Evaluating woodchip bioreactors for mitigating drainage nitrate levels from a municipal wastewater land treatment site

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This PhD thesis has been affected by the Covid-19 pandemic, resulting in impacts on both data collection and delays in the commencement of experiments. Specifically, government restrictions on the movement and access to university facilities during the second lockdown (August - September 2021), disrupted the column bioreactors study when 'Experiment 3' was taking place, leading to missed data collection for the 2:1 C:N dosing rate evaluation. Additionally, the Covid-19 pandemic influenced the commencement of the pilot-scale woodchip bioreactor construction and start of experiments. Confronted with the absence of electricity at the experiment site, a decision was made to install solar panels to operate the water pump. However, due to the impact of the Covid-19 pandemic on international shipping, the delivery of the solar panels and related equipment sourced overseas, was delayed, consequently postponing the start of the experiment. The pilot-scale bioreactor experiment was originally planned for a two-year duration, however, the delays meant that the monitoring period was limited to one year.

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Abstract

Woodchips bioreactors are a well-established end-of-drain treatment technology that has been widely used to reduce nitrate (NO_3^-) from agricultural drainage water. However, their application to municipal wastewater land treatment sites remains less explored, despite potential advantages. In New Zealand, land application of pre-treated wastewater is a growing practice to mitigate excessive nutrient discharges to the aquatic environment. Land treatment can prove effective when operated correctly, but challenges arise when large volumes of wastewater, and small areas available for irrigation, necessitate high application rates, which can result in NO_3^- enrichment of drainage water. The Levin Wastewater Land Treatment Site (LWLTS) is an example of where relatively high annual volumes of municipal wastewater are irrigated over an under-sized application area, resulting in high application depths (4667 mm/year). Consequently, surface drains and shallow groundwater transfer NO_3^- to the Waiwiri Stream continually all year. In order to reduce the impact of the LWLTS on the water quality of the Waiwiri Stream, one of its resource consent requirements involves reducing the NO_3^- levels in the Waiwiri Stream, downstream from the site. The objective of this thesis was to evaluate the potential use of woodchip bioreactors for reducing NO_3^- concentrations in drainage water from LWLTS, including an assessment of the ability of soluble C dosing to enhance NO_3^- removal.

Initial experiments used small-scale column woodchips bioreactors, which simulated similar water temperatures and NO_3^- concentrations to those at the LWLTS. The effect of different water hydraulic retention times (HRT) and the use of dosing with two soluble C sources, liquid sugar, and ethanol, were assessed. Under warm temperature conditions, the column bioreactors achieved 99% NO_3^- removal efficiency with a 10-hour HRT. In contrast, under cool water temperatures at the same HRT, the NO_3^- removal efficiency decreased to 31%. Soluble C dosing was an effective strategy for enhancing NO_3^- removal, with the choice of C source proving to be crucial. Ethanol demonstrated to be more efficient than liquid sugar. Additionally, it was determined that dosing with ethanol at a C:N dosing rate of 1.5:1 achieved high removal efficiencies of 77% under warm conditions and at a 3.3-hour HRT, and 82% under cool conditions and at a 10-hour HRT.

Based on the results of the column bioreactor study, the performance of pilot-scale woodchip bioreactors at reducing NO_3^- levels in drainage water were evaluated at the

LWLTS under field conditions. These experiments involved quantifying the effects of different HRTs and dosing with ethanol at different C:N ratios. Operating the bioreactors, at a 10-hour HRT achieved average NO_3^- removal efficiencies of 43% and 59% during the cool and warm seasons, respectively. While, at a 20-hour HRT, the removal efficiencies were 69% and 85%, respectively. The variations in NO_3^- removal efficiency between both seasons demonstrated that during the cool season the bioreactors were on average about one-third less effective. When bioreactors, operating at 6.6-hour HRT in cool conditions, were dosed with ethanol at a C:N ratio of 0.75:1, the NO_3^- removal efficiency improved from 24% to 93%. This result demonstrates that under field conditions ethanol dosing proved to be a higher effective strategy for enhancing the performance of woodchip bioreactors, particularly during cool periods.

Based on the findings of the pilot-scale bioreactors, two woodchip bioreactor designs were proposed for the LWLTS: a non-dosed woodchip bioreactor of 645 m^3 operating at a long HRT (20 hours), and an ethanol-dosed woodchip bioreactor of 197 m^3 operating at a short HRT (6.6 hours). The two proposed designs provide contrasting approaches, although both are expected to achieve the same annual NO_3^- load removal (1174 kg N/year) and have similar annualised NO_3^- removal costs ($6.90 and $6.50/kg N, respectively). In the long term, it is expected that the NO_3^- removal of the larger non-dosed bioreactor will decline at a faster rate compared to the ethanol-dosed bioreactor due to relying solely of woodchips as the C source. However, it would be less susceptible to the risk of bioclogging and has greater capacity to increase NO_3^- removal. In addition, ethanol dosing could be introduced to the larger non-dosed bioreactor in the future, when a decline in NO_3^- removal efficiency is observed. Therefore, the overall flexibility of the larger bioreactor design is an advantage but comes with higher initial set-up cost.

The results of this research demonstrate that woodchips bioreactors are effective treatment methods for mitigating drain water NO_3^- levels at a municipal wastewater land application site. Additionally, C dosing using ethanol proved to be a promising cost-effective alternative to enhance bioreactor performance, allowing the use of relatively short HRTs, especially during cool conditions. This increases the daily volume of water that can be effectively treated.

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1. Introduction

Nitrogen (N) pollution of aquatic ecosystems is one of the most pervasive global environmental challenges, leading to water eutrophication and posing threats to both aquatic biodiversity and human health (Abascal et al., 2022; Bhatnagar & Sillanpää, 2011; Gómez et al., 2020). Nitrate (NO_3^-) is the most common form of inorganic N in freshwater. Naturally, NO_3^- occurs in water at low concentrations (<0.45 NO_3^--N mg/L) (Gómez et al., 2020), however, anthropogenic activities in recent decades have substantially escalated NO_3^- pollution, resulting in concentrations that can be more than 10 times higher than natural baseline levels (Gómez et al., 2020; Gutiérrez et al., 2018). Some of the major anthropogenic sources of inorganic N in aquatic ecosystems include intensive agricultural practices, such as cultivation, N fertiliser use, livestock grazing and dairy farm effluent discharges. While agriculture is commonly perceived as the primary contributor to elevated fresh water inorganic N concentrations, urban wastewater also constitute a notable source of N, as the result of inadequately treated industrial and domestic wastewater discharges (Abascal et al., 2022; Katz, 2020). The rapid population growth and the associated expansion of urban areas produce significant volumes of wastewater, which require extensive treatment to minimise pollutant load before its release into the wider environment (Price et al., 2018). Nitrogen enrichment of freshwater from wastewater discharges occurs when treatment does not reach the tertiary level (Chakravarthy et al., 2019; Gücker et al., 2006). Increased awareness of inorganic N pollution and eutrophication has led to the development of advanced treatment technologies, although many of these technologies can be expensive to install and operate.

Throughout New Zealand, there are 323 municipal wastewater plants treating over 500 million cubic meters of wastewater annually. Of these treatment plants, 46% of them discharge to freshwater, 33% discharge to land, and the remaining discharge into the ocean (Water New Zealand, 2020). Furthermore, only 27.6% of the wastewater treatment plants discharging into freshwater employ tertiary treatment methods (Chakravarthy et al., 2019). In order to improve water quality, the New Zealand Government developed more stringent policies, as outlined in the National Policy Statement for Freshwater Management 2020 (NPS-FM) (Ministry for the Environment, 2023), with the primary goal of stopping, reversing and improving the deteriorating water quality. Consequently, district councils face the need to improve their municipal wastewater treatment. However, as many district councils have limited financial resources, the technologies that they require to treat wastewater to a tertiary standard need to be more affordable and effective.

Land application of pretreated domestic wastewater is increasingly recognised as a strategy to reduce the transfer of contaminants, including N, to surface fresh waters, as it offers a passive treatment via the filtering capacity of the soil and plant system (Chaudhry et al., 2005). However, successful land treatment frequently demands extensive land areas to handle the significant quantities of wastewater generated by urban centres. As a result, common constraints to the adoption of land treatment include the availability of suitable land, the associated costs, the challenges associated with managing wastewater applications, and the limits that municipal wastewater applications place on land use (Ministry for the Environment, 2020; Robb et al., 2000). Consequently, in certain instances, these limitations lead to insufficient land area for effective treatment, resulting in increased NO_3^- concentrations in surface drains and/or groundwater (Gutierrez-Gines et al., 2020; Lal et al., 2015). Given this, there is a need to identify an effective end-of-drain treatment method for drainage leaving the land irrigation area.

Woodchip bioreactors are recognised as being a well-established, end-of-drain technology that has been widely used to reduce NO_3^- levels in agricultural drainage waters (Christianson et al., 2021). Within woodchip bioreactors, the growth of heterotrophic denitrifying microorganisms is enhanced by the addition of woodchips as a carbon (C) source and by providing anoxic conditions (Hang et al., 2016; Schipper et al., 2010). This treatment method has gained significant interest because it is simple to construct, is cost-effective and requires minimal maintenance (Christianson et al., 2021; Schipper et al., 2010).

While woodchip bioreactors are primarily used for treating agricultural drainage, their application for drainage from municipal wastewater land treatment areas, remains relatively unexplored, despite the potential advantages. Drainage from land treatment sites, where municipal wastewater is consistently applied at high depths throughout the year, often exhibit relatively consistent drainage water flow rates and NO_3^- concentrations throughout the year. This stands in contrast to agricultural drainage, which typically occurs during cooler periods of the year, such as winter or early spring, and is characterised by fluctuating flow rates and NO_3^- concentrations. The more consistent conditions in municipal wastewater land application settings suggest that woodchip bioreactors may operate effectively, as Christianson et al. (2011) demonstrated that woodchips bioreactors achieve greater NO_3^- removal at constant influent flow rates compared to fluctuating flow rates. Nonetheless, a comprehensive understanding of the performance of woodchip bioreactors in these conditions is essential before considering the implementation of full-scale bioreactors.

1.1. The research problem

In Levin, New Zealand, the municipal wastewater is treated to a secondary level and, subsequently, directed to the Levin Wastewater Land Treatment Site (LWLTS), where it is placed in an unlined storage pond. From there, the wastewater is irrigated at a very high annual application depth of 4,667 mm/year (Mains & Douglass, 2018), due to the limited land area of 40.5 ha. The drainage from the irrigated area and the pond seepage are intercepted by the shallow groundwater system. A portion of this shallow groundwater is captured in the surface drains located around the irrigation area, which then direct the drainage water into the Waiwiri Stream (Cass et al., 2018). Consequently, surface water serves as the receiving environment for some of the wastewater-derived contaminants.

In 2020, the LWLTS was granted with a 25-year resource consent, subject to specific conditions. One of these conditions requires the reduction of NO_3^- levels in the surface water of the Waiwiri Stream downstream from the site. Given the substantial volumes of wastewater currently managed at the facility, coupled with anticipated increases due to projected population growth, expanding the wastewater irrigation area to meet the consent's requirements would result in significant financial implications. Therefore, there is an urgent need to identify and adopt a cost-efficient end-of-drain treatment method to mitigate NO_3^- discharges into the Waiwiri Stream.

Woodchips bioreactors may effectively reduce the NO_3^- levels from the drainage water from the LWLTS. However, although using woodchips as a C source have a number of advantages, the main disadvantage is that labile C production declines with temperature and over time, limiting the NO_3^- removal rate (Addy et al., 2016; Nordström & Herbert, 2019; Schipper et al., 2010). The NO_3^- removal in woodchips bioreactors can be enhanced by increasing the hydraulic retention time (HRT), but this approach reduces the volume of water that can be treated per day. Therefore, some studies (Feyereisen et al., 2018;

Hartz et al., 2017; Jansen et al., 2019; Moghaddam et al., 2022; Palomo et al., 2013; Roser et al., 2018) have suggested the addition of soluble C sources as a strategy to enhance bioreactors performance. To date, the assessment of the effectiveness of a wide variety of soluble C sources remains limited. Furthermore, research on C dosing in woodchips bioreactors has primarily concentrated on their application in treating drainage from agricultural areas, with minimal research being available for municipal wastewater land applications sites.

1.2. Research objectives

The main goal of this research is to evaluate the potential use of woodchip bioreactors for treating drainage water from the LWLTS, including assessments of soluble C dosing of woodchip bioreactors to enhance NO_3^- removal. To accomplish this main goal, the specific research objectives are as follows:

1. To conduct an initial assessment of the potential ability of woodchip bioreactors to remove NO_3^- from drainage water using small-scale column bioreactors and investigate the effect of water temperature, HRT and soluble C dosing. The results of this preliminary study inform the design of the experimental pilot-scale bioreactors installed at the LWLTS.
2. To assess the ability of pilot-scale woodchip bioreactors to reduce NO_3^- levels in drainage water from the LWLTS and evaluate how NO_3^- removal efficiency varies with water temperature and HRT.
3. To evaluate the ability of ethanol dosing of pilot-scale woodchip bioreactors to enhance NO_3^- removal, particularly during the cooler season.
4. To use the findings of the pilot-scale study to design a full-scale woodchip bioreactor capable of removing enough NO_3^- from the LWLTS drainage water to achieve a 10% reduction in the site's contribution to the Waiwiri Stream, including a design that incorporates ethanol dosing for enhanced denitrification efficiency.

1.3. Thesis outline and structure

This PhD thesis is subdivided into six chapters which are briefly described as follows:

Chapter 1

This chapter presents the research context, describes the research objectives and presents an outline of the theses.

Chapter 2

This chapter provides a literature review to establish a comprehensive and robust theoretical framework for the thesis. The section commences with an overview of urban wastewater management in New Zealand. This is followed by a review of NO_3^- removal strategies, leading into a detailed exploration of woodchip bioreactors, including the factors influencing their performance, potential adverse effects, design and installation considerations, as well as the potential benefits of C dosing.

Chapters 3 - 5

Each of the research chapters are structured as journal articles.

Chapter 3 describes experiments involving the use of nine small-scale column woodchip bioreactors to investigate the first research objective.

Chapter 4 describes experiments involving four pilot-scale woodchip bioreactors, located at the LWLTS, to study the second and third research objectives.

Chapter 5 uses the main results from the pilot-scale bioreactors to achieve the fourth research objective involving the design of full-scale bioreactors for LWLTS.

Chapter 6

This chapter is a general summary of the results obtained in the three research chapters and provide recommendations for future research.

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2. Literature review

2.1. Urban wastewater management in New Zealand

Cities and towns have the potential to consume vast quantities of natural resources and cause significant impacts on natural ecosystems (Suren & Elliott, 2004). The cities and towns in New Zealand are no exception; as the population grows (annual growth rate 1.6%), urban land use expands (Statistics New Zealand, 2023). Consequently, waterways have been adversely affected, frequently exhibiting poor water quality and degraded biological communities (Suren & Elliott, 2004). In a study assessing water quality in relation to urban land use intensity, Gadd et al. (2020) suggested that if the current form of urban development continues, it is likely to result in both a further decline in water quality and the reduced likelihood of achieving water quality objectives in impacted locations.

The primary urban pollutants that contaminate freshwater bodies include heavy metals (e.g. zinc and copper), nutrients (primarily nitrogen and phosphorus), total suspended solids, polyaromatic hydrocarbons, pharmaceuticals and pathogens (e.g. *E. coli*) (Chakravarthy et al., 2019). Sources of urban pollutants are various, with municipal wastewater discharges representing one of the major contributors (Chakravarthy et al., 2019). In New Zealand, most cities and towns have centralised wastewater treatment plants responsible for treating wastewater produced by about 85% of the country's population (Water New Zealand, 2019). The New Zealand Wastewater Treatment Plant Inventory discloses the existence of 323 municipal wastewater treatment plants across New Zealand, treating over 500 million cubic meters of wastewater annually.

The discharge system of municipal wastewater treatment plants can be classified into four main categories: discharge to freshwater, land application, and discharge to the ocean. Some of these systems may be combined discharge (e.g. water and land application). Among all of the treatment plants in New Zealand, 46% discharge to freshwater and 33% discharge to land, with the remaining being discharged into the ocean (Water New Zealand, 2020). While wastewater treatment plants effectively reduce the contaminant load and biochemical oxygen demand (BOD), the discharged effluent typically fails to match the water quality standards of the receiving environment (Chakravarthy et al., 2019).

Wastewater treatment plants can operate at three levels: primary, secondary and tertiary (Chakravarthy et al., 2019). Due to the lack of standardisation of wastewater treatment in New Zealand, variations in effluent quality, based on the treatment level, have been observed (Ministry for the Environment & Stats NZ, 2023). Of the total of 323 currently existing treatment plants, only 29% (95 treatment plants) have tertiary treatment. Of the 152 wastewater treatment plants discharging into freshwater, only 42 (27.6%) employ tertiary treatment, indicating that more than 70% (110 treatment plants) of the treatment plants discharging into freshwater treat sewage only to the primary and secondary level (Chakravarthy et al., 2019). According to Gücker et al. (2006), effluents treated at the primary or secondary level can increase the nutrient load and contribute to eutrophication in the receiving aquatic environment.

Environmental problems arising from nutrient loading in New Zealand are primarily associated with agricultural production (Follett & Follett, 2008). However, human population growth may also lead to increased nitrogen (N) and phosphorus (P) inputs (Follett & Follett, 2008). Municipal wastewater can contain high levels of inorganic N and P (Hang et al., 2016). In aquatic ecosystems, the high levels of these nutrients stimulate the growth of phytoplankton, which triggers eutrophication and alters water quality (Boyd, 2015). Eutrophication is associated with reduced dissolved oxygen (DO) levels in water, increased sedimentation in waterway beds due to the decomposition of dead algae, and decreased sunlight penetration caused by higher turbidity (Boyd, 2015). These adverse effects can, in turn, endanger the native biodiversity of the aquatic ecosystem and pose a risk to human health. Elevated nitrate (NO_3^-) concentrations in drinking water can affect oxygen transport in the blood, causing methemoglobinemia, a condition with life-threatening potential (Follett & Follett, 2008). Furthermore, excessive ingestion of NO_3^- has been linked to an increased risk of respiratory infections, thyroid problems, birth defects, childhood diabetes and certain cancers, such as colon, ovarian and stomach cancers (Bibi et al., 2016; Follett & Follett, 2008).

The poor water quality associated with high inorganic N and P concentrations can also significantly affect cultural uses, beliefs, and practices. For instance, in the Māori culture, water is valued as a taonga (treasure), and water bodies, such as rivers, lakes and wetlands have their own mauri (life force) and are essential elements in the identity, whakapapa (genealogy) and mana (status) of the hapu (group associated with a geographic region, typically a water catchment) (Morgan, 2006).

According to the Ministry for the Environment & Stats NZ (2023), many rivers, lakes, and groundwater sources exhibit abnormally high nutrient levels. Therefore, improvements in urban wastewater treatment or land treatment systems will likely enhance freshwater quality at many locations.

2.2. Nitrate removal

Numerous strategies and technologies have been developed and applied in municipal treatment plants to mitigate N loads from wastewater into aquatic ecosystems (Oakley et al., 2010; Schipper et al., 2010b). These strategies and technologies include physicochemical and biological processes (Ahn, 2006). While physicochemical methods exist, they tend to be expensive to both operate and maintain, and often generate secondary waste, making them less desirable to implement (Rajta et al., 2020). In contrast, biological methods offer effective and cost-efficient means of removing nitrogenous compounds without waste production, rendering them more favourable (Abascal et al., 2022; Ahn, 2006; Rajta et al., 2020).

Among the biological processes, denitrification is the most widely understood process for bioavailable N removal from natural and human-altered systems (Rajta et al., 2020; Rivett et al., 2008; Seitzinger et al., 2006). Denitrification is a biological process in which heterotrophic and autotrophic microorganisms reduce dissolved NO_3^- to atmospheric N_2. Heterotrophic organisms use a carbon (C) source as an electron donor while autotrophic organisms use iron, sulphur or manganese (Ahn, 2006; Rajta et al., 2020). Autotrophic microorganisms rely on inorganic C sources, while heterotrophic denitrifiers utilise organic C sources for cell synthesis (Rajta et al., 2020).

One popular biotechnology for treating NO_3^- enriched water through denitrification is denitrification bioreactors, which can be operated using various C sources such as methanol, acetate, glucose, ethanol and others (Ahn, 2006). Woodchips are a commonly used C source in denitrification treatments, particularly for treating agricultural drainage water (Christianson et al., 2021). Woodchips denitrification bioreactors are a cost-effective and relatively simple technology (Addy et al., 2016; Christianson & Schipper, 2016).

2.3. Woodchip denitrification bioreactors

Denitrification bioreactors are designed systems that enhance the microbial-mediated process of denitrification (Cheesman et al., 2023). The essential conditions required for NO_3^- attenuation by heterotrophic microorganisms are a physical substrate for microbial growth, an anoxic environment, and a source of liable organic C (Cheesman et al., 2023; Robertson & Cherry, 1995). These conditions are met in woodchip bioreactors, where saturated conditions result in low levels of atmospheric O_2, and the woodchips serve as the physical substrate and the source of dissolved organic carbon (DOC) that acts as an electron donor (Christianson et al., 2021; Robertson & Cherry, 1995).

Denitrification bioreactors can have different designs based on their hydrological connections and are classified as either denitrification walls or beds (Schipper et al., 2010b). Denitrification walls incorporate the C substrate vertically into shallow groundwater, perpendicular to the flow, with the water source primarily limited to boundaries of the wall perpendicular to the flow direction (Schipper et al., 2010b). On the other hand, denitrification beds are containers filled with a C substrate and receive NO_3^- contaminated water from drains which will mostly be artificial (Schipper et al., 2010b). Bed designs, particularly in-ditch variations, have become increasingly popular for woodchip bioreactors (Christianson et al., 2021). Integrating bed-style bioreactors into drainage ditches or streams offers a practical approach to minimise the risks and costs associated with land removal (Christianson et al., 2021).

While most studies of woodchip bioreactors for NO_3^- removal have focused on agricultural tile drainage (Audet et al., 2021; Christianson et al., 2021; Rivas et al., 2020; Schipper et al., 2010b), some researchers have also explored the application of this technology to treating aquaculture wastewater (Aalto et al., 2022; Christianson et al., 2016; Lepine et al., 2020), stormwater (Ashoori et al., 2019), municipal wastewater (Rambags et al., 2016, 2019) and mine drainage (Nordström & Herbert, 2018, 2019).

Studies have demonstrated that woodchips bioreactors can consistently achieve NO_3^- reductions. However, since woodchip bioreactors rely on denitrification as the dominant mechanism to remove NO_3^- (Greenan et al., 2006; Greenan et al., 2009; Robertson et al., 2000; Schipper et al., 2010a), their performance is influenced by a range of factors that affect denitrification in most environments. Some of these environmental factors include; temperature, C availability, NO_3^- concentrations, DO concentrations, and the activity of the microbial community (Seitzinger et al., 2006). Additionally, flow rate or retention time, which are related to the design and operational parameters of the bioreactor, will also impact its performance (Christianson et al., 2011c). Consequently, the efficiency of NO_3^- removal in woodchip bioreactors is largely affected by environmental conditions and retention time, leading to variations in removal rates between studies. For example, Schipper et al. (2010b) reported NO_3^--N removal rates ranging from 2 to 22 g N/m^3/d in most denitrification bed studies. Meanwhile, a meta-analysis by Addy et al. (2016) revealed common NO_3^--N removal rates ranging from 0.5 to 10 g N/m^3/d. Similarly, Christianson et al. (2021) reported NO_3^--N removal rates in line with those presented by Addy et al. (2016), with an average of 5.1 g N/m^3/d. According to Schipper et al. (2010a), in most studies, NO_3^- removal rates from woodchips bioreactors are generally below 10 g N/m^3/d, but higher removal rates may be achieved through the management of factors that influence bioreactor performance.

2.3.1. Factors affecting denitrification bioreactors

2.3.1.1. Dissolved oxygen

Anoxic conditions in the bioreactors are essential for denitrification to occur (Robertson et al., 2000). Therefore, the depletion of DO must take place before denitrification starts (Robertson & Merkley, 2009). In laboratory column tests, Robertson (2010) found that one hour retention time was necessary to substantially deplete influent DO. Meanwhile, Halaburka et al. (2019) employed a DO model to demonstrate that, at different temperatures (4, 15, 21 and 30 °C) and with a constant influent NO_3^- concentration (5 mg N L^{-1}), only 5-7% of the linear fraction of the bioreactor was required to consume DO and enable subsequent denitrification. The authors also concluded that under normal flow and loading conditions, DO was unlikely to affect the operation of denitrification bioreactors. Nevertheless, modelling results have shown that as the influent NO_3^- concentration decreased, the linear fraction of bioreactor required to consume DO increased (Halaburka et al., 2019). For instance, at different porewater velocities (1.4, 3.4 and 8.2 cm/h), and 21°C and an influent NO_3^- concentration of 2 mg N/L, the linear fraction required to reduce DO to less than 0.1 mg/L, accounted for 8 - 12% of the reactor length, while at influent NO_3^- concentrations of 0.6 mg N/L, the fraction of bioreactor needed to consume DO was higher (c. 25 - 37%) (Halaburka et al., 2019), indicating that under low influent NO_3^- concentrations, a significant portion of C (approximately 3 - 35%) will be consumed through aerobic respiration. Additionally, woodchips degrade much faster under aerobic conditions than under anoxic conditions; therefore, more frequent replenishment will be necessary (Halaburka et al., 2019). However, this will not pose a problem for wastewater treatment, as wastewater typically exhibits concentrations ranging from 15 to 20 mg N/L (Tchobanoglous et al., 2003).

2.3.1.2. Temperature

As it is a biologically mediated transformation, denitrification is significantly affected by water temperature (Halaburka et al., 2019). It is widely recognised that biological reaction rates exhibit a positive correlation with temperature (Schipper et al., 2010b). Several studies have consistently demonstrated that NO_3^- removals rates in denitrification bioreactors generally increase as the temperature rises (Cameron & Schipper, 2010; David et al., 2015; Maxwell et al., 2020; Soupir et al., 2018; Thapa et al., 2023). The reaction rate increase for every 10°C temperature increment value (Q_{10}) of woodchip bioreactors ranges from less than 1 to slightly over 3, with most values around 2 (Addy et al., 2016; David et al., 2015; Hoover et al., 2015; Warneke et al., 2011a).

Most studies have examined NO_3^- removal rates in bioreactors up to 25°C, consistently showing better performances around this temperature. However, the upper temperature threshold at which removal rates begin to decline remains unidentified. Liao et al. (2018) determined that complete denitrification with a high influent NO_3^--N concentration can be achieved within a reaction temperature range of 15°C to 35°C. Although, studies have reported that NO_3^- removal remains active at temperatures as low as 2°C to 4°C (Robertson & Merkley, 2009).

The relationship between temperature and NO_3^- removals rates is so relevant that Cameron and Schipper (2011) attempted to increase the temperature of a denitrification bed through passive solar heating. However, despite raising the bed temperature by 3.4°C, they did not report a measurable increase in NO_3^- removal rate.

The impact of temperature on bioreactor efficiency is significant. Although altering water temperature prior to treatment is often challenging, a comprehensive understanding of the operational parameters of the bioreactor can help mitigate temperature sensitivity (Christianson et al., 2012b). For instance, lower NO_3^- removal rates associated with low temperatures can be partly compensated by extending the hydraulic retention time in bioreactors (Addy et al., 2016).

The major factors contributing to the overall temperature response include the degradability of the C source (Schipper et al., 2010b) and the activity of the microbial communities in charge of the C source degradation and denitrification process (Halaburka et al., 2019).

2.3.1.3. Hydraulic retention time

Hydraulic retention time (HRT) of drainage water within bioreactors is primarily determined by the incoming flow rate (Schipper et al., 2010b). It is also defined by design factors and media porosity (Christianson et al., 2011c). The HRT of water inside a bioreactor is a critical factor affecting reactor performance (Christianson et al., 2011a), as very short retention times can result in poor NO_3^- removal (Christianson et al., 2011c). Denitrification, being an anoxic reaction, is inhibited by aerobic environments. Therefore, the retention time inside bioreactors needs to be long enough to consume the DO in the influent water first (Robertson, 2010) and then to facilitate the reduction of NO_3^-. Longer retention times can lead to complete NO_3^- removal; whereas excessively long retention times can generate high levels of DOC and other undesirable by-products (Schipper et al., 2010b). Once complete NO_3^- removal is achieved, additional oxidation-reduction (redox reaction) can occur, resulting in the formation of by-products, such as hydrogen sulfide (Robertson & Merkley, 2009) and methyl mercury (Woli et al., 2010). Longer retention times also reduce the volume of drainage water treated at any given time.

Several studies have demonstrated that longer HRTs results in higher NO_3^- removal efficiency (measures in %). For example, in a laboratory study, Hoover et al. (2015) reported that NO_3^- removal efficiency increased from 7 to 55% as HRT increased from 1.7 to 21.4 hours. However, they found that above 10.9 hours, the increase in NO_3^- removal rate slowed down. Similarly, Christianson et al. (2011a) found in a pilot-scale bioreactor experiment that performance improved with increased HRT, achieving 30 to

70% mass reduction between 4 to 8 hours of HRT, while 10 hours was required for 90% removal. Meanwhile, Martin et al. (2019) reported in their study that the optimal HRT for woodchip bioreactors was 8 hours, considering all factors, including greenhouse gas emissions and NO_3^- removal rates.

It is important to note that while some studies have shown that long HRTs achieve higher NO_3^- removal efficiency, analysing load reductions reveals that short HRTs may allow better overall reductions (i.e. removal rate) on a daily basis, as more NO_3^- enters a fixed-size bioreactor per day (Hoover et al., 2015; Hua et al., 2016; Soupir et al., 2018). For example, Soupir et al. (2018) found that at 21.5°C, with 12 hours of HRT, 67% NO_3^- removal was observed, while the average NO_3^--N removal rate was 22.5 g N/m^3/d. In contrast, at 24 hours HRT, the removal efficiency was 96%, but the NO_3^--N removal rate decreased to 14.6 g N/m^3/d (Soupir et al., 2018). Moreover, Jéglot et al. (2022) evaluated the effect of different HRT (from 5 to 30 hours) on NO_3^- removal in woodchip bioreactors at low water temperatures (3.9 - 5.8°C) and found that the relationship between HRT and NO_3^- removal efficiency followed a linear model with an excellent fit ($R^2_{adj} = 0.94$), with the highest efficiency of 75% observed at 30 hours of HRT. However, the rates of NO_3^- removal peaked at 20 hours of HRT (5.2 g NO_3^--N/m^3/d) and did not increase further with longer HRT. Therefore, the optimal HRT to maximise NO_3^- removal rate may not be the same as the optimal HRT to maximise NO_3^- removal efficiency (Lepine et al., 2016). Balancing these removal metrics is crucial for determining HRT design in woodchip bioreactors.

2.3.1.4. Influent flow rate

Drainage water flow rate, together with bioreactor size, are key parameters controlling water retention time within a bioreactor (Schipper et al., 2010b). Therefore, when designing a bioreactor, it is crucial to characterise the flow rate and its variation, in order to achieve the desired retention time. Generally, for a given bioreactor size, the removal efficiency of the bioreactor decreases as flow rates increase (Greenan et al., 2009). This can be attributed, in part, to the increased transport of DO at higher flow rates, resulting in shorter retention times that are insufficient for complete DO removal from the water, thus limiting the denitrification process (Christianson et al., 2011c; Greenan et al., 2009). Furthermore, higher flow rates lead to an increased N load entering the bioreactor and insufficient time for NO_3^- removal (Greenan et al., 2009). Another negative effect of high flow rates is the potential washout of microbes and available soluble C, which can result in incomplete denitrification (Zhao et al., 2018). Additionally, Christianson et al. (2011c) demonstrated that steady flows entering the bioreactors resulted in better removal rates compared to fluctuating flows.

2.3.1.5. Influent nitrate concentrations

In general, high influent NO_3^- concentrations allow better NO_3^- removal rates in bioreactors (Schipper et al., 2010b). For instance, Husk et al. (2017) reported a significant correlation between the reduction of NO_3^-- concentration in the outflow of a denitrification bioreactor and the influent NO_3^- concentration ($r = 0.71$, $p < 0.001$, $n = 19$). They observed that NO_3^- removal generally increased as the influent concentration increased. Additionally, Schipper et al. (2010a) indicated that low influent NO_3^- concentrations resulted in denitrification being NO_3^- limited.

In a performance evaluation of four field-scale denitrification bioreactors conducted by Christianson et al. (2012a), multiple regression analyses revealed that removal rate (g $N/m^3/d$) was significantly affected by influent NO_3^- concentration. The parameter estimates indicated that a 1 mg NO_3^--N/L increase in influent concentration led to an increase in the removal rate ranging from 0.44 to 1.25 g $N/m^3/d$, assuming other parameters remained constant. Furthermore, in a meta-analysis of 26 published studies, Addy et al. (2016) reported that higher NO_3^- removal rates were observed in bioreactors with influent N concentrations greater than 30 mg N/L compared to bioreactors with intermediate (10 - 30 mg N/L) or low (<10 mg N/L) influent N concentrations.

However, it is important to mention that percentage load reductions will decrease with higher influent NO_3^- concentrations (Hoover et al., 2015). For example, in a column study with a retention time of 12 hours, Hoover et al. (2015) investigated NO_3^- removal at three different influent NO_3^--N concentrations (10.4, 29.4 and 47.8 mg N/L). They found that at the lower concentration, bioreactors were able to reduce effluent concentrations to as low as 2.9 mg N/L (72% reduction). While for the 29.4 and 47.8 mg N/L influent concentrations, the NO_3^--N concentrations after treatment were 17.4 and 34.9 mg N/L (41 and 27% reduction), respectively (Hoover et al., 2015). Based on these findings, Hoover et al. (2015) concluded that the NO_3^- removal pattern indicated N saturation occurring at concentrations between 30 and 50 mg N/L.

2.3.1.6. Microbial community

As mentioned previously, denitrification is the dominant mechanism of NO_3^- removal in bioreactors, and this is carried out by denitrifier microorganisms (Greenan et al., 2009; Hellman et al., 2021; Robertson et al., 2000; Schipper et al., 2010b). These microorganisms are naturally present in the environment; and therefore, no inoculation is required for woodchip bioreactors operation (Schipper et al., 2010b). Moreover, woodchips provide favourable conditions for the growth of denitrifiers (Moorman et al., 2010; Warneke et al., 2011b). For instance, Warneke et al. (2011b) compared the abundance of microbial denitrification genes across different substrates (green waste, maize cobs and woodchips). Although green waste and maize cobs showed, on average, higher proportions of denitrifiers per gram of substrate than woodchips, the proportion of denitrification genes relative to total bacterial DNA was higher in woodchips. This suggests that a significant portion of the C in green waste and corn cobs was consumed by non-denitrifying bacteria, while denitrifying bacteria predominantly consumed the C in the woodchips. Thus, woodchips appear to be a more suitable substrate for denitrifying bacteria (Warneke et al., 2011b). While bacterial populations predominantly mediate denitrification, fungi can also contribute significantly to the enhancement of denitrification through the decomposition of C sources and the release of soluble C (Appleford et al., 2008).

Microbial community structure and population dynamics in woodchip bioreactors are variable and influenced by various factors such as DO, HRT, temperature amongst others (Hartfiel et al., 2022). For instance, in a two-year study tracking the microbial community composition of pilot-scale bioreactors, Porter et al. (2015) observed variations in the community response to seasons and bioreactor depth. These variations were correlated with changes in temperature and woodchip moisture content, which can vary with seasons and bioreactor depth. Similarly, Schaefer et al. (2022) compared microbial communities from nine pilot-scale woodchip bioreactors operating at different HRTs and sampled over two years. The authors found significant shifts in microbial community composition between years and HRTs. They also noted that microbial communities within the same year were more similar compared to different years but with the same HRT. Despite differences in microbial communities, they identified phylogenetic patterns and persistent taxa, indicating the preservation of denitrification potential.

Carbon substrates also play a significant role in microbial community composition and bioreactor performance (Hartfiel et al., 2022) as they determine the microbial community's composition and activity (Hellman et al., 2021). Furthermore, C degradation and fermentation, along with denitrification, are synergistic processes in woodchip bioreactor systems (Zhao et al., 2018). Carbon-degrading and fermentative microorganisms provide denitrifiers with the necessary soluble C (Zhao et al., 2018). Likewise, Nordström and Herbert (2019) highlighted the dependence of NO_3^- removal in woodchip denitrification bioreactors on the cross-feeding between the fermentative microorganisms (C suppliers) and the denitrifying microorganisms (NO_3^- removers). According to the authors, the stability of cross-feeding between these two microbial communities is more important for long-term stable NO_3^- removal than the total C content in woodchips. Several studies suggest that the NO_3^- removal rate is primarily limited by the decomposition of C sources rather than the abundance of denitrifying microorganisms (Feyereisen et al., 2016; Roser et al., 2018; Warneke et al., 2011a). For instance, Feyereisen et al. (2016) reported that the low NO_3^- removal rate observed at 1.5°C was not due to insufficient denitrifying bacterial communities but rather to reduced microbial activity. Similarly, Warneke et al. (2011a) suggested that increased NO_3^- removal at high temperatures is due to higher microbial activity, providing more available C to denitrifying microorganisms. Additionally, Roser et al. (2018) investigated the effect of acetate, as soluble C source added to woodchips, on NO_3^- removal rates. Their study showed that the improved NO_3^- removal at 5°C was due to the availability of soluble C (provided by the acetate treatment), indicating that denitrifying microorganisms are abundant enough at low temperatures but limited by the lack of an electron donor (soluble C).

2.3.1.7. Carbon source

The performance of denitrification bioreactors is significantly influenced by C availability, as it stimulates the denitrifying microbial community (Hartfiel et al., 2022; Rivas et al., 2020). Wood particle media is the most commonly used C source in field-scale denitrification bioreactors (Schipper et al., 2010b). Wood has demonstrated consistent NO_3^- removal capabilities, is a cost-effective C source, provides high permeability, has a high C:N ratio (Schipper et al., 2010b), exhibits long durability (Robertson et al., 2005; van Driel et al., 2006) and requires low maintenance (Robertson et al., 2008; van Driel et al., 2006).

In their review on denitrifying bioreactors, Schipper et al. (2010b) discussed studies investigating NO_3^- removal rates using different C substrates. The highest reported NO_3^- removal rates (19 - 105 g N/m^3/day) were achieved with alfalfa, wheat straw, and rice husk as C sources in column studies (Schipper et al., 2010b; Vogan, 1993). Warneke et al. (2011b) measured NO_3^- removal rates of six different C sources (*Pinus radiata* woodchips, eucalyptus woodchips, sawdust (*P. radiata*), maize cobs, green waste and wheat straw) in large barrels over 23 months. They found that the more easily degradable C sources (maize cobs, green waste and wheat straw) exhibited higher removal rates than wood media, with the maize cob showing the best NO_3^- removal (6.5-fold higher NO_3^- removal than wood media). However, the use of more easily degradable C substrates in denitrification bioreactors may require more frequent replacement due to rapid C depletion (Schipper et al., 2010b), leading to higher maintenance costs. Additionally, more easily degradable C source like maize cobs may have adverse effects, such as a higher release of DOC and other contaminants (Schipper et al., 2010b; Warneke et al., 2011b), as well as a faster decline in saturated hydraulic conductivity (Schipper et al., 2010b).

Denitrification rates, and thus NO_3^- removal rates, do not only rely on substrate type but also on the quantity of microbially available C, which varies between C sources (Warneke et al., 2011b). Therefore, the exceptionally high NO_3^- removal rates observed with C substrates like maize cobs can be attributed to their rapid decomposition and subsequent release of large amounts of available C. In contrast, wood decomposition inside the bioreactors is slow, resulting in a gradual release of microbially available C (Warneke et al., 2011b). This slow release of available C offers advantages such as a sustained NO_3^- removal over time, providing an extended lifespan for the bioreactor (Warneke et al., 2011b).

Estimations of bioreactors performance longevity often span a few decades, with empirical observations indicating at least ten years of operation (Christianson et al., 2012b). For example, Robertson et al. (2008) measured NO_3^- removal rates from a 15-year-old media in a septic treatment wall and found that the rates were about 50% lower than those measured in the first year. Additionally, Moorman et al. (2010) measured the denitrification potential from a 9-year-old woodchip wall operating in a tile drainage system and reported that wood loss was less than 20%, estimating a potential lifespan of 37 years based on the half-life of saturated woodchips. Similarly, from a mass balance calculation, Robertson et al. (2009) found that by year 7, only 10% of the initial C mass in the woodchips was consumed by denitrification.

While the slow release of available C offers advantages in terms of lifespan, it can also limit denitrification over time (Nordström & Herbert, 2019; Rivas et al., 2020; Warneke et al., 2011b). The DOC supply from woodchips is generally abundant in the early years of operation, as under anoxic conditions, cellulose and hemicellulose get easily transformed into sugars that are the preferable energy source consumed for microbial transformations within woodchip bioreactors (Hartfiel et al., 2022; Rivas et al., 2020). However, over time, the cellulose and hemicellulose contents in the bioreactors decrease, leaving a relatively higher proportion of lignin, which is more recalcitrant to microbial degradation (Ghane et al., 2018; Hartfiel et al., 2022). Additionally, it has been found that the release of available C slows down under low-temperature conditions (Feyereisen et al., 2016; Warneke et al., 2011a), primarily due to a reduction in the decomposition rate of the woodchips. Therefore, the greatest efficiency losses in woodchip bioreactors over time are expected to occur at the lowest temperatures, likely due to the changes in the C quality of the woodchips over time (Maxwell et al., 2020).

2.3.2. Potential adverse effects of bioreactors

A concern associated with enhancing denitrification using bioreactors is the issue of pollution swapping, which refers to the generation of contaminants in soluble, particle or gaseous forms in the bioreactor (Fenton et al., 2014). Studies have reported the leaching of dissolved contaminants during start-up, as well as emissions of greenhouse gases (GHG), ammonia (NH_3), sulphate (SO_4^{2-}) reduction and mercury methylation (Fenton et al., 2016; Healy et al., 2015; Rivas et al., 2020; Schipper et al., 2010b). While denitrifying bioreactors can contribute to pollution-swapping, these concerns can be minimised through different management or control approaches, which are significant considerations when designing denitrifying bioreactors (Healy et al., 2012).

2.3.2.1. Losses to the receiving environment

Many studies have noted that during the start-up of denitrifying bioreactors, soluble compounds such as DOC may be released, which is of great concern for water quality. High concentrations of DOC in surface water may indeed promote biological activities leading to oxygen depletion (Gibert et al., 2008; Pluer et al., 2016; Robertson et al., 2005; Schipper et al., 2010b). The initial dark-coloured effluent from bioreactors can contain high concentrations of DOC (Schipper et al., 2010b). However, during long-term operations, these concentrations usually stabilise at much lower levels (Robertson et al., 2005). Consistent with this, Abusallout and Hua (2017) found that in column bioreactors with a 24-hour HRT, the DOC concentrations in the effluent decreased rapidly from 71.8 to 21.7 mg/L during the first week of operation and then gradually decreased to 3 mg/L after 240 days of operation. They also noted that during the last 50 days of operation, the DOC concentrations did not change significantly, suggesting that a near-steady state condition was reached.

In woodchip bioreactors, the duration and magnitude of leaching depend on factors such as retention time, temperature, C source and NO_3^- concentration (Robertson, 2010; Robertson et al., 2005; Schipper et al., 2010b), indicating that start-up flushing can be controlled. For instance, in bioreactors with short retention times, the dissipation of initial DOC leachate is faster (Schipper et al., 2010b). However, the effluent will have substantial NO_3^- concentrations. Therefore, it is necessary to find middle ground in management of the start-up period. In addition to HRT, influent NO_3^- concentration is another important factor affecting the magnitude of DOC losses, as low NO_3^- concentration can limit the use of soluble C for the denitrification process. In contrast, in a full-scale woodchip bioreactor with high NO_3^- concentration inflows, Warneke et al. (2011a) did not observe DOC losses.

Implementing additional management practices can also reduce DOC losses into the environment. For example, Hoover et al. (2015) reported lower concentrations of total organic carbon (TOC) released from weathered woodchips compared to fresh woodchips while maintaining similar denitrification rates. Furthermore, Fenton et al. (2016) found that prewashing woodchips before installation in the bioreactor can reduce initial losses of TOC. Other control measures may include the collection of the initial effluent for disposal elsewhere or for irrigation to agricultural fields, or installing a post-bioreactor treatment and recirculating the effluent (Schipper et al., 2010b).

Phosphorus (P) is another soluble compound that can be released from woodchips bioreactors (Cameron & Schipper, 2010; Healy et al., 2012). This is particularly important because many waters treated in these bioreactors to reduce NO_3^- levels may also have elevated P concentrations (Sharrer et al., 2016). For instance, Healy et al. (2012) found that P concentrations in the discharge from column woodchip bioreactors reached up to 1.10 mg PO_4-P/L over an evaluation period of 100 days. Meanwhile, Herbstritt (2014) observed that P concentrations and loads in field-scale bioreactors (approximately 117 m^3 and about one year old) were higher in the discharge than in the influent, estimating a loss of around 0.2 g PO_4-P/m^3 of bioreactor per day. Conversely, some lab-scale bioreactor studies have reported reductions between 5-10% of orthophosphate or dissolved reactive phosphorus (DRP) by woodchip bioreactors (Goodwin et al., 2015; Zoski et al., 2013). Additionally, Warneke et al. (2011a) found that total phosphorus (TP) concentrations decreased over the operating duration of an in-field woodchip bioreactor treating hydroponic greenhouse wastewater, demonstrating the P-sink capability of woodchips bioreactors. Sharrer et al. (2016) reported findings of a study involving four pilot-scale bioreactors. All the bioreactors initially released DRP, and the shift from DRP leaching to removal depended on the HRT. For example, at the shortest HRT (12 hours), no DRP removal was observed, while the longest HRTs (42 and 55 hours) resulted in the highest removal efficiencies (54%). Overall, the studies summarised above demonstrate that woodchip bioreactors can act both as sources and sinks for P. However, the duration and magnitude of P losses from woodchips bioreactors can be influenced by modifying the HRT.

2.3.2.2. Greenhouse gas production

Apart from the leaching of soluble compounds, biophysical and biogeochemical processes occurring inside denitrifying bioreactors can generate gaseous contaminants, such as nitrous oxide (N_2O), carbon dioxide (CO_2), and methane (CH_4) (Fenton et al., 2014). Studies have found that, generally, N_2O emissions from woodchips bioreactors are not greater than emissions from agricultural soils (Elgood et al., 2010; Rivas et al., 2020). N_2O emissions from bioreactors represent 0.002% to 0.89% of the total NO_3^- removed by the bioreactors (David et al., 2015; Davis et al., 2019; Elgood et al., 2010), with most N_2O emissions being in the dissolved form (Hartfiel et al., 2022). Furthermore, N_2O production is significantly higher at low HRT, likely due to insufficient time for denitrification (Davis et al., 2019; Jéglot et al., 2022). In a pilot-scale study, total N_2O emissions corresponded to 5.19%, 0.38% and 0.50% of the total NO_3^- removed at HRTs of 2, 8 and 16 hours, respectively (Davis et al., 2019). Similarly, Jéglot et al. (2022) found that gaseous emissions of N_2O were the highest at an HRT of 5 hours and the lowest at 30 hours. However, no significant difference was found in N_2O emission at HRTs between 10 and 30 hours, suggesting an HRT threshold above which N_2O production is minimised.

In contrast to the low release of N_2O by woodchip bioreactors, CO_2 and CH_4 were found to be the dominant emissions in column studies (Healy et al., 2012). However, the highest CO_2 emissions were observed during the initial phase of the bioreactor operation, but once a steady-state was achieved, the emissions decreased to 1.2 g CO_2-C/m^2/day. In contrast, there was an increasing trend of CH_4 emissions over time, making CH_4 emissions the most pressing issue in relation to greenhouse gas (GHG) emissions from bioreactors (Healy et al., 2012). In line with this, Hartfiel et al. (2022) suggested that the concern for CH_4 emissions in woodchips bioreactors is generally more significant because the release of CO_2 is primarily due to the decomposition of the media, which would degrade over time regardless of its use in bioreactors, meaning there is no net increase in CO_2 emissions.

Similar to N_2O emission, it has been observed that HRT influences CH_4 emissions. However, contrary to N_2O emissions, higher CH_4 emissions are generated at longer HRTs (Davis et al., 2019; Healy et al., 2015; Healy et al., 2012). This is because larger CH_4 emissions are associated with full removal of NO_3^-, which enables CH_4 production from methanogens using CO_2 as the electron acceptor for the oxidation of hydrogen (H_2) (Jéglot et al., 2022). Therefore, when NO_3^- concentrations remain sufficiently high, methanogenesis is suppressed, and CH_4 fluxes are reduced (Schipper et al., 2010b).

Based on this, GHG emissions by woodchips bioreactors can be controlled and minimised by managing the HRT on a case-by-case basis. Additionally, other studies suggest that covering the bioreactor with a layer of soil can reduce GHG emissions, as similar capping of landfill systems can reduce the CH_4 generated by up to one-third (Healy et al., 2012; Stern et al., 2007).

2.3.2.3. Sulphate reduction

Denitrifying bioreactors have been found to exhibit SO_4^{2-} reduction under specific conditions, such as low flows, long HRT, extremely low NO_3^- concentrations and high temperatures (Blowes et al., 1994; Rivas et al., 2020; Robertson & Merkley, 2009; van Driel et al., 2006; Woli et al., 2010). When NO_3^- is completely removed, SO_4^{2-} acts as an electron acceptor, leading to the release of hydrogen sulfide gas (H_2S) (Robertson & Merkley, 2009). This process raises concerns for several reasons, including the loss of C for denitrifiers, the potential toxicity of H_2S gas, and its association with methylmercury production (Christianson et al., 2012b; Hartfiel et al., 2022; Woli et al., 2010).

Numerous studies have confirmed the occurrence of SO_4^{2-} reduction and methylmercury production in woodchip bioreactors operating at warm conditions, with NO_3^--N concentrations reduced to below 0.5 mg/L (Hudson & Cooke, 2015; Robertson & Merkley, 2009; Shih et al., 2011). However, as for other pollution swapping issues discussed earlier, it is possible to mitigate the risks associated with SO_4^{2-} reduction. Bioreactors can be designed and managed in a way that retains small concentrations of NO_3^- in the effluent, preventing complete NO_3^-removal and minimising the occurrence of SO_4^{2-} reduction (Robertson & Merkley, 2009).

2.3.3. Bioreactors design and installation considerations

The optimum design of denitrification bioreactors is crucial to maximise NO_3^- removal rates and ensuring cost-effectiveness (Christianson et al., 2013a). According to Schipper et al. (2010b), information about flow rates and NO_3^- flux is necessary to design denitrification beds. One of the main challenges in the design and performance of agricultural drainage bioreactors is the high variability of drainage flow rates (Christianson et al., 2012b). However, when using denitrification bioreactors for treating wastewater with steady flows, such as a tertiary treatment, flow rates are generally controllable. Nonetheless, Christianson and Schipper (2016) mention that for bioreactors treating wastewater, NO_3^- removal design criteria may need to consider the C inputs with the wastewater going into the bioreactor.

As mentioned in Section 2.3.1.3, HRT is defined by the bioreactor design (Christianson et al., 2011c). For instance, the retention time can be increased by expanding the bioreactor size at a given flow rate (Christianson et al., 2013a). However, to minimise the side effects associated with extremely high retention times and to reduce installation costs, a balanced design approach is required (Christianson et al., 2013a). Therefore, Christianson et al. (2011b) developed a design method to determine bioreactor dimensions (length and width) that consider retention time at a designed flow rate. The design method combines Darcy's equation for flow through porous media and a retention time equation to iteratively develop a length and width for a denitrification bioreactor designed to treat agricultural drainage. The Darcy's equation determines the bioreactor flow rate given an iteratively chosen length and width, and this flow rates is then used in the retention time model to refine the appropriate length and width. While this is one design approach, other designs are based on other factors, such as drainage area (for agricultural drainage treatment) (Wildman, 2001) or mass removal (Schipper et al., 2010b), which calculate the required bioreactor volume using published reaction rates.

Regarding bioreactor dimensions, it is critical to note that the depth below the ground surface is usually set by the depth of the drainage main (typically 1.2 to 1.5 m) (Christianson et al., 2011a). The desired retention time determines the length of the reactor, and the width is a function of the expected peak flow rate through the system (Christianson et al., 2011a). In general, to achieve a retention time of at least four hours at the design flow rate, bioreactors need a length-width ratio of at least 5:1 (Christianson et al., 2011b). Apart from dimensions, different bioreactor design geometries have been studied, but NO_3^- removal was not significantly affected by different cross-section shapes (Christianson et al., 2011a; Christianson et al., 2010).

Other bioreactor configurations and structures to improve performance have been investigated. For instance, Fenton et al. (2016) suggested the inclusion of baffles inside the bioreactor to create a zig-zag flow pattern to encourage more uniform flow through the entire bioreactor volume. As part of a bioreactor design, the implementation of inflow and outflow structures is crucial to manage retention times effectively (Christianson et al., 2012b). The inflow structure directs water into the bioreactor and allows for diversion through a bypass line during high-flow events, balancing the treatment capacity with the ability to maintain adequate retention times (Christianson et al., 2011a; Chun et al., 2010).

The substrate, source of C, because its properties (C:N ratio, porosity, particle size and hydraulic conductivity), plays a significant role in factors such as retention times, longevity, start-up flushing and hydraulics (Christianson et al., 2012b). When installing a bioreactor, several factors need to be considered, including site evaluation, drainage characteristics and construction details (Christianson et al., 2012b). Typically, installation involves excavating and filling the trench, laying liner and geofabrics, positioning the control structures, mounding the soil cover and re-seeding the site (Christianson et al., 2012b). In highly permeable soils, the use of bioreactor liners is recommended before filling with the C source (Woli et al., 2010). A soil cover is also advised to mitigate N2O emissions from the bioreactor surface (Christianson et al., 2012b).

When considering the costs of woodchip bioreactors, the most significant expenses are related to design and installation fees (Christianson et al., 2013b). Maintenance costs are minimal, with bioreactor material being replaced once in 10 to 20 years and flow control structures replaced every approximately eight years (Christianson et al., 2013b). Moreover, the time for adjusting the control structures is minimal. The cost-efficiency of woodchip bioreactors is site-specific, but Christianson et al. (2013b) estimated an average costs of US$2.10 ± 0.90 per kg of N removed per year for bioreactors treating agricultural drainage.

2.3.4. Overcoming the carbon limitation in woodchip bioreactors

Considering the slow decomposition of wood inside woodchip bioreactors and the consequent slow release of available C, which is one of the main constraints on bioreactors performance, researchers have explored different approaches to address this problem. These approaches include drying-rewetting cycling, combining woodchips with other plant C sources, bioaugmentation and woodchips dosing with soluble C sources.

2.3.4.1. Bioreactor drying-rewetting cycling

Anoxic conditions are commonly believed to be necessary for denitrification in denitrifying bioreactors. However, research has shown that some microorganisms involved in the breakdown of lignin require oxygen (Hartfiel et al., 2022). Therefore, studies on denitrifying bioreactors have investigated the effects of cyclical drying and rewetting periods, which allow for aerobic conditions that can enhance the breakdown of organic matter and stimulate NO_3^- removal by increasing the availability of C (Maxwell et al., 2019a, 2019b).

In woodchip column bioreactors, Maxwell et al. (2019a) conducted experiments in which the woodchips were exposed to aerobic conditions once a week for 8 hours. This approach significantly increased NO_3^- removal rates by up to 80% compared to continuous saturated columns. In continuation of this study, Maxwell et al. (2019b) further investigated the effect of the duration of unsaturated conditions on NO_3^- removal after rewetting. They tested three different aerobic periods, exposing the woodchips to unsaturated conditions once a week for either 2, 8 or 24 hours. The longest drying-rewetting duration (24 hours) resulted in the highest increase in NO_3^- removal rates compared to control columns bioreactors, with mean removal rate increases reaching 172% by the end of the study (287 days).

2.3.4.2. Woodchip bioreactors amended with other plant carbon sources

Studies have investigated the use of a combination of corn cobs (CC) and woodchip (WC) as an alternative to enhance NO_3^- removal in denitrifying bioreactors. Warneke et al. (2011b) conducted a study to investigate the NO_3^- removal rates and the microbial removal mechanisms associated with different C substrates, including woodchips and corn cobs. They found that CC had the highest NO_3^- removal rate (6.2 g N/m^3/day). However, this treatment resulted in significant C consumption by non-denitrifying microorganisms and adverse effects such as high concentrations of TOC and dissolved CH_4 release. On the other hand, WC removed less than half (1.3 g N/m^3/day) of the NO_3^- removed by CC but provided favourable conditions for denitrifying bacteria without observed adverse effects. Therefore, they concluded that a combination of these two substrates would maximise NO_3^- removal rates, with corn cobs providing readily available C and woodchips minimising detrimental TOC leaching.

Similarly, Feyereisen et al. (2016) tested the NO_3^- removal performance of CC, WC and a combination of CC followed by WC (CC at inlet two-thirds and WC at outlet one-third) in a laboratory denitrifying bioreactor column study. They found that at a temperature of 15.5°C the CC substrate presented the highest average NO_3^- removal rate (34.9 g N/m^3/day), while WC had the lowest NO_3^- removal rate (2.2 g N/m^3/day), and the combination of CC and WC fell between the two, but closer to the removals of just CC

(29.3 g N/m^3/day). Correspondingly, the release of TOC and total carbon (TC) was higher for CC (15.7 and 23.7 g/column), lower for WC (1 and 1.2 g/column) and intermediate for the mixture (6 and 12.2 g/column). Thus, they concluded that overall, the performance of a compartment of CC followed by WC was promising at the laboratory scale.

Furthermore, Law et al. (2023) estimated the performance and cost of full-scale bioreactors amended with CC based on data collected from pilot-scale bioreactors. They found that when CC was mixed with WC in a proportion of 75/25%, respectively, the estimated N removal rates were 1.6 to 10.1 times higher than in bioreactors with 100% WC. Additionally, a cost assessment over a 15-year period indicated that the corn cob and woodchip mixture treatment (CC75/WC25) was more cost-efficient (US$ 10.56 to 13.89/kg N) compared to the use of 100% woodchips (US$ 13.30 to 88.11/kg N).

2.3.4.3. Bioaugmentation

Bioaugmentation involves the introduction of microorganisms capable of performing the desired bioremediation into the contaminated site or source to enhance microbial activity (Tyagi et al., 2011). This technique has been applied in woodchip bioreactor studies, such as the one conducted by Feyereisen et al. (2023). This study aimed to assess whether the addition of cold-adapted, locally isolated bacterial denitrifying strains could improve NO_3^- removals of field-scale woodchip bioreactors operating at cold temperatures. The results demonstrated that the inoculation led to increased NO_3^- removal rates. However, the positive effects were not sustained for long periods, possibly due to the washing of the inoculated bacteria from the bioreactor (Feyereisen et al., 2023). Consequently, the authors recommended further research on improving inoculation procedures to better retain and distribute bacterial cells within the bioreactors.

While denitrifying bacteria have been the primary focus in most studies exploring microbial communities in woodchip bioreactors, recent investigations have also examined the role of fungi (Aldossari & Ishii, 2022). Aldossari and Ishii (2022) conducted a study to investigate the effectiveness of fungal inoculation to enhance NO_3^- removal in woodchip bioreactor microcosms under cold conditions. In this study, a cold-adapted nitrate-reducing and cellulose-degrading fungi strain was inoculated to woodchip bioreactor microcosms. The results showed that at 5°C and after 72 hours the NO_3^- concentrations reductions were higher in the inoculated (82%) than uninoculated (62%)

microcosms. Furthermore, the NO_3^- concentrations decreased 1.5 times faster in the inoculated woodchip microcosms than in the control group. These findings suggest that fungi inoculation enhances NO_3^- reduction through denitrification and by providing additional labile C to other denitrifiers (Aldossari & Ishii, 2022). Consequently, this promising approach holds the potential to improve the performance of woodchip bioreactors operating under cold conditions and requires further assessment on a field scale scenario (Aldossari & Ishii, 2022).

2.3.4.4. Bioreactor dosing with soluble carbon

The addition of soluble C sources to woodchips bioreactors has been found to improve NO_3^- removal rates (Feyereisen et al., 2023; Hartz et al., 2017; Moghaddam et al., 2023; Moghaddam et al., 2022; Roser et al., 2018). Liquid C sources such as glycerin, acetate and methanol increased denitrification rates, providing denitrifiers with a more readily available energy source (soluble C).

In laboratory-scale column bioreactors, Roser et al. (2018) explored the NO_3^- removal efficiency of woodchips with the addition of sodium acetate at different temperatures (15°C and 5°C) and HRTs (1.5, 8, 12 and 24 hours). They found that the addition of acetate improved the NO_3^- removal rate by more than an order of magnitude compared to woodchips alone. For example, at 5°C and with an 8-hour HRT, the NO_3^--N removal rate for the woodchip-acetate bioreactor was 29.8 g N/m^3/day, while for the woodchip-only treatment was 0.9 g N/m^3/day. Similarly, at 15°C and at the same HRT, the acetate treatment achieved NO_3^- removal rates of 26.7 g N/m^3/day, compared to the control treatment with a removal rate of 5.8 g N/m^3/day. In another laboratory-scale column experiment, Hartz et al. (2017) evaluated the effects of glycerin and methanol at two different dosing loads, resulting in similar NO_3^- removal rates of 29 – 40 g N/m^3/day and 31.5 – 40 g N/m^3/day respectively, compared to 2.25 g N/m^3/day in the woodchip control treatment. Additionally, they further investigated the effect of constant methanol dosing of field-scale bioreactors, achieving NO_3^- removal rates of approximately 36 g N/m^3/day, which was much higher than the rates achieved by the control bioreactor (~9 g N/m^3/day). From this study, Hartz et al. (2017) determined that the methanol dosing required to consistently reduce NO_3^--N in outlet water to < 1 mg/L had a 1.48:1 C:N ratio. Similarly, Moghaddam et al. (2022) studied the effects of methanol dosing on NO_3^- removal rates in microcosm-scale bioreactors. In this study, methanol was maintained at a C:N ratio of 1.48:1, as previously suggested by Hartz et al. (2017). Methanol dosing significantly increased NO_3^- removal rates by almost four times, from 7 to 27 g N/m^3/day, compared to the woodchips control treatment. Moghaddam et al. (2023) also investigated the potential of constant methanol dosing in a full-scale bioreactor and reported that when the bioreactor was continuously dosed, 10.8 g NO_3^--N/m^3/day was removed, compared to 0.67 – 1.60 g NO_3^--N/m^3/day when the same bioreactor was not dosed (Rivas et al., 2020).

In a different study, Jansen et al. (2019) tested the difference in removal efficiency using two different organic matter sources and dosing strategies. The organic matter used were woodchips-enveloped drains and ethanol (alone) supplied to a flow-through reactor by passive dosing. From this study, Jansen et al. (2019) found that ethanol is a fast electron donor with a short lifespan, allowing rapid NO_3^- removal. Meanwhile, woodchips represent a slow electron donor with a longer lifespan. Initially, woodchips stimulated high NO_3^- removal efficiency (60-80%), but this decreased after two years of operation, along with a decrease in C availability (Jansen et al., 2019). Thus, Jansen et al. (2019) suggested that the combination of woodchip and ethanol would be effective because the woodchip provides a constant source of slow-release bioavailable C, and the additional dosage of ethanol has the potential to increase the denitrification rate in a controlled manner when needed. For example, ethanol could be added in winter when the availability of C from woodchips is lower due to low temperatures.

Although the addition of soluble C sources has been shown to improve NO_3^- removal in woodchip bioreactors, the evaluation of a diverse range of C sources remains limited. Additionally, there is a lack of understanding concerning the possible secondary effects of C dosage. For instance, C dosing could exacerbate the loss of soluble C into aquatic environments (Roser et al., 2018). It could also enhance higher SO_4^{2-} reduction rates (Moghaddam et al., 2022) and stimulate the bio-clogging of the bioreactor (Feyereisen et al., 2018). Therefore, further work is necessary to identify optimum and economical C sources, in addition to determining the optimal doses and the conditions or periods when its use is the most effective (Feyereisen et al., 2018).

2.3.5. Potential of sugar and ethanol for woodchip bioreactors dosing

In wastewater treatment plants, liquid organic C sources are traditionally used in denitrification biofilters for NO_3^- removal (Jiang et al., 2022). Organic compounds of low molecular weights (e.g. methanol, ethanol or acetate), saccharides (e.g. glucose, fructose or sucrose), as well as industrial by-products such as molasses, are some of the most common organic C sources employed (Jiang et al., 2022; Ortmeyer et al., 2021; Ueda et al., 2006). Therefore, these substances have the potential to enrich woodchip bioreactors and improve their performance, particularly during cold seasons. As described in the previous section, studies have successfully proved that dosing woodchip bioreactor with compounds such as methanol and acetate can improve NO_3^- removal rates. However, the effectiveness of other compounds such as ethanol, saccharides and industrial by-products remains unproven. When selecting an additional C source for woodchips bioreactors, factors such as acquisition and operational costs, health and safety considerations, and NO_3^- removal capabilities of the compound should be considered.

The use of sugar as an effective C source for denitrification has been demonstrated in a few studies treating groundwater, drinking water and wastewater, showing good NO_3^- removal capabilities and overall stable and satisfactory operation characteristics (Horová et al., 2020; Karanasios et al., 2016; Nurizzo & Mezzanotte, 1992). Based on this, and considering that sugar is readily available, its low-cost, and that it poses no health and safety risks; sugar is a promising alternative for dosing woodchip bioreactors.

Another source of soluble C that has been demonstrated to be a highly effective stimulant for denitrification is ethanol. Ethanol has successfully reduced NO_3^- in the biological denitrification of groundwater (Cao et al., 2014; Gómez et al., 2000; Grassi et al., 2007; Jansen et al., 2019; Ortmeyer et al., 2021) and in synthetic wastewater simulating nitrified effluents from domestic sewage treatment plants (dos Santos et al., 2004). While methanol is commonly used as the external C source in wastewater treatment plants, ethanol represents a feasible alternative, as some studies have shown that it can be a more effective electron donor compared to methanol in certain cases (Bill et al., 2009; dos Santos et al., 2004). Additionally, Ortmeyer et al. (2021) reported that ethanol presented a more effective NO_3^- degradation at 10°C than at room temperature (approx. 21.5°C), making it an even more attractive dosing agent for woodchip bioreactors.

Considering these points, sugar and ethanol have the potential to increase denitrification rate in woodchip bioreactors, especially during cold seasons, when C availability from woodchips is low due to cool temperatures. Soluble C sources may also allow higher NO_3^- removal rates at shorter hydraulic retention times (HRT), enabling the treatment of larger volumes of water for a given bioreactor size.

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3. The effect of hydraulic retention time and soluble carbon dosing on the nitrate removal performance of column woodchip bioreactors

3.1. Introduction

Nitrate (NO_3^-), which is not strongly held by the soil matrix and is highly mobile in water, is one of the most common pollutants in surface and groundwaters. In recent decades anthropogenic activities have contributed to the dramatic increase of NO_3^- in aquatic ecosystems, promoting water eutrophication and posing a threat to both the aquatic biodiversity and human health (Abascal et al., 2022; Bhatnagar & Sillanpää, 2011; Gutiérrez et al., 2018). Two of the main anthropogenic sources of NO_3^- pollution include agricultural activities and discharges from wastewater treatment plants (Gutiérrez et al., 2018; Katz, 2020). Numerous strategies and technologies have been developed and applied to mitigate NO_3^- loads to aquatic ecosystems. For wastewater, some strategies include the land application of pre-treated wastewater, the establishment of riparian and buffer zones, controlled drainage systems, constructed wetlands and denitrification bioreactors (Carstensen et al., 2020; Christianson et al., 2013; Gutierrez-Gines et al., 2020).

Denitrification bioreactors are a technology used to remove NO_3^- from water, in which the growth of heterotrophic denitrifying microorganisms is enhanced by the addition of a carbon (C) source and by providing anoxic conditions for denitrification (Hang et al., 2016; Schipper et al., 2010). The most widely employed C source in denitrification bioreactors is woodchips, due to their affordability, availability, relatively high permeability, high C:N ratio and durability (Robertson, 2010; Schipper et al., 2010). Woodchip bioreactors have been broadly used to reduce NO_3^- discharges from water draining from farmland (Christianson et al., 2021; Lee et al., 2023). However, bioreactors may also be used to treat drainage waters from municipal wastewater land treatment areas. In fact, the conditions for their use will be particularly favourable at many treatment sites. For instance, drainage waters originating from municipal wastewater land treatment areas, where wastewater is applied at high application depths year-round, can result in relatively consistent drainage water flow rates and NO_3^- concentrations throughout the year. In comparison, drainage from farmland is typically restricted to cooler times of the year, primarily during the winter or early spring, and is often characterised by highly variable flow rates and NO_3^- concentrations.

While woodchips have a number of advantages, the main disadvantage is that labile C production declines with temperature (Nordström & Herbert, 2019), which can limit NO_3^- removal rates (Addy et al., 2016; Robertson, 2010; Schipper et al., 2010). Woodchips are made of relatively recalcitrant organic compounds and, therefore, require microbial processes (i.e. hydrolysis and fermentation) before they can be utilised for denitrification (Nordström & Herbert, 2019). There is a direct relationship between the fermentative and denitrifying community, and at low temperatures the NO_3^- removal in bioreactors is primarily limited by the decomposition of C sources rather than by the abundance of denitrifying microorganisms (Feyereisen et al., 2016; Roser et al., 2018; Warneke et al., 2011).

Although increasing the hydraulic retention time (HRT) can enhance NO_3^- removal efficiency at low temperature, this approach reduces the volume of drainage water that can be treated per day. Therefore, the addition of soluble C sources, such as succinate, sodium acetate and methanol, to woodchip bioreactors has been studied to evaluate their potential to enhance NO_3^- removal (Feyereisen et al., 2018; Hartz et al., 2017; Jansen et al., 2019; Moghaddam et al., 2022; Palomo et al., 2013; Roser et al., 2018). Palomo et al. (2013) found that the addition of succinate as an external C source to a field denitrification wall treating groundwater, led to a substantial increase in NO_3^- removal from 15% to 73%. In a laboratory column experiment, Roser et al. (2018) investigated the effect of sodium acetate addition on NO_3^- removal efficiency from woodchips bioreactors. They found that the addition of acetate increased NO_3^- removal efficiency from 31% to 80%. Through a controlled long-term mesocosm experiment, Moghaddam et al. (2022) demonstrated the effectiveness of methanol dosing, and reported a NO_3^- removal efficiency of 87% when methanol was added, in comparison to a 28% removal in the control treatment. Two other soluble C sources that remain unexplored for enhancing denitrification rates in woodchip bioreactors are sugar and ethanol. Sugar was reported to be an effective C source for denitrification in drinking water, showing high NO_3^- removal capabilities (Karanasios et al., 2016; Nurizzo & Mezzanotte, 1992). Similarly, ethanol has successfully reduced NO_3^- concentrations when used to enhance biological denitrification of NO_3^- in groundwater (Cao et al., 2014; Grassi et al., 2007).

Although the evidence supports the enhancement of NO_3^- removal in woodchip bioreactors through the addition of soluble C, the evaluation of a diverse range of C sources remains limited. Additionally, studies assessing C dosing in woodchip bioreactors have predominantly focused on their implementation in treating drainage water from agricultural landscape, and there is limited research evaluating their use for treating drainage water from municipal wastewater land treatment sites.

The main objectives of this study were to evaluate the potential use of woodchip bioreactors for treating drainage water from a municipal (Levin) wastewater land treatment site, including the assessment of soluble C dosing to woodchip bioreactors to enhance NO_3^- removal. To achieve these objectives, a preliminary study was conducted using woodchip column bioreactors to evaluate NO_3^- removal under varying water temperatures and water hydraulic retention times (HRT), and dosing with soluble C sources at different C:N ratios. The experimental conditions aimed to achieve similar conditions, in terms of water NO_3^--N concentrations and temperatures, of drainage water at a municipal wastewater land treatment site.

3.2. Methodology

The study was conducted between October 2020 and August 2022. Over this period, column bioreactors were evaluated under warm and cool conditions. The warm conditions experiments were conducted during March and June of each year, while the cool conditions experiments were conducted during July and August of each year.

3.2.1. Experiment design

Column experiments were conducted following methodologies adapted from previous studies (Christianson et al., 2014; Hoover et al., 2015; Roser et al., 2018). Nine columns of polyvinyl chloride (PVC) pipes measuring 43 cm in height with an internal diameter of 15 cm were used for these experiments. A PVC cap was fitted at the bottom of each column, while a plastic plate covered the top. Each column was filled with approximately 1500 g (wet weight) of pine woodchips, with particles size ranging from 10 to 40 mm. The media pore volume was found to be 46%, determined by filling the columns with water, allowing the woodchips to soak for 24 hours, and then by measuring the outflow volume. On the top of the packed woodchips, 5 g of moist soil was added as an inoculant for denitrifiers. To promote even water distribution inside the columns, three plates were placed inside each column, between the woodchips, spaced approximately 10 cm apart, and covering about three quarters of each column’s cross-sectional area. The middle plate was installed on the opposite side of the column in relation to the first and third plates.

The columns were supplied with an NO_3^- solution (magnesium nitrate added to groundwater), which was stored in two 200 L tanks (Figure 3.1). This solution was gravity fed into the top of each column with individual tubes and taps to control flow rate. An outflow tube was installed at the bottom of each column, which was installed up the side of each column to maintain constant saturated conditions within each column. For the experiments involving soluble C addition, 4 L containers were connected to the tops of the columns using tubes and fluid flow rate regulators, enabling gravity feed of solutions into the columns. The flow rate regulators were checked at least once daily to ensure that the required solution dripping rate was maintained. Soluble C was diluted in a solution with the same NO_3^- concentration as used in the tanks providing the main supply of water to the columns, which helped to maintain consistent NO_3^- concentrations in the solution entering the columns.

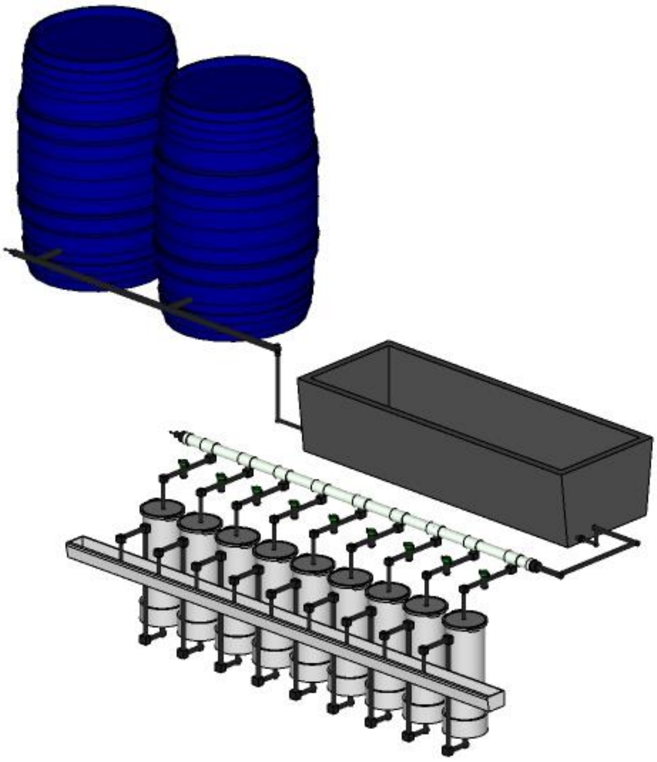

Figure 3.1. The main column bioreactor experiment setup (Note: the soluble carbon dosing component is not shown).

3.2.2. Experiment procedure and treatments

A series of five experiments, and two calibration evaluations, using woodchip column bioreactors were conducted to assess the impact of water temperatures, HRT, and different soluble C sources and C:N ratios on NO_3^- removal. The experiments used water with high average inflow NO_3^--N concentrations (range of 8.4 – 21 mg N/L), with most of the experiments using average concentrations in the range of 11-12.5 mg N/L. These latter range of concentrations are similar to the highest concentrations measured in drainage water at the Levin Wastewater Land Treatment Site (LWLTS). The experiments were conducted outdoors, to evaluate the effect of seasonal temperature fluctuations. Therefore, for the purpose of the study, water temperatures ranging from 15°C to 22°C were considered 'warm' conditions, while water temperatures between 8°C and 15°C were considered to correspond to 'cool' conditions. Temperature sensors recording data every three hours were installed in the storage tanks as well as in each column to measure water temperature. At the end of the description of each experiment, Table 3.1 presents the NO_3^--N inflow concentrations and temperature data for each experiment and C:N ratio tested.

Woodchip column bioreactors calibration evaluations

An initial observational period of 10 days (October 2020) was conducted, which was used to facilitate the development of denitrifying microorganisms in the columns, to detect potential setup faults, and to evaluate the performance of the nine columns under the same conditions. During his initial phase, design flaws were corrected, and it was established that the performance of all nine columns were consistent.

Following this initial setup period, two calibration evaluations were conducted. The first evaluation was carried out under warm conditions (November 2020) and the nine columns received NO_3^- solution at an inflow rate of 5.5 ml/min over 12 days. This flow rate corresponded to an HRT of approximately 10 hours, in line with studies suggesting that, under moderate temperature conditions, increases in the rate of NO_3^- removal start to diminish at HRT above 10 hours (Christianson et al., 2011; Hoover et al., 2015; Martin et al., 2019). Column outflow samples were collected daily, starting 48 hours after the start of the evaluation, this resulted in a total of 10 samples per column.

The second calibration evaluation, also over a period of 12 days and using a 10-hour HRT, was conducted under cool conditions (July 2021). Outflow samples were collected every two days, commencing 72 hours after the start of the evaluation, resulting in a total of 5 samples per column.

The results from these two evaluations were used as a baseline for determining the conditions used in the subsequent experiments.

Experiment 1

In Experiment 1, the performance of the column bioreactors at different HRTs was assessed under warm conditions (February 2021). The nine columns were divided into three treatments, each with three replicates. The treatments involved three different HRTs: 3.3, 6.6 and 10 hours. To achieve these HRTs, the inflow rates of the NO_3^- solution were adjusted to 16.4, 8.2 and 5.5 ml/min, respectively. HRTs exceeding 10 hours were not considered, as the literature suggest that NO_3^- removal rates tend to stabilise beyond this retention time (Christianson et al., 2011; Hoover et al., 2015). The experiment was conducted over a period of 8 days, with samples collected every two days, beginning 24 hours after the start of the experiment.

Experiment 2

In Experiment 2, the assessment focused on the effect of soluble C sources on the NO_3^- removal performance of the column bioreactors under warm conditions (March-April 2021). Building upon the findings of the previous experiment, the shortest HRT previously assessed of 3.3 hours was selected for this experiment. In this experiment, the column bioreactors were subjected to one of three treatments (each replicated three times): woodchips dosed with ethanol (95%, denatured), woodchips dosed with liquid sugar (common table sugar: 50% glucose and 50% fructose), and no added soluble C source, denoted as the control. Within this experiment, the two soluble C sources were added at three different C:N ratios: 0.5:1, 1:1 and 2:1. These ratios were based on the results of Hartz et al. (2017), who established that a C:N ratio of 1.4:1, using methanol, achieved near complete NO_3^- removal in solutions added to woodchip bioreactors operating at 48-hour HRT and under temperatures around 17°C.

The influent solution maintained an average NO_3^--N concentration of approximately 12 mg N/L and was delivered at an inflow rate of 16.4 ml/min (equating to a 3.3-hour HRT). To achieve soluble C solutions with C:N ratios of 0.5:1, 1:1 and 2:1, ethanol (52.5% C) was dosed at rates of 11.7, 23.4 and 46.7 mg/L, and liquid sugar (40% C) was dosed at rates of 15.0, 30.0 and 60.0 mg/L. Samples were collected from each column every second day over a period of 8 days, beginning 24 hours after the start of the experiment, resulting in 4 samples per column for each C:N ratio assessed.

Experiment 3

The objective of Experiment 3 was to evaluate the effect of soluble C sources on the NO_3^- removal rates of the column bioreactors under cool conditions (July-August 2021). The same soluble C sources and C:N ratios, as in Experiment 2, were used. However, for this experiment an HRT of 10 hours was used rather than the 3.3 hours used in Experiment 2. Under cool conditions the NO_3^- removal efficiency of the bioreactors at a 10-hour HRT was lower than under warmer conditions. Therefore, it was decided that a 10-hour HRT would be suitable for assessing the effect of soluble C addition under cool conditions. An influent solution with an average NO_3^--N concentration of approximately 12 mg N/L was used for this experiment.

Experiment 3 was conducted for a total period of 12 days, with sampling occurring every two days, commencing 72 hours after the start of each evaluation, resulting in 5 samples per column for each C:N ratio examined. It is important to note that due to Covid-19 restrictions the evaluation with the 2:1 C:N ratio could not be completed as originally planned. It had to be suspended on Day 6, and samples were only collected on two occasions. Nevertheless, some results are provided.

Experiments 4 and 5

These experiments were conducted to gain a better understanding of the effect of ethanol dosing, using different C:N ratios, on NO_3^- removal and at different water temperatures. As there was a large difference in the NO_3^- removal efficiency between the ethanol 1:1 and 2:1 C:N ratios in Experiment 3, it was considered useful to assess the effectiveness of a 1.5:1 C:N ratio. Additionally, the 1:1 and 2:1 C:N ratios were re-evaluated. Experiment 4 was conducted during warm conditions (March-April 2022) with a 3.3-hour HRT and Experiment 5 was conducted during cool conditions (July-August 2022) with a 10-hour HRT.

For these two experiments, a total of eight columns were allocated to two treatments: woodchips dosed with ethanol and control treatment (no dosing), with four replicates each. The C:N ratios tested included 1:1, 1.5:1 and 2:1. The influent solutions were maintained at the same NO_3^--N concentration (approximately 12 mg N/L) as in the previous experiments. Ethanol dosing rates were the same than those used in Experiment 2 and 3, except for the 1.5:1 C:N ratio treatment, for which the dosing rate was of 35.1 mg ethanol/L. The sampling approach and frequency were the same as those employed in Experiment 3.

Table 3.1. Minimum, maximum and average (Standard deviation) NO_3^--N inflow concentration y temperature during each experiment and corresponding C:N ratio tested.

Experiment	**NO_3^--N inflow concentration (mg N/L)**			**Temperature (°C)**		
	Min	**Max**	**Average**	**Min**	**Max**	**Average**
Calibration 1	5.2	9.9	8.6 (1.4)	12.8	19.5	16.7 (2.2)
Calibration 2	11.8	12.6	12.4 (0.3)	7.0	12.1	9.7 (2.4)
Experiment 1 HRT	17.6	20.9	19.0 (1.4)	19.6	22.8	21.0 (1.3)
Experiment 2 C:N 0.5:1	15.1	19.8	11.8 (0.5)	15.1	19.8	17.7 (2.2)
Experiment 2 C:N 1:1	12.0	14.0	12.9 (0.8)	15.0	18.3	17.2 (1.5)
Experiment 2 C:N 2:1	10.0	11.2	10.7 (0.5)	12.1	17.1	14.9 (2.1)
Experiment 3 C:N 0.5:1	10.6	12.9	11.8 (0.9)	10.4	12.4	11.8 (0.8)
Experiment 3 C:N 1:1	10.5	12.7	11.9 (0.9)	8.4	11.3	9.8 (1.2)
Experiment 3 C:N 2:1	11.0	11.1	11.1 (0.04)	10.7	11.3	11.0 (0.4)
Experiment 4 C:N 1:1	11.6	12.9	12.4 (0.5)	15.2	18.7	16.1 (1.5)
Experiment 4 C:N 1.5:1	12.0	13.0	12.5 (0.5)	16.7	20.0	18.5 (1.2)
Experiment 4 C:N 2:1	11.7	13.1	12.5 (0.6)	16.6	20.6	17.9 (1.7)
Experiment 5 C:N 1:1	12.1	13.2	12.6 (0.4)	11.7	15.7	13.1 (1.4)
Experiment 5 C:N 1.5:1	11.0	12.5	11.7 (0.6)	8.0	13.7	10.6 (2.4)
Experiment 5 C:N 2:1	12.7	13.8	13.1 (0.5)	8.2	13.9	11.5 (2.3)

3.2.3. Temperature monitoring and nitrate analyses

The solution temperature was automatically logged at three-hour intervals using Thermochron Temperature Logging iButtons. The average daily temperature variations of each experiment are presented in Appendix 2. Each column and the main tank, which supplied the influent NO_3^- solution to the columns, were equipped with individual iButtons. Samples of influent and column-treated solutions were analysed for NO_3^- using Ion Chromatography (ThermoFisher Scientific - Dionex Aquion).

3.2.4. Statistical analyses

All data were analysed with a linear mixed effects model with repeated measures using the PROC MIXED procedure of SAS version 9.4 (Statistical Analysis System, version 9.4; SAS Institute Inc., 2016). The NO_3^--N concentrations in the water samples were analysed separately for each evaluation, using a model where treatments were defined as fixed effects and temperature and sampling day as random effects. The model also included replicates (columns) nested within the treatments. The normal distribution of the data was checked. If statistical treatment differences were found, they were further examined by running Tukey-Kramer multiple comparisons tests using the “lsmeans” statement.

3.3. Results

3.3.1. Woodchip column bioreactors calibration evaluations

In the first calibration evaluation, the efficacy of column bioreactors at reducing NO_3^- concentrations under warm conditions and with a 10-hour HRT was assessed. The average water temperature was 16.7°C (SD 2.2), while the average inflow NO_3^--N concentration of the experiment was 8.6 mg N/L (SD 1.4). The bioreactor columns reduced the NO_3^--N concentration in the inflow water to an average of 1.6 mg N/L (SD 1.3) in the outflow. These results indicate that, at warm temperatures, a 10-hour HRT was sufficient for the column bioreactors to achieve a high average NO_3^- removal efficiency of 81% (range 50 - 95%), and an average NO_3^- removal rate of 7.8 g N/m^3/day (range 3.9 – 10.6 g N/m^3/day).

At the second calibration evaluation, column bioreactors were evaluated using a 10-hour HRT, but under cool conditions. The average water temperature was 9.7°C (SD 2.4) and the average inflow NO_3^--N concentration was 12.4 mg N/L (SD 0.3). Outflow NO_3^--N concentrations from the bioreactors averaged 8.6 mg N/L (SD 1.5), which achieved a low average NO_3^- removal efficiency of 31% (range 23 - 41%), and an average NO_3^- removal rate of 4.2 g N/m^3/day (range 1.5 - 8.3 g N/m^3/day).

3.3.2. Experiment 1: Effect of HRT on nitrate outflow and removal efficiency

Throughout this experiment, the average water temperature was 20.2°C (SD 3.8), while the average inflow NO_3^--N concentration was 19.0 mg N/L (SD 1.4) (Figure 3.2). The three HRT treatments had a significant ($P<0.0001$) effect on the average outflow NO_3^--N concentrations. The outflow NO_3^--N concentrations for the treatment with a 3.3-hour HRT was an average of 15.3 mg N/L (range 12.4 – 17.9 mg N/L). Meanwhile, using a 6.6-hour HRT resulted in an average outflow NO_3^--N concentration of 5.2 mg N/L (range 3.2 – 8.2 mg N/L). Notably, with a 10-hour HRT, the outflow NO_3^--N concentrations remained consistently below 1 mg N/L for all four sampling days, with an average of 0.2 mg N/L (range 0.0 – 0.7 mg N/L) (Figure 3.2). The average NO_3^- removal efficiency observed for the 3.3, 6.6 and 10-hour HRT treatments were of 19%, 72% and 99%, respectively. The NO_3^- removal rates were 12.3, 23.3 and 20.9 g N/m^3/day, respectively.

Under the experimental conditions of this study, the relationship between HRT and NO_3^- removal efficiency was linear, with an R^2 of 0.90, reaching the highest efficiency (99%) at a 10-hour HRT and declining at a rate of 12% per 1-hour reduction in HRT. In contrast, the NO_3^- removal rate (g N/m^3/day) peaked at an HRT of 6.6 hours and did not show further improvement at an HRT of 10 hours.

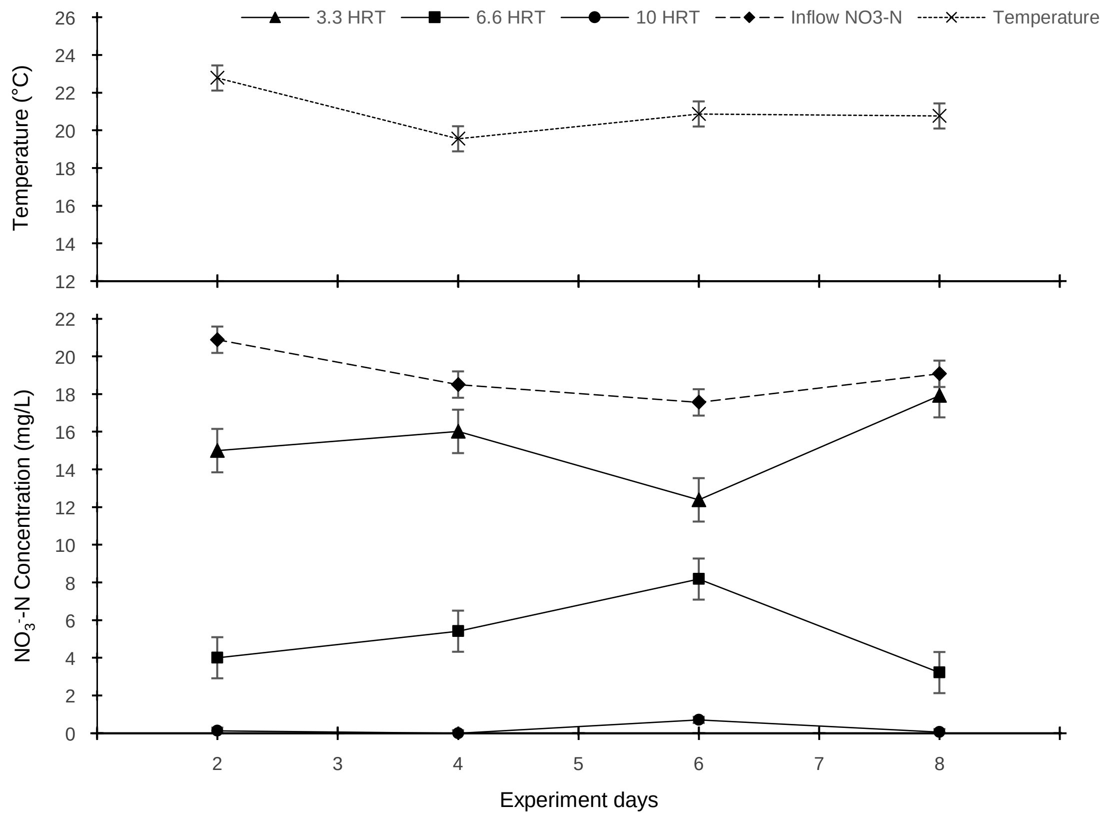

Figure 3.2. Experiment 1 (HRT – warm conditions) - Average NO_3^--N concentration and temperature per sampling day for each treatment. Error bars represent standard error.

3.3.3. Experiment 2: Effect of soluble carbon addition on nitrate removal under warm conditions

To evaluate the ability of soluble C addition to enhance the removal of NO_3^-, this experiment was conducted at an HRT with a low NO_3^- removal efficiency. As identified previously in Experiment 1 under warm conditions, an HRT of 3.3 hours achieved an average NO_3^- removal of only 19%. Therefore, for this experiment the three evaluations were performed at a 3.3-hour HRT, using three different C:N ratios of two soluble C sources (liquid sugar and ethanol) added to the woodchip column bioreactors. During this experiment the average temperature was 16.6°C (SD 2.1), while the average inflow NO_3^-

-N concentration was 11.8 mg N/L (SD 1.1). Table 3.2 presents the average NO_3^--N removal efficiencies and removal rates achieved at each C:N ratio evaluated and for each treatment.

During the 0.5:1 C:N ratio evaluation the temperatures ranged from 15.1 to 19.8°C and the average inflow NO_3^--N concentration was 11.8 mg N/L (SD 0.5). The average outflow NO_3^--N concentrations for the Control, Sugar and Ethanol treatments were 7.7 mg N/L (range 5.0 – 11.0 mg N/L), 6.6 mg N/L (range 4.4 – 9.0 mg N/L) and 6.0 mg N/L (range 4.2 – 8.5 mg N/L), respectively. The three treatments displayed similar responses to the daily variations in temperature over the experimental period (see Appendix 1.1). While the Ethanol treatment achieved the highest average NO_3^--N removal efficiencies and removal rate, the difference between treatments was not large enough to be significant different (P>0.05).

Table 3.2. Average (Standard deviation) NO_3^--N removal efficiency and removal rate for each soluble C concentration (C:N) during Experiment 2 (warm conditions, 3.3-hour HRT).

C:N Ratio	**NO_3^--N Removal Efficiency (%)**			**NO_3^--N Removal Rate (g N/m^3/day)**		
	Control	**Sugar**	**Ethanol**	**Control**	**Sugar**	**Ethanol**
0.5:1	35.4 (23.8) *a*	44.5 (24.1) *a*	49.3 (27.1) *a*	13.7 (9.1) *a*	17.3 (9.0) *a*	19.2 (10.3) *a*
1:1	32.0 (20.3) *a*	38.5 (18.1) *a*	61.4 (19.9) *a*	13.8 (9.0) *a*	16.6 (8.3) *a*	26.7 (9.8) *a*
2:1	17.5 (14.9) *a*	24.5 (13.8) *a*	89.8 (4.6) *b*	6.3 (5.6) *a*	8.8 (5.1) *a*	32.2 (2.2) *b*

Within each C:N evaluation, averages followed by the same lowercase letter are not significantly different (P> 0.05).

When soluble C dosing was evaluated at a 1:1 C:N ratio, temperatures ranged between 15.0 and 18.3°C, the average inflow NO_3^--N concentration was 12.9 mg N/L (SD 0.8), and the average outflow NO_3^--N concentrations for the Control, Sugar and Ethanol treatments were 8.7 mg N/L (range 8.3 – 9.1 mg N/L), 7.9 mg N/L (range 6.7 – 8.9 mg N/L) and 4.9 mg N/L (range 2.4 – 6.8 mg N/L), respectively. The outflow NO_3^--N concentrations for the three treatments followed similar trends as the evaluation progressed (see Appendix 1.2). As was also the case for the previous lower C:N ratio, the Ethanol treatment achieved the highest NO_3^- removal efficiencies and removal rates, but the difference was not large enough to be significantly different (P>0.05) from the other two treatments.

During the 2:1 C:N ratio evaluation, the temperatures were between 12.1 and 17.1°C, the average inflow NO_3^--N concentration was 10.7 mg N/L (SD 0.5), while the average outflow NO_3^--N concentrations for the Control, Sugar and Ethanol treatment were 8.9 mg N/L (range 7.5 – 10.1 mg N/L), 8.1 mg N/L (range 7.1 – 9.4 mg N/L) and 1.1 mg N/L (range 0.8 – 1.5 mg N/L), respectively. The Ethanol treatment achieved outflow NO_3^--N concentrations that remained consistently below 1.5 mg N/L throughout the experiment, regardless of the temperature variations (Figure 3.3). Even when the temperature decreased to 12°C (i.e. below the warm condition range) on the final day of the evaluation, the use of ethanol dosing at a 2:1 C:N ratio was still able to maintain a NO_3^- removal of 86%. The Control and Sugar treatment presented similar trends over the assessment period. However, the Ethanol treatment achieved an average NO_3^--N removal efficiency of 89% and an average NO_3^--N removal rate of 32.2 g N/m^3/day, which were significantly different ($P<0.0001$) from the other treatment. The differences between the Sugar and Control treatments were not significant ($P>0.05$).

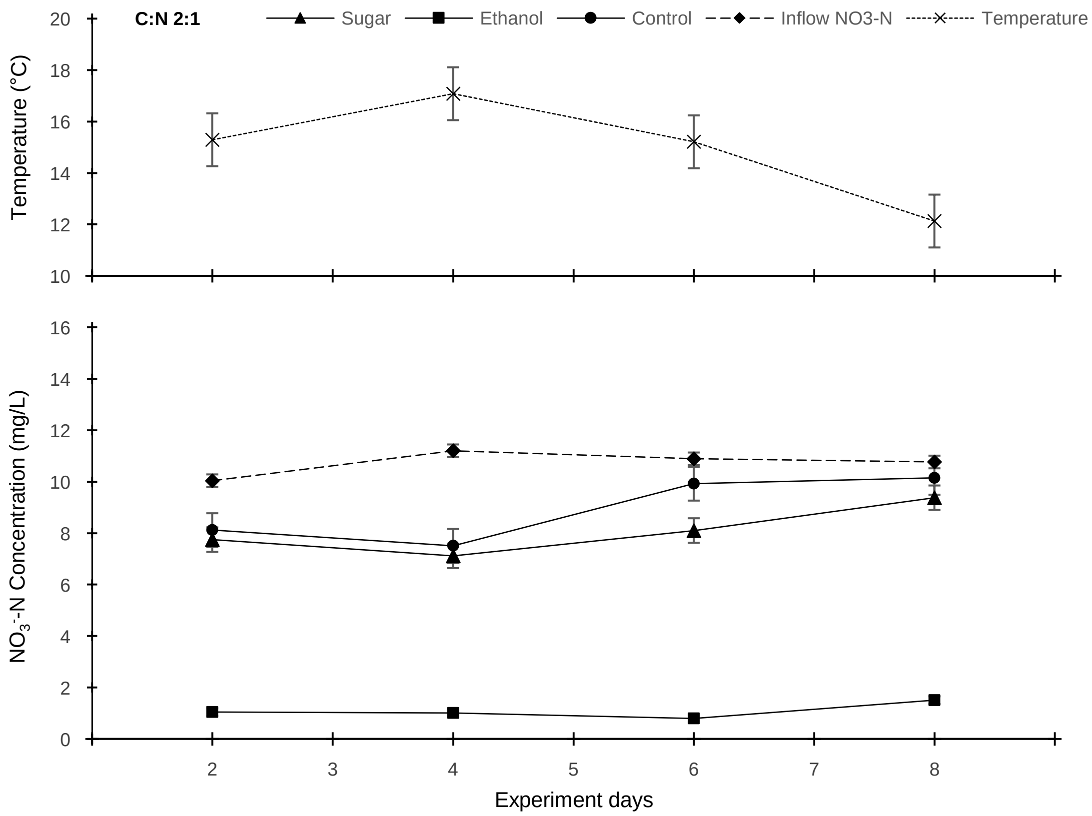

Figure 3.3. Experiment 2 (warm conditions, 3.3-hour HRT) - Average NO_3^--N concentrations and temperatures on each sampling day for each treatment at the 2:1 C:N ratio evaluation. Error bars represent standard error.

The average NO_3^--N removal efficiencies and removal rates for the Ethanol treatment increased significantly ($P<0.05$) as the C:N dosing ratio increased (Table 3.2). Notably, there was a very strong linear relationship ($R^2=0.99$) between C:N ratio and NO_3^--N removal efficiency, with removal efficiency increasing by increments of approximately 30% with each 1 unit increase in C:N ratio ($y = 30.2x + 30.6$; y = removal efficiency (%); x = C:N ratio). In contrast, the NO_3^--N removal efficiencies and removal rates for the Sugar treatment did not increase with the C:N ratio, in fact they decreased as the C:N ratio increased (Table 3.2). This decrease in NO_3^--N removal can be attributed to variations in temperature between C:N ratio trials (Table 3.1). A similar decreasing trend was observed in the Control treatment, indicating the influence of temperature. This suggest that the sugar C:N dosing ratios evaluated were insufficient to counteract the effects of temperature, unlike ethanol, which was less affected by temperature changes.

3.3.4. Experiment 3: Effect of soluble carbon addition on nitrate removal under cool conditions

This experiment employed a 10-hour HRT to assess the effect of soluble C addition on NO_3^- removal under cool conditions. When evaluating soluble C dosing at a 0.5:1 C:N ratio, the water temperature ranged between 10.4 and 12.4°C and the average inflow NO_3^--N concentration was 11.8 mg N/L (SD 0.9). The average outflow NO_3^--N concentrations for the Control, Sugar and Ethanol treatment were 8.3 mg N/L (range 7.8 – 8.7 mg N/L), 8.1 mg N/L (range 7.2 – 9.2 mg N/L) and 5.7 mg N/L (range 4.4 – 6.6 mg N/L), respectively. Notably, the NO_3^--N concentrations in the outflow from the Sugar and Control treatments showed more variation with temperature changes than the Ethanol treatment (Appendix 1.3). The Ethanol treatment achieved an average NO_3^--N removal efficiency of 51.1%, which was significantly higher ($P<0.0001$) than the Control and Sugar treatment values of 29% and 31%, respectively (Table 3.3). The removal rate was also significantly higher for the Ethanol treatment, compared to the other two treatments.

Table 3.3. Average (Standard deviation) of NO_3^--N removal efficiency and removal rate at each soluble C concentration (C:N) during Experiment 3 (cool conditions, 10-hour HRT).

C:N Ratio	**NO_3^--N Removal Efficiency (%)**			**NO_3^--N Removal Rate (g N/m^3/day)**		
	Control	**Sugar**	**Ethanol**	**Control**	**Sugar**	**Ethanol**
0.5:1	29.9 (7.2) *a*	31.6 (8.9) *a*	51.1 (10.5) *b*	4.0 (1.1) *a*	4.2 (1.2) *a*	6.8 (1.6) *b*
1:1	26.9 (12.4) *a*	33.5 (10.6) *a*	64.4 (18.0) *b*	3.6 (1.7) *a*	4.4 (1.4) *a*	8.5 (2.3) *b*
2:1	30.6 (6.7) *a*	48.8 (6.8) *b*	97.5 (0.2) *c*	3.8 (0.8) *a*	6.0 (0.8) *b*	12.0 (0.05) *c*

Within each C:N evaluation, averages followed by the same lowercase letter are not significantly different ($P > 0.05$).

During the evaluation using a C:N ratio of 1:1, temperature varied between 8.4 and 11.3°C and the average inflow NO_3^--N concentration was 11.9 mg N/L (Figure 3.4). The average outflow NO3--N concentrations were 8.6 mg N/L (range 7.4 – 9.4 mg N/L), 7.9 mg N/L (range 7.0 – 8.5 mg N/L) and 4.3 mg N/L (range 1.6 – 6.2 mg N/L) for the Control, Sugar, and Ethanol treatments, respectively. Over the experimental period, the outflow NO_3^--N concentration from the Control and Sugar treatments were similar and remained stable (Figure 3.4). In contrast, the Ethanol treatment maintained a stable outflow NO_3^--N concentration, above 5 mg N/L during the initial eight days of the evaluation, then showed a continual decrease over the following two sampling days, to a concentration of 1.6 mg N/L by Day 12. This improvement in NO_3^- removal for ethanol, over the duration of the experiment, did not solely relate to temperature changes and, therefore, was more likely related to this treatment becoming more effective over time. This observation suggests that, following an acclimation period, the addition of ethanol can further enhance NO_3^- removal. On average over the five sampling days, the Ethanol treatment presented a 64.4% NO_3^--N removal efficiency, which was significantly higher ($P<0.0001$) than the Control and Sugar treatment values of 26% and 33%, respectively (Table 3.3). When averaged over the last two sampling days, the Ethanol treatment achieved an even higher NO_3^--N removal efficiency of 82%. The removal rate was also significantly higher for the Ethanol treatment, compared to the other two treatments.

During the evaluation using a C:N ratio of 2:1, water temperatures ranged between 10.7 and 11.3°C and the average inflow NO_3^--N concentration was 11.1 mg N/L. The average outflow NO_3^--N concentrations from the Control, Sugar, and Ethanol treatments were 7.7 mg N/L (7.88 and 7.48 mg N/L), 5.7 mg N/L (5.92 and 5.40 mg N/L) and 0.3 mg N/L (0.27 and 0.29 mg N/L), respectively. The Ethanol treatment achieved a very high NO_3^--

N removal efficiency of 97%, which was significantly higher (P<0.0001) than the Control and Sugar treatment values of 30% and 48%, respectively (Table 3.3). The removal rate was also significantly higher for the Ethanol treatment, compared to the other two treatments. In addition, the average NO_3^--N removal efficiency and removal rate achieved by the Sugar treatment were significantly higher (P=0.001) than the Control treatment. In this experiment, this was the only C:N ratio where the woodchip columns treated with sugar achieved statistically higher NO_3^--N removals compared to the Control treatment.

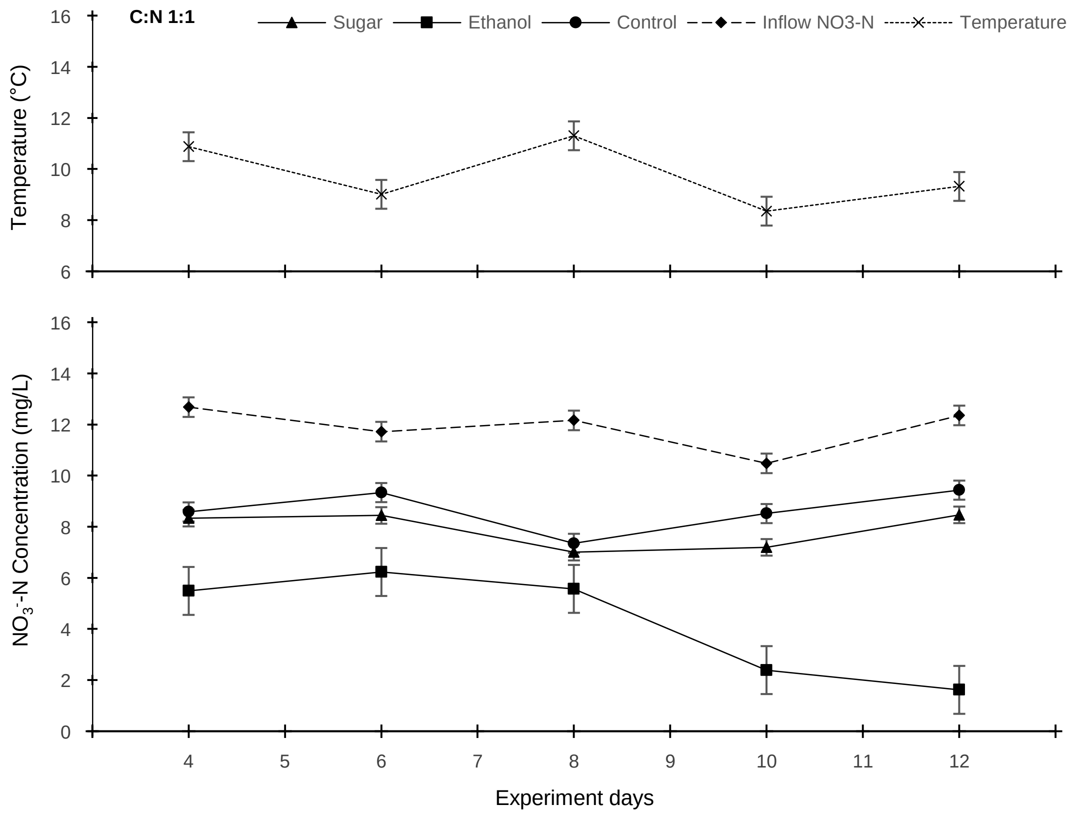

Figure 3.4. Experiment 3 (cool conditions, 10-hour HRT) - Average NO_3^--N concentrations and temperature per sampling day for each treatment at the 1:1 C:N ratio evaluation. Error bars represent standard error.

The experiment provided evidence that under cool conditions, the addition of ethanol at all three C:N ratios significantly (P<0.0001) influenced NO_3^--N removal efficiency and removal rate in comparison to the Control and Sugar treatments. In addition, there was a larger increase in NO_3^--N removal efficiency between the 0.5:1 C:N ratio and 2:1 C:N treatments for the Ethanol treatment, compared to the Sugar treatment. The NO_3^--N removal efficiency and removal rate achieved with the Ethanol treatment at a 2:1 C:N ratio were statistically different (P<0.0001) from the lower C:N ratios. Like with the previous experiment, there was also very strong linear relationship (R^2=0.99) between C:N ratio and NO_3^--N removal efficiency for the Ethanol treatment. Removal efficiency increased by increments of approximately 33% with each 1 unit increase in C:N ratio (y = 33.4x + 31.3; y = removal efficiency (%); x = C:N ratio). Interestingly, this relationship, which was for cool conditions and using a 10-hour HRT, was similar to the relationship achieved in Experiment 2, which was for warm conditions but used a shorter HRT of 3.3 hours.

3.3.5. Experiments 4 and 5: Effect of ethanol addition on nitrate outflow and removal efficiency

During Experiment 4 (warm conditions - 3.3-hour HRT), temperatures ranged between 15.2 and 20.6°C and the average inflow NO_3^--N concentration of the three evaluations combined was 12.4 mg N/L (Table 3.4). The average outflow NO_3^--N concentration for the Control treatment was 10.2 mg N/L (SD 0.6). Across all three C:N ratios assessed, the Ethanol treatment achieved significantly ($P<0.0001$) lower outflow NO_3^--N concentrations compared to the Control treatment. When ethanol was used to dose the column bioreactors at the C:N ratios of 1:1, 1.5:1 and 2:1, the average outflow NO_3^--N concentration were 5.5 mg N/L (range 4.2 – 6.8 mg N/L), 3.2 mg N/L (range 0.7 – 5.7 mg N/L) and 0.9 mg N/L (range 0.3 – 1.4 mg N/L). Over the 12-day duration of the 1.5:1 C:N ratio evaluation, there was a general trend of outflow NO_3^--N concentrations declining for the Ethanol treatment, which was not fully explained by water temperature variation (Figure 3.5). By the end of the evaluation, the outflow NO_3^--N concentration for this treatment had decreased to 0.7 mg N/L (i.e. 94% removal rate). This highlights that, in some cases, it can take time for the effect of the ethanol dosing to show further improvements in NO_3^- removal. The Ethanol treatment achieved significantly higher ($P<0.0001$) average NO_3^--N removal efficiencies and removal rates compared to the Control treatment for all three C:N ratios (Table 3.4). The Ethanol treatment resulted in average NO_3^--N removal efficiencies of 59%, 77% and 91% for the 1:1, 1.5:1 and 2:1 C:N ratios, respectively. This compares to values of 22% or less for the Control treatment. There was a very strong linear relationship (R^2=0.99) between C:N ratio and NO_3^--N removal efficiency, with removal efficiency increasing by increments of approximately 37% with each 1 unit increase in C:N ratio (y = 37.1x + 20.0; y = removal efficiency (%); x = C:N ratio).

Table 3.4. Average (SD) NO_3^--N removal efficiency and removal rate from experiments 4 and 5.

C:N Ratio	NO_3^--N Removal Efficiency (%)		NO_3^--N Removal Rate (g N/m^3/day)	
	Control	**Ethanol**	**Control**	**Ethanol**
Experiment 4: Warm conditions (3.3-hour HRT)				
1:1	12.9 (7.5) *a*	59.6 (11.8) *b*	5.0 (2.8) *a*	23.3 (2.1) *b*
1.5:1	22.5 (9.0) *a*	77.8 (19.5) *b*	9.1 (3.6) *a*	31.4 (7.9) *b*
2:1	19.1 (10.8) *a*	91.3 (7.7) *b*	8.2 (4.6) *a*	39.1 (3.3) *b*
Experiment 5: Cool conditions (10-hour HRT)				
1:1	40.0 (14.5) *a*	85.1 (11.6) *b*	6.4 (2.0) *a*	12.3 (1.6) *b*
1.5:1	30.0 (13.0) *a*	82.8 (9.9) *b*	4.2 (1.8) *a*	11.5 (1.7) *b*
2:1	37.3 (11.9) *a*	98.4 (1.7) *b*	5.3 (1.7) *a*	14.2 (0.5) *b*

Within each C:N evaluation, averages followed by the same lowercase letter are not significantly different ($P > 0.05$).

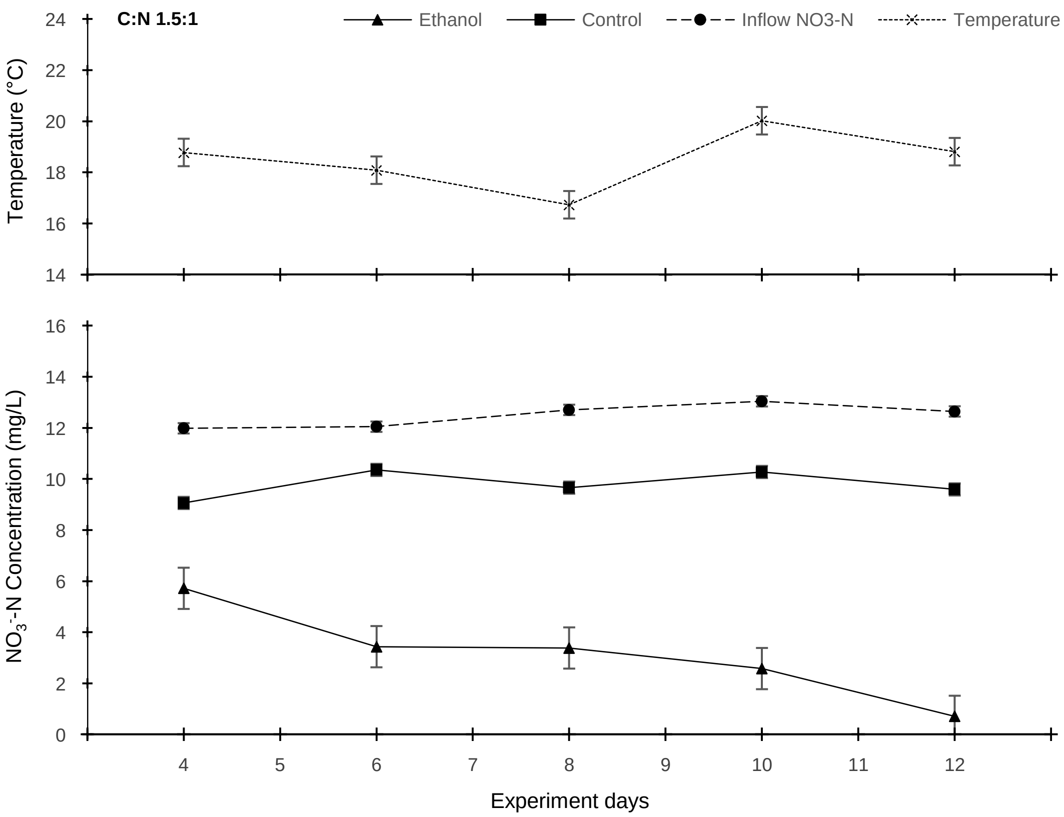

Figure 3.5. Experiment 4 (warm conditions, 3.3-hour HRT) - Average NO_3^--N concentrations and temperature per sampling day for each treatment at the 1.5:1 C:N ratio evaluation. Error bars represent standard error.

During Experiment 5 (cool conditions - 10-hour HRT), temperature varied between 8.0 and 15.7°C, and the average inflow NO_3^--N concentration was 12.5 mg N/L (SD 0.8), while the average outflow NO_3^--N concentration for the Control treatment was 8.1 mg N/L (Table 3.4). The Ethanol treatment presented lower (P<0.0001) outflow NO_3^--N concentrations compared to the Control treatment across all three C:N ratios tested. When ethanol was dosed at a C:N ratio of 1:1, between the third and seventh day of the evaluation, the temperatures were above the criteria for cool conditions. Therefore, the data collected on Day 4 and Day 6 were not included in the data analysis (Appendix 1.6). Based on this, the average outflow NO_3^--N concentrations of the Ethanol treatment at a 1:1 C:N ratio was 2.2 mg N/L (range 1.0 – 4.2 mg N/L).

When ethanol was used to dose the woodchip bioreactors at a C:N ratio of 1.5:1, the average outflow NO_3^--N concentration was 1.3 mg N/L (range 1.0 – 1.6 mg N/L). At this dosing ratio, the outflow NO_3^--N concentrations of the Ethanol treatment were consistently low, despite the temperature variation and temperatures decreasing to an average of only 8.2°C at the last two sampling days (Figure 3.6). In contrast, the Control treatment outflow NO_3^--N concentrations appeared to be more influenced by temperature changes, with average concentration increasing from 5.9 to 8.9 mg N/L, when temperature decreased from 13.7°C on Day 6, to 8.0°C on Day 12.

When the column bioreactors were dosed with ethanol at a C:N ratio of 2:1 the average outflow NO_3^--N concentration was 0.3 mg N/L (range 0.3 – 0.4 mg N/L), and there was minimal variation between sampling days, regardless of variations in temperature. In contrast, the outflow NO_3^--N concentrations in the control treatment fluctuated with temperature changes and remained above 7.1 mg N/L (Appendix 1.7).

During Experiment 5 (cool conditions), NO_3^- removal efficiencies and NO_3^--N removal rates from the Ethanol treatment (Table 3.4) significantly increased (P<0.01) with an increase in C:N dosing ratio from 1:1 to 2:1. However, the removal efficiency and removal rate achieved by the 1:1 and 1.5:1 C:N ratios were not significantly different from each other (P>0.05). This observation may be attributed to the warmer average temperature of 14.0°C during the 1:1 C:N ratio evaluation, compared to the average of 10.7°C during the 1.5:1 CN ratio evaluation. Additionally, inflow NO_3^--N concentration slightly higher the 1:1 C:N ratio evaluation (Table 3.1), which could have also had a bearing on bioreactor performance.

The 1:1 C:N ratio removal efficiency being an overestimation for cool conditions, is supported by the results for this treatment in Experiment 3, which had a removal efficiency of only 64% compared to 85% in Experiment 5. Considering the results from the 1:1 C:N ratio as an outlier, and factoring in a 64.4% removal efficiency, reveals a very strong linear relationship (R^2=0.99) between C:N ratio and NO_3^--N removal efficiency, with removal efficiency increasing by increments of approximately 31% with each 1 unit increase in C:N ratio (y = 37.4x + 34.9; y = removal efficiency (%); x = C:N ratio).

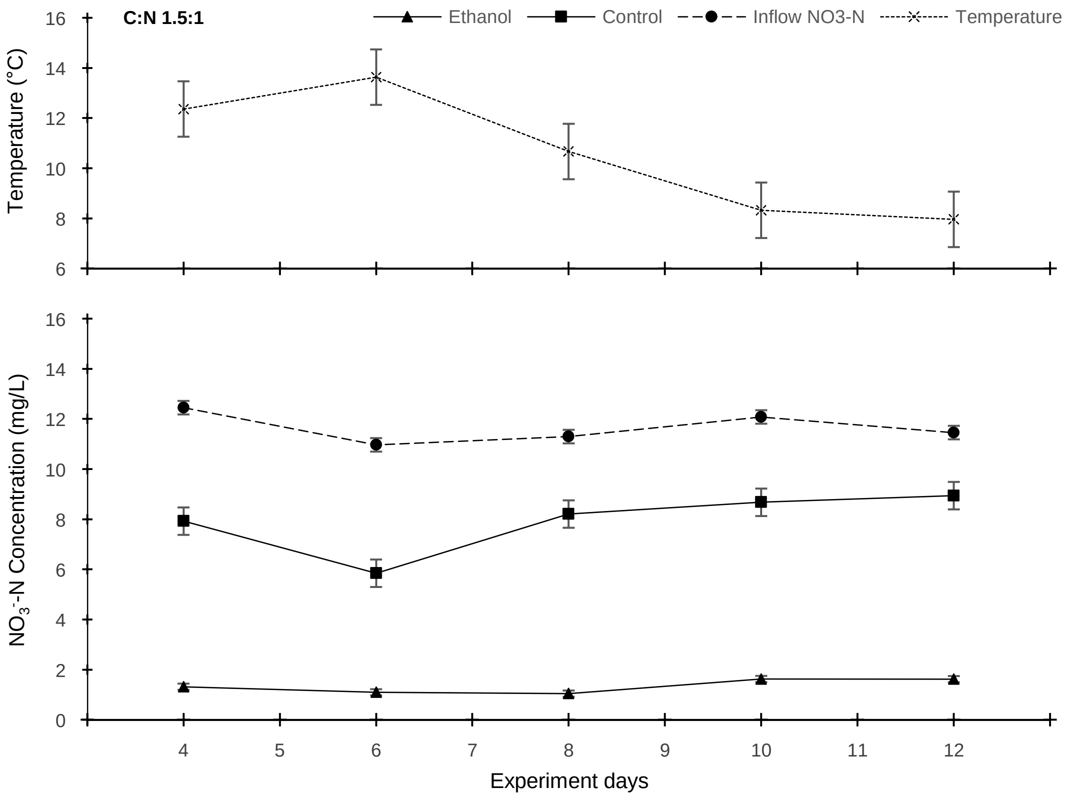

Figure 3.6. Experiment 5 (cool conditions, 10-hour HRT) - Average NO_3^--N concentrations and temperature per sampling day for each treatment at the 1.5:1 C:N ratio evaluation. Error bars represent standard error.

When comparing the NO_3^- removal efficiency of the ethanol dosed column bioreactors, it is important to account for the effects of temperature and retention time. Under warm conditions, the 3.3-hour HRT in the Control treatment, led to an average NO_3^--N removal efficiency of 18%. Conversely, under cool conditions, the HRT was extended to 10 hours, resulting in an average NO_3^--N removal efficiency of 36%. This illustrates the effect of HRT, as a longer 10-hour HRT under cool conditions was able to achieve a higher NO_3^- removal efficiency compared to the use of a shorter HRT under warm conditions.

Therefore, it is important to note that this effect is also affecting the performance of the ethanol-dosed woodchip bioreactors. However, when comparing the regression equations for both warm and cool conditions (Experiments 4 and 5), it was observed that the increase in removal efficiency for a unit increase in C:N ratio was similar (~30%). This highlights the impact of ethanol dosing on NO_3^--N removal and demonstrates that this effect remains relatively consistent across both warm and cold conditions.

Regarding NO_3^--N removal rate, under warm conditions (Experiment 4), the HRT of 3.3 hours, in the Control treatment, led to an average of 7.5 g N/m^3/day, while under cool conditions (Experiment 5), the HRT of 10 hours, resulted in an average of 5.3 g N/m^3/day. This shows the effect of HRT, where shorter retention times generally result in higher NO_3^--N removal rates because it allows for the treatment of larger volumes of water and NO_3^- per day. This effect was also observed in the ethanol-dosed column bioreactors, as under warm conditions, when the HRT was of 3.3 hours, the removal rates were higher than under cool conditions at a 10-hour HRT, for all the C:N dosing ratios. This indicates that ethanol dosing can improve woodchip bioreactors not only by improving the NO_3^- removal efficiency but also by the ability of allowing shorter HRT and consequently increasing the NO_3^- removal rates.

3.4. Discussion

The drainage water from the LWTS maintains relatively constant flow rates and NO_3^- concentrations throughout the year, however, water temperature varies with season. One of the primary challenges in sustaining high rates of NO_3^- removal in woodchip bioreactors is the maintenance of a continuous supply of soluble C from the woodchips in the cooler season. Therefore, at cooler water temperatures, soluble C dosing offers a method of sustaining NO_3^- removal levels without the need to change the HRT and, therefore, the volume of water treated. This research examined the potential of woodchips bioreactors to treat water with high NO_3^- concentrations, using small-scale column woodchip bioreactors and dosing them with two different soluble C sources: liquid sugar and ethanol. This study identified the NO_3^- removal efficiencies and removal rates achievable at different C:N ratios of soluble C, under different water temperatures and using a range of HRT.

Hydraulic retention time

The conditions of warm water temperatures (average of 20°C) and high NO_3^--N concentrations (average of 19.5 mg N/L) during the HRT experiment (Experiment 1) were ideal to determine the effect of HRT on the NO_3^- removal capacity of the columns. These conditions are known to generally allow favourable NO_3^- removal in woodchip bioreactors (Schipper et al., 2010).

Consistent with several other studies (Hoover et al., 2015; Martin et al., 2019; Soupir et al., 2018), the column bioreactors showed increased NO_3^- removal efficiency at longer HRT. While the NO_3^- removal rates also increased between 3.3 hours and 6.6 hours of HRT, they did not increase further when HRT was raised to 10 hours. This is mainly because at longer retention times the total volume of water and the associated total NO_3^- load that can be treated per day is restricted. These results are in line with findings reported by Jéglot et al. (2022), who observed that the efficiency of NO_3^- removal reached its maximum at the longer HRT tested, while the highest NO_3^- removal rate was not achieved at the longest HRT, but instead peaked at a certain HRT and did not further increase with extended HRT. Given this, achieving higher NO_3^- removal efficiency is feasible at longer HRT, but comes at the cost of reducing the volume of water that can be effectively treated. In practical terms, treating a substantial volume of water often restricts the implementation of very long HRT periods. Moreover, other studies have also demonstrated that lower HRT allow better overall daily reductions, as more NO_3^- enters a fixed-size bioreactor per day (Hoover et al., 2015; Hua et al., 2016; Soupir et al., 2018). In addition, Jéglot et al. (2022) suggested that the evaluation of NO_3^- removal rates to describe woodchip bioreactor performance is essential for operational purposes and can assist in determining the minimum HRT for achieving the maximum removal rate. Therefore, the use of C dosing of woodchip bioreactors can be considered as a method to enhance removal rates at lower HRT, allowing for the treatment of larger volumes of water.

Temperature

The effect of temperature on NO_3^--N removal was assessed based on all the experiments (Experiments 1, 3 and 5) when the column bioreactors were evaluated at an HRT of 10 hours, including data from the initial evaluation, the HRT evaluation, as well as the control treatments of the soluble C addition assessment. The average inflow NO_3^--N concentration was 12.1 mg N/L, with a range between 8.0 to 18.0 mg N/L. As expected, increasing temperature significantly improved NO_3^--N removal efficiency (Figure 3.7). For instance, removal efficiencies remained below 50% when the temperature was below 13.5°C (or below 14.0°C for some individual values). However, at temperatures above 13.5°C, removal efficiencies exceeded 50%, approaching 100% removal at temperatures of about 21.2°C. This demonstrated that under cool conditions, an HRT of 10 hours in the column bioreactors is not long enough to remove the majority of the NO_3^-. Therefore, these results suggest a strong response to temperature, consistent with trends observed in many other woodchip bioreactor studies (David et al., 2015; Halaburka et al., 2019; Maxwell et al., 2020; Soupir et al., 2018; Thapa et al., 2023).

At lower temperatures, for example less than 13°C, significant enhancements can be achieved by increasing the HRT. However, as mentioned previously, this reduces the volume of water that can be treated for a given bioreactor size. Therefore, during times of the year when temperatures are cooler, soluble C dosing provides another option for improving the NO_3^- removal performance of woodchip bioreactors.

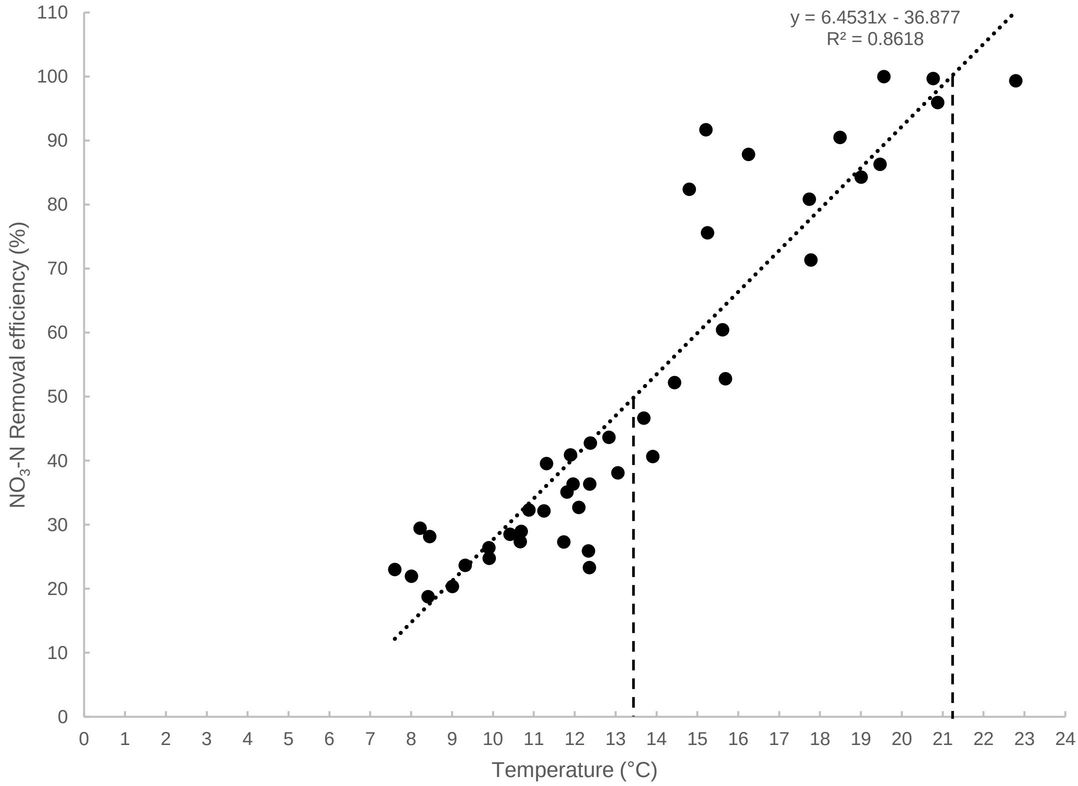

Figure 3.7. NO_3^--N removal efficiency in woodchip column bioreactors operating at a 10-hour HRT, under different temperatures, including data from the initial evaluation, the HRT evaluation, and the control treatment from the soluble C addition assessment. Dashed lines indicating the temperatures at which 50% and 100% removal efficiency were achieved.

Soluble carbon dosing

This study is the first to compare the use of ethanol and sugar as additional C source for dosing woodchips bioreactors. Nevertheless, some other studies have evaluated the use of similar C sources (sucrose, glucose, ethanol and methanol) as electron donors for denitrification in contaminated groundwater or synthetic wastewater (dos Santos et al., 2004; Gómez et al., 2000; Jiang et al., 2022; Ortmeyer et al., 2021). These studies reported that sucrose and glucose were less efficient compared to other C sources and required high C:N ratios to remove NO_3^- effectively. For instance, Gómez et al. (2000) found that sucrose required the highest C:N ratio (2.5:1) for complete removal of 100 mg NO_3^--N/L from groundwater, compared to ethanol and methanol (1.1:1). In their study, they also observed a higher density of denitrifying bacteria in the biofilm when ethanol and methanol were added to the influent, suggesting that these C sources increased denitrification activity compared with sucrose. Similarly, Jiang et al. (2022) found lower denitrification rates when glucose was utilised as C source to treat simulated secondary effluent, in comparison to methanol, attributing it to the complex mechanisms involved in glucose utilisation by denitrifying bacteria. These studies support the findings of the present study, that ethanol is more effective for dosing woodchip bioreactor than sugar. Furthermore, it is suggested that the chemical structure of the soluble C sources added to the woodchip bioreactor has an important influence on denitrification performance (Jiang et al., 2022).

Since ethanol was more efficient than sugar in the current study, further evaluations were conducted with ethanol at different C:N ratios and under warm and cool conditions. The results revealed a significant impact of ethanol dosing on NO_3^--N removal. The data demonstrated an increase in NO_3^--N removal with increasing C:N ratio, which was consistently observed under both warm and cool water conditions. Analysing data from all the Ethanol treatments within the warm (Experiments 2 and 4) and cool conditions (Experiment 3 and 5) revealed that, under both conditions, the Control treatment presented comparable NO_3^--N removals efficiencies of about 30%. In addition, the removal efficiency of the ethanol dosed treatments, under warm and cool conditions, increased by approximately 30% for each 1 unit increase in C:N ratio. These findings suggest a similar effect of ethanol dosing on NO_3^--N removal efficiency under warm conditions and short HRT (3.3 hours) in comparison to cool conditions and longer HRT (10 hours).

Among the three C:N ratios tested, the dosing rate of 1.5:1 appeared to be sufficient, for both warm and cool conditions, to achieve high removal efficiencies close to 80%. Furthermore, after an acclimation period, removal efficiencies could potentially be higher. Notably, Hartz et al. (2017) also identified that a C:N ratio of about 1.5:1 was sufficient for near to complete NO_3^- removal when adding methanol to woodchip bioreactors operating under water temperatures (e.g. average of 17°C) similar to the warm conditions of the present study, but at much longer HRT of 48 hours.

The addition of ethanol at a C:N ratio of 1.5:1 significantly increased NO_3^- removal efficiency compared to the Control treatment. Ethanol dosing provided a 3.4-fold (from 23 to 78%) increase in NO_3^- removal under cool conditions, and a 2.8-fold increase (from 30 to 83%) under warm conditions. This improvement in NO_3^--N removal efficiency in woodchips bioreactors due to ethanol dosing confirms the expected benefits of ethanol proposed by Jansen et al. (2019). These results are comparable to the findings of

Moghaddam et al. (2022) using methanol as a C source. Moghaddam et al. (2022) reported a 3.1-fold increase in average NO_3^--N removal efficiencies, from 28.3% without C dosing to 87.4% with methanol dosing of large (30 L) column woodchip bioreactors. In their study, Moghaddam et al. (2022) also used a similar C:N ratio (1.48:1) for the methanol treatment. However, they used a longer HRT (27 hours) and higher inflow NO_3^--N concentrations (20 - 30 mg N/L), while water temperature was not reported.

Since smaller HRTs potentially allow for better overall NO_3^- removal rates, ethanol dosing can enhance the performance of woodchip bioreactor during warm conditions by reducing the HRT. The NO_3^--N removal rates of the column bioreactors (non-dosed), operating at an HRT of 10 hours were improved, from 20.9 g N/m^3/day to 31.4 g N/m^3/day when they were dosed with ethanol (C:N ratio of 1.5:1) and the HRT was reduced to 3.3 hours.

Based on this lab-scale experiments, ethanol dosing appears to be a promising strategy to enhance the performance of woodchip bioreactors. Therefore, ethanol should be evaluated in field-scale bioreactors to validate its efficacy.

3.5. Conclusions

This study demonstrated the suitability of woodchips bioreactor to treat drainage water, especially in warmer conditions. For example, NO_3^- removal efficiencies of up to 99% were achieved under warm conditions with an HRT of only 10 hours. Furthermore, the study established that C dosing can be an effective method to enhance the NO_3^- removal performance of woodchip bioreactors, especially under cooler conditions. However, it was observed that the choice of the C source significantly impacts denitrification performance, with ethanol being more efficient than liquid sugar. Not only did ethanol dosing prove to be effective in enhancing NO_3^- removal under unfavourable conditions, such as low temperatures, but it can also further reduce the HRT required to optimise woodchip bioreactors performance at warmer temperatures, which increases the daily volume of water that can be treated for a given bioreactor size.

The results of the present study show that woodchip bioreactors are a promising NO_3^- removal method for wastewater land treatment sites, such as the LWLTS, where high NO_3^- drainage water is generated year-round. Further research on the performance of woodchip bioreactors and ethanol dosing is required to focus on field testing, optimising

HRTs and C:N ratios based on the field conditions. Therefore, the following chapter of the present research focuses on evaluating the performance of pilot-scale woodchip bioreactors installed at the LWLTS, aiming to assess the range of HRTs and optimal C:N dosing ratios informed by the findings of the column study.

3.6. References

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4. Evaluating the effect of hydraulic retention time and ethanol dosing on the nitrate removal performance of pilot-scale woodchip bioreactors at a municipal wastewater land application site

4.1. Introduction

The release of nitrogen (N) compounds into aquatic ecosystems poses a human health hazard in drinking water and leads to eutrophication in freshwater ecosystems. Consequently, New Zealand central government has developed policies, as outlined in the NPSFM (2020), with the goal of improving deteriorating water quality. With on-going population growth and expansion of urban areas, it is a challenge to manage discharges from municipal wastewater treatment plants in a way that minimises their contribution to excessive N in surface and ground waters (Price et al., 2018).

Wastewater treatment plants can operate at three levels of treatments: primary, secondary, and tertiary. Nitrate (NO_3^-) enrichment in freshwater can be particularly problematic when treatment does not reach the tertiary level (Chakravarthy et al., 2019; Gücker et al., 2006). In New Zealand, land application is becoming a common method to remove excessive N concentrations from pretreated domestic wastewater. However, to be effective, land treatment often requires substantial areas of land to accommodate the large volumes of wastewater produced by urban communities. Therefore, common constraints to the adoption of land treatment are the availability of suitable land, the associated costs, and the limits that municipal wastewater application places on other land uses. Consequently, the application of wastewater to land areas that are insufficient in size, can cause elevated NO_3^- levels in drainage, which in turn, contribute to NO_3^- enrichment of the receiving aquatic environment.

The Levin Wastewater Land Treatment Site (LWLTS) is an example of a site with an under-sized application area (40.5 ha). Hence, the relatively high annual volumes of wastewater (2,314,469 m^3/year) received by the site result in high application depths (4,667 mm/year). Consequently, drainage and groundwaters with elevated NO_3^- levels are transferred from the site into the Waiwiri Stream. Therefore, there is a need to identify an effective treatment method for drainage water before it enters the stream. One approach that can be applied in this context is the use of denitrification bioreactors. This method involves boosting the growth of heterotrophic denitrifying microorganisms by introducing solid carbon (C) sources (e.g. woodchips) and by creating the ideal conditions for the microorganisms to convert NO_3^- into N_2 gas (Hang et al., 2016; Schipper et al., 2010). Woodchip bioreactor technology is widely applied in the treatment of agricultural drainage water, due to their simplicity in construction, cost-effectiveness, and minimal maintenance requirements (Christianson et al., 2021; Schipper et al., 2010). While denitrification bioreactors are a well-established technology for treating agricultural drainage water, there is currently limited research on their application to drainage water originating from municipal wastewater land treatment areas. This is significant because the conditions in these settings can vary from those encountered most commonly in agriculture. For instance, due to the year-round application of wastewater to the soil, drainage water originating from municipal land treatment areas may provide more consistent drainage flow rates and NO_3^- concentrations throughout the year, including the summer season.

To determine the suitability of woodchip bioreactors to treat water with the characteristics of the drainage originating from the LWLTS, a lab-scale column experiment was conducted (Chapter 3). The column bioreactors achieved up to 99% removal efficiency under warm conditions and 32% under cool conditions with a hydraulic retention time (HRT) of 10 hours. Therefore, the results from the column bioreactor study support that woodchips bioreactors are likely to be a suitable method for removing NO_3^- from wastewater land treatment sites where drainage with high NO_3^- concentrations is generated year-round. Additionally, in the same study, ethanol dosing of woodchip columns was found to be an effective method to improve NO_3^- removal performance, especially during the cooler time of year, increasing removal by about 30% points for each 1 unit increase in C relative to N. However, it is essential to validate these findings further on a larger scale using pilot-scale bioreactors and actual drainage water from the LWLTS, to determine if comparable results can be attained under field conditions. This will assist in designing the appropriate full-scale bioreactor required for the site.

The main objectives of the present study were to (1) assess the ability of pilot-scale woodchip bioreactors to reduce NO_3^- levels in drainage water from the LWLTS and characterise how NO_3^- removal efficiency varies with hydraulic residence time and temperature, and (2) evaluate the ability of ethanol dosing of pilot-scale woodchip bioreactors to enhance NO_3^- removal, particularly during the cooler season.

4.2. Methodology

To achieve the objectives of this study, a field experiment was conducted, assessing pilot-scale woodchip bioreactors at various retention times during both warm and cool seasons. Ethanol was dosed at different C:N ratios to identify the optimal dosing rate for increasing NO_3^- removal but aiming to avoid excess soluble C that would result in sulphate reduction.

4.2.1. Study site

This study was conducted at the LWLTS, located approximately 7 km west of the township of Levin, New Zealand, from October 2022 to October 2023. The site receives wastewater, which is treated to a secondary level. On arrival, wastewater is stored in a pond and subsequently irrigated to the land. The soils at the site consist of sand dunes (approximately 75%), inter-dune areas (about 22%) and a sand plain. Wastewater is irrigated primarily to the sand dunes, which have a vegetation cover that consists of grasses, weeds and young pine trees. Annually, approximately 1,890,509 m^3 of wastewater are irrigated to an area of 40.5 hectares. Additionally, an estimated 448,801 m^3/year are lost from the unlined pond through seepage. The drainage from both the irrigated area and the pond seepage are primarily intercepted by the shallow groundwater system. Subsequently, surface drains located around the pond and irrigation area capture some of the shallow groundwater and deliver it to the Waiwiri Stream.

4.2.2. Bioreactor design

Four pilot-scale woodchips bioreactors were installed at the start of the study. Each of the bioreactors measured approximately 5 m in length, 1 m in width, and 1 m in depth. The bioreactors were installed in the ground, with 1 m of separation between each other. The bioreactors were lined with two layers of heavy-duty plastic liner, and then filled with approximately 5 m^3 of pine woodchips. Water from Drain 3 was pumped to a 5000 L storage tank using a submersible pump installed in the drain. Drain water was gravity fed from the tank through a main supply pipe and then to an individual inflow pipe at the top of each bioreactor, with a tap installed to control flow rate. Outflows pipes were installed at the bottom of each bioreactor, allowing for passive effluent collection into tipping bucket flow meters. These buckets facilitated outflow monitoring, and the treated water was then returned to the drain. Temperature sensors were installed in the storage tank and within the bioreactors themselves. An illustration of the experimental setup is presented in Figure 4.1, while some of the installation procedures are presented in Figure 4.2.

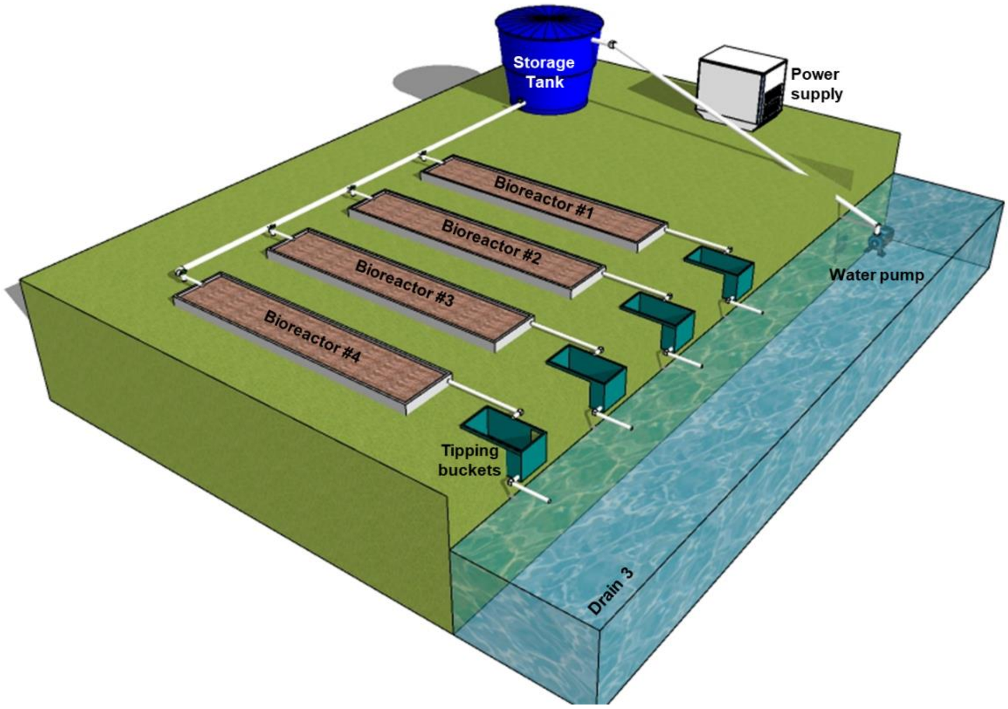

Figure 4.1. A schematic diagram showing the experimental setup and general arrangement of the components.

4.2.3. Experiment procedure and treatments

Treatments

The woodchip bioreactors were monitored over a 12-month period. During this time, the performance of the bioreactors was evaluated using a range of HRTs (5.5 – 22.5 hours), and with the addition of ethanol at a range of C:N ratios (0.5 - 1.5). Throughout the experiment, the bioreactors and drain water were exposed to seasonal temperature fluctuations.

Initially, four bioreactors were installed with the intention of assessing two replicates of two treatments at any given time. However, it was not possible to implement this design during the experiment due to a number of constraints. One of these constraints was the small size of the solar panel and battery storage system used to generate power to operate the water pump. This restricted the volume of water that could be pumped per day. An insufficient amount of daily sunshine hours was also a problem at certain times. This limited the number of bioreactors that could be operated at high flow rates (i.e. short HRTs). Additionally, another constraint encountered in the field was the high concentration of suspended organic matter observed in the drainage water, resulting from herbicide spraying and decomposition of weeds in the drain. This excess of organic matter was mostly distributed into Bioreactor 4 (B4) at the end of the supply pipe, which resulted in restricted hydraulic conductivity of the chips in this bioreactor. This limited its usefulness and, therefore, it was not used for the remainder of the experiment.

Due to these field-related constraints, it was not possible to operate two treatments with replication simultaneously. However, adjustments were made throughout the experimental period in order to test the effect of a range of HRTs and ethanol C:N ratios on the removal of NO_3^- from drainage water. The adjustments of the treatments were made based on the data collected as the experiment progressed. Different evaluations made over the experimental period can be divided into three stages. The first stage tested different HRTs (6, 10, 15 and 20 hours) during the warm season (October 2022 to April 2023, average water temperature was 18.1°C). The second stage tested the ethanol C:N ratio treatments at an HRT of 6 hours during the cool season (April to August 2023, average water temperature was 13.6°C). And lastly, the third stage involved a short period during the cool season (August to October 2023, average water temperature was 13.2°C) in which the bioreactors were tested at longer HRTs (15 and 20 hours) with no dosing, to compare their performance with those operating with ethanol dosing at 6 hours of HRT. A summary of the different treatments implemented in each bioreactor and the periods of the year in which they were applied is presented in Table 4.1, and the average daily temperature variations for each experimental stage are presented in Appendix 3.

Flow rate monitoring

The inflow rate into the bioreactors varied according to the HRT. Each bioreactor had its own tap that was manually adjusted to control the desired water flow rate and corresponding HRT. As previously mentioned, each bioreactor had an outlet pipe connected to an individual tipping bucket flow rate meter. These tipping buckets were instrumented with data loggers to provide continuous measurements of flow rates.

Ethanol dosing

The ethanol solution (10% ethanol and 90% water) used for dosing was delivered into the inflow pipe of the designated bioreactors using a peristaltic pump. The ethanol solution was stored in a 20 L plastic bottle and replenished as needed. Throughout the experiment, three target C:N ratios were tested: 0.5:1, 0.75:1 and 1.5:1. The ethanol concentration to be applied was calculated based on the annual average NO_3^--N concentration of water in Drain 3 as measured between July 2020 and June 2021 (i.e. 8.9 mg N/L). Given that the NO_3^- concentration of the drainage water presented some seasonal variation, the actual C:N ratios were slightly different from the target C:N ratios. The selection of C:N ratios was based on previous lab experiments (Chapter 3), which identified a C:N ratio of 1.5 as an effective ethanol dosing ratio to achieve NO_3^--N removal efficiencies above 80%, at an HRT of 3.3 hours during the warm season (18.6°C) and at an HRT of 10 hours during the cool season (10.7°C). The other C:N ratios tested here were selected to optimise NO_3^- removal and prevent sulphate (SO_4^{2-}) reduction.

4.2.4. Sampling and analyses

Bioreactor water inflow and outflow samples were collected twice a week, every three to four days. After collection, samples were frozen until analysis. Prior to analysis, the samples were defrosted and then filtered through 0.45 μm membranes. The samples were analysed for NO_3^- and SO_4^{2-} using Ion Chromatography (ThermoFisher Scientific - Dionex Aquion).

4.2.5. Statistical analysis

RStudio software (R programming language), as well as the R external package ‘Tidyverse’ (Wickham et al., 2019) were used for all statistical analyses and data modelling. A paired *t*-test was used to compare the mean inflow and outflow NO_3^- concentrations from the woodchip bioreactors, assessing the significance of treatment effects on NO_3^- reductions. Regression models were performed to evaluate the relationship between NO_3^- removals and HRT and C:N dosing ratios.

Table 4.1. Average hydraulic retention time (HRT) and ethanol dosing C:N ratio (EtOH C:N) treatments tested in the pilot-scale bioreactors, and periods in which they were evaluated.

Dates	Bioreactor and treatment			
	B1	**B2**	**B3**	**B4**
Stage 1 - HRT treatments - Warm season (average temperature = 18.1°C)				
28 Sept - 9 Dec 2022	HRT - 19 hours	HRT - 11 hours	HRT - 14 hours	HRT - 15 hours
10 Jan - 31 Jan 2023	HRT - 6 hours	Off [a]	Off	HRT - 7 hours
31 Jan - 24 Feb 2023	HRT - 11 hours	Off	Off	HRT - 13 hours
24 Feb - 24 Mar 2023	Off	HRT - 10 hours	HRT - 9 hours	Off
24 Mar - 31 Mar 2023	HRT - 6 hours	Off	Off	HRT - 11 hours [b]
31 Mar - 18 Apr 2023	HRT - 6 hours	HRT - 7 hours	Off	Off
Stage 2 - Ethanol C:N ratio treatments - Cool season (average temperature = 13.6°C)				
18 Apr - 2 May 2023	HRT - 6 hours EtOH C:N - 1.5	HRT - 7 hours No EtOH (control)	Off	Off
2 May - 16 May 2023	HRT - 7 hours No EtOH (control)	HRT - 7 hours EtOH C:N - 1.5	Off	Off
19 May - 30 May 2023	HRT - 6 hours EtOH C:N - 0.75	HRT - 7 hours EtOH C:N - 1.5	Off	Off
30 May - 2 June 2023 [c]	HRT - 7 hours EtOH C:N - 0.75	HRT - 7 hours No EtOH (control)	Off	Off
5 July - 25 July 2023 [c]	HRT - 10 hours	HRT - 8 hours	Off	Off
25 July - 11 Aug 2023	HRT - 7 hours EtOH C:N - 0.75	HRT - 7 hours No EtOH (control)	Off	Off
Stage 3 - Ethanol C:N treatments - HRT treatments - Cool season (average temperature = 13.2°C)				
11 Aug - 18 Aug 2023	HRT - 7 hours EtOH C:N - 0.75	HRT - 22 hours	HRT - 13 hours	Off
18 Aug - 1 Sept 2023	HRT - 7 hours EtOH C:N - 0.5	HRT - 22 hours	HRT - 16 hours	Off
1 Sept - 19 Sept 2023	HRT - 6 hours	HRT - 12 hours	HRT - 21 hours	Off
19 Sept - 13 Oct 2023	HRT - 6 hours EtOH C:N - 0.5	HRT - 7 hours EtOH C:N - 0.5	HRT - 6 hours	Off

a Power provided by solar panels and batteries was not sufficient to operate the four bioreactors continuously.
b On the 31st of March 2023, B4 was found overflowing due to restricted hydraulic conductivity. It was not operated again after this date.
c Solar panel and battery storage system were stolen from the field study. Bioreactors did not operate for a month, waiting for a replacement power supply system to be installed.

Figure 4.2. Images of experiment installation, procedures, and instrumentation. a) Drain 3 and site selected for the installation of the pilot-scale bioreactors. b) Excavation for the bioreactors. c) Land levelling inside the bioreactor. d) Lining. e) Bioreactor outflow tube. f) Bioreactor filled with woodchips. g) Manual woodchip filling and surface levelling. h) Experimental setup view. i) Water pump installation in Drain 3. j) Tipping bucket. k) Ethanol dosing station. l) Inflow water sampling.

4.3. Results

4.3.1. Inflow drainage water characteristics

Over the duration of this study, the concentration of the inflow drainage water ranged from 5.3 to 9.8 mg N/L, with an average of 7.6 mg N/L (SD 1.4) (Figure 4.3). Meanwhile, the temperature of the inflow drainage water was between 10.1 and 20.5°C (average value of 15.4°C (SD 2.8)). On average, NO_3^--N concentrations between May and October 2023 were higher, reaching 8.7 mg N/L (SD 1.0), corresponding to the period of the year with cooler temperatures (average 13.2°C (SD 1.5)). The NO_3^--N concentrations were lower during the warmer season (average 18.1°C (SD 1.2)), from November 2022 to April 2023, with an average value of 6.4 mg N/L (SD 0.7).

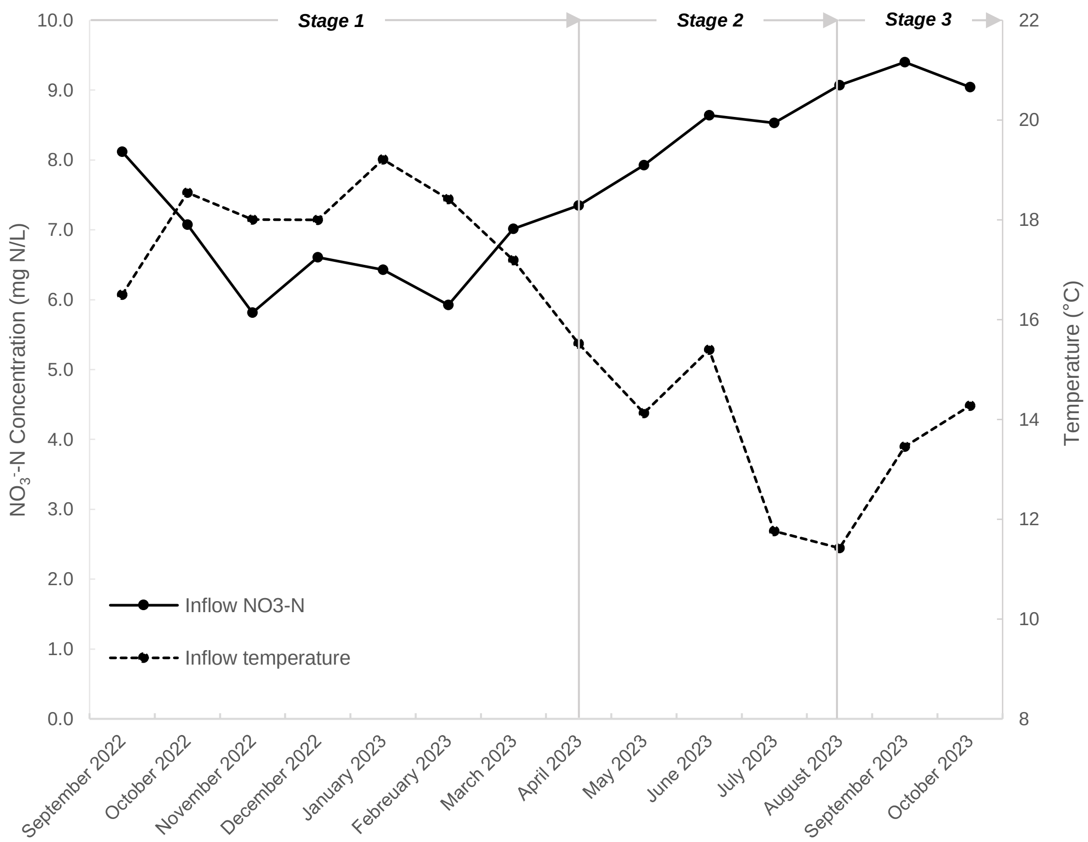

Figure 4.3. Monthly average NO_3^--N concentrations and temperatures of the inflow drainage water over the duration of the experiment.

4.3.2. Hydraulic retention time effect on nitrate removal

The evaluation of the HRT effect on NO_3^- removal was divided into three stages, based on the season and corresponding temperatures. Stage 1 covered the warm season of the experiment, while Stages 2 and 3 were both conducted during the cool season (Figure 4.3). As found in the column experiment study (Chapter 3) and other published works on woodchips bioreactors, temperature has a significant influence on NO_3^- removal and how NO_3^- removal responded to different HRT. Nonetheless, for convenience, in this study the effect of HRT was analysed by seasons; the warm season corresponding to temperatures above 15°C and the cool season corresponding to temperatures below 15°C. This temperature-dependent subdivision was adopted with the aim to better understand the seasonal HRT management for the conditions at the site.

The drainage water NO_3^- concentrations were consistently lower ($P<0.0001$) in the bioreactor outflows compared to the inflows, during both seasons and across all the HRTs tested. In the warm season, the removal efficiency increased from 35% to 100% with increasing HRT from 5.5 to 22.4 hours, while during the cool season the removal efficiencies increased from 13% to 85%, as HRT increased from 5.5 to 21.9 hours. The effect of HRT on NO_3^--N removal efficiency presented significant correlations ($P<0.0001$) for both seasons, with the cool season showing a stronger correlation ($R^2 = 0.77$) compared to the warm season ($R^2 = 0.66$) (Figure 4.4). As anticipated, although the NO_3^--N removal efficiencies increased with prolonged HRT, the rate at which the efficiency increased slowed down at higher HRTs.

Using the correlation between NO_3^--N removal efficiencies and HRTs, in the warm season the HRTs of 6, 10, 15 and 20 hours achieved removal efficiencies of 40%, 59%, 74% and 85%, respectively. While during the cool season, the corresponding efficiencies for each HRT were 24%, 43%, 57% and 68%, respectively. In general, under cooler conditions, the bioreactors presented approximately a one-third decrease in removal efficiency compared to warmer conditions. On average, the NO_3^--N removal efficiencies of the warm and cool data combined were 32%, 51%, 66% and 77% for the respective HRTs.

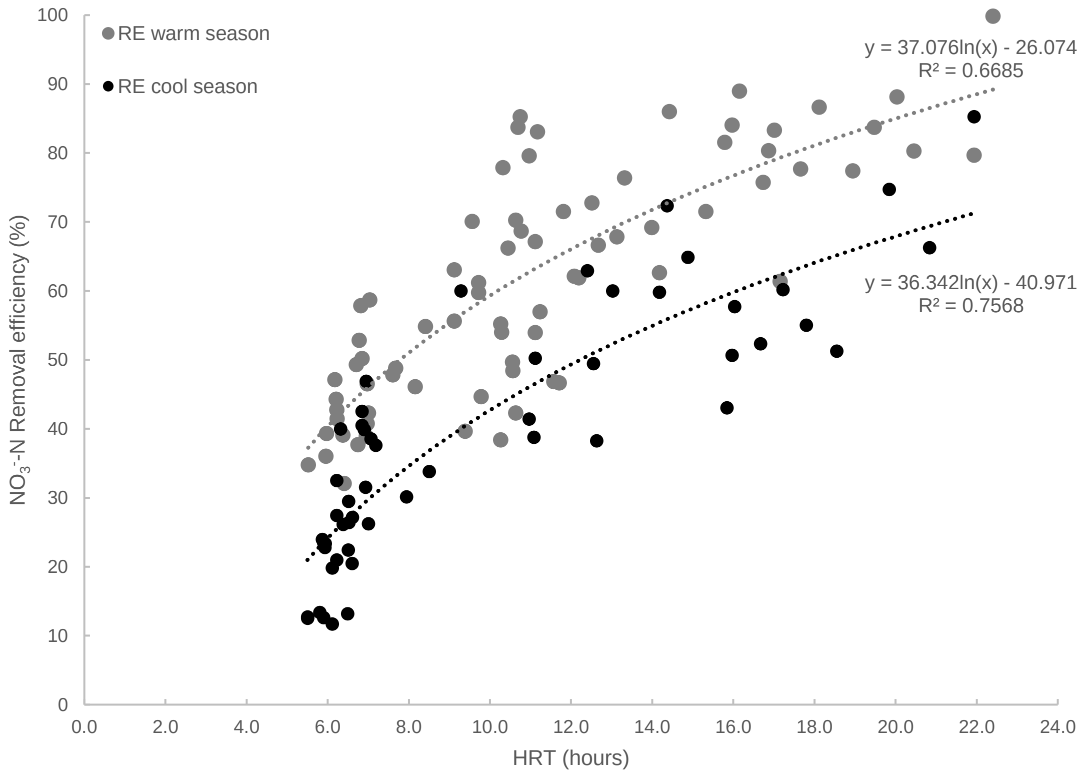

Figure 4.4. Correlation between HRT and nitrate removal efficiency of woodchip bioreactors operating under warm and cool conditions.

Regarding NO_3^--N removal rate, on a per m^3 of bioreactor basis, a range between 1.8 and 6.8 g N/m^3/day was observed. However, no clear correlation was found with HRT during both warm and cool seasons (Figure 4.5). In addition, a high variation in NO_3^- removal rates was observed at shorter HRTs of approximately 6 hours, particularly during the cool season. This variability can be attributed to additional factors influencing the system performance. Based on this, short residence times may not always be sufficient to counteract the influence of other factors. Therefore, careful consideration should be given when operating under this condition. On average, NO_3^--N removal rate of both seasons together remained relatively consistent across changes in HRT. The removal rates at the HRTs of 6, 10, 15 and 20 hours were 4.2 (SD 1.2), 4.2 (1.1), 4.0 (0.8) and 3.5 (0.9) g N/m^3/day, respectively.

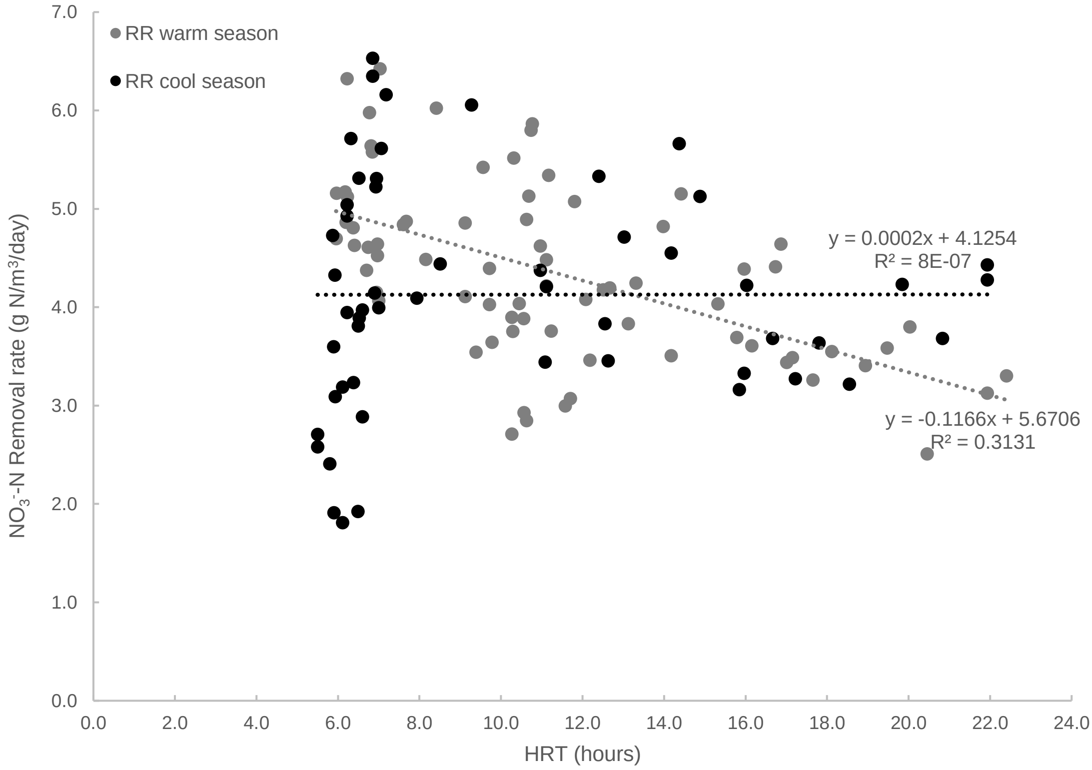

Figure 4.5. Correlation between HRT and nitrate removal rate of woodchip bioreactors operating under warm and cool conditions.

4.3.3. Ethanol dosing effect on nitrate removal

The evaluation of ethanol dosing took place during Stage 2 of the experimental period, which was during the cool season, and with short HRT (average value of 6.6 hours) (Figure 4.3). When the woodchips bioreactors were dosed with ethanol, the NO_3^--N concentrations in the outflow water were consistently lower (P<0.0001) than in the influent drainage water, and concentrations generally decreased as the C:N ratio increased. Moreover, a clear effect of C:N dosing ratio on NO_3^--N removal efficiency was observed as it increased from 44% to 100% with increasing C:N dosing ratio from 0.3:1 to 1.4:1. The effect of C:N dosing ratio on the removal efficiency was described by a significant (P<0.0001) logistic fit (R^2 = 0.95), which accounted for the complete removal of NO_3^- in the drainage water (Figure 4.6). Maximum (>95%) removal efficiency was achieved at C:N dosing ratios greater than about 0.8:1.

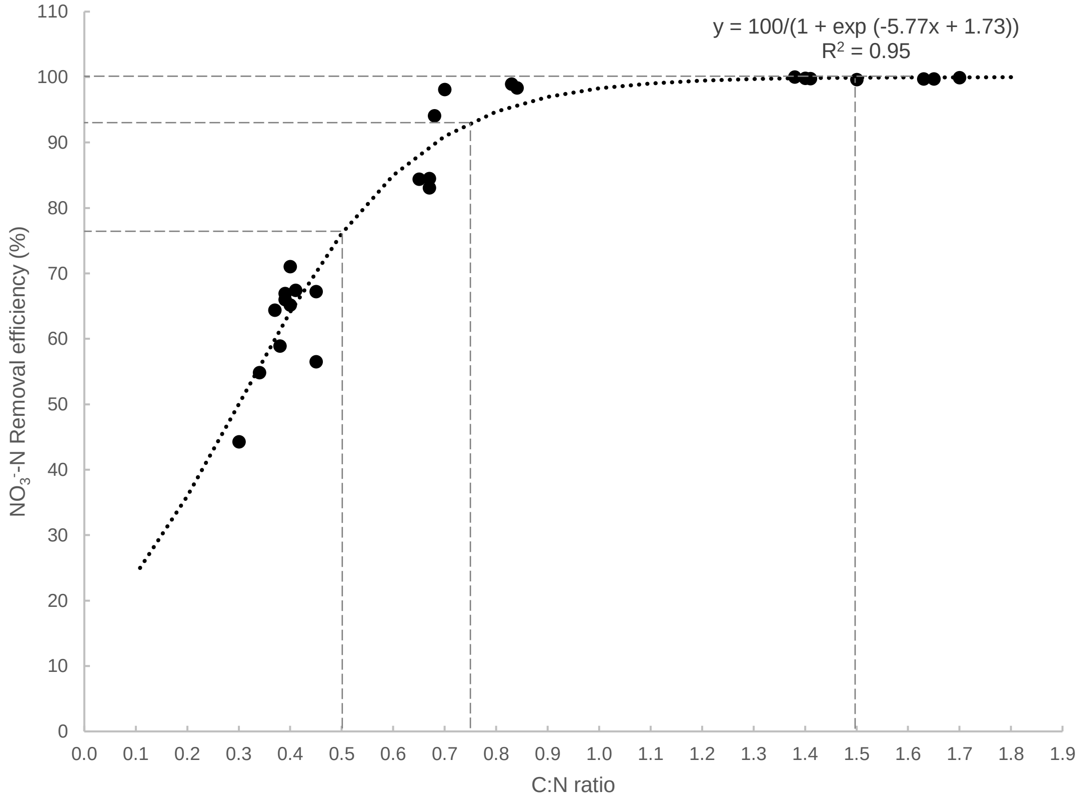

Figure 4.6. Correlation between ethanol C:N dosing ratio and nitrate removal efficiency of woodchip bioreactors operating under cool conditions and at HRT of 6.6 hours. Dashed lines indicating the removal efficiencies achieved at the C:N ratios of 0.5, 0.75 and 1.5.

Based on the model described in Figure 4.6, dosing the bioreactors with ethanol at C:N ratios of 0.5:1, 0.75:1 and 1.5:1 achieved NO_3^--N removal efficiencies of 76%, 93% and 99.9%, respectively. Meanwhile, based on the cool season HRT model described in Figure 4.4, when the bioreactors did not receive any ethanol dosing, at an HRT of 6.6 hours, the removal efficiency was 27%. This means that dosing the woodchip bioreactors with ethanol achieved removal efficiencies that were 2.8, 3.4 and 3.7 times greater for the 0.5:1, 0.75:1 and 1.5:1 C:N dosing ratios, respectively.

The NO_3^--N removal rates also increased with higher C:N dosing ratios (Figure 4.7) However, apparent reductions in removal rates were observed at specific C:N ratios, such as 0.45:1 and 0.84:1, as well as those above 1.4:1. These reductions were attributed to fluctuations of other factors, including small changes in influent NO_3^- concentration and HRT, rather than the C:N ratio itself. For instance, at C:N dosing ratios exceeding 1.4:1, HRTs progressively increased from 6 to 7 hours, which influenced the variation in removal rates. Hence, these fluctuations in HRT are attributed to small variations in the inflow due to changing temperatures. The effect of C:N dosing ratio on NO_3^--N removal rates was also described by a significant logistic fit (P<0.0001, R^2 = 0.73); however, the correlation was less strong. The model accounted for the maximum removal rate, which coincides with the complete removal of NO_3^- by the system (Figure 4.6). Based on the model (Figure 4.7), the removal rates at ethanol C:N dosing rates of 0.5:1, 0.75:1 and 1.5:1 were 12.7, 14.3 and 15.0 g N/m^3/day, respectively. In comparison, when the woodchips bioreactors were not dosed, the average removal rate was 4.0 g N/m^3/day (SD 1.5) (6.6 hours HRT).

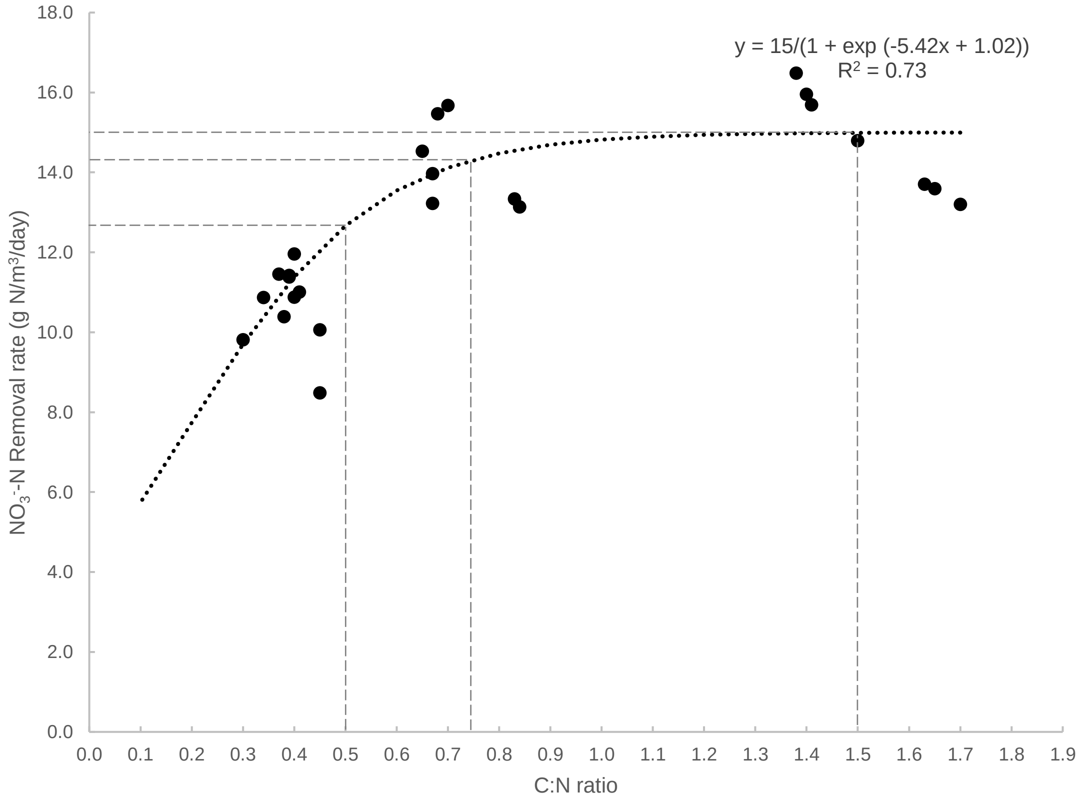

Figure 4.7. Correlation between ethanol C:N dosing ratio and nitrate removal rate of woodchip bioreactors operating under cool conditions and HRT of 6.6 hours. Dashed lines indicating the removal rates achieved at the C:N ratios of 0.5, 0.75 and 1.5.

At cooler temperatures and at a short HRT of approximately 6.6 hours, ethanol dosing at a ratio of 0.75:1 C:N resulted in a higher removal efficiency than when operating at 20-hour HRT without dosing. This highlights the benefit of using ethanol dosing to enable high levels of NO_3^- removal using short HRTs, achieving comparable or, in some cases, better results than from longer HRTs without dosing.

4.3.4. Effect of ethanol dosing on sulphate reduction

Sulphate concentrations in the bioreactor inflow water usually ranged between 20.7 and 30.1 mg SO_4^{2-}-S/L, with an average of 26.4 (SD 1.8) mg SO_4^{2-}-S/L. As outflow concentrations in all the non-dosed HRT treatments were similar to the inflow levels, there was no indication of significant reduction of SO_4^{2-} in these bioreactors. In contrast, in the bioreactors with ethanol dosing, substantial reduction of SO_4^{2-} was observed when ethanol was dosed at the higher C:N ratios. Significant decreases in SO_4^{2-} concentrations in the outflow began to occur when removal efficiencies approached 100%, with C:N ratios >0.8:1. At these higher C:N ratios, the average decrease in SO_4^{2-}-S water concentrations was 8.2 mg SO_4^{2-}-S /L (range 3.7 to 12.0 mg SO_4^{2-}-S/L). Hydrogen sulfide (H_2S) odor was detected in the bioreactors outflow samples when dosed at these higher C:N ratios. However, the C:N dosing ratios <0.8:1 resulted in only minor reductions in water SO_4^{2-}-S concentrations.

4.4. Discussion

The LWLTS receives large volumes of wastewater annually, resulting in high irrigation depths. This leads to increased levels of NO_3^- in surface drainage water, which then elevate the NO_3^- concentrations in the Waiwiri Stream. Pilot-scale woodchip bioreactors were used to assess the management practices, through adjusting HRT and ethanol dosing, that would enable high rates of NO_3^- removal by the bioreactors year-round.

Hydraulic retention time effect

The consistently lower NO_3^- concentrations in the outflow of the pilot-scale bioreactors compared to the inflows across the different HRTs indicate the ability of woodchips bioreactors to treat the drainage water from the LWLTS. Over the duration of the experiments, including the warm and cool seasons, a clear positive relationship between increasing HRT and NO_3^- removal efficiency was observed. This relationship between NO_3^- removal efficiency and HRT was nonlinear, as the rate at which the efficiency increased slowed down at higher HRTs. These findings are consistent with both the previous column experiment (Chapter 3) and other published studies (Audet et al., 2021; Christianson et al., 2012; Fan et al., 2023; Hoover et al., 2015; Martin et al., 2019).

Also, increasing HRT resulted in relatively constant NO_3^- removal rates, which align with the column experiments and other published studies (Christianson et al., 2011a; Hoover et al., 2015). A common feature of these studies and the present one is that influent NO_3^- loads were more consistent over time at each HRT, leading to more constant removal rates.

Overall, it was found that an HRT of 10 hours is sufficient to achieve an average removal efficiency for cool and warm conditions of 50%. For this level of removal, a HRT of 10 hours is relatively short compared to other pilot-scale studies treating agricultural drainage water. For instance, Christianson et al. (2011b) reported that an approximately 15-hour HRT were required for 50% NO_3^- reduction when operating under fluctuating flow rates and under cool conditions (temperature range 5°C to 13°C). Similarly, Martin et al. (2019) found that, to a achieve an average NO_3^- removal of 53.8%, under warm conditions (temperature range 12°C to 20°C), a 16-hour of HRT was required. However, in a study by Lepine et al. (2016) evaluating the effect of HRT in pilot-scale bioreactors treating wastewater from aquaculture system, they observed that a 12-hour HRT reached a 45% removal under a temperature range between 12°C and 23°C, which is similar to the findings in the present study. This similarity may be attributed to constant flows being treated in both studies. In terms of NO_3^- removal rate, the pilot-scale bioreactor presented a range between 1.8 and 6.8 g N/m^3/day, which is comparable to the range of 2.9 to 7.3 g N/m^3/day reported for field woodchips bioreactors in a meta-analysis published by Addy et al. (2016).

When analysing the seasonal performance of the pilot-scale bioreactors, a regression model revealed that the correlation between HRT and removal efficiency was stronger during the cool season compared to the warm season, with the bioreactors being approximately one-third less effective under the cooler season. Thapa et al. (2023) also reported that warm water temperatures appeared to induce one-third more NO_3^- removal, compared to cooler temperatures, when four field bioreactors were monitored at temperatures between 5°C to 20°C. Furthermore, better NO_3^- removal has been observed at increased influent water temperatures in other woodchip bioreactor studies (Christianson et al., 2012; David et al., 2015; Halaburka et al., 2019; Hassanpour et al., 2017). This highlights the importance of managing NO_3^- removal efficiencies through adjusting HRT, especially during cooler periods.

In the present pilot-scale woodchip bioreactor study, the differences in warm and cool seasons were smaller compared the column woodchip bioreactors, previously described on Chapter 3. The columns exhibited a 2.6-fold increase in effectiveness under warm conditions (82% removal efficiency) compared to cool conditions (31% removal efficiency). The differences in removal efficiency between the column study and the pilot-scale study may be attributed to various conditions in the field. While small-scale experiments are valuable for understanding the relationships among the parameters being studied, and for narrowing down the treatments to evaluate in the field, these differences emphasise the importance of evaluating mitigation technologies, like bioreactors, under field conditions.

In relation to the NO_3^--N removal rate, very low correlation coefficients with HRT were found for both cool and warm seasons, which could be attributed to the variability observed in the removal rate across the HRTs tested. Fan et al. (2023) also observed that the variation in removal rates was substantially higher in field experiments. Therefore, it is likely that in the field, woodchips bioreactors are exposed to other factors influencing the removal rate, including fluctuations in influent NO_3^- concentrations, C availability and microbial activity within the woodchip media.

The seasonal variability in the performance of woodchips bioreactors has been linked to seasonal variations in microbial communities and activity, which in turn may be linked to organic C availability, which varies with temperature (Aalto et al., 2022; Jéglot et al., 2021; Porter et al., 2015). Therefore, identifying management methods to increase C availability during the cooler months of the year is important to improve overall woodchip bioreactor performance. This need becomes increasingly significant as the woodchip bioreactor ages, when even greater decreases in efficiency will occur at lower temperatures (Maxwell et al., 2020). Significant improvements can be easily achieved by increasing HRT over the cooler periods. However, as noted previously, increasing HRT does not always result in higher NO_3^- removal rates, as longer HRTs also reduce the volume of water that can be treated at a time for a given bioreactor size. Therefore, during the cooler months of the year, ethanol dosing may be used to enhance NO_3^- removal by woodchip bioreactors, without needing to decrease the volume of water treated or increase the size if the bioreactor.

Ethanol dosing effect

Consistently lower NO_3^- concentrations in the outflow of the woodchip bioreactors compared to the inflow, for all the different ethanol dosing C:N ratios tested, support that ethanol dosing can be an effective and practical means of improving bioreactor performance, especially during cooler periods. The results of this study demonstrated that the NO_3^- removal efficiency and NO_3^- removal rates of the bioreactors generally increased with higher C:N dosing ratios. However, the removal efficiency and rate showed a diminishing marginal improvement with increases in the C:N dosing ratio above 0.8:1. Given this, and that near complete removal efficiency (>95%) and high removal rate (14.5 g N/m^3/day) was achieved at this C:N ratios >0.8:1, it is expected that a further increase in dosing ratio would only yield very minor additional benefits.

As NO_3^- removal efficiency approached 100% when ethanol was dosed at C:N ratios >0.8:1, SO_4^{2-} reduction was observed and H_2S odor was detected in the bioreactors outflow samples, indicating potential risks of pollution swapping. However, when the

C:N ratio was below 0.8:1, SO_4^{2-} reduction was minimal. Based on this and considering that at a C:N dosing ratio of 0.75:1 both high removal efficiency (93%) and removal rate (14.3 g N/m^3/day) were achieved, this could be the optimal C:N ratio for ethanol dosing under cool conditions. Indeed, it maximises bioreactor performance while reducing the risk of supplying excess soluble C, which allows sulphate reduction to occur.

Ethanol dosing of the pilot-scale bioreactors at a C:N ratio of 0.75:1 resulted in a significant enhancement (3.4-fold average increase) in NO_3^- removal efficiency compared to the control treatment. This improvement is comparable to that achieved by the column bioreactors (Chapter 3), but when using ethanol dosing at double the C:N dosing ratio of 1.5:1. Moghaddam et al. (2022) also observed a similar 3.1-fold improvement (from 28% to 87%) when 30 L column woodchip bioreactors were dosed with methanol at a C:N dosing rate of 1.5:1. It is not possible to identify why the pilot-scale woodchip bioreactors in the present study requires ethanol dosing at a lower C:N, however, the C present in the drain water could be a factor. As mentioned in the methods section, a high concentration of suspended organic matter, due to herbicide spraying and decomposition of weeds in the drain, was observed in the drainage water during the evaluation, which could have contributed C for denitrification.

The NO_3^- removal rate achieved by the woodchip bioreactors when dosed with ethanol, is an effective method of reducing HRT. For example, when ethanol dosing at a 0.75:1 C:N ratio during the cool season at an HRT of 6.6 hours, the removal efficiency was 93% and the removal rate was 14.3 g N/m^3/day. Without ethanol dosing, an HRT of 20 hours is needed to achieve a similar removal efficiency of 85%, but at a much reduced removal rate of 3.5 g N/m^3/day. This indicates that ethanol dosing can improve woodchips bioreactor performance without the need for extending the HRT, allowing a larger volume of drainage water to be treated for a certain bioreactor size, due to the potential higher removal rate.

4.5. Conclusions

This study demonstrated the suitability of woodchip bioreactors to treat drainage water from LWLTS at a pilot scale. An average NO_3^- removal efficiency of approximately 50% for the cool and warm seasons was achieved with an HRT of only 10 hours. Nonetheless, there was a variation in the NO_3^- removal efficiency of the bioreactors between the cool and warm seasons, being 43% and 59%, respectively. Doubling the HRT to 20 hours increased the average removal efficiency to an approximate of 69% and 85% under cool and warm seasons, respectively (average 77%). However, the improvements were not proportional to the increase in HRT.

The study established that ethanol dosing represents a promising strategy for enhancing the performance of woodchip bioreactors, particularly during the cooler periods of the year. Ethanol dosing at a C:N ratio of 0.75:1 was sufficient to achieve high NO_3^- removal while minimising the risk of SO_4^{2-} reduction. Ethanol dosing at this C:N ratio increased NO_3^- removal efficiency from 24% to 93% under cool conditions, when the HRT was 6.6 hours. However, it is important consider that this ethanol C:N dosing ratio will be specific to conditions at this site, including the composition of the drainage water being treated. The drainage water at the site contained high concentrations of suspended organic matter, which may have supplied additional C to the system, possibly influencing the results of the C:N dosing ratio. Nonetheless, this study also demonstrated that dosing with ethanol during the cool season can be used as a strategy to reduce the HRT required, allowing the treatment of larger volumes of water for a given bioreactor size.

The following chapter focuses on designing full-scale woodchip bioreactors for the LWLTS, based on findings of the pilot-scale bioreactor study such as the removal efficiency achieved at certain HRTs, as well as the optimal C:N dosing ratio, which served as guidelines to determine the size of the designed bioreactors

Further research on the performance of woodchip bioreactors treating drainage water from a wastewater land treatment site should prioritise full-scale testing over a longer period (i.e. > 1 year). This would allow the effect of continuous year-round use on any changes in the C supply from the woodchips and on bioreactor performance.

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5. Modelling woodchip bioreactors designs for the Levin Municipal Wastewater Land Treatment Site

5.1. Introduction

Nitrogen (N) pollution of freshwater resources is often associated with agricultural activities. However, another significant anthropogenic source of N includes inadequately treated municipal wastewaters (Abascal et al., 2022; Katz, 2020). In New Zealand, an increasingly common practice to reduce N before discharging pre-treated wastewater directly into the aquatic environment, is the use of land application. Irrigation of wastewater to land uses the soil and plants as contaminant filters, while providing them with nutrients and water. This treatment method can be highly efficient if operated and managed properly. For many soils, best management practice involves using irrigation depths that take into account the soil water deficit. In this context, land application of farm wastewater can be very effective given the relatively small volumes that need to be applied to the land. In contrast, the application of municipal wastewater can very often be a challenge due to the large volumes generated daily, and the associated requirement for large land areas. Therefore, in some cases, application depths may exceed the optimal levels, leading to unwanted environmental effects including elevated drainage water nitrate (NO_3^-) concentrations. To address this issue, an end-of-drain treatment system, like woodchip bioreactors, can be applied to the water leaving the treatment area, in order to reduce the risks of NO_3^- enrichment of the receiving aquatic environment.

In Levin, a town with a population of 19,800, wastewater is treated to a secondary level before being applied to land at a notably high application depth of 4,667 mm/year. Additionally, direct drainage from the wastewater containment pond (7 ha) is, on average, 6,400 mm/year. Consequently, drainage water with elevated NO_3^- concentrations reaches the shallow groundwater. A portion of this groundwater is also directed to surface drains, eventually flowing into the Waiwiri Stream.

In 2020, the Levin Wastewater Land Treatment Site (LWLTS) was granted a 25-year resource consent. However, this consent is subject to certain conditions, including a requirement to reduce NO_3^- levels in the surface water of the Waiwiri Stream downstream from the site . The large volumes of wastewater currently managed at the site, and the potential increases due to projected population growth, create challenges with expanding the application area. These challenges include limitations in the accessibility of additional suitable land close to the existing site and the substantial associated costs. Therefore, an end-of-drain treatment offers a cost-effective method of reducing the transfer of NO_3^- to the Waiwiri Stream.

Considering the consistent flow rates and NO_3^- concentrations observed in the drainage water at the site throughout the year, woodchip bioreactors are a promising end-of-drain treatment option at the LWTS. The previous lab-scale and pilot-scale woodchip bioreactors studies (Chapters 3 and 4) support the use of woodchip bioreactors as a suitable technology to treat the drainage water from the LWLTS. The pilot-scale bioreactors showed average (including warm and cool conditions) NO_3^- removal efficiencies of around 50% with an HRT of 10 hours. Moreover, high NO_3^- removal efficiencies of up to 85% were achieved in the field under warm conditions, with an HRT of 20 hours. Finally, both studies established that ethanol dosing is an effective strategy to enhance the performance of woodchip bioreactors, without the need of increasing the HRT, thereby allowing the treatment of larger volumes of water for a given bioreactor size.

The primary objective of the present study was to design a woodchip bioreactor capable of removing enough NO_3^- from LWLTS drainage water to achieve a 10% reduction of the site's contribution to the Waiwiri Stream, including a design that incorporates ethanol dosing for enhanced denitrification efficiency. Additionally, a comprehensive cost analysis was conducted, and practical recommendations were proposed to address potential implementation challenges.

5.2. Methodology

2.1.1. Study site

The LWLTS is located 7 km west of the Levin township in the Manawatu-Whanganui region of New Zealand. The Tasman Sea is about 0.5 km away from the western boundary of the property, and the southern boundary is defined by the Waiwiri Stream.

5.2.1.1. Wastewater management and discharge

Wastewater from Levin undergoes treatment at the local wastewater treatment plant to a secondary level. Following treatment, the wastewater is directed to LWLTS, where it is stored in an unlined pond with a capacity of 425,000 m^3. From there, the wastewater is irrigated to land (Cass et al., 2018).

At the LWLTS, discharges to the land include seepage from the unlined storage pond and spray irrigation (Mains & Douglass, 2018). Annually, approximately 1,890,509 m^3 of wastewater is discharged through spray irrigation, covering 40.5 ha of land at an average application depth of 4,667 mm/year. Meanwhile, an estimated 448,801 m^3/year is lost from the pond with a surface of 7 ha, which corresponds to a discharge depth of approximately 6,400 mm/year (Mains & Douglass, 2018).

Considering that the wastewater applied to the land undergoes treatment up to only a secondary level, certain contaminants have the potential to cause adverse effects on the receiving environment. One of these contaminants is nitrogen (N), and approximately 88,543 kg N/year arrive at the site in the wastewater. From this total N load, about 14,813 kg N/year enter the shallow groundwater system through pond seepage, while the N load entering though the irrigated area is around 73,730 kg N/year (Mains & Douglass, 2018).

5.2.1.2. Receiving environment system: soil, groundwater and surface water

The study area consists of sand dunes (approximately 75%), inter-dune areas (about 22%) and a sand plain. Wastewater irrigation primarily takes place on the sand dunes. Soils in the sand dunes are typically well drained, allowing irrigated wastewater to move through the soil (Cass et al., 2018). The drainage from both the irrigated area and pond seepage is primarily intercepted by the shallow groundwater system. Groundwater depth at the LWLTS varies across the site and is influenced by season, distance from the pond and used of irrigation. As mentioned before, the pond is unlined and is considered to be hydraulically connected to the shallow groundwater (Mains & Douglass, 2018). A significant portion of the groundwater from the site is intercepted by the surface drains on-site, and ultimately flows into the Waiwiri Stream (Mains & Douglass, 2018). Consequently, surface water acts as the receiving environment for some of the wastewater-derived contaminants. Mains and Douglass (2018) developed a numerical groundwater model to simulate the interaction between the pond, the groundwater system and the surface water (i.e. drains and Waiwiri Stream). With this model, they determined that variations in pond level are the main factor influencing groundwater fluxes and travel times. In addition, the W-E transect of this groundwater model shows that Drain 3 plays a critical role in intercepting groundwater from the pond seepage and irrigation area, while Drain 4 collects groundwater from the Western half of the irrigation area (Figure 5.2). Therefore, these two drains are important interceptors of contaminated water, which they then transfer to the Waiwiri Stream. In contrast, the SW-NE transect of the model shows that pond leakage enters groundwater and flows directly towards the Waiwiri Stream and other minor surface drains (Figure 5.2). The modelling also indicates a relatively long groundwater travel time of approximately five years for groundwater to travel from the pond to the nearest drain (Drain 3), a distance of 200 m (Mains & Douglass, 2018).

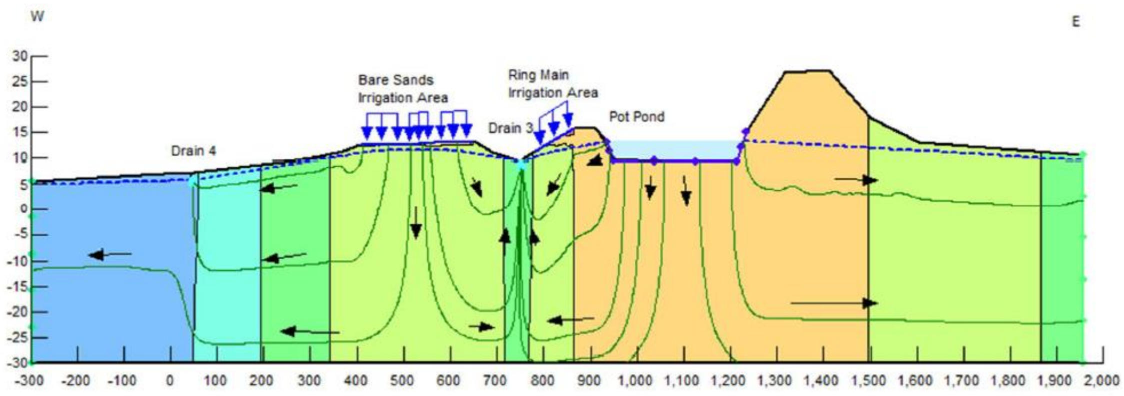

Figure 5.1. Groundwater flow paths model (W-E transect) (Mains & Douglass, 2018).

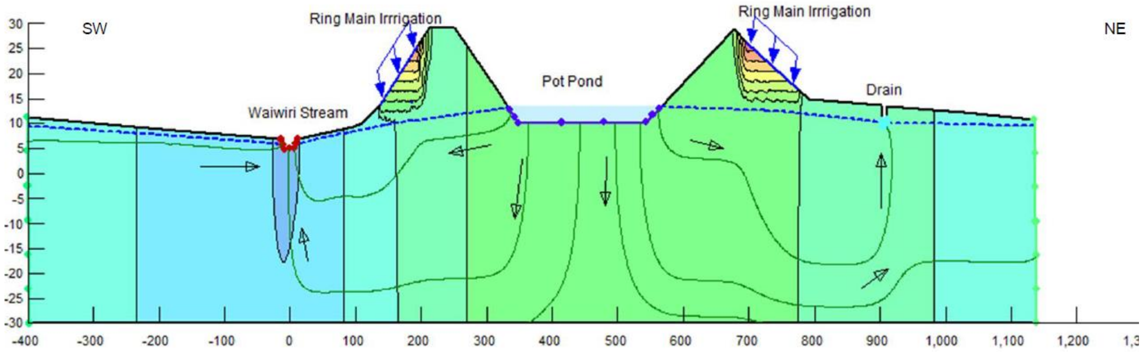

Figure 5.2. Groundwater flow paths model (SW-NE transect) (Mains & Douglass, 2018).

Water quality data for the groundwater between the pond and the drains does not show any significant evidence of attenuation of N concentrations. However, the data indicates a transformation of ammoniacal-N (NH_4-N) to NO_3^--N in the groundwater (Mains & Douglass, 2018). Surface water quality data collected from 2013 to 2017 by Ausseil et al. (2017), revealed no significant increase of NH_4-N concentrations in the Waiwiri stream downstream of LWLTS. However, there was a significant increase in concentrations of NO_3^- and total N (TN) downstream of LWLTS in the Waiwiri Stream, compared to upstream (Ausseil et al., 2017). Additionally, NO_3^- concentrations in Drain 3 exceeded the threshold for ecosystem health, outlined in the New Zealand National Policy Statement for Freshwater Management (NPSFM, 2014) (Ausseil et al., 2017).

5.2.2. Nitrate concentration, flow and temperature characterisation

Given that Drains 3 and 4 are the main point-sources of intercepted groundwater, from the pond seepage and irrigation area, the present study aimed to monitor water quality in these drains between July 2020 to June 2021. Water samples were collected once a month, and water temperature was measured at the time of sample collection. The samples were collected from the drains near the discharge point to the Waiwiri Stream (Figure 5.3). Collected samples were filtered through a 0.45 μm diameter membrane filter and stored in a freezer (<0°C) until analysis. Nitrate-N concentration was analysed using Ion Chromatography (ThermoFisher Scientific - Dionex Aquion). The monitoring data was compared with existing water quality data provided by the Horowhenua District Council (HDC). The HDC also provided water flow data from the drains for the years 2013 to 2017, which were compared with more recent flow rate measurements conducted as part of this study from August to October 2023, with a Valeport EM Flow Meter model 80, using the velocity-area method.

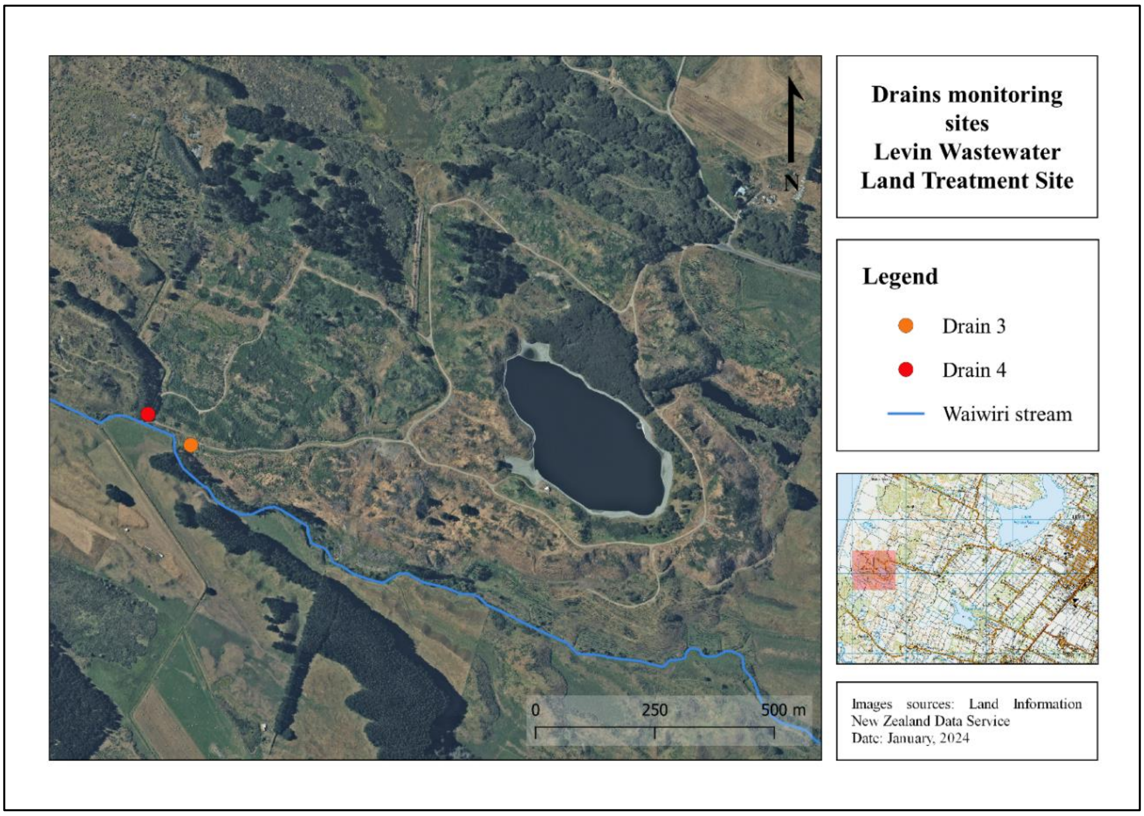

Figure 5.3. Drains 3 and 4 monitoring sites.

5.2.3. Nitrate nitrogen contribution from LWLTS into the Waiwiri Stream and drain selection for treatment

A N balance was constructed to assess the contribution of the LWLTS to the NO_3^- load in the Waiwiri Stream and to determine the proportion originating from Drains 3 and 4, and groundwater. Based on this N balance, the most suitable drain for treatment with a woodchip bioreactor was identified. The calculations of NO_3^- loading were based on historical (years 2013 - 2017) water quality monitoring data gathered from both the Waiwiri Stream and from Drains 3 and 4. Three monitoring sites along the Waiwiri Stream were considered (Figure 5.4). The first monitoring site (Stream 1) was located upstream of the LWLTS, the second (Stream 2) was located upstream from Drains 3 and 4 at a position adjacent to LWLTS, and the third (Stream 3) was positioned downstream of Drains 3 and 4, and the LWLTS. Using the monitoring data, the NO_3^- load at each stream location was determined. If the NO_3^- load in the Waiwiri Stream increased between monitoring sites, and if no surface drains were present, then the contribution was attributed to groundwater. This approach is supported by the groundwater model proposed by Mains and Douglass (2018), who suggested that a portion of the pond leakage infiltrates the groundwater and subsequently flows directly into the Waiwiri Stream.

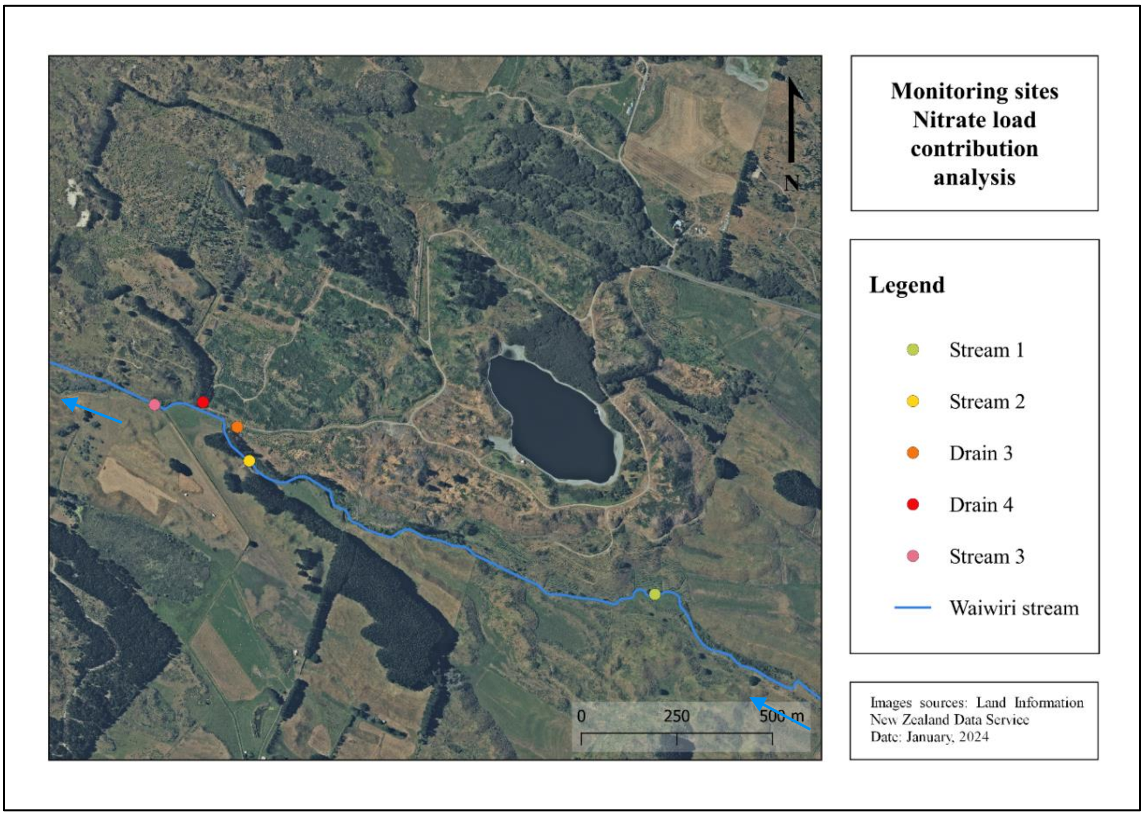

Figure 5.4. Monitoring sites in the Waiwiri Stream and Drains 3 and 4 used to calculate the nitrate load contribution from LWLTS.

5.2.4. Woodchip bioreactor volume required to achieve nitrate reduction goal

The size of the woodchip bioreactor to be installed at LWLTS was determined based on a target reduction of 10% of the NO_3^- load contribution from the site to the Waiwiri Stream. Given that most of the existing research on woodchip bioreactor performance focuses on agricultural applications, pilot-scale bioreactors were constructed and monitored at LWLTS with the aim of measuring the removal efficiencies of bioreactors treating drainage water from a wastewater land application site. In addition, the ability of ethanol dosing to enhance the performance of bioreactors, particularly at shorter HRTs, was also quantified (Chapter 4). Based on the data obtained from the experiments discussed in Chapter 4, two full-scale bioreactors (non-dosed and ethanol dosed) were designed for a NO_3^- removal efficiency performance of approximately 80%. Additional design criteria included a bioreactor porosity of 50%. Once the size of each bioreactor was determined, their NO_3^- removal rate potential was estimated based on their expected average removal efficiency, the NO_3^- load to be treated, as well as the bioreactor size.

2.1.2. Cost analysis

To evaluate the cost effectiveness of the bioreactors, the per unit cost (NZ$/kg N/year) to remove NO_3^--N was determined. Typically, bioreactor costs include design costs, excavation costs, materials (liners, woodchip, piping, pumps, etc.), installation labor and in some cases, costs associated with obtaining a resource consent. However, for this project, design costs were excluded, and no resource consent is required. Additionally, operational costs were not included because it is assumed that minimal active management will be required.

Because bioreactor volume is the primary factor determining costs and removal efficiency, the costs were estimated for two contrasting potential bioreactors volumes. In addition, for one of the bioreactors options the costs for ethanol dosing were estimated. Variable costs that depend on the bioreactor size include material costs (mainly liner and woodchips), excavation costs and labour for installation. The total present cost of each bioreactor volume was then converted to a total annualised cost, assuming a lifespan of 10 years at a 6% discount rate. Subsequently, the annualised costs were then divided by the NO_3^- removal rate estimates in order to get the costs per unit of NO_3^--N removed (NZ$/kg N/year).

5.3. Results

5.3.1. Nitrate concentration, flow, and temperature characterisation

The water quality monitoring, for the period of July 2020 to June 2021, demonstrated that Drain 3 had an annual average NO_3^- concentration of 8.9 mg N/L, which was more than double the Drain 4 value of 4.0 mg N/L. Notably, the NO_3^- concentration in the drains exceeded both the NPS-FM (2022) New Zealand national bottom line for ecosystem health of 2.40 mg N/L (Ministry for the Environment, 2023) and the trigger value for physiochemical stressors in New Zealand lowland rivers of 0.44 mg N/L (ANZECC & ARMCANZ, 2000). Both drains had a range of monthly NO_3^- concentrations, with the largest concentration occurring during the winter and the lowest during the summer (Figure 5.5). Historical monitoring (2013 to 2017) also found that NO_3^- concentrations were higher in Drain 3 than in Drain 4 (Table 5.1). However, the more recent water NO_3^- concentrations in Drain 3 were 15% lower compared to the historical monitoring data. This decrease in NO_3^- concentration may be attributed to a number of reasons, such as improvements to the irrigation system and changes in the vegetation cover, including the removal and replanting of pine trees. These changes may affect nutrient removal within the irrigation area from which Drain 3 intercepts drainage water (Figure 5.1).

Historical monitoring data (years 2013-2017) was also used to determine drain flow rates, which showed that Drain 3 had a higher average annual flow rate of 13 L/s compared to the Drain 4 value of 8 L/s. When more recent (August and October 2023) flow rate measurements were made in Drain 3, similar values to the historical monitoring were found. The higher water flow rates and NO_3^- concentrations measured in Drain 3, support the findings of the groundwater numerical model proposed by Mains and Douglass (2018), which suggests that out of the two drains, Drain 3 is expected to intercept a higher proportion of the groundwater from pond seepage and the irrigation area, compared to Drain 4.

As anticipated, seasonal fluctuations were observed in water temperatures, with only small differences between the two drains (Figure 5.6). The highest temperatures were both recorded in February (16.7°C for Drain 3 and 15°C for Drain 4), while the lowest temperatures were both recorded in August (11°C for Drain 3 and 10.7°C for Drain 4).

The higher temperatures recorded for Drain 3 were likely due to the greater length of the drain, compared to Drain 4.

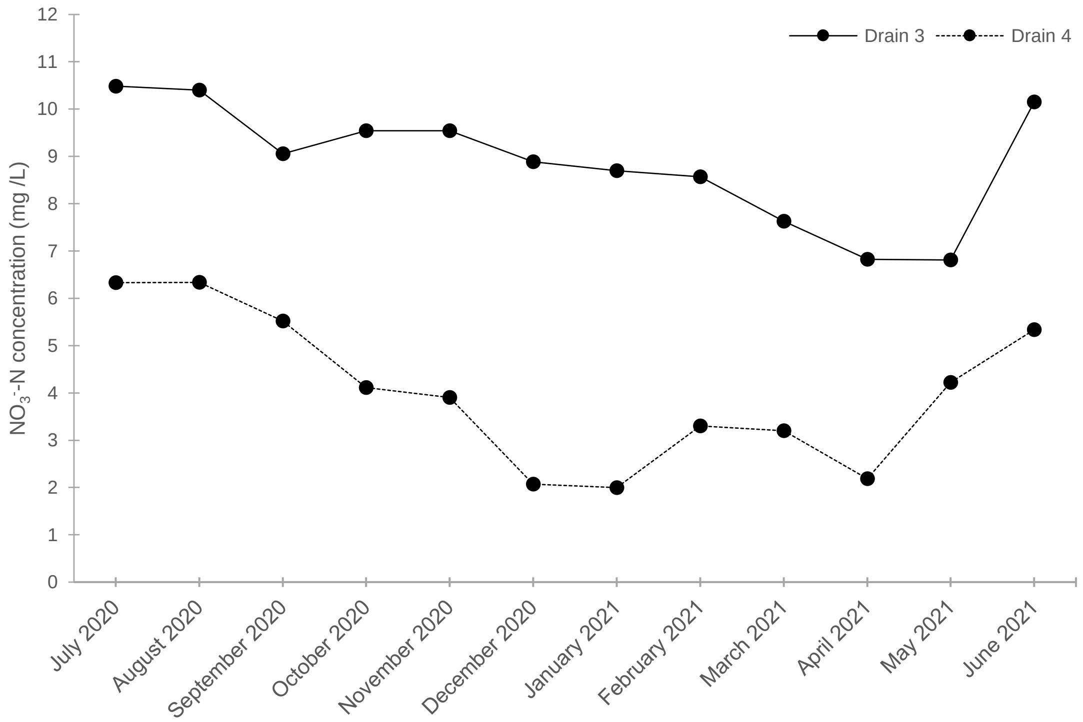

Figure 5.5. Nitrate nitrogen concentrations in Drain 3 and 4, from July 2020 to June 2021.

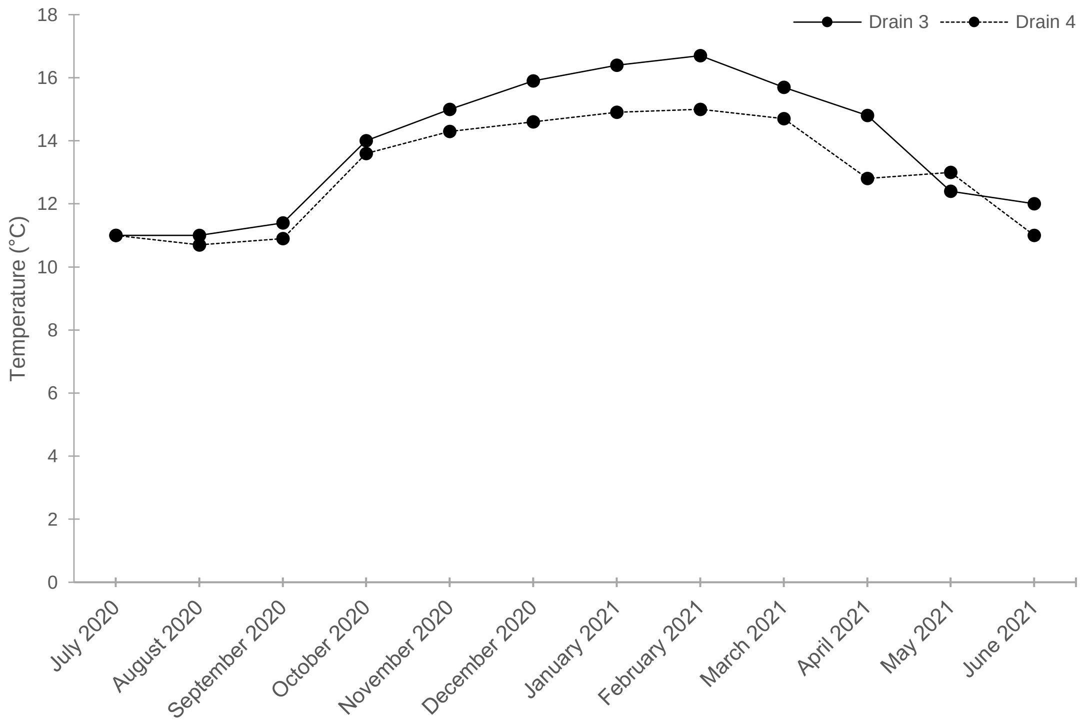

Figure 5.6. Water temperatures in Drain 3 and 4, from July 2020 to June 2021.

5.3.2. Nitrate nitrogen contribution from LWLTS into the Waiwiri Stream and drain selection for treatment

Based on the analysis of the historical average NO_3^- concentrations and the flow rates at the different monitoring sites, the Waiwiri Stream total NO_3^--N load downstream of the LWLTS (Stream 3 monitoring site) is estimated to be 32 kg N/day. In comparison, the upstream NO_3^--N load is 1.9 kg N/day (Stream 1 monitoring site). Therefore, the NO_3^--N load contribution from the site into the Waiwiri Stream was quantified as being 30.2 kg N/day. It is important to note that the contribution measured at the Stream 2 monitoring site, of 13.9 kg N/day, is considered to be predominantly from groundwater, as no significant contribution from surface drains was identified. Table 5.1 summarises the NO_3^--N concentrations and loads at the different monitoring sites and their respective contributions.

Based on the NO_3^- load analysis, the LWTS's primary contributors to the NO_3^--N load in the Waiwiri Stream are groundwater inflows, which account for 49% of the total contribution of the site, and Drain 3, which contributes to 38%. Given these findings, and because it is preferable to use a point-source for treatment with woodchip bioreactors, Drain 3 was considered to be the most suitable source of NO_3^- for treatment. In addition, the constant water flow rates and high NO_3^- concentrations make Drain 3 an ideal source of water for a bioreactor treatment setup (Addy et al., 2016; Christianson et al., 2012; David et al., 2015; Schipper et al., 2010).

Table 5.1. Average nitrate concentration and load at the different monitoring sites and their contribution into the Waiwiri Stream.

Monitoring site	Average NO_3^--N Concentration (mg/L) *	Average NO_3^--N Load (kg/d)	Contribution from LWLTS into the Waiwiri Stream (%)
Stream 1	0.2	1.9	6
Stream 2	1.4	15.8	49
Drain 3	10.8	12.1	38
Drain 4	3.3	2.3	7
Stream 3	2.2	32.1	-

* Average NO_3^--N concentration based on historical data between years 2013 and 2017.

5.3.3. Woodchip bioreactor volume without dosing

When designing a bioreactor, the interactions, and sometimes tension, between removal efficiency, HRT and the NO_3^--N concentration and rate of inflow need to be considered. In this case study, the target HRT was determined based on the data collected from the pilot-scale bioreactors (Chapter 4). To select the HRT, a near 80% NO_3^- removal efficiency was set as the design performance criterion. The data provided by the pilot-scale bioreactors showed that during the warmer months of the year (November – April), an 85% removal was attained with an HRT of 20 hours, while during the cooler months (May – October) the same HRT achieved a 69% removal efficiency. Based on this, an HRT of 20 hours would be necessary to achieve an average removal efficiency of 77%, which is close to the 80% performance criterion.

Given the target of a 10% reduction in the site's contribution to the NO_3^- load in the Waiwiri Stream, a removal of 3.2 kg N/day is needed. Assuming the bioreactor will achieve a 77% removal efficiency at a 20-hour HRT, the total NO_3^- load that needs to enter the bioreactor is 4.2 kg N/day. Considering the average NO_3^--N load in Drain 3 of 12.1 kg N/day, approximately 387 m^3 (34% of daily drain volume) of drainage water would require treatment daily. This means that 322 m^3 of drain water will be treated every 20 hours (HRT), and assuming the woodchips have a ~50% pore volume, then a bioreactor with a volume of 645 m^3 is needed at LWLTS to achieve a 10% reduction in the NO_3^- load in the Waiwiri Stream downstream from the site.

5.3.4. Woodchip bioreactor volume with ethanol dosing

The results from the pilot-scale bioreactors (Chapter 4), indicate that ethanol dosing is an effective management strategy to enhance the performance of woodchip bioreactors without the need for longer HRTs, allowing the treatment of larger volumes of water for a given bioreactor size. Therefore, an ethanol-dosed bioreactor design is also proposed here.

The results from the pilot-scale suggest that year-round dosing with ethanol at a C:N ratio of 0.5:1 would be required to achieve the design performance criterion of close to 80% NO_3^- removal efficiency. At this C:N ratio, the pilot-scale bioreactors achieved a 76% removal efficiency during the cool season. However, ethanol dosing was not evaluated under warm conditions. During the warm season, the pilot-scale bioreactors without ethanol dosing exhibited removal efficiencies of approximately 15% greater than those observed in the cool season (Figure 4.4). Considering an assumption of approximately 15% increase in removal efficiency during the warm season, the removal efficiency is expected to reach approximately 91%. Consequently, the average annual NO_3^--N removal efficiency is projected to be around 83%.

A bioreactor with a 6.6-hour HRT and using ethanol dosing at a C:N ratio of 0.5:1, would require a total NO_3^--N load of 3.9 kg N/day to remove 3.2 kg N/day. This equates to about 32% of the drainage water from Drain 3, or approximately 359 m^3 per day. As 99 m^3 of drainage water will be treated every 6.6 hours and, taking into consideration a ~50% pore volume of the bioreactor, a 197 m^3 ethanol-dosed bioreactor at LWLTS is required to attain a 10% reduction in the NO_3^- load in the Waiwiri Stream downstream from the site.

5.3.5. Nitrate nitrogen removal estimates for the designed bioreactors

As explained above, it is anticipated that the larger non-dosed bioreactor (20-hour HRT), and the smaller ethanol-dosed bioreactor (6.6 HRT) will have similar average NO_3^- removal efficiencies of 77% and 83%, respectively. Based on these removal efficiencies, the expected NO_3^- removal rate and associated annual NO_3^- load removal for the designed bioreactors were estimated. It is important to note that estimates of the NO_3^- removal rates depend on the transferability of removal efficiency between the pilot-scale and the full-scale system. This transferability is not straightforward, as larger bioreactors can often be less efficient than smaller ones. This reduced efficiency in larger bioreactors is attributed to challenges in achieving uniform water distribution inside the bioreactor, which becomes more difficult due to a greater cross-sectional area. However, this challenge can be mitigated by implementing specific structural design features, such as baffles, that allow a more even distribution of water inside the bioreactor (de Oliveira et al., 2023; Dougherty et al., 2020).

Another significant consideration when estimating NO_3^- removal rate and annual NO_3^- load removal is the NO_3^- concentration in the drainage water. As mentioned previously, there was a 15% reduction in NO_3^- concentration measured in Drain 3 during recent monitoring compared to historical monitoring data. However, additional monitoring of the stream, including flow rates will be needed to confirm whether the site's contribution to the stream NO_3^- load has also decreased. Thus, the estimated NO_3^- removal rates of the designed bioreactors are based on the historical data (presented in Table 5.1) and are presented in Table 5.2.

Both bioreactors were designed for the same level of removal (~80%), therefore, the estimated annual NO_3^--N load removal is the same for both bioreactors, indicating that both can meet the resource consents requirements. However, the daily rate per m^3 of bioreactor volume at which this annual load is achieved differs between the bioreactors, as the ethanol-dosed bioreactor will present removal rates 3.3 times greater than the no-dosed bioreactor. This confirms the ability of ethanol dosing to allow short HRTs and treat large volumes of water daily.

Table 5.2. Average nitrate nitrogen removal efficiency and rate (per m^3 of bioreactor volume) and annual load removal estimates for the two bioreactors designs.

Bioreactor alternative	**Bioreactor size (m^3)**	**Average Annual NO_3^--N Removal Efficiency (%)**	**Estimated NO_3^--N Removal Rate (g N/m^3/day)**	**Estimated Annual NO_3^--N Load Removal (kg N/year)**
20 hours HRT No ethanol dosing	645	77	5.0	1174
6.6 hours HRT Ethanol dosing 0.5 C:N ratio	197	83	16.3	1174

Another potential advantage of ethanol dosing is the potential to maintain more consistent NO_3^- removal efficiencies and rates over time, compared to solely using woodchips. The ethanol provides a consistent supply of soluble C, which is immediately available for the denitrification process. In contrast, the removal efficiency of bioreactors that rely only on woodchips as a C source, declines over time due to the reduction in the supply of soluble C from the woodchips (Robertson, 2010; Robertson et al., 2008). Additionally, it is important to consider that a bioreactor at the LWLTS will be used continuously, 24 hours per day and year-round, treating water with relatively high NO_3^- concentrations. This continuous year-round use, which is not typical of bioreactors used in agriculture, has the potential to cause a faster decline in the supply of soluble C from the woodchips. Therefore, it is conceivable that the removal efficiency, and consequently the removal rate, of the non-dosed bioreactor design could decline with the 10-year time frame of the project. While the rate of decline is difficult to predict, it is important to prepare for this possible eventuality. While more frequent replacement of woodchips is one response to the short life span of woodchips at the LWLTS, a more practicable option may be to introduce dosing with a soluble C source at some stage.

5.3.6. Nitrate removal cost analysis

It is important to note that the following costs do not include the cost of infrastructure related to supplying power to the site or for the pump and pipes required to deliver water from Drain 3 to a bioreactor. Also, the cost of equipment required to remove organic material from the water has not been accounted for. However, it is assumed that these costs will be similar for both bioreactor designs.

To determine the annual NO_3^- removal costs of both bioreactor designs, the NO_3^- removal estimates were combined with the total annualised cost estimates. Installation costs are primarily dependent on bioreactor size, with the main components being woodchips and excavation costs. Consequently, the larger non-dosed woodchip bioreactor incurs a higher installation cost, compared to the smaller ethanol-dosed bioreactor (Table 5.3). However, the ethanol-dosed bioreactor has a higher operational cost, due to the on-going cost of ethanol, and the associated labour cost for maintenance and operation of the ethanol tank and pumps. Based on the cost estimations, the total annualised cost of the non-dosed bioreactor is NZ$ 8,085, which is 47% lower than of the ethanol-dosed bioreactor (NZ$ 15,388). Based on an expected annual removal load of about 1174 kg NO_3^- N/year by both bioreactors, the NO_3^- removal cost for the non-dosed bioreactor will be approximately NZ$6.9/kg NO_3^--N/year, compared to a removal cost of around NZ$13.1/kg NO_3^--N/year for the ethanol-dosed bioreactor. However, it is important to consider that, as mentioned earlier, the removal efficiency and removal rate of the non-dosed bioreactor are likely to decline over time at a faster rate, compared to the ethanol-dosed bioreactor, because it relies on the woodchips as the only C source. Consequently, the NO_3^- removal cost will likely increase due to a decrease in the NO_3^- removal of the bioreactor. For example, if the bioreactor efficiency decreases from 77% to 50%, the removal cost will increase from NZ$6.90 to NZ$10.6/kg NO_3^--N/year. A further reduction to a removal efficiency of only 25% would increase the removal cost to NZ$21.2/kg NO_3^--N/year (Figure 5.7). Moreover, Figure 5.7 illustrates that the cost will increase exponentially with the decline of the removal efficiency. This is attributed to the constant annualised cost (NZ$8,085), while the annual NO_3^- load removal decreases with declining removal efficiency. As removal efficiency declines, along with the increase in costs per kg of NO_3^--N removed, the bioreactor will also fail to achieve the required NO_3^- removal to comply with the resource consent condition. Therefore, introducing ethanol dosing once a decline in efficiency is observed may be a practical option to maintain the required removal while minimising costs. For example, if ethanol dosing is applied at the same C:N ratio (0.5:1) as suggested for the small bioreactor design, it is assumed that a removal efficiency of 80% will be maintained, then the cost would not be more than NZ$~11/kg NO_3^--N/year. However, considering the long HRT (20 hours), dosing year-round may not be needed, or lower C:N dosing ratios could be sufficient, potentially reducing the required ethanol volume and associated costs. Nevertheless, further research is needed to confirm this.

Table 5.3. Woodchip bioreactors cost-estimate.

Bioreactor component	**Cost/ Unit**	**Quantity**	**Non-dosed Bioreactor (645 m^3)**	**Quantity**	**Ethanol dosed Bioreactor (197 m^3)**
Inflow piping	75	20 m	1495	12 m	897
Outflow piping	10	30 m	300	15 m	150
Polyethylene liner	125	7 rolls	875	2 rolls	250
Liner tape	40	2 units	80	1 unit	40
Woodchips	35	645 m^3	22575	197 m^3	6895
Excavation (Equipment + Labour)	33	645 m^3	21285	197 m^3	6501
Lining and piping work (Labour)	20	645 m^2	12900	197 m^2	3940
		Total installation cost =	59,510		18,673
Ethanol cost (annual)	2.8	0 L	0	1804 L	5051
Maintenance and operation (Labour)	150	0	0	52 weeks	7,800
		Total annualised cost (10-year, 6% interest rate) =	8,085		15,388
		Nitrate removal cost (NZ$/kg NO_3^-/year) =	6.9		13.1

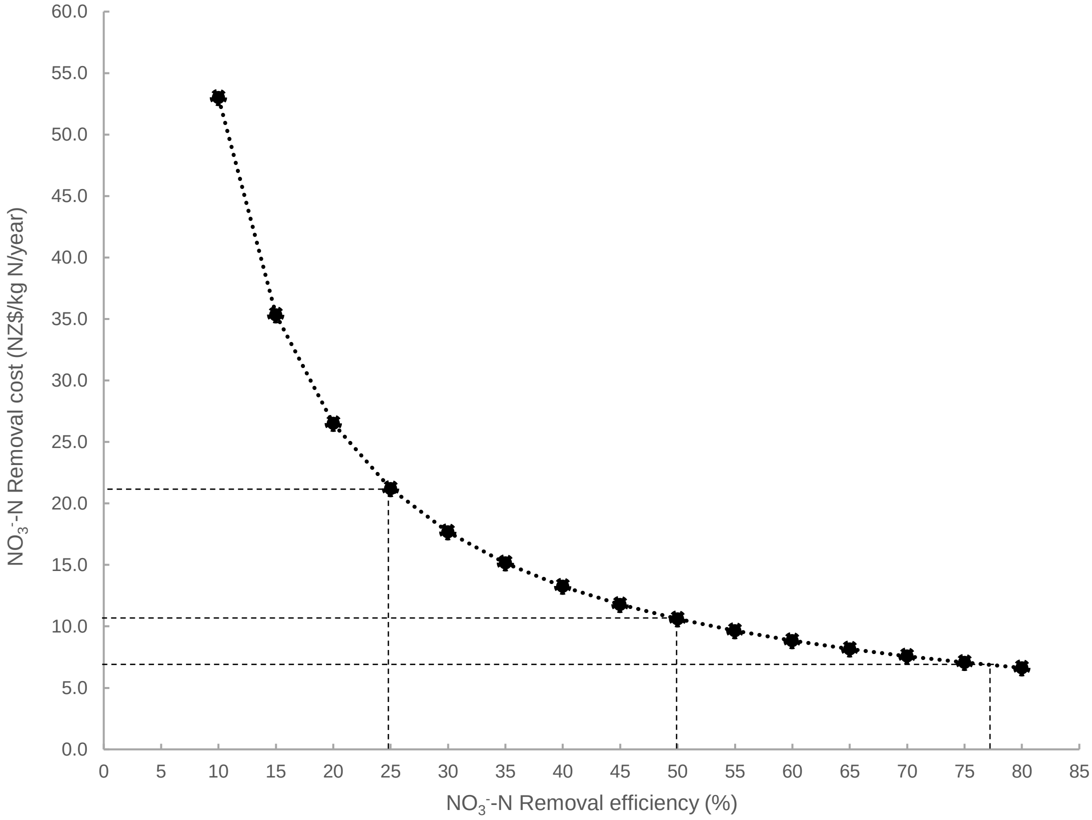

Figure 5.7. Sensitivity curve of nitrate-nitrogen removal cost increase with decreasing nitrate nitrogen-removal efficiency for the non-dosed bioreactor, 20-hour HRT design. Dashed lines indicating the price increase when removal efficiency decreased from 77% to 50% and 25%.

5.4. Discussion

Estimates of NO_3^- contributions from the LWLTS to the Waiwiri Stream indicate a contribution of approximately 30.2 kg N/day, which is 94% of the total load downstream from the site of 32.1 kg N/day. Based on this, a 10% reduction in the NO_3^--N load equates to 3.2 kg N/day. Drain 3 is the most suitable for treatment with woodchip bioreactors, because it is the surface drain with the highest NO_3^- load. To achieve NO_3^- removals close to 80%, two bioreactor designs are proposed. The first design is a non-dosed 645 m^3 woodchip bioreactor using a long HRT of 20 hours, and the second design is an ethanol-dosed (0.5:1 C:N ratio) 197 m^3 woodchip bioreactor using a short HRT of 6.6 hours. These two designs were based on a conservative assumption of using no more than about a third of the water from Drain 3. The shallow water level in the drain, combined with a sand base, may make it difficult to pump a larger proportion of the drain water. The installation of a weir or a dam may improve the ability to pump more of the drain water, but this will require further assessment. While this study proposes two contrasting bioreactors designs, it is likely that there are other potential combinations of bioreactor size, HRT, and ethanol dosing level for the LWLTS, in between the here proposed designs.

While the proposed bioreactors differ in size, it is anticipated that both bioreactors will exhibit similar annual NO_3^--N removal loads of an estimated 1174 kg N/year. However, the NO_3^--N removal rate per m^3 of bioreactor volume will differ between the two designs. The non-dosed bioreactor is expected to remove approximately 5.0 g N/m^3/day, whereas the ethanol-dosed bioreactor is expected to remove 16.3 g N/m^3/day, which is more than three times higher. This difference is primarily attributed to the utilisation of the ethanol dosing for the smaller bioreactor, allowing for a shorter HRT, while maintaining high removal efficiency, making the ethanol-dosed bioreactor more efficient.

Regarding the annualised removal costs, based on the initial removal efficiencies of each bioreactor design, the large non-dosed dosed bioreactor will remove NO_3^--N at a cost of NZ$6.90/kg N, while the small ethanol-dosed bioreactor will have a cost of NZ$13.1/kg N. The cost of removal for the ethanol-dose design is almost double than of the non-dosed one. However, as previously mentioned, the ethanol-dosed bioreactor offers a NO_3^--N removal rate that is more than three times higher compared to the non-dosed bioreactor.

Another difference between the two bioreactors is the C source for denitrification. In the non-dosed bioreactor, woodchips serve as the primary C source, which must first undergo decomposition to release available soluble C. It is understood that the decomposition of woodchips into available C precedes the utilisation of soluble C by denitrifiers (Zhao et al., 2018). Therefore, the removal performance of the bioreactors operating without ethanol dosing is primarily influenced by the HRT, with longer retention time providing the necessary contact time between denitrifying bacteria and organic matter. Conversely, in the ethanol-dosed bioreactor, ethanol provides soluble C immediately for denitrification, thus, avoiding the requirement for longer retention times.

Another difference between the two bioreactors, potentially favoring the ethanol-dosed option, is the anticipated performance decline of the non-dosed bioreactor continuously operating at a 20-hour HRT year-round. Although no definitive studies have precisely outlined the rate at which removal efficiency declines over time, it is understood that C supply from woodchips is generally more abundant in the initial years of operation, and gradually diminishes thereafter. Initially, cellulose and hemicellulose are readily converted into soluble C for denitrification. However, over time, their content diminishes, leaving more recalcitrant compounds, which reduces the C supply, resulting in a decline of removal efficiency (Ghane et al., 2018; Hartfiel et al., 2022). In contrast, the decline in removal efficiency is expected to be lower for the ethanol-dosed system, as only a proportion of the soluble C is supplied by woodchips. While it is not feasible within this study to estimate the proportion of soluble C derived from woodchips versus ethanol dosing, it is anticipated that the addition of soluble C through ethanol will enable a more consistent removal efficiency in the long-term operation of the system, although some depletion of soluble C from the woodchips is also expected. However, any decline in removal efficiency due to reduced C supply from the woodchips can be compensated by increasing the C:N dosing ratio.

The depletion of C availability in the non-dosed bioreactor will not only affect the NO_3^- removal performance, but it will also impact the associated removal cost. Since the NO_3^- removal cost depends directly on the NO_3^- removal rate of the bioreactor, a reduction in efficiency will lead to increased costs. For example, if the initial removal efficiency of 77% decreases to 50%, the removal cost will increase from NZ$6.90 to NZ$10.60/kg NO_3^--N/year. In addition to the increasing cost, the required N removal is no longer being achieved. However, the introduction of ethanol dosing at a C:N dosing ratio of 0.5:1, when the removal efficiency declines to a 50%, will incur in a higher cost of NZ$~11/kg NO_3^--N/year but would achieve greater NO_3^- removal. Comparatively, as the removal efficiency in the dosed bioreactor is expected to decline much more slowly, the expected increase in NO_3^- removal costs will be lower compared to the increase in the non-dosed bioreactor. In addition, the removal efficiency could be easily enhanced by increasing the C:N dosing ratio. This will, however, require the use of a larger volume of ethanol, which in turn will affect the removal cost.

Another consideration to make regarding the two bioreactor designs is their ability to increase the total quantity of NO_3^- removed from the drain water, if necessary, in the future. An advantage of the large non-dosed bioreactor is that it provides greater flexibly to increase NO_3^- removal. Introducing ethanol dosing with this design can not only compensate the potential decline in the C supply from the woodchips overtime, but also allow for the treatment of a larger volume of drain water. This would be possible if, in the future, the flow of drain water ceases to be a limiting factor. In contrast, opting for a smaller bioreactor presents challenges to adapting the system to increased volumes of drain water due to the initial short HRT. Therefore, there is limited flexibility to further reduce the HRT. Additionally, bioclogging due to microbial biomass growth could potentially compromise the long-term efficiency of both bioreactors, but particularly the smaller ethanol-dosed bioreactor. Further research is needed on the long-term performance of ethanol-dosed woodchip bioreactors to determine their longevity and the potential for bioclogging.

Either of the two described bioreactor designs are initially expected to remove enough NO_3^- from the water in Drain 3 at the LWLTS to achieve the required reduction in NO_3^- load in the Waiwiri Stream. However, constant monitoring of the bioreactor will be necessary to detect potential reductions in removal efficiency over time, and to adjust the operational conditions as necessary.

Prior to installing a full-scale woodchip bioreactor at the LWLTS, there are several considerations to take into account to ensure optimal performance, longevity and environmental effectiveness of the system: Firstly, it is necessary to establish baseline values of NO_3^- concentration and water flow in the Waiwiri Stream downstream from the site (Stream 3 monitoring site). Given the importance of the NO_3^- load at this point in the stream, determining the current value with actual measurements will provide more reliable data than the calculations presented here. A second consideration is the selection of the bioreactor installation site within the LWLTS. Since the water level in Drain 3 is about 2 meters below ground level, water supply to the bioreactor through gravity feeding becomes challenging. Therefore, selecting a suitable location involves considering the proximity to Drain 3, in order to limit the distance that drainage water needs to be pumped to reach the bioreactor. Additionally, the available land surface must be sufficient to accommodate the selected bioreactor size, and ideally the installation site should allow for a bioreactor with a length to width ratio of 4:1. This recommendation is based on findings by Christianson et al. (2013), who suggested that this ratio provides uniform distribution of flow through the bioreactor, thereby optimising the bioreactor performance. Another important consideration for ensuring optimal operation of the bioreactor is the implementation of an organic matter removal system. One of the primary challenges encountered during the pilot-scale experiments was the presence of suspended organic matter in the drainage water, which was the result of weed spraying in the drain. This organic matter poses a risk of clogging the bioreactor, as other studies have demonstrated that drainage water containing sediments can reduce the hydraulic performance of woodchip bioreactors and can shorten their operational lifespan (Christianson et al., 2020; Feyereisen et al., 2023). This risk could be mitigated by installing a rotating screen or a similar filtration system. Additionally, organic matter generation can be reduced by mechanically removing weeds from the drain.

To effectively track bioreactor performance over time, consistent monitoring is important. For instance, the installation of a flow control equipment will facilitate regular monitoring of flow rates, entering and exiting the bioreactor, thereby enabling the assessment of hydraulic performance, and ensuring an adequate retention time within the woodchips. A practical monitoring approach involves the installation of in-pipe flow meters. In addition, monitoring NO_3^- concentrations in the inflow and outflow of the bioreactor is essential for evaluating the system performance. Due to the relatively uniform NO_3^- concentrations in the drain water, sample collection once every 1-2 weeks should be adequate. Additionally, combining this data with water flow data allows to calculate NO_3^- load reductions. In addition to monitoring the bioreactor performance, it is important to periodically monitor water flows and NO_3^- concentrations in the Waiwiri Stream downstream from the site, at least once a month, in order to determine the NO_3^- load and the compliance with the 10% reduction requirement.

It is important that the additional costs, associated with these further considerations, are also factored into the total cost of using a woodchip bioreactor treatment system at the LWLTS and, therefore, are budgeted for before installation.

5.5. Conclusions

Woodchip bioreactor are a suitable mitigation option for treating drainage water from LWLTS to a standard that will meet resource consent conditions. Two contrasting woodchip bioreactor designs have been proposed for the LWLTS. The designs include a woodchip bioreactor of 645 m^3 operating at a long HRT (20 hours), and an ethanol-dosed woodchip bioreactor of 197 m^3 operating at a short HRT (6.6 hours). Both bioreactors are expected to achieve the same annual NO_3^- load removal of 1174 kg N/year. The smaller ethanol-dosed design has the potential to be more cost-efficient over time, as it is less likely to be affected by a decline in removal efficiency due to a depletion in C availability. However, this design has limited capacity to increase NO_3^- removal if this would be required in the future. Therefore, it would be an advantage to implement the larger non-dosed bioreactor design but also planning for the potential need for gradually introducing ethanol dosing, in case of a decline in NO_3^- removal efficiency and/or a demand to treat more water in the future. Regular monitoring of the system is required to facilitate the necessary adjustments in the operational conditions.

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6. Summary and future research

6.1. Introduction

Population growth together with the ongoing expansion of urban areas continue to increase the generation of wastewater that needs to be managed in centralised treatment plants. At the same time, there are increasing expectations from communities that the treatment of wastewater will improve and that its negative impacts on the aquatic environment will be minimised (Price et al., 2018; Zagklis & Bampos, 2022). In New Zealand, land application has been shown to be an effective treatment for pre-treated urban wastewater (secondary level) (Sparling et al., 2006). Application to land offers a passive treatment via the filtering capacity of the soil and plant system (Chaudhry et al., 2005). However, successful land treatment of urban wastewater frequently requires large areas of land to accommodate the large volumes generated by towns. Therefore, common barriers to the adoption of land treatment include the availability of suitable land, the associated costs, and restrictions with land use under application of municipal wastewater. In certain cases, these limitations have led to land treatment sites with insufficient area to achieve satisfactory treatment, resulting in increased nutrient concentrations in artificial drains and/or groundwater, which subsequently contribute to the nutrient enrichment of the receiving aquatic environment.

The Levin Wastewater Land Treatment Site (LWLTS) is an example of an under-sized site, where the volumes of wastewater being applied over an insufficient land area result in very high application depths. As a result, there is a large quantity of drainage that is intercepted by the groundwater system, from which a significant portion is captured by surface drains that flow into the Waiwiri Stream. Therefore, the Waiwiri Stream has become a receiving environment for some of the wastewater-derived contaminants, especially nitrate (NO_3^-) (Mains & Douglass, 2018). In 2020, the LWLTS obtained a resource consent, subject to specific conditions, one of which requires a 10% reduction in NO_3^- levels in the Waiwiri Stream downstream from the site. Expanding the irrigation area would be the most straightforward method of meeting the consent condition. However, this strategy would entail considerable financial costs, not to mention the difficulty of locating suitable and available land close to the current land application site.

Consequently, the District Council is exploring alternative, cost-efficient treatment methods for mitigating drainage water NO_3^- discharges to the Waiwiri Stream. This study was centred on assessing the effectiveness and applicability of woodchips bioreactors in treating drainage water originating from the LWLTS. In addition, the objective was to design a bioreactor capable of meeting the NO_3^- reduction requirements for the LWLTS. A woodchip bioreactor was selected as a treatment method, because it has been shown to be an effective end-of-drain treatment for reducing NO_3^- discharges from agricultural drainage water (Christianson et al., 2021). Moreover, this technology is cost-effective, simple to construct and requires minimal maintenance (Christianson et al., 2021; Schipper et al., 2010).

Woodchip bioreactors have been widely studied for their effectiveness in reducing NO_3^- discharges from agricultural drainage. However, their potential application to drainage water from municipal wastewater land treatment areas remains relatively unexplored. Unlike agricultural drainage, which typically occurs during cooler periods of the year, and is characterised by fluctuating flow rates and NO_3^- concentrations, drainage from the LWLTS exhibits relatively consistent flow rates and NO_3^- concentrations throughout the year, due to constant application of wastewater at high application depths. These consistent conditions suggest that denitrification bioreactors may operate effectively in municipal wastewater land application settings. Nonetheless, before considering the implementation of full-scale bioreactors in these locations, a thorough understanding of the performance of woodchips bioreactors under these conditions is necessary.

The main objective of this research was to evaluate the potential use of woodchip bioreactors for treating drainage water from the LWLTS, including the assessment of soluble C dosing of woodchip bioreactors to enhance NO_3^- removal. To accomplish this, the specific research objectives were to:

i. conduct an initial assessment of the potential ability of woodchip bioreactors to treat drainage water from a municipal wastewater land treatment site using small-scale column bioreactors and investigate the likely effects of hydraulic residence time (HRT) and temperature on NO_3^- removal and the potential impact of soluble C dosing (Chapter 3),

ii. assess the ability of pilot-scale woodchip bioreactors to reduce NO_3^- levels in drainage water from the LWLTS, and how NO_3^- removal efficiency varies with HRT and temperature, and evaluate the ability of ethanol dosing to enhance NO_3^- removal, particularly during cooler seasons (Chapter 4) and,

iii. use the findings of the pilot-scale study to design woodchip bioreactors capable of removing sufficient NO_3^- from LWLTS drainage water to meet the NO_3^- reduction requirements for the Waiwiri Stream, including a design that incorporates ethanol dosing for enhanced denitrification efficiency (Chapter 5).

6.2. Main findings of the research

6.2.1. Small-scale column bioreactor study (Chapter 3)

This study used small-scale column woodchip bioreactors, and similar conditions (i.e. water temperature and consistent water NO_3^- concentrations) to those at the LWLTS, to investigate the ability of bioreactors to remove NO_3^- from water. This included assessing the effect of HRT and soluble C dosing on enhancing NO_3^- removal.

The woodchip column bioreactors achieved NO_3^- removal efficiencies of up to 93% under warm conditions and operating at 10 hours of HRT. However, at the same HRT the efficiency decreased to an average of 31% when the bioreactors operated under cooler conditions. These findings emphasise the need to enhance the performance of woodchips bioreactors in cool conditions. One possible strategy for improvement is extending the HRT. However, this approach comes at the cost of reducing the volume of water that can be treated at a time for a given bioreactor size.

This study also determined that C dosing represents an effective strategy for enhancing the NO_3^- removal performance of woodchip bioreactors, without compromising the volume being treated. However, it was observed that the selection of the soluble C source has an important influence on denitrification performance, with ethanol proving to be more effective than liquid sugar. During the experiment, ethanol dosing was tested at a range of C:N ratios. As the C:N ratio increased, there was a corresponding increase in NO_3^- removal, and this trend was consistent in both warm and cool water conditions. Of the C:N ratios tested, a dosing rate of 1.5:1 was effective at achieving high removal efficiencies at relatively short HRTs. For instance, under cool conditions and at 10-hour HRT, this dosing rate increased the NO_3^- removal efficiency from 23% to 78%. Whereas under warm conditions the same ethanol dosing rate increased NO_3^- removal from 30%

to 83% with a 3.3-hour HRT. This demonstrated that ethanol dosing under warm conditions can also improve bioreactor performance by allowing shorter HRTs to be used, thereby increasing the daily volume of water treated for a given bioreactor size. On average for cool and warm conditions, there was a consistent relationship between the level of ethanol dosing and level of NO_3^- removal efficiency achieved, with removal increasing by approximately 30 percentage points for every 1 unit increase in ethanol C, relatively to N.

Overall, this preliminary study established the effect of different HRTs and ethanol dosing rates on NO_3^- removal, for a range of water temperatures, which helped to identify the treatments to be evaluated in the pilot-scale study at the LWLTS.

6.2.2. Field pilot-scale woodchip bioreactor study (Chapter 4)

This study used pilot-scale bioreactors located at the LWLTS to assess the ability of woodchip bioreactors to reduce drainage water NO_3^- levels. This included evaluating the effect of HRT on NO_3^- removal across a range of warm and cool season water temperatures, and the influence of ethanol dosing on NO_3^- removal, particularly during the cool season.

There were variations in NO_3^- removal efficiency between the cool and warm seasons with removal efficiencies being approximately one-third less under cool conditions. For example, when the bioreactors operated at a 6.6-hour HRT, the average removal efficiencies for the cool and warm season were 24% and 40%, respectively. When operating at a 10-hour HRT, the average removal efficiencies for the cool and warm season were 43% and 59%, respectively. While, at a 20-hour HRT, the removal efficiencies were 69% and 85%, respectively. The pilot-scale bioreactors achieved an average annual NO_3^- removal efficiency of approximately 50% i.e. over both the warm and cool seasons while operating at a 10-hour HRT, which increased to 77% for a 20-hour HRT.

To improve the removal efficiency during the cool season, the pilot bioreactors, operating at a 6.6-hour HRT, were dosed with ethanol at different C:N ratios. Dosing at a C:N ratio of 0.75:1 improved the NO_3^- removal efficiency from 24% to 93%, compared to no dosing. Importantly, secondary effects such as sulphate reduction were minimised in these conditions. However, it is important to note that this ethanol C:N dosing ratio is specific to the conditions at LWLTS, as this result may have been influenced by the pre-existing C content in the drainage water from the site.

The study demonstrated the ability of woodchip bioreactors to treat drainage water from LWLTS as the pilot-scale bioreactors achieved relatively good NO_3^- removal efficiencies with HRTs ranging between 10 and 20 hours. The NO_3^- removal rates observed here were comparable to those reported for full-scale woodchips bioreactors used for treating agricultural drainage waters. This current study also confirmed that woodchip bioreactors have potential to achieve high NO_3^- removals at the LWLTS using relatively short HRTs during the cool season when they were dosed with ethanol.

6.2.3. Full-scale woodchip bioreactor designs (Chapter 5)

This assessment involved scaling-up the results from the pilot study. Two contrasting woodchip bioreactors were designed with a capacity to treat drainage water from the LWLTS to meet the resource consent requirement. The total NO_3^- load in the Waiwiri Stream, downstream of the LWLTS is estimated to be 32.1 kg N/day, of which about 30.2 kg N/day is contributed by the site. The resource consent for the LWLTS requires a 10% reduction in the stream's total NO_3^- load, which is 3.2 kg N/day. Drain 3 was identified as being the most suitable drain on the site for achieving the required NO_3^- load reduction.

The proposed bioreactors for the LWLTS included a non-dosed 645 m^3 woodchip bioreactor, operating at a 20-hour HRT, and an ethanol-dosed 197 m^3 bioreactor, operating at a 6.6-hour HRT. Both bioreactors had a target average NO_3^- removal efficiency of approximately 80%. There are clearly other potential combinations of bioreactor size, HRT and ethanol dosing that would achieve the same NO_3^- removal at the LWLTS, but for the purpose of the study the proposed ones were discussed for comparison purposes. While the two bioreactor designs have the same target NO_3^- removal efficiency, and they will remove the same NO_3^- load, they have different NO_3^- removal rates per m^3 of bioreactor. Both designs have a similar NO_3^- removal cost annualised over a 10-year period, being NZ$6.90 kg NO_3^-/year for the larger non-dosed bioreactor and NZ$ 6.50 kg NO_3^-/year for the smaller ethanol-dosed bioreactor. However, the larger non-dosed bioreactor will have a 218% greater installation cost. With the bioreactors intended for continuous, year-round, use a potential limitation of the non-dosed bioreactor is that it is more prone to a decline in soluble C supply, and consequently decrease in NO_3^- removal efficiency, due to its full reliance on woodchips as the C source. Whereas a potential limitation of the ethanol-dosed bioreactor is that it has limited capacity to further increase NO_3^- removal, and has a higher risk of bioclogging, which would reduce its hydraulic conductivity over time. Because the larger non-dosed bioreactor provides greater flexibility to increase NO_3^- removal, and is less vulnerable to losses in hydraulic conductivity, it is a more flexible option. In addition, any potential decline in NO_3^- removal efficiency in the future, could be compensated for by the introduction of ethanol dosing. While this would increase the cost of removal, it is expected to not exceed NZ$ 11 kg NO_3^-/year, and it would ensure continuous compliance with the resource consent requirement. Regular monitoring of the bioreactor performance will be important to identify any reductions in removal efficiency over time, so that operational conditions can be adjusted accordingly.

When designing a woodchip bioreactor, there are a number of factors to be considered if optimal performance is to be achieved. One of these considerations is the dimensions of the bioreactors. Christianson et al. (2013) suggests that a bioreactor with a length to width ratio of 4:1 provides good distribution of water flow through the bioreactor. For the larger 645 m^2 bioreactor, this means recommended dimensions of approximately 50 m long, 13 m wide and 1 m deep. Location is another important design consideration. As discussed in Chapter 5, several factors must be considered when selecting a suitable location within the LWLTS. These factors include proximity to Drain 3, ensuring that drainage water collection occurs downstream of the wastewater holding pond and near the drain outlet into the Waiwiri stream, availability of sufficient land surface to accommodate the selected bioreactor size and dimensions. Considering these factors, Figure 6.1 provides an example of a suitable location at the LWTS, which is close to the site of the pilot-scale bioreactors and is close to Drain 3. Moreover, the site is downstream of the pond and situated close (approximately 400 meters) to the drain discharge point into the Waiwiri Stream. Another advantage of this location is that is easy to access and is not far from other infrastructure, facilitating the installation of electricity and equipment for pumping water from the drain.

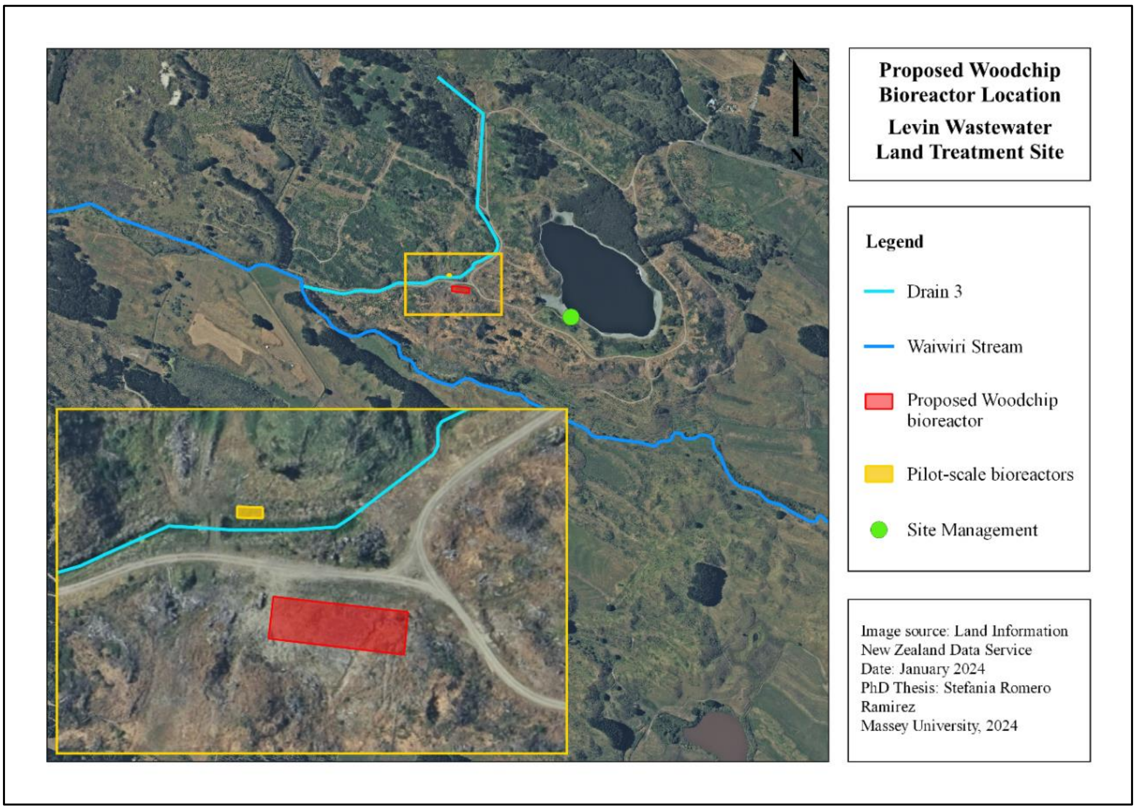

Figure 6.1. Proposed location for the designed bioreactor at the Levin Wastewater Land Treatment Site.

The conditions at the LWLTS are specific to the landscape, soils, and hydraulic characteristics of the site. However, woodchip bioreactors, as an end-of-drain treatment method, may be applicable to other sites where municipal wastewater is currently land applied or where land treatment is proposed. Currently, approximately 46% of wastewater treatment plants in New Zealand still discharge partially treated wastewater to freshwater rivers (Water New Zealand, 2020). However, the practice of land treatment, while not widespread, is increasingly becoming a more favoured method of wastewater treatment. This results from growing community awareness of the associated environmental impacts of wastewater discharges, including Māori cultural concerns. Therefore, it is likely that land treatment will become more common, which may provide more opportunities for the use of woodchip bioreactors in the future, especially in situations where an additional level of protection is needed to meet NO_3^- discharge standards.

6.3. Recommended future research

This PhD thesis concluded that woodchip bioreactors are a suitable end-of-drain treatment strategy for drainage water derived from municipal wastewater land treatment sites, and that ethanol dosing of these bioreactors can enhance NO_3^- removal. However, areas that have been identified that could benefit from further research include:

1. Assess the long-term supply of soluble C in woodchips bioreactors, treating drainage water from wastewater land application sites, that are under continuous year-round use.
2. Analyse the long-term performance of ethanol-dosed woodchip bioreactors, including flow analysis and hydraulic performance to determine the possible risks of bioclogging associated with the dosing.
3. Evaluate how the microbial communities inside woodchip bioreactors changes when ethanol is added as soluble C and determine in what proportion the microbial communities utilise the soluble C derived from ethanol dosing and from the decomposition of the woodchips.
4. Explore the viability of utilising alternative substrates, distinct from woodchips, for treatment solely relying on ethanol dosing as the C source. Ideally, identify an inert low-cost medium resistant to breakdown, yet favourable for microbial growth.

6.4. References

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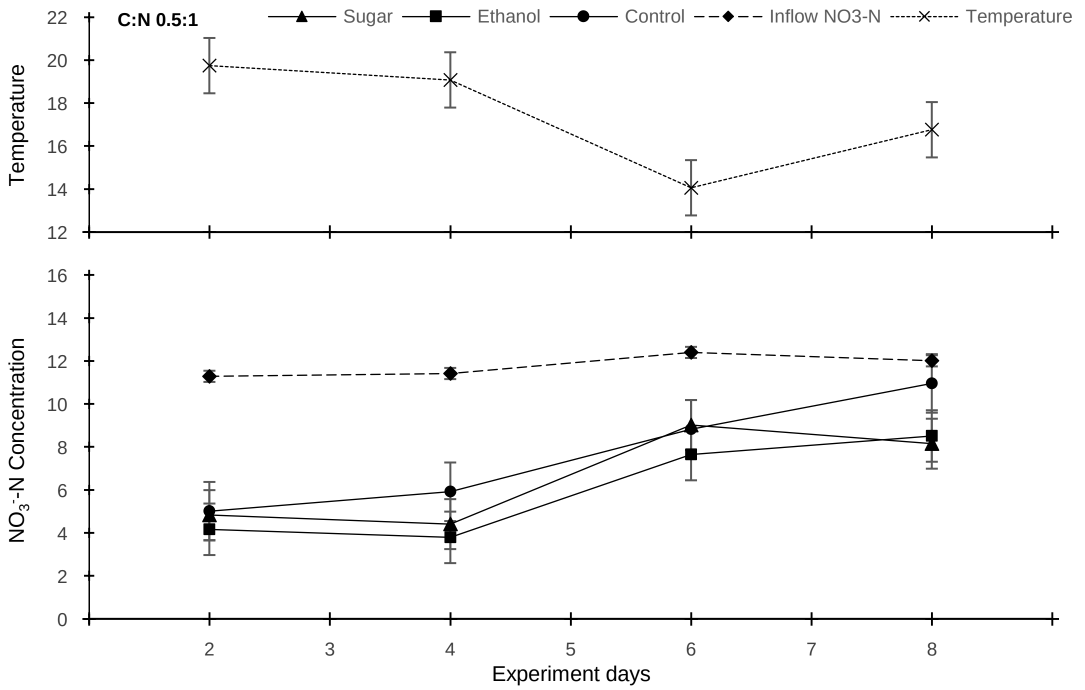
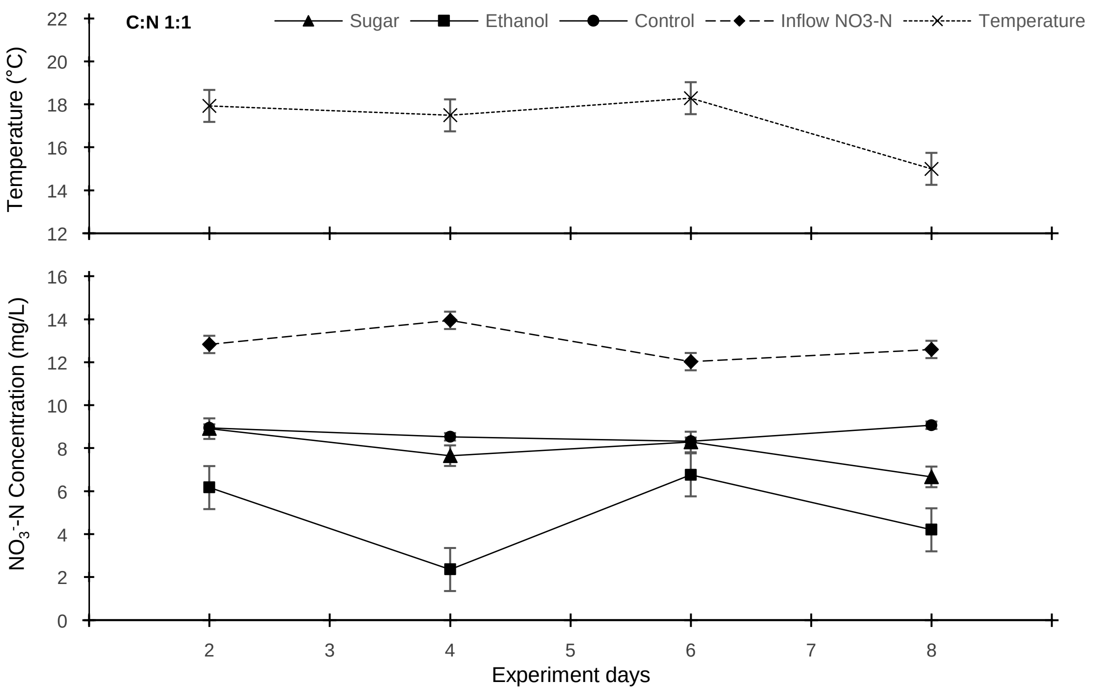
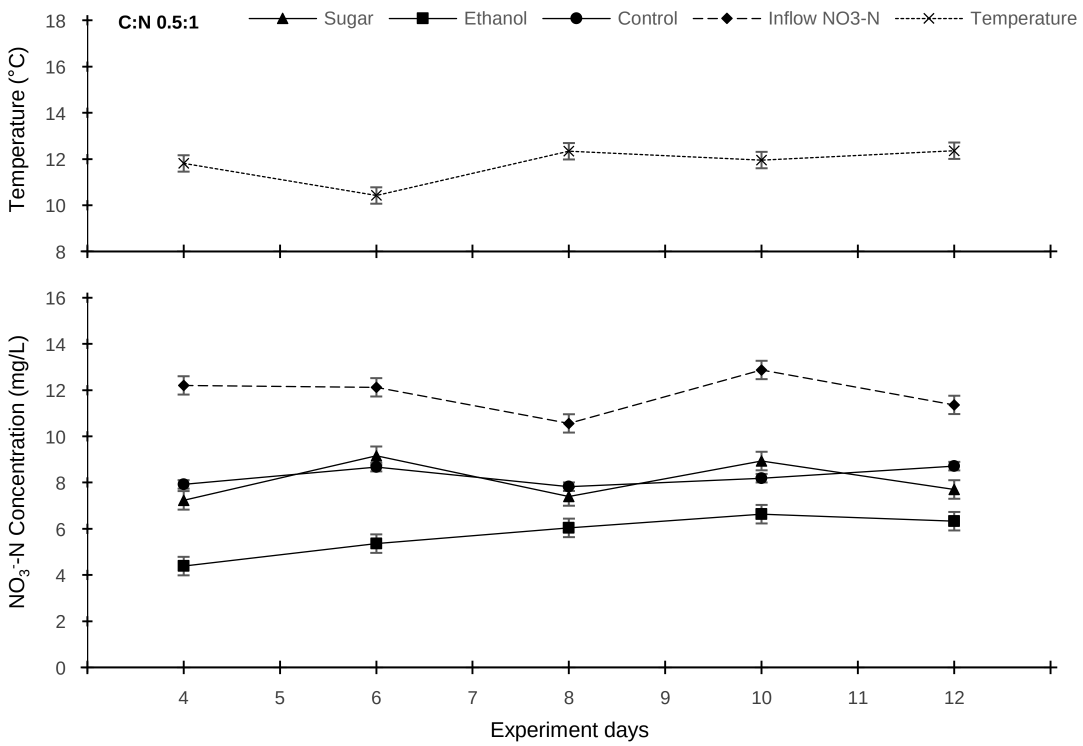
Appendix 1: Column bioreactors - Average nitrate concentrations and temperatures per experiment



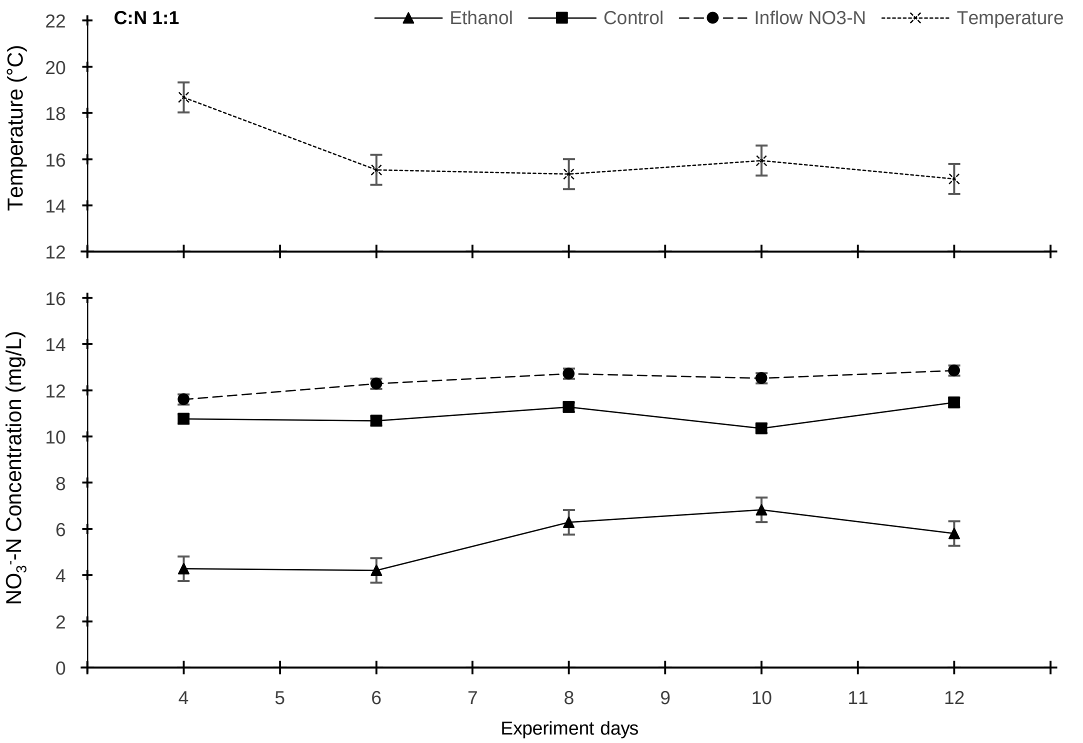
Appendix 1.1. Experiment 2 (warm conditions, 3.3-hour HRT) - Average NO_3^--N concentrations and temperature per sampling day for each treatment at the 0.5:1 C:N ratio evaluation. Error bars represent standard error.



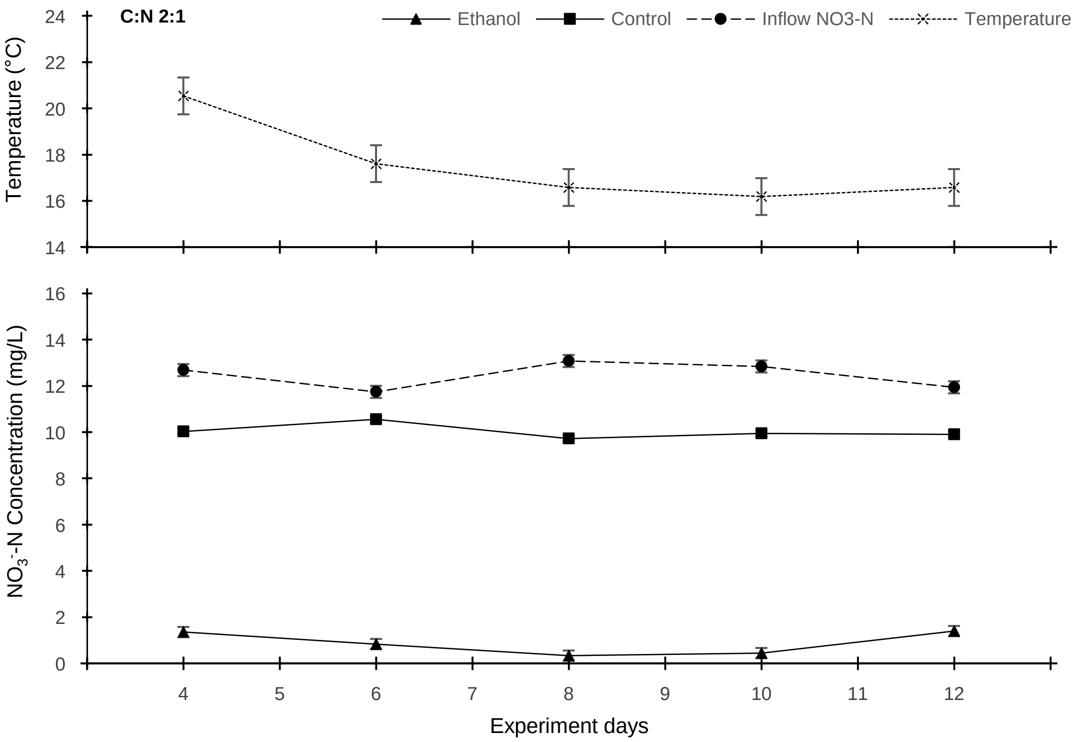
Appendix 1.2. Experiment 2 (warm conditions, 3.3-hour HRT) - Average NO_3^--N concentrations and temperature per sampling day for each treatment at the 1:1 C:N ratio evaluation. Error bars represent standard error.



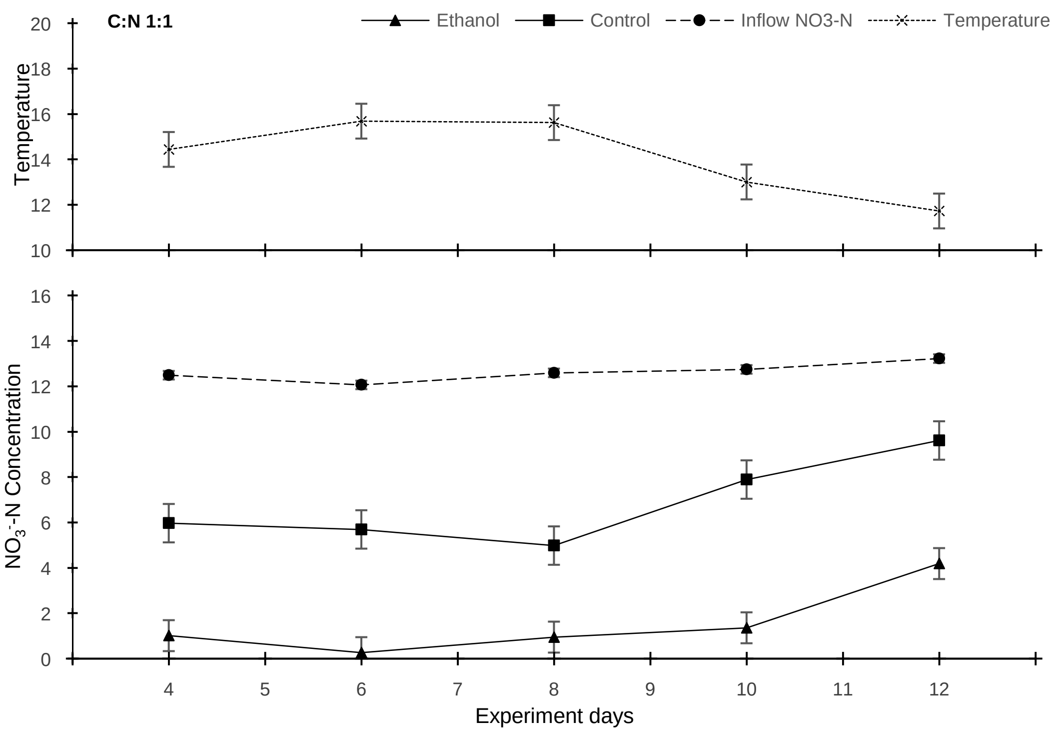
Appendix 1.3. Experiment 3 (cool conditions, 10-hour HRT) - Average NO_3^--N concentrations and temperature per sampling day for each treatment at the 0.5:1 C:N ratio evaluation. Error bars represent standard error.



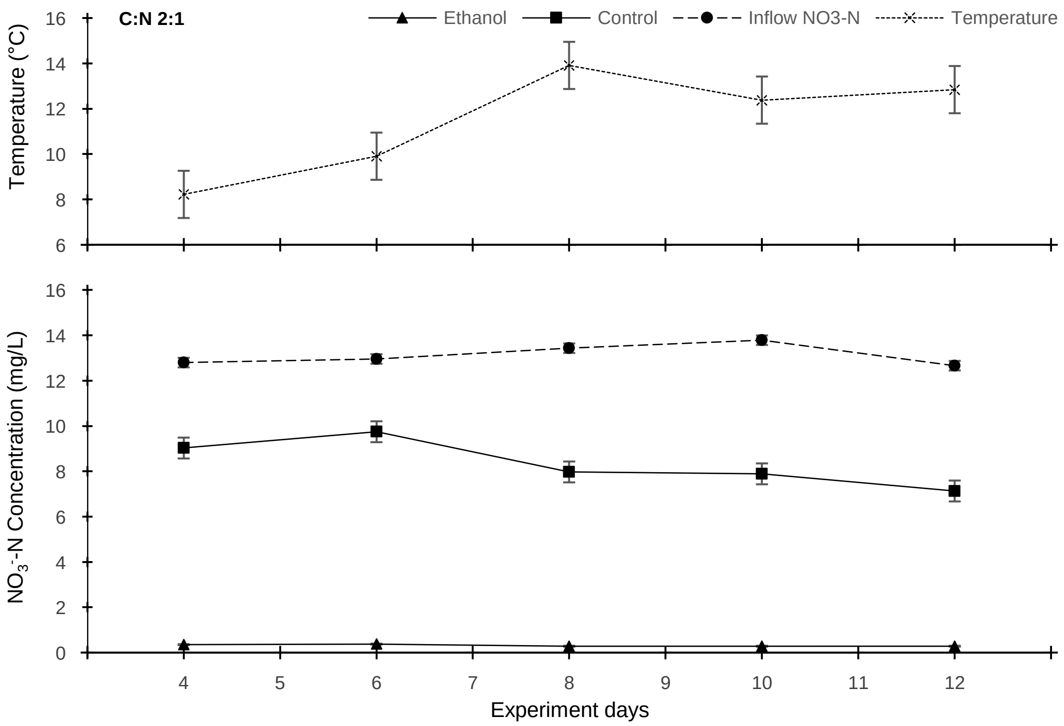
Appendix 1.4. Experiment 4 (warm conditions, 3.3-hour HRT) - Average NO_3^--N concentrations and temperature per sampling day for each treatment at the 1:1 C:N ratio evaluation. Error bars represent standard error.



Appendix 1.5. Experiment 4 (warm conditions, 3.3-hour HRT) - Average NO_3^--N concentrations and temperature per sampling day for each treatment at the 2:1 C:N ratio evaluation. Error bars represent standard error.

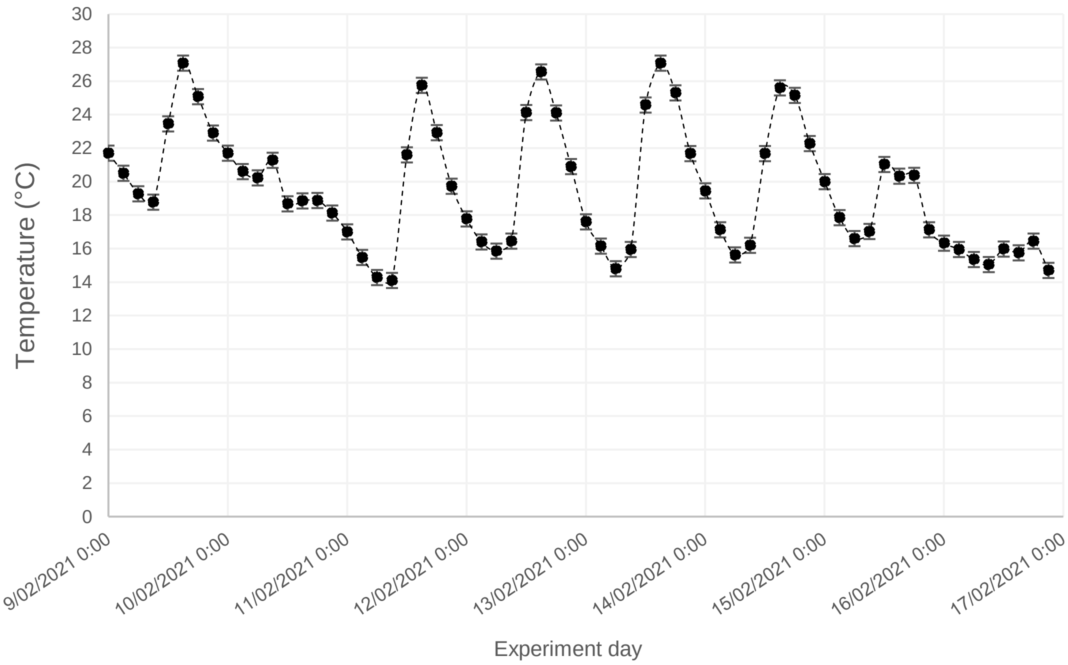


Appendix 1.6. Experiment 5 (cool conditions, 10-hour HRT) - Average NO_3^--N concentrations and temperature per sampling day for each treatment at the 1:1 C:N ratio evaluation. Error bars represent standard error.

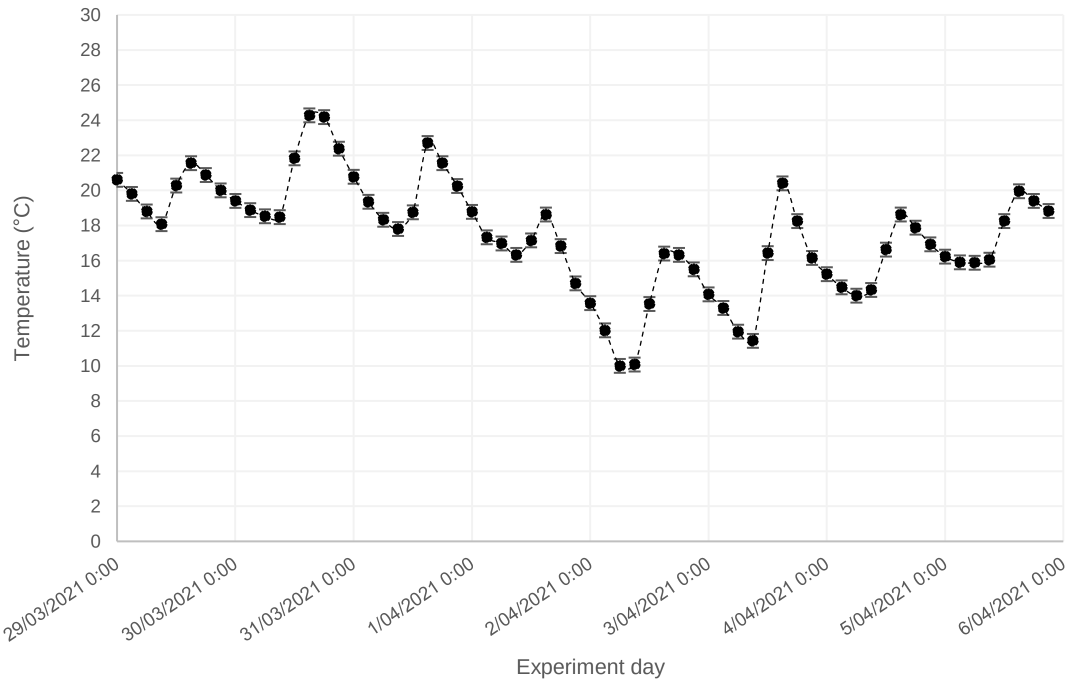


Appendix 1.7. Experiment 5 (cool conditions, 10-hour HRT) - Average NO_3^--N concentrations and temperature per sampling day for each treatment at the 2:1 C:N ratio evaluation. Error bars represent standard error.

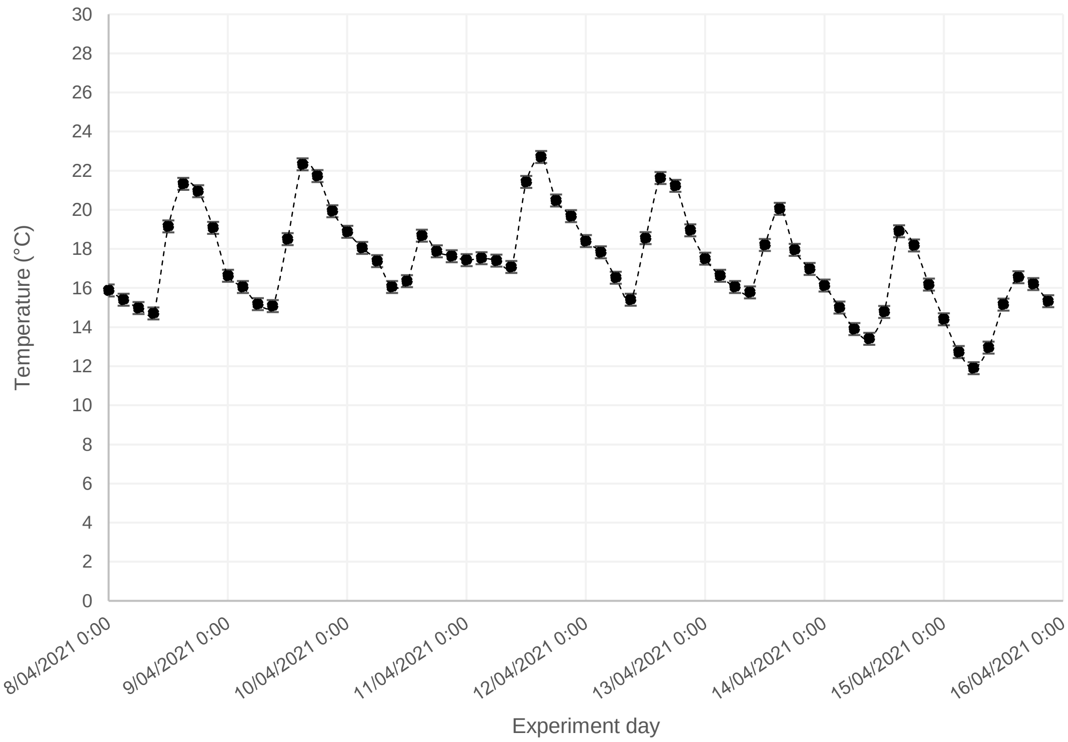
Appendix 2: Column bioreactors - Daily temperature variations per experiment



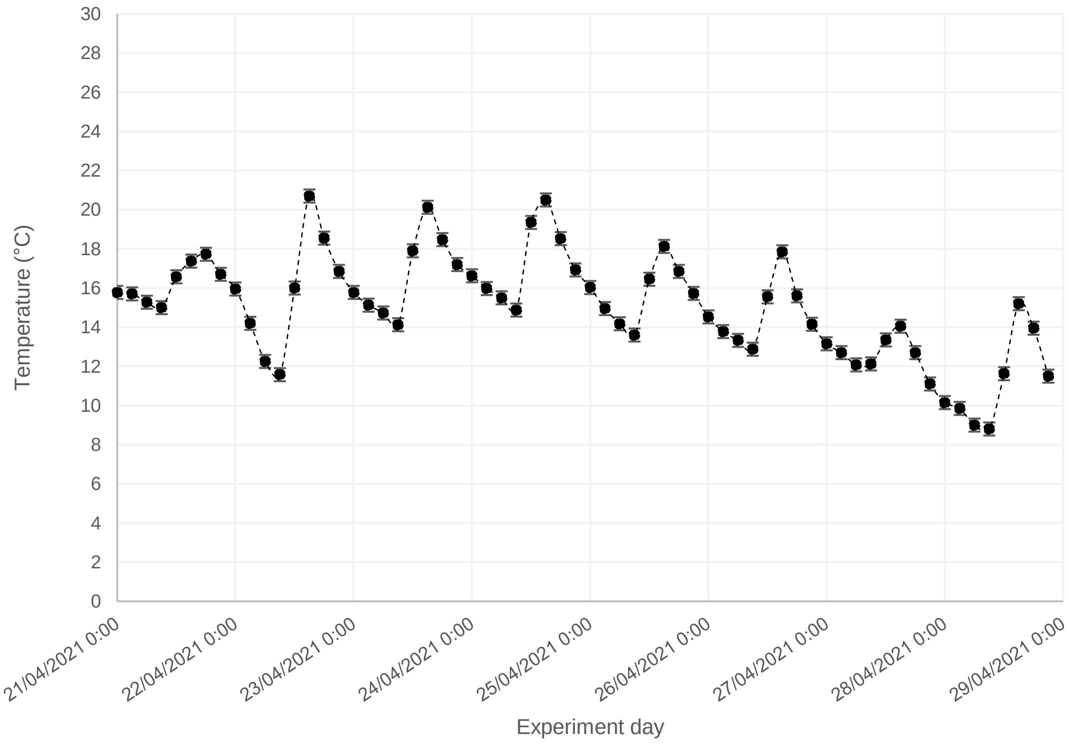
Appendix 2.1. Experiment 1 (HRT – warm conditions) – Average daily temperature variations recorded every three hours inside the column bioreactors. Error bars represent standard error.



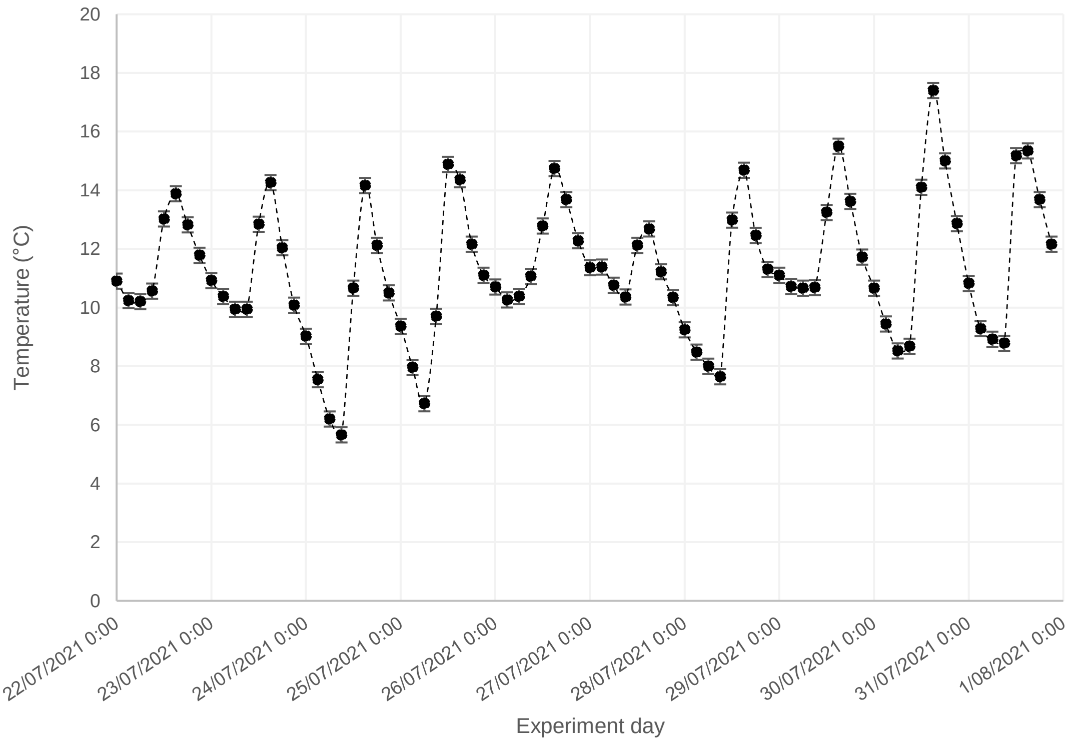
Appendix 2.2. Experiment 2 (0.5:1 C:N ratio, warm conditions, 3.3-hour HRT) - Average daily temperature variations recorded every three hours inside the column bioreactors. Error bars represent standard error.



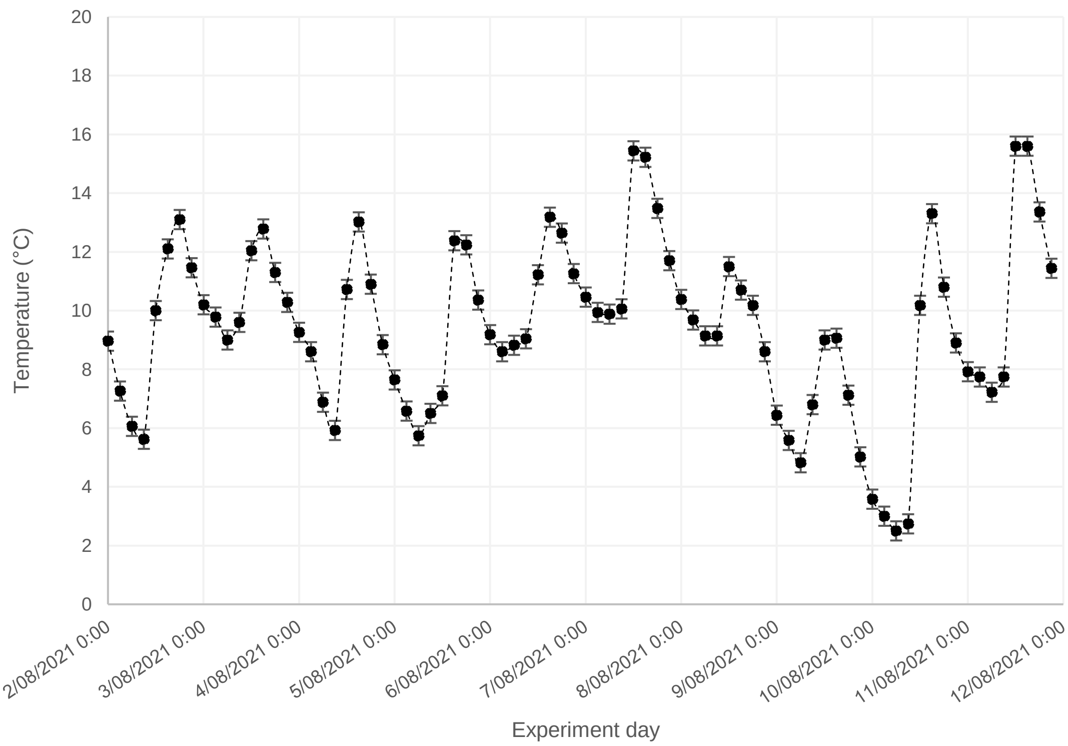
Appendix 2.3. Experiment 2 (1:1 C:N ratio, warm conditions, 3.3-hour HRT) - Average daily temperature variations recorded every three hours inside the column bioreactors. Error bars represent standard error.



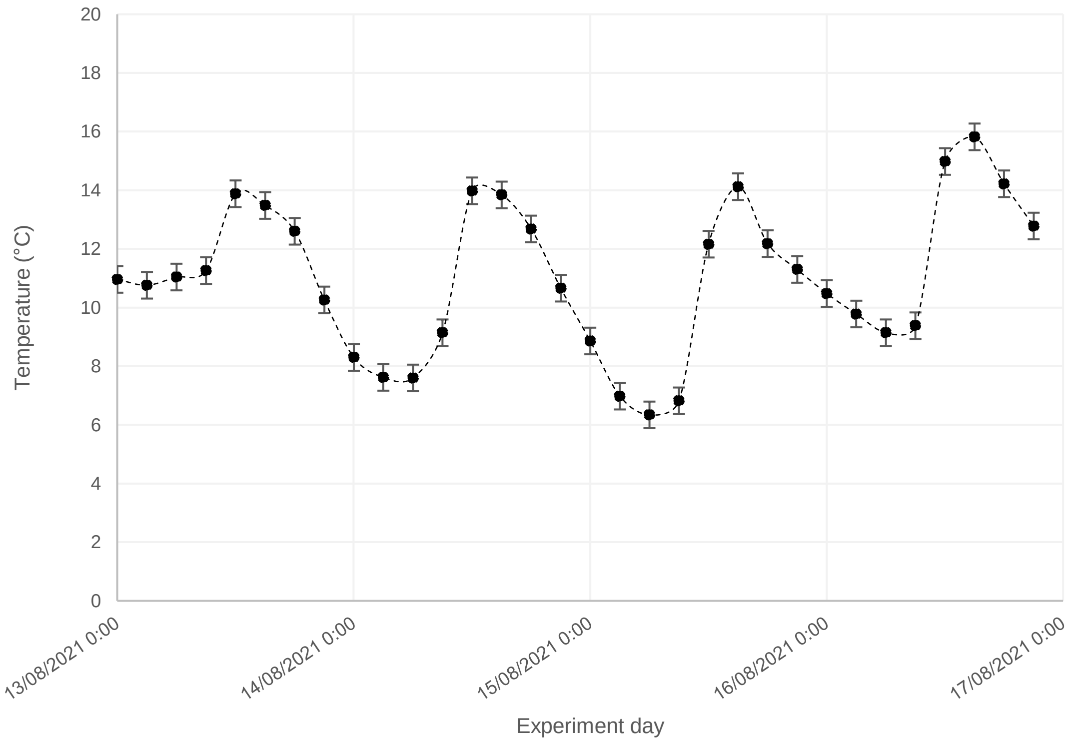
Appendix 2.4. Experiment 2 (2:1 C:N ratio, warm conditions, 3.3-hour HRT) - Average daily temperature variations recorded every three hours inside the column bioreactors. Error bars represent standard error.



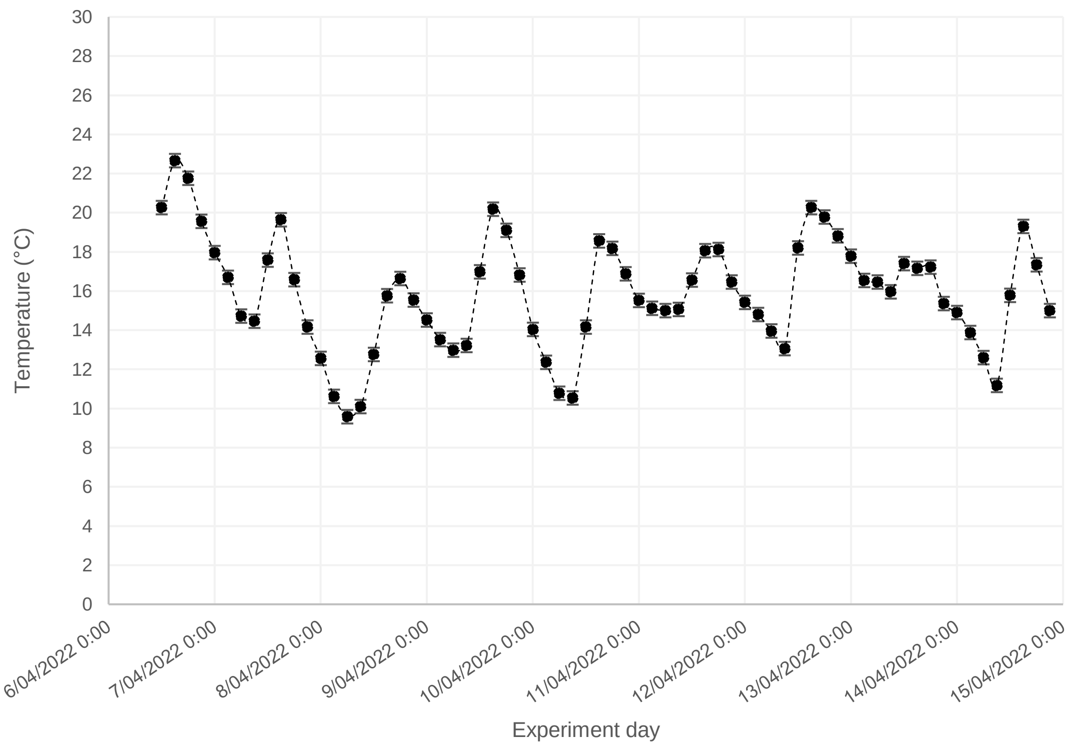
Appendix 2.5. Experiment 3 (0.5:1 C:N ratio, cool conditions, 10-hour HRT) - Average daily temperature variations recorded every three hours inside the column bioreactors. Error bars represent standard error.



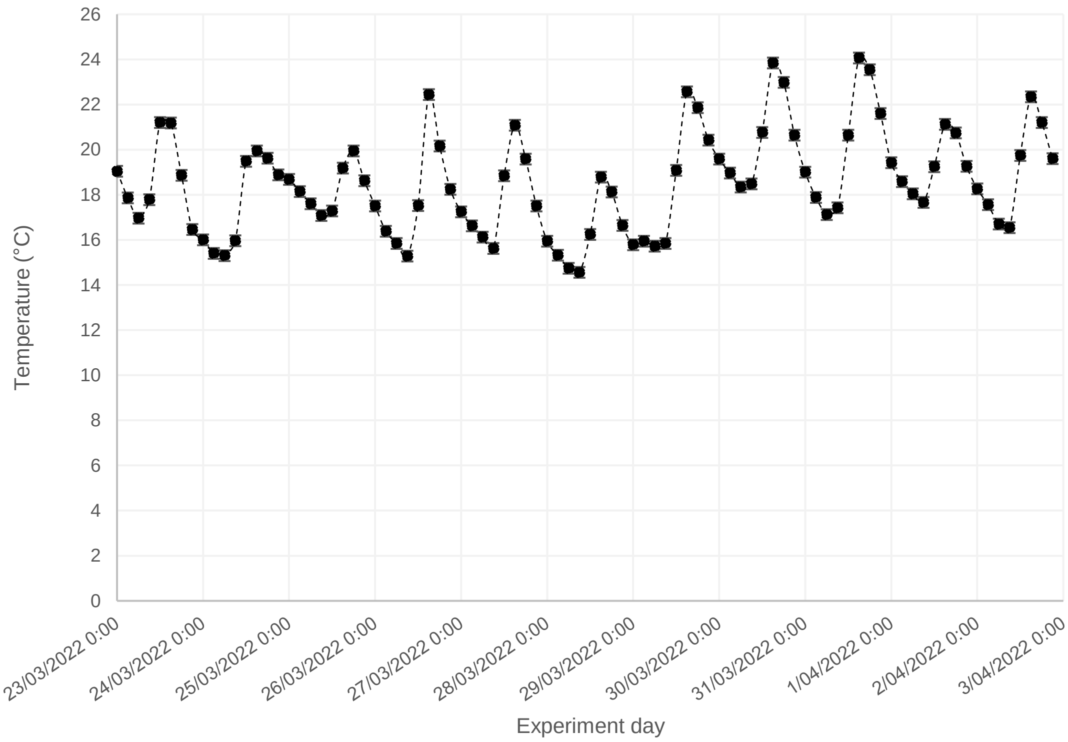
Appendix 2.6. Experiment 3 (1:1 C:N ratio, cool conditions, 10-hour HRT) - Average daily temperature variations recorded every three hours inside the column bioreactors. Error bars represent standard error.



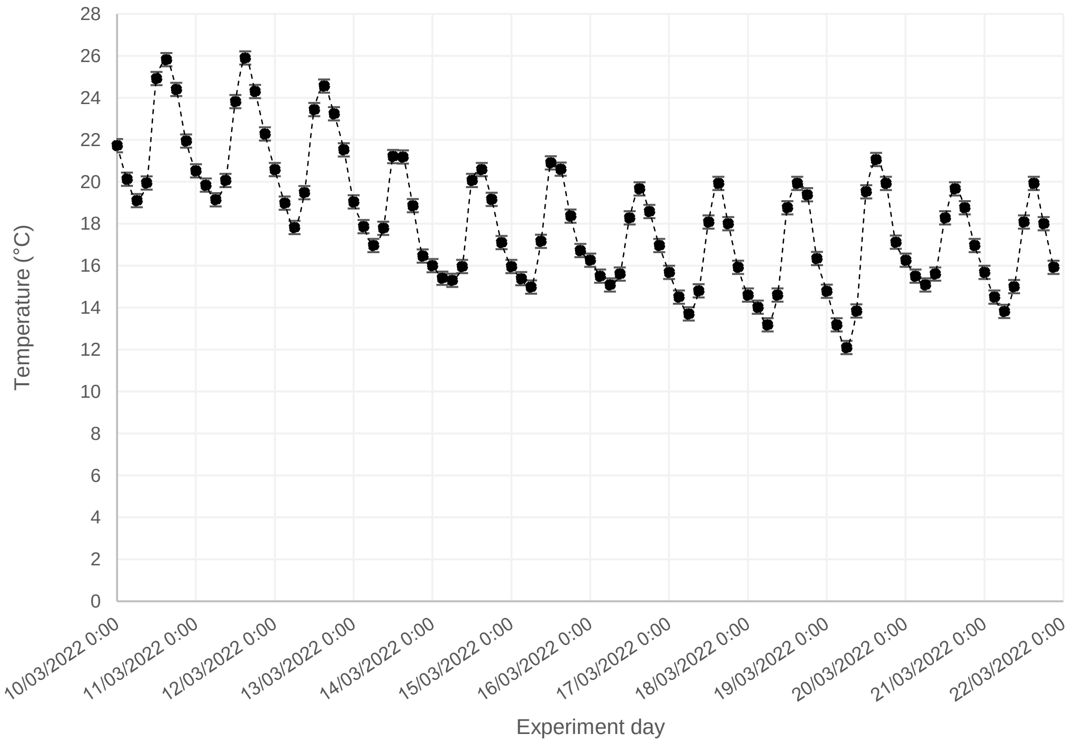
Appendix 2.7. Experiment 3 (2:1 C:N ratio, cool conditions, 10-hour HRT) - Average daily temperature variations recorded every three hours inside the column bioreactors. Error bars represent standard error.



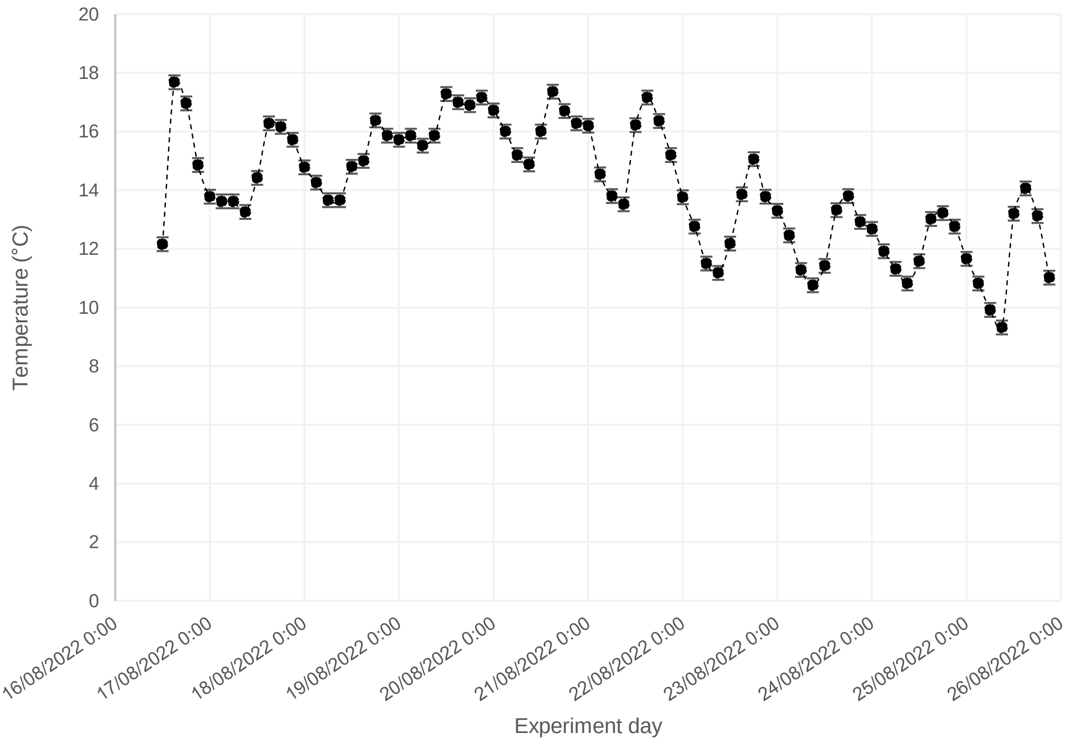
Appendix 2.8. Experiment 4 (1:1 C:N ratio, warm conditions, 3.3-hour HRT) - Average daily temperature variations recorded every three hours inside the column bioreactors. Error bars represent standard error.



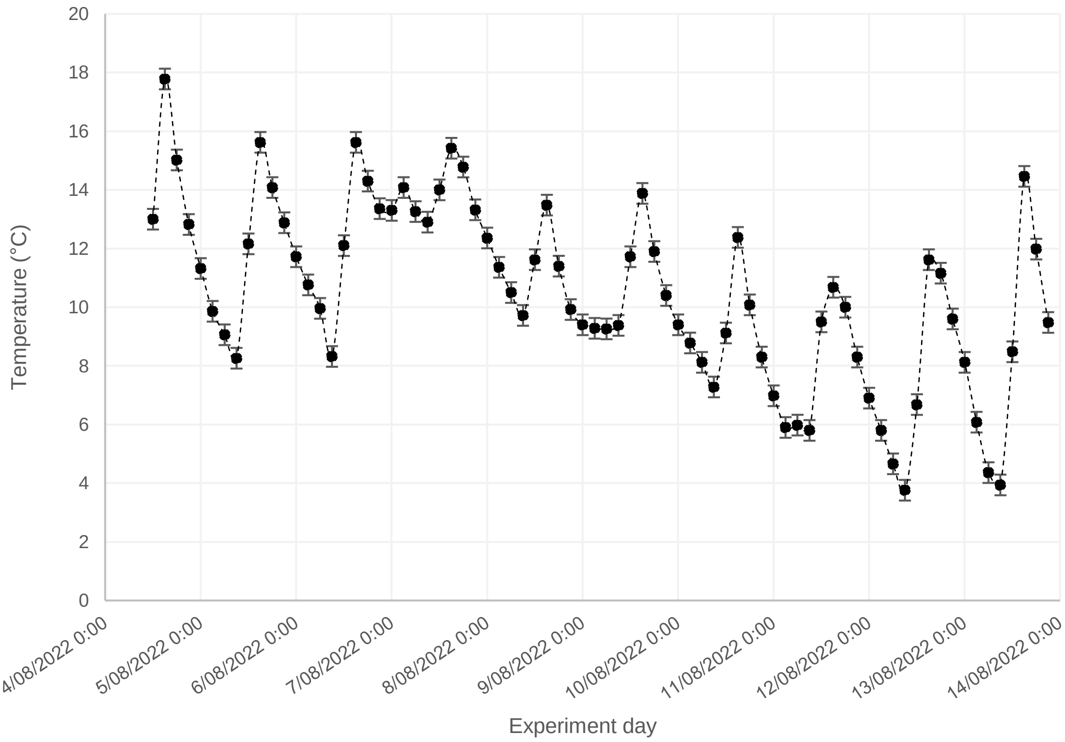
Appendix 2.9. Experiment 4 (1.5:1 C:N ratio, warm conditions, 3.3-hour HRT) - Average daily temperature variations recorded every three hours inside the column bioreactors. Error bars represent standard error.



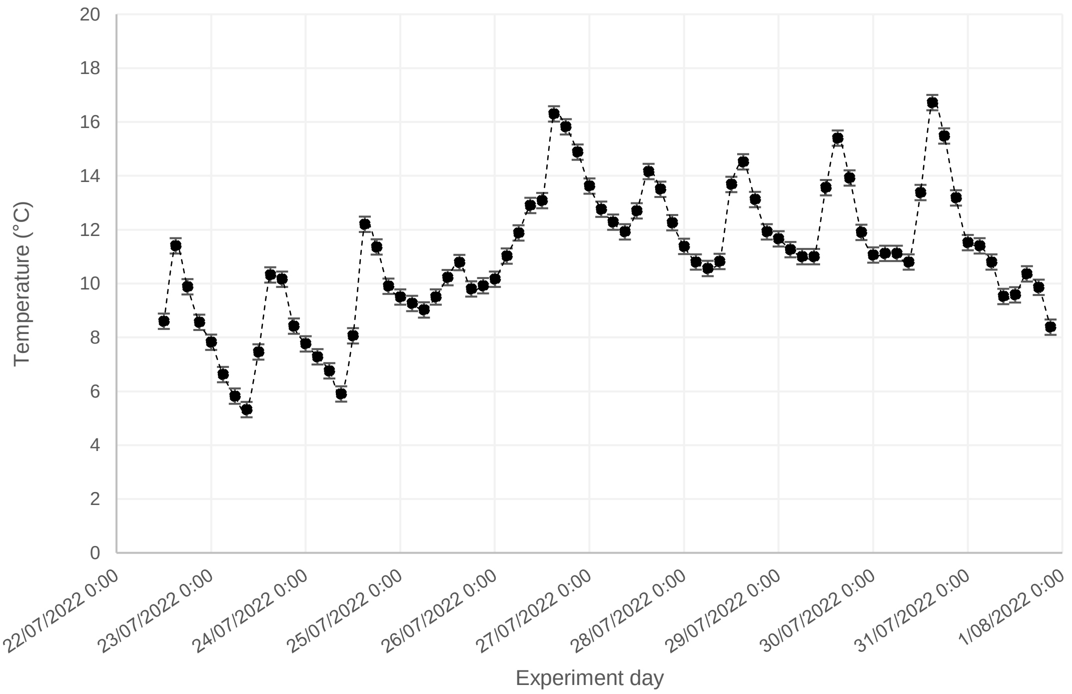
Appendix 2.10. Experiment 4 (2:1 C:N ratio, warm conditions, 3.3-hour HRT) - D Average daily temperature variations recorded every three hours inside the column bioreactors. Error bars represent standard error.



Appendix 2.11. Experiment 5 (1:1 C:N ratio, cool conditions, 10-hour HRT) - Average daily temperature variations recorded every three hours inside the column bioreactors. Error bars represent standard error.

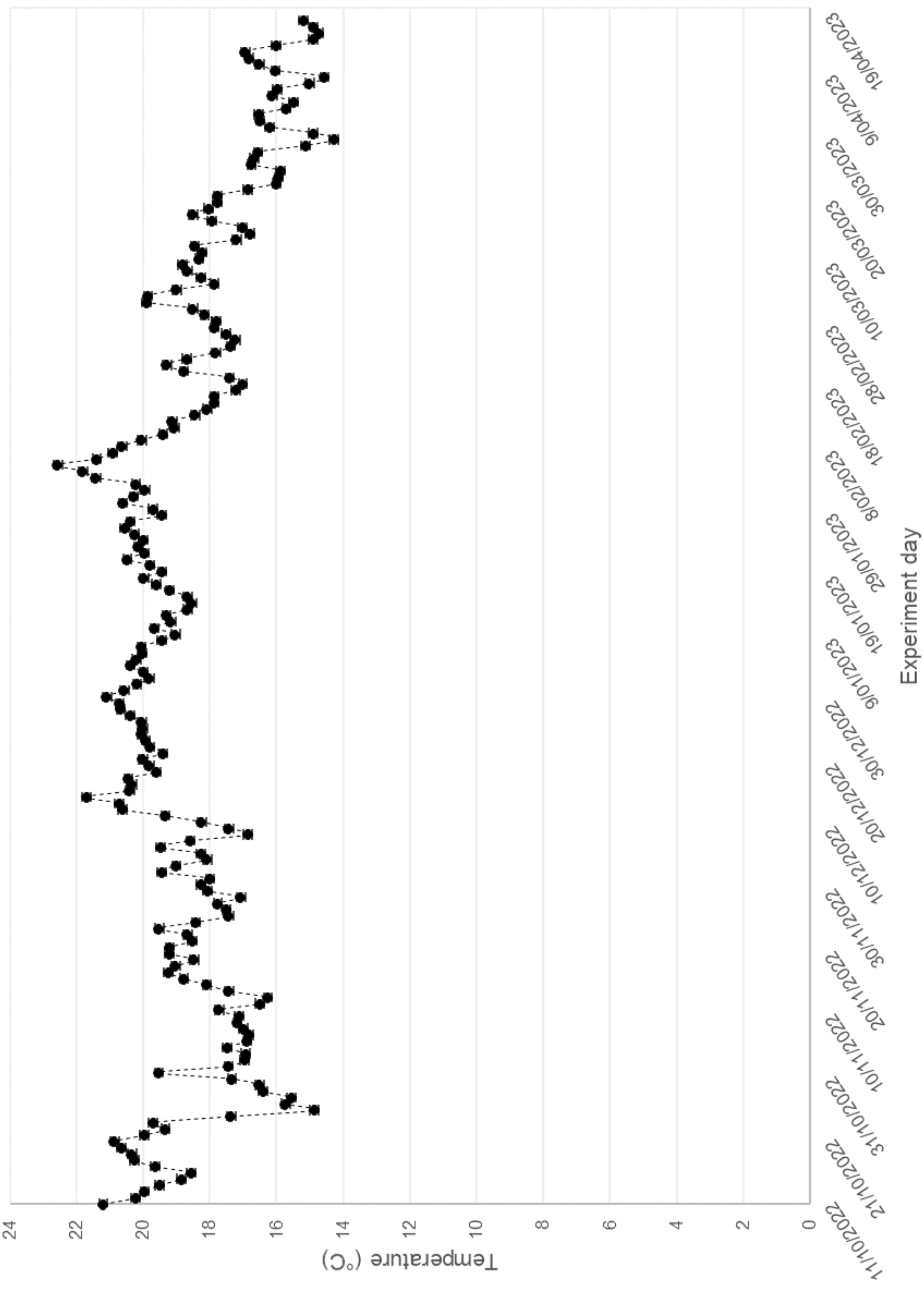


Appendix 2.12. Experiment 5 (1.5:1 C:N ratio, cool conditions, 10-hour HRT) - Average daily temperature variations recorded every three hours inside the column bioreactors. Error bars represent standard error.

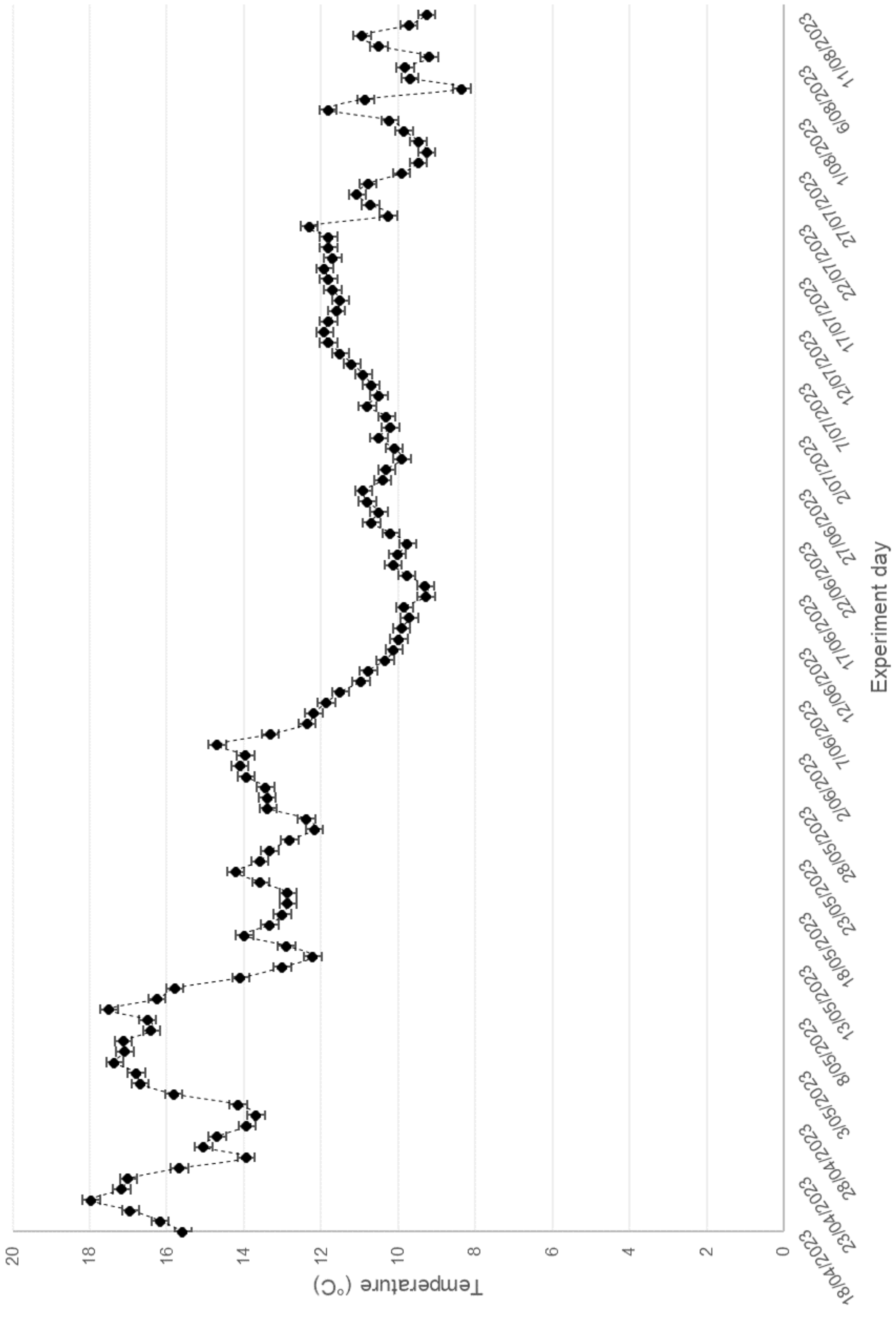


Appendix 2.13. Experiment 5 (2:1 C:N ratio, cool conditions, 10-hour HRT) – Average daily temperature variations recorded every three hours inside the column bioreactors. Error bars represent standard error.

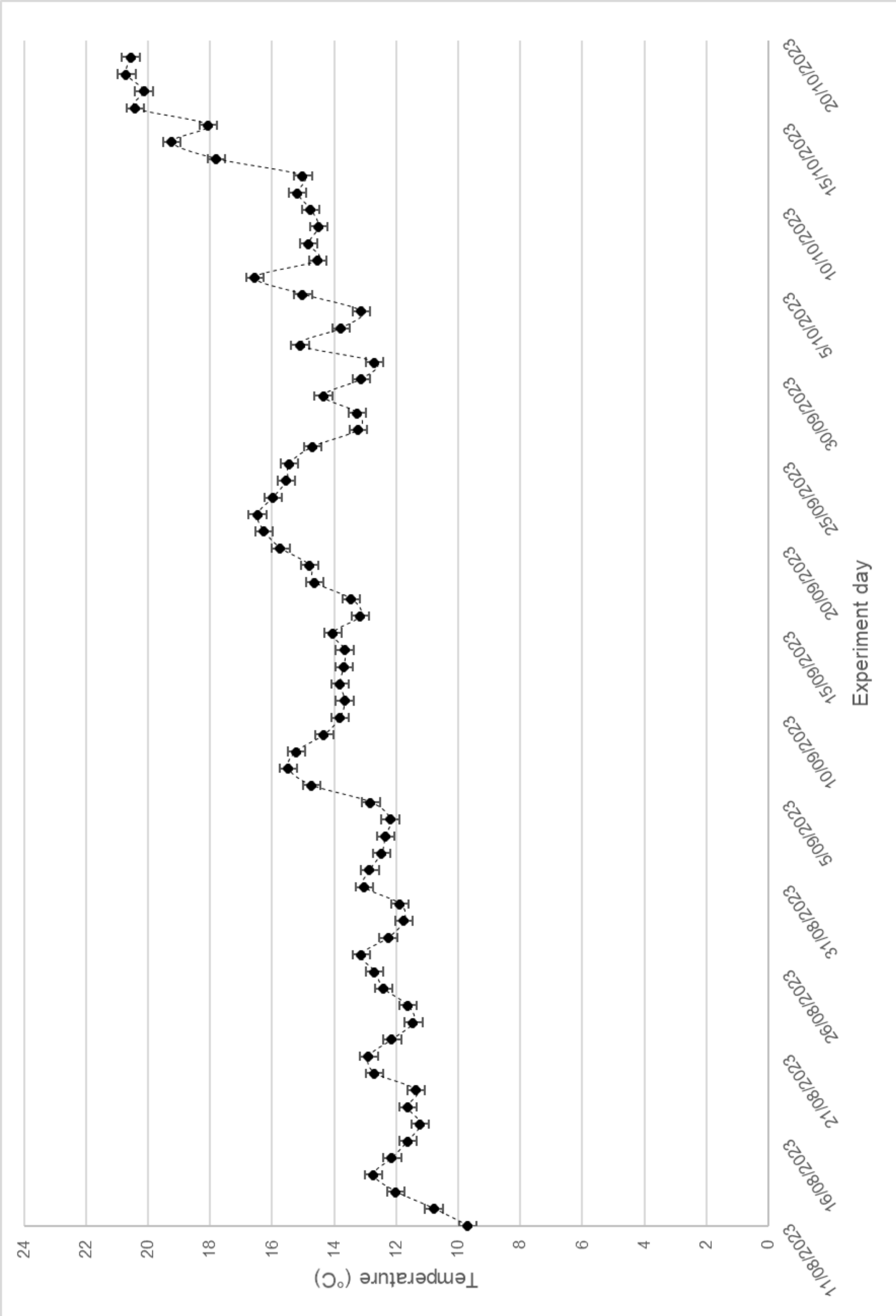
Appendix 3: Field pilot-scale bioreactors - Daily temperature variations



Appendix 3.1. Stage 1 experiments (HRT treatments - warm conditions) – Average daily temperature recorded inside the pilot bioreactors. Error bars represent standard error.



Appendix 3.2. Stage 2 experiments (Ethanol C:N ratio treatments - cool conditions) – Average daily temperature recorded inside the pilot bioreactors. Error bars represent standard error



Appendix 3.3. Stage 3 experiments (Ethanol C:N ratio treatments - HRT treatments - cool conditions) – Average daily temperature recorded inside the pilot bioreactors. Error bars represent standard error