Bacterial Inoculation of *Pseudomonas fluorescens* in Aquaponic Systems

An Assessment of its Potential Influence on Nitrification

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Abstract

Aquaponic systems are increasingly recognized as sustainable alternatives to conventional agricultural systems, offering enhanced resource efficiency in terms of both water and nutrients. However, key factors that could further improve the productivity and resilience of these systems, such as improved nitrification, remain largely underexplored. This dual case study primarily investigated the effects of bacterial inoculation with two strains of *Pseudomonas fluorescens* on nitrogen dynamics in a coupled aquaponic system, while also examining the relative amount and distribution of microbial communities across system compartments. Water samples used in this study were collected from the fish tank and the biofilter at different time points and inoculated under controlled conditions. The two strains were originally isolated from a recirculating aquaculture system at Gårdsfisk located in Kristianstad, Sweden. Concentrations of ammonium, nitrite, nitrate and total inorganic nitrogen (TIN) were measured over two consecutive weeks to examine nitrogen transformations and assess overall nitrification activity. Bacterial inoculation resulted in significantly increased nitrate and TIN production in biofilter water relative to controls. In contrast, these effects were not consistently observed in fish tank water, where nitrification activity was presumed low. Analysis of culturable microbial groups revealed significant differences in microbial community structure between system compartments, however interpretation of these results was limited. Overall, the results indicate that the influence of *P. fluorescens* on nitrogen dynamics is strongly context dependent and likely mediated through indirect mechanisms such as enhanced mineralization or synergistic interactions with already existing nitrifying autotrophs. While the findings of this study suggest potential benefits of inoculating with *P. fluorescens* during biofilter establishment, further controlled studies are required to evaluate its applicability and consistency across varying water conditions.

Keywords: Aquaponics, Nitrification, *Pseudomonas fluorescens*, Nile tilapia, Inoculation, Ammonium, Nitrite, Nitrate, Total inorganic nitrogen (TIN)

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Abbreviations

AOB	Ammonia oxidizing bacteria
DWC	Deep water culture
NFT	Nutrient film technique
NOB	Nitrate oxidizing bacteria
PGPR	Plant growth-promoting rhizobacteria
RAS	Recirculating aquaculture system
TAN	Total ammonia nitrogen
TIN	Total inorganic nitrogen

1. Introduction

One of the most pressing challenges of the 21st century is how to sustainably feed a growing global population. As the number of people on Earth continues to rise, which is expected to reach nearly 10 billion by 2050, the demand for food will increase significantly (FAO 2017; 2025). It is estimated by the Food and Agriculture Organization (FAO) that global food production must increase by approximately 50 % compared with 2012 levels to meet this demand and enhance global food security (FAO 2017).

Currently, the majority of agricultural systems that feed the world are straining under their own environmental impacts. Although crucial for global food security, traditional farming methods are major contributors to freshwater depletion, soil degradation, environmental pollution, and greenhouse gas emissions (Smith et al. 2007; Abbasi et al. 2014; Hussain et al. 2021; Ingrao et al. 2023). Hence, agriculture becomes a significant threat to natural ecosystems and a major driver of climate change. These issues highlight a fundamental contradiction. While there is an urgent need to increase food production, we cannot rely solely on practices that degrade the very ecosystems on which agriculture depends. In addition, ongoing urbanization is expected to intensify in the coming decades, increasing demand for locally produced food in areas where large-scale traditional food systems are often unsuitable (Eigenbrod & Gruda 2015; United Nations 2025). Taken together, environmental pressures and rising urbanization call for diversification of our food production methods and the development of innovative and more sustainable systems.

In response to these limitations of conventional agriculture, aquaponics has emerged as a possible solution. Aquaponics represent a production method that integrates tank-based aquaculture (the farming of aquatic animals) and hydroponics (soilless plant cultivation) within a coupled system (Baganz et al. 2022). It emphasizes environmental responsibility and minimal resource utilization, while operating independently of arable land. Furthermore, it can be effectively implemented into space-limited urban environments. The combination of sustainability and spatial adaptability makes aquaponics a compelling approach for producing fresh, local food efficiently in urban settings.

Despite its apparent advantages, the productivity of aquaponic systems can vary considerably and are strongly influenced by factors such as system configuration, energy input, environmental conditions, animal and plant stocking densities, as well as the load and diversity of microflora within the system. Particularly the development of microbial communities affecting the nitrogen cycle can be a slow process, which may delay stabilization and reduce early productivity of newly installed systems. This challenge forms the basis of the present study and underscores the need for improved understanding of how bacterial inoculation may influence nitrogen transformation processes and overall system performance.

1.1. Aim of study

The aim of this study is to primarily investigate the effects of bacterial inoculation of two different strains of *Pseudomonas fluorescens* on the nitrogen dynamics in water samples from a two month old aquaponic system. Additionally, this study explores the diversity and relative amount of three different microbial communities within varying compartments of the same system. This study could potentially provide more knowledge surrounding the effect of *P. fluorescens* as a bacterial inoculum and its effect on the rate of nitrification, which could further enhance the productivity of aquaponic systems.

1.1.1. Research questions

1. How does the addition of a bacterial inoculum affect the rate of nitrification in water sampled from an aquaponic system?
2. How does the relative amount of three different microbial communities vary in different compartments of the system?

1.1.2. Hypothesis

The hypothesis for this study is that the addition of bacterial inoculum will alter the nitrogen dynamics in aquaponic water, leading to an increased rate of nitrification compared to uninoculated control. Moreover, it is hypothesized that microbial populations will generally be greater in the biofilter compared to the fish tank, while the relative proportions of major microbial groups of general bacteria, pseudomonads and fungi will remain largely consistent across the system.

2. Background

2.1. Aquaponics

While there are claims of more ancient practices linking aquaculture and plant cultivation, modern aquaponics was developed and established in the United States during the 1970s and 1980s, most notably through research led by Dr. James Rakocy and his colleagues at the University of the Virgin Islands (Lennard & Goddek 2019). Since then, it has garnered increasing scientific and commercial interest, leading to continuous refinement of system configurations and operating strategies (Kotzen et al. 2019; Nair et al. 2025). Although a wide range of aquatic animals and plant species can be cultivated, commercial production largely focuses on freshwater fish species and high value crops such as leafy greens (Love et al. 2015; Thorarinsdottir 2015). A common alternative for fish is tilapia, among which Nile tilapia (*Oreochromis niloticus*) is considered one of the hardiest species, capable of tolerating high nutrient loads and stocking densities (Pompa & Masser 1999; Lim & Carl D 2006; Abd El-Hack et al. 2022).

The main principle of aquaponics is that nutrient-rich wastewater from fish-tank culture can be repurposed as a natural fertilizer for plant growth. Through nutrient uptake, the plants contribute to water purification, allowing the treated water to be recirculated back to the fish tanks. Central to this process is the innate microflora, in particular nitrifying bacteria, which is responsible for converting solid and soluble fish waste into readily available nutrients that plants can use (Rakocy 2012).

Given the system's extensive dependence on biological processes, maintaining the health of fish, plants and microflora is vital for achieving high productivity. Ensuring good water quality and stable operating conditions is therefore essential. Several parameters such as pH, dissolved oxygen (DO), temperature and nutrient levels are important to monitor and control daily. Fish feed acts as the main input of essential macro- and micronutrients required for plant growth. Feed composition is predominantly based on the type of fish but can be refined further to accommodate plant needs. Nevertheless, iron (Fe) and potassium (K) often need to be supplemented depending on plant species and life stage (Bittsánszky et al. 2016).

Levels of nitrogen (N) are especially critical to manage in aquaponics, not only for plant growth but even more so for fish health. Approximately 30% of all nitrogen in consumed feed is assimilated into fish biomass, while the majority is diffused via gills directly into the water (Rafiee & Saad 2005; Rakocy 2012). This process raises levels of total ammonia nitrogen (TAN) which consists of ammonia (NH_3) and ammonium (NH_4^+). A substantial part is also excreted as organic nitrogen via urine and feces, that can further break down into TAN (Wongkiew et al. 2017). Nitrogen in forms of TAN and nitrite (NO_2^-) can be highly toxic to fish at elevated concentrations. To reduce levels in the system, a consortium of nitrifying bacteria is required to convert these compounds into less toxic forms, such as nitrate (NO_3^-), that is readily taken up by plants (Wongkiew et al. 2017).

Aquaponics is most often implemented by coupling a recirculating aquaculture system (RAS) with a hydroponic unit (DWC, media bed, NFT, etc) in a single, closed loop (Lennard & Goddek 2019). The components of a typical system are usually arranged in the following sequence: fish tank, mechanical filter, biofilter, hydroponic unit and reservoir (sump). A more detailed description of each unit is provided in Table 1. Depending on system configuration, some units may be omitted or combined into a single functional module (Rakocy 2012; Krastanova et al. 2022).

Table 1. General description commonly included units in a typical aquaponic system.

Fish tank	The highest point in the system and the main rearing unit for fish (or other aquatic animals). This is where nutrients enter the system through the addition of fish feed. Solid particles rich in organic nitrogen from feces and uneaten feed are generated here. Air pumps are typically installed to maintain high DO levels, which are essential for optimal fish health.
Mechanical filter	By removing solid particles, this filter helps prevent clogging in the biofilter and other downstream components. It also reduces total particle load within the system, as decomposing solids can lower DO levels and negatively affect root growth and fish health (Rakocy et al. 2006; Krastanova et al. 2022). Solid particles accumulate at the bottom of the filter as sludge. Recurrent sludge removal is necessary, and the nutrient-rich sludge can be re-introduced as liquid fertilizer to the system after aerobic digestion (Khiari et al. 2019).

Biofilter	The biofilter plays a crucial role in converting toxic TAN and NO_2^- into NO_3^- through nitrification. It generally consists of a separate unit filled with water and a substrate with a large surface area. This surface supports colonization and biofilm formation by a large population of nitrifying bacteria. Common substrates include inert materials made from plastic, rock or ceramics (DeLong & Losordo 2012; Rakocy 2012). The biofilter should be sized based on system dimensions and expected nitrogen load. Continuous use of air pumps is required to maintain DO levels high (> 2.5 mg/L) for optimal nitrification (Ruiz et al. 2020a).
Hydroponic unit	The hydroponic unit is where the plants are situated in the system. Deep water culture (DWC), nutrient film technique (NFT) and media bed-based systems are some of the most common types used in aquaponics. Aeration is only required in DWC to maintain the health of continuously submerged roots. In cases where a media bed (e.g. expanded clay, rocks) is used, it serves a dual function as both hydroponic unit and a biofilter, eliminating the need for a separate biofilter (Rakocy 2012; Krastanova et al. 2022).
Reservoir (sump)	Often positioned at the lowest part of the system, this unit serves as the site for nutrient supplementation and pH adjustments. The water pump is typically installed here, returning treated water to the fish tank. In cases where NFT troughs are used, the sump is usually placed before the hydroponic unit (Rakocy 2012).

2.2. Nitrification

TAN is produced via fish metabolism or breakdown of organic matter, and accumulates in water as a fraction of NH_3 and NH_4^+. NH_3 is highly toxic to fish at elevated concentrations as it readily diffuses across biological membranes, whereas NH_4^+ is far less permeable and thereby less toxic (Randall & Tsui 2002; Ip & Chew 2010). As NH_3 is released from the gills or through microbial degradation, it rapidly reacts with free H^+ to form the less toxic NH_4^+. The equilibrium of TAN is mainly dependent on the pH and temperature of water, but also to a lesser extent on water hardness. A rise in pH and temperature leads to an increase in the NH_3/NH_4^+-ratio

(Emerson et al. 1975; Edwards et al. 2024). In aquaponics, TAN can be reduced in two ways. The first is direct plant uptake of NH_4^+ which indirectly reduces NH_3 through equilibrium shift. The other way is through aerobic nitrification, which is predominantly attributed to several species of autotrophic nitrifying bacteria (Wongkiew et al. 2017). Nitrifying bacteria may be found throughout the whole system, either suspended in solution or adhered on to walls of the system, roots or solid particles where they can produce biofilms. Nevertheless, they are most abundant in the biofilter, where extensive surface area and heavily oxygenated conditions are present (Rakocy 2012).

The first step of nitrification (1), where NH_4^+ is oxidized into NO_2^-, is performed mainly by ammonia oxidizing bacteria (AOB) within different genera (*Nitrosomonas*, *Nitrosoccocus*, *Nitrosospira* etc.). Secondly (2), toxic NO_2^- can be further oxidized to NO_3^- by nitrite oxidizing bacteria (NOB), also belonging to different genera (*Nitrobacter*, *Nitrococcus*, *Nitrospira* etc.) (Wongkiew et al. 2017; Ruiz et al. 2020a). In addition, some members of the genus *Nitrospira* are complete ammonia oxidizers (comammox) and can perform both steps of nitrification (Daims et al. 2015; Heise et al. 2021).

(1) $$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + 2H^+ + H_2O$$

(2) $$NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e^-$$

In newly installed systems, nitrification activity is typically low because the colonization and establishment of nitrifying microbial communities may require several weeks or months. Once established, well-functioning aquaponic systems are maintained within defined ranges of specific nitrogen species to support the optimal performance of fish, plants, and microbial communities. TAN usually ranges around 1-3 mg/L, NO_2^- below 1 mg/L, and NO_3^- between 1-100 mg/L. The system is also generally maintained at a neutral pH of 7.0 (Rakocy 2012).

Although autotrophic nitrifying bacteria are considered the principal agents of nitrification, they usually coexist in biofilms with heterotrophic bacteria that also can play an important role in influencing nitrogen transformations (Qi et al. 2022). Heterotrophic bacteria exhibit relatively faster growth rates and are known to be the most abundant and diverse microbial consortium in aquaponic systems, where they primarily degrade organic waste to obtain carbon and energy (Ruiz et al. 2020b; Kasozi et al. 2021). Through degradation, they can mineralize organically bound elements into inorganic forms, such as the production of NH_3. They can further alter the concentration of different nitrogen species through several other processes. For example, heterotrophic bacteria can conversely assimilate inorganic nitrogen directly into biomass, resulting in temporary nitrogen immobilization (Wongkiew et al. 2017). In addition, during organic carbon metabolism they can oxidize NH_4^+

and NO_2^- into NO_3^- through a process called heterotrophic nitrification. There are numerous species of heterotrophic bacteria that may also couple nitrification with partial aerobic denitrification, which reduces NO_3^- into gaseous nitrogen forms such as nitrous oxide (N_2O) and dinitrogen (N_2). Nevertheless, the understanding of heterotrophic nitrification remains limited, with many involved species and oxidation pathways yet to be fully elucidated (Preena et al. 2021; Martikainen 2022a).

2.3. *Pseudomonas fluorescens*

Pseudomonas fluorescens has traditionally been classified as a single species, but more recent genomic studies suggest that it is more accurately described as a species complex, comprising multiple closely related yet highly diverse bacterial strains (Silby et al. 2009; 2011; Taylor et al. 2025). Members of this group are Gram-negative, rod-shaped and predominantly heterotrophic. They are widely distributed and adapted to a broad range of habitats, including soil, freshwater and marine environments (Taylor et al. 2025). Many strains of *P. fluorescens* are also motile and efficient colonizers of plant roots, a trait attributed to the presence of multiple flagella (Barahona et al. 2016; Bouteiller et al. 2021).

Much of the scientific interest in *P. fluorescens* stems from its well-documented abilities as a plant growth-promoting rhizobacteria (PGPR) and biocontrol agent. As PGPRs they can contribute to induced systemic resistance in plants, resulting in faster and more robust responses of plant defenses upon pathogen infection (Hol et al. 2013). They can also enhance plant growth through the solubilization of nutrients and production of phytohormones such as indole-3-acetic acid (IAA) (Garrido-Sanz et al. 2016; David et al. 2018). As biocontrol agents, their main mode of action is production of secondary metabolites that can suppress root pathogens. These metabolites include phenazines, pyrrolnitrin, pyoluteorin, hydrogen cyanide and siderophores, which can mediate both direct antagonistic effects and indirect inhibition of a broad range of pathogens (e.g. bacteria, nematodes, fungi and oomycetes) (Ganeshan & Manoj Kumar 2005; Haas & Défago 2005).

While *P. fluorescens* and other *Pseudomonas* spp. are primarily studied for their interaction with plants and pathogens, there is evidence that certain strains are able to alter concentrations of different nitrogen species via heterotrophic nitrification and aerobic denitrification (Zhang et al. 2015; Duan et al. 2022; Hastuti et al. 2023; Huang et al. 2023). Moreover, the presence of *Pseudomonas* spp. has been shown to promote biofilm formation by autotrophic nitrifiers, thereby indirectly enhancing nitrification activity (Blanc et al. 1986; Petrovich et al. 2017).

3. Methodology

3.1. Study site and aquaponic system

The experimental work was primarily conducted in a laboratory (~22°C), while samples were collected from an aquaponic system located in a temperature-controlled greenhouse chamber. The temperature in the chamber ranged from 22-24°C during the day and 22-20°C during the night.

A closed, recirculating aquaponic system was used in this study and is shown schematically in Figure 1. The system consisted of one fish tank, a particle filter, a biofilter unit, and three parallel nutrient film technique (NFT) troughs connected in sequence. Water flowed from the fish tank to the particle filter, then to the biofilter, and was subsequently pumped upward to the NFT troughs where the plants were grown. The NFT troughs were installed at a slight slope, allowing a thin film of water to pass beneath the plant roots before returning to the fish tank by gravity, thereby completing a full cycle within the system. A submersible pump located in the biofilter maintained water flow throughout the system, and air pumps were used to aerate both the fish tank and biofilter unit to ensure adequate dissolved oxygen levels.

Prior to the start of this study, the aquaponic system had been independently operated for a total of two months following its initial setup. During this period, the system was maintained in a separate climate-controlled grow room with artificial lighting (16:8 h light:dark, ~22°C, ~60% RH). Nile tilapia (*Oreochromis niloticus*) fingerlings were reared in the fish tank, while juvenile Pak choi (*Brassica rapa* subsp. *chinensis*) plants were cultivated in the NFT troughs.

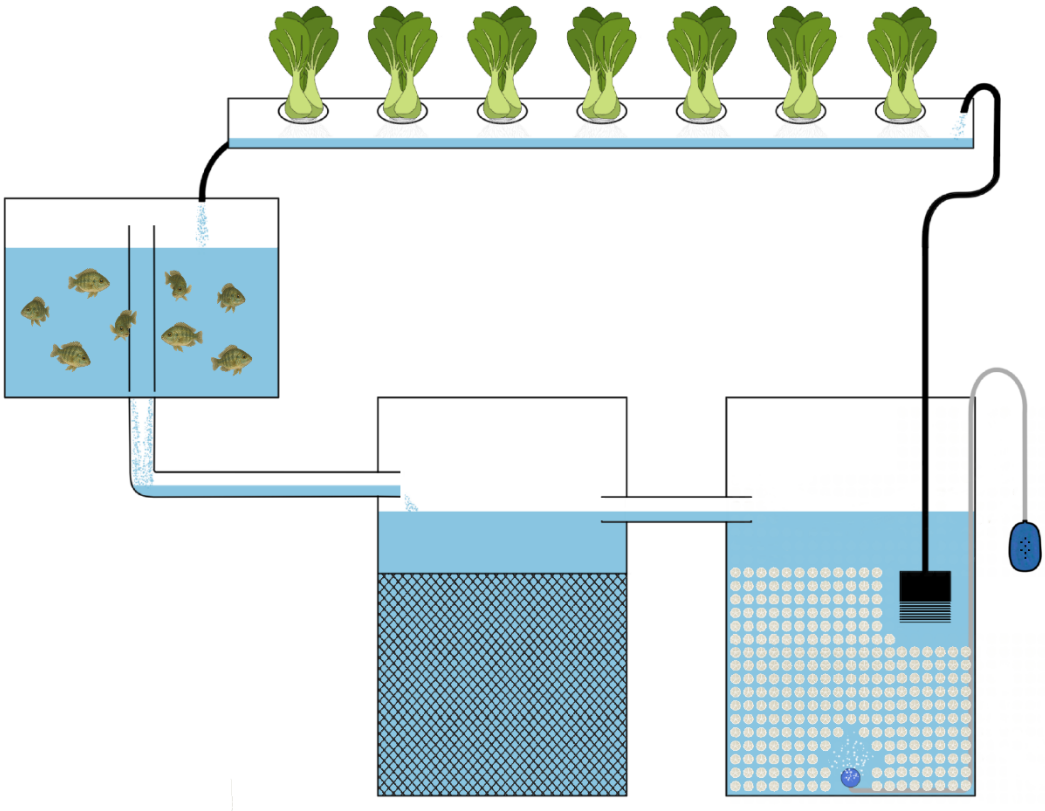

Figure 1. Schematic overview of aquaponic system. By August Lundqvist, 2025.

3.2. Nitrogen dynamics experiment

3.2.1. Sampling and treatments

Three types of water samples were prepared, representing both different locations and operational stages of the aquaponic system.

1. **Fish tank (8 weeks):** Subsamples taken from a 25 L water sample originally collected from the fish tank after eight weeks of continuous operation. The sample was stored in a sealed plastic container for two weeks before use, representing water from an earlier operational stage of the system.
2. **Biofilter (8 weeks):** Subsamples taken from a 25 L water sample collected from the biofilter after eight weeks and were stored in a sealed plastic container for two weeks prior to use.

3. **Biofilter (10 weeks):** Water collected directly from the biofilter after ten weeks of continuous operation in the relocated system, representing the system at a later operational stage.

Table 2. Overview of water sources and treatments used. FT-8 refers to fish tank water sampled after 8 weeks, B-8 and B-10 denote biofilter samples collected after 8 and 10 weeks respectively. C (control) refers to non-inoculated treatments, whereas SK2 and SK3 represent bacterial inoculation treatments. Each treatment and water source combination was represented by four replicates.

Water source	Treatment	Replicates	Label
Fish tank (8 weeks)	C, SK2, SK3	4	FT-8
Biofilter (8 weeks)	C, SK2, SK3	4	B-8
Biofilter (10 weeks)	C, SK2, SK3	4	B-10

Two different strains of *P. fluorescens* (SK2 and SK3) were used as treatments. These were isolated from water samples collected in the sump of a Nile tilapia RAS system, located at Gårdsfisk facility in Kristianstad, Sweden. Isolation and identification was conducted by Khalil et al. (2021). The control (C) treatment consisted of water samples with no addition of bacterial inoculum.

3.2.2. Preparation of inoculum

15 g of Tryptic Soy Broth (TSB) media was mixed with 500 ml of distilled water and was autoclaved for 15 minutes at 121°C. After sterilization, 20 ml of TSB was pipetted in two 50 ml screw cap tubes. Bacteria isolates were then transferred from vials and mixed into the tubes with an inoculation loop, and placed on a shaker (200 rpm) to incubate for 24 h at 22°C. The tubes were later stored in a fridge prior to use.

3.2.3. Experimental design

A total of 36 water tubes (50 ml) were prepared for the experiment under a laminar flow hood. Holes with a diameter of ~2 mm were pierced in each lid using a needle to allow insertion of an airline. A volume of 45 ml of water sample was pipetted in each tube, and depending on treatment, 200 µl of inoculant was further added. Lids were then closed and sealed with parafilm.

Randomization was performed using the RAND() function in Excel, and sealed tubes were arranged accordingly in a tube rack that was subsequently placed on a shaker (135 rpm). To ensure uniform oxygenation across all tubes, the airlines were positioned at the 5 ml mark and secured to the lids using additional parafilm. Air was supplied by three air pumps (Rena 301; ~1.5 L/min) connected to a main air manifold with multiple outlets, resulting in approximately 0.13 L/min/tube. Lastly, the entire rack was covered with a sheet of black plastic to prevent any light exposure.

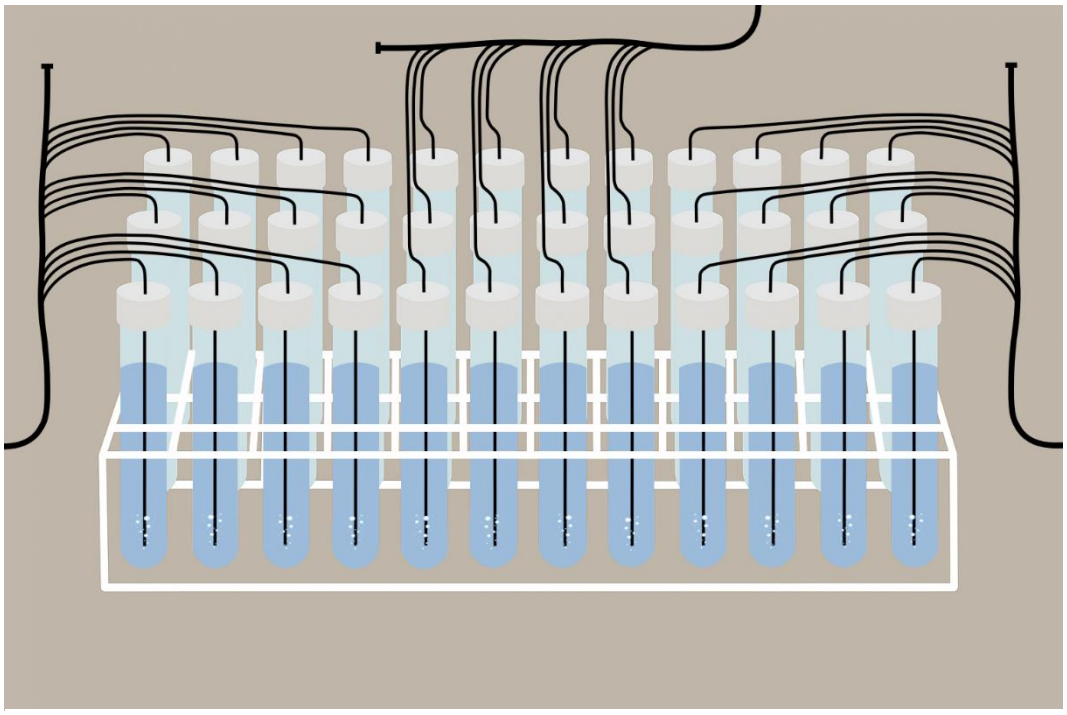

Figure 2. Schematic overview of the experimental setup. A total of 36 individual tubes were used for the experiment. Oxygen was supplied with air lines for each tube. By August Lundqvist, 2025.

3.2.4. Data collection

Measurements of NH_4^+, NO_2^-, NO_3^- and total inorganic nitrogen (TIN) concentrations (mg/L) were taken repeatedly four times from each tube throughout the experiment (days 0, 1, 7 and 14). A Hach DR3900 laboratory spectrophotometer was used in combination with the corresponding Hach reagent kits for each analyte, requiring 0.5 ml sample per measurement. The measurements taken on day 0 were taken for each water type without any addition of inoculum, thereby serving as baseline values representing normal conditions.

3.2.5. Data processing and analysis

The preliminary dataset was compiled using Excel, while all analytical computations and graphical outputs were generated in R (ver. 4.3.1). After data cleaning and prior to modelling, visualization of raw treatment means over time was performed to assess data quality and characterize preliminary temporal patterns. Because of the nested repeated-measures structure and the observation that treatment effects seemed to differ across water sources, linear mixed-effects models were fitted separately for each water source to account for within-tube correlation and treatment specific temporal trends. Model assumptions were evaluated using residual diagnostics, and extreme observations were identified via studentized residuals. Observations were deemed true outliers when studentized residuals exceeded |3|, and these were removed before refitting the models.

Treatment effects were analyzed using Type III ANOVA tests of fixed effects from the linear mixed-effect models. Sidak-adjusted ($p < 0.05$) comparisons of estimated marginal means were used to conclude differences among treatments, at the final sampling day (day 14). To further quantify treatment responses over time, model derived slopes were extracted to estimate the change in concentration per day for each nitrogen species (Sidak-adjusted ($p < 0.05$)). Slopes were not estimated for NO_2^- because its concentrations exhibited transient, non-directional dynamics that were not well described by linear trends.

3.3. Microbial load analysis

3.3.1. Sample collection & preparation of dilution series

A total of three individual 1 ml samples were taken from each water source (B-8, B-10 and F-8). To prepare a dilution series, each sample was pipetted into tubes containing 9 ml of NaCl (0.85%), followed by four successive tenfold dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} ,10^{-5}).

3.3.2. Inoculation and enumeration of agar plates

Petri dishes containing Tryptone Soy Agar (TSA), King's B Agar (KB) and Malt Extract Agar (MEA) were prepared with the following ingredients listed in Table 3. TSA is a general, non-selective medium for culturable bacteria, KB is a selective medium for pseudomonads, and MEA is a selective medium for fungi. A volume of 100 µl from each dilution was spread onto the agar plates in three replicates. The solution was evenly distributed across the agar surface using sterile glass beads, after which the plates were sealed with parafilm and incubated at 30°C for 48 h. Following incubation, colonies were enumerated through visual inspection.

Table 3. Ingredients of different culture mediums to support growth of cultivable bacteria (TSA), pseudomonas (KB) and fungi (MEA).

Media	Ingredients	
Tryptone Soy Agar (TSA)	Tryptone Soy Agar (Difco)	40 g
	Bacto Agar (Difco)	15 g
	Aq dest	1000 ml
King's B Agar (KB)	Proteose peptone (no. 3, Difco)	20 g
	K_2HPO_4	1.5 g
	$MgSO_4 * 7H_2O$	1.5 g
	Glycerol (99%)	15 ml
	Bacto Agar (Difco)	15 g
	Aq dest	1000 ml
Malt Extract Agar (MEA) (diluted; half-strength)	Malt Extract (Difco)	10 g
	Bacto Agar (Difco)	20 g
	Aq dest	1000 ml

3.3.3. Data processing and analysis

Consistent with the workflow described earlier in section 2.2.4, data processing and analysis were again conducted using Excel and R. Colony counts outside the countable range (30-300 colonies per plate) were excluded from further analysis. Valid colony counts were converted to colony-forming units per ml (CFU/mL) by multiplying the value by the corresponding dilution factors, followed by $\log_{10}$-transformation.

Differences in microbial load were first assessed using a two-way ANOVA with agar type and water source as fixed factors, to account for medium-dependent variation in growth. One-way ANOVAs were then performed within each agar type to compare water sources, followed by Tukey's HSD ($p < 0.05$) for pairwise comparisons.

4. Results

The results are organized into two sections. Section 4.1 examines nitrogen dynamics in water sources subjected to different treatments, whereas Section 4.2 presents microbial load in the different water sources.

4.1. Nitrogen dynamics

4.1.1. Mean concentration of nitrogen species

The three water sources exhibited distinct baseline compositions of different nitrogen species at the start of the experiment (day 0) (Fig. 3). FT-8 water showed relatively high concentrations of NH_4^+ (~17.5 mg/L) and NO_2^- (~1.25 mg/L), alongside reduced levels of NO_3^- (~2 mg/L). In contrast, water from B-8 and B-10 was characterized by lower NH_4^+ levels (~9 and 5 mg/L respectively), moderate to low NO_2^- (~0.4 and 0 mg/L respectively) and higher NO_3^- (~15 and 17.5 mg/L respectively).

Concentration patterns of NH_4^+ varied depending on water source and treatments. In FT-8, C showed a continuous decline over the experiment, while SK2 and SK3 showed an initial increase on day 1, followed by an overall reduction in concentration. SK2 was reduced more gradually compared to SK3, which at first exhibited a steep decline that was followed by a modest increase after day 7. For B-8 and B-10 water, C treatments reduced concentrations initially at day 1 and subsequently stabilized (~5 and 4 mg/L respectively). Conversely, SK2 and SK3 treatments first raised concentration levels, followed either by stabilization in B-8 or a slight reduction in B-10 water.

Levels of NO_2^- remained highest around ~1.25 mg/L in FT-8 water throughout the experiment, with little difference between the treatments. All treatments in B-8, particularly C and SK3, peaked during day 1, before finishing around 0 mg/L on day 14. In B-10 there was a similar transient peak on day 7 of SK2 and SK3 before returning to ~0 mg/L, but on the contrary, C stayed close to 0 mg/L during the entire experiment.

NO_3^- concentrations stayed relatively low in FT-8 water over the entire experiment. C treatment only saw a slight increase in NO_3^- after day 7, whereas SK2 and SK3 treatments stayed flat. In contrast, B-8 and B-10 waters started off with relatively high levels of NO_3^- (~15 and 17 mg/L respectively). The SK2 treatment resulted in the largest increase in biofilter waters, with the most pronounced increase observed in B-10 water. The SK3 treatment also led to a rise in NO_3^- concentrations in both waters, while C exhibited a comparatively smaller increase in B-8 and B-10.

Initial TIN concentrations were approximately 20 mg/L in FT-8 and B-10 waters and about 25 mg/L in B-8. There was general decrease in TIN for all treatments in FT-8 water, with no apparent difference among treatments them. By comparison, TIN concentrations in B-8 and B10 waters increased over time in all treatments, with C displaying the smallest increase.

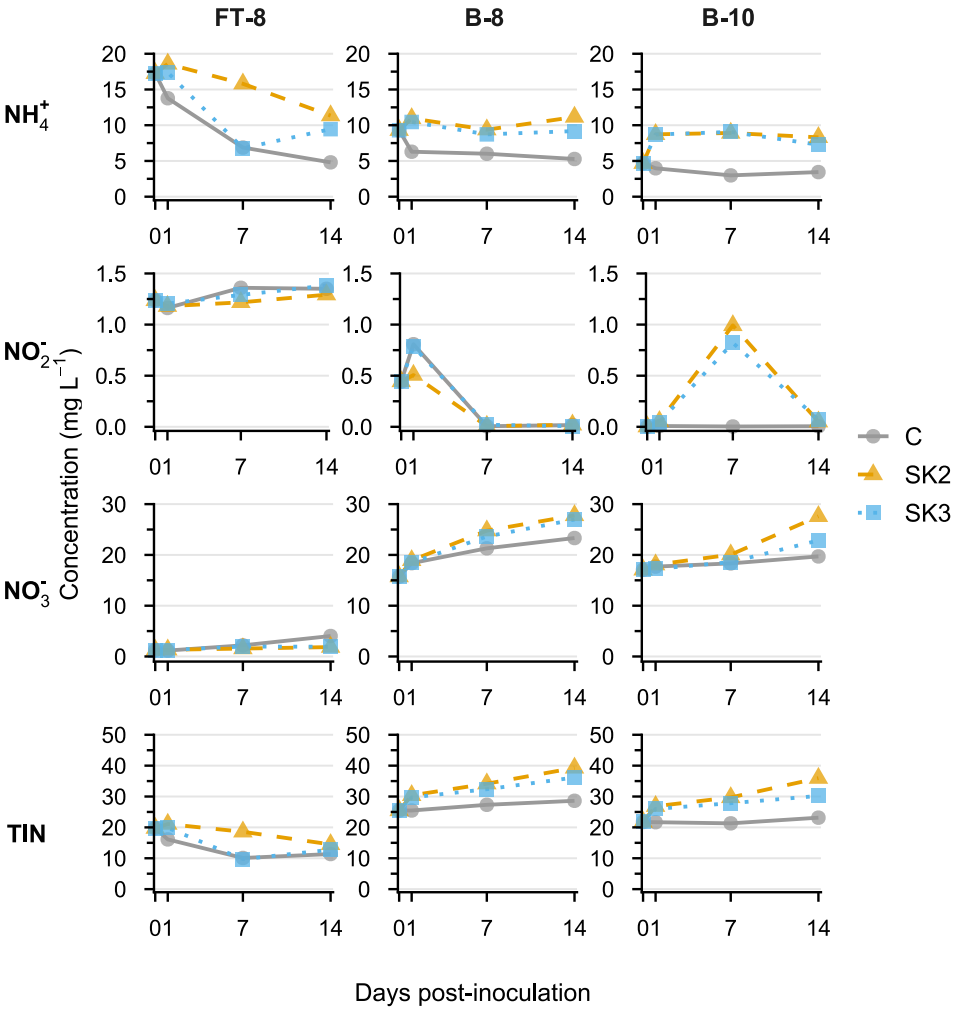

Figure 3. Temporal dynamics of dissolved inorganic nitrogen species over two weeks across water sources and treatments. Mean concentrations (mg L^{-1}) of ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-) and total inorganic nitrogen (TIN) are shown for three water sources (FT-8, B-8 and B-10) subjected to control (C), SK2 and SK3 treatments.

4.1.2. Linear mixed-effects models of nitrogen species

To formally assess treatment- and time dependent effects while accounting for repeated measurements over the course of the experiment, nitrogen species were analyzed using linear mixed-effects models. Each model tested the fixed effect of treatment, time, and their interaction within individual water sources. Model-estimated trajectories (except for NO_2^-) are shown in Figure 4-6. Overall treatment effects are summarized using estimated marginal means (EMMs) for concentrations on day 14 (Fig. 7). Furthermore, model-derived slopes (mean net change, mg/L/day) are reported in Table 4.

On day 14, NH_4^+ concentrations differed significantly among treatment in all waters (Fig. 7). In FT-8 water, the control exhibited significantly lower concentrations than SK2, while SK3 did not differ from either treatment. In biofilter waters (B-8 and B-10), NH_4^+ were also lowest in C, with both SK2 and SK3 leading to significantly higher concentrations. There was only a significant difference between SK2 and SK3 in B-8 water. Estimated slope trends varied among water types and treatments but were only significantly different in B-8 water (Table 4).

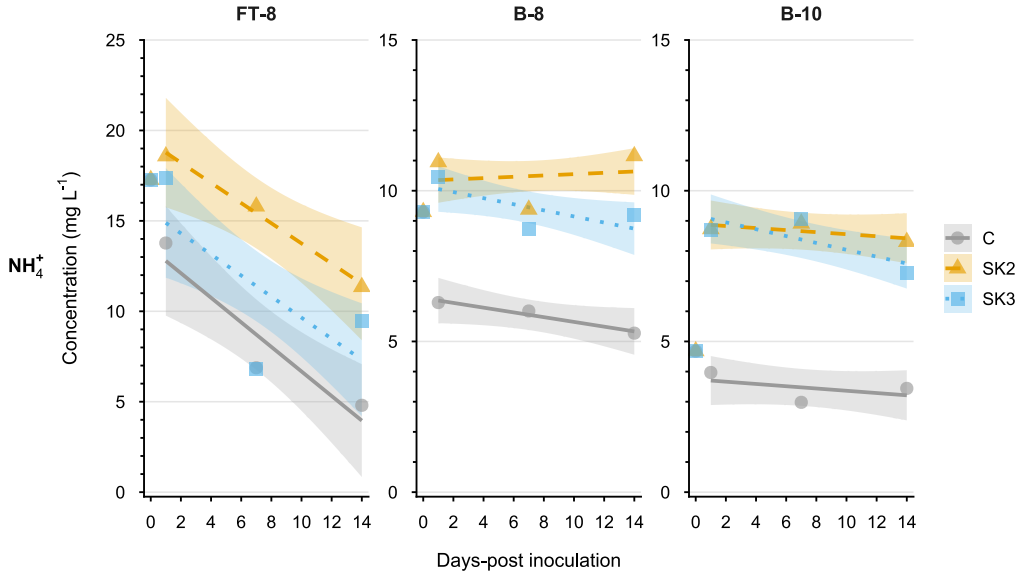

Figure 4. Linear mixed-effects model predictions of ammonium (NH_4^+) concentrations. Points show observed means, while lines and shaded bands represent model estimated mean trajectories and their 95% confidence intervals. Note: Y-axis scale differs among panels.

NO_2^- concentrations did not differ significantly between treatments in any water type on day 14 (Fig. 7). However, concentrations were consistently higher in fish tank water and comparatively lower in biofilter waters. Given the transient, non-linear dynamics of NO_2^-, slopes estimates are not reported.

Significant differences in NO_3^- among treatments were evident across all waters on day 14, although treatment responses differed depending on water types (Fig. 7). In F-8 water, C showed significantly higher NO_3^- compared to SK2 and SK3. In B-8 water, both SK2 and SK3 resulted in significantly higher NO_3^- than C. In B-10 water, SK2 treatment produced the highest NO_3^- concentrations, significantly exceeding both C and SK3, while SK3 also remained higher than C. Estimated slopes were significantly different among treatments in all waters (Table 4).

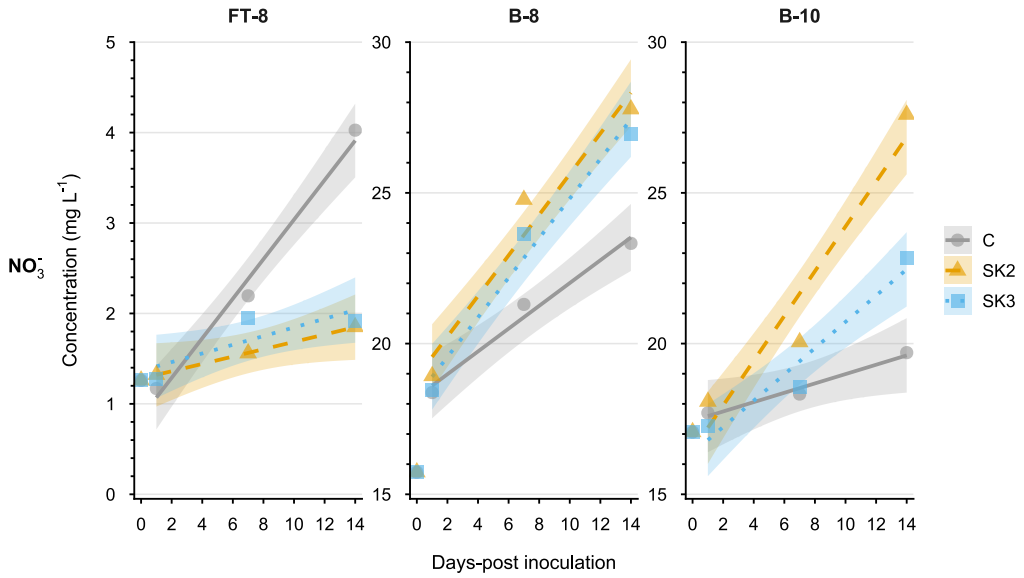

Figure 5. Linear mixed-effects model predictions of nitrate (NO_3^-) concentrations. Points show observed means, while lines and shaded bands represent model estimated mean trajectories and their 95% confidence intervals. Note: Y-axis scale differs among panels.

TIN concentrations differed significantly among treatments in all waters on day 14 (Fig. 7). In FT-8 water, SK2 resulted in significantly higher TIN than C, while SK3 did not differ significantly from either treatment. In biofilter waters, TIN concentrations were lowest in the C treatment and significantly higher in both SK2 and SK3. In both biofilter waters, SK2 treatment produced the highest TIN concentrations, significantly higher than SK3. Estimated slopes were significantly different among treatments in all waters except FT-8 (Table 4).

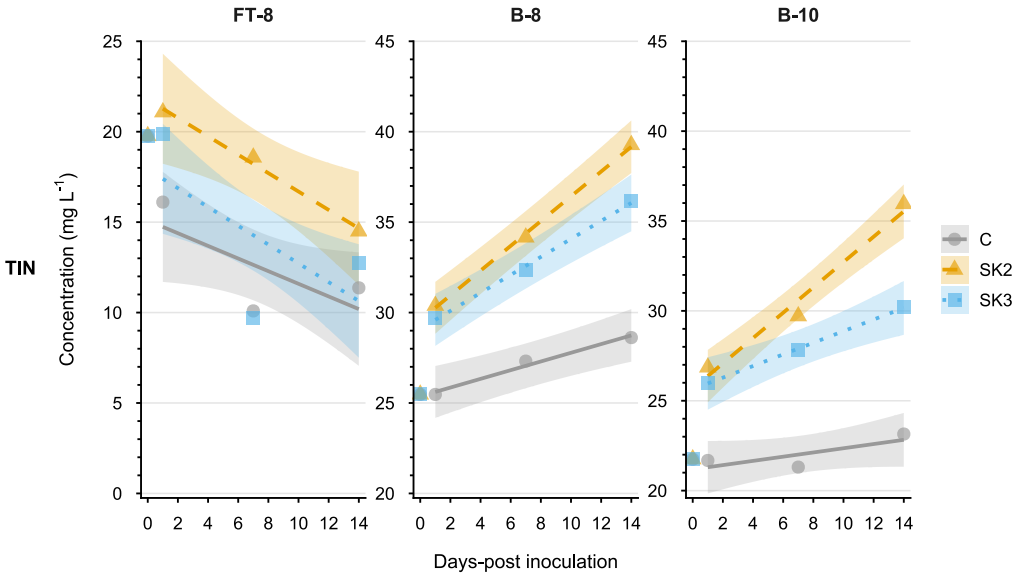

Figure 6. Linear mixed-effects model predictions of total inorganic nitrogen (TIN) concentrations. Points show observed means, while lines and shaded bands represent model estimated mean trajectories and their 95% confidence intervals. Note: Y-axis scale differs among panels.

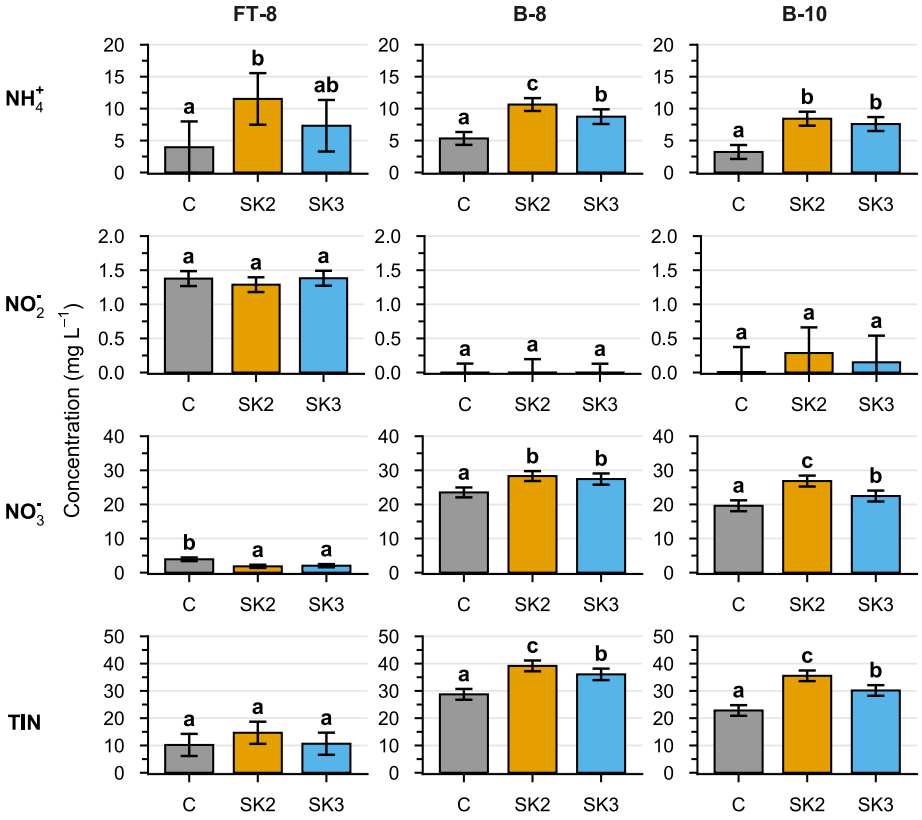

Figure 7. Model-estimated marginal means (EMMs) at day 14 representing overall treatment effects for ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-) and total inorganic nitrogen (TIN). Error bars represent standard errors, and different letters indicate significant differences ($p < 0.05$).

Table 4. Model-estimated slopes describing the net change in concentration (mg/L/day) over 14 days. Different letters indicate significant differences ($p < 0.05$).

	Water type	Control (C)	SK2	SK3
NH_4^+	FT-8	-0.847 ± 0.138 a	-0.467 ± 0.138 a	-0.661 ± 0.138 a
NH_4^+	B-8	-0.205 ± 0.045 a	0.067 ± 0.045 b	-0.062 ± 0.049 ab
NH_4^+	B-10	-0.079 ± 0.070 a	0.152 ± 0.070 a	0.084 ± 0.070 a
NO_3^-	FT-8	0.200 ± 0.017 b	0.041 ± 0.016 a	0.053 ± 0.016 a
NO_3^-	B-8	0.488 ± 0.065 a	0.812 ± 0.065 b	0.775 ± 0.072 b
NO_3^-	B-10	0.171 ± 0.045 a	0.728 ± 0.045 c	0.405 ± 0.045 b
TIN	FT-8	-0.555 ± 0.141 a	-0.420 ± 0.141 a	-0.601 ± 0.141 a
TIN	B-8	0.236 ± 0.077 a	0.866 ± 0.077 b	0.665 ± 0.084 b
TIN	B-10	0.092 ± 0.082 a	0.877 ± 0.082 c	0.494 ± 0.082 b

4.2. Microbial load in water sources

The amount of culturable microflora varied depending on water samples and the growth media used (Fig. 8). CFU (CFU/mL) measurements were consistently higher on TSA and KB media, whereas MEA yielded substantially lower CFU across all water sources.

Among the water sources, differences in CFU were most pronounced on MEA medium. B-8 water exhibited significantly higher CFU on MEA compared with FT-8 and B-10, while FT-8 had the lowest CFU on this medium. CFU on TSA and KB medium were high and showed less variation, indicating similar levels of total culturable bacteria and pseudomonads among water sources. However, significant differences were still observed. B-10 water had the significantly highest CFU on TSA, followed by FT-8 and then B-8. Similarly, CFU on KB were significantly higher in B-10 and FT-8 compared with B-8.

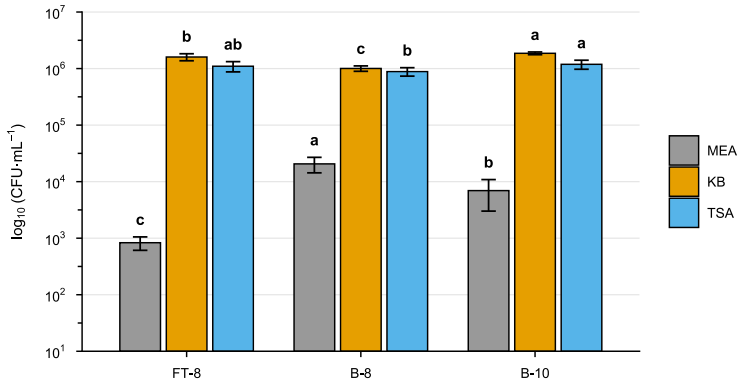

Figure 8. Overview of culturable microflora expressed as colony forming units (CFU/mL) in different water sources (FT-8, B-8, and B-10) grown on TSA, KB and MEA. Bars represent mean CFU values and error bars indicate standard deviation. Different letters correspond to significant differences ($p < 0.05$).

5. Discussion

Findings from this study demonstrate that inoculation of *P. fluorescens* may influence nitrogen dynamics and processes involved in nitrification in aquaponic systems. However, the magnitude and consistency of these effects appear to be strongly dependent on prevailing water conditions.

In biofilter waters (B-8 and B-10), bacterial inoculation resulted in a significant increase in NO_3^- production relative to control. Out of both strains (SK2 and SK3), SK2 was shown to have the highest increase. Surprisingly, NH_4^+ concentrations were reduced less in inoculated treatments than in control, suggesting that although nitrification occurred, increased mineralization of organic nitrogen into NH_4^+ may be attributed to the extra addition of heterotrophic bacteria. Divergence in NH_4^+ concentrations between inoculated treatments and the control was first observed on day 1, after which the concentration within each treatment appeared to stabilize, slightly fluctuating around a certain level. At the same time, total net production of TIN remained positive throughout the experiment, indicating active heterotrophic mineralization in all treatments, with significantly higher rates observed in inoculated treatments. Transient peaks of NO_2^- were detected in almost all treatments in both biofilter waters. Nonetheless, NO_2^- concentrations generally remained low, pointing to active nitrification.

Several mechanisms may explain the observed increase of NO_3^- in the biofilter waters after bacterial inoculation. One possibility is that *P. fluorescens* may have indirectly promoted nitrification by increasing the availability of NH_4^+ for subsequent oxidation by autotrophic AOB. Alternatively, the addition of heterotrophic bacteria could have improved biofilm formation, thereby conditioning autotrophic bacteria to perform nitrification more efficiently (Tsuneda et al. 2001). Biofilms are complex microbial aggregates in which multiple species coexist, providing protection from turbulent water and predation, and conditions conducive to rapid growth. They also enable close cell-to-cell interactions, allowing metabolic cooperation, signaling and interspecies synergy (Luo et al. 2022).

Information on species-specific interactions between heterotrophic bacteria and nitrifying autotrophs that promote nitrification is rather scarce. However, in an

experiment conducted by Blanc et al. (1986), the addition of *Pseudomonas* sp. was shown to increase the growth and activity of *Nitrobacter* sp.. Moreover, siderophores produced by heterotrophic bacteria, such as *P. fluorescens*, can act as an essential source of iron for the metabolism of autotrophic nitrifiers (Keluskar et al. 2013; Luján et al. 2015). Siderophores were demonstrated by Keluskar et al. (2013) to be crucial for the activity and survival of *Nitrosomonas* sp. under iron-limited conditions, which often occur during microbial competition. In addition to indirect effects on autotrophic nitrification, it is possible that heterotrophic nitrification may also have contributed to the observed increase of NO_3^-. But this is highly speculative, as there is a great variability in the capacity to perform this between species and strains. However, some *Pseudomonas* sp. has proven to be able to convert NH_4^+ directly into both NO_2^- and NO_3^- (Duan et al. 2022; Hastuti et al. 2023).

A contrasting trend was observed in the fish tank water, which was anticipated, although not in the expected manner. NH_4^+ concentrations decreased across all treatments, with a significantly greater reduction in the control. Concurrently, NO_3^- concentrations only showed a marginal increase, which was significantly highest in control. NO_2^- also remained substantially high for all treatments over the experiment. These dynamics put together indicate low nitrification activity, expected for fish tank water containing less autotrophic nitrifiers and large amounts of organic matter rich in carbon. A high carbon:nitrogen ratio (C:N) can lead to overgrowth of heterotrophic bacteria, which in turn increases competition for oxygen and assimilation of nitrogen sources (Michaud et al. 2006; Wongkiew et al. 2017). Such conditions can be detrimental for AOB and NOB and reduce nitrification efficiency, leading to low production of NO_3^-, and accumulation of TAN and NO_2^- (Rurangwa & Verdegem 2015).

It is probable that the reduction of NH_4^+ in the fish tank water is attributed mainly to assimilation into microbial biomass. This is further supported by decreasing TIN levels for all treatments during the experimental period. Interestingly this was not seen to be exacerbated by addition of *P. fluorescens* which would have been expected. With respect to the control treatment, it can be assumed that the significantly different NH_4^+ and NO_3^- dynamics observed may be attributed to the absence of *P. fluorescens*, which likely resulted in reduced competition for the relatively small population of autotrophic nitrifiers present in the water, thereby permitting limited NO_3^- production. Overall, the lack of NO_3^- production in fish tank water weakens the hypothesis that heterotrophic nitrification by *P. fluorescens* could be a contributor to the observed treatment effects across all water types.

CFU measurements provided an indication on how the microbial community structure differed between water types. Significant differences of all microbial

groups were seen across all waters, however it is difficult to draw any concrete conclusions from it. Although, general bacteria and pseudomonas was significantly highest in the biofilter unit which had run for the longest time, as is expected (Rakocy 2012; Maier & Pepper 2015). Furthermore, when the CFU results are considered alongside the initial concentrations of nitrogen species in each water type (Fig. 3), some assumptions can be made. Fish water was characterized by relatively high NH_4^+ and NO_2^- concentrations, and low NO_3^- levels, indicating a low amount of nitrifying microflora. Hence, the majority of general bacteria measured here is presumably heterotrophic and non-nitrifying. The CFU of fungi was also significantly low and may represent an additional influencing factor. Fungi are commonly not considered an important driver of nitrification in aquaponic systems, nevertheless, it has been proven that several species of fungi can perform heterotrophic nitrification to produce NO_2^- or NO_3^- (Martikainen 2022b; Fang et al. 2023). In contrast, both biofilter water types originally exhibited lower NH_4^+ and NO_2^-, and high levels of NO_3^-, consistent with a greater amount of nitrifying microflora. Accordingly, a substantial proportion of the general bacteria community in these waters is likely contributing to nitrification. The number of fungi was also greater here.

5.1. Limitations

This study is subject to multiple limitations that may have influenced the results or constrained the contextual interpretation of the findings. Several important water quality parameters were not monitored, including pH, alkalinity, hardness, and dissolved oxygen. These are factors known to influence processes involved in nitrification, and the absence of monitoring means they may have varied during the experimental period. Another possible source of error is contamination of air lines, which could have occurred when measurements were taken. Moreover, microbial community structure, nutrient concentrations, and general water quality may have been altered in water samples F-8 and B-8 during the two-week storage period.

6. Conclusions

The results of this study are not conclusive enough to suggest that *P. fluorescens* directly enhances nitrification across different aquaponic water conditions. While bacterial inoculation led to increased NO_3^- production and higher TIN turnover in biofilter water, these effects were not consistently observed in fish tank water. This implies that the influence of *P. fluorescens* on nitrogen dynamics is strongly dependent on coexisting microbial communities and prevailing water conditions, particularly the presence of autotrophic nitrifiers and the organic carbon load.

An indirect influence of *P. fluorescens* on nitrification in biofilters is more consistent with the observed results, potentially mediated through increased mineralization of organic nitrogen, biofilm development, or synergistic interaction with autotrophic nitrifiers, rather than heterotrophic nitrification alone. However, the lack of comprehensive monitoring of water quality and further characterization of microbial community structure limits a deeper interpretation of the results. It is also important to consider that the treatment effects appear to be highly context dependent, and more controlled experiments are required to draw robust conclusions. Future research should also investigate more closely whether heterotrophic nitrification could have positive effect on the establishment of newly installed systems.

Both *P. fluorescens* strains showed similar effects, however the SK2 strain gave a slightly stronger response. Although the present study was limited in scope, the observed differences indicate that the effects of *P. fluorescens* might be highly context dependent. If further research demonstrates consistently positive effects of *P. fluorescens* on nitrification and overall system productivity, particularly in newly established systems, the development of an inoculum product may be warranted. This could be formulated either as a standalone preparation of specific strains or in combination with other synergistic species. Numerous commercially available inoculum products are currently used in both aquaculture and aquaponics to support nitrification during system startup, often consisting of mixtures of autotrophic nitrifying bacteria (DeLong & Losordo 2012; Rurangwa & Verdegem 2015; Derikvand et al. 2021). Furthermore, recent research has been increasingly focusing on the use of inoculum and “probiotics” to improve productivity, as well as their potential as biocontrol agents, mitigating disease in both fish and plants (Day et al.

2021; Derikvand et al. 2024; Fachri et al. 2024; Kasozi et al. 2024; Rahayu et al. 2024; Rudoy et al. 2025). Notably, *Pseudomonas* spp., including *P. fluorescens*, are widely recognized for their biocontrol and plant growth-promoting capabilities (Ganeshan & Manoj Kumar 2005; Haas & Défago 2005; Khalil et al. 2021; Khatri et al. 2024). In light of these properties, together with the findings of the present study, further investigation into the application of *P. fluorescens* as a multifunctional inoculum in aquaponic systems is encouraged.

References

Abbasi, A., Sajid, A., Haq, N., Rahman, S., Misbah, Z., Sanober, G., Ashraf, M. & Kazi, A.G. (2014). Agricultural Pollution: An Emerging Issue. In: Ahmad, P., Wani, M.R., Azooz, M.M., & Tran, L.-S.P. (eds) *Improvement of Crops in the Era of Climatic Changes: Volume 1*. Springer. 347–387. https://doi.org/10.1007/978-1-4614-8830-9_13

Abd El-Hack, M.E., El-Saadony, M.T., Nader, M.M., Salem, H.M., El-Tahan, A.M., Soliman, S.M. & Khafaga, A.F. (2022). Effect of environmental factors on growth performance of Nile tilapia (Oreochromis niloticus). *International Journal of Biometeorology*, 66 (11), 2183–2194. https://doi.org/10.1007/s00484-022-02347-6

Baganz, G.F.M., Junge, R., Portella, M.C., Goddek, S., Keesman, K.J., Baganz, D., Staaks, G., Shaw, C., Lohrberg, F. & Kloas, W. (2022). The aquaponic principle—It is all about coupling. *Reviews in Aquaculture*, 14 (1), 252–264. https://doi.org/10.1111/raq.12596

Barahona, E., Navazo, A., Garrido-Sanz, D., Muriel, C., Martínez-Granero, F., Redondo-Nieto, M., Martín, M. & Rivilla, R. (2016). Pseudomonas fluorescens F113 Can Produce a Second Flagellar Apparatus, Which Is Important for Plant Root Colonization. *Frontiers in Microbiology*, 7. https://doi.org/10.3389/fmicb.2016.01471

Bittsánszky, A., Uzinger, N., Gyulai, G., Mathis, A., Junge, R., Villarroel, M., Kotzen, B. & Kőmíves, T. (2016). Nutrient supply of plants in aquaponic systems. *Ecocycles*, 2 (2). https://doi.org/10.19040/ecocycles.v2i2.57

Blanc, J., Audic, J.M. & Faup, G.M. (1986). Enhancement of *Nitrobacter* activity by heterotrophic bacteria. *Water Research*, 20 (11), 1375–1381. https://doi.org/10.1016/0043-1354(86)90135-1

Bouteiller, M., Dupont, C., Bourigault, Y., Latour, X., Barbey, C., Konto-Ghiorghi, Y., Merieau, A., Bouteiller, M., Dupont, C., Bourigault, Y., Latour, X., Barbey, C., Konto-Ghiorghi, Y. & Merieau, A. (2021). Pseudomonas Flagella: Generalities and Specificities. *International Journal of Molecular Sciences*, 22 (7). https://doi.org/10.3390/ijms22073337

Daims, H., Lebedeva, E.V., Pjevac, P., Han, P., Herbold, C., Albertsen, M., Jehmlich, N., Palatinszky, M., Vierheilig, J., Bulaev, A., Kirkegaard, R.H., von Bergen, M., Rattei, T., Bendinger, B., Nielsen, P.H. & Wagner, M. (2015). Complete nitrification by Nitrospira bacteria. *Nature*, 528 (7583), 504–509. https://doi.org/10.1038/nature16461

David, B.V., Chandrasehar, G. & Selvam, P.N. (2018). Pseudomonas fluorescens: A Plant-Growth-Promoting Rhizobacterium (PGPR) With Potential Role in Biocontrol of Pests of Crops. In: *Crop Improvement Through Microbial Biotechnology*. Elsevier. 221–243. https://doi.org/10.1016/B978-0-444-63987-5.00010-4

Day, J.A., Diener, C., Otwell, A.E., Tams, K.E., Bebout, B., Detweiler, A.M., Lee, M.D., Scott, M.T., Ta, W., Ha, M., Carreon, S.A., Tong, K., Ali, A.A., Gibbons, S.M. & Baliga, N.S. (2021). Lettuce (Lactuca sativa) productivity influenced by microbial inocula under nitrogen-limited conditions in aquaponics. *PloS One*, 16 (2), e0247534. https://doi.org/10.1371/journal.pone.0247534

DeLong, D.P. & Losordo, T.M. (2012). *How to start a biofilter*. (SRAC Publication No. 4502). Southern Regional Aquaculture Center.

Derikvand, P., Sauter, B., Keddie, A. & Stein, L.Y. (2024). Inoculum and pH effects on ammonium removal and microbial community dynamics in aquaponics systems. *iScience*, 27 (3), 109073. https://doi.org/10.1016/j.isci.2024.109073

Derikvand, P., Sauter, B. & Stein, L.Y. (2021). Development of an aquaponics microbial inoculum for efficient nitrification at acidic pH. *Applied Microbiology and Biotechnology*, 105 (18), 7009–7021. https://doi.org/10.1007/s00253-021-11529-y

Duan, S., Zhang, Y. & Zheng, S. (2022). Heterotrophic nitrifying bacteria in wastewater biological nitrogen removal systems: A review. *Critical Reviews in Environmental Science and Technology*, 52 (13), 2302–2338. https://doi.org/10.1080/10643389.2021.1877976

Edwards, T.M., Puglis, H.J., Kent, D.B., Durán, J.L., Bradshaw, L.M. & Farag, A.M. (2024). Ammonia and aquatic ecosystems – A review of global sources, biogeochemical cycling, and effects on fish. *Science of The Total Environment*, 907, 167911. https://doi.org/10.1016/j.scitotenv.2023.167911

Eigenbrod, C. & Gruda, N. (2015). Urban vegetable for food security in cities. A review. *Agronomy for Sustainable Development*, 35 (2), 483–498. https://doi.org/10.1007/s13593-014-0273-y

Emerson, K., Russo, R.C., Lund, R.E. & Thurston, R.V. (1975). Aqueous Ammonia Equilibrium Calculations: Effect of pH and Temperature. *Journal of the Fisheries Research Board of Canada*, 32 (12), 2379–2383. https://doi.org/10.1139/f75-274

Fachri, M., Amoah, K., Huang, Y., Cai, J., Alfatat, A., Ndandala, C.B., Shija, V.M., Jin, X., Bissih, F. & Chen, H. (2024). Probiotics and paraprobiotics in aquaculture: a sustainable strategy for enhancing fish growth, health and disease prevention-a review. *Frontiers in Marine Science*, 11. https://doi.org/10.3389/fmars.2024.1499228

Fang, C., Xie, J., Yang, X., Yang, R., Fransson, P., Sheteiwy, M.S., Weih, M. & Yang, H. (2023). Arbuscular mycorrhizal fungi drive soil nitrogen

transformation under wheat varieties with different nitrogen utilization efficiencies. *Rhizosphere*, 27. https://doi.org/10.1016/j.rhisph.2023.100775

FAO (2017). *The future of food and agriculture: trends and challenges*. Food and Agriculture Organization of the United Nations.

FAO (2025). *The State of the World's Land and Water Resources for Food and Agriculture 2025. The potential to produce more and better*. FAO. https://doi.org/10.4060/cd7488en

Ganeshan, G. & Manoj Kumar, A. (2005). Pseudomonas fluorescens, a potential bacterial antagonist to control plant diseases. *Journal of Plant Interactions*, 1 (3), 123–134. https://doi.org/10.1080/17429140600907043

Garrido-Sanz, D., Meier-Kolthoff, J.P., Göker, M., Martín, M., Rivilla, R. & Redondo-Nieto, M. (2016). Genomic and Genetic Diversity within the Pseudomonas fluorescens Complex. *PLOS ONE*, 11 (2), e0150183. https://doi.org/10.1371/journal.pone.0150183

Haas, D. & Défago, G. (2005). Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nature Reviews Microbiology*, 3 (4), 307–319. https://doi.org/10.1038/nrmicro1129

Hastuti, Y.P., Siregar, A., Fatma, Y.S. & Supriyono, E. (2023). Application of a nitrifying bacterium Pseudomonas sp. HIB_D to reduce nitrogen waste in the Litopenaeus vannamei cultivation environment. *Aquaculture International*, 1–17. https://doi.org/10.1007/s10499-023-01123-6

Heise, J., Müller, H., Probst, A.J. & Meckenstock, R.U. (2021). Ammonium Removal in Aquaponics Indicates Participation of Comammox Nitrospira. *Current Microbiology*, 78 (3), 894–903. https://doi.org/10.1007/s00284-021-02358-3

Hol, W.H.G., Bezemer, T.M. & Biere, A. (2013). Getting the ecology into interactions between plants and the plant growth-promoting bacterium Pseudomonas fluorescens. *Frontiers in Plant Science*, 4. https://doi.org/10.3389/fpls.2013.00081

Huang, X., Luo, L., Luo, Y., Huang, J., Jiang, D., Wu, X. & Li, Z. (2023). *Pseudomonas fluorescens* 2P24: A root beneficial bacterium with an excellent ability to remove multiple nitrogen sources. *Journal of Water Process Engineering*, 55, 104138. https://doi.org/10.1016/j.jwpe.2023.104138

Hussain, S., Hussain, S., Guo, R., Sarwar, M., Ren, X., Krstic, D., Aslam, Z., Zulifqar, U., Rauf, A., Hano, C. & El-Esawi, M.A. (2021). Carbon Sequestration to Avoid Soil Degradation: A Review on the Role of Conservation Tillage. *Plants*, 10 (10), 2001. https://doi.org/10.3390/plants10102001

Ingrao, C., Strippoli, R., Lagioia, G. & Huisingh, D. (2023). Water scarcity in agriculture: An overview of causes, impacts and approaches for reducing the risks. *Heliyon*, 9 (8). https://doi.org/10.1016/j.heliyon.2023.e18507

Ip, Y. & Chew, S.F. (2010). Ammonia production, excretion, toxicity, and defense in fish: a review. *Frontiers in Physiology*, 1. https://doi.org/10.3389/fphys.2010.00134

Kasozi, N., Abraham, B., Kaiser, H. & Wilhelmi, B. (2021). The complex microbiome in aquaponics: significance of the bacterial ecosystem. *Annals of Microbiology*, 71 (1), 1. https://doi.org/10.1186/s13213-020-01613-5

Kasozi, N., Iwe, G.D., Walakira, J. & Langi, S. (2024). Integration of probiotics in aquaponic systems: an emerging alternative approach. *Aquaculture International*, 32 (2), 2131–2150. https://doi.org/10.1007/s10499-023-01261-x

Keluskar, R., Nerurkar, A. & Desai, A. (2013). Mutualism between autotrophic ammonia-oxidizing bacteria (AOB) and heterotrophs present in an ammonia-oxidizing colony. *Archives of Microbiology*, 195 (10), 737–747. https://doi.org/10.1007/s00203-013-0926-2

Khalil, S., Panda, P., Ghadamgahi, F., Rosberg, A. & Vetukuri, R.R. (2021). Comparison of two commercial recirculated aquacultural systems and their microbial potential in plant disease suppression. *BMC Microbiology*, 21 (1), 205. https://doi.org/10.1186/s12866-021-02273-4

Khatri, S., Sazinas, P., Strube, M.L., Ding, L., Dubey, S., Shivay, Y.S., Sharma, S. & Jelsbak, L. (2024). Pseudomonas is a key player in conferring disease suppressiveness in organic farming. *Plant and Soil*, 503 (1–2), 85–104. https://doi.org/10.1007/s11104-023-05927-6

Khiari, Z., Kaluthota, S. & Savidov, N. (2019). Aerobic bioconversion of aquaculture solid waste into liquid fertilizer: Effects of bioprocess parameters on kinetics of nitrogen mineralization. *Aquaculture*, 500, 492–499. https://doi.org/10.1016/j.aquaculture.2018.10.059

Kotzen, B., Emerenciano, M.G.C., Moheimani, N. & Burnell, G.M. (2019). Aquaponics: Alternative Types and Approaches. In: Goddek, S., Joyce, A., Kotzen, B., & Burnell, G.M. (eds) *Aquaponics Food Production Systems: Combined Aquaculture and Hydroponic Production Technologies for the Future*. Springer International Publishing. 301–330. https://doi.org/10.1007/978-3-030-15943-6_12

Krastanova, M., Sirakov, I., Ivanova-Kirilova, S., Yarkov, D. & Orozova, P. (2022). Aquaponic systems: biological and technological parameters. *Biotechnology & Biotechnological Equipment*, 36 (1), 305–316. https://doi.org/10.1080/13102818.2022.2074892

Lennard, W. & Goddek, S. (2019). Aquaponics: The Basics. In: Goddek, S., Joyce, A., Kotzen, B., & Burnell, G.M. (eds) *Aquaponics Food Production Systems: Combined Aquaculture and Hydroponic Production Technologies for the Future*. Springer International Publishing. 113–143. https://doi.org/10.1007/978-3-030-15943-6_5

Lim, C. & Carl D, W. (eds) (2006). *Tilapia : biology, culture, and nutrition*. https://doi.org/10.1201/9781003578482

Love, D.C., Fry, J.P., Li, X., Hill, E.S., Genello, L., Semmens, K. & Thompson, R.E. (2015). Commercial aquaponics production and profitability: Findings from an international survey. *Aquaculture*, 435, 67–74. https://doi.org/10.1016/j.aquaculture.2014.09.023

Luján, A.M., Gómez, P. & Buckling, A. (2015). Siderophore cooperation of the bacterium Pseudomonas fluorescens in soil. *Biology Letters*, 11 (2), 20140934. https://doi.org/10.1098/rsbl.2014.0934

Luo, A., Wang, F., Sun, D., Liu, X. & Xin, B. (2022). Formation, Development, and Cross-Species Interactions in Biofilms. *Frontiers in Microbiology*, 12. https://doi.org/10.3389/fmicb.2021.757327

Maier, R.M. & Pepper, I.L. (2015). Bacterial Growth. In: *Environmental Microbiology*. Elsevier. 37–56. https://doi.org/10.1016/B978-0-12-394626-3.00003-X

Martikainen, P.J. (2022a). Heterotrophic nitrification – An eternal mystery in the nitrogen cycle. *Soil Biology and Biochemistry*, 168, 108611. https://doi.org/10.1016/j.soilbio.2022.108611

Martikainen, P.J. (2022b). Heterotrophic nitrification – An eternal mystery in the nitrogen cycle. *Soil Biology and Biochemistry*, 168, 108611. https://doi.org/10.1016/j.soilbio.2022.108611

Michaud, L., Blancheton, J.P., Bruni, V. & Piedrahita, R. (2006). Effect of particulate organic carbon on heterotrophic bacterial populations and nitrification efficiency in biological filters. *Aquacultural Engineering*, 34 (3), 224–233. https://doi.org/10.1016/j.aquaeng.2005.07.005

Nair, C.S., Manoharan, R., Nishanth, D., Subramanian, R., Neumann, E. & Jaleel, A. (2025). Recent advancements in aquaponics with special emphasis on its sustainability. *Journal of the World Aquaculture Society*, 56 (1), e13116. https://doi.org/10.1111/jwas.13116

Petrovich, M., Wu, C.-Y., Rosenthal, A., Chen, K.-F., Packman, A.I. & Wells, G.F. (2017). Nitrosomonas europaea biofilm formation is enhanced by Pseudomonas aeruginosa. *FEMS Microbiology Ecology*, 93 (5). https://doi.org/10.1093/femsec/fix047

Pompa, T. & Masser, M. (1999). *Tilapia: Life History and Biology*. (SRAC Publication No. 283). Southern Regional Aquaculture Center, United States Department of Agriculture.

Preena, P.G., Rejish Kumar, V.J. & Singh, I.S.B. (2021). Nitrification and denitrification in recirculating aquaculture systems: the processes and players. *Reviews in Aquaculture*, 13 (4), 2053–2075. https://doi.org/10.1111/raq.12558

Qi, W., Skov, P.V., de Jesus Gregersen, K.J. & Pedersen, L.-F. (2022). Estimation of nitrifying and heterotrophic bacterial activity in biofilm formed on RAS biofilter carriers by respirometry. *Aquaculture*, 561, 738730. https://doi.org/10.1016/j.aquaculture.2022.738730

Rafiee, G. & Saad, C.R. (2005). Nutrient cycle and sludge production during different stages of red tilapia (Oreochromis sp.) growth in a recirculating aquaculture system. *Aquaculture*, 244 (1–4), 109–118. https://doi.org/10.1016/j.aquaculture.2004.10.029

Rahayu, S., Amoah, K., Huang, Y., Cai, J., Wang, B., Shija, V.M., Jin, X., Anokyewaa, M.A. & Jiang, M. (2024). Probiotics application in aquaculture: its potential effects, current status in China and future prospects. *Frontiers in Marine Science*, 11. https://doi.org/10.3389/fmars.2024.1455905

Rakocy, J.E. (2012). Aquaponics—Integrating Fish and Plant Culture. In: *Aquaculture Production Systems*. John Wiley & Sons, Ltd. 344–386. https://doi.org/10.1002/9781118250105.ch14

Rakocy, J.E., Masser, M.P. & Losordo, T.M. (2006). *Recirculating Aquaculture Tank Production Systems: Aquaponics—Integrating Fish and Plant Culture*. (SRAC Publication No. 454). Southern Regional Aquaculture Center.

Randall, D.J. & Tsui, T.K.N. (2002). Ammonia toxicity in fish. *Marine Pollution Bulletin*, 45 (1), 17–23. https://doi.org/10.1016/S0025-326X(02)00227-8

Rudoy, D., Olshevskaya, A., Shevchenko, V., Prazdnova, E., Odabashyan, M., Teplyakova, S., Rudoy, D., Olshevskaya, A., Shevchenko, V., Prazdnova, E., Odabashyan, M. & Teplyakova, S. (2025). Prospects for the Application of Probiotics to Increase the Efficiency of Integrated Cultivation of Aquatic Animals and Plants in Aquaponic Systems. *Fishes*, 10 (6). https://doi.org/10.3390/fishes10060251

Ruiz, P., Vidal, J.M., Sepúlveda, D., Torres, C., Villouta, G., Carrasco, C., Aguilera, F., Ruiz-Tagle, N. & Urrutia, H. (2020a). Overview and future perspectives of nitrifying bacteria on biofilters for recirculating aquaculture systems. *Reviews in Aquaculture*, 12 (3), 1478–1494. https://doi.org/10.1111/raq.12392

Ruiz, P., Vidal, J.M., Sepúlveda, D., Torres, C., Villouta, G., Carrasco, C., Aguilera, F., Ruiz-Tagle, N. & Urrutia, H. (2020b). Overview and future perspectives of nitrifying bacteria on biofilters for recirculating aquaculture systems. *Reviews in Aquaculture*, 12 (3), 1478–1494. https://doi.org/10.1111/raq.12392

Rurangwa, E. & Verdegem, M.C.J. (2015). Microorganisms in recirculating aquaculture systems and their management. *Reviews in Aquaculture*, 7 (2), 117–130. https://doi.org/10.1111/raq.12057

Silby, M.W., Cerdeño-Tárraga, A.M., Vernikos, G.S., Giddens, S.R., Jackson, R.W., Preston, G.M., Zhang, X.-X., Moon, C.D., Gehrig, S.M., Godfrey, S.A.C., Knight, C.G., Malone, J.G., Robinson, Z., Spiers, A.J., Harris, S., Challis, G.L., Yaxley, A.M., Harris, D., Seeger, K., Murphy, L., Rutter, S., Squares, R., Quail, M.A., Saunders, E., Mavromatis, K., Brettin, T.S., Bentley, S.D., Hothersall, J., Stephens, E., Thomas, C.M., Parkhill, J., Levy, S.B., Rainey, P.B. & Thomson, N.R. (2009). Genomic and genetic analyses of diversity and plant interactions of Pseudomonas fluorescens. *Genome Biology*, 10 (5), R51. https://doi.org/10.1186/gb-2009-10-5-r51

Silby, M.W., Winstanley, C., Godfrey, S.A.C., Levy, S.B. & Jackson, R.W. (2011). Pseudomonas genomes: diverse and adaptable. *FEMS Microbiology Reviews*, 35 (4), 652–680. https://doi.org/10.1111/j.1574-6976.2011.00269.x

Smith, P., Martino, D., Cai, Z., Gwary, D., Janzen, H., Kumar, P., McCarl, B., Ogle, S., O'Mara, F., Rice, C., Scholes, B., Sirotenko, O., Howden, M., McAllister, T., Pan, G., Romanenkov, V., Schneider, U., Towprayoon, S., Wattenbach, M. & Smith, J. (2007). Greenhouse gas mitigation in agriculture. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 363 (1492), 789–813. https://doi.org/10.1098/rstb.2007.2184

Taylor, T.B., Silby, M.W. & Jackson, R.W. (2025). Pseudomonas fluorescens. *Trends in Microbiology*, 33 (2), 250–251. https://doi.org/10.1016/j.tim.2024.11.005

Thorarinsdottir, R.I. (ed.) (2015). *Aquaponics guidelines*. 2015, 2nd printing. ed. Eco-innovation Initiative of the European Union.

Tsuneda, S., Park, S., Hayashi, H., Jung, J. & Hirata, A. (2001). Enhancement of nitrifying biofilm formation using selected EPS produced by heterotrophic bacteria. *Water Science and Technology*, 43 (6), 197–204. https://doi.org/10.2166/wst.2001.0374

United Nations (2025). *World Urbanization Prospects 2025: Summary of Results*. (UN DESA/POP/2025/TR/No. 12). United Nations.

Wongkiew, S., Hu, Z., Chandran, K., Lee, J.W. & Khanal, S.K. (2017). Nitrogen transformations in aquaponic systems: A review. *Aquacultural Engineering*, 76, 9–19. https://doi.org/10.1016/j.aquaeng.2017.01.004

Zhang, S., Sha, C., Jiang, W., Li, W., Zhang, D., Li, J., Meng, L. & Piao, Y. (2015). Ammonium removal at low temperature by a newly isolated heterotrophic nitrifying and aerobic denitrifying bacterium *Pseudomonas fluorescens* wsw-1001. *Environmental Technology*, 36 (19), 2488–2494. https://doi.org/10.1080/09593330.2015.1035759