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Effect of Fertigation with Struvite and Ammonium Nitrate on Substrate Microbiota and N_2O Emissions in a Tomato Crop on Soilless Culture System

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Abstract: Struvite and ammonium nitrate (AN), as wastewater-recovered products, are possible alternatives as raw materials for nutrient solutions. However, their impact on the rhizosphere microbiota and N_2O emissions is scarcely known. Therefore, the present research studies the ecological changes in the bulk-substrate microbiome and its correlation with N_2O emissions in a perlite-based system tomato crop under (i) conventional synthetic fertigation management; (ii) fertigation with struvite; and (iii) struvite and AN. A high bacterial diversity and the natural presence of plant-growth-promoting rhizobacteria in a soilless system are highlighted. However, the different $N\text{-}NH_4^+$:$N\text{-}NO_3^-$ ratios influence the ecological niches of ammonia-oxidizing archaea (AOA) and bacteria (AOB), with a stronger response by AOB community, while AOA kept constant regarding the fertilization applied. Despite this, enrichment of N-transforming bacterial phylotypes was relatively enhanced (mainly Nitrosomonas, Nitrosospira, and Nitrospira) concomitant with the production of N_2O emissions when ammonium fertilization was overapplied. In the absence of a plant, N_2O emissions were positively correlated, respectively, with Nitrosospira and AOB:AOA ratio, suggesting potential indicators for ammonium availability in the substrate. Fertilizer blends using recovered nutrients are a feasible alternative for increasing circularity in horticulture. Nevertheless, optimum fertilizer management is needed due to its influence on rhizosphere microbiota and N_2O emissions.

Keywords: circular horticulture; nitrification; denitrification; rhizosphere; microbiome; ammonia; nitrate; struvite

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1. Introduction

Horticulture hydroponic growing systems have gained worldwide popularity during the last few decades and provide horticultural products in great amounts, like what happens in Almeria; in this Spanish province, in 2016, the surface devoted to greenhouses was 28.500 ha, 10% being areas devoted to soilless culture [1,2]. These systems usually use water and soluble fertilizer to feed plants. Inorganic fertilizers in soilless systems have been an important input for increasing agricultural production during recent decades. Although their use is constantly growing [3], the concerns regarding their negative environmental effects (greenhouse gas emissions (GHG), soil and water pollution, phosphate rock reserves, and energy consumption) do too [4]. In addition, due to the current crisis (e.g., low raw material availability and increase in energy costs) that Western society is facing, the production of fertilizers is being limited.

To minimize the environmental impacts and move towards a more circular horticulture model in soilless systems, the use of innovative, and more sustainable fertilizing

products recycled from bio-waste, should be promoted after being studied in depth. In this regard, recovered struvite ($MgNH_4PO_4 \cdot 6H_2O$) and ammonium nitrate (AN) from urban wastewaters seem to be a feasible alternative to be used in agriculture as alternative phosphorous (P) and nitrogen (N) sources due to their concentrated source of nutrients delivered to the plants in a highly socially acceptable form [5–9].

The rhizosphere microbial community plays pivotal roles in maintaining key plant functions, such as nutrient cycling [10]. Although the perlite growing medium usually shows an absence [11] or lower microbial load and diversity than in soil crops due to the substrate fabrication process or sterilization, it constitutes a potential support for bacterial biofilms. The microorganisms present in soilless crops are primarily those that come from nursery plants, fertigation water (groundwater well), and additional allochthonous microbial biomass added for plant growth promotion and disease suppression. Fertigation can greatly impact microbial activity and community structure [12]. The initial abiotic condition of perlite, since it is obtained at high temperatures [13], is a good scenario to test the initial effects of different types of fertilization on the microbial colonization of crops' rhizosphere.

Some major processes driven by microorganisms in the rhizosphere of both soil or substrate media or composting are included within the N-cycle [14]. Most plants take up inorganic N available in the substrate essentially in the form of nitrate (NO_3^-) but also of ammonium (NH_4^+) [15]. Although NH_4^+ assimilation is less energy-demanding than NO_3^-, it has been described that high NH_4^+ concentration can cause severe toxicity symptoms [16]. Therefore, depending on the plant species and environmental conditions, the combination of NH_4^+ and NO_3^- may affect plant growth and yield [17]. Therefore, the N-transforming microorganisms interact with plants through the nitrification process, converting the NH_4^+ or ammonia (NH_3) to nitrite (NO_2^-) by ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA), and further transforming it into NO_3^- by nitrite-oxidizing bacteria (NOB) [18]. Moreover, in the case of reducing conditions and electron source availability, a denitrification process can occur, and then NO_3^- can be reduced and ultimately produce N_2 from N_2O, this being the last step mediated by the nitrous oxide reductase genes (*nosZ*). The microbial processes of nitrification and (incomplete) denitrification are the major sources of N_2O emissions, known as a strong CO_2 equivalent GHG [19]. However, several authors [20,21] reported that an adequate fertilizer application also governs N_2O fluxes in hydroponics.

Nevertheless, the understanding of the microbial community of soilless culture systems, its interaction with the environmental factors, and its functions related to the N-cycle, among others, are scarcely known and might help to work out approaches to progress toward a more sustainable horticulture.

The general objective of the present research was to study and promote the circularity and sustainability of horticultural production by using solubilized recovered struvite and AN through fertigation on a soilless tomato crop, gaining deeper insight into microbiological and environmental aspects. Previous studies have focused on agronomic performance [8,9]. Thus, the present research aimed at studying the effect of using recovered nutrient sources as a fertigation solution on the ecological changes in the rhizosphere microbiome in a soilless tomato crop (perlite-based), focusing mainly on the N-cycle-related phylotypes and functional genes, as well as its relation with N_2O emissions, under the influence of fertigation, different $N\text{-}NH_4^+$:$N\text{-}NO_3^-$ composition ratios of the NS, and the effect of plant presence/absence.

2. Materials and Methods

2.1. Greenhouse Soilless Trial: Experimental and Fertilization Conditions

The present study was conducted in a 200 m^2 greenhouse, located at the IRTA research facilities in Cabrils, Barcelona, Spain. Tomato seedlings (*Solanum lycopersicum* L. Cv "Egara") were produced in an organic substrate under nursery standard conditions

and then transplanted into 30 L perlite bags (brand PERLINDUSTRIA®), each providing substrate for three plants (3.33 plants·m^{-2}). Plants were grown from April to August 2020.

The culture was conducted in an open hydroponic system. Nutrients were provided through fertigation, mixing concentrated nutrient solution (cNS) with irrigation water in a proportion 1:100 through a 2 L·h^{-1} nominal flow dripper per plant [9]. The triggering of irrigation was based on crop evapotranspiration (ETc) estimation and the leaching fraction; using these criteria, a mean of 7–8 irrigations per daytime was applied.

To study the agronomic and microbiological effects of the fertigation and the different N-NO_3^-:N-NH_4^+ composition ratios of the NS manufactured with recovered products as raw materials, three fertilization treatments were applied; these consisted of supplying three different NS, differing in the P and N sources and the mineral N fraction applied as N-NH_4^+, with the rest applied as N-NO_3^-: (i) struvite treatment (STR), with 100% and 17 ± 4% of P and N-recovered source, respectively, and 25 ± 8% N-NH_4^+:N-total; (ii) struvite and ammonium nitrate treatment (SAN), with 100% and 39 ± 11% of P and N-recovered source, respectively, and 34 ± 5% N-NH_4^+:N-total; and (iii) control treatment (CON), with 5.8 ± 6.5% N-NH_4^+:N-total, using solely synthetic mineral fertilizers. The recovered nutrients were the P and N-NH_4^+ from ground struvite and the N-NH_4^+ from liquid AN. The struvite and AN used in this study were recovered from urban wastewater (provided by Murcia Este WWTP and Universitat Politècnica de Catalunya (UPC), respectively). The characterization of the recovered products (struvite and AN) was carried out in terms of macro/micronutrients, organic carbon, and heavy metals, meeting the EU-wide quality standards of the new European fertilizer regulation (Table S1). The conventional P fertilizer used in the CON nutrient solution was monopotassium phosphate (KH_2PO_4). Other commercial fertilizers were used to complete the NS: potassium nitrate, potassium sulfate, calcium nitrate, magnesium nitrate, micronutrients, and nitric acid. The different compound concentrations that made up the cNS for each treatment are shown in Carreras-Sempere et al., 2021. The chemical composition of the NS applied differs in the N-concentration along the crop, with a dynamic 70-112-70 mg N·L^{-1} depending on the crop development stage (initial–development–final). NS composition is detailed in Table S2.

Each treatment (CON, STR, and SAN) was replicated three times, with 5 perlite bags/15 plants per replication. Moreover, each treatment was also applied to other perlite bags without plants (one bag per treatment), following the same irrigation schedule (WP group).

2.2. Agronomic and Environmental Parameters (Leachates and GHG Emissions)

Fruit yield (total and marketable), fruit quality (g·$fruit^{-1}$, caliber, and total soluble solids (TSS)), nutrient content of fruits and leaves (N, P, Mg, K, and Ca), and N and P uptake were determined at the end of the crop. Moreover, the regulated heavy metals concentration (cadmium, lead, and mercury) was analyzed for fruits and leaves. ICP-OES and Kjeldahl methods were used.

The volume and composition of the NS supplied and the drainage from one replicate per treatment collected separately were determined and analyzed weekly in the laboratory for chemical parameters (pH, EC, P, NO_3^-, NO_2^-, and NH_4^+).

Furthermore, greenhouse gas (N_2O, CO_2, and CH_4) emissions were measured using a non-steady-state static gas chamber with 331 cm^3 of headspace volume (6.5 cm diameter × 25 cm height with 15 cm buried). The chambers were made of a polyvinylchloride (PVC) structure and rubber septa. Three sampling times during the 111-day crop cycle, with three replicas each (t1 (26–28 May 2020—development stage), t2 (7–9 July 2020—fruit formation), and t3 (11–13 August 2020—mature fruiting)), were determined during the crop growing for both of the factors (fertilization treatment and plant presence/absence). WP group was not determined in t2. Six gas samples (12.5 cm^3) per chamber were taken every time (t0, 10 min, 30 min, 60 min, 120 min, and 180 min), being consistent in the hour (11 a.m.) to minimize variability derived from the daily emission variation [22]. Each gas sample was transferred over-pressured to pre-evacuated 12.5 mL vials (Labco Ltd.,

Buckinghamshire, UK). CH_4, CO_2, and N_2O were analyzed simultaneously by a CG 7820A Agilent (Santa Clara, CA, USA) system equipped with a single channel and 2 valves of ten-port gas sampling with back-flush to vent and 6-port to change between the FID and micro-ECD detectors using 2 packed columns Hayesep-Q 80–100 mesh 2 m × 1/8 × 2.0 mm Ultimetal Agilent. Rates of the GHG emissions were calculated from the linear regression slope between the increase in gas concentration and the three-hour period of sampling.

Other details on the experiment setup, fertigation treatments, and agronomic and environmental parameters were the same as elsewhere described in a previous study [9].

2.3. Bulk-Substrate (Perlite): Sampling and Microbial Quantification and Metabarcoding Assessment

DNA extraction: Regarding the microbiological assessment, for each fertigation treatment (CON, STR, and SAN), perlite samples were taken in triplicate from the bulk zone (2 cm distant from the stem and 0–15 cm depth) throughout four different times during the tomato crop: t0 (15 April 2020—initial point, common for all treatments) and t1, t2, and t3, same date and samples as for gas sampling (see Section 2.2).

Total DNA from 48 bulk samples was extracted from the substrate using DNeasy® PowerSoil® (Qiagen), following the manufacturer's instructions.

Quantification of microbial populations by qPCR: To elucidate the microbial community changes, especially related to the N cycle, a DNA-based assessment was carried out by quantitative polymerase chain reaction (qPCR) (Mx3000P, Stratagene, Bellingham, WA, USA) in order to quantify total bacterial population (16S rRNA), ammonia-oxidizing prokaryotes (AOP) by *amoA* of AOB and AOA [23–25], and complete bacterial denitrifiers (*nosZ*-clade I) [26].

The 16S-metabarcoding assessment: Moreover, the diversity structure of bacterial and archaeal populations, as well as taxonomy assignment, were assessed by Next Generation Sequencing by means of V3-V4 hypervariable region of 16S rRNA genes. The pair of primers used for bacteria were V3_341F (5′-CCTACGGGNGGCWGCAG-3′)/V4_R805 (5′-GACTACHVGGGTATCTAATCC-3′) and specifically for archaea 349F (5′-GYGCASCAGK CGMGAAW-3′)/806R (5′-GGACTACVSGGGTATCTAAT-3′) [27]. The PCR products were sequenced on a 2 × 300 bp (v3) paired-end format using the Illumina Miseq platform at Molecular Research DNA (San Diego, CA, USA), following the standard instructions of the 16S Metagenomic Sequencing Library Preparation protocol. The raw sequence data (demultiplexed fastq files R1 and R2) were deposited in the sequence read archive of NCBI under the BioProject accession number PRJNA914541.

Diversity assessment: Bioinformatic tools were used to determine the microbial diversity. Raw data (fastq files) from 16S rRNA-metabarcoding assessment of bacteria and archaea were further processed using Cutadapt 1.9 [28] and R package DADA2 [29]. Primers were removed from the demultiplexed forward (R1) and reverse (R2) reads and the resulting paired reads were filtered and trimmed, denoised, and merged. R1 and R2 reads were truncated to 270 and 250 for 16S. In all samples, reads with ambiguities or an expected error (maxEE) higher than 2 were discarded. The DADA2 denoising algorithm was applied to determine an error rates model to infer true sequence variants (ASVs). The full denoised ASVs were obtained after merging the denoised R1 and R2 sequences. Finally, chimeras were detected and removed as described elsewhere in the DADA2 1.16 tutorial (https://benjjneb.github.io/dada2/tutorial.html; accessed on 20 December 2023). The taxonomic affiliations of the ASVs for total bacteria and archaea were assigned by using the naïve Bayesian classifier method [30] using the RDP database training set 18 and compiled into each taxonomic level [31]. For the taxonomical assignment, a bootstrap cut-off of 80% was set.

To assess alpha diversity, Phyloseq, Microbiome, and ggpubr R packages were utilized [32–34]. Four different alpha diversity indexes were determined: (i) the number of species or richness (Chao 1 index), (ii) the relative abundance of each of these species or evenness (Pielou's index), (iii) the pool of species or diversity (Shannon index), and

(iv) the relative dominance of the most abundant species (dominance) from the final ASVs distribution matrix.

To examine microbial community dissimilarities throughout different treatments and experimental conditions, beta diversity assessment was performed through permutational multivariate analyses of variance (PERMANOVA) of ASV distributions based on Bray–Curtis distances with 999 permutations. Principal coordinate analysis (PCoA) based on Bray–Curtis dissimilarity and Canonical Analysis of Principal Coordinates Plot (CAP) [35] were used to identify the separation pattern in microbial communities to visualize the differences among samples and the influence of environmental parameters. Comparisons between community groups were conducted in Vegan R [36] and pairwiseAdonis [37] packages.

Spearman's correlation coefficient was used to test the correlations among environmental parameters and N-cycle-related phylotypes and functional genes ($p < 0.05$) using corrplot R packages [38].

All the analyses were performed on rarefied data (using Phyloseq R package) by the minimum number of reads both in bacteria and archaea kingdoms.

2.4. Statistical Analysis

The analyzed data were tested for normality and homogeneity of variance using the Shapiro–Wilk test and Levene's test ($p > 0.05$), respectively. Once these parameters were validated, a parametric statistical analysis was carried out (ANOVA and post hoc Tukey's test with a significance level of 5%). Alternatively, non-parametric data were analyzed for significance using Kruskal–Wallis (significance level of 5%) and post hoc Wilcoxon test (R-studio Workbench software, R-4.2.1).

3. Results

3.1. Fertilization and Agronomic Parameters

The fertilization applied and agronomic performance results details are described in a previous study [9].

Briefly, regarding the NS composition of the three treatments, nutrients from recovered products (P, N, and Mg^{2+}), in STR and SAN, displayed a similar supply to the plants as the CON (Table S2). However, due to the struvite composition and the different commercial fertilizers used to complete the NS, CON showed lower values than STR and SAN in Mg^{2+} and $SO_4{}^{2-}$ concentration in the NS development. In addition, pH (6.4 ± 0.2, 6.8 ± 0.1, and 6.9 ± 0.1), N-$NO_3{}^-$ (106 ± 16, 86 ± 8, and 76 ± 8 mg·L^{-1}), and N-$NH_4{}^+$ concentrations (6 ± 4, 23 ± 4, and 37 ± 8 mg·L^{-1}) differed statistically significantly between treatments (CON, STR, and SAN, respectively) for NS crop development (Table S2). Furthermore, the applied N concentration was different over time due to the different N needs for the plant growth stages and then manufacturing a dynamic NS composition, being 0.6-fold higher during the plant vegetative development and fruit formation (t1 and t2) than the initial (t0) and final stages (t3) of the crop.

The agronomic parameters results [9] (Table S3) showed that both the STR and SAN treatments were equally effective compared to synthetic fertilizers (CON) in all the parameters measured at the end of the crop ($p > 0.05$): production parameters such as total (23 ± 2 kg·m^{-2}) and marketable yield (20 ± 2 kg·m^{-2}), quality product parameters such as fruit weight (233 ± 22 g), caliber (81 ± 3 cm), and TSS (4.5 ± 0.3 °Brix), and nutritional parameters such as fruit (except for Mg, $p < 0.05$) and leaves nutritional content and N (51 ± 6 g·m^{-2}) and P (16 ± 5 g·m^{-2}) uptake. Moreover, the concentration of heavy metals regulated in fruits and leaves was below the permissible limits.

3.2. Environmental Parameters

The leachate results are shown in Table S4. The N-nitrate (50.9 ± 47 mg·L^{-1}) and N-ammonium (4.1 ± 5.1 mg·L^{-1}) concentrations in leachates were not different among treatments, except for ammonium in August ($p < 0.05$). However, it is important to highlight that SAN had the higher mean percentage of N-$NH_4{}^+$ from the total N leached, followed

by STR and CON, with 11, 8, and 5%, respectively. The concentration of nitrites measured was negligible (<2 mg·L^{-1}).

The GHG emissions results are shown in Figure 1 and Table S5. The N-N_2O gas emissions measured during the three sampling campaigns, in the plant group, showed no significant differences between treatments and sampling times (5.6 ± 3.3 mg N-N_2O·m^{-2}·h^{-1}). Even the differences were not statistically significant; higher values were measured in t1 (6.6 ± 4.1 mg N-N_2O·m^{-2}·h^{-1}) and t2 (5.7 ± 3.7 mg N-N_2O·m^{-2}·h^{-1}) compared to t3 (4.6 ± 2.5 mg N-N_2O·m^{-2}·h^{-1}) (p-value > 0.05). The N-concentration applied during t3 was 0.6-fold lower than t1 and t2 due to the different N needs for the plant growth stages.

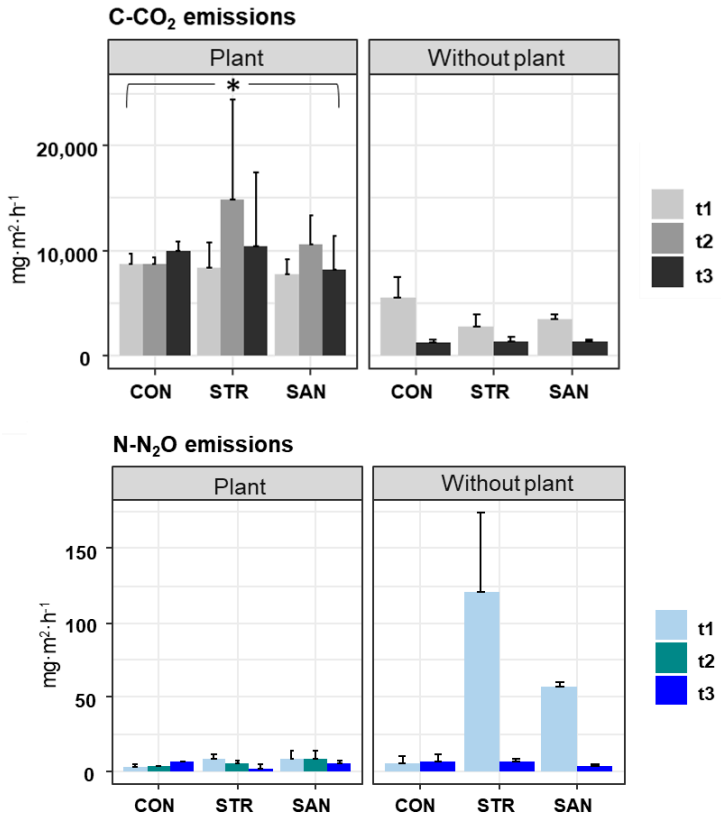

Figure 1. N_2O and CO_2 gas emissions (mean + SD) for the fertilization treatments and plant presence/absence groups during the crop cycle (sampling times: t1, t2, and t3). Gaseous samples in without plant (WP) group were not determined in t2. Significance p-value codes (* $p < 0.05$) indicate statistical differences according to Wilcox test. Within WP group, STR showed significantly higher N_2O emissions. The plant group released significantly higher CO_2 emissions than WP group.

Moreover, when plant and without plant (WP) data are compared together, in t1, the interaction between plant and treatment (p-value 0.018) influenced the N-N_2O emissions, while t3 showed no differences (5.7 ± 3.0 mg N-N_2O·m^{-2}·h^{-1}). Among t1 without plant, clearly STR and SAN showed higher values (121.2 ± 53.1 and 56.9 ± 3.3 mg N-N_2O·m^{2}·h^{-1}, respectively) than CON (5.6 ± 4.8 mg N-N_2O·m^{-2}·h^{-1}), STR even being significantly different (p-value < 0.01).

Furthermore, among the plant group, C-CO_2 showed no significant differences by time or treatment. Moreover, with plant presence, C-CO_2 emissions were significantly higher in both sampling times. Among the WP group, t1 showed higher C-CO_2 emissions than t3 (p-value < 0.001). CH_4 gas was not detected in any of the samples due to the presence of O_2 in the porous perlite [13], achieved by the irrigation management, and therefore there was an absence of redox reductive conditions, as described by [39].

3.3. Microbial Community Description

3.3.1. Sequence Data

Illumina MiSeq 16S rRNA for bacteria and archaea yielded 89,350 ± 20,779 and 93,963 ± 23,844 raw reads per sample, respectively. After discarding chimeras, singletons, chloroplasts, and kingdom selection and rarefying data by the minimum number of reads, overall rarefied clean reads and ASVs were 6633 and 5308 for bacteria and 2749 and 692 for archaea, respectively.

3.3.2. Microbiome Composition Comparison by Different Factors

Beta diversity shown in PCoA plots derived from Bray–Curtis dissimilarity distances (and compared by PERMANOVA) was performed to test the following factors (effects): (i) different N-NH_4^+:N-NO_3^- ratios of the nutrient solution (CON, STR, and SAN) on plant group (by fertilization); (ii) fertigation along the crop on plant group (by time); (iii) the effect of the plant (presence vs. absence) (on plant and without plant (WP) groups using data from t1 and t3) (by plant presence).

Rhizosphere microbial communities assessed by Bray–Curtis PERMANOVA revealed an important impact of the fertilization treatment, time, and plant presence in both bacterial and archaeal communities' structure (Table 1 and Figure 2).

Table 1. Summary of the principal coordinate analysis (PCoA) plot derived from Bray–Curtis distance showing variation in bacterial and archaeal communities' structure (PERMANOVA test with 999 permutations). Tests were performed (i) on plant group using data from t1 to t3 (by fertilization); (ii) on plant group using data from t0 to t3 (by time); (iii) on plant and without plant (WP) groups using data from t1 and t3 (by plant presence).

		BACTERIA					ARCHAEA				
Test Performed by	**Variable**	**Df**	**SumOfSqs**	**R2**	**F**	**Pr (>F)**	**Df**	**SumOfSqs**	**R2**	**F**	**Pr (>F)**
(i) Fertilization	Treatment	2	1.27	0.17	3.07	**0.001**	2	0.98	0.35	10.89	**0.001**
	Time	2	1.34	0.18	3.24	**0.001**	2	0.62	0.22	6.89	**0.001**
	Treatment * Time	4	1.01	0.14	1.22	0.08	4	0.37	0.13	2.07	0.008
	Residual	18	3.72	0.51			18	0.81	0.29		
	Total	26	7.34	1			26	2.79	1		
(ii) Time	Treatment	3	2.79	0.31	4.47	**0.001**	3	2.2	0.51	13.62	**0.001**
	Time	2	1.34	0.15	3.41	**0.001**	2	0.62	0.15	5.78	**0.001**
	Treatment * Time	4	1.01	0.11	1.28	0.06	4	0.37	0.09	1.74	0.025
	Residua	20	3.94	0.43			20	1.08	0.25		
	Total	29	9.07	1			29	4.27	1		
(iii) Plant Presence	Treatment	2	1.64	0.13	5.62	**0.001**	2	1.38	0.14	13.76	**0.001**
	Plant	1	2.84	0.23	19.53	**0.001**	1	1.68	0.16	33.42	**0.001**
	Time	1	1.15	0.09	7.94	**0.001**	1	2.22	0.22	44.24	**0.001**
	Treatment * Plant	2	1.18	0.09	4.05	**0.001**	2	0.64	0.06	6.36	**0.001**
	Treatment * Time	2	0.66	0.05	2.27	**0.004**	2	0.53	0.05	5.29	**0.001**
	Plant * Time	1	0.86	0.07	5.91	**0.001**	1	1.74	0.17	34.71	**0.001**
	Treatment * Plant * Time	2	0.62	0.05	2.14	**0.008**	2	0.79	0.08	7.88	**0.001**
	Residual	24	3.49	0.28			24	1.21	0.12		
	Total	35	12.43	1			35	10.19	1		

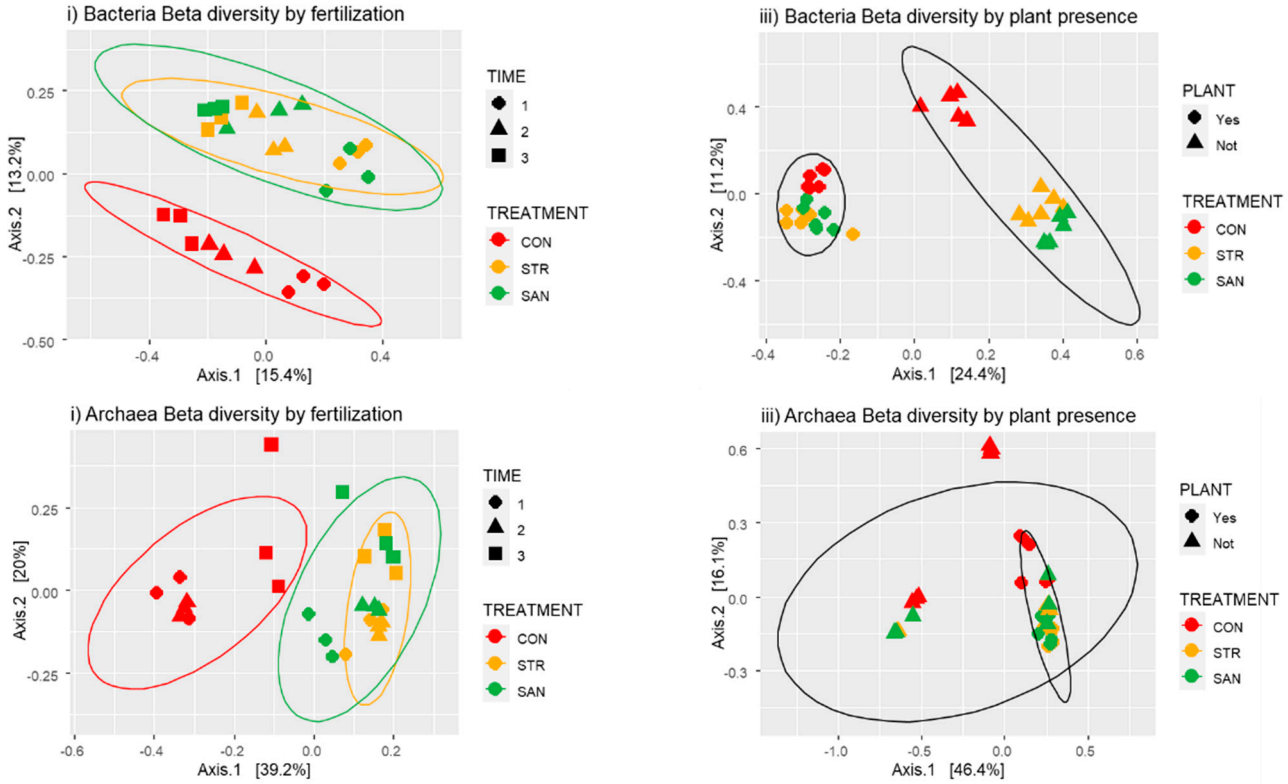

Figure 2. Beta-diversity principal coordinate analysis (PCoA) plots derived from Bray–Curtis distances of bacterial and archaeal communities' structure on (**i**) plant group using data from t1 to t3 (by fertilization); (**iii**) plant and without plant (WP) groups using data from t1 and t3 (by plant presence) (PERMANOVA test, see Table 1).

Treating data by (i) fertilization (*p*-value 0.001) showed significant differences in beta diversity between CON and both recovered-nutrient treatments (STR and SAN), while no differences were detected among them; (ii) time (*p*-value 0.001) t0 was separate from the other sampling times along the crop, showing a clear influence from the nursery substrate. Thus, t1, t2, and t3 data are treated as time, separated from t0. t1 showed significant differences from t2 and t3 in bacteria communities' dispersion, while archaea communities pointed out that t3 was significantly different from t1 and t2; (iii) regarding plant presence (*p*-value 0.001), a significant distinction was observed between samples with and without plant (WP).

3.3.3. Effect of Fertilization and Time on Plant Group Microbial Community

Microbial alpha diversity (Figure 3). While richness was kept constant during the assay in both bacteria (416 ± 98) and archaea (24 ± 7) kingdoms studied, the rest of the alpha diversity metrics (Figure 3 and Table S6) revealed differences among them. Bacteria showed no differences in any of the indices despite the fertilization strategy. On the other side, archaea showed differences among CON, with higher evenness and diversity and lower relative dominance index (0.56 ± 0.1, 1.8 ± 0.5, and 0.37 ± 0.1, respectively) than both recovered nutrients' treatments (STR: 0.38 ± 0.08, 1.1 ± 0.2, and 0.65 ± 0.1; SAN: 0.4 ± 0.1, 1.2 ± 0.3, and 0.61 ± 0.1, respectively).

In addition, along the crop (from t1 to t3), bacterial diversity (from 5.0 to 5.3) and evenness (from 0.85 to 0.88) showed a significant increase, while relative dominance (from 0.08 to 0.04) decreased over time. However, archaea acted oppositely to bacteria in these same metrics.

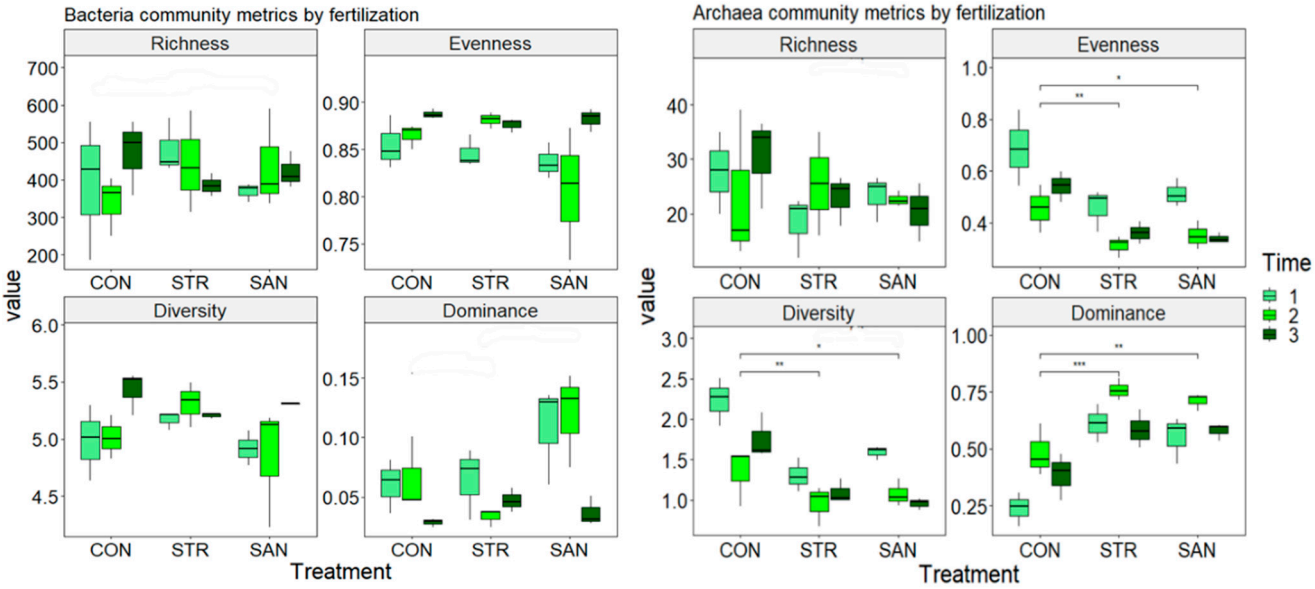

Figure 3. Bacteria and archaea community metrics: richness (Chao1), evenness (Pielou's), diversity (Shannon), and relative dominance index. Significance *p*-value codes (*** $p < 0.001$, ** $p < 0.01$; * $p < 0.05$) indicate statistical differences according to Wilcox test between the fertilization treatments (CON: conventional fertilization treatment; STR: struvite fertilization treatment; SAN: struvite + ammonium nitrate fertilization treatment).

Main taxa composition. The relative abundance (RA) of the taxonomic groups of both bacteria and archaea kingdom and the *p*-value for the different variables (time, fertilization treatment, and plant presence) are shown in Table S7.

The dominant bacterial phyla (Figure 4), for all the samples **with plants** (mean RA ± standard deviation (SD)), were *Proteobacteria* (54 ± 7%) (predominantly alpha), *Bacteriodetes* (12 ± 4%), *Actinobacteria* (5.3 ± 4%), *Planctomycetes* (2.9 ± 1%), *Nitrospirae* (2.7 ± 2%), *Acidobacteria* (2.5 ± 1%), *Verrucomicrobia* (2.4 ± 1%), *Candidatus_Saccharibacteria* (2.3 ± 3%), *Parcubacteria* (2.1 ± 1%), and *Chloroflexi* (2 ± 1%). The most abundant genuses in the bulk environment in most of the samples were *Sphingobium*, *Nitrospira*, *Cellvibrio*, *Hydrogenophaga*, *Flavobacterium*, *Shinella*, *Acidovorax*, *Devosia*, *Sphingopyxis*, *Streptomyces*, *Tahibacter*, *Rhizobium*, *Arthobacter. Sediminibacterium*, and *Methylophilus*, although being found at different concentrations depending on the treatment.

Throughout the crop growing (from t1 to t3), *Acidobacteria*, *Nitrospirae*, *Candidatus Saccharibacteria*, and *Latescibacteria* significantly increased their RA, while *Bacteroidetes* and *Spirochaetes* decreased it, following the same tendency in all the treatments.

Furthermore, the different fertilization treatments exhibited significant changes between them in *Nitrospirae* and *Deinococcus-Thermus* phyla (*p*-value < 0.01). Especially, Nitrospirae RA was higher in STR (3.2 ± 2.2%) and SAN (3.6 ± 2.0%) compared to CON (1.1 ± 0.7%). *Chlamydiae* and *Planctomycetes* also show significantly lower values in CON compared to STR (*p*-value 0.01 and $p < 0.01$, respectively).

Regarding the archaeal communities, the dominant phyla (RA ± SD) were *Thaumarchaeota* (85 ± 16%), *Euryarchaeota* (7.8 ± 11%), and *Woesearchaeota* (3.6 ± 7%), *Nitrosopumilaceae* (77.5 ± 24%) being the dominant family. Throughout the crop time, few differences were detected. While *Euryarchaeota* and *Pacearchaeota* significantly decreased (*p*-value < 0.001), *Thaumarchaeota* increased their RA (*p*-value 0.001). No differences were detected among treatments.

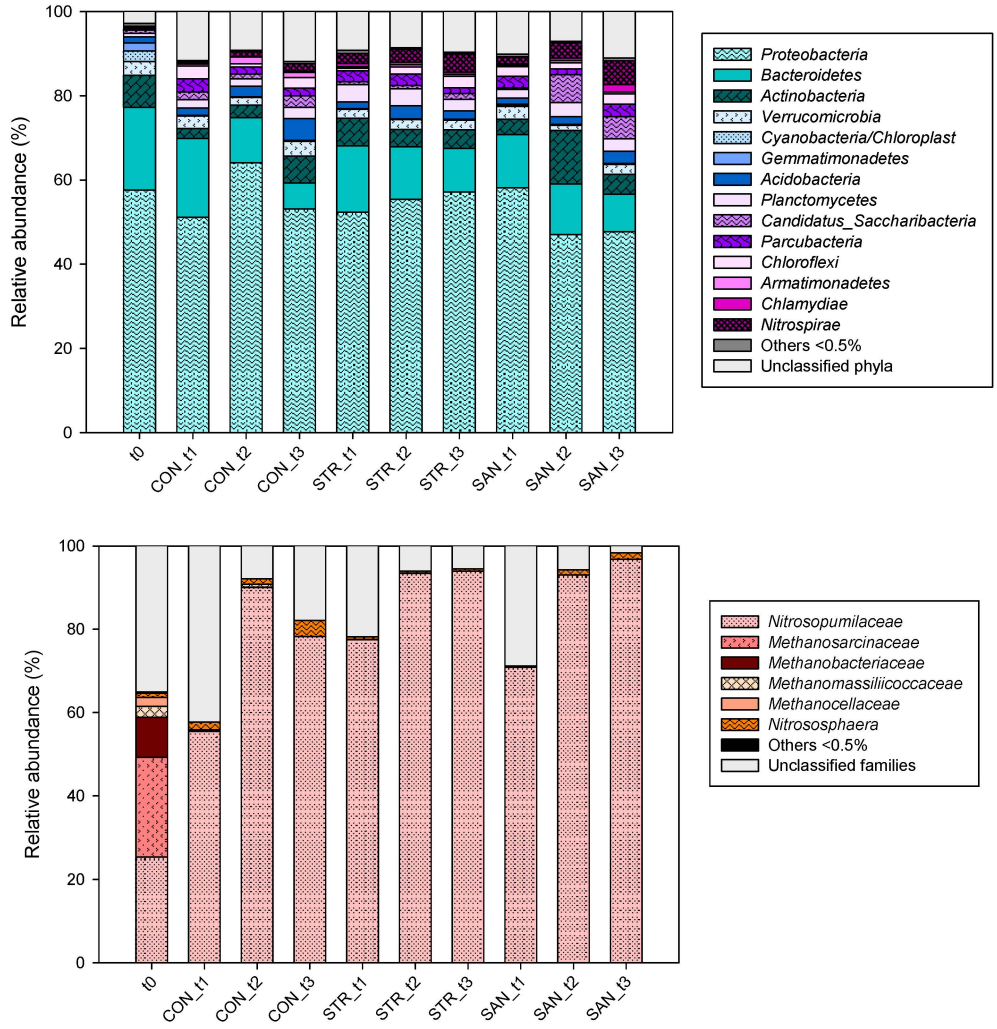

Figure 4. Bacterial phylum (**upper**) and archaeal family (**lower**) relative abundance (%) for plant group.

Functional diversity related to N cycle. Quantification of functional genes and 16S rRNA-metabarcoding data related to N cycle are shown in Tables S7 and S8. The total bacterial population (16S rRNA) showed no differences over time and treatments ($1.2 \times 10^9 \pm 7.5 \times 10^8$ copies·g^{-1}). Among the AOP:16SrRNA ratio, a significant increase from t1 to t3 was observed in all the treatments (p-value 0.001), while no differences were detected among them; even STR and SAN showed higher values in t3.

Furthermore, among AOP, the ratios of the bacteria (AOB) and archaea (AOA) populations varied widely by the time and fertilization applied (Figure 5). Within plant group, CON (0.0001 ± 0.0002) showed significantly lower AOB:AOA ratios than the treatments with higher ammonium concentration, STR (0.15 ± 0.22), and SAN (0.06 ± 0.05) (p-value < 0.001).

Concerning the AOA population, no significant differences were detected among treatments, while it displayed an increase over time for all the treatments from t1 to t3 (x 6.5 folds) (p-value < 0.001). On the other hand, AOB manifested changes depending on the fertilization strategy. While CON treatment kept constant over time (10^2 copies·g^{-1}), STR and SAN showed an increase (from 10^4 to 10^5–10^6 copies·g^{-1}) (p-value < 0.001). Moreover, denitrifiers (*nosZ*) showed a slightly significant increment over time despite the fertilization applied.

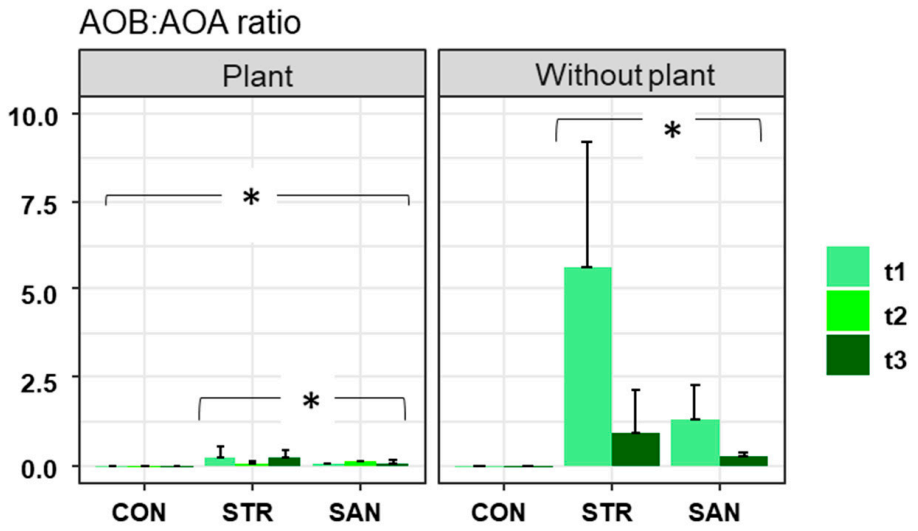

Figure 5. AOB:AOA ratios (mean + SD) for the fertilization treatments and plant presence/absence groups during the crop cycle (t1, t2, and t3). Without plant (WP) group was not determined in t2. Significance *p*-value codes (* $p < 0.05$) indicate statistical differences according to Wilcox test. Plant group showed an AOB:AOA significantly lower (*p*-value 0.008) than WP group. Within each group, plant presence (*p*-value 0.0003) and without plant (*p*-value 0.002) group, STR, and SAN treatments showed significantly higher AOB:AOA ratios compared to CON.

From the total microbial community detected by 16S rRNA-metabarcoding, regarding bacteria species related to the N cycle, three of them were assigned as nitrifying bacterial communities, *Nitrosomonas* and *Nitrosospira* as AOB, and *Nitrospira* as nitrite-oxidizing bacteria (NOB). Moreover, two ammonium-oxidizing archaea families, *Nitrosopumilaceae* and *Nitrososphaera*, were also detected.

Nitrosomonas was only depicted in STR and SAN treatments, while *Nitrosospira* was only identified with low RA in STR-t1 and STR-t2 (0.02 and 0.03%, respectively). These results illustrate an absence of AOB in CON treatment, where AOA were more predominant. Likewise, *Nitrospira* showed significantly higher (approximately 2× fold) RA in STR and SAN compared to CON. Thus, significant differences in RA of *Nitrosomonas* (*p*-value < 0.001) and *Nitrospira* (*p*-value < 0.01) were detected between CON and both fertilization treatments with higher ammonium concentrations. Even though no significant differences were detected, SAN had higher RA mean values for both genera within total bacteria (0.6 ± 0.6% and 3.6 ± 2%) compared to STR (0.5 ± 1.1% and 3.2 ± 2.2%).

Furthermore, RA within total archaea of *Nitrosopumilaceae* (83 ± 16%) and *Nitrososphaera* (1.3 ± 1.7%) showed no significant differences among treatments. However, *Nitrosopumilaceae* revealed a significant increase throughout time (*p*-value < 0.01).

3.3.4. Effect of Plant Presence on Microbial Community

Microbial alpha diversity. Microbial alpha diversity metrics (Figure 6 and Table S6) showed significant differences by plant presence.

On one side, bacteria metrics revealed higher significant diversity (5.2 ± 0.2) and evenness (0.86 ± 0.02) and lower relative dominance (0.06 ± 0.03) compared to the without plant (WP) group (4.4 ± 0.4, 0.78 ± 0.06 and 0.14 ± 0.08, respectively). On the other side, archaea showed lower diversity (1.5 ± 0.5), evenness (0.5 ± 0.1), and richness (24 ± 6.6) values when the plant was present than when it was absent (3.2 ± 1.6, 0.7 ± 0.2, and 107 ± 74, respectively).

Main taxa composition affected by the plant presence. The phyla and genera relative abundance of both the bacteria and archaea kingdom are shown in Table S7. The dominant bacterial phyla (>1% RA) for samples without plants were similar to those with plants as mean values for the three treatments, except for the inclusion of *Ignavibacteriae* and

Chlamydiae and the exclusion of *Candidatus Saccharibacteria*. In addition, *Nitrospirae* decreases its RA with plants, while *Actinobacteria*, *Planctomycetes*, *Proteobacteria*, and *Verrucomicrobia* increase it.

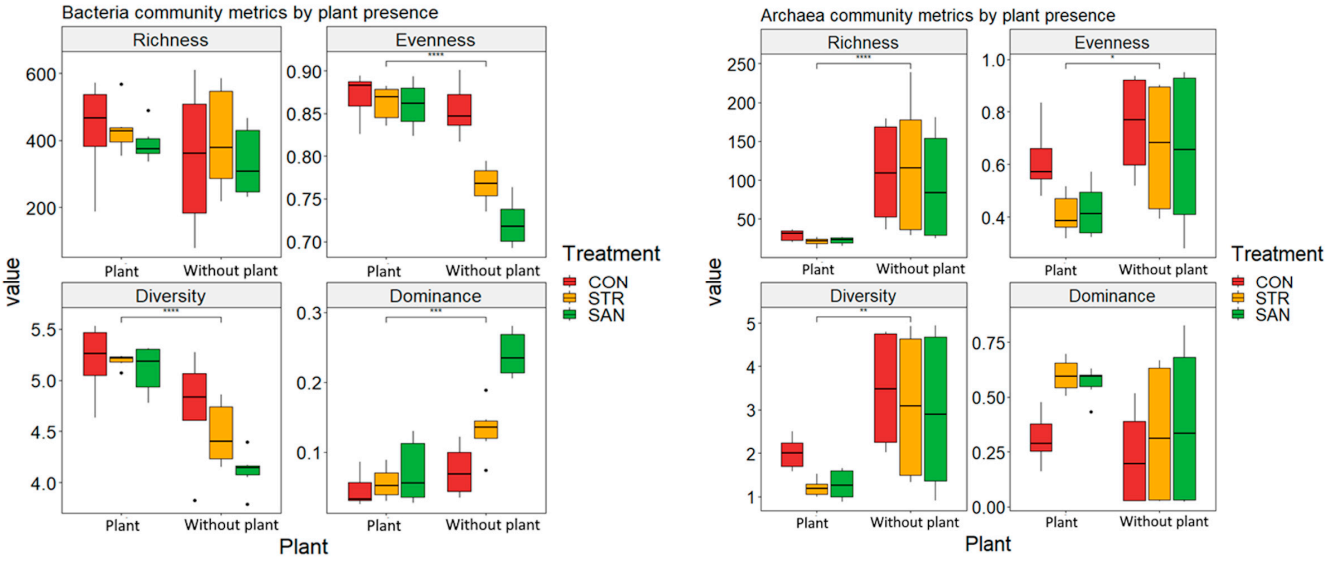

Figure 6. Bacteria and archaea community metrics: richness (Chao1), evenness (Pielou's), diversity (Shannon), and relative dominance. Significance *p*-value codes (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$) indicate statistical differences according to Wilcox test between plant and without plant (WP) samples. CON: conventional fertilization treatment; STR: struvite fertilization treatment; SAN: struvite + ammonium nitrate fertilization treatment.

The differential relative abundance analysis (Table S7) showed that the most abundant bacterial taxa (RA > 1%) enriched with plants compared to the WP group were *Sphingobium*, *Cellvibrio*, *Flavobacterium*, *Hydrogenophaga*, *Acidovorax*, *Sphingopyxis*, *Rhizobium*, and *Methylophilus*. Other genera with lower RA, such as *Pseudomonas* and *Streptomyces* (in t3), were also enriched in the rhizosphere when plants were present. Some genera (*Rhizobium*, *Flavobacterium*, *Pseudomonas*, *Streptomyces*, etc.) are highlighted due to their plant-growth-promoting rhizobacteria (PGPR) functions, such as nitrogen fixers, P solubilizers, and plant growth promoters, as well as plant-associated methanol-consuming bacteria (methylotrophs such as *Methylophilus* and *Methyloversatilis*), which are able to regulate methanol levels in the rhizosphere contributing to the carbon cycle [40]. Moreover, methanotrophic bacteria are stimulated by rhizobia genus, such as *Rhizobium* and *Mesorhizobium* present in the study, by a cobalamin-dependent stimulation.

On the other hand, *Nitrospira*, *Nitrosomonas*, *Nitrosospira*, *Bdellovibrio*, and *Ignavibacterium* were enriched in an environment WP and only nutrient solution application.

Regarding the archaea communities (Table S7), the dominant phyla for samples WP were similar to those with plants (*Thaumarchaeota and Euryarchaeota*), finding a significant increment of *Pacearchaeaota*, especially in t1, and a decrease in *Methanomassiliicoccus* genus in the plant group.

Functional diversity related to N cycle. qPCR and 16S rRNA-metabarcoding data are shown in Figure 5, Tables S7 and S8. The plant presence promoted a higher total bacterial population (16S rRNA *p*-value < 0.0001), while the AOP:16S rRNA ratio was significantly lower (*p*-value 0.0006). Moreover, in the treatments with plants, a lower AOB:AOA ratio was detected (*p*-value 0.008) due to the lower AOB population (*p*-value 0.053) (Figure 5). As stated above, also without plant, STR and SAN treatments showed higher AOB presence than CON. In addition, the *nosZ* community showed a slightly significant increment with plant presence (*p*-value < 0.0001).

Regarding the 16S rRNA-metabarcoding, the same nitrifying genera/family were found in the WP group. Nitrifying bacteria RA was influenced by the fertilizer and plant presence. The WP group showed significantly higher values than plant treatments on the three nitrifying communities, except for *Nitrosospira*-CON, which is absent in all cases. The WP group also showed a predominance of *Nitrosomonas* among AOB. Furthermore, *Nitrospira* RA was higher in SAN than other treatments, being higher at the highest NS NH_4^+ concentration (CON < STR < SAN). Regarding the nitrifying archaea families, no differences were detected by plant presence.

3.3.5. Factors Affecting the Structure of Microbial Communities and N_2O Correlations

Canonical analysis of principal components (CAP) plot (Figure 7, Table S9) separated plant presence from without plant group (archaea plot joined plant group with STR-WP-t3 and SAN-WP-t3) along the first axis and CON from STR and SAN along the second axis in both kingdoms. Both bacteria and archaea community distributions were significantly correlated to 16SrRNA, AOA, AOB:AOA ratio, *nosZ*, Nitrogen, and N-NO_3^- concentrations supplied with the NS and N_2O and CO_2 emissions. Moreover, bacteria communities were also influenced by AOB population. CAP plot confirmed that microbial communities linked to plant presence are characterized by the increase in total bacterial, *nosZ* genes, and C-CO_2 emissions, while microbial communities grown without plants tend to increase AOB population, AOB:AOA ratio, and N-N_2O emissions.

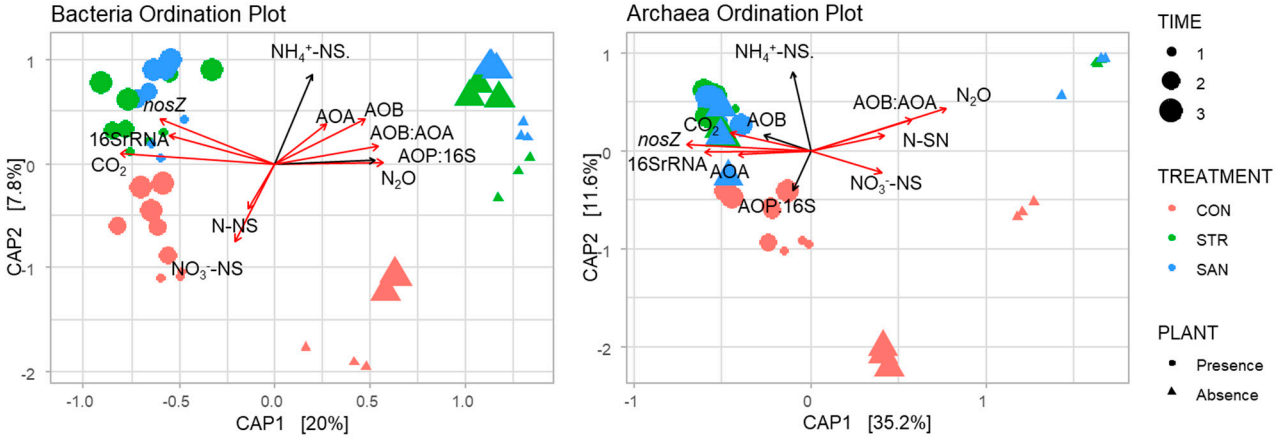

Figure 7. Canonical analysis of principal components (CAP) plot ordination (based upon Bray–Curtis distance), visualizing the differences in bacteria and archaea communities and the effect of the parameters represented: qPCR data (16SrRNA, AOA*amoA*, AOB*amoA*, nosZ, AOP:16SrRNA, AOB:AOA), emissions (N-N_2O and C-CO_2), and N concentration and forms applied through the NS (N, N-NO_3^-, and N-NH_4^+ concentrations). Arrows in red mean significant parameters, while arrows in black are not significant.

In addition, Sperman's correlation (Figure 8) showed (i) with plant presence: positive correlations between N_2O emissions and N-NH_4^+ concentration applied with the NS; (ii) with plant absence (WP): N_2O emissions correlate positively with AOB:AOA ratio and *Nitrosospira* RA, while a negative correlation was detected with AOA:16SrRNA.

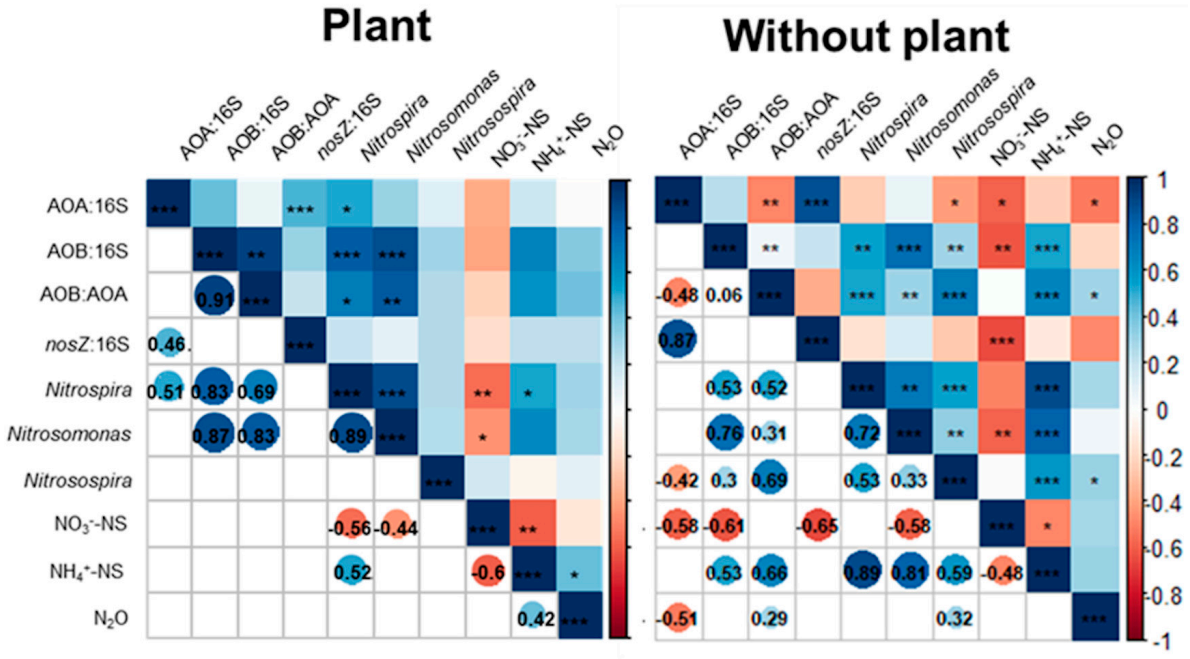

Figure 8. Heat map of correlation based on Spearman's correlation method among different parameters (AOA:16SrRNA, AOB:16SrRNA, AOB:AOA, and *nosZ*:16SrRNA ratios, *Nitrospira*, *Nitrosomonas*, and *Nitrosospira* relative abundances, NO_3^- and NH_4^+ applied with the NS and the N_2O emissions ratio). The correlation coefficient is shown as a number and statistical level at *p*-values < 0.05 *, $p < 0.01$ **, $p < 0.001$ ***.

4. Discussion

Our research aim was to promote circularity and sustainability of horticultural crops in soilless growing media (perlite) by using recovered products as fertilizers through fertigation on a tomato crop. Thus, the main objective of our study was to maintain the agronomic and environmental effectiveness of a tomato crop by using recovered struvite and ammonium nitrate instead of synthetic fertilizers and to gain deeper insight into the influence of the fertilization on N_2O emissions and rhizosphere microbiota in a soilless crop system, especially the N-cycle-related ones, and thus regarding the fate of the different mineral N forms.

4.1. Influence of Recycled Fertilizers on Agronomic and Environmental Parameters

Our previous results showed that both struvite and ammonium nitrate (AN) are suitable raw materials for nutrient solution manufacture as they can deliver plant-available nutrients properly and be equally effective and safe in agronomic and human and soil health parameters as synthetic fertilizers [9]. Satisfactory agronomic results on fertigated horticultural crops were also obtained with the same treatments as this manuscript on a soil horticultural crop rotation [8] with a combination of struvite and K-vinasse in a nutrient film technique system with tomato crop [41] and with struvite use as a slow-releasing P source in hydroponics [42]. However, it is important to consider the ammonium tolerance of the plant species/variety [9].

Regarding the environmental parameters (for both N-leached and GHG emissions), plant group samples showed no differences between recovered-nutrient and control treatments. Regarding the N-leached emissions, the lack of differences despite the different mineral N forms used in the NS suggests that a nitrification process of the ammonium and/or uptake by the plant occurs to the same extent among treatments on the soilless cropping system, as [43] reported. Regarding the GHG emissions, similar or lower-range values to the ones obtained in the present study from hydroponic growing media on tomato crops were already reported [20,41,44,45]. As the obtained emissions data are precise short

interval measurements over the crop and fluctuations were observed within replicates, only comparison among treatments can be conducted instead of reporting emissions values.

The CO_2 emissions from the root zone were 10 times higher than the N_2O emissions converted to CO_2 equivalents, as other authors reported [45]. Nonetheless, the CO_2 emissions from the root zone are considered to be in balance with photosynthetic CO_2 fixation by the aboveground biomass [46]. Furthermore, the prominent differences obtained between plant presence/absence (×2.7 fold higher with plant) may be explained by organic matter decomposition (plant litter and root exudates) and autotrophic and heterotrophic microbial and root respiration in the plant group. However, as CO_2 emissions were also present in the WP group, being higher in t1, we hypothesize that green algae grown on the substrate could also be a C source [47], and a limited N-source may be the cause of time differences.

While N_2O emissions showed no differences among the treatments when plants were present, the plant absence (in the WP group) and the higher N-NH_4^+ concentration input from STR and SAN treatment during t1, compared to CON and t3, increased the emission rates.

Therefore, we speculate that, when the NH_4^+ availability for microbial N-transformation processes is low due to the plant uptake/competition (plant presence group), despite the different N-NH_4^+:N-NO_3^- composition ratios of the NS used, no increase in N_2O emissions is detected. Similar results were reported by [41] with the use of mineral recycling fertilizers with different N-NH_4^+:N-NO_3^- ratios for hydroponic tomato production and by [48], showing that N_2O emissions were significantly different by soilless substrate type but not by the N form content.

However, without plant presence, the NH_4^+ availability was higher; this is the reason why the N_2O emissions were higher. Several authors [44,49–51] reported that N_2O augmented when N fertigation was increased. Such results indicate that mineral NH_4^+ provided by excessive fertilization (mainly in the absence of plants), and its associated water and nutrients substrate content and microbial activity, may be a major factor influencing N_2O generation from the studied soilless culture system.

Therefore, the correlations between N-transforming microbes' functional gene abundances and N_2O fluxes, among other factors, may provide information to improve fertilization strategies.

4.2. Influence of N-NH_4^+:N-NO_3^- Composition Ratios of the Nutrient Solution on Community Metrics, Functional Genes, and Its N_2O Fluxes Correlations

Coherent significant correlations have been found between 16S-metabarcoding and functional N-genes analysis. The higher N-NH_4^+ concentration applied with STR and SAN related to CON promoted a relative abundance increase in AOB community and some taxonomic bacterial phyla, especially nitrifiers *Nitrosomonas* and *Nitrospira*. However, the community metrics measured were not affected. On the other hand, archaea seem to be more sensitive to the high ammonium concentration (Cáceres et al., 2018), driving its community to a specialization, suggested by the lower diversity and evenness in these two treatments, mainly represented by the ammonium oxidizer *Nitrosopumilaceae* (88 ± 12% of RA). However, AOA abundance was not affected by the treatment applied, showing in STR and SAN higher AOB:AOA ratios versus CON as a consequence.

As in the results of our studies, there are already several pieces of evidence, most of them in soil studies [52,53], that ammonia concentrations contribute to the definition of distinct ecological niches of AOA and AOB. Ammonia-oxidizing archaea, due to their high ammonia affinity [54], are thought to be predominant in most natural environments with very low ammonia concentration presence, as in our CON treatment, even not being altered with higher concentrations [55]. However, AOB shows a stronger response and higher tolerance to high NH_4^+ concentrations [56].

These differences among treatments (STR and SAN versus CON) were more evident when the plant was absent due to the higher ammonium availability, the AOB:AOP ratios

and the *Nitrosomonas* and *Nitrospira* RA being higher compared to the plant group and coincident with higher N_2O emission (in t1, when the amount of N is higher).

Moreover, [43] found that *Nitrosomonas communis* was the dominant AOB genus in soilless media under neutral and slightly acidic pH, which is defined as r strategist strain with low substrate affinity and maximum activity. However, OTUs putatively related to *Nitrosomonas ureae*, *N. marina*, and *N. nitrosa* found in the present study seem to be high-affinity/low-activity populations, being more active under low ammonium concentrations [57,58]. Contrarily, in most agricultural soils [59,60], *Nitrosospira* dominates due to its higher stress tolerance. As an NOB community, *Nitrospira* was on the whole more abundant than ammonia oxidizers, as [43] also reported in soilless media, while Nitrobacter was not detected. This *Nitrospira*-like NOB dominance profile, published also in some soil [61,62] and marine aquaculture biofilm studies [57], plays a major functional role in low-potential NO_2^- oxidation activity environments, contrary to *Nitrobacter* [63]. However, the *Nitrospira* genus could also be composed of the recently discovered complete ammonia oxidizers (comammox), such as *Nitrospira inopinata*, which produce less N_2O during nitrification than AOB [64].

Studies describing the AOA taxonomy in soilless cultures are relatively rare. The dominance of *Nitrosopumilacea* versus *Nitrosophaera* in the present tomato experiment, contrary to the usual soil studies [8,65], may be the consequence of its adaptation to growing under extreme nutrient limitation (without minerals from soil) due to having the highest ammonium affinity reported for any ammonia-oxidizing microorganisms to date [66], suggesting that *Nitrosopumilacea* might successfully compete with AOB at low NH_4^+ concentrations.

These nitrifiers' community kinetic characteristics suggest that all of them may be able to live under limiting ammonium concentrations, such as the short and frequent fertigation management used in this experiment, especially when this is available for a limited time as plants are better competitors for NH_4^+ than AOB [67].

However, further studies are needed to confirm this hypothesis due to the insufficiently studied linkages between ammonia-oxidizing and NOB communities in soilless media. In addition, the origin of the high predominance of *Nitrosopumilacea* among AOA seems to be the utilization of groundwater to prepare the nutrient solution because *Nitrosopumilus (Nitrosopumilacea)* has been described as one of the main AOA in coastal groundwater, as described elsewhere [68].

Other than nitrification processes, N_2O consumption/denitrification also occurs simultaneously in a soilless substrate [41,48], which is a complex environment including both aerobic and anaerobic sites. Several authors [48,69] showed that *amoA* and *nosZ* genes are the best explanation for the variation in nitrification and denitrification, respectively.

Thus, the relative abundance of *nosZ* and *amoA* genes in our study supports that perlite can potentially enhance both nitrification and denitrification, even when the plant was absent (green algae growth on the substrate could be the C source in this case). Even both genes increase slightly along the crop campaign; the high porosity, oxygen availability, and low water-filled pore space (WFPS) of the substrate and the continuous circulation of NS in the system may suggest the dominance of the nitrification process, as other authors reported in perlite [49], coir substrate [48], and nutrient film technique [41]. Moreover, the use of an appropriate N concentration to optimize the fertilizer assimilation and the limited C availability may also result in a reduction in the heterotrophic denitrifying activity. Hence, nitrification could have been the major source of N_2O.

Additionally, the correlations between N-transforming microbes' functional gene abundances and N_2O fluxes showed distinct results with the plant presence/absence factor. When a plant was present, a non-significant relationship between N_2O emissions and functional gene abundance was found, as other authors revealed in hydroponics [20] and grasslands [70]. However, without a plant, when the NH_4^+ concentration is available for microorganisms, N_2O emissions correlated positively with AOB:AOA and *Nitrosospira* RA and negatively with AOA:16SrRNA. Similarly, [71] found a negative and positive

correlation with AOA *amoA* and AOB *amoA*, respectively, in tropical soil, while [72] revealed positive correlations with both ammonia-oxidizing kingdoms in fertilized soils. Moreover, correlations between N_2O emissions and *amoA* and some denitrification gene abundances were also demonstrated in soilless tomato culture systems (Lin et al., 2023) and long-term-fertilized soils [73], suggesting a relation with irrigation patterns and wet/dry season.

Overall, our results indicate that adequate fertilization management, with low NH_4^+ concentration available for microorganisms, may control the rhizosphere microbiota and its associated N_2O emissions. Furthermore, the AOB:AOA ratio may be a good indicator for the ammonia availability in soilless crop systems and its potential transformation to N_2O gas.

4.3. Influence of Recycled Fertilizers on the Bulk-Substrate Microbiome

The microbiome diversity in soil and soilless tomato crops is likely to be different due to the different growing conditions and substrate types, as has been demonstrated by several authors [11,74,75], suggesting a facility-specific microbiome form within the rhizosphere for hydroponics systems, as well as other growing media [76,77]. Moreover, under soilless conditions, plant species can exert a stronger discriminatory influence on their rhizosphere composition [78]. Even the microbiology of the hydroponics rhizosphere and the substrate's effect remain understudied; to discuss our microbial community structure, we are going to mainly focus on soilless culture and/or tomato literature.

Similar dominant rhizosphere phyla (>1% RA) were found among treatments, sampling times, and plant presence/absence samples, being reported as bacterial and archaeal keystone taxa for tomato plants [74,79]. While bacteria r-strategists, which grow fast when the substrate is abundant, were dominant (*Alpha- and Beta-Proteobacteria*, *Actinobacteria*, *Firmicutes*, and *Candidatus Saccharibacteria*), k-strategists, which grow when resources are limited, were present too (*Acidobacteria*, *Gamma-* and *Delta-Proteobacteria*, *Gemmatimonadetes*, *Verrucomicrobia*, and *Chloroflexi*). Many archaea of the dominant phylum Thaumarchaeota are ammonia-oxidizing archaea (AOA), playing a role in marine and terrestrial N and C cycles [80]. In addition, *Euryarchaeaota* represents most of the methanogens, which are mainly located in anoxic niches in the rhizosphere of the plants [81]. Even the similarities between samples, some dominant phyla, as well as less-well-documented rhizosphere colonizers' abundance were impacted by different factors. Even though it is well-known that rhizosphere-associated microorganisms coexist, few studies discuss bacteria and archaea interactions, probably due to the high influencing factors (host plant cultivar, root zone, plant growth stage, disease emergence, or environmental conditions) [82,83].

Along the crop season, shifts in community structure were detected earlier in bacteria than in the archaea kingdom, in concordance with a rhizosphere process formation study [84] where archaea were included in the last stages of plant development as "late colonizers" compared to bacteria. The microbial richness, diversity, and evenness were highest among bacterial communities concerning archaea, both within the range determined for tomato in hydroponics [74] but lower than that reported in soil crops [8,82] due to the presence of a natural soil ecosystem [75]. Moreover, an increase over time in the last two indexes was observed in the bacteria kingdom, probably due to fertilization, as has been reported in a long-term soil study across the globe [85]. However, several studies reported that mineral fertilization would reduce microbial diversity, including plant-beneficial microbial taxa [12].

Furthermore, the microbiome comparison among plant presence/absence groups highlights the higher diversity and evenness, as well as the natural presence of some genera, especially plant-growth-promoting rhizobacteria (PGPR), associated with plants. The establishment of beneficial microbiota for plant growth in the perlite growbags, which produce certain chemicals, including phytohormones required for plants, increases plant resistance and promotes the absorption of certain minerals [86] and boosts the reuse of the substrate for further cropping years while reducing the environmental impacts associated with their production. [87] reported that the main physical properties of 5-year-old reused

perlite growbags remained steady and had no negative effect on the fertigation, growth, and productivity of sweet pepper and melon crops. Further studies following the microbiome evolution over time and crops and the N_2O emissions in reused soilless cropping systems should be promoted.

5. Conclusions

Previous publications regarding this experiment demonstrated that recovered struvite and ammonium nitrate used as raw materials for a nutrient solution manufactured on a tomato soilless crop under greenhouse Mediterranean conditions had similar agronomic performance to conventional synthetic fertilization. The present study on microbiological and environmental parameters demonstrates that these alternative fertilization strategies had no negative impact on N_2O emissions, nitrogen leached, and bacteria community indexes, which were not strongly affected.

However, distinct ecological niches for ammonia-oxidizing archaea and bacteria were found when different $N\text{-}NH_4^+$:$N\text{-}NO_3^-$ concentrations of the nutrient solution were applied, with a stronger response and higher tolerance to ammonium concentration by AOB community. Moreover, the nitrogen-cycle-related microbiota analysis suggested a nitrification process dominance on the perlite growing media, even one that can also support denitrification.

In spite of that, when this ammonium fertilization is applied in the absence of a plant (or ammonia is overapplied), as there is no uptake of nutrients from plants immediately, NH_4^+ remained available for microorganisms. This promoted an increase in nitrogen-transforming bacteria (mainly nitrifying bacteria such as *Nitrosomonas*, *Nitrosospira*, and *Nitrospira*), while archaea (AOA) showed no differences regarding the fertilization applied. Simultaneously, the generation of nitrous oxide emissions from the soilless culture system was also being boosted. As a result, correlations were found between N-transforming microbes' functional gene abundances and N_2O fluxes, being positively correlated with AOB:AOA ratio and *Nitrosospira* relative abundance. These results suggest potential indicators for ammonium availability in the substrate, which would be necessary to investigate to guide the best fertilization management practices.

Moreover, our results highlight the high bacterial diversity indexes and the natural presence of some PGPR associated with tomato plants in a soilless culture system, considering a potential benefit for further cropping years by reusing the perlite growbags substrate while reducing the environmental impact associated with their production.

Overall, fertilizer blends for nutrient solution manufacture using recovered nutrients are a feasible alternative to synthetic fertilizers for increasing circularity in horticulture. Nevertheless, adequate fertilizer management is needed due to its influence on rhizosphere microbiota and its associated N_2O emissions.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/agronomy14010119/s1, Table S1. Main characteristics of recovered struvite and ammonium nitrate batches used in the assays. Characterization of recovered struvite products based on the current legal framework in the EU-wide quality standards of the new European fertilizer regulation for CMC12-precipitated phosphate salts and derivates; Table S2. Nutrient solution composition for the initial/final NS (during the first and the last month of the crop) and the development NS (during 2 months, the vegetative and fruit formation plant development stages) (*p*-value SN development) (mean ± SD); Table S3. Agronomic parameters results (mean ± SD); Table S4. Nitrogen-leached concentration measured along the experiment (mean ± SD); Table S5. Greenhouse gas emissions (mean ± SD). Greenhouse gas emissions for the fertilization treatments and plant presence/absence groups during the crop cycle (t1, t2, and t3). Without plant (WP) group was not determined during t2; Table S6. Bacteria and archaea community metrics: richness (Chao1), evenness (Pielou's), diversity (Shannon), and relative dominance. Significance *p*-value (codes ** p <0.01; * p < 0.05) indicate statistical differences by time, fertilization treatment, time * treatment, and plant presence/absence; Table S7. Taxonomic categories' relative abundance (RA) of both bacteria and archaea kingdom. Statistical tests were performed (i) within plant group using data from t1 to t3

(by time and fertilization) and (ii) between plant and without plant (WP) groups using data from t1 and t3 (by plant presence); Table S8. qPCR results of total bacteria (16S rRNA), ammonia-oxidizing prokaryotes (AOP) community (*amoA* of ammonia-oxidizing bacteria (AOB), and ammonia-oxidizing archaea (AOA)) and nitrous oxide reductase genes (nosZ) and their respective ratios. Statistical tests were performed (i) within plant group using data from t1 to t3 (by time and fertilization treatment) and (ii) between plant and without plant (WP) groups using data from t1 and t3 (by plant presence); Table S9. Permutation analysis of variance (PERMANOVA) on constrain axes used in canonical analysis of principal components bi-plot ordination (CAP) based upon Bray–Curtis distance for bacteria and archaea communities. Parameters represented: qPCR data (16SrRNA, AOA, AOB, nosZ, AOP:16SrRNA, AOA:AOB), emissions ($N.N_2O$ and $C.CO_2$), and N concentration and forms applied to the plants though the NS.

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