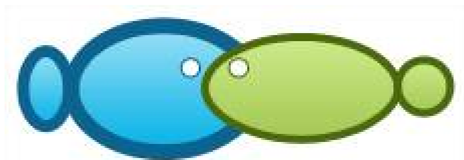

Nitrous oxide production and microbial diversity in an aquaponic system

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Abstract. The concentration and N_2O production and consumption rates in an aquaponic system (fish tank, settling tank, and biofilter) were quantified during a culture cycle to determine if this type of system contributes to the emission of N_2O and the microbial communities involved. Chemical inhibition (allylthiourea and acetylene) and metataxonomic techniques were applied. The results indicated that the highest dissolved N_2O concentrations were recorded in the biofilter (1.99±0.74 μM), and a net N_2O production rate was observed in all the compartments, reaching the highest value (324.4 nM d^{-1}) in the settling tank. The N_2O production was associated with nitrification (rates up to 168.6 nM d^{-1}) and denitrification (rates up to 372±180 nM d^{-1}). Nitrification was predominantly related to ammonia-oxidizing bacteria and archaea. However, Comammox bacteria were present in the three compartments. Alphaproteobacteria, Bacteroidota, and Actinobacteria were the most abundant phyla shared between containers, including some genera reported as denitrifiers capable of producing N_2O.
Key Words: aquaponics, denitrification, greenhouse gas, metataxonomic, nitrification.

Introduction. Aquaponic aquaculture is a particular form of recirculating polyculture system for fish and plants (Hu et al 2012). Small-scale aquaponic systems are environmentally friendly farming technologies that allow the intensive and simultaneous production of high-quality animal and plant food in small spaces. Compared to soil cultivation and aquaculture, the advantages of aquaponics are a more efficient use of water, a reduction in the release of heavy metals, nitrates, fertilizers, pesticides, hormones, and antibiotics to the environment, and a reducing in ecosystem degradation (Junge et al 2017).

Aquaculture development in Colombia has been remarkable, increasing from 9,200 tons in 1990 to 82,733 tons in 2011, which means an annual growth rate of 12%, higher than other Latin American countries. Production corresponded to red and silver tilapia at 58.5%, cachamas at 19.25%, rainbow trout at 6.8%, and other native and exotic continental fish species at 5.04% (Merino et al 2013). However, cleaner alternatives, such as aquaponic systems, lower greenhouse gas emissions, efficient production (fish and vegetables), quality, and profit for rural populations, are currently being implemented.

In the country, aquaponic systems on a small scale with goldfish, lettuce (Ramírez et al 2016a), carp-oregano (Ramírez et al 2016b), red tilapia-mint (Cardenas et al 2022), rainbow trout-basil (Igua-Galindo 2020), tilapia and different varieties of chard (Alturo 2016), and commercial aquaponics systems for the production of tilapia, cachama and three varieties of basil (Santos & Pandales 2017), rainbow trout-mint (Valencia-Ramirez 2019) and rainbow trout for the production of different types of vegetables (López 2020) have been developed.

In this type of crop, the transformations of N depend on the relationships between bacteria, fish, and plants. Fish in aquaculture tanks excrete total ammonia nitrogen

(TAN), and biofilters' bacteria and plant roots maintain good water quality for fish by oxidizing TAN to nitrite and nitrate by nitrification (Wongkiew et al 2017a). Plants grow because they assimilate nitrate and ammonium from the system (Hu et al 2012, 2013). In this way, aquaponic systems have a high nutrient recycling capacity and minimize the concentrations of ammonium, nitrite, nitrate, and N_2O production. This latter is a potent greenhouse gas with a higher global warming capacity than carbon dioxide (Ravishankara et al 2009). Currently, N_2O emission estimations from aquaponics indicate that they represent between 0.6 and 2.0% of the N input in fish feed (Hu et al 2015; Wongkiew et al 2017b). In general, hydroponic and aquaponic systems emit less nitrous oxide, i.e., up to 24.1 mg-N d^{-1} (Wongkiew et al 2018), compared to intensive aquaculture systems, i.e., up to 46 mg $N\text{-}N_2O$ d^{-1} (Paudel et al 2015). Fish production generates approximately 1.69 g $N_2O\text{-}N$ Kg^{-1} fish (Hu et al 2012), and 9.30×10^{10} $N_2O\text{-}N$ g^{-1} emission estimates account for aquaculture globally (Paudel et al 2015).

Microbial processes associated with nitrification, nitrifier denitrification, denitrification, and coupled nitrification and denitrification contribute to N_2O production in aquaculture systems (Hu et al 2012; Wongkiew et al 2018). Moreover, microbial activities resulting in N_2O emissions from aquaponic systems can be affected by several parameters such as feeding rate, pH, dissolved oxygen (DO) levels, plant species, and densities (Hu et al 2012, 2015; Zou et al 2016; Paudel et al 2019; Paudel 2020). Although the biochemical mechanisms of N_2O production in aquaculture have been extensively studied, a comprehensive understanding of the N_2O production mechanisms in aquaponic systems, as well as the contribution of each compartment (fish tank, biofilter, and settling tank) to N_2O emission, is not readily available. For this reason, our working hypothesis proposes differences in the production of N_2O between the compartments of the aquaponic system, given that the microbial processes and even the microbial communities could be specific to each compartment because of different environmental conditions.

This is the first study focused on analyzing whether or not aquaponics contributes to the production of nitrous oxide and which communities and microbial processes may be associated with each compartment of the system throughout the cultivation cycle, combining chemical inhibition and meta-taxonomic techniques. The objectives were to examine the differences in N_2O production from fish tanks, biofilters, and settling tanks and the associated microbial communities. The findings could be helpful for the better management of aquaponic systems for lower N_2O emissions.

Material and Method. Two parallel replicates of a 1.7 m^3 aquaponics system were operated in university greenhouses (4°56'24''N and 4°57'0''N; 74°1'12''W and 74°0'36''W) from November 15th, 2020, to June 30th, 2021. In this period, a freshwater polyculture was maintained with Nile tilapia (*Oreochromis niloticus*) and white cachama (*Piaractus brachypomus*); both had an average initial weight of 438±25 g, reaching a stocking density of 20±0.2 Kg m^{-3}. The vegetables used were 63 watercress (*Nasturtium officinale*).

Each aquaponic system (system 1 and system 2) consisted of a fish tank (1000 L), a settling tank (500 L), an up-flow biofilter (100 L), and a sump tank (100 L). The water collected in a sump tank was pumped (Resun® King 5, 6000 L h^{-1}) to the NFT vertical hydroponic units. The system had constant aeration in the fish tank and biofilter by a blower (Resun® GF 250).

Sampling methods for N_2O determination in each aquaponic compartment. Samplings (n = 3) for dissolved N_2O concentration determination were carried out from fish tank water, the settling tank, and the biofilter of two aquaponic systems mounted in parallel. Gas sampling was conducted every two weeks for four months until the end of the plant harvesting cycle. The samples from each compartment were taken in 100 mL amber bottles (n = 3) at a depth of 0.5 m without shaking the water and eliminating the bubbles inside the bottle. The vials were capped with butyl stoppers and sealed with metal lids, and 1 mL of saturated $ZnCl_2$ solution (50% v/v) was injected into each bottle to inhibit bacterial activity until quantification by gas chromatography.

Sampling and experiments for N_2O production estimation. Water samples (2 L) were taken from the fish tank, biofilter, and settling tank and distributed in 100 mL amber glass bottles that were sealed with butyl rubber and metal caps to carry out the in-situ experiments for 24 hours at room temperature (18°C) and in the dark to inhibit phytoplankton activity.

To determine the production of N_2O by nitrification, inhibition experiments were carried out using allylthiourea (ATU) at 100 mg L^{-1} (Ginestet et al 1998) and acetylene injection at 10% v/v (Wrage et al 2004), both inhibitors of ammonium monooxygenase (AMO). N_2O production rate by nitrification was estimated as the difference between the rates estimated in the control (net N_2O production by nitrification and denitrification) and the ATU or acetylene experiment below in situ O_2 levels.

A total of 27 bottles (n = 9 per each treatment) considering three replicates for ATU, acetylene, and a control (bottles without inhibitor addition) and endpoint incubations at 0, 12, and 24 h were required for each compartment (fish tank water, settling tank, and biofilter) and each aquaponic system mounted in parallel (systems 1 and 2 as was described in methods) and during three sampling times along the aquaponic culture: the beginning (November 2020), four months later and seven months later. Incubation was stopped by injection of 1 mL of saturated $ZnCl_2$ solution (50% v/v) to inhibit microbial activity and the production of N_2O into the bottles until the analysis. The vials were stored upside down under refrigeration until quantification.

Chemical analyses. Water temperature and pH in the fish tank were monitored daily using a multiparameter probe (HI 9829 Hanna®). In this compartment, the ammonium, nitrite, and nitrate were analyzed weekly by spectrophotometric quantification with Spectro quant Multy®, following the supplier's instructions. Dissolved N_2O determinations in water samples from each aquaponic compartment and from the N_2O production experiments were carried out using the gas-liquid phase equilibrium procedure, injecting 40 mL of ultrapure He into each bottle and incubating for 1 hour at 40°C with shaking (McAuliffe 1971). Subsequently, 25 mL of the gas phase was injected into vacuum vials. The N_2O gas concentration was analyzed using a Gas Chromatography Electron Capture Detector (GC-ECD) from Shimadzu at 350°C with a high purity nitrogen mobile phase. Scotty N_2O standards were used with an accuracy of ±5%.

The dissolved N_2O concentration was calculated from the headspace gas concentration using the solubility formula of Weiss & Price (1980). The coefficient of variation of dissolved N_2O measurements between replicates was < 3% and the average precision was always better than 3%.

Statistics analyses. The estimation of the evolution of N_2O production (production or consumption rates) during 24 hours of incubation for each treatment and compartment of both aquaponic systems during the three stages of the culture was carried out through linear regression analysis from the concentration change measured in 3 replicates at each sampling time (0, 12, and 24 h). The N_2O concentrations in the samples were plotted against time and fitted to the linear model ($At = A0 \pm mt$) using the method of least squares, where t is the incubation time, A0 is the N_2O concentration at t = 0, and m is the linear slope. The rates were calculated from the slope and expressed in nM h^{-1}. Rate uncertainties (±) were calculated from the errors in the linear regression estimation. Positive values represented N_2O accumulation through time, whereas negative values indicated its consumption. The normal distribution of the data was tested using the Shapiro-Wilk test and previous data transformation with Box-Cox. The N_2O rate comparison between experiments and compartments of the aquaponic system, as well as the comparison of the dissolved N_2O concentration (μM) in water (mean±standard deviation) between compartments during the cultivation time, was made through an ANOVA analysis and Tukey's test using a significance level of 5% ($p < 0.05$). All statistical analyses were run with the R software 4.0.2 for Windows (R Core Team 2018).

Microbial community analysis*.* To obtain insight into archaeal and bacterial community profiles, water samples from different compartments of the aquaponic system (fish tank, biofilter plus its biofilm, and settling tank) were taken and analyzed by 16S rRNA gene deep sequencing. The relative abundance of oxidant ammonium and oxidizing nitrite communities (Bacteria and Archaea) associated with Comammox was analyzed with the amoA gene encoding ammonia monooxygenase, which was involved in ammonia oxidation. The quantification was developed by quantitative PCR (Q-PCR) using a real-time PCR system (LightCycler-480; Roche, CH) under the method described in Molina et al (2018).

During all stages of cultivation, samples were taken to analyze the microbial community. Two liters of water were collected in sterile Pyrex bottles for the fish and settling tanks. In order to concentrate the bacteria, the samples were filtered through 0.45 μm filters (Millipore) with a vacuum pump. For the biofilter samples, 20 biochips were collected and carefully washed into 100 mL of biofilter water to ensure a maximum quantity of bacteria was harvested. After that, the samples were filtered through 0.45 μm filters (Millipore) with a vacuum pump. The filters were frozen to -80°C with a lysis buffer (20 mM Tris HCl, 50 mM EDTA, 20 Mm NaCl) until DNA extraction. DNA extraction was performed with phenol-chloroform following the protocol described by Castro-González et al (2005). The DNA extracts were cleaned with the One Step PCR Inhibitor Removal Kit (Zymo Research) before quantification in a Nanodrop (Thermo Fisher Scientific) and stored at -20°C until further analysis.

Sequencing*.* Novogene (California, USA) carried out library preparation and sequencing. The library preparation was done using the NovaSeq 6000 reagent kit (Illumina, CA, USA), while sequencing was conducted on an Illumina NovaSeq 6000 PE250 (2 × 250 bp). Sequencing primers were chosen to cover the hypervariable regions V3-V4 of the 16S ribosomal RNA gene with the universal primers MiCSQ_343FL and MiCSQ_806R (Bukin et al 2019). Data is available under the accession PRJNA 847209 on the Sequence Read Archive database (SRA) of the National Center for Biotechnology Information (NCBI).

Bioinformatic analysis*.* The bioinformatic analysis of the sequences included a sequence filtering step that consisted of primers, barcodes, short, low quality, and chimeric sequence elimination following standard protocols using MOTHUR (Schloss et al 2009). The community composition was analyzed using the SILVAngs platform from http://www.arb-silva.de/ (Quast et al 2013).

Results

Physicochemical parameters*.* During the crop cycle, the physicochemical conditions in the tank fish remained stable with a temperature of 21.6±1.6°C, pH 6.6±0.7, DO 4.6± 0.5 mg L^{-1}, ammonium 0.3±0.4 mg L^{-1}, nitrite 0.03±0.02 mg L^{-1} and nitrate 82.1±39.8 mg L^{-1}.

N_2O concentration in the surface waters of aquaponic systems. The concentration of N_2O in the surface waters of the tank, biofilter, and settling tank showed significant differences and values from 0.02±0.00 up to 1.99±0.74 μM in the three compartments during the crop cycle. In general, the highest N_2O concentrations were recorded after three months of culture in the biofilter (1.99±0.74 μM), followed by a settling tank (1.12±0.58 μM) and fish tank (0.10±0.05 μM) and decreased by 82.4%, 91.5%, and 95.5 % in the fish tank, biofilter, and settling tank, respectively, at the end of the culture (Figure 1).

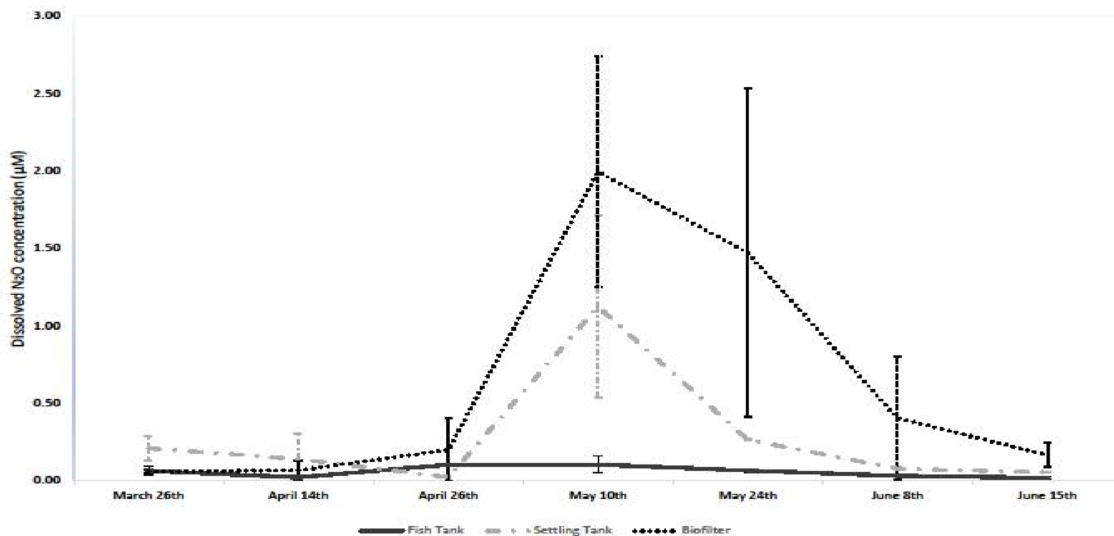

Figure 1. Concentration of dissolved N_2O (mean±standard deviation) in water of aquaponic system compartments during the cycle of culture (n = 6).

N_2O production and consumption rates in the aquaponic system. The results of the experiments to estimate the N_2O production by the microbial community in each compartment over seven months of culture (Table 1) showed substantial differences between both aquaponic systems for which they were analyzed independently. In general, system 1 (S1) showed low rates of N_2O consumption (between -0.008 and -0.058 nM h^{-1}) during the length of the culture. In the S1, the N_2O was consumed at the beginning of the culture in the fish tank (-0.058 nM h^{-1}) and the biofilter (-0.014 nM h^{-1}), and this consumption decreased by approx. 50% at the end of the culture in both compartments. Likewise, low net N_2O production (control experiments) was recorded only at four months of culture in the fish tank (0.044±0.052 nM h^{-1}) and the biofilter (0.118±0.064 nM h^{-1}) in comparison with the system 2 (S2) which showed higher net N_2O production rates during all the culture cycles in the tree compartments. The highest value was reached in the fourth month in the settling tank (13.517±2.258 nM h^{-1}), followed by the biofilter (0.696±0.436 nM h^{-1}) and fish tank (0.130±0.146 nM h^{-1}). However, in the S2 at the end of culture, the net N_2O production in the settling tank and the biofilter decreased by 98% and 38%, respectively, but in the fish tank, there was a considerable increment (3.113±2.987 nM h^{-1}).

The addition of inhibitors caused varied effects on the microbial community. The ATU addition inhibited N_2O production from nitrifiers in the fish tank and settling tank of S2 at the fourth month of culture, with N_2O production rates between 2.0±0.4 and 7.0±1.1 nM h^{-1} respectively by ammonium oxidant microorganisms. Contrary to expectations, the ATU increased the N_2O production between 232% (fish tank) and 1386% (biofilter) in the fourth month of culture and between 26% (biofilter) and 46% (settling tank) at the end of culture in the S2 in comparison with control experiments, suggesting its use as an N-substrate by the microbial community. Contrarily, in S1, even at the end of the culture, it was observed that ATU increased the N_2O consumption in the biofilter, followed by the fish tank and settling tank.

In S2, the addition of acetylene increased the N_2O production by 2229% (biofilter) and 155% (fish tank) in the fourth month of culture concerning control experiments, and the N_2O production decreased to 50% in the biofilter and settling tank and to 100% in the fish tank at the end of cultivation. These results indicated the presence of an active denitrifying community in the biofilter with denitrification rates that decrease from 15.5±7.5 to 7.5±4.2 nM h^{-1} in comparison with low rates recorded in the fish tank to the middle of culture (0.20±0.12 at S2 and 0.08±0.0 nM h^{-1} at S1) and the settling tank at the end of culture (of 3.2±1.8 nM h^{-1}).

Table 1

N_2O production and consumption rates (nM h^{-1} ± typical error) estimated for each experiment (n = 9) carried out (to 0, 12, and 24 hours) from different compartments (fish tank, biofilter, and settling tank) at three times of the culture (0, 4, and 7 months) and two systems (system 1 and 2)

Sampling month	*Compartment*	*Experiment*	*System 1*	*System 2*
0	Fish tank[a]	Control	-0.058±0.118	0.001±0.015
		ATU	-0.058±0.029	0.018±0.017
		Acetylene	-0.007±0.077	0.003±0.018
	Biofilter[b]	Control	-0.014±0.021	-0.012±0.018
		ATU	-0.035±0.012	0.025±0.009
		Acetylene	-0.206±0.097	-0.014±0.023
4	Fish tank[a]	Control	0.044±0.052	0.130±0.146
		ATU	0.107±0.040	0.432±0.067
		Acetylene	0.127±0.050	0.331±0.274
	Biofilter[b]	Control	0.118±0.064	0.696±0.436
		ATU	0.111±0.026	10.342±4.410
		Acetylene	0.304±0.085	16.210±7.965
	Settling tank[c]	Control	ND	13.517±2.258
		ATU	ND	6.499±1.129
		Acetylene	ND	7.031±1.733
7	Fish tank[a]	Control	-0.029±0.017	3.113±2.987
		ATU	-0.042±0.013	1.073±2.612
		Acetylene	-0.168±0.033	-0.168±0.033
	Biofilter[b]	Control	-0.008±0.047	0.431±1.542
		ATU	-0.152±0.093	0.547±0.833
		Acetylene	-0.204±0.152	7.947±5.794
	Settling tank[c]	Control	0.028±0.126	0.260±1.256
		ATU	-0.011±0.030	0.380±1.721
		Acetylene	-0.137±0.128	3.461±3.114

Superscripts a, b and c represent statistically significant differences ($p < 0.05$); ND = not determined.

Microbial communities′ composition. The results showed that the microbial communities shared several phyla between compartments. However, they presented differences in their relative abundance (Figure 2).

Figure 2. Relative abundance of the taxonomic groups detected in the aquaponic system based on 16S rRNA gene sequencing.

The most abundant operational taxonomic units (OTUs) in the fish tank and settling tank were members of Alphaproteobacteria (32.7-40.4% read abundance) and Bacteroidota (9.3-30.1% read abundance), while Alphaproteobacteria (19-36% read abundance) and Actinobacteria (23.7% read abundance) were dominant in the biofilter. The Gammaproteobacteria were detected in lower abundance (12.5%) in all compartments, and Firmicutes (15%) and Nitrospirota (6.1%) were detected only in the biofilter.

A high richness of bacterial genera (33) was detected in the aquaponics system, with differences in relative abundance in each compartment. The most abundant genera were *Zymomonas*, *Flavobacterium*, *Xanthobacter*, *Sphingomonas*, *Acinetobacter*, *Nitrospira*, uncultured bacteria, and other bacteria that could be classified but do not belong to the most abundant bacterial genera (Figure 3).

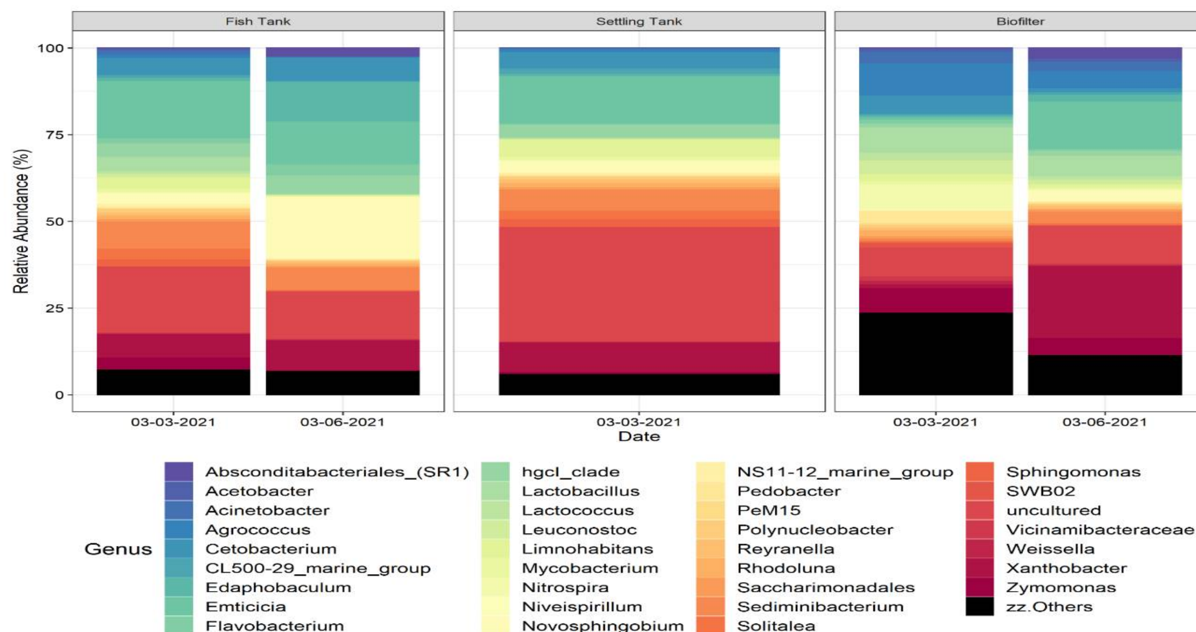

Figure 3. Relative abundances of the bacterial genera detected in the aquaponic system based on 16S rRNA gene sequencing. Uncultured depicts all putative OTUs that could not be assigned to cultured taxa.

The analysis of the ammonia-oxidizing community (Figure 4) showed the dominance of *Nitrospira* in the three compartments since the fourth to seventh month of culture. Contrary to expectation, *Nitrosomonas* was only detected in very low abundance in the biofilter (< 5%). Ammonia-oxidizing Archaea Candidatus *Nitrosopelagicus*, Candidatus *Nitrosopumilus*, and Candidatus *Nitrosotalea* were also detected in low abundance (< 10%) only in the fish tank.

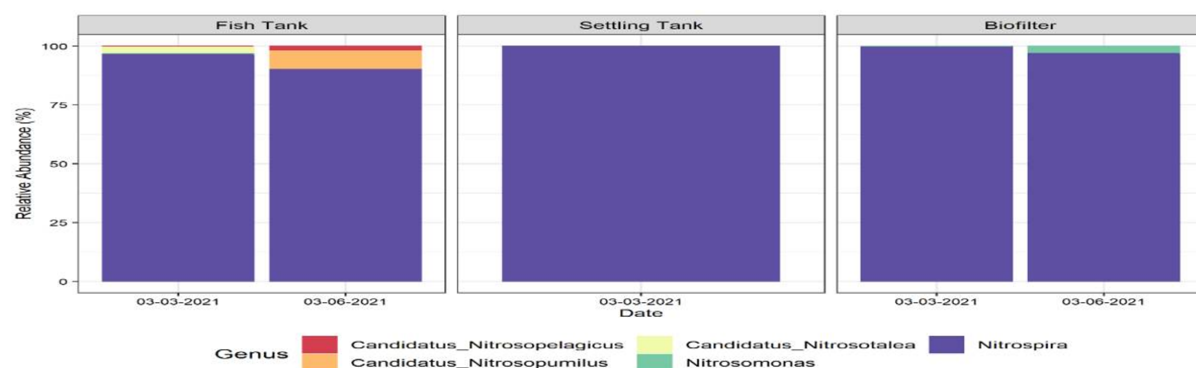

Figure 4. Relative abundances of the ammonia-oxidizing Archaea and Bacteria in the aquaponic system based on 16S rRNA gene sequencing.

Given the dominance of *Nitrospira* sequences in the compartments, the abundance of oxidizing ammonium communities (Archaea-AOA, bacteria-AOB) and Comammox was quantified by qPCR (Table 2). Although the quantification was unsuccessful for several samples, the results indicated that the AOA was detected only in the fish tank and in lower abundance than the AOB, supporting the 16S rRNA sequence analysis results. The AOB showed greater abundance in the fish tank (1.15×10^3) than in the settling tank and biofilter (up to 3.19×10^2) at the fourth month of culture.

Likewise, in the three compartments, in the middle (March) of the culture, *Nitrospira* was detected by 16S rRNA sequencing, and we were able to determine that this corresponds to Comammox (Clade A) by qPCR. The data indicated a higher abundance of Comammox than beta AOB in all the compartments, with a predominance of Comammox in the fish tank. However, its abundance was similar to that recorded for the settling tank. At the end of the culture (June), an increase of up to 2.0×10^5 copies μL^{-1} in Comammox abundance in the fish tank was detected. Although *Nitrospira* sequences were found in the middle (March) and end of culture (June) at the biofilter (Figure 4), their detection by qPCR in this compartment was possible only at the beginning of the culture (November).

Table 2

Average (copies μL^{-1}) and standard deviations of oxidizing ammonium and oxidizing nitrite communities

Compartment	*Comammox (Clade A)*	*beta AOB*	*AOA*
Fish tank-June	$2.0 \times 10^5 \pm 2.0 \times 10^4$	$1.7 \times 10^3 \pm 5.6 \times 10^2$	ND
Fish tank-March	$1.4 \times 10^4 \pm 9.9 \times 10^2$	$1.1 \times 10^3 \pm 6.3 \times 10^1$	$2.8 \times 10^2 \pm 3.9 \times 10^1$
Biofilter-November	$1.5 \times 10^3 \pm 4.2 \times 10^2$	$1.1 \times 10^2 \pm 3.2 \times 10^1$	ND
Biofilter-March	ND	$2.6 \times 10^2 \pm 5.8 \times 10^1$	ND
Settling tank-March	$1.1 \times 10^3 \pm 1.4 \times 10^2$	$3.2 \times 10^2 \pm 4.3 \times 10^1$	ND
Settling tank-March	$1.8 \times 10^4 \pm 7.0 \times 10^2$	ND	ND

Notes: Archaea (AOA), Bacteria (beta AOB), and Comammox (Clade A) detected in each compartment of the aquaponic system; ND = not determined.

Discussion. In this study, the N_2O concentration and production and consumption rates in three compartments of a small-scale aquaponic system were quantified during a 7-month culture cycle of watercress with tilapia and black cachama to determine if this type of culture contributes to the emission into the atmosphere of this greenhouse gas and what microbial processes and microbial communities could be involved.

Monitoring the water quality parameters in the fish tank showed their stability during the culture cycle. The temperature (21.6±1.6°C) and the pH remained within the ranges required for good fish growth (6.5-8.5) and plants growth (5.5-6.5), and although the pH was slightly low (5.9-7.3) for optimal metabolism of nitrifying bacteria (7-8.5) following Sallenave (2016), the low concentrations of nitrite and ammonium suggest active nitrification to nitrate, the latter with levels continuously lower than 120 mg N L^{-1}.

Few studies report the concentration of N_2O in the water of aquaponic systems; one of them is Hu et al (2015), who reported values between 0.6 and 1.3 $\mu g\ L^{-1}$, which are similar to our values up to 1.99±0.74 $\mu g\ L^{-1}$ suggesting active nitrification in the system. However, our results differ from those reported by Wongkiew et al (2018) who observed higher N_2O emissions from the fish tank than the biofilter, probably because of higher turbulence and N_2O stripping from the water in this compartment. Our results are consistent with previous reports that indicate that nitrification and denitrification can coincide in the anoxic microenvironments in the biofilm and biofilter sediments (Zou et al 2016), which would explain the high levels of N_2O measured in our biofilter.

Our results corroborate previous information indicating that aquaponics is not an absolute solution to N_2O emissions; but, it reduces these emissions significantly (Hu et al 2010, 2015; Paudel 2020). However, this varies with changes in environmental and operational parameters like water exchange rate, feed type, feeding rate, fish stocking density, pH, DO, water quality, and temperature (Hu et al 2012).

Microbial processes associated with N_2O production in the aquaponic system. The results of laboratory experiments to analyze the participation of different microbial groups in the generation of N_2O from the aquaponic system showed that in all compartments, there is a net N_2O production during the entire culture cycle, which is exceptionally high at four months. The latter could be associated with the fact that three days prior to the experiments, potassium nitrate (50 g), DPTA-Fe (3.6 g), and sodium bicarbonate (7g) were added to each aquaponic system (S1 and S2). However, these showed significant variation between them throughout the growing cycle. From the experiments with inhibitors, it could be inferred that N_2O production corresponds to nitrification at 16% in the biofilter (ATU experiments) with rates of 1.8 nM d^{-1} and of at 50% in the settling tank (ATU and acetylene experiments) with rates up to 168.6 nM d^{-1}.

The bacterial community facilitates nitrification in aquaponics in two intermediate and sequential steps. First, TAN is oxidized to nitrite by ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB). Sequentially, nitrite is oxidized to nitrate by nitrite-oxidizing bacteria (NOB) (Wongkiew et al 2018). It has also been reported that the AOA oxidizes ammonia in low and high concentrations, up to 100 µg g^{-1} (Zhao et al 2020). Although this is rare in aquaponic systems, they were founded in the fish tanks where N_2O production could be attributed to its presence since the ATU inhibitor does not affect them (Sauder et al 2011) or high concentrations (193-500 µM) are required for some inhibition (Ruser & Schulz 2015). The production of nitrous oxide has been directly observed in the pure cultures of Ca. *Nitrosopumilus maritimus* and other marine AOA enrichment cultures, despite the lack of genes homologous to the HAO and NOR (nitric oxide reductase) responsible for N_2O formation in AOB (Santoro et al 2011; Löscher et al 2012). However, to our knowledge, archaeal communities and their role in N_2O production have not been studied in aquaponics systems. Therefore, studies should be expanded using inhibitors such as PTIO (Martens-Habbena et al 2015) and Simvastatina (Zhao et al 2020), more specific to AOA, to demonstrate their role in N_2O production.

The data also suggest that other processes, such as denitrification and denitrifying nitrification or other microbial communities unaffected by nitrification inhibitors (such as heterotrophic bacteria and/or fungi), could contribute to the net N_2O production in this system. The acetylene experiments showed inhibition of N_2O reduction by denitrifies, with the subsequent N_2O accumulation associated with high ammonium levels (up to 240 mg L^{-1}, perhaps by a dissimilatory nitrate reduction to ammonium. However, this hypothesis should be tested in further studies, as this process was not measured. In addition, it was observed that the microbial communities could be very active and generate large amounts of N_2O within each compartment, such as in the settling tank where the highest net N_2O production rates were recorded (324.4±66.3 nM d^{-1}), probably due to a variety of microorganisms associated with particulate matter working to suboptimal O_2 concentration. Previous studies showed that high quantities of N_2O can be produced under conditions that are suboptimal for both nitrifiers and denitrifiers and that 75.2-78.5% of N_2O emission from aquaponics can be attributed to denitrification or the coupled nitrification and denitrification in oxic and anoxic zones in the non-turbulent area or inside the bio flocs (Tallec et al 2006; Hu et al 2010; Zou et al 2016).

However, it is also necessary to keep in mind that some artifacts could affect our results, such as the variation in sensitivity of different ammonia oxidizer phylotypes to nitrification inhibitors, as reported by Shen et al (2013); the acetylene inhibition on some crenarchaeota (Ofree et al 2009); and the stimulation of some Archaea by ATU (Lehtovirta-Morley et al 2013); or its growing on urea or cyanate with subsequent ammonia production, which can even happen spontaneously (Palatinszky et al 2015), which could explain the increment in ammonium along with the N_2O production in the presence of this inhibitor in some experiments.

On the other hand, the results of N_2O consumption recorded in the fish tank and biofilter indicate that the denitrifying community is also active during the entire cultivation cycle, contributing to the purification of this greenhouse gas, especially in the biofilter, where the highest consumption rates were recorded (-3.2±2.2 nM d^{-1}) by inhibition with ATU of oxidizing ammonium bacteria at the end of the culture and where, besides the N_2O it could be used as an electron acceptor because of low nitrate levels

recorded in these experiments (5 mg L^{-1}). Our results are in accordance with previous studies that indicate that nitrogen loss occurs in the aquaponic system by denitrification (Monsees et al 2017; Wongkiew et al 2017b) or via nitrifier denitrification (Wongkiew et al 2018) and that each compartment of the aquaponic system representes a different microenvironment with specific microbial processes (Schmautz et al 2021a, b).

Microbial community at an aquaponic system compartment. The analysis of the microbial communities in each compartment of the aquaponic system showed a richness of phyla and genera. At the phylum level, the bacterial communities from all compartments were relatively similar, with a predominance of Proteobacteria, Bacteroidetes, and Acidobacteria similar to those reported by Heise et al (2021) and Eck et al (2019). At the genus level, the communities in the compartments were similar. The biofilter samples harbored a higher OTU than the settling tank and fish tank, similar to those reported by Eck et al (2019), Schmautz et al (2021a), and Bartelme et al (2019). Our data also support the hypothesis that a common bacterial base may exist between all aquaponic systems despite differences in fish species, system layout, or fish feed proposed by the last researcher.

On the other hand, several dominant genera were found in the compartments, such as *Zymomonas*, *Flavobacterium*, *Xanthobacter*, *Sphingomonas*, and *Acinetobacter*, some of which have been previously reported in other aquaponic systems (Eck et al 2019; Adhikari et al 2020). The presence of bacteria with autotrophic (*Leuconostoc*), heterotrophic (*Agrococcus*, *Edaphobaculum*, *Flavobacterium*), mixotrophic (*Rhodoluna*) and fermentative (*Zymomonas*, *Pedobacter*, Absconditabacteriales, *Acetobacter*, *Acinetobacter*, *Cetobacterium*) metabolisms suggests an active organic and inorganic nutrient cycling in the aquaponic system. Microorganisms involved in the N cycling, such as SWB02 and *Sphingomonas* as AOB; *Xanthobacter*, *Lactococcus*, *Leuconostoc*, *Niveispirillum* and *Zymomonas* as N-fixer; *Agrococcus* as an ammonifier (White et al 2018) besides of phosphorus solubilizers bacteria such as *Sediminibacterium*, *Emticicia*, Hgcl_clade and *Pedobacter* (Kim et al 2016) and bacteria involved in the degradation of complex carbon sources (biopolymers and aromatic compounds) such as Saccharimonada (Starr et al 2018), Hgcl clade (Ghylin et al 2014), *Flavobacterium* (Kirshman 2002) and *Novosphingobium* (Takeuchi et al 2001) were recorded in this study and could be contributing to improving the water quality for plant and fishes growth.

However, there were also detected some bacteria that produce fish diseases, such as *Flavobacterium* and *Weissella* (Avendaño-Herrera 2014) or that are common in its gastrointestinal tract, such as *Lactococcus* (Zatán et al 2020) and *Cetobacterium* (Tsuchiya et al 2008). This genus could reduce nitrate during denitrification (Tsuchiya et al 2008), contributing to the N_2O loss in aquaponic systems. Besides, in the context of this study and according to our experiment results, the Alphaproteobacteria genera *Acetobacter, Acinetobacter, Flavobacterium*, *Novosphingobium*, *Reyranella*, *Solitalea*, and *Sphingomonas* could be contributing to N_2O production in the aquaponic system because they have been reported as denitrifiers or N_2O producers (Takeuchi et al 2001; Weon et al 2009; Cua & Stein 2014; Li et al 2018; Chen et al 2020), and some perform heterotrophic nitrification and denitrification simultaneously at low dissolved oxygen ($<$ 0.3 mg L^{-1}) as *Sphingomonas* spp. (Fitzgerald et al 2015).

High throughput 16S rRNA gene sequencing also reveals the prokaryotic community composition related to ammonia oxidizers. The archaeal (Thaumarchaeota) genera related to ammonia oxidizers were Ca. *Nitrosopelagicus*, Ca. *Nitrosopumilus*, and Ca. *Nitrosotalea*. The genus Ca. *Nitrosopumilus* was dominant in the fish tank only, accounting for 70% of the archaeal sequences and 10% of the ammonia oxidizer related sequences. Representatives of the Ca. *Nitrosopumilus* cluster have been detected in marine (Könneke et al 2005), estuarine (Li et al 2018), freshwater (Ye et al 2009; Liu et al 2013), groundwater (van der Wielen et al 2009), aquarium filters (Sauder et al 2011), wastewater treatment plants (Mussmann et al 2011), and sediments of mesotrophic lakes (French et al 2012). The Ca. *Nitrosotalea* has been detected in freshwaters (Li et al 2018) and lake sediments (Bollmann et al 2014), and the Ca. *Nitrosopelagicus* has been detected in the open ocean (Santoro et al 2015). Most AOA can be found in environments

with low ammonium concentrations (Bollmann et al 2014). However, as observed in previous studies, the freshwater Ca. *Nitrosopumilus* strains could be adapted to different trophic states in their environment. Our results follow the study of Schmautz et al (2021a), who also report a low taxonomic richness of OTUs of archaeal in biofilms from different compartments of an aquaponic system analyzed with terminal restriction fragment length polymorphism, as well as the study of Bartelme et al (2017), who have reported only the archaea *Nitrososphaera* in the biofilter.

For 16S-based bacterial community composition, the genera *Nitrosomonas* and *Nitrospira* were related to ammonia oxidizers. *Nitrosomonas* was found in very low abundance at the biofilter, similar to that reported by Hovanec & Delong (1996). *Nitrospira* was the most dominant AOB, accounting for 6.1% of the whole bacterial sequences and 95% of the ammonia oxidizers related sequences. The genus *Nitrospira* was also detected in biofilter samples by Bartelme et al (2017), Hovanec et al (1998), and in fish tank sediments by Heise et al (2021). *Nitrospira* is known as a NOB (Daims et al 2015; Rurangwa & Verdegem 2015). Daims et al (2015) showed that certain strains of the *Nitrospira* genus could be complete nitrifiers, i.e., able to oxidize ammonia to nitrate without the help of AOB, a process now known under the name Commamox. Our results demonstrated that the *Nitrospira* sub lineage II is present and is dominant with respect to AOB in all compartments, similar to the study conducted on the Wädenswil Aquaponics system by Schmautz et al (2017, 2021a) and at different aquaponics systems by Eck et al (2019) that indicate that the Commamox process is common in diverse aquatic culture systems, as well as in soils with pH 6.5-7.8 (Daims et al 2015; Zhao et al 2020) which would explain its dominance in our aquaponic system with a pH of 6.6±0.7 during the growing cycle.

In general, our results from microcosm experiments showed that nitrification contributes to the generation of N_2O, and the sequencing and qPCR data indicated that Bacteria and Archaea are contributing to it. However, a lower number of copies of amoA genes (Commamox, AOB, and AOA) (up to 2×10^5) were detected in this aquaponic system in comparison with the study of Fang et al (2017), who quantified up to 5×10^{17} copies in the fish tank with semi aeration associated with emissions of N_2O (4.19±0.79 mg N d^{-1}). However, our qPCR values are similar to those reported in lake sediments, where it has been detected between 1×10^5 up to 1×10^9 copies/g for AOA and AOB (Bollmann et al 2014).

The results of this study add the understanding of the N_2O production and associated microbial communities in separate compartments of aquaponic systems during seven months of culture. This can help designers and operators optimize existing designs and configurations. However, further research is needed to find the exact pathways of nitrogen transformation and N_2O production in the system using nitrogen isotopic analysis in combination with new inhibitors of specific functional groups.

Conclusions. The emission of N_2O from this polyculture system of white cachama, Nile tilapia, and watercress was low compared to previous reports of other aquaponic systems. Its net production was associated with nitrifiers and denitrifiers, including ammonia-oxidizing Bacteria and Archaea, Comammox bacteria, Alphaproteobacteria, Bacteroidota, Actinobacteria, and some genera reported as denitrifiers with the capacity to produce N_2O.

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Conflict of interest. The authors declare that there is no conflict of interest.

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