Recovered fertilizers from urban wastewater treatment plants used in fertigation systems for horticultural crops

Maria del Mar Carreras Sempere

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Benvinguts al llarg viatge...
Tenim el riure a favor, cada rialla del món,
que avança que avança, no tinguis por
que avui una plaça s'omple de festa entre les llums
la mateixa que m'ha vist créixer llàgrimes als ulls

Txarango

Taula de continguts

Estructura de la tesi doctoral

El contingut de la tesis doctoral ha estat preparat en concordança amb la regulació interna de la Universitat Autònoma de Catalunya per a la presentació de la Tesis Doctoral. Tanmateix, aquesta tesis presenta la següent estructura:

- **Introducció,** on es presenta l'estat de l'art de la temàtica de la tesi i la interrelació entre els diferents capítols, en els següents apartats: i) Horticultura i fertilització actuals: consideracions generals; ii) La fertilització amb nitrogen i fòsfor: situació actual i alternatives per a la recuperació de nutrients; iii) Les sinèrgies entre els sectors de l'aigua i l'agricultura: El projecte LIFE ENRICH
- **Objectius**, on es detallen els objectius globals i específics de la recerca.
- **Capítols**. S'inclouen les quatre publicacions científiques presentades com a capítols d'aquesta tesi doctoral (tres incloses en el primer i segon quartil de l'índex SCI i una pendent de sotmetre)
    - El tercer capítol (*Agronomy* 2020, 10, 1039; DOI:10.3390/agronomy10071039, Accés obert), és una revisió dels criteris de qualitat establerts en la regulació de fertilitzants de la UE per a les sals fosfatades precipitades recuperades de corrents de residus, i l'eficiència agronòmica d'aquestes com a fertilitzants alternatius.
    - En el quart capítol (*Agriculture* 2021, 11, 1063; DOI:10.3390/agriculture11111063, Accés obert, seleccionat com a portada del volum 11, 2021) es presenta l'avaluació agronòmica i ambiental de la utilització de l'estruvita i el nitrat d'amoni recuperats com a matèries primeres per a la fertirrigació d'un cultiu de tomàquet hidropònic.
    - En el cinquè capítol (pendent de sotmetre) s'estudia l'efecte de la fertirrigació, de les diferents solucions nutritives utilitzades i de la presència de la planta en l'evolució de la microbiota del substrat, centrant-se en els gèneres i gens funcionals relacionats amb el cicle del nitrogen, així com la seva relació amb les emissions de N_2O, en el cultiu hidropònic de tomàquet.
    - En el sisè capítol (*Environmental Technology* 2022, 12, 0959-3330, DOI: 10.1080/09593330.2022.2154172, Accés restringit) es mostra la utilització de l'estruvita i el nitrat d'amoni recuperats per al seu ús en fertirrigació en una rotació de cultius hortícoles en sòl, des d'un aspecte agronòmic, ambiental i pel que fa al seu efecte sobre la microbiota del sòl.
- **Discussió general**, on els resultats més interesants i importants son analitzats i la interrelació entre els diferents capítols és discutida.
- **Conclusions** de la tesi doctoral
- **Referències**, on es citen les referències usades en totes les seccions de la tesi doctoral.

Abreviacions i símbols

AOA	Arquees amoni oxidants
AOB	Bacteris amoni oxidants
AOP	Procariotes amoni oxidants
BNR	Eliminació biològica de N
CE/EC	Conductivitat elèctrica
CEC	Capacitat d’intercanvi catiònic
CFU	Unitats formadores de colònies
CMC	Categoria de materials components
cNS	Solució nutritiva concentrada
CON	Tractament de fertilització control amb fertilitzants sintètics convencionals
EDAR/WWTP	Estacions depuradores d’aigües residuals
ETc	Evapotranspiració del cultiu
EUA	Estats Units d’Amèrica
DM	Matèria seca
DW	Pes sec
GEH/GHG	Gasos d’efecte hivernacle
ICP-OES	Espectrometria òptica de plasma
LLMC	Contactors de membrana líquid-líquid (sigles en anglès)
MAP	Fosfat monoamònic
N	Nitrogen
NA/AN	Nitrat d’amoni
NDN	Nitrificació-desnitrificació
NGS	Next Generation Sequencing
NH_3	Amoníac
NOB	Bacteris nitrit oxidants
P	Fòsfor
P_{Ol}	Fòsfor Olsen
P_T	Fòsfor Total
PFC	Categoria funcional del producte
PNA	Nitrificació parcial Anamox

RAE	Eficiència agronòmica relativa
SA/AS	Sulfat d’amoni
SAN	Tractament de fertilització amb estruvita i nitrat d’amoni recuperats
SD	Desviació Standard
STR	Tractament de fertilització amb estruvita recuperada
SE	Sud-est
SN/NS	Solució nutritiva
TOC	Carboni Orgànic Total
TSP	Triple superfosfat
TSS	Sòlids solubles Totals
UE/EU	Unió Europea

Llista de figures

Llista de taules

Agraïments

Aquest llarg viatge sembla que arriba a destí... amb el cor ple d'alegries i llàgrimes als ulls.

Són molts els aprenentatges i emocions viscudes i molts els fars que m'heu il·luminat el camí per ajudar-me a veure-hi més clar i a gaudir de la ruta. Si el destí és un bon port és, en gran part, gràcies a la vostra llum.

Començo per agrair als meus tres directors de tesi, la Dra. Carme Biel, la Dra. Rafaela Cáceres i el Dr. Marc Viñas tot el coneixement i energia positiva transmesa. Gràcies per confiar en mi en tot moment, recolzar-me amb paraules i actituds i donar-me ales per volar lliure i segura. Sou els fars que, tot i estar exposats a les inclemències del temps, les afronten amb fortalesa, positivisme i molta paciència.

Moltes gràcies a les meves tres tècniques preferides, la Mari Carmen, la Miriam i l'Anna, sou els meus fars com a punt de referència permanent. Durant el dia, visibles des de gran distància i els quatre punts cardinals i durant la nit, amb la vostra inconfusible llum. Gràcies per ser-hi sempre i amb aquesta actitud tan positiva que tants aprenentatges i somriures m'ha donat, i algun que altre plor. Continuarem sent més que companyes de feina.

Els fars s'assenten a llocs increïbles que porten a viure moments ben bonics, com tots els que he viscut envoltada dels i les grans companys/es i amics/gues de l'IRTA.

Gràcies a tota la família de Cabrils, passant per totes les generacions, per totes les estones d'ajuda i suport per seguir avançant i no tirar-se dels cabells, de riures, creacions artístiques i demés a la sala de becàries i becarios, pels esmorzars i dinars compartits amb bones converses i molta comèdia a les taules de fora, per les feines de laboratori i camp com la collita de tomàquet o plantar enciams dins dels hivernacles ja que una feina compartida sempre és una feina més agradable, tot i estar a altes temperatures a l'estiu, per les platges, birres, festes, paelles, bromes, excursions i mil més fora de l'IRTA.

Gràcies a tota la família de Caldes per acollir-me dins de l'equip sense cap mena de dubte. Agraeixo, especialment a les noies, que dia a dia m'heu fet sentir part d'un grup on l'alegria, la sinceritat, l'empatia, el companyerisme, i les cançons al laboratori són els pilars fonamentals. Gràcies per les estones al lab de micro, pels dinars compartits a les taules de TM i a la gespa, per tots els pastissos d'aniversari, per les quedades fora de l'IRTA, inclús aquella en la que un virus ens va fer una mala jugada, pels missatges que ajuden a pujar l'estima i fer que la vida sigui més fàcil.

Em sento molt afortunada d'haver compartit aquests anys amb dues famílies tan increïbles, amb les quals he pogut gaudir de la millor part de cadascuna d'elles. Així que no em cansaré de repetir-vos que milions de gràcies per fer que recordi aquesta aventura amb plenitud i un gran somriure.

Aquesta tesi doctoral s'ha pogut realitzar gràcies a la institució de l'IRTA, ICTA i la UAB i tots els professionals que hi treballen, així com al projecte europeu LIFE-ENRICH i tots els seus partners. Gràcies per fer-ho possible.

Un enorme agraïment també us el mereixeu les persones estimades que heu permès que avui en dia sigui on soc, i que durant aquests anys m'heu envoltat i heu gaudit de les alegries, com els dinars de celebració de cada publicació, però també heu sofert les baixades. Sou aquells fars que m'heu guiat per arribar a destí i evitar que naufragi, ajudant sempre que es podia a calmar la mar, mai millor dit. Una part d'aquest llibre és vostre família... pare, mare, tieta, sogres, germà, amics-germans i especialment del meu faroner Rubén. Gràcies per aquest final de viatge tan màgic i especial voltant la península amb la nirVANa.

I voldria acabar recordant i agraint als meus avis, allà on siguin, tot el que m'han donat al llarg de la vida perquè sense ells, no seria qui soc.

Resum

L'horticultura és una de les principals activitats de producció d'aliments i béns primaris d'origen vegetal, essencial per al desenvolupament i benestar humà. Espanya és el país d'Europa amb la major superfície d'agricultura protegida destinada al cultiu hortícola, tant en sòl com en sistemes sense sòl. L'aportació d'aigua i nutrients per a la producció hortícola intensiva es realitza majoritàriament per fertirrigació, i els nivells d'aplicació de fertilitzants minerals de fòsfor (P) i nitrogen (N) en sòls agrícoles ascendeixen, en l'àmbit mundial, a més de 40 i 100 milions de tones anuals, respectivament. L'obtenció, fabricació i subministrament d'aquests fertilitzants sintètics té un alt impacte ambiental i econòmic. Aquest, unit a l'augment del cost dels fertilitzants i al sostingut consum creixent, mostra la necessitat de recerca i ús d'alternatives de fonts de nutrients més sostenibles i renovables.

Una de les activitats de recerca estudiada abastament en els darrers anys a la Unió Europea, dins de l'horticultura circular, és la recuperació de nutrients o subproductes de corrents de residus per a la producció de fertilitzants alternatius segurs, des del punt de vista agronòmic i de salut humana i del sòl. Les tècniques de recuperació més estudiades i aplicades són la precipitació químicament induïda dels fosfats dissolts per obtenir sals fosfatades, com ara l'estruvita, i la combinació de l'intercanvi iònic i els sistemes de membrana de contacte per obtenir sals d'amoni líquides. Les sals fosfatades precipitades recuperades estan incloses en el reglament de productes fertilitzants UE 2019/1009. Recentment, el reglament d'agricultura ecològica de la UE ha estat modificat (UE 2023/21) per autoritzar també el seu ús, sota les condicions de l'esmentat reglament.

L'eficiència agronòmica de l'ús de l'estruvita com a fertilitzant sòlid d'alliberament controlat s'ha demostrat en diferents cultius, emprant sòls amb diferents pH, diferents mides de partícula del fertilitzant, etc. La recerca sobre les sals d'amoni recuperades s'ha centrat en el procés d'obtenció més que en la qualitat i potencial ús d'aquests productes. No obstant, el ús d'ambdós productes recuperats com a matèria primera per a la fabricació de solucions nutritives (SN) líquides per a fertirrigació no ha estat estudiat anteriorment.

Així mateix, existeix poc coneixement sobre l'efecte de la fertirrigació en la microbiota de la rizosfera, especialment en cultius sense sòl, tot i l'elevada influència de les comunitats microbianes en el creixement dels cultius, el cicle del N i les emissions de GEH.

En relació a aquests antecedents, l'objectiu general d'aquesta tesi doctoral és aconseguir un maneig sostenible i eficient dels productes recuperats d'aigües residuals urbanes, l'estruvita i el

nitrat d'amoni, com a fertilitzants alternatius als convencionals en sistemes de fertirrigació de cultius hortícoles tot estudiant el seu impacte en el creixement del cultiu i la microbiota de la rizosfera, des d'una visió agronòmica, microbiològica i ambiental.

Per tal d'assolir l'objectiu, s'ha realitzat una revisió de l'estat de l'art dels criteris de qualitat i eficiència agronòmica de diferents productes fosfatats recuperats pel seu ús com a fertilitzants. En aquesta s'ha trobat que les sals fosfatades de magnesi, com l'estruvita, tenen un major potencial fertilitzant per la seva elevada puresa, contingut i disponibilitat de fòsfor per a les plantes en un rang ampli de pH i medis de cultiu, comparat amb altres sals fosfatades precipitades recuperades.

Aquesta tesi doctoral ha establert una nova metodologia per a l'ús d'estruvita recuperada d'aigües residuals urbanes en sistemes de fertirrigació de cultius hortícoles en condicions protegides i a escala semicomercial, obtenint una resposta homogènia i satisfactòria de la seva dissolució en l'aigua de reg testada. Aquest nou ús de l'estruvita i el nitrat d'amoni com a matèries primeres per a la fabricació de solucions nutritives ha estat provat en el cultiu de tomàquet en substrat sense sòl (perlita) i en una rotació de tres cultius hortícoles (tomàquet, coliflor i enciam) en sòl, obtenint resultats satisfactoris, comparat amb fertilitzants convencionals sintètics, des d'una visió i) agronòmica, no mostrant diferències significatives en el rendiment dels cultius, la qualitat dels fruits i l'absorció de P i N. Tanmateix, per al seu ús, és necessari considerar la tolerància a l'amoni de l'espècie vegetal a cultivar; ii) de salut humana, no superant els nivells regulats de metalls pesants en fruits; iii) ambiental, ja que l'afectació en la lixiviació/acumulació en el sòl de N i P, en la generació de gasos d'efecte hivernacle (GEH) i en els índexs d'alfa-diversitat bacterians de la rizosfera ha estat similar al us de fertilitzants convencionals sintètics. A més, el maneig de la fertirrigació en el cultiu de tomàquet sense sòl, sota condicions d'hivernacle a la regió Mediterrània, ha permès demostrar que la concentració de N òptima de la SN pot ser reduïda, respecte a l'aplicació habitual -igual o superior a 9 mM-, a una solució nutritiva "dinàmica" d'aportació de 5-8-5 meq·N·L^{-1} -segons l'estadi fenològic de la planta- minimitzant la lixiviació de N mantenint el rendiment del cultiu i la qualitat del fruit.

Per altre costat, aquesta tesi doctoral ha aprofundit en l'impacte de l'aplicació de les diferents estratègies de fertirrigació sobre la composició i la diversitat de la microbiota (bacteris i arquees) de la rizosfera, en sòl i perlita, i la seva relació amb les emissions de N_2O sota la influència de la fertirrigació, de diferents ràtios de N-NH_4^+:N-NO_3^- en la composició de la solució nutritiva i l'efecte de la presència/absència de planta. Aquest estudi en el cultiu de perlita permet concloure que existeix una correlació positiva entre la ràtio de bacteris amoni-oxidants (AOB) : arquees amoni-oxidants (AOA) i els fluxos d'emissions de N_2O. Una aplicació en excés de fertilització amoniacal, on les plantes no són capaces d'absorbir l'amoni immediatament i roman disponible per a les poblacions microbianes, promou un increment de l'abundància relativa dels microorganismes transformadors del N respecte la població total bacteriana, i simultàniament, d'emissions d'òxid nitrós. L'estudi de la microbiota en els diferents medis de cultiu sota condicions de fertilització similars mostra les diferencies significatives en la beta diversitat de les comunitats microbianes i en la composició dels amoni-oxidants. A més, s'han detectat gèneres bacterians promotors del creixement vegetal associats a la presència de planta.

El maneig plantejat i avaluat en aquesta tesi doctoral, a més de reduir el impacte ambiental associat a l'ús de fertilitzants recuperats substituint als minerals sintètics, tant a la seva producció, transformació i transport, promou la millora de l'eficiència en l'ús dels nutrients aplicats als cultius, contribuint a minimitzar la lixiviació i conseqüent eutrofització de les masses d'aigua. També disminueix la generació d'emissions de N_2O, un dels principals GEH, i es restringeix la pèrdua de biodiversitat microbiana de la rizosfera. Els resultats obtinguts posen de manifest la necessitat de properes investigacions per treballar cap a una gestió dels cultius hortícoles més sostenible, reduint la demanda d'inputs no renovables/contaminants, així com gestionant eficientment els nutrients aportats per minimitzar l'impacte ambiental, preservant i/o millorant la qualitat del sòl i els seus serveis ecosistèmics.

Resumen

La horticultura es una de las principales actividades de producción de alimentos y bienes primarios de origen vegetal, esencial para el desarrollo y bienestar humano. España es el país de Europa con la mayor superficie de agricultura protegida destinada al cultivo hortícola, tanto en suelo como en sistemas sin suelo. El aporte de agua y nutrientes para la producción hortícola intensiva se realiza mayoritariamente por fertirrigación, y los niveles de aplicación de fertilizantes minerales de fósforo (P) y nitrógeno (N) en suelos agrícolas ascienden, a nivel mundial, a más de 40 y 100 millones de toneladas anuales, respectivamente. La obtención, fabricación y suministro de estos fertilizantes sintéticos tiene un alto impacto ambiental y económico. Éste, unido al aumento del coste de los fertilizantes y al sostenido consumo creciente, muestra la necesidad de búsqueda y uso de alternativas de fuentes de nutrientes más sostenibles y renovables.

Una de las actividades de investigación ampliamente estudiada en los últimos años en la Unión Europea, dentro de la horticultura circular, es la recuperación de nutrientes o subproductos de corrientes de residuos para la producción de fertilizantes alternativos seguros, desde el punto de vista agronómico y de salud humana y del suelo. Las técnicas de recuperación más estudiadas y aplicadas son la precipitación químicamente inducida de los fosfatos disueltos para obtener sales fosfatadas, como la estruvita, y la combinación del intercambio iónico y los sistemas de membrana de contacto para obtener sales de amonio líquidas. Las sales fosfatadas precipitadas recuperadas están incluidas en el reglamento de productos fertilizantes UE 2019/1009. Recientemente, el reglamento de agricultura ecológica de la UE ha sido modificado (UE 2023/21) para autorizar también su uso, bajo las condiciones de dicho reglamento.

La eficiencia agronómica del uso de la estruvita como fertilizante sólido de liberación controlada se ha demostrado en diferentes cultivos, empleando suelos con diferentes pH, diferentes tamaños de partícula del fertilizante, etc. La investigación sobre las sales de amonio recuperadas se ha centrado en el proceso de obtención más que en la calidad y potencial uso de estos productos. Sin embargo, el uso de ambos productos recuperados como materia prima para la fabricación de soluciones nutritivas (SN) líquidas para fertirrigación no ha sido estudiado anteriormente.

Asimismo, existe poco conocimiento sobre el efecto de la fertirrigación en la microbiota de la rizosfera, especialmente en cultivos sin suelo, a pesar de la elevada influencia de las comunidades microbianas en el crecimiento de los cultivos, el ciclo del N y las emisiones de gases de efecto invernadero (GEI).

En relación a estos antecedentes, el objetivo general de esta tesis doctoral es conseguir un manejo sostenible y eficiente de los productos recuperados de aguas residuales urbanas, la estruvita y el nitrato de amonio, como fertilizantes alternativos a los convencionales en sistemas de fertirrigación de cultivos hortícolas, estudiando su impacto en el crecimiento del cultivo y la microbiota de la rizosfera, desde una visión agronómica, microbiológica y ambiental.

Para conseguir el objetivo, se ha realizado una revisión del estado del arte de los criterios de calidad y eficiencia agronómica de diferentes productos fosfatados recuperados por su uso como fertilizantes. En ésta se ha encontrado que las sales fosfatadas de magnesio, como la estruvita, tienen un mayor potencial fertilizante por su elevada pureza, contenido y disponibilidad de fósforo para las plantas en un rango amplio de pH y medios de cultivo, comparado con otras sales fosfatadas precipitadas recuperadas.

Esta tesis doctoral ha establecido una nueva metodología para el uso de estruvita recuperada de aguas residuales urbanas en sistemas de fertirrigación de cultivos hortícolas en condiciones protegidas y a nivel semicomercial, obteniendo una respuesta homogénea y satisfactoria de su disolución en el agua de riego testada. Este nuevo uso de la estruvita y el nitrato de amonio como materias primas para la fabricación de soluciones nutritivas ha sido probado en el cultivo de tomate en sustrato sin suelo (perlita) y en una rotación de tres cultivos hortícolas (tomate, coliflor y lechuga) en suelo, obteniendo resultados satisfactorios, comparado con fertilizantes convencionales sintéticos, desde una visión i) agronómica, no mostrando diferencias significativas en el rendimiento de los cultivos, la calidad de los frutos y la absorción de P y N. Sin embargo, para su uso, es necesario considerar la tolerancia al amonio de la especie vegetal a cultivar; ii) de salud humana, no superando los niveles regulados de metales pesados en frutos; iii) ambiental, ya que la afectación en la lixiviación/acumulación en el suelo de N y P, en la generación de GEI y en los índices de alfa-diversidad bacterianos de la rizosfera ha sido similar al uso de fertilizantes convencionales sintéticos. Además, el manejo de la fertirrigación en el cultivo de tomate sin suelo, bajo condiciones de invernadero en la región Mediterránea, ha permitido demostrar que la concentración de N óptima de la SN puede ser reducida, respecto a su aplicación habitual -igual o superior a 9 mM-, a una solución nutritiva “dinámica” de aportación de 5-8-5 $meq \cdot N \cdot L^{-1}$ -según el estadio fenológico de la planta- minimizando la lixiviación de N manteniendo el rendimiento del cultivo y la calidad del fruto.

Por otro lado, esta tesis doctoral ha profundizado en el impacto de la aplicación de las diferentes estrategias de fertirrigación sobre la composición y diversidad de la microbiota (bacterias y arqueas) de la rizosfera, en suelo y perlita, y su relación con las emisiones de N_2O bajo la influencia de la fertirrigación, de diferentes ratios de $N\text{-}NH_4^+$:$N\text{-}NO_3^-$ en la composición de la solución nutritiva y el efecto de la presencia/ausencia de planta. Este estudio en el cultivo de perlita permite concluir que existe una correlación positiva entre el ratio de bacterias amonio-oxidantes (AOB): arqueas amonio-oxidantes (AOA) y los flujos de emisiones de N_2O. Una aplicación en exceso de fertilización amoniacal, donde las plantas no son capaces de absorber el amonio de inmediato y permanece disponible para las poblaciones microbianas, promueve un incremento de la abundancia relativa de los microorganismos transformadores del N respecto a la población total bacteriana, y simultáneamente, de emisiones de óxido nitroso. El estudio de la microbiota en los distintos medios de cultivo bajo condiciones de fertilización similares muestra las diferencias significativas en la beta diversidad de las comunidades microbianas y en la composición de los amonio-oxidantes. Además, se han detectado géneros bacterianos promotores del crecimiento vegetal asociados a la presencia de planta.

El manejo planteado y evaluado en esta tesis doctoral, además de reducir el impacto ambiental asociado al uso de fertilizantes recuperados sustituyendo a los minerales sintéticos, tanto a su producción, transformación y transporte, promueve la mejora de la eficiencia en uso de los nutrientes aplicados a los cultivos, contribuyendo a minimizar la lixiviación y consecuente eutrofización de las masas de agua. También disminuye la generación de emisiones de N_2O, uno de los principales GEI, y se restringe la pérdida de biodiversidad microbiana de la rizosfera. Los resultados obtenidos ponen de manifiesto la necesidad de próximas investigaciones para trabajar hacia una gestión de los cultivos hortícolas más sostenible, reduciendo la demanda de inputs no renovables/contaminantes, así como gestionando eficientemente los nutrientes aportados para minimizar el impacto ambiental, preservando y /o mejorando la calidad del suelo y sus servicios ecosistémicos.

Abstract

Horticulture is one of the main activities for the production of food and primary goods of vegetable origin, essential for human development and well-being. Spain is the country in Europe with the largest area of protected agriculture intended for horticultural cultivation, both in soil and soilless systems. The water and nutrients supply for intensive horticultural production is mostly carried out by fertigation, and the application levels of phosphorus (P) and nitrogen (N) mineral fertilizers in agricultural soils rise, worldwide, to more than 40 and 100 million tons per year, respectively. Obtaining, manufacturing and supplying these synthetic fertilizers has a high environmental and economic impact. This, together with the fertilizers cost increase and its sustained growing consumption, shows the need for the research and use of more sustainable and renewable sources of nutrients.

One of the research activities recently studied in the European Union, within circular horticulture, is the recovery of nutrients or by-products from waste streams for the production of safe alternative fertilizers, from an agronomic and human and soil health point of view. The most studied and applied recovery techniques are the chemically induced precipitation of dissolved phosphates to obtain phosphate salts, such as struvite, and the combination of ion exchange and contact membrane systems to obtain liquid ammonium salts. The recovered precipitated phosphate salts are included in the EU fertilizer product regulation 2019/1009. Recently, the EU organic farming regulation has been modified (EU 2023/21) to also authorize its use, under the conditions of the aforementioned regulation.

The agronomic efficiency of the use of struvite as a slow-release solid fertilizer has been demonstrated in different crops, using soils with different pH, different fertilizer particle sizes, etc. Research on recovered ammonium salts has focused on the production process rather than on the quality and potential use of these products. However, the use of both recovered products as raw materials for the manufacture of liquid nutrient solutions (NS) for fertigation has not been previously studied.

Likewise, there is little knowledge about the effect of fertigation on the rhizosphere microbiota, especially in soilless crops, despite the high influence of microbial communities on crop growth, the N cycle and greenhouse gases emissions (GHG).

Concerning these antecedents, the general objective of this doctoral thesis is to achieve a sustainable and efficient management of the products recovered from urban wastewater, struvite and ammonium nitrate, as alternative fertilizers to conventional ones, in horticultural

crops fertigation systems, while studying its impact on the crop growth and the rhizosphere microbiota, from an agronomic, microbiological and environmental point of view.

In order to achieve the objective, a state-of-the-art review of the quality and agronomic efficiency criteria of different phosphate products recovered for their use as fertilizers has been carried out. It has been found that magnesium phosphate salts, such as struvite, have a greater fertilizer potential due to their high purity, phosphorous content and availability for plants in a wide range of pH and growing media, compared with other recovered precipitated phosphate salts.

This doctoral thesis has established a new methodology for the use of recovered struvite from urban wastewater in fertigation systems of horticultural crops under protected conditions and on a semi-commercial scale, obtaining a homogeneous and satisfactory response from its dissolution in the irrigation water tested. This new use of struvite and ammonium nitrate as raw materials for the manufacture of nutrient solutions has been tested in tomato crops in a soilless substrate (perlite) and in a three horticultural crops rotation (tomato, cauliflower and lettuce) in soil, obtaining satisfactory results, compared to conventional synthetic fertilizers, from i) an agronomic point of view, showing no significant differences in crop yield, fruit quality and P and N absorption. However, for its use, it is necessary to consider the ammonium tolerance of the plant species to be cultivated; ii) human health, not exceeding the regulated levels of heavy metals in fruits; iii) environmental, since the impact on the leaching/accumulation in the soil of N and P, on the generation of GHG and on the bacterial alpha-diversity indices of the rhizosphere has been similar to the use of conventional synthetic fertilizers. In addition, the management of fertigation in a tomato soilless crop, under greenhouse conditions in the Mediterranean region, allowed us to demonstrate that the optimal N concentration of the NS can be reduced, compared to the usual application -equal to or higher than 9 mM-, to a "dynamic" nutrient solution of 5-8-5 $\text{meq}\cdot\text{N}\cdot\text{L}^{-1}$ -according to the phenological stage of the plant- minimizing the N leaching while maintaining the crop yield and the fruit quality.

On the other hand, this doctoral thesis has delved into the impact of the different fertigation strategies' application on the composition and diversity of the rhizosphere microbiota (bacteria and archaea), in soil and perlite, and their relationship with the emissions of N_2O under the influence of fertigation, different ratios of $\text{N-NH}_4^+\text{:N-NO}_3^-$ in the composition of the nutrient solution and the effect of the plant presence/absence. This study in perlite growing media allows us to conclude that there is a positive correlation between the ratio of ammonium-oxidizing bacteria (AOB): ammonium-oxidizing archaea (AOA) and N_2O emission flows. An excess application of ammonia fertilization, where plants are unable to absorb ammonium immediately and it remains available to microbial populations, promotes an increase in the relative abundance of N-transforming microorganisms relative to the total bacterial population, and simultaneously, of nitrous oxide emissions. The study of the microbiota in the different growing media under similar fertilization conditions shows significant differences in the beta diversity of the microbial communities and the ammonium-oxidizers composition. In addition, plant growth-promoting bacterial genera associated with the plant presence have been detected.

The management strategies suggested and assessed in this doctoral thesis support the reduction of the environmental impact associated with the use of recovered fertilizers replacing synthetic minerals, both in their production, transformation and transport. Moreover, it also promotes the improvement of nutrient use efficiency, contributing to minimize nutrient leaching and consequent eutrophication of water bodies. It also reduces the generation of N_2O emissions, one of the main GHG, and restricts the loss of rhizosphere microbial biodiversity. The results obtained highlight the need for further research to work towards a more sustainable horticultural crops management, reducing the demand for non-renewable/polluting inputs, as well as efficiently managing the nutrients provided to minimize the environmental impact, preserving and/or improving the quality of the soil and its ecosystem services.

Capítol 1.

Introducció

1.1. Horticultura i fertilització actuals: consideracions generals

L'**horticultura** és la part de la ciència agrícola que s'ocupa de la producció d'hortalisses, fruites, plantes ornamentals, aromàtiques o medicinals destinades al consum o ús humà. L'horticultura és essencial per al desenvolupament i benestar de la vida humana, ja que és una de les principals activitats de producció d'aliments saludables i béns primaris, aportant, a més, confort, plenitud i esperança de vida a les persones. És una activitat que té un gran impacte econòmic a escala mundial.

Els grans països productors d'hortalisses europees són Espanya i Itàlia, representant aproximadament el 42% del total (Eurostat, 2019). En termes de volum de producció, el tomàquet és la verdura més important, representant el 31%, seguit per altres cultius com la ceba, pastanaga, col, cogombre, pebrot, coliflor, enciam i carabassó (Eurostat, 2019).

A Europa, el desenvolupament de **l'horticultura protegida** es localitza en els països mediterranis, principalment a les comarques costaneres, on les bones condicions d'insolació, suaus temperatures hivernals i l'estabilitat del clima derivada de la proximitat del mar determinen unes condicions molt favorables. La major eficiència en l'ús de l'aigua, majors rendiments i avançament de l'entrada en producció dels cultius protegits enfront d'aquells situats a l'exterior, ha promogut la ràpida expansió de les grans àrees d'hivernacles en la regió mediterrània, passant de 68000 ha a finals de la dècada dels vuitanta a més de 200.000 ha en l'actualitat, suposant aproximadament un terç de la superfície dels cultius protegits mundials (Pérez-Parra&Céspedes, 1998; Antón, 2004). Dins d'Europa, Espanya és el país amb la major superfície d'hivernacles, túnels i umbracles, principalment la regió sud-est (SE), Almeria, la majoria dels quals estan dedicats al cultiu hortícola, representant el 19% de la superfície total cultivada d'hortalisses a Espanya (MAPA, 2021). En l'àrea Mediterrània, la major part dels cultius en hivernacle (80% en el SE d'Espanya) es cultiven directament en sòl, mentre que la resta és cultivat en medis sense sòl o hidroponia (p. ex. perlita i fibra de coco), tot i que l'ús d'aquests darrers està augmentant en tot el món (Thompson et al., 2007; Blok&Urrestarazu, 2010). El **cultiu sense sòl** està guanyant popularitat per una combinació de factors com són un major rendiment, control del sistema i possibilitat de prevenir o evitar malalties fitopatògenes, així com la facultat d'evitar/disminuir l'emissió de nutrients i agroquímics fitosanitaris (fertilitzants, herbicides, fungicides, etc.). A més, aquest sistema permet una major eficiència de l'aigua de reg, aspecte urgent en certes parts del món, inclòs el sud d'Europa (Blok&Urrestarazu, 2010).

La **fertilitat d'un sistema hortícola** no només depèn dels fertilitzants que s'aporten a aquests cultius, sinó que és el resultat del conjunt de característiques físiques, químiques i biològiques del medi de cultiu on creixen les plantes, a més dels aspectes associats a les condicions climàtiques i al control de plagues i patògens (Figura 1.1).

La **fertilitat química** és la capacitat del sòl o del substrat de posar a disposició de les plantes els nutrients necessaris i en forma assimilable per al seu correcte desenvolupament. En aquest aspecte, els fertilitzants tenen un paper fonamental, especialment en hidroponia/cultiu sense sòl on el substrat sol ser inert. L'aportació d'aigua i nutrients per a la producció de cultius hortícoles es realitza majoritàriament emprant un sistema de reg per degoteig i aportant els nutrients en una solució nutritiva (fertirrigació); el seu maneig depèn de les característiques del medi de cultiu i de l'aigua de reg. La fertirrigació, doncs, és la tècnica que permet l'aplicació simultània d'aigua i fertilitzants a través del sistema de reg; els elements necessaris són: dipòsits amb solucions nutritives, dosificadors (p. ex. bomba d'injecció) i canonades. Nombroses solucions nutritives (SN) universals (per a la majoria de cultius) amb composicions químiques diferents han estat formulades des dels anys seixanta (Smith, 1983). En els darrers anys, s'ha fet palesa la necessitat d'incrementar l'eficiència en l'ús dels fertilitzants per reduir els impactes econòmics i ambientals (p. ex. per lixiviació), tot mantenint o augmentant els rendiments de la collita (Narváez et al., 2012; 2013); per això s'han estudiat noves formulacions de SN amb concentracions de nutrients específiques per als requeriments de cada espècie vegetal, estadi fenològic i microclima (Ruano, 2010). Per assegurar resultats nutricionals satisfactoris d'una SN s'han de controlar diversos factors com la qualitat de l'aigua de reg, el pH (idealment, entre 5.5 i 6.5), la conductivitat elèctrica (CE) (entre 1 i 3.5 dS/m), el balanç de cations i anions, sinèrgies i antagonismes entre nutrients, dosi de micronutrients i la seva disponibilitat, etc., sempre considerant les concentracions de nutrients aportades per l'aigua, el sòl i el possible adobat de fons (Castellanos et al., 2014). Per disminuir el pH, en el cas que sigui necessari com en les zones amb aigües molt carregades de bicarbonats, s'utilitzen comunament adobs de reacció àcida o àcid nítric (Thompson et al., 2007). Els fertilitzants emprats en fertirrigació han de complir certes característiques bàsiques com l'alta solubilitat en medi líquid, puresa per no obturar els goters i baix índex de salinitat.

Per altre costat, les propietats biològiques del sòl o substrat, contribueixen al desenvolupament de múltiples funcions, ja que són un dels principals reservoris mundials de biodiversitat (FAO et al., 2020). Aquest reservori inclou les comunitats microbianes (microbiota del sòl/substrat) com els bacteris, arqueobacteris, fongs i protists (protozous i microalgues), així com micro, meso, macro i megafauna com els nematodes, àcars, cucs de terra, escarabats, etc. La gran varietat de nínxols ecològics en el sòl o substrat, en termes de mida i de recursos proveïts, condueix a una diferenciació funcional dels organismes. Cadascun d'ells té la seva importància, tot i que la majoria d'indicadors biològics de qualitat del sòl fan èmfasi en les mesures microbianes (p.ex. índex de diversitat i abundància microbiana), ja que estan associades a una gamma més àmplia de processos (Norris et al., 2020; Creamer et al., 2022).

La **microbiologia del sòl o substrat** és la disciplina que estudia les comunitats microbianes presents, les seves funcions i la importància d'aquestes poblacions per a la nutrició i salut de les plantes i el rendiment de les collites. S'estima que una cullerada de sòl alberga més microorganismes (1e^{10} cèl·lules/g-sòl) que éssers humans hi ha en el nostre planeta (Torsvik&Ovreas, 2002); es tracta d'una biodiversitat "amagada" entre la que trobem milions de bacteris, fongs i arqueobacteris, entre d'altres. La diversitat microbiana en aquests hàbitats és essencial per a garantir l'estabilitat i el correcte funcionament de l'ecosistema, possibilitant una àmplia varietat de funcions per a mantenir la fertilitat del medi de cultiu a través de la transformació de la matèria orgànica (Tang et al., 2022) i la transformació i disponibilitat dels nutrients (mineralització, nitrificació, fixació de N atmosfèric, solubilització de fosfats, etc.). A més, poden contribuir a la millora de l'estructura del sòl, donar suport al creixement saludable de la planta a través de l'alliberació d'hormones promotores del creixement i d'antibiòtics per a prevenir el creixement de patògens, així com degradar contaminants presents al sòl, entre d'altres (Dincâ et al., 2021). S'ha descrit que els microorganismes no es distribueixen en el sòl a l'atzar, sinó que segueixen patrons especials d'agregació, governats per la presència d'arrels, agregats, nutrients i porus (Ettema et al., 2002). A través de l'exsudació i rizodeposició de les plantes, entre d'altres factors, es seleccionen les comunitats de microorganismes que s'estableixen a la zona de la rizosfera (Sasse et al., 2018). La rizosfera es descriu com la zona que envolta l'aparell radicular més jove de les plantes i que està directament influenciada per les secrecions de les arrels.

Actualment, la microbiologia ambiental s'ha establert com una àrea d'estudi fonamental i de gran importància en àmbits com la bioremediació, biocombustibles, control biològic i els biofertilitzants amb l'ús i control de poblacions microbianes promotores del creixement vegetal (PGPR), entre d'altres. En les darreres dues dècades ha sorgit un nou camp de la ciència, la metagenòmica, que persegueix obtenir seqüències del genoma dels diferents microorganismes que componen una comunitat, així com els seus trets funcionals, extraient i analitzant el seu material genètic de forma global, sense necessitat d'haver d'aïllar i cultivar de forma individual els microorganismes del sòl. La possibilitat de seqüenciar directament els ADN i ARN, obre noves possibilitats d'estudi com la identificació i quantificació de les poblacions microbianes o les seves funcions de major interès (p. ex. els gens que intervenen en el cicle del nitrogen, índex de diversitat microbiana, control de plagues (supressió de patògens), producció d'hormones vegetals com el IAA, solubilització de fòsfor orgànic i mineral, fixació de N_2) (Thiele-Bruhn et al., 2020). Tot i això, el coneixement sobre la distribució i ecologia dels microorganismes del sòl, i encara més del cultiu sense sòl, és encara escàs, a causa de l'elevada diversitat de les poblacions microbianes, les complexes interaccions entre la biota del medi, els processos i la funcionalitat i les dificultats associades a la seva identificació i estudi (Creamer et al., 2022). A més, s'ha realitzat poca recerca comparant la composició i evolució microbiana durant un cultiu en sòl i un cultiu sense sòl, complementats amb fertilitzants (Grunert et al., 2020). Així, es millora la comprensió dels factors que influencien en la diversitat microbiana general i com aquesta pot ajudar al desenvolupament de sistemes de cultiu més sostenibles, interpretant les seves funcions lligades a la disponibilitat de nutrients, a temes ambientals com ara l'emissió de gasos d'efecte hivernacle o gasos acidificants com l'amoníac (Grunert et al., 2020), a la contribució en la salut humana, animal i ambiental (Banerjee&Van der Heijden, 2023), etc.

Figura 1.1. Representació de la fertilitat d'un sistema hortícola considerant el conjunt de propietats físiques, químiques i biològiques del medi de cultiu i la seva interrelació. Font: Shutterstock i ONU, 2018.

Així doncs, es considera necessari mantenir la fertilitat dels sistemes agrícoles mitjançant el maneig i l'ús de tècniques que permetin sustentar els rendiments dels cultius, a l'hora que es millora la sostenibilitat ambiental dels mateixos (qualitat del sòl, lixiviació de nutrients, emissions de gasos d'efecte hivernacle (GEH), etc.), sobretot amb l'actual augment de la població mundial i en conseqüència, la creixent demanda d'aliments.

1.2. La fertilització amb fòsfor i nitrogen

1.2.1. Situació actual: consum, impacte ambiental i tendències

Actualment, els nivells d'aplicació de **fertilitzants minerals nitrogenats i fosfatats** en sòls agrícoles ascendeixen, en l'àmbit mundial, a més de 100 i 40 milions de tones anuals, respectivament, essent, pel N, un ordre de magnitud superior que en l'any 1960 (Tubiello et al., 2021). L'obtenció, fabricació i subministrament d'aquests fertilitzants té un alt impacte ambiental i econòmic.

Per una banda, els dipòsits de roca fosfòrica constitueixen la major font de fòsfor per al seu ús en agricultura i indústria. Tanmateix, aquest és un recurs finit distribuït en punts concrets del planeta (principalment Marroc, Xina i EUA), el qual està inclòs en la llista de matèries primeres crítiques de la Unió Europea per: la seva importància en l'economia, la situació geopolítica de les mines i l'alt risc associat al seu subministrament (CE, 2017). A més, després de ser extreta, la roca fosfòrica és processada per a la producció de fertilitzants com ara el triple superfosfat (TSP de les sigles en anglès) o el fosfat monoamònic (MAP), deixant muntanyes de runes i deixalles de desenes de quilòmetres al voltant de les zones mineres.

Per altra banda, aproximadament el 2% de l'ús de l'energia mundial és dedicat a la fabricació industrial d'amoníac (NH_3) a partir d'hidrogen (H_2) i nitrogen atmosfèric a través del procés Haber-Bosch (Sutton et al., 2013). Concretament, per produir una tona de fertilitzant NH_3, es necessiten 949 m^3 de gas natural i s'emeten 1.6 tones de diòxid de carboni (Beckinghausen et al., 2020); la producció de NH_3 representa el 87% de l'energia total utilitzada en la indústria dels fertilitzants.

Davant d'aquest alt cost ambiental unit a l'augment del cost monetari dels fertilitzants minerals sintètics i al sostingut consum creixent (Figura 1.2), es fa palesa la necessitat de recerca i ús d'alternatives més sostenibles i renovables.

Una de les activitats de recerca àmpliament estudiada en els darrers anys a la Unió Europea, dins de l'horticultura circular, és la **recuperació de nutrients o subproductes** de corrents de residus per a la producció de fertilitzants alternatius (Sutton et al., 2013). Les aigües residuals, siguin d'origen municipal, industrial o ramader, són un dels reservoris de nutrients més important per a ser recuperats (Saliu&Oladoja, 2021). La tecnologia estudiada darrerament podria transformar les estacions depuradores d'aigües residuals (EDAR) en biorefineries, possibilitant la conversió de residus en bioproductes (Tobin et al., 2020). També hi ha la possibilitat de tractar les aigües residuals provinents de sistemes hortícoles sense sòl, amb la qual s'augmentaria la disponibilitat d'aigua de reg i l'eficiència de l'ús de l'aigua i dels nutrients (Cáceres et al., 2017; Cleanleach - YouTube).

L'aprofitament dels nutrients presents en les aigües residuals d'estacions depuradores permet reduir les distàncies entre els punts de producció i consum d'aquests nutrients, assegurant el seu subministrament, així com disminuir la seva lixiviació a les masses d'aigües i problemàtiques d'obturació de canonades de les plantes de tractament (en el cas del P), essent una recomanació de la comissió europea per promoure el canvi cap a la circularitat (Comissió Europea, 2020).

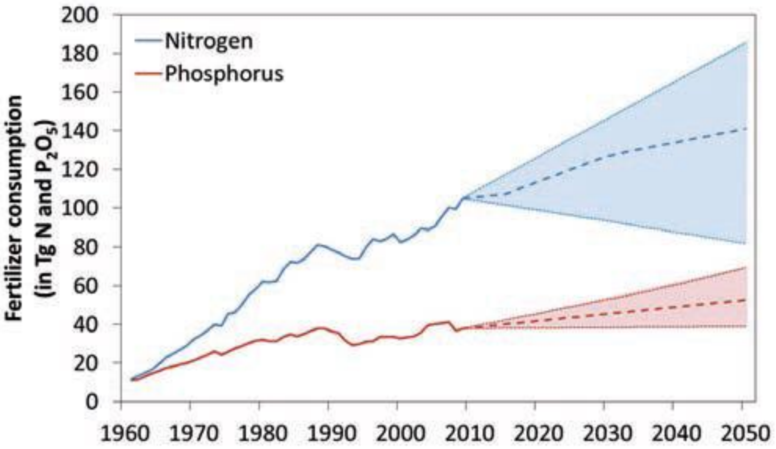

Figura 1.2. Tendències del consum mundial de fertilitzants minerals de nitrogen i fòsfor i futurs possibles projectats. Font: Sutton et al., 2013.

1.2.2. Alternatives per a la recuperació de nutrients

La història revela mètodes tradicionals d'utilització dels nutrients presents en les aigües residuals com ara l'ús d'aigües regenerades (depurades amb tractament terciari) per al reg dels cultius que poden contenir certs nutrients com el fòsfor, l'aplicació dels fangs de depuradora en forma d'adob o la desviació de l'orina per a la seva aplicació directa, entre d'altres (Kirchmann et al., 2005). Tot i que aquestes pràctiques es realitzen en diversos països, hi ha certa problemàtica d'acceptació professional i social degut, principalment, a la possible contaminació per alta concentració de compostos tòxics (p. ex. metalls pesants o contaminants emergents) i patògens, la variabilitat en la composició nutricional dels productes i a les olors emeses en la seva aplicació (Tur-Cardona et al., 2018; Pradel et al., 2020).

Actualment, s'estan introduint noves **tecnologies en el tractament d'aigües residuals** per a recuperar nutrients produint productes més segurs, des del punt de vista agronòmic i de salut, que puguin ser usats com a fertilitzants.

Entre les tècniques que permeten la recuperació del fòsfor dels corrents de residus, la precipitació químicament induïda dels fosfats dissolts és una de les alternatives més comunes (Cardoso et al., 2019). La precipitació/cristal·lització de P de corrents rics d'aquest nutrient, com són els llots concentrats a la sortida d'un reactor biològic o els concentrats del llot digerit, es produeix en un reactor (Figura 1.3). El procés requereix del control del pH, mitjançant la dosificació de sosa càustica, i l'addició de ions metàl·lics (p. ex. magnesi (Mg^{2+}), calci (Ca^{2+}) o ferro (Fe^{2+})), proporcionant les condicions de precipitació adequades per obtenir, com a producte final, una sal fosfatada com ara l'estruvita (Kumar&Pal, 2015). L'aplicació d'aquesta tecnologia en les EDAR permet un major control de la precipitació incontrolada d'aquest compost en les canonades, colzes, bombes, etc. reduint problemes operacionals i augmentant l'eficiència dels processos de la mateixa planta de tractament (Le Corre et al., 2009). Actualment, la producció d'estruvita a la Unió Europea (UE) cobreix el 0.5% de les importacions de fertilitzants fosfatats, podent arribar a una substitució del 13% en un futur (Muys et al., 2021). A més, s'ha simulat que el procés de precipitació d'estruvita reduiria d'un 3 a un 38% els efectes sobre l'escalfament global (Zhang et al., 2020).

L'estruvita o MAP ("magnesium ammonium phosphate") és el nom pel qual es coneix el fosfat de magnesi i amoni hexahidratat ($(NH_4)MgPO_4 \cdot 6H_2O$). És un compost cristal·lí, amb una composició teòrica de 12.6% P, 5.5% N i 9.9% Mg, sobre pes sec, sent constituïda tant per P com per N recuperat. L'estruvita és tèrmicament inestable a temperatures superiors als 55ºC, podent perdre totes o part de les molècules d'amoni i aigua (Bayuseno et al., 2020). A més, la seva solubilitat varia en funció del pH, aspecte clau a considerar en el seu ús com a fertilitzant, sobretot si és aplicat com a component d'una solució nutritiva, en agronomia, explicat en l'apartat següent.

Respecte a la recuperació de nitrogen d'aigües residuals, l'ús de tècniques com la combinació de l'intercanvi iònic (p.ex. zeolita) i els sistemes de membranes de contacte (p.ex. líquid-líquid) (Figura 1.3) són tecnologies prometedores estudiades actualment (Sancho et al., 2017; Beckinghausen et al., 2020; Reig et al., 2021; Jang et al., 2022). La zeolita és un mineral porós natural que té una adsorció preferent (i reversible) per l'amoni per la seva alta capacitat d'intercanvi catiònic i alta selectivitat. Les zeolites carregades són regenerades usant solucions de NaOH o NaCl, obtenint concentrats rics d'amoni/amoníac, els quals són l'alimentació per al sistema de membranes de contacte. Aquest és un instrument que intercanvia molècules de gas dissolt entre els dos costats d'una membrana hidròfoba, permetent la transferència de l'amoni/amoníac a una solució d'stripping (p. ex. àcid nítric o sulfúric) aconseguint una sal d'amoni líquida (p. ex. nitrat d'amoni o sulfat d'amoni).

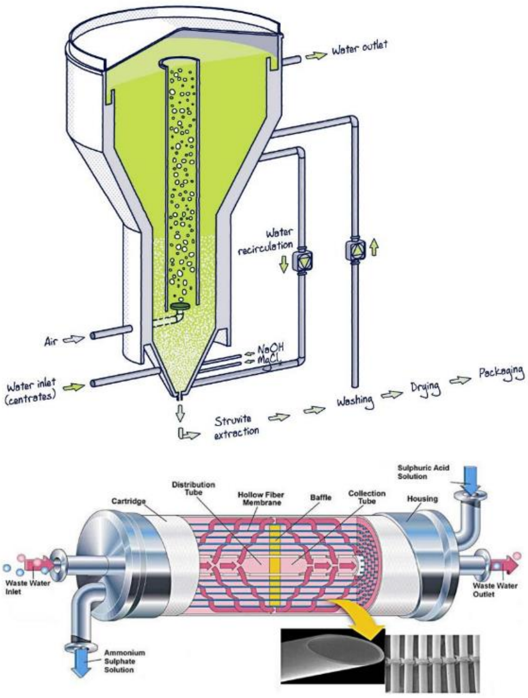

Figura 1.3. Esquema del reactor de precipitació/cristal·lització que permet la recuperació del P de les aigües residuals en forma d'estruvita (imatge superior). Esquema del contactor de membrana líquid-líquid (LLMC de les sigles en anglès) on a través del contacte entre dues fases líquides i una membrana hidròfoba, les espècies químiques son transportades per difusió, permetent la recuperació de N en forma de sals d'amoni (imatge inferior). Font: Projecte LIFE-ENRICH

1.2.3. Productes recuperats com a fertilitzants i normativa

L'ús com a fertilitzants de l'estruvita i les sals d'amoni obtinguts de la valorització de residus, com les aigües residuals, els llots de depuradora o purins, suposen un clar exemple d'agricultura circular i responen als objectius fixats en el Pla d'acció per a una economia circular a Europa (CE, 2015). Aquest últim estableix el següent respecte dels nutrients reciclats i el seu ús com a fertilitzants:

"Els nutrients reciclats són una categoria diferent i important de les matèries primeres secundàries, respecte de les quals cal elaborar normes de qualitat. Són presents en els residus orgànics, per exemple, i es poden retornar al sòl com a fertilitzants. El seu ús sostenible en l'agricultura redueix la necessitat de fertilitzants sintètics, la producció dels quals té efectes negatius per al medi ambient i, en el cas del P, depèn de la importació de roca fosfatada, un recurs limitat, és a dir, no renovable.

Fins fa poc, la circulació de certs fertilitzants compostos per nutrients reciclats era obstaculitzada per la regulació i les normes mediambientals i de qualitat diferents entre els estats membres. Per fer front a aquesta situació, la Comissió Europea va proposar una revisió del Reglament de la UE relatiu als fertilitzants. Es tracta de noves mesures a escala de la UE per facilitar el reconeixement dels fertilitzants orgànics i en base de residus, fomentant així el desenvolupament sostenible d'un mercat a escala de la UE".

A data 25 de juny de 2019 es va publicar en el Diari Oficial de la Unió Europea el nou Reglament (UE) 2019/1009 del Parlament Europeu i del Consell (EU, 2019), per la qual cosa s'estableixen criteris relatius a la posada a disposició en el mercat europeu dels productes fertilitzants i es modifiquen els Reglaments (CE) 1069/2009 i (CE) 1107/2009 i es deroga el Reglament (CE) 2003/2003. El nou Reglament 2019/1009 estableix requisits i normes d'alta qualitat i seguretat sobre els productes fertilitzants i la seva comercialització en tot el territori de la Unió Europea.
Entre altres objectius, el Reglament pretén establir condicions harmonitzades per a la disposició en el mercat dels fertilitzants produïts a partir de materials reciclats o orgànics, amb la finalitat de promoure un major ús dels nutrients reciclats. Alguns dels nous productes fertilitzants recuperats inclosos en el Reglament són l'estruvita (i altres sals fosfatades precipitades), el biochar i productes a base de cendres. El reglament inclou requisits per als residus utilitzats com el material de base en l'operació de valorització, també les tècniques i processos de tractaments, així com criteris de qualitat per als productes fertilitzants resultants -amb la finalitat de garantir que l'ús d'aquests productes no causin efectes negatius generals per al medi ambient o la salut humana. Per tant, a partir del moment de la conformitat amb tots els requisits del Reglament 2019/1009, aquests productes deixen de considerar-se residus, podent accedir al mercat de fertilitzants. Recentment, el reglament d'agricultura ecològica de la UE ha estat modificat (UE 2023/121) per autoritzar l'ús com a fertilitzant de les sals fosfatades precipitades recuperades, sota les condicions de l'esmentat reglament 2019/1009.

L'**estruvita** (Figura 1.4), producte descrit anteriorment, té un alt potencial com a alternativa als fertilitzants fosfatats degut: i) a l'elevada puresa del producte en la seva producció; ii) alt contingut nutricional en forma de fòsfor, nitrogen i magnesi, macronutrients rellevants en el procés de la nutrició vegetal; iii) elevat valor d'eficiència agronòmica en un rang ampli de sòls i medis de cultiu; iv) baixa salinitat i reduït contingut en metalls pesants. Un estudi comparatiu de les estruvites produïdes comercialment a la UE destaca la idoneïtat de la majoria de productes per entrar en el mercat de fertilitzants, essent Holanda (35-43%), Bèlgica (16-20%) i Alemanya (15%) els principals productors actuals d'estruvita a Europa (Muys et al., 2021). De les 80 plantes existents de producció d'estruvita estimades mundialment, 39 són identificades a la UE (Spiller et al., 2022).
A més, la seva solubilitat en aigua (constants de solubilitat de $4.37 \cdot 10^{-14}$ a $3.89 \cdot 10^{-10}$ (Rahaman et al., 2006)), baixa comparada amb fertilitzants convencionals (totalment solubles) i alta comparada amb fosfat de calci (constant de solubilitat de 2.07×10^{-33}), fan que l'estruvita, aplicada en estat sòlid, es comporti com un fertilitzant de lent alliberament o alliberament

controlat, subministrant els nutrients a la planta de forma gradual i reduint la seva susceptibilitat a pèrdues en condicions intenses de precipitació, etc. No obstant, sota condicions acídiques, com l'entorn creat pels exsudats de les arrels (p.ex. citrat, malat, oxalat), la dissolució de l'estruvita es veu incrementada, fet que podria millorar el moment de l'alliberament dels nutrients (Talboys et al., 2016).

Diversos meta-anàlisis (Huygens et al., 2018; Ahmed et al., 2018; Hertzberger et al., 2020, Möller et al., 2018) han estudiat l'eficiència agronòmica de l'estruvita com a fertilitzant d'alliberament controlat en diversos cultius, pH i textura del sòl, mida de partícula, origen del residu, etc. En general, s'han obtingut resultats satisfactoris de l'ús de l'estruvita com a fertilitzant d'alliberament controlat, obtenint rendiments i absorcions de P similars als fertilitzants convencionals comercials, generalitzables entre tipus de sòls i de cultius rellevants per a l'agricultura europea (Huygens et al., 2018; Möller et al., 2018). Per exemple, Gonzalez-Ponze et al. (2009), Cabeza et al. (2010), Gell et al. (2011), Liu et al. (2011), Antonini et al. (2012), Uysal et al. (2014), Cerrillo et al. (2015) i Robles-Aguilar et al. (2020) en els seus estudis amb cultius hortícoles i extensius (blat de moro, tomàquet i enciam) en diferents sòls i substrats, avaluen positivament l'ús d'estruvita, aplicada en sòlid, enfront un fertilitzant fosfatat comercial.

Malgrat això, Hertzberger et al. (2020) destaca una major absorció del fòsfor en sòls amb pH inferior a 6, minvant la resposta del cultiu, sobretot la concentració de P en el teixit vegetal, amb l'increment del pH. Aquest fet podria ser important a Europa, on la majoria de pH són superiors a 6 (Reuter et al., 2008). Existeix una controvèrsia en aquest aspecte, ja que a més, la mida de partícula de l'estruvita i el mètode d'aplicació (barrejat o en superfície), promouen diferències en la seva dissolució (Ahmed et al., 2018). Per un costat, Achat et al. (2014) mostren que el P intercanviable isotòpicament de l'estruvita molta és similar al triple superfosfat en rang de pH de 5.2 a 8.1 i Talboys et al. (2016) prova una dissolució de l'estruvita granulada a curt termini (<42 dies) similar al control en un rang de pH de 5 a 8. D'altra banda, Degryse et al. (2017) indica una dissolució de l'estruvita granulada, en un període de 60 dies, superior al 80% en un sòl àcid (pH 5.9), però inferior al 10% en un sòl bàsic (pH 8.5); no es mostraven diferències quan l'estruvita era molta. Altres estudis mostren, en cultiu de blat en contenidors amb sòls de pH 5.3 (Rech et al., 2019) i en cultius de *Lolium multiflorum* en sòls alcalins (Meyer et al., 2018), eficiències relatives de l'estruvita granulada i molta, respectivament, del 75%, comparada amb fertilitzants fosfatats solubles comercials.

Tot i que la majoria d'estudis s'han centrat en el potencial de l'estruvita com a fertilitzant de lenta alliberació aplicada en sòls, Arcas-Pilz et al. (2021, 2022) han estudiat el seu ús en forma granulada en cultiu hidropònic de mongetera, pebrot i enciam, obtenint rendiments comparables als fertilitzants convencionals a més d'una reducció de la majoria de categories ambientals, com ara l'eutrofització de les masses d'aigües.

Tanmateix, **l'ús d'estruvita com a matèria primera per a la fabricació de solucions nutritives líquides per a fertirrigació, no ha estat estudiat anteriorment.**

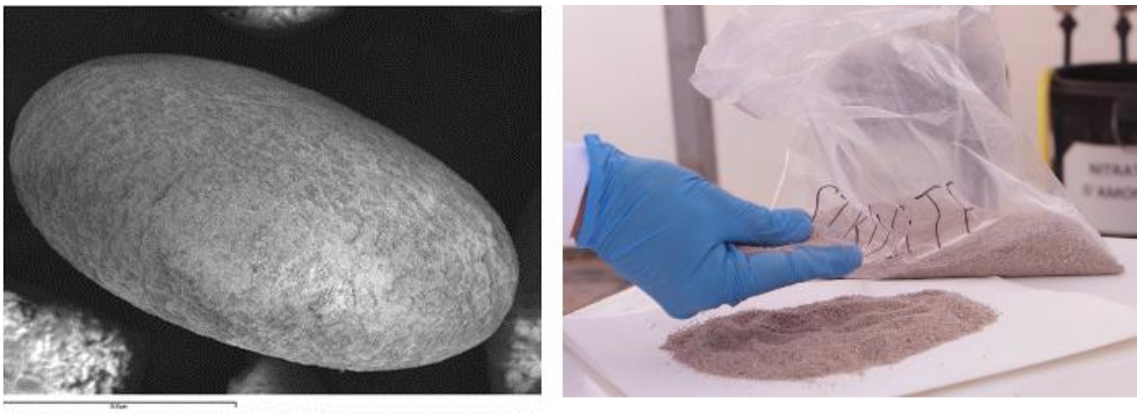

Figura 1.4. Ampliació X100 de mostra d'estruvita granulada (esquerra) i estruvita molta per a ser dissolta en la solució nutritiva (dreta). Font: Projecte LIFE-ENRICH

Respecte a les **sals d'amoni recuperades d'EDARs**, existeix força investigació sobre els aspectes tecnològics per a la recuperació del nitrogen, però els productes resultants, la seva qualitat i el seu potencial ús com a fertilitzants han estat poc discutits. A més, **no hi ha estudis descrits sobre l'ús d'aquests productes com a matèries primeres de solucions nutritives per fertirrigació.**

Actualment, les sals d'amoni recuperades, com el nitrat d'amoni (NA), són catalogades per la Comissió Europea com a productes d'alta prioritat pel seu potencial de substitució dels fertilitzants sintètics nitrogenats, tot i que l'acceptació legal d'aquests productes dependrà de l'evidència científica, en la caracterització i rendiment d'aquests fertilitzants alternatius (Sigurnjak et al., 2019). Així mateix, en ser generats amb aire ric en amoníac, el seu contingut en carboni o contaminants hauria de ser mínim.

Per afegiment, Beckinghausen et al. (2020) destaca que, mundialment, la majoria de tècniques de recuperació produeixen sulfat d'amoni (SA) en forma cristal·lina o en solució a causa del menor preu de l'àcid sulfúric front a l'àcid nítric. Però, tenint en compte el tipus de fertilitzants majoritàriament usats (urea, nitrat d'amoni i calci sintètics), el SA constitueix un petit percentatge, considerant necessària la comunicació entre productors i usuaris finals, per conèixer el mercat real. A més, l'ús de NA incrementa la concentració de N en el producte recuperat, tot i ser força variable (Sigurnjak et al., 2019). Altrament, un meta-anàlisis basat en la gestió de nutrients, suggereix que el nitrat i el fosfat d'amoni són els fertilitzants, a part de la urea, més efectius en reduir la volatilització d'amoníac després de la seva aplicació (Pan et al., 2016).

Assajos realitzats amb NA recuperat en enciam i blat de moro (Sigurnjak et al., 2019) i espinacs i rave (Rodrigues et al, 2022), ambdós realitzats en sòls naturals, mostren un efecte similar en el rendiment del cultiu i en el risc de lixiviació de nitrats, comparat amb fertilitzants nitrogenats sintètics.

Així mateix, Robles-Aguilar et al. (2022) van preparar barreges de fertilitzants de fonts reciclades de P i N com l'estruvita, estruvita de potassi, SA i NA pel cultiu de *Viola x wittrockiana L*. en contenidor -emprant torba com a substrat. Els seus resultats mostren que el NA afavoreix el creixement saludable i concentracions òptimes de N en la planta. En canvi, la majoria de barreges amb el SA augmenten l'amoni en el medi de cultiu a una concentració limitant per al creixement de l'espècie vegetal emprada.

Així doncs, la integració d'aquests fertilitzants recuperats en fertirrigació i l'estudi de barreges òptimes, té un elevat ús potencial per la gran extensió de cultius sota aquest maneig, l'actual context de la promoció d'una economia circular a Europa, la recent publicació de la nova normativa europea sobre fertilitzants i l'acceptació social d'aquests productes (Tur-Cardona et al., 2018).

1.3. Les sinèrgies entre els sectors de l'aigua i l'agricultura: El projecte LIFE ENRICH

Davant del context anteriorment descrit, neix el projecte LIFE-ENRICH (Enhanced Nitrogen and phosphorus Recovery from wastewater and Integration in the value Chain, LIFE16ENV/ES/000375), a partir del qual s'ha desenvolupat aquesta tesi doctoral.

L'objectiu general del LIFE-ENRICH era implementar el concepte d'economia circular mitjançant la demostració de tota la cadena de valor per a la recuperació de nutrients a les estacions depuradores d'aigües residuals i la seva posterior reutilització per a la valoració de productes fertilitzants. La figura 1.5 mostra de manera senzilla i gràfica la situació actual del sector del tractament d'aigües residuals i el sector de l'agricultura (producció de fertilitzants). És evident que avui en dia ambdós sectors són independents entre si. Mentre les EDAR descarreguen nutrients en el medi ambient (rius, llots deshidratats i atmosfera), la indústria dels fertilitzants consumeix grans quantitats de nutrients procedents de reserves naturals o de processos de fabricació complexos. El projecte tenia com a objectiu abordar diversos problemes ambientals, relacionats amb les diferents etapes del procés, des de la recuperació de matèries primeres fins a la creació del producte final i la seva utilització per a activitats agrícoles.

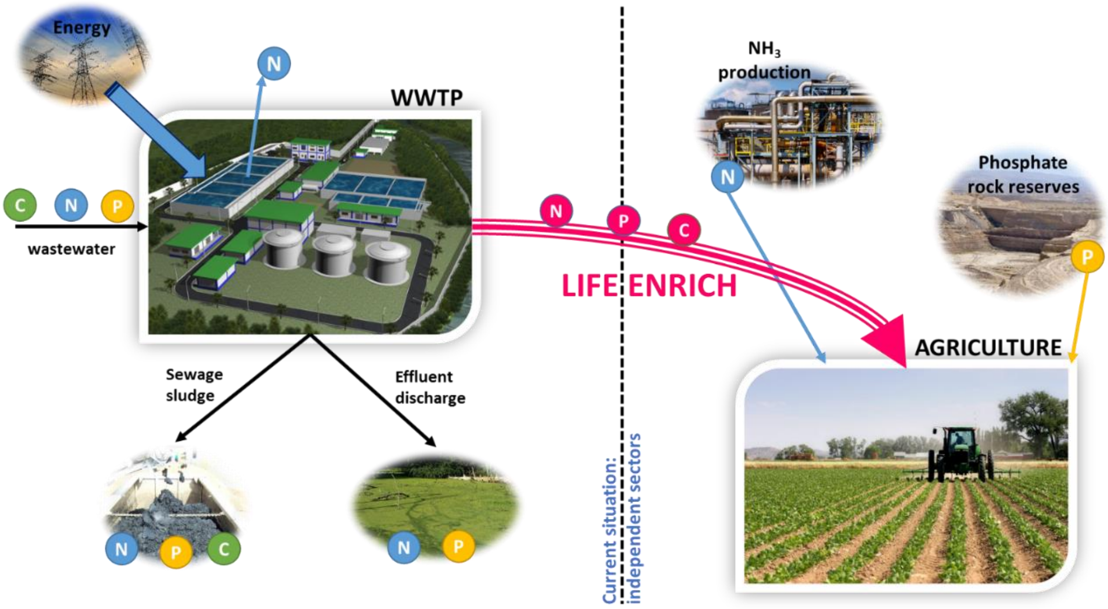

Figura 1.5. Problemes ambientals més rellevants en els que es va centrar el projecte LIFE-ENRICH

Els problemes mediambientals més rellevants en els que es va orientar el projecte es representen en la figura 1.5 i són els següents: i) Gran quantitat d'impactes en la producció i eliminació de fangs de depuradora; ii) Risc d'eutrofització pels nutrients presents en els efluents abocats; iii) Petjada de carboni de la producció i ús de fertilitzants químics sintètics; iv) Esgotament de les reserves de roca fosfatada.

El LIFE-ENRICH va treballar en tota la cadena de valor: la recuperació de nutrients a l'EDAR, la caracterització dels productes obtinguts, la barreja òptima dels mateixos per obtenir fertilitzants de valor afegit i la validació del seu rendiment en casos reals. A més, paral·lelament va dissenyar el model de negoci adequat per tal de garantir que les solucions proposades estiguin llestes per a ser transferides i replicades en altres llocs/regions.

La recuperació de nutrients es va aconseguir integrant diferents tecnologies innovadores a l'EDAR de Murcia Este. La recuperació de N s'ha basat en l'adsorció d'amoni en zeolites combinades amb contactors de membrana. El N es recupera com a sal, amb una capacitat de produir fins a 40L/dia d'una dilució amb 10-20% de N. La recuperació de P s'ha assolit mitjançant l'elutriació de P a gran escala seguida d'una unitat de cristal·lització pilot que produeix al voltant de 5 kg/dia d'estruvita. La combinació d'ambdós productes s'ha avaluat en dos tipus d'assaigs diferents: proves de camp a l'aire lliure (realitzat per l'empresa Aigües del Segarra Garrigues a Agramunt i Castelldans) i proves de petita superfície en condicions controlades sota hivernacle (realitzat per l'Institut de Tecnologia Agroalimentària (IRTA) i inclosos en aquesta tesi doctoral).

Capítol 2.

Objectius

Aquesta tesi doctoral pretén aportar innovació i coneixement per augmentar la circularitat i sostenibilitat dels sistemes hortícoles mitjançant l'ús eficient de macronutrients (fòsfor i nitrogen) recuperats de corrents d'aigua residual urbana, en forma d'estruvita i nitrat d'amoni, com a font de fertilitzants alternatius.

Aquesta tesi doctoral s'estructura, segons els objectius, en diversos capítols, connectats entre si per aconseguir l'objectiu principal: **El maneig sostenible dels productes recuperats d'aigües residuals urbanes, estruvita i nitrat d'amoni, com a fertilitzants alternatius en sistemes de fertirrigació de cultius hortícoles, i l'estudi del seu impacte, des d'una visió agronòmica, ambiental i microbiològica.**

Els objectius específics de la tesis doctoral són els següents:

1) Realitzar una revisió dels criteris de qualitat i eficiència agronòmica dels productes fosfatats recuperats pel seu ús com a fertilitzants alternatius (Capítol 3).

2) Establir una metodologia per a l'ús d'estruvita i nitrat d'amoni recuperats d'aigües residuals urbanes en sistemes de fertirrigació i avaluar la seva resposta en:
   i) L'eficiència agronòmica sobre el rendiment i la qualitat del cultiu;
   ii) L'impacte ambiental associat a la lixiviació de nitrogen i fòsfor; a la generació de gasos d'efecte hivernacle (N_2O i CO_2); i/o a la composició i evolució de les comunitats microbianes de la rizosfera

   en:
   - El cultiu de tomàquet en substrat sense sòl (perlita) (Capítol 4 & 5)
   - La rotació de tres cultius hortícoles en sòl (Capítol 6).

3) Estudiar l'impacte de l'aplicació de les diferents estratègies de fertirrigació en la composició i diversitat de la microbiota (bacteris i arquees) de la rizosfera, i la seva relació amb les emissions de N_2O, sota la influència de la fertirrigació, de diferents ràtios de $N\text{-}NH_4^+$:$N\text{-}NO_3^-$ en la composició de la solució nutritiva i l'efecte de la presència/absència de planta, en un cultiu sense sòl (perlita) de tomàquet (Capítol 5).

El maneig plantejat i avaluat en aquesta tesi, a més de reduir el impacte ambiental associat a l'ús de fertilitzants recuperats substituint als minerals sintètics, tant a la seva producció, transformació i transport, pretén promoure la millora de l'eficiència en l'ús dels nutrients aplicats als cultius, contribuint a minimitzar la lixiviació i conseqüent eutrofització de les masses d'aigua, la generació d'emissions de N_2O, principal causant de l'esgotament de la capa d'ozó, i la pèrdua de biodiversitat microbiana.

Capítol 3

Recovery of Phosphorus from Waste Water Profiting from Biological Nitrogen Treatment: Upstream, Concomitant or Downstream Precipitation Alternatives

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Magrí A, Carreras-Sempere M, Biel C, Colprim J. 2020. Recovery of Phosphorus from Waste Water Profiting from Biological Nitrogen Treatment: Upstream, Concomitant or Downstream Precipitation Alternatives**.** Agronomy, *10*, 1039.
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3.1. Introduction

Phosphorus (P) is an essential element for all living organisms as a constituent of nucleic acids, energy-transfer molecules in metabolism, cell membranes, and body building blocks. Phosphorus is also an irreplaceable nutrient, non-manufacturable nor destroyable. Its shortage limits crop growth and agri-food production (Cordell&White, 2011). Yet, when P is discharged in excess to aquatic and terrestrial ecosystems, it acts as a pollutant, causing eutrophication and nutrient imbalances (Penuelas et al., 2009). Phosphorus has no significant gaseous phase, so it cannot flow freely in the atmosphere. This is in contrast with the nature of the other essential elements supporting life—carbon (C), nitrogen (N), oxygen (O) and hydrogen (H). Nowadays, mined phosphate rock is the largest source of P for use in both agriculture and industry. However, this is a finite resource irregularly distributed around the world. This means that P availability is linked to geopolitical considerations that may lead to uncertainties about supplies (Cordell et al., 2009; Gilbert, 2009). In this regard, the creation of (inter)national strategic P reserves has already been suggested to stabilise commodity prices (Elser&Bennet, 2011)) and the European Union (EU) has identified phosphate rock and P as two of the 27 critical raw materials of high importance to the EU economy and of high risk associated with their supply (EC, 2017).

As an alternative to mined phosphate rock, organic waste and waste water are renewable sources of P, typically available at the local scale. The P present in these waste streams is chemically or organically bound—i.e., forming complex molecules—or dissolved as orthophosphate. The recovery of P from secondary streams and its subsequent reuse, either directly or after intermediate processing, represent a major opportunity for exploiting new and more sustainable pathways for producing P fertilisers. Phosphorus has no substitute but can be reused continuously, and thus, it is a good example of a critical resource that can be utilised more efficiently in the circular economy framework to support sustainable growth with less pollution (Withers et al., 2015). Methods potentially applicable for P recovery from waste water and organic waste have been reviewed elsewhere (Rittmann et al., 2011; Desmidt et al., 2015; Egle et al., 2016).

Among the procedures allowing for P recovery from waste streams, chemically induced crystallisation/precipitation/mineralisation of the already dissolved phosphate in the form of low soluble salts is one of the most common alternatives. Precipitation is achieved by appropriately supplying metal ions to the liquid phase, typically magnesium (Mg^{2+}), to form magnesium phosphate minerals (MgP) (Snoeyink&Jenkins, 1980; Le Corre et al., 2009; Kataki et al., 2016); calcium (Ca^{2+}) to form calcium phosphate minerals (CaP) (Song et al., 2002; Law&Pagilla, 2018); or iron (Fe^{2+}) to form iron phosphate minerals (FeP) (Wilfert et al., 2015, 2018; Wang et al., 2018; Wu et al., 2019; Prot et al., 2020). In waste water treatment plants (WWTPs), this kind of process can be implemented at different locations (Desmidt et al., 2015; Cornel&Schaum, 2009) in order to foster resource recovery by producing a specific P-rich stream while meeting water quality standards of the receiving water bodies. Additionally, these processes may also involve additional benefits linked to overall plant performance and energy balance such as: (1) prevention of uncontrolled formation of scale deposits in pipelines and recirculation pumps (Borgerding, 1972; Buchanan et al., 1994; Doyle&Parsons, 2002; Sharp et al., 2013); (2) improvement in sludge dewaterability, allowing for producing a dryer solid product, which is advantageous from the point of view of subsequent transport and thermal processing (Geerts et al., 2015; Marchi et al., 2015); (3) reduction in P backflows (Marchi et al., 2015; Ueno&Fujii, 2001).

Biological N removal (BNR) is typically applied to reduce the ammonium (NH_4^+) content in waste water through its transference to the atmosphere as dinitrogen gas (N_2). Origin of the waste water—either from municipal, industrial or agricultural sources—will determine its composition, and thus, treatment particularities. In this context, treatment based on the combination of autotrophic nitrification—aerobic oxidation of ammonium to nitrite (NO_2^-) (i.e., nitritation), and subsequently, to nitrate (NO_3^-) (i.e., nitratation)—, plus heterotrophic denitrification—anoxic reduction of nitrate to nitrite, and finally, to N_2 (NDN)—, has commonly been considered. In recent years, the fully autotrophic treatment, based on the combination of partial nitritation (i.e., 57%) and anaerobic ammonium oxidation (anammox) (PNA), is attracting the interest of the water industry as a more energy efficient strategy (Van Hulle et al., 2010; Magrí et al., 2013). Hence, the number of new PNA facilities operating worldwide is increasing fast (Lackner et al., 2014; Kumwimba et al., 2019). Some encouraging reasons are the lower energy demand for aeration in partial nitritation and there being no need for an organic C source in anammox (which favours its integration with the anaerobic digestion process to produce biogas as a renewable energy source). Alternatively, nitrification can be applied individually in view of converting ammonium to nitrate, thereby mitigating ammonia (NH_3) emission from storage facilities and enabling N recovery from high loaded waste water streams (Ndegwa et al., 2008; Fumasoli et al., 2016). Other biological N treatment processes based on its assimilation and immobilization and aiming at the recovery of N products, such as single cell proteins, amino acids, and protein-rich aquaculture plants (e.g., duckweed and algae), are out of the scope of this work.

Phosphate precipitation can be applied before (upstream configuration), together with (concomitant configuration), and after (downstream configuration) biological N treatment; this is chemically induced as a conditioning pre-treatment, biologically induced inside the reactor, and chemically induced as a refining post-treatment. The aim of this contribution is to perform a review, in view of producing materials easily usable as green fertilisers, of the quality criteria and agronomic performance for the products obtain trough the different alternatives for P recovery from waste water flows when biological N treatment is coupled with phosphate precipitation. Particular attention is given to the analysis of the implications of the sequence in which biological treatment and phosphate precipitation are combined. The potential requirements to be complied by the recovered P-rich materials in order to be covered by the EU fertiliser regulation will also be overviewed.

3.2. Use of recovered phosphate products

The use of the recovered phosphate salts as fertilisers must promote plant growth and nutrient uptake without leading to overall adverse environmental or human health impacts (Huygens&Saveyn, 2018). Recovered MgP products (i.e. MAP) have been proved as equivalent to regular mineral P fertilisers and more effective than recovered CaP products (Johnston et al., 2003; Römer&Steingrobe, 2018). Besides, recovered CaP products are very similar in composition to the mined phosphate rock, so they can be mixed with ores at the beginning of an industrial P production process (Schipper et al., 2001). Currently, precipitated phosphate salts are not typified in the European fertiliser regulation, and this fact limits marketability.

3.2.1. Quality criteria for the precipitated phosphate salts to be covered by the EU fertiliser regulation

In the EU, those materials used as fertilisers must comply with the requirements stated by the fertilising products regulation (EU) 2019/1009 (EU 2019). Currently, the precipitated phosphate salts, as well as other derivate products, are not typified as Component Material Category (CMC) within this regulation. Nonetheless, the topic is on progress. The requirements to be complied by these materials in view of their possible inclusion in an updated version of the rule are overviewed below, according to the criteria fixed by the Joint Research Centre (JRC) of the European Commission (Huygens et al., 2019), who is in charge of establishing a regulatory framework, enabling the production of fertilising products. In this regard, to be accepted as EU fertiliser, the recovered precipitated phosphate salts will have to comply with the requirements laid down for a new CMC *"precipitated phosphate salts and derivates"* as well as with those for the already stablished Product Funtion Category (PFC) *"1C-Inorganic fertilisers"*. The newly proposed CMC *"precipitated phosphate salts and derivates"* aims to cover a wide range of phosphate-based compounds of high purity produced through a precipitation process with the intention to be used as fertilising materials, or as intermediates in the manufacturing processes of P-fertilisers, and which are sufficiently effective at providing P to plants. The technical proposal for the inclusion of precipitated phosphate salts and derivates in the (EU) fertilising products regulation includes the following items: (1) input materials and reagents; (2) production process conditions; (3) agronomic value; (4) environmental and human health aspects; (5) physicochemical properties; and (6) handling and storage. As a fertilising product, the use of this material must not lead to overall adverse environmental or human health impacts and provide plants/mushrooms with nutrients or improve their nutrition efficiency, either on its own or mixed with another material, among other market aspects.

As input materials to the precipitation process, common waste water flows to be taken into account are main- and side-streams in municipal WWTPs and effluents from food processing. Animal manure liquid fractions have also been considered as promising input materials (Ginger-Santonja et al., 2017). Concerning the production process, it has been suggested to differentiate between those facilities specifically designed to produce fertilising materials and those others where the precipitated phosphate salts are recovered as a by-product resulting from a process aimed at producing different primary outputs (e.g. energy and treated water) as long as the material quality criteria are fulfilled. The agronomic value of the precipitated phosphate salts has been proposed not to be assessed on the basis of the minimum extractable P content (due to the misinformation on the agronomic efficiency), which is in line with the criterion used for other CMCs. However, it was suggested to label the ratios water-extractable P to total P, and acid-extractable P to total P. Recommended minimum P solubility values are as follows: 25% in water, 30% in neutral ammonium citrate, and 35% in formic acid.

From the point of view of environmental and human health, and regarding the presence of inorganic, organic and biological toxic elements, safety must be assured by a combination of requirements in the production process and parameter assessment on the precipitate. Most input materials intended for P recovery through precipitation have high contents of inorganic and organic (micro)pollutants (Boxall, 2012), which can be transferred to the precipitate, usually adhered onto the organic matter (Lou et al., 2018; Ye et al., 2018). Yet, available data concerning presence of contaminants in the precipitates, which have often been measured for high-purity precipitated phosphate salts with low organic C content (TOC), tend to indicate safe levels (Huygens et al., 2019; Uysal et al., 2010). To prevent risks associated with particular organic (micro)pollutants (e.g. flame retardants, pharmaceutical compounds), it has been proposed to limit the organic C content in precipitated phosphate salts to a maximum of 3% of the dry matter content (Huygens et al., 2019; STOWA, 2015). By applying satisfactory operational conditions during the production process and considering a maximum threshold for the organic C content, the risk associated to the accumulation of microbial pathogens in the precipitate will be reduced. In turn, this may help reducing the risk of appearance of microbial resistances in the agrifood chain. Standard microbial testing is proposed, considering as limit values the absence of *Salmonella spp*. in a 25 g sample and 1000 CFU g^{-1} fresh mass (CFU is colony-forming unit) for *Escherichia coli* or *Enterococcus*. Additional tests would be required when manure or municipal waste water were used as input material for the production process, ensuring absence of viable *Ascaris* sp. eggs in a 25 g fresh mass and concentrations of *Clostridium perfringens* (as a spore-forming bacteria) below 100 CFU g^{-1} fresh mass. Potentially, the precipitated phosphate salts may contain significant levels of trace elements, including heavy metals and metalloids, when such elements are available in the water flows under processing (Eriksson, 2001; Gendebien et al., 2001). When managing biosolids in WWTPs, organic C has commonly been shown as an important metal-sorption phase (Karvelas et al., 2003). Thus, low organic contents in the precipitate may also help reducing associated metal/metalloid levels. Since typical levels of these contaminants (Ewert et al., 2014) are one or two orders of magnitude below the limit values fixed by the EU fertiliser regulation for the PFC level "*inorganic macronutrient fertiliser*" (EU, 2019) - i.e. cadmium (Cd): 60 mg kg^{-1} P_2O_5; chromium (Cr) VI: 2 mg kg^{-1} dry matter; nickel (Ni): 100 mg kg^{-1} dry matter; lead (Pb): 120 mg kg^{-1} dry matter; arsenic (As): 40 mg kg^{-1} dry matter; copper (Cu): 600 mg kg^{-1} dry matter; zinc (Zn): 1500 mg kg^{-1} dry matter- it has not been suggested to include additional specific limit values for metals and metalloids, regardless of the input material.

In relation to the physicochemical properties of the precipitated phosphate salts, the regulation proposal for the new CMC have mostly concerned on the total P content, the micronutrients content (Al and Fe), and the presence of physical impurities. Thus, the precipitated salts have been suggested to have a total P content of 16% (P_2O_5 equivalent) or more, on dry weight basis. According to available data from running plants (Huygens et al., 2019), a 20% P_2O_5 limit value seems an achievable target. Moreover, the assessed material should contain less than 10% dry matter of Al plus Fe (elemental forms), assuming a Fe/P molar ratio not compromising plant P-availability (Kahiluoto et al., 2015). Finally, visually detectable physical impurities (above 2 mm) should be less than 0.3% dry matter and total macroscopic impurities less than 0.5% dry matter, in line with the provisions stablished for other CMCs (EU, 2019). As handling and storage conditions, it is proposed to avoid physical contact between input and output materials in the production plant after the precipitation process and to store the precipitated phosphate salts in dry conditions. All the above referred considerations seek ensuring appropriate material quality and chemical composition for the intended functions of the fertilising materials.

3.2.2 Expected characteristics of the precipitated phosphate salts depending on the production process

Overall, the characteristics of the recovered end-products are strongly dependent on the input material and the precipitation process applied (Hao et al., 2013; Kratz et al., 2019). Although final decision on the criteria to be fulfilled in order to include precipitated phosphate salts in the EU fertilising products regulation (EU, 2019) is still pending, characteristics of these materials must be aligned with the above mentioned quality criteria. Thus, the main aspects to be specified will likely be related to the environment, human health and the physicochemical properties, and focused on features such as the organic C content, toxicity limit values (concerning both chemical and biological agents), and particular contents of P, Al, Fe and physical impurities.

When P precipitation is conducted before biological N treatment (upstream configuration), phosphate is frequently crystallised heterogeneously within a wet sludge. Depending on the waste water source, the resulting product may contain a high amount of impurities including solid particles, organic C –easily above the aforementioned 3% dry matter– and heavy metals. The availability of organic C (mostly if biodegradable or not previously digested) will condition the later handling and storage of this P rich material. Simultaneous phosphate precipitation and biological N treatment (concomitant configuration) is still under development and not applicable at the large scale, but it is known that frequently leads to the formation of a heterogeneous precipitate mixed with organic C (basically constituted by the microbial cells catalysing the process). Although the nature of this organic C will be different from the case of the upstream configuration, organic contents will be still high. Washing and drying the recovered solids may help improving their characteristics in view of fitting the requirements needed for a marketable fertiliser product (Ewert et al., 2014). When P precipitation is applied after biological N treatment (downstream configuration), the resulting product will have a higher purity and contain less organic C. Risk of chemical and biological toxicity will likely be reduced due to the lower availability of organic matter, potential biodegradation and adsorption phenomena occurring within the bioreactor and the alkaline environment applied to achieve P precipitation, which will promote pathogens destruction (Vanotti et al., 2018; Vanotti et al., 2005) (Table 3.1).

Table 3.1. Major expected characteristics for the recovered precipitated phosphate salts depending on the production process configuration (upstream, concomitant and downstream alternatives)

Characteristics	Upstream	Concomitant	Downstream
Purity	Presence of solids and other compounds will disturb crystallisation and affect purity of precipitate	Heterogeneous precipitate	Increased effective ion availability favoring crystal formation
Organic content	High	High	Low
Toxicity	Potential presence of metals, organics and pathogens depending on the waste water origin	-	Pathogen destruction depending on process pH
P form (most probable)	MAP and CaP. Carbonate forms may co-precipitate together with phosphates	CaP and MAP	Absence of ammonium will favor formation of alternative MgP salts such as MPP

It is important to ensure that any treatment alternative to be implemented at the full-scale will be able to produce valuable materials according the upcoming definition for the CMC *"precipitated phosphate salts and derivates"*. The aim of the EU regulation is to cover a wide range of phosphate-based compounds of high purity, sufficiently effective at providing P to plants, while reducing the risk of contamination by the long-term application to agricultural soils (Weissengruber et al., 2018). Thus, any material that fulfil the proposed quality criteria should be considered. The data summarised in the previous sections of this review indicate the potential occurrence of a wide range of mineral forms in the end-products of the precipitation processes (Table 3.1). Most of the time, the precipitated P is in the form of struvite, calcium phosphate or a mixture of Ca- and Mg-salts (Kratz et al., 2019). The form, in which P is combined with other elements to form the precipitated phosphate salts, has a strong effect on the later accessibility to P for plants.

3.2.3. Agronomic efficiency achieved when using precipitated phosphate salts

Plants can only absorb P if it occurs in specific chemical forms, the most relevant of which is orthophosphate ($H_2PO_4^-$, HPO_4^{2-}, and PO_4^{3-} as soluble ions). However, in soils, only a small portion of the total P is available as PO_4 dissolved in the soil solution, while the largest amount is bound to chemical compounds of different solubility. The amount of P dissolved, and thereby accessible for plant roots during the life cycle of a crop, is defined as plant-available P (Yli-Halla et al., 2016). Typical relative solubilities for different recycled fertilisers can be found in the literature, referring to the most frequently used extractant chemicals and according to the EU fertiliser regulation (EU, 2019). However, the highly variable chemical composition and structure of the P-compounds and other components available in the recycled material, together with its inherent characteristics (e.g. granule size), may affect the solubility in a given extracting chemical (Kratz et al., 2019; Cabeza, 2011), leading to an unsuitable assessment of the plant-available P. Besides dominant chemical form and structure, soil-plant-fertiliser interactions may also affect P release dynamics, and availability for crops, depending on: (1) soil properties (e.g. pH, texture, existence of potential P sorption sites); (2) plant characteristics (e.g. plant species, root traits, strategies for mobilising P from soil, temporary variations in nutrient demand); (3) fertiliser properties (e.g. crystal size, granule size, application method, presence of impurities, occurrence of co-precipitations); and (4) type of trial conducted (e.g. properties of the growing substrate, trial duration, pot / field trial, pot size, basic / multi- nutrient supply, weather conditions) (Degryse et al., 2017; Möller et al., 2018). Despite such a long list of factors potentially inducing variability when assessing recycled fertilisers efficiency, there are some common conclusions that can be distilled from the data available in the literature (Römer&Steingrobe, 2018; Huygens&Saveyn, 2018; Kratz et al., 2019; Möller et al., 2018). Otherwise, the agronomic efficiency of a fertiliser can be assessed with respect to a reference (or control) –i.e. relative agronomic efficiency (RAE)– on the basis of plant biomass yield (dry matter increase per unit of nutrient supply), or plant nutrient uptake (dry matter yield*biomass nutrient concentration) (Brod et al., 2015).

The objective for those materials that could be included in the newly proposed CMC *“precipitated phosphate salts and derivates”* is to supply P to plants as a macronutrient. Nowadays, precipitated salts such as (relatively pure) struvite, several forms of CaP, or a mixture of CaP + MgP are gaining relevance as a byproduct for agronomy. Struvite (MAP) is the most common precipitated phosphate salt, with a high potential to be marketed as a recycled nutrient source since its production is feasible according to a relatively high purity standard (only containing trace amounts of impurities), and also, it has a high P-content and a demonstrated value as a P-fertiliser (Johnston&Richards, 2003; Römer&Steingrobe, 2018; Antonini et al., 2012). Nonetheless, other CaP and MgP are also registered under the REACH Regulation (Regulation (EC) No 1907/2006) as fertilisers (ECA, 2017). Constituent molecules and ions, and how they are arranged in the recovered materials, have been proved to influence on the plant-available P. In this regard, P will be unavailable for crops if it is strongly bound to certain bi- and trivalent ions. Beyond plant nutrition, this aspect is also relevant because of the potential accumulation of P in soil at the long-term, and social aspects linked to farmer’s confidence and market acceptance of innovative P fertilisers derived from secondary raw materials.

Concerning the agronomic efficiency of the struvite as P fertiliser, published data show variable values for the RAE depending on the origin of the struvite, even when it has been tested under identical growing conditions (Cabeza, 2010; Wollman et al., 2018). As it has been mentioned above, this is probably because of the presence of co-precipitates and impurities, or the different sizing of crystals and product granules. Nonetheless, good agronomic performance has been reported for a wide range of soil pH values (Huygens&Saveyn, 2018; Möller et al., 2018; Hall et al., 2020), even though, in some cases, limited availability of P was found in basic soils (Degryse et al., 2017; Ackerman et al., 2013). It has also been suggested that the high agronomic efficiency of the struvite as P fertiliser is favored by naturally-occurring local acidifying processes in roots, the uptake of ammonium by the roots and the nitrification of ammonium to nitrate by the soil microbial community (El Diwani et al., 2007; Vaneeckhaute et al., 2016; Vogel et al., 2018). Moreover, several authors did not find significant differences in the RAE for other MgP (Johnton&Richards, 2003; Römer&Steingrobe, 2018; Huygens&Saveyn, 2018), showing better P availability than when considering recycled CaP.

The precipitation of CaP may involve the formation of different compounds. While mono- and di-calcium phosphates show the highest P availability for plants, the formation of compounds with an increasing Ca content in the binding forms –i.e. with higher values for the Ca/P molar ratio– generally results in lower values for P availability (Wang&Nancollas, 2008). In addition, the RAE of the CaP varies widely depending on factors such as the aggregation size, crystalline structure, cation content (e.g. Mg^{2+}) and presence of co-precipitates (Bauer et al., 2007). In this regard, several authors have obtained lower values for the RAE of the CaP than for the RAE of other conventional fertilisers, as well as of the struvite (Johnston&Richards, 2003; Römer&Steingrobe, 2018; Huygens&Saveyn, 2018; Möller et al., 2018; Wollman et al., 2018). Generally speaking, CaP tend to show better P availability for plants under (moderately) acidic conditions than under neutral or alkaline conditions since acidic environments favor the transformation of P into reactive forms (Cabeza et al., 2011; Wollman et al., 2018). In calcareous soils, the Ca present in the soil solution will tend to precipitate on the surface of the CaP granules (Meyer et al., 2018). After soil application, CaP can evolve and transform into more stable mineral phases (Arai&Sparks, 2007), which could potentially justify the wider range of RAEs observed for CaP than for struvite and other MgP (Huygens&Saveyn, 2018). According to the current state-of-the-art for technologies that seek to recover P from waste waters, it is difficult to guide the precipitation processes in a way that the formation of mono- and di-calcium phosphates become dominant (Vasenko&Qu, 2018). Thus, the CaP commonly formed are unsuitable for most of the soils in Europe, which pH value varies between 5 and 8 (Reuter et al., 2008), at least, in the short-term. Regarding FeP, they are not currently registered as fertilisers under the REACH Regulation (ECA, 2017). Ferric phosphates were initially proposed as end materials to be included in the newly proposed CMC but finally discarded due to limited testing of the agronomic value (i.e. concerns over the plant availability of Fe-complexed phosphates and risk of Al/Fe forms inducing plant toxicity have been reported) (Huygens et al., 2019). Yet, there may be potential for P recovery from waste water as precipitated FeP mineral salts, which could be used as new input materials or intermediates (Wilfert et al., 2015), or as alternative to other Fe fertilisers used to prevent Fe chlorosis (Diaz et al., 2010).

Indeed, all the precipitated phosphate salts can behave as multi-nutrient fertilisers since they contain a broad range of elements, including not only P, but also other nutrients for plants. This fact may help justifying the wide range of RAEs reported for these products. Nonetheless, those products recovered as MgP have been proved to supply equivalent amounts of plant-available P to other regular, high water-soluble, phosphate rock-based fertilisers, and to be more effective than other products recovered as CaP (Johnston&Richarards, 2003; Römer&Steingrobe, 2018; Huygens&Saveyn, 2018). It is challenging to distinguish between effective and ineffective fertilisers due to all the factors influencing nutrient availability and release dynamics. From an agronomic point of view, MgP (e.g. struvite) are a desirable product due to the higher amount of plant-available P that they provide and the independence of its dissolution with respect to the soil pH value. Moreover, those CaP products recovered as hydroxyapatite are very similar in composition to the mined phosphate rock (i.e. apatite-type ores) so they can be used as feedstock in an industrial P production process (Wilfert et al., 2018; Schipper et al., 2001). The use of these secondary raw materials are not expected to result in large discharges of new contaminants into the environment. This scenario contrasts with the current import-based model of phosphate rock-derived P-fertilisers which is associated with high consumptions of chemicals and new inputs of mobilised metals into the environment. Yet, local conditions should be taken into account in forthcoming scenarios based on closing nutrient cycles, working in close collaboration with all relevant stakeholders, since social and market acceptance of the technologies and recovered products is crucial for their successful implementation (Díaz et al., 2010).

3.3. Conclusions

The recovery of precipitated phosphate salts from waste water may help in reducing dependence on phosphate rock as a critical raw material, while preventing environmental pollution and promoting more sustainable development. Several technological alternatives are feasible for this purpose. The increase in pH value and the dosage of metal ions (such as Mg^{2+}, Ca^{2+}, and Fe^{2+}) are factors commonly considered. In an integrated approach, the precipitation process can be applied before, during, or after biological N treatment. Some potential targets are: lowering the consumption of energy and chemical reagents, lowering the treatment cost, minimising the risk of toxicity for the microorganisms involved in the biological treatment, and recovering new valuable and marketable high-quality products in the framework of the circular economy. The typification of the end-products, in order to be covered by the EU fertilizer regulation, is currently ongoing. Phosphorus and organic carbon contents are the primary factors to be taken into account when assessing the characteristics of the precipitated phosphate salts. Those materials fulfilling the technical criteria under discussion will be directly usable as agricultural fertiliser, or as a by-product in the P-fertilisers industry.

Capítol 4

Use of recovered struvite and ammonium nitrate in fertigation in tomato production for boosting circular and sustainable horticulture

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4.1. Introduction

Phosphorous (P) and Nitrogen (N) are major constraints on the yield and quality of food production. However, about 90% of commercially available P is sourced from phosphate rock, a non-renewable and geographically restricted resource, with no meaningful reserves in the European Union (EU) (Chowdhury et al., 2017). Moreover, the production of nitrogen fertilizers through the Haber-Bosch process is associated with a negative environmental impact due to its high energy demand. On the other hand, wastewater streams contain large amounts of nutrients, especially P and N, that are polluting the water bodies (Preisner et al., 2021). The production of renewable and high-quality fertilizers from waste streams should be promoted, and the products should be tested in field conditions in order to boost circularity in horticulture systems. Among the most important recovering processes, the precipitation of P as struvite ($MgNH_4PO_4 \cdot 6H_2O$) should be highlighted (Li et al., 2019; Magrí et al., 2020); in the last years, the production of ammonium nitrate (AN) (or ammonium sulphate) from WWTPs through liquid-liquid membrane contactors (LLMC), where N is captured into an acid, has also been investigated (Vecino et al., 2020).

In terms of application, several studies on struvite agronomic efficiency have been focused on its potential as slow-release fertilizer applied to the soil, finding similar crop responses to mined or synthetic fertilizers (Huygens & Saveyn, 2018). Even so, some authors have reported highly variable struvite dissolution rates in soils with alkaline pH (Degryse et al., 2017). Besides, some autors (Arcas-Pilz et al., 2021) assessed the struvite performance as slow-release fertilizer in hydroponics. Nevertheless, the use of struvite in fertigation as a raw material (fertilizer) for nutrient solution (NS) manufacture has not been studied so far. Nowadays, this topic acquires relevance in the context of the promotion of a circular economy of P by EU initiatives and the recent publication of the new European fertilizer regulation that is setting EU-wide quality standards for struvite and hereby facilitating its EU-wide trade (EU, 2019). In addition to the regulation, it has been stated that physical parameters and the solubilization rate of struvite are critical for further commercial use of struvite (Huygens et al, 2017).

To date, many researchers have focused on technological aspects for N recovery, and little has been reported on the resulting products such as recovered AN and their potential to be used as N fertilizers (van der Hoek et al., 2018). Moreover, nothing has been described on its use as raw material for a NS. Nowadays, recovered AN is a high-priority product for its potential of replacing synthetic N fertilizers being highly dependent on science-based knowledge on characterization and fertilizer performance of recovered end-products (Sigurnjak et al., 2019). The effective N is the amount of N from an applied bio-based material that is expected to be available for crop uptake in the season of application. For AN, 100% of N-effective is accepted (VLM, 2016). This is similar to what is expected from the application of synthetic N fertilizer. The agronomic efficiency of recovered products with different chemical characteristics must be known to optimize their use as fertilizers.

The agrosystems vegetable crops grown with fertigation in combination with drip irrigation is continually increasing and an important research effort has been developed in fertilization management techniques to reduce leachate losses (i.e. NS management strategies, planting material, models, greenhouse structures, technology (sensors, soil and plant monitoring)) (Narváez et al., 2012, 2013). Even this combination provides the technical capacity to precise N and irrigation management especially in soilless growing (Massa et al., 2020), commonly, the N and irrigation supplied to vegetable crops are excessive to crop requirements (Thompson et al., 2017). While this practice prevents growth from being limited by nutrient supply, it exacerbates the release of nutrients into the environment with impacts on drinking water and the eutrophication of fresh water and marine ecosystems (Good&Beatty, 2011), and the increment of plant disease (Veresoglou et al., 2013). There are various standard NS that are general guidelines, yet they are not adapted to specific growing conditions, which mainly concern the climatic conditions, irrigation patterns, and the development stages of the crop. With the climatic conditions, the light intensity and the transpiration rate are detected as being important. With irrigation, the fraction drain to waste and the reuse rate of drain water are the main factors. With the development stages, the change from the vegetative to the reproductive phase is important (Sonneveld & Voogt, 2009). To further hone the fertilizer recipes, periodic sampling is a must, helping to determine the nutritional status of the plants (Cáceres&Marfà, 2013). Many field studies have analyzed the optimum best nutrient management practices (BNMP) for the Mediterranean region, including fertilizer rate for a variety of crops (Gallardo et al., 2020; Martínez-Gaitán et al., 2020; Massa et al., 2020; Muñoz et al., 2008). However, choices to achieve optimal irrigation and nutrient management require complex decision-making. Numerous factors regarding climate, substrate characteristics, field infrastructures, and crop characteristics need to be considered (Gallardo et al., 2020).

Tomato cultivation is the most important horticultural crop in Spain, in terms of area and production (MAPA, 2020). The composition of NS used for intensive production of this culture is high in nutrients; therefore, growers have increasing pressure to minimize water and nutrient management. The horticulture sector should cope with the challenge of protecting water bodies from nitrate and P pollution and find alternative renewable sources of fertilizers. Although there is a need to increase crop yields to feed the growing global population, this needs to be done in an environmentally sustainable way.

The general objective of the study is to contribute to increase the circularity and sustainability of horticultural crops, particularly in a tomato soilless crop cultivated with fertigation in greenhouse conditions. The specific objectives are (a) to assess the effects of using solubilized recovered struvite and ammonium nitrate through fertigation upon the tomato plants and (b) to assess the feasibility of reducing the N concentration in nutrient solution and the possible mitigation of N leaching.

The variables measured (yield, fruit quality, biomass, P and N uptake) in the treatments with nutrient-recovered fertilizers were compared to the correspondent control treatment (synthetic fertilizers). Moreover, two different N concentrations (10mM and a dynamic 5-8-5mM) of the NS were tested to evaluate the environmental impact on the N-leaching of tomato soilless crop. Particularly, to our knowledge, the use of struvite and ammonium nitrate as a raw material for nutrient solution manufacture has not been studied so far.

4.2. Materials and methods

4.2.1. Greenhouse experimental set-up and climate data measurement

Experiments were carried out, during two growing seasons, in a passively ventilated multi-span single-layer polyethylene greenhouse, 200 m^2 surface area, located at the IRTA research facilities in Cabrils, Barcelona, Spain. Tomato (*Lycopersicum esculentum*) seedlings were transplanted into new perlite bags (brand PERLINDUSTRIA®) of 30L and 0.75*0.25 m in length, and five bags, each providing substrate for three plants, were placed in lines (Figure 4.1a). The plant density was 3.33 plants·m^{-2}, achieved by using a 25 cm plant-spacing and 120 cm row-spacing. Each treatment was replicated three times, with 15 plants per replication (Figure S4.1). Tomato plants were cultivated during the spring-summer season, March-August 2019 and April-August 2020. Since the used cultivar in 2019, Bond®, produced a high number of non-commercial fruits, Egara® cultivar was selected for 2020 campaign. An open hydroponic system was used for irrigation, providing the nutrient solution (NS) through one dripper of 2 L·h^{-1} of nominal flow per plant. Irrigation decisions (timing and volume) were primarily based on estimation of crop evapotranspiration (ET_c), but the overriding factor was a target drainage volume of about 20%, as a surplus of 20-30% leaching fraction is commonly used to avoid salt accumulation in the root zone (Massa et al., 2020). The irrigation strategy was to apply the daily doses in 7-8 irrigations, to reduce the risk of water and nutrients losses (Thompson et al., 2017). Climate data inside the greenhouse was recorded every hour using a Hortimax sensor. Table 4.1 summarizes the monthly mean and standard deviation (SD) indoor global radiation, temperature, maximum temperature, and relative humidity during both crop periods.

Figure 4.1. Tomato trial design. (a) Plants are placed in lines with five bags per replication, each providing substrate for three plants; (b) fertigation system containing each concentrated nutrient solution x100 (cNS) per treatment, to be released into passing irrigation water through venturi system with automatic control of irrigation; (c) drainage system from one replicate per treatment for volume measurement with water-meters and sample collection.

Table 4.1. Average indoor global radiation (MJ·m^{-2}·day^{-1}), temperature (ºC), maximum temperature (ºC), and relative humidity (%) (mean ± SD).

Campaign 2019	**March**	**April**	**May**	**June**	**July**	**August**
Indoor global radiation (MJ·m^{-2}·day^{-1})	9.7±2	9.2±3.3	11.6±4.4	13.7±4	12.6±2.6	13.7±1.9
Temp. (ºC)	16.6±5.1	17.6±4.2	20.1±4.7	25.4±5.3	28±4	28.9±4
Maximum temp. (ºC)	24±2.2	24±1.7	26.4±2.6	31.3±3.7	33.3±1.4	33.5±3
Relative humidity (%)	52±18.9	58.9±15.8	61.3±15.5	56.1±16.4	64.7±13	60.3±14.7
Campaign 2020						
Indoor global radiation (MJ·m^{-2}·day^{-1})		9.2±4	11.2±4.6	13.4±2.4	13.1±3.5	13±1.6
Temp. (ºC)		19.8±4	23.2±5	25.6±4.5	29±4.3	29.8±4.3
Maximum temp. (ºC)		25.7±2.4	30.6±3.9	31.2±2.3	31.4±10.6	35.6±2.6
Relative humidity (%)		66.5±16.6	58.9±17.5	62.2±14.4	58.1±13.3	59.8±14

4.2.2. Characterization of recovered products, struvite and ammonium nitrate, based on the current legal framework

A new European fertilizer regulation is setting EU-wide quality standards for struvite (EU, 2019), which defines 17 physicochemical and 5 microbiological parameters to be utilized as a fertilizer or component material in fertilizers. P-recovered products used in this study were recovered by Århusvand A/S company (Denmark) and Murcia Este WWTP (Spain) in 2019 and 2020 respectively, through P-elutriation at full-scale followed by a crystallization unit from the sludge line. These samples were identified as highly pure struvite and accomplish the new legislative requirements for precipitated phosphate salts in the revised fertilizer directive (Magrí et al., 2020) (Table S4.1). Moreover, N, P and Mg^{2+} content of struvite are close to the theoretical values (Table 4.2), within the range detected in a systemic comparison of commercially produced struvite (Muys et al., 2021), and accomplish for the prescription of the current legislation being the P content higher than 7% of the dry matter (DM). Furthermore, the total organic carbon (TOC) content is below 0.25%, being 3% DW the legal limit, and the heavy metal and the biological contaminants are well below the threshold legal limit. Organic pollutants concentrations are also shown (Table S4.1).

According to the current Fertilizer regulation EU2003/2003, AN is considered a nitrogen fertilizer solution if the N-concentration is at least 15% (w/v) (EC, 2003). The current draft of the new European fertilizer regulation for “inorganic liquid compound macronutrient fertilizer” proposes lower N-concentration criteria (1.5 or 3%; EC, 2016), which could meet the quality criteria of the products used in this study. Similar to synthetic mineral N fertilizers produced via the Haber-Bosch process, recovered AN contains total N entirely in mineral form, which can be found in the form of $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$. AN liquid batches used in this study are an end-product of an ion-exchange with zeolites and further treated in a pilot plant in Universitat Politècnica de Catalunya (Spain) of liquid-liquid membrane contactors where N from wastewater is captured into nitric acid (Vecino et al., 2020). All AN batches were collected in sampling bottles (1L), stored (-20ºC), and characterized to determine the required fertilizer dosage. These samples showed lower N content (Table 4.2) than ranges reported in other studies (13.2-19.8%), with similar $N\text{-}NH_4^+$ / $N\text{-}NO_3^-$ ratios (Sigurnjak et al., 2019). Nevertheless, high N variability was found depending on the recovering technology used. The use of ammonium nitrate instead of ammonium sulfate is adopted in this experiment because of its higher N concentration. Moreover, as AN is obtained from NH_3 rich air, it should not contain contaminants associated with carbon (Sigurnjak et al., 2019).

Table 4.2. Average phosphorous (P-PO_4^{3-}), nitrogen (N-NH_4^+), and magnesium (Mg^{2+}) content of struvite batches (mass %) and N-NH_4^+, N-NO_3^- and N-total content of ammonium nitrate batches (mass % w/v) (mean ± standard deviation (SD)).

Struvite batch	**P-PO_4^{3-}**	**N-NH_4^+**	**Mg^{2+}**
DK 2018	12.9 ± 0.03	7.2 ± 0.3	10.3 ± 0.6
DK 2019	10.77 ± 0.4	7.3 ± 0.2	9.8 ± 0.3
MU 2020	13.1	5.6	8.2
Ammonium nitrate batch	**N-NO_3^-**	**N-NH_4^+**	**N-total**
AN 2019	4 ± 0.7	6.1 ± 0.8	10.1 ± 1.5
AN 2020	3.3 ± 1.0	3.8 ± 1.1	7.1 ± 2.1

4.2.3. Struvite dissolution assays

Struvite has low solubility in water, with published pK_{sp} values from 9.41 to 13.36 at 25ªC and pH 7 (Bhuiyan et al., 2007), and its dissolution is increased with decreasing pH (Rahaman et al., 2006). In order to carry out a fertigation trial, a dissolution struvite experiment was performed to evaluate the impact of pH solution on P release rates from the three different struvite batches. The test was done under different pH conditions, kind of acid (citric/nitric acid), and struvite size (granular/ground). Seven grams of struvite were suspended in 250 mL-irrigation water (Table S4.2) and pH was adjusted to achieve the target pH values (pH 6, 4, and 1), being continuously stirred. The suspension concentration and pH values were chosen due to agronomic interests. pH, electrical conductivity (CE), P, and NH_4^+ content were determined after 24h to ensure that the equilibrium was reached. The conclusions of these tests were used to made up a concentrated NS (cNS) that would have all nutrients concentrated before being diluted (1:100) and adjusted to the final NS. From the practical point of view, the use of struvite in fertigation can be performed when preparing the fertilizers in an intermediate tank with concentrated nutrient solution and the irrigation water composition must be considered.

4.2.4. Struvite and ammonium nitrate fertigation treatments

Three different compositions of NS were tested, differing on the P and N sources: (i) struvite (STR), with 100% and 17±2% of P and N-recovered source, respectively; (ii) struvite and ammonium nitrate (SAN), with 100% and 34±6% of P and N-recovered source, respectively; (iii) the conventional fertilization (CON) using solely synthetic fertilizers. The recovered sources were the P and N from ground struvite (batches DK 2018, DK 2019 and MU 2020) and the N-NH_4^+ from liquid AN. The reference P fertilizer used in the CON nutrient solution was KH_2PO_4. Other commercial fertilizers were used to complete the NS and to lower the pH, such as nitric acid, potassium nitrate, potassium sulfate, calcium nitrate, and micronutrients.

Fertigation system was established by 2 tanks per treatment, containing concentrated nutrient solution x100 (cNS) to be released into passing irrigation water (pH 7,7 and CE 1,3µS·cm^{-1}) through venturi system with automatic control of irrigation (Figure 4.1b). The concentration of the different compounds that made up the cNS for each treatment is shown in Table 4.3.

Table 4.3. Concentrated Nutrient Solution (cNS) composition (g·L^{-1}) for each treatment and campaign. CON: conventional fertilization treatment; STR: struvite fertilization treatment; SAN: struvite + ammonium nitrate fertilization treatment

		g·L^{-1} Concentrated Solution								
		Campaign 2019			Campaign 2020					
					Initial / final NS			Development NS		
Conventional fertilizers		**CON**	**STR**	**SAN**	**CON**	**STR**	**SAN**	**CON**	**STR**	**SAN**
Nitric Acid	HNO_3	27.2	28.4	20.7	5.1	26.1	19.2	26.8	26.8	20.7
Potassium Nitrate	KNO_3	39.4	30.3		20.2			23.2	10.1	
Potassium Sulfate	K_2SO_4	9.1	26.1	52.2		26.1	26.1	22.6	42.6	51.3
Monopotassium Phosphate	KH_2PO_4	13.6			13.6			13.6		
Calcium nitrate	$Ca(NO_3)_2$	16.4	16.4	16.4				16.4	16.4	16.4
Magnesium nitrate	$Mg(NO_3)_2$				12.8					
Recovered fertilizers										
Struvite	$NH_4MgPO_4·6H_2O$		28.7	28.7		23.7	23.7		23.7	23.7
Ammonium nitrate	NO_3NH_4			60			17.8			32

Regarding the dripper nutrient solution (NS), the concentration of nutrients provided to the crops over the two growing seasons was guided by the agronomic expertise of the authors, following similar criteria explained in previous studies. Phosphorous was tried to adjust to 1 meq·L^{-1}. Since the Nitrogen concentration of 10 meq·L^{-1} used in 2019 involved a high N runoff, in 2020 a dynamic and lower N concentration of the NS was used, starting the first month with 5 meq·L^{-1}, with 8 meq·L^{-1} during the next two months, and ending the crop cycle with 5 meq·L^{-1} again, 5-8-5, as is recommended by some authors to lower the concentration and provide dynamic responses to temporal N requirements, generally fixed for individual phenological phases (Muñoz et al., 2008; Sanjuan-Delmás et al., 2020). The maximum N concentration used in this study of 10 meq·L^{-1} is similar or slightly lower than the commonly adopted for the cultivation of soilless tomato (Massa et al., 2020). Since K^+ concentrations in the drainage during 2019 were high, in 2020, the concentration in the NS was reduced from 6 to a dynamic 3-5-3 meq·L^{-1}. As struvite is formed by Mg^{2+}, it was considered as an extra input, so it was not matched in the reference treatment, being 3 and 5 meq·L^{-1} the concentrations for CON and STR/SAN treatments, respectively. All other micro and macro-elements (except sulfur) were prepared for being identical for all treatments. Effects of nitrate (NO_3^-) and ammonium (NH_4^+) ratio nutrition have been compared for many years with many horticultural plant species, having effects on plant growth, development, chemical composition, and metabolism (Britto & Kronzucker, 2013). Therefore, that aims to highlight the different fraction of mineral N applied as N-NO_3^- with the NS development among treatments, being 99±1, 83±6, and 65±3% as mean values for CON, STR and SAN, respectively, with the rest applied as N-NH_4^+.

4.2.5. Sampling and chemical characterization

The principal chemical properties of the recovered products were determined by the supplying company. The volume of the NS supplied and the drainage from one replicate per treatment collected separately was measured with water-meters (Figure 4.1c). Samples of dripper and drainage solution were collected weekly and 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 total amount of nutrients leached were determined by multiplying the monthly drainage volume by the mean monthly nutrient concentration. 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^+ content were analyzed by APHA Standard Method 4500-P C. Vanadate-molybdate Method, Spectroquant®Nitrate and Spectroquant®Ammonium Reagent Test, respectively using SPECTROQUANT nova 60 Spectrophotometer.

4.2.6. Fruit yield, quality, biomass, and agronomic efficiency

Red tomato fruits were harvested at a maximum 7-day interval, with a total of 14 harvests from June to August in both years, 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, and three samples (with 10 representative tomatoes each) per treatment, from different harvest periods, were graded according to their caliber, total suspended solids (TSS), individual weight, and color. At the end of the crop, the biomass (leaves, stem, and root) from five plants per repetition and treatment was dried at 60ºC after the fresh weight was determined. Three fruit and leaves samples per treatment were assessed for the concentration of nutrients and heavy metals by ICP-OES and Kjeldahl method. These last have been analyzed just in one replicate per treatment.

The parameters used to evaluate the agronomic efficiency of the two recovered treatments compared with reference fertilizers were total and marketable yield, aboveground biomass, fruit quality, fruit and leaves nutrients concentration and P and N uptake, which are shared among several studies. Crop N and P uptake were estimated by considering two components: (i) the harvested fruit and (ii) the “standing” biomass. During the crop, some pruning (removal of stems and leaves that are not part of the main stem) was not accounted. For each component, the N and P uptake was determined from the total weight of yield/dry matter in $kg \cdot m^{-2}$ and the nutrient concentration in the fruit/leaves.

4.2.7. Statistical analysis

The analyzed data were tested for normality and homogeneity of variance using Shapiro-Wilk test $p>0.05$ and Levene’s test $p>0.05$. Once these parameters were validated, a parametric statistical analysis was performed (one-way ANOVA and post hoc Tukey’s test with a significance level of 5%). Alternatively, non-parametric data were analysed for significance using Kruskal-Wallis and Wilcoxon test (SAS version 9.4 and R-studio software) (Table S4.3).

4.3. Results and discussion

4.3.1. Struvite dissolution assays

Struvite dissolution assays were found to be consistent among the different struvite batches, even the different technologies implemented to recovered it and influent type may affect struvite quality (Muys et al., 2021).

Table 4.4. Percentage of P-solubilized from struvite under different sizes, pH and kind of acid conditions (mean ± SD of n = 3)

Sample	**pH**	**P-solubilized (%)**	
		Citric Acid	**Nitric Acid**
Granular	6 ± 0.3	22 ± 8	20 ± 3
Ground		22 ± 5	19 ± 7
Granular	3.7 ± 0.7	86 ± 4	79 ± 9
Ground		87 ± 13	81 ± 6
Granular	1.2 ± 0.3	-	88 ± 6
Ground		-	87 ± 4

To evaluate the struvite dissolved, the concentration of phosphate ($P\text{-}PO_4^{-3}$) was used. The percentage of soluble P obtained from struvite under different sizes, pH and kind of acid conditions is shown in Table 4.4. Citric acid does not allow to achieve pH 1. Struvite was nearly fully solubilized at pH 4 and 1 with both acids and struvite sizes, with a mean percentage of P-obtained (percentage of P-solubilized from the total struvites’ P) of 85±4%, meaning 3.1±0.1 $g\text{-}P \cdot L^{-1}$ and 24±1 $g\text{-}struvite \cdot L^{-1}$. They had a significantly higher percentage than pH 6, while no differences were obtained between struvite batches and the acid used. However, the use of citric acid allows certain microbial activity at pH 4 and nitrite formation that should be minimized to prevent plant toxicity.

4.3.2. Struvite and ammonium nitrate fertigation treatments

Struvite solubilization results lead to prepare a concentrated nutrient solution (cNS) using nitric acid as acidifying agent (Thompson et al., 2007), boosted by the low pH of the ammonium nitrate in SAN treatment (Soto et al., 2015), at pH range 1-2 to obtain an appropriate pH in the final dripper NS, considering the irrigation water properties. The cNS were kept constant during the assays, with pH values 1.2±0.4 and 1.6±0.5 for STR and SAN treatments, respectively.

Table 4.5. Average measured nutrient concentration (meq · L^{-1}) and pH and EC (dS/m) in the nutrient solution for each treatment and year.

Treatments	**Nutrient concentration (meq·L^{-1})**								**dS/m**	
	N	**$H_2PO_4^{2-}$**	**K^+**	**Ca^{2+}**	**Mg^{2+}**	**Na^+**	**SO_4^{2-}**	**Cl^-**	**EC**	**pH**
Campaign 2019										
CON	9.1 b	0.9 b	5.3	8.6	2.8 b	3.8	5.9 c	5.6	1.9 b	6.4 c
STR	10.7 a	1.2 a	6.6	8.9	5.2 a	3.8	9.1 b	5.6	2.2 a	6.5 b
SAN	10.3 ab	1.1 a	6.6	8.8	5.2 a	3.8	13.6 a	5.8	2.2 a	7.0 a
p-value	**0.0058**	**<.0001**	N.S	N.S	**0.0003**	N.S	**0.0001**	N.S	**0.0023**	**<.0001**
Campaign 2020										
CON	4,3 -8,2 - 5	1	5.5	8.8	3.1 b	3.8	5.7 b	4.6	2.1	6.4 c
STR	4,6 - 7,8 - 5,2	0.9	5.0	8.2	4.7 a	3.5	7.9 a	4.6	2.1	6.8 b
SAN	5 - 8,1 -5,3	0.9	3.3	8.6	4.5 a	3.1	8.7 a	4.6	2.2	6.9 a
p-value	N.S	N.S	N.S	N.S	**<.0001**	N.S	**<.0001**	N.S	N.S	**<.0001**

For 2020, except for N, the values are from the development NS. Within each year letters indicate statistical differences according to Tukey test ($P < 0.05$) followed by the P-value. N.S.: Not significantly different

Regarding the dripper NS, nutrients from recovered products (P, N and Mg^{2+}) were detected and supplied to the plants, manifesting a good performance of struvite dissolution under field conditions. However, as the dissolution of struvite is not total and the percentage of P-struvite can vary, is important to be aware of the P-obtained from the dripper. The N-NO_3^-/N-NH_4^+ concentrations in the NS were kept constant (Table S4.4), which exhibits a non-transformation of the ammonium while is stored in the cNS. Table 4.5 shows the mean measured nutrient concentration provided to the crop for each treatment and year. In the 2019 campaign, CON treatment had some problems with the dosing dispenser over the experiment, suppling a 9-12% less total N and P than the other treatments, showing significant differences. Mg^{2+} supplied was significant for both years, due to the struvite composition. In order to keep the same K^+ concentration among the different NS of the treatments, the use of potassium sulphate was needed; this is the reason why the different concentration of sulphate was obtained. Results from the 2020 campaign show a better adjustment for the NS composition among the three applied treatments; only three out of 10 parameters were different significantly.

4.3.3. Leachates: volume, composition and nutrient losses (N and P).

Considering that the fertigation management (irrigation time, water applied (834±79 and 816±77 L·m^{-2}, in 2019 and 2020, respectively) and climatic conditions) were similar for all treatments within a year, the leaching of water, P and N concentrations have been compared. Moreover, considering the two different N concentrations (10 mM and a dynamic 5-8-5mM) supplied with the NS and the non-statistically significant total yield for both crop seasons, even being different tomato varieties, the N and P dynamics were compared between years. However, all the comparison results for the years of the study can be the result of the genetic characteristics of the tomato varieties and other studies should be performed to confirm the issue.

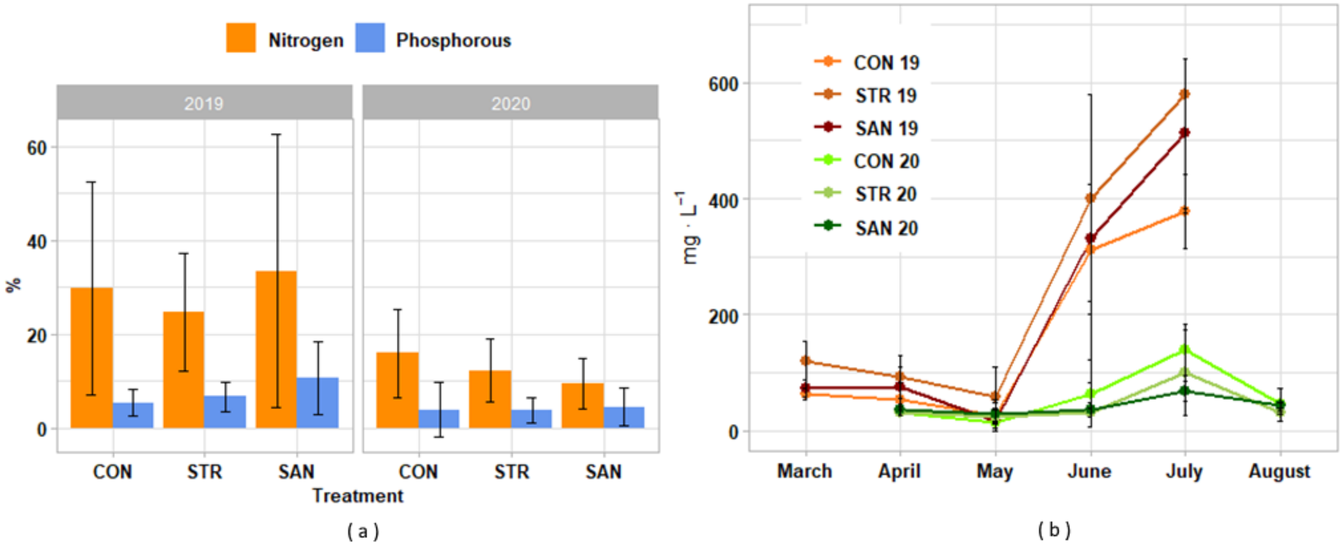

Figure 4.2. (a) P and N leached percentage per treatment and year (mean ± Standard Error (SE) of n = 5); (b) monthly average N concentration leached along the growing season per treatment and year (mean ±SE of n = 5 per treatment). The number next to the treatment name indicates the year campaign.

Firstly, the leached volume was 186±41 and 168±24 L·m^{-2} for 2019 and 2020, respectively, with mean leached volumes percentage in the range of 19-31% of the water supplied for all treatments and years. Regarding the P concentration leached within a year, no significant differences were found, except for April-2019, with higher values in STR treatment than SAN and CON (11±4, 8±3 and 4±1 mg-P·L^{-1}, respectively) and April-2020, with higher values in SAN treatment than CON and STR (12±2, 9±2 and 6±2 mg-P·L^{-1}, respectively). However, when considering the total amount leached, SAN and STR had higher values than CON in both years (3.9, 1.9, and 1.2 g-P·m^{-2} in 2019 and 0.8, 0.9, and 0.5 g-P·m^{-2}, in 2020, representing a 13.4, 6.7, 4.6, 3.4, 3.7 and, 2.2% of the total P-supplied, respectively). Data reveals that the percentage of P-leached is lower when P concentration input is closer to 1 meq·L^{-1} and that most of the P has been either taken up by the plants or remains in the substrate. Figure 4.2a shows the monthly mean P-percentage leached per treatment and year, appreciating no differences among treatments.

Regarding the N concentration leached within the same year, no significant differences were found between treatments, except for April-2019 with higher N concentration in STR than SAN and CON, with 93±36, 76±35, and 54±28 mg·L^{-1}, respectively. However, when considering the total amount of N leached in 2019, STR had the lower value with 38 g-N·m^{-2}, followed by CON and SAN with 43 and 60 g-N·m^{-2}, respectively, representing de 28, 36 and 45 % of the N-leached. The dispersion among months is quite high, yet all the treatments showed a higher concentration of N leached in July and June (Figure 4.2b; Table S4.5), on fruit development stage. Nevertheless, considering the N-leached percentage, the initial development stage (March and April) had also high values (19-37%) due to the small size of the plant. Thus, in 2020 campaign, the N supplied was lower and dynamic, 5-8-5 meq·L^{-1}. In 2020 assay, the total amount of N leached was much lower, with values of 13, 11 and 8 g-N·m^{-2} for CON, STR and SAN, respectively, representing the 16, 14 and 9% of the total N-applied. Both years followed a similar dynamic, July and April of 2020 having higher N-leached percentages. Nevertheless, in the 2020 campaign, the periods of high N-leached were reduced in nutrient concentration and time. Figure 4.2a illustrates the mean N-percentage leached per treatment and year and Figure 4.2b the N concentration leached evolution along both campaigns. Vegetable crops are particularly susceptible to having low N uptake efficiencies caused by several characteristics of vegetable cropping (i.e., excess N input, shallow rooting, wide row spacing, short growing cycle, climate conditions) (Soto et al., 2015; Thompson et al., 2017), being associated with N losses to the environment and subsequent negative environmental impacts. However, this study highlights the fact that N leached may be reduced by lowering the standard N concentration in NS to approximately 8 mM for a greenhouse soilless tomato crop under Mediterranean climatic conditions (Massa et al., 2019; Muñoz et al., 2008; Soto et al., 2015) and using a dynamic nutrient solution, due to its reduction in nutrient runoff, as other authors demonstrated (Muñoz et al., 2008; Vázquez et al., 2006). Besides, some authors observed that a nutrient depletion at the end of the crop drives a fruit loading at the cost of N leaves reserves, suggesting an alternative strategy to limit N-waste (Siddiqi et al., 1998).

However, when recommending a N concentration, it is important to consider the irrigation amount supplied, since it seems pointless to determine a critical N concentration in solution since high or low rates of nutrient combined with low or high N concentrations in solution may lead to similar plant growth rates and environmental pollution. Thus, in our study, the mean amount of N leached was 47±11 and 11±3 $g\text{-}N\cdot m^{-2}$ in 2019 and 2020, respectively. Even the N leached by 10 mM-N treatment is lower than that estimated by the regional N balance for the main greenhouse growing area in SE Spain, which suggested that N supplied annually by all sources, in soil and soilless trials, exceeds crop N uptake by 517-1058 $kg\text{-}N\cdot ha^{-1}$ (Bot et al., 2001; Jadoski et al., 2013). In soil crops, is it suggested the use of technology to schedule irrigation to reduce drainage amount, which substantially restricts the N losses. However, in a soilless trial, is it important to maintain a certain percentage of drainage to avoid salt accumulation in the "wet bulb".

In the 2020 campaign, the $N\text{-}NH_4^+$ was analysed. About the effects of the different $N\text{-}NO_3^-/N\text{-}NH_4^+$ ratio applied by the different treatments in the leachates, even no significant differences were detected except for August-20, the mean percentage of $N\text{-}NH_4^+$ from the total N leached in 2020 was higher in SAN, followed by STR and CON, with 15, 8 and 3.5%, respectively. These results suggest that there was a partial nitrification process of the ammonium in the soilless cropping system, probably due to the low retention of the perlite. However, the growing season effect is remarkable, thus, $N\text{-}NH_4^+$ does not usually have an adverse effect in summer weather due to rapid transformation and vigorous plant growth (Atherton&Rudich, 1986).

4.3.4. Fruit yield, quality, and biomass.

In both years' assays, there were no significant differences in total yield between treatments. That means that the recovered products rich in N and P can substitute the conventional fertilizers without bad effects in tomato production. However, the marketable yield varies, being SAN treatment lower than CON in 2019, and STR treatment lower than SAN (but without differences with CON) in 2020 (Table 4.6). Even so, no significant differences were observed in fruit quality ($g\cdot fruit^{-1}$, caliber, and total soluble solids (SST)). The percentage of non-marketable fruits in 2019 was remarkable, but it was not influenced by treatment (results not showed). The tomato variety and high mean temperatures could explain that, mostly due to blossom-end rot (Muñoz et al., 2008). Moreover, a high NH_4^+/NO_3^- ratio could produce a reduction in marketable fruit for SAN treatment, in agreement with other authors (Halbert-Howard et al., 2020), being important to consider the ammonium tolerance of the plant species. As struvite use as raw material for a nutrient solution has not been investigated to our knowledge, no comparison to other studies can be done. Still, some autors (Sigurnjak et al., 2019) found no significant differences in respect to fertilizer performance of AN as compared to the conventional use of synthetic N fertilizers in lettuce and maize crop, indicating that recovered AN are valuable N sources and therefore might be used as N fertilizers in crop cultivation.

Table 4.6. Total and marketable fruit yield, quality of tomato (weight, caliber (mm) and Total Soluble Solids SST (ºBrix)) and total biomass cv.Bond and Egara for 2019 and 2020, respectively. Average macronutrient concentration in fruits and leaves. Within years, letters indicate statistical differences ($p<0.05$) followed by the p-value. N.S.: Not significantly different

	Treatment	**Total fruit**	**Marketable fruit**				**Biomass**	**Fruit**					**Leaves**				
		yield	**yield**	**fruit quality**			**total**	**N**	**P**	**Mg**	**K**	**Ca**	**N**	**P**	**Mg**	**K**	**Ca**
		Kg·m^{-2}	**Kg·m^{-2}**	**g/fruit**	**Caliber**	**SST**	**Kg·m^{-2}**	**mg/100g wet basis**					**%, dry basis**				
Campaign 2019	CON	22.8	14.7 a	278.8	81.5	5.3	1.16 b	110	25	7.4	199	5.8	2.2 b	0.6 b	1.3	5.6 a	4.4 b
	STR	23.3	13.3 ab	253.9	79.8	5.4	1.38 a	96	22.7	6.5	183	5	2.8 a	1.2 a	1.8	3.2 b	7.8 a
	SAN	21.6	12.5 b	240.9	78	5.5	1.20 ab	103	23.3	6.1	178	4.8	3.0 a	1.6 a	1.5	4.2 ab	5.5 b
	p-value	N.S	**0.04**	N.S	N.S	N.S	**0.03**	N.S.	N.S.	N.S.	N.S.	N.S.	**0.008**	**0.003**	N.S.	**0.01**	**0.003**
Campaign 2020	CON	23	20.1 ab	248.5	81.4	4.5	1,08	106	22.7	6.9b	213	7.2	2.4	0.9	1	1.7	8.8
	STR	22	18.8 b	223.6	78.9	4.6	0,93	126	25.4	7.7ab	211	6.9	2.1	0.7	1.5	1.5	8.1
	SAN	23	20.7 a	230.6	79.7	4.6	0,9	135	27.3	8.3a	226	6.3	2.4	1	1.5	1.6	8.3
	p-value	N.S.	**0.008**	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	**0.04**	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

Regarding total biomass (Table 4.6), STR treatment shows more weight per unit of surface then the rest in 2019, being the treatment that received higher N input. Moreover, in 2020, when the N input was reduced, the total biomass did it too. According to these results, several authors correlate positively the N availability with dry matter accumulation (Tei et al., 2003) and the tendency to allocate biomass to vegetative tissue while there is no increase or decrease in fruit production, the well-known phenomena of excess N favoring vegetative growth (Elia&Conversa, 2012).

All the fruits' nutrients concentrations obtained are in concordance with published data (Colla et al., 2002; Söylemez&Pakyurek, 2018) without detecting any type of deficiency or stress, being the magnesium the only nutrient that showed significant differences within the same year, in 2020 trial, with a higher amount in recovered treatments (STR and SAN) due to struvite composition (Table 4.4). However, nutrient leaves' content exhibited more dispersion in 2019, with a higher content of P and N in STR and SAN treatments (Table 4.4), probably due to the higher amount supplied as other authors reported (Arcas-Pilz et al., 2021). These results confirm a good performance of the recovered products in the NS. Moreover, the analysis of heavy metals on fruit showed lower values than the ICP-AES detection threshold for Cd, Cr, Hg, Ni and, Pb. For

Cu and Zn, CON had similar values than the other treatments (Table S4.6), evidencing the security for health and environmental risk.

4.3.5. P and N crop uptake

P uptake by tomato plants increased significantly in STR and SAN, compared with CON in 2019, due to a higher P concentration in the NS (Figure 4.3; Table S4.7). However, what particularly increased was the P content in crop aerial biomass, but not in fruits, with no effects on total yield, as reported by other authors (Ylivainio et al., 2018). Thus, in 2020 campaign, when the P concentration between treatments was similar, no differences in the amount of P uptake were detected. Besides, the mean percentage for all treatments of P uptake from the total P supplied in 2020 was 54±9% (30±6% for biomass and 24±7% for fruit), similar to other studies in hydroponics (Sanjuan-Delmás et al., 2020).

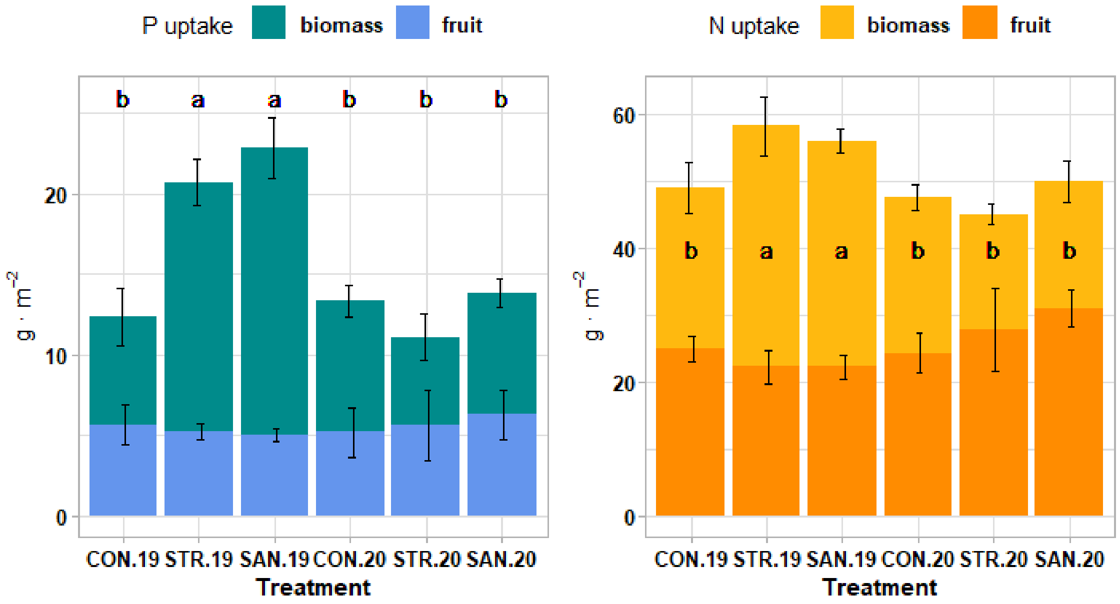

Figure 4.3. P (left) and N (right) allocation in fruits and aerial biomass (g·m^{-2}) (mean ± SE of n = 3). Within each nutrient, letters indicate statistical differences according to Tukey test (p>0.05)

N uptake showed significant differences only in N-aerial biomass, mainly in STR and SAN in 2019, indicating a similar tendency as P when the concentration in the NS is higher. However, when comparing the percentage of N uptake by the biomass from the total N applied, no differences are found within a year, meaning that the more nitrogen is applied, the more is absorbed by the biomass. These results agree with the conclusions obtain when comparing amongst both crop seasons, where the percentage of N uptake by the biomass is similar, being 24±4% for both years, as other authors reported for tomato crops (Sanjuan-Delmás et al., 2020). However, there are significant differences among the fruits and the total N uptake, this last with values of 42±3 and 59±5% for 2019 and 2020, respectively (Figure 4.4), considering a better N uptake efficiency with the 5-8-5 meq-N·L^{-1} NS. Even so, some studies associate environmental pollution to the limited crop uptake of applied nutrients, often 30-40% of applied N, by fast-growing vegetable species (Colla&Rouphael, 2015).

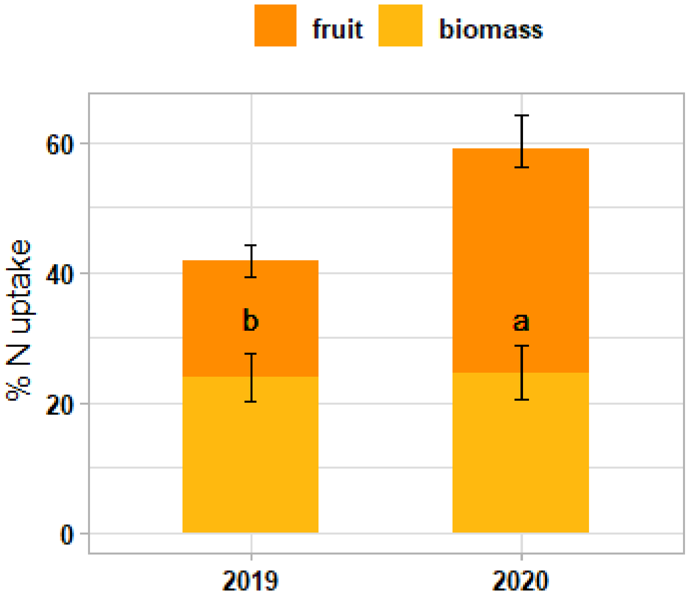

Figure 4.4. Percentage of N uptake by biomass and fruits per year (mean ±SE of n = 9). Letters indicate statistical differences according to Tukey test (p>0.05) between years.

4.4. Conclusions

This study showed that struvite and ammonium nitrate products used as fertilizers in fertigation systems for tomato crops were equally effective in total yield and quality product to conventional fertilizers. For the first time, struvite has been used in fertigation in edible crops and this use has been fully successful. However, there were some differences in marketable yield for SAN treatment.

Furthermore, our results show that for soilless tomato cultivation under Mediterranean climatic conditions, the concentration of N in the nutrient solution can be reduced to a dynamic 5-8-5 meq·L^{-1} without reducing yield or physical quality, which may cause the reduction of nitrogen leaching.

These results give insight into the urgent need for more sustainable crop management reducing the fertilizer demand. Further studies should investigate the agronomic and environmental fertilizer performance of struvite and ammonium nitrate under varying plant species, substrates, climatic and geographical conditions, and keep focusing on sustainable crop management.

Capítol 5.

Effect of fertigation with struvite and ammonium nitrate on substrate microbiota and N_2O emissions in a tomato crop on soilless culture system

5.1. Introduction

The 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 greenhouse was 28.500 ha, being the 10% the area devoted to soilless culture (Gruda, 2019; Garcia et al., 2016). 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 the last decades. Whereas their use is constantly growing (FAO, 2017), the concern regarding their negative environmental effects (greenhouse gas emissions (GHG), soil and water pollution, phosphate rock reserves, energy consumption) does too (UN, 2022). In addition, due to the current crisis (e.g. low raw material availability, increase of energy costs) that Western society are 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 social acceptable form (Huygens et al., 2018; Rodrigues et al., 2022, Magrí et al., 2020, Carreras et al., 2021, 2022).

The rhizosphere microbial community plays pivotal roles in maintaining key plant functions such as nutrient cycling (Lankau et al.,2022). Although the perlite growing medium usually shows an absence of microbiota (Grunert et al., 2016), it constitutes a potential support for bacterial biofilms; and fertigation can greatly impact microbial activity and community structure (Dincă et al., 2022). The initial abiotic condition of perlite, since it is obtained at high temperatures (Orozco, 1995), 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 (Cáceres et al., 2018). Most plants take up inorganic N available in the substrate essentially in the form of nitrate (NO_3^-) but also of ammonium (NH_4^+) (Bothe et al., 2007). 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 (Britto and Kronzucker, 2002). Therefore, depending on the plant species and environmental conditions, the combination of NH_4^+ and NO_3^- may affect plant growth and yield (Britto and Kronzucker, 2013). 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) (Kuypers et al., 2018). Moreover, in case of reducing conditions and electron source availability, a denitrification process can occur, and then, NO_3^- can be reduced and ultimately produces N_2 from N_2O, being this 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 (Mathieu et al., 2006). However, several authors (Hashida et al., 2014; Baggs, 2011) 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 (Carreras-Sempere et al., 2021; 2022). Thus, the present research aimed at studying the effect of using recovered nutrient sources as fertigation solution on the evolution of 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.

5.2. Material and methods

5.2.1. 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 (Latitude 41° 25’N, longitude 2° 23’ E, altitude of 85 m), Barcelona, Spain. Tomato seedlings (*Solanum lycopersicum* L. Cv “Egara”) were produced in an organic substrate under nursery standard conditions and then transplanted into 30L-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 given through fertigation, mixing concentrated nutrient solution (cNS) with irrigation water in a proportion 1:100 through a 2 $L \cdot h^{-1}$ nominal flow dripper per plant (Carreras-Sempere et al., 2021). 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 $N\text{-}NO_3^-$:$N\text{-}NH_4^+$ 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 $N\text{-}NH_4^+$, with the rest applied as $N\text{-}NO_3^-$: i) struvite treatment (STR), with 100% and 17±4% of P and N-recovered source, respectively, and 25±8% $N\text{-}NH_4^+$:N-total; ii) struvite and ammonium nitrate treatment (SAN), with 100% and 39±11% of P and N-recovered source, respectively, and 34±5% $N\text{-}NH_4^+$:N-total; and iii) control treatment (CON), with 5.8±6.5% $N\text{-}NH_4^+$:N-total, using solely synthetic mineral fertilizers. The recovered nutrients were the P and $N\text{-}NH_4^+$ from ground struvite and the $N\text{-}NH_4^+$ 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, accomplishing the EU-wide quality standards of the new European fertilizer regulation (Table S5.1). 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 compounds 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 $N \cdot L^{-1}$ depending on the crop development stage (initial-development-final). NS composition is detailed in Table S5.2.

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).

5.2.2. Agronomic and environmental parameters (leachates and GHG emissions)

Fruit yield (total and marketable), fruit quality ($g \cdot fruit^{-1}$, 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. Besides, 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 was 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 days crop cycle, with three replicas each (t1 (26-28/05/20 – development stage), t2 (07-09/07/20 – fruit formation) and t3 (11-13/08/20 – 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, 10min, 30min, 60min, 120min and 180min) being consistent in the hour (11 am) to minimize variability derived from the daily emission variation (Hatala et al., 2012). Each gas sample was transferred over-pressured to pre-evacuated 12.5mL vials (Labco Ltd., Buckinghamshire, UK). CH_4, CO_2 and N_2O were analyzed simultaneously by a CG 7820A Agilent (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 x 1/8" x 2.0 mm Ultimetal Agilent. Rates of the GHG emissions were calculated from the linear regression slope between the increase of 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 (Carreras-Sempere et al., 2021).

5.2.3. 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 (15/04/20 – initial point, common for all treatments) and t1, t2 and t3, same date and samples as for gas sampling (see section 4.2.2).

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), in order to quantify total bacterial population (16S rRNA), ammonia-oxidizing prokaryotes (AOP) by *amoA* of AOB and AOA (Pelissari et al 2017), and complete bacterial denitrifiers (*nosZ*-clade I) (Calderer et al., 2014).

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 ʹ) / V4_R805 (5'-GACTACHVGGGTATCTAATCC-3 ʹ) and specifically for archaea 349F (5'-GYGCASCAGKCGMGAAW-3') / 806R (5’–GGACTACVSGGGTATCTAAT-3’) (Klindworth et al 2012). The PCR products were sequenced on a 2×300 bp (v3) paired-end format using the Illumina Miseq platform at Molecular Research DNA (Texas, 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 (Martin, 2011) and R package DADA2 (Callahan et al., 2016). 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). The taxonomic affiliations of the ASVs for total bacteria and archaea were assigned by using the naïve Bayesian classifier method (Wang et al., 2007), using the RDP database and compiled into each taxonomic level (DeSantis et al. 2006). For the taxonomical assignment, a bootstrap cut-off of 80% was set.

To assess alpha diversity, Phyloseq, Microbiome and ggpubr R packages were utilized (McMurdie and Holmes, 2013; Lahti et al., 2017; Kassambara et al., 2022). 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) (Anderson and Willis, 2003) 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 (Oksanen, 2017) and pairwiseAdonis (Martinez-Arbizu, 2020) 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 (Wei and Simko, 2021).

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

5.2.4. Statistical Analysis

The analyzed data was 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 was analyzed for significance using Kruskal-Wallis (significance level of 5%) and Post-hoc Wilcoxon test (R-studio Workbench software).

5.3. Results

5.3.1. Fertilization and Agronomic parameters

Fertilization applied and agronomic performance results details are described in a previous study (Carreras-Sempere et al., 2021).

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 S5.2). 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), $N\text{-}NO_3^-$ (106±16, 86±8 and 76±8 $mg{\cdot}L^{-1}$) and $N\text{-}NH_4^+$ concentrations (6±4, 23±4 and 37±8 $mg{\cdot}L^{-1}$) differ statistically significant between treatments (CON, STR and SAN, respectively, for NS crop development) (Table S5.2). 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-folds 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 (Table 4.6 and Table S4.7-Campaign 2020) showed that both, 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 $kg{\cdot}m^{-2}$) and marketable yield (20±2 $kg{\cdot}m^{-2}$), quality product parameters such as fruit weight (233±22 g), caliber (81±3 cm) and TSS (4.5±0.3 ºBrix), and nutritional parameters such as fruit (except for Mg, $p < 0.05$)) and leaves nutritional content and N (51±6 $g{\cdot}m^{-2}$) and P (16±5 $g{\cdot}m^{-2}$) uptake. Besides, the concentration of heavy metals regulated in fruits and leaves was below the permissible limits.

5.3.2. Environmental parameters

The leachate results are shown in Table S5.3. The N-nitrate (50.9±47 $mg{\cdot}L^{-1}$) and N-ammonium (4.1±5.1 $mg{\cdot}L^{-1}$) 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 $N\text{-}NH_4^+$ from the total N leached, followed by STR and CON, with 11, 8 and 5%, respectively. The concentration of nitrites measured was negligible (<2 $mg{\cdot}L^{-1}$).

The GHG emissions results are shown in Figure 5.1 and Table S5.4. The $N\text{-}N_2O$ 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-$N_2O \cdot m^{-2} \cdot h^{-1}$). Even the differences were not statistically significant, higher values were measured in t1 (6.6±4.1 mg N-$N_2O \cdot m^{-2} \cdot h^{-1}$) and t2 (5.7±3.7 mg N-$N_2O \cdot m^{-2} \cdot h^{-1}$) compared to t3 (4.6±2.5 mg N-$N_2O \cdot m^{-2} \cdot h^{-1}$) (p-value >0.05). The N-concentration applied during t3 was 0.6-folds lower than t1 and t2, due to the different N needs for the plant growth stages.

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-$N_2O \cdot m^{-2} \cdot h^{-1}$). Among t1 without plant, clearly STR and SAN showed higher values (121.2±53.1 and 56.9±3.3 mg N-$N_2O \cdot m^{2} \cdot h^{-1}$, respectively) than CON (5.6±4.8 mg N-$N_2O \cdot m^{-2} \cdot h^{-1}$), even being only STR significantly different (p-value 0.009)

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 WP group, t1 showed higher C-CO_2 emissions than t3 (p-value 0.0003). CH_4 gas was not detected in any of the samples, due to the presence of O_2 in the porous perlite (Orozco, 1995), achieved by the irrigation management, and therefore the absence of redox reductive conditions as described by Martinez-Eixarch et al. (2018).

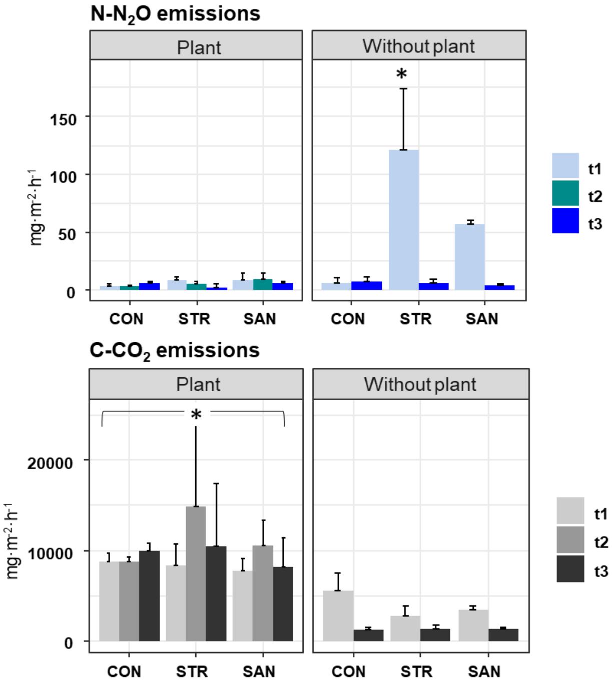

Figure 5.1. N_2O and CO_2 gas emissions (mean+SD) for the fertilization treatments and plant presence/absence groups during the crop cycle (sampling times: t1, t2 and t3). Gaseous samples in Without plant (WP) group were not determined in t2. Significance p-value codes (*<0.05) indicate statistical differences 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.

5.3.3. Microbial community description

5.3.3.1. Sequence Data

Illumina MiSeq 16S rRNA for bacteria and archaea yielded 89350±20779 and 93963±23844 raw reads per sample, respectively. After discarding chimeras, singletons, chloroplasts and kingdom selection and rarefying data by the minimum number of reads, overall rarefied reads and ASVs were 6633 and 5308 for bacteria and 2749 and 692 for archaea, respectively.

5.3.3.2. 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, the time and the plant presence, in both, bacterial and archaeal communities structure (Table 5.1 and Figure 5.2).

Table 5.1. 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	0.078	4	0.37	0.13	2.07	**0.008**
	Residual	18	3.72	0.51			18	0.81	0.29		
	Total	26	7.34	1.00			26	2.79	1.00		
ii) TIME	Treatment	3	2.79	0.31	4.47	**0.001**	3	2.20	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	0.062	4	0.37	0.09	1.74	**0.025**
	Residual	20	3.94	0.43			20	1.08	0.25		
	Total	29	9.07	1.00			29	4.27	1.00		
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	1.00			35	10.19	1.00		

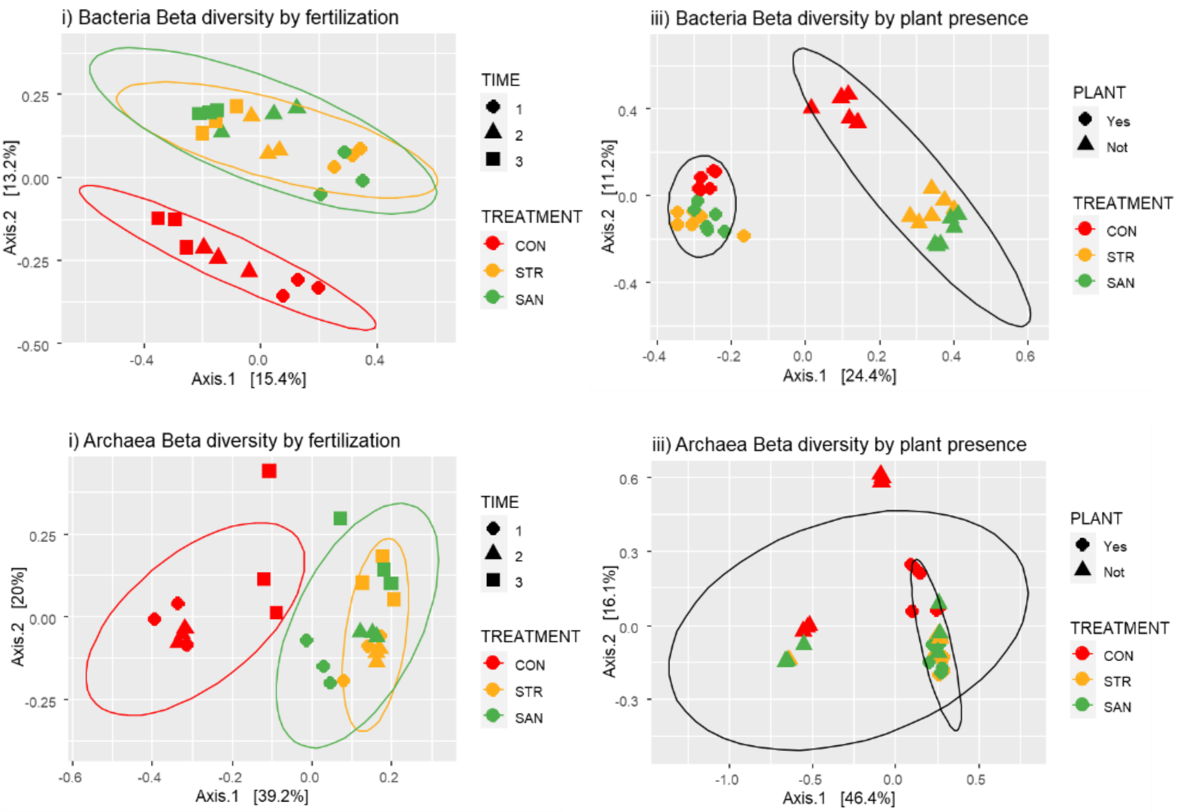

Figure 5.2. Beta-diversity Principal Coordinate Analysis (PCoA) plots derived from Bray-curtis distances of bacterial and archaeal communities structure on: i) Plant group using data from t1 to t3 (by fertilization); iii) Plant and without plant (WP) groups using data from t1 and t3 (by plant presence) (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) plant presence (p-value 0.001), a significant distinction was observed between samples with and without plant (WP).

5.3.3.3. Effect of fertilization and time on Plant group microbial community

Microbial alpha diversity (Figure 5.3). 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 5.3 and Table S5.5) revealed differences among them. Bacteria showed no differences in none 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.

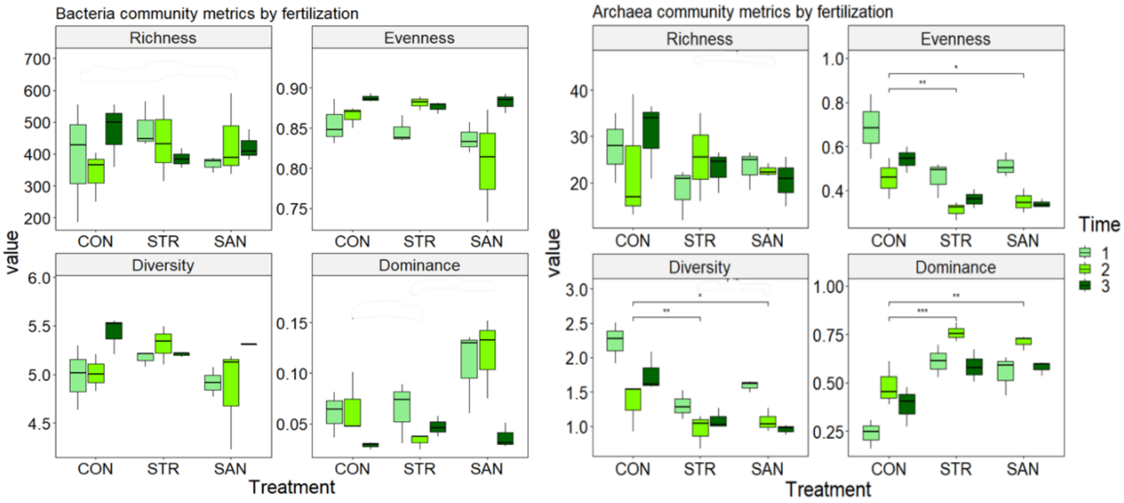

Figure 5.3. Bacteria and Archaea community metrics: Richness (Chao1), evenness (Pielou's), diversity (Shannon) and relative dominance index. Significance p-value codes (**<0.01; *<0.05) indicate statistical differences according to Wilcox test between the fertilization treatments (CON: Conventional fertilization 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 S5.6.

The dominant bacterial phyla (Figure 5.4), 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 genus 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.

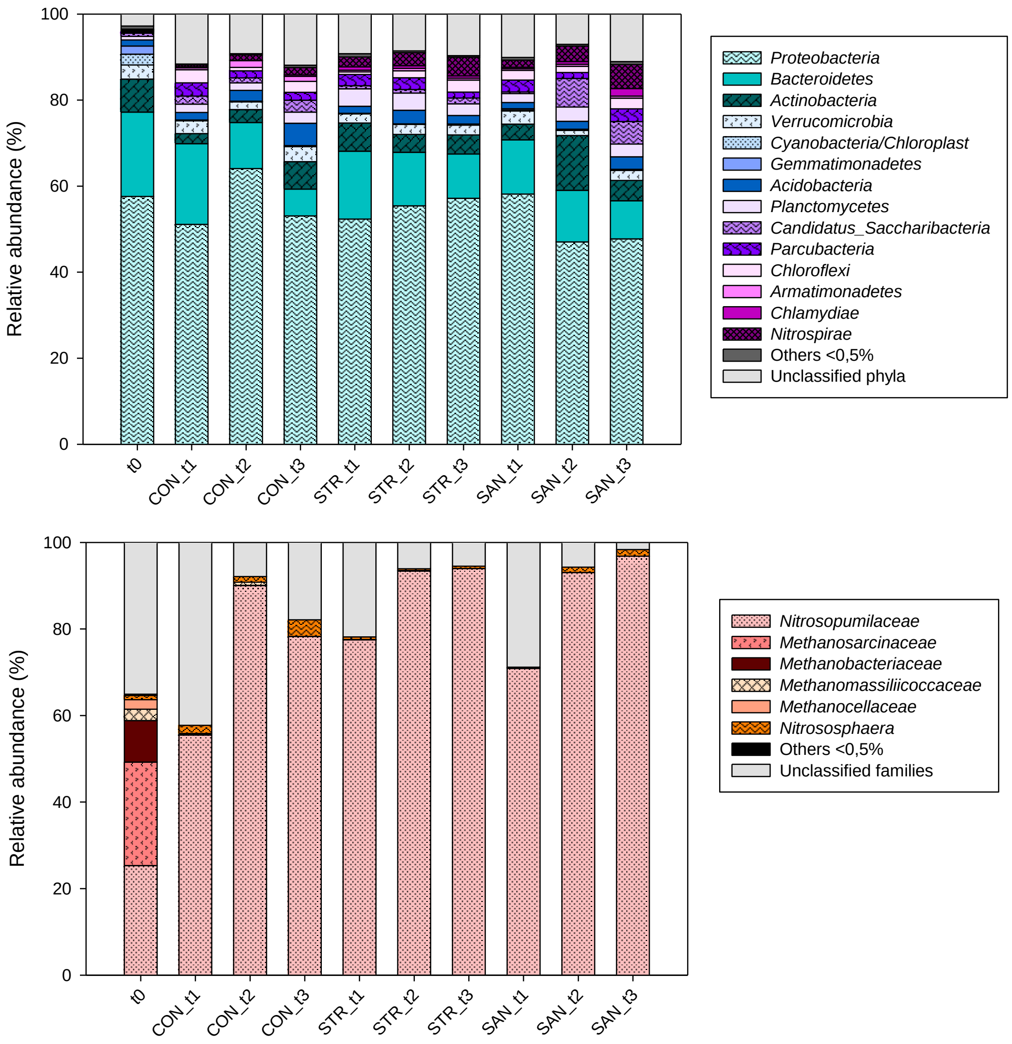

Figure 5.4. Bacterial phylum (upper) and archaeal family (lower) relative abundance (%) for plant group

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 0.007 and 0.004, respectively). Specially 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 0.006, respectively).

Regarding the archaeal communities, the dominant phylum (RA±SD) were *Thaumarchaeota* (85±16%), *Euryarchaeota* (7.8±11%) and *Woesearchaeota* (3.6±7%), being *Nitrosopumilaceae* (77.5±24%) the dominant family. Throughout the crop time, few differences were detected. While *Euryarchaeota* and *Pacearchaeota* significantly decreased (p-value 0.0001 and 0.0003, respectively), *Thaumarchaeota* increased their RA (p-value 0.001). No differences were detected among treatments.

Functional diversity related to N cycle. Quantification of functional genes and 16S rRNA-Metabarcoding data related to N cycle are shown in Table S5.6 and S5.7. The total bacterial population (16S rRNA) showed no differences over time and treatments ($1.2\cdot10^9 \pm 7.5\cdot10^8$ 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 are detected among them, even STR and SAN showed higher values in t3.

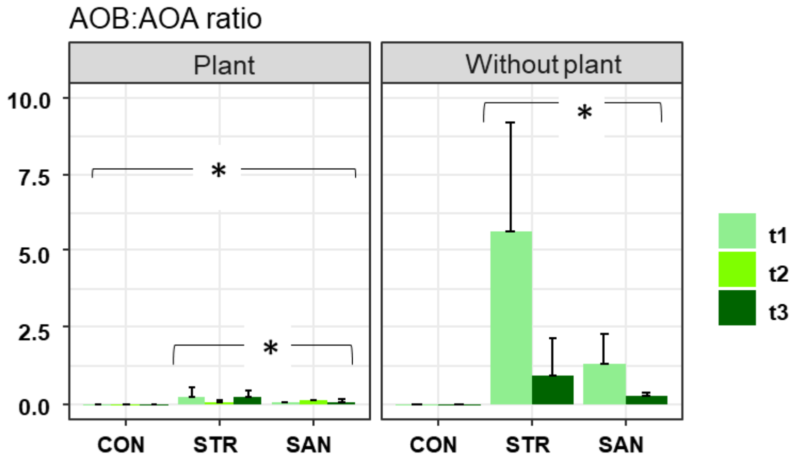

Figure 5.5. AOB:AOA ratios (mean+SD) for the fertilization treatments and plant presence/absence groups during the crop cycle (t1, t2 and t3). Without plant (WP) group was not determined in t2. Significance p-value codes (*<0.05) indicate statistical differences according to Wilcox test. Plant group 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.

Furthermore, among AOP, the ratios of the bacteria (AOB) and archaea (AOA) population varied widely by the time and fertilization applied (Figure 5.5). 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 0.0003).

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 0.0008). 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 0.0002). Moreover, denitrifiers (*nosZ*) showed a slightly significant increment over time, despite the fertilization applied.

From the total microbial community detected by 16S rRNA-Metabarcoding, regarding bacteria species related to the N cycle, three out of which 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 2x fold) RA in STR and SAN compared to CON. Thus, significant differences in RA of *Nitrosomonas* (p-value 0.0003) and *Nitrospira* (p-value 0.007) were detected between CON and both fertilization treatments with higher ammonium concentrations. Even 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 0.002).

5.3.3.4. Effect of plant presence on microbial community

Microbial alpha diversity. Microbial alpha diversity metrics (Figure 5.6 and Table S5.5) showed significant differences by plant presence.

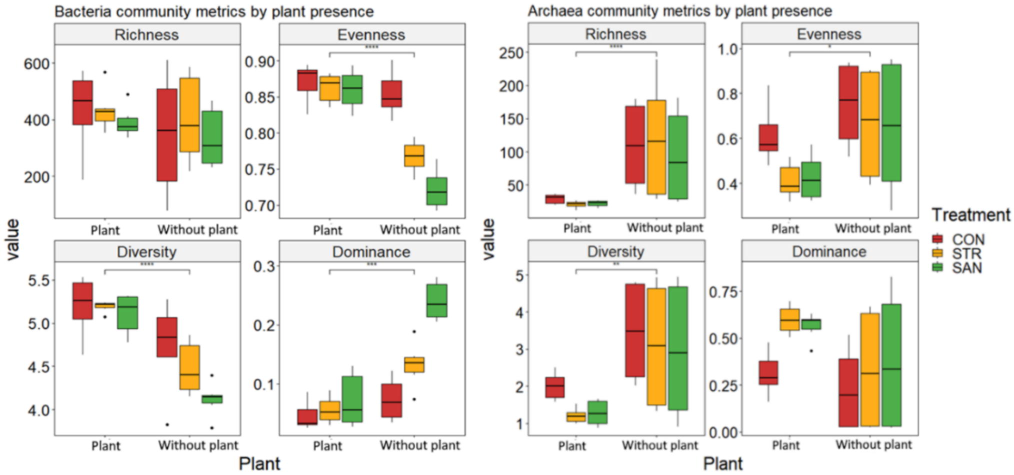

Figure 5.6. Bacteria and Archaea community metrics: Richness (Chao1), evenness (Pielou's), diversity (Shannon) and relative dominance. Significance p-value codes (**<0.01; *<0.05) indicate statistical differences according to Wilcox test between Plant and without plant (WP) samples. CON: Conventional fertilization treatment; STR: Struvite fertilization treatment; SAN: Struvite + ammonium nitrate fertilization treatment.

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 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 bacteria and archaea kingdom are shown in Table S5.6. The dominant bacterial phyla (>1% RA) for samples without plant 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.

The differential relative abundance analysis (Table S5.6) showed that the most abundant bacterial taxa (RA > 1%) enriched with plants compared to WP group were *Sphingobium, Cellvibrio, Flavobacterium, Hydrogenophaga, Acidovorax, Sphingopyxis*, *Rhizobium* and *Methylophilus*. Other genera with lower RA such *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 on the rhizosphere contributing to the carbon cycle (Macey et al. 2020). 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 S5.6), 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 of *Methanomassiliicoccus* genus in the plant group.

Functional diversity related to N cycle. qPCR and 16S rRNA-Metabarcoding data are shown in Figure 5.5, Table S5.6 and Table S5.7. The plant presence promoted a higher total bacterial population (16S rRNA p-value <.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 its lower AOB population (p-value 0.053) (Figure 5.5). As stated above, also without plant, STR and SAN treatments showed higher AOB presence than CON. In addition, *nosZ* community showed a slightly significant increment with plant presence (p-value <.0001).

Regarding the 16S rRNA-Metabarcoding, the same nitrifying genera/family were found in 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. 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.

5.3.3.5. Factors affecting the structure of microbial communities and N_2O correlations

Canonical analysis of principal components (CAP) plot (Figure 5.7, Table S5.8) 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 of total bacterial, *nosZ* genes and C-CO_2 emissions, while microbial communities grown without plant tend to increase AOB population, AOB:AOA ratio and N-N_2O emissions.

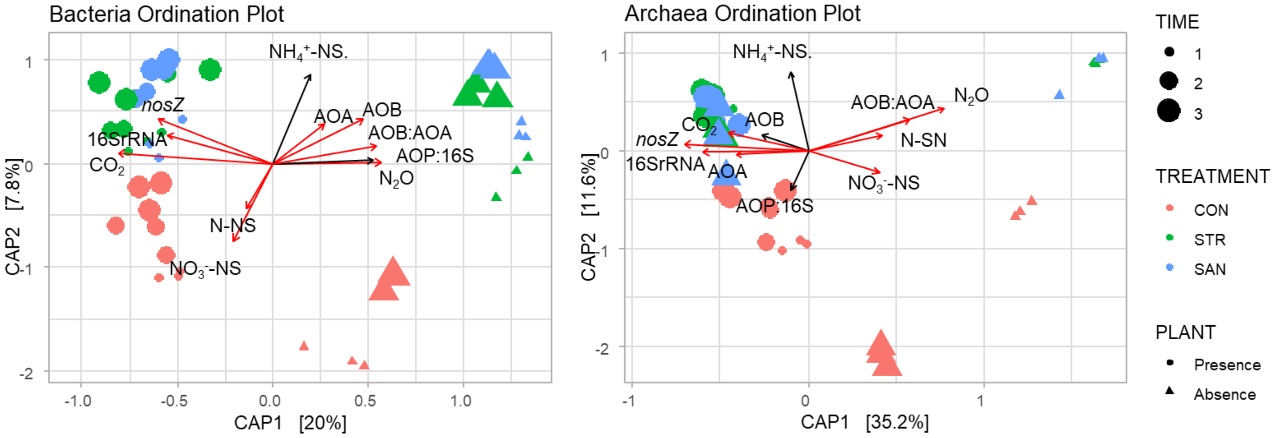

Figure 5.7. Canonical analysis of principal components (CAP) plot ordination (based upon Bray-Curtis distance), visualizing the differences in bacteria and archaea communities and the effect of the parameters represented: qPCR data (16SrRNA, AOAamoA, AOBamoA, nosZ, AOP:16SrRNA, AOB:AOA), emissions (N-N2O and C-CO2) and N concentration and forms applied through the NS (N, N-NO3- and N-NH4+ concentrations).

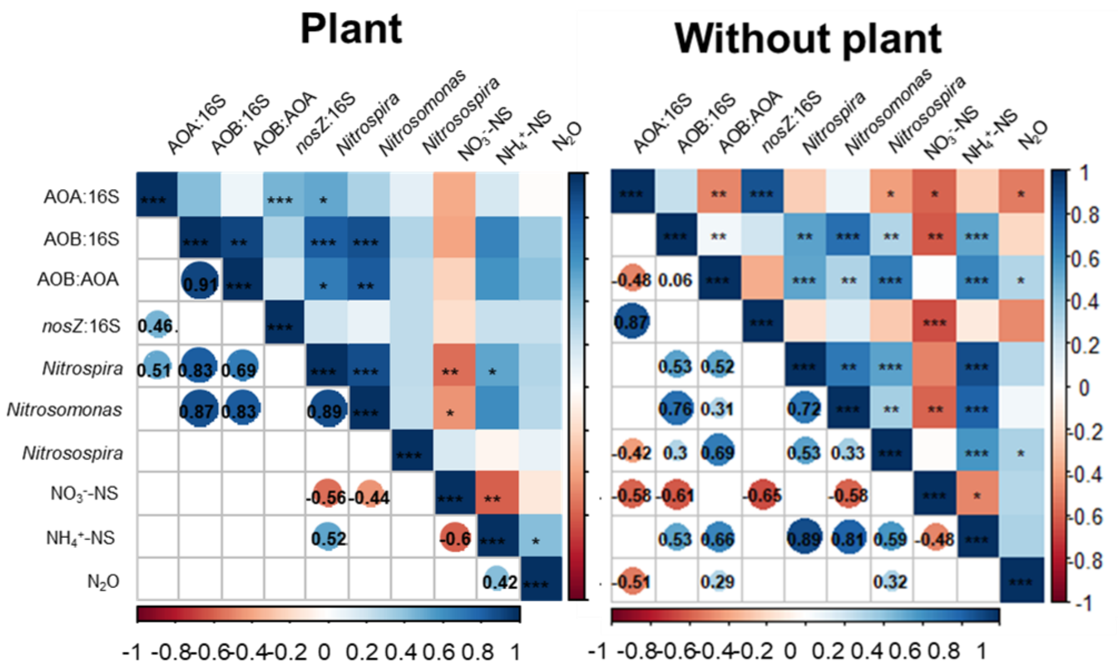

Figure 5.8. Heat map of correlation based on Spearman's correlation method among different parameters (AOA:16SrRNA, AOB:16SrRNA, AOB:AOA and *nosZ*:16SrRNA ratios, *Nitrospira, Nitrosomonas* and *Nitrosospira* relative abundances, NO_3^- and NH_4^+ applied with the NS and the N_2O emissions ratio). The correlation coefficient is shown as a number and statistical level at p-value <0.05*, <0.01**, <0.001***.

In addition, Sperman's correlation (Figure 5.8) 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.

5.4. 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, thus on the fate of the different mineral N forms.

5.4.1. 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 (Carreras-Sempere et al., 2021). Satisfactory agronomic results on fertigated horticultural crops were also obtained with the same treatments as this manuscript on a soil horticultural crop rotation (Carreras-Sempere et al., 2022), with a combination of struvite and K-vinasse in a nutrient film technique system with tomato crop (Halbert-Howard et al., 2020) and with struvite use as slow-releasing P source in hydroponics (Arcas-Pilz et al., 2022). However, it is important to consider the ammonium tolerance of the plant species/variety (Carreras-Sempere et al., 2021); on the other hand, the nitrification of the ammonia could be linked to the environmental conditions, since temperature is a key parameter that drives this process (Cáceres et al., 2018).

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, 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 Cytryn et al. (2012) reported. About the GHG emissions, similar or lower range values than the ones obtained in the present study from hydroponic growing media on tomato crops were already reported (Hashida et al, 2014; Yoshihara et al., 2014; Karlowsky et al., 2021, Halbert-howard et al., 2020). The different variables that influence GHG emissions generation, especially nitrification and denitrification processes and their consequent N_2O gases (fertigation schedule, environmental and substrate temperature and humidity/moisture, amount of total-N applied, etc.), can strongly vary over time, environmental conditions and with the plant growth stage (Yoshihara et al., 2016; Daum and Schenk, 1996). Therefore, 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 done, 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 (Karlowsky et al., 2021). Even though, the CO_2 emissions from the root zone are considered to be in balance with photosynthetic CO_2 fixation by the aboveground biomass (Smith et al, 2014). Furthermore, the prominent differences obtained between plant presence/absence (x 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 WP group, being higher in t1, we hypothesize that green algae grown on the substrate could also be the C-source (Daum and Schenk, 1996) and a limited N-source may be the cause of time differences.

While N_2O emissions showed no differences among treatments when plants were present, the plant absence (in 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 Halbert-Howard (2020) with the use of mineral recycling fertilizers with different N-NH_4^+:N-NO_3^- ratios for hydroponic tomato production and by Lin et al. (2023), 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 (Llorach-Massana et al., 2017; Yoshihara et al, 2014; Zhang et al., 2016, Zhang et al., 2018) 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.

5.4.2. 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 of the AOB community and some taxonomic bacterial phyla, especially the nitrifiers *Nitrosomonas* and *Nitrospira*. Even though, 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, being 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 a higher AOB:AOA ratios versus CON as a consequence.

As the results of our studies, there are already several evidences, most of them in soil studies (Verhamme et al., 2011; Carey et al., 2016), that ammonia concentrations contribute to the definition of distinct ecological niches of AOA and AOB. Ammonia-oxidizing archaea, due to their high ammonia affinity (Stahl et al., 2012), are thought to be predominant in most natural environments with very low ammonia concentration presence, as our CON treatment, even being not alter with higher concentrations (Simonin et al., 2015). However, AOB shows a stronger response and higher tolerance to high NH_4^+ concentrations (Di et al., 2009).

These differences among treatments (STR and SAN versus CON) were more evident when the plant was absent, due to the higher ammonium availability, being AOB:AOP ratios higher compared to plant group, and coincident with a higher N_2O emission (in t1, when the amount of N is higher).

Moreover, Cytryn et al. (2012) 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 population, being more active under low ammonium concentrations (Foesel et al., 2008; Koops et al., 1991). Contrary, in most agricultural soils (Avrahami et al., 2002; Shaw et al., 2006), *Nitrosospira* dominates due to its higher stress tolerance. As NOB community, *Nitrospira* was on the whole more abundant than ammonia-oxidizers, as Cytryn et al. (2012) also reported in soilless media, while Nitrobacter was not detected. This *Nitrospira*-like NOB dominance profile, published also in some soil (De Gannes et al. 2015; Ouyang and Norton, 2020) and marine aquaculture biofilm studies (Foesel et al., 2008), plays a major functional role in low potential NO_2^- oxidation activity environment, contrary to *Nitrobacter* (Attard et al., 2010). However, *Nitrospira* genus could also be composed of the recently discovered complete ammonia oxidizers (comammox) such *Nitrospira inopinata,* which produce less N_2O during nitrification than AOB (Kits et al., 2019).

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 (Carreras-Sempere et al., 2022; Clark et al., 2020), 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 (Martens-habbena et al., 2009), suggesting that *Nitrosopumilacea* might successfully compete with AOB at low NH_4^+ concentrations.
These nitrifiers' community kinetic characteristic suggests 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 (Verhagen et al., 1995).

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 elsewhere described (Rogers and Casciotti, 2010).

Other than nitrification processes, N_2O consumption/denitrification also occurs simultaneously in a soilless substrate (Lin et al., 2023; Halbert-Howard et al., 2020), which is a complex environment including both aerobic and anaerobic sites. Several authors (Petersen et al., 2012; Lin et al., 2023) 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 (Llorach-Massana et al., 2017), coir substrate (Lin et al., 2023) and nutrient film technique (Halbert-Howard et al., 2020). Moreover, the use of an appropriate N concentration to optimize the fertilizer assimilation and the limited C availability may also result in a reduction of the heterotrophic denitrifying activity. Hence, nitrification could have been the major source of N_2O, which is to be expected at less than 60% of water-filled pore space (Bateman and Baggs, 2005).

Besides, the correlations between N-transforming microbes' functional gene abundances and N_2O fluxes showed, also, distinct results with the plant presence/absence factor. When plant was present, a non-significant relationship between N_2O emissions and functional gene abundance was found, as other authors revealed in hydroponics (Hashida et al., 2014) and grasslands (Yin et al., 2020). However, without plant, when NH_4^+ concentration is available for microorganisms, N_2O emissions correlated positively with AOB:AOA and *Nitrosospira* RA, and negatively with AOA:16SrRNA. Similarly, Soares et al., (2016) found a negative and positive correlation with AOA *amoA* and AOB *amoA,* respectively, in tropical soil, while Castellano-Hinojosa et al., (2018) revealed positive correlations with both ammonia-oxidizing kingdoms in fertilized soils. Moreover, correlations between N_2O emissions and *amoA* and some denitrification genes abundance were also demonstrated in soilless tomato culture systems (Lin et al., 2023) and long-term fertilized soils (Dong et al., 2015) suggesting a relation with irrigation patterns and wet/dry season.

Overall, our results indicate that an 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.

5.4.3. Influence of recycled fertilizers on the bulk-substrate-microbiome

The microbiome diversity in soil and soilless tomatoes crops is likely to be different due to the different growing conditions and substrate types, as have been demonstrated by several authors (Anzalone et al, 2022; Grunert et al., 2016; Grunert et al., 2020), suggesting a facility-specific microbiome form within the rhizosphere for hydroponics systems, as well as other growing mediums (Schreiter et al., 2014; Edmonds et al., 2020). Soil crops grown in natural soil tend to enclose a higher microbial diversity than in soilless systems, due to the complex mixture of minerals, organic matter and native soil microbiota, whereas soilless crop systems, due to the substrate fabrication process or sterilization to prevent the growth of pathogens and weeds, result in a reduced microbiome initial load and diversity. The microorganisms present in soilless crops are primarily those that come from the nursery plants, fertigation water (groundwater well) and additional allochthonous microbial biomass added for plant growth promotion and disease suppression. Moreover, under soilless conditions, plant species can exert a stronger discriminatory influence on their rhizosphere composition (Lobanov et al., 2022). Even the microbiology of the hydroponics rhizosphere and the substrate's effect remains 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 archaea keystone taxa for tomato plants (Anzalone et al., 2022; Taffner et al., 2020). 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 (Pester et al., 2011). In addition, *Euryarchaeaota* represents most of the methanogens, which are mainly located in anoxic niches in the rhizosphere of the plants (Magnus et al., 2019). 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, environmental conditions) (Lee et al., 2018; Odelade et al., 2019).

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 (Edwards et al., 2018) where archaea were included in the last stages of plant development as “late colonizers” compared to bacteria. The microbial richness, diversity and evenness was highest among bacterial communities concerning archaea, both within the range of that determined for tomato in hydroponics (Anzalone et al., 2022) but lower than that reported in soil crops (Lee et al., 2018; Carreras-Sempere et al., 2022) due to the presence of a natural soil ecosystem (Grunert et al., 2020). 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 (Dai et al., 2018). However, several studies reported that mineral fertilization would reduce microbial diversity, including plant-beneficial microbial taxa (Dincă et al., 2021).

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 grow-bags, which produce certain chemicals, including phytohormones required for plants, increase plant resistance and promote the absorption of certain minerals (Aioub et al., 2022), boost the reuse of the substrate for further cropping years, while reducing the environmental impacts associated to their production. Acuña et al. (2013) reported that the main physical properties of 5-year-old reused perlite grow-bags 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.

5.5. Conclusions

Previous publications regarding this experiment demonstrated that recovered struvite and ammonium nitrate used as raw materials for a nutrient solution manufacture 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 that can also support denitrification.

In spite of that, when this ammonium fertilization is applied in the absence of 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 of 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 into 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 reusing the perlite grow-bags 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, an adequate fertilizer management is needed due to its influence on rhizosphere microbiota and its associated N_2O emissions.

Capítol 6.

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

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6.1. 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) 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&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.

6.2. Materials and methods

6.2.1. 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 6.2.2 section), mixing concentrated nutrient solution (cNS) with irrigation water (Table S4.2) in a proportion 1:100 through a drip irrigation system with a 2 L·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 S6.1, highlighting the basic pH, accumulation of carbonates and bicarbonates, low organic matter (<1.1%), and Cation exchange capacity (CEC) of 5.3 meq·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 S6.2).

6.2.2. 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 (EC 2019; Magrí et al., 2020) (Table S5.1). 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-PO_4^{3-}, 6.4±1% N-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-NH_4^+ and 4.3±2.8% N-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-NO_3^-:N-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-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 S6.3.

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 6.1). 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-NH_4^+:N-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-NH_4^+ concentrations. The nutrient concentrations of the different treatments provided to each crop are shown in table S6.4.

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 6.1. 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).
NS composition was based on those described in previous studies (adaptation of Muñoz et al., 2008; Bianco et al., 2015; Silber et al., 2003).

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

6.2.3. 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&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., 2022; Meyer et al., 2018). Besides, Total Phosphorus (PT) (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_{OI} 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 S6.5.

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.

6.2.4. 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 S6.6. 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 $N\text{-}NH_4^+$:$N\text{-}NO_3^-$ ratios applied).

6.2.5. 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).

6.3. Results and discussion

6.3.1. 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 $N\text{-}NO_3^-$ and $N\text{-}NH_4^+$ 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 S6.4) 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 S6.4). 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.

6.3.2. 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 6.2).

The crop yield, fruit/inflorescence quality and aerial biomass weight results of the 2-year crop rotation experiment (Table 6.2) 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 6.2), 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 S6.4) 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).

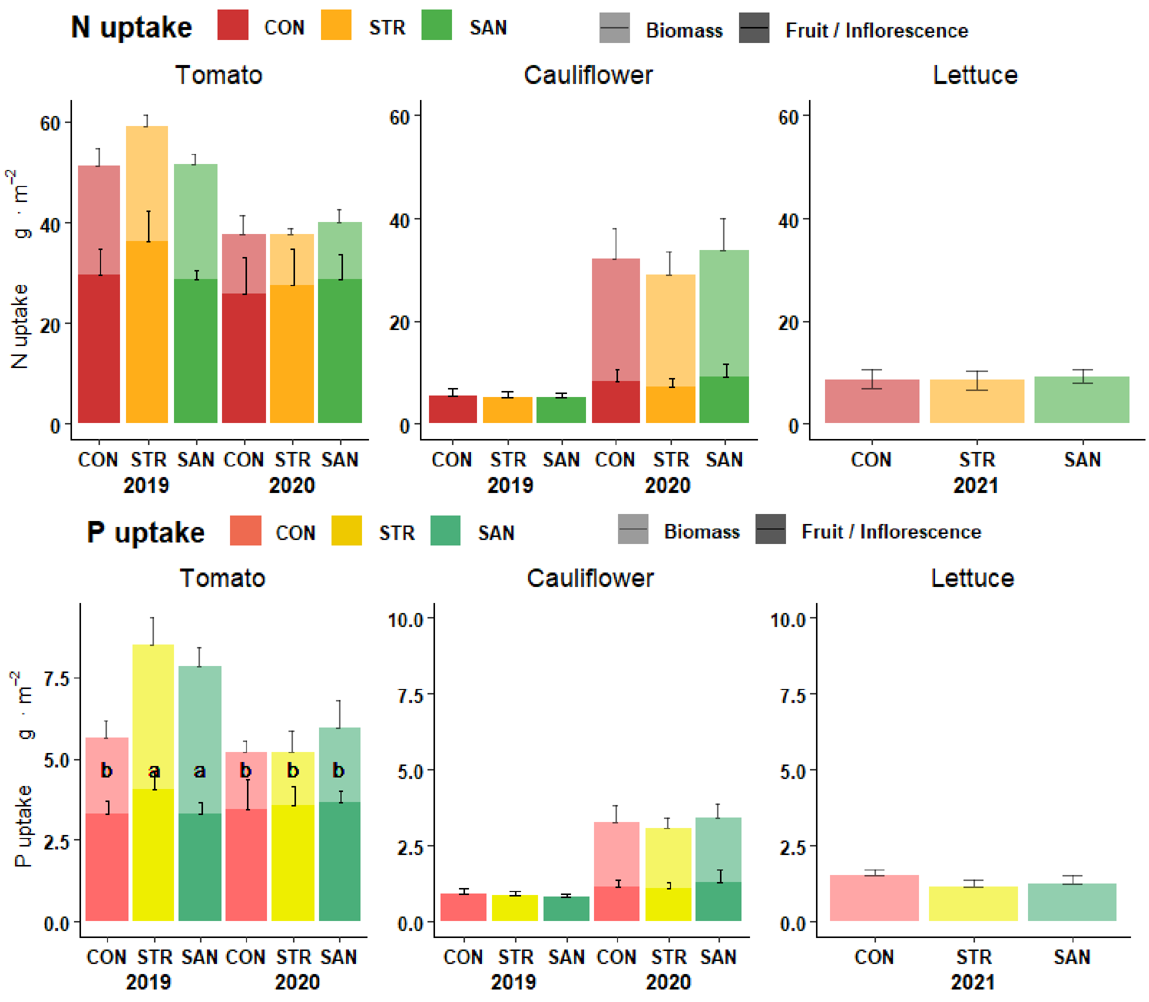

Figure 6.1. Nitrogen and Phosphorous uptake by the aerial non-edible part (Biomass) and the fruit/inflorescence of the different treatments for the crops grown during both campaigns (mean+SD). Cauliflowers leave analysis and lettuce crop during the first campaign couldn't be performed. Letters indicate statistical differences according to Tukey test ($p < 0.05$) CON: control; STR: struvite; SAN: struvite with ammonium nitrate.

Table 6.2. 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·m^{-2})**		**Fruit/inflorescence quality**			**Biomass (kg·m^{-2})**	**Fruit nutritional values (mg·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.
			Fresh	**Dry**	**Diameter (cm)**			**Aerial**	**N**	**P**	**Mg**	**K**	**Ca**	**N**	**P**	**Mg**	**K**	**Ca**
cauliflower	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.					
			Fresh	**Dry**	**Diameter (cm)**	**SPAD**								**N**	**P**	**Mg**	**K**	**Ca**
lettuce	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.

Moreover, the concentration of heavy metals (Table S6.7) 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·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&Terry (2003), typical values of Cr in plants growing in non-contaminated soils rarely exceed 5 mg·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 S6.8; Figure 6.1), 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., 2014). 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).

6.3.3. Soil nutrient content

The study of the soil phosphorus dynamics, in particular, the “plant-available P” (P_{Ol}) and the total P (PT) 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 PT content decreases with depth (Wan der wal et al., 2007), only the 0-30 cm depth was analyzed in this experiment.

Figure 6.2 and Table S6.1 show the concentration of P_{Ol} and PT in soil over the 2-year crop rotation at two soil depths. Firstly, no significant differences among treatments were detected neither in P_{Ol} and PT, 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_{Ol} 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 6.2, 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 S6.1), 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&Sikora, 2002; Van 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 PT 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 PT 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).

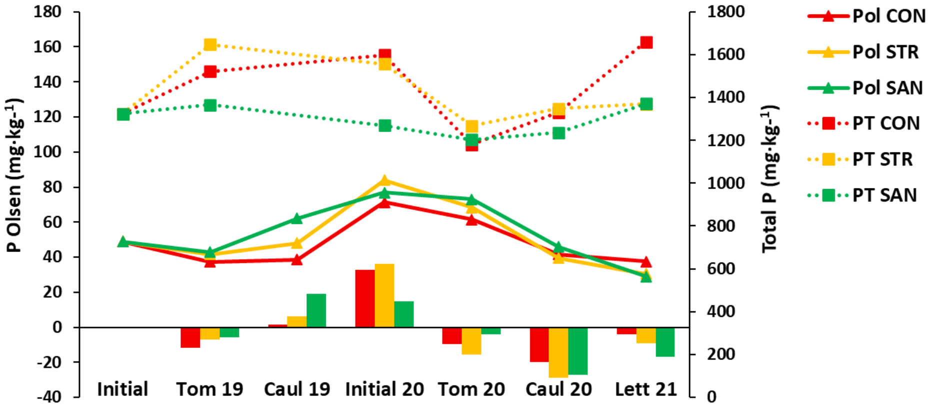

Figure 6.2. Phosphorous Olsen (P_{Ol}) and Total P (PT) soil content at 0-30 cm depth during 2-year crop rotation. Lines present Pol (left axis) and PT (right axis) values, while bars present the differences between the initial and final Pol content for each crop.

Furthermore, the mean value of all the other nutrients measured (Table S6.1) 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.

6.3.4. 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 ($1.7\cdot10^9$ and $3.7\cdot10^9$ 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 6.3, Table S6.9) 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&Ismail, 1999).

In the case of the diversity indices of the archaea kingdom (Figure 6.3, Table S6.9), 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.

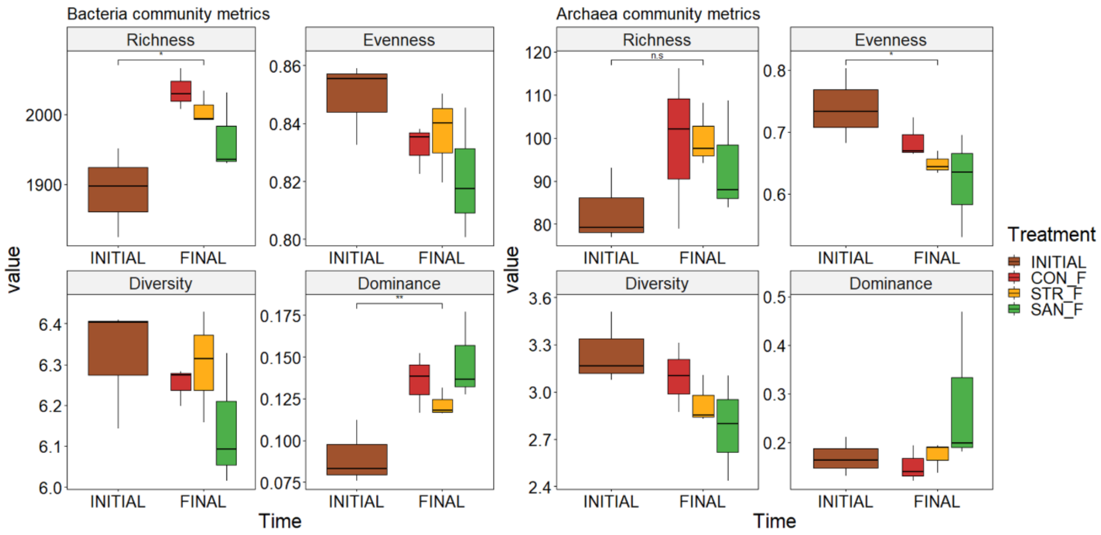

Figure 6.3. Bacteria and Archaea community metrics: Richness (Chao1), evenness (Pielou's), diversity (Shannon) and relative dominance. Significance p-value codes (**<0.01; * <0.05) indicate statistical differences according to Wilcox test between initial (INI) and pooled final samples (FIN).

Beta diversity analysis (Table S6.10) 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 S6.11) 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 6.4).

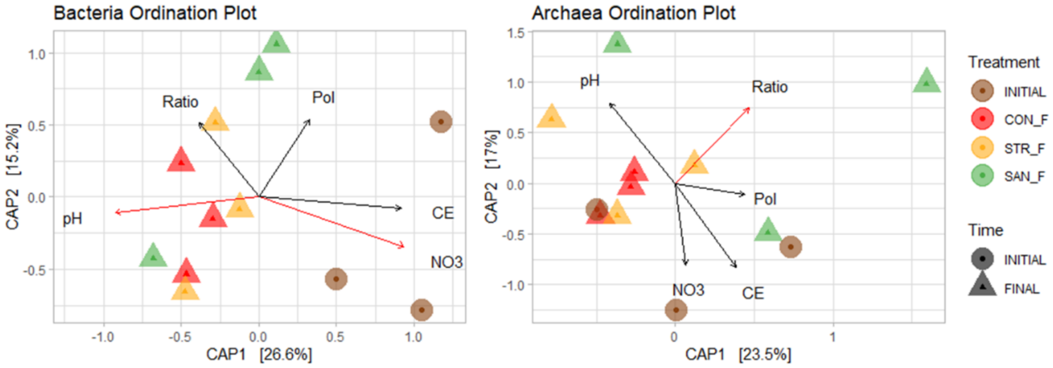

Figure 6.4. Canonical analysis of principal components (CAP) bi-plot ordination (based upon Bray-Curtis distance), visualizing the differences in bacteria (a) and archaea (b) communities among the four experimental conditions and the effects of soil chemical parameters (pH, Pol, CE and NO_3^-) and the N-NH_4^+:N-total ratio applied with the NS. Red arrows indicate the significant variables for each plot (Table S6.13).

Results showed that a 2-year crop rotation with fertigation promotes a microbial richness increase and a selection effect of the bacterial communities (Figure 6.3, Table S6.9), 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 S6.12. 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-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., 2021). qPCR and 16S rRNA-Metabarcoding data are shown in Table S6.13.

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·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&Conrad, 2009; Pratscher et al., 2011; Sun et al., 2021), with a greater ammonia inhibition of cultivated AOA as a potential explanation (Prosser&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&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., 2019).

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.

6.4. 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.

Capítol 7.

Discussió general

La introducció de la tesi doctoral mostra la necessitat de promoure una agricultura més sostenible davant de la situació ambiental, econòmica i social que existeix actualment. Des d'una perspectiva d'agricultura circular, un dels plans d'acció és la substitució d'inputs d'origen no renovables o que són contaminants, en agricultura, per fertilitzants renovables més sostenibles, amb millor empremta de carboni, recuperats de corrents de residus, sempre que es garanteixi la salut humana i del medi ambient (CE, 2015).

Així doncs, al llarg d'aquesta tesi doctoral, s'ha aprofundit en la recuperació dels nutrients fòsfor i nitrogen, dos dels macronutrients més importants per a les plantes, i el seu ús sostenible com a fertilitzants alternatius als comercials d'origen sintètic o d'extracció minera en sistemes de fertirrigació de cultius hortícoles, des d'una visió agronòmica, microbiològica i ambiental.

El capítol 3 inclou un article de revisió que mostra breument les diferents tecnologies i configuracions del procés de tractament de corrents de residus, aplicades a la recuperació de fòsfor, i es focalitza en les **característiques de qualitat i d'eficiència agronòmica dels productes fosfatats** resultants. Els resultats mostren que la precipitació de P aplicada després del tractament biològic de nitrogen (configuració aigües avall o "downstream"), proporciona un producte amb major puresa i menor contingut de contaminants; aquestes, són característiques requerides per a què els productes recuperats siguin considerats en la nova regulació de fertilitzants de la Unió Europea (EU 2019/1009) i pel seu ús en fertirrigació. Els criteris de qualitat i composició química establerts per a la incorporació de les sals fosfatades precipitades en el mercat de fertilitzants europeu pretenen cobrir una àmplia gamma de compostos basats en fosfats d'alta puresa que garanteixin, a més de les funcions previstes dels materials fertilitzants, seguretat en la salut humana i ambiental. D'altra banda, el capítol 3 mostra que les sals fosfatades de magnesi (p.ex. estruvita) tenen un major potencial de comercialització com a font de nutrients recuperats; es tracta de productes amb una puresa relativament alta, alt contingut en P, i una composició química que allibera el P més fàcilment en un rang més ampli de pH, comparada amb els productes precipitats de fosfat de calci, els quals són més adequats per a ser usats com a matèria prima en processos de producció de P industrials. Tot i això, la presència de co-precipitats i impureses, la mida de partícula del producte i les diferents espècies vegetals i medis de cultius emprats poden influenciar en l'eficiència agronòmica de l'ús d'aquests productes com a fertilitzants. En conseqüència, és necessari avaluar l'eficiència agronòmica d'aquests productes recuperats en diferents condicions.

Existeix força literatura (Huygens et al., 2018; Möller et al., 2018) sobre l'ús de l'estruvita com a fertilitzant testat en diversos cultius i sòls o substrats, tot i que sempre aplicat com a fertilitzant d'alliberament lent i controlat, en estat sòlid, aprofitant les seves característiques de baixa solubilitat en aigua i conseqüentment, el seu potencial per a minimitzar la lixiviació de nutrients en el medi i els impactes ambientals associats.

L'aportació científica innovadora d'aquesta tesi doctoral és l'ús de l'estruvita i el nitrat d'amoni, ambdós productes recuperats d'aigües residuals municipals, com a fertilitzants de P i N alternatius als convencionals sintètics en sistemes de fertirrigació pel cultiu d'hortícoles, tant en cultiu en sòl com en hidroponia, emprant perlita com a medi de cultiu. Com s'ha comentat en la introducció, l'ús de sistemes de fertirrigació, cultiu sense sòl i agricultura protegida està augmentant mundialment, particularment a zones properes a ciutats o amb escassetat d'aigua, mala qualitat del sòl i problemes amb malalties transmeses pel sòl (Gruda et al., 2022). Aquests permeten un major control i una major eficiència de l'ús de l'aigua i dels fertilitzants. Per a oferir una alternativa més sostenible en aquest tipus de maneig, la substitució dels fertilitzants de síntesi per fertilitzants recuperats es presenta com a una alternativa possible.

El capítol 4 mostra una **nova metodologia per a l'ús d'estruvita i nitrat d'amoni (NA) recuperats en sistemes de fertirrigació**, realitzant prèviament assajos de dissolució de l'estruvita en dos àcids (àcid nítric i cítric) en el laboratori, per a posteriorment implementar la millor opció per a la seva incorporació a la solució nutritiva (SN) en els dipòsits de fertirrigació, a camp.

Els resultats dels assajos de dissolució mostren una composició i resposta homogènia de les diferents estruvites utilitzades, complint els requisits descrits per la regulació de fertilitzants (EU, 2019), tal com descriu Muys et al. (2021) en la comparació sistemàtica de la qualitat de diverses estruvites comercials produïdes a Europa.

Els assajos de dissolució, juntament amb les propietats (pH, CE i carbonats) de l'aigua de reg emprada, condueixen a obtenir una SN concentrada (SNc) a pH 1-2, usant àcid nítric com a agent acidificant. En el cas del tractament SAN, el baix pH del nitrat d'amoni recuperat també contribueix a reduir el pH de la SNc (Vaneeckhaute et al., 2013). Aquesta SN es manté estable al llarg dels cultius, suplementant a les plantes els nutrients calculats, **en concordança amb el segon objectiu**. Diversos autors ja destaquen la major dissolució de l'estruvita en medis àcids, tant del pH del sòl (Hertzberger et al., 2020) com dels àcids orgànics (àcid oxàlic, acètic, cítric i malic) exsudats per les arrels (Talboys et al., 2016), però en cap cas s'havia provat la dissolució de l'estruvita en un medi àcid per a fabricar una solució nutritiva. Cal remarcar que l'ús d'àcid nítric per ajustar el pH de les SN és habitual en la regió Mediterrània per a neutralitzar l'alt contingut en bicarbonats presents a l'aigua (Thompson et al., 2007) i que el seu impacte ambiental segons el mètode d'anàlisis del cicle de vida (LCA) és similar a la resta de fertilitzants amb nitrat (p.ex. nitrat de calci, de magnesi, d'amoni) (Brentrup et al., 2004). A més, l'acidificació de la SN a pH 1-2 permet l'eliminació de potencials patògens i inclús, certs contaminants emergents orgànics com els gens de resistència a antibiòtics detectats en l'estruvita (Chen et al., 2017; An et al., 2014). A banda, la concentració de nitrat d'amoni recuperat aplicada a la SN concentrada és un factor limitant, a causa del seu baix potencial acidificant (si la barregem amb estruvita) i a la toxicitat potencial de l'aportació del 50% o més del N en forma d'amoni, com sostenen certs estudis amb espècies hortícoles (Song et al., 2021), tot i que existeixen publicacions amb resultats oposats (Ferreira et al., 2017).

Un cop la metodologia per a l'ús d'estruvita i NA com a matèries primeres per a la fabricació de SN ha estat establerta, s'ha estudiat **l'eficiència agronòmica i la qualitat del fruit** en cultiu de tomàquet en substrat sense sòl (perlita) (capítol 4) i en una rotació de tres cultius hortícoles en sòl (capítol 5), **en concordança amb l'objectiu 2.i).** Els resultats d'ambdós medis de cultiu mostren bastants similituds en la resposta dels tractaments, tot i que els valors de rendiment i qualitat siguin diferents, com mostren altres autors (Maboko et al., 2009). El tractament STR, en el qual s'empra estruvita com a única font de P, ha obtingut, en tots els cultius realitzats durant dos anys en perlita i sòl, rendiments totals i comercials i valors de qualitat dels fruits similars al tractament control, amb fertilitzants convencionals sintètics. D'altra banda, el tractament SAN, amb estruvita i NA, mostra menor rendiment de producció de tomàquet total (en sòl) i comercial (en ambdós medis) respecte del control, en funció de la varietat de tomàquet plantada. Aquesta diferència probablement és deguda a la tolerància a l'amoni de cada espècie/varietat (Vega-mas et al., 2015) i a l'associació entre un major rati NH_4^+:NO_3^- i una major susceptibilitat al blossom-end-rot, deguda a la reducció de l'absorció i translocació de calci per part de les plantes (Halbert-Howard et al., 2020). Els altres cultius que es varen realitzar sobre sòl (espècies coliflor i enciam) van mostrar valors de qualitat del fruit i rendiments similars o majors que el control (igualment, emprant SN estàndard, amb l'ús de fertilitzants de síntesi). Cal destacar que l'ús d'estruvita, que és rica en Mg, ha promogut una major o similar absorció d'aquest element en els fruits de tomàquet, comparat amb el control, com detecten altres autors (Cerrillo et al., 2015) i dins de valors acceptats (Soylemez&Pakyurek et al., 2018). A més, els nivells de metalls pesants detectats, tant en fruit com en fulla, es troben per sota dels nivells regulats. **Així doncs, des del punt de vista agronòmic i de salut humana, sempre considerant el possible efecte de la intolerància a l'amoni de la planta, tant l'estruvita com el nitrat d'amoni són una alternativa**

factible als fertilitzants convencionals com a matèries primeres per a la fabricació de SN, tant pel cultiu en sòl com en perlita en les condicions experimentades.

Ara bé, per promoure una horticultura circular sostenible, **l'estudi i aplicació de noves estratègies ha de comportar una no afectació o minimització dels impactes ambientals** associats. **En concordança amb l'objectiu 2.ii)**, s'ha avaluat l'impacte associat: I) a la lixiviació de nitrogen i fòsfor en perlita (capítol 4) i acumulació de nutrients en sòl (capítol 6); II) a la generació de gasos d'efecte hivernacle (N_2O i CO_2) en perlita (capítol 5) i en sòl (aspecte no inclòs com a capítol); III) a la composició i evolució de les comunitats microbianes de la rizosfera, tant en perlita (capítol 5) com en sòl (capítol 6). Posteriorment, es discutirà sobre la interrelació entre aquests tres factors.

Respecte de **la lixiviació en perlita (capítol 4) i acumulació en el sòl de N i P (capítol 6)**, els resultats demostren que l'ús d'estruvita i NA recuperats com a matèries primeres no té incidència sobre la concentració d'aquests elements, respecte del control; Això significa que ambdós nutrients són absorbits per la planta i actuen de manera similar als fertilitzants convencionals. A més, el P de l'estruvita no té tendència a re-precipitar (abans de ser absorbit), si més no, en les condicions experimentades. De manera similar, Meyer et al., (2018) informen de l'elevada similitud dels productes resultants de la transformació de l'estruvita molta i els grànuls de fosfat monoamònic, mostrant mobilitat i solubilitat comparables en sòls calcaris. Com es comenta en la introducció, en els darrers anys s'ha fet palesa la necessitat d'incrementar l'eficiència en l'ús dels fertilitzants per reduir els impactes econòmics i ambientals (p.ex. nutrients lixiviats o acumulats en el sòl), tot mantenint o augmentant els rendiments de la collita. Com refereix Massa et al. (2020), el cultiu sense sòl permet una alta precisió en la gestió dels nutrients i de l'aigua, controlant-se a través de la combinació d'estratègies com la fertilització eficient. En aquesta tesi doctoral s'ha demostrat que una disminució de la concentració de nitrogen, respecte a la usada en la majoria d'estudis -igual o superior a 9 mM- (Sonneveld&Voogt, 2009; Massa et al., 2019; Sanjuan-Delmas et al., 2020), a una solució nutritiva "dinàmica" d'aportació de 5-8-5 meq-N·L^{-1}, segons l'estadi fenològic de la planta, en un cultiu de tomàquet sota condicions mediterrànies en hivernacle, causa la reducció del N lixiviat en perlita i la no acumulació de N-NO_3^- en sòl, sense reduir el rendiment o la qualitat del fruit. Cal mencionar que els resultats de la comparació entre anys d'estudis podrien ser el resultat de les característiques genètiques de les diferents varietats de tomàquet sembrades. Tanmateix, Muñoz et al., (2008) demostren, en un cultiu de tomàquet en perlita en hivernacle a Cabrils (Barcelona), que una reducció d' 11 a 7 mM de N, implica una reducció del 70% dels nitrats lixiviats sense alterar paràmetres agronòmics. També mostren que, durant un any molt calorós, el tractament amb SN dinàmica de 5-7-5 mM-N produeix una disminució del fruit comercial, així com del pes individual del tomàquet. Massa et al. (2019) consideren que una reducció d'11 a 4.5 mM-N en tomàquet és massa dràstica si es desitgen obtenir rendiments i qualitat de fruit suficients. **Així doncs, podem resumir que l'ús d'estruvita i NA recuperats no té afectació en la lixiviació/acumulació de N i P; també que la concentració de nitrogen en les SN habitualment utilitzades en el cultiu de tomàquet fertirrigat en hivernacle en el Mediterrani pot ser disminuïda, i que estratègies de maneig del cultiu com l'anàlisi periòdic dels lixiviats (volum i concentració) i l'ús de sensors d'humitat del sòl per a gestionar el reg permeten una major sostenibilitat de la producció hortícola.**

L'estudi de la **generació de gasos d'efecte hivernacle** (GEH, N_2O i CO_2) durant el cultiu de tomàquet en perlita (capítol 5) i en sòl (Taula S7.1) ens ofereix la possibilitat de comparar entre diferents tractaments de SN i entre medis de cultiu; segons alguns autors, aquest tema ha estat poc investigat (Karlowsky et al., 2021). Així mateix, en ambdós medis de cultiu es van analitzar les emissions GEH en absència de planta. Els resultats mostren que, tant en perlita com en sòl, en presència de planta i una fertilització òptima, no hi ha diferències entre tractaments, determinant que **l'ús de productes recuperats (estruvita i NA en aquest cas) com a fertilitzants, no té un impacte ambiental en els GEH diferent dels fertilitzants convencionals**. De manera similar, Halbert-Howard et al. (2020) mostra que l'ús de dues orines nitrificades com a fertilitzants d'un cultiu hidropònic de tomàquet genera emissions de GEH gairebé indetectables i Wang et al. (2023) inclús apunta que l'ús d'estruvita com a fertilitzant sòlid en sòl disminueix del 41 al 58% les emissions de N_2O respecte a les que generen l'ús de fertilitzants solubles, ja que el lent alliberament de l'amoni permet satisfer les necessitats del cultiu sense deixar nitrogen en excés en el sòl.

Contràriament, **quan hi ha excés d'amoni en el medi de cultiu, com és el cas de la sobrefertilització amb estruvita i NA (o tractaments sense planta en el nostre estudi), en la perlita es detecten majors emissions de N_2O comparats amb el tractament control, mentre que en el sòl no s'observen diferències entre tractaments**. Aquest fet el podem relacionar potencialment amb els índex de diversitat microbians del cultiu de tomàquet dels dos medis de cultiu. En la perlita sense planta s'observa una major dominància relativa i menor diversitat (Figura 7.1), així com un augment de les comunitats bacterianes amonioxidants, especialment en els tractaments STR i SAN (Figura 5.5); això duu a interpretar una sobrespecialització de les comunitats bacterianes a l'aportar amoni en la SN, i en conseqüència, majors emissions de N_2O, escenari comparat amb el sòl sense planta, i inclús amb la perlita amb planta (on els mateixos exsudats de la planta promouen una major diversitat microbiana (Steinauer et al., 2016)).

Aquest fet és degut a la pròpia naturalesa de cada medi de cultiu. Així com la perlita és un substrat inert que, quan el sac de perlita és nou, és estèril i, per tant, les poblacions microbianes comencen a colonitzar des de zero, el sòl té una matriu mineral, matèria orgànica i població microbiana ja associada des de l'inici dels cultius, sent més resilient als canvis poblacionals microbians per factors externs (Banerjee et al., 2023).

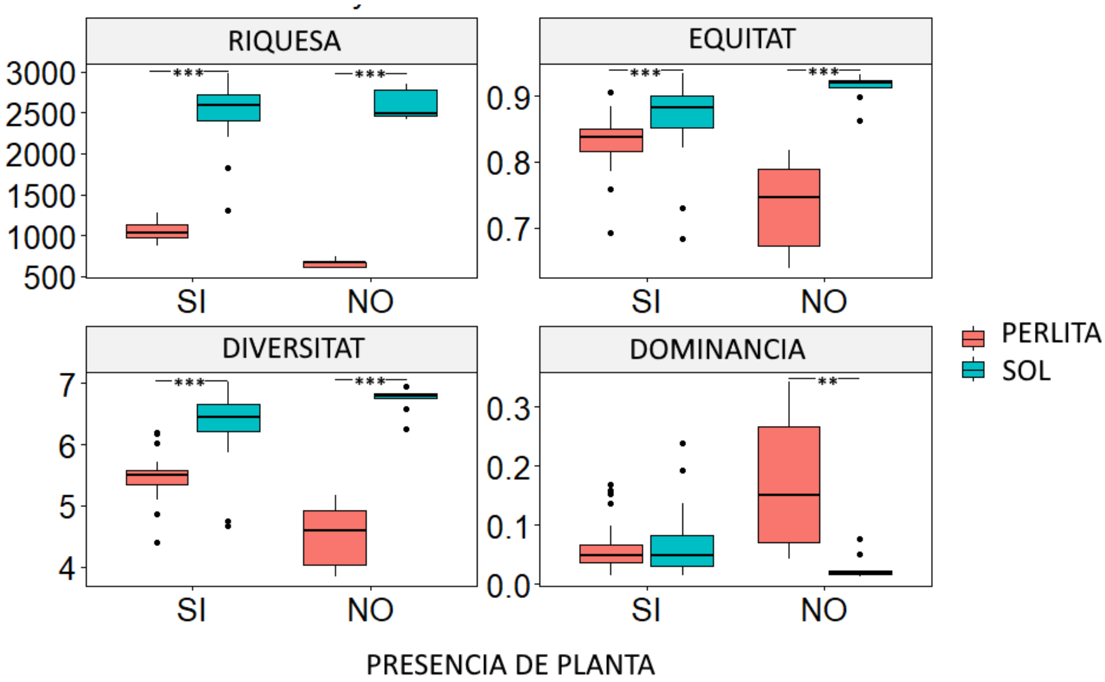

Figura 7.1. Índex d'alfa diversitat de la comunitat bacteriana del cultiu de tomàquet en sòl i perlita. Riquesa (Chao1), equitat (Pielou's), diversitat (Shannon) i dominància relativa. Codis de significància del p-valor (***<0.001; **<0.01; * <0.05) indiquen les diferències significatives segons el test de kruskal wallis entre ambdós medis de cultius (perlita i sòl), en condicions de presència i d'absència de planta.

Respecte a les emissions de GEH, també es destaca que la menor concentració de N aportada al final del cultiu (5meq·L^{-1}) en tots els tractaments genera significativament menors emissions de N_2O, en el grup sense planta en perlita i en el grup amb planta en sòl; la possible causa podria ser la limitació en l'aportació de N.

A més, en ambdós medis de cultiu, són significativament majors les emissions de CO_2 en els grups en planta respecte als grups sense planta (en sòl, només en el període amb major concentració de N aportada), degut a la descomposició de la matèria orgànica i la respiració microbiana i de les arrels.

Les dades de GEH mesurades en cadascun dels cultius de tomàquet, tant en perlita com en sòl, són mesures precises d'intervals curts, i s'observa variabilitat entre rèpliques. Per a un estudi comparatiu adequat entre medis de cultiu, determinant valors anuals d'emissions per àrea, es considera que serien necessaris més mostrejos al llarg del cultiu, a diferents moments del dia, o fins i tot en continu, per la forta influència de l'estadi de cultiu, meteorologia, etc. (Yoshihara et al., 2016). Així i tot, un estudi preliminar de les dades mostra que les emissions de N-N_2O en presència de planta, són significativament majors en sòl (28±25 mg·m^{-2}·h^{-1}) que no pas en perlita (5.7±3.5 mg·m^{-2}·h^{-1}) (Taula S7.2). Aquests resultats podrien ser deguts a diversos factors que depenen de la configuració dels sistemes com la taxa de reg, drenatge i capacitat d'intercanvi catiònic del substrat, temperatura, característiques dels exsudats, etc. Cal remarcar que el maneig del cultiu de tomàquet és diferent depenent del medi de cultiu (Incrocci et al., 2020). Per exemple, en sòl, l'aplicació de fertirrigació és menys freqüent i la dosi per esdeveniment de reg més alta que en perlita, a causa de les característiques de retenció d'aigua i nutrients pròpies; les quantitats de fertilització i aigua, els rendiments, i les característiques pròpies de cada hivernacle també són diferents. Diversos autors citen que la menor taxa de reg i major drenatge del substrat (Abalos et al., 2014; Yoshihara et al., 2014), en el cas de la perlita, podria proporcionar un ambient més aeròbic i reduir les emissions de N_2O de la desnitrificació; per contra, una major concentració de C orgànic en el sòl, juntament amb certa anòxia, possibilitaria una desnitrificació parcial, augmentant els òxids de N (Lin et al., 2023); mesures de GHG a diferents hores del dia mostren una reducció de les emissions quan la dosis de solució nutritiva és menor (Zhang et al., 2018), com en el cas de la perlita respecte del cultiu en sòl, o absent (Karlowsky et al., 2021); Varis autors (Karlowsky et al., 2021; Llorach-Massana et al., 2017) determinen que, tot i que el cultiu hidropònic té un elevat potencial per emissions de N_2O per causa de la intensa aplicació de fertilitzants nitrogenats, els resultats mostren que, si la gestió de la fertirrigació és òptima, els factors d'emissions en cultiu hidropònics (0.3-0.9%) són menors que els valors generals estimats per sòls agrícoles (1% segons el Grup Intergovernamental sobre el Canvi Climàtic (IPCC, 2019)).

Per altre costat, les emissions de CO_2 en presència de planta mostren valors superiors en perlita (9736±4145 mg·m^{-2}·h^{-1}) que en sòl (3623±1872 mg·m^{-2}·h^{-1}), potencialment degut a la diferència en el marc de plantació (1.66 plantes·m^{-2} en sòl i 3.33 en perlita).

L'estudi de la composició i evolució de les comunitats microbianes de la rizosfera permet determinar l'impacte ambiental de la fertirrigació i els fertilitzants alternatius utilitzats. A més, la comparativa entre els resultats descrits en els capítols entre medis de cultiu, perlita (capítol 5) i sòl (capítol 6), tot i ser cultius diferents (tomàquet durant un any i rotació d'hortícoles (tomàquet, coliflor i enciam) durant dos anys, respectivament), permet visualitzar característiques pròpies de cada sistema. A part, s'ha comparat els índex d'alfa diversitat i la beta diversitat dels cultius de tomàquet, en perlita (capítol 6) i en sòl (no descrit en cap capítol), amb planta i sense.

L'avaluació del microbioma en presència de planta dels dos assajos realitzats en aquesta tesi doctoral mostra que la fertirrigació comporta una evolució de les comunitats bacterianes diferent entre medis de cultiu (perlita vs sòl). La perlita (Taula S5.5) mostra un increment de la diversitat (índex de Shannon) i equitat (índex de Pielou) de les comunitats bacterianes al llarg del cultiu de tomàquet, tot i no mostrar diferències en els valors de riquesa (Chao1). En canvi, el cultiu en sòl, després de la rotació de dos anys de cultius, manté aquests dos índexs força estables. En canvi, els arqueobacteris o arquees, tant en perlita com en sòl, tendeixen a disminuir la diversitat i l'equitat. Així i tot, els valors d'alfa diversitat (riquesa, equitat i diversitat), tant de bacteris com d'arquees, són superiors en sòl respecte de cultiu sense sòl (Figura 7.1; Taula S7.3), com citen altres autors (Anzalone et al., 2022). Aquestes dades mostren una major sensibilitat de la perlita als canvis, ja que aquesta, inicialment, conté nuls o baixos nivells de comunitats microbianes, però un cop introduïdes les plantes i el reg, la colonització es produeix ràpidament i al cap d'unes setmanes, les comunitats s'estabilitzen (Calvo-Bado et al., 2006). De manera contrària, Grunert et al. (2016) mostra que hi ha un major dinamisme de les comunitats bacterianes en sòl que no pas en un medi de cultiu confinat orgànic.

Per altre costat, **el tractament de fertilització utilitzat també mostra diferències significatives en els índex de diversitat d'arquees, amb menor diversitat i equitat en els tractaments amb altes concentracions d'amoni (STR i SAN), significativament en perlita i com a tendència en el sòl, però no en els índexs d'alfa-diversitat bacterians**. Cal remarcar que la majoria d'arquees pertanyen al grup AOA, mentre que en el cas dels bacteris, els AOB estan per sota del 3% i, per tant, tenen menor influència en els índex d'alfa diversitat. A més, així com la majoria de comunitats bacterianes són més tolerants a altes concentracions d'amoni, algunes espècies d'arquees són més sensibles (Cáceres et al., 2018), el que condueix a una especialització de la comunitat. La sensibilitat o tolerància a l'amoni varia entre les diferents espècies d'arquees i depèn de la seva capacitat de metabolitzar i fer ús de l'amoni (Naitam et al., 2021). Els microorganismes autotròfics responsables de l'oxidació de l'amoni (AOP) són els bacteris (AOB) i arquees (AOA) amoni-oxidants. En general, el ratio AOB:AOA és influenciat per una varietat de factors com la disponibilitat d'amoni, pH, la temperatura, la humitat (Prosser&Nicol, 2012; Cáceres et al., 2018). Entendre la dinàmica d'aquest ràtio pot proporcionar informació sobre el funcionament dels ecosistemes i optimitzar processos que depenen del cicle del nitrogen.

En la comparativa entre medis de cultiu, **el creixement d'AOP, especialment AOB, en els tractaments amb major concentració d'amoni és observat tant en sòl com en perlita, tot i que**

es detecten clares diferències en la composició dels dos regnes. En els tractaments en planta, es veu en tots els tractaments del sòl una clara dominància d'AOB, mentre que en perlita, dominen els AOA. El ràtio AOB:AOA és major (p-valor <0.0001) en el sòl (19±18.2) que en la perlita (0.07±0.1), amb valors similars reportats en altres assajos (Du et al., 2022; Taylor et al., 2012; Verhamme et al., 2011) associats a mostrejos del sòl a poca profunditat, fertilització nitrogenada, alta concentració d'amoni, etc. Per altra costat, existeixen diversos estudis que mostren dominància d'AOA en rangs de sòls diversos (Mukhtar et al., 2019; Di et al., 2010). **A més, així com en el sòl s'observa una clara dominància de *Nitrosospira* i *Nitrososphaera*, en la perlita trobem major abundància relativa de *Nitrosomonas* i *Nitrosopumilus*. Respecte les comunitats oxidants de nitrit (NOB), ambdós medis tenen dominància de *Nitrospira*.**

Aquest perfil de nitrificants tan diferent entre el cultiu en sòl i en perlita, potencialment pot anar associat al diferent maneig de la fertirrigació que rep cada medi de cultiu, així com les característiques singulars de cadascun. Com s'ha descrit anteriorment, l'aplicació de fertirrigació en sòl més llarga i més freqüent que en perlita, fa que les quantitats d'amoni aplicades d'un cop, siguin majors. Existeixen nombroses evidències que les concentracions d'amoni contribueixen a la definició dels diferents nínxols dels AOA i AOB, sobretot en estudis en sòl (Verhamme et al., 2011; Carey et al., 2016). Els AOB mostren una major tolerància a concentracions elevades, mentre que els AOA predominen en ambients amb concentracions baixes, a causa de la seva alta afinitat a l'amoni (Stahl et al., 2012). Així mateix, la major abundància de *Nitrosospira* i *Nitrososphera* en sòls, també ha estat reportada en diversos estudis (Kowalchuk et al., 2000; Avrahami et al., 2002; Liu et al, 2018; Chen et al., 2013; Clark et al., 2020), relacionant-la amb una major capacitat de viure i utilitzar altes concentracions de nitrogen (Tourna et al., 2011; Xu et al., 2022). En canvi, els gèneres *Nitrosomonas* i *Nitrosopumilus* han estat destacats en cultius hidropònics amb perlita (Cytryn et al., 2012) i biofilms d'aqüicultura marina (Foesel et al., 2008), mostrant, algunes de les espècies, certs requisits d'ambients salins i concentracions d'amoni baixes/moderades (Foesel et al., 2008).

Complementàriament, ha estat demostrat (Sager et al., 2007) que les plantes cultivades en perlita poden produir diferents tipus i quantitats d'exsudats d'arrels en comparació amb les plantes cultivades en sòl, veient-se influenciats per varietat de factors com la comunitat microbiana, disponibilitat de nutrients i propietats físiques del medi de cultiu. La diferent tipologia d'exsudats podria promoure la distinció entre els gèneres microbians d'amonioxidants detectats. Caldrien futures investigacions al respecte.

D'altra banda, la major abundància relativa de *Nitrospira* respecte als AOB, tant en sòl com en perlita, porta a valorar la possibilitat que en un elevat percentatge sigui *Nitrospira* comammox, un gènere bacterià capaç d'oxidar directament l'amoni a nitrat (Kits et al., 2019). Hu et al. (2021) han demostrat, en un conjunt de sòls a Xina, que la *Nitrospira* comammox era el microorganisme amoni oxidant més present i que dominava en 76 de 108 mostres.

I per acabar, es compara la composició general de les comunitats microbianes. El gràfic de beta-diversitat (Figura 7.2) i significança en els PERMANOVA (Taula 7.1) dels cultius de tomàquet en sòl i perlita, mostra una clara diferenciació de les comunitats bacterianes. S'observa com els temps 0 d'ambdós substrats són força similars entre sí, i a mesura que creixen les plantes, les comunitats bacterianes es van diferenciant segons el medi de cultiu (perlita vs sòl). A més, en la perlita es detecta una diferència clara entre les comunitats crescudes amb planta i les d'absència de planta (mostrat també en la Figura 5.2), i en canvi, en sòl, hi ha més homogeneïtat.

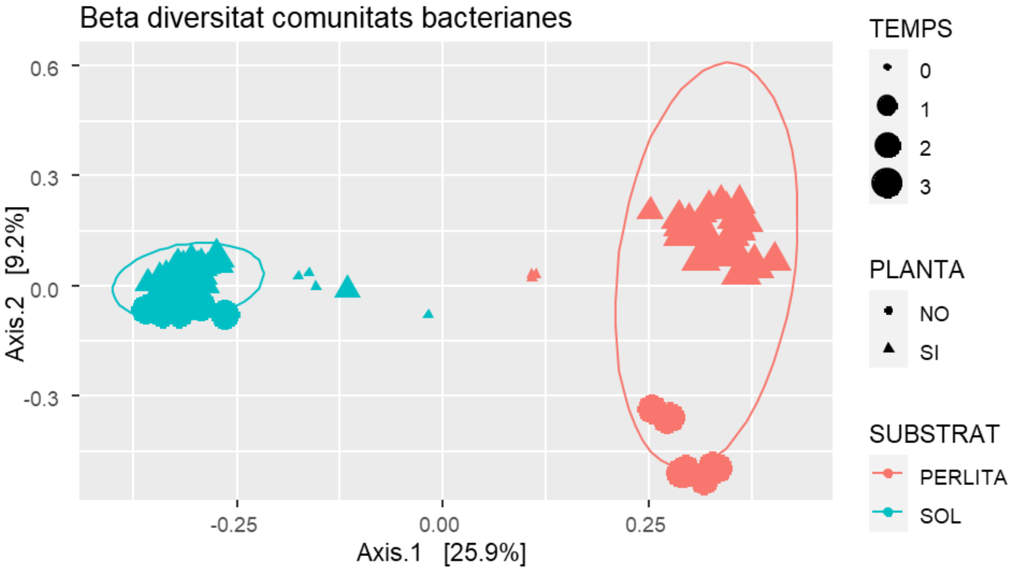

Figura 7.2. Beta diversitat de les comunitats bacterianes derivada de la distància Bray-curtis dels cultius de tomàquet en sòl i perlita, amb i sense planta, en els diferents temps del cultiu (temps 0, 1, 2 i 3).

Taula 7.1. PERMANOVA de les comunitats bacterianes derivada de la distància Bray-curtis dels cultius de tomàquet en sòl i perlita, amb i sense planta, en els diferents temps del cultiu (temps 0, 1, 2 i 3).

Variable	Df	SumOfSqs	R2	F	Pr(>F)
Substrat	1	7.1088	0.24757	32.6051	**0.001**
Planta	1	1.9683	0.06855	9.0279	**0.001**
Temps	1	1.2248	0.04266	5.6179	**0.001**
Substrat*planta	1	1.4987	0.05219	6.8739	**0.001**
Substrat*temps	1	0.7795	0.02715	3.5754	**0.001**
Residual	74	16.134	0.56188		
Total	79	28.7142	1.0000		

Diversos autors (Anzalone et al., 2022; Grunert et al, 2020, Edmonds et al., 2020) suggereixen que el microbioma associat a la rizosfera és específic per a cada tipus de medi de cultiu o substrat. Tot i que les espècies vegetals també exerceixen una influència discriminatòria forta en la composició de la rizosfera (Lobanov et al., 2022).

La majoria de fílums predominants descrits en els capítols són comuns en ambdós medis de cultiu (p.ex. *Proteobacteria, Actinobacteria, Bacteroidetes* i *Acidobacter*ia de bacteris i *Thaumarchaeota* i *Euryarchaeota* d'arquees), els quals han estat identificats com a taxons representatius de la rizosfera del tomàquet (Lee et al., 2016; Lee et al., 2019). Però quan aprofundim en els gèneres majoritaris (>1% abundància relativa), es destaquen les diferències, a excepció de *Nitrospira*, que és un gènere comú. Així i tot, gèneres bacterians promotors del creixement vegetal (p.ex. *Rhizobium, Flavobacterium, Pseudomonas i Streptomyces*) (Bhattacharyya&Jha, 2012; Banerjee&Van der Heijden, 2023) s'han detectat (<1% abundància relativa) associats a les arrels de les plantes tant en perlita com en sòl.

Ara bé, un cop explicada l'eficiència agronòmica i els diferents impactes ambientals associats a la fertirrigació i a les diferents estratègies de fertilització per separat, la seva interrelació es fa evident, i ha estat estudiada **en concordança amb l'objectiu 3 (capítol 5)**. La fertirrigació aporta nutrients i aigua promovent un creixement òptim del cultiu i de la microbiota beneficiosa associada. No obstant, una dosificació inadequada dels nutrients que conté la SN, comporta problemes ambientals com la baixa eficiència en l'ús de fertilitzants nitrogenats (Lassaletta et al., 2014), l'emissió de N_2O (Smil, 1999), la contaminació per nitrats de les aigües subterrànies (Saadi&Maslouhi, 2003) i la pèrdua de biodiversitat (Dincâ et al., 2021).

Així doncs, una gestió adequada de la fertilització conserva o incrementa certs índexs de diversitat bacteriana, els quals ajuden al manteniment de les funcions clau per a les plantes, com ara el cicle dels nutrients mitjançant la seva transformació (Lankau et al., 2022). Concretament, la transformació de l'amoni a nitrat (parcial o total) afavoreix una millor tolerància i absorció del nitrogen per part dels cultius, minimitzant així, la lixiviació d'aquests nutrients i per tant, la contaminació de les aigües subterrànies, sempre que l'aportació de N no sigui excessiva (Ortuzar-Iragorri et al., 2018). A més, el procés de transformació/nitrificació de l'amoni comporta la generació d'òxid nitrós (N_2O), tot i que la composició de la microbiota (AOA, AOB i Nitrospira-Comammox emeten diferents taxes de N_2O) (Kits et al., 2019), així com el maneig de la fertilització (Hashida et al., 2014), i el tipus d'exsudats de les arrels, poden influenciar simultàniament en la quantitat de GEH final generats. Diversos estudis destaquen una major relació positiva entre els AOB i les emissions de N_2O (Soares et al., 2016; Hink et al., 2017; Wang et al., 2016). Similar als resultats del capítol 5, on s'observa correlació positiva entre el ràtio AOB:AOA i els gasos N_2O.

Els diferents capítols de la tesi s'interrelacionen entre sí per mostrar aquesta visió holística de l'horticultura circular, considerant l'origen de la fertilització utilitzada, l'ús eficient d'aquests nutrients, el creixement òptim del cultiu i l'avaluació de l'impacte ambiental associat. Així doncs, l'ús de nutrients recuperats d'aigües residuals com a matèries primeres per a la fabricació de solucions nutritives ha demostrat ser una alternativa factible més sostenible que els fertilitzants sintètics, tant en el cultiu d'hortícoles en sòl com en perlita. El maneig òptim d'aquesta fertilització comporta un creixement adequat dels cultius i de la microbiota beneficiosa associada, minimitzant la lixiviació/acumulació de nutrients i la generació d'emissions de N_2O.

Projecte LIFE-ENRICH

El projecte LIFE-ENRICH considera tots els processos de la cadena de valor, des de la recuperació dels nutrients a la seva valorització en l'agricultura, i compara l'eficiència tècnica, impacte ambiental i viabilitat econòmica, considerant una escala a mida industrial implantada a l'EDAR de Múrcia Este, amb la situació actual, on s'eliminen els nutrients de les EDARs i s'utilitzen fertilitzants químics sintètics. La comparació s'ha elaborat en termes d'eficiència tècnica, avaluació del cicle de vida (ACV/LCA) i cost del cicle de vida (CCV/LCA), i ha estat realitzada per l'equip de CETAQUA (www.cetaqua.com). Se'n poden obtenir els detalls a través de la web del projecte (www.life-enrich.eu). El projecte ha inclòs l'anàlisi del cicle de vida (ACV) de tota la cadena de valor. L'ACV és una metodologia àmpliament utilitzada per a l'estimació dels impactes ambientals d'un producte, procés o servei al llarg del seu cicle de vida. L'ACV considera el cicle de vida complet d'un producte, des de l'extracció de recursos i el processament de la matèria primera, passant per la producció, l'ús i el reciclatge, fins a l'eliminació dels residus restants (JRC, 2010).

La recerca realitzada en aquesta tesi doctoral adquireix major viabilitat mostrant els resultats globals de l'aplicació del model LIFE-ENRICH, els quals mostren que, tant amb la recuperació de P com de P i N, es redueix l'impacte ambiental global respecte la situació actual. Hi ha una millora per a la majoria de les categories d'impacte (potencial d'escalfament global, esgotament de l'ozó estratosfèric, acidificació terrestre, eutrofització d'aigua dolça i escassetat de recursos minerals), principalment associada a la reducció de fangs generats a l'EDAR, la substitució dels adobs convencionals i la recuperació de nutrients. Tot i això, s'ha observat que la recuperació de nutrients, sobretot en forma de nitrat amònic, requereix l'ús de químics que tenen un elevat impacte associat i que l'optimització del procés de recuperació de N ha de ser més investigada. A més, es destaca que en altres països o llocs, la situació pot ser diferent pel que fa a la replicabilitat (p.ex. major concentració de N en el sistema de recuperació) o al marc legal (p.ex. límits de descàrrega de N més estrictes), millorant l'eficiència i l'impacte del sistema. Respecte del CCV, es detecta que l'escenari LIFE-ENRICH té un potencial per generar un marge econòmic, tot i els elevats costos invertits en la recuperació de N en un corrent amb baixa concentració de N.

El projecte també ha permès realitzar un estudi de mercat a partir de les dades del projecte. El marc legal va tenir un paper clau en el desenvolupament del model de negoci, tant en les analítiques, normatives, qualitat dels fertilitzants, o la percepció i posicionament dels actors clau sobre fertilitzants alternatius. Per l'anàlisi de mercat, l'estruvita i el nitrat d'amoni es comparen amb els seus homòlegs de la indústria dels fertilitzants convencionals, fosfat monoamònic (adob sòlid) i nitrat d'amoni sintetitzat químicament (adob líquid), amb els quals es considera que competeixen. L'anàlisi de mercat destaca un gran mercat de fertilitzants, en el qual, tant l'estruvita com el NA recuperats tindríem cabuda. A més, amb la pujada constant dels preus dels fertilitzants convencionals, arran de les diferents crisis socials, els adobs alternatius recuperats podrien ser més competitius i, a més, evitar el consum de recursos escassos de matèries primeres i energètics.

Com s'ha mencionat a la introducció, les sals fosfatades precipitades recuperades de diferents fonts (incloses les aigües residuals), com l'estruvita, són acceptades, des del juliol de 2022, com a matèries primeres secundàries o fertilitzants en el Reglament europeu de productes fertilitzants (EU 2019/1009) i posteriorment, per a agricultura ecològica (EU 2023/121), sota certs criteris de qualitat, i en conseqüència, se'n permet la seva comercialització. La incorporació de l'estruvita com a fertilitzant a la nova regulació de productes fertilitzants de la UE suposa un incentiu important tant per a la indústria dels fertilitzants com per al sector agrícola. Per ara, el major preu dels fertilitzants alternatius comparat amb els convencionals pels costos de producció, l'adaptació lenta del marc legal i la certa reticència en l'ús de fertilitzants alternatius pel que fa a qüestions de seguretat són les principals debilitats d'aquest sistema alternatiu. Tot i que, les noves polítiques mediambientals i d'economia circular tant per l'àmbit de les aigües residuals com dels fertilitzants, s'encaminen cap a aquesta visió.

Capítol 8.

Conclusions i futura recerca

Aquesta tesi doctoral aporta innovació i coneixements sobre l'ús alternatiu de fertilitzants recuperats de corrents de residus del tractament d'aigua residual urbana, l'estruvita i el nitrat d'amoni.

Les principals conclusions es podrien resumir de la següent manera:

- La precipitació de fòsfor aplicada després del tractament biològic de nitrogen ("aigües avall" o downstream) en una planta de tractament d'aigües residuals és la configuració més adequada per recuperar sals fosfatades precipitades amb la qualitat requerida per a la seva acceptació com a fertilitzants en la regulació europea de productes fertilitzants EU 2019/1009, respecte d'altres configuracions del sistema.
- Les sals fosfatades precipitades recuperades de magnesi tenen un major potencial fertilitzant per la seva elevada puresa, contingut i disponibilitat de fòsfor per a les plantes en un rang ampli de pH i medis de cultiu, comparat amb altres sals fosfatades precipitades.
- Diferents mostres d'estruvita tenen una resposta homogènia i satisfactòria en la seva dissolució en aigua de reg, sent gairebé completa a pH 1 i 4, usant àcid nítric com a agent acidificant, així com dues mides de partícula (granular i molta).
- L'ús d'estruvita i nitrat d'amoni com a matèries primeres per a la fabricació de solucions nutritives ha estat investigat per primera vegada, obtenint resultats satisfactoris tant en cultiu sense sòl (perlita) com en sòl. Ambdós productes recuperats són una alternativa factible als fertilitzants convencionals, des d'una visió i) agronòmica, pel fet que no han mostrat diferències significatives en el rendiment dels cultius, la qualitat dels fruits i l'absorció de P i N. Tanmateix, per al seu ús, és necessari considerar la tolerància a l'amoni de l'espècie vegetal a cultivar; ii) de salut humana, perquè en cap cas els fruits han superat els nivells regulats de metalls pesants; iii) ambiental, ja que no han tingut major afectació en la lixiviació/acumulació en el sòl de N i P, en la generació de gasos d'efecte hivernacle ni en els índexs d'alfa-diversitat bacterians, que els fertilitzants convencionals sintètics.
- La concentració de nitrogen òptima de la solució nutritiva per al cultiu de tomàquet sense sòl a la regió Mediterrània sota condicions d'hivernacle pot ser reduïda, respecte a l'aplicació habitual, a una solució nutritiva "dinàmica" d'aportació de 5-8-5 meq·N·L^{-1} -segons l'estadi fenològic de la planta- minimitzant la lixiviació de N mantenint el rendiment del cultiu i la qualitat del fruit.

- Existeix una correlació positiva entre la ràtio de bacteris amoni-oxidants (AOB) : arquees amoni-oxidants (AOA) i els fluxos d'emissions de N_2O, en l' aplicació en excés de fertilització amoniacal en el cultiu en perlita. Aquests resultats suggereix un potencial indicador de disponibilitat d'amoni en el substrat i generació de N_2O.
- En el sòl predominen els bacteris amoni-oxidants (AOB), principalment *Nitrosospira* i entre els amoni-oxidants arquees (AOA) domina *Nitrososphaera*. En el medi de cultiu perlita, predominen les arquees amoni-oxidants (AOA), i els generes *Nitrosomonas* i *Nitrosopumilus* son majoritaris*.* Respecte a les comunitats oxidants de nitrit (NOB), ambdós medis tenen dominància de *Nitrospira*. Aquest perfil de nitrificants tan diferent entre el cultiu en sòl i en perlita, potencialment pot anar associat al diferent maneig de la fertirrigació, així com les característiques singulars de cada medi de cultiu i dels exsudats de les plantes.
- Els fílums predominants en la microbiota del substrat són comuns en ambdós medis (sòl i perlita) i són identificats com a taxons representatius del tomàquet. Ambdós medis difereixen en la majoria de gèneres predominants, excepte *Nitrospira*.
- En el cultiu sense sòl (perlita) es destaquen els alts índex de diversitat bacteriana i la presència de gèneres bacterians promotors del creixement vegetal com Rhizobium, Flavobacterium, Pseudomonas i Streptomyces associats a la planta, considerant un benefici potencial per als següents cultius.

Els resultats obtinguts posen de manifest la necessitat de noves investigacions en la temàtica de l'ús de nutrients recuperats d'aigües residuals en sistemes de fertirrigació, especialment en el nou marc d'acceptació legal per al seu ús i comercialització com a fertilitzants. Es fa palesa la necessitat de treballar cap a una gestió dels cultius hortícoles més sostenible, reduint la demanda d'inputs no renovables/contaminants i gestionant eficientment els nutrients aportats per minimitzar l'impacte ambiental. Futurs estudis tècnics serien requerits per a confirmar la idoneïtat de l'ús d'estruvita i nitrat d'amoni recuperats en fertirrigació en diferents medis de cultiu, espècies hortícoles, qualitats d'aigua de reg i climatologies. A més, un enfocament holístic, considerant el nexe aigua-tecnologia ambiental-agronomia i la comunicació entre fabricants i usuaris finals, és rellevant per conèixer el mercat real, aconseguir una producció optimitzada dels productes fertilitzants, i la seva acceptació social.

Capítol 9.

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Figure S4.1: Experimental scheme of the study

growing season	Season	Cultivar	Nutrient Solution
2019 campaign	March - August 2019	Bond®	One NS for all the crop stages
2020 campaign	April - August 2020	Egara®	Initial + development + final NS

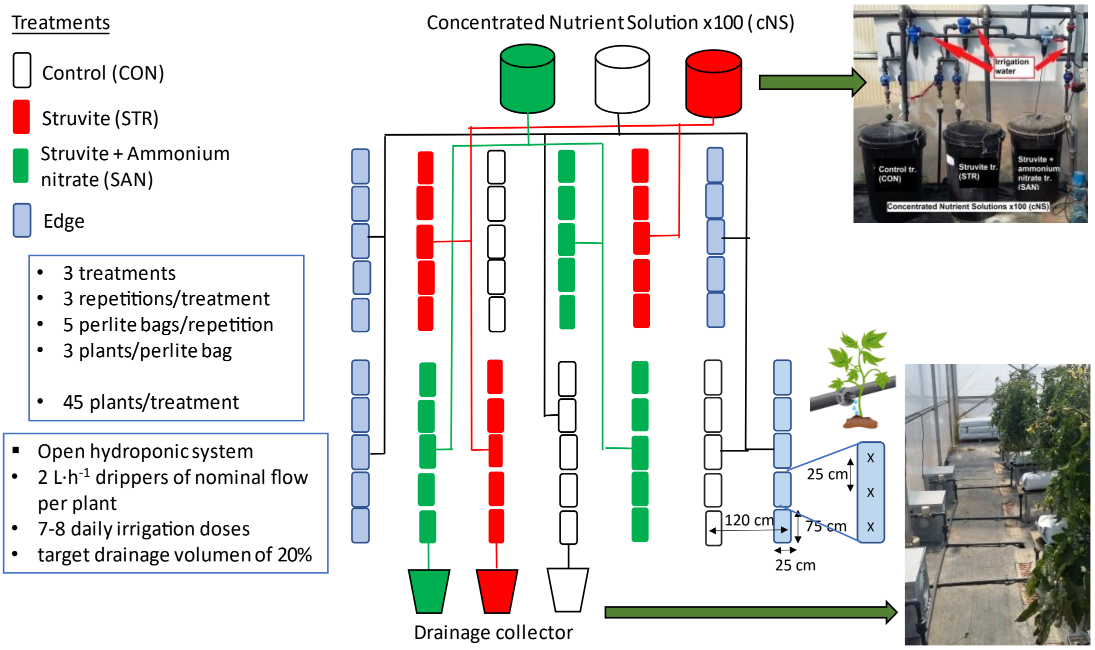


Table S4.1. Characterization of recovered struvite product based on the current legal framework in the revised fertilizer directive and in the temporary STRUBIAS document (Huygens et al., 2017)

*Sum of napthalene, acenaphythylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, dibenzo(a,h)anthracene and benzo(ghi)perylene.

**Diazepam, naproxeno, diclofenaco, 4-octilfenaco, 4-ninilfenol, triclosan, eritromicina, fluoxetina, roxitromicina, sulfametoxazol, citalopram, estrona, estradiol

***Naftaleno, 1-Metilnaftaleno, 2-Metilnaftaleno, Acenafteno, Acenaftileno, Fluoreno, Fenantreno, Antraceno, Pireno, Benzo(a)antraceno, Criseno, Benzo(a)fluoranteno, benzo(k)fluoranteno, Benzo(a)pireno, Indeno(1,2,3-cd)pireno, dibenzo(a,h)antraceno, Benzo(g,h,i)perileno, etc.

ND: Not detected; LD: Limit detection

Parameter	**Value**	**Remarks**	**DK 2018**	**DK 2019**	**MU 2020**
P_2O_5 content	16% (7% P)	Minimum	59.1 (12.9)	49.5 (10.8)	60.1 (13.1)
Organic carbon	3%	Maximum		0.02	0.2
Macroscopic impurities	5 g/kg	Maximum		< 1	< 1
Dry matter content	90%	Minimum		99.9	99.65
Heavy metals					
Cadmium (Cd)	3 $mg \cdot kg^{-1}$ DM	If <5% P2O5			
	60 $mg \cdot kg^{-1}$ DM		1.3	0.07	<0.01
Chromium (Cr VI)	2 $mg \cdot kg^{-1}$ DM		ND	0.4	2.2
Mercury (Hg)	1 $mg \cdot kg^{-1}$ DM		ND	ND	
Nickel (Ni)	100 $mg \cdot kg^{-1}$ DM		1.7	0.4	0.34
Copper (Cu)	600 $mg \cdot kg^{-1}$ DM		7.3	4	1.4
Zinc (Zn)	1500 $mg \cdot kg^{-1}$ DM		563.7	11	36
Lead (Pb)	120 $mg \cdot kg^{-1}$ DM		4.8	1	0.14
Inorganic Arsenic (As)	40 $mg \cdot kg^{-1}$ DM		ND	4	<0.01
Fe + Al content	10% DM	Maximum	ND	0.02	0.038
Others					
Biuret ($C_2H_5N_3O_2$)	12 $mg \cdot kg^{-1}$ DM			Not measured; no risk in struvite based on literature (Muys et al., 2021)	
Perchlorate (ClO_4)	50 $mg \cdot kg^{-1}$ DM				
Polycyclic aromatic hydrocarbons (PAH16*)	6 $mg \cdot kg^{-1}$ DM	Maximum; only for wastewater and sludge from municipal WWTP			
Biological contaminants					
E.coli	1000 $CFU \cdot g^{-1}$ fresh mass	Maximum		0	
Enterococcaceae	1000 $CFU \cdot g^{-1}$ fresh mass	Maximum		0	
Clostridium perfringens	100 $CFU \cdot g^{-1}$ fresh mass	Maximum		0	
*Ascaris sp.*eggs	Absent in 25 g fresh mass			Absence	
Salmonella spp.	Absent in 25 g fresh mass			Absence	
Organic pollutants - not set in the regulatory limits					
Galaxolide	$ng \cdot g^{-1}$ DM		2456	0.14	
Bisphenol A	$ng \cdot g^{-1}$ DM		987	<0.12	
Celestolide	$ng \cdot g^{-1}$ DM		<0.12	0.39	
Tonalide	$ng \cdot g^{-1}$ DM		<0.12	0.22	
Carbamazepina	$ng \cdot g^{-1}$ DM		<0.04	0.08	
ibuprofeno	$ng \cdot g^{-1}$ DM		<0.05	0.2	
trimetoprim	$ng \cdot g^{-1}$ DM		96	<0.02	
etinilestradiol	$ng \cdot g^{-1}$ DM		26	<0.05	
others	$ng \cdot g^{-1}$ DM		<LD**	<LD**	<LD***

Table S4.2. Chemical analysis of irrigation water

Parameter	Unit	Value
pH		7.7
CE (25ºC)	$\mu S\cdot cm^{-1}$	1.27
Bicarbonate	$mg\cdot L^{-1}$	337
Clorur		178
Sulphate		135
Nitrate		10
Phosphorous		0.45
Ammonia		1.3
Magnesium		35
Calcium		145
Sodium		84
Potassium		3.4
Nitrite		0.03

Table S4.3: Non-parametric variables (analysed for significance using Kruskal-Wallis p<0,05 and Wilcoxon test)

Variable	
$H_2PO_4^{2-}$ ($meq\cdot L^{-1}$)	Table 6
pH	Table 6
Nitrogen leached %	Figure 1
Phosphorous leached %	Figure 1
ppm-N leached May 2019	Figure 2
ppm-N leached May 2020	Figure 2
ppm-N leached June 2020	Figure 2
ppm-N leached July 2019	Figure 2
ppm-N leached July 2020	Figure 2
Biomass total $Kg\cdot m^{-2}$	Table 7
Caliber Campaign 2020	Table 7
Mg fruit content mg/100g wet basis campaign 2019	Table 8
P fruit content mg/100g wet basis campaign 2020	Table 8
P leaves content mg/100g wet basis campaign 2020	Table 8
K leaves content mg/100g wet basis campaign 2020	Table 8
P-biomass	Figure 3

Table S4.4: N-NH_4^+, N-NO_3^- and N-total concentration of the nutrient solutions (NS) along the experiment (mean ± SD)

		N-NO_3^-	**N-NH_4^+**	**N-total**
			meq·L^{-1}	
Campaing 2019	CON	9.03±1.84	0.1±0.09	9.13±0.83
	STR	9.34±2.30	1.36±0.28	10.7±1.06
	SAN	6.43±1.74	3.85±0.34	10.3±0.77
Campaign 2020 Initial / Final NS	CON	4.17±0.66	0.34±0.53	4.51±0.33
	STR	3.43±0.38	1.39±0.74	4.82±0.40
	SAN	3.28±0.64	1.86±0.27	5.13±0.57
Campaign 2020 Development NS	CON	7.56±1.13	0.42±0.31	8.0±0.28
	STR	6.16±0.61	1.61±0.29	7.8±0.33
	SAN	5.45±0.55	2.65±0.56	8.1±0.46

Table S4.5. Nitrogen leached concentration measured along the experiments (mean ± SD)

	mg·L^{-1} N leached					
	Campaign 2019			**Campaign 2020**		
	CON	**STR**	**SAN**	**CON**	**STR**	**SAN**
March	63.8±9.3	120.8±34.1	72.4±15.1			
April	54.1±28.0	92.9±36.4	76.2±35.0	31.9±5.1	31.7±4.2	36.8±10.2
May	27.2±26.7	57.3±53.4	17.4±22.2	15.3±1.0	23.5±11.6	29.6±19.0
June	312.5±112.2	400.9±178.6	330.9±248.0	63.8±58.0	31.0±6.9	35.8±12.4
July	378.8±64.3	580.6±204,7	512.3±130.0	139.6±44.5	100.3±73.6	68.0±18.1
August				45.0±28.5	30.1±2.4	43.3±5.3

Table S4.6: Analysis of heavy metals on fruit and leaves (n = 1)

Treatments		**Fruit content**							**Leaves content**						
		Cd	**Cu**	**Cr**	**Hg**	**Ni**	**Pb**	**Zn**	**Cd**	**Cu**	**Cr**	**Hg**	**Ni**	**Pb**	**Zn**
		mg·kg^{-1} fresh basis							**mg·kg^{-1} fresh basis**						
Campaing 2019	CON	<0.1	0.45	<0.1	<0.4	<0.5	<0.5	1.24	0.01	2.37	0.04	<0.4	<0.5	<0.5	7.07
	STR	<0.1	0.29	<0.1	<0.4	<0.5	<0.5	1.06	0.01	1.94	0.08	<0.4	<0.5	<0.5	3.48
	SAN	<0.1	0.3	<0.1	<0.4	<0.5	<0.5	1.08	0.01	1.8	0.06	<0.4	<0.5	<0.5	4.14
Campaing 2020	CON	<0.1	0.34	0	<0.4	0	<0.5	0.76	<0.1	1.30	0.01	<0.4	<0.5	<0.5	1.15
	STR	<0.1	0.36	<0.1	<0.4	<0.5	<0.5	0.96	<0.1	1.27	<0.1	<0.4	<0.5	<0.5	1.57
	SAN	<0.1	0.36	<0.1	<0.4	<0.5	<0.5	0.96	<0.1	1.39	<0.1	<0.4	<0.5	<0.5	1.47

Table S4.7. P and N uptake in aerial biomass (stems and leaves), fruit and both (total) for all treatments and years (mean ± SD)

Treatments		**P uptake**			**N uptake**		
		Aerial biomass	**fruit**	**total**	**Aerial biomass**	**fruit**	**total**
		$g \cdot m^{-2}$					
Campaing 2019	CON	6.7±1.8 b	5.7±1.3	12.4±2.2 b	24.1±3.8 b	24.9±1.9	49.0±5.5
	STR	15.5±1.5 a	5.3±0.5	20.8±1.6 a	35.9±4.3 a	22.2±2.5	58.2±6.6
	SAN	17.8±1.9 a	5.0±0.4	22.8± 2.3 a	33.7±1.8 a	22.2±1.8	55.9±0.2
Campaing 2020	CON	8.1±1.0 b	5.2±1.6	13.3±2.5 b	23.2±2.0 b	24.3±2.9	47.5±2.4
	STR	5.5±1.4 b	5.6±2.2	11.1±0.8 b	17.2±1.6 b	27.8±6.1	45.0±6.1
	SAN	7.6±0.9 b	6.3±1.5	13.9±1.7 b	19.0±3.0 b	30.9±2.7	50.0±2.5
	p-value	0.01813	N.S.	0.01919	0.0005	N.S.	N.S.

Table S5.1. 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 (Regulation EU 2019; Magrí et al., 2020)

Struvite				
mass %	**DK 2018**	**DK 2019**	**MU 2020**	**MU 2021**
Crop Campaign	Dissolution laboratory tests	Tomato & Cauliflower 2019	Tomato & Cauliflower 2020	Lettuce 2021
PO_4^{3-}	12.9 ± 0.03	10.8 ± 0.4	13.1	12.3
$N\text{-}NH_4^+$	7.2 ± 0.3	7.3 ± 0.2	5.6	5.7
Mg^{2+}	10.3 ± 0.6	9.8 ± 0.3	8.2	9.6

Parameter	**Regulation Values**	**Remarks**	**DK 2018**	**DK 2019**	**MU 2020**	**MU 2021**
P_2O_5 content	16% (7% P) DM	Minimum	59.1 (12.9)	49.5 (10.8)	60.1 (13.1)	56.3 (12.3)
Organic carbon (Corg)	3% DM	Maximum		0.02	0.2	0.56
Macroscopic impurities	5 g/kg DM	Maximum		< 1	< 1	< 1
Heavy metals (according to PFC 1(C)(I): Inorganic macronutrient Fertiliser						
Cadmium (Cd)	3 mg/kg DM	If <5% P2O5				
	60 mg/kg DM		1.3	0.07	<0.01	<0.001
Hexavalent chromium (Cr VI)	2 mg/kg DM		3.07*	0.4*	2.2*	4*
Mercury (Hg)	1 mg/kg DM		ND	ND		1
Nickel (Ni)	100 mg/kg DM		1.7	0.4	0.34	<0.001
Copper (Cu)	600 mg/kg DM		7.3	4	1.4	<0.001
Zinc (Zn)	1500 mg/kg DM		563.7	11	36	13
Lead (Pb)	120 mg/kg DM		4.8	1	0.14	4
Inorganic Arsenic (As)	40 mg/kg DM		0.5	4	<0.01	<0.001
Fe + Al content	10% DM	Maximum	0.02	0.02	0.038	0.0299
Others						
Biuret ($C_2H_5N_3O_2$)	12 g/kg DM		Not measured; no risk in struvite based on literature (Muys et al., 2021)			
Perchlorate (ClO_4)	50 mg/kg DM					
Polycyclic aromatic hydrocarbons (PAH16**)	6 mg/kg DM	Maximum; only for wastewater and sludge from municipal WWTP				
Biological contaminants						
E.coli	1000 CFU/g fresh mass	Maximum		0		<10
Enterococcaceae	1000 CFU/g fresh mass	Maximum		0		
Clostridium perfringens	100 CFU/g fresh mass	Maximum		0		<10
Ascaris sp .eggs	Absent in 25 g fresh mass			Absence		
Salmonella spp.	Absent in 25 g fresh mass			Absence		Absence
Organic pollutants - not set in the regulatory limits						
Galaxolide	ng/g DM		2456	0.14		
Bisphenol A	ng/g DM		987	<0.12		
Celestolide	ng/g DM		<0.12	0.39		
Tonalide	ng/g DM		<0.12	0.22		
Carbamazepina	ng/g DM		<0.04	0.08		
ibuprofeno	ng/g DM		<0.05	0.2		
trimetoprim	ng/g DM		96	<0.02		
etinilestradiol	ng/g DM		26	<0.05		
others	ng/g DM		<LD***	<LD***	<LD****	

*Chromium total measured (Cr III and Cr VI)

**Sum of napthalene, acenaphythylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, dibenzo(a,h)anthracene and benzo(ghi)perylene.

***Diazepam, naproxeno, diclofenaco, 4-octilfenaco, 4-ninilfenol, triclosan, eritromicina, fluoxetina, roxitromicina, sulfametoxazol, citalopram, estrona, estradiol

****Naftaleno, 1-Metilnaftaleno, 2-Metilnaftaleno, Acenafteno, Acenaftileno, Fluoreno, Fenantreno, Antraceno, Pireno, Benzo(a)antraceno, Criseno,

ND: Not detected; LD: Limit detection; DM:dry matter

Ammonium nitrate					
w/v %	**UPC 1**	**UPC 2**	**UPC 3**	**CET 1**	**CET 2**
Crop Campaign	Tomato 2019	Cauliflower 2019	Tomato 2020	Cauliflower 2020	Lettuce 2021
$N\text{-}NO_3^-$	3.5	3.9	3.5	1.4	9
$N\text{-}NH_4^+$	7	3.9	7	0.75	4
N-total	10.5	7.8	10.5	2.15	13

Table S5.2. 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) (Carreras-Sempere et al., 2021)

Nutrient Solution	Fertilization treatments	Nutrient concentration ($mg\cdot L^{-1}$)											
		N	**$N\text{-}NO_3^-$**	**$N\text{-}NH_4^+$**	**P**	**K^+**	**Ca^{2+}**	**Mg^{2+}**	**Na^+**	**SO_4^{2-}**	**Cl^-**	**EC(dS/m)**	**pH**
INITIAL/FINAL	**CON**	63.1±10.3	58.3±9.3	4.7±7.4	34.2±5.2	106.8±0.6	143.5±0.3	48.3±5.1	79.6±2.6	170.2±3.7	168±7.1	1.7±0.1	7±0
	STR	72.8±6.7	48±5.3	24.8±8.8	32.4±5.7	99.5±11.6	141.8±0.1	65.6±11	80.7±3.6	330±28.3	168.5±6.4	1.8±0.1	6.9±0.1
	SAN	72.8±10.4	46.8±10.3	26±3.7	29.6±2.7	95.7±7.3	156±28.8	58.3±2.4	79±0.2	321.1±3.6	171.5±2.1	1.8±0.1	7.1±0.1
DEVELOPMENT	**CON**	114.5±16.7	105.8±15.9 **a**	5.9±4.4 **c**	30.8±8.7	214.9±34.5	176.6±24.3	37.6±3.2 **b**	86.3±12.5	272.5±5.9 **b**	164.9±6.5	2.1±0.2	6.4±0.2 **c**
	STR	108.7±10.4	86.2±8.5 **b**	22.6±4.1 **b**	28.1±1.7	194.9±15.1	164.5±22.3	56.4±2.2 **a**	79.4±1.9	381.3±4.7 **a**	163±1	2.1±0.1	6.8±0.1 **b**
	SAN	113.4±14.3	76.3±7.7 **b**	37.1±7.8 **a**	27.9±3.1	129.2±60.6	172±60.3	55.0±0.1 **a**	71.4±22.7	418.5±10.6 **a**	162.8±1	2.2±0.2	6.9±0.1 **a**
	p-value	N.S	**<.0001**	**<.0001**	N.S	N.S	N.S	**<.0001**	N.S	**<.0001**	N.S	N.S	**<.0001**

CON: Conventional fertilization treatment; STR: Struvite fertilization treatment; SAN: Struvite + ammonium nitrate fertilization treatment.

Table S5.3. Nitrogen leached concentration mesured along the experiment (mean ± SD) (Carreras-Sempere et al., 2021)

	Nitrogen leachead ($mg\cdot L^{-1}$)								
	Nitrogen			**$N\text{-}NO_3^-$**			**$N\text{-}NH_4^+$**		
	CON	**STR**	**SAN**	**CON**	**STR**	**SAN**	**CON**	**STR**	**SAN**
April	31.9±5.1	31.7±4.2	36.8±10.2	31.4±5	27.3±10.9	32.1±10.7	0.5±0.8	4.4±6.7	4.7±2
May	15.3±1.0	23.5±11.6	29.6±19.0	14.7±0.7	22.7±11.6	24.3±14	0.6±0.6	0.8±0.7	5.3±5.1
June	63.8±58.0	31.0±6.9	35.8±12.4	63.2±58.4	30.6±6.9	32±13.2	0.6±1.2	0.3±0.4	3.7±3.8
July	139.6±44.5	100.3±73.6	68.0±18.1	136.8±43.9	91.8±77.2	61.7±19.6	2.8±2.1	8.5±8.8	6.2±3
August	45.0±28.5	30.1±2.4	43.3±5.3	37.5±26	26.3±0.5	26.9±5.9	7.5±2.6 **ab**	3.8±3 **b**	17.3±2.7 **a**

Table S5.4. 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.

Plant presence	**Time**	**Fertilization treatments**	**$N\text{-}N_2O$**	**$C\text{-}CO_2$**	**GWP (CO_2 eq.)**
				$mg\cdot m^{2}\cdot h^{-1}$	
PLANT	**t1 (1/6/2020)**	**CON**	3±1.7	8692±1041	9596±741
		STR	8.6±2.4	8316±2368	10879±3068
		SAN	8.3±5.5	7762±1362	10245±3005
	t2 (1/7/2020)	**CON**	3.2±0.4	8774±476	9727±493
		STR	5.2±1.8	14838±9507	16397±9419
		SAN	8.7±5.5	10570±2727	13152±1104
	t3 (1/8/2020)	**CON**	6.2±0.4	10035±816	11892±764
		STR	2±2.7	10369±7057	10965±6495
		SAN	5.7±1.5	8266±3212	9954±3059
	p-value	**Time**	N.S.	N.S.	N.S.
		Treatment	N.S.	N.S.	N.S.
		Time*Treatment	N.S.	N.S.	N.S.
WITHOUT PLANT	**t1 (1/6/2020)**	**CON**	5.6±4.8	5515±1965	7184±3388
		STR	121.2±53.1	2818±1029	38975±16571
		SAN	56.9±3.3	3489±402	20375±771
	t3 (1/8/2020)	**CON**	6.8±4.7	1173±373	3199±1053
		STR	6.1±2.7	1394±371	3221±641
		SAN	4.1±0.6	1362±191	2584±161
	p-value	**Time**	**0.02**	**0.0003**	**0.0003**
		Treatment	N.S.	N.S.	N.S.
		Time*Treatment	**0.034**	**0.012**	**0.01**

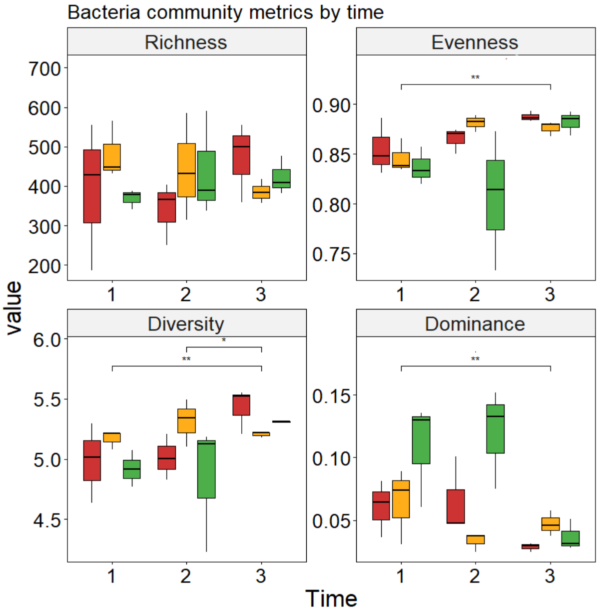
			N-N$_2$O	**C-CO$_2$**	**GWP (CO$_2$ eq.)**
t1 (1/6/2020)	**p-value**	**Plant**	**0.038**	**0.0013**	N.S.
		Treatment	**0.017**	N.S.	N.S.
		Plant*Treatment	**0.018**	**0.021**	**0.029**
t3 (1/8/2020)	**p-value**	**Plant**	N.S.	**0.0003**	**0.0003**
		Treatment	N.S.	N.S.	N.S.
		Plant*Treatment	N.S.	**0.023**	**0.02**

CON: Conventional fertilization treatment; STR: Struvite fertilization treatment; SAN: Struvite + ammonium nitrate fertilization treatment.

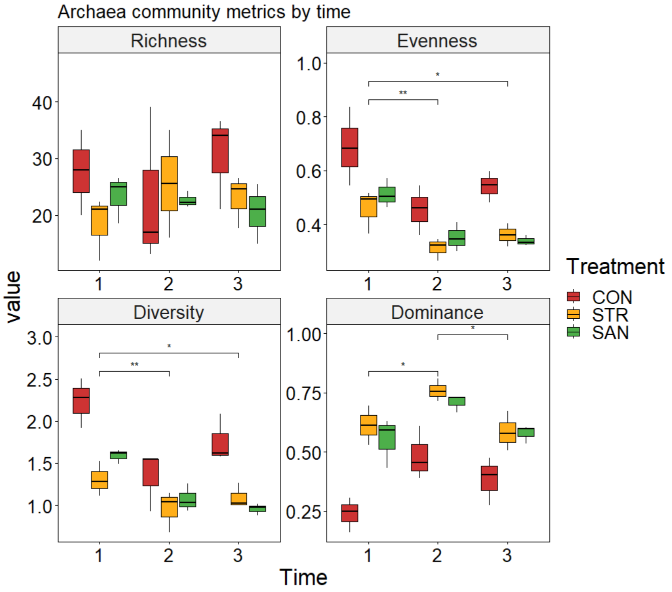
GPW: Global Warming Potential

Table S5.5. 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 by time, fertilization treatment, time*treatment and plant presence/absence.

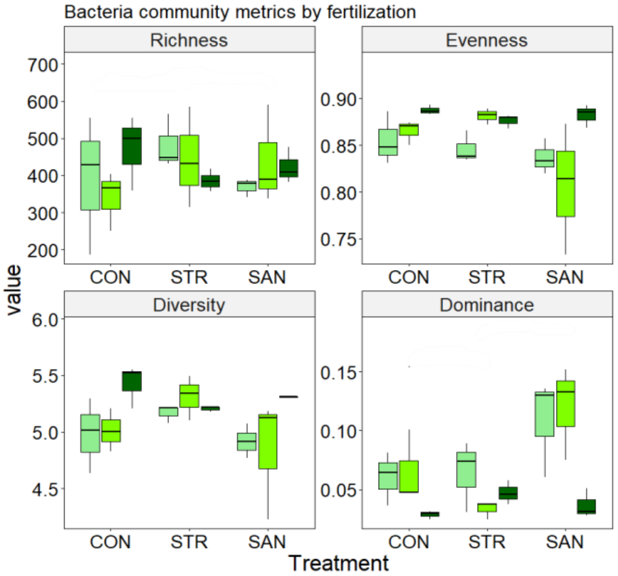
Kingdom	**Plant presence**	**Time**	**Fertilization treatments**	**Shannon**	**Relative dominance**	**Pielou's**	**Chao1**
Bacteria	**Plant**	1	CON	5±0.3	0.06±0.02 **abc**	0.86±0.03 **ab**	390±187
			STR	5.2±0.1	0.06±0.03 **abc**	0.85±0.02 **ab**	482±73
			SAN	4.9±0.2	0.11±0.04 **ab**	0.84±0.02 **b**	369±25
		2	CON	5±0.2	0.07±0.03 **abc**	0.87±0.01 **ab**	340±79
			STR	5.3±0.2	0.03±0.01 **c**	0.88±0.01 **ab**	444±136
			SAN	4.8±0.5	0.12±0.04 **a**	0.81±0.10 **ab**	439±133
		3	CON	5.4±0.2	0.03±0.00 **c**	0.89±0.00 **a**	472±101
			STR	5.2±0	0.05±0.01 **abc**	0.88±0.01 **ab**	386±30
			SAN	5.3±0	0.04±0.01 **b**	0.88±0.01 **ab**	422±48
	p.adj	**Time**		**0.015**	**0.025**	**0.009**	N.S.
			Treatment	N.S.	N.S.	N.S.	N.S.
		Time*Treatment		N.S.	**0.003**	**0.03**	N.S.
	Without Plant (WP)	1	CON	4.4±0.5	0.1±0.02	0.88±0.02	104±187
			STR	4.2±0.1	0.2±0.03	0.8±0.03	266±43
			SAN	4±0.2	0.2±0.04	0.7±0.03	249±28
		3	CON	5.1±0.1	0.04±0.01	0.83±0.01	108±189
			STR	4.7±0.2	0.1±0.03	0.8±0.02	516±76
			SAN	4.2±0.2	0.2±0.04	0.7±0.02	436±90
	dj Plant presence			**<.001**	**<.001**	**<.001**	N.S.
Kingdom	**Plant presence**	**Time**	**Fertilization treatments**	**Shannon**	**Relative dominance**	**Pielou's**	**Chao1**
Archaea	**Plant**	1	CON	2.2±0.3	0.2±0.1	0.7±0.15	28±8
			STR	1.3±0.2	0.6±0.08	0.5±0.08	18±6
			SAN	1.6±0.1	0.6±0.1	0.5±0.05	23±4
		2	CON	1.3±0.4	0.5±0.11	0.5±0.09	23±14
			STR	1±0.2	0.8±0	0.3±0.04	26±10
			SAN	1.1±0.2	0.7±0	0.4±0.1	23±1
		3	CON	1.8±0.3	0.4±0.1	0.5±0.06	31±8
			STR	1.1±0.1	0.6±0.08	0.4±0.08	23±5
			SAN	1±0.1	0.6±0.04	0.3±0.02	21±5
	p.adj	**Time**		**<.0001**	**0.0003**	**0.009**	N.S.
			Treatment	**<.0001**	**<.0001**	**0.008**	N.S.
		Time*Treatment		N.S.	N.S.	N.S.	N.S.
	Without Plant (WP)	1	CON	4.7±0.1	0.03±0.00	0.92±0.01	171±8
			STR	4.7±0.2	0.03±0.01	0.9±0.01	198±36
			SAN	4.7±0.3	0.03±0.02	0.9±0.03	159±23
		3	CON	2.2±0.2	0.43±0.08	0.58±0.06	48±10
			STR	1.5±0.2	0.6±0.04	0.4±0.05	38±14
			SAN	1.2±0.3	0.7±0.11	0.4±0.08	29±4
	dj Plant presence			**0.008**	N.S.	**0.02**	**<.0001**



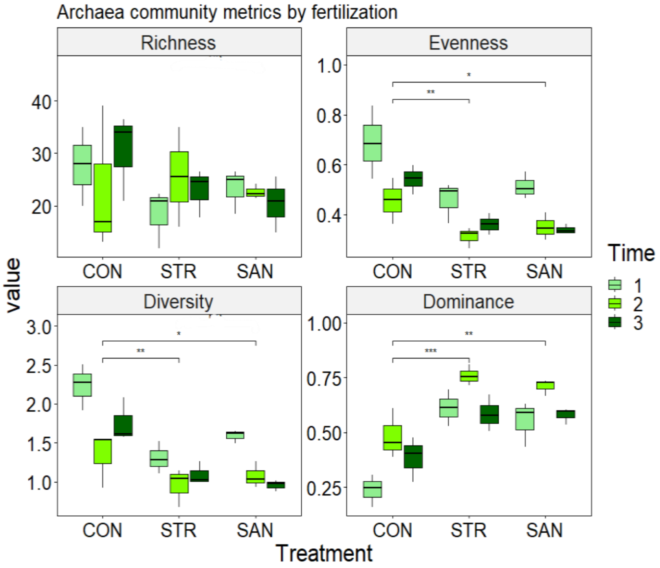
Bacteria community metrics by time



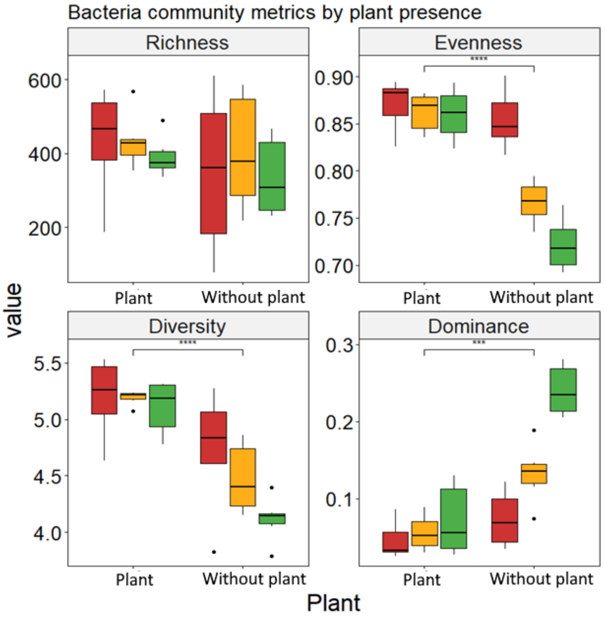
Archaea community metrics by time



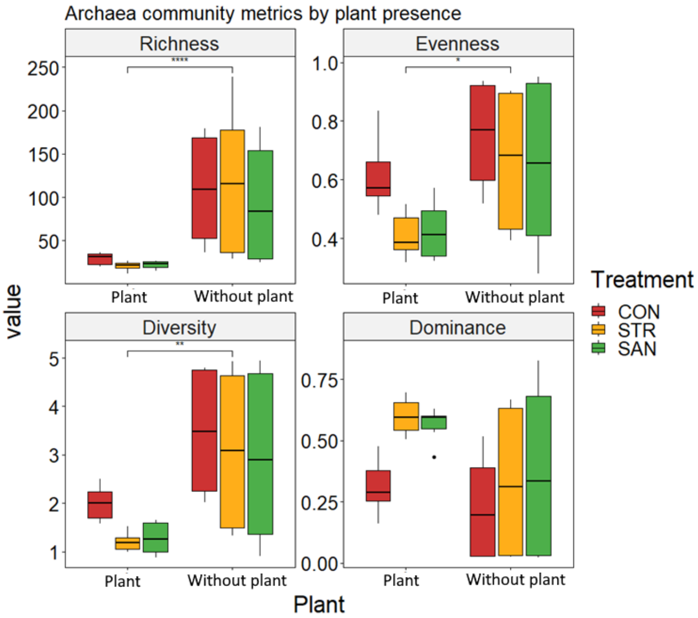
Bacteria community metrics by fertilization



Archaea community metrics by fertilization



Bacteria community metrics by plant presence



Archaea community metrics by plant presence

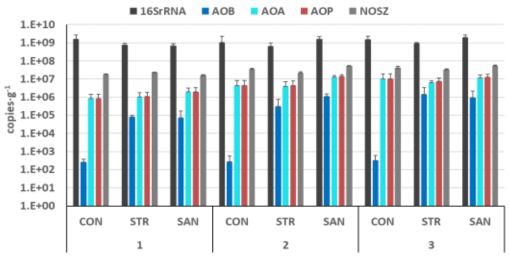
Table S5.6. Taxonomic categories relative abundance (RA) of both bacteria and archaea kingdom. Statistical tests were performed: i) Within plant group using data from t1 to t3 (by time and fertilization) and ii) between plant and without plant (WP) groups using data from t1 and t3 (by plant presence)

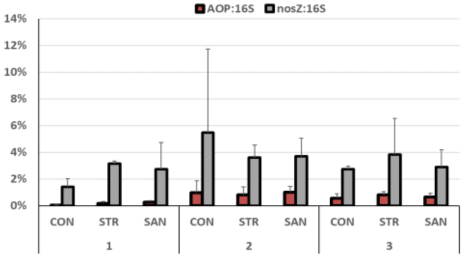
Kingdom	Taxonomic level	Plant presence	Time	Fertilization Treatment	Acidobacteria	Actinobacteria	Armatimonadetes	Bacteroidetes	candidate_division_WPS-1	Candidatus_Saccharibacteria	Chlamydiae	Chloroflexi	Cyanobacteria	Deinococcus-Thermus	Firmicutes	Gemmatimonadetes	Hydrogenedentes	Ignavibacteriae	Latescibacteria	Microgenomates	Nitrospirae	Parcubacteria	Planctomycetes	Proteobacteria	Spirochaetes	SR1	Unclassified	Verrucomicrobia	candidate_division_ZB3	candidate_division_WPS-2
BACTERIA	Phylum	Plant	1	CON	1.7±1	2.1±0.3	0.4±0.3	18.8±1.8	0±0	1.8±0.7	0.3±0.2	2.9±2.4	0±0	0±0	0.1±0.1	0.1±0.2	0±0.1	0.1±0.1	0±0	0±0	0.5±0.4	3.3±1.4	2±0.2	51.1±8.2	0.1±0.1	0.1±0.1	11.8±6.6	2.9±1.1	0±0	0±0
				STR	1.7±0.9	6.5±2.4	0.5±0.3	15.4±1	0±0	0.8±0.2	0.8±0.7	0.8±0.2	0±0.1	0.2±0.2	0.1±0	0.1±0	0.2±0.1	0±0.1	0±0	0±0	2.4±1.1	2.7±0.9	3.9±0.9	52.7±3.2	0.3±0.1	0±0	8.9±1.2	2.1±1	0±0	0±0
				SAN	1.4±0.5	3.7±2.2	0.4±0.3	12.8±2.9	0±0.1	0.5±0.2	0.3±0.3	2.2±2.1	0.2±0.3	0.1±0.1	0.2±0.3	0.3±0.2	0.1±0.1	0.2±0.1	0±0	0±0	1.7±1.7	2.8±0.5	2.1±0.4	57.8±1.5	0.1±0	0±0	10.3±2.1	3±2.3	0±0	0±0
			2	CON	2.5±1.5	3.1±1.3	1.6±1.3	10.6±3.5	0±0	1.1±0.1	0.1±0	0.8±0.1	0±0	0±0	0.1±0.1	0.1±0.1	0±0	0±0	0±0.1	0±0	1.2±0.3	1.6±0.3	2±0.3	63.7±2	0.1±0.1	0±0.1	9.7±0.8	1.8±0.4	0±0	0±0
				STR	3±1	4.2±1.7	0.6±0.2	12.6±2.1	0±0	0.9±0.3	0.6±0.2	1.5±1.1	0±0	0.1±0.1	0.1±0.1	0.1±0.1	0.1±0.1	0±0	0±0.1	0±0	2.9±2.2	2.5±0.3	4.1±0.5	55.7±6.1	0±0	0±0	8.6±2.1	2.5±1.5	0±0	0±0
				SAN	1.7±0.9	12.9±7.9	0.5±0.4	11.8±2.2	0±0	6.5±8.1	0.7±0.4	1.6±0.4	0±0	0±0	0.2±0.2	0.3±0.2	0.1±0	0±0	0±0.1	0±0	3.6±0.8	1.2±0.7	3.6±2.6	47±5.5	0±0	0±0	7.3±1.9	1.2±1.3	0±0	0±0
			3	CON	5.2±1.7	6.3±4.9	1.2±0.7	6.4±3.9	0±0	2.8±1.8	0.3±0.1	2.3±0.6	0±0	0±0	0.2±0.1	0.2±0.1	0.1±0	0±0	0.1±0.1	0±0	1.7±0.6	1.3±0.4	2.6±0.2	53.1±4.1	0±0	0±0	12.6±2.3	3.4±1.3	0±0	0±0
				STR	2.1±0.4	4.5±2.4	0.4±0.2	10.1±1.1	0±0	1.5±0.3	0.5±0.3	2.9±1.3	0±0	0.1±0.1	0.1±0.1	0.3±0.1	0.1±0.1	0±0	0.2±0.1	0±0	4.3±3.2	1.1±0.5	2.9±0.5	57.2±4	0±0	0±0	9.6±2.1	2.3±0.4	0±0	0±0
				SAN	3.1±1.3	4.7±2	0.5±0.3	8.9±1.1	0±0	5.2±4.2	1.8±1.7	2.8±0.9	0±0	0±0	0±0	0.4±0.2	0.1±0	0.1±0	0.4±0.4	0±0	5.6±1.1	2.4±1.5	3±0.7	47.3±5.4	0±0	0±0	11.4±1.5	2.3±1.3	0±0	0±0
		p-value	Time		**0.03**	N.S.	N.S.	**0.008**	N.S.	**0.013**	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	**0.009**	N.S.	**0.047**	N.S.	N.S.	N.S.	**0.012**	N.S.	N.S.	N.S.	N.S.	N.S.
				Fertilization	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	**0.012**	N.S.	N.S.	**0.004**	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	**0.007**	N.S.	**0.006**	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
		Without Plant (WP)	1	CON	0.3±0.4	2.1±0.4	0.1±0.2	16.9±7.1	0±0	0.8±0.8	1.8±1.7	1.2±1.3	0±0	0.2±0.3	1.4±0.3	0.1±0.1	0.1±0.1	0±0	0±0	0±0	1.5±1.1	0.4±0.1	0.9±0.2	54.7±2.1	0±0	0±0	15.6±5.3	2±1.2	0±0	0±0
				STR	0.9±0.8	0.9±0.5	0.1±0	18.8±8.1	0±0	0.5±0.2	0.8±0.5	0.4±0.1	0±0	0.1±0	2±1.3	0±0	0.1±0.1	1.1±0.3	0±0	0±0	18.7±3.6	1.9±1.2	2.5±1.7	40.4±4.9	0±0	0±0	9.9±2	0.8±0.4	0±0	0±0
				SAN	1.1±0.3	0.6±0.5	0.1±0.1	14.9±3.6	0±0	0.1±0	3±0.9	0.5±0.5	0±0	0.1±0	0.4±0.2	0.3±0	0.1±0.1	5.4±2.3	0±0	0±0	27.2±3	1±0.8	1.8±0.6	33.4±1.8	0±0	0±0	9.4±2.2	0.7±0.3	0±0	0±0
			3	CON	2.6±1	2.4±0.7	0.2±0.1	7.1±0.8	0.1±0.1	1.1±0.6	2.3±1.2	2.6±1.3	0±0	0±0	0.1±0	1±0.4	0.1±0.1	0.1±0.1	0.4±0.3	0±0	6.3±1	5.2±1	0.9±0.2	46.6±6.6	0±0.1	0±0	18.4±5.5	2.4±0.3	0±0	0±0
				STR	7.4±1.2	0.8±0.4	0.3±0.2	8.9±0.5	0.2±0.1	0.5±0.1	1.6±0.2	2.3±0.5	0.2±0.1	0.3±0.1	0±0.1	0±0	0.1±0.1	1±0.2	0.1±0	0±0	16.9±3.4	3.6±0.2	1±0.2	41.3±2.3	0±0	0±0	12.2±0.8	1.4±0.4	0±0	0±0
				SAN	3.5±0.3	0.6±0.3	0.2±0.1	9±0.7	0±0	0.5±0.2	1.3±0.6	1.1±0.2	0±0	0.2±0.1	0±0	0.4±0.2	0.1±0.1	6.3±2	0.8±0.7	0±0	29±3.3	3.8±0.8	1.9±0.6	29.1±1.6	0±0	0±0	11±1.6	1±0.3	0±0	0±0
	p-adj	Plant presence			N.S.	**<.0001**	**0.005**	N.S.	N.S.	**0.006**	**0.014**	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	**0.016**	N.S.	N.S.	**0.004**	N.S.	**0.002**	**0.002**	**0.026**	N.S.	N.S.	**0.011**	N.S.	N.S.
	Taxonomic level	**Plant presence**	**Time**	**Fertilization**	**Acidovorax**	**Arthrobacter**	**Azospirillum**	**Bdellovibrio**	**Cellvibrio**	**Devosia**	**Flavobacterium**	**Hydrogenophaga**	**Ignavibacterium**	**Ilumatobacter**	**Methylophilus**	**Nitrosomonas**	**Nitrosospira**	**Nitrospira**	**Pseudomonas**	**Rhizobium**	**Sediminibacterium**	**Shinella**	**Sphingobium**	**Sphingopyxis**	**Streptomyces**	**Tahibacter**	**Terrimonas**	**Unclassified**		
	Dominant genus (Relative abundance >1%)	Plant	1	CON	1.4±0.3	0.9±0.4	0.03±0.1	1.1±0.6	3.8±2.2	1.3±1	6.5±0.7	3.8±0.8	0±0	0.1±0.1	2.4±0.7	0±0	0±0	0.5±0.4	0.7±0.9	1.8±1.9	1.5±0.7	3.3±3	4.7±2.8	0.7±0.3	0±0	0.2±0.1	0.4±0.1	43.3±9.5		
				STR	1.6±0.5	3.8±1.7	0±0	0.3±0.1	1.8±0.3	1.4±0.3	2.9±2.6	1.6±0.5	0±0.1	0.3±0.1	0.9±0.1	0.1±0	0.01±0.02	2.4±1.1	1.3±0.2	1.1±0	1.7±0.1	2.9±1	5.9±0.9	0.8±0.4	0±0	0±0.1	0.4±0.3	51±5.4		
				SAN	1.2±0.8	2.6±1.8	0±0	0.5±0.1	6.3±6	1.1±0.2	1.9±0.5	2.3±0.9	0.1±0.1	0.2±0.2	1.6±1.2	0.2±0.2	0±0	1.7±1.7	1.1±0.3	1±1	2.5±1.4	1.3±0.9	2.8±0.6	1.7±0.4	0±0	0±0.1	0.5±0.2	48.5±4.6		
			2	CON	1.4±0.7	0.6±0.1	0±0	0.6±0.2	3.6±1.7	2.1±0.4	2.7±2.7	3.5±0.6	0±0	0.6±0.5	1.3±1.2	0±0	0±0	1.2±0.3	2±2.3	1.1±0.6	0.6±0.4	2.4±1.1	5.4±0.5	1.2±0.5	0±0	4.4±4.9	0.3±0.5	46±2.4		
				STR	1.7±0.5	0.9±0.5	0±0	0.4±0.5	2.1±2.1	1.9±0.5	1.4±0.9	2.8±1	0.02±0.03	0.8±0.4	0.4±0.2	0.3±0.5	0.02±0.03	2.9±2.2	0.2±0.2	0.4±0.2	0.8±0.1	2±0.6	5.1±2	3.4±1.3	0±0	2±1.9	1.1±0.7	53.1±5.9		
				SAN	3.9±1.3	0.5±0.1	0±0	0.2±0.1	1.2±0.4	1.4±0.2	2.4±1.5	1.5±0.9	0.02±0.03	0.5±0.2	0.2±0.3	0.6±0.3	0±0	3.6±0.8	0.8±0.5	2.1±0.9	0.4±0.1	1.5±0.2	4.2±1.9	1.3±0.5	9.7±8	0.5±0.8	0.5±0.3	49.9±8		
			3	CON	1.6±0.6	0.3±0.1	0.02±0.03	0.3±0.2	1.1±1.2	1.7±0.1	0.3±0.2	1.7±0.4	0±0	2.4±1.9	1.2±0.9	0±0	0±0	1.7±0.6	0.2±0.2	0.8±0.2	0.2±0.2	1.3±0.4	3.2±2.4	2±1.2	0.8±1.4	0.7±0.2	0.3±0.2	61.5±5.5		
				STR	1.9±0.3	0.2±0.1	0±0	0.5±0.4	1.3±1	1.6±0.1	0.6±0.5	1.8±0.8	0±0	0.8±0.1	0.4±0.1	1.3±1.9	0±0	4.3±3.2	0.5±0.8	1.5±0.3	0.8±0.2	2±1.3	4.4±1.4	1.7±0.6	0.4±0.4	1.4±1.3	0.7±0.5	57.1±5		
				SAN	1.5±0.6	0.1±0.1	0.07±0.1	0.3±0.3	0.9±0.4	1.6±1	0.4±0.1	1.4±0.9	0.01±0.01	1.7±0.8	0.7±0.6	1.1±0.7	0±0	5.6±1.1	0.2±0.3	0.9±0.5	0.7±0.3	0.8±0.4	1.9±1	1.2±0.3	0.2±0.1	1.7±1.5	0.6±0.5	61.6±7.7		
		p-value	Time		N.S.	**<.0001**	N.S.	N.S.	**0.02**	N.S.	**0.002**	**0.04**	N.S.	**0.0005**	**0.049**	N.S.	N.S.	**0.047**	N.S.	N.S.	**0.002**	N.S.	N.S.	N.S.	N.S.	**0.006**	N.S.	N.S.		
				Fertilization	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	**0.0003**	N.S.	**0.007**	N.S.	N.S.	N.S.	**0.03**	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.		
		Without Plant (WP)	1	CON	0±0	0.5±0.5	0±0	15.6±2.1	0±0	3.6±1	1.6±1.7	0.3±0.3	0±0	0±0	0±0	1.1±1.1	0±0	1.5±1.1	0±0	1.2±0.3	0.9±1.1	2.9±1.8	0.2±0.3	0±0	0±0	0±0	0.01±0.01	47.3±6.4		
				STR	0±0.1	0.2±0.1	0±0	2.7±1.7	0±0	1.5±0.5	0.1±0.1	0.2±0.1	0.1±0.1	0±0	0±0	7.1±1.1	1.1±0.3	18.7±3.6	0.1±0.1	0±0.1	6.4±3.5	2.7±0.6	0.03±0.04	0.01±0.02	0±0	0±0	3.2±2	39.9±6.1		
				SAN	0.1±0.1	0.4±0.4	0±0	0.8±0.3	0±0	1.5±0.4	0.04±0.05	0.4±0.1	2.4±0.8	0±0	0±0	11.7±0.6	2.5±2.6	27.2±3	0.1±0.1	0±0	2.6±0.5	2.6±0.6	0±0	0±0	0±0	0±0	1.7±1.5	31.4±5.4		
			3	CON	0±0	0.02±0.02	0±0	2±0.6	0±0	3.4±2.6	0±0	1.2±0.3	0.1±0	0±0	0±0	1.7±0.1	0±0	6.3±1	0.2±0.2	0.01±0.01	2±0.5	4±1.9	0.3±0.2	0.04±0.05	0±0	0±0	2.7±1.3	61.8±7.9		
				STR	0±0	0±0	0±0	2.7±0.4	0±0	0.9±0.2	0.03±0.01	0.05±0.04	0.5±0.1	0.1±0.1	0±0	15.3±1	0.3±0.2	16.9±3.4	0.02±0.03	0±0	1.8±0.2	1.4±0.4	0.05±0.05	0±0	0±0	0.02±0.03	3.9±0.7	47.1±3		
				SAN	0±0	0.2±0.3	0±0	1.4±0.3	0±0	0.5±0.2	0±0	0.1±0	5.8±1.6	0.08±0.03	0±0	10.2±4.4	0.7±0.6	29±3.3	0.01±0.01	0±0	1.3±0.3	0.9±0.1	0±0	0±0	0±0	0±0	3.7±0.5	39.8±3.3		
	p-adj	Plant presence			**<.0001**	N.S.	N.S.	**0.001**	**<.0001**	N.S.	**0.013**	**0.0004**	**0.015**	**0.011**	**<.0001**	**0.001**	**0.024**	**0.035**	**0.041**	**0.007**	N.S.	N.S.	**<.0001**	**<.0001**	N.S.	**0.003**	N.S.	N.S.		

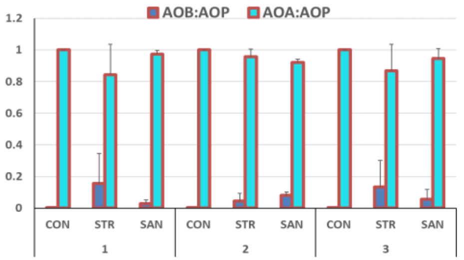
Kingdom	Taxonomic level	Plant presence	Time	Fertilization	Euryarchaeota	Pacearchaeota	Thaumarchaeota	Unclassified	Woesearchaeota				
ARCHAEA	Phylum	Plant	1	CON	30.4±5.67	6.7±7.76	57.4±14.13	0±0.08	5.4±8.89				
				STR	8.8±12.06	8.1±7.92	78.1±14.93	0±0.04	5±5.26				
				SAN	19±9.65	5.9±0.76	71±10.89	0.1±0.11	4±2.46				
			2	CON	5.5±9.45	0.7±0.02	91.3±8.32	0.1±0.1	2.4±4.07				
				STR	3.6±5.18	0.8±0.53	93.8±6.79	0.5±0.44	1.3±1.44				
				SAN	0.4±0.38	0.5±0.39	94.3±4.56	3.5±3.71	1.3±0.88				
			3	CON	2.1±3.72	1.4±0.7	82.1±24.61	3.1±2.67	11.3±19.12				
				STR	0.1±0.12	0.4±0.3	94.5±5.59	4±5.73	0.9±0.46				
				SAN	0±0	0.3±0.28	98.3±1.02	0.4±0.39	1±0.53				
		p-value	Time		**0.0001**	**0.0003**	**0.001**	**0.002**	N.S.				
				Fertilization	N.S.	N.S.	N.S.	N.S.	N.S.				
		Without Plant (WP)	1	CON	3.8±1.9	0±0	95.6±1.4	0.3±0.6	0.3±0.5				
				STR	18.3±7.6	0.5±0.4	80.5±6.9	0.5±0.5	0.2±0.4				
				SAN	41.8±25.4	1.6±1.4	53.5±21.8	1.8±3.1	1.4±2.2				
			3	CON	18.1±9.1	0.2±0.3	80.2±9.3	0.9±0.5	0.6±0.3				
				STR	7.1±7.7	0.8±0.8	83.2±7.7	7.9±0.9	1.1±0.6				
				SAN	1.5±0.7	0.2±0.1	92.1±2.2	3.8±0.9	2.3±0.8				
	p-adj	Plant presence			N.S.	**0.01**	N.S.	N.S.	N.S.				
	Taxonomic level	**Plant presence**	**Time**	**Fertilization**	**Methanobacteriacea**	**Methanocellaceae**	**Methanomassiliicoccaceae**	**Methanoregulaceae**	**Methanosarcinaceae**	**Methanotrichaceae**	**Nitrosopumilaceae**	**Nitrososphaera**	**Unclassified**
	Dominant family (Relative abundance >1%)	Plant	1	CON	0.01±0.02	0±0	0.3±0.48	0±0	0.05±0.08	0±0	55.5±13.11	1.9±1.18	42.3±13.86
				STR	0.07±0.13	0±0	0±0	0±0	0.02±0.04	0±0	77.5±14.91	0.6±0.53	21.8±14.98
				SAN	0.05±0.08	0±0	0.01±0.02	0±0	0.1±0.23	0±0	70.9±10.81	0.1±0.08	28.8±10.94
			2	CON	0±0	0±0	0.8±1.3	0±0	0.04±0.06	0±0	90±8.38	1.3±1.96	7.9±7.17
				STR	0±0	0±0	0.1±0.21	0±0	0.02±0.02	0±0	93.4±6.59	0.4±0.39	6.1±6.58
				SAN	0±0	0±0	0±0	0±0	0.01±0.02	0±0	93±4.46	1.2±1.26	5.7±4.57
			3	CON	0±0	0±0	0.01±0.02	0±0	0±0	0±0	78.3±21.64	3.8±3.48	17.9±24.59
				STR	0±0	0±0	0.02±0.04	0±0	0±0	0±0	93.9±5.5	0.6±0.43	5.5±5.55
				SAN	0±0	0±0	0±0	0±0	0±0	0±0	96.8±1.5	1.5±2.09	1.7±1.02
		p-value	Time		**0.03**	N.S.	N.S.	N.S.	N.S.	N.S.	**0.002**	N.S.	N.S.
				Fertilization	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
		Without Plant (WP)	1	CON	0±0	0±0	0.5±0.2	0±0	0±0	0±0	93.6±0.9	2±1.8	3.9±1.3
				STR	0±0	0±0	2±1.3	0±0	0±0	0±0	80.5±6.9	0±0	17.5±5.5
				SAN	0±0	0±0	1.3±1.2	0±0	0±0	0±0	53.5±21.8	0±0	45.2±20.6
			3	CON	0±0	0±0	1.4±0.6	0±0	0±0	0±0	60.6±8.3	19.6±10.7	18.5±9.1
				STR	0±0	0±0	0.2±0.2	0±0	0±0	0±0	79.5±5.6	3.6±2.4	16.6±7.7
				SAN	0±0	0±0	0.4±0.6	0±0	0±0	0±0	90.5±2.9	1.6±1	7.5±1.7
	p-adj	Plant presence			N.S.	N.S.	**<.0001**	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

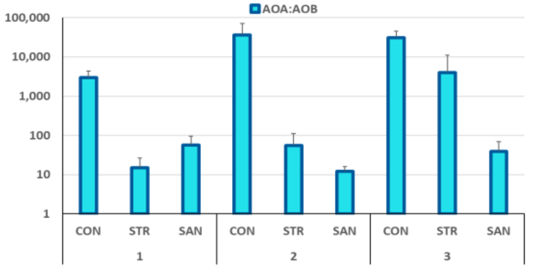
Table S5.7. qPCR results of total bacteria (16S rRNA), ammonia-oxidizing prokaryotes (AOP) community (amoA of ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA)) and nitrous oxide reductase genes (nosZ) and its respective ratios. Statistical tests were performed: i) Within plant group 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)

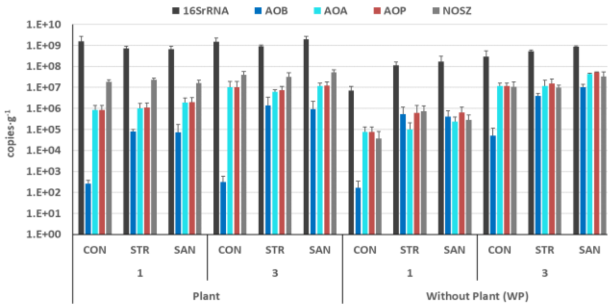
Plant presence	Time	Treatment	16SrRNA	AOB	AOA	AOP	nosZ	AOB:16S	AOA:16S	AOB:AOP	AOA:AOP	AOA:AOB	AOB:AOA	AOP:16S	nosZ:16S	C.CO2	N.N2O
Plant	1	CON	1.60E+9 ± 1.08E+9	2.63E+2 ± 1.12E+2	8.39E+5 ± 5.50E+5	8.39E+5 ± 5.50E+5	1.83E+7 ± 5.30E+6	0 ± 0	0.0005 ± 0.0002	0 ± 0	1 ± 0	2963.2 ± 1430.2	0.007 ± 0.003	0.0005 ± 0.0002	0.014±0.006	8692±1041	3.03±1.65
		STR	7.37E+8 ± 1.82E+8	7.99E+4 ± 2.11E+4	1.03E+6 ± 7.52E+5	1.11E+6 ± 7.38E+5	2.31E+7 ± 4.86E+6	0.0001 ± 0	0.0016 ± 0.0013	0.16 ± 0.19	0.84 ± 0.19	14.8 ± 12.1	3.27 ± 4.46	0.0017 ± 0.0013	0.032±0.002	8316±2368	8.6±2.35
		SAN	6.77E+8 ± 2.18E+8	7.19E+4 ± 9.69E+4	1.92E+6 ± 1.22E+6	1.99E+6 ± 1.30E+6	1.58E+7 ± 6.28E+6	0.0001 ± 0.0001	0.0026 ± 0.0011	0.03 ± 0.03	0.97 ± 0.03	57.5 ± 38.7	0.42 ± 0.36	0.0027 ± 0.0012	0.027±0.02	7762±1362	8.33±5.52
	2	CON	1.03E+9 ± 1.28E+9	2.69E+2 ± 3.06E+2	4.41E+6 ± 3.80E+6	4.41E+6 ± 3.80E+6	3.50E+7 ± 2.90E+7	0 ± 0	0.0097 ± 0.0087	0 ± 0	1 ± 0	36287 ± 34686	0.0008 ± 0.0005	0.0097 ± 0.0087	0.055 ± 0.063	8774±476	3.2±0.44
		STR	6.33E+8 ± 3.43E+8	3.03E+5 ± 4.59E+5	3.94E+6 ± 2.97E+6	4.25E+6 ± 3.43E+6	2.18E+7 ± 9.43E+6	0.001 ± 0.001	0.0075 ± 0.0054	0.04 ± 0.05	0.96 ± 0.05	56 ± 55	0.5 ± 0.8	0.0081 ± 0.0061	0.036 ± 0.009	14838±9507	5.23±1.76
		SAN	1.57E+9 ± 6.55E+8	1.11E+6 ± 4.22E+5	1.26E+7 ± 3.04E+6	1.37E+7 ± 3.38E+6	5.25E+7 ± 1.53E+7	0.001 ± 0.001	0.009 ± 0.0039	0.08 ± 0.02	0.92 ± 0.02	12 ± 4	1.15 ± 0.3	0.0099 ± 0.0045	0.037 ± 0.014	10570±2727	8.67±5.5
	3	CON	1.53E+9 ± 7.36E+8	3.21E+2 ± 2.86E+2	1.00E+7 ± 9.39E+6	1.00E+7 ± 9.39E+6	4.06E+7 ± 1.67E+7	0 ± 0	0.0057 ± 0.0031	0 ± 0	1 ± 0	31126.9 ± 13764.4	0.0006 ± 0.0004	0.0057 ± 0.0031	0.027±0.002	10035±816	6.23±0.4
		STR	9.04E+8 ± 1.42E+8	1.40E+6 ± 1.98E+6	6.15E+6 ± 1.70E+6	7.54E+6 ± 3.56E+6	3.25E+7 ± 1.83E+7	0.0014 ± 0.0019	0.0067 ± 0.001	0.13 ± 0.17	0.87 ± 0.17	4033.6 ± 6973.9	2.34 ± 3.18	0.0081 ± 0.0026	0.038±0.027	10369±7057	2±2.71
		SAN	1.97E+9 ± 6.94E+8	9.09E+5 ± 1.29E+6	1.15E+7 ± 4.63E+6	1.25E+7 ± 5.81E+6	5.30E+7 ± 1.53E+7	0.0004 ± 0.0005	0.0062 ± 0.0027	0.05 ± 0.06	0.95 ± 0.06	39.2 ± 29.6	0.81 ± 0.93	0.0066 ± 0.0028	0.029±0.013	8266±3212	5.67±1.46
p.adj	**Time**		N.S.	N.S.	**0.0008**	**0.0008**	**0.025**	N.S.	**0.0008**	N.S.	N.S.	N.S.	N.S.	**0.001**	N.S.	N.S.	N.S.
		Treatment	N.S.	**0.0002**	N.S.	N.S.	N.S.	**0.0002**	N.S.	**0.0003**	**0.0003**	**0.0003**	**0.0003**	N.S.	N.S.	N.S.	N.S.
	Time * Treatment		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Without Plant (WP)	1	CON	7.24E+6 ± 3.81E+6	1.65E+2 ± 1.85E+2	7.49E+4 ± 5.48E+4	7.51E+4 ± 5.50E+4	3.70E+4 ± 4.21E+4	0 ± 0	0.0105 ± 0.0063	0 ± 0	1 ± 0	875 ± 663.1	0.05 ± 0.02	0.0105 ± 0.0063	0.004 ± 0.003	5515±1965	5.6±4.79
		STR	1.13E+8 ± 4.72E+7	5.25E+5 ± 6.35E+5	1.02E+5 ± 1.08E+5	6.27E+5 ± 7.40E+5	7.29E+5 ± 5.89E+5	0.0046 ± 0.0051	0.001 ± 0.0009	0.81 ± 0.12	0.19 ± 0.12	0.3 ± 0.2	22.59 ± 16.03	0.0055 ± 0.0059	0.006 ± 0.004	2818±1029	121.33±53.15
		SAN	1.68E+8 ± 1.33E+8	4.12E+5 ± 3.44E+5	2.42E+5 ± 1.41E+5	6.54E+5 ± 4.84E+5	2.78E+5 ± 2.10E+5	0.002 ± 0.0011	0.0021 ± 0.0012	0.49 ± 0.27	0.51 ± 0.27	1.9 ± 2.3	94.9 ± 104.06	0.004 ± 0.0006	0.002 ± 0.001	3489±402	56.67±3.51
	3	CON	2.92E+8 ± 2.54E+8	5.12E+4 ± 6.44E+4	1.15E+7 ± 4.42E+6	1.16E+7 ± 4.42E+6	1.09E+7 ± 7.59E+6	0.0003 ± 0.0003	0.0525 ± 0.0224	0 ± 0.01	1 ± 0.01	572.8 ± 516.2	0.13 ± 0.13	0.0528 ± 0.0225	0.041 ± 0.007	1173±373	6.8±4.71
		STR	5.39E+8 ± 7.22E+7	3.90E+6 ± 1.17E+6	1.16E+7 ± 1.03E+7	1.55E+7 ± 9.38E+6	9.59E+6 ± 3.27E+6	0.0072 ± 0.0015	0.0236 ± 0.0233	0.36 ± 0.3	0.64 ± 0.3	3.5 ± 3.3	8.6 ± 7.7	0.0308 ± 0.0227	0.018 ± 0.007	1394±371	6.13±2.69
		SAN	8.78E+8 ± 7.10E+7	1.04E+7 ± 3.85E+6	4.31E+7 ± 4.98E+6	5.35E+7 ± 1.40E+6	3.26E+7 ± 2.12E+7	0.0117 ± 0.0037	0.0495 ± 0.0088	0.2 ± 0.08	0.8 ± 0.08	4.7 ± 2.1	21.9 ± 28.1	0.0613 ± 0.0065	0.038 ± 0.024	1362±191	4.1±0.62
p-adj Plant presence			**<.0001**	**0.053**	N.S.	N.S.	**<.0001**	**0.0006**	**0.03**	**0.008**	**0.008**	**0.008**	**0.008**	**0.0006**	**0.036**	**<.0001**	N.S.

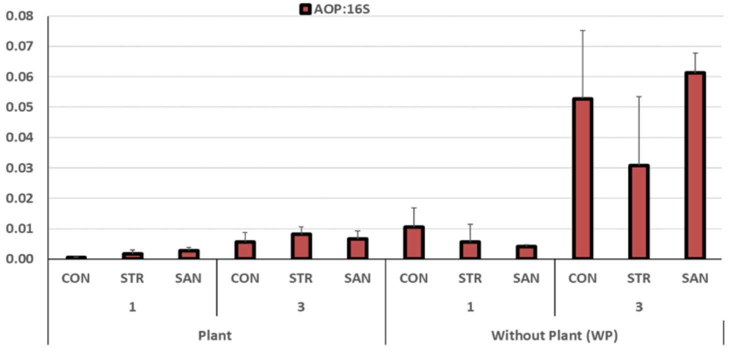












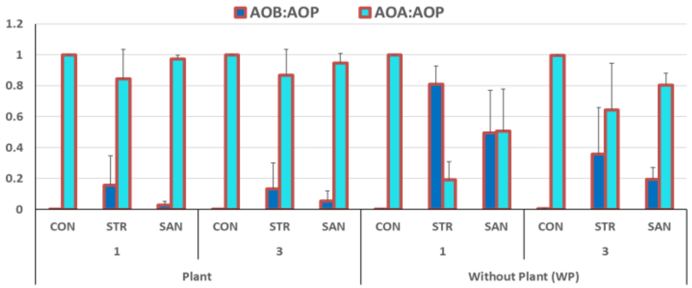


Table S5.8. Permutation analysis of variance (PERMANOVA) on constrain axes used in canonical analysis of principal components bi-plot ordination (CAP) based upon Bray-Curtis distance for bacteria and archaea communities. Parameters represented: qPCR data (16SrRNA, AOA, AOB, nosZ, AOP:16SrRNA, AOA:AOB), emissions (N.N2O and C.CO2) and N concentration and forms applied to the plants though the NS

Kingdom	Variable	Df	SumOfSqs	F	Pr(>F)
Bacteria	AOA	1	0.7354	3.2894	**0.001**
	AOB	1	1.4362	6.424	**0.001**
	16SrRNA	1	1.0236	4.5783	**0.001**
	nosZ	1	0.665	2.9743	**0.002**
	N-NS	1	0.596	2.6659	**0.005**
	N-NO_3^--NS	1	0.9669	4.3246	**0.001**
	N-NH_4^+-NS	1	0.3077	1.3763	0.154
	C-CO_2	1	0.517	2.3124	**0.004**
	N-N_2O	1	0.7669	3.4303	**0.001**
	AOP:16S	1	0.5972	2.6712	0.108
	AOB:AOA	1	0.4562	2.0406	**0.018**
	Residual	30	7.378		
Archaea	AOA	1	1.0133	7.1353	**0.004**
	AOB	1	0.2603	1.833	0.124
	16SrRNA	1	1.1516	8.1086	**0.001**
	nosZ	1	0.6263	4.4102	**0.002**
	N-NS	1	0.5957	4.1947	**0.004**
	N-NO_3^--NS	1	0.8583	6.0433	**0.001**
	N-NH_4^+-NS	1	0.1457	1.0261	0.306
	C-CO_2	1	0.4557	3.2086	**0.034**
	N-N_2O	1	1.2904	9.0866	**0.001**
	AOP:16S	1	0.286	2.0141	0.058
	AOB:AOA	1	0.3051	2.1481	**0.005**
	Residual	30	4.6866		

Table S6.1. Soil analysis at the beginning (Initial) and along the 2-year crop rotation, for each crop, campaign and treatment. pH (extract 1:2.5 H_2O); EC: electrical conductivity (extract 1:5 H_2O); Letters indicate statistical differences ($p < 0.05$) between the mean values of the treatments initial, CON, STR and SAN.

Sampling depth	Crop	Treatment	CE	pH	P Total	P Olsen	$N\text{-}NO_3^-$	K	Mg	Ca	Na	Carbonates	Bicarbonates
			$dS·m^{-1}$				$mg·kg^{-1}$ dry basis					$meq·L^{-1}$	
							0-30 cm depth						
0-30 cm	initial 19	initial	1.0	8.2	1324	48.8	121.6	355.0	312.3	3383.0	406.0	1.00	3.6
	tom 19	CON	0.8	8.5	1522	37.2	61.2	738.0	268.0	3325.7	272.3		
		STR	1.8	8.2	1647	41.6	169.5	940.3	446.3	3856.7	479.7		
		SAN	1.5	8.3	1366	43.0	182.8	724.0	437.0	3855.0	521.7		
	caul 19	CON	0.4	8.5		38.6	27.8	623.3	242.7	3642.3	168.3		
		STR	0.2	8.4		48.0	5.6	493.3	290.3	2219.0	86.0		
		SAN	0.6	7.7		62.1	34.5	562.3	317.7	2605.0	164.3		
	initial 20	CON	0.4	8.7	1599	71.3	22.8	534.0	237.3	2926.0	130.7		
		STR	0.4	8.5	1556	84.0	20.7	508.0	276.3	2457.3	104.7		
		SAN	0.6	8.4	1270	77.0	44.4	413.3	278.0	3050.3	228.7		
	tom 20	CON	0.5	8.8	1180	61.6	34.2	769.7	292.0	3726.3	223.7		
		STR	0.7	8.6	1267	68.4	40.0	646.3	430.0	3323.3	306.7		
		SAN	1.0	8.6	1203	73.1	83.9	631.3	447.0	3944.7	455.0		
	caul 20	CON	0.4	8.7	1331	41.6	13.7	563.0	298.3	3435.3	142.7		
		STR	0.5	8.6	1348	39.6	8.5	493.7	337.7	2869.0	125.0		
		SAN	0.8	8.6	1237	45.9	39.2	484.7	391.3	3562.7	267.0		
	lett 21	CON	0.3	8.7	1659	37.5	14.0	327.0	249.3	3168.0	134.3		
		STR	0.4	8.7	1369	30.5	7.8	358.7	265.0	2755.7	125.0		
		SAN	0.4	8.7	1370	28.9	8.8	303.0	258.7	2971.3	129.7		
	mean values	initial	0.96	8.2b	1324	48.8	121.5a	355.0	312.3ab	3383.0	406a		
		CON	0.48	8.6a	1458	48.0	28.9b	592.5	264.6b	3370.6	178b		
		STR	0.64	8.5ab	1437	52.0	42.0b	573.4	340.9a	2913.5	204ab		
		SAN	0.81	8.3b	1289	55.0	65.6ab	519.8	354.9a	3331.5	294ab		
	p-value	**Treatment**	N.S.	**0.007**	N.S.	N.S.	**0.005**	N.S.	**0.003**	N.S.	**0.03**		
							30-60 cm depth						
30-60 cm	initial 20	CON				23.2	10.9	89.7	269.0	2364.0	292.7		
		STR				33.5	19.4	142.0	267.3	2074.3	118.3		
		SAN				33.5	36.5	162.7	268.7	2512.0	316.7		
	tom 20	CON				41.0	9.6	144.0	289.7	2529.0	243.3		
		STR				45.4	15.0	102.7	351.3	3026.0	373.3		
		SAN				46.2	44.2	75.7	372.7	3390.3	417.0		
	caul 20	CON				17.8	5.5	153.7	269.3	2377.3	138.0		
		STR				18.2	3.8	252.0	279.7	1873.0	139.7		
		SAN				20.4	17.7	191.0	331.3	2710.7	257.3		
	lett 21	CON				24.5	7.1	173.3	223.3	2449.0	174.0		
		STR				17.2	2.7	156.0	260.3	2004.3	176.0		
		SAN				18.9	5.0	111.7	273.0	2236.3	247.7		
	mean values	CON				26.6	8.3	140.2	262.8	2429.8	212.0		
		STR				28.6	10.2	163.2	289.7	2244.4	201.8		
		SAN				29.8	25.8	135.3	311.4	2712.3	309.7		
	p-value	**Treatment**				N.S.	N.S.	N.S.	N.S.	N.S.	N.S.		

Table S6.2. Monthly average indoor global radiation (MJ·m^{-2}·day^{-1}), temperature (ºC), maximum temperature (ºC), and relative humidity (%) (monthly mean ± SD) during 2-year crop rotation

Campaign 2019			**March**	**April**	**May**	**June**	**July**	**August**	**September**	**October**	**November**	**December**
Indoor Global Radiation (MJ·m^{-2}·day^{-1})			13.3±1.8	14.3±5.9	25.8±8.8	30.3±5.9	26.4±5.8	23.0±4.4		11.8±6.1	5.9±6.3	5.3±4.3
Temp. (ºC)			14.3±4.9	15.8±4.4	18.2±5	23.7±5.9	27±4.7	27.7±5.7		20.2±4.6	14.4±4.8	12.6±4.1
Maximum Temp. (ºC)			22.7±1.7	23.9±2.2	26.4±3.1	31.8±4.1	34.7±1.9	37.1±5		29.1±4.5	23.3±2.8	20.8±2.8
Relative humidity (%)			58.3±16.5	67.3±14.7	70.2±14	64.2±15.5	69.9±12.8	65.4±15		74.3±14.9	67.5±14.2	78.9±11.0
Campaign 2020	**January**	**February**	**March**	**April**	**May**	**June**	**July**	**August**	**September**	**October**	**November**	**December**
Indoor Global Radiation (MJ·m^{-2}·day^{-1})	9.2±2.2			23.2±8.9	29.9±7.8	28.4±7.0	28.0±3.7	25.7±2.4	18.4±3.7	13.9±4.0	8.1±2.7	6±2.7
Temp. (ºC)	10±4.8			19.0±5.2	22.0±5.2	22.8±4.7	26.5±4.0	27.6±4.2	20.9±5	18.5±5.6	15.8±4.6	11±4.5
Maximum Temp. (ºC)	19.8±2.2			28.3±3.9	30.3±3.41	29.8±2.7	33.1±1.5	34.61±2.0	29.5±2.7	29.1±2.6	24±3.2	19.3±3.1
Relative humidity (%)	79.3±10.7			71.6±16.2	66.5±14.9	73.1±11.4	69.7±10.7	69.0±10.6	61.7±16	69.2±16.5	78.8±11.8	75.1±13.6
Campaign 2021	**January**	**February**	**March**	**April**								
Indoor Global Radiation (MJ·m^{-2}·day^{-1})	6±2.8		15.8±5.4	19±8.5								
Temp. (ºC)	6.7±4.5		14.5±6.1	15.8±5.6								
Maximum Temp. (ºC)	15.5±3.9		25.8±4.1	25.5±3.7								
Relative humidity (%)	74.1±9.9		65.5±16.4	64.2±16.4								

Table S6.3. Concentration of the different compounds that made up the cNS (Concentrated Nutrient Solution) for each treatment, campaign and crop. Units are g·L^{-1}, except for ammonium nitrate and Nitric acid (mL·L^{-1}).

		g·L^{-1}	Recovered fertilizers		Convencional fertilizers					
Crop	Campaign	Treatment	Struvite $NH_4MgPO_4·6H_2O$	Ammonium nitrate NO_3NH_4	Nitric Acid 60% HNO_3	Potassium Nitrate KNO_3	Potassium Sulfate K_2SO_4	Monopotassium Phosphate KPO_4H_2	Calcium nitrate $Ca(NO_3)_2$	Magnesium nitrate $Mg(NO_3)_2$
Tomato	2019	CON			27.2	39.4	9.1	13.6	16.4	
		STR	28.7		28.4	30.3	26.1		16.4	
		SAN	28.7	60	20.7		52.2		16.4	
	2020 Initial&final NS	CON			5.1	20.2		13.6		12.8
		STR	23.7		26.1		26.1			
		SAN	23.7	17.81	19.16		26.1			
	2020 Development NS	CON			26.8	23.2	22.6	13.6	16.4	
		STR	23.7		26.8	10.1	42.6		16.4	
		SAN	23.7	32	20.69		51.3		16.4	
Broccoli	2019	CON			27.2	42.42	6.1	16.3		
		STR	29.4		34.5	16.7	37.4			
		SAN	29.4	44	23.0		52.2			
	2020	CON			26.4	40.4	8.7	13.6		
		STR	23.8		33.7	20.2	34.8			
		SAN	23.8	247.8	20.7		52.2			
Lettuce	2021	CON			18.4	9.1		3.4		7.4
		STR	7.4		21.5	12.1				
		SAN	7.5	33.4	7.7		10.4			

Table S6.4. Nutrient Solution composition used for each treatment for the different crops and campaigns (mean value±SD in meq·L-1) CON: control; STR: struvite; SAN: struvite with ammonium nitrate. Letters indicate statistical differences ($p < 0.05$) between treatments within the same crop and campaign, followed by the p-value. For cells in italics, n=2

Crop	**Campaign**		**N**	**$H_2PO_4^-$**	**K^+**	**Ca^{2+}**	**Mg^{2+}**	**Na^+**	**SO_4^{2-}**	**Cl^-**	**EC(dS/m)**	**pH**	**N-NH4+ : N-NO3-**
							meq·L^{-1}						
Tomato	2019	CON	9.1b	0.9b	5.3±0.9	8.6±1	2.8b	3.8±0.2	5.9c	5.6±0.1	1.9b	6.4b	0.01 c
		STR	10.7a	1.2a	6.6±0.8	8.9±1.2	5.2a	3.8±0.1	9.1b	5.6±0.2	2.2a	6.5b	0.15 b
		SAN	10.3ab	1.1a	6.6±0.9	8.8±1.6	5.2a	3.8±0.4	13.6a	5.8±0.3	2.2a	7a	0.60 a
	p-value		0.006	<0.0001	N.S.	N.S.	0.0003	N.S.	0.0001	N.S.	0.002	<0.0001	<0.0001
	2020 Initial / final NS	CON	4.5±0.7	1.1±0.2	*2.7±0*	*7.2±0*	*4±0.4*	*3.5±0.1*	*3.5±0.1*	*4.7±0.2*	1.8	7ab	0.08 c
		STR	5.2±0.5	1±0.2	*2.6±0.3*	*7.1±0*	*5.5±0.9*	*3.5±0.2*	*6.9±0.6*	*4.8±0.2*	1.8	6.9b	0.52 b
		SAN	5.2±0.7	1±0.1	*2.5±0.2*	*7.8±1.4*	*4.9±0.2*	*3.4±0*	*6.7±0.1*	*4.8±0.1*	1.8	7.1a	0.56 a
	p-value		N.S.	N.S.							N.S.	<0.0001	<0.0001
	2020 Development NS	CON	8±1.2	1±0.3	5.5±0.9	8.8±1.2	3.1b	3.8±0.5	5.7c	4.7±0.2	2.1	6.4c	0.06 c
		STR	7.8±0.7	0.9±0.1	5±0.4	8.2±1.1	4.7a	3.5±0.1	7.9b	4.6±0	2.1	6.8b	0.26 b
		SAN	8.1±1	0.9±0.1	3.3±1.6	8.6±3	4.5a	3.1±1	8.7a	4.6±0	2.2	6.9a	0.49 a
	p-value		N.S.	N.S.	N.S.	N.S.	<0.0001	N.S.	<0.0001	N.S.	N.S.	<0.0001	<0.0001
Broccoli	2019	CON	7.9±0.5	1±0.1	*6.1±0*	*7.4±0.3*	*3.1±0*	*4.1±0.3*	*4.3±0.6*	*5.3±0.3*	2.0b	6.6b	0.03 c
		STR	8.5±0.6	1.1±0.1	*6.6±0.2*	*7.5±0.4*	*5.9±0.5*	*3.9±0.1*	*9.1±0.3*	*5.3±0.6*	2.4a	6.6b	0.27 b
		SAN	8.8±1.4	1.1±0.1	*6.5±0.2*	*7.3±0.2*	*5.7±0*	*4±0*	*10.1±1.7*	*5.5±0.4*	2.4a	7.0a	0.77 a
	p-value		N.S.	N.S.							<0.0001	<0.0001	<0.0001
	2020	CON	7±0.7	0.9±0.1	*5.4±0.1*	*7±0.5*	*3.1±0.4*	*3.8±0.4*	*4.3±0.2*	*4.5±0.1*	1.7	6.6	0.03 c
		STR	6.8±0.8	0.8±0.1	*6.1±1.4*	*6.3±1.5*	*4.8±1*	*3.9±0.6*	*7.7±0.2*	*4.4±0.1*	1.7	6.7	0.23 b
		SAN	7.4±0.6	0.8±0.1	*4.6±0.1*	*5.1±2.7*	*4±0.1*	*4±0.2*	*9.3±0.6*	*4.5±0.2*	1.9	6.9	0.56 a
	p-value		N.S.	N.S.							N.S.	N.S.	<0.0001
Lettuce	2021	CON	4.4±0.3	0.2±0	*1.3±0*	*5.7±1.1*	*3.9±0*	*4.1±0.4*	*3.1±0.1*	*4.3±0.1*	1.4	6.7b	0.01 c
		STR	4±0.7	0.3±0	*0.8±0.7*	*5.5±1.8*	*3.2±0.7*	*4.6±1*	*3.3±0.1*	*4.5±0.2*	1.5	6.8b	0.09 b
		SAN	3.8±0.3	0.3±0	*1.1±0.1*	*5.4±1.6*	*3.4±0*	*3.8±0*	*4.2±0.2*	*4.4±0*	1.4	7.5a	0.52 a
	p-value		N.S.	N.S.							N.S.	<0.0001	<0.0001

Table S6.5. Soil categories for different nutrients based on Mediterranean soils values (Villar&Villar, 2016)

Classification	**N-NO$_3^-$**	**P(Olsen)**	**K$^+$**	**Mg^{2+}**	**Ca^{2+}**	**Na$^+$**
	mg·kg^{-1}					
Deficit	<10				<700	
Low	10-15	<12	<125	<100	700-2000	<100
Medium	15-20	12-24	125-175	100-175		100-300
Optimum	20-25	24-36	175-250	175-250	2000-4000	
High	>25	36-80	250-350	250-600	>4000	300-1000
Very high		>80	>350	>600		>1000

Table S6.6. NGS data analysis and 16SrRNA Metabarcoding sequencing data.

Raw data (R1 and R2 demultiplexed FASTQ files) from 16S rRNA of bacteria and archaea were further processed using Cutadapt and DADA2 software. Primers were removed from the demultiplexed forward (R1) and reverse (R2) reads using Cutadapt (Martin, 2011) and the resulting paired reads were filtered and trimmed, denoised and merged using the R package DADA2 (Callahan et al., 2016). R1 and R2 reads were truncated (truncLen) to 260 and 240 for 16S. In all samples, reads with ambiguities or an expected error (maxEE) higher than 2 were discarded (script for V3-V4 16S: filterAndTrim(fnFs, filtFs, fnRs, filtRs, truncLen=c(260,240), maxN=0, maxEE=c(2,2), truncQ=2, rm.phix=TRUE, compress=TRUE, multithread=FALSE). 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 using a minimum overlap of 12 bp. Finally, chimeras were detected and removed using the function "removeChimeraDenovo" as described elsewhere in the DADA2 1.16 tutorial (https://benjjneb.github.io/dada2/tutorial.html).

The taxonomic affiliations of the ASVs for total bacteria and archaea were assigned by using the naïve Bayesian classifier method (Wang et al., 2007), using the RDP database and compiled into each taxonomic level (DeSantis et al. 2006). For the assignment, the RDP Bayesian Classifier was set with a bootstrap cut-off of 80%.

	Bacteria	**Archaea**
minimum number of reads	22157	10898
maximum number of reads	26352	25039
average number of reads	24959	18059
overall number of OTUs	2233	165

Table S6.7. Concentration of heavy metals (mg·kg-1 dry matter)in fruit and leaves for each treatment, crop and campaign (n=1). Lettuce crop during the first campaign couldn't be performed.

Crop	**Year campaign**	**Treatment**	**Plant part**	**Cadmium (Cd)**	**Lead (Pb)**	**Mercury (Hg)**	**Chromium (Cr)**	**Copper (Cu)**	**Nickel (Ni)**	**Zinc (Zn)**	**%dry matter**
							mg·kg^{-1} dry matter				
tomato	2019	CON	Fruit	<0.100	<0.500	<4.0	<0.100	3	<0.500	13	5.3
		STR		<0.100	<0.500	<4.0	<0.100	3	<0.500	11	5.6
		SAN		<0.100	<0.500	<4.0	<0.100	3	<0.500	13	6.2
	2020	CON	Fruit	<0.100	<0.500	<4.0	0.11	5	<0.500	15	6
		STR		<0.100	<0.500	<4.0	<0.100	5	<0.500	17	6.6
		SAN		<0.100	<0.500	<4.0	<0.100	5	<0.500	15	6.6
cauliflower	2019	CON	nflorescence	<0.100	<0.500	<4.0	<0.100	3	<0.500	25	6.8
		STR		<0.100	<0.500	<4.0	<0.100	2	<0.500	18	7.3
		SAN		<0.100	<0.500	<4.0	<0.100	3	<0.500	22	7.3
	2020	CON	nflorescence	0.043	0.098	<4.0	0.463	3	0.435	45	7.1
		STR		0.0398	0.179	<4.0	0.409	4	0.292	47	7.1
		SAN		0.0443	0.065	<4.0	1,618	3	0.747	34	6.6
lettuce	2021	CON	Leaves	0.22	0.41	<0.5	0.2	8	0.34	32	5.5
		STR		0.22	0.5	<0.5	0.92	7	0.57	34	5.5
		SAN		0.4	0.77	<0.5	4.51	8	2.08	37	4.3

Table S6.8. P and N content ($g \cdot m^{-2}$) in fruit/inflorescence and aerial biomass (and total) for each treatment, crop and campaign. 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	N-Biomass	N-Fruit	N-Total	P-Biomass	P-Fruit	P-Total
			$g \cdot m^{-2}$					
tomato	2019	CON	21.68	29.61	51.29	2.33b	3.29	5.62
		STR	22.75	36.4	59.15	4.46a	4.04	8.51
		SAN	22.98	28.76	51.75	4.52a	3.31	7.83
	2020	CON	11.69	25.96	37.65	1.77b	3.45	5.21
		STR	10.19	27.52	37.72	1.65b	3.56	5.22
		SAN	11.15	28.84	39.99	2.29b	3.65	5.94
	p-value	Treatment	N.S.	N.S.	N.S.	<.0001	N.S.	0.0017
		Year	<.0001	N.S.	0.0005	0.0092	N.S.	0.036
		Treatment*year	N.S.	N.S.	N.S.	0.0286	N.S.	N.S.
cauliflower	2019	CON		5.62			0.9	
		STR		5.2			0.88	
		SAN		5.23			0.83	
	2020	CON	23.79	8.37	32.15	2.12	1.16	3.28
		STR	21.64	7.23	28.87	2	1.1	3.1
		SAN	24.4	9.27	33.66	2.14	1.27	3.4
	p-value	Treatment	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
		Year		0.0029			0.005	
		Treatment*year		N.S.			N.S.	
lettuce	2021	CON			8.72			1.51
		STR			8.5			1.14
		SAN			9.29			1.24
	p-value	Treatment			N.S.			N.S.

Table S6.9. Alfa diversity indexes assessment for the initial (INI) and pooled final samples (FIN), and for each of the three final treatments samples for bacteria and archaea communities (mean±SD values). Significance p-value ($p < 0.05$) indicate statistical differences according to Wilcox test between initial (INI) and pooled final samples (FIN) and within the three final treatments (CON, STR and SAN).

Kingdom	Time	Final treatments	Shannon	Relative dominance	Pielou's	Chao1
Bacteria	INITIAL		6.3±0.15	0.09±0.02	0.85±0.01	1980±64
	FINAL		6.2±0.13	0.13±0.02	0.83±0.02	2002±46
		CON	6.2±0.05	0.14±0.02	0.83±0.01	2034±30
		STR	6.3±0.14	0.12±0.01	0.84±0.02	2007±23
		SAN	6.1±0.16	0.15±0.03	0.82±0.02	1965±57
p-value	Time		N.S.	0.01	N.S.	**0.03**
		Final treatments	N.S.	N.S.	N.S.	N.S.
Archaea	INITIAL		3.25±0.23	0.17±0.04	0.74±0.06	83±9
	FINAL		2.93±0.25	0.20±0.10	0.65±0.05	97±12
		CON	3.09±0.22	0.15±0.04	0.69±0.03	99±19
		STR	2.9±0.15	0.17±0.03	0.65±0.02	100±7
		SAN	2.78±0.33	0.28±0.16	0.62±0.08	94±13
p-value	Time		N.S.	N.S.	**0.03**	N.S.
		Final treatments	N.S.	N.S.	N.S.	N.S.

Table S6.10. Permutation analysis of variance (PERMANOVA) and Principal Correspondance Analysis (PCoA) plots of the microbial communities based on Bray-Curtis distances of bacterial and archaeal communities. Points in the PCoA plot represent the different samples each colored and shaped according to the treatments and the sampling time, respectively.

Kingdom	Variable	Df	SumOfSqs	R2	F	Pr(>F)
Bacteria	Final treatments	2	0.168	0.221	0.849	0.7
	Residual	6	0.595	0.779		
	Total	8	0.764	1.000		
	Time	1	0.322	0.246	3.2577	0,006**
	Residual	10	0.987	0.754		
	Total	11	1.309	1.000		
Archaea	Final treatments	2	0.325	0.286	1.2016	0.293
	Residual	6	0.811	0.714		
	Total	8	1.136	1.000		
	Time	1	0.163	0.106	1.1915	0.299
	Residual	10	1.368	0.894		
	Total	11	1.531	1.000		

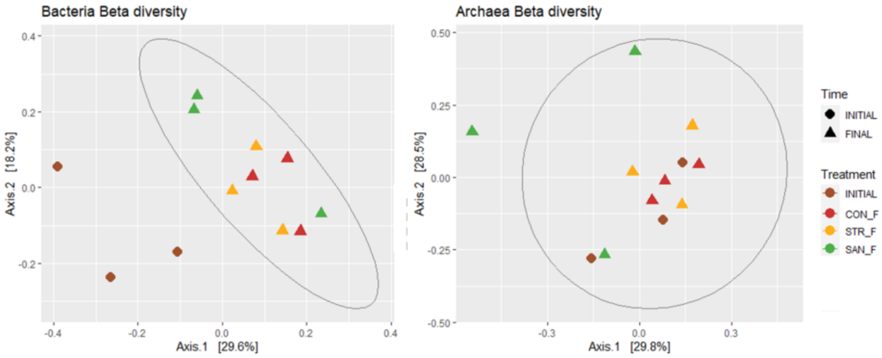


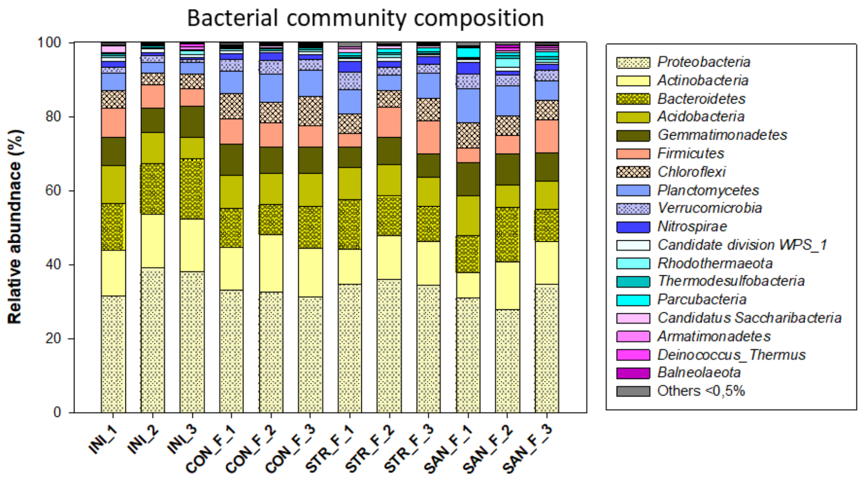
Table S6.11. Permutation analysis of variance (PERMANOVA) on constrain axes and bi-plot used in canonical analysis of PCoA bi-plot ordination (based upon Bray-Curtis distance). Significance p-value of the soil chemical parameters (pH, Pol, CE and NO_3^-) and the N-NH_4^+:N-total ratio applied with the NS for bacteria and archaea communities.

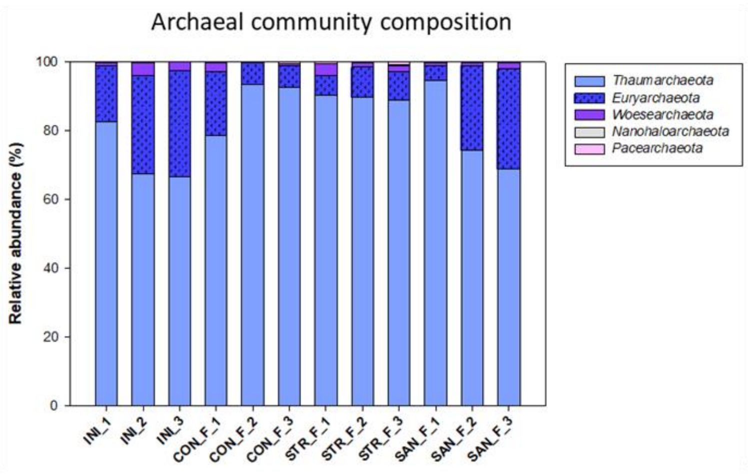
Kingdom	Variable	SumOfSqs	F	Pr(>F)
Bacteria	NO3	0.326	3.682	0.001**
	pH	0.170	1.920	0.042*
	CE	0.073	0.826	0.625
	RATIO	0.083	0.938	0.459
	Pol	0.126	1.423	0.141
	Residual	0.531		
Archaea	CE	0.251	2.244	0.053
	pH	0.085	0.763	0.620
	RATIO	0.263	2.352	0.02*
	Pol	0.170	1.523	0.168
	NO3	0.105	0.939	0.531
	Residual	0.671		

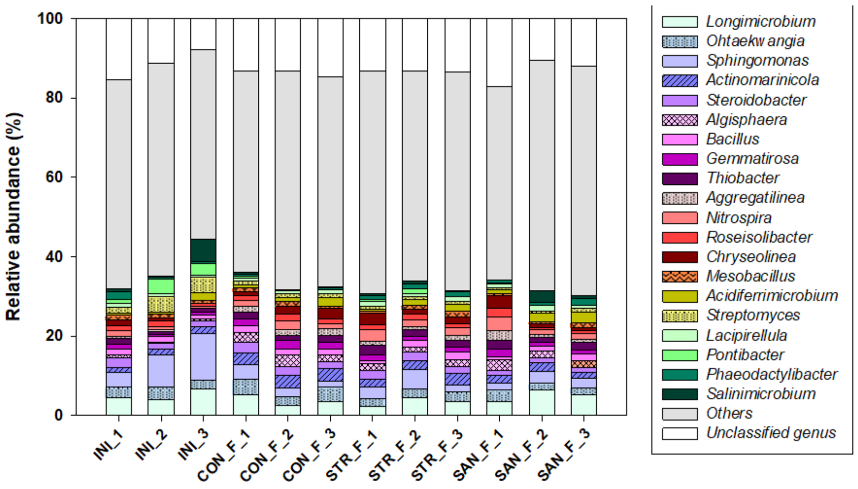
Table S6.12. Table and representation of the relative abundance of taxonomic assignation at the phylum and genus level of the bacterial and archaeal population. Significance p-value ($p < 0.05$) indicate statistical differences according to Kruskal-Wallis (KW) and Wilcox test between initial (INI) and pooled final samples (FIN) and within the three final**.**

Kingdom	Taxonomic level	Time	Final treatments	*Proteobacteria*	Unclassified	*Actinobacteria*	*Bacteriodetes*	*Acidobacteria*	*Firmicutes*	*Planctomycetes*	*Gemmatimonadetes*	*Chloroflexi*	*Verrucomicrobia*	*Nitrospirae*	Others <1%	*Parcubacteria*	*Candidatus Saccharibacteria*
bacteria	**Phylum**	FIN	CON	30.56%	13.77%	12.43%	10.65%	7.77%	5.51%	5.10%	4.16%	3.47%	3.19%	1.54%	1.37%	0.28%	0.21%
		FIN	CON	31.59%	11.71%	15.20%	8.32%	7.96%	5.62%	6.04%	3.24%	3.20%	3.50%	2.08%	0.68%	0.17%	0.69%
		FIN	CON	28.38%	15.24%	13.14%	11.46%	8.57%	3.90%	5.30%	3.55%	4.17%	2.95%	1.15%	1.45%	0.43%	0.31%
		FIN	STR	33.11%	11.78%	9.30%	12.60%	8.06%	2.11%	4.78%	2.74%	3.81%	4.74%	2.85%	1.70%	1.16%	1.27%
		FIN	STR	37.45%	11.59%	11.18%	10.11%	7.12%	6.66%	3.73%	2.88%	2.38%	1.79%	1.64%	1.60%	1.02%	0.85%
		FIN	STR	32.25%	13.29%	11.85%	9.13%	7.54%	7.55%	5.26%	3.12%	3.27%	2.35%	1.74%	1.23%	1.05%	0.40%
		FIN	SAN	28.37%	17.73%	6.80%	9.85%	9.12%	2.32%	6.28%	4.18%	4.11%	3.56%	3.08%	1.26%	2.95%	0.39%
		FIN	SAN	28.45%	13.69%	11.39%	14.79%	5.01%	3.67%	6.51%	2.65%	3.30%	2.83%	1.11%	4.61%	0.74%	1.25%
		FIN	SAN	33.17%	12.75%	11.42%	9.76%	6.61%	6.78%	4.35%	2.99%	2.58%	2.60%	1.30%	2.64%	2.66%	0.37%
		INI		31.35%	11.33%	11.98%	12.56%	10.27%	6.15%	4.01%	2.96%	2.55%	1.60%	1.42%	1.39%	0.41%	2.03%
		INI		38.08%	7.56%	14.17%	15.34%	7.51%	5.19%	2.51%	3.13%	2.15%	1.65%	0.67%	1.39%	0.07%	0.58%
		INI		36.39%	8.42%	13.61%	19.66%	5.25%	3.64%	2.76%	3.25%	2.56%	0.79%	0.51%	2.95%	0.05%	0.17%
	p-value	**Time**		N.S.	N.S.	N.S.	**0.03**	N.S.	N.S.	**0.02**	N.S.	N.S.	**0.01**	N.S.	N.S.	N.S.	N.S.
			Final treatments	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
		Time	**Final treatments**	***Acidiferrimicrobium***	***Actinomarinicola***	***Algisphaera***	***Bacillus***	***Chryseolinea***	***Nitrospira***	***Unclassified***	***Ohtaekwangia***	**Others**	***Sphingomonas***	***Steroidobacter***	***Streptomyces***	***Thiobacter***	**unclassified**
	Genus	FIN	CON	1%	3%	2%	2%	1%	1%	11%	4%	51%	3%	3%	1%	2%	14%
		FIN	CON	1%	3%	3%	1%	2%	2%	13%	2%	54%	2%	2%	1%	1%	12%
		FIN	CON	2%	3%	2%	1%	2%	1%	13%	4%	50%	1%	2%	1%	2%	16%
		FIN	STR	0%	2%	1%	1%	3%	3%	12%	2%	54%	3%	2%	1%	2%	13%
		FIN	STR	1%	2%	1%	2%	1%	2%	11%	2%	55%	5%	2%	1%	2%	13%
		FIN	STR	2%	3%	1%	2%	1%	2%	12%	2%	54%	2%	2%	1%	2%	14%
		FIN	SAN	1%	2%	2%	1%	3%	3%	15%	3%	47%	2%	1%	1%	2%	18%
		FIN	SAN	2%	2%	1%	1%	1%	1%	9%	2%	59%	3%	2%	1%	1%	15%
		FIN	SAN	2%	2%	1%	2%	1%	1%	11%	2%	58%	3%	1%	1%	2%	14%
		INI		1%	1%	1%	1%	1%	2%	14%	2%	55%	4%	2%	1%	1%	12%
		INI		0%	1%	0%	1%	1%	1%	10%	3%	59%	8%	2%	3%	1%	8%
		INI		2%	2%	0%	1%	0%	1%	7%	2%	58%	12%	1%	4%	1%	9%
	p-value	**Time**		N.S.	**0.02**	**0.01**	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	**0.02**	N.S.	**0.01**	**0.03**	N.S.
			Final treatments	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

		Time	Final treatments	*Woesearchaeota*	*Thaumachaeota*	*Euryarcheaota*	Unclassified	Others <1%					
Archaea	**Phylum**	FIN	CON	2.22%	69.90%	26.95%	0.86%	0.01%					
		FIN	CON	0.15%	93.64%	6.11%	0.07%	0.00%					
		FIN	CON	1.13%	90.37%	6.30%	1.27%	0.92%					
		FIN	STR	3.43%	89.64%	6.06%	0.26%	0.58%					
		FIN	STR	1.06%	88.89%	9.54%	0.23%	0.21%					
		FIN	STR	2.51%	84.83%	7.53%	2.98%	2.08%					
		FIN	SAN	1.29%	91.98%	4.77%	1.58%	0.36%					
		FIN	SAN	1.18%	72.78%	25.71%	0.15%	0.17%					
		FIN	SAN	2.24%	66.61%	29.30%	1.62%	0.19%					
		INI		1.06%	80.22%	17.88%	0.67%	0.04%					
		INI		4.06%	63.27%	30.95%	1.47%	0.00%					
		INI		2.51%	64.50%	29.88%	3.10%	0.00%					
	p-value	**Time**		N.S.	N.S.	N.S.	N.S.	N.S.					
			Final treatments	N.S.	N.S.	N.S.	N.S.	N.S.					
		Time	**Final treatments**	***Haladaptatus***	***Halalkalicoccus***	***Halococcus***	***Haloprofundus***	***Methanomassiliicoccus***	***Nitrosopumilus***	***Nitrososphaera***	**Others**	**Unclassified**	***Woesearchaeota_Incertae_Sedis_AR16***
	Genus	FIN	CON	4.76%	1.36%	0.45%	0.45%	9.76%	5.94%	72.30%	1.40%	0.91%	2.66%
		FIN	CON	3.80%	0.58%	0.45%	0.25%	0.88%	0.03%	93.40%	0.31%	0.07%	0.24%
		FIN	CON	3.48%	0.25%	0.69%	0.37%	0.31%	5.46%	85.94%	1.46%	1.32%	0.72%
		FIN	STR	0.71%	0.02%	0.26%	0.17%	4.37%	1.26%	88.99%	0.79%	0.20%	3.25%
		FIN	STR	5.32%	1.20%	0.56%	0.52%	0.94%	0.06%	89.57%	0.66%	0.23%	0.95%
		FIN	STR	3.45%	0.70%	2.39%	0.35%	0.40%	9.76%	77.10%	1.33%	3.05%	1.45%
		FIN	SAN	0.26%	0.14%	0.01%	0.00%	4.00%	19.82%	73.18%	0.23%	1.48%	0.87%
		FIN	SAN	12.35%	0.43%	8.96%	0.16%	0.22%	2.87%	71.61%	2.45%	0.23%	0.73%
		FIN	SAN	4.22%	0.21%	21.75%	0.23%	1.42%	48.44%	19.42%	1.02%	1.52%	1.77%
		INI		2.42%	0.59%	1.97%	1.73%	8.59%	0.79%	81.35%	0.86%	0.72%	0.99%
		INI		6.85%	0.50%	2.26%	3.43%	12.82%	0.33%	66.08%	2.43%	1.69%	3.61%
		INI		11.57%	1.90%	11.84%	2.05%	0.30%	0.14%	64.52%	2.00%	3.38%	2.30%
		Time		N.S.	N.S.	N.S.	**0.01**	N.S.	N.S.	N.S.	N.S.	N.S.	**0.01**
			Final treatments	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.


Bacterial community composition


Archaeal community composition



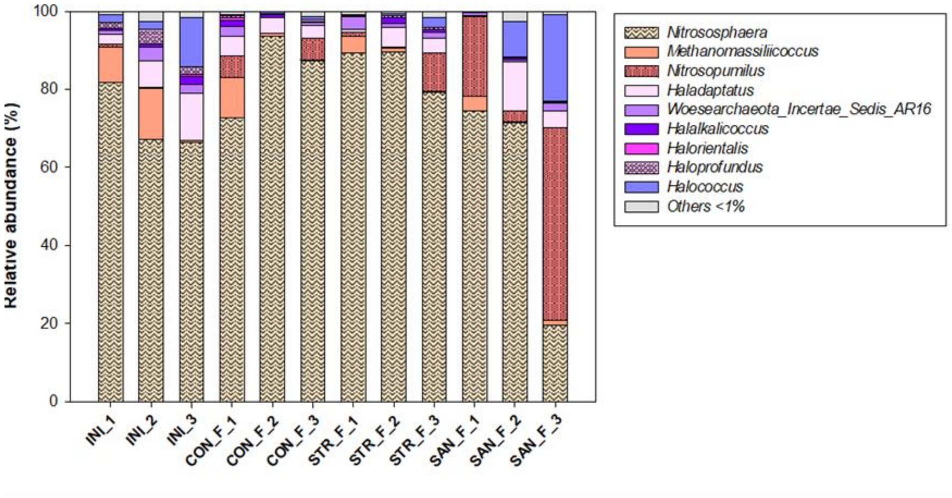


Table S6.13. qPCR and MiSeq data. qPCR: 16S rRNA, amoA_AOB, amoA_AOA gene abundance and further ratio calculations. AOP (ammonium oxidizing prokariote; amoA_AOB + amoA_AOA). MiSeq: relative abundance of nitrifying microbiota genus. Mean values for initial (INI) and pooled final samples (FIN), followed by statistical significant differences p-value (p<0.05) between Time variable.

Final treatment	**Time**	**16S rRna**	***amoA* _AOB**	***amoA* _AOA**	**AOP**	**AOP:16S rRNA**	**AOB:AOP**	**AOA:AOP**	**AOB:AOA**	**AOA:16S rRNA**	**AOB:16S rRNA**
		qPCR data (gen copies·g^{-1})									
CON	**FIN**	2.25E+09	6.97E+06	1.68E+06	8.65E+06	0.004	0.805	0.195	4.14	0.001	0.003
CON	**FIN**	8.38E+09	5.00E+07	5.15E+06	5.52E+07	0.007	0.907	0.093	9.71	0.001	0.006
CON	**FIN**	1.99E+09	4.25E+07	2.78E+06	4.53E+07	0.023	0.939	0.061	15.33	0.001	0.021
STR	**FIN**	2.37E+09	3.88E+07	2.31E+06	4.12E+07	0.017	0.944	0.056	16.83	0.001	0.016
STR	**FIN**	4.43E+09	2.65E+07	2.70E+06	2.92E+07	0.007	0.908	0.092	9.81	0.001	0.006
STR	**FIN**	6.49E+09	7.05E+07	9.68E+06	8.02E+07	0.012	0.879	0.121	7.29	0.001	0.011
SAN	**FIN**	2.55E+09	3.55E+07	8.11E+06	4.36E+07	0.017	0.814	0.186	4.37	0.003	0.014
SAN	**FIN**	2.80E+09	6.74E+07	3.09E+06	7.05E+07	0.025	0.956	0.044	21.84	0.001	0.024
SAN	**FIN**	2.94E+09	8.51E+07	1.83E+07	1.03E+08	0.035	0.823	0.177	4.66	0.006	0.029
	INI	1.60E+09	2.65E+07	9.65E+05	2.75E+07	0.017	0.965	0.035	27.47	0.001	0.017
	INI	1.93E+09	3.07E+07	4.84E+05	3.12E+07	0.016	0.985	0.015	63.53	0.000	0.016
	INI	1.60E+09	3.01E+07	6.88E+05	3.07E+07	0.019	0.978	0.022	43.69	0.000	0.019
mean	**INI**	1.71E+09	2.91E+07	9.95E+05	3.01E+07	0.018	0.966	0.034	29.25	0.001	0.017
values	**FIN**	3.72E+09	4.70E+07	4.61E+06	5.17E+07	0.017	0.908	0.092	10.21	0.002	0.016
p-value	**Time**	0.147	0.242	0.132	0.2	0.849	**0.029**	**0.029**		0.233	0.186

Final treatment	**Time**	***Nitrosomonas***	***Nitrosospira***	***Nitrobacter***	***Nitrospira***	**AOB *(Nitrosomonas +Nitrosospira)***	**NOB *(Nitrobacter +Nitrospira)***	***Nitrosopumilus***	***Nitrososphaera***
		16S rRNA-Metabarcoding data (% bacteria relative abundance)						**barcoding data (% archaea relati**	
CON	**FIN**	0.00%	0.03%	0.06%	1.54%	0.03%	1.60%	5.94%	72.30%
CON	**FIN**	0.00%	0.16%	0.10%	2.08%	0.16%	2.18%	0.03%	93.40%
CON	**FIN**	0.00%	0.18%	0.07%	1.15%	0.18%	1.22%	5.46%	85.94%
STR	**FIN**	0.11%	0.22%	0.09%	2.85%	0.33%	2.94%	1.26%	88.99%
STR	**FIN**	0.00%	0.09%	0.11%	1.64%	0.09%	1.76%	0.06%	89.57%
STR	**FIN**	0.00%	0.31%	0.08%	1.74%	0.31%	1.82%	9.76%	77.10%
SAN	**FIN**	0.02%	0.30%	0.04%	3.08%	0.32%	3.12%	19.82%	73.18%
SAN	**FIN**	0.00%	0.24%	0.03%	1.11%	0.24%	1.14%	2.87%	71.61%
SAN	**FIN**	0.01%	0.43%	0.09%	1.30%	0.45%	1.39%	48.44%	19.42%
	INI	0.00%	0.29%	0.03%	1.42%	0.29%	1.44%	0.79%	81.35%
	INI	0.00%	0.22%	0.04%	0.67%	0.23%	0.71%	0.33%	66.08%
	INI	0.00%	0.28%	0.02%	0.51%	0.28%	0.53%	0.14%	64.52%
mean	**INI**	0.00%	0.27%	0.03%	0.87%	0.27%	0.89%	0.42%	70.65%
values	**FIN**	0.02%	0.22%	0.08%	1.83%	0.24%	1.91%	10.40%	74.61%
p-value	**Time**	0.53	0.48	**0.02**	0.06	0.7	**0.04**	0.307	0.776

Table S7.1. Greenhouse Gas emissions (mean ± SD) for tomato crop in soil trial during 2020 campaign. 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.

Plant presence	**Time**	**Fertilization treatments**	**N-N_2O**	**C-CO_2**	**GWP (CO_2 eq.)**
				mg·m^2·h^{-1}	
PLANT	**t1 (1/6/2020)**	**CON**	43.7±9.5	5659±800.4	18683.3±3623
		STR	20.4±7	4735.1±2567.2	10828.7±2952.4
		SAN	40.1±31.1	4815±1881.3	16764.8±11119.2
	t2 (1/7/2020)	**CON**	66.1±38.1	3567±2653.6	23254.9±10986.8
		STR	12.7±3.8	3586±479	7380.5±1087.1
		SAN	34.6±19.7	4188.3±1383.6	14499.1±6728.8
	t3 (1/8/2020)	**CON**	7.2±2	1738±914.6	3873.7±1393.8
		STR	17±19.1	2296.7±1002.3	7352.7±6521.4
		SAN	10.3±7.2	2018±1107.4	5097.3±3206.7
	p-value	**Time**	**0.01**	**0.0003**	**0.004**
		Treatment	N.S.	N.S.	N.S.
		Time*Treatment	N.S.	N.S.	N.S.
WITHOUT PLANT	**t1 (1/6/2020)**	**CON**	23.5±18.1	1070±519.9	8064.5±5836.7
		STR	13.3±11.2	1145±678.9	5095.5±3734.8
		SAN	21.8±3.8	1599±261.8	8101.4±864.5
	t3 (1/8/2020)	**CON**	9.9±0.7	1513.3±170.2	4473.5±257.4
		STR	11.7±2.4	1335.7±291	4832.2±972.2
		SAN	8.4±2.5	1417±289.4	3910.3±673
	p-value	**Time**	N.S.	N.S.	N.S.
		Treatment	N.S.	N.S.	N.S.
		Time*Treatment	N.S.	N.S.	N.S.
t1 (1/6/2020)	**p-value**	**Plant**	N.S.	**0.0003**	**0.009**
		Treatment	N.S.	N.S.	N.S.
		Plant*Treatment	N.S.	N.S.	N.S.
t3 (1/8/2020)	**p-value**	**Plant**	N.S.	N.S.	N.S.
		Treatment	N.S.	N.S.	N.S.
		Plant*Treatment	N.S.	N.S.	N.S.

CON: Conventional fertilization treatment; STR: Struvite fertilization treatment; SAN: Struvite + ammonium nitrate fertilization treatment.

GPW: Global Warming Potential

Table S7.2. Greenhouse Gas emissions (mean ± SD) for tomato crop with plant presence in soil and soilless trial during 2020 campaign.

Plant presence	**Growing media**	**$N\text{-}N_2O$**	**$C\text{-}CO_2$**	**GWP (CO_2 eq.)**
			$mg\cdot m^2\cdot h^{-1}$	
PLANT	Perlite	5.7±3.5	9736±4145	11423±4101
	Soil	28±25	3623±1872	11971±8343
p-value	Growing media	<0.0001	<0.0001	N.S.

Table S7.3. Bacterial Alfa-diversity indexes: Richness (Chao1), evenness (Pielou's), diversity (Shannon) and relative dominance.

Plant presence	**Substrate**	**Variable**	**mean ± SD**
NO	**perlite**	Diversity	4.5±0.5
		Dominance	0.2±0.1
		Evenness	0.7±0.1
		Richness	659±46
	soil	Diversity	6.7±0.2
		Dominance	0.03±0.02
		Evenness	0.9±0.02
		Richness	2588±176
YES	**perlite**	Diversity	5.5±0.3
		Dominance	0.1±0.04
		Evenness	0.8±0
		Richness	1042±113
	soil	Diversity	6.4±0.5
		Dominance	0.1±0.1
		Evenness	0.9±0.1
		Richness	2523.5±323.9