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Struvites with comparable nitrogen and phosphorus composition have similar agronomic response but shape cherry tomato rhizosphere bacterial community structure differently

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ABSTRACT

Struvites are circular-economy based fertilisers which are set to reduce some of the demand on finite phosphate rock in the European Union. While they are near similar in P and N content, with low P water solubility, subtle chemical differences exist between recovered struvites from different wastewaters. The objectives of this study were to assess if two struvites similar in N and P, but differing feedstock material, similarly effect tomato growth and tomato rhizosphere bacteria. A glasshouse experiment was setup using low-P soil and applications of struvite from municipal wastewater (MWS), struvite from potato wastewater (PWS) and compared to single superphosphate (SSP). While Tomato aboveground biomass yields were similar between the struvites (39.5 and 37.4 g/pot), they were 17–22 % lower in comparison to the SSP treatment (47.8 g/pot) when harvested during the early fruiting stages. However, P uptake was not significantly different among all the treatments ranging 21.6 to 25.4 mg P/pot. The PWS treated soil had a plant available P of 5.52 mg P/L at the fruiting stage that was comparable to that of SSP (4.74 mg P/L) but significantly greater than that of the MWS treatment (4.37 mg P/L). PWS and SSP tomato rhizosphere bacteria were ecologically similar but ecologically distant to the MWS treatment. Slow growing oligotrophic bacteria of the *Xanthobacteraceae*, *Planococcaceae*, and *Bacillaceae* families were abundant in MWS possibly because of the presence of Fe which limited P dissolution from the struvite. We concluded that while tomato had a similar agronomic response to PWS and MWS, the struvites affected the soil bacteria in the tomato rhizosphere differently. Long-term studies at field scale are recommended to identify the long-term suitability of struvites for horticulture crops.

1. Introduction

The main source of phosphorus fertiliser in current agriculture is finite phosphate rock which is only available in a few countries and not necessarily of a good quality for commercial use. Major phosphorus rock producers are China, United States of America, Morrocco, and Russia whose total production account for >72 % of global supply (Jasinski, 2022; Vaccari et al., 2019). The finite nature of these reserves creates a distinct need of more sustainable phosphorus fertiliser. As a result, the fertiliser landscape in the EU is changing towards circular economy-based technologies to manufacture alternative phosphorus fertilisers (Carreras-Sempere et al., 2021). Recently, the interest in recycled derived fertilisers such as struvites has risen because of their potential to avail multiple benefits in terms of country self-sufficiency and environmental sustainability.

Struvite fertilisers are made from recovered phosphorus, generally precipitated as magnesium ammonium phosphate ($NH_4MgPO_4{\cdot}6H_2O$) from various wastewaters. Struvites with potential agricultural application have been recovered from various P and ammonium containing wastewaters such as semiconductor wastewater (Ryu et al., 2012), phosphogypsum wastewater (Miroslav et al., 2021), yeast industry anaerobic effluent (Uysal et al., 2014), wood based activated carbon wastewater (Wang et al., 2023) and urine (Kabdasli et al., 2022). The chemical properties of recovered struvite can vary depending on the chemical composition of the wastewater, the chemical additives used (e.g. $MgSO_4$, $MgCl_2.H_2O$), and the precipitation conditions. As shown by several studies, various minerals and elements (in various chemical forms) such as $MgHPO_4.7H_2O$, $KMgPO_4.6H_2O$, $NH_4MgAsO_4.6H_2O$, K,

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Zn, Cu, Al, and Fe can be absent or present in varied concentrations (Kabdasli et al., 2022; Miroslav et al., 2021; Uysal et al., 2014). Regardless of these chemical variances, all struvites are sparingly soluble in water with <3 % of the P water soluble (Jama-Rodzeńska et al., 2022; Wang et al., 2023).

When applied to soil, struvites only liberate P slowly compared to conventional P fertilisers made from acidulated apatite rocks. Considering a struvite product with similar intrinsic chemical properties, the amount of P and other elements that eventually become available for plant uptake per unit time is a function of the soil condition (mainly pH, temperature, organic matter content and soil microbial composition and activity), plant root activity and the interaction of plant and soil activity. To generalize, it has been shown that acidic soil conditions result in higher struvite dissolution rates (~0.43 mg struvite/d) compared to alkaline soils (~0.03 mg struvite/d) (Degryse et al., 2016; Valle et al., 2022) and addition of microorganisms capable of solubilising P greatly improve struvite dissolution (Hernández Jiménez et al., 2021; Jokkaew et al., 2022). Given that root activity is at its lowest during the early growth stages of plants, the effect of the soil condition on struvite dissolution becomes the major factor. In the initial stages of plant establishment, microbial activity therefore presumably takes precedence in driving struvite dissolution.

The application of struvite fertilisers to soil may directly or indirectly affect soil microbiota. Direct effects could result from soil solution chemistry that favours particular microbial groups to proliferate, while adsorbed pharmaceuticals (i.e. antibiotics from wastewater) could result in an opposite effect. It has been shown, through spiking urine, that up to 26 % of pharmaceuticals may get adsorbed onto struvite crystals during precipitation. While these do not pose a significant risk to humans or animals through plant uptake (de Boer et al., 2018), they may affect the proliferation of some soil bacteria especially in the case of antibiotics. Such studies present a “worst case scenario” as the concentration of pharmaceuticals in wastewater and the resultant recovered struvite in a real-world setting will be much lower. Other direct effects of struvite application to soil include soil pH increase and release of potentially toxic adsorbed elements such as Cu, Al, and Fe which in turn affect soil microbiota (Wang et al., 2023). It is therefore prudent to investigate the interactions of struvite fertilisers with soil microbiota to shed light into the environmental effects of struvite application.

Most research on the use of struvite as a fertiliser has focused on its agronomic efficiency on various field crops and perennials (Hernández Jiménez et al., 2021; Hertzberger et al., 2021; O’Donnell et al., 2021; Omidire and Brye, 2022; Valle et al., 2022) with much less focus on resultant soil microbiota changes (Carreras-Sempere et al., 2022). A paucity of studies have looked at soil microbiota effects and this is in particular the case for horticultural crops which are economically important. One of these horticultural crops is tomato which has a trade value of over a billion euros in the EU (~ 360,000 t of exports and ~ 790,000 t of imports per year) (Eurostat, 2023).

The objectives of this study were therefore to assess two struvites with similar N and P content, but differing manufacturing processes alongside the use of superphosphate on tomato plant growth and the corresponding tomato rhizosphere bacteria. Our hypothesis was that while struvites have high nutrient use efficiencies, their short-term application may be less beneficial than fast P releasing superphosphate and that differing P fertilisers will have a distinctive effect on the bacterial rhizosphere community structures.

2. Materials and methods

2.1. Potting soil

The soil used for plant growth was collected from a grassland site in County Limerick, Ireland. The soil was sieved through a 5.6 mm mesh size sieve to remove stones, twigs, and gravel, and kept moist (approximately 10 % water *w*/w) before being used as potting soil. This soil was a sandy loam soil (54 % sand, and 8.6 % clay) that had a bulk density of 1037 kg/m^3 and a water holding capacity (WHC) of 22.4 %. The soil was chosen because it had a low available P content (Irish soil P index 2, Table 1), which is ideal for robust P application agronomic comparisons. Other chemical attributes of the soil are given in Table 1. Briefly, the soil was slightly alkaline and had low exchangeable Na, Mn, and Zn.

2.2. Experimental setup

The experiment consisted of 4 treatments (3 - P fertiliser types and a no P control) replicated 6 times and arranged in a randomised complete block design (RCBD). Phosphorus treatments consisted of single super phosphate - SSP (7.6 % P, 0 % N, 0 % K), struvite from municipal wastewater - MWS (14.8 % P, 5.5 % N, 0.06 % K), and struvite from potato processing wastewater - PWS (15.3 % P, 5.1 % N, 1.9 % K). About 1.75 kg of soil was placed in clean plastic bags and the various fertilisers were added at 80 mg P/ kg soil and then thoroughly mixed by shaking before placing the soil into a 3 L pot. N and K were applied at planting as NH_4NO_3 and KNO_3 at 182 mg N/ kg soil and 142 mg K/ kg soil respectively. Micronutrients were applied routinely once every week to each pot as a 100 mL micronutrient solution. This solution contained the following per litre 2.22 g - $MgSO_4.7H_2O$, 1.80 g - $CaCO_3$ (anhydrous), 3.29 g - NaCl, 3.0 mg - H_3BO_3, 1 mg - $CuCl_2.H_2O$, 20.0 mg - $MnCl_2.H_2O$, 0.4 mg - $(NH_4)Mo_7O_{24}.4H_2O$, and 1.5 mg - $ZnCl_2$ (Middleton and Toxopeus, 1973). Physicochemical properties of PWS and MWS struvites are available online (https://www.nmi-agro.nl/wp-content/uploads/2019/09/21022022_WPT1_D3.4-Product-factsheets.pdf).

2.3. Planting and experimental management

Germination tray seeded healthy cherry tomato (var. *Mr Fothergill's Garden Pearl*) seedlings at the 3-leaf stage were transplanted into pots (2 seedlings per pot) and initially watered with collected rainwater to field capacity. Watering was done routinely to approximately 75 % WHC using average soil water depletion values for each treatment obtained from weighing the pots once a week. Average day (d) temperatures in the glasshouse ranged between 19 and 34 °C and night temperatures ranged between 14 and 25 °C during the experiment (Supplementary Fig. S1).

2.4. Plant and soil sampling

Aboveground biomass harvesting of tomato plants was done by cutting the stems at soil level, at 60 d after transplanting (DAT) during the early fruiting stages. At this time, fruits were at various stages of development so the fruit yield, though useful for comparison, was not representative of the final yield if the plants were allowed to grow longer. All fruit was separated from the above ground biomass, and both were weighed after being dried to a constant mass in an oven at 75 °C.

Soil sampling was done in three stages and not pooled together (for enzymatic, molecular, and chemical analysis). For enzymatic analysis, rhizosphere soil was sampled by taking loosely root-attached soil after gently shaking the roots and this was put in a fridge at 4 °C until analysis. For DNA extraction and subsequent molecular analysis, the rhizosphere/rhizoplane soil used was sampled by gently scrapping the roots, using a flamed spatula. The obtained soil was transferred into 2 mL collection vials and immediately stored at −20 °C until DNA extraction was performed. For chemical analysis (P, pH), bulk soil in each pot was mixed and sampled by coning and quartering, then further air-dried and sieved through a 2 mm sieve before chemical analysis.

2.5. Plant chemical analysis and soil chemical and enzymatic analysis

Dried tomato aboveground biomass (stem and leaves) was ground and digested in concentrated nitric acid (70 %) (1 g sample: 10 mL acid) on a DigiBlock digester and diluted to 25 mL before chemical analysis.

Table 1
Properties of soil used for pot trial.

pH	OM (%)	Total N (%)	Avail. P (mg P/L)	CEC (meq/100 g)	Exchangeable cations (ppm)					
					K	Mg	Ca	Zn	Mn	B
7.5	7.5	0.43	4.6	15.0	154	217	4252	4.9	43	1.38

OM - organic matter, CEC - cation exchange capacity.

These were then analysed for P, Ca, K, S, Mg, Fe and Na content via inductively coupled plasma optical emmission spectroscopy (ICP-OES). Nutrient uptake was then calculated according to eq. (1):

$$Nutrient\ uptake\ (mg/pot) = \frac{\%Nutrient\ content\ x\ Biomass\ yield\ (mg/pot)}{100} \tag{1}$$

Soil available P was measured colorimetrically at 882 nm using a UV–Vis Spectrophotometer (Murphy and Riley, 1962) after a 30-min extraction from soil (1:5 *v*/*v*) in Morgan's extractant (10 % NaOAc pH 4.8) (Peech and English, 1944). Soil pH was measured using a pH meter in a 1:5 (*w*/*v*) soil: water mixture.

Soil alkaline and acidic phosphatase activity of soil was analysed by measuring *p*-nitrophenol released using a UV-Vis spectrophotometer after incubating fresh soil with *p*-nitrophenol phosphate (PNPP) solution (Tabatabai, 1994).

2.6. Tomato plant and soil data analysis

Tomato biomass production, fruit yield and nutrient uptake and soil chemical attributes data were analysed using IBM SPSS Statistics software (version 28). Data were tested for normality and homogeneity of variance using the Shapiro-Wilkinson and Levene tests respectively before running a one-way analysis of variance (ANOVA) at 95 % confidence interval. The normality assumption was met for all the data ($p_{\text{Shapiro-Wilkinson}} > 0.05$), while the assumption of homogeneity of variance was met for all the data except for Mg and S uptake data. The Tukey HSD statistic was used to separate significantly different means for all data that met normality and homogeneity of variance assumptions. For Mg and S uptake, data that had significant heterogenous variances, a robust test of equality of means was done using the Brown-Forsythe test and separation of means was done using the Games-Howell statistic.

2.7. Molecular microbiological analysis

Soil DNA extraction from 0.25 g of rhizosphere soil was performed using a DNeasy® PowerSoil® Pro Kit (Qiagen GmbH) according to the manufacturer's guidelines. DNA quantification and quality were assessed using a microplate spectrophotometer (Eon plate reader with Take 3 plate, BioTek, Winooski, VT). DNA of a 260/280 nm range of above 1.8 and above 20 ng/µl were subjected to amplicon based sequencing.

16S rRNA gene amplicon sequencing was caried out at the Novogene Bioinformatics Technology Co., Ltd. to determine changes in bacterial structure. The DNA was amplified in the 16S rRNA gene region V3-V4, using the 341F/806R primer sets (5′- CCTAYGGGRBGCASCAG - 3′, 5′ - GGACTACNNGGGTATCTAAT - 3′) (Hjelmso et al., 2014). The PCR products of correct size were initially assessed via 2 % agarose gel electrophoresis. Suitable PCR products were ligated with Illumina adapters. Library quality was checked using a Qubit Fluorometer and real-time PCR for quantification, while a bioanalyzer was used for size distribution detection. Libraries were then sequenced on a paired-end Illumina platform (NovaSeq) to generate 250 bp paired-end raw reads which were then merged using FLASH (version 1.2.11) (Magoc and Salzberg, 2011) and pre-treated to obtain clean tags following QIIME2 protocols. Amplicon Sequence Variants (ASVs) were generated by denoising through de-duplicating sequences using the DADA2 parameter in QIIME2. Annotation of each ASV was done using a Naive Bayes classifier that applies the QIIME2's classify-sklearn algorithm (Bokulich et al., 2018; Bolyen et al., 2019) to obtain abundance tables at kingdom, phyla, class, order, family, genus, and species levels. Sequences were deposited in the Sequence Read Archive (BioProject ID: PRJNA996060, accession numbers SAMN36511351-74).

Alpha and beta diversities were determined with QIIME2 (Version 2022–02). Principal coordinates analysis (PCoA) was performed and graphed in R software (version 4.0.3) using the ggplot2 package (Wickham, 2016). Bacterial abundance heat maps were created in R. Functional Annotation of Procaryotic Taxa (FAPROTAX) analysis (Louca et al., 2016) was done with the Python software (version 2.7.15). Differences of abundances between treatments were assessed for significance using pairwise *t*-tests and metastat ($\alpha = 0.05$) (Paulson et al., 2011) in R software. The correlation between alpha diversity and plant biomass, soil pH, Morgans extractable P, and soil alkaline phosphatase activity was assessed using Spearman rank correlation in R software.

Phosphorus cycling genes (*phoD*) were quantified via real time amplification using the primers ALPS-F730 (5'-CAGTGGGACGACCACGAGGT-3′) and ALPSR1101 (5′-GAGGCCGATCGGCATGTCG-3′) (Sakurai et al., 2008) in a LightCycler 96 Real-time PCR System. The following recipe was used for PCR reactions: 5 µl of qPCR Universal Master Mix (KAPA SYBR FAST - Kapa Biosystems, Boston, MA), 3 pmol of each primer, 1 µl template (10 ng/µl), 2 µl of 5 M Betaine and 1.4 µl PCR grade water to make a 10 µl reaction. The cycling conditions used consisted initial denaturation and activation at 95 °C for 300 s, 45 amplification cycles consisting of denaturation at 95 °C for 3 s, annealing at 60 °C for 20 s and an extension at 72 °C for 20 s. Samples, standards and non-template controls were run in triplicates on 96-well microtiter plates and a melt curve analysis was done to check the specificity of the reaction. Tests for significance were conducted as described above for tomato plant data.

3. Results

3.1. Tomato growth and fruit yield

At the time of harvest, struvite treatments PWS and MWS produced total aboveground biomass that was 17 and 22 %, below that of SSP (significant, $p < 0.001$; Fig. 1). However, addition of PWS and MWS fertilisers led to significantly more biomass than the control (31 and 24 % respectively). No significant difference was observed between the two struvite treatments at the aboveground biomass level. The similar outcome to aboveground biomass was observed for dry fruit yield where PWS, MWS and SSP produced significantly ($p < 0.001$) more dry fruit mass at 69, 51 and 128 % respectively compared to the No P control. Overall, SSP had the highest yields (Fig. 1).

3.2. Phosphorus, Ca, Na, K, Mg and S uptake in tomato biomass (leaves and stems)

Phosphorus uptake was not significantly different ($p = 0.08$) between the four treatments per pot (Fig. 2a). Though Na and S uptake was not significantly different between the control, PWS, and MWS, the SSP treatment had significantly ($p < 0.001$) greater Na (at least 40 %) and S (at least 46 %) uptake (Fig. 2b,f). Calcium uptake significantly varied (p

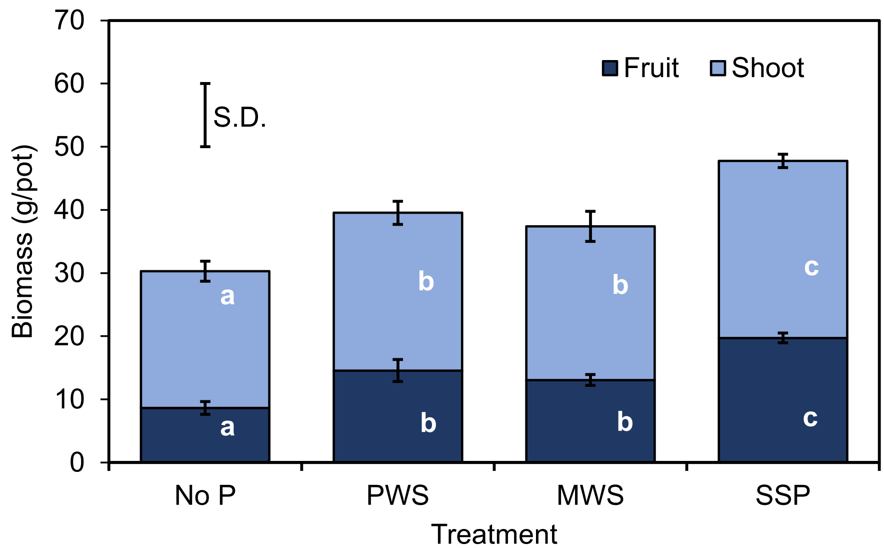

Fig. 1. Total aboveground dry mass of tomato (fruit and shoots) after growth with various phosphorus sources. S.D. – standard deviation, $n = 6$. Different letters below bars indicate statistical significance at $\alpha = 0.05$.

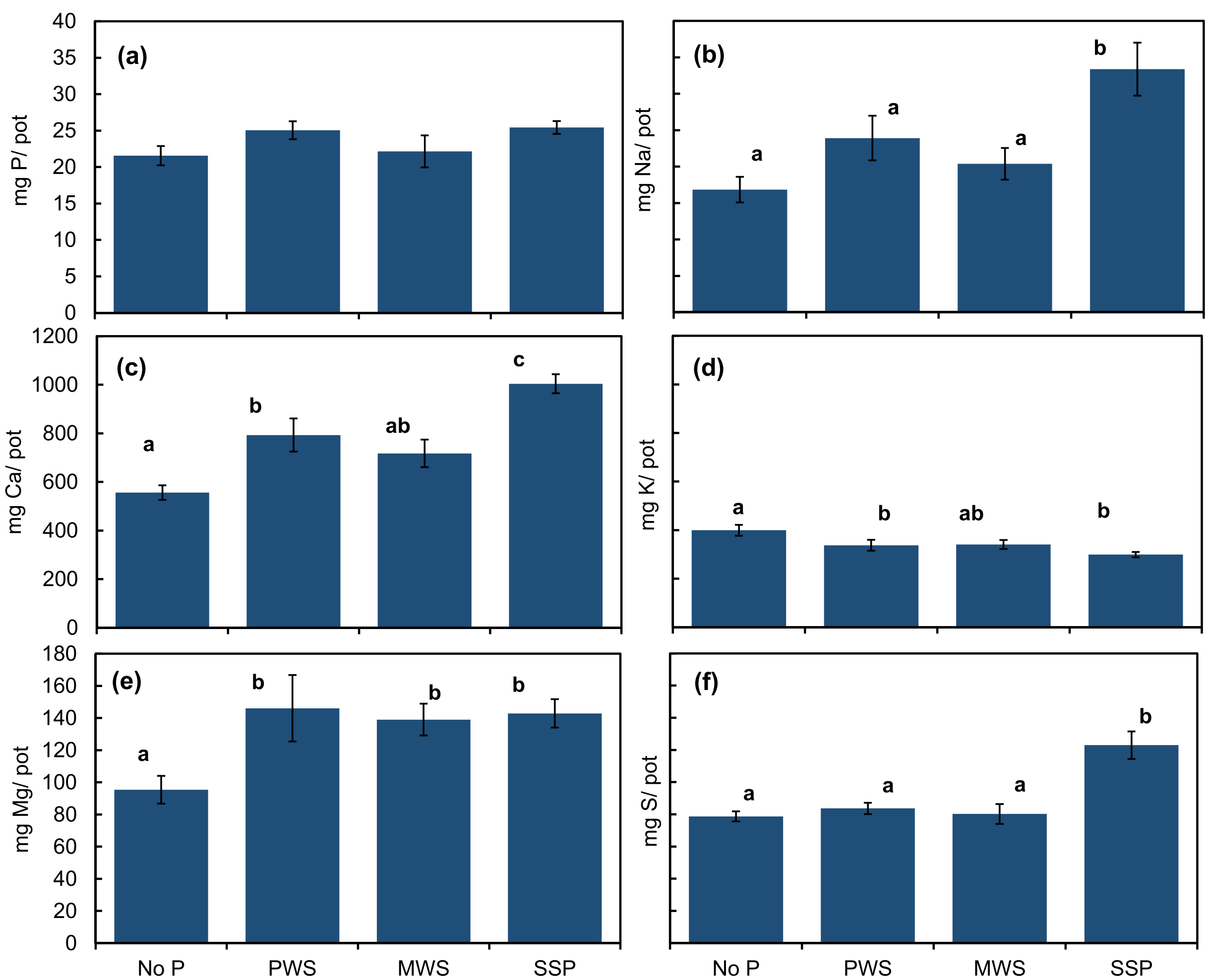

Fig. 2. Plant P, Na, Ca, K, Mg and S uptake in tomato shoots (stem and leaf only) after application of various P sources. Error bars are standard deviation, $n = 6$. Different letters above bars indicate statistical significance at $\alpha = 0.05$.

< 0.001) with treatments following a similar trend to aboveground biomass in the order: No P < MWS = PWS < SSP (Fig. 2c). While P treatments had at least 45 % more Mg uptake compared to the control, Mg uptake did not vary among the P fertilized treatments (Fig. 2e). Potassium uptake was greatest in the No P control (Fig. 2d), about 15 % less in the struvite fertilisers and SSP had the lowest K uptake (25 % less compared to the control, $p < 0.05$).

3.3. Selected soil attributes after tomato growth

Soil available P was lowest in the No P control at 2.32 mg P/L (Table 2) and was significantly higher ($p < 0.001$) by factor 1.9, 2 and 2.4 when MWS, SSP and PWS were applied, respectively. Comparing with the initial available P of 4.6 mg P/L (Table 1), tomato growth without P application depleted available P, while SSP and MWS maintained a similar range to initial available P and PWS increased available P at time of harvest. Compared to the No P control, addition of MWS and SSP significantly ($p = 0.004$) reduced soil pH by about 0.23 units while PWS had similar pH to the No P control (Table 2). Soil acidic and alkaline phosphatase activity was however not significantly different across treatments ($p = 0.96$ and 0.46 respectively) at time of harvest.

3.4. Treatment effects on phoD gene abundance and bacterial community structure

Copy numbers of the *phoD* gene in rhizosphere soils were high overall, exceeding 10^9 copies per gram soil (fresh weight). There were however, no significant differences ($p = 0.63$) in the *phoD* gene copy numbers across all treatments (Table 2).

Alpha diversity community metrics (Chao1, Shannon, Pielou and Simpson) significantly varied with treatment (Table S1). The MWS treatment had the lowest ($p < 0.05$) ASV richness as measured by the Chao1 and Shannon indices (Table S1) while the SSP and PWS treatments were statistically similar to each other ($p_{\text{Chao1}} = 0.84$ & $p_{\text{Shannon}} = 0.13$) and had the highest ASV richness. The same constellation was observed for ASV evenness (Pielou and Simpson indices). Principal coordinate analysis of beta diversity (weighted unifrac distance matrix) revealed that bacterial community composition in the treatments could be distinguished on the first axis (Fig. 3). Bacterial community composition of the SSP and PWS treatments appeared to overlap. Both SSP and PWS were separated on the first axis to the No P treatment and were most distant to the MWS treatment. No further separation was visible on the second axis. Statistical pairwise comparisons Anosim ($R^2 \geq 0.13$), Adonis ($R^2 \geq 0.12$), and MRPP ($A \geq 0.01$)) of soil bacterial community differences confirmed significant differences ($p < 0.05$) in bacterial community structure between all the treatments with the exemption of SSP and PWS treatments (Table S2).

Further analysis of taxa with significant intra-group variation was done via meta-analysis (Fig. 4). Compared to No P, MWS resulted in significantly higher relative abundance of *Xanthobacteraceae*, *Planococcaceae*, *Bacillaceae and Methyloligellaceae,* while *Peptostreptococcaceae*, *Alicyclobacillaceae* and *Streptococcaceae* were significantly reduced. When compared to No P, out of the ten most abundant bacterial families, application of PWS resulted in significantly reduced abundances of only *Bacillaceae* and *Chthoniobacteraceae*. However, the application of SSP significantly reduced abundances for *Xanthobacteraceae*, *Planococcaceae*, *Bacillaceae*, *Alicyclobacillaceae*, and *Streptococcaceae,* while *Peptostreptococcaceae* significantly increased in abundance. Comparison of the two struvites (MWS and PWS) revealed significant opposite effects on abundances of *Xanthobacteraceae*, *Planococcaceae*, *Bacillaceae, Peptostreptococcaceae*, *Alicyclobacillaceae* and *Streptococcaceae*, with the first three families showing higher relative abundance and the last three families being lower in relative abundance in MWS when compared to PWS.

Table 2
Soil available P, pH, phosphatase activity and *phoD* gene measured after tomato growth.

	No P	PWS	MWS	SSP
pH	7.32[c] ± 0.05	7.23[abc] ± 0.08	7.09[ab] ± 0.17	7.09[a] ± 0.11
Morgan's P (mg P/L)	2.32[a] ± 0.32	5.52[c] ± 0.45	4.37[b] ± 0.97	4.74[bc] ± 0.54
ACP (ug PNP/ g soil)	1078 ± 210	1071 ± 202	1118 ± 218	1066 ± 62
ALP (ug PNP/ g soil)	691 ± 139	788 ± 151	790 ± 76	756 ± 97
phoD (copies/ g soil)	$8.8 \times 10^9 \pm 2.1 \times 10^9$	$1.0 \times 10^{10} \pm 1.8 \times 10^9$	$1.0 \times 10^{10} \pm 2.1 \times 10^9$	$9.2 \times 10^9 \pm 1.9 \times 10^9$

± values represent standard deviation, $n = 6$. ACP - Acidic phosphatase, ALP - Alkaline phosphatase, PWS - struvite from potato processing wastewater, MWS - struvite from municipal wastewater and SSP – single superphosphate. pH and Morgan's P measured in bulk soil, ACP, ALP and *phoD* measured in rhizosphere soil.

Functional Annotation of Procaryotic Taxa (FAPROTAX) analysis revealed that MWS usually had the lowest abundance of functional categories (Supplementary Fig. S2). In comparison to PWS, MWS was lower in N-fixation, N-respiration, nitrite respiration, nitrification, S-respiration, Fe-respiration functional attributes, and higher in cellulolytic, methylotrophic, methanotrophic and nitrate respiration attributes (Suppl. Fig. S2). Compared to No P, addition of MWS significantly ($p < 0.01$) reduced chemoheterotrophic and fermentation bacteria while addition of SSP significantly reduced ($p = 0.03$) ureolysis bacteria (Suppl. Fig. S2 & S3). Tomato aboveground dry weight was strongly positively correlated with the taxon group *X67.14* and *Chitinophagaceae* abundances (Fig. 5). Soil Morgan's P content was positively correlated with *Gaiellaceae* and negatively correlated with *Solibacteraceae*. Soil alkaline phosphate activity was positively correlated to *Mycobacteriaceae*, *Micrococcaceae* while it was negatively correlated to *Sphingobacteriaceae* families.

4. Discussion

The present study investigated tomato plants and their rhizosphere bacteria when fertilized with struvites, superphosphate or no P. At the outset we hypothesized that different types of P fertilisers will result in distinguishable short-term biomass gains and bacterial community structures but did not consider minor differences in the elemental struvite composition to be relevant. Here, the PWS fertiliser contained 31 times more K (19 vs. 0.6 g/kg), as well as three times more S and Ca content than MWS (Table S3). These differences could have had limited influence on the P dissolution kinetics, albeit not enough to achieve significant agronomic differences between the struvites. Other studies have shown that increasing ionic strength can increase struvite solubility (Rech et al., 2018). In addition, MWS had considerably more (17 times) Fe content which could potentially precipitate with released phosphorus to form insoluble iron phosphates. It is likely that the higher Fe content in MWS could be a result of Fe that is generally used to immobilize arsenic during municipal wastewater treatment (Miroslav et al., 2021). The effect of high Fe and Al content limiting P availability is well known in conventional fertilisers (Tumbure et al., 2019). Tomato root exudates have limited ability to dissolve Fe bound P as shown by Khashi u Rahman et al. (2021), where exogenously applied tomato root exudates solubilised about eight times less Fe bound P compared to Ca, Al and organically bound P. Tomato plants secrete majorly oxalic acid in the rhizosphere, among formic, malic, malonic, lactic, acetic, citric, succinic, and propionic acids (Yang et al., 2016). This likely contributed to more P release from the PWS than the MWS in this study because oxalic acid is better at solubilising Ca bound P than Fe bound P and the acid can remove the Ca reaction product from solution which drives the reaction forward (Tumbure et al., 2022). The ability to solubilise Fe bound P is also less common in bacteria, limited to siderophore producing and phytate solubilising bacteria that can chelate the Fe such as those that belong to the *Burkholderia* genus (Tumbure et al., 2023; Wang et al., 2021).

The observed delayed growth vigour in the struvite treatments suggests that P supply could initially be out of synchrony with plant requirements in the early growth stages of tomato (Supplementary Fig. S4) which then improves to similar levels to SSP by the fruiting stage as

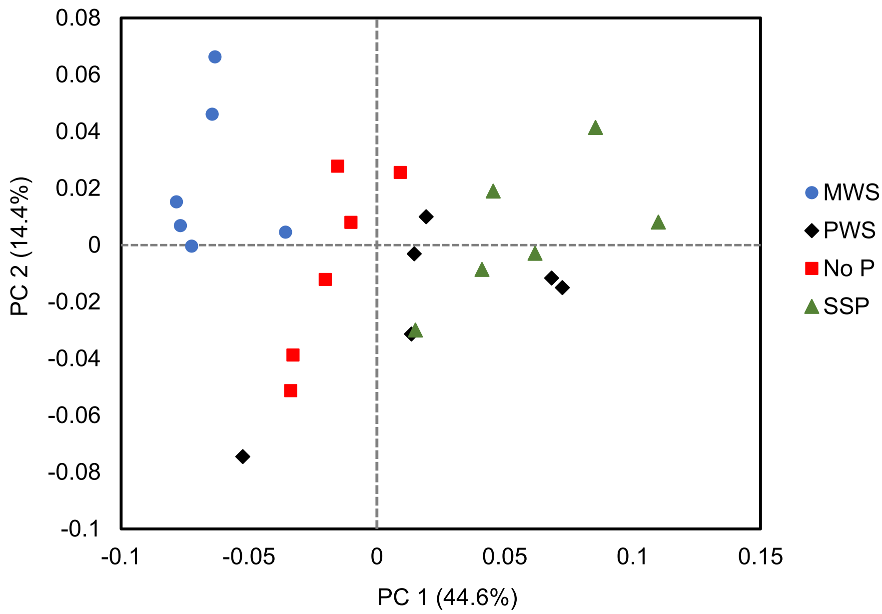

Fig. 3. Principal co-ordinate analysis (PCoA) of bacterial communities using weighted unifrac distance matrix ($n = 6$), MWS = blue disk, PWS = black diamond, SSP = green triangle, No P = red square.

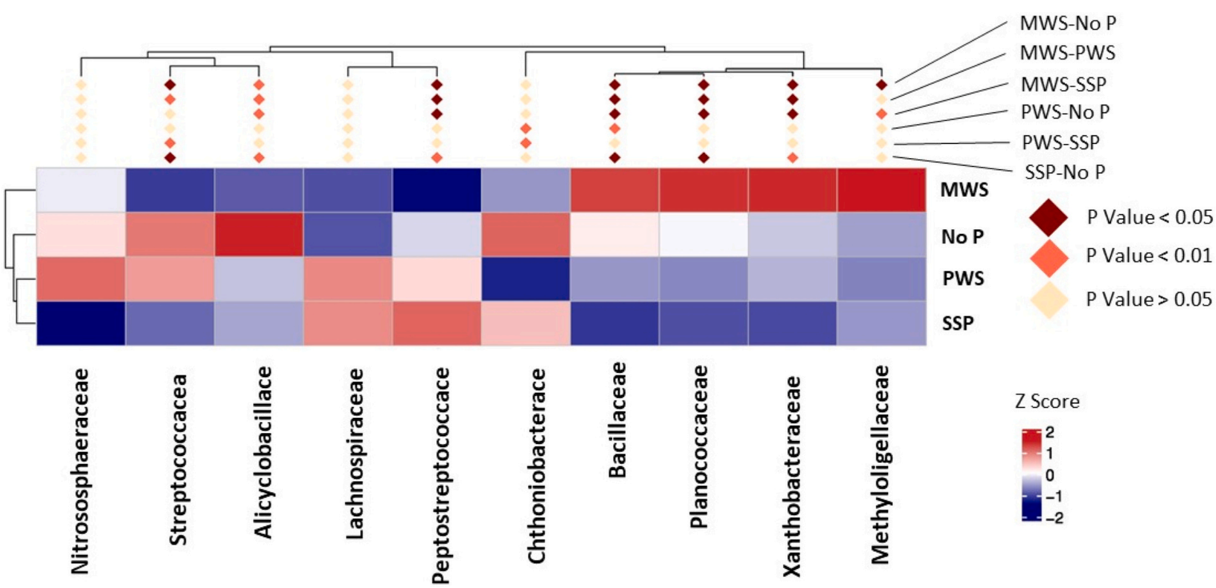

Fig. 4. Cluster heatmap of the identified ten most abundant bacterial families based on 16S RNA gene sequence analysis. Red colour indicates an increase in relative abundance per treatment, while blue colours indicate a reduction. Diamonds identify significant (light red), highly significant (dark red) or no significant (cream) differences.

measured by tomato P uptake (Fig. 2). Other studies report slow P release rates in struvite compared to conventional fertilisers and attribute this to the chemical composition which varies from one struvite to another (Rech et al., 2018). It is likely that the struvite treatments could have performed agronomically the same or better than SSP if the tomato plants were cultivated over a complete growing season. Similar to this study, a pot study by Deinert et al. (2023) reported increases in available P in soil after MWS struvite addition and 54 d of ryegrass growth compared to a No P control. The measured high Morgan's P content after plant harvest (Table 2) in PWS applied soil implies that the struvite could be better at supplying P over longer periods providing a long-term benefit. Indeed, during a three-year grassland field trial with PWS, MWS and SSP, both struvites achieved higher nutrient recovery rates in the harvested grass biomass than SSP over the three-year period (Patrick Forrestal, oral communication). Hence, longer growth periods could have led to improved fruit and plant biomass from the PWS treatments with the potential to exceed SSP applications.

The lower soil pH at the end of the experiment in all treatments (Table 2) compared to initial soil pH (7.5) supports the assumption that rhizospheric soil acidification took place. An ensuing rhizospheric acidification has been noted resulting in better P availability from struvites (Robles-Aguilar et al., 2019). While initial vegetative vigour was reduced, it is likely that the tomato plants were able to adapt to low initial soil P conditions in the struvite treatments and prolonged P limitations on the no P treatment. It as has been shown by Wang et al. (2022a) that tomato plants can increase root H^+-ATPase activity and H^+ secretion under low available P conditions to improve P availability.

While this study did not analyse P accumulation in tomato fruit and

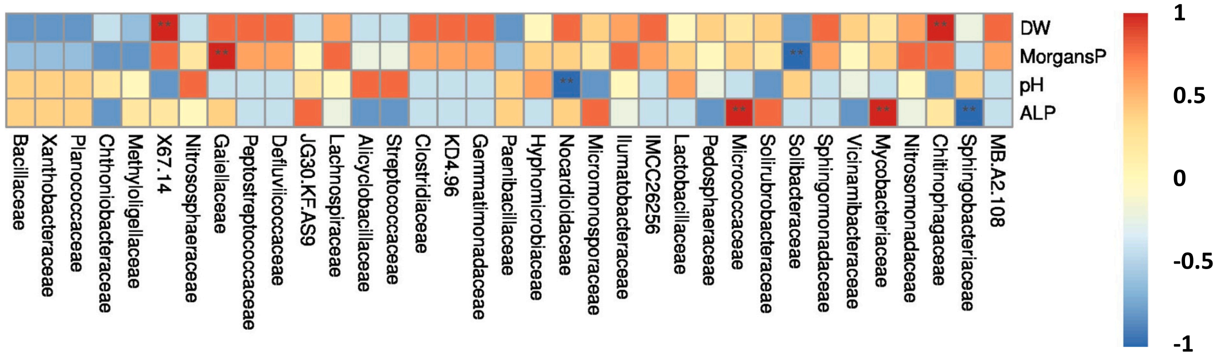

Fig. 5. Spearman correlation of environmental variables and bacterial abundances at family level with positive correlations appearing in red and negative correlations appear in blue. Significant differences ($p < 0.05$) are highlighted as **.

roots, P uptake values in stem and leaves has been documented to constitute ~85 % of total uptake at 60 DAP by other researchers who analysed total P accumulation (Zhu et al., 2018). The same authors reported that total P uptake was not significantly different over two seasons, in field tomatoes at 95 DAP which received varying amounts of P. Even though nutrient solution was used in the present study to provide all the treatments with sufficient amounts of Ca and S, the observed greater Ca and S uptake in tomatoes which received SSP (Fig. 2c & f) is likely a result of extra Ca and S added through SSP (45 % *w*/w > $CaSO_4$).

Soil microbial action also influences total P dissolution in addition to the mentioned chemical constitution of a P source and plant root exudates. The major microbially-led P availing processes are extracellular enzyme actions (i.e. phosphatases), reductive dissolution and organic acid/metal chelation activity (Tumbure et al., 2023; Wang et al., 2021). However, since phosphatase enzymes and *phoD* gene abundance were not significantly different between treatments in this study, it is probable that secretion of low molecular weight organic acids (LMWOA) by microbes and the tomato plant had a greater effect on struvite dissolution than P-solubilising bacteria. The resulting availability of P and other mineral elements from the different P sources affected microbial proliferation in the short term as witnessed by significant changes of bacterial community composition in the MWS treatment.

The increased abundances of *Xanthobacteraceae*, *Planococcaceae*, and *Bacillaceae* in the MWS treatment compared to No P, could have been encouraged by the presence of the less chemically soluble MWS because many genera in these families have the ability to solubilise sparingly soluble inorganic P (Li et al., 2019). Proliferation of *Xanthobacteraceae* is reported by other researchers as indicative of low P conditions that favour slow growing oligotrophic bacteria (Oliverio et al., 2020). Particular genera within the *Xanthobacteraceae* family such as *Bradyrhizobium* are known to produce siderophores that solubilise Fe bonded P. Similar reasons as above could explain when the two struvites are compared to each other, where MWS led to increased abundances of *Xanthobacteraceae*, *Planococcaceae*, and *Bacillaceae.* The PWS struvite could have been relatively more readily broken down by microbes and plant exudates compared to the MWS struvite. An opposite trend to that of MWS was observed when SSP is compared to No P, where *Xanthobacteraceae*, *Planococcaceae*, and *Bacillaceae* abundances were reduced probably as a result of increased available P that increases microbial competition from non-P solubilising microbes. This result agrees with findings from Ikoyi et al. (2018) who found a reduction of genera such as *Bacillus* and *Bradyrhizobium* with increasing rates of soluble inorganic P fertiliser. The Reduction in *Alicyclobacillaceae* abundance in MWS compared to PWS is likely a result of MWS's higher Fe content compared to PWS. A recent study by Liu et al. (2022) showed that increased content of poorly crystalline Fe(II) minerals in soil negatively impacted *Alicyclobacillaceae* abundance.

The significant positive correlation with available P and *Gaiellaceae* abundance found in this study has been reported by other researchers in different soils and is attributed to genera in this family having the ability to produce alkaline and acid phosphatase (Wang et al., 2022b). *Solibacteraceae* are known to proliferate in low P conditions (Lang et al., 2021; Oliverio et al., 2020) and the negative correlation between available P and *Solibacteraceae* found in this study supports this.

Bacterial community shifts in MWS applied pots observed through functional analysis to result in a reduction of chemoheterotrophic and fermentative soil functions may indicate reduced rates of organic matter breakdown. This is likely through the presence of Fe that may lead to Fe-organic complex formations that are difficult to decompose. Reductions in chemoheterotrophic function in the MWS treatment is likely to have been led by the significant reduction of *Peptostreptococcaceae* abundance that was observed earlier.

On the other hand, compared to No P, addition of SSP led to a decrease in ureolytic function. Ureolysis is known to increase soil pH resulting in the precipitation of calcium carbonate (Bibi et al., 2018). It is unlikely that the reduced ureolytic function tipped the balance of soil acid/alkaline processes resulting in low soil pH because rhizospheric soil acidification was noted for all treatments. At the same time, the effect of pH on ureolytic function is also unlikely because, while the ureolysis enzymes are negatively affected by low soil pH (Singh and Nye, 1984), the measured pH at the end of the experiment was similar to what several researchers have noted to be in the optimum range for bacterial urease secretion and activity (Hasan, 2008). The reason for reduced ureolytic function after SSP addition as observed in this study is therefore unknown. In comparison to PWS, MWS had higher cellulolytic, methylotrophic, methanotrophic and nitrate respiration attributes which are likely a result of different chemical compositions of the two struvites. In particular, the higher Fe content of MWS may have promoted the methane pathway, which has been reported recently in soils after addition of Fe (Zhang et al., 2022).

Results from this study show that while struvite fertilisers from various sources can be agronomically effective P sources for horticultural plants such as tomatoes, they could have different short-term impacts on soil microbiota. Further studies that lead to full fruiting till senescence are recommended. The short-term bacterial community shifts noted in this study are essential as a starting point of enquiry, and potential future long-term studies on struvite use effects on soil microbial communities are recommended to improve our mechanistic understanding on lasting environmental impacts of struvite fertilisers.

5. Conclusion

Results from this study demonstrated that variations in quantitatively minor elements between struvites may not result in significant differences in terms of agronomic response of tomato. However, distinctions in the bacterial community structure were observed and the two struvites tested resulted in bacterial community structures that were ecologically distant to each other. In the long-term, these rather subtle differences in struvite composition could lead to agronomically different outputs, when one considers that bacterial rhizosphere communities influence plant growth. In addition, while in the short-term the struvites could have provided near similar amounts to a growing tomato plant, the PWS soil amendment resulted in more plant available P at the tomato fruiting stage which indicates that PWS could be more agronomically effective in the long-term. Further long-term field studies should shed additional light on the present observations.

CRediT authorship contribution statement

Akinson Tumbure: Formal analysis, Methodology, Software, Writing – original draft, Investigation, Validation, Visualization. **Achim Schmalenberger:** Conceptualization, Data curation, Funding acquisition, Investigation, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2024.105276.

References

Bibi, S., Oualha, M., Ashfaq, M.Y., Suleiman, M.T., Zouari, N., 2018. Isolation, differentiation and biodiversity of ureolytic bacteria of Qatari soil and their potential in microbially induced calcite precipitation (MICP) for soil stabilization. RSC Adv. 8 (11), 5854–5863. https://doi.org/10.1039/c7ra12758h.

de Boer, M.A., Hammerton, M., Slootweg, J.C., 2018 Apr 15. Uptake of pharmaceuticals by sorbent-amended struvite fertilisers recovered from human urine and their bioaccumulation in tomato fruit. Water Res. 133, 19–26. https://doi.org/10.1016/j.watres.2018.01.017.

Bokulich, N.A., Kaehler, B.D., Rideout, J.R., Dillon, M., Bolyen, E., Knight, R., Huttley, G. A., Gregory Caporaso, J., 2018. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. Microbiome 6 (1), 90. https://doi.org/10.1186/s40168-018-0470-z.

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J.E., Bittinger, K., Brejnrod, A., Brislawn, C.J., Brown, C.T., Callahan, B.J., Caraballo-Rodriguez, A.M., Chase, J., Cope, E.K., Da Silva, R., Diener, C., Dorrestein, P.C., Douglas, G.M., Durall, D.M., Duvallet, C., Edwardson, C.F., Ernst, M., Estaki, M., Fouquier, J., Gauglitz, J.M., Gibbons, S.M., Gibson, D.L., Gonzalez, A., Gorlick, K., Guo, J., Hillmann, B., Holmes, S., Holste, H., Huttenhower, C., Huttley, G.A., Janssen, S., Jarmusch, A.K., Jiang, L., Kaehler, B.D., Kang, K.B., Keefe, C.R., Keim, P., Kelley, S.T., Knights, D., Koester, I., Kosciolek, T., Kreps, J., Langille, M.G.I., Lee, J., Ley, R., Liu, Y.X., Loftfield, E., Lozupone, C., Maher, M., Marotz, C., Martin, B.D., McDonald, D., McIver, L.J., Melnik, A.V., Metcalf, J.L., Morgan, S.C., Morton, J.T., Naimey, A.T., Navas-Molina, J.A., Nothias, L.F., Orchanian, S.B., Pearson, T., Peoples, S.L., Petras, D., Preuss, M.L., Pruesse, E., Rasmussen, L.B., Rivers, A., Robeson 2nd, M.S., Rosenthal, P., Segata, N., Shaffer, M., Shiffer, A., Sinha, R., Song, S.J., Spear, J.R., Swafford, A.D., Thompson, L.R., Torres, P.J., Trinh, P., Tripathi, A., Turnbaugh, P.J., Ul-Hasan, S., van der Hooft, J.J.J., Vargas, F., Vazquez-Baeza, Y., Vogtmann, E., von Hippel, M., Walters, W., Wan, Y., Wang, M., Warren, J., Weber, K.C., Williamson, C.H.D., Willis, A.D., Xu, Z.Z., Zaneveld, J.R., Zhang, Y., Zhu, Q., Knight, R., Caporaso, J.G., 2019, Aug. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature Biotechnolgy 37 (8), 852–857. https://doi.org/10.1038/s41587-019-0209-9.

Carreras-Sempere, M., Caceres, R., Viñas, M., Biel, C., 2021. Use of recovered struvite and ammonium nitrate in fertigation in tomato (*Lycopersicum esculentum*) production for boosting circular and sustainable horticulture. Agriculture 11 (11). https://doi.org/10.3390/agriculture11111063.

Carreras-Sempere, M., Biel, C., Vinas, M., Guivernau, M., Caceres, R., 2022, Dec 12. The use of recovered struvite and ammonium nitrate in fertigation in a horticultural rotation: agronomic and microbiological assessment. Environ. Technol. 1–17. https://doi.org/10.1080/09593330.2022.2154172.

Degryse, F., Baird, R., da Silva, R.C., McLaughlin, M.J., 2016. Dissolution rate and agronomic effectiveness of struvite fertilizers – effect of soil pH, granulation and base excess. Plant Soil 410 (1–2), 139–152. https://doi.org/10.1007/s11104-016-2990-2.

Deinert, L., Ikoyi, I., Egeter, B., Forrestal, P., Schmalenberger, A., 2023. Short-term impact of recycling-derived fertilizers on their P supply for perennial ryegrass (*Lolium perenne*). Plants *12* Article 2762. https://doi.org/10.3390/plants12152762.

Eurostat. (2023). Tomatoes trade https://agridata.ec.europa.eu/extensions/DashboardTomato/TomatoTrade.html.

Hasan, H.A.H., 2008. Ureolytic microorganisms and soil fertility: a review. Commun. Soil Sci. Plant Anal. 31 (15–16), 2565–2589. https://doi.org/10.1080/00103620009370609.

Hernández Jiménez, J.E., Nyiraneza, J., Fraser, T.D., Peach Brown, H.C., Lopez-Sanchez, I.J., Botero-Botero, L.R., Naeth, M.A., 2021. Enhancing phosphorus release from struvite with biostimulants. Can. J. Soil Sci. 101 (1), 22–32. https://doi.org/10.1139/cjss-2019-0147.

Hertzberger, A.J., Cusick, R.D., Margenot, A.J., 2021. Maize and soybean response to phosphorus fertilization with blends of struvite and monoammonium phosphate. Plant Soil 461 (1–2), 547–563. https://doi.org/10.1007/s11104-021-04830-2.

Hjelmso, M.H., Hansen, L.H., Baelum, J., Feld, L., Holben, W.E., Jacobsen, C.S., 2014, Jun. High-resolution melt analysis for rapid comparison of bacterial community compositions. Appl. Environ. Microbiol. 80 (12), 3568–3575. https://doi.org/10.1128/AEM.03923-13.

Ikoyi, I., Fowler, A., Schmalenberger, A., 2018 Jul 15. One-time phosphate fertilizer application to grassland columns modifies the soil microbiota and limits its role in ecosystem services. Sci. Total Environ. 630, 849–858. https://doi.org/10.1016/j.scitotenv.2018.02.263.

Jama-Rodzeńska, A., Chohura, P., Gałka, B., Szuba-Trznadel, A., Falkiewicz, A., Białkowska, M., 2022. Effect of different doses of phosgreen fertilization on chlorophyll, K, and Ca content in butterhead lettuce (*Lactuca sativa* L.) grown in peat substrate. Agriculture 12 (6). https://doi.org/10.3390/agriculture12060788.

Jasinski, S. M. (2022). *Phosphate rock* (U.S. Geological Survey, Mineral Commodity Summaries, Issue. https://pubs.usgs.gov/periodicals/mcs2022/mcs2022-phosphate.pdf.

Jokkaew, S., Jantharadej, K., Pokhum, C., Chawengkijwanich, C., Suwannasilp, B.B., 2022. Free and encapsulated phosphate-solubilizing bacteria for the enhanced dissolution of swine wastewater-derived struvite—an attractive approach for green phosphorus fertilizer. Sustainability 14 (19). https://doi.org/10.3390/su141912627.

Kabdasli, I., Kuscuoglu, S., Tunay, O., Siciliano, A., 2022. Assessment of K-struvite precipitation as a means of nutrient recovery from source separated human urine. Sustainability *14* (3). https://doi.org/10.3390/su14031082. Article 1082.

Khashi u Rahman, M., Wang, X., Gao, D., Zhou, X., Wu, F., 2021. Root exudates increase phosphorus availability in the tomato/potato onion intercropping system. Plant Soil 464 (1–2), 45–62. https://doi.org/10.1007/s11104-021-04935-8.

Lang, M., Zou, W., Chen, X., Zou, C., Zhang, W., Deng, Y., Zhu, F., Yu, P., Chen, X., 2021. Soil microbial composition and *phoD* gene abundance are sensitive to phosphorus level in a long-term wheat-maize crop system. Front. Microbiol. 11 Article 605955 https://doi.org/10.3389/fmicb.2020.605955.

Li, Y., Zhang, J., Zhang, J., Xu, W., Mou, Z., 2019. Characteristics of inorganic phosphate-solubilizing bacteria from the sediments of a eutrophic lake. Int. J. Environ. Res. Public Health 16 (12). https://doi.org/10.3390/ijerph16122141. Article 2141.

Liu, M., Wang, J., Xu, M., Tang, S., Zhou, J., Pan, W., Ma, Q., Wu, L., 2022. Nano zero-valent iron-induced changes in soil iron species and soil bacterial communities contribute to the fate of Cd. J. Hazard. Mater. 424 https://doi.org/10.1016/j.jhazmat.2021.127343 (Pt A), Article 127343.

Louca, S., Parfrey, L.W., Doebeli, M., 2016. Decoupling function and taxonomy in the global ocean microbiome. Science 353 (6305), 1272–1277.

Magoc, T., Salzberg, S.L., 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27 (21), 2957–2963. https://doi.org/10.1093/bioinformatics/btr507.

Middleton, K., Toxopeus, M., 1973. Diagnosis and measurement of multiple soil deficiences by a subtractive technique. Plant Soil 38, 219–226.

Miroslav, H., Pavel, H., Josef, B., Jarmila, K., 2021. Arsenic as a contaminant of struvite when recovering phosphorus from phosphogypsum wastewater. J. Taiwan Inst. Chem. Eng. 129, 91–96. https://doi.org/10.1016/j.jtice.2021.10.005.

Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27, 31–36. https://doi.org/10.1016/S0003-2670(00)88444-5, 1962/01/01/.

O'Donnell, C., Barnett, D., Harrington, J., Power, N., 2021. The extended effect of top-dressed recovered struvite fertiliser on residual Irish grassland soil phosphorus levels compared to commercial phosphorus fertiliser. Agronomy 12 (1). https://doi.org/10.3390/agronomy12010008. Article 8.

Oliverio, A.M., Bissett, A., McGuire, K., Saltonstall, K., Turner, B.L., Fierer, N., 2020, Oct 27. The role of phosphorus limitation in shaping soil bacterial communities and their metabolic capabilities. mBio 11 (5). https://doi.org/10.1128/mBio.01718-20.

Omidire, N.S., Brye, K.R., 2022. Wastewater-recycled struvite as a phosphorus source in a wheat–soybean double-crop production system in eastern Arkansas. Agrosyst., Geosci. Environ. 5 (2) https://doi.org/10.1002/agg2.20271.

Paulson, J.N., Pop, M., Bravo, H.C., 2011. Metastats: an improved statistical method for analysis of metagenomic data. Genome Biol. 12, P17. https://doi.org/10.1186/gb-2011-12-s1-p17.

Peech, M., English, L., 1944. Rapid microchemical soil tests. Soil Sci. 57 (3), 167–196. https://journals.lww.com/soilsci/Fulltext/1944/03000/RAPID_MICROCHEMICAL_SOIL_TESTS.1.aspx.

Rech, I., Withers, P., Jones, D., Pavinato, P., 2018. Solubility, diffusion and crop uptake of phosphorus in three different struvites. Sustainability *11* (1). https://doi.org/10.3390/su11010134. Article 134.

Robles-Aguilar, A.A., Schrey, S.D., Postma, J.A., Temperton, V.M., Jablonowski, N.D., 2019. Phosphorus uptake from struvite is modulated by the nitrogen form applied. J. Plant Nutr. Soil Sci. 183 (1), 80–90. https://doi.org/10.1002/jpln.201900109.

Ryu, H.D., Lim, C.S., Kang, M.K., Lee, S.I., 2012. Evaluation of struvite obtained from semiconductor wastewater as a fertilizer in cultivating Chinese cabbage. J. Hazard. Mater. 221-222, 248–255. https://doi.org/10.1016/j.jhazmat.2012.04.038.

Sakurai, M., Wasaki, J., Tomizawa, Y., Shinano, T., Osaki, M., 2008. Analysis of bacterial communities on alkaline phosphatase genes in soil supplied with organic matter. Soil Sci. Plant Nutr. 54 (1), 62–71. https://doi.org/10.1111/j.1747-0765.2007.00210.x.

Singh, R., Nye, P.H., 1984. The effect of soil pH and high urea concentrations on urease activity in soil. J. Soil Sci. 35 (4), 519–527. https://doi.org/10.1111/j.1365-2389.1984.tb00609.x.

Tabatabai, M.A., 1994. Soil Enzymes. In *Methods of soil analysis*, pp. 775–833. https://doi.org/10.2136/sssabookser5.2.c37.

Tumbure, A., Bretherton, M.R., Bishop, P., Hedley, M.J., 2019. Updated characterization of Dorowa phosphate rock mined in Zimbabwe. Nat. Resour. Res. 29 (3), 1561–1570. https://doi.org/10.1007/s11053-019-09567-5.

Tumbure, A., Bretherton, M.B., Bishop, P., Hedley, M.J., 2022. Phosphorus recovery from an igneous phosphate rock using organic acids and pyrolysis condensate. Scientific African 15. https://doi.org/10.1016/j.sciaf.2022.e01098. Article e01098.

Tumbure, A., Dube, S., Tauro, T.P., 2023. Insights of microbial inoculants in complementing organic soil fertility management in African smallholder farming systems. In: Fanadzo, M., Dunjana, N., Mupambwa, H.A., Dube, E. (Eds.), Towards Sustainable Food Production in Africa. Springer Nature, pp. 59–83. https://doi.org/10.1007/978-981-99-2427-1_4.

Uysal, A., Demir, S., Sayilgan, E., Eraslan, F., Kucukyumuk, Z., 2014. Optimization of struvite fertilizer formation from baker's yeast wastewater: growth and nutrition of maize and tomato plants. Environ. Sci. Pollut. Res. 21 (5), 3264–3274. https://doi.org/10.1007/s11356-013-2285-6.

Vaccari, D.A., Powers, S.M., Liu, X., 2019. Demand-driven model for global phosphate rock suggests paths for phosphorus sustainability. Environ. Sci. Technol. 53 (17), 10417–10425. https://doi.org/10.1021/acs.est.9b02464. , Sep 3.

Valle, S.F., Giroto, A.S., Guimaraes, G.G.F., Nagel, K.A., Galinski, A., Cohnen, J., Jablonowski, N.D., Ribeiro, C., 2022. Co-fertilization of sulfur and struvite-phosphorus in a slow-release fertilizer improves soybean cultivation. Frontiers Plant Sci. 13 https://doi.org/10.3389/fpls.2022.861574. Article 861574.

Wang, B., Gao, Z., Shi, Q., Gong, B., 2022a. SAMS1 stimulates tomato root growth and P availability via activating polyamines and ethylene synergetic signaling under low-P condition. Environ. Exp. Bot. 197 https://doi.org/10.1016/j.envexpbot.2022.104844. Article 104844.

Wang, L., Ye, C., Gao, B., Wang, X., Li, Y., Ding, K., Li, H., Ren, K., Chen, S., Wang, W., Ye, X., 2023. Applying struvite as a N-fertilizer to mitigate N_2O emissions in agriculture: feasibility and mechanism. J. Environ. Manage. 330 https://doi.org/10.1016/j.jenvman.2022.117143. Article 117143.

Wang, S., Walker, R., Schicklberger, M., Nico, P.S., Fox, P.M., Karaoz, U., Chakraborty, R., Brodie, E.L., 2021. Microbial phosphorus mobilization strategies across a natural nutrient limitation gradient and evidence for linkage with iron solubilization traits. Front. Microbiol. 12 https://doi.org/10.3389/fmicb.2021.572212. Article 572212.

Wang, W., Wang, J., Wang, Q., Bermudez, R.S., Yu, S., Bu, P., Wang, Z., Chen, D., Feng, J., 2022b. Effects of plantation type and soil depth on microbial community structure and nutrient cycling function. Front. Microbiol. 13 https://doi.org/10.3389/fmicb.2022.846468. Article 846468.

Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis, 2 ed. Springer-Verlag. https://doi.org/10.1007/978-3-319-24277-4.

Yang, Z., Li, Y., Li, P., Zhang, F., Thomas, B.W., 2016. Effect of difference between day and night temperature on tomato (*Lycopersicon esculentum* Mill.) root activity and low molecular weight organic acid secretion. Soil Sci. Plant Nutr. 62 (5–6), 423–431. https://doi.org/10.1080/00380768.2016.1224449.

Zhang, C., Liu, S., Hussain, S., Li, L., Baiome, B.A., Xiao, S., Cao, H., 2022, Mar 2. Fe(II) addition drives soil bacterial co-ocurrence patterns and functions mediated by anaerobic and chemoautotrophic taxa. Microorganisms 10 (3). https://doi.org/10.3390/microorganisms10030547. Article 547.

Zhu, Q., Ozores-Hampton, M., Li, Y.C., Morgan, K.T., 2018. Phosphorus application rates affected phosphorus partitioning and use efficiency in tomato production. Agron. J. 110 (5), 2050–2058. https://doi.org/10.2134/agronj2018.03.0152.