavelength of 655 nm, which required preparation of an alkaline solution and a dyeing solution. The alkaline solution consists of 16 g of sodium hydroxide (NaOH) dissolved in 250 mL of deionised water and 1g of sodium dichloroisocyanurate dihydrate ($C_3N_3O_3Cl_2Na_2H_2O$) which afterwards followed a period of incubation until this solution reached room temperature. The dyeing solution contains 32.5g of sodium salicylate ($C_7H_5O_3Na$) and 32.5g of sodium citrate dihydrate ($Na_3C_6H_5O_72H_20$) added to 250 ml of deionised water. After the dissolution, 0.238g of sodium nitroprusside dihydrate ($Na_2[Fe(CN)5NO]\bullet 2H_2O$) was added to the solution allowing complete dissolving.

The measurement of NH_4 includes the preparation of a blank solution and the sample solutions (containing the inflow and outflow measurements). The blank solution is prepared by pipetting 8.4 ml of ultra-pure water, 0.8 ml of the alkaline solution and 0.8 ml of the dyeing solution. Furthermore, the sample solutions for inflow measurements are 8x diluted meaning they are prepared with pipetting 7.4 ml of ultra-pure water, 1 ml of the sample and 0.8 ml of each alkaline and dyeing solution. On the other hand, the sample solutions for the outflow measurements are prepared by pipetting 8 ml of ultra-pure water, 0.4 ml of the sample itself and 0.8 ml of each alkaline and dyeing solution. This results in having 10 ml solutions into each test tube.

After all test tubes are prepared, they are closed with a cap and allowed to stand for 60 minutes. Subsequently, each test tube was measured in the before-mentioned spectrophotometer using a 1 cm cuvette. Results are recorded.

4.2.3 Total Organic Carbon, Total Carbon, Inorganic Carbon and Total Nitrogen

Thirdly, TOC, TC, IC and TN were all measured using the primacy SERIES TOC analyzed (Skylar, Dutch). This machine provides accurate results and analysis due to its large autosampler, which exposes the samples to high-temperature catalyst combustion. The TN is converted into Nitric Oxide (NO) and TOC is measured by subtracting the IC from the TC. This process catches the particles, which are then transferred into a detector that uses infrared light to detect the measurements.

When the machine is turned on, 10 mL of H_3PO_3 solution needs to be added to the pipes using a syringe. Afterwards, the programme is turned on and the analyzer needs to warm up, then the samples are ready to be measured. Firstly, a test tube containing tap water is added, secondly, 50 mg/L of IC standard solution, thirdly 50 mg/L of TC standard solution and after that 5 mg\L of TN standard solution is added, and tap water is placed at the end. After this, the water samples are placed in a numbering order. The machine starts analyzing the samples and the results are recorded.

4.2.4 Phosphates, Nitrates and Nitrites

Lastly, the phosphates, nitrates and nitrites were measured using the 883 Basic IC plus (Metrohm, Switzerland). The process starts by preparing the two mobile phases: the phosphoric acid and sodium bicarbonate solution. Phosphoric acid is prepared using 6.78 ml of H_3PO_4 to 1 L of ultra-pure water and the sodium bicarbonate solution consists of 25 mL of $NaHCO_3$ solution to 0.5 L of ultra-pure water.

The machine is turned on and needs 30 minutes for the conductivity to stabilize, so in the meantime, the water samples are prepared. Each sample is filtered using a 0.22 um filter. There should be tap water, 5 mg/L standard solution and 25 mg/L standard solution placed before the water sample test tubes. Additionally, there should be a test tube filled with ultra-pure water at the end of the measurements. After the conductivity stabilizes, the machine starts analyzing the samples and results are recorded.

5. Results

5.1 Water Sample Analysis Results

The results from the water sample analysis include information on pH, ORP, TOC, TC, IC, NH_4, TN, NO_2^-, NO_3^- and PO_4^{3-} for the inflow and outflow measurements. The results from the raw data for NH_4, NO_2^- and NO_3^- were converted into NH_4-N, NO_2 -N, and NO_3 -N using the following formulas where NH_4-N is given as an example:

a) Conversion Factor

$$Mr(NH_4) = \frac{Ar(N)}{Ar(N)+4Ar(H)}$$

Where:

Mr = molar mass

Ar = atomic mass

$$(NH_4\text{-}N) = conc.(NH_4) \times Mr(NH_4)$$

Where:

conc.(NH_4) = values from raw data

The same calculations were done on the raw data for PO_4^{3-} where it was converted into PO_4^{3-} -P.

Moreover, removal efficiency was calculated for all water sample analysis results using the following formula:

b) Removal Efficiency

$$Removal\ efficiency = \frac{(Cin \times Vin) - (Cout \times Vout)}{Cin \times Vin} \times 100$$

Where:

Cin = concentration of inflow

Vin = volume of inflow

Cout = concentration of outflow

Vout = volume of outflow

Afterwards, graphs in the style of lines and columns were created in order to show the visual representation of the results. All figures shown in the results section of this thesis are made by the author.

5.1.1 pH and Oxidation/Reduction Potential

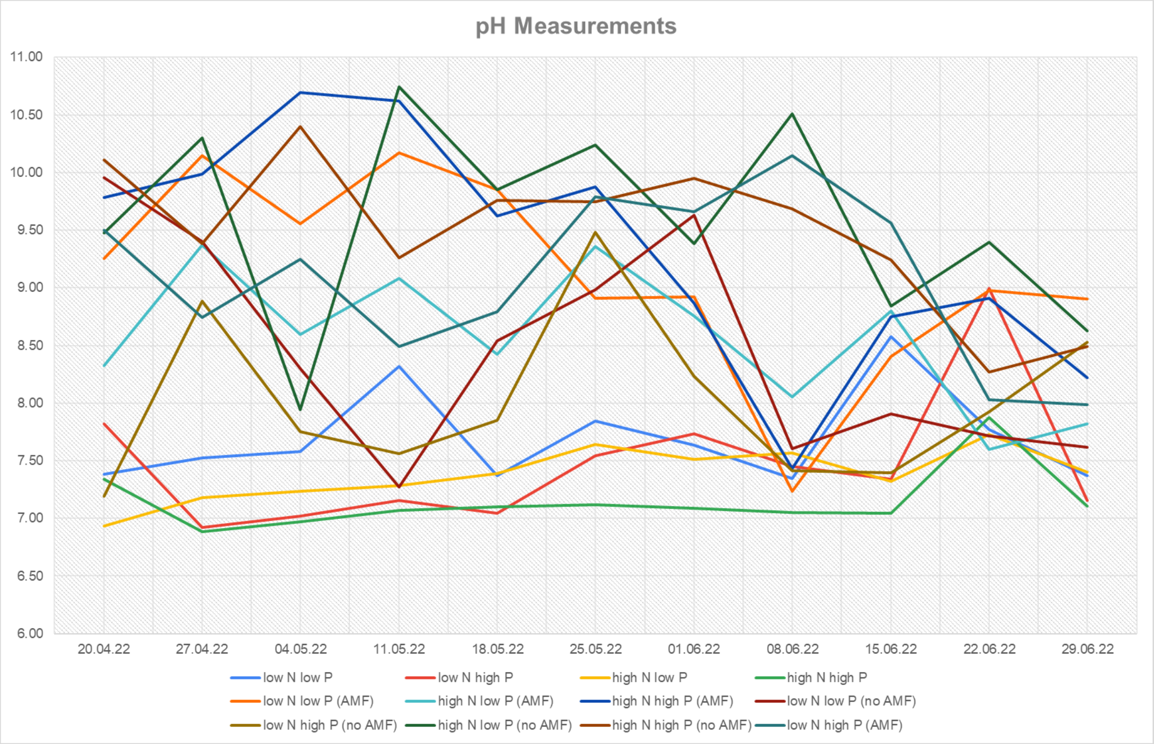

***Figure 8**: pH measurements for inflow (low N low P, low N high P, high N low P, high N high P) and outflow (AMF and no AMF) measurements throughout the experiment.*

pH is referred to the process of measuring hydrogen ions concentration in the wastewater. This measurement has a significant impact on wastewater treatment processes, which is usually treated at pH levels from 6.5 to 8.5. Some of the issues related to wastewater purification are high alkalinity levels and accumulation of pollutants.

The results from the pH measurements in **Figure 8** demonstrate a range of values, where the inflow measurements indicate lower pH levels specifically from 6.88 in high N high P concentration, to 8.58 in low N low P concentration. Additionally, outflow measurements show higher values than inflow measurements ranging from 7.27 in low N low P without mycorrhizal fungi to 10.69 in high N high P containing mycorrhiza.

Moreover, all outflow measurements containing mycorrhizal fungi demonstrated a decrease in pH levels over time. Similarly, the measurements not containing mycorrhiza decreased over time, for example, reactor with low N low P concentrations started at 9.96 pH and decreased to a value of 7.62 pH toward the end of the experiment. Furthermore, there is a significant drop in pH levels on the 08.06 for low N low P measurements regardless of the mycorrhizal presence. In contrast, when the reactor with high N low P concentration ratios containing mycorrhizal fungi dropped in pH levels, the same concentration ratio reactor, however not containing AMF, increased to a significant 10.51 value.

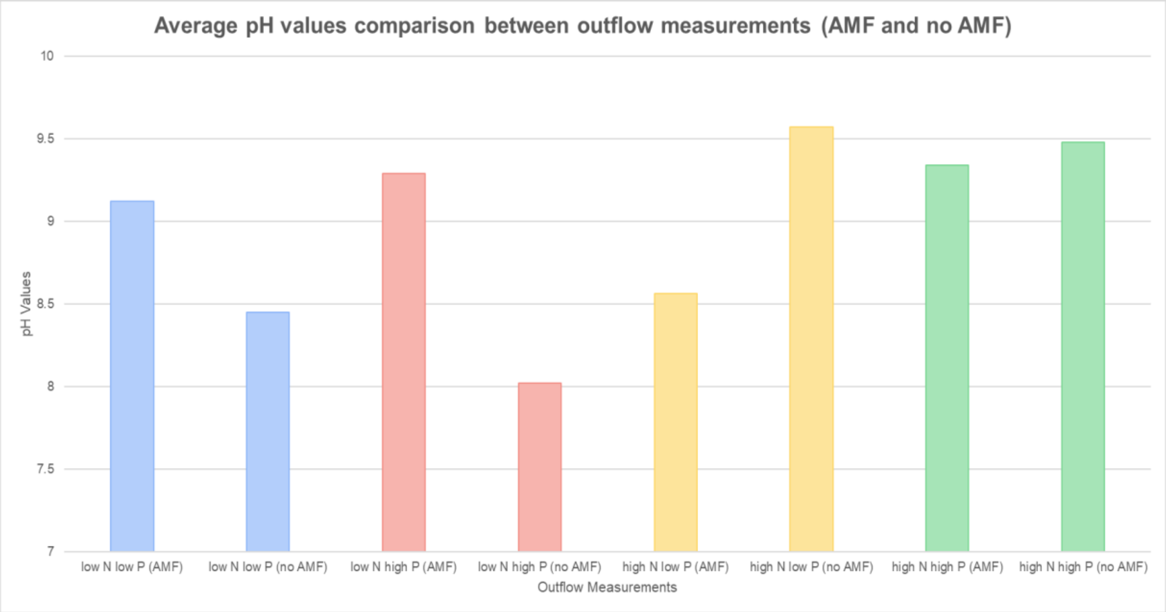

Figure 9*: Average pH values comparison between outflow measurements (AMF or no AMF). Same colors represent same N:P concentration ratios*.

Figure 9 emphasizes the difference in the average of pH values between reactors with the same N:P concentration ratios, however different AMF presence. The reactor with ratios low N low P as well as low N high P demonstrated higher pH values where there was a presence of mycorrhizal fungi. On the other hand, the reactors with concentrations high N low P and high N high P indicate lower pH values where there is a presence of mycorrhiza. The graph highlights that the higher concentration of N where there is also a presence of mycorrhizal fungi, lowers the pH level in the reactors.

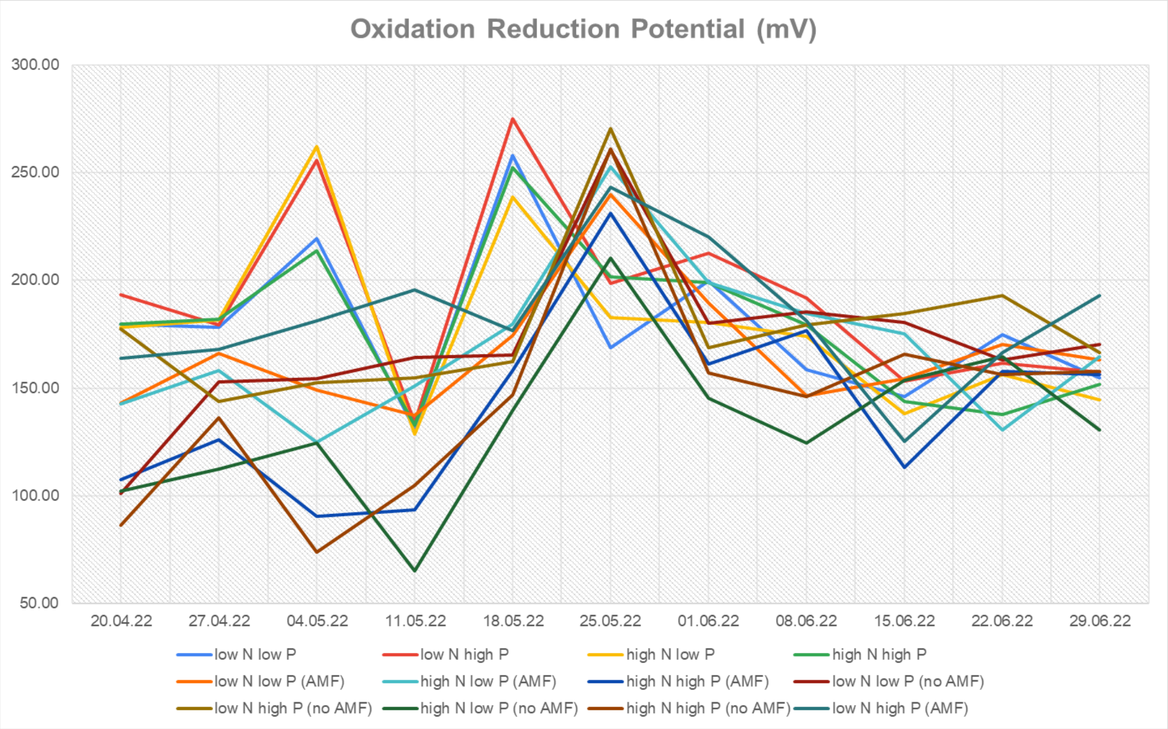

***Figure 10**: ORP results for inflow (low N low P, low N high P, high N low P, high N high P) and outflow (AMF and no AMF) measurements.*

Oxidation-reduction potential (ORP) acts as a measurement of sanitizing effectiveness of water. The measuring of ORP is commonly used in wastewater treatment in order to monitor the occurrence of different chemical processes and reactions. Some important reactions in wastewater purification include nutrients such as C, N, P, S which change oxidations states such as nitrate and sulphate and reduced states such as ammonia and sulfides (Gerardi, 2008).

The inflow measurements for the ORP level shown in **Figure 10** demonstrated high values throughout the experiment, ranging from 137.5 mV to 275.1 mV. Additionally, outflow measurements show a similar pattern in ORP levels, starting at lower values, increasing during the middle of the experiment and gradually stabilizing at lower values toward the end of the experiment.

Generally, outflow measurements indicated high values, meaning processes such as nitrification, cBOD degradation and biological phosphorus removal occurred. Nitrification is one of the most important processes in wastewater treatment, and values above 100 mV demonstrate high oxidation of ammonia into nitrates (Higgins, 2013). The ORP results indicate increasing values from 20.04 to 25.05 and then stabilizing values for the rest of the experiment length.

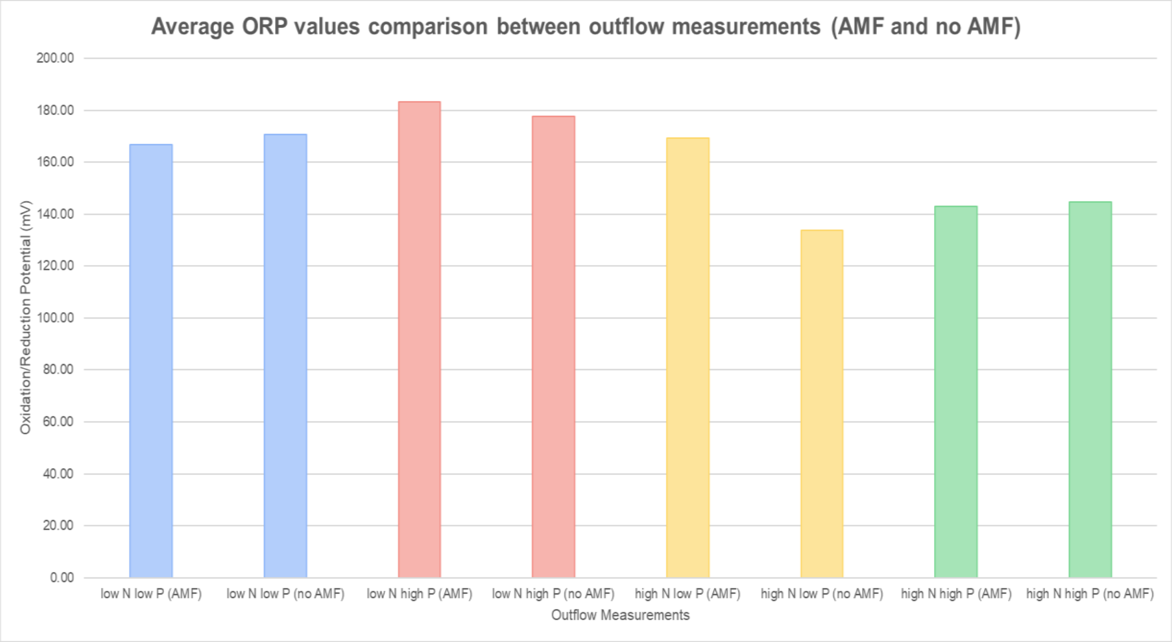

Figure 11*: Average ORP values comparison between outflow measurements (AMF or no AMF). Same colors represent same N:P concentration ratios.*

When comparing values from the outflow measurements with and without the presence of mycorrhizal fungi, **Figure 11** demonstrates similar results between the reactors. However, there is a significant difference in the reactors with high N low P, where the presence of mycorrhizal fungi indicates higher ORP values, specifically 169.35 mV, than the reactor not containing AMF with 133.87 mV. Furthermore, the reactors with high N high P concentrations show similar values in oxidation-reduction potential regardless of the mycorrhizal fungi presence.

5.1.2 Carbon

5.1.2.1 Total Organic Carbon (TOC)

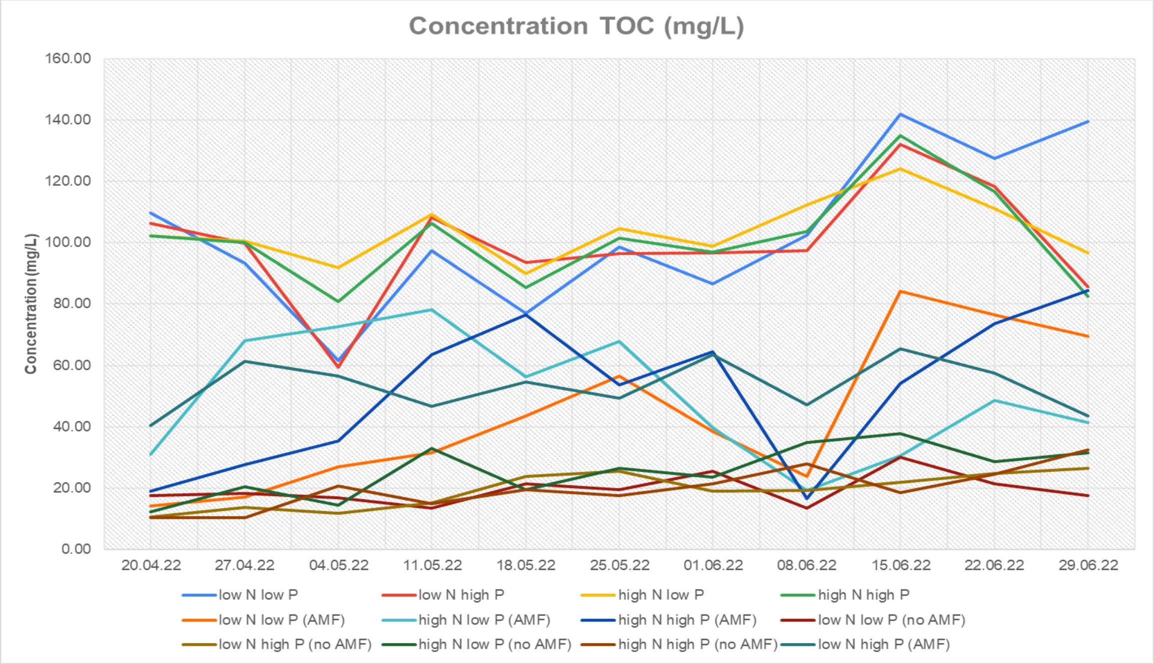

***Figure 12**: Concentration of TOC (mg/L) for inflow (low N low P, low N high P, high N low P, high N high P) and outflow (AMF and no AMF) measurements.*

Total Organic Carbon (TOC) represents the concentration number of organic compounds in the water sample, and it is used in wastewater treatment for ensuring the wetland plants comply with the TOC amount and regulations.

Figure 12 represents the results for the ongoing concentration values for TOC throughout the experiment for inflow and outflow measurements. The inflow values demonstrate higher concentration values than the outflow due to loss and removal of organic carbon through the process of wastewater treatment in the CWs.

Moreover, the concentration of TOC for outflow measurements containing mycorrhizal fungi indicates significantly larger values than the outflow measurements without mycorrhizal fungi. In addition, the reactors not containing AMF demonstrate more constant values ranging from 10.45 mg/L to 32.94 mg/L than the reactors containing AMF, which range from 23.77 mg/L to 76.54 mg/L. Furthermore, the values for outflow measurements, especially reactors not containing mycorrhizal fungi, indicate an exponential growth over time throughout the experiment.

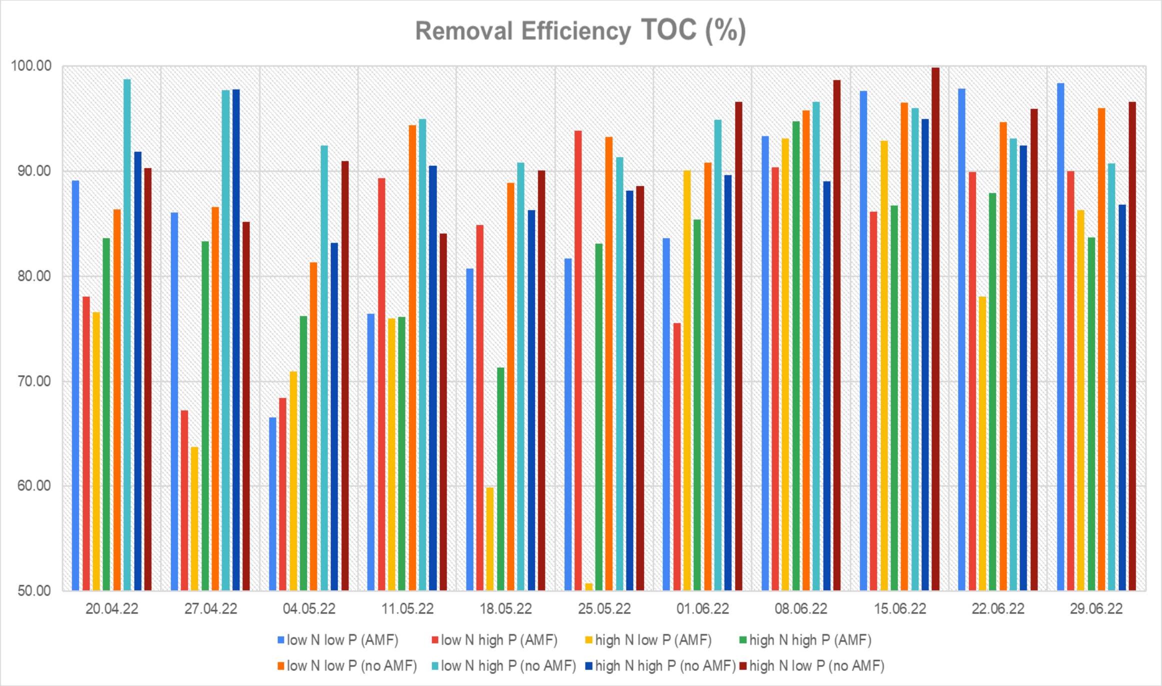

Figure 13*: Removal Efficiency of TOC (%) for outflow (AMF and no AMF) measurements.*

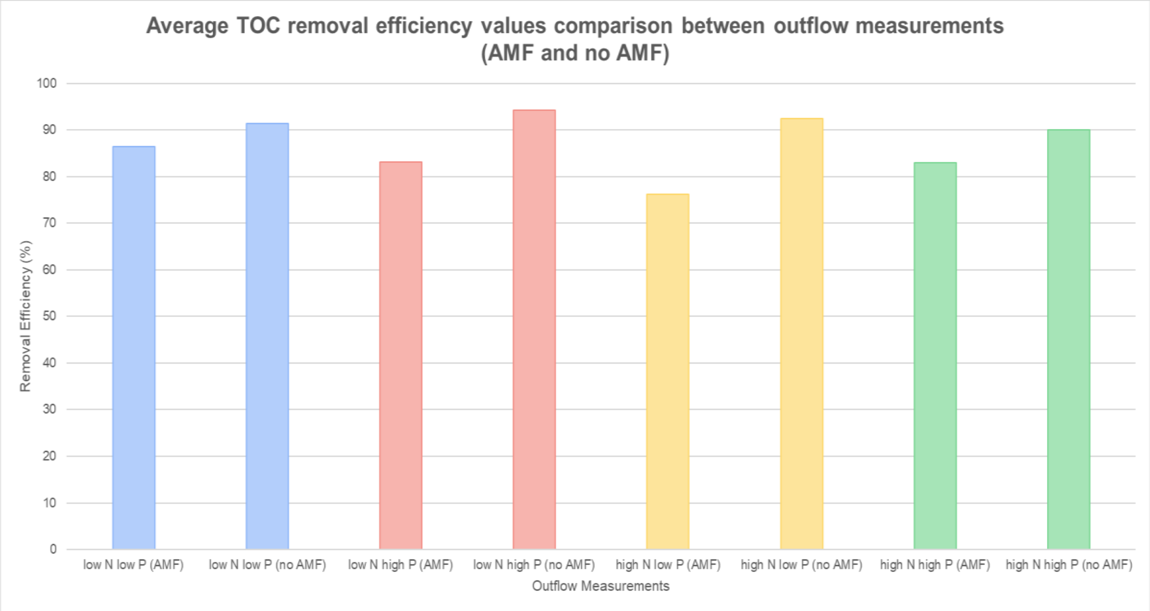

Figure 14*: Average TOC removal efficiency for outflow measurements (AMF or no AMF). Same colors represent same N:P concentration ratios.*

Figure 13 emphasizes the removal efficiency of TOC throughout the experiment in all outflow measurements. The reactors without a presence of mycorrhizal fungi demonstrate higher removal efficiency than the reactors containing mycorrhiza. Additionally, the rate of removal efficiency has been shown to be increasing over time during the experiment.

The graph under **Figure 14** more clearly demonstrates the differences in TOC removal efficiency between the outflow measurements with different N:P concentrations and AMF presence. The reactors where there is no presence of mycorrhizal fungi show a larger removal efficiency rate than the reactors containing mycorrhiza.

Additionally, the highest removal efficiency rate is shown by the reactor with low N high P and without a presence of AMF with 94.2%. In contrast, the lowest removal efficiency rate is demonstrated by reactor high N low P with a presence of mycorrhiza with a value of 76.2%.

5.1.2.2 Total Carbon (TC) Results

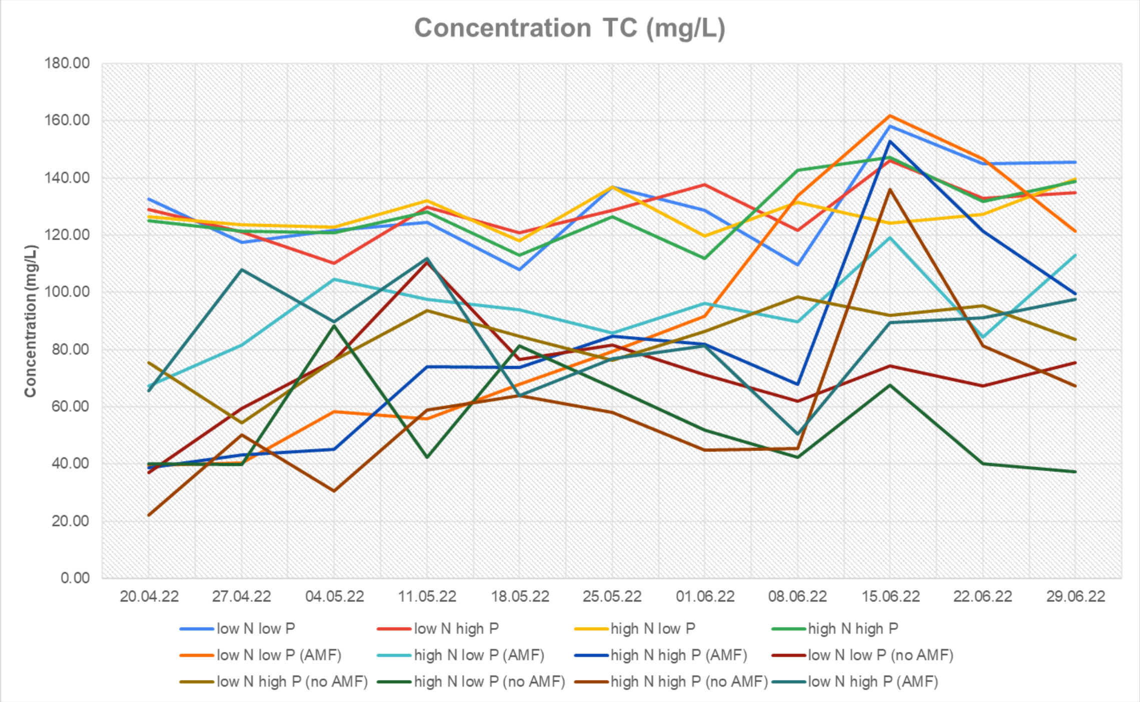

***Figure 15**: Concentration of TC (mg/L) for inflow (low N low P, low N high P, high N low P, high N high P) and outflow (AMF and no AMF) measurements.*

Total Carbon (TC) in wastewater includes both inorganic and organic carbon, which consists of carbon found in ores and minerals as inorganic and carbon found in nature and living organisms as organic.

The results shown in **Figure 15** demonstrate a range of values describing the TC concentration for inflow and outflow measurements over time. Similarly, as previously shown in the TOC concentration graph, the inflow concentrations indicate larger values throughout the experiment than outflow concentrations, meaning removal of TC occurred in the wastewater treatment process as well.

However, there are points where the outflow measurements surpassed or approximated the inflow concentration values, specifically on the 15.06 where values for high N high P concentrations increased up to 152.76 mg/L for the reactor with presence of mycorrhizal fungi, and 136 mg/L for the reactor without mycorrhiza.

Similarly, the reactor with concentrations low N low P containing mycorrhizal fungi increased to a value of 161.72 mg/L, reaching the highest value of TC concentrations for outflow measurements throughout the experiment.

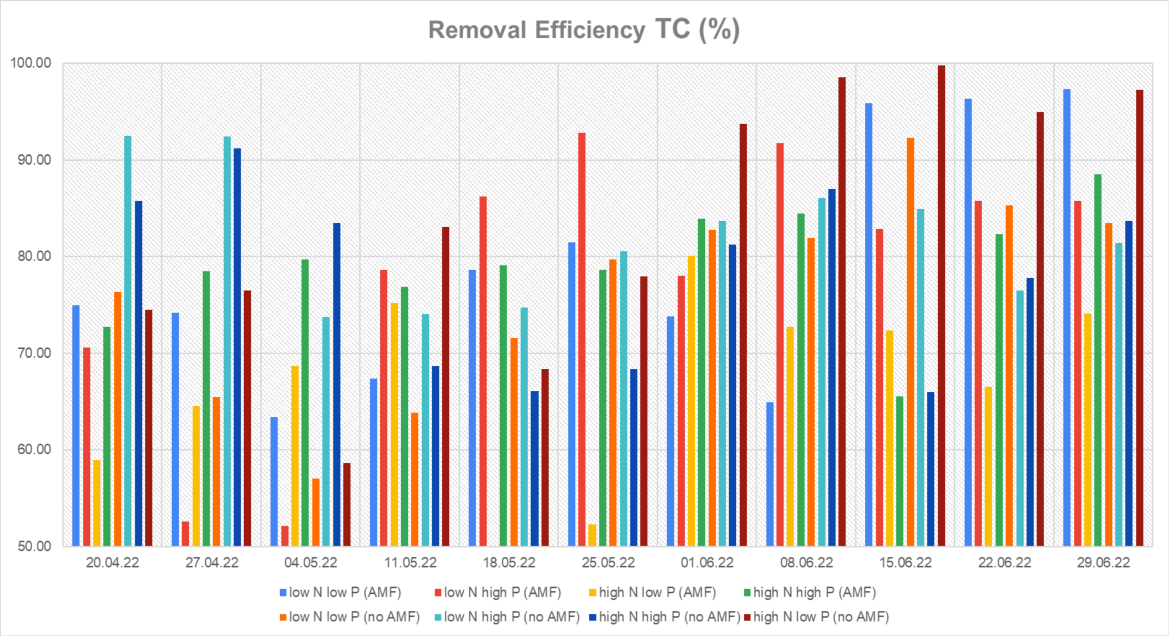

Figure 16: *Removal Efficiency of TC (%) for outflow (AMF and no AMF) measurements.*

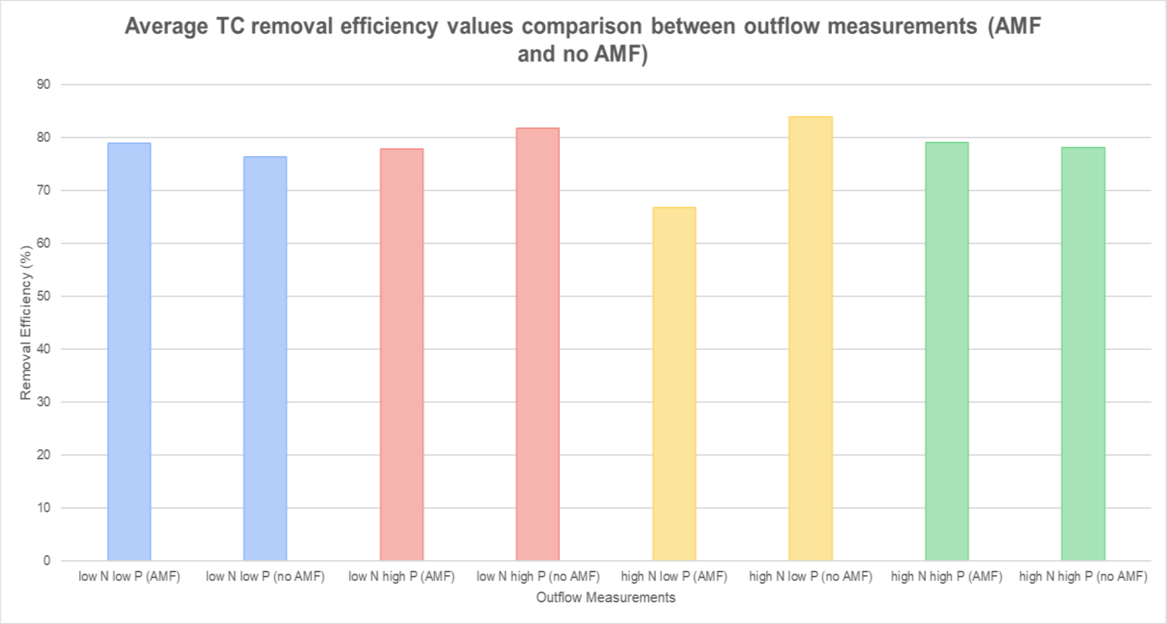

Figure 17: *Average TOC removal efficiency for outflow measurements (AMF or no AMF). Same colors represent same N:P concentration ratios.*

The highest removal efficiency for TC is represented in **Figure 16** from the 1st of June until the end of the experiment in reactor high N low P without a presence of mycorrhizal fungi. On the other hand, the lowest removal efficiency is shown in reactor with high N low P concentrations with a presence of mycorrhiza, where this reactor reached the lowest removal efficiency of 52.3%.

Moreover, there is a clear increasing trend in removal efficiency throughout the experiment as the weather became warmer, especially in reactor high N low P without AMF, where it has reached a value of 99.7% removal efficiency on the 15th of June.

Additionally, **Figure 17** demonstrates similar values of average TC removal efficiency for reactors containing and not containing mycorrhizal fungi. The most significant difference is emphasized in reactor with high N low P concentrations, where no presence of mycorrhiza indicated higher values of removal efficiency than the reactor with AMF. Subsequently, reactor with low N high P also demonstrated higher removal efficiency where AMF was not present. In contrast, in reactors low N low P and high N high P, higher removal efficiency was reached by reactors with a presence of mycorrhiza.

5.1.2.3 Inorganic Carbon (IC) Results

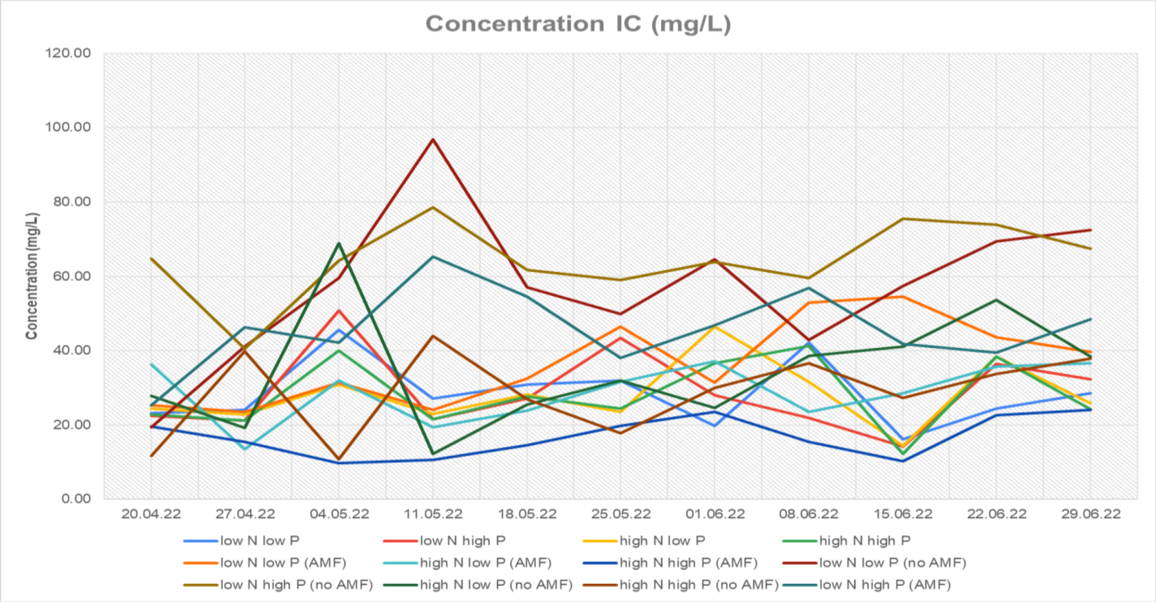

***Figure 18**: Concentration of IC (mg/L) for inflow (low N low P, low N high P, high N low P, high N high P) and outflow (AMF and no AMF) measurements.*

Inorganic carbon (IC) summarizes all simple carbon compounds such as carbon dioxide, carbonate, bicarbonate and carbonic acid.

The inflow values shown in **Figure 18** demonstrate values ranging from 12.27 mg/L to 45.58 mg/L of IC concentration. Additionally, the outflow measurements containing mycorrhizal fungi show significantly higher concentration values than the measurements without a presence of AMF.

The lowest concentration of IC is demonstrated by the reactor with high N high P concentrations containing mycorrhiza, with 9.69 mg/L. In contrast, the highest concentration is shown in reactor low N low P without a presence of mycorrhiza with a value of 78.52 mg/L.

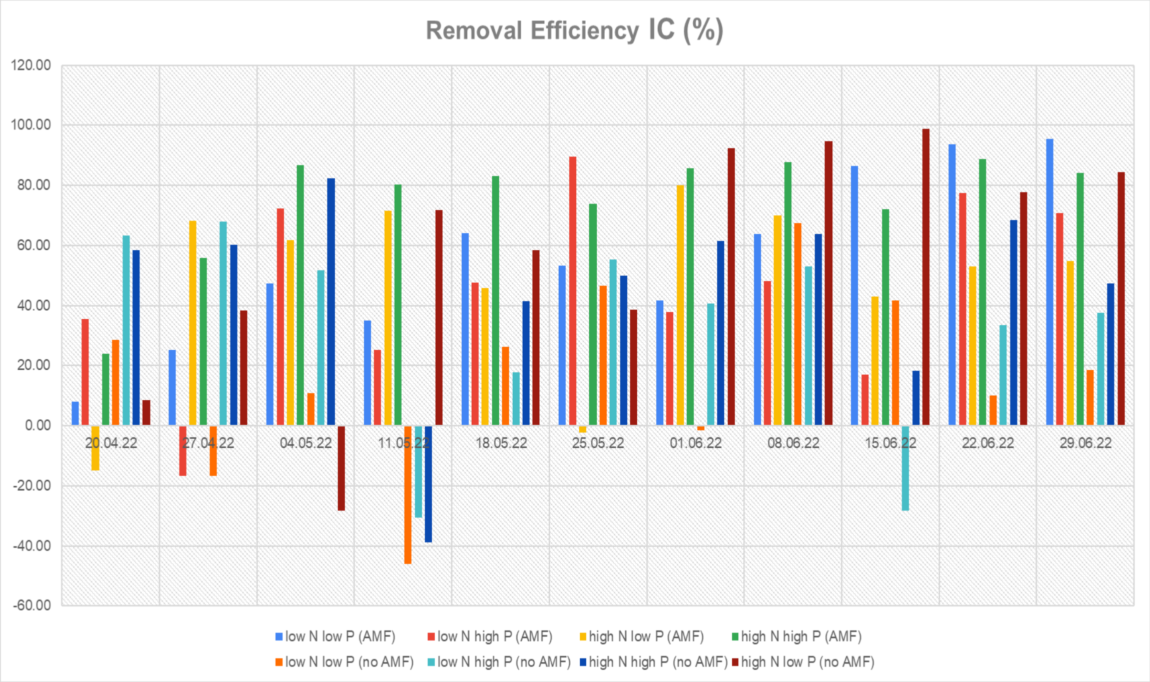

Figure 19*: Removal Efficiency of IC (%) for outflow (AMF and no AMF) measurements.*

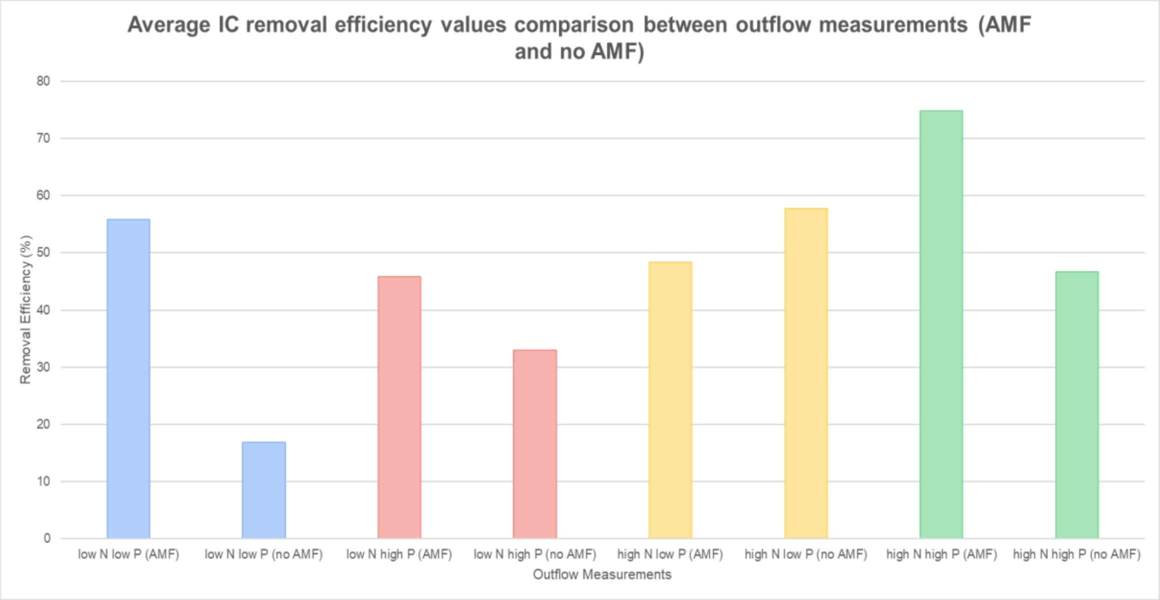

Figure 20: *Average IC removal efficiency for outflow measurements (AMF or no AMF). Same colors represent same N:P concentration ratios.*

Furthermore, removal efficiency for IC presented in **Figure 19** demonstrates an increase in values over time for the reactor with low N low P concentrations containing mycorrhizal fungi. Similarly, reactor with high N low P without mycorrhiza and reactor high N high P with mycorrhiza shows an exponential increase throughout the experiment.

Additionally, removal efficiency for reactors low N high P and high N low P with AMF have negative values in the first four weeks of the experiment which represent a gain in IC rather than removal efficiency. At the same time, all reactors not containing mycorrhizal fungi had negative values mostly in the first four weeks of the experiment, and reactor low N high P without AMF indicates a gain in IC on the 15th of June.

Moreover, when comparing values for IC removal efficiency in reactors with and without mycorrhizal fungi in **Figure 20**, reactor low N low P demonstrates higher removal efficiency on average where mycorrhiza was present. In addition, similar results are presented in reactor with concentrations low N high P and reactor high N high P, where mycorrhizal fungi increased the removal efficiency for IC.
In contrast, reactor with concentrations high N low P has higher removal efficiency where there was not a presence of AMF.

5.1.3 Nitrogen

5.1.3.1 Ammonia (NH4+)

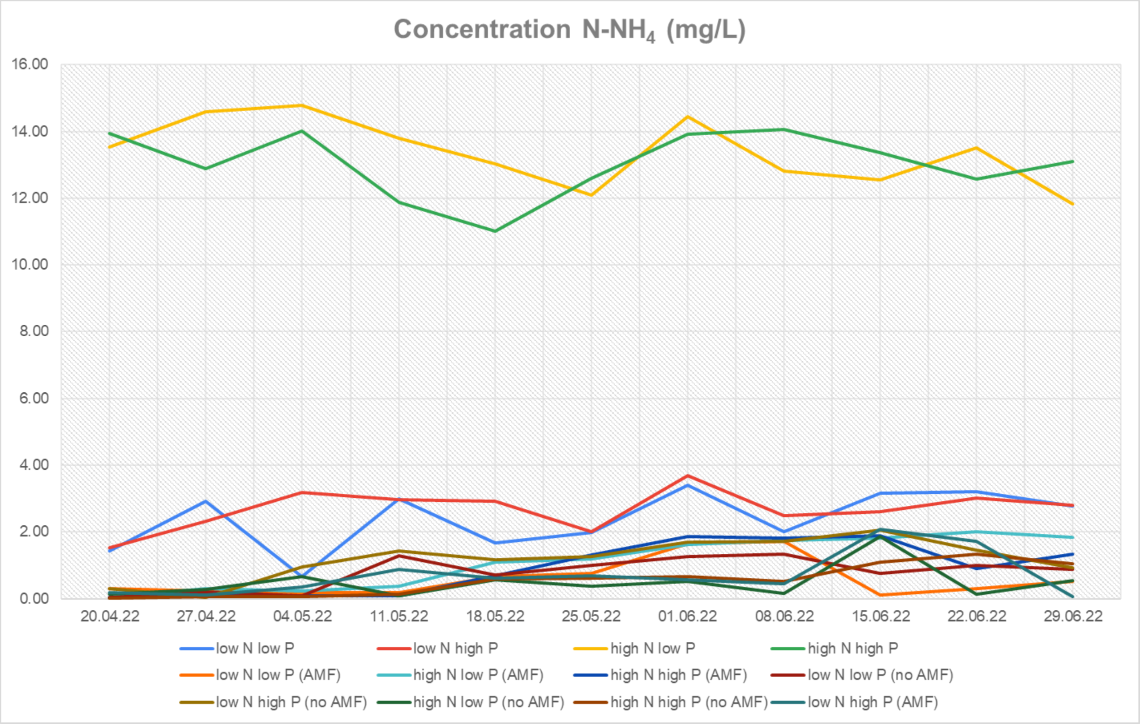

***Figure 21**: Concentration of N-NH$_4$ (mg/L) for inflow (low N low P, low N high P, high N low P, high N high P) and outflow (AMF and no AMF) measurements.*

Ammonia Nitrogen is a critical nutrient in biological processes during wastewater treatment, where nitrification is an essential process in which ammonia gets converted into nitrates.

From the results shown in **Figure 21** demonstrating the concentration of NH$_4$-N throughout the experiment, values show more constant trends. The measurements for inflow, specifically where N concentrations are high, demonstrate a significantly higher concentration of NH$_4$-N with values ranging from 11.02 mg/L to 14.78 mg/L respectfully. On the other hand, measurements for inflow where N concentrations are low indicate values ranging from 0.67 mg/L to 3.70 mg/L.

Outflow measurements show a range of values below the inflow values, meaning the concentration of NH_4-N during wastewater treatment in the reactors decreased as the experiment progressed. Additionally, the graph demonstrates an increase in outflow measurement concentrations in time. Values for high N high P containing mycorrhizal fungi started at 0.19 mg/L and progressed to a value of 2.07 mg/L over time. Similarly, values for high N high P without AMF started at 0.19 mg/L at the beginning of the experiment and increased to 1.33 mg/L towards the end of it.

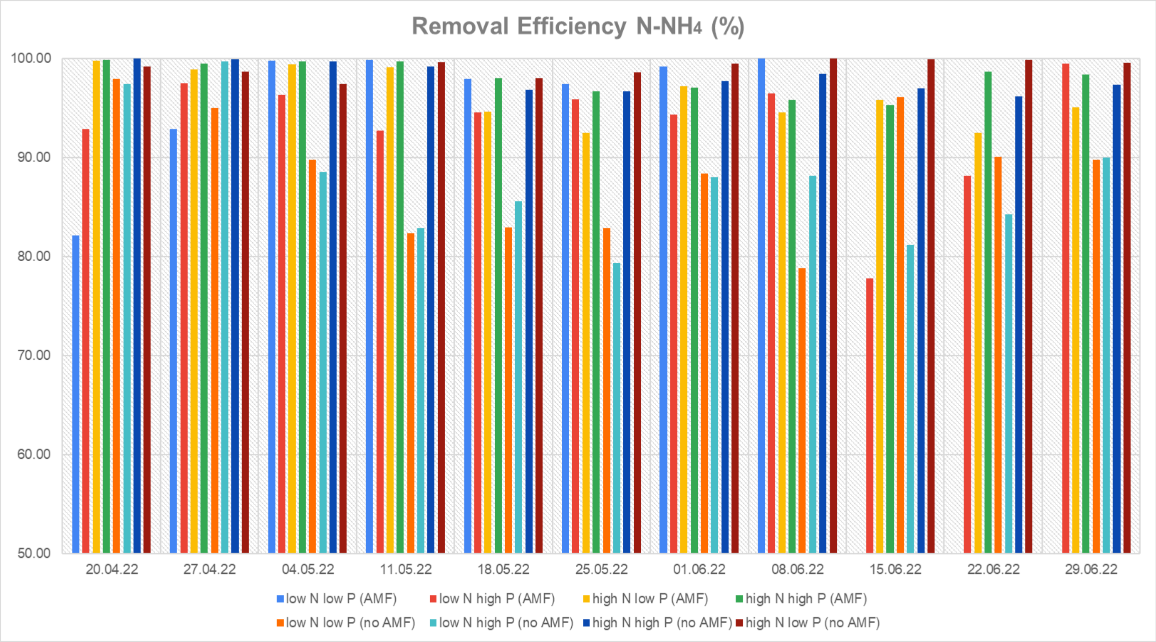

***Figure 22**: Removal Efficiency of N-NH_4 (%) for outflow (AMF and no AMF) measurements*

Furthermore, **Figure 22** demonstrates results for outflow measurements containing all removal efficiency for NH_4-N throughout the experiment. The reactor with the highest removal efficiency is high N low P without mycorrhizal fungi and the lowest removal efficiency is low N high P without mycorrhizal fungi. The measurements indicate a constant trend of values over time for each reactor with different N:P concentrations and mycorrhiza presence.

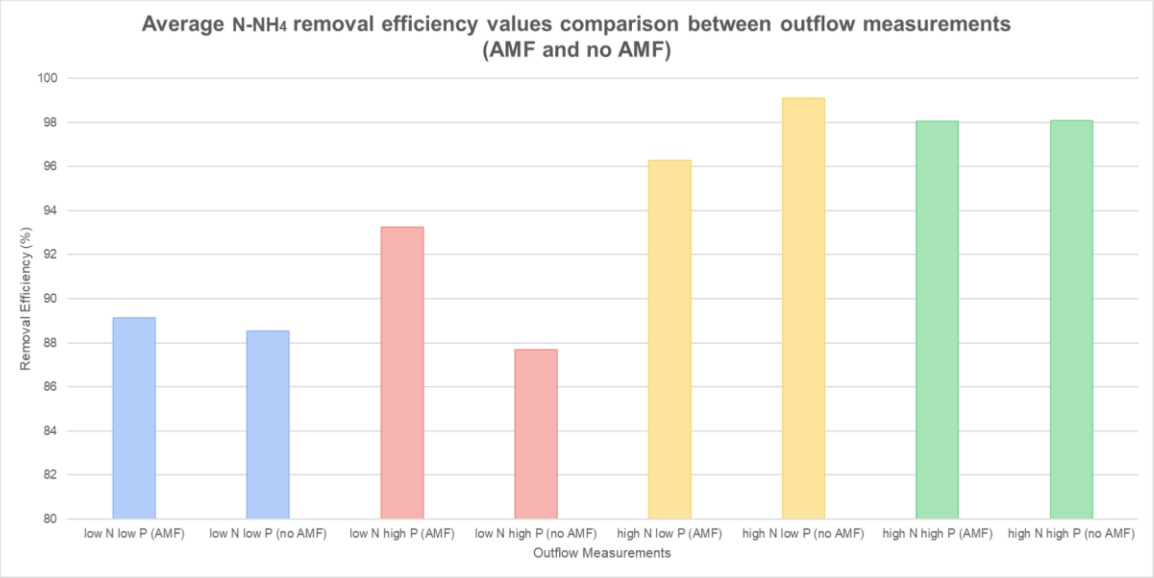

Figure 23*: Average N-NH_4 removal efficiency for outflow measurements (AMF or no AMF). Same colors represent same N:P concentration ratios.*

Additionally, **Figure 23** shows the difference in efficiency removal for NH_4-N for reactors considering the presence of AMF. The highest removal efficiency is shown in reactors where N concentrations are high, for example 99.1% in high N low P without AMF and high N high P containing mycorrhizal fungi with 98%.

In contrast, the least removal efficiency is demonstrated in reactors where N concentrations are low, specifically where there is not a presence of AMF. The reactor low N low P and low N high P without mycorrhizal fungi have values of 88.5% and 87.6% removal efficiency on average, and the same reactors however containing mycorrhizal fungi showed values of 89.1% and 93.2% removal efficiency on average, demonstrating a relationship where higher removal efficiency is achieved with the presence of mycorrhizal fungi in the CWs.

5.1.3.2 Total Nitrogen (TN) Results

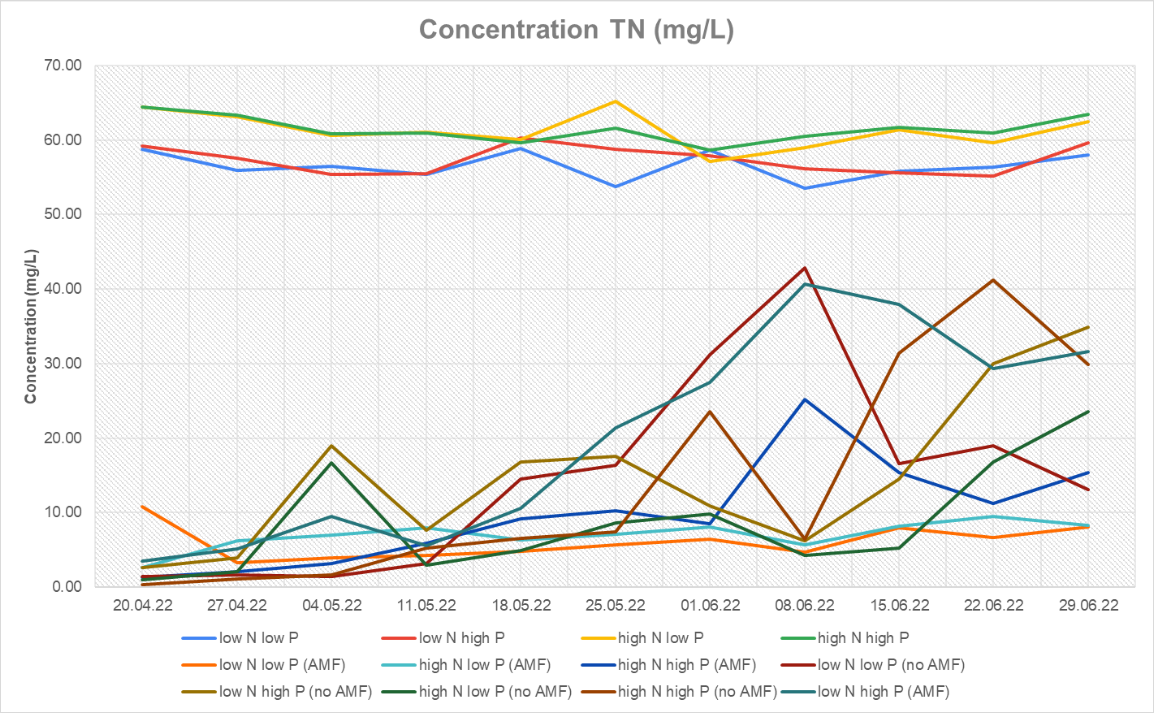

Figure 24*: Concentration of TN (mg/L) for inflow (low N low P, low N high P, high N low P, high N high P) and outflow (AMF and no AMF) measurements.*

Total Nitrogen in wastewater treatment processes can be present by having total organic nitrogen and ammonia which is added with municipal wastewater discharge.

Figure 24 demonstrated high values throughout the experiment for inflow measurements, where the average for the reactor with low N low P is 56.51 mg/L of TN, low N high P has an average of 57.37 mg/L, high N low P has 61.27 mg/L and high N high P has an average of 61.45 mg/L.

Additionally, outflow measurements demonstrated lower values of TN concentration than inflow values, meaning removal of TN occurred during the wastewater purification process. Moreover, outflow measurements indicated a gradual increase in TN concentration over time, especially in reactors without a presence of mycorrhizal fungi. For example, reactor with concentrations low N high P without mycorrhiza started off with a value of 3.53 mg/L and towards the end of the experiment reached a value of 34.9

mg/L of TN concentration. In contrast, reactor with concentrations high N low P however containing mycorrhiza kept more constant values throughout the experiment with 3.53 mg/L at the beginning of the experiment and 8.32 mg/L at the end of it.

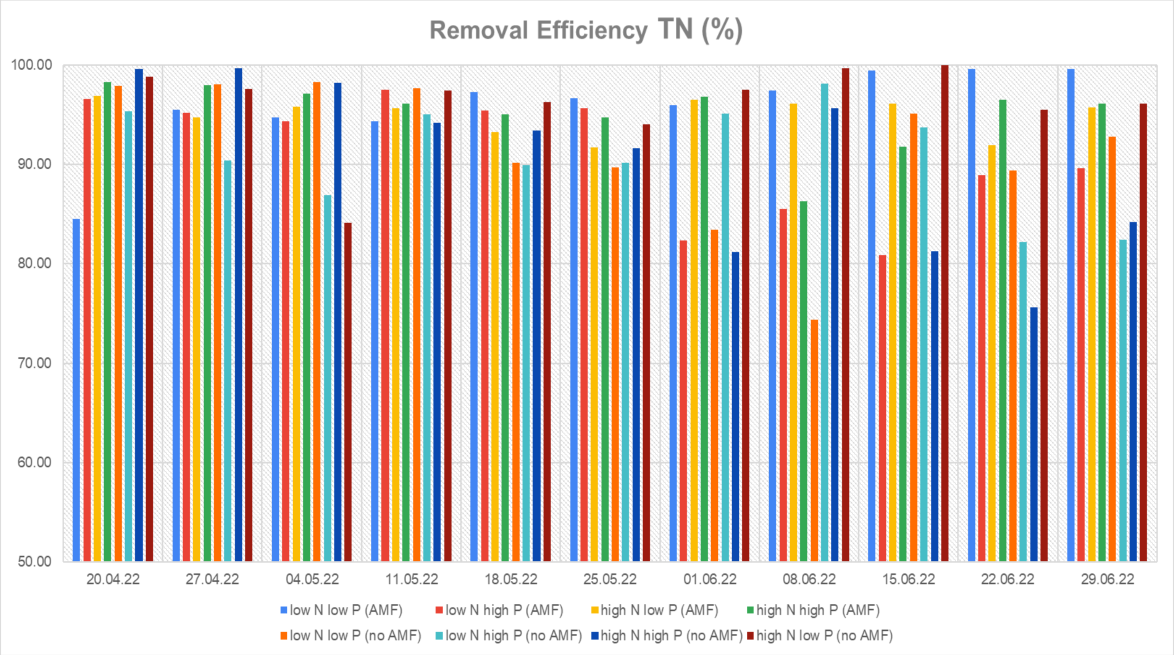

***Figure 25**: Removal Efficiency of TN (%) for outflow (AMF and no AMF) measurements.*

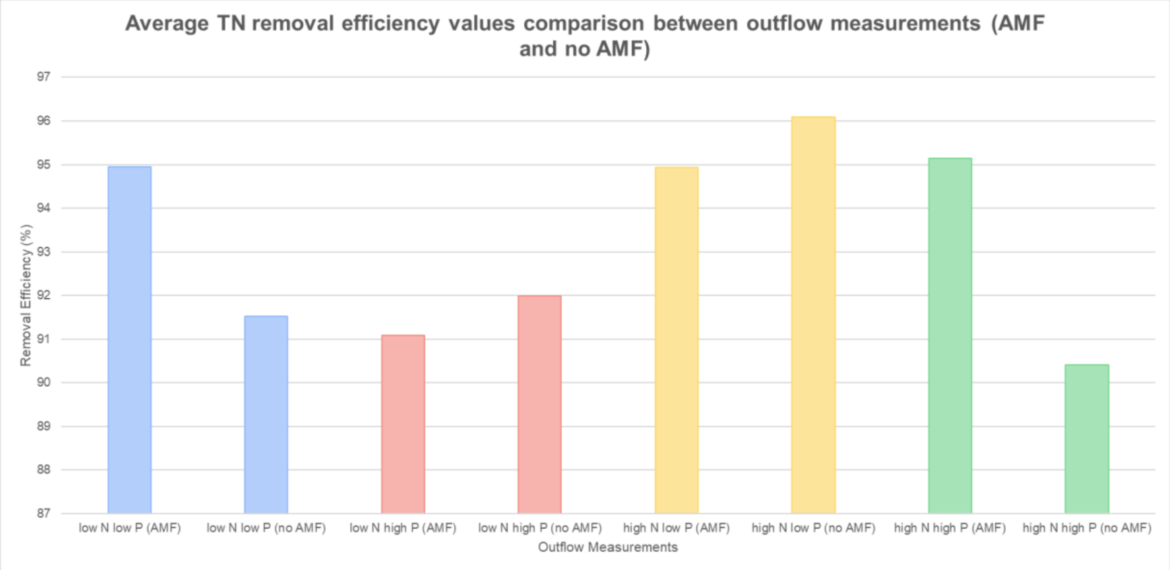

***Figure 26**: Average TN removal efficiency for outflow measurements (AMF or no AMF). Same colors represent same N:P concentration ratios.*

Furthermore, **Figure 25** demonstrates all removal efficiency values from TN for outflow measurements throughout the experiment, where reactors with a presence of AMF indicated constant or increasing values over time, and reactors without mycorrhiza revealed a gradual decrease of removal efficiency over time. For example, reactor with high N high P concentrations with AMF presence has an initial value of 97.8 % and at the end of the experiment has 96.1 % and the reactor with the same concentrations but without AMF starts with values 99.5 % and towards the end of the experiment has a value of 84.2 % of removal efficiency respectfully.

Additionally, **Figure 26** more visually represents the difference of TN removal efficiency in reactors with and without mycorrhizal fungi. The highest average removal efficiency is demonstrated by reactor high N low P without AMF with 96%, and the lowest removal efficiency is shown by reactor high N high P without AMF with 90%.

Moreover, reactor low N low P indicates higher removal efficiency where mycorrhizal fungi is present, as well as reactor with high N high P concentrations. In contrast, reactor with concentrations high N low P and low N high P demonstrates higher removal efficiency where mycorrhiza was not present.

Overall, the lowest removal efficiency was shown in reactor with concentrations low N high P, where the average TN removal efficiency for the reactor containing AMF is 91% and the reactor without AMF presence with a value of 92%.

5.1.3.3 Nitrates (NO_3) and Nitrites (NO_2) Results

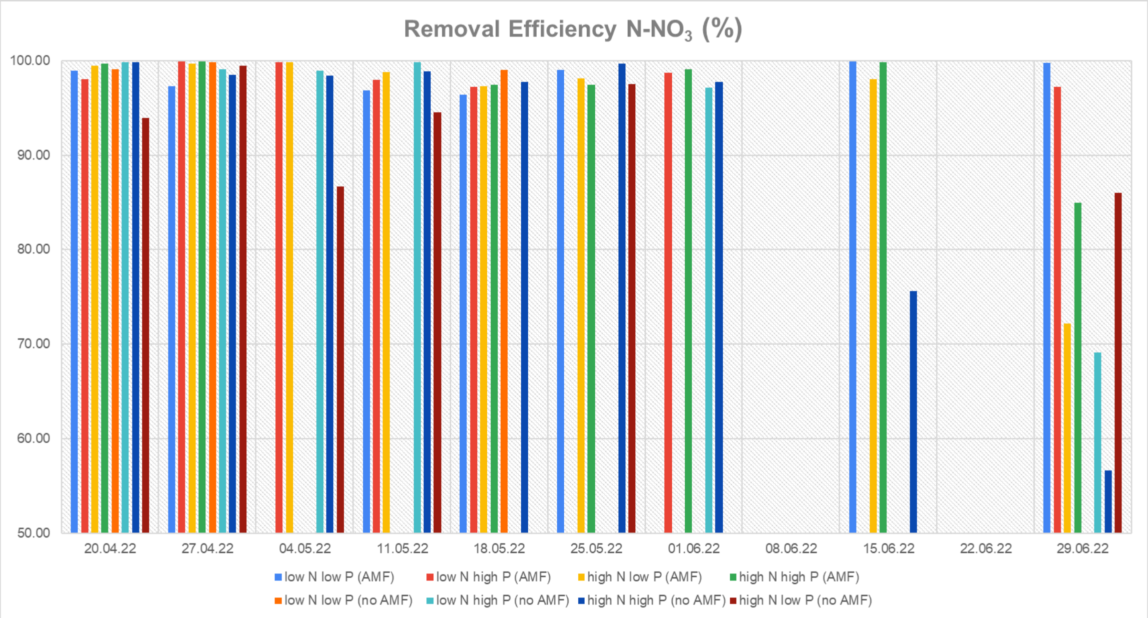

***Figure 27**: Removal Efficiency of N-NO_3 (%) for outflow (AMF and no AMF) measurements.*

Nitrates are an essential source of N for plants which is absorbed through their roots and assists in their growth and metabolism processes, such as creating amino acids and then proteins. Nitrates are commonly found in agricultural fertilizers where they appear as NH_4. Ammonification, nitrification and denitrification are processes which contribute to nitrogen transformation and removal in wastewater purification, where NH_4 is converted into nitrates by nitrifying bacteria.

Concentration values for NO_3 are not shown because there is limited data due to systematic error concerning the machine measurements. However, **Figure 27** demonstrates a variety of values for the removal efficiency of N-NO_3 in outflow wastewater measurements throughout the experiment. The highest removal efficiency is shown to be in reactor with low N high P concentrations containing mycorrhiza with an average of 98.4%. On the other hand, the lowest removal efficiency is indicated by reactor low N low P without AMF with an average removal rate of 86.7%.

Overall, higher removal efficiency of NH_4-N is demonstrated by reactors with a presence of mycorrhizal fungi, and in contrast, lower removal efficiency is shown by reactors without mycorrhiza.

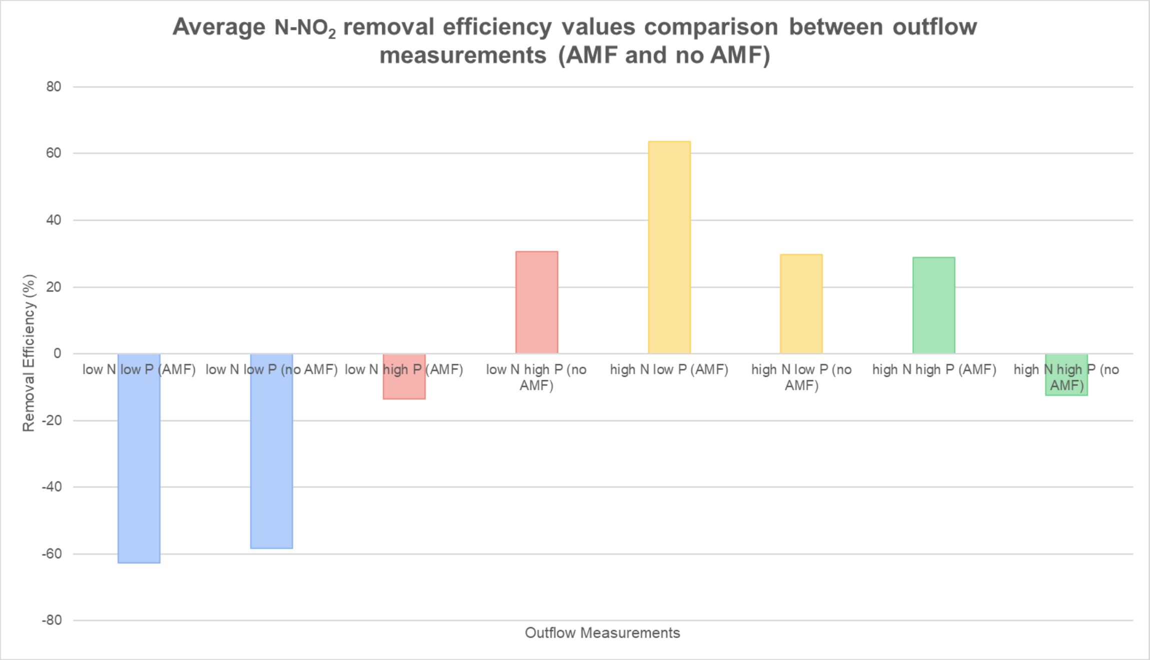

Figure 28: *Average Removal Efficiency of N-NO₂ (%) for outflow (AMF and no AMF) measurements.*

In the process of nitrification, ammonium is converted into nitrites and later can be reduced to ammonia during the N ammonification process and converted into N gas in the process of denitrification.

Figure 28 shows a variety of positive and negative values for average removal efficiency of each outflow measurement. Reactors with concentrations low N low P indicate negative values during the experiment, meaning more NH_4-N was created rather than removed during the wastewater purification process. Similar results occurred in reactor low N high P with mycorrhiza and high N high P without AMF.

In contrast, reactors with concentrations low N high P without mycorrhiza and high N low P with and without mycorrhiza demonstrate positive removal efficiency reaching 60.5% in high N low P with AMF.

5.1.4 Phosphorus

5.1.4.1 Phosphates (PO_4^{3-}) Results

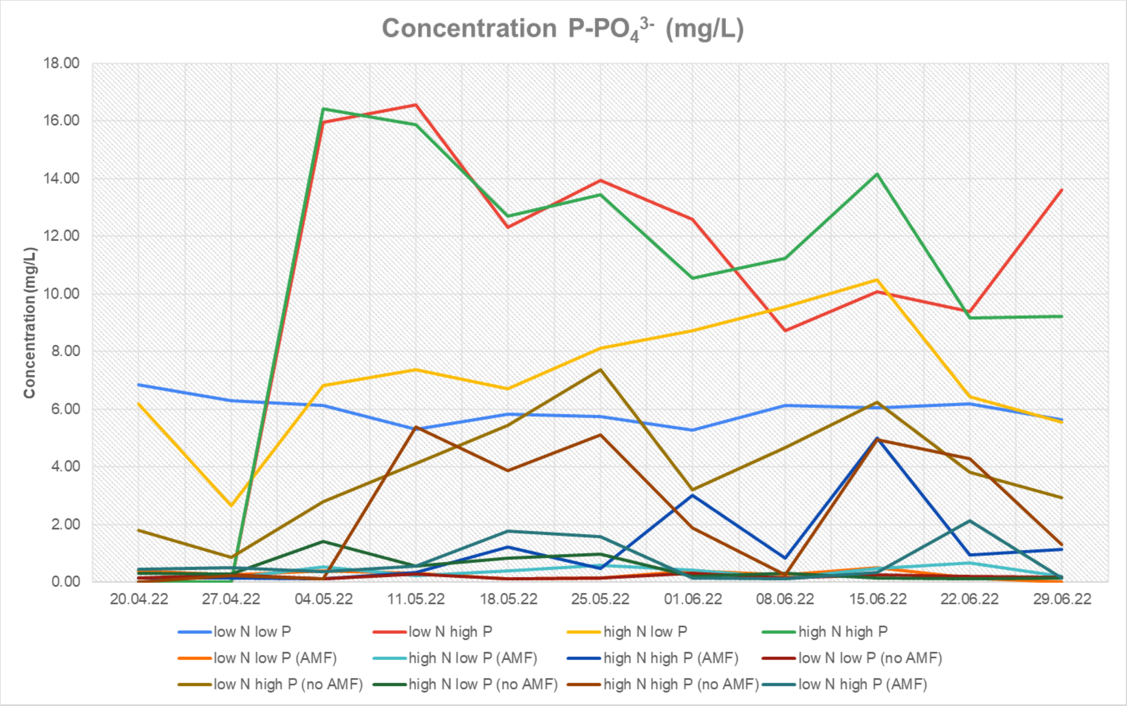

***Figure 29**: Concentration of P-PO_4^{3-} (mg/L) for inflow (low N low P, low N high P, high N low P, high N high P) and outflow (AMF and no AMF) measurements.*

Phosphorus, which most often appears in wastewater as phosphates derives from human and animal waste as well as agricultural runoff and industrial and household chemicals. P is essential for plant's growth and development, however, when large quantities of P enter water bodies, they could cause algae species to overgrow and cause eutrophication. This is why this nutrient should be removed from wastewater inflow, in this case, by biological processes in CWs.

Figure 29 presents concentration of PO_4^{3-}-P for inflow and outflow measurements over time, where some results are missing due to systematic error concerning the machine measurements. However, from the given values, it's clear that inflow measurements for concentration of PO_4^{3-}-P are higher than outflow measurements throughout the experiment, especially inflow with concentrations low N high P, where the average is 12.57 mg/L and high N high P with an average concentration value of 12.53 mg/L.

Moreover, values for reactors where P is low, regardless of AMF presence have low concentrations, where low N low P with mycorrhiza averages at 0.29 mg/L and low N low P without mycorrhizal fungi averages at 0.20 mg/L.

In contrast, reactors where P concentrations were initially high such as low N high P and high N high P gradually increase over time. For example, the reactor with concentrations low N high P without the presence of AMF increased from a value of 1.79 mg/L to a concentration of 7.36 mg/L on the 25th of May, and then decreased to 2.92 mg/L at the end of the experiment.

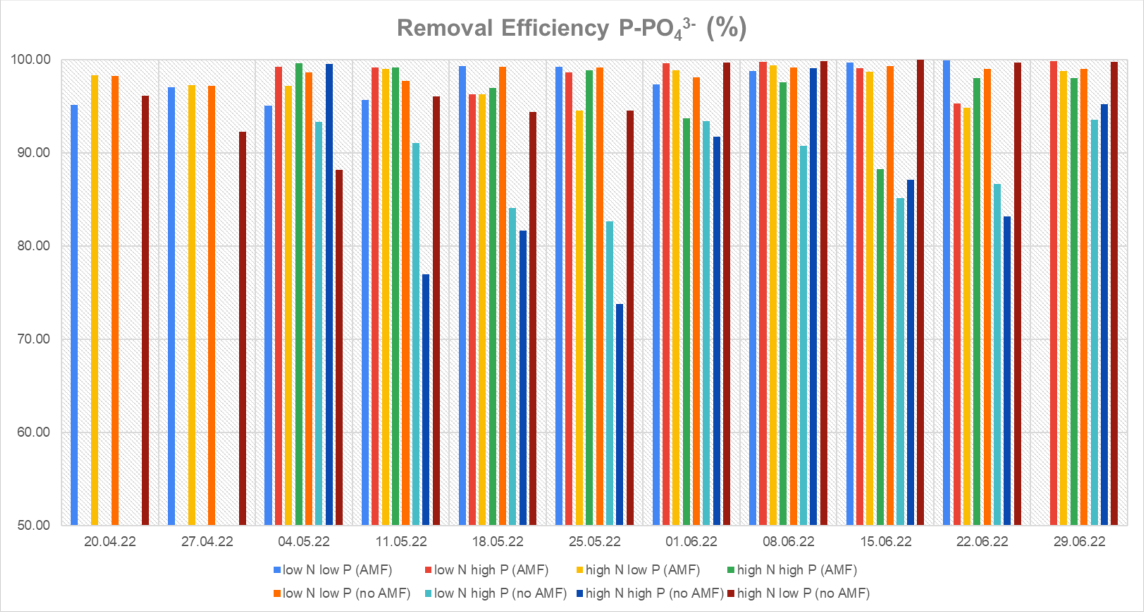

***Figure 30**: Removal Efficiency of P-PO_4^{3-} (%) for outflow (AMF and no AMF) measurements.*

The removal efficiency of PO_4^{3-}-P in **Figure 30** demonstrates high values for all reactors where mycorrhiza is present. Additionally, removal efficiency is high where P concentrations in all reactors are low, for example low N low P reactor has a constant removal efficiency throughout the experiment.

In contrast, reactors where P concentrations are high, the removal efficiency is lower, especially where there is no mycorrhizal fungi present, for example in low N high P and high N high P reactors.

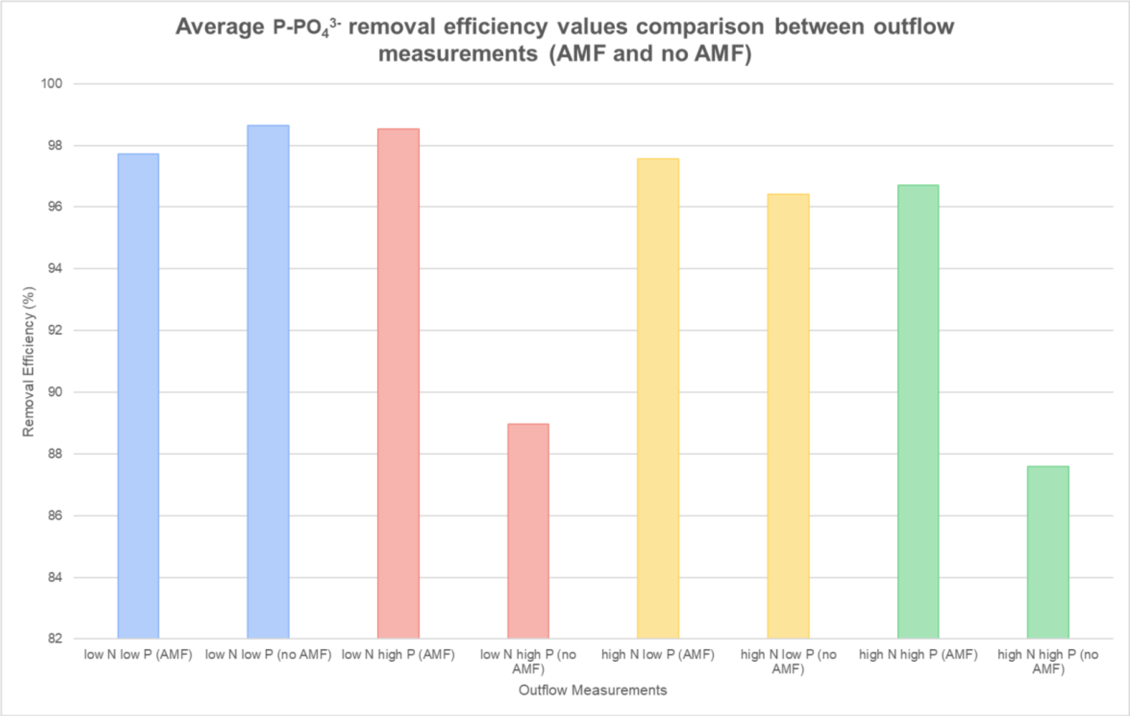

Figure 31*: Average P-PO$_4^{3-}$ removal efficiency for outflow measurements (AMF or no AMF). Same colors represent same N:P concentration ratios.*

When comparing PO$_4^{3-}$-P average removal efficiency for reactors with and without mycorrhizal fungi, **Figure 31** demonstrates the highest removal in reactor low N high P containing mycorrhiza with an average of 98.6%, and the lowest in high N high P without mycorrhiza with 87.5%. Moreover, in these reactors, it’s clear to see that removal efficiency is higher where mycorrhizal fungi are present.

Additionally, reactor with concentrations high N low P shows higher removal efficiency where mycorrhiza is present as well, specifically with 97.5%, and 96.4% in the reactor without mycorrhiza.

In contrast, reactor low N low P without mycorrhiza demonstrates slightly higher average removal efficiency with 98.6%, and 97.7% where mycorrhiza is present.

6. Discussion

6.1 Carbon Removal

Constructed wetlands (CWs) can be a source or a sink for Carbon (C), depending on the type of CW, its age and operational capacity (Zemanova et al. 2010), environmental surroundings like climate conditions (Scholz et. 2011), and vegetation diversity (Sun et al. 2019). There are two important processes that contribute to C removal from wastewater in CWs which are microbial uptake and transformation by microorganisms in different carbon compounds (Barbera et al. 2014).

This thesis demonstrates the highest removal efficiency of a C compound which is Total Organic Carbon (TOC) with a removal rate of 87%. According to an article by Sgroi et al. 2017, where the TOC removal efficiency in vertical flow CWs indicated 72%, the high removal rate could be achieved by relatively high oxidation/reduction potential (ORP) and the availability for oxygen to pass through the vertical substrate surface. This is further supported by the high ORP levels throughout this experiment, meaning reactions where molecules are reduced by an oxidizing agent occurred in the wastewater purification process.

Furthermore, the removal efficiency for Total Carbon (TC) demonstrated an overall removal rate of 79% throughout this experiment. This includes all forms of carbon including organic, inorganic, dissolved, and suspended. These high levels of TC removal could have occurred because of uptake by wetland vegetation during the wastewater purification process (Barbera et al 2014). Additionally, the plant uptake could be influenced by weather changes throughout the experiment (Yang et al. 2020), for example sunny weather provides photosynthesis conditions and therefore more C is absorbed. Moreover, according to an article by Hu et al. 2023, mycorrhizal fungi as part of the wetlands’ substrate could enhance the removal efficiency of TC.

Inorganic Carbon (IC) is an essential component which is extracted from the soil and minerals in CWs and plays a significant role in the C cycle (Nanzyo and Kanno, 2018). This experiment reported an overall average removal efficiency of 47%, however, the IC removal rate fluctuated throughout the experiment depending on the different nutrient ratios. Specifically, during the springtime, the IC removal efficiency demonstrated negative values, meaning more IC was created rather than removed. This could be enhanced by the microbial impact on the wetland vegetation in the CW, assisting its photosynthesis production. Additionally, according to a study by Liu et al. 2022, the degree of IC content in the soil depended on the depth, where the topsoil indicates smaller amounts than the ground soil.

6.2 Nitrogen Removal

Nitrogen (N) compounds occur in most types of wastewaters, such as agricultural runoff, industrial and urban drainage, and landfill leachate, which can cause various negative impacts in water habitats, such as wetlands. N can appear in many forms such as inorganic compounds like NO_3, NO_2, NH_3 or NH_4^+, which have the biggest impact on aquatic life due to their availability to be absorbed by microorganisms (Holmes et al. 2018). Various compounds of N can be removed with biological treatment processes like nitrification, denitrification, ammonification, and a combination of them (Holmes et al. 2018).

In this thesis experiment, the average inflow concentration of Total Nitrogen (TN) showed 59 mg/L and the average outflow concentration indicated 12 mg/L, which results in an average removal efficiency of 93%. Moreover, the average removal efficiency of ammonia-N (NH_4-N) in the wastewater was 93%, as well as for nitrates-N (NO_3-N), which TN comprises of. According to a research article by Vymazal et al. 2020, the NH_4-N in the simulated wastewater that is being deposited in the CWs becomes available for the wetland vegetation to use which promotes growth and development and takes an important role in photosynthesis. Additionally, the NH_4 which is not available for plant uptake is being nitrified by nitrifying bacteria into NO_3-N, which then proceeds to leach into the soil and drainage systems (Goswami et al. 2009). Reasoning for the high TN removal efficiency in this experiment could be due to denitrification processes that might have taken place during the wastewater purification process (Vymazal et al. 2020), which is supported by the similar pattern of removal rate of NO_3-N and NH_4-N.

6.3 Phosphorus Removal

Phosphorus (P) is an essential macronutrient that takes part in many biological and geological processes to enhance the plants' ability to grow and develop (Chen and Graedel, 2016). P can exist in many organic as well as inorganic compounds which provide various benefits and functions that are crucial for plants and microorganisms, which is why this nutrient is an important component in the production of fertilizers (Gubernat et al. 2023). However, the increased usage of these fertilizers for agricultural purposes and the unmonitored regulation can lead to surface runoff which could result in eutrophication, for which the main cause is uncontrolled amounts of P and N in wastewater. Therefore, CWs stand as a viable option to use biogeochemical processes in order to treat phosphorus removal in wastewater.

In this experiment, the average inflow concentration of phosphate-P (PO_4^{3-}-P) amounted to 9.25 mg/L whereas the average outflow concentration resulted in 1.23 mg/L. Therefore, the removal efficiency of PO_4^{3-}-P demonstrated an average of 95% throughout the experiment. According to a research article by Krzeminska et al. 2023, the removal efficiency of P could be affected by the different seasons, where the highest removal rate was shown to be in the springtime, and the lowest rate was indicated in the summer, which is further supported by the decreased removal efficiency rates over time throughout the experiment which was conducted during spring and summer. Additionally, despite the concentration of PO_4^{3-}-P fluctuating during the course of the experiment, the removal efficiency rate remained consistently high.

Furthermore, wetland vegetation could have an essential effect on the removal efficiency of PO_4^{3-}-P, which significantly increases the retention time and promotes sedimentation (Kill et al. 2022). Moreover, the research paper by Kill et al. 2022, suggests that the presence of dense wetland vegetation can increase the P removal efficiency as well as promote greater resilience to extreme conditions. Additionally, according to a research article by Hu et al. 2023, the presence of mycorrhizal fungi could directly affect the removal efficiency of P along with other nutrients, which would lead to an increased positive bio filter performance in CWs.

6.4 Effect of N:P Ratios in Constructed Wetlands on Pollutant Removal

The results from this thesis experiment demonstrated a difference in removal efficiencies of pollutants according to the different nutrient N:P ratios. The removal of Total Carbon (TC) indicated the highest removal rate in reactors with low Nitrogen (N) and high Phosphorus (P) with an efficiency of 80%. In contrast, the lowest removal rate was shown as 75% in reactors with high N and low P, which would suggest that high concentrations of P could cause greater removal efficiency of TC, and low concentrations of N decrease the removal rate of TC. Moreover, CWs support C removal through the usage of wetland vegetation and microorganisms, in this case, mycorrhizal fungi. Macrophytes minimize C being released into the atmosphere by reusing the carbon dioxide which is released by the mycorrhiza during the process of photosynthesis (Mthembu et al. 2013).

Furthermore, the highest removal rate of Total N (TN) was found in reactors with high N and low P concentrations with a removal efficiency of 96%, and the lowest was in reactors with high concentrations of N and P with an efficiency of 93%. These results could indicate that higher concentrations of N correspond to higher removal efficiency of N. Moreover, a research article by Zheng et al. 2016 suggests that warmer seasons such as spring or summer during which this thesis experiment was conducted can indicate higher production of nitrates and higher removal of N compared to cooler seasons such as winter or even during nighttime. Therefore, the changes in removal efficiency for N throughout the experiment could be due to seasonal changes and increased temperature. Additionally, more concentration of N would suggest more processes such as nitrification and denitrification take place, which further continues the recycling rate of the N cycle, allowing higher N removal efficiency to occur (McCarty et. 2018).

On the other hand, the removal of phosphate phosphorus (PO_4^{3-}-P) has demonstrated the highest removal rate in reactors with low concentrations of N and P with an efficiency of 98%. In contrast, the lowest removal rate of 92% was shown in reactors with high concentrations of N and P. These results indicate that lower concentrations of N and P lead to higher removal efficiency of PO_4^{3-}-P, and in contrast high concentrations of N and P demonstrate a lower removal rate of PO_4^{3-}-P. Some of the processes that could have taken place in the P removal process include natural sedimentation, plant absorption, and most often, absorption by the substrate in the CWs (Gao and Zhang, 2022). Furthermore, according to a research article by Ji et al. 2021, most of the P removal processes take place in the substrates, where specifically 50-70% of the P is removed by abiotic processes rather than biotic ones.

6.5 Effect of Arbuscular Mycorrhizal Fungi in Constructed Wetlands on Pollutant Removal

The evidence from this thesis experiment distinguishes pollutant removal efficiencies between reactors with arbuscular mycorrhizal fungi (AMF) and without. The highest average removal rate for TC is shown in reactors without the presence of AMF at 80%, while reactors containing AMF indicated a lower average removal rate of 76%. This suggests that reactors without AMF presented a more effective method for removing TC in CWs, specifically by 4%. This contradicts the previously stated idea of AMF having the capacity to increase the removal rate of TC in CWs when present. Mycorrhizal functions could also be affected by the wetlands' substrate structure, which could influence AMF development and symbiosis with plants (Hu et al. 2023). This could further modify or reduce the pollutant removal capacity in CWs, which could be a reason why TC removal was lower with the presence of AMF during wastewater purification. Additionally, during the aqueous phase of the microbial degradation process, as well as uptake by plants during a short retention time, pollutants are not removed very easily and quickly (Jia et al. 2010).

On the other hand, the presence of AMF in reactors in terms of TN removal suggests higher removal efficiencies than in reactors without AMF. Reactors with AMF demonstrated a removal efficiency of 94% and reactors without AMF showed a 92% removal rate, meaning the presence of mycorrhizal fungi increased the TN removal efficiency by 2%. According to a research article by Hu et al 2023, AMF inoculation can significantly enhance TN removal, where the results indicated a 10% increased removal efficiency of TN when mycorrhiza was present. This enhancement of pollutant removal is most likely due to AMF being able to promote plant growth and resilience (Hu et al. 2021) while also providing nutrient uptake which further supports N removal during wastewater purification.

Similarly, the removal rate for P was increased with the presence of mycorrhiza, where reactors with AMF indicated 98% removal efficiency, while reactors without AMF demonstrated a 93% removal rate, meaning the presence of mycorrhiza in the reactors was 5% more effective in the process of P removal. When comparing these results to a recent research article by Hu et al. 2023, where the PO_4^{3-}-P removal rates demonstrated a 0.2-5.0% increase, it is clear that mycorrhizal fungi enhance PO_4^{3-}-P removal. Moreover, previous studies have suggested that mycorrhizal symbiosis may form extra radical hyphae that can lead to a clearer path to acquiring nutrients such as N and P, which results in higher removal and absorption of P in wetland plants (Mader et al. 2000).

6. Conclusion

This experimental thesis aimed to evaluate the effects of different nutrient concentrations in wastewater purification processes in subsurface flow constructed wetlands (CWs), as well as the influence of arbuscular mycorrhizal fungi (AMF) on pollutant removal. Based on the wastewater sample analysis results and their further evaluation, it can be concluded that different concentrations of nitrogen (N) and phosphorus (P) can affect removal efficiency of carbon (C), N and P respectively. The results indicate a correlation between total carbon (TC) and total nitrogen (TN) removal and high N concentrations, as well as phosphate phosphorus (PO_4^{3-}-P) removal and low concentrations of N and P. Additionally, the presence of AMF enhanced TN and PO_4^{3-}-P removal, however C removal was shown to have higher removal efficiency without mycorrhizal presence. This research clearly illustrates a better understanding of the relationship between different nutrient concentrations and AMF presence in CWs for wastewater purification, however it also raises questions regarding the composition and colonization of microbial communities in these nutrient conditions. This thesis opens a wider understanding in the field of CWs assisted with AMF that are used for wastewater purification, where the effects of different nutrient concentrations are considered.

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