Desalination 616 (2025) 119388

Contents lists available at ScienceDirect

Desalination

journal homepage: www.elsevier.com/locate/desal

Pilot-scale membrane bioreactor for source-separated urine: Impact of hydraulic retention time on fertiliser production

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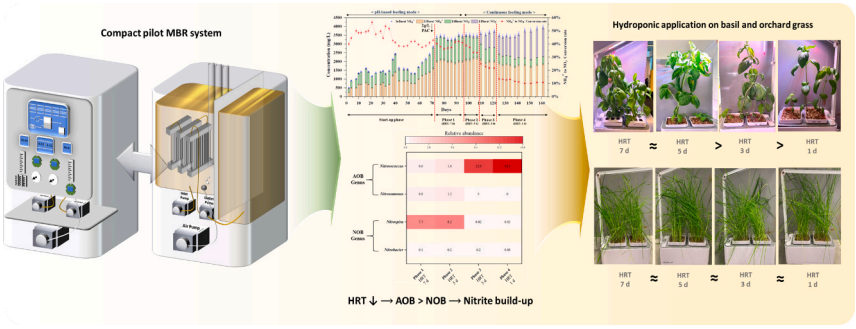
HIGHLIGHTS

- Pilot-scale compact MBR system was developed for urine to fertiliser conversion.
- pH-based urine feeding achieved stable nitrification with 7-day of HRT.
- Systematic HRT reduction led to gradual nitrite accumulation and AOB enrichment.
- Hydroponic basil and orchard grass growth was optimal at HRTs of up to 5 days.
- Orchard grass tolerated high-nitrite solution, showing potential at suboptimal HRT.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Urine
Nitrification
Membrane bioreactor
Nutrient
Hydroponic
Fertiliser
Circular economy

ABSTRACT

Prolonged hydraulic retention time (HRT) in urine-treating membrane bioreactors (MBR) remains a challenge as it increases system footprint and costs. This study investigated the effects of HRT conditions in a pilot-scale compact MBR system on urine nitrification performance, aiming to determine the optimal HRT threshold ensuring the effectiveness of the produced liquid fertiliser on hydroponic plant growth. The start-up phase of the MBR successfully achieved stable nitrification at a 7-day HRT under pH-controlled feeding, with a high enrichment of *Nitrospira* as the predominant nitrite-oxidising bacteria (NOB) and *Nitrosococcus* as the dominant ammonia-oxidising bacteria (AOB). However, the transition to continuous urine feeding at systematically reducing HRTs of 5 days, 3 days, and 1 day resulted in a decreasing ammonia-to-nitrate conversion rate, dropping from 40 % to 10 % along with a significant nitrite accumulation caused by the high enrichment of AOB over NOB. The urine fertiliser produced under each HRT condition presented distinctive formulations, with a fixed total nitrogen concentration and varying nitrogen species proportions. The fertilisers were applied to hydroponic growth of basil and orchard grass. Both basil and orchard grass showed optimal growth, in terms of roots-to-shoots ratio, at HRTs of up to 5 days. However, orchard grass showed more resilience to the variations in HRT, displaying similar fresh biomass yields across the different conditions. This study offers valuable insights

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https://doi.org/10.1016/j.desal.2025.119388
Received 1 August 2025; Received in revised form 2 September 2025; Accepted 4 September 2025
Available online 5 September 2025

into optimising HRT in urine MBR systems to enhance nutrient recovery as a liquid fertiliser, paving the way for more compact and cost-efficient on-site nutrient recovery and fertiliser application at scale.

1. Introduction

In recent years, there has been a significant paradigm shift from traditional linear economic models to sustainable circular approaches that focus on minimising waste while managing resources effectively. Although wastewater treatment plants are traditionally designed to remove nutrients found in wastewater, strategies to recover and reuse such nutrients are now gaining traction [1–3]. Source-separation of urine from the municipal wastewater and its separate treatment for nutrient recovery has been recognised as one of the most promising strategies. By recovering, rather than removing, nutrients contained in urine, approximately 33 % of the aeration energy in conventional wastewater treatment plants can be saved, and 25 % of direct carbon dioxide emissions through biological oxidation processes can be reduced [4]. Source-separated urine is a significant nutrient source of for agricultural cultivation, however, its direct application is generally not recommended because of unpleasant odour, high pH (~9.5) and substantial nutrient losses through ammonia volatilisation. Among the various technologies explored for treating urine, biological nitrification stands out for its efficacy in reclaiming essential nutrients from source-separated urine, transforming it into a liquid fertiliser that supports the circular economy framework. Nitrification addresses the major limitations of direct use by converting ammonium to nitrate, lowering the pH to around 6.2, and thereby reducing ammonia volatilisation. The process also eliminates odour, decreases organic matter content, and produces a safe and stable fertiliser. Importantly, for hydroponic application, a mixed ammonium–nitrate nitrogen form is desirable, since both species can be readily taken up by plants. Ammonium supplies a quick and energy-efficient nitrogen source, while nitrate provides stability and pH balance, together ensuring more reliable plant performance. This makes biologically nitrified urine an agronomically suitable and operationally stable fertiliser product [5].

The integration of ultrafiltration membrane bioreactors (UF-MBR) has emerged as a promising technological advancement, which effectively reduces odorous compounds and stabilises pH without chemical additives by partially converting NH_3/NH_4^+ into NO_3^-. When considered on a full-scale implementation, the nitrification process of source-separated urine in MBR systems requires more than 70 % less energy compared to the Haber-Bosch process used in traditional fertiliser synthesis [6]. This substantial reduction in energy use presents a great opportunity to recover nutrients efficiently and sustainably, paving the way for their use in various applications such as public parks, and urban agriculture systems. Furthermore, the addition of powdered activated carbon (PAC) in urine MBR system as an adsorbent significantly removes micropollutants over 99 %, while the incorporation of an ultrafiltration (UF) membrane facilitates virus removal in the permeate [7]. As a result, the produced nitrogen, phosphorous, and potassium (NPK) liquid fertiliser showed a comparable performance to commercial fertilisers, making it suitable for various applications [8].

During the biological nitrification process in the MBR, ammonia (NH_3) oxidised to nitrite (NO_2^-) by ammonia oxidising bacteria (AOB) and then nitrite oxidised to nitrate (NO_3^-) by nitrite oxidising bacteria (NOB) [9]. Maintaining suitable operational conditions, particularly pH, is essential to ensure a balanced interaction between the AOB and NOB activities. If the NOB process is unable to keep pace with AOB activity, there can be accumulation of nitrite in the reactor, leading to inhibition of NOB activity. The relationship between AOB and NOB kinetics can be influenced by several inhibitory factors including pH, dissolved oxygen (DO), temperature, free ammonia (FA) and free nitrous acid (FNA) concentrations [10–12]. Among these, effective pH control is crucial, as it regulates the NH_4^+/NH_3 and the HNO_2/NO_2^- equilibria, affecting FA and FNA concentrations, which in turn maintain nitrifier activity and prevent inhibition [13]. Moreover, this proper pH control mitigates accumulation of nitrite that is considered undesirable from an agronomic perspective due to its negative effects on plants [8]. As such, urine-treating MBRs have traditionally operated under pH-based feeding modes to effectively maintain a pH setpoint. The consumption of alkalinity during nitrification results in decline in pH. When this accurs, a pH-dosing pump automatically adds high-pH urine (pH 9.2–9.5) to maintain the pH set-point to promote balanced action between AOB and NOB. Consequently, in this typical operational mode, the hydraulic retention time (HRT) is directly influenced by the nitrification rate and cannot be independently controlled [14].

The HRT, as a factor dependant on nitrification rates, poses a significant challenge for urine MBR systems. The high salinity and elevated ammonia concentrations (4–5 g/L) in urine, which are substantially higher than those found in municipal wastewater, result in operational bottlenecks that lead to prolonged HRTs [5,15]. This limitation increases the system footprint, capital costs, and operational complexity, thereby hindering the commercialisation of urine-treating MBR systems [1,16]. Previous studies have investigated the biological nitrification of a source separated urine in both lab-scale and pilot-scale MBRs, showing that the HRTs were 14 ± 5 days and 10 ± 5 days, respectively, during stable operation [17,18]. These durations are significantly longer than the average HRTs for MBRs used in municipal wastewater treatment ranging between 2 and 12 h [19–22]. The integration of biofilm carriers into MBR systems to support the stable growth of nitrifying bacteria has been proved effective in reducing the HRT. This strategy successfully enhanced the nitrification rate by 36 %, resulting in a 40 % reduction in HRT, ultimately achieving an average 6 days of HRT [23]. However, the scaling up of such systems may still face challenges in managing variations in urine flow, which are common in real-world applications. In this context, moving away from the conventional strategy of maintaining a fixed pH setpoint introduces additional complexity in the control of nitrification, presenting an important research gap. In particular, it is necessary to explore whether alternative strategies for managing process conditions can reduce HRT while still supporting biological nitrification. Addressing this question is critical, as a lower HRT could enhance the overall feasibility and scalability of urine treatment systems. Moreover, it is essential to evaluate how such changes in operational strategy would affect the composition and agronomic quality of the resulting fertilisers. A deeper understanding of these relationships will provide valuable insights for the design and optimisation of future urine MBR systems, while also establishing a foundation for predicting and addressing system variations and identifying opportunities for HRT reduction.

In this study, a novel pilot-scale MBR system was developed, featuring a 200 L MBR tank and 100 L fertiliser tank in a compact, fridge-like design for building-scale urine treatment and fertiliser production. In this compact MBR system with PAC incorporation, stable nitrification was achieved after successful sludge acclimation by typically adopting pH-based feeding mode. The system then transitioned to continuous feeding mode under fixed HRT conditions, with a systematic reduction in HRT throughout the operational period. This study explored the impacts of HRT under continuous feeding conditions, focusing on the nitrification performance, including ammonia-to-nitrate conversion rates. In addition, this study evaluated the effectiveness of urine-derived fertilisers with varying ammonium-to-nitrate ratios in promoting hydroponic growth of basil and orchard grass. Previous research has reported that nitrite concentrations of 120 mg/L in the hydroponic solution adversely affect basil growth, although the precise threshold for nitrite toxicity remains unexplored [8]. To address this knowledge gap,

this study aimed to investigate the threshold nitrite concentration by applying treatment solutions with increasing nitrite levels under progressively reduced HRT conditions in hydroponic systems. By identifying the optimal HRT threshold for the efficient application on hydroponics as a fertiliser, this study aims to provide practical insights for enhancing the commercial viability of compact MBR systems for source-separated urine treatment.

2. Materials and methods

2.1. Experimental set-up

2.1.1. Pilot-scale compact membrane bioreactor system

A fully automated pilot-scale MBR has been developed in a compact design and customised for urine treatment, manufactured by OriginWater Ltd., China. The equipment is 768x623x1550 mm dimensions and consists of main and inner controller panels, a 200 L MBR tank and 100 L permeate (fertiliser) tank, as shown in Fig. 1. A photo of the system is presented in Fig. S1. The bottom of the equipment is equipped with wheels for easy movement. The main display panel that appears on the outer door enables monitoring the water levels of each tank as well as controlling the main operational parameters such as the water level range and pH limits. In the inner controller panel, there are display screens for real-time monitoring of total suspended solids (TSS) concentration, dissolved oxygen (DO), and pH value of the MBR tank as well as electrical conductivity (EC) from the permeate tank. In addition, inlet and outlet water flow meters as well as two air flow meters for coarse and fine air bubbling are installed for controlling the water and air flow rates together with pressure gauges and transmitter to monitor. Moreover, the feed and permeate water pumps and air blower have been installed in the inner bottom shelf.

The MBR tank was filled with inoculative activated sludge which was collected from a decentralised wastewater treatment plant (Central Park Sydney, Ultimo, NSW Australia). The feed urine was collected throughout this study in Building 11 at the University of Technology Sydney (UTS) where urine diversion pipes are installed facilitating the collection of source-separated urine from male urinals on each floor. The collected urine is stored in a 100 L water tank to ensure a complete urea hydrolysis before feeding to the pilot MBR system.

The MBR was operated with an effective working volume of 140 L during phases 1–3 and 100 L in phase 4, with the volume maintained constant within each operational phase. The water level in the system was automatically regulated by sensors that controlled the permeate pump connected to the submerged membrane modules. Three ultrafiltration membrane modules (PVDF, inner and outer diameter of 0.65 mm and 1.35 mm, 0.05 μm pore size, OriginWater, China) having 0.36 m^2 of total surface area were submerged in the MBR tank. During the start-up phase and phase 1, the MBR was operated at pH-based dosing mode. The pH set point was set at 6.2, and when the pH drops under this set point due to nitrification, the influent pump was automatically activated to dose high-pH hydrolysed urine into the MBR. As such, the hydraulic retention time (HRT) was therefore determined by the feeding rate, dependant factor of nitrification rate. The HRT was calculated according to the equation of $HRT = V/Q_{in}$, where V is the effective working volume (L) and Q_{in} is the influent flow rate (L/day). In phase 1, the membrane was operated at a flux of 6 L/m^2/h (LMH) with a 3-min on/5-min off cycle, corresponding to an average HRT of 7 days. To investigate the effect of HRT on the MBR performance, the feeding mode was changed to continuous feeding at fixed HRT from phase 2 onwards by adjusting the influent pump flow rate, resulting in fixed HRTs of 5, 3, and 1 day in phases 2, 3, and 4, respectively. During these phases, an extra pH dosing pump was installed outside the system and the pH level was maintained by automatically dosing 0.01 M hydrochloride solution when the pH increased above 6.2. In phase 2, the membrane flux remained at 6 LMH with a cycle of 5-min on/4-min off. In phase 3, the flux was slightly increased to 8 LMH with a cycle of 4-min on/2-min off. In phase 4, the effective volume was reduced to 100 L owing to the maximum flow rate capacity of influent pump. The membrane flux was further increased to 14 LMH with a cycle of 5-min on/1-min off. Physical membrane cleaning was performed by flushing with DI water between operational phases, and fortnightly physical cleaning was additionally conducted during phase 1 to remove foulants accumulated on the membrane surface. Furthermore, fine and coarse air bubbling from the bottom of the MBR facilitated membrane scouring, allowing for more effective mitigation of reversible fouling by reducing biofilm thickness. Consequently, transmembrane pressure (TMP) development remained stable throughout the study (<10 kPa in phases 1–3 and <20 kPa in phase 4), indicating that membrane fouling was minimal under the relatively low flux operation, which was further mitigated by regular physical cleaning and aeration-assisted scouring.

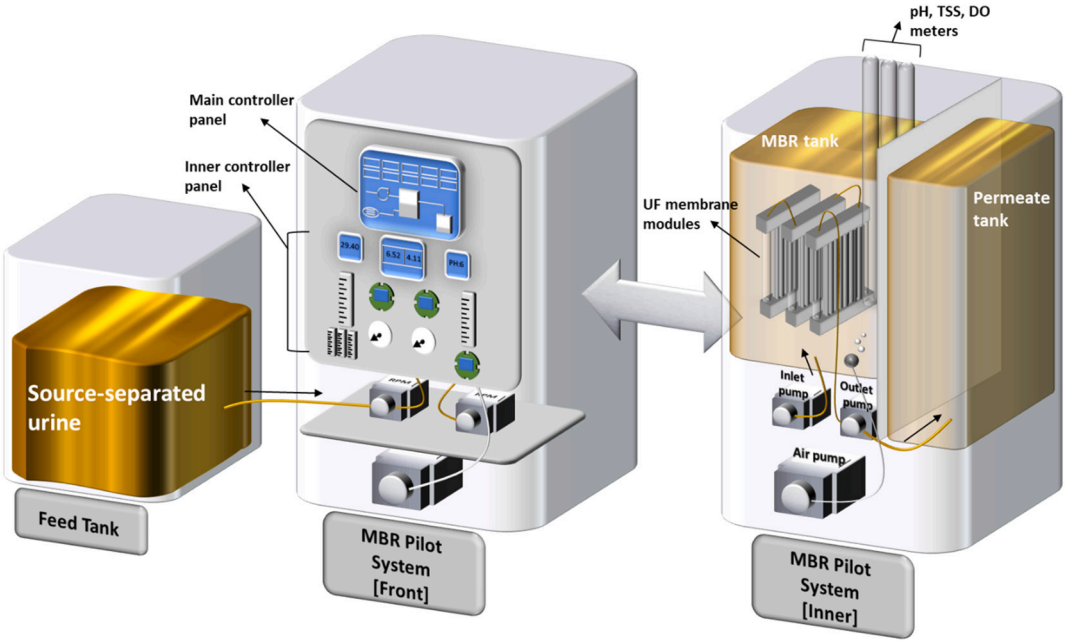

Fig. 1. Schematic diagram of pilot scale compact MBR system.

The dissolved oxygen (DO) level was maintained at 4.5 ± 1.0 mg/L and initial mixed liquor suspended solids concentration (MLSS) was 5.5 ± 0.1 g/L. Moreover, 2 g/L of PAC (100–325 mesh of particle size, 500–1000 m^2/g of surface area, Darco KB-B, Norit, US) was added at the end of the start-up period. To achieve this concentration in phase 1, 40 g of PAC was introduced daily over the last 7 days of the start-up phase, totalling 280 g. This stepwise dosing approach was adopted to avoid sudden increases in suspended solids concentration that could cause process instability, thereby allowing the microbial community to adapt progressively to the presence of PAC. With PAC addition, the MLSS was controlled at 7.5 ± 0.1 g/L. At the beginning of each phase, approximately 5–6 % of the sludge mixture was withdrawn to reset the MLSS to around 7.5 g/L, thereby standardising biomass conditions across phases. PAC was correspondingly topped up to compensate for this withdrawal and maintain its concentration, while no further replenishment was conducted during phase operation. Since sludge was only removed at the start of each phase and not during subsequent operation, the sludge retention time (SRT) was effectively infinite during each phases. As a result, an average MLSS of 7.7 ± 0.3 g/L and MLVSS of 6.1 ± 0.3 g/L were maintained throughout the whole operation, corresponding to a stable MLVSS/MLSS ratio of 0.75–0.80. It should be noted that the transition from Phase 1 to Phase 2 involved a change from pH-triggered feeding to continuous feeding with external pH dosing, which may represent a potential confounding factor. However, this influence was minimised in the present study as other operational parameters, including pH, DO, biomass concentration, and temperature, were maintained constant. Thus, the observed performance was interpreted as primarily reflecting the impact of HRT.

2.1.2. Hydroponic system

Basil (*Ocimum basilicum*, Johnsons seeds, Australia) and orchard grass (*Dactylis glomerata*, Mr. Fothergill's seed, Australia) were cultivated for 12 weeks and 6 weeks respectively in a deep-water culture indoor hydroponic system, called 'HydroGarden', at the environmental laboratory at the University of Technology Sydney. A total of 5 hydrogarden units were set up for each plant, and each unit fertigated with different fertiliser solutions. Vermiculite was used as the growing medium, and each unit was equipped with built-in LED lights operating on an automatic 16 h light-on/8 h light-off daily cycle to provide efficient illumination for plant growth. A commercial fertiliser (Hydro Plus for All Plants, Manutec garden care, Australia) was applied in hydrogarden 1, labelled as "HG1", and benchmarked with urine fertilisers produced from the pilot MBR system in different operational phases. Commercial fertilisers such as HG1 are typically formulated with a higher proportion of nitrate than ammonium, since nitrate is chemically stable. In contrast, urine-derived fertilisers are produced from biological nitrification, and under stable operation the expected final composition is close to an ammonium nitrate solution with an approximate 1:1 ratio. Previous studies have demonstrated that such urine fertilisers can achieve comparable performance to commercial formulations [8,18]. In the present study, this comparison was extended by evaluating fertilisers produced under different HRT conditions, which resulted in varying ammonium, nitrite, and nitrate concentrations. The urine fertilisers produced from phase 1 to 4 were applied to hydrogarden 2 to 5, labelled "HG2", "HG3", "HG4", and "HG5". To ensure comparability, each fertiliser solution was diluted with deionised (DI) water to achieve a total nitrogen concentration of around 250 mg TN/L, consistent with the commercial fertiliser formulation. The diluted fertiliser solutions in each hydroponic unit were fully replaced with freshly prepared solutions on a weekly basis to maintain consistent water levels and electrical conductivity (EC), as well as to prevent the accumulation of sodium. The compositions of the treatment solutions applied in the hydrogardens are described in Table 2.

2.2. Analytical methods

2.2.1. Nutrient, and total organic carbon analysis

Nutrients concentrations, sampled from source-separated urine, MBR permeate, and each treatment solutions from hydrogardens, such as ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), nitrite nitrogen (NO_2^--N), total phosphate (PO_4^{3-}-P), and sulphate (SO_4^{2-}) were analysed using standard test kits (Merck Millipore, Burlington, USA) and a photometer (Spectroquant NOVA 60, Merck, Germany). Inductively coupled plasma mass spectrometry (ICP-MS) was used to measure the concentration of cations (K^+, Ca^{2+}, Mg^{2+}) and heavy metals (Fe and Mn) in the hydroponic treatment solutions. Total organic carbon (TOC) concentration was determined by a TOC analyser (Analytik Jena Multi N/C 2000).

2.2.2. Amplicon sequencing and bioinformatics analysis

The suspended sludge was sampled at the end of each phase from 1 to 4 for the microbial community analysis. The samples were delivered to the UTS Next Generation Sequencing Facility, Sydney, Australia, for DNA extraction and amplicon sequencing. The V3-V4 regions of bacterial and archaeal 16S rRNA genes were amplified using the universal primer set Pro341F (5'-CCTAYGGGRBGCASCAG-3') and Pro806R (5'-GGACTACNNGGGTATCTAAT-3'), to obtain a comprehensive profile of the microbial community [24]. Paired-end amplicon sequencing (2 × 300 bp) was carried out using the Illumina MiSeq platform at the UTS sequencing facility. The raw sequence data were initially processed with the Illumina bcl2fastq pipeline. The raw reads underwent microbial community and diversity analysis using on CJ Bioscience's bioinformatics cloud platform EzBioCloud with 16S-based metagenome taxonomic profiling (MTP) [25].

2.2.3. Growth of basil and orchard grass in hydroponic systems

At the conclusion of each experiment, plant shoots and roots were divided to evaluate fresh biomass. Roots were carefully extracted from the growing medium by gently rinsing them under a stream of water. Both shoot and root samples were enclosed in paper bags and dried in an oven at 60° C for 72 h [26,27]. After drying, the samples were weighed to determine their dry biomass. The root-to-shoot ratio was calculated by dividing the dry weight of the roots by that of the shoots. Additionally, the height of the main stems was measured using a 50 cm ruler, from the base to the tip of the shoot. The number of stems were recorded for each system by counting those branching from 5 cm above the media surface. For data analysis, a paired *t*-test was performed in Microsoft Excel to assess the statistical significance of observed differences. A 5 % significance level ($p \leq 0.05$) was used to determine whether treatment effects were statistically significant.

3. Results and discussion

3.1. Effect of HRT on the performance of MBR

The pilot-scale compact MBR system was continuously operated for 160 days. The operation can be divided into five distinct phases which includes the start-up phase for sludge acclimation; phase 1, where the MBR was operated in a traditional pH-based feeding mode with full-strength hydrolysed urine; phase 2 to 4 when the MBR was operated at a continuous urine feeding mode under the systematically reduced HRT conditions. From day 1 to day 72, indicated as a start-up phase, the inoculated sludge was acclimatised by initially feeding hydrolysed urine with 10-fold dilution having around 400 mg/L of total nitrogen. The feed nitrogen concentration was then gradually increased by reducing the dilution rate according to the stable nitrification rate. When the nitrogen concentration reached ~2 g/L, a slight nitrite accumulation occurred from day 40 to day 50. This might be attributed to the increased ammonia loading, which promoted a higher growth rate of AOB than that of NOB, resulting in an imbalance between the two [28].

The nitrite accumulation was resolved by immediately reducing the feed nitrogen strength to 1.5 g/L, as described in the previous lab-scale studies [14]. After that, the feed urine dilution was progressively decreased to reach undiluted hydrolysed urine from day 73. Overall, the 72-day start-up phase carried out following our established protocols led to the successful sludge acclimation as well as stabilisation of the pilot-scale MBR in agreement with our previous lab-scale urine MBR studies [7,9,14,17].

In phase 1, following the successful sludge acclimation in the start-up phase, the MBR was operated by adopting the conventional pH-based feeding method with automatic feeding of full-strength hydrolysed urine based on the pH set-point. During phase 1 (28 days), the MBR showed stable operation with undiluted urine feeding, in alignment with previous studies on MBR systems operated in pH setpoint mode [9,14,17]. On average, 42 % of NH_4^+ was converted to NO_3^-, resulting in a nitrification rate of 194 ± 11 mgN/L·d, and an average HRT of 7 days. While the HRT during phase 1 was controlled by the nitrification rate according to the pH-based feeding, afterwards, the system was set to continuous feeding mode at fixed HRT conditions to investigate the impact of systematically reduced HRT on the nitrification performance of the MBR. In order to do that, the system settings were changed to enable a continuous feed of urine at fixed flow rate for each operational phase. As the stable operation at phase 1 resulted in 7-day HRT, the HRT was firstly reduced to 5 days in phase 2, corresponding to a fixed 5.8 L/h flow rate of urine. As a result, due to the higher nitrogen loading, nitrite concentration increased to 81 mg/L and the NH_4^+ to NO_3^- conversion rate dropped to 34 % on average for 14-day operational period. When the HRT was set at 3 days on day 110, the nitrite concentrations started to accumulate, reaching 580 mg/L in the early stage of phase 3, then it further increased up to 890 mg/L by the end of the phase. This significant nitrite accumulation led to a decrease in nitrate concentration from 2012 ± 64 mg/L (phase 1) to 820 ± 49 mg/L on average, resulting in the reduction of the NH_4^+ to NO_3^- conversion rate down to 22 %. Upon further reduction of the HRT to 1 day in phase 4 on day 125, the nitrite concentration surged to around 1 g/L, peaking at 1.6 g/L at the end of the phase. This substantial nitrite build-up in phase 4 led to a steady decline in nitrate concentration in the effluent, averaging 452 ± 44 mg/L which was nearly half of what was observed in phase 3. As a result, the NH_4^+ to NO_3^- conversion rate dropped to 11 %, likely due to severely inhibited NOB activity. It is worth noting that nitrite concentration increased progressively within each phase, by 305 %, 54 %, and 59 % from the first to the last day of phases 2, 3, and 4, respectively. However, a more significant increase was observed during the transitions between phases, primarily attributed to changes in HRT. Specifically, nitrite concentration increased sharply from 81 mg/L to 580 mg/L during the transition from phase 2 to 3, and from 890 mg/L to 1030 mg/L during the transition from phase 3 and 4. Importantly, this nitrite accumulation is itself a key result, demonstrating that AOB activity outpaced NOB under short HRTs, consistent with the microbial community analysis (Section 3.3). However, as reflected by the relatively stable ammonium-to-nitrate conversion, phases 1 and 4 represented steadier operation, whereas phases 2 and 3 can be regarded as transitional regimes shaped by the imposed stepwise reductions in HRT. Moreover, given that the ammonium concentration in the effluent remained relatively stable at an average of 1950 ± 144 mg/L throughout the entire operation period, it is noteworthy that the ammonium oxidation also remained constant at 44 ± 5 %. When normalized to the biomass concentration (MLVSS = 6.1 ± 0.3 g/L), the ammonium oxidation rate to NOx ($NO_2^- + NO_3^-$) showed a clear dependence on HRT, increasing from 30.9 mgN /gVSS·d at 7 d HRT (phase 1) to 52.4 mgN /gVSS·d at 5 d HRT (phase 2), 85.4 mgN /gVSS·d at 3 d HRT (phase 3), and 313.1 mgN /gVSS·d at 1 d HRT (phase 4). This trend might be attributed to the increased abundance of AOB, as discussed in Section 3.3, which intensified the daily ammonium oxidation efficiency under shorter HRT conditions. Consequently, the effluent from different phases exhibited varying ratios of ammonium, nitrite, and nitrate, with a decline in nitrate and an increase in nitrite concentrations as a function of the system HRT.

In terms of TOC removal efficiency, as shown in Fig. 2b, the addition of 2 g/L of PAC significantly improved the TOC removal rate from 85 % in the start-up phase up to 95 % in phase 1, owing to the superior adsorption capacity of PAC for organic matters [24]. During phase 2 and 3, this high organic removal rate was maintained at similar levels, ranging from 92 % to 94 %. This effectiveness might be due to the formation of biologically activated carbon (BAC) which was developed through the proliferation of microorganisms attached to the initially adsorbed organics on the PAC, and continued to facilitate organic removal [24,29]. In phase 4 with a reduced HRT, the TOC removal rate decreased to 88 % on average. This slight reduction could be due to a combination of factors. On one hand, the progressive saturation of PAC adsorption capacity may have reduced its contribution to organic removal, since PAC was only topped up at the beginning of each phase to offset sludge withdrawal, with no further replenishment during the operation phases. Although previous research on urine MBR system indicated that 1.6 % of daily PAC replenishment can be beneficial, further studies are required to optimise the replenishment strategies in urine-treating MBRs to sustain both adsorption and biological enhancement effects [30]. On the other hand, the shortened HRT might have also played a crucial role in the reduction in TOC removal rates. The transition from phase 3 to phase 4 showed a slight drop of 4 %, from 92 % to 88 % on average, while previous phases remained relatively steady at 95 %, 94 %, and 92 % for phase 1 to 3. It was reported that the HRT significantly influences hydraulic shear forces and affects the contact time between the substrate and bacteria [31]. These phenomena can explain the evolution of the TOC removal rate as a function of the HRT, with low HRT causing a decreased in contact time between the organic matter and the heterotrophic bacteria, thereby reducing the TOC removal effectiveness.

3.2. Microbial community analysis

3.2.1. Microbial diversity

The microbial diversity was investigated by analysing the number of sequences and observed operational taxonomic units (OTUs), as well as alpha indices for microbial diversity and richness including ACE, Chao1, Shannon, and Simpson, as presented in Table 1. ACE and Chao1 indices are indicators of species richness, while Shannon and Simpson measure species evenness. Higher ACE, Chao1, and Shannon indices indicate greater microbial diversity, whereas a lower Simpson index reflects higher diversity. Based on these indices, the activated sludge from phase 1 and 2 showed comparable microbial richness and evenness. However, the HRT reduction from 7 days in phase 1 to 5 days in phase 2 slightly increased microbial diversity, with phase 2 showing the highest diversity levels. In contrast, further HRT reduction to 3 days in phase 3 resulted in more than halved values in the number of OTUs, ACE, and Chao1 indices, indicating the significant reduction in microbial richness. Shannon and Simpson indices also indicated a significant decline in microbial evenness, with a 22 % decrease in Shannon index and a 233 % increase in the Simpson index. When the HRT was further reduced to 1 day in phase 4, both richness and evenness declined even more. The number of OTUs, ACE, and Chao1 indices decreased by a factor of 1.3, while the Shannon index decreased by 1.1-fold, and the Simpson index increased by 1.4-fold. This reduction is likely due to the increased substrate loading caused by the shortened HRT, which adversely impacted microbial diversity while selectively enriching certain species, making them predominant in the system. These results align with previous studies that reported reduced microbial diversity after the shock loading of substrates in the feed [32].

3.2.2. Microbial composition

The relative abundances from different operational phases at the phylum and genus levels were investigated. The functional bacterial communities were characterised by assigning sequence reads to known

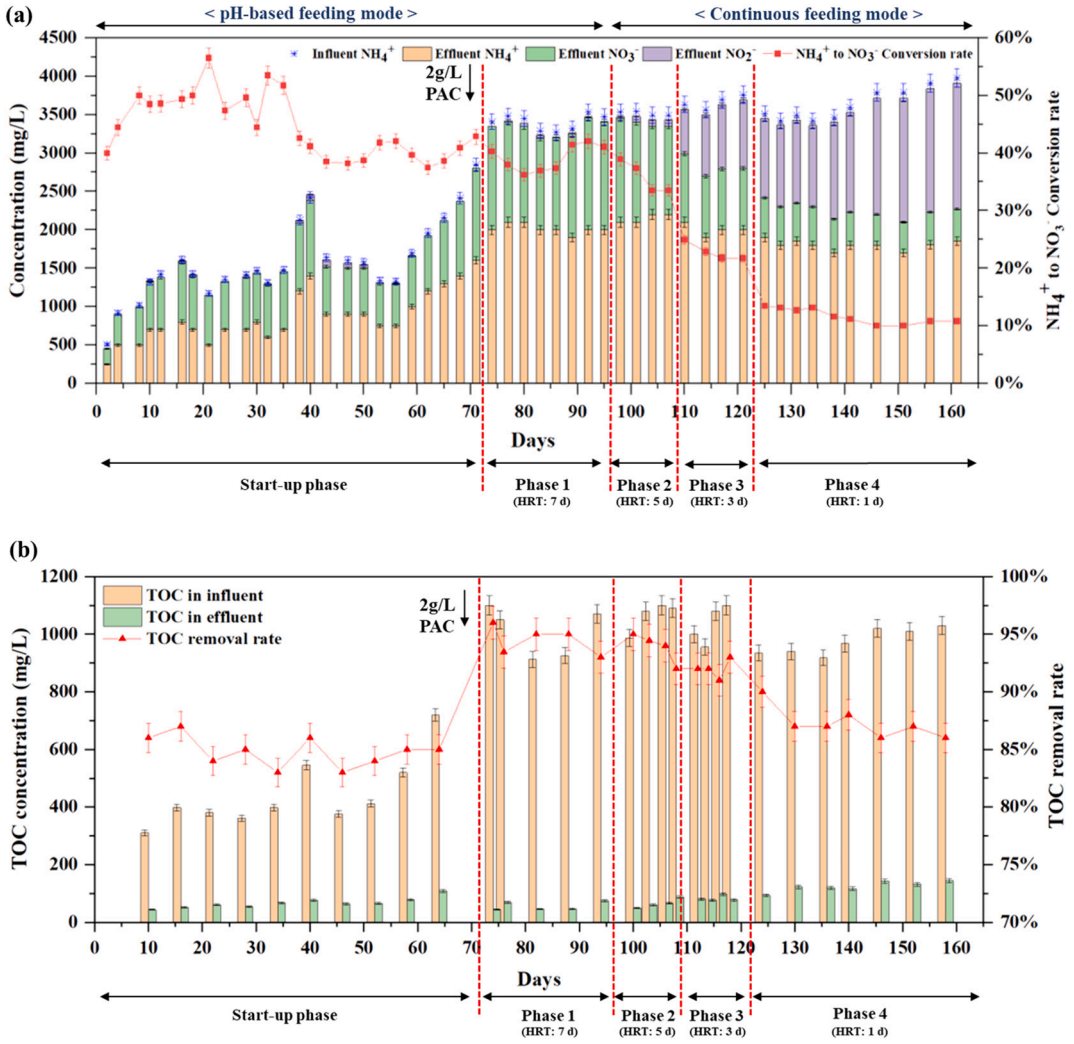

Fig. 2. (a) Nitrification performance of pilot-scale MBR system; (b) total organic carbon (TOC) removal efficiency over the operational period.

Table 1
Alpha indices for microbial diversity.

	Sequences	No. of OTUs	ACE	Chao1	Shannon	Simpson
Phase 1 (HRT 7 d)	55,778	1650	1977	1897	5.3	0.018
Phase 2 (HRT 5 d)	61,222	1849	2267	2155	5.4	0.015
Phase 3 (HRT 3 d)	60,551	910	1047	1016	4.2	0.05
Phase 4 (HRT 1 d)	51,879	694	789	774	3.8	0.07

phyla, resulting in the detection of 12 major phyla, as shown in Fig. 3a. Phyla with a relative abundance of less than 1 % were grouped under 'others' in Fig. 3a. During the stable nitrification stage (phase 1), the order of dominant bacteria in phylum level was *Proteobacteria* (25.5 %), *Planctomycetes* (21.4 %), *Chloroflexi* (18.0 %), *Actinobacteria* (16.2 %), and *Nitrospirae* (7.7 %). After continuous feeding of hydrolysed urine began with a 5-day HRT condition (phase 2), the dominant phyla sequence remained unchanged, but the proportion of *Proteobacteria* and *Nitrospirae* increased to 31.1 % and 8.2 %, respectively, while other phyla decreased by 2–5 %. When the urine feed rate was further increased under a 3-day HRT condition (phase 3), the dominant sequence of the top five phyla significantly changed to: *Proteobacteria* (55.3 %) > *Actinobacteria* (17.3 %) > *Bacteroidetes* (11.6 %) > *Chloroflexi* (7.7 %) > *Firmicutes* (4.1 %). During phase 4 under the shortest HRT condition, the biomass was largely dominated by *Proteobacteria* (67 %), followed by *Bacteroidetes* (14.8 %), *Actinobacteria* (8.5 %), and *Firmicutes* (3.3 %). Interestingly, the *Proteobacteria* phylum, which is known to include diverse functional bacteria playing important roles in pollutants biodegradation as well as organics removal, was the most abundant across all phases, a trend that is consistent with the high TOC removal observed in this study [33]. However, the order of subdominant phyla showed significant changes with HRT conditions. Notably, the *Nitrospirae* phylum, which ranked among the top five dominant phyla in phase 1 and 2, significantly lost its dominance in phase 3 and 4, alongside severe nitrite accumulation. The dominant phyla identified in each sludge sample align with their ubiquity in biological reactors found in wastewater treatment plants as well as urine treating MBR systems, as previously reported [23,34].

Further taxonomic analysis at the genus level was conducted to gain more insight into the evolution of the bacterial communities as a function of the HRT. Fig. 3b illustrates the top 42 bacterial genera, displaying only those with a relative abundance above 1 % in at least one sample. The remaining genera are grouped under 'Others'. The predominant genus in both phases 1 and 2 was *AF234694_g*, which belongs to the

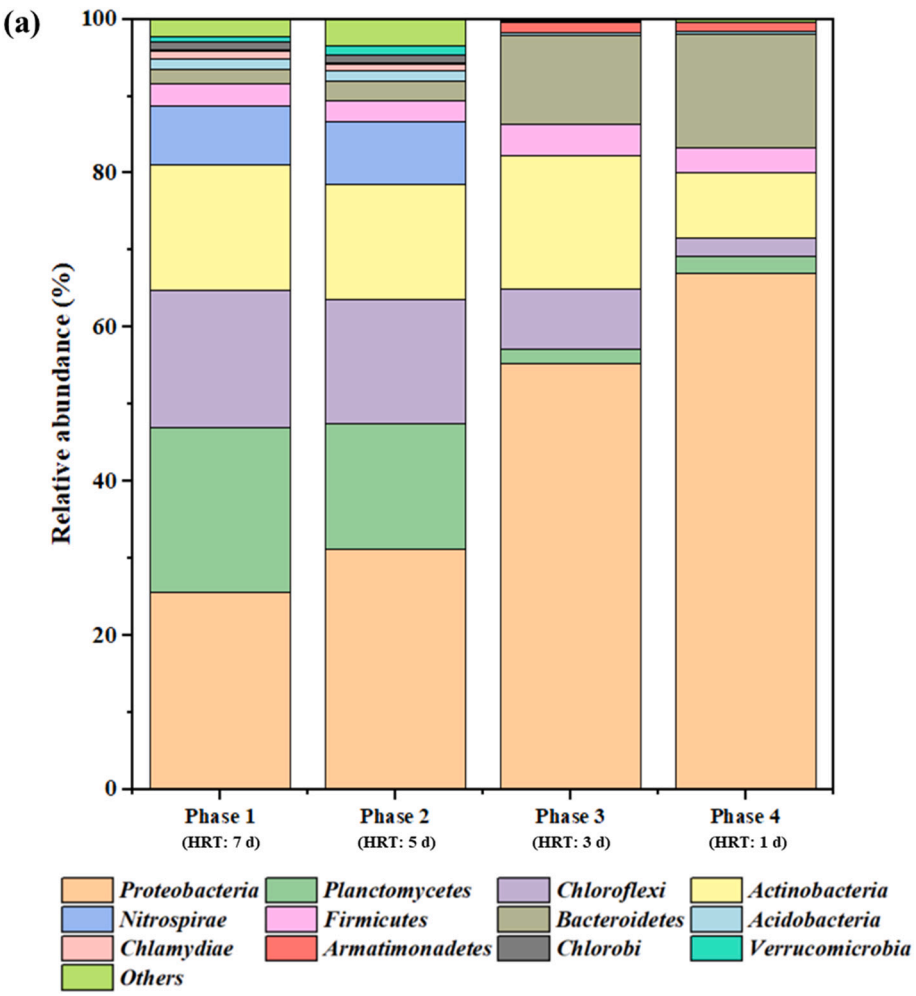

Fig. 3. Microbial compositions at (a) phylum and (b) genus level in each sample. Only phyla and genera with relative abundance >1 % in at least one sample are shown individually, the rest are grouped into "Others".

Caldilineae class of the *Chloroflexi* phylum. This genus has been reported as an enriched taxon in wastewater treatment processes, where it plays an important role in COD removal [35]. It is also known for filamentous bacteria involved in surface proliferation, and its dominance may be attributed to the addition of PAC at the start of phase 1, as the porous surface of PAC likely provided a favourable habitat for their growth [36]. However, the gradual decrease in *Chloroflexi* abundance from phase 1 to phase 4, reaching only 2.3 % in phase 4, likely contributed to the decline in TOC removal efficiency observed in later phases, in conjunction with PAC saturation. In the *Proteobacteria* phylum during phases 1 and 2, the prevailing genera were *Bradyrhizobium*, *Hyphomicrobium*, and *HQ003485_g*, belonging to the *alpha-* (α-) and beta- (β-) *Proteobacteria* classes. However, this dominance shifted greatly to gamma- (*γ-) Proteobacteria* in phases 3 and 4, with *Rhodanobacter* (23.0 %) and *Chujaibacter* (20.5 %) becoming the most abundant genera, respectively. Furthermore, *Mycobacterium* emerged as the most abundant genus in the *Actinobacteria* phylum across phases. The *Mycobacterium* genus is known for its excellent pollutants biodegradation and high tolerance to harsh environments such as high salinity contributing to the system's TOC removal performance regardless of the urine feed strength and flow rate [37–39]. This finding aligns with our previous study, which reported a notable increase in *Mycobacterium* abundance in PAC-added urine-treating MBR compared to that without PAC [23]. However, the relative abundance of *Actinobacteria* declined markedly in Phase 4, from 15 to 17 % in Phases 1–3 to 8.5 %, which may have contributed to the reduction in TOC removal observed under shortened HRT conditions. Notably, reducing the HRT to 3 and 1 day (phase 3–4) increased the relative abundance of *Firmicutes*. The *Firmicutes* genus ranked as the fifth and fourth predominant phylum in phases 3 and 4, respectively, when the HRT was intentionally shortened. *Firmicutes* are known for enzyme production for various organic degradation pathways as well as high resistance to extreme conditions such as high ammonia loadings [40,41].

To shed light into the nitrification performance of the system, a closer look at the taxonomic analysis of nitrifying bacteria was necessary. Fig. 4 presents a heatmap of the distribution of the nitrifying bacterial genera of AOB and NOB from different operational phases. Overall, two AOB genera were detected, which were *Nitrosococcus* from *γ-Proteobacteria* class, and *Nitrosomonas* from the *β-Proteobacteria* class, both responsible for the oxidation of ammonia to nitrite [42–44]. While *Nitrosococcus* was the predominant AOB in this study, it is rarely found in conventional wastewater treatment plants, similar to the *Nitrosococcaceae* family in previous research, but has frequently been reported as a primary AOB in saline aquaculture systems [45–47]. This prevalence can be attributed to the high salinity levels present in source-separated urine, which are 5 to 30 times greater than those in typical municipal wastewater. In terms of NOB genera, *Nitrospira* from the *Nitrospirae* phylum was predominant, as widely distributed worldwide, responsible for the oxidation of nitrite to nitrate [48,49]. In contrast, *Nitrobacter*, another well-known NOB genus within the *Proteobacteria*,

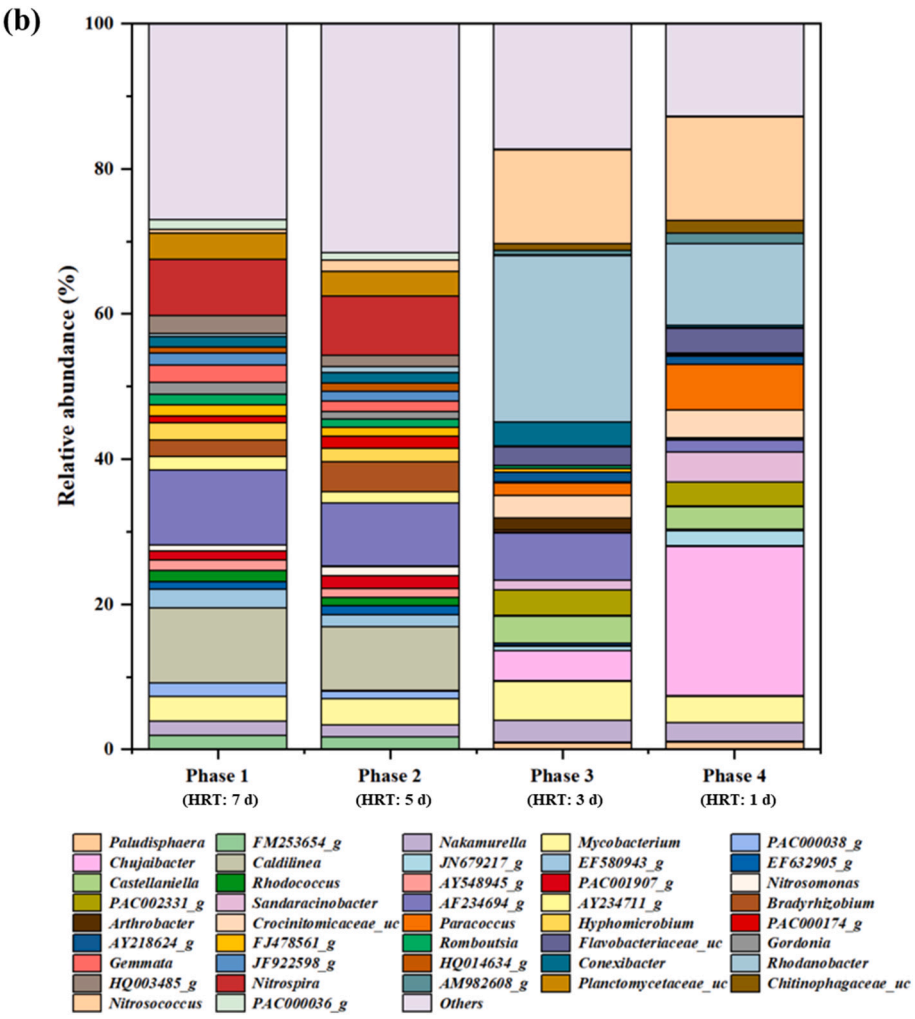

Fig. 3. (*continued*).

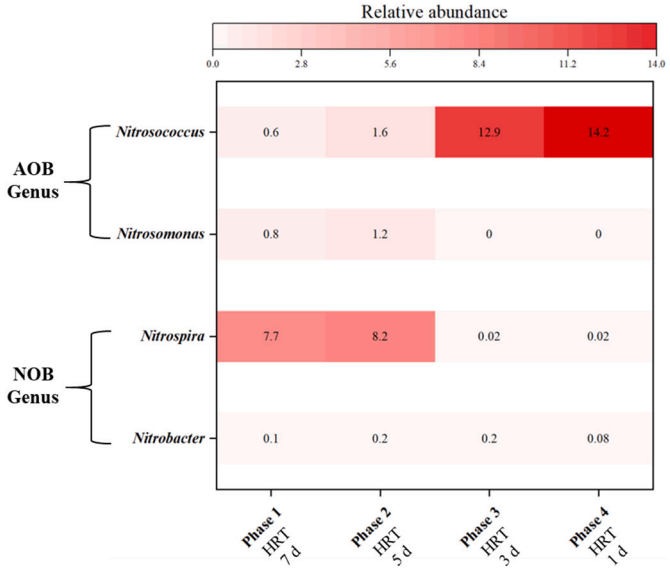

Fig. 4. Heatmap of relative abundances of ammonia-oxidising bacteria (AOB) genus and nitrite-oxidising bacteria (NOB) genus in different phases.

was also detected in MBR, with a relative abundance of around 0.1–0.2 %. This low occurrence may be due to its lower substrate affinity compared to *Nitrospira,* as well as the unfavourable conditions for its growth, likely owing to low nitrite concentrations during phase 1 and 2 [50].

In phase 1, both *Nitrosococcus* and *Nitrosomonas* were present as AOB genera, with a combined relative abundance of 1.4 %. Conversely, *Nitrospira* was the predominant NOB genus at 7.7 %, while *Nitrobacter* was present at only 0.1 %. In phase 2, the increased feeding rate and greater substrate availability led to higher abundances of *Nitrosococcus* and *Nitrosomonas*, at 1.6 % and 1.2 %, respectively. At the same time, *Nitrospira* and *Nitrobacter* showed slight increases as well, to 8.2 % and 0.2 %, respectively, owing to the higher substrate availability. During these two phases, the NOB-to-AOB ratio was 5.6 and 3.0, respectively. It has been reported that this ratio typically ranges between 1.3 and 5.9 during stable nitrification, likely due to NOB having higher substrate uptake rates compared to AOB [51–54]. The findings in this study suggest that NOB-to-AOB ratio of 5.6 can achieve stable urine nitrification without nitrite accumulation, resulting in the HRT of 7 days. This result is in line with previous reports, where stable nitrification was observed at NOB:AOB ratio of 3.5 in a urine-treating MBR and 5.1 in an MBR treating nutrient-rich liquid anaerobic digestate, with *Nitrospira* identified as the dominant NOB genus in both systems [23,54]. However, further investigation into the abundance ratios of NOB and AOB is necessary for deeper insights into optimising the nitrifying community in urine MBR systems.

Further increase in urine feeding rate under 3-day HRT, however, caused significant shifts in AOB and NOB abundances. *Nitrosococcus* were highly enriched by 8-fold up to 12.9 %, whereas *Nitrospira* was highly inhibited dropping to 0.02 %, and *Nitrobacter* remained stable at 0.2 %. This trend continued in phase 4 under the shortest HRT condition, where *Nitrosococcus* further enriched to 14.2 %, with *Nitrospira* still inhibited at 0.02 % and *Nitrobacter* reducing to 0.08 %. These findings explain the observed nitrification performance, where nitrite accumulation was significant during phases 3 and 4. Since AOB generally have a higher maximum specific growth rate (0.9 d^{-1}) than NOB (0.7 d^{-1}), they can proliferate and oxidise ammonium more rapidly under favourable conditions [55]. This kinetic advantage explains the significant increase in AOB abundance under shortened HRTs with elevated ammonium loading, their primary substrate. In phase 3, AOB rapidly outcompeted NOB, leading to substantial nitrite accumulation, which in turn further inhibited NOB activity. Consequently, AOB activity was maintained while NOB were suppressed, resulting in progressive nitrite build-up. Moreover, nitrite accumulation influenced the NOB community structure, with *Nitrobacter* replacing *Nitrospira* as the dominant genus. This shift likely occurred because *Nitrobacter* is better adapted to high-nitrite environments [50].

In addition to growth kinetics, FNA exerted a significant influence on AOB and NOB during nitrite accumulation. NOB are known to be more sensitive to FNA than AOB, with inhibition beginning at concentrations above 0.06 mg HNO_2-N/L, and stable inhibition occurring at 1 mg HNO_2-N/L [56,57]. It has been also reported that *Nitrospira* activity decreased to 35 % of its original level at 0.25 mg HNO_2-N/L, and to 10 % at 0.87 mg HNO_2-N/L, whereas AOB activity remained unaffected at 0.25 mg HNO_2-N/L and still retained 64 % activity at 0.87 mg HNO_2-N/L [58]. The observations in this study aligns with these earlier reports. As shown in **Fig. S2**, FNA concentrations remained low during phases 1 and 2 (< 0.04 mg HNO_2-N/L), which explains why both AOB and NOB maintained stable activity despite slight nitrite accumulation. From phase 3 onwards, however, nitrite accumulation increased significantly, resulting in FNA levels of 0.28–0.42 mg HNO_2-N/L in phase 3 and 0.49–0.78 mg HNO_2-N/L in phase 4. These elevated concentrations strongly inhibited NOB, *Nitrospira*, while AOB remained the predominant nitrifier. The persistence of AOB activity under high FNA conditions may be partly explained by the enrichment of *Nitrosococcus*, an acidophilic AOB genus known for its tolerance to acidic conditions and resistance to FNA inhibition. In terms of FA, the concentrations remained at 0.89–1.1 mg NH_3-N/L throughout the operation. Previously reported thresholds suggest that FA in the range of 1–10 mg NH_3-N/L can inhibit NOB activity without negatively affecting AOB, whereas AOB inhibition generally begins only at much higher levels (10–150 mg NH_3-N/L) [59]. Thus, in this study, FA likely had minimal influence on NOB inhibition compared to the dominant role of FNA. Overall, the microbial community dynamics reflected the process performance trends. The persistence of *Proteobacteria* supported stable TOC removal, while the decline in *Chloroflexi* and *Actinobacteria* during phase 4 coincided with reduced organic matter degradation capacity. In parallel, the enrichment of AOB (*Nitrosococcus*) and suppression of *Nitrospira* under elevated nitrite demonstrated that HRT reduction reshaped the balance between nitrifying groups. These findings highlight that both heterotrophic and autotrophic populations jointly influenced reactor performance under varying HRTs.

3.3. Hydroponic application - basil and grass growth in commercial indoor hydroponic system

The compositions of five treatment solutions used for hydroponic cultivation of basil and orchard grass in indoor hydroponic systems are presented in Table 2. Although the total nitrogen concentrations were standardised across all treatments, the relative proportions of nitrogen species (ammonium, nitrate, and nitrite) differed significantly ($p < 0.05$). Notably, ammonium and nitrate concentrations were similar only between HG4 and HG5 ($p > 0.05$), while all other pairwise comparisons showed significant differences. For EC and TDS, only HG2 and HG4 were not significantly different ($p > 0.05$), whereas other treatments differed significantly ($p < 0.05$). Concentrations of other nutrients and trace elements were largely comparable across treatments ($p > 0.05$), except for potassium, which was significantly higher in HG5 ($p < 0.05$). It is worth to note that under shorter HRT conditions, nitrite concentrations significantly increased, reaching 50 mg/L and 80 mg/L under 3-day and 1-day HRT conditions, respectively ($p < 0.05$). Basil and orchard grass seeds germinated after two weeks and one week, respectively, and growth responses were measured after cultivation periods of 12 weeks for basil and 6 weeks for orchard grass.

Table 2
Composition of treatment solutions.

Hydrogardens	1	2	3	4	5
Treatment solution	Commercial fertiliser	HRT 7	HRT 5	HRT 3	HRT 1
Total nitrogen (mg/L)			250		
Ammonium (mg/L)	50	140 ± 5	150 ± 5	130 ± 5	135 ± 5
Nitrate (mg/L)	200	100 ± 5	85 ± 5	60 ± 5	30 ± 5
Nitrite (mg/L)	0	0	5 ± 1	50 ± 1	80 ± 1
Phosphorous (mg/L)	50	13 ± 1	12 ± 2	11 ± 1	15 ± 2
Potassium (mg/L)	200	63 ± 5	65 ± 5	65 ± 5	74 ± 5
Calcium (mg/L)	100	4.1 ± 0.5	4.2 ± 0.5	4.1 ± 0.5	4.0 ± 0.5
Magnesium (mg/L)	25	2.4 ± 0.2	2.3 ± 0.2	2.6 ± 0.2	2.4 ± 0.2
Sulphur (mg/L)	n.a	82 ± 5	85 ± 5	81 ± 5	82 ± 5
Iron (mg/L)	3	0.06 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.06 ± 0.01
Copper (mg/L)	0.3	n.a	n.a	n.a	n.a
Zinc (mg/L)	0.75	n.a	n.a	n.a	n.a
Boron (mg/L)	0.9	n.a	n.a	n.a	n.a
Manganese (mg/L)	1	0.007	0.006	0.006	0.007
Molybdenum (mg/L)	0.1	n.a	n.a	n.a	n.a
EC (μS/cm)	2030 ± 15	1880 ± 15	1850 ± 20	1892 ± 20	1780 ± 20
TDS (mg/L)	1015 ± 10	986 ± 12	967 ± 10	987 ± 10	897 ± 10

*n.a: not analysed.

The plant responses to each fertiliser formulation were assessed by analysing stem numbers, the height of main stems, and the fresh and dry weights of shoots and roots of each plant type, as illustrated in Figs. 5 and 6**.** The number of basil stems was the highest in the HG1 and HG2 treatments returning 10 and 8 germinated stems, respectively. The number of basil stems gradually decreased with the reduction in HRT among the urine fertiliser treatments, from 8 stems in HG2 to 3 stems in HG5. Conversely, orchard grass stem numbers remained relatively consistent, ranging from 79 to 82 for HG1 to HG4, with a slight decrease to 75 under HG5. Regarding the average height of main stems, although variations in average heights were observed among the basil plants, neither basil nor orchard grass showed statistically significant differences across the five treatments ($p > 0.05$), with orchard grass stems heights remaining steady across all conditions.

The fresh biomass of basil shoots grown using urine fertilisers under 7-day HRT and 5-day HRT conditions (HG2 and HG3) resulted in the two highest yields of 66 g, and 61 g, respectively. The slight reduction from HG2 to HG3 might be associated with the presence of 5 mg/L nitrite in the treatment solution in HG3 ($p < 0.05$). Conversely, the fresh weight of basil shoots cultivated with commercial fertiliser (HG1) had significantly lower yields compared to HG2 and HG3, averaging 50 g ($p < 0.05$). Despite this, the fresh biomass of roots in HG1 exhibited the

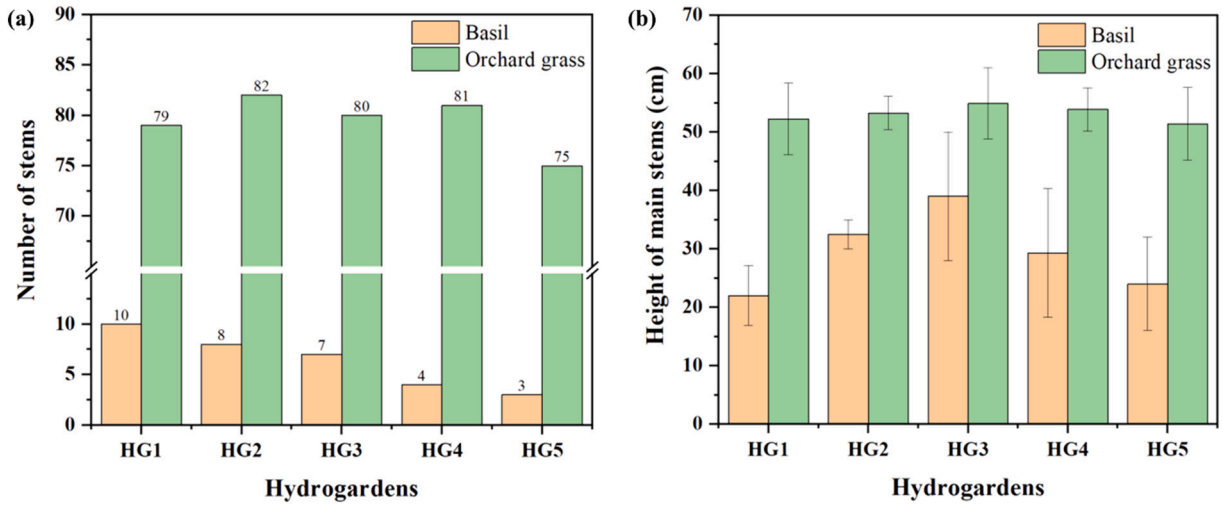

Fig. 5. Plant growth performance under different fertiliser treatments: (a) number of stems and (b) height of main stems of basil and orchard grass. Error bars indicate standard deviation of replicate measurements ($n = 3$ for basil; $n = 5$ for orchard grass) for stem height. The number of stems was recorded per unit as a single representative measurement; therefore, error bars are not applicable. Statistical analysis showed that differences in height of main stems between treatments were not significant ($p > 0.05$).

highest weights among all treatments, showing an average weight of 21 g, which is 1.1–1.8 times higher than HG2 and HG3. This observation may be attributed to the higher concentrations of phosphorous and potassium in the commercial fertiliser compared to the urine fertiliser [54]. Previous studies have shown that increased availability of these two nutrients promoted in root development such as mass density. Particularly, the roots can absorb potassium (K^+) ions, which regulate osmotic pressure and water utilisation, enhancing nutrient uptake and protein synthesis, thereby supporting overall plant health [60–62]. Thus, the comparatively higher levels of phosphorus and potassium in HG1 may have promoted greater root development rather than shoot biomass, resulting in a different nutrient allocation pattern compared with HG2 and HG3. Further reduction of HRT to 3 days resulted in a significant decline in basil biomass to 34 g (HG4), and a further decrease to 23 g under the 1-day HRT condition (HG5) ($p < 0.05$). These findings highlight the negative impacts of elevated nitrite concentrations in hydroponic solutions, as previously reported [8]. High nitrite concentrations are known to be toxic to plants because excessive accumulation in plant tissues can disrupt enzymatic reduction pathways, induce oxidative stress, and degrade chlorophyll, ultimately resulting in inhibited biomass production and stunted growth. In this study, basil growth remained stable at nitrite concentrations of approximately 5 mg/L, but was significantly stunted when nitrite concentrations in the treatment solution exceeded 50 mg/L. In contrast, the fresh biomass yield of orchard grass showed similar values across HG2, HG3 and HG4, ranging from 22 g to 24 g, which were comparable to or slightly exceeded the yield of grass grown with commercial fertiliser (22 g) ($p \leq 0.05$). A minor decline in grass biomass yield was observed in HG5, decreasing to approximately 19 g ($p < 0.05$). Although declines in fresh biomass were observed in both plants under HG5, it is noteworthy that basil biomass decreased by 62–65 % (from HG2/HG3 to HG5), whereas orchard grass showed only a 21 % decline (from HG3 to HG5). This indicates that orchard grass exhibited relatively greater tolerance in maintaining fresh biomass yield compared to basil under elevated nitrite conditions.

The root-to-shoot ratio of dried plant biomass is an important indicator of plant growth response, as it helps to assess the optimal allocation of nutrients between root and shoot development. A balanced ratio can reflect the ability of plants to efficiently utilise nutrients, adapt to environmental conditions, and support overall growth [63]. The root-to-shoot ratio of basil from 7-day and 5-day HRT conditions (HG2 and HG3) ranged from 0.12 to 0.13, which is comparable to the ratio of 0.13 observed in the previous study from the basil grown with commercial fertiliser solution [8]. However, the ratio observed with commercial fertiliser (HG1) in this study was slightly higher at 0.15 ($p < 0.05$), due to the enhanced root growth resulting from higher phosphorous and potassium levels as discussed above. The dried root-to-shoot ratio for orchard grass grown under 7-day and 5-day HRT conditions (HG2 and HG3) was 0.23 and 0.22, respectively. The ratio observed in HG2 did not differ significantly from that of the commercial fertiliser (HG1) ($p > 0.05$), whereas HG3 showed a significant difference ($p < 0.05$). These findings suggest balanced growth between roots and shoots in both plants under these conditions. However, under 3-day and 1-day HRT conditions, the root-to-shoot ratio increased significantly compared to HG1–3, reaching 0.17 for basil and 0.25 for grass ($p < 0.05$). This increase may indicate greater nutrient allocation to roots as a stress response, potentially triggered by nutrient imbalance or deficiency, such as elevated nitrite levels in this study [64,65]. Nonetheless, no significant difference was observed between HG4 and HG5 for either plant species. ($p > 0.05$).

Overall, urine-based fertiliser formulations obtained with an HRT of 7 or 5 days supported the hydroponic growth of basil with a comparable outcome to the commercial fertiliser in terms of fresh shoot weight of basil. This is particularly important as the profitability of basil production is closely tied to market dynamics, with bunch size largely determined by branch number per plant and fresh shoot biomass yield [66,67]. This result suggests that basil could withstand up to 5 mg/L of nitrite concentration as a threshold for optimal growth. In contrast, reduced HRTs of 3 days and 1 day inhibited basil growth, likely due to elevated nitrite concentrations, indicating the need for additional treatment to mitigate nitrite toxicity [68]. Meanwhile, unlike basil, orchard grass demonstrated higher tolerance to systematically reduced HRTs and so higher concentration of nitrite in the fertiliser formulations, with minimal variations in growth metrics such as biomass yield and height of main stems, even under 3-day and 1-day conditions. Given its original use as an aesthetic and functional groundcover for parklands and golf courses, particularly for lawns and greens, the findings of this study highlight the potential of urine-based fertilisers, containing up to approximately 80 mg NO_2^--N/L under shortened HRT conditions, to promote grass growth. These findings lay the foundations for the application of urine fertilisers in supporting sustainable and efficient grass cultivation for landscaping purposes.

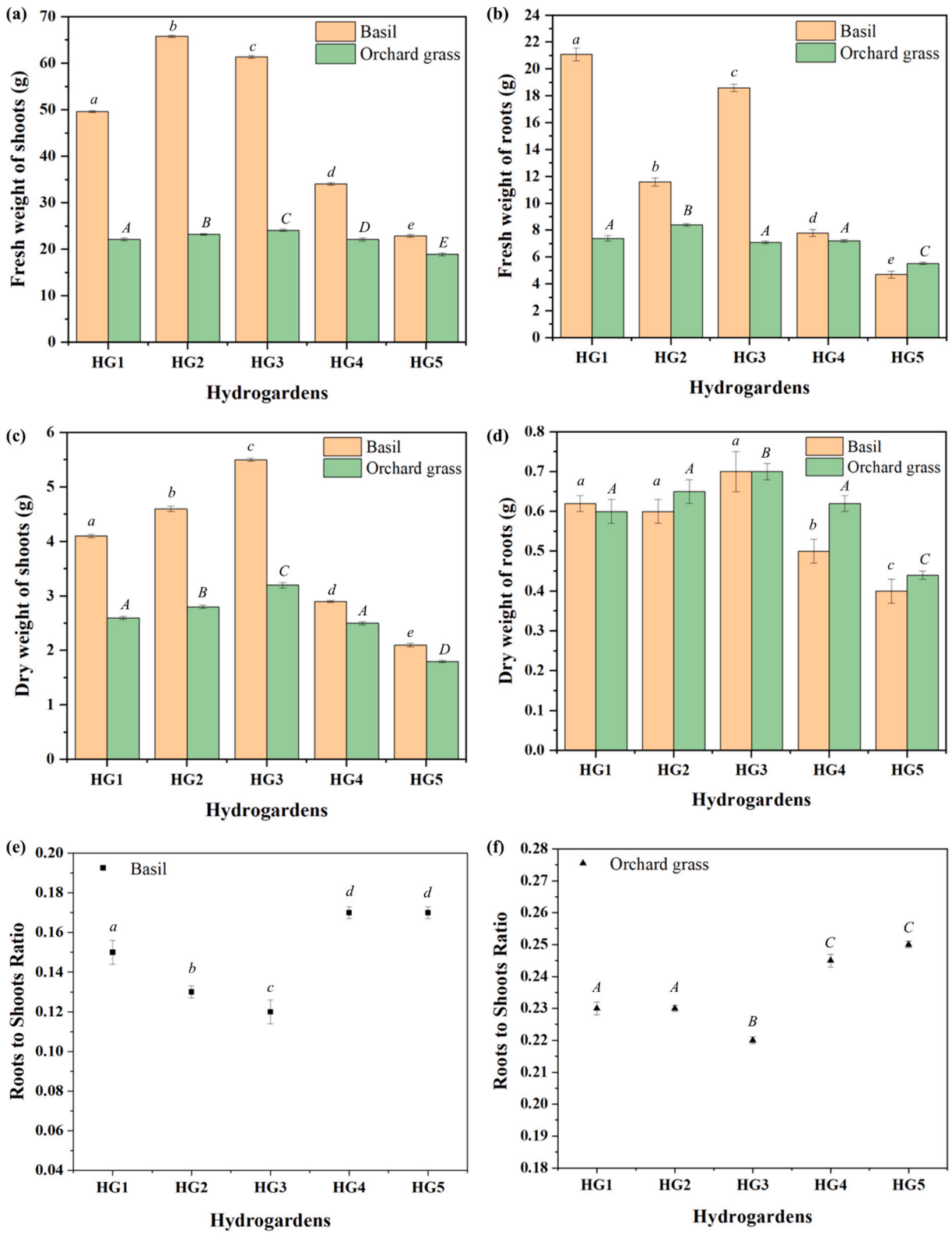

Fig. 6. Growth responses of basil and orchard grass in terms of (a) fresh weight of shoots; (b) fresh weight of roots; (c) dry weight of shoots; (d) dry weight of roots; (e) roots to shoots ratio of basil; and (f) roots to shoots ratio of orchard grass. Error bars indicate standard deviation of replicate measurements ($n = 3$). Different letters indicate statistically significant differences between treatments within each plant species (lowercase letters refer to basil and uppercase letters to orchard grass, $p < 0.05$). Treatments sharing a letter are not significantly different.

4. Conclusion

This study evaluated the impact of varying HRT conditions on the nitrification performance of a compact MBR system and the potential urine fertiliser effectiveness as a function of the fertiliser formulation. The findings demonstrate that the system successfully acclimated to a 7-day HRT under a pH-based feeding regime, achieving stable nitrification at 194 ± 11 mgN/L·d. However, as the HRT was progressively reduced, the nitrite and nitrate concentrations varied as a function of the HRT, while the ammonium oxidation rate remained stable, suggesting an increasing abundance of AOB which were able to adapt and thrive even under highly reduced HRT conditions. In hydroponic applications, urine-derived fertiliser produced at HRTs of up to 5 days showed a similar performance in terms of basil growth and health compared to the commercial fertiliser, indicating the system's effectiveness for high-value crops. Conversely, grass growth showed higher tolerance to variations in HRT, suggesting its potential as a target crop for fertiliser produced under less optimal conditions with high nitrite contents. These results underscore the feasibility of employing compact MBR systems for producing effective urine-derived fertiliser while highlighting the importance of optimising HRT to balance nitrification efficiency and agricultural performance.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. Ho Kyong Shon serves as a co-Editor-in-Chief for the Desalination journal, while the editorial handling and review of this manuscript were overseen by a different Editor.

Acknowledgements

This project is supported by the Australian Research Council (ARC) through the ARC Research Hub for Nutrients in a Circular Economy (NiCE) (IH210100001).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.desal.2025.119388.

Data availability

Data will be made available on request.

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