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Nutrient removal and nitrogen recovery from aquaculture effluents by the edible halophyte *Sesuvium portulacastrum* (L.) in hydroponics and in sand substrate

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ABSTRACT

Aquaculture is important for food security and livelihoods, especially in the developing tropics. Many nutrients supplied by feed degrade water quality, waste resources and lead to nutrient loss. Halophytes have the potential to recover these nutrients from aquaculture effluents. Most studies have focused on temperate species, but there is a need for extractive species suitable for integration in tropical saline aquaculture. Sea purslane *Sesuvium portulacastrum* is a promising candidate providing biomass for human consumption with potential pharmaceutical applications, but to date, nutrient uptake has not been studied in this species. This study investigated the nutrient removal from milkfish wastewater by systems planted with *S. portulacastrum* in hydroponics versus planted in sand. Most nutrients were removed from the wastewater within 48 h. Phosphorus and nitrogen were most efficiently removed in treatments with plants and sand and on average lower in the hydroponic incubations. However, in hydroponic systems, plants assimilated 50.2% of the added labelled N with no significant difference in recovery after 48 h when ^{15}NH4 or ^{15}NO3 was added. The recovery of wastewater N into plant biomass was several-fold higher in hydroponics than in sand substrate. Integration of *S. portulacastrum* into aquaculture turns aquaculture effluents into a valuable resource.

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Introduction

Nitrogen (N) and phosphorus (P) are essential nutrients for cultured organisms and supplied via feed or fertilizer (Verdegem 2013). With clear limits to global P deposits and the high energy costs and CO_2 emissions of artificial N-fertilizer production, we need to increase our nutrient use efficiency for food production systems, including aquaculture (Erisman et al. 2008; Cordell et al. 2009; Verdegem 2013; Huang et al. 2020). Up to 50% of feed supplied can go uneaten and be lost as particulate waste and of the feed consumed by the target species, around 25% of N and 30% of P are retained with the rest excreted in faeces or dissolved form (Piedrahita 2003; Ballester-Moltó et al. 2017). N not assimilated by the cultivated organism is mainly released in the form of ammonium (NH_4^+) (Lefebvre et al. 2001; Neori et al. 2007), which in aerobic environments is quickly converted to nitrite (NO_2^-) and further oxidized to nitrate (NO_3^-) by bacteria and archaea (Blackburn and Blackburn 1992; Martens-Habbena et al. 2009). Closed recirculating aquaculture systems (RAS) apply this microbial activity in nitrifying biofilters to prevent harmful concentrations of NH_4^+, NH_3^- and NO_2^-, and some further apply denitrifying biofilters where NO_3^- is ultimately released as N_2 gas (Van Rijn 1996). An intermediate product of denitrification is however nitrous oxide (N_2O), which is a greenhouse gas with 296 times the global warming potential of CO_2 and a further challenge to sustainable N treatment in aquaculture (IPCC 2007; Hu et al. 2013, 2015). In a mariculture context, sandy marine sediments have been shown to very efficiently remove inorganic nitrate from eutrophic coastal systems (Rao et al. 2008; Gao et al. 2012), but the ratio of N_2O vs. N_2 production is also very high here due to the characteristic variable oxygen conditions in permeable sands (Marchant et al. 2018). Unlike fixed N, P can only be

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removed by assimilation into new biomass or retained in the sediment through adsorption, leading to blooms of dinitrogen fixing algae regaining the fixed nitrogen, or other harmful algal bloom contributing agents (Conley et al. 2009). Under high nutrient loads, sediments eventually become anoxic where anaerobic respiration prevails and produces environmentally harmful compounds such as hydrogen sulphide and methane, a strong greenhouse gas (Jorgensen 1982; Capone and Kiene 1988). Continuous supply of dissolved inorganic N and P from aquaculture effluents has been observed to favour the growth of micro- and macroalgae, leading to eutrophication and imbalances in natural ecosystems (Diaz and Rosenberg 2008; Herbeck et al. 2014; Thomsen et al. 2020). Consequently, these nutrients need to be removed from effluents, ideally through mechanisms other than denitrification in the case of N, to prevent eutrophication of receiving waterbodies.

Constructed wetlands planted with salt-tolerant halophytes treat wastewater while further producing valuable crops for human consumption, pharmaceutical application or biomass for biogas production (Glenn et al. 1999; Buhmann and Papenbrock 2013a; Custódio et al. 2017). Edible halophytes from both temperate and tropical regions have been successfully integrated for the treatment of saline aquaculture effluents (Webb et al. 2012; Shpigel et al. 2013). Removal of nutrients from wastewater in these systems is highly variable, but can achieve >99% (Brown et al. 1999; Shpigel et al. 2013; Webb et al. 2013, 2012; De Lange and Paulissen 2016). N is, however, primarily removed through denitrification and ammonia volatilization and only a small fraction is converted into harvestable plant biomass (Kadlec and Knight 1996; Lin et al. 2002; Vymazal 2010). In terrestrial soils, microbes can outcompete plants for inorganic N (Hodge et al. 2000; Lipson and Näsholm 2001; Bardgett et al. 2003; Harrison et al. 2008). In freshwater RAS, aquaponic systems are already well established, where plants are integrated in hydroponic (without soil) culture (Diver and Rinehart 2000; Hu et al. 2015; Ogah et al. 2020). While plants in hydroponic systems can suffer nutrient deficiencies, it has been recommended for some halophyte species as a practical method with higher biomass production compared with plants in sand culture (Singh et al. 2014; Buhmann et al. 2015).

The selection of edible plants that can grow in a saline environment is far smaller than for glycophytes that depend on fresh water. However, edible halophytes have been successfully integrated in tropical RAS with fish and/or shrimp and increased system yield and nitrogen recovery without adverse effects on the fed species (Pinheiro et al. 2017; Poli et al. 2019). Liu et al. (2019) found good growth of the tropical halophyte *Sesuvium portulacastrum* (Linnaeus) planted in floating structures in coastal waters and suggest that it can be cultivated to reduce N and P loads around net cages. *S. portulacastrum* significantly lowered N concentrations, accounting for maximum removal of 75% of aqueous N in a RAS with fish (Boxman et al. 2017) and acted as a net sink of N in a RAS with milkfish and sea cucumbers (Senff et al. 2020). *S. portulacastrum* or sea purslane is a perennial halophyte with a wide distribution in coastal habitats of the tropics and sub-tropics between 34° north and 42° south (Lonard and Judd 1997). It has potential as a valuable crop for saline soils and is consumed fresh, used as a traditional medicinal plant with antibacterial and antioxidant activities and can be cultivated for the commercial production of secondary metabolites (Magwa et al. 2006; Lokhande et al. 2009). *S. portulacastrum* and other edible halophytes can be considered functional foods due to high antioxidant activity and protein content (Boestfleisch et al. 2014; Barreira et al. 2017; Pinheiro et al. 2017) and it has been shown that marine aquaponics produced halophytes with improved n-3 fatty acid content compared with harvests from wild populations (Maciel et al. 2020).

Experiments testing plant filters in RAS in addition to other water cleaning mechanisms, such as biofilters, do not give insight into the performance of the plants in unfiltered fish effluents (Webb et al. 2012; Waller et al. 2015) or yield information on nutrient uptake and assimilation by the plants, as water is continuously cycled in a closed system (Boxman et al. 2017). This information is however needed to determine the effectiveness of edible halophytes as extractive species, where a lack of research exists especially for tropical species (Custódio et al. 2017).

This study thus investigates *S. portulacastrum* as an edible extractive species for saline aquaculture in the tropics by evaluating its potential for nutrient removal and optimized assimilation of nutrients into new biomass from milkfish effluents. It aims to provide new insights into its application in integrated aquaculture and optimize utilization of resources by (1) determining the removal efficiency of dissolved inorganic nitrogen (DIN) and phosphorus (DIP) from aquaculture wastewater and (2) comparing how plants cultivated hydroponically versus in sand perform in terms of N recovery into new valuable biomass. It is hypothesized that *S. portulacastrum* assimilates more N in hydroponics, but that more N is removed in the cultivation system in sand.

Materials and methods

Experimental setup

Experimental plants were propagated from plants provided by the Institute of Botany at the Leibniz University Hannover, planted in silicate sand (Bauhaus, Germany) and irrigated with tap water (Buhmann et al. 2015). At a height of approximately 10 cm, the plants were transferred to boxes, each planted with six plants.

Two different types of plant boxes of different sizes were prepared for the hydroponic (Figure 1A) and sand (Figure 1B) treatments to allow for the same volume of water to be applied for the two treatments. The boxes for sand culture were 17.5 cm wide and 32 cm long. The bottom of the box was covered by a 3 cm layer of glass marbles, covered by a layer of pond fleece, on which 8 kg of sand was placed. The hydroponic boxes were of 15 cm width and 25 cm length with a 3 cm layer of glass marbles on the bottom. For each treatment, eight replicates were used, resulting in a total of 32 boxes. For the hydroponic treatment, the plants were held on a styrofoam raft into which six holes were cut. Foam strips held the plants in place in the holes, and the walls of the boxes were covered in black, opaque foil. Before planting, the plants were weighed. Aeration was provided by air stones placed into the water of the hydroponic treatments and into the water layer on top of the sand. Light was supplied with rows of 3W LED ('Reef', JMB Aqua Light) lamps at an average of 240 µmol m^{-2} s^{-1} and a light:dark period of 12:12 h.

Treatments:	Plants cultivated hydroponically	(HP)
	Hydroponic control without plants	(HC)
	Plants cultivated in sand	(SP)
	Sand control without plants	(SC)

Pre-experimental period

All boxes were filled with 2500 ml of water sourced from a recirculating system with milkfish (*Chanos chanos* Forskal) and left to establish for a period of about two months. The water was exchanged weekly. Average nutrient concentrations (mg l^{-1} ± SD) in the milkfish water were 0.00 ± 0.00 NH_4^+-N, 0.02 ± 0.01 NO_2^--N, 2.78 ± 0.32 NO_3^--N and 1.18 ± 0.56 PO_4^+-P. Salinity was 34 ± 0 PSU.

Experiments

Two separate experiments were performed to evaluate bioremediation using different types of wastewater from cultivating a total biomass of 125 g milkfish: direct effluent (Figure 2A) and RAS water (Figure 2B). In both systems, the fish were fed at 5% body weight day^{-1}. Prior to the experiments, the water was filtered through a 300 µm gauze to remove larger solids. Before each experiment, the sand boxes were left to completely drain overnight while the hydroponic boxes were drained on the day of the experiment.

Pucher et al. (2014) recommended adding between 0.1 and 1% of total N present in the system (in inorganic and organic form) as ^{15}N tracer. Based on a rough budget estimate, two stock solutions of 150 mg 15N L^{-1} were prepared by dissolving either 860 mg ^{15}N-$NaNO_3$ or 544.86 mg $^{15}NH_4Cl$ in 100 ml of Milli-Q water. To each box from both experiments (direct effluent or RAS water), 2.345 ml of this stock solution were pipetted into a glass beaker of 1500 ml of water from the fish tank, well mixed and poured into the plant boxes. In total, each replicate of the direct effluent or RAS water received 3.518 mg of ^{15}N.

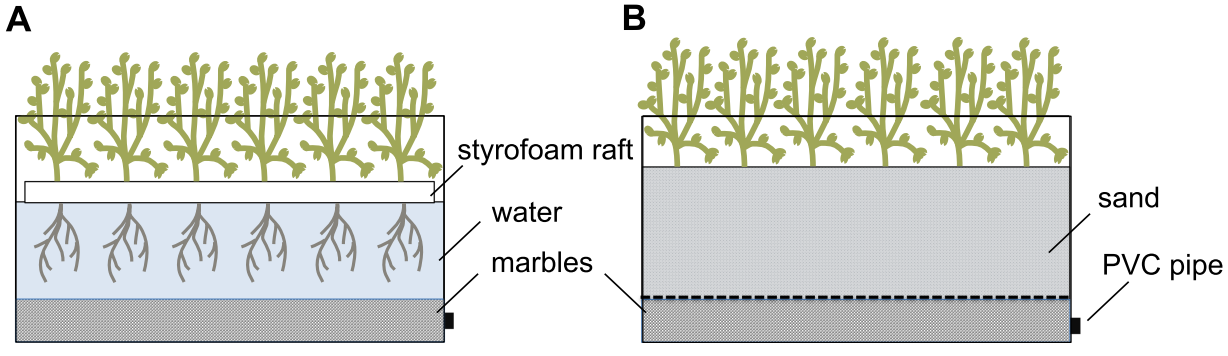

Figure 1. Schematic drawing of the two different boxes planted with *Sesuvium portulacastrum* grown (A) in hydroponics or (B) in sand substrate in the experiments.

Dissolved inorganic nutrients	Direct effluent	RAS water
NH_4^+-N (mg L^{-1})	2.32 ± 0.03	2.37 (added as $^{15}NH_4Cl$-N)
NO_2^--N (mg L^{-1})	0.14 ± 0.00	0.02 ± 0.00
NO_3^--N (mg L^{-1})	2.18 (added as $^{15}NaNO_3$-N)	14.64 ± 0.07
PO_4^+-P (mg L^{-1})	0.55 ± 0.01	2.05 ± 0.03
N:P	8.30	8.31

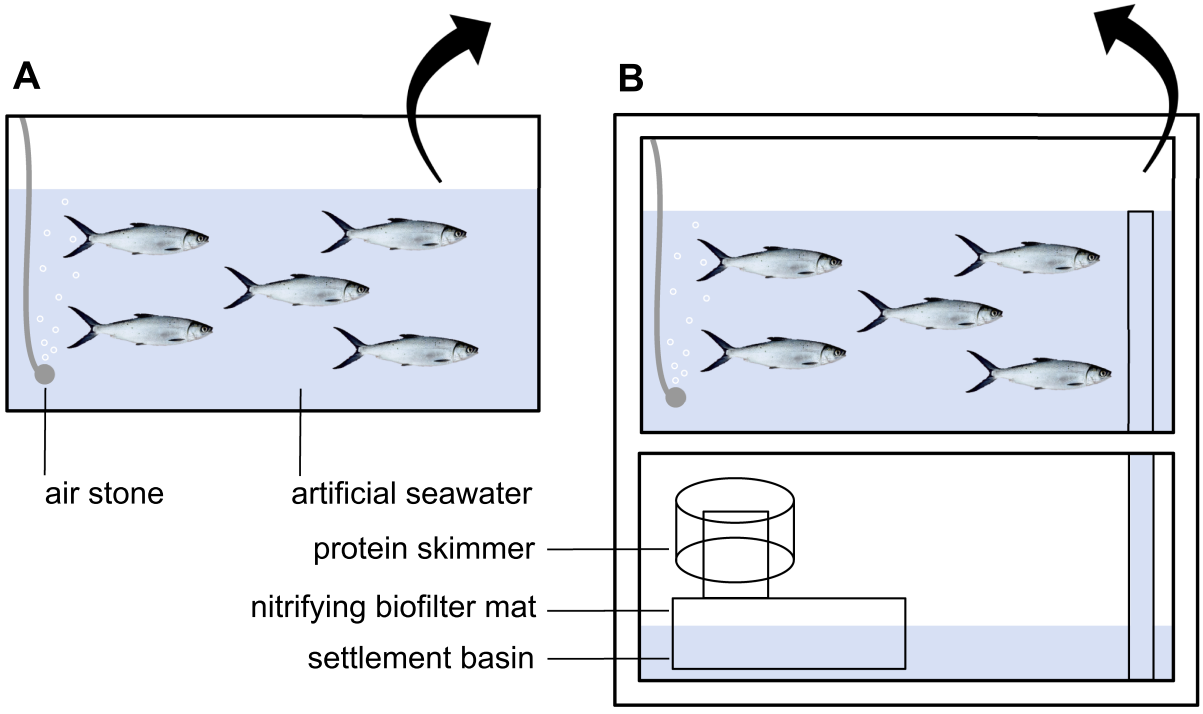

Figure 2. Concentrations (mean mg l^{-1} ± SD) of dissolved inorganic nutrients, ^{15}N tracer and N:P in the aquaculture wastewater supplied to the boxes planted with *Sesuvium portulacastrum* during the two experiments and conceptual set-up of sources of milkfish wastewater used: (A) milkfish kept in a 300 l aquarium without a filtration system for 48 h (=direct effluent) and (B) milkfish kept in an established 300 l recirculating system (=RAS water).

Sample collection and analysis

Before the start of the experiment (at 0 h) and after 3, 6, 12, 24 and 48 h, one plant from each box was sampled, weighed as a whole, divided into above ground ('shoots') and below ground ('roots') biomass and weighed again. The plant relative growth rate (RGR) was calculated as

$$\mathrm{RGR} = (\ln \mathrm{WW}_2 - \ln \mathrm{WW}_1)/(t_2 - t_1),$$

where WW = wet weight of the entire plants (g), t = time (d) and subscripts 1 and 2 indicate at planting (1) and at the end of the experiment (2).

The plant parts were placed in plastic bags and stored at −20°C until lyophilization and homogenization for isotope analysis. Plant parts were weighed again to determine dry weight (DW) before homogenizing roots with a mortar and pestle and shoots in an IKA Benchtop Tube Mill (Cole-Parmer, Vernon Hills, USA). Water volume, pH, temperature and salinity of the drainage water were measured using a 1500 ml beaker and a multiparameter probe (WTW, Germany). Water samples were taken from the milkfish wastewater before and after the addition of the isotope tracer and after 48 hours, when the plant boxes were drained. All water samples were taken with 10 ml syringes through a 4.5 µm filter and immediately stored at −20°C. Concentrations of NH_4^+-N, NO_x-N, NO_2^--N and PO_4^+-P (DIP) were analysed following the protocol of Strickland and Parsons (1972) and photometrically determined using an Infinite 200 PRO microplate reader (TECAN, Austria). Concentrations were

compared against standard solutions (Merck) and lower detection limits (mg l^{-1}) were 0.05, 0.02, 0.01 and 0.02 for NH_4^+-N, NO_x-N, NO_2^--N and PO_4^+-P, respectively. NO_3^--N concentrations were subsequently determined as $NO_x - NO_2^-$ and DIN was calculated as NH_4^+-N + NO_3^--N + NO_2^--N.

Stable isotope analysis was done by combustion on a Thermo Flash EA 1112 elemental analyser coupled to an isotopic ratio mass spectrometer (Thermo Delta Plus XP, Thermo Fisher Scientific, Germany) at the Max Planck Institute for Marine Microbiology in Bremen (Halm et al. 2012). The nitrogen isotopes are measured as dinitrogen gas ($^{29}N_2$/total N_2) and expressed as atomic % ^{15}N. Caffeine (Sigma-Aldrich) was used as a standard for isotope calibration and quantification. Based on this, the excess ^{15}N in the sample ($^{15}N_{Excess}$) was determined as

$$^{15}N_{excess} = (atom\%^{15}N\ sample - 0.37)/100$$

where 0.37 = mean atomic % of ^{15}N of baseline plants sampled before label addition. The $^{15}N_{excess}$ was then quantified (into g or mol) using a known standard (caffeine) and used to calculate the uptake rate of the isotopic label in mg ^{15}N g dw^{-1} h^{-1}. These were calculated separately for the shoots and roots. ^{15}N label measured in shoots and roots were combined to determine the % mg ^{15}N added that was recovered cumulatively in plant biomass at the different sampling times.

Statistical analysis

All statistical analyses were performed in R (R Core team 2019). The data were evaluated for normality and homoscedasticity by Shapiro–Wilk and Levene's test, respectively. Water parameters (pH, temperature and Salinity) at the end of the experiments were compared between treatments by one-way analysis of variance (ANOVA) or non-parametric Kruskal–Wallis in case assumptions of normality and homoscedasticity were not met. Weights of the plants at stocking and relative growth rate were compared between the hydroponic and sand treatments with two-sample t-test. Per cent removal of total N and P was compared between all treatments by Kruskal–Wallis test. Concentrations of phosphate and inorganic N species in the water were compared between the treatments with plants and their corresponding controls by Wilcoxon signed-rank test.

N uptake in the shoots and roots were compared between the hydroponic and sand treatment by linear mixed effect model (lme) with treatment and time of sampling as independent factors and the boxes taken into account as random factor in the repeated measures design. Box–Cox transformation was applied to the data on mg ^{15}N g^{-1} dw h^{-1} and data on atom % ^{15}N cumulative ^{15}N uptake were log transformed. Normality and homoscedasticity of model residuals were tested by Shapiro–Wilk and Levene's test. Post-hoc comparison between treatments was performed by pairwise comparison of estimated marginal means. Statistical significance was evaluated at $p < 0.05$.

Results

Plant growth

The plants in the experiment with direct effluent weighed 10 ± 3 g (mean ± SD) at stocking and grew at 0.01 day^{-1} (RGR). There was no significant difference in the size of plants at stocking (df = 14, $p = 0.23$) or in the relative growth rate ($t = -2.156$, df = 14, $p = 0.05$) between the hydroponic and sand treatment. Average biomass density at the start of the experiment was 2862 g m^{-2} and biomass subsequently decreased over the course of the experiment, as plants were sampled (Table I).

The plants receiving RAS water weighed 16 ± 5 g at stocking and grew at 0.01 day^{-1} (RGR). There was no significant difference in weight at stocking (df = 14, $p = 0.27$) or relative growth rate ($p = 0.73$, df = 14) between hydroponic and sand cultivation. Average biomass density at the start of the experiment was 4676 g m^{-2} and the biomass subsequently decreased over the course of the experiment as plants were sampled (Table II).

Water parameters

At the final sampling of the 48-hour experiment, there were small differences between treatments in pH and

Table I. Plant biomass (mean wet weight (g ± SD) and density (g m^{-2} ± SD)) at the six sampling times of *Sesuvium portulacastrum* grown in hydroponics or sand substrate and irrigated with direct effluent from milkfish. Growth over the course of the 48 h experiment was not taken into account.

Treatment	0 h	3 h	6 h	12 h	24 h	48 h
Hydroponic (HP) weight	119 (±13)	100 (±13)	81 (±11)	59 (±11)	40 (±9)	20 (±5)
Density	3170 (±338)	2679 (±355)	2171 (±301)	1584 (±304)	1081 (±236)	532 (±140)
Sand (SP) weight	144 (±22)	120 (±20)	95 (±20)	71 (±18)	47 (±9)	25 (±7)
Density	2555 (±398)	2139 (±357)	1692 (±351)	1268 (±326)	845 (±168)	445 (±132)

Table II. Plant biomass (mean wet weight (g ± SD) and density (g m^{-2} ± SD)) at the six sampling times of *Sesuvium portulacastrum* grown in hydroponics or sand substrate and irrigated with RAS water. Growth over the course of the 48 h experiment was not taken into account.

Treatment	0 h	3 h	6 h	12 h	24 h	48 h
Hydroponic (HP) weight	214 (±26)	180 (±17)	147 (±14)	106 (±11)	70 (±12)	40 (±8)
Density	5723 (±690)	4790 (±458)	3933 (±361)	2826 (±305)	1869 (±307)	1075 (±225)
Sand (SP) weight	203 (±33)	167 (±26)	133 (±21)	95 (±17)	65 (±17)	36 (±14)
Density	3628 (±583)	2988 (±461)	2367 (±374)	1699 (±296)	1162 (±312)	639 (±244)

temperature. Salinity increased in the hydroponic boxes compared with the boxes with sand (Table SI, SII). Changes in the concentrations of DIN species and PO_4-P in the systems varied across treatments and time (Tables SIII, SIV).

DIN removal from the direct effluent experiment was not significantly different for plants in hydroponics or in sand, but the planted systems removed more than the unplanted controls (Figure 3A). Final P removal was lowest in the treatments with plants in hydroponics (Figure 3B). In the experiment with RAS water, removal of DIN was significantly higher in the treatments with plants cultivated in sand than in the plants cultivated hydroponically after 24 h, but neither of the treatments with plants performed significantly better than their control treatments without plants. N removal in the hydroponic control treatments however showed high variability, and after 48 h overall concentrations increased relative to initial concentrations of the milkfish wastewater (Figure 4A). The planted and unplanted systems removed the same P. The sand treatment however removed more than the hydroponic plant treatment, both after 24 and 48 hours (Figure 4B).

^{15}N tracer uptake

Atomic % ^{15}N increased over time in the plants grown hydroponically in both experiments. In the plants supplied with $Na^{15}NO_3$ in the direct effluent experiment, isotopic label concentrations were significantly higher in hydroponics than in sand after 24 and 48 h (Figure 5A). The rate of ^{15}N label uptake did not show a clear trend, but was generally higher in the hydroponic treatment (Figure 5B).

In the plants supplied with $^{15}NH_4Cl$ in RAS water, atomic % ^{15}N was significantly higher in the plants grown hydroponically than in sand substrate. In hydroponics, the concentration was consistently high in the roots and increased throughout the experiment in the leaves and stem (Figure 6A). Also, the uptake rate of the ^{15}N tracer was significantly higher in hydroponics than in sand. The highest uptake rate was measured at the 3 h sampling point and it decreased thereafter (Figure 6B).

In both experiments, a significantly higher proportion of ^{15}N was recovered in total plant biomass in the hydroponic treatment than the sand substrate treatment. From 3.518 mg ^{15}N supplied as $Na^{15}NO_3$

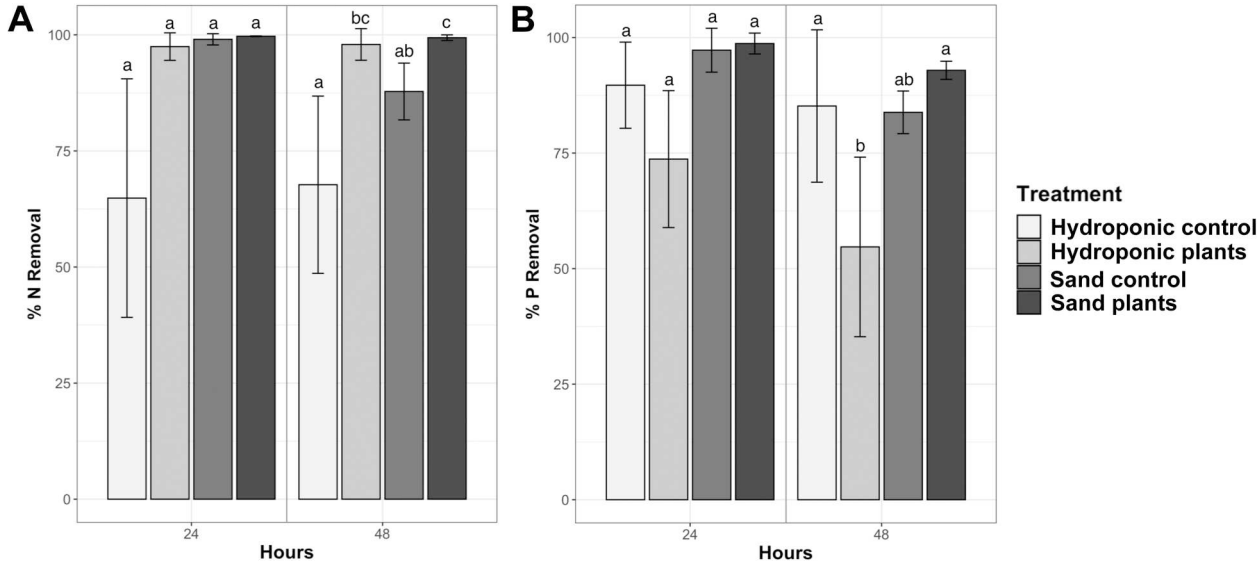

Figure 3. Change in concentrations (% of initial) of dissolved inorganic (A) N and (B) P in direct effluent from milkfish after 24 h in boxes with *Sesuvium portulacastrum* or unplanted control boxes and in the drainage water after 48 h. Different letters indicate statistically significant differences based on multiple comparison test after Kruskal–Wallis test.

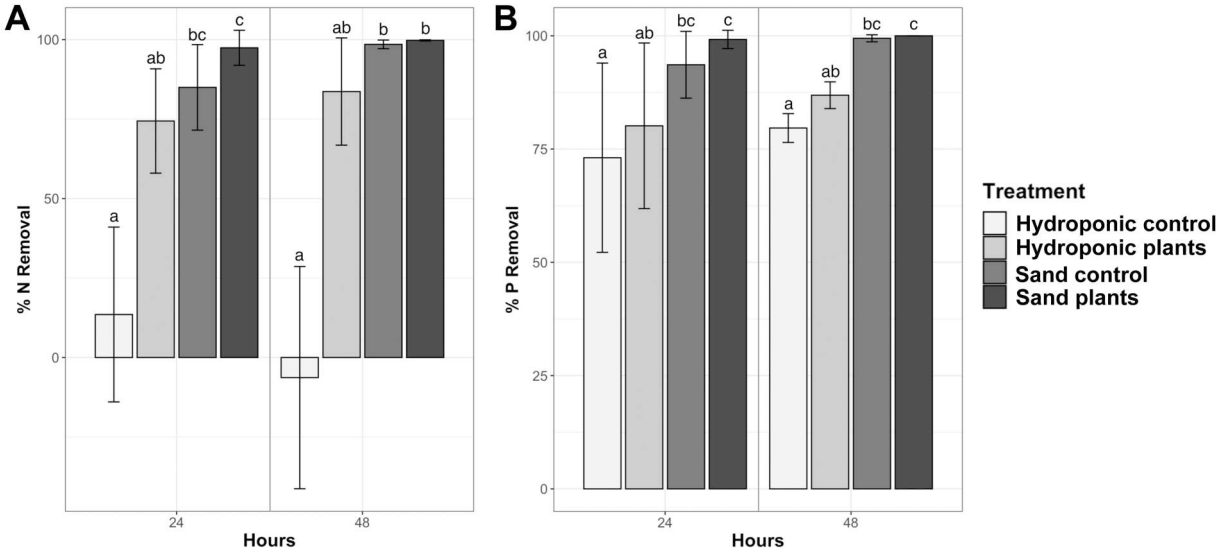

Figure 4. Change in concentrations (% of initial) of dissolved inorganic (A) N and (B) P in RAS water after 24 h in boxes with *Sesuvium portulacastrum* or unplanted control boxes and in the drainage water after 48 h. Different letters indicate statistically significant differences based on multiple comparison test after Kruskal–Wallis test.

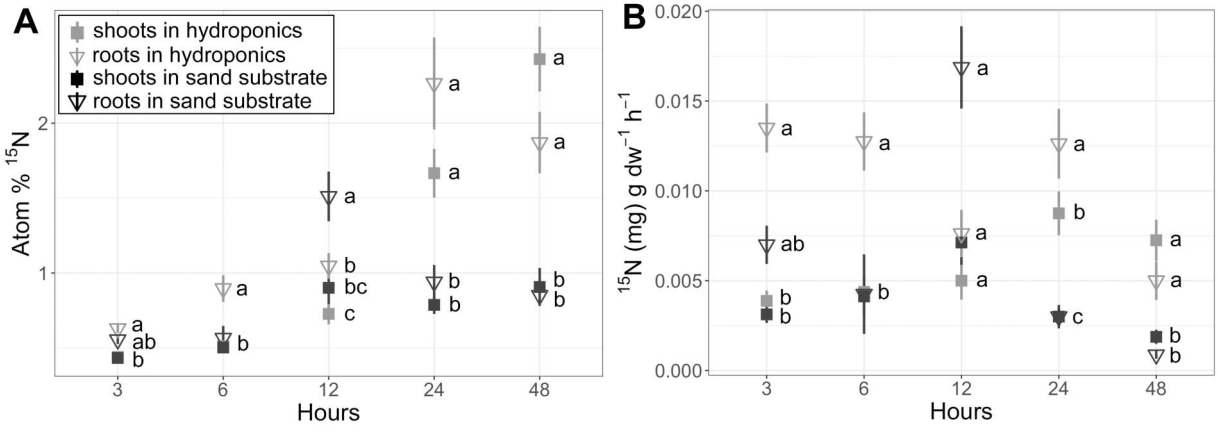

Figure 5. Mean (±SD) ^{15}N assimilated in the stem and leaves (shoots) and roots of *Sesuvium portulacastrum* grown in hydroponics or sand substrate and irrigated with direct effluent from milkfish labelled with $Na^{15}NO_3$. Lower case letters indicate statistically significant differences at the same time point identified by pairwise comparison between estimated marginal means at $p < 0.05$.

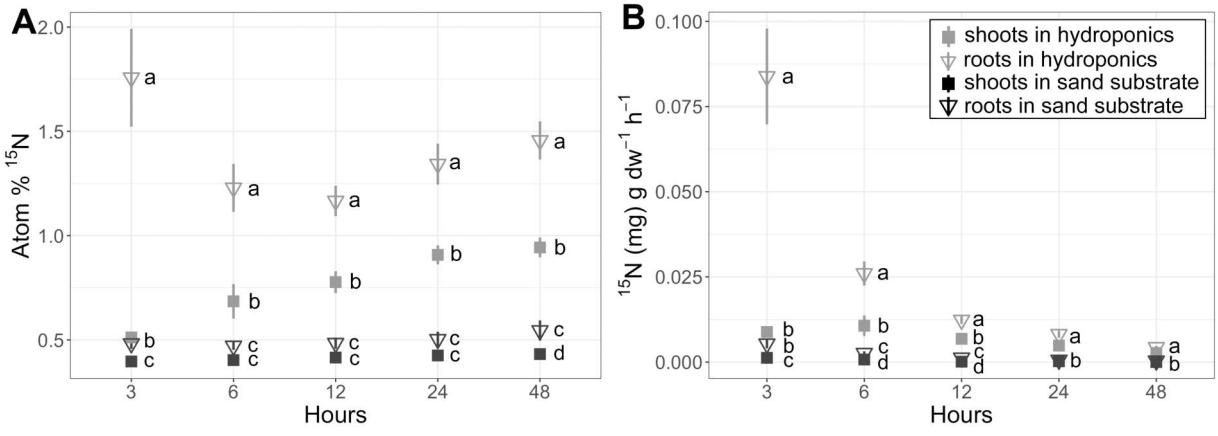

Figure 6. Mean (±SD) ^{15}N assimilated in the stem and leaves (shoots) and roots of *Sesuvium portulacastrum* grown in hydroponics or sand substrate and irrigated with RAS water labelled with $^{15}NH_4Cl$. Lower case letters indicate statistically significant differences at the same time point identified by pairwise comparison between estimated marginal means at $p < 0.05$.

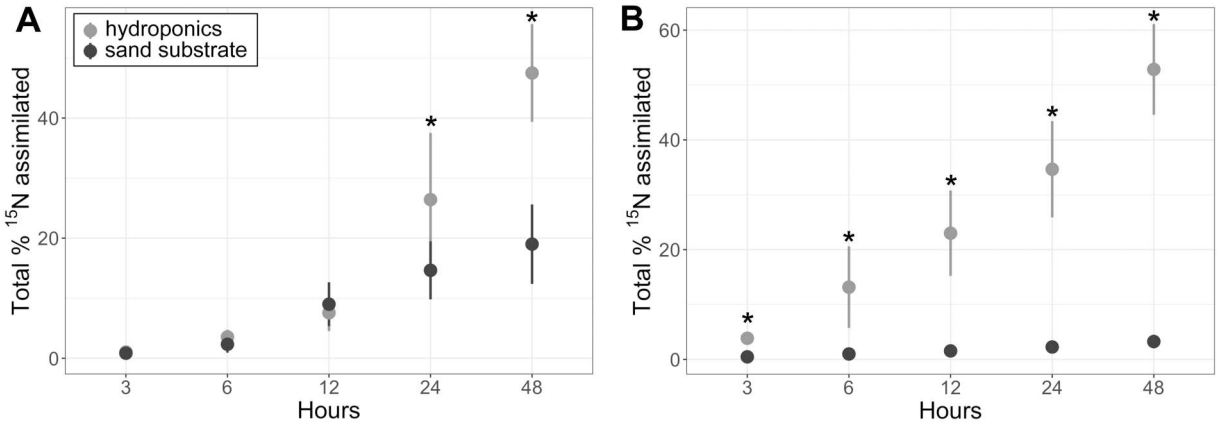

Figure 7. Mean (± SD) % assimilated cumulatively to whole plants of *Sesuvium portulacastrum* grown in hydroponics or sand substrate. ^{15}N label was supplied as (A) $Na^{15}NO_3$ in direct effluent or (B) $^{15}NH_4Cl$ added to RAS water. * = statistically significant differences at the same time point identified by pairwise comparison between estimated marginal means at $p < 0.05$.

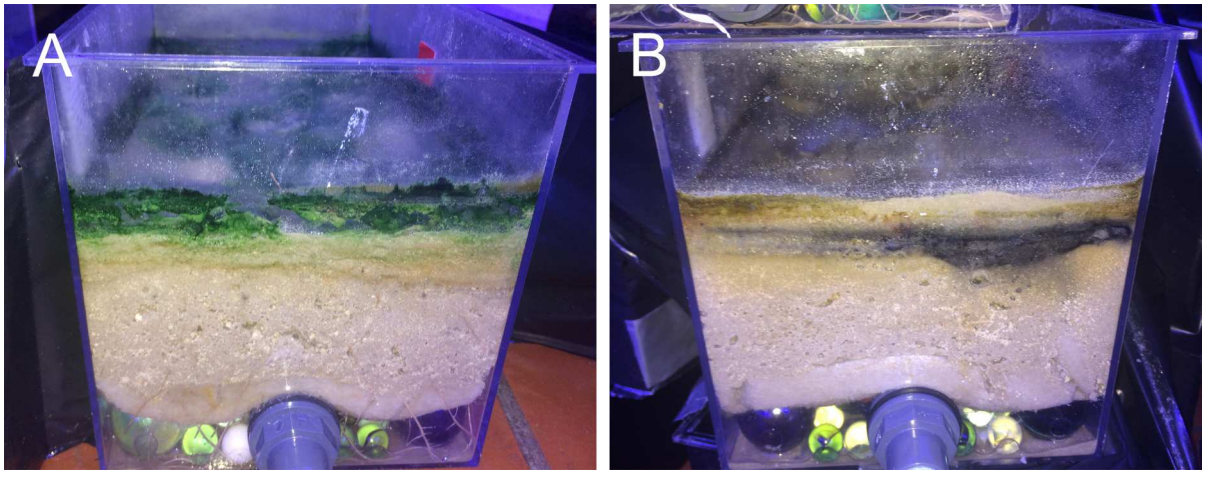

Figure 8. Example of the sand in the boxes that had received RAS water (A) planted with *S. portulacastrum* and (B) unplanted control treatment after black cover was removed upon termination of the experiment.

in direct effluent, the plants in hydroponics assimilated 47.50 ± 8.14% (mean ± SD) and plants in sand assimilated 18.77 ± 6.53% (Figure 7A). From 3.35 mg ^{15}N supplied as $^{15}NH_4Cl$ in RAS water, plants in hydroponics assimilated 52.84 ± 8.27% while the plants in sand substrate assimilated 3.26 ± 1.25% (Figure 7B).

System observations

Upon removal of the wall cover from the boxes of the sand treatment, it was noted that the substrate showed clear differences in its microbial and/or algal community. While the sand surface in the boxes planted with halophytes was overgrown with green and filamentous algae, the control treatments only showed small patches of brown or red microalgae and/or cyanobacteria. The control treatments also showed a clear anoxic layer at an approximate depth of 3 cm, while the boxes with plants did not (Figure 8).

Discussion

This study investigated nutrient removal from milkfish wastewater by systems planted with *S. portulacastrum* in hydroponics, with the roots in water, versus planted in sand. The two experiments allowed comparing nutrient removal and nitrogen recovery by the plants in the two systems when exposed to wastewater from fish cultivation.

Nutrient removal

The results of both experiments presented here show efficient removal of N and P from milkfish wastewater, irrespective of the system being hydroponic or with

sand substrate, unplanted or planted with *Sesuvium portulacastrum*. N removal was fastest in the systems with sand, which comes as no surprise as sands have been shown to be extremely efficient in terms of N-loss by denitrification (Rao et al. 2008; Gao et al. 2012), but especially within the first 24 h and at lower N and P concentrations, there was little to no difference between the treatments. In the systems without plants, N removal would have to be driven by assimilation into un-harvestable biomass growing on the sediment and tank walls and/or anaerobic processes like anammox and denitrification in anaerobic environments that developed in the tanks (Figure 8). The high variability and slight net-increase of DIN concentration observed in the hydroponic control treatment of the second experiment may be due to remineralization of a part of the nitrogen formerly bound in suspended particulate matter (Yoshikawa et al. 2017; Gichana et al. 2018). As P cannot be decayed into a gaseous form like N, P would have to have been removed from the wastewaters by adsorption to the sediment and assimilation into un-harvestable biomass and thus remained in the tank in a different form (Barak et al. 2003). At lower concentrations these pathways appear to have been sufficient to remove most nutrients from the wastewater. The fact that no difference in P removal between the planted systems and their unplanted controls was observed in the second experiment may indicate that these possible pathways, involving assimilation by plants or not, had the same capacity.

Removal of up to 99% DIN and 100% DIP from initial concentrations of 16.73 and 2.05 mg l^{-1}, respectively is comparatively high (Brown et al. 1999; Lymbery et al. 2006; Webb et al. 2012, 2013; Shpigel et al. 2013; De Lange and Paulissen 2016). High removal occurred despite NH_4^+ and NO_3^- concentrations in the milkfish RAS water being similar or even higher than effluents in other studies (Carton-Kawagoshi et al. 2014; Senff et al. 2020), showing that *S. portulacastrum* can remediate wastewater with concentrations higher than what has been shown previously (Boxman et al. 2017). This differs from performances of other halophytes such as *Salicornia europaea*, which, in constructed wetlands, removed only about 0.7% N at concentrations of 14 mg N l^{-1}, but 59% N at low nutrient loads of 0.2 mg N l^{-1} (Shpigel et al. 2013). The wastewater in this experiment remained in the systems for a total of 48 h, comparable with filter systems receiving effluents in batches or operated at low water flow (Webb et al. 2012; Shpigel et al. 2013). A similar removal efficiency of 36–98% P and N was achieved with constructed wetlands planted with *S. europaea* receiving aquaculture discharge water once a day in a flood and drain system, but concentrations were lower at 1.5–5.4 mg N l^{-1} and 1.05–2.79 mg P l^{-1} (Webb et al. 2012). Nutrient removal from wastewater by systems planted with *S. portulacastrum* can be influenced by numerous factors including the availability of carbon substrates and the established microbial community (Ma et al. 2021, Wang and Sheng 2023). Although not measured, such factors may have contributed to the efficient removal observed in this experiment.

Nitrogen recovery

The focus of this study was not on the overall removal of nutrients from wastewaters, but to determine the most efficient way of using these nutrients and assimilating them into valuable biomass. As P does not have stable isotopes that can be easily traced (Schoffelen et al. 2018), we focused on adding a specific amount of 3.518 mg labelled NO_3-N or NH_4-N to milkfish waste water, allowing to identify which condition would result in the highest N recovery into halophyte biomass. In the systems with sand substrate, the plants assimilated only a low percentage of the added ^{15}N label, whereas in the hydroponic systems around 50% could be recovered. This trend was clear and not affected by the form in which ^{15}N was supplied or overall DIN concentration. Recovery of 50% DIN is comparatively high especially when considering the relatively small plant biomass that was effectively maintained throughout the experiment while plants were being harvested (Buhmann et al. 2015; Waller et al. 2015).

High ^{15}N concentrations in root biomass showed fast N uptake. $^{15}N/^{14}N$ ratios were higher in the roots where it is taken up, but due to the far higher biomass, most of the assimilated ^{15}N was retrieved in the leaves and stems and thus converted into a harvestable resource. As only a portion of the DIN available to the plants in this experiment was in the form of ^{15}N, resulting uptake rates are not necessarily representative of overall N uptake. Where label was added as $^{15}NH_4Cl$, $^{15}N/^{14}N$ ratios continued to increase even after NH_4^+ was removed from the water, showing that *S. portulacastrum* was able to take up the tracer in different nitrogen forms. This is further supported by the fact that equally large proportions of ^{15}N were taken up by hydroponic plants in both experiments and may indicate that *S. portulacastrum* can take up different forms of dissolved nitrogen equally and that overall rates of DIN uptake are similar to what has been determined for temperate species of edible halophytes (Quintã et al. 2015a, 2015b). The fact that the

$^{15}N/^{14}N$ ratio in plants decreased after a certain point in the roots of the hydroponic treatments indicates that N uptake continued and *S. portulacastrum* was able to take up other available forms of N. While the focus of this study was on the phytoremediation performance in effluents from fish, further labelling studies with dissolved inorganic and organic N tracers are needed to reveal specific uptake rates and possible preferences for N species.

Recovery of ^{15}N label in plant biomass was up to 17 times higher in hydroponics compared with sand substrate. Similar differences have been found with other species of halophytes (Quintã et al. 2015a). This shows the better recovery of dissolved N in hydroponics and the suitability to integrate *S. portulacastrum* into RAS to improve N-use efficiency (Pinheiro et al. 2017). N uptake by plants in hydroponic cultivation is supported by the large root surface area developed in the absence of substrate, facilitating nutrient uptake and, to some extent, nutrient storage (Wallace and Pate 1967; Hu et al. 2015). When subtracting the effect of the unplanted controls, the planted hydroponic systems removed 407 mg N m^{-2} in the first 24 hours. This is higher than the 117–303 mg N m^{-2} day^{-1} found in cultivation experiments in floating structures (Xiaojie et al. 2011; Liu et al. 2019), indicating that *S. portulacastrum* can be used to remediate waters impacted by more concentrated effluents.

The removal determined here is however a rough estimate and does not consider possible mineralization of dissolved and particulate organic N into the DIN pool. It further does not account for the differences in N-loss due to the presence or absence of plants. In addition to N assimilation, halophytes can contribute to faster nitrification and better N removal by supplying oxygen to the rhizosphere and maintaining an oxic layer in otherwise anoxic, flooded substrates, supporting environmental conditions favourable to microbial activity (summarized by Reddy and Patrick 1984). The roots of salt marsh *Salicornia* sp. were for example found to possess aerenchyma, which allow the exchange of gases between the shoot and the root and indirectly aerate the surrounding soil zone, resulting in increased nitrification/denitrification efficiency (Brix 1994; Haberl et al. 1995; Faulwetter et al. 2009). While dark anoxic layers were observed in the unplanted sand treatment, the absence of such a layer in the planted systems indicates that *S. portulacastrum* may have increased oxygen availability in the substrate. Furthermore, aquaponic systems have been found to harbour more nitrite oxidizing bacteria than conventional RAS and the roots of halophytes grown hydroponically in aquaculture effluents were enriched in bacterial taxa involved in nutrient cycling (Bartelme et al. 2019; Oliveira et al. 2020).

Potential for efficient resource-use

While continuous exposure to excess DIN from aquaculture effluents has clear adverse environmental impacts, microbial processes have the ability to quickly remove excess N from eutrophic systems (Gao et al. 2012; Sokoll et al. 2016; Thomsen et al. 2020). P however remains and is mostly removed from the water column by sorption to sediment and other particles (Krom et al. 1991; Jia et al. 2015). Weakly bound in sediments, it can be mobilized easily, fuelling the growth of primary producers with the potential to cause eutrophication (Jia et al. 2015). Harvesting organically bound P is thus the only strategy to remove it from the aquaculture system and effluents. The significantly higher DIP removal observed in the sand treatments, suggests that this was achieved partly due to mechanisms supported by the sand substrate (Kadlec and Knight 1996; Barak et al. 2003; Cronk and Fennessy 2016). In hydroponics, P removal was also high, possibly driven by immobilization and uptake (Rubio et al. 1997; Barak et al. 2003). As phosphate uptake in halophytes has been found to increase with high N availability, the N:P ratio of 8.31 in the aquaculture effluent of this experiment may have supported good uptake of DIP (Webb 2005). The productivity of modern aquaculture relies on our limited global P reservoirs that are projected to become depleted within a century (Cordell et al. 2009). Between fisheries and aquaculture, we are currently using more P to produce fish than we are harvesting in fish biomass, and aquaculture is thus contributing to the anthropogenic flux of this valuable nutrient from the terrestrial to the aquatic realm (Huang et al. 2020). Therefore, recapture of P from food production waste streams is imperative and the integration of halophytes is an appropriate strategy to do so in saline aquaculture.

Since we have been able to produce fertilizer from inert atmospheric N_2, we are able to use N luxuriously in our food production systems, but at the cost of high greenhouse gas emissions and discharge to natural ecosystems (Erisman et al. 2008). Unlike N, P is of finite supply and we are becoming increasingly aware of the urgency to reduce its waste (Cordell et al. 2009). As aquaculture production is ever increasing, the contribution of fertilizer use in the sector is increasing as well (Huang et al. 2020). Minimizing the waste of nutrient resources is seen as a necessary step to turn aquaculture into a sustainable food production

sector contributing to food security and recovery of nutrients from effluents into valuable biomass is an approach to significantly contribute to this goal (Huang et al. 2020). Rising interest and demand provide an option to produce for local and/or international niche markets (Buhmann and Papenbrock 2013b). The ability to produce high quality health food with consistent nutritional conditions can be a strategy to profitably include *S. portulacastrum* in aquaculture systems and valorize nutrients from aquaculture effluents (Custódio et al. 2020; Maciel et al. 2020).

Conclusions

Integrating extractive species in aquaculture to treat wastewater and increase nutrient recovery into usable biomass is an important step towards sustainability. Cultivating the edible halophyte *Sesuvium portulacastrum* in hydroponics or sand substrate effectively treated milkfish effluents and removed dissolved inorganic nutrients. Our direct result of recovering half of the added ^{15}N nutrient back into new edible biomass is very high and hydroponic cultivation would thus be the recommended cultivation system to prevent losses through denitrification or discharge. The contribution of plants towards effluent treatment appears to go beyond uptake and assimilation by possibly enabling conditions that support microbial nitrification, favouring immobilization over denitrification and potentially reducing nitrous oxide emissions. Determining uptake rates for different N forms could aid in further optimizing the hydroponic growth method applied in this study and move towards more complete resource recovery in closed recirculation systems and integrated aquaculture.

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