The use of recovered struvite and ammonium nitrate in fertigation in a horticultural rotation: agronomic and microbiological assessment

Mar Carreras-Sempere [1,2,*], Carmen Biel[1], Marc Viñas[2], Miriam Guivernau[2] and Rafaela Caceres[2]

[1] Sustainable Plant Protection Program, Institute of Agrifood Research and Technology (IRTA), E-08348 Cabrils, Spain; carmen.biel@irta.cat
[2] Sustainability in Biosystems Program, Institute of Agrifood Research and Technology (IRTA), E-08140 Caldes de Montbui, Spain; marc.vinas@irta.cat (M.V.); miriam.guivernau@irta.cat (M.G.); rafaela.caceres@irta.cat (R.C.);
[*] Correspondence: mar.carreras@irta.cat

ABSTRACT

Phosphorus and nitrogen recovery from wastewater as struvite and ammonium nitrate (AN) may be viable alternative fertilizers to boost circularity in horticulture. A 2-year fertigated crop rotation in soil under greenhouse conditions was evaluated to determine the efficiency of both recovered products as raw materials for a nutrient solution (NS) manufacture. The effects of these treatments versus synthetic fertilizers were compared in terms of crop performance, plant nutrient uptake, soil chemistry and microbiota. This is the first study to implement struvite through fertigation as the sole source of P in soil crops. Results showed that both recovered products can be used as fertilizers in NS, due to the similar response to the control for different parameters and crops (tomato, lettuce, and cauliflower). However, the AN treatment showed lower yield in the first tomato crop, which results may depend on the cultivar ammonium tolerance. Besides, the concentration of heavy metals in fruits/leaves was below the permissible limits. Total and Olsen phosphorus soil analysis revealed no differences among treatments, resulting in a similar performance of P-struvite to commercial phosphate. Bulk soil bacteria structure, richness and relative dominance were increased over time, while archaea only showed lower evenness, both despite the fertilization strategy. Shannon diversity was not significantly affected. A predominance of ammonia-oxidizing bacteria (AOB) versus archaea (AOA) was observed, while nitrite-oxidizing bacteria (NOB), dominated by *Nitrospira*, increased with fertigation. Our results demonstrate that fertilizer blends for NS containing recovered nutrients are a feasible alternative to synthetic fertilizers.

Keywords: Circular horticulture, struvite, ammonium nitrate, resource recovery, soil microbiota

INTRODUCTION

The horticultural sector is of paramount importance in agriculture, representing 14% of the value of all the agricultural goods and services produced in the EU (Cicco, 2018). Tomato, lettuce and cauliflower are some of the most produced horticultural crops in Spain, with 36% of the total vegetable production cultivated under greenhouse conditions (MAPA, 2020). On the other hand, horticultural products are also recognized as healthy within the Mediterranean diet (Bagetta et al., 2020). Selected varieties and management techniques should improve the quality of the products and the mitigation of the agricultural activities, respectively (Erba et al., 2013). In the last years, circularity in horticultural systems are being strengthened by adopting strategies such as the reuse of substrates (Acuña et al., 2013), the re-use of leachates (Prenafeta et al., 2017; Cáceres et al.,2017) in soilless agro-systems and the use of alternative fertilizers or fertilization strategies (Narváez et al., 2012, 2013).

Even though the high level of fertilization in the last century has promoted, overall, nutrient enrichment of the topsoil, intensive horticulture still relies on the input of fertilizers to sustain food production (Yu et al., 2021). Phosphorus (P) and Nitrogen (N) are essential plant nutrients, and their deficiency in soils severely restricts crop yields and soil fertility. Rock phosphate, the P fertilizers source, is a non-renewable and geographically restricted resource included in the 'critical raw material' list by the European Commission (EU, 2020). N fertilizers are produced from the N_2 present in the atmosphere through the Haber-Bosch process, which implies a high energetic demand, being linked to resource depletion and greenhouse gas (GHG) emissions. Both nutrient fertilizers are highly demanded, associated with a high environmental footprint, and strongly linked to key feedstocks prices (FAO, 2017). Moreover, the excessive use of these fertilizers is recognized as one of the most important causes of water bodies pollution (Huang et al., 2017).

Therefore, in order to move towards a more circular horticulture model, new agricultural practices should be promoted to reduce the use of synthetic fertilizers by finding ways to recycle and use these nutrients more efficiently and safely (regarding human and soil health).

In this regard, new technologies to recover N and P are being introduced in the treatments of wastewater for the production of high-quality fertilizers, being the precipitation of P as struvite ($NH_4MgPO_4{\cdot}6H_2O$) and the production of ammonium salts through liquid-liquid membrane contactors (LLMC) some of the most important ones (Perera et al., 2019; Magrí et al., 2020).

In terms of their application as fertilizers, several studies on struvite agronomic efficiency have been focused on its potential as slow-release fertilizer (as sparing water-soluble), applied to different types of growing media, soil pH and crops with successful results (Huygens et al., 2018, Arcas-Pilz et al., 2022). Besides, the potential use of recovered ammonium salts as fertilizers has been claimed by most studies while few have been performed on crops. The use of ammonium sulfate and nitrate produced by stripping has been effective on maize, lettuce, spinach and radish crops (Sigurnjak et al., 2019; Rodrigues et al., 2022). Moreover, the blend of both recovered products and other fertilizers has shown good agronomic performance in an organic growing medium (Robles-Aguilar et al., 2022). To our knowledge, the use of struvite and AN in fertigation as raw material for NS manufacture in soil trials has not been studied so far, which would be a way to improve sustainability and circularity in these systems. The use of struvite in fertigation has been used in soilless crops with successful agronomic and environmental results (Carreras-Sempere et al., 2021). Yet, such an experiment is imperative in the context of the ongoing publication of the new European fertilizer regulation (EU 2019/1009) that is setting EU-wide quality standards for struvite and ammonium salts, helping to develop circular agriculture, while reducing dependence on synthetic fertilizers.

Nowadays, drip fertigation is widely adopted by vegetable growers to achieve higher nutrient uptake and water use efficiencies. It represents a flexible tool to adjust the fertilizers rate according to the crop's nutritional status with precise irrigation and NS management while avoiding superfluous costs and environmental pollution (Priya et al., 2017). Another agronomical practice adopted for sustainable crop management is crop rotation. It gives direct benefits to soil fertility with the use of crop species differing in root architecture, the ability to take nutrients from the soil, and the potential symbiosis with certain microorganisms (Benincasa et al., 2017), influencing the soil-plant-rhizosphere microbial communities.

The former (i.e. bacteria, archaea and fungi) are the primary components of the soil food web and play a key role in the functioning, balance and stability of the soil ecosystem (Hillel, 2008; Delgado-Baquerizo et al., 2016). Thus, soil microbial biomass, activity and diversity are indicators of potential soil fertility and ecosystem productivity (Schloter et al., 2018). One major process in nitrogen cycling driven by soil microorganisms is the nitrification, divided into two steps, the conversion of ammonium (NH_4^+) or ammonia (NH_3) to nitrite (NO_2^-), which is called 'ammonia-oxidation', and the further transformation of NO_2^- into nitrate (NO_3^-), called 'nitrite-oxidation'. Ammonia-oxidizing archaea (AOA), ammonia-oxidizing bacteria (AOB) and Nitrite-oxidizing bacteria (NOB) drive soil nitrification and appear to be sufficient physiological diverse within each group for growth in most terrestrial ecosystems (Prosser & Nicol, 2012) and other nature-based processes of organic matter transformation as composting (Cáceres et al., 2018). Then, most of the horticultural crops take up N in the form of NH_4^+ or NO_3^- (Nasholm et al., 2000). NH_4^+ uptake and assimilation are less energy demanding, indicating a competitive advantage for plants that possess a higher ammonium absorption capacity. However, high ammonium concentrations can cause severe toxicity symptoms (Britto and Kronzucker, 2002). Therefore, the dynamic N cycle involves the synergistic interaction between plants and microbial communities in the soil.

The general objective of this study is to promote the circularity and sustainability of horticultural crops by using solubilized recovered struvite and AN through fertigation on a two-year soil rotation trial with tomato, cauliflower and lettuce crops. The agronomic effectiveness of the nutrient-recovered fertilizers treatments was measured in terms of response parameters (yield and biomass production, vegetable quality, and P and N uptake) compared to control treatment with synthetic fertilizers. Besides, soil parameters such as P concentration and the soil-plant-rhizosphere microbiota were monitored to study the behavior of the fertilizers and the fertilization management impact. Particularly, to our knowledge, the use of struvite and AN as raw materials for NS manufacture applied in soil trials has not been studied so far.

MATERIALS AND METHODS

2.1. Greenhouse crop rotation: Experimental conditions

The 2-year crop rotation experiment was performed on loamy sandy soil under greenhouse conditions, located at the IRTA research facilities in Cabrils, Barcelona, Spain (Latitude 41° 25'N, longitude 2° 23' E, altitude of 85 m). The horticultural crops grown were tomato (*Lycopersicum esculentum*, Bond® and Egara® in 2019 and 2020, respectively) during the spring-summer season (March-August 2019 and April-August 2020), cauliflower (*Brassica oleracea convar. Botrytis*, Trevi®) during the autumn season (October-January 2019 and 2020) and lettuce (*Lactuca sativa*, Maravilla®) in the spring season (March-April 2021). Tomato and cauliflower seedlings were transplanted in lines, each for 15 plants. The plant density was 1.66 plants·m^{-2}, achieved by using a 50-cm plant-spacing and 120-cm row-spacing. Each treatment was replicated three times, with 45 plants in total. Lettuce plants were transplanted in lines with 58 plants each. The plant density was 13.3 plants·m^{-2}, achieved by using a 25-cm plant-spacing and 30-cm row-spacing. Each treatment was replicated three times, with a total of 174 plants.
Nutrients were given through fertigation (see 2.2 section), mixing concentrated nutrient solution (cNS) with irrigation water (Table 1S) in a proportion 1:100 through a drip irrigation system with

a 2 $L \cdot h^{-1}$ nominal flow per plant. The irrigation schedule was 2-4 daily irrigation doses based on the estimation of crop evapotranspiration (ETc) and the soil volumetric water content at 2 depths (20 and 40cm) measured with Teros 10 sensors (Meter Group, USA) to keep the soil at a constant water volume (Segal et al., 2006).
The main loamy sandy soil characteristics of the field experiment are presented in table 2S, highlighting the basic pH, accumulation of carbonates and bicarbonates, low organic matter (<1.1%), and Cation exchange capacity (CEC) of 5.3 $meq \cdot 100g^{-1}$. Even though the soil has a slightly high calcium concentration, it is considered non-calcareous (Villar and Villar, 2016).
Radiation, air temperature and relative humidity inside the greenhouse were measured during crop campaigns with a pyranometer (SP-110 Apogee Instruments, USA), a temperature and relative humidity sensor (RH/Temp, Decagon, USA), and recorded every hour with a datalogger (Em50, Decagon, USA) (Table 3S).

2.2. Recovered Nutrients and Fertigation Treatments

The characterization of the recovered products (struvite and AN) was carried out in terms of macro/micronutrients, organic carbon and heavy metals, accomplishing the EU-wide quality standards of the new European fertilizer regulation (EU 2019/1009; Magrí et al., 2020) (Table 4S). P-recovered products used in this study were obtained and analyzed by Århusvand A/S company (Denmark) and Murcia Este WWTP (Spain) through P-elutriation at full-scale followed by a crystallization unit from the sludge line (Roldán et al., 2020; Castro et al., 2020). The mean mass % (±SD) composition of struvite samples was 12.3±1% $P\text{-}PO_4^{3-}$, 6.4±1% $N\text{-}NH_4^+$ and 9.5±1% Mg^{2+}. The recovered AN used was an end-product of ion exchange with zeolites and hollow fiber liquid-liquid membrane contactors (HF-LLMCs) treated in a pilot plant in Universitat Politècnica de Catalunya (Spain) where N from wastewater is captured into nitric acid (Reig et al., 2021). The mean w/v % (±SD) AN liquid fertilizer composition used was around 8.8±4 % N (4.5±2.6% $N\text{-}NH_4^+$ and 4.3±2.8% $N\text{-}NO_3^-$).
The methodology for the efficient use of P from struvite in fertigation has been set up in a previous experiment (Carreras-Sempere et al., 2021). Briefly, the struvite was diluted into nitric acid to solubilize the P, with a final pH around 1-2; then, the other fertilizers were added and this was the concentrated nutrient solution (cNS). The final nutrient solution (NS) applied to the crops was diluted 1:100 to achieve a pH 6.5-7 considering the irrigation water properties. This nutrient solution management in southern Europe is commonly carried out (Massa et al., 2020). Moreover, lowering the pH of the cNS reduces the risk of potential phytopathogens, while increasing sanitation in irrigation management.
To assess the effectiveness of the recovered products as raw materials for fertilizer blends, three fertigation treatments were applied throughout a crop rotation trial to compare the agronomic performance of the crops and their environmental effects. The treatments consisted of supplying three different NS, differing on the P and N sources and the $N\text{-}NO_3^-$:$N\text{-}NH_4^+$ ratio: i) struvite (STR) treatment, with 100% and 17±4% of P and N-recovered source, respectively; ii) struvite and ammonium nitrate (SAN) treatment, with 100% and 39±11% of P and N-recovered source, respectively; and iii) control (CON) treatment, using solely synthetic mineral fertilizers. The recovered nutrients were the P and N from ground struvite and the $N\text{-}NH_4^+$ from liquid AN. The reference P fertilizer used in the CON nutrient solution was monopotassium phosphate (KH_2PO_4). Other commercial fertilizers were used to complete the nutrients needed for the cNS and to diminish the pH: potassium nitrate, potassium sulfate, calcium nitrate, magnesium

nitrate, micronutrients, and nitric acid (respectively). The fertigation system was established with 2 tanks per treatment, containing the mentioned cNS to be released into passing irrigation water through venturi system with automatic control of irrigation (Dosatron, France). The concentration of the different compounds that made up the cNS for each treatment and crop is shown in table 5S.

Regarding the final NS, the concentration of nutrients provided to the different crops over the two growing seasons was based on those described in previous studies (adaptation of Muñoz et al., 2008; Bianco et al., 2015; Silber et al., 2003) (Table 1). The second growing campaign's NS composition for each crop was adjusted based on the results obtained, reducing the N and P concentrations during the 2nd growing campaign, and, in the case of the tomato crop, applying different nutrient concentrations depending on the plant's development stages. Moreover, as the response to ammonium nutrition varies between plant species and environmental conditions (Britto & Kronzucker, 2002), it is important to highlight the different $N\text{-}NH_4^+$:$N\text{-}NO_3^-$ ratios applied (0.04±0.03, 0.25±0.1 and 0.58±0.1 for CON, STR and SAN, respectively, as mean±SD values for all the crops) and consequently, different $N\text{-}NH_4^+$ concentrations. The nutrient concentrations of the different treatments provided to each crop are shown in table 6S.

Overall, the three treatments had similar fertigation management (irrigation time, amount of water and nutrients applied, harvesting time), environmental conditions (soil pH, texture, soil water content) and climatic conditions (temperature, humidity, CO_2 concentration).

Table 1. Nutrient Solution composition applied to the different crops and campaigns (mean value and standard deviation (SD) in meq·L^{-1} of the three treatments CON, STR and SAN).

Crop	Campaign	N	$H_2PO_4^-$	K^+	Ca^{2+}	Mg^{2+}	Na^+	SO_4^{2-}	Cl^-	pH	EC
					meq·L^{-1}						dS·m^{-1}
tomato	2019	10±0.8	1.1±0.1	6.2±0.8	8.8±0.2	4.4±1.4	3.8±0.0	9.5±3.9	5.7±0.1	6.6±0.3	2.1±0.2
	2020 initial & final	4.6±0.4	1±0.1	2.6±0.1	7.4±0.4	4.7±0.7	3.5±0.0	5.7±1.9	4.8±0.1	7.0±0.1	1.8±0.0
	2020 development	8.0±0.1	0.9±0.1	4.6±1.1	8.6±0.3	4.1±0.9	3.4±0.3	7.4±1.6	4.6±0.0	6.7±0.3	2.1±0.1
cauliflower	2019	8.4±1	1.1±0.1	6.4±0.2	7.4±0.1	4.8±1.5	4±0.1	7.8±3.1	5.3±0.1	6.7±0.2	2.3±0.2
	2020	7±0.7	0.8±0.1	5.4±0.7	6.2±0.9	3.9±0.9	3.9±0.1	7.1±2.5	4.5±0.0	6.7±0.1	1.8±0.1
lettuce	2020	4±0.3	0.3±0.0	1.0±0.2	5.5±0.1	3.5±0.4	4.2±0.4	3.5±0.6	4.4±0.1	7.0±0.4	1.4±0.1

NS composition was based on those described in previous studies (adaptation of Muñoz et al., 2008; Bianco et al., 2015; Silber et al., 2003).

2.3. Chemical, soil and plant material sampling and analytical procedure

The NS applied was quantified through water meters and collected weekly to be analyzed in the laboratory for chemical parameters (pH, EC, P, NO_3^-, and NH_4^+). The concentration of nitrites was negligible (<2 mg·L^{-1}). The pH and EC were determined using a selective ion analyzer (Thermo Scientific Orion model Dual Star selective ion) and Crison conductivity meter (model GLP31), respectively. The P, NO_3^-, and NH_4^+ concentration was analyzed by APHA Standard Method 4500-P C. Vanadatemolybdate method, Spectroquant®Nitrate and Spectroquant®Ammonium Reagent Test, respectively, using a SPECTROQUANT nova 60 Spectrophotometer.

Soil samples in each plot were collected at 0-30 and 30-60 cm intervals depth at planting time and the end of each growing period. Three samples per treatment were sent to an external laboratory to assess the concentration of nutrients by UV-VIS spectrophotometry and ICP-OES.

Organic matter, pH and CE were analyzed by Walkey-Black method, a suspension of 1:2.5 soil:water and a suspension 1:5 soil:water, respectively.

To assess soil fertility and P fertilizers management, two different soil P tests were done. Phosphate is the main inorganic form of P that is available to plants and exists in complex equilibria within all the P forms, from very stable, sparingly available, to labile and solution P (Shen et al., 2011). The amount of water-soluble P is very low relative to the total P pool and can rapidly be fixed in occluded forms unavailable to plants, such as Ca-phosphates in alkaline soils. To determine the P fraction that gives more relevant information on plant-available soil P, P-Olsen (P_{Ol}) method (Olsen and Sommers, 1982) was chosen due to its widespread use, well performance in basic/alkaline soils, and its detection of different P fertilizer sources including struvite (Battisti et al., 2021; Meyer et al., 2018). Besides, Total Phosphorus (P_T) (with aqua regia digestion) have been also measured as a P background value assumed to include most of the inorganic forms of P-phosphate. The ranges for establishing the soil categories for P_{Ol} were based on Mediterranean soils (Villar and Villar, 2016), defined as low (<12 $mg \cdot kg^{-1}$), medium (12-24 $mg \cdot kg^{-1}$), optimum (24-36 $mg \cdot kg^{-1}$), high (36-80 $mg \cdot kg^{-1}$) and very high (>80 $mg \cdot kg^{-1}$). Other nutrients soil category ranges are shown in table 7S.

About the plant material, the red tomatoes were harvested at a maximum 7-day interval for all the treatments, with a total of 14 harvests from June to August in both years (2019-2020), and fresh production was weighed to obtain the total yield. Fruits that were deformed or showed symptoms of blossom-end rot were weighed separately as "non-marketable yield". Marketable fruit yield consisted of tomato fruit that showed no signs of disease or deformation. Three samples (with 10 representative tomatoes each) per treatment, from different harvest periods, were graded according to their caliber, total soluble solids (TSS), individual weight, and color to evaluate fruit quality. At the end of the crop, the non-edible aerial part (leaves and stem), referred to as aerial biomass, from 15 plants per treatment was dried at 60ºC after the fresh weight was determined. For the cauliflower crop, 15 plants per treatment were harvested, fresh and dry inflorescence production and aerial biomass were weighed, and inflorescences diameter was measured. For the lettuce crop, the fresh and dry aerial part weight of 45 plants per treatment was determined, and diameter and relative chlorophyll content (SPAD) were measured. For all crops, three fruits and leaves composite samples per treatment were assessed for the concentration of nutrients and heavy metals by optical spectrometry (ICP-OES) and Kjeldahl method, after acid digestion.

In order to establish the performance of the recovered fertilizers compared with the reference one for each crop and year, yield and aboveground biomass weight, fruit/inflorescence quality and nutrient concentration and N and P uptake by the aboveground plant are presented. The former was estimated by considering the nutrient concentration and the weight of the harvesting fruit/inflorescence and aerial biomass at the end of each crop. For the tomato crop, some suckers pruning was not taken into account.

2.4. Soil-plant-rhizosphere microbiome assessment

In order to have a better understanding of the effect of the fertigation and the different composition of the NS (raw material origin and $N\text{-}NH_4^+:N\text{-}NO_3^-$ ratios applied) on the changes in the microbial community, a characterization of the rhizosphere-associated microbial community structure and its functionality has been done. Other effectors of change (i.e. soil type, crop species, soil disturbance) were held constant among the three treatments.

For the microbiological assessment, samples were taken in triplicate from the rhizosphere zone (2 cm distant from the stem and 0-15 cm depth) at the beginning (INI) (after transplanted and irrigated with water) and the end of the 2nd-year crop rotation for each of the treatments (CON, STR and SAN). Total DNA from 12 samples was extracted from the soil using DNeasy® PowerSoil® (Qiagen), following the manufacturer's instructions. To elucidate the microbial communities changes occurring in the agronomic trials, especially the ones related to the nitrogen cycle, a DNA-based assessment was carried out quantifying total bacteria and functional genes by quantitative polymerase chain reaction (qPCR) (Mx3000P, Stratagene) of total bacteria (16S rRNA) and ammonia-oxidizing prokaryotes (AOP) community (*amoA* of ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA)). Moreover, bacteria and archaea microbial communities' structure and taxonomy classification were assessed by Next Generation Sequencing (NGS). 16S-Metabarcoding paired end amplicon sequencing of the V3-V4 hypervariable region of 16S rRNA gene and library generation were performed. The pair of primers used for bacteria were V3_341F (5'-CCTACGGGNGGCWGCAG-3 ') / V4_R805 (5'-GACTACHVGGGTATCTAATCC-3 ') and specifically for archaea 349F (5'-GYGCASCAGKCGMGAAW-3') / 806R (5'–GGACTACVSGGGTATCTAAT-3') (Klindworth et al 2013). Sequences were obtained on the Illumina MiSeqTM platform in a 2 × 300 bp (v3) paired-end run (Molecular Research DNA, Texas, USA) , following the standard instructions of the 16S Metagenomic Sequencing Library Preparation protocol. Raw data (R1 and R2 demultiplexed FASTQ files) from 16S rRNA of bacteria and archaea were further processed using Cutadapt and DADA2 software. NGS data analysis and 16S rRNA-Metabarcoding sequencing data is detailed in table 8S. The raw sequence data were deposited in the sequence read archive of NCBI under the BioProject accession number PRJNA900046.

To assess alpha and beta diversity, phyloseq, microbiome and ggpubr R packages were utilized. The microbial community metrics of alpha diversity determine the number of species or richness (Chao 1 index), the relative abundance of each of these species or evenness (Pielou's index), the pool of species or diversity (Shannon index) and the relative dominance of the most abundant species (dominance) from the final ASVs distribution matrix. Besides, community composition and functional diversity related to N-cycle were also studied.

To examine community dissimilarities, beta diversity assessment was performed by means of permutational multivariate analyses of variance (PERMANOVA) of ASV distributions between treatments, based on Bray–Curtis distances with 999 permutations. Comparisons between community groups were conducted in Vegan R Package (Oksanen, 2007). PCoA and CAP plot were employed to visualize the differences among samples. Alpha and beta diversity and ordination plot analyses were performed on data rarefied by the minimum number of reads both in bacteria and archaea. All the data was analyzed within two sample groups: i) Initial (INI) and pooled final samples (all the treatments at the end of the experiment analyzed together) (FIN) to elucidate the effect of the fertigation; and ii) the three treatments of the final samples (CON, STR, SAN) to study the effect of the different composition of the Nutrient Solution (NS) (raw material origin and N-NH_4^+:N-NO_3^- ratios applied).

2.5. Statistical Analysis

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

RESULTS and DISCUSSION

3.1. Struvite and ammonium nitrate fertigation treatments

Regarding the final NS, nutrients from recovered products (P, N and Mg^{2+}) were detected and effectively supplied to the plants, manifesting a good performance of struvite dissolution under the established field conditions as stated in a previous study (Carreras-Sempere et al., 2021). However, as the dissolution of struvite is not total and the percentage of P-struvite can vary, it is important to be aware of the P-obtained from the final NS applied, which can be sampled in the drippers. The N-NO_3^- and N-NH_4^+ concentrations in the NS were kept constant, which exhibits a non-transformation of the ammonium to nitrate, instead of that, this N form remains in its reduced form.
The nutrient concentrations applied for each crop (Table 6S) have been similar for the three test treatments, except for magnesium due to the struvite's elemental composition and sulfate due to the use of potassium sulfate to keep the same K^+ concentration among treatments. However, in the tomato crop 2019 campaign, CON treatment infrastructure had some problems with the dosing dispenser over the experiment, supplying around 8-17% less total N and P than the other treatments, showing significant differences with STR and SAN. Moreover, as the cNS compositions vary just slightly among treatments, also the pH and EC of the final NS differ (Table 6S). The lower amount of nitric acid in SAN treatment is revealed with a higher pH in all the crops' NS compared to CON and STR. EC also varies slightly among treatments in the tomato and cauliflower crop of the 2019 campaign.

3.2. Crop performance and N and P uptake

In order to establish the agronomic performance of the recovered fertilizers (STR and SAN treatments), a comparison with the control treatment has been done for each crop and year. Due to technical problems linked to extreme meteorological events, cauliflower leaves analysis and lettuce crop couldn't be performed during the first crop rotation. Apart from that, all the treatments in all the crops grown achieved the objective yields (12, 1.7 and 3.5 kg·m^{-2} for tomato, cauliflower and lettuce, respectively) (Ruano, 2010; Doltra, 2010). Moreover, the quality characteristics obtained for all the crops (individual weight, caliber, color and total soluble solids (TSS) for tomato fruits; inflorescence diameter for cauliflower and lettuce diameter and relative chlorophyll content (SPAD)) were in concordance with published data (Gastelum-Barrios et al., 2011; Cotrina, 2020; Mendoza-Tafolla et al., 2019) (Table 2).
The **crop yield, fruit/inflorescence quality and aerial biomass weight** results of the 2-year crop rotation experiment (Table 2) demonstrate that no differences were found between STR treatment and the use of synthetic fertilizers (CON) in any of the crops grown within each year. However, few significant differences with SAN treatment have been detected. On the one hand, the total and commercial yield of the tomato crop on 2019, in SAN, was lower than CON and STR (p-value 0.0007 and 0.006, respectively) even no differences were found in the second growing cycle. As the tomato variety was different in both years and similar results have been obtained in a soilless trial assay with the same NS composition and tomato strains (Carreras-Sempere et al., 2021), the ammonium assimilatory capacity by the plant variety seems to be a key factor to

contribute to its accumulation and therefore to NH_3 toxicity. However, further studies should be performed to confirm the issue. On the other hand, for the cauliflower crop, the diameter of SAN treatment was bigger than CON in the 2020 campaign (p-value 0.002), even finding similar values in yield (fresh and dry weight). For the lettuce crop, the production was higher in SAN compared with CON and STR in terms of fresh weight (p-value <.0001). However, it has a significantly less percentage of dry matter (p-value <.0001), which means that those plants had just higher water content. There is conflicting data concerning the effect of increasing the concentration of NH_4^+ in the nutrient solution. For example, with a 50% ammonium supply from total N on the NS, a higher fresh weight for lettuce and cabbage (Song et al., 2021) and lower dry weight for cauliflower (Ferreira et al., 2017) have been reported, while a small degree effect on yield was found in tomato crops (Bialczyk et al., 2007), compared with 100% nitrate supply. Nevertheless, the sensitivity to NH_4^+ nutrition depends on the particular crop species or varieties and multiple environmental and climate conditions such as the external N concentration, the N-NH_4^+:N-NO_3^- availability, other nutrients concentration, soil pH and CO_2 concentration (Vega-Mas et al., 2015; Chaignon et al., 2002).

Additionally, **fruit, inflorescence and leaves nutrient concentrations** have been assessed (Table 2), being most of them in concordance with published data (Villar and Villar, 2016; Söylemez & Pakyurek, 2018). While similar values were found in most of the nutritional analysis results among treatments within years, few differences were detected in the tomato crop on Mg^{2+} in fruit (p-value 0.04) and P concentration in leaves (p-value 0.047), this last only during the first campaign; STR and SAN treatments had higher values of both nutrients. The higher concentration of these nutrients applied with the NS in these treatments (Table 6S) can explain the differences obtained, being also found in similar experiments with soilless growing media and others (Carreras-Sempere et al., 2021; Zhang et al., 2015).

Moreover, the **concentration of heavy metals** (Table 9S) regulated by FAO/WHO (2014) (Cadmium, Lead and Mercury) in fruits was below the permissible limits. Even chromium is not under regulation, the maximum value obtained was in lettuce crop (4.5 $mg \cdot kg^{-1}$ dry basis). These results agree with the fact that the struvite used in the NS of this crop showed the highest Cr content, and as Raptis et al. (2018) reported, Cr applied with irrigation water significantly increases Cr concentration and accumulation in shoots and roots of lettuce samples. According to Zayed andTerry (2003), typical values of Cr in plants growing in non-contaminated soils rarely exceed 5 $mg \cdot kg^{-1}$, even Cr is rarely toxic in plants under field conditions. Copper and Zinc concentrations did not show differences between nutrient-recovered treatments and control.

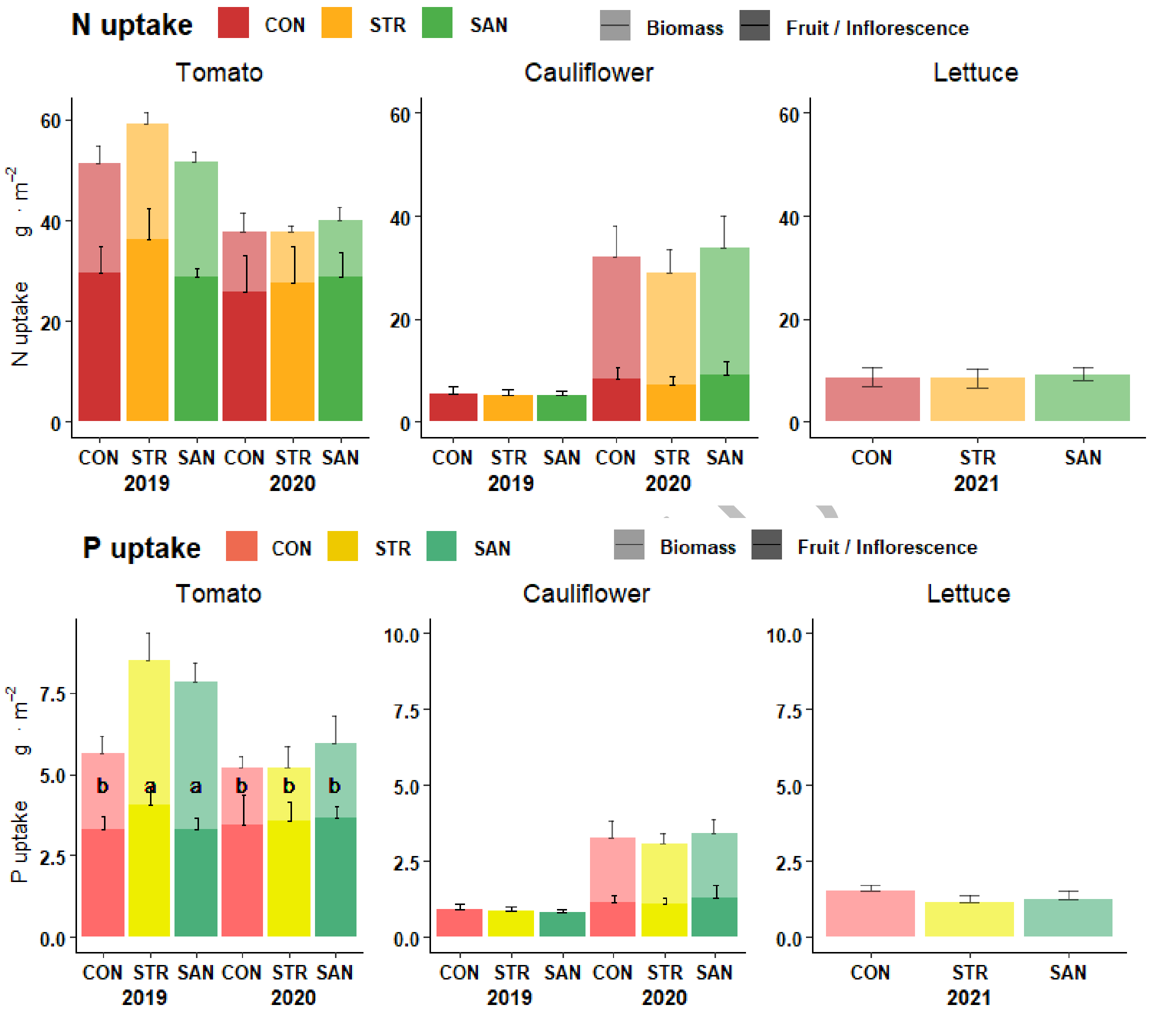
Concerning the **P and N uptake by the fruit/inflorescence and aerial biomass** (Table 10S, Figure 1), no significant differences have been revealed among treatments within a campaign in lettuce and cauliflower crops, being the values in concordance with reported data elsewhere (Gonzalez-Ponce et al., 2009; Tempesta et al., 2019; Dhakal et al., 2009). For the tomato crop in 2019, the nutritional leaf values also contributed to the major P content in the biomass of STR and SAN treatment (p-value 0.0286).

As investigated in this study, we may observe that fertilizer blends using recovered nutrients such as P and N from struvite and ammonium nitrate can successfully substitute the use of synthetic fertilizer to grow fertigated horticultural plants species in the soil such as tomato, cauliflower, and lettuce, as it has been previously reported in ornamental plants (Robles-Aguilar et al., 2022).

Table 2. Agronomic parameters (yield, fruit/inflorescence quality, aerial biomass, fruit and leaves nutritional composition) of the 2-year crop rotation for each crop, campaign and treatment. CON: control; STR: struvite; SAN: struvite with ammonium nitrate. Letters indicate statistical differences ($p < 0.05$) when Treatment*year interaction is significant, followed by the p-value for each variable. N.S.: not significantly different. Cauliflowers leaves analysis and lettuce crop during first campaign couldn't be performed.

Crop	Year campaign	Treatment	Yield ($kg \cdot m^{-2}$)		Fruit/inflorescence quality			Biomass ($kg \cdot m^{-2}$)	Fruit nutritional values ($mg \cdot 100g^{-1}$ wet basis)					Leaves nutritional values (%, dry basis)				
			Total	**Marketable**	**g·fruit^{-1}**	**Caliber (mm)**	**TSS**	**Aerial**	**N**	**P**	**Mg**	**K**	**Ca**	**N**	**P**	**Mg**	**K**	**Ca**
tomato	2019	CON	25.3 a	19.6 a	291.3	83.1	6.1	0.83	116.7	13.0	6.9	200	8.2	2.6	0.3 b	1.8	2.7	8.6
		STR	24.2 a	19.2 a	260.7	80.3	6.0	0.81	150.2	17.3	8.3	230	7.2	2.8	0.6 a	2.1	2.4	8.9
		SAN	20.6 b	16.5 b	268.9	81.8	6.4	0.82	139.4	16.0	7.6	225.7	7.5	2.8	0.6 a	2.1	2.2	8.9
	2020	CON	20.0 b	17.6 ab	279.0	85.4	5.1	0.62	128.1	17.0	8.6	274.7	9.0	1.9	0.3 b	2	2	9.3
		STR	20.9 b	18.3 ab	267.5	84	5.3	0.55	130.6	17.0	9.4	278.3	8.0	1.9	0.3 b	1.8	1.8	8.2
		SAN	20.9 b	18.5 ab	257.7	82.5	5.4	0.63	137.4	17.7	9.6	276.0	9.5	1.8	0.4 ab	2.2	1.6	9
	p-value	Treatment	**0.01**	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	**0.04**	N.S.	N.S.	N.S.	**0.007**	N.S.	N.S.	N.S.
		Year	**<.0001**	N.S.	N.S.	N.S.	**<.0001**	**<.0001**	N.S.	N.S.	**0.0002**	**0.03**	**0.047**	**<.0001**	**0.003**	N.S.	**0.003**	N.S.
		Treatment*year	**0.0007**	**0.006**	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	**0.047**	N.S.	N.S.	N.S.
cauliflower			**Fresh**	**Dry**	**Diameter (cm)**			**Aerial**	**N**	**P**	**Mg**	**K**	**Ca**	**N**	**P**	**Mg**	**K**	**Ca**
	2019	CON	1.77 b	0.12	21.3 ab			4.9	313.9	50.7	18.7	372.0	18.9					
		STR	1.75 b	0.09	21.6 a			5.1	294.3	50.0	18.2	364.7	16.4					
		SAN	1.71 b	0.13	20.8 ab			5	307.0	48.3	17.9	374.3	18.7					
	2020	CON	1.81 ab	0.10	18.7 d			8.2	456.7	63.7	19.1	378.7	17.5	4.9	0.43	0.38	4.4	3.2
		STR	1.83 ab	0.12	19.0 cd			7.7	393.3	60.0	18,0	367.0	17.1	4.8	0.45	0.35	5,0	3.6
		SAN	2.20 a	0.10	20.2 bc			9.0	416.7	53.7	18.1	366.7	18.1	4.9	0.43	0.41	4.5	3.2
	p-value	Treatment	N.S.	N.S.	N.S.			N.S.	N.S.	N.S.	N.S.	N.S.	N.S.					
		Year	**0.02**	**<.0001**	**<.0001**			**<.0001**	**0.0002**	**0.008**	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
		Treatment*year	**0.04**	N.S.	**0.002**			N.S.	N.S.	N.S.	N.S.	N.S.	N.S.					
lettuce			**Fresh**	**Dry**	**Diameter (cm)**	**SPAD**								**N**	**P**	**Mg**	**K**	**Ca**
	2021	CON	5.7 b	0.30	34.0	31.6								2.9	0.5	0.3	9.7	1.0
		STR	5.8 b	0.30	33.7	30.6								2.8	0.4	0.3	9.9	1.1
		SAN	7.0 a	0.29	34.3	30.3								3.2	0.4	0.3	8.5	0.9
	p-value	Treatment	**<.0001**	N.S.	N.S.	N.S.								N.S.	N.S.	N.S.	N.S.	N.S.

Figure 1. Nitrogen and Phosphorous uptake by the aerial non-edible part (Biomass) and the fruit/inflorescence of the different treatments for the crops grown during both campaigns (mean+SD). Cauliflowers leave analysis and lettuce crop during the first campaign couldn't be performed. Letters indicate statistical differences according to Tukey test ($p < 0.05$) CON: control; STR: struvite; SAN: struvite with ammonium nitrate.



3.3. Soil nutrient content

The study of the soil phosphorus dynamics, in particular, the "plant-available P" (P_{OI}) and the total P (P_T) over time is an essential prerequisite for providing adequate P fertilizer recommendations, evaluating the benefits derived from the applied P fertilizer and the balance with the background P values. As the P_T content decreases with depth (Wan der wal et al., 2007), only the 0-30 cm depth was analyzed in this experiment.

Figure 2 and table 2S show the concentration of P_{OI} and P_T in soil over the 2-year crop rotation at two soil depths. Firstly, no significant differences among treatments were detected neither in P_{OI} and P_T, meaning that P from recovered struvite acts similar to that from commercial potassium phosphate. Meyer et al. (2018) reported the high similarity of the reaction products in calcareous soil of non-water soluble milled struvite and monoammonium phosphate granules, showing comparable mobility and solubility in soil.

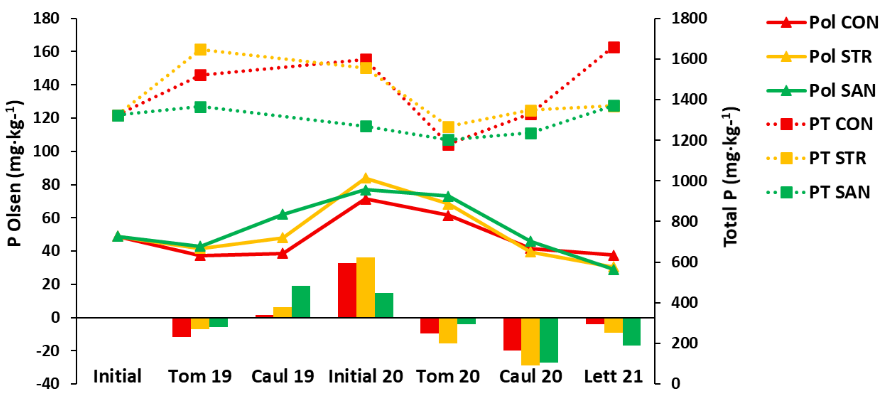
Secondly, the initial soil samples (49±22 mg·kg^{-1}) and most of the P_{OI} concentrations measured at 0-30 cm depth during the experiments were in the high P fertility category (>36 mg·kg^{-1}), except for the last measures after the 2-year crop rotation with average values of 32.3±15.7 mg·kg^{-1} (mean value categorized as optimum P fertility).

There is a highly significant linear relationship between changes in soil test P Olsen and the P content in the plant (Messiga et al., 2010), even being found that with high mineral fertilizer P rates (in calcareous soil), the P_{Ol} variations are lower (Morari et al., 2008). Thus, we report changes in the 0-30 cm depth, showing depletion of the P concentrations over each crop growing, except for the cauliflower 2019 campaign. As seen in Figure 2, the initial point in 2020 (Initial 20) shows the highest P_{Ol} concentration. It was measured after one month of rainfall (55 mm) inside the greenhouse without plastic cover due to the extreme meteorological event already mentioned, which may increase the soil soluble P (Shigaki et al., 2007). Still, the wide range of P_{Ol} levels included in this study has been found in many other agricultural soils where intensive horticulture was done (Recena et al., 2019; McDowell et al., 2001).

Besides, P transfers between the plow and deeper layers, apart from the P uptake by plants roots, might influence the results. Thus, 30-60 cm depth soil analysis (only during the second campaign) were done (Table 2S), showing no differences among treatments and a low fluctuance with 28.3±13 mg·kg^{-1} as mean±SD values for all the samples. As the soil water content at 40-cm depth was controlled and kept constant, the proportion of nutrients leached may be scarce (except for the punctual rainfall period mentioned above).

The total P was 1323±231 mg·kg^{-1} at the beginning of the assay and ranged from 921 to 1694 mg·kg^{-1} (average 1387±194) during the crop rotations. Considering already reported values (Zapata and Sikora, 2002; Wan der wal et al., 2007), the soil employed in this study can be considered to have inherent P fertility. However, it is supposed that with the constant supply of P fertilizers and the tendency for most of the measures along the experiment (except for tom20) to increase its mean P_T value, most of the P uptake by plants must be from the P applied through fertigation. Even though no differences were found between treatments, SAN had a propensity to maintain lower P_T values than STR and CON, which P non-precipitation could be due to the lower soil pH caused by the nitrification process of the ammonium contained in this fertilizer (Anderson et al., 2015).

Figure 2. Phosphorous Olsen (P_{Ol}) and Total P (P_T) soil content at 0-30 cm depth during 2-year crop rotation. Lines present P_{Ol} (left axis) and P_T (right axis) values, while bars present the differences between the initial and final P_{Ol} content for each crop.



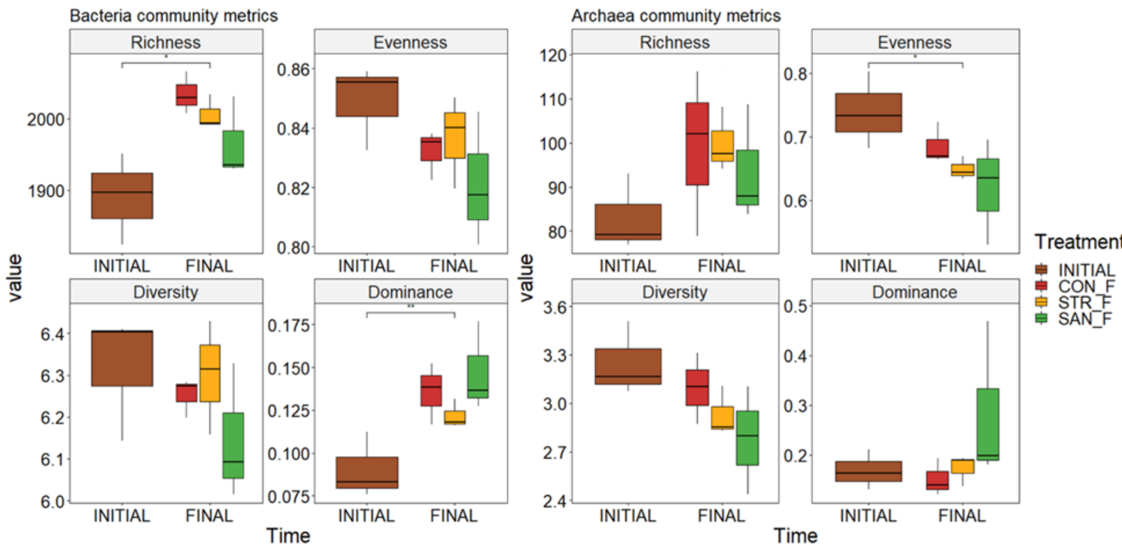
Furthermore, the mean value of all the other nutrients measured (Table 2S) was lower at the end of the crop rotation compared with the initial sampling and the EC declined from high (1±0.5) to not limitant (0.4±0.1) values, showing a good fertilization performance during the 2-

year crop rotation. However, the use of struvite as fertilizer increased the soil Mg^{2+} content, being higher than CON. N-NO_3^- and Na^+ showed higher significant values on the initial samples. The pH increased at the end of the crop rotation, changing from basic to alkaline due to fertilization as other authors reported (Radulov et al., 2011). Besides, treatments exhibited significant differences among them (p-value 0.007), corresponding the lower pH to the treatments with NH_4^+, especially SAN. It is well known that during the nitrification process, NH_4^+ releases H^+ ions which determine soil acidification (Barak et al., 1997). It has been described that the level of N-NH_4^+:N-total that affect growth and tomato yield may be mainly dictated by its impact on the rhizosphere pH (Chaignon et al., 2002). As has been stated, even the plant variety and other environmental factors (i.e., soil buffer capacity, NS concentration) must be considered when AN fertilizers are used, the soil pH and its related microbial community play an important role in the availability of certain nutrients such as P and the ammonia volatilization risk.

3.4. Soil-plant-rhizosphere microbial assessment

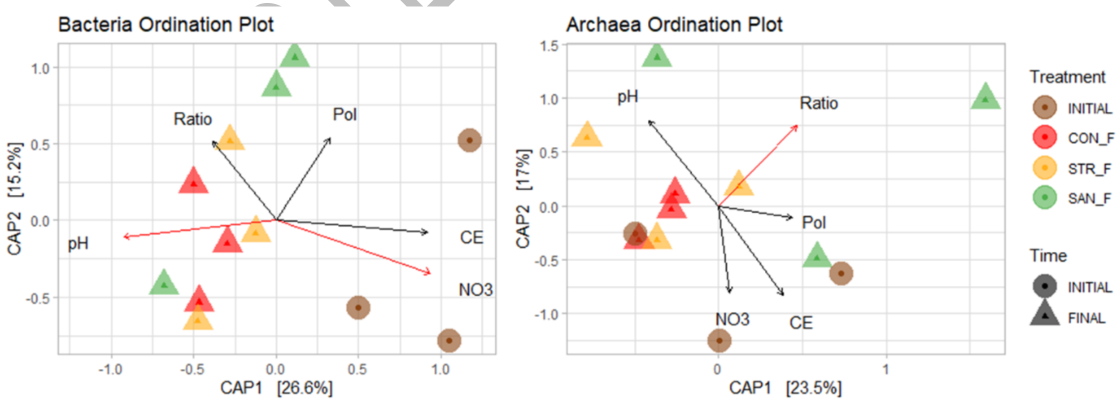
Regarding bulk soil bacterial populations abundance at the beginning and the end of the 2-year crop rotation with daily fertigation during the growing seasons, a tendency to increase the total bacterial population over time ($1.7{\cdot}10^9$ and $3.7{\cdot}10^9$ 16SrRNA gene copy number·g^{-1} for initial (INI) and pooled fertilized final samples (FIN), respectively), was revealed even being not statistically significant. Moreover, alpha diversity assessment (Figure 3, Table 11S) showed, in FIN compared to INI ones, a higher richness (Chao1) (2002 and 1890, respectively) and relative dominance (0.13 and 0.09, respectively). However, evenness (0.83±0.02) and diversity (Shannon) (6.25±0.13) showed no differences between them. Regarding the three final treatments samples, even no significant differences were found due to the low number of samples and its high dispersity, SAN treatment displayed lower values in richness, evenness and diversity, while STR and CON treatments had a similar tendency. The higher ammonium concentration of SAN may have exerted a selective pressure that does not allow the growth of as many bacterial species as the other treatments (Omar and Ismail, 1999).
In the case of the diversity indices of the archaea kingdom (Figure 3, Table 11S), the patterns are quite similar to bacterial communities, except for dominance. In the final samples compared to the INI ones, richness tends to increase (97 and 83, respectively) while evenness decreases significantly (0.65 and 0.74, respectively). Diversity (3.01±0.28) and relative dominance (0.19±0.09) showed no differences, as well as the comparison among FIN samples.

Figure 3. Bacteria and Archaea community metrics: Richness (Chao1), evenness (Pielou's), diversity (Shannon) and relative dominance. Significance p-value codes (<0.01; * <0.05) indicate statistical differences according to Wilcox test between initial (INI) and pooled final samples (FIN).**



Beta diversity analysis (Table 12S) of bacteria communities' resulted in significant differences between the initial and final samples ($R2 = 0.246$, p-value 0.006). However, no differences were found between the different fertigation treatments. The archaeal communities did not show differences neither throughput time nor between the different final treatments. Canonical analysis of principal components (CAP) bi-plot ordination and PERMANOVA analysis (based upon Bray-Curtis distance) (Table 13S) showed that soil pH and NO_3^- concentration were the significant variables (p-value 0.041 and 0.001, respectively) that explain bacterial community distribution, while the N-NH_4^+:N-total ratio applied with the NS (p-value 0.02) is the only one for the archaeal community distribution (Figure 4).

Figure 4. Canonical analysis of principal components (CAP) bi-plot ordination (based upon Bray-Curtis distance), visualizing the differences in bacteria (a) and archaea (b) communities among the four experimental conditions and the effects of soil chemical parameters (pH, P_{ol}, CE and NO_3^-) and the N-NH_4^+:N-total ratio applied with the NS. Red arrows indicate the significant variables for each plot (Table 13S).



Results showed that a 2-year crop rotation with fertigation promotes a microbial richness increase and a selection effect of the bacterial communities (Figure 3, Table 11S), indicating that some species dominate the site, even diversity is not significantly affected.

It is known that the use of mineral fertilizers changes the abundance of microbial populations and stimulates their growth thanks to the nutrient supply added (Dincă et al., 2021). However, controversial evidence has been published on its effect on the community metrics parameters. On one hand, a long-term study across the globe (Dai et al., 2018) reported that bacterial taxonomic diversity was increased by NPK fertilization. However, its response varies with soil texture and water management, being independent of crop type or N application rate. On the other hand, a tendency to diminish alpha diversity and evenness, but not richness, when struvite as slow-release fertilizer was applied on tomato crops (Grunert et al., 2019), and no effect on richness and diversity on extensive and horticultural crops (Francioli et al., 2016; Ge et al., 2008; Cai et al., 2017; Bei et al., 2018) have also been described. In our study, it is important to highlight that crop rotation, among other practices, favours preserving natural microbial communities (Dincă et al., 2021). Moreover, the diversity values obtained in our trials are included in published ranges (Cesaro et al., 2021) even after the long-term agricultural history of the soil used. High microbial community diversity is positively related to multifunctionality and adaptability to environmental changes (Delgado-Baquerizo et al., 2016), and therefore, a positive driver for plant growth, soil health and ecosystem functioning. Nevertheless, soil functional approaches, described as different roles of ecological units in the functioning of natural systems, may provide information on the microbiome strategy in front of the different fertilization conditions.

Microbial community composition

The phyla and genera relative abundance (RA) of both bacteria and archaea kingdoms are shown in Table 14S. The dominant bacterial phyla RA, for all the samples, were *Proteobacteria* (34±3%), *Actinobacteria* (12±2%), *Bacteriodetes* (12±3%), *Acidobacteria* (8±1%), *Firmicutes* (5±1%) and *Gemmatimonadetes* (3±0.5%), in agreement with previous studies (Bei et al., 2018). In the case of *Proteobacteria*, *Alpha-Proteobacteria* (15±4%) and *Gamma-Proteobacteria* (9±2%) were predominant in all cases. The taxonomic analysis showed similar profiles between INI and FIN samples, although a higher percentage of all final treatments stands out in the Phylum Planctomycetes (p-value 0.02) and Verrucomicrobia (p-value 0.01). At genus level, the most abundant on average are 9 genera, of which *Actinomarinicola, Algisphaera*, *Thiobacter*, *Nitrospira* and *Chryseolinea* increased with fertigation after 2-year crop rotation (being significant the first three). Instead, *Sphingomonas* and *Streptomyces* were the most predominant genera in the initial sampling. No significant differences were observed regarding *Ohtaekwangia* and *Steroidobacter* genera.

Regarding the archaea community, Thaumarchaeota (80±11%) phyla was predominant, followed by Euryarchaeota (17±11%) and Woesearchaeota (2±1%). The dominant phylum was identified as a chemolitoautotrophically ammonium-oxidizer, being found in nearly all environments, including fertilized soils (Kuypers et al., 2018). *Nitrososphaera* (74±20%) and *Nitrosopumilus* (8±14%) genera, described as AOA, were the ones with higher relative abundance, followed by *Haladaptatus, Halococcus, Methanomassilicoccus, and Woesearcheaota Incertae Sedis AR16*. The former was significantly higher in INI samples (p-value 0.01). No differences were found in other archaea RA, neither phylum nor genus, between samples.

Functional diversity related to N-cycle

This study has focused on one aspect of soil ecosystem function, the potential of the soil microbial community to perform the first step of nitrification, to study the performance of the $N\text{-}NH_4^+$ of the recovered fertilizers. Ammonium is one of the plant absorbable N forms and its transformation may play an important role in the interaction between plants and microbial communities (He et al., 2022). qPCR and 16S rRNA-Metabarcoding data are shown in Table 15S. Among the ammonia-oxidizing prokaryote (AOP) community, a clear predominance (x 7-36 folds) of the bacterial population (AOB) was observed concerning the archaea population (AOA) in all samples. The AOP:16S rRNA ratio showed an increasing trend in the final samples related to a higher ammonium concentration applied with the NS (CON<STR<SAN), similar to other studies (Hu et al., 2021), even though no significant differences were found (p-value 0.13). Neither the population of AOB ($4.3 \pm 2.2 \cdot 10^7$ gene copy number$\cdot g^{-1}$) and AOA ($3.7 \pm 4.9 \cdot 10^6$ gene

copy number·g^{-1}) showed significant differences between samples, as well as for relative abundance data by 16S rRNA-Metabarcoding, even they tend to increase in FIN samples, especially for SAN treatment.

Several authors also reported greater growth and activity of AOB in soils treated with ammonia fertilization (Jia et al., 2009; Pratscher et al., 2010; Sun et al., 2021), with a greater ammonia inhibition of cultivated AOA as a potential explanation (Prosser and Nicol, 2012). Moreover, a meta-analysis of ammonia-oxidizing microbiota on soil (Carey et al., 2016) reveals that AOB responds more strongly to N addition than AOA, even archaea also increased its abundance. Besides, AOB showed a greater response to fertilization in soils derived from wildlands than agricultural soils, with a reported background population size of $2.55 \pm 4.65 \cdot 10^7$ *amoA* gene copies·g^{-1} soil in unmanipulated agricultural control soils. As AOB are predominant in our study, their population size is in the same range and our soils have long fertilization history with several crops, it is possible that AOB communities are adapted to repeat fertilization events and that additional N has less effect as Griffiths and Philippot (2013) already reported.

From the total bacterial species detected by 16S rRNA-Metabarcoding, four out of which were assigned as nitrifying bacterial communities, *Nitrosomonas* and *Nitrosospira* as AOB, and *Nitrobacter* and *Nitrospira* as nitrite-oxidizing bacteria (NOB). On one hand, the AOB communities (0.2±0.1% of relative abundance (RA)) do not show significant differences between treatments, with a predominance of *Nitrosospira* in all of them. On the other hand, a significant increase of the NOB population is observed in the final fertilized samples (1.9±0.7% RA) versus the initial ones (0.9±0.5% RA), with a clear dominance of *Nitrospira* in all the samples. Other studies also reported this NOB profiling in surface rice paddy soil (Ke et al., 2013) and maize rhizosphere (Sun et al., 2021). However, most of the previous studies reported that *Nitrobacter* had a lower affinity than *Nitrospira* for N-NO_2 substrate and could be stimulated by high N levels (Attard et al., 2010; Nowka et al., 2015). The recent discovery of a complete ammonia oxidizer (termed "comammox") within the *Nitrospira* genus and its demonstrated active role in nitrification of agricultural soils amended with nitrogen fertilizers (Kits et al., 2019; Li et al., 2019) highlight the potential *Nitrospira* Commamox clade on our study system, with a low relative abundance of AOB and *Nitrobacter* genus compared to *Nitrospira* and the AOB dominance in front of AOA. However, the response of nitrite oxidizers to N fertilization is likely dependent on soil type, pH and nutrient availability and needs to be more thoroughly investigated (Sun et al., 2021). Regarding the archaea nitrifiers, *Nitrososphaera* is the most abundant archaeal genus in our soils (70-74% among total archaea, whereas *Nitrosopumilus* accounted for 0.4-10.4% of RA), but representing only 0.1-0.2% of total microbial populations, being below the predominance of AOB (1.6-1.7% RA), and *Nitrospira* (0.9-1.8% RA). *Nitrososphaera* has been detected with high abundance in most agricultural soils, finding a strong positive correlation with agricultural management, in particular with soil pH and ammonium levels (Villamil et al., 2021; Wang et al., 2018).

To deeply study the effect of the fertigation, the different NS compositions, and soil parameters on the microbial community, a bigger sampling size along with the crop rotation and longer period trials are needed due to the influence of assay duration (Bei et al., 2018), the correlations among soil microbiota and soil properties (Zarraonaindia et al., 2020; Carey et al., 2016) and even just the plant presence (Grunert et al., (2019). Moreover, the study of the active populations rather than the total community, that contains dormant taxa, may elucidate information about ecosystem functioning in real environmental conditions.

CONCLUSIONS

The present study aimed to provide viable alternative fertilizers to boost circularity in horticulture by using recovered struvite and ammonium nitrate as raw materials for nutrient solution manufacture on a two-year soil crop rotation. The effect of these fertilization strategies was considered from a holistic perspective, including crop performance, soil nutrients content and a microbiota assessment. For the first time, struvite and ammonium nitrate has been used as raw materials for nutrient solution manufacture in soil trials and this utilization has been fully successful.

The results showed that (i) both recovered products were equally effective in the agronomic parameters such as yield, vegetable quality, and N and P uptake compared to synthetic mineral fertilizers, with the exception for some differences detected in the tomato yield with SAN treatment that may depend on the ammonium tolerance of the plant variety; (ii) Application of recovered products from wastewater treatment plants did not exceed the heavy metals permissible concentrations in fruit and leaves; (iii) Soil nutrients content analysis revealed similar performances of the N and P from the diverse sources (recovered and synthetic); (iv) Bulk soil microbiota showed differences over the crop period, despite the fertilization treatment used. While richness and relative dominance bacteria's indexes increased over time, archaea evenness decreased. However, Shannon diversity was not significantly affected by none of both kingdoms. In addition, an increase over time of NOB, mainly *Nitrospira*, and a dominance of AOB, mostly *Nitrosospira*, versus AOA, principally *Nitrososphaera*, were observed.

Therefore, fertilizer blends for nutrient solution manufacture using recovered nutrients are a feasible alternative to synthetic fertilizers for enhancing sustainability in horticultural systems.

These results give deeper insights into the future potential use of nutrient-recovered products, especially under the ongoing process of the future EU quality standards for the use of struvite as fertilizer.

Supplementary Materials: The following are available online at XXXX, Table 1S. Chemical composition of irrigation water; Table 2S. Soil analysis at the beginning and along the 2-year crop rotation, for each treatment; Table 3S. Monthly average indoor global radiation, temperature, maximum temperature and relative humidity during 2-year crop rotation; Table 4S. Main characteristics of recovered struvite and ammonium nitrate batches; Table 5S. Concentration of the different compounds that made up the cNS for each treatment and crop; Table 6S. Nutrient Solution composition used for each treatment for the different crops and campaigns; Table 7S. Soil categories for different nutrients based on Mediterranean soils values; Table 8S. 16S rRNA-Metabarcoding sequencing data; Table 9S.Concentration of heavy metals in fruit and leaves for each treatment, crop and campaign; Table 10S. P and N content ($g{\cdot}m^{-2}$) in fruit/inflorescence and aerial biomass (and total) for each treatment, crop and campaign; Table 11S. Alfa diversity assessment for the initial (INI) and pooled final samples (FIN), and for each of the three final treatments samples for bacteria and archaea communities; Table 12S. Permutation analysis of variance (PERMANOVA) and Principal Correspondance Analysis (PCoA) plots of the microbial communities based on Bray-Curtis distances of bacterial communities and archaeal communities ; Table 13S. Permutation analysis of variance (PERMANOVA) on constrain axes used in canonical analysis of PCoA bi-plot ordination (based upon Bray-Curtis distance); Table 14S. Table and representation of the relative abundance of taxonomic assignation at the phylum and genus level of the bacterial and archaeal population; Table 15S. qPCR and MiSeq data. Author

Contributions: Conceptualization, methodology: all authors; data curation, writing—original draft preparation: M.C.-S.; supervision and writing—reviewing and editing: C.B, M.V., M.G and R.C. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors report there are no competing interests to declare.

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