Programa de Doctorado en Recursos y Tecnologías Agrarias, Agroambientales y Alimentarias

El fósforo como nutriente vegetal limitante en la agricultura mediterránea: Estudio y validación de nuevas formas de fertilización fosfórica biótica y abiótica

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La presente Tesis Doctoral, titulada “El fósforo como nutriente vegetal limitante en la agricultura mediterránea: Estudio y validación de nuevas formas de fertilización fosfórica biótica y abiótica”, se presenta bajo la modalidad de **tesis por compendio** de las siguientes **publicaciones**:

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Que Dña. *Lucía Valverde Vozmediano* ha realizado bajo nuestra supervisión el trabajo titulado **“El fósforo como nutriente vegetal limitante en la agricultura mediterránea: Estudio y validación de nuevas formas de fertilización fosfórica biótica y abiótica”** conforme a los términos y condiciones definidos en su Plan de Investigación y de acuerdo al Código de Buenas Prácticas de la Universidad Miguel Hernández de Elche, cumpliendo los objetivos previstos de forma satisfactoria para su defensa pública como tesis doctoral.

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A ti, papá

“Y ahora que estamos llegando, veo que esto no es ninguna carrera”

Amaia, “Tocotó”

Resumen

La fertilización de los cultivos supuso una revolución al permitir incrementar de forma notoria su rendimiento y, así pues, la producción de alimentos necesaria para acompañar el crecimiento de la población a nivel global. El fósforo es un nutriente esencial para la nutrición de las plantas. La fertilización fosfórica ha sido tradicionalmente mineral, siendo los fertilizantes desarrollados fundamentalmente a partir de roca fosfórica. Sin embargo, el empleo de este tipo de fertilización tiene varios problemas y riesgos asociados. Por una parte, la roca fosfórica es un recurso limitado cuya extracción es costosa y, adicionalmente, se encuentra geográficamente muy concentrada. Esto sitúa a muchos países, entre ellos los países europeos, en una situación de alta dependencia de este recurso. Por otra parte, los fertilizantes minerales tienen el fósforo en forma inorgánica. Esto ha resultado ventajoso debido a su rápida disolución y absorción por parte de los cultivos, pero, al mismo tiempo, una gran parte del fósforo contenido en estos fertilizantes que no es inmediatamente absorbido por las plantas acaba siendo fijado en los suelos. Esto afecta a la calidad de los suelos y acarrea riesgos medioambientales como la eutrofización de las aguas. Ante este contexto, son cada vez mayores los esfuerzos por encontrar estrategias de fertilización fosfórica alternativas. En este trabajo se abordan dos estrategias de fertilización fosfórica alternativa.

La primera estrategia de fertilización fosfórica alternativa validada en este trabajo fue el desarrollo de fertilizantes órgano-minerales (OMFs) de fósforo basados en compost, en un ensayo en maceta con lechuga (*Lactuca sativa* L.) como cultivo modelo bajo condiciones controladas de producción. En este ensayo se estudiaron comparativamente fuentes de fósforo convencionales (roca fosfórica (RP), fosfato monoamónico (MAP) y recuperadas a partir de residuos orgánicos (estruvita comercial (STR) y harina de hueso (BM). Como resultados más relevantes, se confirmó el efecto beneficioso de la adición de compost a las fuentes inorgánicas MAP y STR, que permitió mejorar propiedades del suelo como los contenidos en materia orgánica y fósforo disponible, así como aumentar significativamente el rendimiento del cultivo. Además, la estruvita tanto en forma simple como compleja (OMF con compost) se comportó de forma similar al fertilizante convencional MAP, lo que confirma su potencial como fuente alternativa de fósforo.

La segunda estrategia estudiada fue la recuperación de fósforo en forma de estruvita a partir de residuos orgánicos ricos en este nutriente: estiércol y lodos de depuradora. Este procedimiento es habitual en las plantas de tratamiento de aguas residuales para reducir la concentración de fósforo de las aguas. El enfoque de este trabajo resulta novedoso al aplicar este tipo de procedimiento a los lodos de depuradora. Básicamente, para conseguir la extracción del fósforo de los residuos es necesario realizar una extracción en condiciones ácidas, lo cual se realiza habitualmente con reactivos químicos. En este trabajo se explora la generación del medio ácido necesario para la

extracción de fósforo a través de un proceso fermentativo, es decir, de forma biótica. Para ello, se incubaron los residuos con una fuente de azúcar simple y una suspensión de la bacteria acido-láctica *Lactobacillus acidophilus*. Tras una fase de centrifugación, se precipitó estruvita a partir de la fase líquida recuperada mediante dosificación de $MgCl_2$ y NaOH. A través del procedimiento implementado, se obtuvo una mezcla de minerales de fósforo recuperado con predominio de la estruvita, lo que fue confirmado a través de difracción de rayos X.

Posteriormente, los minerales de fósforo recuperado obtenidos se validaron en el cultivo modelo empleado en el anterior ensayo (lechuga) mediante un ensayo en maceta en condiciones controladas de producción, durante dos ciclos de cultivo consecutivos, evaluando el efecto de la fuente de fósforo aplicada además de la influencia sobre la disponibilidad de fósforo de un factor abiótico, el pH del suelo, y uno biótico, la inoculación del suelo con bacterias solubilizadoras de fósforo. Los resultados obtenidos destacan el potencial de los fertilizantes órgano-minerales con fuentes de fósforo derivadas de residuos orgánicos como sustitutos alternativos a las fuentes inorgánicas convencionales de fósforo, ya que mostraron efectos similares respecto al rendimiento y la calidad del cultivo de lechuga, así como en cuanto al fósforo disponible y las actividades enzimáticas relacionadas con la mineralización de fósforo en el suelo. Por otra parte, el factor abiótico (pH del suelo) tuvo un claro efecto sobre el rendimiento y calidad de la planta, obteniendo los mejores resultados en condiciones ácidas. Sin embargo, la inoculación con bacterias solubilizadoras de fósforo no pareció tener un claro efecto durante los dos ciclos de cultivo en los parámetros estudiados en la planta y el suelo.

Abstract

Crop fertilization revolutionized agriculture by significantly increasing crop yield and, thus, the food production necessary to maintain global population growth. Phosphorus is an essential nutrient for plant nutrition. Phosphorus fertilization has traditionally been mineral-based, the fertilizers being mainly developed from phosphate rock. However, the use of this type of fertilization has several associated problems and risks. On the one hand, phosphate rock is a limited resource that is costly to extract and, in addition, is geographically concentrated. This places many countries, including European countries, in a situation of a high dependence on this resource. On the other hand, mineral fertilizers contain phosphorus in inorganic form. This has been advantageous due to its rapid dissolution and absorption by crops, but at the same time, a large part of the phosphorus contained in these fertilizers that is not immediately absorbed by plants ends up being fixed in the soil. This affects soil quality and implies environmental risks such as water eutrophication. In this context, the efforts to find alternative phosphorus fertilization strategies are increasing. Thus, this paper addresses two alternative phosphorus fertilization strategies.

The first alternative phosphorus fertilization strategy validated in this study was the development of compost-based organo-mineral phosphorus fertilizers in a pot trial with lettuce (*Lactuca sativa* L.) as model crop under controlled production conditions. In this trial, inorganic conventional phosphorus sources (phosphate rock (RP) and monoammonium phosphate (MAP)) and recovered phosphorus sources (commercial struvite (STR) and bone meal (BM) were studied comparatively. The most relevant results confirmed the beneficial effect of adding compost to the inorganic sources MAP and STR, which improved soil properties, such as the content in organic matter and available phosphorus, and significantly increased plant yield. In addition, struvite in both simple and complex forms (OMF with compost) performed similarly to conventional MAP fertilizer, confirming its potential as an alternative source of phosphorus.

The second strategy studied was the recovery of phosphorus in the form of struvite from organic waste rich in this nutrient: manure and sewage sludge. This procedure is common in wastewater treatment plants to reduce the concentration of phosphorus in water. The approach of this study is novel in that it applies this type of procedure to sewage sludge. Basically, to achieve phosphorus extraction from organic wastes is necessary to carry out an extraction in acidic conditions, which is usually developed using chemical reagents. This study explores the generation of the acidic medium necessary for the phosphorus extraction through a fermentation process, i.e., biotically. To this end, organic wastes were incubated with a simple sugar source and a suspension of the lactic acid bacterium *Lactobacillus acidophilus*. After a centrifugation phase, struvite was precipitated from the recovered liquid phase by dosing $MgCl_2$ and NaOH. Through the procedure implemented, a mixture of recovered phosphorus minerals mainly based on struvite were obtained, which was confirmed by

X-ray diffraction. Later, the recovered phosphorus materials obtained were validated in the model crop used in the previous assay (lettuce) through a pot trial under controlled production conditions during two consecutive crop cycles, evaluating the effect of the type of phosphorus source used and the influence on phosphorus availability of an abiotic factor, soil pH, and a biotic factor, the soil inoculation with phosphorus solubilizing bacteria (PSB). The results obtained highlights the potential of the organo-mineral fertilizers with organic waste-derived phosphorus sources as alternative substitutes for conventional inorganic phosphorus sources, since these materials showed similar effects on the yield and quality of the lettuce crop, as well as in relation to the available phosphorus and the soil enzymatic activities related to the soil phosphorus mineralization. On the other hand, the abiotic factor (soil pH) had a clear effect on the plant yield and quality, with best results in acidic conditions. However, the PSB inoculation seemed not to have a clear effect across the two growing cycles in the parameters studied in the plant and in the soil.

ÍNDICE

1. INTRODUCCIÓN

1. Introducción

1.1. Fósforo como nutriente esencial vegetal

El fósforo es un elemento que resulta fundamental para todos los seres vivos pues es necesario para formar importantes moléculas implicadas en procesos celulares. Es el caso de los ácidos nucleicos ADN y ARN que almacenan la información genética de las células, los fosfolípidos que constituyen las membranas celulares y participan en rutas de transducción de señales, y el adenosín trifosfato (ATP), encargado de almacenar la energía generada en la fotosíntesis y respiración (Karunanithi et al., 2015). En el caso de las plantas, forma el fitato, ácido orgánico que actúa como fuente de P durante la germinación de las semillas (Barker y Pilbeam, 2015).

A la par que esencial para los seres vivos y, concretamente, las plantas, las reservas de fósforo son limitadas. El fósforo para fertilización procede en su totalidad de reservas minerales de este elemento. Adicionalmente, las reservas de este mineral en territorio europeo son muy reducidas, lo que hace a Europa dependiente de las importaciones.

1.1.1. Biogeoquímica del P

En lo que respecta a la reserva rocosa de P, en torno al 90–95% de P está almacenado en forma de apatita $Ca_5(PO_4)_3X$, (X = OH, F, Cl), principalmente fluorapatita (Geng et al., 2022). El fósforo entra en los ecosistemas a través de la erosión de dichos minerales (A. Pradhan et al., 2017). Otro aporte de P al suelo procede de la acción de las enzimas de tipo fosfatasa secretadas por las raíces de las plantas y micorrizas arbusculares asociadas sobre la materia orgánica del suelo (A. Pradhan et al., 2017), liberando fosfato (PO_4^{3-}) que es directamente absorbido por las raíces sin que llegue a interaccionar con la fase mineral del suelo. Estas enzimas son inducidas por bajos niveles de fosfato en el suelo y son dependientes de altos niveles de N, necesario para su síntesis.

El ciclo del P, a diferencia del de otros elementos como C y N, presenta una fase gaseosa poco relevante con un volumen de emisiones de fosfina (PH_3) poco significativo. Así, el ciclo natural del P implica la erosión de los minerales de P en suelos, de donde es tomado por microorganismos y plantas y por consiguiente por animales que se alimentan de dichas plantas. El P retorna a los suelos a través de las excreciones de los animales y la descomposición de plantas y animales. A lo largo del tiempo se repite cíclicamente este proceso. Parte del P puede perderse hacia las corrientes de agua llegando eventualmente al mar donde después de largos periodos de tiempo se reincorpora a los depósitos de roca sedimentaria. Sin embargo, hay que tener en cuenta el impacto sobre este ciclo de actividades humanas como la deforestación y la consecuente erosión de los suelos, la extracción extensiva de roca fosfórica para producción de fertilizantes o la deposición de excrementos animales en el medio ambiente (Jupp et al., 2021). En global, solo el 15% del P extraído de las minas y empleado en la producción de alimentos es consumido por los humanos mientras que el resto se pierde hacia el medio ambiente (Karunanithi et al., 2015).

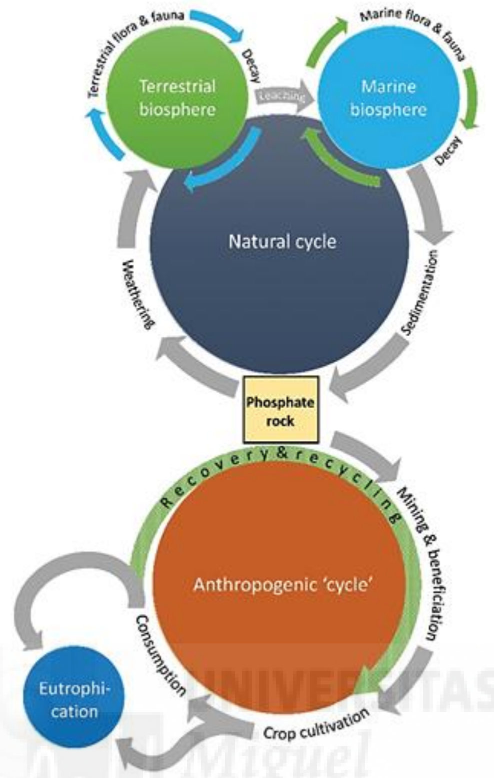

Figura 1. Ciclos natural y antropogénico de P (Jupp et al., 2021).

1.1.2. Rol del P en el sistema suelo-planta

El fósforo en las plantas, así como en el resto de seres vivos, cumple una importante función de acúmulo y transferencia de energía mediante su rol central en el proceso de fotofosforilación. Además de esta función, también ejerce un papel importante en la construcción de biomoléculas como fosfolípidos, ácidos nucleicos, coenzimas y fosfoproteínas. El ácido fítico, el éster hexafosfato de mioinositol fosfato, es la reserva de fósforo más común en las semillas (Barker y Pilbeam, 2015).

En cuanto a los efectos del P en distintas partes de la planta, a nivel de la raíz, este nutriente estimula el desarrollo radicular, la formación de pelos radiculares, y el aumento de la superficie de absorción de agua y nutrientes. El P también regula la absorción de otros nutrientes y la degradación de compuestos orgánicos y aumenta la tolerancia a condiciones de estrés. A nivel del tallo, el P también está implicado en su crecimiento, así como en la activación de enzimas fotosintéticas, transferencia de energía a nivel celular siendo componente del adenosín difosfato (ADP) y adenosín trifosfato (ATP). En las hojas, el P está involucrado en la regulación de la apertura y cierre estomatal y en la regulación del balance hídrico y absorción de nutrientes. También interviene en la formación de cloroplastos e incrementa la eficiencia de la fotosíntesis, y aumenta la producción de carbohidratos necesarios para el crecimiento de la planta (Khan et al., 2023).

Las plantas absorben P en forma de ortofosfatos inorgánicos ($H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-}). En suelos ácidos, $H_2PO_4^-$ es la forma dominante; en suelos de pH neutro a alcalino, HPO_4^{2-}, y en suelos alcalinos, PO_4^{3-} (Tisdale et al.,1990). La absorción de HPO_4^{2-} parece ser más lenta que la de $H_2PO_4^-$ (Pradhan et al., 2017). El 60% del fósforo orgánico que hay en el suelo se encuentra en forma de inositol polifosfato y se sugiere que es un componente importante de la fracción húmica de los suelos. Los microorganismos del suelo se encargan de transformar los polifosfatos en ortofosfato inorgánico disponible para las plantas, mediante la secreción de enzimas fosfatasas. El fósforo inorgánico que entra a formar parte de la solución del suelo mediante mineralización o el aporte de fertilizantes, rápidamente se convierte en formas menos disponibles mediante reacciones de adsorción o precipitación (Barker y Pilbeam, 2015).

Así, las reacciones de transformación del fósforo en los suelos se dividen en bióticas: mineralización, inmovilización y absorción por las plantas, y abióticas: lixiviación, adsorción y desorción, meteorización y precipitación. En el proceso de mineralización, los microorganismos degradan la materia orgánica liberando P inorgánico disponible para las plantas. La inmovilización es el proceso contrario, en el que el P inorgánico del suelo es empleado por los microorganismos para favorecer su crecimiento pasando a estar no disponible. El proceso de inmovilización biológica no es tan importante como la inmovilización química o adsorción. El P lábil es adsorbido por Ca en suelos calcáreos y Fe, Al y silicatos en suelos ácidos, mayoritariamente (Devau et al., 2009). Los óxidos hidratados y óxidos de hierro y aluminio generalmente se encuentran como cubiertas en superficies minerales arcillosas. En los suelos calcáreos los óxidos hidratados también intervienen en la adsorción de P, aunque es más importante la adsorción a carbonatos cálcicos (Barker y Pilbeam, 2015).

El carbonato cálcico ($CaCO_3$) puede influir en el ciclo del fósforo del suelo en tres niveles diferentes. A nivel elemental, el Ca^{2+} y el Mg^{2+} disueltos de los minerales carbonatados pueden provocar la precipitación de fosfatos. A nivel superficial, los iones de fosfato tienen una gran capacidad para ocupar sitios con carga positiva o desplazar a otros aniones debido a la unión electrostática. A nivel medioambiental, el $CaCO_3$ regula el pH del suelo hacia valores más alcalinos, lo que favorece la precipitación de fosfatos.

La adsorción del P lábil a óxidos hidratados, carbonatos y coloides arcillosos está regulada por pH, mineralogía, contenido de materia orgánica, temperatura, porcentaje de arcilla y limo, superficie y cantidad de Fe y Al en el suelo (Alovisi et al., 2020). Un mayor contenido de materia orgánica reduce la adsorción de P. Existe un equilibrio adsorción-desorción. La adición de enmiendas orgánicas y la alcalinización del pH del suelo aumentan la desorción de P (Karunanithi et al., 2015). Cuando aumenta la concentración de P en solución, se produce su precipitación. La tasa de precipitación es proporcional a las cantidades de fosfato soluble por encima del nivel de equilibrio y a la cantidad de Al y Fe disponibles para fijar P.

Cuando el fosfato forma fuertes asociaciones con óxidos metálicos se reduce su eficiencia de recuperación y aumenta el riesgo de agotamiento del fósforo a largo plazo. En estos sistemas, el fósforo se encuentra predominantemente ligado a la materia orgánica y debe sufrir mineralización a través de fosfatasas microbianas para liberar P_i biodisponible para su absorción por las plantas. Este proceso de mineralización depende del carbono y requiere la inversión microbiana de nitrógeno para la síntesis de enzimas fosfatasa, lo que establece mecanismos de retroalimentación entre los ciclos del nitrógeno y el fósforo (Hayat et al., 2025).

En el suelo, el P está tanto en forma inorgánica como orgánica. En su forma orgánica, se encuentra covalentemente unido a un átomo de carbono, bien directamente o mediante enlace fosfodiéster. El P orgánico representa el 30-50% del P total en la mayor parte de suelos (Pradhan et al., 2017). El P orgánico del suelo incluye biomoléculas de P procedentes de plantas y microorganismos como ácidos nucleicos, fosfolípidos, ácidos fíticos, fosfonatos, polifosfonatos y azúcares fosfato (Aliyat et al., 2024). Sin embargo, todos estos compuestos son rápidamente degradados en el suelo. Los inositol-fosfatos representan el porcentaje mayoritario (42-67%) de P orgánico (Fuentes et al., 2008).

En cuanto al fósforo inorgánico, se encuentran los aniones ortofosfato en la solución del suelo, aunque el P también puede encontrarse formando sales minerales o incluido en la matriz orgánica del suelo (Silva et al., 2023). La concentración de P inorgánico en los suelos varían desde 684 μg g^{-1} suelo en Entisoles a 200-430 μg g^{-1} suelo en Ultisoles y Oxisoles (Tian et al., 2021). Así, aproximadamente un 0,1% del P total presente en el suelo está disponible para su asimilación por las plantas (Li et al., 2020).

1.1.3. Tipos de fertilizantes fosfóricos

Tradicionalmente, los residuos domésticos y derivados de la actividad ganadera se reaplicaban a los campos con el consecuente reciclaje de nutrientes, favoreciendo un sistema agrícola sostenible. Con el aumento de la demanda de alimentos propiciado por el crecimiento poblacional en la década de los 70, este tipo de abonos se reemplazaron por los fertilizantes químicos. Este fenómeno se denominó “Revolución verde” y cambió el modelo de agricultura hacia las prácticas intensivas que se han extendido hasta la actualidad. De acuerdo a Chen et al. (2022), el consumo global de fertilizantes aumentó 34,5 millones de toneladas (Mt) de 1961 a 2019 (según datos estadísticos del IFASTAT). El fosfato diamónico (DAP) y fosfato monoamónico (MAP) son los fertilizantes inorgánicos más comunes, junto con el superfosfato simple (SSP) y el superfosfato triple (TSP). Los NPK, o fertilizantes compuestos, que contienen nitrógeno, fósforo y potasio, también se utilizan ampliamente (Bhattacharya, 2019; de Boer et al., 2018).

La mayor parte del fósforo empleado en la producción de fertilizantes procede de reservas de roca fosfórica. Existen tres principales reservas de roca fosfórica, siendo la más común la fosforita marina, roca sedimentaria que se origina en los fondos marinos. Este tipo de roca tiene principalmente apatita en su composición. Además existen rocas ígneas y metamórficas, aunque la explotación de estas últimas no es económicamente viable (Ohtake y Tsuneda, 2018).

La roca fosfórica se procesa para obtener fertilizantes inorgánicos basados en ortofosfato como fosfato monoamónico (MAP), fosfato diamónico (DAP) y superfosfato triple (TSP) (Chtouki et al., 2024). En la actualidad, existe una creciente preocupación debido a la concentración de las reservas de roca fosfórica. Cinco países poseen el 83 % de las reservas mundiales: Marruecos (68 %), China (5 %), Egipto (4 %), Argelia (3 %) y Túnez (3 %). En 2023, cuatro países dominaban su extracción: China (41 %), Marruecos (16 %), Estados Unidos (9 %) y Rusia (7 %) (Brownlie et al., 2024). Las estimaciones indican que, al ritmo actual de extracción, China, Estados Unidos y Rusia agotarán sus reservas en un plazo de 40 años (Blackwell et al., 2019), lo que concentrará aún más el mercado.

Los fertilizantes minerales tienen una alta concentración de P altamente soluble en agua, por lo que consiguen una rápida disolución de P en el suelo. Este aspecto fue el responsable de su popularidad y auge, debido a su rentabilidad económica. Sin embargo, y debido a los procesos de inmovilización desarrollados anteriormente, la eficiencia de los fertilizantes minerales es de tan sólo el 30% (Aliyat et al., 2024), mientras el resto del P queda retenido en el suelo e inaccesible a las plantas (Weeks y Hettiarachchi, 2019). Tras aplicaciones sostenidas en el tiempo, esto condiciona la biodisponibilidad del P en aplicaciones sucesivas de estos fertilizantes y puede incluso provocar problemas medioambientales como la eutrofización de las aguas si se producen fenómenos de lixiviación (Carpenter, 2008). Otra problemática asociada al uso de fertilizantes minerales es su contenido en cadmio (Cd) que, tras aplicaciones sucesivas, puede terminar acumulándose en los suelos y eventualmente en los cultivos, que son en última instancia consumidos por los humanos. El cadmio es un conocido carcinógeno humano, y se ha estimado que el 55% del total de consumo de Cd en la dieta proviene precisamente de esta vía (Gómez-Gallego et al., 2025). Así, el Reglamento EU 2019/1009 sobre Fertilizantes de la Unión Europea establece un límite de Cd en fertilizantes de P inorgánicos y órgano-minerales de 60 mg Cd kg^{-1} P_2O_5 (EU, 2019). Por otra parte, la roca fosfórica es una de las pocas fuentes de P junto con compost admitida en prácticas de agricultura ecológica. Sin embargo, su aplicación directa presenta limitaciones debido a su baja reactividad limitando la cantidad de P que llega a estar disponible para las plantas tras su aplicación (Bustamante et al., 2016).

En vista de todos los factores descritos anteriormente, resulta crucial el desarrollo de fertilizantes de P alternativos a los fertilizantes minerales y la roca fosfórica, que sean más sostenibles a nivel medioambiental y agronómicamente más eficientes. Actualmente se está investigando el uso de biofertilizantes, descritos por Wester-Larsen et al (2022) como «materiales o productos derivados de biomateriales (de origen vegetal, animal o microbiano, a menudo residuos, desechos o subproductos de la agricultura, la industria o la sociedad) con un contenido de nutrientes vegetales biodisponibles adecuado para servir como fertilizante para cultivos».

1.1.4. Flujos residuales orgánicos como fuente alternativa de P

La aplicación agrícola de compost, lodos estabilizados, estiércoles, digeridos procedentes de digestión anaerobia o cenizas de lodo son estrategias extendidas para mejorar la fertilidad de los suelos que están siendo limitadas a causa de la preocupación por las pérdidas de P y los riesgos de contaminación por elementos traza tóxicos, patógenos y/o xenobióticos (Günther et al., 2018). Una buena parte de los lodos urbanos son incinerados, resolviendo el problema de su higienización y reducción de volumen. Una alternativa que se presenta y que se desarrollará en capítulos posteriores es la recuperación de P a partir de residuos orgánicos por procesos de precipitación-cristalización mediante adición de compuestos de Mg o Ca, obteniendo estruvita y fosfatos cálcicos (brushita, fosfato octacálcico y, principalmente, hidroxiapatita), respectivamente. De acuerdo a las estimaciones, en 2030 en torno al 60% de sales de fosfato recuperadas derivarán de aguas residuales urbanas y el 39%, de estiércoles (Witek-Krowiak et al., 2022).

Por otra parte, la harina de carne y de hueso, subproductos de la industria cárnica, se solían emplear en alimentación animal pero a consecuencia de los brotes de la enfermedad de las vacas locas (Yamamoto et al., 2006), este uso quedó prohibido en la Unión Europea por la Regulación 1774/2002 de subproductos de origen animal. En el territorio de la UE se generan 18 millones de toneladas de residuos cárnicos anualmente que se procesan mayoritariamente en forma de harinas de carne y de hueso hasta producir 4,5 Mt/año (Gil et al., 2025). Es su alto contenido en materia orgánica, nitrógeno y fósforo lo que hace a este residuo un buen candidato para su uso como fertilizante. Además, tiene un alto contenido proteico lo que le confiere valor añadido debido al papel bioestimulante y promotor del crecimiento de los aminoácidos. A modo de ejemplo, en la figura 2 se muestran algunas de las principales fuentes residuales de P y el contenido en P que presentan.

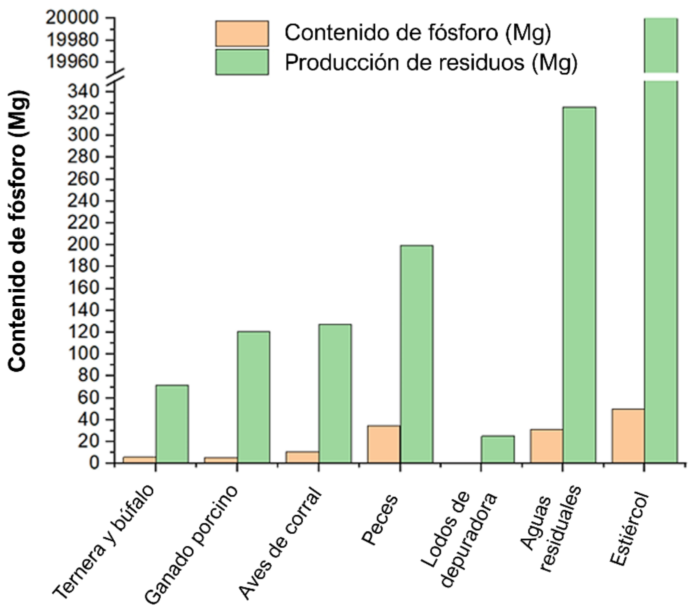

Figura 2. Fuentes residuales de P y la cantidad de P (en mega gramos, Mg) que contienen. Adaptado de Witek-Krowiak et al. (2022).

1.1.5. Procesos de recuperación de fósforo

En los países de la Unión Europea, los residuos orgánicos de origen animal (purín, estiércol y harinas de carne y de hueso) y los residuos municipales (lodos de depuradora) son los que se generan en mayor cantidad (Union, 2017). De acuerdo a las estimaciones de Recena et al. (2022), respecto a la demanda de P en suelos agrícolas del territorio europeo, dicha demanda podría ser satisfecha a partir del P reciclado de estiércoles, aguas residuales y residuos municipales. En este sentido, existen diferentes tipos de tecnologías o métodos de tratamiento aplicados a los diferentes residuos que permiten una potencial recuperación del P.

Con respecto a la potencial recuperación de P a partir de las aguas residuales de origen urbano y los residuos derivados de su tratamiento en las plantas depuradoras, existen una serie de tecnologías que se aplican en las plantas de tratamiento a gran escala, que incluyen procesos fisicoquímicos o biológicos. La incineración es uno de los métodos utilizados para la gestión de los lodos (Pasalari et al., 2024), con el que se consigue la reducción de volumen e higienización de los mismos, pudiendo obtener biochar como producto final. Este método tiene un alto potencial de recuperación de P, pudiendo alcanzar rendimientos del 85-95% (Ohtake y Tsuneda, 2018). A partir de las cenizas de los lodos obtenidas tras su incineración también es posible llevar a cabo la recuperación de P. Sin embargo, este procedimiento exige unas condiciones (reactivos ácidos para solubilizar el P e instalaciones más costosas), que encarecen los costes del proceso, lo que hace que esta tecnología todavía se encuentre en fase piloto (Hayat et al., 2025).

La precipitación de estruvita ($MgNH_4PO_4 \cdot 6H_2O$) o hidroxiapatita ($Ca_5(PO_4)_3(OH)$), que se desarrollará en el siguiente apartado, son de las formas más frecuentes de recuperación de P a partir de aguas residuales de origen urbano y/o lodos de depuradora (Pasalari et al., 2024). Además de los métodos químicos, existen los biológicos como la eliminación biológica mejorada de fósforo o EBPR por sus siglas en inglés (enhanced biological phosphorus removal) en el que intervienen un tipo de microorganismos, los organismos acumuladores de polifosfatos (PAOs), los cuales trabajan en condiciones aerobias. Bajo condiciones anaerobias, los microorganismos liberan el P acumulado en forma de ortofosfatos. Este procedimiento resulta interesante para la concentración de P en los efluentes, lo cual rinde hasta un 40% del P entrante en la planta de tratamiento de aguas residuales urbanas (Jupp et al., 2021).

Otras tecnologías para la recuperación de P en este tipo de residuos incluyen la carbonización hidrotérmica (HTC, por sus siglas en inglés), consistente en un proceso termoquímico en el que se aplican temperaturas entre 180 y 250ºC y presiones entre 2 y 10 MPa, del que se obtiene hidrochar y una fracción líquida con una alta carga orgánica (Suárez-Iglesias et al., 2017), o el uso de resinas de intercambio aniónico y sorbentes como biochar y zeolita por su alta capacidad de adsorción, aunque su aplicabilidad es limitada debido a la competición por parte de otras especies iónicas presentes en los lodos así como los altos costes de estos materiales (Hayat et al., 2025). Actualmente su aplicación se limita al tratamientos de las aguas residuales más que a la recuperación de P para un posterior uso.

1.1.6. Obtención de minerales de P: estruvita

La estruvita ($MgNH_4PO_4 \cdot 6H_2O$) es un cristal formado de concentraciones equimolares de magnesio, amonio y fosfato. Su peso molecular es 245,43 g mol^{-1}. El valor de solubilidad es de 0,018 g/100 ml a 25 °C en agua, 0,033 g/100 ml a 25 °C en HCl 0,001 N y 0,178 g/100 ml a 25 °C en HCl 0,01 N (Le Corre et al., 2009). La cristalización de este mineral en las plantas de tratamiento de aguas residuales ayudaría a reducir costes de mantenimiento y aumentar la eficiencia operativa de la planta al reducir la ocurrencia de acúmulos de estruvita que precipita espontáneamente durante el proceso de depuración, bloqueando las tuberías (Petzet y Cornel, 2012).

El proceso de cristalización de la estruvita se ha estudiado fundamentalmente en aguas residuales, siendo los factores críticos en el proceso los siguientes: pH, temperatura, grado de sobresaturación, energía de mezcla, ratio molar Mg/P y N/P, ratio molar Ca/P y tiempo de retención hidráulica (TRH) (Krishnamoorthy et al., 2021). El rango de pH óptimo para promover la cristalización de la estruvita es 8-9, aunque dependiendo del tipo de agua residual el valor óptimo puede variar de 7,5 a 10,5 (Yan et al., 2025). En general, es necesario ajustar el pH mediante adición de NaOH o “stripping” de CO_2 (B. Li et al., 2019). Es importante monitorizar el pH durante el proceso ya que un aumento de pH por encima de los valores óptimos puede provocar el desajuste de los ratios molares debido a la conversión de NH_4^+ en NH_3 y el aumento de la concentración de ion fosfato PO_4^{3-}.

Para la formación de la estruvita se requiere un ratio molar Mg^{2+}:P-PO_4^{3-}:N-NH_4^+ 1:1:1, pero el Mg^{2+} debe estar en exceso en solución para minimizar la reacción de los iones fosfato con iones competitivos como Ca^{2+}. El ratio óptimo Mg:P descrito en la bibliografía es de 1,2:1 (Li et al., 2020) o 1,5:1 (Wang et al., 2018; Yan et al., 2023). Para conseguir este exceso, por lo general es necesario adicionar Mg externamente, siendo las sales de Mg más habitualmente utilizadas sulfato de magnesio, óxido de magnesio y cloruro de magnesio (Krishnamoorthy et al., 2021). Sin embargo, la necesidad de dosificación de estas sales encarece el escalado del proceso. Por ello, se está investigando el uso de fuentes alternativas ricas en Mg como el subproducto derivado de la producción de cloruro sódico “bittern”(Sanghavi et al., 2020), el residuo en polvo de magnesita derivado de la producción de óxido de magnesio (Al-Mallahi et al., 2020) o la salmuera resultante de la desalinización de agua marina (Kim et al., 2016). Todas estas fuentes son a su vez residuos de procesos industriales por lo que su uso como fuente de Mg en la precipitación de estruvita contribuiría a su gestión y, así, a impulsar la economía circular. Asimismo, el mantenimiento del ratio molar N/P óptimo es crucial para la cristalización de estruvita (Mayer et al., 2016).

Por otra parte, el calcio, es el inhibidor más importante de la cristalización de la estruvita (Hakimi et al., 2020). El calcio reduce la eficacia de la eliminación del ortofosfato-P y disminuye el rendimiento de estruvita obtenido. La presencia de Ca^{2+} favorece la formación de precipitados alternativos como el fosfato cálcico amorfo (Ca_xPO_{4y}), la hidroxiapatita ($Ca_5(PO_4)_3(OH)$), la brushita ($CaHPO_4 \cdot 2H_2O$), el carbonato cálcico ($CaCO_3$) y el fosfato dicálcico hidroxílico ($Ca_2(HPO_4)(OH)_2$). Las relaciones molares de Ca^{2+}:PO_4^{3-} en torno a 0,25 (Huang y Liu, 2014) y de Ca^{2+}:Mg^{2+} superiores a 0,20 (Huchzermeier y Tao, 2012) inhiben la cristalización. Por otra parte, los contenidos de materia orgánica y metales pesados (Cu, Zn, Fe…) (Tarragó et al., 2018; Bai et al., 2023) también pueden interferir con la cristalización, que estará por tanto influenciada por la composición del material de origen (aguas residuales o residuos orgánicos sólidos, lodos de depuradora o estiércoles).

Diferentes tecnologías, tales como Ostara PEARL®, AirPrex®, PHOSPAQ®, ANPHOS®, NuReSys® y PhosphoGREEN inducen la precipitación de estruvita de flujos residuales ricos en nutrientes mediante adición de magnesio bajo condiciones alcalinas (Hayat et al., 2025) (Tabla 1).

Tabla 1. Características de las principales tecnologías de recuperación de P a partir de flujos de aguas residuales. Adaptado de Witek-Krowiak et al. (2022).

Tecnología	Tipo de lodo	Eficiencia recuperación (%)	Eficiencia recuperación P influente (%)	Tipo de reactor	Producto	Plantas operativas	Capacidad obtención de producto (kg día^{-1})
PHOSPAQ	Tipo I	10-15	80	Reactor agitado mixto	Estruvita	4	80-1200
ANPHOS	Tipo I	10-15 (municipal) 90% (industria de patata)	63-90	Reactor agitado mixto, en discontinuo	Estruvita	5	400-1800
NuReSys	Tipo I	10-15	85	Reactor agitado mixto, en continuo	Estruvita BioSTRU	10	55-2000
Ostara PEARL™	Tipo I	10-15	85	Reactor lecho fluidizado	Estruvita	22	325-6350
PhosphoGREEN (Suez)	Tipo I	10-15	80-90	Reactor lecho fluidizado	Estruvita PhosphoGREEN	3	230-270
AirPrex	Tipo II	10-50	80-90	Corriente de salida del digestor anaerobio, reactor cilíndrico	Estruvita Berliner Pflanze	13	500-4550

Tipo I - lodo líquido tras deshidratación de lodos de la unidad EBPR; Tipo II – lodo deshidratado.

La mayor parte de estas instalaciones se construyeron del año 2010 en adelante, siendo bastante recientes. La tecnología Pearl se aplica a 22 instalaciones en el mundo. Hay 10 instalaciones NuReSys® distribuidas por Bélgica, Holanda y Alemania. 13 instalaciones AirPrex® trabajan en Alemania, Holanda y China. El resto de instalaciones no alcanzan la decena de plantas. PHOSPAQ, ANPHOS y NuReSys emplean reactores agitados mixtos mientras Ostara PEARL y PhosphoGREEN emplean reactores de lecho fluidizado. En general, la eficiencia de recuperación P a partir del influente es del 80-90% (Witek-Krowiak et al., 2022) pero la recuperación del P total entrante en la planta de tratamiento de aguas residuales es del 10-20%. Para aumentar la eficiencia de recuperación de P hasta el 50% se propone la incorporación a la línea de fangos de reactores de hidrólisis térmica para el pretratamiento de los lodos. Este tipo de sistemas sólo son aplicables a plantas de tratamiento grandes (>150.000 habitantes equivalentes). En Europa, tan sólo se recuperan y comercializan ~15000 Mg año^{-1} de estruvita (CrystalGreen, BioSTRU, PhosphoGREEN y Berliner Pflanze) de 635000 Mg año^{-1} estimados de P recuperable. Estos productos se venden a precios de 188-763 euro/Mg

(Witek-Krowiak et al., 2022). Por otra parte, otro tipo de mineral de P recuperado que puede precipitarse en las plantas de tratamiento de aguas residuales es la vivianita ($Fe_3(PO_4)_2 \cdot 8H_2O$). Comparado con la estruvita, la precipitación de vivianita ofrece una serie de ventajas operativas, como un rango de pH de trabajo más amplio (pH 6-9), por lo que puede precipitar a pH neutro, sin necesidad de adicionar reactivos para aumentar el pH. Otra ventaja es el abaratamiento en la compra de reactivos ya que Fe(II) es más económico que las fuentes de Mg empleadas en la precipitación de estruvita como $MgCl_2$ o MgO (Y. Zhang et al., 2023). Adicionalmente, el hierro habitualmente se usa como coagulante en la depuración de aguas residuales y está naturalmente presente en los residuos urbanos. La vivianita es una buena opción para recuperar P de aguas residuales con contenido de Fe, en las que los lodos se tratan mediante digestión anaerobia (P.Wilfert et al., 2018). Así, se ha demostrado en diversas especies de cultivo que la estruvita es capaz de reemplazar a los fertilizantes convencionales en términos de absorción de P en un 98% y la vivianita, en un 75% (Faller et al., 2025).

Por otra parte, la recuperación de fósforo en forma de estruvita ha sido estudiada en diferentes residuos orgánicos con altas concentraciones de N y P, como residuos industriales, municipales y de origen animal (Kataki et al., 2016), tal como se muestra a modo de resumen en la Tabla 2, así como en aguas residuales de diferentes orígenes, aparte de las aguas residuales de origen urbano, como efluentes de industrias cárnicas (Ramaswamy et al., 2022), agroalimentarias (Turker et al., 2010) e incluso textiles (Huang et al., 2013), entre otros.

Tabla 2. Estudios sobre recuperación de fósforo en forma de estruvita a partir de fuentes orgánicas en los últimos 15 años.

Fuente orgánica	**Rendimiento recuperación P (%)**	**Referencia**
Residuos animales		
Estiércol porcino	74	(Su et al., 2025)
	100	(Calvo-de Diego et al., 2025)
Purín porcino	60-90	(Piveteau et al., 2017)
	No declarado	(Daumer et al., 2010)
Aguas residuales porcinas	97	(Ryu et al., 2010)
	96	(Huang, Xiao et al., 2011; Huang, Xu et al., 2011)
	99	(Zhang et al., 2012)
Estiércol de ganado vacuno	69	(Shen et al., 2011)
	≤ 82	(Zhao et al., 2010)
Residuos municipales		
Lodo de depuradora	No declarado	(Munir et al., 2019)
	89	(Ovsyannikova et al., 2021)
	60	(Cañas et al., 2023)
	93	(Xu et al., 2021)
Digerido anaerobio de FORSU	43	(Oliveira et al., 2018)
Otros residuos orgánicos		
Residuos textiles	≤ 94	(Huang et al., 2012)
Residuos alimentarios	No declarado	(Turker et al., 2010)
Residuos de matadero	85	(Ramaswamy et al., 2022)

FORSU: fracción orgánica de residuos sólidos urbanos.

Así, la recuperación de estruvita en residuos de origen animal se ha estudiado principalmente en estiércoles porcinos (Ryu and Li, 2010; Liu et al., 2011) y sus correspondientes digeridos obtenidos por digestión anaerobia (Nagarajan et al., 2023) así como en estiércoles vacunos (Zhao et al., 2010; Shen et al., 2011). Sin embargo, cabe mencionar que, pese al gran número de estudios relacionados con la obtención de estruvita a partir de aguas residuales de origen urbano, el número de estudios de obtención de este mineral a partir de lodos de depuradora es bastante más limitado, estando la mayor parte basados en procesos químicos como la oxidación húmeda (Munir et al., 2019; Ovsyannikova et al., 2021; Cañas et al., 2023).

1.1.7. Implementación de procesos microbiológicos de recuperación de P

En los procesos de recuperación de fósforo a partir de residuos orgánicos, el primer paso fundamental es la solubilización de los sólidos inorgánicos presentes en el flujo residual que se utiliza como fuente de fósforo, los cuales contienen la mayor parte del P del residuo, y para ello se suelen utilizar procesos de acidificación química o biológica (Piveteau et al., 2017).

A nivel químico, diferentes reactivos se han utilizado para disolver el fósforo contenido en los residuos, tales como ácido clorhídrico (Zhang et al., 2012), formato (sal procedente del ácido fórmico) (Daumer et al., 2010) y citrato (sal procedente del ácido cítrico) (Szögi et al., 2015). Sin embargo, el proceso de acidificación mediante reactivos químicos tiene un coste económico muy significativo, así como impactos asociados a nivel medioambiental (Daumer et al., 2010, Molinos-Senante et al., 2012; Egle et al., 2015).

En este contexto, la acidificación biológica, conocida también como bioacidificación, constituye una alternativa innovadora y medioambientalmente sostenible en la que el proceso de acidificación es llevado a cabo por microorganismos, presentes en el propio residuo o posteriormente añadidos (Nykänen et al., 2010; Regueiro et al., 2022), y normalmente basado en un metabolismo fermentativo en condiciones anaerobias (Bareha et al., 2022). Para favorecer e inducir el proceso, en diferentes estudios se contempla la incorporación de sustratos orgánicos fácilmente biodegradables, como azúcares (Bareha et al., 2022; Braak et al., 2016) o residuos agroalimentarios (Wu et al., 2020). Sin embargo, no hay disponible mucha información respecto a este tipo de procesos de acidificación a nivel biológico para la recuperación de fósforo a partir de residuos orgánicos, existiendo sólo algunos antecedentes de investigaciones en lodos de depuradora y purín porcino, que se detallan a continuación.

Piveteau et al. (2017) realizaron un estudio para la disolución de fósforo particulado en purín porcino mediante acidificación biológica como paso previo a su recuperación como estruvita. Para ello, utilizaron sacarosa como fuente de azúcares fermentables y la microbiota naturalmente presente en el purín, que suele presentar un contenido relativamente alto de bacterias acido-lácticas (Snell-Castro et al., 2005). El residuo se incubó en botellas de 1 L selladas, a una temperatura de 38ºC y con agitación continua a 300 rpm. Se testaron concentraciones de sacarosa de 10, 20, 30, 40, 50 y 60 g L^{-1} de purín. Los principales resultados obtenidos mostraron que la mayor parte del fósforo (60-90% del fósforo total) presente en el purín porcino podía ser solubilizado utilizando el proceso biológico sencillo conocido como acidogénesis. Así, una acidificación limitada a un pH por debajo del rango 5,5-6 utilizando sacarosa permitía la máxima solubilización del P, así como proporcionaba condiciones óptimas para su posterior recuperación como estruvita.

Moyo et al. (2022) estudiaron el proceso de recuperación de P a partir de diversas concentraciones de purín de cerdo y utilizando restos de cáscara de plátano como co-sustrato orgánico para el aporte de los azúcares fermentables y de los microorganismos fermentadores. La fermentación se llevó a cabo en recipientes de 1 L a 37ºC y con agitación manual cada 12 horas. Los resultados mostraron una solubilización del 80% del fósforo total del residuo, aunque las sustancias como la lignina, presentes en el residuo de cáscara de plátano, podrían haber dificultado la transferencia de masa del sólido a la fase líquida, lo que se pudo traducir en menores porcentajes de solubilización.

Sobre la base de estudios sobre biolixiviación de roca de fosfato usando microorganismos acidogénicos (Bhatti y Yawar, 2010; Calle-Castañeda et al., 2018; Priha et al., 2014), plantearon su estudio Pradhan et al. (2021). El estudio se basó en la incubación de lodo de depuradora con dos bacterias acidófilas: *Acidithiobacillus ferrooxidans*, oxidante de hierro y azufre, y *Acidithiobacillus thiooxidans*, oxidante de azufre. Ambas tienen actividad productora de ácido sulfúrico, mayor en el caso de *A. thiooxidans.* Para realizar el ensayo, utilizaron botellas de cristal de 250 mL conteniendo 90 mL de inóculo y 10 mL de lodo. La incubación se llevó a cabo a 30ºC, con agitación a 150 rpm y aireación, durante 63 días, añadiendo 10 g/L de azufre elemental (S) en el día 42. Durante el proceso, se estudió el efecto de cultivos puros de cada una de las bacterias, así como de un cultivo mixto de ambas. Los resultados obtenidos mostraron que tras la adición de azufre elemental en la fase 3, *A. thiooxidans* redujo el pH más que *A. ferrooxidans* o un cultivo mixto de *A. ferrooxidans* y *A. thiooxidans,* observando el mayor grado de solubilización de P (92%) a pH=0,9 en el lodo tratado con *A. thiooxidans*.

1.2. Uso de P en el marco de la economía circular y la agricultura sostenible

1.2.1. Calidad del suelo y fertilidad orgánica

Un factor importante en los ecosistemas mediterráneos es la modificación de los mismos para el desarrollo de campos de cultivo (Khanghahi et al., 2019). En el contexto actual de cambio climático, al incremento de la aridez se suma la creciente frecuencia de eventos climatológicos extremos como inundaciones, sequía e incendios forestales (Peñuelas et al., 2018). En este sentido, nitrógeno y fósforo son nutrientes limitantes en los suelos mediterráneos, estando los fosfatos inmovilizados en buena medida por los iones Ca^{2+}.

Por otra parte, los bajos niveles de humedad en el suelo, especialmente en temporada estival, aunque cada vez más durante buena parte del año, afecta a la concentración de las enzimas extracelulares como β-glucosidasas, ureasas o fosfatasas, la cual disminuye y, consecuentemente, también lo hace la biodisponibilidad de los nutrientes del suelo. Adicionalmente, también disminuye la tasa de respiración de los suelos (Sardans y Peñuelas, 2013). Así, el nivel de humedad de los suelos influye en la difusión de P y, por tanto, en su disponibilidad. Los suelos arenosos por lo general tienen menos capacidad de retención hídrica que los arcillosos (Bhadoria et al., 1991).

Por otra parte, los niveles de materia orgánica de los suelos son importantes a la hora de frenar su erosión. Los suelos mediterráneos tienen un índice de humificación bajo-moderado (Sardans y Peñuelas, 2013). Además, el ascenso de las temperaturas máximas debido al impacto del cambio climático y la consecuente desertificación acelera la tasa de degradación de la materia orgánica, siendo la recuperación de la materia orgánica un proceso mucho más lento y costoso (Diacono y Montemurro, 2010).

Por tanto, con el objetivo de mejorar la fertilidad de los suelos mediterráneos, cobra importancia la modificación de las prácticas agrícolas intensivas que agravan la erosión de los suelos y de los fertilizantes minerales por fertilizantes alternativos que permitan aportar los nutrientes necesarios a los suelos mediterráneos y revertir los daños en el suelo y, en general, en el ecosistema.

1.2.2. Eficiencia y problemática vinculada al uso de fósforo

El fósforo está irregularmente distribuido en los suelos, con excesos de concentración en las áreas próximas a explotaciones ganaderas y zonas urbanas (Cordell et al., 2009). Además, tradicionalmente se ha llevado a cabo una sobrefertilización de los cultivos ligada a la aplicación de fertilizantes minerales en zonas con una alta actividad agrícola, que contrasta con déficits en otras regiones a nivel global en las que el acceso a estos fertilizantes es limitado por razones de distinta índole, entre ellas, económicas. Varios estudios han descrito el aumento de las fracciones de P lábil y moderadamente lábil (adsorbido a fosfatos de Al y Fe) tras años de sobrefertilización con fertilizantes minerales (Chen et al., 2022; Biassoni et al., 2023).

Debido a los procesos de inmovilización y fijación de P (desarrollados en el apartado 1.1.2.), la disponibilidad de P para las plantas en el corto plazo tras la aplicación de fertilizantes minerales es del 7,5-12,4% (Sica et al., 2025), hasta el 30% el primer año (Korkmaz et al., 2009). Este problema se agudiza en suelos calcáreos, como los suelos de la región mediterránea, en los que se produce la precipitación de fosfatos cálcicos, disminuyendo aún más la concentración de P en la solución del suelo (da Gama et al., 2021).

Por otra parte, procesos tales como la deforestación o las prácticas de manejo intensivo en la agricultura (labranza, uso de monocultivo…) afectan significativamente a la regulación del ciclo del P en los suelos al modificar las propiedades del suelo. Este tipo de procesos reducen los inputs de materia orgánica y biomasa microbiana en los suelos y aceleran su erosión (Iqbal et al., 2025). La reducción en el contenido de materia orgánica afecta directamente a la microbiota del suelo, lo que impacta negativamente al proceso de mineralización del fósforo, que es un aporte de este elemento crucial en los suelos. El tipo y la topografía de los suelos o el clima afectan al proceso de erosión

(Xiong et al., 2019), que puede ser importante en zonas sometidas a eventos de lluvias torrenciales, como es el caso de algunas regiones de la cuenca mediterránea española. El uso de fertilizantes minerales de fósforo para suplir esta deficiencia causa saturación de este elemento en los suelos, que igualmente pasa a estar no disponible.

Las soluciones para minimizar estos efectos incluyen prácticas agronómicas conservativas como el uso de cubiertas, el encalado o la aplicación localizada de los fertilizantes, junto a la aplicación de fertilizantes con una base orgánica como compost o estiércol y de inóculos microbianos. Los residuos orgánicos permiten que el aporte de fósforo (en su mayoría orgánico) al cultivo sea más lento y sostenido, minimizando el problema de fijación de P en el suelo y su lixiviación y problemas asociados de contaminación de las aguas. El aporte nutricional a los suelos tras aplicar residuos orgánicos contribuye a mejorar su estructura y aumentar su porosidad y permeabilidad (Iqbal et al., 2025), así como una mayor infiltración de agua reduce el riesgo de erosión y las pérdidas de P asociadas.

1.2.3. Uso de P en agricultura sostenible

Ejemplos de aplicación de fuentes alternativas de P

En los últimos años han proliferado los estudios de validación agronómica de fertilizantes de fósforo alternativos, muchos de los cuales presentan resultados interesantes que sitúan a estos fertilizantes como potenciales candidatos a limitar y eventualmente reemplazar el uso de fertilizantes minerales convencionales. En este sentido, incluso la normativa europea ya contempla su uso regulado en el sector agrícola, incluso en uno de los más exigentes, como es el de la agricultura ecológica, donde ‘la estruvita recuperada y sales de fosfato precipitadas’ se han aprobado para su uso en la producción ecológica en la Unión Europea a través de su inclusión en el anexo II de la Regulación 2021/1165 (Comisión Europea, 2021). Así, los diferentes estudios recientes que se detallan en este apartado muestran los principales resultados obtenidos respecto al uso de fertilizantes de fósforo alternativos sobre diferentes tipos de cultivos en diferentes condiciones (condiciones controladas en invernadero y en ensayos en campo) y periodos de tiempo (a corto y medio-largo plazo).

En un estudio en maceta con raigrás (*Lolium perenne L.)* como planta modelo y un suelo calcáreo degradado bajo condiciones controladas de producción (T=25ºC, humedad 70%, fotoperíodo 16h), García-Díaz et al. (2025) compararon los fertilizantes alternativos estruvita y harina de carne-hueso con roca fosfórica con el fertilizante mineral convencional fosfato monoamónico. Los resultados obtenidos mostraron que la estruvita incrementó significativamente el fósforo disponible en el suelo tras 30 días de ensayo, lo que se correlacionó con niveles significativamente superiores de fósforo en la biomasa vegetal. Los tres fertilizantes alternativos produjeron un rendimiento de planta significativamente mayor que el fertilizante convencional.

Por otra parte, Sharma et al. (2024), en un estudio en maceta utilizando como cultivos trigo y garbanzo, compararon los efectos del uso de estruvita respecto a fosfato monopotásico (KH_2PO_4). Los resultados mostraron que a dosis bajas y medias las plantas tratadas con ambos fertilizantes acumulaban concentraciones similares de fósforo, pero al aumentar la dosis, el fósforo acumulado era dos veces superior en las plantas fertilizadas con estruvita.

Meneghetti et al. (2025), en un estudio en condiciones controladas en invernadero con un cultivo de crisantemo, observaron que el suelo enmendado con el fertilizante órgano-mineral compuesto de cenizas de madera y un fertilizante mineral compuesto por una mezcla de superfosfato simple, cloruro de potasio y micronutrientes presentó una concentración significativamente superior de fósforo, así como de otros macronutrientes como K, Ca y Mg, que el suelo fertilizado únicamente con el fertilizante mineral.

En un estudio en campo, Frick et al. (2025) evaluaron 8 fertilizantes alternativos de fósforo, incluyendo estruvita, cenizas, harina de carne y hueso, y pellets a base de gallinaza, durante dos temporadas consecutivas en cinco parcelas distribuidas en el norte, centro y sur de Europa, con rotación de cereales (trigo, maíz o cebada) o girasol. Durante la segunda temporada de cultivo, se dividieron las parcelas en 2 subparcelas para estudiar el efecto residual, así como el efecto de una reaplicación de fertilizante. En el se concluyó que ninguno de los fertilizantes alternativos provocaba pérdidas de rendimiento o absorción de P respecto al fertilizante mineral de referencia utilizado, superfosfato triple.

Por otra parte, en otro ensayo de campo bianual en trigo, Bo et al. (2025) observaron que el uso combinado de un fertilizante orgánico y un fertilizante mineral incrementó la biomasa del cultivo y el rendimiento de grano significativamente, aumentando la eficiencia de uso de P en un 21,1% y mejorando las propiedades del suelo (materia orgánica y contenido en nutrientes), respecto a la aplicación de fertilizante mineral. Asimismo, Nogalska et al. (2024), en un estudio en maíz durante tres años no consecutivos, comparando diferentes dosis de aplicación de harina de carne y huesos respecto a un fertilizante inorgánico (NPK), concluyeron que el uso de harina de carne y hueso en la dosis más baja utilizada (1 Mg/ha) era capaz de cumplir los requerimientos de fósforo del cultivo de maíz seleccionado, con un efecto similar al del fertilizante inorgánico convencional utilizado.

Microbioma beneficioso en la nutrición fosfórica

El papel de los microorganismos presentes en el suelo es especialmente relevante en la biodisponibilidad del fósforo y, por tanto, en la nutrición fosfórica, ya que la liberación de ácidos orgánicos y enzimas al medio contribuyen a la degradación del P orgánico y a su solubilización para hacerlo disponible para la planta (Geng et al., 2022).

La rizosfera es la región con mayor concentración de microorganismos beneficiosos para el desarrollo de las plantas. Se trata de bacterias con actividades de fijación de N, solubilización de P, secuestro de Fe, síntesis de fitohormonas, control de microorganismos patógenos, etc. Las rizobacterias se pueden clasificar en extracelulares, localizadas en la rizosfera o en los espacios entre las células del córtex de la raíz, e intracelulares, localizadas en los nódulos radiculares. Los géneros *Agrobacterium*, *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Caulobacter*, *Chromobacterium*, *Erwinia*, *Flavobacterium*, *Micrococcus*, *Pseudomonas*, y *Serratia* son géneros de rizobacterias extracelulares (Pradhan et al., 2017). Las rizobacterias intracelulares corresponden a bacterias fijadoras de N como rizobios, entre otras.

Por otra parte, los hongos de los géneros *Aspergillus*, *Penicillium*, *Rhizopus*, *Fusarium* y *Sclerotium*, y las bacterias solubilizadoras de fósforo (BSF) de los géneros *Arthrobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Microbacterium*, *Pseudomonas*, *Rhizobium*, y *Rhodococcus* son usadas como inoculantes en los suelos para mejorar la disponibilidad de P y el rendimiento de las plantas (Bhattacharyya y Jha, 2012), siendo las bacterias de los géneros *Bacillus*, *Pseudomonas* y *Acinetobacter* las que se han estudiado de forma más amplia (Pang et al., 2024). En este sentido, las bacterias solubilizadoras de P representan hasta el 50% de la microbiota del suelo (Chen et al., 2006). Estas bacterias solubilizan el P fijado en los suelos mediante un mecanismo de acción consistente en la liberación de ácidos orgánicos como ácido glucónico, láctico, isovalérico, isobutírico, acético, glicólico, oxálico, malónico y succínico (Pradhan et al., 2017; Kaur et al., 2024). Los grupos carboxilo e hidroxilo de estos ácidos son capaces de quelar los cationes unidos al fósforo liberando así formas solubles de este elemento. Además, la secreción de estos ácidos también conlleva un descenso del pH del suelo, que resulta asimismo beneficiosa para la solubilización de P.

Así, la generación de ácido glucónico es el principal mecanismo de solubilización del fósforo mineral. Este ácido orgánico se obtiene a partir de glucosa por la acción de la enzima glucosa deshidrogenasa a través de una reacción de oxidación, que requiere asimismo del cofactor pirroloquinolina quinina, compuesto aromático derivado de los aminoácidos tirosina y ácido glutámico (Pradhan et al., 2017). Además, ciertas bacterias solubilizadoras de P también tienen una acción promotora del crecimiento vegetal por medio de la síntesis de hormonas como auxinas, citoquininas y giberelinas, particularmente ácido indolacético (IAA), derivado del metabolismo del triptófano. Adicionalemnte, algunos géneros, entre ellos *Bacillus* sp., son capaces de secretar compuestos antifúngicos, por lo que este tipo de bacterias también son relevantes en el control biológico frente a plagas (Aliyat et al., 2024).

También existen cepas de microorganismos solubilizadores de P (PSMs) adaptadas a ciertos estreses abióticos. Es el caso de diferentes cepas del género *Bacillus* como *Bacillus atrophaeus* GQJK17S8 y *Bacillus* YMX5, adaptadas a condiciones de alta salinidad, *Bacillus subtilis* TPB4, adaptado al calor, *Bacillus subtilis* IA6, resistente a condiciones de sequía o *Bacillus licheniformis* PSB3, resistente a concentraciones elevadas de metales pesados. Todas estas cepas confieren ventajas adaptativas a las plantas, además de contribuir a la adquisición de P (Pang et al., 2024).

Por otra parte, ciertos microorganismos secretan enzimas fosfatasas y fitasas que favorecen la mineralización del P orgánico del suelo. Las enzimas de tipo fosfatasa se dividen en fosfatasas ácidas y alcalinas. La abundancia de cada una de ellas está condicionada por el pH del suelo. Las fosfatasas alcalinas pueden inhibirse por altas concentraciones de fosfatos inorgánicos en el entorno (Pang et al., 2024). Así, se ha descrito la eficiencia de las bacterias solubilizadoras de fósforo de los géneros *Bacillus*, *Streptomyces* y *Pseudomonas* en acelerar la mineralización de P orgánico (Jilani et al., 2009). En este sentido, Shi et al. (2017) describieron un aumento del 35,1% de los niveles de P disponible en suelos inoculados con bacterias solubilizadoras de fósforo.

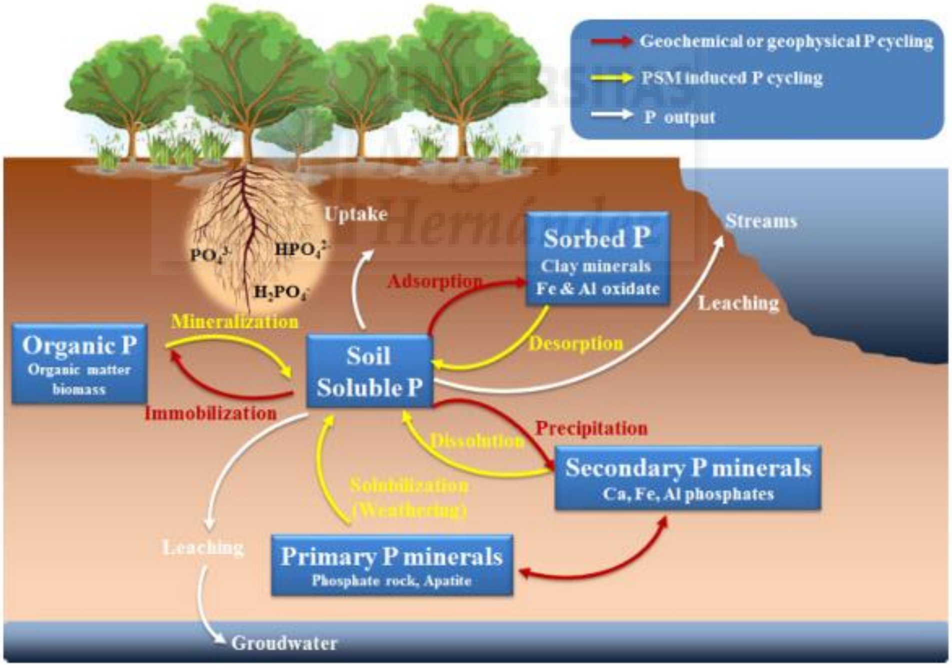

Figura 3. Acción de los microorganismos solubilizadores de P sobre el ciclo biogeoquímico del P. Fuente: Tian et al. (2021).

Por otra parte, diversos estudios han mostrado que la aplicación de microorganismos solubilizadores de P como biofertilizantes podría minimizar la aplicación de fertilizantes fosfóricos en un 50% sin pérdidas de rendimiento del cultivo significativas (Yazdani et al., 2009). Así, en un ensayo en maceta usando soja como planta modelo, Alam et al. (2023) estudiaron la aplicación de fertilizantes fosfóricos (fosfato diamónico) junto con el efecto de la inoculación de bacterias solubilizadoras de P

(PSB) del género *Enterobacter* sp. Tras la inoculación de PSB, se registró un aumento significativo en la concentración del fósforo disponible en el suelo, acompañado del aumento en la actividad de la enzima fosfatasa alcalina, efecto también descrito por Behera et al. (2017). Como consecuencia de un ensayo de fraccionamiento de P se observó que, mientras tras la aplicación del fertilizante se produjo un aumento en las fracciones Fe-P y Al-P, con la inoculación de PSB, estas fracciones junto a la fracción Ca-P disminuyeron significativamente, como resultado de la producción de ácidos orgánicos por parte de estas bacterias. Los autores concluyeron que la inoculación de PSB permitía solubilizar el P inmovilizado en el suelo. En última instancia esto se tradujo en el aumento del rendimiento de las plantas inoculadas con PSB respecto al de las plantas control. El mayor rendimiento correspondió a las plantas aplicadas con la dosis más alta de fertilizante e inoculadas con PSB (Alam et al., 2023).

Asimismo, Kaur et al. (2024) observaron que al inocular las bacterias *Enterobacter* sp. J49 y *Serratia* sp. S119 aisladas de la rizosfera de plantas de cacahuete a plantas de soja y maíz, aumentó su rendimiento, lo que muestra la utilidad de la inoculación de PSB en distintas especies vegetales para mejorar su eficiencia de uso de fósforo.

Por otra parte, en un estudio de comparación de un biofertilizante con inóculo de *Bacillus* sp. respecto a un fertilizante inorgánico convencional en un cultivo de maíz y uno de soja, Zhang et al., (2025) observaron que se incrementó el rendimiento del cultivo de maíz en un 16,07% y el del cultivo de soja en un 17,48%. Además, los suelos tratados con el biofertilizante presentaron niveles significativamente superiores de P disponible. Los investigadores también apuntaron a un aumento en la complejidad de la población microbiana y presencia de microorganismos beneficiosos en la rizosfera en comparación al fertilizante mineral. En este sentido, García-Díez et al. (2025) también observaron en un cultivo de raigrás cultivado en un suelo calcáreo degradado inoculado con *Pseudomonas* sp., *Bacillus* sp. y *Azospirillum* sp. y fertilizado con estruvita que, tras 30 días, las plantas mostraban valores de eficiencia de uso de P (PUE) mayores que el fertilizante convencional fosfato monoamónico.

2. OBJETIVOS

2. Objetivos

El objetivo principal de esta Tesis Doctoral fue el estudio de estrategias de fertilización fosfórica alternativas a los fertilizantes minerales convencionales, basadas, por un lado, en la recuperación de minerales de fósforo a partir de flujos residuales orgánicos mediante la implementación de un proceso de acidificación biológico, y por el otro, en el diseño y validación de fertilizantes órgano-minerales empleando las fuentes de fósforo recuperado a partir de residuos orgánicos. Para ello, se establecieron los siguientes objetivos específicos:

Objetivo 1. Validación agronómica en un sistema suelo-planta modelo de fertilizantes órgano-minerales basados en compost combinado con diferentes fuentes de fósforo, en comparación con fertilizantes inorgánicos convencionales. Estudio de los efectos inducidos por las diferentes fuentes de fósforo sobre las propiedades del suelo, el rendimiento del cultivo, la eficiencia de uso de nutrientes y el estado metabólico de la planta.

Objetivo 2. Implementación de un proceso biológico de extracción de fósforo a partir de residuos orgánicos (lodos de depuradora y fracción sólida de purín porcino) mediante fermentación láctica empleando la bacteria acidoláctica *Lactobacillus acidophilus*. Estudio del potencial de precipitación/cristalización de minerales de fósforo recuperado (estruvita).

Objetivo 3. Validación agronómica de fertilizantes órgano-minerales utilizando fuentes de fósforo recuperado, mediante su aplicación a un sistema suelo-planta modelo. Evaluación de la influencia de factores bióticos (inoculación de bacterias solubilizadoras de fósforo) y abióticos (pH del suelo) en el sistema suelo-planta y la biodisponibilidad de fósforo.

3. RESUMEN DE MATERIALES Y MÉTODOS

3. Resumen de materiales y métodos

En este apartado se desarrolla un resumen de los materiales y métodos utilizados en cada uno de los bloques experimentales que forman esta tesis y que se han publicado o enviado a las diferentes revistas mencionadas al inicio de esta memoria. Estas publicaciones, incluidas en los Anexos de esta Tesis Doctoral, presentan las tablas y figuras que se comentan en este apartado.

En el **primer bloque experimental** del trabajo se llevó a cabo la **validación agronómica de nuevos fertilizantes abióticos** de naturaleza órgano-mineral. Los resultados de este bloque experimental han sido publicados en la revista *Agronomy* bajo el título "*Looking for new P fertilizers: comparative study of mineral-, organomineral- and organic-based fertilizers for lettuce (Lactuca sativa L.)*".

Se establecieron dos grupos de tratamientos: simples y complejos u órgano-minerales (OMFs, *organomineral fertilizers*). Los tratamientos simples incluían tratamientos inorgánicos (roca fosfórica, fosfato monoamónico (MAP) y estruvita comercial) y un tratamiento orgánico (harina de hueso). Los tratamientos complejos incorporaban los tratamientos simples pelletizados con compost (alpechín:gallinaza:hojas de olivo 60:20:20%) al 50%. Las dosis de aplicación fueron 200 kg N ha^{-1} y 120 kg P ha^{-1}. El fertilizante convencional ComplexIN (15-15-15) se empleó como referencia a 3 dosis de aplicación: IN100 (100 kg NPK ha^{-1}), IN200 (200 kg NPK ha^{-1}) e IN300 (300 kg NPK ha^{-1}). Estos tratamientos se aplicaron a un sistema de suelo franco-arcillo-arenoso de pH 6,5 y lechuga (*Lactuca sativa* var. baby leaf) como planta modelo, bajo condiciones controladas de producción (T=21 ºC, humedad relativa del 60% y 12h de fotoperíodo). El diseño experimental fue aleatorizado (n=36) para 11 tratamientos por triplicado junto a un control sin fertilizar.

Las plantas se regaron al 60% de capacidad de campo y se midieron parámetros morfológicos de forma no destructiva cada 15 días hasta cosecha: factor de cobertura vegetal (fCOVER) mediante *Canopeo* app (Patrignani y Ochsner, 2015) y contenido en clorofila (CCC) mediante el medidor de clorofila portátil SPAD Minolta 502, (Konica Minolta Sensing, Inc., Sakai, Osaka Prefecture, Japón) (Casella et al., 2022). Tras cosechar las lechugas en estadío fenológico de 45 días, la parte aérea se cortó y pesó para determinar rendimiento en peso fresco, se secó a 60ºC, se anotó su peso seco y posteriormente se molieron. Una vez procesadas, se midió C y N totales en un microanalizador elemental EuroVector. Tras ser digeridas con HNO_3 67% en un digestor Mars 6, se determinó P total mediante colorimetría en un espectrofotómetro visible-UV, Na y K mediante fotómetro de llama y otros macronutrientes (Ca, S, Mg) y micronutrientes (Cu, Fe, Mn, Zn) mediante espectrometría de masas con plasma acoplado inductivamente (ICP-MS). Las clorofilas de las hojas se extrajeron en acetona 80% (Ritchie, 2008) y se determinaron espectrofotométricamente en un lector de placas

Thermoscientific Multiskan. También se llevó a cabo un estudio metabolómico no dirigido en el que se evaluaron 29 metabolitos entre aminoácidos, ácidos orgánicos, azúcares y otros metabolitos. Se realizó una extracción por el protocolo de Van der Sar et al. (2013)) y las muestras se procesaron mediante resonancia magnética nuclear (RMN) con un RMN Ascend 500 MHz AVANCE III HD H–NMR (Weinheim, Alemania).

Los suelos se extendieron para su secado al aire y una vez secos, se tamizaron mediante tamiz de 2 mm, tras lo cual se determinó pH y conductividad eléctrica (CE) utilizando un ratio 1:2,5 y 1:5 p/v, respectivamente; N total Kjeldahl (TN) (Paredes et al., 2015) y P disponible mediante el método Olsen-Watanabe (Pext) (Olsen et al., 1954), mientras que el carbono orgánico oxidable se evaluó de acuerdo al método descrito por Yeomans y Bremner (1988) para determinación del contenido de materia orgánica (OM).

En cuanto al análisis estadístico de los datos, se empleó el software R, versión 4.3.1, junto al software Infostat v.2020 asociado a R. Para evaluar diferencias entre parámetros del tejido vegetal y del suelo entre tratamientos, se llevó a cabo un análisis de la varianza (ANOVA) con nivel de significancia $p < 0.05$. Para confirmar la normalidad y la homocedasticidad de los datos se aplicaron los test de Shapiro-Wilks y Levene. Para comparaciones múltiples entre medias se empleó el test LSD de Fisher ($\alpha = 0{,}05$).

En el **segundo bloque experimental** de esta memoria se abordó la **recuperación de estruvita** a partir de residuos orgánicos mediante un proceso de extracción biológica de fósforo. Los resultados de este bloque experimental han sido publicados en la revista *Agronomy* bajo el título "*Sustainable phosphorus and protein recovery from different organic wastes: process optimization and struvite precipitation potential*".

En este estudio se utilizaron diversas muestras de residuos orgánicos: tres lodos de depuradora de aguas residuales urbanas (SS, por sus siglas en inglés) y la fracción sólida de un purín porcino (M). SS1 y SS2 procedían de estaciones depuradoras de aguas residuales con tratamiento de lodos activados, estabilizados posteriormente mediante digestión anaerobia, mientras que SS3 fue obtenido tras en una estación depuradora con tratamiento aerobio, seguido de estabilización en condiciones aerobias. Todas las muestras de lodo se deshidrataron mediante centrifugación. La fracción sólida de purín porcino (M) se obtuvo en una explotación porcina de engorde tras una separación sólido-líquido mediante una prensa de tornillo.

Para la caracterización de los residuos se determinaron diversos parámetros físico-químicos y químicos. El pH y la conductividad eléctrica (CE) se determinaron en un extracto acuoso 1:10 (p/v), mientras que los contenidos totales de C y N se determinaron utilizando un microanalizador elemental automático (EuroVector, Milán, Italia). El nitrato (NO_3^-) y el amonio (NH_4^+) se midieron en un extracto

1:5 (p/v) con KCl 0,2 M utilizando un analizador multiparamétrico K-365 Dist Line (BÜCHI Labortechnik AG). En el extracto ácido obtenido tras la digestión por microondas con HNO_3 al 69 % de los residuos, el P se evaluó colorimétricamente como ácido fosfórico molibdovanadato, el K y el Na se determinaron mediante fotometría de llama (fotómetro de llama Jenway PFP7, Jenway Ltd., Felsted, Dunmow, Essex, Reino Unido), mientras que el Ca, Mg, Fe, Mn, Cd, Cr, Cu, Pb, Zn, Ni y Hg se determinaron mediante espectroscopía de masas con plasma acoplado inductivamente (ICP-MS), siguiendo los métodos descritos por Morales et al. (2016).

Para la extracción del fósforo de los residuos orgánicos seleccionados mediante fermentación se empleó una versión modificada del procedimiento de Vanotti y Szogi (2018). Para ello, se llevó a cabo la acidificación biológica de los residuos orgánicos por medio de *Lactobacillus acidophilus*, una bacteria acido láctica Gram positiva ampliamente utilizada en la industria (Huang et al., 2021). La suspensión de *L. acidophilus* se preparó disolviendo 1,1 g de liófilo Lacto10, FORZA VITALE (cepa *L. acidophilus* SGL11) en 25 ml de agua desionizada, seguido de una centrifugación a 3600 rpm durante 10 minutos y redisolución del precipitado en 20 mL de agua desionizada. La cepa de *L. acidophilus* se utilizó en forma liofilizada a una dosis equivalente de 10^8 UFC/ml.

El procedimiento general para la extracción de fósforo que se realizó mediante fermentación en todos los ensayos fue el siguiente: se mezcló una solución precursora de ácido constituida por un peso optimizado de una fuente de azúcar y la suspensión de *L. acidophilus,* con el residuo orgánico correspondiente (2 g en base seca) en una proporción 10:1 (volumen:peso). La mezcla se incubó en un baño termostatizado a temperatura constante (37 ºC), en oscuridad y con agitación continua (frecuencia de agitación de 35 rpm) durante un tiempo de incubación optimizado, controlando la disminución del pH durante el proceso fermentativo. A continuación, la mezcla se centrifugó a 3600 rpm durante 30 minutos para obtener el sobrenadante ácido y un primer precipitado (precipitado ácido, pp1). Se realizó un lavado de este precipitado mediante la adición de un volumen equivalente (20 ml) de agua y se centrifugó en las mismas condiciones, obteniendo un sobrenadante y un segundo precipitado (pp2). A continuación, se determinó la concentración de fósforo en el sobrenadante ácido y en el sobrenadante resultante del lavado del precipitado, expresándose como la suma de ambas contribuciones.

Para el proceso de fermentación se optimizaron parámetros clave: tipo y cantidad de la fuente de azúcar y tiempo de incubación. Se evaluaron dos fuentes de azúcar diferentes (sacarosa y melaza) en diferentes cantidades (0,25, 0,5, 1, 2 y 3 g). Una vez establecidas la cantidad y fuente de azúcar óptimas, se probaron diferentes tiempos de incubación (0 h, 6 h, 12 h, 24 h, 36 h y 48 h).

Los sobrenadantes ácidos obtenidos se utilizaron para la precipitación de P como estruvita ($(NH_4)MgPO_4 \cdot 6H_2O$), según la metodología de Vanotti y Szogi (2018). En resumen, se añadió $MgCl_2$ en una dosis que mantuviera una relación molar Mg:P de 1,2:1. También fue necesaria la alcalinización con una solución de NaOH 4M para alcanzar un pH de 9. Los sobrenadantes ácidos recogidos de la fase de extracción de P se colocaron en un vaso de precipitados con agitación continua. Se añadió gota a gota la solución de NaOH 4M y se controló el pH durante todo el proceso. Los precipitados se separaron de la fase líquida utilizando filtros de microfibra y un sistema de vacío, y se secaron en un horno a una temperatura no superior a 60 ºC durante 24 h.

Para el estudio de la composición química de los precipitados, el contenido total de C y N se determinó utilizando un microanalizador elemental automático (EuroVector, Milán, Italia). En el extracto ácido obtenido tras la digestión por microondas con HNO3 al 69 %, el P se evaluó colorimétricamente como ácido fosfórico molibdovanadato; el K y el Na se determinaron mediante fotometría de llama; y el Ca, Mg, Fe, Mn, Cd, Cr, Cu, Pb, Zn, Ni y Hg se determinaron mediante espectroscopia de masas con plasma acoplado por inducción (ICP-MS), siguiendo los métodos descritos por Morales et al.

Para el estudio de la estructura morfológica de estos precipitados, estos se analizaron mediante difracción de rayos X (DRX) utilizando un equipo Bruker D8-Advance equipado con un espejo Goebel (muestras no planares), una cámara de alta temperatura (hasta 900ºC), un generador de rayos X KRISTALLOFLEX K 760-80F (potencia: 3000 W, voltaje: 20-60 KV, corriente: 5-80 mA) y un tubo RX con ánodo de cobre (Bruker Corporation, Billerica, MA, USA). Para determinar su composición elemental, se analizaron mediante un microscopio electrónico de barrido de emisión de campo (FESEM) Sigma 300 VP de tipo Schottky con un sistema de rayos X de dispersión de energía (EDX) acoplado (Carl Zeiss Microscopy GmbH, Oberkochen, Alemania). Las muestras se visualizaron bajo una alta tensión de electrones (EHT) de 15 kV y utilizando un detector de electrones secundarios retrodispersados (BSE). Todas las muestras se recubrieron con una capa de Cr de 10 nm. Los resultados obtenidos también se estudiaron mediante un análisis mineralógico racional (Coelho et al., 2002) para establecer las proporciones relativas de estruvita e hidroxiapatita en las muestras.

En el **tercer y último bloque experimental** se llevó a cabo la **validación agronómica de nuevos fertilizantes en condiciones bióticas y abióticas**, entre ellos los materiales de P recuperado obtenidos en el anterior bloque experimental. Los resultados de este bloque experimental se han desarrollado en la publicación científica con título "*Assessment of P availability in lettuce crop production using alternative P sources under different biotic and abiotic soil conditions*", enviada a la revista *Resources, Conservation and Recycling*.

Como parte de este bloque de trabajo, los materiales de P recuperado se utilizaron como ingredientes en fertilizantes órgano-minerales basados en un compost elaborado con alperujo, hojas de olivo y gallinaza (proporción 3:2:1 en volumen). Estos fertilizantes se utilizaron en un cultivo de lechuga (*Lactuca sativa* var. baby leaf) desarrollado mediante un ensayo en maceta bajo condiciones controladas de producción (T=21ºC, humedad 60%, fotoperíodo 12h) durante dos ciclos de cultivo. Se empleó un suelo calcáreo franco-arcillo-arenoso clasificado como Xerofluvent (Soil Survey Staff, 2014) obtenido de la capa superficial (0-25 cm) de la granja experimental de la Escuela Politécnica Superior de Orihuela (EPSO), cuyas principales características eran: conductividad eléctrica de 0,4 $dS \cdot m^{-1}$, 0,5% de carbono orgánico, 595 $mg \cdot kg^{-1}$ de N total Kjeldahl y 60,3 $mg \cdot kg^{-1}$ de P disponible. Junto a los materiales de P recuperado, se emplearon como ingredientes de los fertilizantes órgano-minerales basados en compost, fuentes de P inorgánicas (roca fosfórica, fosfato monoamónico, superfosfato triple) y recuperadas a partir de residuos orgánicos (harina de hueso y estruvita comercial), a una dosis normalizada de 80 kg $P \cdot ha^{-1}$. El N se suministró a todos los tratamientos en forma de urea a una dosis normalizada de 150 $kg \cdot ha^{-1}$. El diseño fue completamente aleatorizado de 6 réplicas por tratamiento, que se compararon con un tratamiento con el mismo compost de los fertilizantes órgano-minerales y un tratamiento control sin fertilizar.

Además del efecto de la fuente de P utilizada, se estudió la influencia de un factor abiótico, el pH del suelo, y uno biótico, el efecto de la inoculación de bacterias solubilizadoras de P, sobre la disponibilidad de P. Para ello, se incluyó suelo de dos pH en el estudio: suelo franco-arcillo-arenoso de pH 8,5 y el mismo suelo con pH 6,5 modificado tras la adición de $FeSO_4$ al 4%. Se emplearon dos especies de bacterias solubilizadoras de P: *Bacillus aerophilus* (BM161) y *Pseudomonas putida* (BIO175), ambas aisladas a partir de muestras de compost y cedidas por el grupo de investigación BIO-175 de la Universidad de Almería. Las bacterias se recuperaron a partir de crioviales preservados a -80ºC creciéndolas en placa con medio APHA. Posteriormente, se dejaron crecer durante 24h a 30ºC en caldo de cultivo (Panreac) y se inocularon al suelo una semana tras la fecha de plantación en cada ciclo de cultivo, a una concentración equivalente de 10^7 $UFC \cdot g^{-1}$ de suelo a través del agua de riego.

Las plantas se regaron al 60% de capacidad de campo. No se reaplicaron los tratamientos de modo que en el segundo ciclo se estudió el efecto residual de las fuentes de P aplicadas en el primer ciclo del ensayo. Al final de cada ciclo de cultivo, se obtuvieron las muestras de suelo procedentes de muestras destructivas, se dividieron en dos submuestras, una fue extendida y secada al aire para las mismas determinaciones químicas y físico-químicas que se realizaron en el primer bloque experimental, mientras que la otra fue congelada inmediatamente a -80 ºC para las determinaciones biológicas (respiración edáfica y actividades enzimáticas). La respiración del suelo se realizó mediante la metodología descrita por Bustamante et al. (2011), mientras que las actividades enzimáticas del suelo que se determinaron fueron fosfomonoesterasas alcalina y ácida (Eivazi y Tabatabai, 1977), β-glucosidasa a dos pH (6,5 y 8,5) (Eivazi y Tabatabai, 1988) y ureasa (Nannipieri et al., 1981), en cuyas determinaciones se usó el método adaptado a microplacas de Popova y Deng (Popova y Deng, 2010). También tras cada ciclo de cultivo, la parte aérea de las lechugas se cortó y pesó para determinar rendimiento en peso fresco, se secó a 60ºC, se anotó su peso seco, y posteriormente se molieron. Una vez procesadas, se determinaron parámetros químicos en el tejido vegetal, replicando la metodología aplicada en el primer bloque experimental del trabajo. Adicionalmente, también se determinaron los dos parámetros de calidad estudiados en el primer bloque experimental, de forma no destructiva cada 15 días hasta la finalización del ensayo, el factor de cobertura vegetal fCOVER, mediante CANOPY app © desarrollada por Patrignani y Oschner (Patrignani y Ochsner, 2015) y la intensidad de clorofila, mediante el medidor portátil SPAD-502 (Minolta Co., Ltd., Osaka, Japón) (Casella et al., 2022).

Los análisis estadísticos se realizaron utilizando el paquete de software estadístico Infostat, v.2020, vinculado al programa R (Di Rienzo et al, 2020). La normalidad de los datos se comprobó mediante la prueba de Shapiro-Wilks y la homogeneidad de la varianza se evaluó y confirmó mediante la prueba de Levene ($p > 0,05$). Se realizó una prueba de análisis de varianza (ANOVA) con $p < 0,05$ para todas las variables experimentales utilizando un modelo lineal mixto generalizado, con los tratamientos de fertilizantes y las fechas de muestreo como factores fijos y cada parcela como factor aleatorio. El análisis post hoc se llevó a cabo con la prueba de Tukey ($p < 0,05$).

4. RESUMEN DE RESULTADOS Y DISCUSIÓN

4. Resumen de resultados y discusión

A continuación se detallan los resultados obtenidos en cada uno de los bloques experimentales del trabajo.

En lo que respecta a los resultados del **primer bloque experimental**, recopilados en la publicación "*Looking for new P fertilizers: comparative study of mineral-, organomineral- and organic-based fertilizers for lettuce (Lactuca sativa L.)*", de la revista *Agronomy,* en términos de rendimiento en peso fresco del cultivo se apreciaron diferencias significativas respecto al control de las tres dosis del fertilizante inorgánico convencional, así como MAP y STR en forma simple y compleja-OMF. Los mejores datos de rendimiento correspondieron a OMF(MAP+C) (101,37 g) seguido de OMF(STR+C) (83,21 g), con distancia significativa del fertilizante inorgánico convencional IN300 (60,42 g). Los resultados de rendimiento mostraron correlación con los valores de fCOVER del día 45 (cosecha). En lo que se refiere a la concentración de clorofila en las hojas, esta fue significativamente superior en los tratamientos convencionales inorgánicos IN200 e IN300, seguidos por MAP y STR. En el estudio de Carciochi et al. (2024), los OMF mostraron un aumento del 13 % en el rendimiento del trigo respecto al MAP inorgánico más urea en un experimento de campo de 3 años en un cultivo de trigo. En un ensayo en maceta con lechuga y col, una dosis del 50 % de OMF-P produjo al menos un 11 % más de materia fresca y materia seca que la fertilización 100 % mineral (Vieira et al., 2020). De Morais et al. (2023) descubrieron que los OMF formulados con MAP 25 %, estiércol de pollo 37,5 % y cáscara de café 37,5 % aumentaban la biomasa de los brotes de maíz en un 26 % con respecto a las plantas fertilizadas con MAP. Según Uddin et al. (2025), los fertilizantes órgano-minerales liberan los nutrientes lentamente en función de las necesidades de las plantas a lo largo de la temporada de crecimiento, permitiéndoles absorber los nutrientes de manera más eficiente, lo que se traduce en un mejor crecimiento y desarrollo de las plantas. Sin embargo, este efecto depende de la fuente de P utilizada (Bouhia et al., 2023).

El pH del suelo en todos los tratamientos al final del ensayo fue significativamente superior al del control, con valores ligeramente alcalinos, resultado que también se obtuvo en un estudio similar en lechuga (Mancho et al., 2023). La CE fue significativamente menor en los suelos tratados con los fertilizantes organo-minerales a base de fosfato monoamónico y estruvita respecto al resto de tratamientos y al control. MAP mostró una concentración de P disponible comparable al fertilizante convencional IN300. Aunque no de manera significativa, Mancho et al. (2023) observaron que la mayor concentración de P disponible en el suelo se encontró en el tratamiento MAP. Los niveles de P disponible fueron significativamente superiores en todos los tratamientos complejos respecto a los simples, indicando un potencial efecto beneficioso de la adición de compost en este sentido. Estos resultados concuerdan con los obtenidos por Erenoğlu et al. (2023) en un experimento en macetas con trigo, en el que, tras 90 días, el P disponible en los suelos tratados con el tratamiento OMF en todas las dosis estudiadas fue significativamente mayor que en las plantas tratadas con el fertilizante mineral. En un experimento de campo con *Moringa oleífera*, Adebayo et al. (2017) describieron concentraciones de P en el suelo más altas para el OMF que el resto de los fertilizantes evaluados: NPK (15:15:15), estiércol de vaca y estiércol de aves de corral. Frazão et al. (2019) observaron que se obtenían cantidades significativamente mayores de P disponible para las plantas de maíz utilizando fertilizantes fosfatados órgano-minerales en un estudio en el que se comparaban estos OMF con el superfosfato triple. Del mismo modo, los OMF a base de biocarbón mostraron una mayor eficiencia agronómica y una mayor disponibilidad de P después de la cosecha en maíz (Willian da Silva et al., 2024). El aumento de la disponibilidad de P por los fertilizantes OMF también fue descrito por Borges et al. en la caña de azúcar (Borges et al., 2019). Este efecto puede deberse al aporte adicional de MO con tratamientos complejos de OMF (véase la tabla 4 en el artículo incluido en los Anexos).

Los suelos tratados con el fertilizante órgano-mineral a base de harina de hueso (OMF(BM+C)) presentaron concentraciones significativamente superiores de materia orgánica, nitrógeno total y nitratos. Los suelos fertilizados con los tratamientos simples y complejos exhibieron niveles de materia orgánica en cosecha significativamente superiores a los fertilizantes convencionales, siendo mayor este efecto en el caso de los OMFs. Este efecto de los OMF de fósforo también fue descrito en un experimento en macetas con raigrás perenne (Sánchez-Méndez et al., 2025). Toprak y Seferoğlu describieron un aumento de la materia orgánica del suelo tras la aplicación de OMF-P de un 60,1 % (Toprak y Seferoğlu, 2024).

La eficiencia de uso de fósforo fue significativamente mayor en las plantas tratadas con la dosis más baja del fertilizante NPK convencional (11.60% PUE). De entre los tratamientos empleados en el estudio, destacó el fertilizante órgano-mineral de estruvita OMF(STR+C) (8.33% PUE) seguido por OMF(MAP+C) (7.40 % PUE). La adición de compost contribuyó así a incrementar la eficiencia de uso de fósforo de MAP y STR, mientras que no tuvo efecto en el caso de PR y BM, que presentaron los valores más bajos de eficiencia de uso de nutrientes.

En el estudio de Araújo et al. (2020) en un experimento de invernadero con un cultivo de mijo comparando fertilización con MAP y OMF a base de MAP y compost, descubrieron que el OMF promovía un mayor contenido de nutrientes en comparación con el MAP (Araújo et al., 2020). En el estudio de Erenoğlu et al., (2023) las plantas fertilizadas con OMF-P mostraron mayores concentraciones de P que las fertilizadas con fertilizantes minerales. De Sousa y Alleoni (2024), en un experimento en invernadero con dos cultivos sucesivos de maíz, concluyeron que STR y un OMF compuesto por estiércol de pollo y superfosfato triple (TSP) tenían una mayor eficiencia agronómica que el TSP en suelos arenosos. La absorción de P de OMF(PR+C) y OMF(BM+C) fue menor que la de OMF(MAP+C) y OMF(STR+C), probablemente debido al alto contenido de Ca de estos OMF, que puede formar enlaces con el P en la matriz orgánica, disminuyendo su disponibilidad (Sitzmann et al., 2024).

Se estudiaron los niveles de metabolitos cruciales en el metabolismo (glicolisis y ciclo de los ácidos tricarboxílicos (TCA)). Los niveles de glucosa se vieron significativamente aumentados en plantas tratadas con OMF(STR+C), OMF(MAP+C), STR y MAP. Esto concuerda con los resultados de Hurtado et al., (2017), quienes sugirieron que el uso de fertilizantes con alto contenido de nutrientes promueve la acumulación de carbono. Por otra parte, en las plantas tratadas con PR y BM se detectó acumulación de intermediarios del ciclo TCA como citrato y succinato, lo cual sería debido a una mayor activación de esta ruta en respuesta a la mayor demanda energética. Esto concuerda con los resultados de Matamoros et al., (2021), quienes concluyeron que, en condiciones de baja disponibilidad de nutrientes, la actividad del ciclo de Krebs se regulaba al alza para la producción de ATP y para proporcionar los precursores de muchas vías biosintéticas (Wang et al., 2017; Araújo et al., 2012). Gao et al. (2020) propusieron el aumento de los niveles de citrato y succinato como biomarcadores del estrés por bajo contenido en fósforo en plantas de lechuga.

Los niveles de aminoácidos en las plantas tratadas con tratamientos a base de PR y BM se vieron significativamente aumentados respecto al resto. Este resultado coincide con el obtenido por Chandrou et al. (2024).

Por otra parte, el ácido gamma-aminobutírico (GABA) y mioinositol, moléculas implicadas en la regulación del crecimiento y la resistencia celular a factores de estrés externos, presentaron concentraciones significativamente superiores en plantas tratadas con OMF(MAP+C) y OMF(STR+C) (Donahue et al., 2010; Loewus y Murthy, 2000; Ramesh et al., 2015).

Los niveles de tartrato fueron significativamente superiores en las plantas fertilizadas con los tratamientos simples PR y BM. Estudios previos han observado una regulación al alza de los derivados del ácido tartárico en plantas estresadas (Jacobo-Velázquez y Cisneros-Zevallos, 2012; Sitzmann et al., 2014).

En lo que respecta a los resultados del **bloque experimental 2**, publicados en la revista *Agronomy* en el artículo "*Sustainable phosphorus and protein recovery from different organic wastes: process optimization and struvite precipitation potential",* las dos fuentes de azúcar evaluadas tuvieron un claro efecto acidificante sobre los medios de los residuos orgánicos utilizados. En el caso de la melaza, el aumento de la cantidad de azúcar dio lugar a una acidificación casi lineal del medio de fermentación para todas las fuentes orgánicas, lo que no ocurrió en el caso de la sacarosa, aunque la bajada de pH más significativa se produjo empleando 2 g de sacarosa. Este efecto sobre el proceso de bioacidificación es similar al observado por Piveteau et al. (2017) en un estudio sobre la recuperación de fósforo en forma de estruvita a partir de purines de cerdo mediante acidificación biológica utilizando sacarosa como fuente de azúcar.

Por otra parte, la cantidad de azúcar pareció tener un efecto significativo en la recuperación de P para ambos tipos de azúcares, aumentando la eficiencia con el aumento de las cantidades de azúcar, excepto en los medios de fermentación de SS2 y SS3, en los que se observó incluso el efecto contrario cuando se utilizó melaza como fuente de azúcar. La disminución de la eficiencia de recuperación de P a concentraciones más altas de sacarosa y el pH más bajo asociado podrían atribuirse a la asimilación de P por la biomasa bacteriana, ya que cuando el pH es cercano a 5, no se disuelve P adicional y el crecimiento de la biomasa puede disminuir la concentración de P disuelto (Piveteau et al., 2017).

Las muestras de lodos de depuradora (SS1, SS2 y SS3) mostraron valores iniciales de pH más bajos en comparación con la fracción sólida del purín de cerdo (M). Tras 48 h de incubación, todos los medios de fermentación alcanzaron sus valores de pH más bajos, en el rango de 3,58-5,06. Su et al. (2025) también encontraron una estabilización en las tasas de liberación de fósforo y las tasas más altas de fosfato a las 48 h de fermentación en un estudio sobre la recuperación de fósforo a partir de estiércol porcino utilizando un proceso de co-fermentación con residuos alimentarios.

Al incubar los residuos orgánicos aplicando los parámetros previamente optimizados, el extracto con SS3 mostró el valor de pH más bajo (3,58) y el mayor rendimiento de recuperación de P (69,0 %), efecto que no se observó en el resto de los tratamientos de lodos de depuradora (SS1 y SS2), con valores bajos de pH y bajos rendimientos de recuperación de P. Sin embargo, la fracción sólida del estiércol porcino (M) mostró un rendimiento de recuperación de P estadísticamente similar al de SS3, a un valor de pH de final de proceso más alto (5,06).

La diferente naturaleza de las fuentes orgánicas utilizadas, como sus diferentes contenidos en fósforo orgánico, que puede constituir hasta el 22 % del fósforo total (Daumer et al., 2010), o su contenido en hierro (Wilfert et al., 2015), podrían explicar las diferencias en la eficiencia de recuperación de P. Así, los residuos SS1 y SS2, los cuales presentaban las concentraciones más altas de Fe, obtuvieron bajos rendimientos de recuperación de P.

Los minerales de P precipitados a partir de las muestras de lodos de depuradora (SS1, SS2 y SS3) mostraron un mayor contenido total de C orgánico, Ca, Na y Fe en comparación con el precipitado de la fracción sólida de purín de cerdo (M). Sin embargo, los elementos básicos de la estruvita (N, Mg y P) se encontraban en concentraciones más altas en el precipitado de M, y solo el precipitado de SS3 mostraba contenidos similares de estos elementos. De hecho, sólo los precipitados recuperados de M y SS3 cumplían el criterio de contenido de P_2O_5 (>16 % del contenido de materia seca) establecido en el reglamento de la UE sobre productos fertilizantes (EU, 2021), así como los criterios relativos al contenido de metales pesados (EU, 2019).

El contenido de N más bajo correspondió a las muestras SS1 y SS2, siendo casi insignificante en el caso de SS1 (<0,5 %), lo que excluía la posibilidad de presencia de estruvita en este precipitado. Adicionalmente, en las muestras SS1 y SS2 el Fe podría haber interferido en la precipitación de estruvita, dada la mayor afinidad por el fosfato del hierro respecto al magnesio (Wilfert et al., 2015; Korving et al., 2018). Consecuentemente, las concentraciones de N y Mg en las muestras SS1 y SS2 fueron limitantes para la formación de estruvita.

Los resultados de los análisis DRX de los precipitados mostraron que sólo los de SS3 y M presentaban un patrón (intensidad y posición de los picos de difracción) que coincidía con el difractograma de la estruvita pura (JCPDS 77-2303) (Zeng et al., 2018). Esto también se confirmó con el patrón de la estruvita comercial, que coincidía con el de la estruvita pura (Oliveira et al., 2018). Sin embargo, el precipitado de SS2 mostró un patrón completamente diferente, indicando que no se formó estruvita en esta muestra. El análisis FESEM-EDX de los precipitados confirmó que estos contenían los elementos constituyentes de la estruvita (O, N, P y Mg) junto con trazas de otros elementos, lo que era coherente con la composición de la estruvita, excepto en el caso del precipitado SS2, que tenía un bajo contenido en Mg. Estos resultados coinciden con los de trabajos anteriores (Xu et al., 2021; Oliveira et al., 2018). Con los datos obtenidos de los espectros EDX, se desarrolló un análisis mineralógico racional (RMA), estudio estimativo basado en la combinación de análisis químicos cuantitativos y mineralógicos cualitativos para la determinación cuantitativa de las fases mineralógicas de una muestra (Coelho et al., 2002). En este caso se consideraron las fases de estruvita e hidroxiapatita. Los resultados del RMA mostraron proporciones estimadas del 94 % de estruvita y del 6 % de hidroxiapatita para el precipitado M, y del 83,2 % de estruvita y del 16,8 % de hidroxiapatita para el precipitado SS3. Además, las imágenes FESEM también revelaron que los precipitados eran principalmente cristalizaciones de estruvita gruesas y con forma de ataúd, respectivamente, en consonancia con los hallazgos de Xu et al. (2021) en un estudio sobre la recuperación de fósforo a partir de lodos activados residuales.

En cuanto a los resultados obtenidos en los ensayos del **tercer bloque experimental**, en el artículo "*Influence of lettuce crop production and soil properties of using alternative P sources under different biotic and abiotic soil conditions*", enviado a la revista *Resources, Conservation and Recycling*, los diferentes tratamientos mostraron una influencia significativa en el rendimiento de la lechuga, especialmente en el primer ciclo de cultivo. Al final de la primera temporada de cultivo, todos los grupos de tratamientos mostraron un rendimiento significativamente mayor que el tratamiento control. Los mayores rendimientos de lechuga se obtuvieron con los tratamientos con fuentes de P derivadas de residuos STR, SS1, SS2 y SS3 y los fertilizantes órgano-minerales con fuentes de P inorgánicas TSP y MAP, posiblemente debido a una mayor presencia en estos materiales de nutrientes disponibles para el cultivo (Soudek et al., 2024). Estos resultados coincidieron con los de otros estudios en los que se utilizaron fertilizantes órgano-minerales de estruvita y fuentes minerales de P (de Sousa y Alleoni, 2024; Valverde-Vozmediano et al., 2025). Sin embargo, en el segundo ciclo de cultivo sólo los tratamientos de compost (C), y los fertilizantes órgano-minerales de harina de hueso (C-BM) y roca fosfórica (C-RP) presentaron rendimientos de lechuga superiores al suelo control. El factor pH del suelo influyó significativamente en el rendimiento del cultivo en ambos ciclos ($p < 0{,}001$), mientras que la inoculación de PSB no pareció tener efecto en ninguna temporada de cultivo ($p > 0{,}05$).

Por otra parte, el tipo de tratamiento y los valores de pH del suelo tuvieron un efecto significativo en los valores SPAD. Los valores medios de SPAD más altos se observaron al comienzo de la temporada, en los tratamientos con fuente de P derivada de residuos (C-SS1 y C-SS2) y el tratamiento C-TSP. En la segunda temporada de lechuga, los valores más altos de SPAD se encontraron en los tratamientos C-RP y C-BM. La tendencia decreciente en los valores SPAD podría atribuirse a una rápida liberación de nutrientes en el primer ciclo de cultivo. En cuanto al efecto de los diferentes factores sobre la cobertura foliar media (CANOPY), en el primer ciclo de crecimiento, los valores medios de CANOPY se vieron afectados principalmente por el tipo de tratamiento fertilizante y el pH del suelo, y solo se encontró un efecto significativo de la inoculación de PSB a los 15 días de la siembra. Los valores medios más altos de CANOPY se observaron en los fertilizantes órgano-minerales con fuentes de P derivadas de residuos (C-STR, C-SS1, C-SS2 y C-SS3), con un efecto similar en los tratamientos con fuentes minerales de P C-MAP y C-TSP. Sin embargo, en el segundo ciclo de cultivo, los valores más altos se encontraron en los tratamientos C, C-RP y C-BM, lo que parece implicar una liberación más lenta de P en estos tratamientos, resultado compatible con los de estudios previos de fertilización sobre la producción de lechuga (Reis et al., 2014; Álvarez-Alonso et al., 2025).

Los valores medios de absorción de nutrientes (N, P y K) por parte de la lechuga en cada temporada de cultivo se vieron significativamente afectados por el tipo de tratamiento de fertilización y el pH del suelo, con una mayor absorción al valor de pH ácido, sin observarse un efecto significativo de la inoculación con PSB. En general, los tratamientos C, C-RP y C-BM indujeron la mayor absorción de N y P en ambos ciclos de cultivo y de K en el segundo ciclo de cultivo. Estos resultados concuerdan con los resultados de cobertura foliar (CANOPY), demostrando que la combinación con compost de esos fertilizantes mejora su productividad (Valverde-Vozmediano et al., 2025).

En cuanto al efecto de los distintos factores estudiados sobre los parámetros del suelo, se observó un efecto significativo del tipo de tratamiento y del pH del suelo en la materia orgánica del suelo y el P disponible en los dos ciclos de cultivo. En general, los tratamientos C-MAP y C-TSP indujeron las concentraciones más altas de P disponible en el suelo, con un efecto comparable de los tratamientos con STR, SS1 y SS3. Tras los dos ciclos de cultivo, las concentraciones de P disponible disminuyeron en todos los suelos debido a la absorción de este elemento por las plantas de lechuga. Algunos estudios han descrito un comportamiento similar de la estruvita en comparación con fertilizantes inorgánicos en relación a la absorción de P en suelos ácidos (Achat et al., 2014; Bonvin et al., 2015).

La respiración del suelo, utilizada como indicador de la actividad biológica (Bustamante et al., 2010), se vio influida significativamente en ambos ciclos de cultivo por el tipo de tratamiento, y en el segundo ciclo, también por la inoculación de PSB. En ambas temporadas, todos los tratamientos mostraron una respiración del suelo más alta que el suelo control, y los valores más altos se obtuvieron con los tratamientos C, C-MAP y C-TSP. Mientras en el primer ciclo de cultivo el fertilizante órgano-mineral C-RP mostró valores de respiración del suelo estadísticamente similares a los del suelo control, en el segundo ciclo de cultivo indujo los valores más altos de respiración del suelo junto con los tratamientos mencionados anteriormente. Hernández et al. (2016) observaron en un experimento de producción intensiva de lechuga comparando fertilizantes orgánicos e inorgánicos una mejora en la actividad de la microbiota del suelo al emplear fertilizantes orgánicos.

En cuanto a las actividades enzimáticas del suelo, la influencia de los factores considerados fue heterogénea, con sólo un efecto significativo del tipo de tratamiento en todas las actividades enzimáticas en ambos ciclos de cultivo. En general, todos los fertilizantes órgano-minerales aumentaron la actividad de las enzimas relacionadas con la degradación de fósforo, concretamente la actividad de la fosfatasa ácida durante el segundo ciclo de crecimiento. Cui et al. (2023) confirmaron en un meta-análisis que los acondicionadores orgánicos aumentaban significativamente las actividades enzimáticas relacionadas con la degradación del nitrógeno y el fósforo en el suelo. En este sentido, las fosfatasas pueden constituir un buen indicador del potencial de degradación del P de los compuestos orgánicos y de la actividad biológica del suelo. Además, la intensidad de la secreción de fosfatasas por los cultivos y los microorganismos depende en gran medida de las necesidades de P de los cultivos (Ramut et al., 2025). Por otro lado, el efecto del pH del suelo solo fue significativo en el primer ciclo de crecimiento para la actividad de la fosfatasa ácida, mientras que el efecto de los inoculantes PSB fue significativo en las actividades de la fosfatasa alcalina y la β-glucosidasa en condiciones ácidas (pH 6,5). En el segundo ciclo de crecimiento, el pH del suelo influyó significativamente en todas las actividades enzimáticas, excepto en la actividad de la ureasa, mientras que el inoculante PSB no afectó a ninguna de las actividades enzimáticas. En general, los tratamientos con fuentes de P derivadas de residuos, como C-BM, C-STR, C-SS1, C-SS2 y C-SS3, produjeron valores más altos que el resto de tratamientos, concretamente de la fosfatasa ácida, la β-glucosidasa en condiciones ácidas (pH 6,5) y la ureasa en el primer ciclo de crecimiento, y de la fosfatasa alcalina en el segundo ciclo. La composición heterogénea de los diferentes fertilizantes órgano-minerales produjo claramente efectos diferentes en función del sustrato utilizado por cada enzima.

5. CONCLUSIONES

5. Conclusiones

En esta tesis se han evaluado dos estrategias innovadoras para el desarrollo de fertilizantes alternativos de fósforo: i) la recuperación de minerales de fósforo (estruvita) a partir de residuos orgánicos (lodos de depuradora y fracción sólida de purín porcino) mediante la implementación de un proceso biológico; ii) el desarrollo de fertilizantes órgano-minerales utilizando las fuentes de fósforo recuperado con compost como base orgánica.

De los resultados obtenidos en este trabajo de investigación se deducen las siguientes conclusiones:

Respecto a la validación agronómica de fertilizantes órgano-minerales con diferentes fuentes de fósforo:

- → La aplicación de fertilizantes órgano-minerales a base de compost permitió mejorar las propiedades del suelo, al aumentar los niveles de materia orgánica y fósforo disponible, obteniendo los mejores resultados de rendimiento, absorción y eficiencia de uso de fósforo de las plantas de lechuga con los fertilizantes de estruvita (STR) y fosfato monoamónico (MAP), respectivamente, lo que demuestra el potencial fertilizante de la estruvita, con resultados equivalentes a los obtenidos con fertilizantes convencionales.

- → Se encontraron niveles significativamente superiores de metabolitos protectores (GABA y mioinositol) en plantas tratadas con fertilizantes órgano-minerales basados en estruvita (STR) y fosfato monoamónico (MAP), y de metabolitos vinculados al estrés por deficiencia de fósforo (intermediarios del ciclo del TCA, aminoácidos y tartrato), en plantas tratadas con roca fosfórica (PR) y harina de hueso (BM), sin efecto alguno de la adición de compost.

Respecto a la recuperación de minerales de fósforo (estruvita) a partir de residuos orgánicos:

- → La bioacidificación mediante fermentación láctica utilizando *Lactobacillus acidophilus*, constituye una alternativa viable para la recuperación de fósforo a partir de muestras de lodos de depuradora y fracción sólida de purín porcino, obteniendo rendimientos de recuperación de fósforo del 69 % y el 65,7 %, respectivamente. En ambos casos se confirmó la precipitación de estruvita mediante difracción de rayos X, lo que señala el potencial de este procedimiento para la recuperación de fósforo en forma de estruvita.

→ Las características de los residuos orgánicos influyeron notablemente en el rendimiento de la recuperación de P, especialmente el contenido de Fe de las fuentes orgánicas utilizadas, siendo ésta una de las principales limitaciones para la recuperación de fósforo a partir de muestras de lodos de depuradora mediante la precipitación de estruvita.

Respecto a la validación agronómica de los minerales de fósforo recuperado:

→ Los fertilizantes órgano-minerales alternativos con estruvita, minerales de fósforo recuperado y harina de hueso mostraron efectos comparables a los de los fertilizantes órgano-minerales con fuentes inorgánicas (superfosfato triple y fosfato monoamónico), en el rendimiento y la calidad del cultivo de lechuga, así como en el P disponible en el suelo y las actividades enzimáticas relacionadas con la mineralización del P, apuntando a los fertilizantes órgano-minerales de fósforo recuperado como potenciales sustitutos de las fuentes minerales tradicionales de fósforo.

→ Los fertilizantes órgano-minerales con roca fosfórica y harina de hueso fueron los únicos tratamientos que mostraron un efecto residual positivo en la productividad del cultivo y la absorción de nutrientes por las plantas.

→ El factor abiótico considerado (pH del suelo) tuvo un efecto significativo sobre el rendimiento y calidad de la planta, obteniendo los mejores resultados en condiciones ácidas. Sin embargo, el factor biótico (inoculación con bacterias solubilizadoras de P) pareció no tener un claro efecto a lo largo de los dos ciclos de cultivo en los parámetros estudiados en el suelo y la planta.

6. PERSPECTIVAS DE FUTURO

6. Perspectivas de futuro

Basándonos en los resultados y conclusiones alcanzadas en este trabajo, hay que señalar en primer lugar que este es un campo de investigación con un gran potencial futuro, ya que es necesario ahondar en el estudio de posibles fuentes de P alternativas a las fuentes tradicionales y en las tecnologías de recuperación de este elemento a partir de flujos residuales en forma de minerales de P, especialmente estruvita.

En lo que se refiere a la recuperación de fósforo como estruvita a partir de flujos residuales, sería interesante investigar el alcance de este proceso y en qué otro tipo de flujos residuales de distinta naturaleza podría aplicarse, como los residuos agroalimentarios. Es importante estudiar la posibilidad de emplear algún co-sustrato como fuente de azúcar como algún residuo vegetal o agroalimentario con un alto contenido de azúcares, con el fin de abaratar los costes del proceso de cara a su eventual escalado industrial. Respecto a la implementación de esta tecnología a media y gran escala en pilotos industriales, también habría que abordar el reto de adaptar y optimizar los procesos de fermentación y posterior cristalización de estruvita en un reactor, así como el procesamiento posterior de los minerales obtenidos. Además, sería necesario llevar a cabo un estudio de viabilidad económica de cada una de las fases del proceso.

Ya que el objetivo último sería una eventual comercialización de este producto para su aplicación en campo, la continuación de esta línea de investigación también exigiría el planteamiento de nuevos ensayos de validación agronómica en especies vegetales diferentes tanto en maceta como en campo y dilatar la duración de dichos ensayos para estudiar el efecto a largo plazo de los fertilizantes de fósforo recuperado sobre la planta y el suelo.

El futuro de esta línea de investigación también requiere el coestudio de estrategias de aumento de la biodisponibilidad de fósforo, principalmente la inoculación de microorganismos solubilizadores de fósforo, similares a las planteadas en este trabajo. Aunque en este caso no se observó un efecto significativo de la inoculación de bacterias solubilizadoras de fósforo en el aumento del P disponible y, consecuentemente, en la eficiencia de uso de fósforo del cultivo modelo de lechuga, existen numerosos estudios que apuntan lo contrario. Ya que hay una gran variedad de bacterias y hongos solubilizadores de fósforo, estudiar el efecto de la inoculación con otras especies sería muy útil para arrojar luz en este campo, y comprobar si, efectivamente, esta es una estrategia útil para mejorar la solubilización del fósforo aplicado a través de los fertilizantes fosfóricos permitiendo reducir las dosis de aplicación, tal y como sugieren otros estudios. Un estudio más exhaustivo de las fracciones de fósforo en el suelo y de la microbiota del suelo mediante técnicas metagenómicas aportaría información adicional para aumentar el conocimiento sobre las dinámicas del fósforo en el suelo y, en concreto, en la rizosfera de las plantas.

7. REFERENCIAS

7. Referencias

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8. ANEXOS

8.1. Publicación 1: Looking for New P Fertilizers: Comparative Study of Mineral-, Organomineral- and Organic-Based Fertilizers for Lettuce (*Lactuca sativa* L.). 2025.

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Looking for New P Fertilizers: Comparative Study of Mineral-, Organomineral- and Organic-Based Fertilizers for Lettuce (*Lactuca sativa* L.)

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Abstract: In this study several phosphorus fertilizers were evaluated under controlled production conditions using *Lactuca sativa* var. baby leaf and a clay-loam soil of pH 6.5 as a plant–soil model system. Various inorganic (phosphate rock, monoammonium phosphate, struvite), organic (bone meal and bone meal pelletized with compost) and organomineral fertilizers (phosphate rock, monoammonium phosphate, struvite pelletized with compost) were compared. The soil properties, crop yield, morphological aspects and metabolomics of the plants were analyzed. After 45 days of the growing cycle, the organomineral fertilizers (OMFs) composed of compost and monoammonium phosphate (OMF_2(MAP+C)) or struvite (OMF_3(STR+C)) exhibited the best yield results: 101.37 g and 83.21 g, respectively. These treatments also exhibited the best phosphorus use efficiency (PUE) results: 7.40% and 8.33%, respectively. The yield of plants treated with MAP was 56.01 g, and its PUE was 5.33%. The yield of plants treated with STR was 62.10 g and the PUE was 4.67%. Accordingly, the development of OMFs with compost had a positive effect regarding MAP and STR fertilization. Lettuce fertilized with organic bone meal fertilizers had the lowest yield and nutrient use efficiency. The non-targeted metabolic study of green tissue revealed an overactivation of the TriCarboxylic Acids-TCA cycle and amino acid biosynthesis in plants fertilized with bone meal and phosphate rock treatments, likely as a plant stress response. The overall conclusion of this work is that the development of OMFs with compost is a good strategy to increase soil P availability and, accordingly, plant P uptake and %PUE.

Keywords: phosphorus; lettuce; struvite; organomineral fertilizers; yield; NUE; PUE; metabolomics

1. Introduction

Phosphorus is essential in crop nutrition and to ensure crop productivity. However, P for fertilizer production is mostly obtained from the mining of phosphate rock (PR) [1], which is a non-renewable resource and is highly concentrated in few countries, generating dependency on imports. The major concentration of these reserves is found in Morocco, accounting for more than 70% of the world's phosphate reserves (50,000 Mt) [2]. Morocco dominates PR mining together with other countries such as China and Russia [3]. It is mandatory to find alternative P supplies to eliminate the uncertainty on P fertilizer availability in the future.

After years of conventional inorganic fertilizer use in an intensive agriculture context, P is considered an ecological contaminant. NPK fertilizers contain usually 60–70% P in its water-soluble form. P not immediately absorbed by plant roots is immobilized by the soil [4]. In acidic soils, most P is readily adsorbed on the surfaces of iron (Fe) and aluminum (Al) (hydr)oxides. In calcareous soils P is predominantly adsorbed on calcite surfaces or precipitated as calcium phosphates, predominantly as dicalciumphosphate. In soils oversaturated with P due to overfertilization practices over prolonged times, it partially leaches towards water bodies. This results in eutrophication, a huge environmental concern [5].

It is urgent to close the agricultural P cycle in order to avoid side-effects caused by excess P accumulating in soil. The strategy to be addressed is to develop sustainable fertilizers which ensure complete P utilization. Slow-release fertilizers reduce leaching losses [6,7]. An interesting slow-release fertilization strategy is the use of organomineral fertilizers (OMFs) [8], which combine a high nutrient dosage in an organic matrix. OMFs provide the soil with organic matter which improves the soil's water retention capacity and decreases erosion [9]. OMFs with compost help to restore soil fertility [10]. The technology employed to develop OMFs is pelletization, which is a widespread strategy in animal feed production [11]. The pelletization of compost enables easier handling, transport and storage. Through pelletization, compost can be combined with other fertilizing materials to achieve nutrient concentration and uniform amending properties [12].

Currently, <50% of phosphorus wastes/residues are recycled back into the global food system [13]. Biowaste, due to its high generation rate, is a critical issue at a global level. P recovery from residues such as sludge or manure is a desirable strategy for recycling nutrients, avoiding environmental pollution and contributing to a circular bioeconomy model. Some available P sources include municipal and industrial wastewater, meat and bone meal (MBM) and other organic wastes [14]. One widespread approach being implemented at lab and industrial scales is struvite crystallization [15].

The aim of this work was to study the potential of compost-based OMFs as slow- release fertilizers in comparison to mineral and organic fertilizers in a pot study, together with the evaluation of the fertilizing capacity of recovered P sources. We comparatively studied the effect of the treatments on (1) soil properties, (2) crop yield, (3) nutrient use efficiency and (4) the plant metabolic state at harvest. For the present research we de- veloped various OMFs with organic matrix compost, combined with several P sources: conventional mineral fertilizers like monoammonium phosphate (MAP) and phosphate rock (PR), organic recovered fertilizers like bone meal (BM), and struvite (STR) recovered from a wastewater treatment plant (WWTP). The plant model employed in this work was *Lactuca sativa* var. baby leaf. According to the most recent data from FAOSTAT [16] corresponding to the year 2023, in Europe, lettuce crop represented an extension of 127,311 ha, accounting for a total production of 3,332,479.7 tons. Specifically in Spain, lettuce crop production was 864,570 tons, which represents 26% of total European lettuce production. *Lactuca sativa* is a desirable plant model, since it has a short vegetative cycle; the duration of the crop is usually between 45 and 60 days.

2. Materials and Methods

Experimental Design

The P treatments studied are classified into 2 groups: simple and complex OMF treat- ments. The simple treatments include inorganic materials, such as phosphate rock (PR, 0-33-0 %N-P_2O_5-K_2O), monoammonium phosphate (MAP, 11-61-0) and struvite recov- ered from a wastewater treatment plant (STR, 5-33-0), and organic materials, including meat industry by-product bone meal (BM, 3-30-0). The complex OMF treatments involve organomineral fertilizers with the addition of compost at a 50:50 ratio. We used the conven- tional inorganic fertilizer ComplexIN (15-15-15) as a reference for 3 doses: 100 (IN100), 200 (IN200) and 300 (IN300) kg NPK ha^{-1}. In all cases an isodose of potassium nitrate (KNO_3, 13-0-46) was supplied to satisfy the N demand of the crop. The N and P sources for all the treatments included in the experiment are shown in Table 1. The fertilizing treatments were applied according to a normalized N application rate of 200 kg N ha^{-1} and 120 kg P ha^{-1}.

Table 1. Treatment summaries and NPK sources

Treatment Group	Treatment	N Source	P Source	K Source
Control	Control	--	--	--
Conventional inorganic treatments	IN100	IN100	IN100	IN100
	IN200	IN200	IN200	IN200
	IN300	IN300	IN300	IN300
Simple treatments	PR	$KNO3$	Phosphate rock	$KNO3$
	MAP	$KNO3$	Monoammonium phosphate	KNO_3
	STR	KNO_3	Struvite	KNO_3
	BM	KNO_3	Bone meal	KNO_3
Complex OMF treatments	OMF_1(PR+C)	KNO_3 + compost	Phosphate rock + compost	KNO_3 + compost
	OMF_2(MAP+C)	KNO_3 + compost	Monoammonium phosphate + compost	KNO_3 + compost
	OMF_3(STR+C)	KNO_3 + compost	Struvite + compost	KNO_3 + compost
	OMF_4(BM+C)	KNO_3 + compost	Bone meal + compost	KNO_3 + compost

A pot trial was performed using lettuce (*Lactuca sativa* var. baby leaf) as a plant model. The soil used was a clay-loam soil collected from the EPS-Orihuela experimental farm (OECD 208:2006). The soil was mixed with 4% $FeSO_4$ to acidify soil pH from a value of 8.5 to 6.5 to improve nutrient availability. The soil had an initial electrical conductivity of 3.75 dS m^{-1}, a total N content of 0.86 g kg^{-1}, 1.62 mg kg^{-1} N-NH_4^+, 26.2 mg kg^{-1} N-NO_3^-, 15.6 mg kg^{-1} available P and 0.59% organic matter. The soil was sieved through 5 mm mesh to remove large particles and roots. The pots used were filled with 1500 g of the soil mentioned. The pots were placed in an environmentally controlled room with an average temperature of 21 °C, 60% relative humidity and a 12 h photoperiod with artificial lightning. The experimental design was randomized (*n* = 36) for 11 treatments in triplicate, in addition to a non-fertilized control.

Treatments Characterization

Previously to the experimental setup, a thorough characterization of the fertilizers to be assayed was performed according to Paredes et al.'s [17] methodology. The results are presented in Table 2.

To obtain the OMFs, organic compost was used (prepared from a mixture of olive mill waste–poultry manure–olive leaf waste in a 60:20:20 ratio). The OMFs were manufactured at Compolab-EPSO UMH (Orihuela, Alicante, Spain) through the extrusion of the mixtures using a low-power (4 kW) three-phase small scale pelletizer machine (50 kg h^{-1} capacity) with two rotating rollers (78 mm) operating on a fixed flat die of 119 mm diameter H-24, with holes of 6 mm diameter. Mixtures were prepared combining compost with various sources of high P content.

Table 2. Treatment characterization.

Nutrient	PR	MAP	STR	BM	OMF$_1$ (PR+C)	OMF$_2$ (MAP+C)	OMF$_3$ (STR+C)	OMF$_4$ (BM+C)
pH	6.5	4.2	7.3	6.9	9.4	5.6	6.7	8.6
EC (dS m^{-1})	0.97	22.8	1.3	0.7	2.05	3.02	3.9	1.9
Total C (%)	0.48	0.031	0.14	15.1	17.5	21.7	17.9	29.4
Total N (%)	0.044	12.1	5.7	3.00	1.16	7.06	3.73	2.76
K (%)	0.11	0.001	0.06	0.05	1.25	1.44	1.20	1.57
P (%)	2.0	2.3	14.2	2.1	6.5	10.3	7.3	4.6
Ca (%)	25.1	0.11	0.09	23.2	16.3	2.0	5.3	11.6
Mg (%)	0.20	0.003	11.7	0.49	0.24	0.22	2.11	0.41
Na (%)	0.07	0.002	0.004	0.44	0.09	0.06	0.22	0.28
Fe (mg kg^{-1})	10,930	16.5	156	47.9	4808	1177	4173	1216
Cu (mg kg^{-1})	78	0.16	0.5	0.24	84.4	51.1	51.3	51.5
Mn (mg kg^{-1})	470	<0.01	642	42.6	388	159	272	165
Zn (mg kg^{-1})	413	1.33	1.2	136	244	120	184	168

EC: Electrical conductivity. Simple treatments: PR: Phosphate rock; MAP: Monoammonium phosphate; STR: struvite; BM: Bone meal. Complex OMF treatments: OMF$_1$(PR+C): Phosphate rock + Compost; OMF$_2$(MAP+C): Monoammonium phosphate + Compost; OMF$_3$(STR+C): Struvite + Compost; OMF$_4$(BM+C): Bone meal + Compost.

Crop Management

The first lettuce cycle began on 4 April 2023. Previously at day 0 the treatments were applied and distributed along the surface of the pot soil followed by irrigation with 200 g of deionized water. Then, lettuce seedlings at 2–3-true-leaves phenological stage were planted. Following the infiltration of the first watering, 75 g was added to each pot. Periodical watering with deionized water was performed in order to keep soil moisture at 60% field capacity.

At days 15, 25, and 45, the green cover factor (fCOVER) was determined using the *Canopeo* app developed for Matlab by Patrignani and Ochsner [18], and the chlorophyll content (CCC) of the plants was determined using a hand-held chlorophyllmeter (SPAD Minolta 502, Konica Minolta Sensing, Inc., Sakai, Osaka Prefecture, Japan) [19].

Lettuce plants were harvested on 19 May 2023 at phenological stage 45.

Plant Analysis

At the time of harvest, the aerial part of the plant was cut. In order to establish the crop yield, fresh biomass was weighed, then it was dried at T = 60 °C for 48 h, and its weight was recorded again for dry biomass determination [20]. The dry lettuces were milled in a RETSCH mill to prepare them for physicochemical determinations.

Physicochemical determinations were conducted as in the work by Martínez-Sabater et al. [21]. The C and N of the green tissue were measured in an automatic elemental micro analyzer

(EuroVector Elemental Analyzer, EuroVector S.p.A., Pavia, Italy). For other nutrients, samples were digested with nitric acid 67% (0.2 g in 10 mL) in a Mars 6 digester [22]. Subsequently, total P (Pt) was determined by colorimetry in an UV-V spectrophotometer, K and Na were determined in a flame photometer and the rest of the macronutrients (Ca, S, Mg) and micronutrients (Na, Cu, Fe, Mn, Zn) were analyzed via induced coupled plasma mass spectroscopy (ICP-MS).

For chlorophyll quantification we performed an extraction in 80% acetone followed by its spectrophotometric determination on a Thermoscientific Multiskan plate reader [23].

Nutrient use efficiency was also calculated for the main nutrients involved, N (NUE), P (PUE) and K (KUE), as the ratio of the nutrient application rate of the fertilizers and the nutrient uptake by the plant tissue, expressed as a percentage [24].

Through a non-targeted metabolomic study, a set of 29 metabolites were studied, belonging to 4 groups: amino acids (glutamate, glutamine, alanine, arginine, GABA, leucine, valine, isoleucine, asparagine, aspartate, phenylalanine, proline and threonine), organic acids (formate, malate, succinate, citrate, fumarate, acetate, ascorbate, 2-oxoglutarate, tartrate) and sugars (glucose, fructose, sucrose, myo-inositol). The samples were extracted following the protocol by Van der Sar et al. [25]. They were analyzed with an RMN Ascend 500 MHz AVANCE III HD H–NMR (Weinheim, Germany). The resulting spectra were evaluated with the ‘Chenomx NMR Suite’ version 8.3 program [26].

Soil Analysis

After harvest, soil from the pots was processed in order to remove the roots and was spread out to dry. Once dried, it was sieved through a 2 mm mesh. The soil parameters analyzed were pH, electrical conductivity (EC), total N (TN), available P (Pext) and organic matter content (OM). The pH and EC of the soil samples were measured in extracts with a 1:2.5 and 1:5 soil–water (*w*/*v*) ratio, respectively. For TN, we used the Kjeldahl method [17] of prior digestion with sulfuric acid. Pext was determined colorimetrically through the Olsen–Watanabe method [27]. For OM determination, organic carbon (COT) oxidization was measured according to Yeomans and Bremmer [28].

Statistical Analysis

Statistical analyses were performed using the Infostat v.2020 statistical software pack- age linked to the R program [29]. Differences in soil and lettuce characteristics between the different fertilization treatment groups were evaluated using Analysis of Variance (ANOVA) models with a significance level of $p < 0.05$. The Shapiro-Wilks test and Levene’s test were previously applied to confirm the normality and homoscedasticity of the data across groups. Multiple comparisons between means were also performed using Fisher's LSD test (α = 0.05). Variables were grouped in chemical parameters of the soil and biochem- ical parameters of the lettuce. R software [version 4.3.1 (16 June 2023 ucrt)] was employed to generate a heat map of correlations between lettuce biochemical variables. Principal components analysis (PCA) was performed to identify patterns and highlight similarities and differences between the different treatments and their outcomes on soil parameters.

3. Results

Yield

All conventional inorganic treatments, MAP, STR, OMF_2(MAP+C) and OMF_3(STR+C), performed significantly better than the control, whereas PR and BM as well as the corre- sponding complex OMF treatments showed yield values comparable to the control. The best yield results corresponded to OMF_2(MAP+C) (101.37 g), followed by OMF_3(STR+C) (83.21 g), with significant differences with respect to the best-performing conventional inorganic treatment: IN300 (60.42 g) (Figure 1).

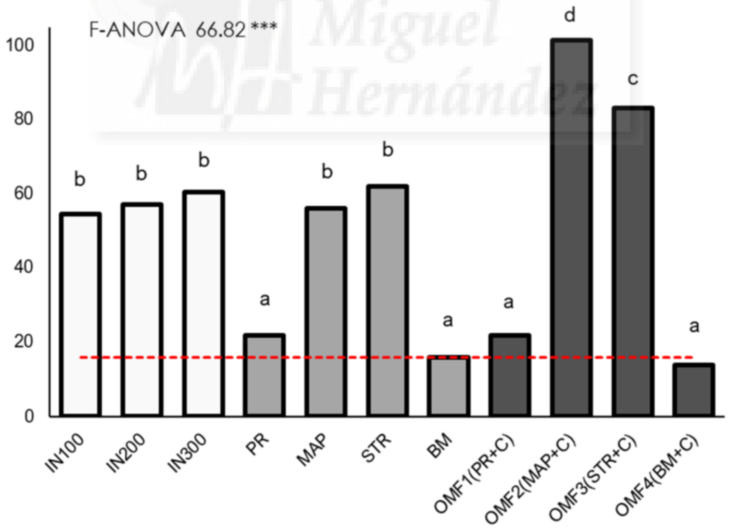

Figure 1. Fresh mass yield (FMY) (g) (t = 45 days). The red dotted line corresponds to the reference value of control plants. ***: significant difference between treatments at $p < 0.0001$. Different letters within a column indicate significant differences between treatments ($p < 0.05$). Values indicate mean ($n = 3$). Conventional inorganic treatments: IN100: 100 kg NPK ha $^{-1}$; IN200: 200 kg NPK ha $^{-1}$; IN300: 300 kg NPK ha $^{-1}$; Simple treatments: PR: Phosphate rock; MAP: Monoammonium phosphate; STR: struvite; BM: Bone meal. Complex OMF treatments: OMF_1(PR+C): Phosphate rock + Compost; OMF_2(MAP+C): Monoammonium phosphate + Compost; OMF_3(STR+C): Struvite + Compost; OMF_4(BM+C): Bone meal + Compost.

Morphological Aspects

The crop's morphological parameters studied during the plant's growing cycle were green cover (fCOVER) and chlorophyll content (CCC). By the end of the study, the chloro- phyll concentration in the plant's leaves was determined by spectrophotometry. The results of these parameters at harvest date are shown in Table 3.

Dynamics of the green cover factor (fCOVER) for the whole duration of this study were established from the measurements performed at days 10, 25 and 45. By day 10, all treatments presented similar fCOVER values, but by day 25, it already demonstrated a divergence of these values, with this tendency being clearly established by the end of this study (Figure 2). Control (C) PR-based simple and complex treatments, and BM- based simple and complex treatments, presented the lowest values of fCOVER during the whole period of study, with a remarkable decrease by the end of the growing cycle. OMF_2(MAP+C) presented the highest fCOVER value by day 45, with significant differences with respect to the rest of treatments. Conventional inorganic treatments, especially IN200, MAP, STR and OMF_3(STR+C), presented more similar fCOVER values by day 45. These results correlate with the yield results.

Table 3. Morphological parameters at harvest.

Treatment group	Treatment	SPAD t_f	Canopy t_f (%)	Chl a (mg g^{-1})	Chl b (mg g^{-1})	Chl (mg g^{-1})
Control	C	5.61 b	2.68 a	0.32 b	0.13 a	0.43 ab
Conventional inorganic treatments	IN100	10.29 c	10.01 b	0.44 c	0.20 b	0.64 c
	IN200	16.03 f	11.67 cd	0.55 e	0.24 cd	0.80 f
	IN300	12.99 d	10.75 bc	0.53 de	0.25 cd	0.78 ef
Simple treatments	PR	3.82 a	2.56 a	0.24 a	0.10 a	0.37 a
	MAP	12.26 d	10.27 b	0.52 de	0.27 d	0.70 cde
	STR	12.87 d	10.73 bc	0.43 c	0.23 bc	0.68 cd
	BM	3.82 a	2.23 a	0.33 b	0.13 a	0.46 ab
Complex-OMF treatments	OMF_1(PR+C)	3.61 a	2.36 a	0.30 ab	0.11 a	0.41 ab
	OMF_2(MAP+C)	14.33 e	13.88 e	0.47 cd	0.26 d	0.73 def
	OMF_3(STR+C)	20.14 g	10.73 bc	0.44 c	0.24 cd	0.68 cd
	OMF_4(BM+C)	4.29 a	2.69 a	0.35 b	0.13 a	0.48 b
F-ANOVA		*177 ****	*116 ****	*17****	*36 ****	*24 ****

SPAD: Non-destructive chlorophyll content measurement, t_f: final time, 45 days, Chl: Chlorophyll. Different letters within a column indicate significant differences between treatments ($p < 0.05$). ***: significant difference between treatments at $p < 0.0001$. Values indicate mean ($n = 3$). C: Control; Conventional inorganic treatments: IN100: 100 kg NPK ha^{-1}; IN200: 200 kg NPK ha^{-1}; IN300: 300 kg NPK ha^{-1}; Simple treatments: PR: Phosphate rock; MAP: Monoammonium phosphate; STR: struvite; BM: Bone meal. Complex OMF treatments: OMF_1(PR+C): Phosphate rock + Compost; OMF_2(MAP+C): Monoammonium phosphate + Compost; OMF_3(STR+C): Struvite + Compost; OMF_4(BM+C): Bone meal + Compost.

The chlorophyll concentration in the plants' leaves measured by spectrophotometry allowed us to study the concentration of chlorophyll a and chlorophyll b separately together with total chlorophyll levels. In general, the levels of chlorophyll a were greater than those of chlorophyll b. The results show that MAP and STR were the best performing simple treatments, with significant differences with respect to the other simple treatments (PR, BM). Among the complex treatments, the chlorophyll concentration in plants treated with OMF_2(MAP+C) and OMF_3(STR+C) was significantly higher than in plants treated with OMF_1(PR+C) and OMF_4(BM+C). There were no significant differences in chlorophyll concentration between simple and complex treatments. Plants treated with the conventional inorganic treatments IN200 and IN300 had the highest chlorophyll concentration (Figure 3)

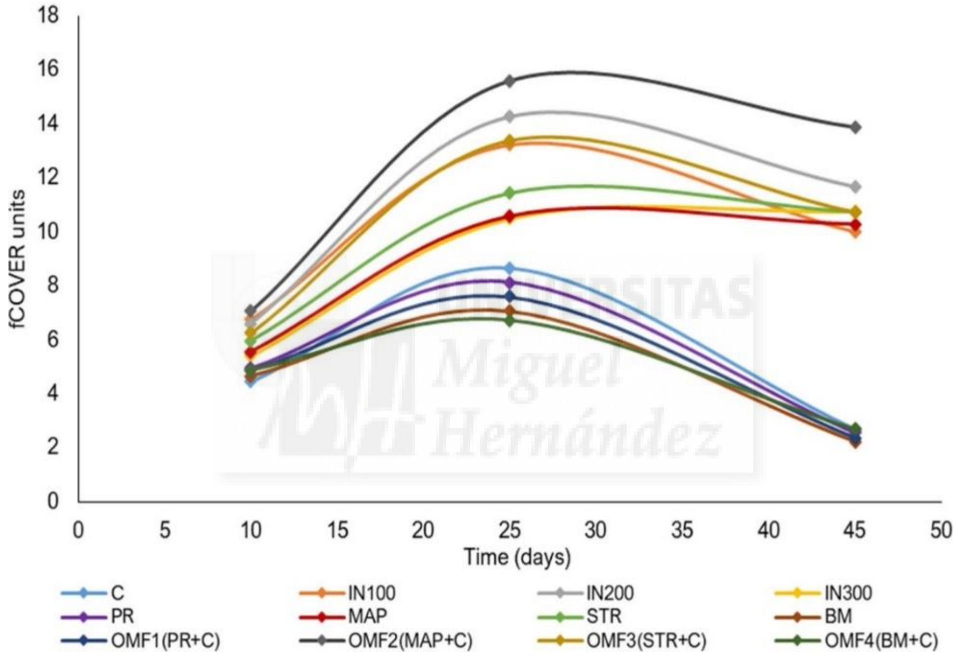

Figure 2. Green cover (fCOVER) dynamics (t1 = 10, t2 = 25, tf = 45 days). C: Control; Conventional inorganic treatments: IN100: 100 kg NPK ha−1; IN200: 200 kg NPK ha−1; IN300: 300 kg NPK ha−1; Simple treatments: PR: Phosphate rock; MAP: Monoammonium phosphate; STR: struvite; BM: Bone meal. Complex OMF treatments: OMF1(PR+C): Phosphate rock + Compost; OMF2(MAP+C): Monoammonium phosphate + Compost; OMF3(STR+C): Struvite + Compost; OMF4(BM+C): Bone meal + Compost.

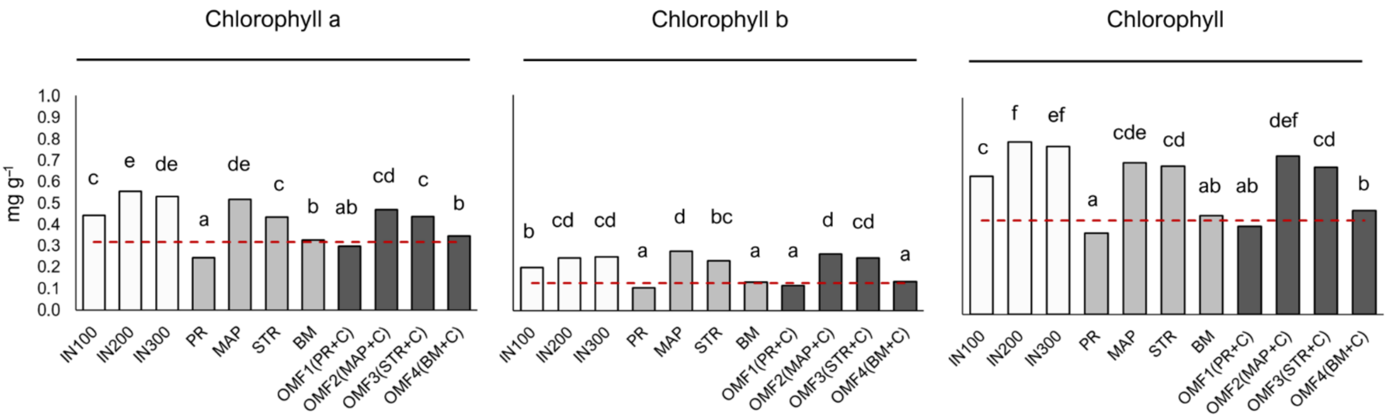

Figure 3. Chlorophyll content in plants at harvest (mg g^{-1}). Chl: chlorophyll. Different letters within a column indicate significant differences between treatments ($p < 0.05$). Values indicate mean ($n = 3$). The red dotted line corresponds to the reference value of control plants. Conventional inorganic treatments: IN100: 100 kg NPK ha^{-1}; IN200: 200 kg NPK ha^{-1}; IN300: 300 kg NPK ha^{-1}; Simple treatments: PR: Phosphate rock; MAP: Monoammonium phosphate; STR: struvite; BM: Bone meal. Complex OMF treatments: OMF_1(PR+C): Phosphate rock + Compost; OMF_2(MAP+C): Monoammonium phosphate + Compost; OMF_3(STR+C): Struvite + Compost; OMF_4(BM+C): Bone meal + Compost.

Soil Parameters

Soil pH at day 45 had values of 7.6–7.8 (Table 4) for all treated soils, with no significant differences between treatments. There were significant differences with the control, with a pH of 7.37. The soil EC was significantly lower in soils treated with OMF_2(MAP+C) and OMF_3(STR+C) with respect to the rest of treatments and the control.

The percentage of soil organic matter at harvest was significantly higher in soils treated with OMF_4(BM+C) than with the rest of the treatments. Soils treated with MAP had the lowest %OM, even lower than the control.

In the case of extractable phosphorus, of soils treated with conventional inorganic treatments, IN300 had the highest P concentration. In the group of simple treatments, the soil of plants treated with MAP had the highest P extractable value, comparable to IN300. In the group of complex treatments, OMF_2(MAP+C) had the highest P extractable value. Organomineral fertilizers OMF_1(PR+C) and OMF_4(BM+C) exhibited significantly higher available *p* values than the corresponding simple treatments. In general, there seems to be an effect of compost addition on P availability increase in the soil.

Table 4. Effect of fertilizer treatments on soil properties at harvest.

Treatment group	**Treatment**	**pH**	**EC (dS m^{-1})**	**OM (%)**	**Pext (mg kg^{-1})**	**TN (g kg^{-1})**	**NH$_4^+$-N (mg kg^{-1})**	**NO$_3^-$-N (mg kg^{-1})**
Control	C	7.37 a	3.25 cd	0.51 a	138 d	0.34 a	2.2 ab	1 a
Conventional inorganic treatments	IN100	7.62 bc	3.17 bc	0.66 d	190 e	0.41 b	5.6 cd	7 ab
	IN200	7.66 bc	3.19 c	0.39 b	218 ef	0.41 b	7.0 de	18 bc
	IN300	7.69 bc	3.14 abc	0.42 bc	260 g	0.49 c	8.5 e	44 d
Simple treatments	PR	7.81 c	3.20 cd	1.12 f	85 ab	0.52 c	7.0 d	85 e
	MAP	7.55 ab	3.18 bc	0.10 a	263 g	0.49 c	1.2 a	55 d
	STR	7.75 bc	3.20 cd	0.90 e	100 abc	0.54 c	11.2 f	43 d
	BM	7.75 bc	3.22 cd	0.98 e	78 a	0.49 c	5.4 c	111 f
Complex-OMF treatments	OMF$_1$(PR+C)	7.77 bc	3.20 cd	1.14 f	128 cd	0.51 c	3.5 b	94 e
	OMF$_2$(MAP+C)	7.77 bc	3.02 a	1.17 f	231 fg	0.42 b	3.0 b	26 c
	OMF$_3$(STR+C)	7.73 bc	3.04 ab	1.11 f	114 bcd	0.41 b	11.1 f	22 c
	OMF$_4$(BM+C)	7.77 bc	3.34 d	1.35 g	112 bcd	0.67 d	5.3 c	111 f
F-ANOVA		*3**	*3**	*120****	*36****	*17****	*37****	*89****

EC: Electrical conductivity; OM: Organic matter; Pext: available phosphorus; TN: total Kjeldahl N; NH$_4^+$- N: ammonium; NO$_3^-$-N: nitrate. *, ***: significant difference between treatments at $p < 0.01$ and $p < 0.0001$, respectively. Different letters within a column indicate significant differences between treatments ($p < 0.05$). Values indicate mean ($n = 3$). C: Control; Conventional inorganic treatments: IN100: 100 kg NPK ha^{-1}; IN200: 200 kg NPK ha^{-1}; IN300: 300 kg NPK ha^{-1}; Simple treatments: PR: Phosphate rock; MAP: Monoammonium phosphate; STR: struvite; BM: Bone meal. Complex OMF treatments: OMF1(PR+C): Phosphate rock + Compost; OMF2(MAP+C): Monoammonium phosphate + Compost; OMF3(STR+C): Struvite + Compost; OMF4(BM+C): Bone meal + Compost.

Soil treated with OMF$_4$(BM+C) had the highest total N value. There were no significant differences between the simple treatments, with values comparable to IN300, which had a significantly higher TN value than the conventional inorganic fertilizers IN100 and IN200. Ammonia levels were significantly higher in soils treated with both STR (11.2 mg kg^{-1}) and OMF$_3$(STR+C) (11.1 mg kg^{-1}), followed only by IN300 (8.5 mg kg^{-1}). The lowest ammonia concentration was detected in soils treated with MAP (1.2 mg kg^{-1}). The soils with the highest content of nitrates were soils treated with BM and OMF$_4$(BM+C). Soils treated with the complex treatments OMF$_2$(MAP+C) and OMF$_3$(STR+C) had a significantly lower NO$_3^-$ concentration than soils treated with the simple treatments MAP and STR (Table 4). When comparing the mean values of each group, differences in soil parameters were observed between groups of treatments (Table 5). The percentage of organic matter in the soil at the end of this study was significantly higher in soils treated with OMFs thanks to the effect of compost, thus helping to improve soil properties. In any case, soils treated with both simple and complex OMF treatments exhibited greater OM content than those treated with conventional inorganic fertilizers, except the inorganic fertilizer MAP.

Table 5. Comparison between treatment groups on soil properties at harvest.

Treatment group	**pH**	**EC ($dS\ cm^{-1}$)**	**OM (%)**	**Pext ($mg\ kg^{-1}$)**	**TN ($g\ kg^{-1}$)**	**NH_4^+-N ($mg\ kg^{-1}$)**	**NO_3^--N ($mg\ kg^{-1}$)**
Control	7.37 a	3.25	0.51 ab	138 a	0.41 a	2.16	1.25 a
Conventional inorganic treatments	7.66 b	3.17	0.49 a	222 b	0.41 a	7.02	22.81 a
Simple treatments	7.72 b	3.20	0.78 b	131 a	0.51 b	6.18	73.65 b
Complex-OMF treatments	7.76 b	3.15	1.19 c	146 a	0.50 ab	5.73	63.27 b
F-ANOVA	*7.8 ****	*0.93 ns*	*14.07 ****	*4.48 ***	*3.93 **	*1.91 ns*	*7.78 ****

EC: Electrical conductivity; OM: Organic matter; Pext: available phosphorus; TN: total Kjeldahl N; NH_4^+-N: ammonium; NO_3^--N: nitrate. *, **, ***: significant difference between treatments at $p < 0.01$, $p < 0.001$ and $p < 0.0001$, respectively; ns: not significant. Different letters within a column indicate significant differences between treatments ($p < 0.05$). Values indicate mean ($n = 3$).

The concentration of available P was significantly higher in the conventional inorganic fertilizer group. Though not significantly, available P (Pext) concentration had a greater value in the complex OMF treatment group (146 mg kg^{-1}) than in the simple treatments group (131 mg kg^{-1}). Soils treated with simple treatments had a significantly higher total N concentration. Nitrate concentration in the soil was significantly higher in both the simple and complex treatment groups when compared to the conventional inorganic fertilizers and the control. Though not significantly, nitrate concentration in soils treated with complex OMF treatments was lower than in those treated with simple treatments.

Nutrient Use Efficiency

OMF_2(MAP+C)- and OMF_3(STR+C)-treated plants presented significantly higher P uptake values compared to the rest of the treatments and the control, followed by inorganic treatments IN200 and IN300 (Table 6). PR and BM in simple and complex form exhibited P uptake values comparable to those of control plants.

Table 6. Lettuce N, P and K nutrient uptake and nutrient use efficiency at harvest.

Treatment group	Treatment	P uptake (g P pot^{-1})	PUE (%)	N uptake (g N pot^{-1})	NUE (%)	K uptake (g K pot^{-1})	KUE (%)
Control	C	0.001 a	-	0.05 a	-	0.012a	-
Conventional inorganic treatments	IN100	0.006 b	11.60 e	0.11 c	71.33 gh	0.032 d	24.80 d
	IN200	0.009 d	9.33 d	0.18 de	69.67 gh	0.053 g	25.80 d
	IN300	0.011 e	7.97 c	0.20 ef	53.67 de	0.039 e	11.17 c
Simple treatments	PR	0.002 a	0.97 a	0.10 c	30.33 c	0.020 c	1.37 a
	MAP	0.007 c	5.33 b	0.20 f	64.33 fg	0.045 f	5.97 b
	STR	0.006 bc	4.67 b	0.16 d	47.00 d	0.040 ef	5.13 b
	BM	0.001 a	0.23 a	0.07 b	13.00 ab	0.014 ab	0.30 a
Complex-OMF treatments	OMF$_1$(PR+C)	0.001 a	0.30 a	0.10 c	18.33 b	0.018 bc	0.77 a
	OMF$_2$(MAP+C)	0.016 g	7.40 c	0.25 g	75.33 h	0.071 h	10.43 c
	OMF$_3$(STR+C)	0.013 f	8.33 cd	0.20 f	59.33 ef	0.054 g	7.37 b
	OMF$_4$(BM+C)	0.001 a	0.13 a	0.07 ab	8.67 a	0.013 ab	0.13 a
F-ANOVA		*241.51* ***	*120.93* ***	*112.31* ***	*57.61* ***	*99.60* ***	*89.64* ***

NUE: Nitrogen use efficiency, PUE: phosphorus use efficiency, KUE: potassium use efficiency. ***: significant difference between treatments at $p < 0.0001$. Different letters within a column indicate significant differences between treatments ($p < 0.05$). Values indicate mean ($n = 3$). C: Control; Conventional inorganic treatments: IN100: 100 kg NPK ha^{-1}; IN200: 200 kg NPK ha^{-1}; IN300: 300 kg NPK ha^{-1}; Simple treatments: PR: Phosphate rock; MAP: Monoammonium phosphate; STR: struvite; BM: Bone meal. Complex OMF treatments: OMF$_1$(PR+C): Phosphate rock + Compost; OMF$_2$(MAP+C): Monoammonium phosphate + Compost; OMF$_3$(STR+C): Struvite + Compost; OMF$_4$(BM+C): Bone meal + Compost.

Regarding nutrient use efficiency indexes, the best phosphorus use efficiency (PUE) results correspond to the complex treatment of OMF$_3$(STR+C) followed by OMF$_2$(MAP+C), with values of 8.33% and 7.40%, respectively. These results are significantly better than those obtained with the simple treatments MAP and STR, with values of 5.33% and 4.67% PUE, respectively. The complex treatments OMF$_3$(STR+C) and OMF$_2$(MAP+C) exhibited

%PUE values close to those of the inorganic treatments IN100, IN200 and IN300 (11.60%, 9.33%, 7.97% PUE, respectively). PR and BM did not present significant differences between their simple and complex forms. BM-based treatments presented the lowest PUE (Table 6). Nitrogen use efficiency (NUE) results are comparable to PUE. The complex treatment OMF$_2$(MAP+C) obtained the best NUE (75.33%), followed by the simple treatment MAP (64.33%) and OMF$_3$(STR+C) (59.33%). As in the case of P, the addition of compost had a significant effect improving N uptake in OMFs with respect to the simple treatments MAP and STR. Pelletization with compost had no effect in the case of the PR and BM fertilizers. The organic fertilizers BM and OMF_4(BM+C) had the lowest NUE (13.00% and 8.67%, respectively. In general, NUE had greater values than PUE. The highest values of potassium use efficiency (KUE) were observed for the inorganic treatments IN 100 and IN 200 (24.80% and 25.80%, respectively). As in the cases of NUE and PUE, the complex treatments OMF_3(STR+C) and OMF_2(MAP+C) showed better KUE values compared to the simple treatments.

Comparing the different treatment groups, the inorganic treatments show the highest NUE, PUE and KUE values. Among the phosphorus treatments, though not significantly, the complex treatments group shows the greatest values, indicating a trend in higher nutrient use efficiency as a consequence of compost addition in OMF treatments (Table 7).

Table 7. Comparison between treatment groups on lettuce N and P nutrient uptake and nutrient use efficiency at harvest.

Treatment	N uptake (g N pot^{-1})	NUE (%)	P uptake (g P pot^{-1})	PUE (%)	K uptake (g N pot^{-1})	KUE (%)
Control	0.05 a		0.001 a		0.01	
Conventional inorganic treatments	0.16 b	7.42 b	0.009 c	9.63 b	0.03	20.59 b
Simple treatments	0.14 b	6.42 a	0.004 ab	2.80 a	0.04	3.19 a
Complex-OMF treatments	0.15 b	6.42 a	0.008 bc	4.04 a	0.04	4.68 a
F-ANOVA	*3.04 **	*4.26 **	*3.10 **	*14.76 ****	*2.49 ns*	*36.03 ****

NUE/PUE/KUE: nitrogen/phosphorus/potassium use efficiency, respectively. *, ***: significant difference between treatments at $p < 0.01$ and $p < 0.0001$, respectively. ns: not significant. Different letters within a column indicate significant differences between treatments ($p < 0.05$). Values indicate mean ($n = 3$).

Metabolomic Study

The metabolomics data obtained provides a picture of the metabolic state of the plants at the moment of harvest.

We studied the levels of fundamental metabolites involved in energetic metabolism (glycolysis and TCA cycle). Sugars like glucose, which is the primary substrate for glycolysis, are significantly augmented in plants treated with OMF_3(STR+C) followed by OMF_2(MAP+C), STR and MAP (Table S3). This means that in these plants glucose is not being degraded through glycolysis, so it is being accumulated. On the contrary, significantly lower glucose levels are detected in plants treated with PR, BM, OMF_1(PR+C) and OMF_4(BM+C), with no significant differences among them, nor with control plants. This result suggests a higher energy demand in these plants. The TCA cycle's initial intermediate citrate levels are significantly higher in plants treated with BM, OMF$_1$(PR+C), OMF$_4$(BM+C) and PR, which reinforces the idea of a more active energetic metabolism in these plants (Table S2). Other TCA cycle intermediates such as succinate also accumulated in these plants.

Among organic acids, tartrate concentration was significantly higher in the simple treatment group than in the complex OMF treatment group.

In general, amino acids presented significantly higher levels at harvest in plants treated with PR and BM simple and complex OMF treatments (Table S1).

GABA and myoinositol presented significantly higher concentrations in plants treated with OMF$_2$(MAP+C) and OMF$_3$(STR+C).

4. Discussion

Effects of Fertilization on Quality and Yield

The organomineral MAP and STR with compost fertilizers showed significantly higher yield with respect to fertilizers in their inorganic form. These results are in concordance with those obtained by Carciochi et al., in which OMFs exhibited a 13% increase in wheat yield with respect to inorganic MAP plus urea in a 3 year field experiment on a wheat crop [30]. In a pot trial using lettuce and cabbage, a 50% dose of OMF-P produced at least 11% higher fresh matter and dry matter than 100% mineral fertilization [31].

De Morais et al. found that OMFs formulated with MAP$^{25\%}$ChickenManure$^{37.5\%}$ CoffeeHusk$^{37.5\%}$ increased maize shoot biomass in ~26%, and shoot P in ~22% over MAP- fertilized plants [32]. Araújo et al. evaluated the growth of millet cultivated in sandy soil in a greenhouse experiment under fertilization with MAP, and OMF prepared with MAP and organic compost of waste from small ruminant production. They found that, considering the total accumulated in the two cuts of millet, the OMF promoted a higher nutrient content compared to MAP [33]. In the study by Erenog˘ lu et al., the OMF-P-fertilized plants showed higher plant P concentrations and P uptake than the mineral fertilizers, with a more noticeable effect at the P rates of 25 and 50 mg kg^{-1} soil [34]. De Sousa and Alleoni, in a greenhouse experiment of two successive maize crops, concluded that STR and an OMF composed of chicken manure and triple superphosphate (TSP) had higher agronomic efficiencies than TSP in sandy soils. STR application increased plant tissue P concentrations. The OMF also benefited P uptake, mainly in the second crop cycle. The authors discuss that this effect may be explained by their slow nutrient release properties that would enable sustained nutrient availability over multiple crop cycles [35].

In general, struvite performed similarly to MAP in terms of yield and P uptake. In various assays using acidic soils, struvite performed similarly to inorganic soluble P fertilizers in terms of

P uptake [36,37]. Erdal et al. also obtained comparable yield and P uptake results in lettuce plants fertilized with struvite and MAP in a 60 day experiment [38].

N-use efficiency (NUE) was higher in the inorganic treatments (IN100, IN200) and in complex fertilizer OMF$_2$(MAP+C), outperforming its correspondent simple fertilizer MAP in 11%; also, OMF$_3$(STR+C) was higher than STR alone. This indicates that simple inorganic fertilizer improves its productivity when combined with compost. This effect has also been observed in other studies comparing urea and blended fertilizers of urea and biochar as organic matter [39,40].

The addition of compost seems to have had a positive effect on P use efficiency in the case of MAP and STR, but not in the case of PR and BM. The effects of the different OMFs are dependent on the P sources used [41]. The P uptake of OMF$_1$(PR+C) and OMF$_4$(BM+C) was lower than that of OMF$_2$(MAP+C) and OMF$_3$(STR+C), probably due to the high Ca content of these OMFs, which can form bonds with P in the organic matrix, thereby decreasing its availability [42].

KUE was higher in the case of conventional inorganic treatments, probably due to overfertilization. As K fertilization was imbalanced, this could lead to the leaching of soluble potassium, which would prevent its absorption by the plant [43]. As reported by Yin et al. [44], higher doses of K present lower KUE.

According to Uddin et al. [10], organomineral fertilizers release nutrients slowly according to plant needs throughout the growing season, which enables the plants to uptake nutrients more efficiently, resulting in better plant growth and development.

Effects of Fertilization on Soil Properties

The pH was slightly alkaline in all the soils, and no significant differences were observed between treatments for pH values. Similar results were observed in a study about the effect of soil quality on lettuce production using alternative sources of P (struvite) compared with the use of other conventional P fertilizers, including monoammonium phosphate (MAP) [45]. The conductivity values were slightly lower in the treatment groups compared to the control group, possibly due to the leaching of salts due to irrigation.

Soils treated with OMFs had the highest OM percentages, higher than those of conven- tional inorganic and simple treatments, probably due to the effect of compost. Phosphorus OMF treatments also had higher OM values than the control in a pot experiment with perennial ryegrass [46]. According to Toprak and Seferoglu's research, p-OMFs increased the soil organic matter (SOM) by 60.1% [47].

Soil nitrate content was significantly lower in soils treated with OMF$_3$(STR+C) and OMF$_2$(MAP+C), matching high NUE values, indicating high nitrate consumption by the plant for these treatments. Nitrate content was higher in organic simple and complex OMF fertilizers, suggesting an immobilization of mineral N in the soil.

The concentration of available phosphorus in the soil after the assay was higher for all treatments and the control compared to the initial soil. The soils fertilized with MAP and OMF-MAP treatments had the highest concentration of available phosphorus at the end of the experiment. The available phosphorus value of the MAP treatment was similar to that of the conventional inorganic treatment IN300. Although not significantly, Mancho et al. observed that the highest concentration of available P in the soil was found for the MAP treatment [45].

In our study, available P significantly increased in the soils treated with the organomin- eral fertilizers OMF_1(PR+C) and OMF_4(BM+C) when compared to simple treatments. In the case of OMF_3(STR+C), there was a slight increase in available P with respect to STR, though it was not significant. These results are in concordance with those obtained by Erenog˘ lu et al. in a pot experiment in wheat [34], in which, after 90 days, available P in soils treated with the OMF treatment at all studied rates was significantly higher than in plants treated with the mineral fertilizer. This effect was also described by Adebayo et al. in a field experiment with *Moringa oleifera*. They described higher P soil concentrations for the OMF applied at 30 tons ha^{-1}, than the rest of the fertilizers evaluated: NPK (15:15:15), cow dung and poultry manure [48]. Frazão et al. observed that significantly higher amounts of available P to maize plants were obtained using organomineral phosphate fertilizers in a study comparing these OMFs with triple superphosphate [49]. Similarly, biochar-based OMFs showed greater agronomic efficiency and greater P availability after harvest in maize [50]. P availability increase by OMF fertilizers was also described by Borges et al. in sugarcane [51]. This effect may be caused by the extra supply of OM with complex OMF treatments (see Table 4).

As seen in the individual PCA of soil parameters (Figure 4), clearly significant differences are observed between treatments. Treatments IN100, IN200, IN300 and OMF_2(MAP+C) are located in the left region, close to the horizontal axis, indicating high amounts of assimilable P. MAP and C treatments are located in the lower left region, which means that they present low amounts of OM, pH and NH_4^+ and high amounts of assimil- able P. The treatments OMF_3(STR+C), STR and PR are located in the upper right region, indicating that these present high amounts of OM, pH and NH_4^+. In addition, a downward diagonal trend is observed between these treatments, suggesting that the OMF_3(STR+C) treatment presents medium levels of assimilable P, and higher amounts of NH_4^+, while the PR treatment presents lower amounts of assimilable P and higher amounts of OM. Finally, BM, OMF_1(PR+C) and OMF_4(BM+C) treatments are located in the lower right region, indicating higher amounts of NO_3^-, TN and EC, as well as lower amounts of extractable P.

Effects of Fertilization on Metabolomics

Plants fertilized with PR and, especially, BM treatments had a significantly lower yield than plants fertilized with MAP and STR treatments at the end of the assay, which is consistent with a greater activation of anabolic metabolism for molecule synthesis and growth promotion in the first plants. This is in concordance with the results by Matamoros et al., who concluded that under low nutrient availability, the activity of the TCA cycle was up-regulated [52], since it is necessary for ATP production and for providing the precursors used in many biosynthetic pathways [53,54]. Gao et al. proposed increased citrate and succinate levels as low-phosphorus stress biomarkers in lettuce plants [55].

Figure 4. Individual PCA of soil parameters. C: Control; Conventional inorganic treatments: IN100: 100 kg NPK ha^{-1}; IN200: 200 kg NPK ha^{-1}; IN300: 300 kg NPK ha^{-1}; Simple treatments: PR: Phosphate rock; MAP: Monoammonium phosphate; STR: struvite; BM: Bone meal. Complex OMF treatments: OMF1(PR+C): Phosphate rock + Compost; OMF2(MAP+C): Monoammonium phos- phate + Compost; OMF3(STR+C): Struvite + Compost; OMF4(BM+C): Bone meal + Compost.

On the contrary, sugars including glucose, fructose and sucrose were found to be significantly augmented in plants treated with MAP and STR simple treatments, and to a greater extent, in the case of complex-OMF treatments. This is consistent with the results of Hurtado et al., who suggested that the use of high-nutrient-content fertilizers promotes carbon accumulation [56]. As is the case in the present study, Chandrou et al. also found augmented amino acid concentrations in plants treated with phosphate rock and bone meal [57], indicating an overactivation of amino acid biosynthesis in these plants. Among the 21 proteinogenic amino acids, arginine has the highest nitrogen-to-carbon ratio, making it especially suitable as a storage form of organic nitrogen [58]. The increase in alanine concentration, as a precursor of acetyl-CoA, is another indicator of metabolism activation [59]. Both amino acids presented significantly higher levels in plants treated with bone meal and phosphate- rock-based treatments.

Myoinositol levels are implicated in growth and development regulation through their participation in key cellular processes, including the biogenesis of the cell wall and membrane structures, phosphate storage, cell signaling, and cell resistance to external stress factors [60,61]. Conventional inorganic treatments, OMF_2(MAP+C) and OMF_3(STR+C), presented the highest levels of this metabolite.

Another crucial molecule in plant growth regulation is the non-protein amino acid gamma-aminobutyric acid (GABA) [62]. Similarly, to myoinositol concentration, a higher GABA concentration was detected in plants treated with OMF_2(MAP+C) and OMF_3(STR+C).

Tartrate is a product of ascorbic acid oxidation. Its increased levels could be an indicator of antioxidant mechanisms. Tartaric acid derivatives have been described as the main up-regulated phenolic acid components in stressed plants [42,63]. Phosphate rock-based treatments presented high tartrate levels. Tartrate concentration was, in general, significantly augmented in the simple treatment group with respect to the complex OMF treatment group. Interestingly, the levels of this metabolite presented a lower concentration in plants treated with MAP and STR complex OMF treatments than in plants treated with MAP and STR simple treatments.

When correlating plant metabolites and macro and microelements at harvest (Figure 5), it was observed that P (%) positively correlated with sucrose, glucose, fructose, myo-inositol, chlorophyll, GABA and K (%), while it correlated negatively with the vast majority of amino acids and organic acids, including TCA cycle intermediates and tartrate.

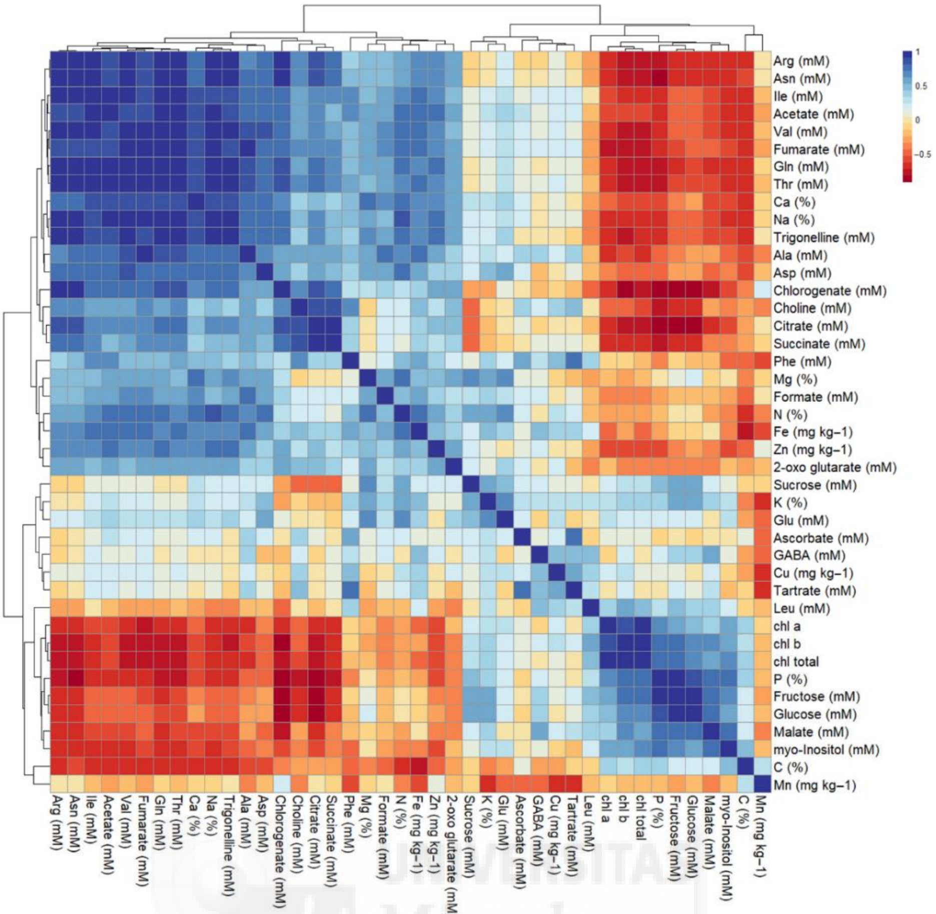

Figure 5. Heatmap correlating plant metabolites and macro and microelements.

5. Conclusions

The complex OMF STR and MAP treatments produced the best results in terms of yield, plant phosphorus (P) uptake and percentage phosphorus use efficiency (PUE), with significant differences with respect to simple MAP and STR treatments, and PR- and BM-based treatments.

The application of complex treatments incorporating compost improved levels of OM and Pext in soils when compared to simple treatments.

Significantly higher levels of GABA and myo-inositol were found in plants treated with OMF_2(MAP+C) and OMF_3(STR+C) than in plants treated with the remaining simple and complex phosphorus treatments. High levels of these metabolites are important biomarkers of plant homeostasis.

High levels of TCA cycle intermediates, amino acids, and tartrate may be indicators of plant low-phosphorus stress at harvest. These metabolites were augmented in plants treated with PR and BM simple and complex-OMF treatments.

According to their metabolic state, plants treated with OMF_2(MAP+C) and OMF_3(STR+C) better activated protective mechanisms towards oxidative stress, while the contrary hap- pened in plants treated with PR- and BM-based treatments.

The results of this work demonstrate the efficiency of compost addition through pelletization as a novel fertilizing strategy for improved plant and soil quality post-harvest, specifically in combination with mineral P sources like monoammonium phosphate (MAP) and struvite (STR). In addition, it proves the fertilizing potential of struvite, with results equivalent to those obtained with the widespread mineral fertilizer MAP.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/agronomy15071661/s1: Figure S1: Experimental setup at EPSOUMH greenhouse; Figure S2: Chlorophyll content dynamics measured non-destructively during this study (t1 = 10 days, t2 = 25 days, tf = 45 days). Table S1: Amino acid concentration (mM); Table S2: Organic acid concentration (mM); Table S3: Water-soluble sugar and alcohol concentration (mM).

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8.2. Publicación 2: Sustainable Phosphorus and Protein Recovery from Different Organic Wastes: Process Optimization and Struvite Precipitation Potential. 2025.

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Sustainable Phosphorus and Protein Recovery from Different Organic Wastes: Process Optimization and Struvite Precipitation Potential

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Abstract: Currently, researchers are exploring alternative phosphorus sources for agricultural production that are more sustainable than rock phosphate. In this context, the recovery of phosphorus from organic wastes as struvite can constitute an important tool for promoting circular economy practices and reducing the risk of phosphorus contamination through eutrophication. Struvite recovery has been widely developed using different organic wastes with high concentrations of N and P, such as industrial, municipal and animal wastes, mainly in the form of effluents. However, little information is available concerning phosphorus recovery in the form of struvite from sewage sludge samples, these processes being mainly based on chemical procedures. Therefore, the main aim of this work was to study phosphorus recovery from three sewage sludge samples from different wastewater treatment plants (SS1, SS2 and SS3), in comparison with the solid fraction of pig manure (M), through an optimized bioacidification process, as well as to evaluate the potential for struvite precipitation from the recovered P-rich supernatants. Protein recovery through alkaline treatment of the remaining precipitates was also studied. The results obtained showed the feasibility of the optimized bioacidification process for P recovery, especially in the samples M and SS3, which showed the highest P recovery yields (65.7% and 69%, respectively) and the best results regarding struvite formation. In addition, the protein recovery efficiency of the remaining solid residues ranged from 59.3% to 67.4%, without showing a clear influence of the type of organic waste used.

Keywords: bioacidification; FESEM-EDX; phosphorus extraction; pig slurry; sewage sludge; struvite; XRD.

1. Introduction

Phosphorus is an essential nutrient in plant nutrition. The availability of phosphorus poses a challenge for food production maintenance in a global context of population growth. The P fertilizer industry relies mostly on P_2O_5 rock (PR), which is a non-renewable resource generally existing in old marine deposits, with the associated physical, economic, energy or legal constraints [1]. PR reserves are concentrated in a few countries. Morocco alone holds 77% of global reserves, estimated at 50,000 tons [2]. PR is manufactured into inorganic orthophosphate-based fertilizers, such as monoammonium phosphate, diammonium phosphate and triple superphosphate [3]. Traditional inorganic P fertilizers use is associated with inefficient P use in soils, with the majority of this nutrient not being used by plants and being immobilized into soils or leached towards water bodies [4]. P is irregularly distributed in soil, with surpluses around extensive farms and urban areas [5].

Therefore, it is crucial to find alternative phosphorus sources to achieve independence from geopolitical factors and to improve phosphorus use efficiency by crops to ensure global food production. The direct application of compost or stabilized sludge, manure, digestate from anaerobic digestion or sludge ashes is a widespread strategy that is being limited due to concerns about P leaks and heavy metals, antibiotic residues and pathogen contamination [6]. Thus, further processing of these residues to obtain environmentally friendly, specific fertilizers is desirable. Recovery of nutrients such as P from these residues is important to promote circular economy practices and reduce the risk of P pollution through eutrophication. In this sense, the current most popular recovered P mineral from organic wastes is struvite ($MgNH_4PO_4 \cdot 6\ H_2O$), a P mineral formed from magnesium (Mg^{2+}), ammonium (NH_4^+) and orthophosphate (PO_4^{3-}) at a molar ratio of 1:1:1. Due to its composition (approximately 10% magnesium (Mg), 7% ammonium (N), 39% phosphate (P) and 44% crystal water by mass [7], this mineral has gained significant interest in recent years as potential substitute for inorganic fertilizers based on PR, which also implies sustainable practices in nitrogen and phosphorus recycling [8]. Furthermore, organic production systems, usually based on organic fertilizers such as manures or composts, demand highgrade natural fertilizers to increase their productivity [9]. In this sense, 'recovered struvite and precipitated phosphate salts' have been approved for use in organic production in the European Union by their inclusion in annex II of Regulation 2021/1165 [10].

The recovery of phosphorus as struvite has been assessed in different organic wastes with high concentrations of N and P, such as industrial, municipal and animal wastes [11] (Table S1). Thus, the recovery of struvite has been studied in the wastewaters from different industries, such as slaughterhouse [12], food processing [13] and even textile [14], among others [11]. In the case of animal wastes, the recovery of P as struvite has been mainly reported in swine manures [15–19] and their anaerobic digestates [20,21], as well as in dairy manures [22,23]. However, municipal wastes, mainly wastewater, have been one of the main feedstock sources studied for struvite recovery, due to the spontaneous precipitation of this mineral in the municipal wastewater treatment plants [11]. Thus, struvite recovery is mainly developed in the effluents from anaerobic sludge digestion of municipal wastewater, since in other wastes, such as municipal wastewater, landfill leachate, human urine and ash from sewage sludge incineration, chemical supplementation or/and pre-treatments are required due to their characteristics [11]. However, studies on phosphorus recovery in the form of struvite from sewage sludge samples are scarce, with most recovery processes being mainly based on chemical processes, such as the wet oxidation [24–26].

In this context, since the main purpose is to recover phosphorus, the first step is to dissolve the inorganic solids present in the organic waste stream used as P source, which contain most of the phosphorus, usually by chemical or biological acidification [27]. Thus, the process of recovering phosphorus as struvite can be mainly based on the following steps: acidic dissolution, solid–liquid separation and pH increase in the liquid phase using magnesium oxide (MgO) or magnesium hydroxide ($Mg(OH)_2$), leading to phosphorus precipitation [28]. Different chemicals have been used to dissolve P, such as hydrochloric acid [19], formate [29] and citrate [30]. However, the acidification process using chemicals has a significant economic cost and negative relative impacts on the environment [29,31,32]. In this context, a new process alternative to chemical acidification is the biological acidification process or bioacidification. Bioacidification constitutes an innovative and environmentally friendly process in which a medium is acidified by the activity of native or added microorganisms [33,34], usually by a fermentative metabolism in anaerobic conditions [35]. To induce the process, the incorporation of easily biodegradable organic substrates such as sugar [35,36] and food waste has been reported [37]. However, very little information is available regarding the use of this process for phosphorus dissolution to facilitate its recovery from organic wastes, having been reported only recently in sewage sludge [35,36] and pig slurry [38].

After phosphorus dissolution, struvite precipitation can usually be developed by adding different salts, such as $MgSO_4$, $MgCl_2$, $Mg(OH)_2$ and MgO [39]. Generally, struvite precipitation is carried out using high concentrations of MgCl2, followed by base application (e.g., NaOH or KOH) or adding Mg in the form of MgO or $Mg(OH)_2$ and allowing the pH to increase naturally [40]. According to previous studies [41,42], the molar ratio of magnesium to phosphate must be adjusted to 1.2:1 for optimal precipitation. At low Mg concentrations, a mixture of struvite and hydroxyapatite results from the process [43]. However, one problem that remains unresolved is the processing of the remaining solids once the P extraction is completed. The principal components in wastewater sludge flocs are polysaccharides and proteins [44]. One possible processing approach is alkaline treatment to recover proteins and other macromolecules of industrial interest. Protein recovery by alkaline treatment with sodium hydroxide (NaOH) has already been implemented in carbohydrate-digested rice.

Therefore, the aims of this study were the following: (i) to accomplish P recovery from organic wastes (solid fraction of pig manure and sewage sludge) through lactic fermentation using Lactobacillus acidophilus; (ii) to study the potential of struvite precipitation from the recovered P-rich supernatants; and (iii) to recover proteins through alkaline treatment of the remaining solid residues.

2. Materials and Methods

2.1. Organic Sources Used

The sewage sludge (SS) samples used in this experiment (SS1, SS2 and SS3) were obtained from the wastewater treatment plants located at the municipalities of Almazora, Castellon and Nules (Castellon, Spain), respectively. SS1 and SS2 were produced using an activated sludge treatment and then stabilized by anaerobic digestion, while SS3 was produced through the aerobic treatment of the wastewater samples, followed by stabilization in aerobic conditions. All the sludge samples were dehydrated by centrifugation. The solid fraction of pig slurry (M) was considered as control treatment to compare the results obtained with the sewage sludge samples and it was collected from a fattening pig farm placed at Todolella (Castellon, Spain) after a solid–liquid separation using a screw press. A complete characterization of these organic materials is shown in Table 1.

Table 1. Main characteristics of the organic wastes used in the experiment. Data values expressed on a dry matter basis.

	M	**SS1**	**SS2**	**SS3**
Dry weight (%)	27.6 ± 0.3	19.8 ± 5.9	22.2 ± 0.1	24.7 ± 0.3
pH	8.9 ± 0.4	6.2 ± 0.3	7.9 ± 0.0	7.2 ± 0.2
EC (dS m^{-1})	2.3 ± 0.0	1.0 ± 0.2	1.7±0.4	1.4 ± 0.7
TC (%)	29.6 ± 0.4	32.7 ± 0.5	30.0 ± 0.4	38.4 ± 0.3
TN (%)	2.3 ± 0.8	6.1 ± 0.2	5.1 ± 0.2	7.1 ± 0.0
NH_4 (g kg^{-1})	0.9 ± 0.1	2.3 ± 0.7	4.0 ± 0.7	2.1 ± 0.5
NO_3 (mg kg^{-1})	185 ± 35	599 ± 20	175 ± 21	109 ± 16
P (%)	3.9 ± 0.2	3.3 ± 0.4	3.3 ± 0.5	2.5 ± 0.5
K (%)	1.6 ± 0.5	0.1 ± 0.0	0.1 ± 0.0	0.5 ± 0.2
Ca (%)	5.9 ± 0.1	4.7 ± 1.7	6.1 ± 0.3	3.2 ± 0.3
Mg (%)	3.1 ± 0.8	0.7 ± 0.3	0.7 ± 0.1	0.8 ± 0.3
Na (%)	0.5 ± 0.1	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0
Fe (g kg^{-1})	3.67 ± 0.01	18.4 ± 0.1	45.7 ± 0.2	4.65 ± 0.01
Cu (mg kg^{-1})	317 ± 1	172 ± 2	222 ± 0	183 ± 0
Mn (mg kg^{-1})	1198 ± 1	108 ± 0	177 ± 1	59 ± 2
Zn (mg kg^{-1})	1355 ± 0	2209 ± 0	1006 ± 1	1206 ± 0
Cd (mg kg^{-1})	0.31 ± 0.04	2.71 ± 0.80	1.35 ± 0.38	1.00 ± 0.25
Cr (mg kg^{-1})	13.2 ± 2.9	196 ± 2	97.3 ± 3.5	33.9 ± 2.1
Hg (mg kg^{-1})	0.66 ± 0.02	2.99 ± 0.03	1.81 ± 0.78	0.94 ± 0.31
Ni (mg kg^{-1})	19.9 ± 1.5	118 ± 2	145 ± 5	35.8 ± 0.3
Pb (mg kg^{-1})	< 0.01 ± 0.00	67.9 ± 1.7	156 ± 0	42.7 ± 1.2

M: solid fraction of pig slurry; SS1, SS2 and SS3: sewage sludge samples from wastewater municipal treatments plants of the municipalities of Almazora, Castellon and Nules, respectively. EC: electrical conductivity; TC: total organic carbon: TN: total nitrogen. Data values are reported as mean value ± standard deviation.

2.2. General Procedure for Phosphorus Extraction Through Fermentation

The general methodology used for the phosphorus acid extraction and protein recovery through fermentation was based on a modified version of the procedure of Vanotti and Szogi [45]. Briefly, biological materials, such as organic wastes, are dissolved in an acidic solution; in this study, the solution was developed by fermentation. The resulting supernatant from the acidic solution (acidic supernatant) may then be separated and used for phosphorus recovery. The resulting precipitate from the acidic treatment (acidic precipitate) may be separated from the supernatant and treated with an alkaline solution, obtaining a supernatant (alkaline supernatant) that can be separated and used to extract proteins. In this study, the phosphorus acid extraction was carried out trying to optimize the fermentation process to obtain the acid precursor solution. For this, a L. acidophilus suspension was studied, along with different factors, such as the sugar source, the sugar to waste ratio and the incubation time for the fermentation.

Lactobacillus acidophilus suspension was prepared by dissolving 1.1 g *L. acidophilus* (Lacto10, L. acidophilus SGL11 strain, Forza Vitale Italia srl., Corato, Italy) in 25 mL of deionized water. L. acidophilus was used in lyophilized form at a dose equivalent to 10^8 UFC/mL. The mixture was centrifuged at 3600 rpm for 10 min, and the supernatant was discarded. Then, 20 mL of water were added to the remaining solid to obtain the L. acidophilus suspension.

The general procedure for the acid extraction of phosphorus through fermentation in all the assays was the following: an acid precursor solution based on the mixture of an optimized weight of a sugar source and the L. acidophilus suspension was mixed with the corresponding organic waste (2 g dry basis) in a 10:1 ratio (volume/weight) of precursor to residue. The different mixtures (depending on the organic waste used) were incubated in a thermostatic bath at a constant temperature (37 ◦C), in darkness and under continuous agitation (shaking frequency of 35 rpm) for an optimized incubation time, monitoring the pH decrease during the fermentative process. Afterward, the mixtures were centrifuged at 3600 rpm for 30 min to obtain the acidic supernatant and a first precipitate (acidic precipitate, pp1). One rinse of this precipitate was performed by the addition of an equivalent volume (20 mL) of water and centrifuged under the same conditions, obtaining a supernatant and a second precipitate (pp2). Afterward, the phosphorus concentration was determined in the cidic supernatant and in the supernatant resulting of the precipitate rinse, being expressed as the sum of both contributions. The resulting precipitate from the rinse procedure (pp2) was pasteurized at 70 ◦C for 1 h and used for the alkaline protein extraction.

2.3. Optimization of Phosphorus Extraction Through Fermentation: Sugar Source, Ratio Sugar/Waste and Incubation Time

The fermentation to extract phosphorus from each selected type of organic waste was optimized using the general procedure previously detailed, but considering two different factors: (a) the sugar source and sugar-to-waste ratio; (b) duration of the fermentation process.

2.3.1. Optimization of the Sugar Source and Sugar-to-Waste Ratio

Two sugar sources, sucrose (Condalab, S.A., Madrid, Spain) and molasse from sugar cane (Poballe, S.A., Barcelona, Spain), were used in the fermentation process for phosphorus extraction. Different amounts (0.25, 0.5, 1, 2 and 3 g) of each type of sugar were added to the fermentation media, respectively. A control assay with no sugar addition was also established.

2.3.2. Incubation Assay to Optimize the Incubation Time

Different incubation times were applied (0 h, 6 h, 12 h, 24 h, 36 h and 48 h), monitoring the pH during the incubation process and considering that fermentation had been completed when pH was stabilized.

2.4. Alkaline Protein Extraction

The precipitate resulting from the rinse procedure (pp2) was mixed with a 0.4 M NaOH solution (pellets for analysis, ACS, ISO, ITW Reagents Panreac, Barcelona, Spain) with stirring for 20 min, followed by homogenization with ULTRA-TURRAX for 10 min and centrifugation at 3600 rpm for 30 min to obtain an alkaline supernatant and an alkaline precipitate (ap). This precipitate was rinsed with 20 mL of water and centrifuged under the same conditions, obtaining a second supernatant (rinse supernatant). The total protein extracted was determined in both supernatants (alkaline and rinse supernatant) using the Bradford assay [46] and expressed as the sum of both contributions.

2.5. Analytical Characterization of the Organic Wastes and Precipitates

The pH and electrical conductivity (EC) of the organic wastes were determined in a 1:10 (w/v) water extract, while nitrate (NO3−) and ammonium (NH4+) were measured in a 1:5 (w/v) extract with 0.2 M KCl using a multiparametric analyzer K-365 Dist Line (Buchi Labortechnik AG, Flawil, Swiss). The rest of the parameters in all the samples (organic wastes and recovered P precipitates, pp1 and pp2) were analyzed. The total C and N contents were determined using an automatic elemental microanalyzer (EuroVector, Milan, Italy). In the acid extract obtained after microwave digestion with HNO3 69% (reagent for analysis, ACS, ISO, ITW Reagents Panreac, Spain), P was assessed colorimetrically as molybdovanadate phosphoric acid; K and Na were determined by flame photometry (Jenway PFP7 Flame Photometer, Jenway Ltd., Felsted, Dunmow, Essex, UK); and Ca, Mg, Fe, Mn, Cd, Cr, Cu, Pb, Zn, Ni and Hg were determined by induced coupled plasma mass spectroscopy (ICP-MS), following the methods described by Morales et al. [47].

2.6. Struvite Precipitation and Mineralogical Characterization

The acidic supernatants obtained were used for P precipitation as struvite ($(NH_4)MgPO_4{\cdot}6{\cdot}H_2O$), according to Vanotti and Szogi [45]. Briefly, MgCl2 was used as the Mg source due to its high solubility, which favors the formation of a precipitate with a high purity degree. The reagent was added at a dose to maintain a molar Mg:P ratio of 1.2:1. Alkalinization with a solution of NaOH 4 M was also needed to reach a pH of 9. The acidic supernatants collected from the P extraction phase were placed in a beaker under continuous stirring. The 4M NaOH solution was added drop by drop and pH was monitored throughout the whole process. Precipitates were separated from the liquid phase using microfiber filters and a vacuum system, and they were dried in an oven at a temperature below 60 ◦C for 24 h.

The morphological structure of these precipitates was analyzed using X-ray diffraction analyses, carried out using a Bruker D8-Advance with a Goebel mirror (non-planar samples) and a high temperature chamber (up to 900 ◦C), equipped with a KRISTALLOFLEX K 760-80F x-ray generator (power: 3000W, voltage: 20–60 KV and current: 5–80 mA) and a copper anode RX tube (Bruker Corporation, Billerica, MA, USA). In addition, the precipitates were analyzed using a Schottky-type Sigma 300 VP field emission scanning electron microscope (FESEM) with a coupled energy dispersive X-ray system (EDX) to determine their element composition (Carl Zeiss Microscopy GmbH, Oberkochen, Germany). Samples were imaged under 15 kV electron high tension and using a backscattered secondary electron detector. The samples were covered with a Cr layer of 10 nm. The obtained results were also analyzed through a Rational Mineralogical Analysis [48] to establish the relative proportions of struvite and hydroxyapatite in the samples.

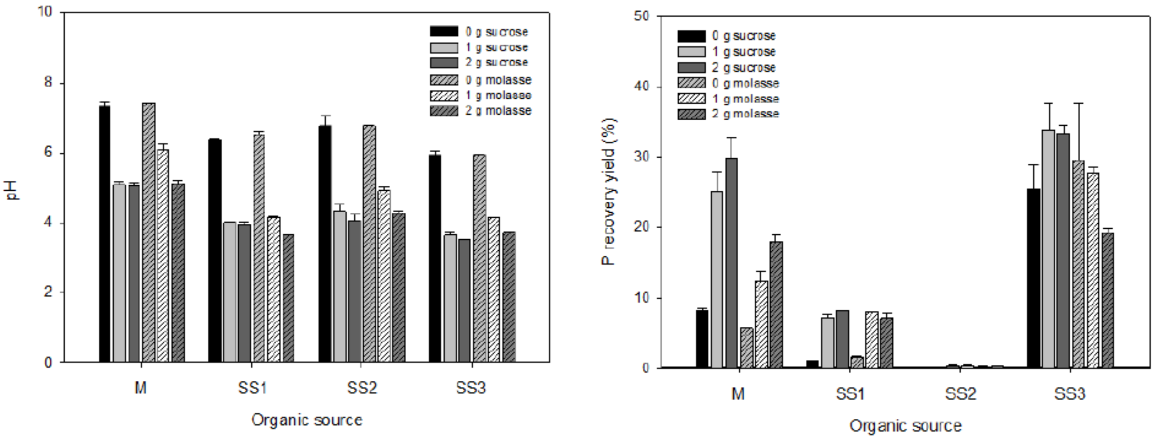
2.7. Statistical Analysis

The statistical analyses conducted to assess the effects of the two factors considered (sugar source and amount) in the fermentation media were conducted using a two-way analysis of variance (ANOVA). The results of the experiment to evaluate the optimal incubation time were analyzed using a repeated-measures analysis of variance within a general linear model. The differences in the phosphorus and protein recovery yields depending on the organic source used were determined using ANOVA. The post hoc analysis was conducted using the Tukey-b test at $p < 0.05$. The statistical analyses were conducted using IBM SPSS Statistics v. 29.0 statistical software package (IBM Corp. Released 2020. Armonk, NY, USA).

3. Results and Discussion

3.1. Optimization of the Phosphorus Extraction: Sugar Source and Ratio

The first step of the procedure optimization in the fermentation process was based on the selection of the best sugar source and the optimal ratio. For this, as described in the previous section, two different sugar sources (sucrose and molasses) were added to the fermentation media, in different amounts (0.25, 0.5, 1, 2 and 3 g), with a control treatment without sugar addition, to study the effects of both factors on the pH of the fermentation media and consequently, on P recovery yield (Figure 1). The different sugar amounts and the type of sugar had a statistically significant effect (two-way analysis of variance, $p < 0.001$) on the pH of all the fermentation media (Figure 1a), showing that both sugars had a clear acidification effect on the media of all the organic wastes used, indicating a low buffer capacity in these wastes. In the case of molasses, increasing the amount of sugar resulted in an almost linear acidification of the fermentation medium for all the organic sources. This behavior differed for sucrose, which did not show linear acidification with increasing sugar amounts. However, in general, sucrose showed the highest acidification effect compared to molasse, revealing the lowest pH values (two-way analysis of variance, $p < 0.001$) at 2 g of this sugar. This effect on the bioacidification process is similar to that observed by Piveteau et al. [27] in a study of phosphorus recovery as struvite from pig slurry by biological acidification using sucrose as the sugar source. However, P recovery efficiency was significantly influenced by both the type and amount of sugar source used for the precipitate from M (two-way analysis of variance, $p < 0.001$). The highest P recovery yields for M and SS1 were obtained from fermentation media with sucrose. For SS1, recovery efficiency was influenced only by the sugar amount (two-way analysis of variance, $p < 0.001$) (Figure 1b).



(a) (b)

Figure 1. Final pH values in the P recovery extract (**a**) and phosphorus recovery yield (**b**) depending on the amount and type of sugar used for the fermentation process. M: solid fraction of pig slurry; SS1, SS2 and SS3: sewage sludge from wastewater municipal treatments plants in the municipalities of Almazora, Castellon and Nules, respectively. Vertical bars represent standard deviation.

Thus, the amount of sugar seemed to show a significant effect on P recovery for both types of sugars, augmenting the efficiency with increasing amounts of sugar, except for the ermentation media of SS2 and SS3, in the latter even showing the opposite effect when molasses was used as the sugar source. The decrease in P recovery efficiency at higher sucrose concentrations and associated lower pH could be attributed to P assimilation by bacterial biomass, since when pH is close to 5, no additional P is dissolved, and biomass growth can decrease the dissolved P concentration [27].

3.2. Optimization of the Phosphorus Extraction: Incubation Time

The incubation time for the fermentation process was the other factor considered for the optimization of the bioacidification process for phosphorus extraction. For this, the pH values were assessed at different incubation times (0 h, 6 h, 12 h, 24 h, 36 h and 48 h), considering the optimal factors obtained in the first assay (2 g of sucrose) for the fermentation. The process was considered finished when the pH values were stabilized. Table 2 shows the evolution of the pH values during the fermentation process, considering the different incubation times.

The pH values significantly decreased with incubation time in the media of all the organic sources used. The sewage sludge samples (SS1, SS2 and SS3) showed lower initial pH values (repeated measures analysis, $p < 0.001$) compared to the solid fraction of pig slurry (M). After 48 h of incubation, all fermentation media reached their lowest pH values, in the range of 3.58–5.06. Su et al. [49] also reported a stabilization in the phosphorus release rates and the highest phosphate rates at 48 h of fermentation in a study of phosphorus recovery from pig manure using a co-fermentation process with food waste.

Table 2. pH values during the incubation at different times.

Organic waste	Initial pH	pH after 6 h	pH after 12 h	pH after 24 h	pH after 48 h
M1	8.73	7.51	6.49	5.24	5.06
SS1	6.13	6.00	5.49	4.43	3.97
SS2	7.10	6.32	5.60	4.87	4.13
SS3	5.90	5.38	4.88	4.12	3.58

M: solid fraction of pig slurry; SS1, SS2 and SS3: sewage sludge samples from municipal wastewater treatment plants in the municipalities of Almazora, Castellon and Nules, respectively.

3.3. Phosphorus Extraction Efficiency with the Optimized Procedure

The phosphorus recovery yield and the final pH values for each extract of the different organic wastes used are shown in Figure 2. As can be observed, the extract with SS3 as the organic source showed the lowest pH value (3.58) and consequently, the highest P recovery yield (69.0%) of all the extracts from the sewage sludge samples, which was also reported by Piveteau et al. [27] in an experiment using biological acidification to recover phosphorus as struvite from pig slurry as the organic source. However, this effect was not observed in the rest of the treatments with sewage sludge samples (SS1 and SS2), which showed low pH values and low P recovery yields, or in the treatment with the solid fraction of pig manure (M), which displayed the opposite behavior: a P recovery yield statistically similar to that of SS3 (Figure 2) but with the highest pH value (5.06).

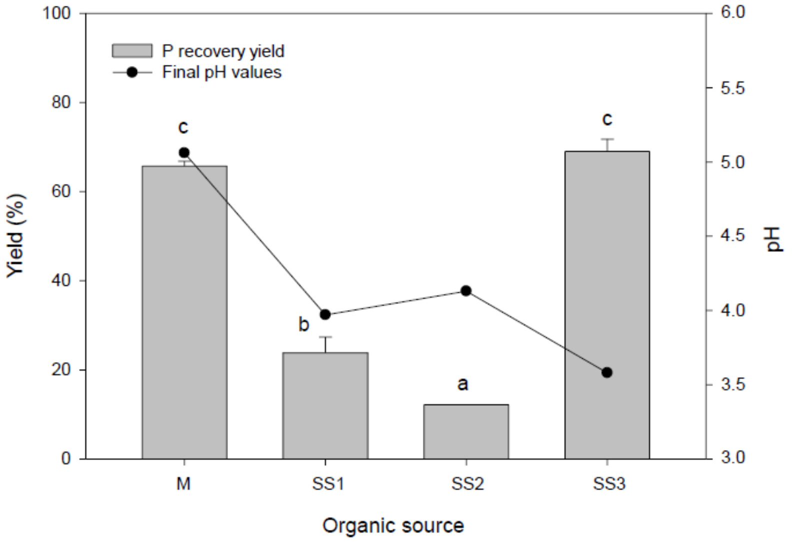


Figure 2. Phosphorus recovery yield with the optimized procedure and final pH values of the extract. Vertical bars represent standard deviation. Mean values with different letters indicate statistical differences between treatments at $p > 0.05$ (Tukey-b test).

The disconnection between dissolved phosphorus and pH could be explained by the processes of hydrolysis of solid organic P and mineralization of dissolved organic matter [27]. Thus, the different nature of the organic sources used and their different contents in organic P, which can constitute up to 22% of total phosphorus [29], could also explain the differences observed. On the other hand, other characteristics of the organic wastes used, such as their iron content, could strongly affect the efficiency of P recovery. Fe has a strong affinity for the P compounds, especially phosphates, forming stable iron–phosphate minerals, which can make P release difficult, depending on the conditions (pH, the presence of organic substances, redox conditions and particle morphology) [50]. Thus, the samples SS1 and SS2 showed the highest Fe concentrations (Table 3), which could explain the low P recovery yields found for these samples (Figure 2).

Table 3. Chemical composition of the recovered precipitates. Data are expressed on a dry matter basis.

	P-M	**P-SS1**	**P-SS2**	**P-SS3**
TC (%)	5.50	19.4	19.0	10.4
TN (%)	4.05	0.44	1.19	2.54
P (%)	7.45	5.75	4.69	7.18
K (%)	1.28	0.38	0.074	0.057
Ca (%)	2.5	10.3	5.3	5.1
Mg (%)	8.97	1.77	0.49	6.28
Na (%)	0.63	2.03	1.31	1.94
Fe (g kg^{-1})	0.73	28.5	17.6	12.0
Cu (mg kg^{-1})	47.0	7.9	20.0	3.6
Mn (mg kg^{-1})	1420	330	884	363
Zn (mg kg^{-1})	490	1079	137	86
Cd (mg kg^{-1})	<0.01	1.64	3.93	<0.01
Cr (mg kg^{-1})	3.76	37.8	108	22.3
Ni (mg kg^{-1})	4.73	103	5.50	7.68
Pb (mg kg^{-1})	2.11	11.6	44.6	6.23

P-M: precipitate from the solid fraction of pig slurry; P-SS1, P-SS2 and P-SS3: precipitates from the sewage sludge samples from municipal wastewater treatment plants in the municipalities of Almazora, Castellon and Nules, respectively. EC: electrical conductivity; TC: total organic carbon: TN: total nitrogen.

3.4. Protein Recovery Efficiency

Regarding the efficiency of protein recovery from the final precipitate (pp2) obtained from the rinse procedure, the values obtained ranged from 59.3%, obtained for the precipitate from SS3, to 67.4% for the precipitate obtained from SS1, showing no significant differences depending on the organic source (Figure 3). These values were similar or even higher to those found in other studies of protein recovery from slurry-fed microalgae using different technologies, such as sonication and alkaline treatment at pH 12 [51], alkaline hydrolysis at pH 12 and acid precipitation [52]. However, the values were lower than those reported by Vanotti and Szogi [45] from spirulina algae, where an acid step (citric acid) at pH 3.1 was followed by a second alkaline extraction at pH 12.8, and that observed by Calvo-de Diego et al. [53] in a study of an integrated biorefinery approach to simultaneously recover nutrients, proteins and energy from pig manure.

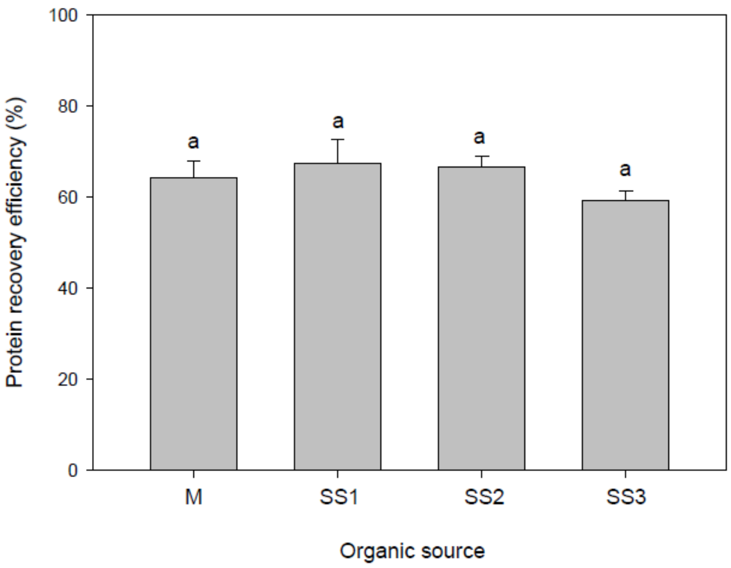


Figure 3. Protein recovery efficiency from the alkaline supernatants obtained with the optimized procedure. Vertical bars represent standard deviation. Mean values with the same letter are not significantly different at p > 0.05 (Tukey-b test).

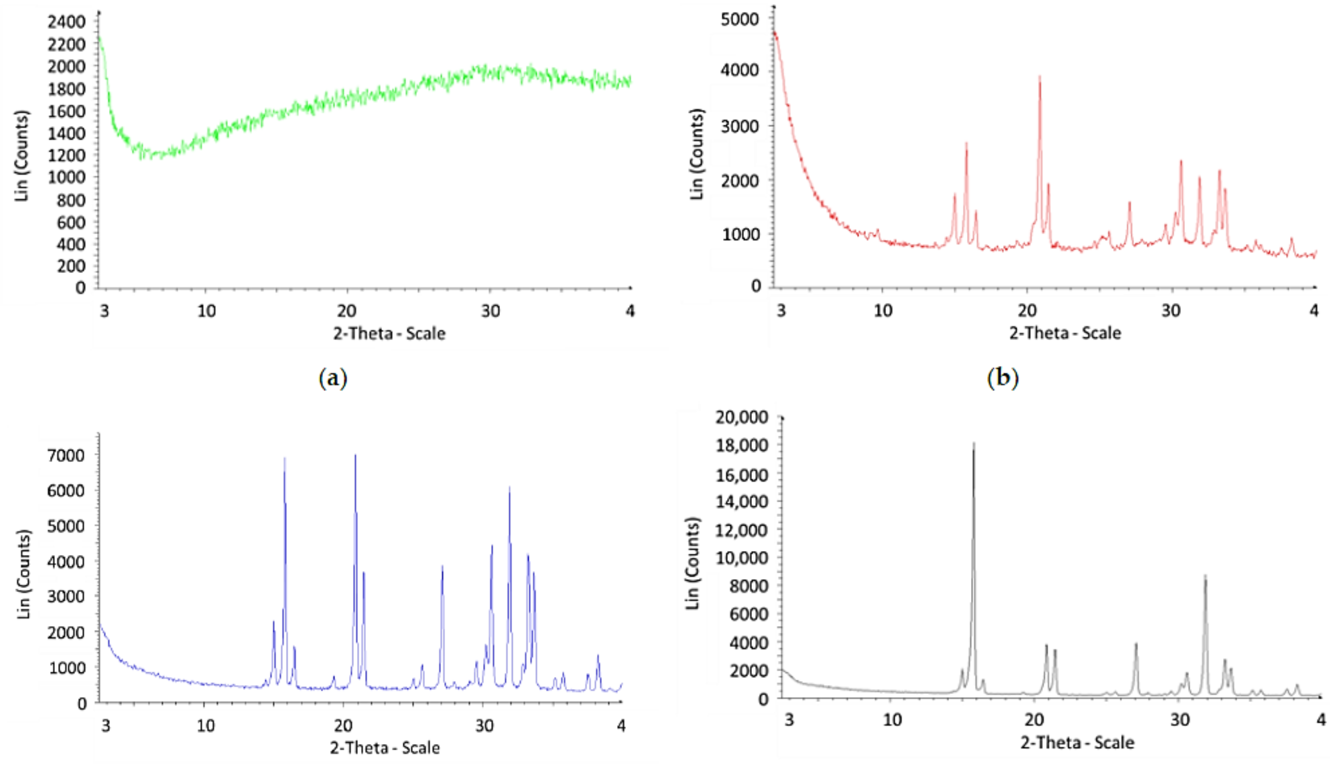
3.5. Struvite Production: Chemical and Mineralogical Characterization of the Precipitates

Table 3 shows the chemical characterization of the struvite precipitates obtained from the acidic supernatants after applying the procedure for struvite precipitation. The precipitates from the sewage sludge samples (SS1, SS2 and SS3) had higher contents of total organic C, Ca, Na and Fe compared to the precipitate from the solid fraction of pig slurry (M). However, the rest of the elements were in higher concentrations in the precipitate from M, especially the basic elements of struvite (N, Mg and P), with only the precipitate from SS3 showing similar contents on these elements (Table 3). The low contents of N, P and Mg could have influenced the efficiency of struvite recovery method, since the process of struvite crystallization depends mainly on two operational factors, pH and the Mg:N:P molar ratio [54]. The pH must be alkaline, with optimal pH values generally between 8.5 and 9.5 [19], while the Mg:N:P molar ratio must be greater than 1:1:1. Shih et al. [55] established the Mg/N/P molar ratio of 1.3:4:1 as optimal, obtaining phosphorus recovery efficiencies of 95.8%. Thus, the limiting reagent is usually magnesium ion, which must be dosed [37,54]. Furthermore, as has been previously commented in the P recovery efficiency, the highest background concentration of iron in the samples SS1 and SS2 could strongly affect the efficiency of this recovery method, especially via struvite precipitation, since iron has a higher affinity for phosphate than magnesium and can bind phosphate, making it unavailable for its recovery as struvite [50,56]. In fact, Fe is used in the municipal wastewater treatment plants to prevent struvite formation [50]. This could partly explain the low recovery efficiencies observed in the samples SS1 and SS2, which showed the highest Fe concentrations. In addition, only the recovered precipitates from M and SS3 fulfilled the criterion of P2O5 contents (>16% of the dry matter content) established in the EU fertilizing products regulation [57], also verifying the criteria concerning heavy metal content (Table 3) [58].

Concerning the mineralogical characterization of the precipitates, the X-ray diffraction (XRD) and the field emission scanning electron microscope with a coupled energy dispersive X-ray system (FESEM-EDX) techniques were applied in all the precipitates except for those from SS1, due to their almost negligible N contents (<0.5%), which limited struvite formation.

The XRD analysis is the only qualitative method used to characterize the nature of struvite [59]. Thus, the results of the XRD analyses of the precipitates showed that only those from SS3 and M (Figure 4b,c) displayed a pattern (intensity and position of the diffraction peaks) that matched the pure struvite diffractogram (JCPDS 77-2303) [60]. This was also confirmed with the pattern of commercial struvite (Figure 4d), which indicates the formation of struvite crystals [61]. However, the precipitate from SS2 showed a completely different pattern, indicating that struvite was not formed in this sample. On the other hand, the FESEM-EDX technique provides surface characterization and identifies the principal components of the precipitates [59]. The FESEM-EDX analysis of the precipitates confirmed the results obtained with the XRD analysis (Figure 5).

The precipitates contained the basic elements of struvite (O, N, P and Mg) (EDX spectra), together with traces of other elements, which was consistent with struvite composition, except for the SS2 precipitate, which had low Mg content and did not yield struvite. These results have also been reported in previous works [59,61]. In addition, with the data obtained from the EDX spectra, a Rational Mineralogical Analysis (RMA) [48] was developed to estimate the proportion of the principal mineralogical phases, with the estimation considering only the presence of struvite and hydroxyapatite. This analysis is an estimative study based on the combination of quantitative chemical and qualitative mineralogical analyses, which allows the quantitative determination of the mineralogical phases of a sample [48]. The results of the RMA carried out in the precipitates, which confirmed the presence of struvite (from M and SS3), showed estimated proportions of 94% of struvite and 6% of hydroxyapatite for the M precipitate, and of 83.2% of struvite and 16.8% of hydroxyapatite for the SS3 precipitate, also indicating the higher proportion of struvite in these precipitates. Furthermore, the FESEM images also revealed that the precipitates were mainly coarse (Figure 5c) and coffin-like (Figure 5e) struvite crystallization, respectively, in line with the findings by Xu et al. [59] in a study of phosphorus recovery from waste activated sludge.



(a) (b)

(c) (d)

Figure 4. X-ray diffraction (XRD) patterns of the precipitates obtained: (**a**) precipitate from SS2 (sewage sludge from Castellon); (**b**) precipitate from SS3 (sewage sludge from Nules); (**c**) precipitate from M (solid fraction of pig slurry) and (**d**) sample of commercial struvite.

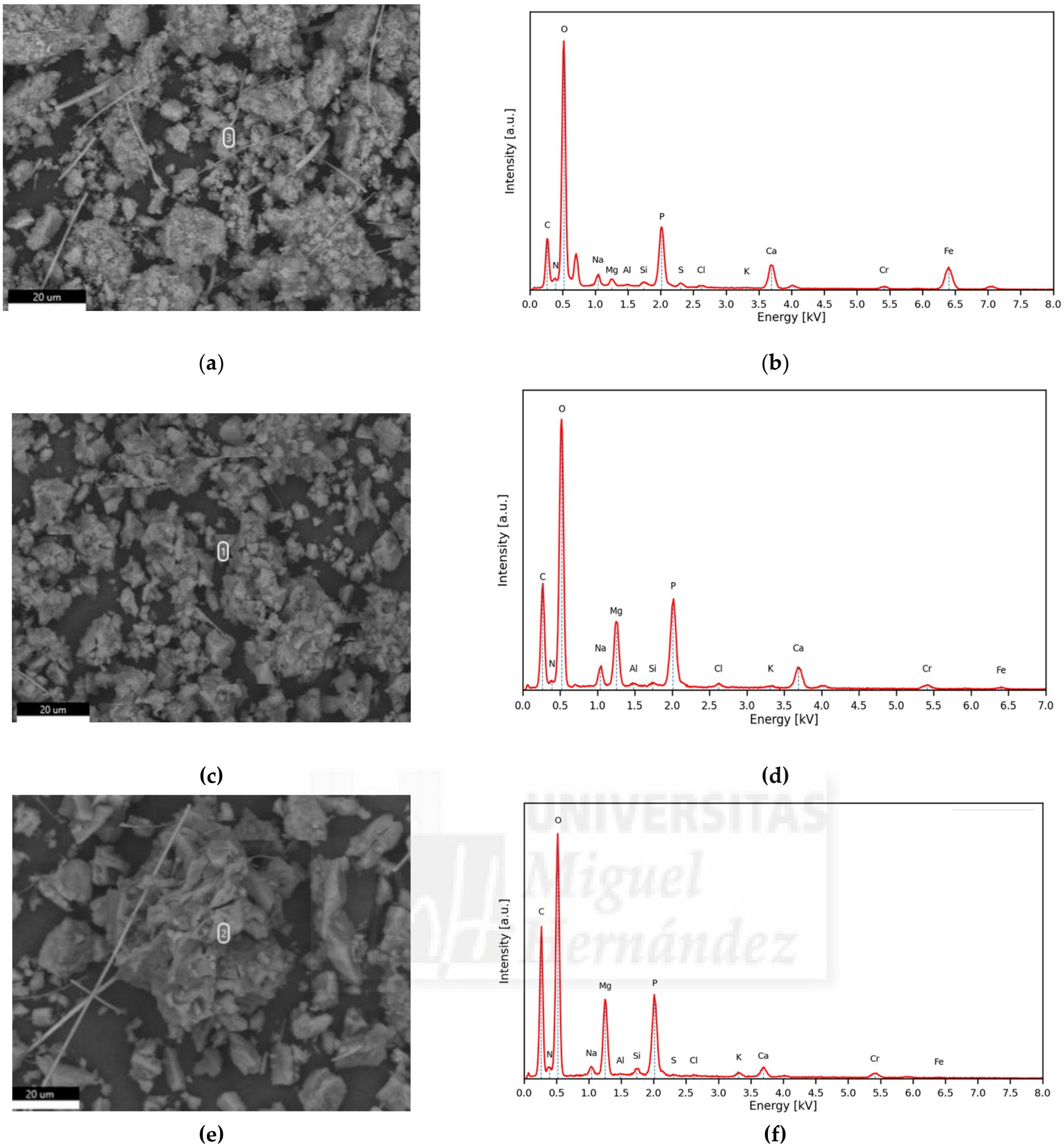


Figure 5. FESEM images with EDX of the precipitates obtained: (**a**,**b**) precipitate from SS2 (sewage sludge from Castellon); (**c**,**d**) precipitate from SS3 (sewage sludge from Nules); (**e**,**f**) precipitate from M (solid fraction of pig slurry).

4. Conclusions

The results obtained show that the phosphorus recovery process based on the modified methodology of Vanotti and Szogi [42], with an optimized bioacidification process through lactic fermentation using Lactobacillus acidophilus, constitutes a feasible alternative for phosphorus recovery from sewage sludge and pig slurry samples. The highest phosphorus recovery yields obtained were of 69% and 65.7%, for sewage sludge SS3 and the solid fraction of pig slurry (M), respectively. Moreover, the precipitates from the recovered P-rich supernatants of SS3 and M also showed the highest potential for struvite precipitation. These results show the strong influence of the organic waste characteristics on P recovery yield, especially the background Fe content of the organic sources used, which constitutes one of the main limitations for phosphorus recovery from sewage sludge samples via struvite precipitation. On the other hand, the efficiency of protein recovery through alkaline treatment of the remaining solid residues ranged from 59.3% to 67.4%, without showing a clear influence of the type of organic waste used. However, despite the promising results obtained, this study constitutes only an initial step for P recovery from sewage sludge using bioacidification processes. Thus, further research is necessary on process upscaling and operational factors, as well as on its economic cost, to confirm the viability of the process to obtain a market-ready product.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy15102305/s1, Table S1: Studies on phosphorus recovery as struvite from different organic sources in the last 15 years.

Author Contributions: Conceptualization: R.M., M.A.B., M.B.V. and E.S.; methodology: L.V.-V., M.B.V., E.M.-S., M.M.J., J.A.S.-T. and E.S.; software: L.V.-V., E.M.-S. and J.A.S.-T.; validation: L.V.-V., R.M., M.B.V., M.A.B. and M.M.J.; formal analysis: L.V.-V., E.M.-S., M.M.J. and J.A.S.-T.; investigation: L.V.-V., R.M., M.B.V., M.A.B., E.S., J.A.S.-T., E.M.-S. and M.M.J.; resources: R.M.; data curation: L.V.-V., R.M., M.A.B., J.A.S.-T., E.M.-S. and M.M.J.; writing—original draft preparation: L.V.-V., M.A.B., E.M.-S. and M.M.J.; writing—review and editing: L.V.-V., R.M., M.A.B., E.M.-S. and M.M.J.; visualization: R.M., M.B.V. and E.S.; supervision. R.M., M.A.B., E.M.-S. and J.A.S.-T.; project administration: R.M. and E.S.; funding acquisition: R.M. and E.S. All authors have read and agreed to the published version of the manuscript.

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Abbreviations

The following abbreviations are used in this manuscript:

FESEM Field emission scanning electron microscope

EDX Energy dispersive X-ray system

XRD X-ray diffraction

SS Sewage sludge

M Solid fraction of pig manure

PR Phosphate rock

N Nitrogen

P Phosphorus

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8.3. Publicación 3: Assessment of P Availability in Lettuce Crop Production Using Alternative P Sources Under Different Biotic and Abiotic Soil Conditions. 2025.

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Influence on Lettuce Crop Production and Soil Properties of using Alternative P Sources under Different Biotic and Abiotic Soil Conditions

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Abstract

The demand of alternative P sources for crop nutrition has become a crucial topic due to the increasing concern on soil contamination linked to mineral fertilizers continuous application, together with the concentration and finiteness of phosphate rock resources. In this study, the effect of several organo-minerals fertilizers based on compost and alternative P sources derived from an organic waste origin (P recovered from sewage sludge (SS1, SS2 and SS3); bone meal from meat waste (BM) and commercial struvite, STR), were compared with the same organo-mineral compost-based fertilizers using traditional P mineral sources (phosphate rock (RP); monoammonium phosphate (MAP) and triple superphosphate (TSP)) on a lettuce crop (*Lactuca sativa* L.) during two consecutive growing seasons. These treatments were also compared to the compost used as base for the organo-minerals fertilizers (C) and with a non-fertilized control treatment (B). In addition, these treatments were studied under two different pH soil conditions (pH 6.5 and 8.5) and applying or not a biotic factor (phosphorus-solubilizing bacteria (PSB)) to study the influence of abiotic and biotic soil conditions on P availability. Lettuce yield and quality aspects were determined, and physico-chemical, chemical and biochemical soil parameters were assessed. The alternative organo-mineral fertilizers with STR, SS1, SS2, SS3 and BM showed comparable effects to the organo-mineral fertilizers with TSP and MAP regarding plant productivity and quality, as well as on soil available P and enzymatic activities related to P mineralization. Soil pH values also significantly influenced plant yield and quality, but PSB inoculation seemed not to have a clear effect across the two growing cycles.

Keywords: phosphorus fertilization; struvite; organo-mineral fertilizers; compost; phosphate-solubilizing bacteria; SPAD; CANOPY; soil pH.

1. Introduction

Phosphorus (P) is an essential nutrient in plant nutrition for plant growth promotion due to its role in important cellular processes as a component of nucleic acids, adenosine triphosphate (ATP), adenosine diphosphate (ADP), and phospholipids (Plaxton and Lambers, 2015). P for fertilizer production has historically been obtained from phosphate rock mining. Some of the most commonly applied P fertilizers manufactured from phosphate rock are monoammonium and diammonium phosphate, single and triple superphosphate and NPK complex fertilizers (de Boer et al., 2018). P application has been characterized by its excessive application and inefficient use by crops leading to substantial losses: around three-quarters of the P in conventional chemical fertilizers can become partially unavailable to crops within months of soil application (van der Bom et al., 2019), binding to the soil mineral matrix (Ahmed et al., 2019) or leaching towards water bodies causing eutrophication (Carpenter, 2008), a huge environmental concern. An added inconvenience of fertilizers manufactured from phosphate rock is that it is the main source of cadmium (Cd) in agricultural soils (Santos et al., 2016). The EU 2019/1009 Fertilizers Regulation establishes a limit cadmium concentration in P inorganic and organo-mineral fertilizers of 60 mg Cd kg^{-1} P_2O_5 (EU, 2019.). Additionally, currently there is an increasing concern, due to the localization of P-rock reserves concentration makes Europe highly dependent on imports. Five countries hold 83% of the planet's phosphate rock reserves: Morocco (68%), China (5%), Egypt (4%), Algeria (3%) and Tunisia (3%). In 2023, four countries dominated phosphate rock mining: China (41%), Morocco (16%), the USA (9%) and Russia (7%) (Brownlie et al., 2024). Estimates indicate that, at current mining rates, China, the USA and Russia will deplete their reserves within 40 years (Blackwell et al., 2019), further concentrating the market. Thus, this situation makes it mandatory to secure alternative P sources. Some available P sources include municipal and industrial wastewater, meat and bone meal and other organic wastes (Scholz et al., 2014). P can be removed from municipal wastewater, more specifically sewage sludge, and sewage sludge ash after the influent treatment, which concentrate 90% of P (Egle et al., 2016). Most efforts are directed towards P precipitation. Phosphoric minerals can be precipitated in the form of struvite ($NH_4MgPO_4{\cdot}6H_2O$), hydroxyapatites [$Ca_5(PO_4)_3(OH)$] or calcium phosphates [$Ca_3(PO_4)_2$].

On the other hand, Mediterranean soil faces a problem regarding soil fertility and crop productivity due to drought, water scarcity, and soil nutrient deficiencies (Chtouki et al., 2024). In Mediterranean calcareous soils, there is a low concentration of hydrogen phosphate in the soil solution because of the precipitation of calcium phosphates (da Gama et al., 2021). Thus, across the last 20 years, some European countries like France, Spain, Italy, and Portugal have reduced their consumption of fertilizers decreasing nutrient application per hectare but enhancing nutrient use efficiency. A strategy for enhancing P use efficiency is the use of P-solubilizing microorganisms. Increasing P bioavailability of P fertilizers is an interesting strategy to optimize P utilization thus enabling to reduce application rates. This can be achieved by the action of P-solubilizing microorganisms. According to Yazdani et al. (Yazdani et al., 2009), applying P-solubilizing microorganisms may minimize P fertilizers application to 50% with no significant yield loss. Phosphate solubilizing bacteria (PSB) are able to mineralize organic P in the soil through the secretion of phosphatase enzymes (Pang et al., 2024) and to solubilize fixed inorganic P through the production of organic acids, mainly gluconic acid (Pradhan et al., 2017). Furthermore, some PSB have also a plant growth promoting role through the secretion of hormones such as indole acetic acid (Aliyat et al., 2024). PSB of genera *Bacillus* sp., *Pseudomonas* sp. or *Enterobacter* sp. have been widely studied (Pang et al., 2024). In a pot experiment in soybean, following *Enterobacter* sp. inoculation, a significant increase in available P and phosphatase activity was described as well as a significant decrease in immobilized P fractions Fe-P, Al-P and Ca-P (Alam et al., 2023).

Therefore, the main aims of this study were twofold: i) to assess the effects on the soil properties and on the lettuce yield and quality of the different organo-mineral fertilizers using as organic waste-derived P sources, compared the organo-mineral treatments using mineral P-sources; ii) to evaluate the influence of abiotic (soil pH) and biotic (soil inoculation with P-solubilizing bacteria) conditions on the effect on the soil-plant system and P bioavailability of these alternative organo-mineral fertilizers.

2. Material and methods

2.1 Treatments and soil characteristics

Different organo-minerals fertilizers prepared by using as basic component a compost from agri-food and livestock organic wastes and different P sources (mineral and waste-derived sources) were assessed. The compost was obtained by mixing olive mill waste, olive leaves and poultry manure in the proportions of volume 3:2:1, showing a suitable development of the process, with a duration of the thermophilic phase of 70 days, with temperatures ranged 50-60ºC during this period. The final compost showed a good degree of maturity and good physical-chemical and chemical properties, with absence of phytotoxins and pathogens. The P sources derived from an organic waste origin were: bone meal obtained from meat industry (BM); commercial struvite (STR) and P recovered (SS1, SS2 and SS3) using a bioacidification process from sewage sludge samples from different municipal wastewaters plants. More detailed information about these materials is described in a previous study (Valverde-Vozmediano et al., 2025). On the other hand, the mineral P sources used were phosphate rock (PR, 0-33-0 % $N-P_2O5-K_2O$), monoammonium phosphate (MAP, 11-61-0) and triple superphosphate (TSP, 0-46-0). The main characteristics of these materials are shown in Table 1.

Table 1. Main characteristics of the phosphorus sources used. Data expressed on a dry weight basis.

		Waste-derived P sources					*Mineral P sources*		
	C	**BM**	**STR**	**SS1**	**SS2**	**SS3**	**RP**	**MAP**	**TSP**
Total C (%)	41.9	15.1	0.14	19.4	19.0	10.4	0.48	0.031	-
Total N (%)	2.71	3.00	5.70	0.44	1.19	2.54	0.04	12.1	<0.01
P (%)	0.73	2.1	14.2	5.75	4.69	7.18	2.0	2.3	20.1
K (%)	2.60	0.05	0.06	0.381	0.074	0.057	0.11	0.001	<0.01
Ca (%)	2.97	23.2	0.09	10.3	5.30	5.10	25.1	0.11	17.8
Mg (%)	0.39	0.49	11.7	1.77	0.49	6.28	0.20	0.003	-
Na (%)	0.11	0.44	0.004	2.03	1.31	1.94	0.07	0.002	-
Fe (mg/kg)	2643	47.9	156	28.5	17.6	12.0	10930	16.5	1000
Cu (mg/kg)	112	0.24	0.5	7.9	20.0	3.6	78	0.16	<0.1%
Mn (mg/kg)	297	42.6	642	330	884	363	470	<0.01	<0.1%
Zn (mg/kg)	227	136	1.2	10797	137	86	413	1.33	<0.1%

C: compost; BM: bone meal; STR: commercial struvite; SS1, SS2 and SS3: recovered phosphorus from sewage sludge; RP: phosphate rock; MAP: monoammonium phosphate; TSP: triple superphosphate.

The soil used was a calcareous sandy clayey-loam soil classified as Xerofluvent (Soil Survey Staff, 2014), obtained from the surface layer (0–25 cm) at the EPS-Orihuela experimental farm, Miguel Hernández University, Orihuela, Alicante) (38°4′0″ N, 0°58′0″ W and elevation 24 m above sea level). The soil had an alkaline pH (8.5), low electrical conductivity values (0.40 dS m^{-1}), very low concentrations in organic C (0.50%), with a concentration of 595 mg kg^{-1} of total Kjeldahl N and 60.3 mg kg^{-1} of available P.

2.2 Bacterial inoculum

Two bacterial strains from the private collection of the BIO-175 research group of the University of Almeria were used. The bacterial strains were *Bacillus aerophilus* (BM161) and *Pseudomonas putida* (BIO175) were isolated from compost samples process and were selected by their demonstrated phosphate-solubilizing activity in media with 2.5% phosphate tricalcium by a clear halo around colony (Jurado et al., 2014; Toribio et al., 2022). The strains were recovered on plates with APHA culture medium (Standard Methods Agar, ISO 4833:2003, Ref. 413799, Panreac Applichem, Spain), from cryovials preserved at - 80°C. For the microbial inocula preparation, the two bacteria were grown for 24 h at 30 ºC in nutrient broth (Panreac) under shaking conditions and added to the soil to reach a final concentration equivalent to approximately 10^7 colony forming units (CFU) g^{-1} soil.

2.3 Experimental design

A pot trial was established using a randomized design with six replicates per treatment during two successive cultivation cycles. The treatments considered were the organo-mineral fertilizers with organic waste-derived P sources (CSS1, CSS2, CSS3, CBM and CSTR) and with mineral P sources (CMAP, CTS and CPR), respectively, compared with the compost used as base (C) and with a non-fertilized control treatment (B), using lettuce (*Lactuca sativa* var. baby leaf) as a plant model. All the treatments were applied at a normalized rate of 80 kg P ha^{-1} and at a normalized N application rate of 150 kg N ha^{-1}, reached by supplying additional N in all the treatments as urea. In addition, two factors were considered to study the influence of biotic and abiotic factors on the effect in the soil-plant system of the treatments studied, an abiotic factor (soil pH) and a biotic factor (inoculation with two different phosphate-solubilizing bacteria, PSB). Thus, different soil pH values were considered in the assay: pH 8.5 (of the original soil) and pH 6.5, obtained adding $FeSO_4$ to the original soil until reaching the pH value of 6.5. In addition, the two different PSBs previously commented were used for the soil inoculation. The pots were filled with 1500 g of soil previously sieved through a 5 mm mesh and the different treatments were applied. Following irrigation, lettuce seedlings at 2-3 true leaves phenological stage were planted and after a week of planting, the microbial inocula were incorporated to the corresponding pots. Plants were placed in an environmentally controlled room with an average temperature of 21 ºC, 60% relative humidity and 12 h photoperiod with artificial lightning. Periodical watering with deionized water was performed at 60% field capacity. Two consecutive lettuce crops were grown. The first crop was harvested 48 days after planting. Following the first harvest, soil samples were collected before planting the second growing cycle, the microbial inocula was incorporated again one week after planting and lettuce plants were harvested 60 days after planting.

2.2 Compost, P-sources and soil and determinations

In the compost and P sources considered, the total C and N contents were assessed with an automatic elemental microanalyzer (EuroVector, Milan, Italy). After acid microwave digestion with HNO_3 69%, P was colorimetrically determined as molybdovanadate phosphoric acid; K and Na were assessed by flame photometry (Jenway PFP7 Flame Photometer, Jenway Ltd., Felsted, Dunmow, Essex, UK); and Ca, Mg, Fe, Cu, Mn and Zn were determined by induced coupled plasma mass spectroscopy (ICP-MS).

Concerning the soil samples, after each harvest, soil from the pots was processed in order to remove roots and divided into two subsamples, one was extended and air-dried for the chemical determinations and the other was immediately frozen at -80ºC for the biological determinations (soil respiration and enzymatic activities). The pH and EC of the soil samples were measured in aqueous extracts at the 1:2.5 and 1:5 soil–water (w/v) ratios, respectively. Available P was determined colorimetrically by the Olsen-Watanabe method (Olsen et al.,1954), while soil organic matter (OM) and soil respiration were assessed following the methods described by Yeomans and Bremmer and Bustamante et al. (2011), respectively.

In the soil samples, four soil enzymatic activities were assessed: acid and alkaline phosphatase according to Eivazi and Tabatabai (1977); β-glucosidase at two pH values (6.5 and 8.5, following the methodology of Eivazi and Tabatabai (1988) and urease, according to Nannipieri et al. (1981). For alkaline phosphatase determination, the microplate adapted method by Popova and Deng (2010) was implemented. First, an extraction was performed with 2 g soil in 25 mL Universal Modified Buffer (pH 8) with agitation for 30 min. After, 1 mL extract was transferred to a 24-well plate plus 200 μL of substrate, 20 mM p-nitrophenyl phosphate, and 300 μL of distilled water. Plates were incubated for 1h at 37ºC. and later, they were put on ice for 15 min for the enzymatic reaction to stop. Plates were centrifuged at 2500 rpm for 5 min at T=4ºC. 250 μL supernatant were transferred to a 96-well plate with addition of 50 μL 0.5M NaOH. Finally, absorbance at 410 nm was measured on a Thermoscientific Multiskan plate reader.

For acid phosphatase determination, 0.5 g of soil were incubated with 2 mL MUB buffer (pH 6.5) and 0.5 mL of substrate, 20 mM p-nitrophenyl phosphate, for 1h at 37ºC on a thermostatic bath with agitation. After incubation, samples were put on ice for 15 min for the enzymatic reaction to stop. 0.5 mL 0.5 M $CaCl_2$, 2 mL 0.5M NaOH and 5 mL of distilled water were added to the tubes which were then centrifuged at 4000 rpm for 4 min. 300 μL were transferred to a 96-well plate and absorbance at 410 nm was measured on a Thermoscientific Multiskan plate reader.

For β-glucosidase determination, 0.5 g of soil were incubated with 2 mL MUB buffer (pH 6.5 for β-glucosidase-a and pH 8.5 for β-glucosidase-b) and 0.5 mL of substrate, 20 mM p-nitrophenyl β-D-glucopyranoside, for 1h at 37ºC on a thermostatic bath with agitation. After incubation samples were put on ice for 15 min for the enzymatic reaction to stop. 0.5 mL 0.5 M $CaCl_2$, 2 mL THAM pH 12 and 5 mL of distilled water were added to the tubes, which were then centrifuged at 4000 rpm for 4 min. 300 μL were transferred to a 96-well plate and absorbance at 410 nm was measured on a Thermoscientific Multiskan plate reader.

For urease determination, 0.5 g of soil were incubated with 2 mL 0.1 M phosphate buffer (pH 7) and 0.5 mL 6.4% urea for 45 min at 30ºC on a thermostatic bath with agitation. After incubation samples were put on ice for 15 min for the enzymatic reaction to stop. Deionized water was added to reach a 10 mL final volume and the samples were then centrifuged at 3400 rpm for 8 min. 1 mL of the resulting supernatant was then mixed with 0.8 mL 0.3 M sodium citrate, 1.6 mL reactant A (sodium salicylate, sodium nitroprusside), 0.8 mL reactant B (sodium dichloroisocyanurate, NaOH) and incubated in darkness for 45 min. 300 μL were transferred to a 96-well plate and absorbance at 665 nm was measured on a Thermoscientific Multiskan plate reader.

2.3 Plant determinations

After each harvest, the aerial part of lettuce plants was cut and weighted to determine crop yield and after, were washed and dried at 60 ºC in an air-force oven for 48 h. After this, lettuce samples were ground using a RETSCH mill to a 0.5 mm particle size for the analytical determinations.

The analytical determinations were conducted with the methodology described by Martínez-Sabater et al. (2022). Briefly, total C and N of the green tissue were measured in an automatic elemental micro analyser (EuroVector Elemental Analyzer). For other nutrients, samples were digested with nitric acid 67% (0.02 g in 10 mL) in a Mars 6 digester (Kabata-Pendias, 2010). Subsequently, total P was determined by colorimetry in an UV-V spectrophotometer, K and Na were determined in a flame photometer and the rest of macronutrients (Ca, Mg) and micronutrients (Na, Cu, Fe, Mn, Zn) were analysed via induced coupled plasma mass spectroscopy (ICP-MS).

In addition, two quality parameters were determined non-destructively in the lettuce plants every 15 days until the end of each growing cycle, the green cover factor (CANOPY), determined using the CANOPY app © developed for Matlab by Patrignani and Ochsner (Patrignani and Ochsner, 2015), and the chlorophyll intensity, assessed using a hand-held chlorophyll meter (SPAD-502, Minolta Co., Ltd., Osaka, Japan) (Casella et al., 2022).

2.4 Statistical analysis

Statistical analyses were performed using the Infostat, v.2020 statistical software package linked to the R programme (Di Rienzo et al, 2020) Normality of the data was tested using the Shapiro-Wilks test and the homogeneity of variance was assessed and confirmed using the Levene test ($p > 0.05$). Analysis of variance (ANOVA) test at $p < 0.05$ was performed for all the experimental variables using a generalized linear mixed model, with fertilizer treatments and sampling dates as fixed factors and each plot as a random factor. The post hoc analysis was carried out with the Tukey test ($p < 0.05$).

3. Results and discussion

3.1 Assessment of the different factors (type of treatment, soil pH and PSB inoculation) on lettuce yield, quality and nutrient uptake

The different treatments showed a significant influence on the lettuce yield, especially in the first growing cycle (Fig. 1). At the end of the first growing season, all the treatment groups showed significantly higher yield than the control treatment without amendment. The greatest lettuce yields were found with the treatments with the waste-derived P sources STR, SS1, SS2 and SS3 and the organo-mineral fertilizers with the inorganic P-sources TSP and MAP, possibly due to a higher presence in these materials of available nutrients for the crop (Soudek et al., 2024). These results coincided with those obtained in different studies using organo-mineral fertilizers with struvite and mineral P-sources (De Sousa and Alleoni, 2024; Valverde-Vozmediano et al., 2025). Furthermore, different studies have reported a similar behaviour of struvite compared to inorganic soluble P fertilizers in relation to P uptake in acidic soils (Achat et al., 2014; Bonvin et al., 2015). Thus, other studies have reported that P availability was similar among struvite sources and mineral fertilizers as MAP, DAP and TSP (Talboys et al., 2016; Cabeza et al., 2011; Hall et al., 2020; Katanda et al., 2016).

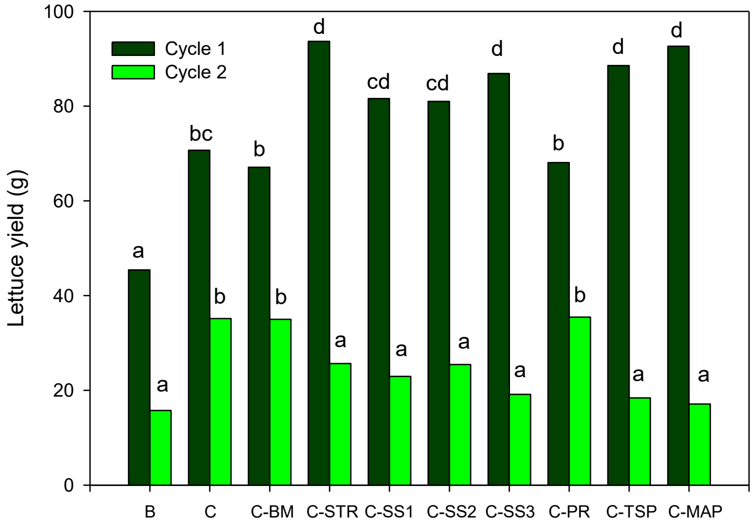

Figure 1. Average values of lettuce yield (g) for the two growing cycles. Average values with different letters in the same growing cycle indicate statistical differences between treatments by Tukey test (p > 0.05).

However, the second growing cycle did not exhibit such differences, only showing the treatments C, C-BM and C-RP lettuce yields higher than the control soil without amendment. In addition, the factor soil pH showed a significant effect in this parameter in both growing cycles ($p < 0.001$), with a more positive effect on yield of alkaline pH in the first cycle (average yield value of 62.6 g at pH 6.5 and 97.1 g at pH 8.5, $p < 0.001$), but the opposite effect in the second cycle (35.3 g at pH 6.5 and 16.1 at pH 8.5, $p < 0.001$). However, the PSB inoculation did not seem to have any effect in any of the lettuce growing seasons ($p > 0.05$).

On the other hand, the effect of the different variables considered (type of treatment, soil pH and PSB inoculation) were also studied in quality parameters in the crop, such as chlorophyll intensity (SPAD) and leaf coverage (CANOPY). As can be seen in Table 2, the type of treatment considered and the soil pH values showed a significant effect on the SPAD values; however, the PSB inoculation seemed not to produce any significant effect on the chlorophyll intensity in any of the growing cycles, except for the second measurement at 30 days in the second growing cycle. The SPAD average values were also higher in the first growing cycle, observing the greatest values at the beginning of the season and in the treatments with waste-derived P source (C-SS1 and C-SS2) and the treatment C-TSP, with inorganic P-source. However, in the second lettuce season, the highest SPAD values were found in the treatments C-RP and C-BM throughout the growing cycle. In general, the SPAD values showed a decreasing trend with the progression of the growing cycles, which could be attributed to a quick release rate of nutrients in the first growing cycle. The opposite behaviour was found by Álvarez-Alonso et al. (2025) in a study of lettuce cultivation using composts from decentralized composting scenarios.

Concerning the effect of the different factors on the mean leaf coverage (CANOPY), in the first growing cycle, the CANOPY average values were mainly affected by the type of fertilizer treatment and the soil pH, only finding a significant effect of the PSB inoculation at 15 days of planting (Table 3). The higher CANOPY average values were observed for the organo-mineral fertilizers with waste-derived P sources (C-STR, C-SS1, C-SS2 and C-SS3), with similar effect of the mineral P-source treatments C-MAP and C-TSP. However, in the second growing cycle, the highest CANOPY average values were found for the treatments C, C-RP and C-BM, which seems to imply a slowest release of P in these treatments. Thus, leaf cover is closely related to crop development, showing the treatments commented a residual effect with a positive influence in the consecutive lettuce growing cycle reported in a previous fertilization studies on lettuce crop production (Reis et al., 2014; Álvarez-Alonso et al., 2025).

Table 2. Effect of each factor considered (treatment, soil pH and PSB inoculation) in the SPAD average values of the lettuce crop during the two growing cycles.

	Growing cycle 1			*Growing cycle 2*			
	15 days	30 days	45 days	15 days	30 days	45 days	60 days
Type of treatment							
B	47.42 ab	41.86 a	35.96 a	26.23 a	22.50 a	20.33 ab	21.25 abc
C	48.95 abc	43.03 a	43.58 bc	27.37 a	28.01 abc	29.29 d	23.73 cd
C-MAP	49.88 bcd	49.00 c	41.84 b	27.88 ab	23.14 a	19.18 a	16.87 a
C-TSP	50.57 cd	50.02 c	43.83 bc	27.98 abc	23.43 a	21.21 ab	17.37 a
C-RP	47.18 a	42.01 a	44.10 bc	29.93 cd	28.51 bc	29.52 d	24.53 cd
C-BM	49.04 bc	42.72 a	43.93 bc	30.78 d	28.31 abc	28.66 cd	25.95 d
C-STR	49.96 bcd	48.80 bc	44.39 c	28.91 abcd	28.66 c	24.42 bc	19.35 ab
C-SS1	50.96 d	49.27 c	43.41 bc	29.64 bcd	26.50 abc	24.39 bc	21.35 bc
C-SS2	50.69 cd	46.15 b	44.30 c	29.88 abcd	27.27 abc	24.14 b	21.33 bc
C-SS3	49.72 bcd	49.02 c	43.36 bc	28.46 abc	24.40 ab	21.46 ab	18.20 ab
ANOVA	**	***	***	*	*	***	***
Soil pH							
6.5	48.54 a	45.33 a	45.58 b	30.73 b	31.06 b	29.00 b	23.39 b
8.5	50.62 b	47.66 b	41.14 a	26.82 a	21.59 a	20.08 a	18.56 a
ANOVA	***	**	***	***	***	***	***
PSB-inoculation							
Without PSB	49.38	45.91	42.77	28.14	24.74 a	23.36	20.28
PSBa	50.08	47.62	44.12	28.86	26.64 ab	24.65	21.61
PSBb	49.31	46.02	43.26	29.40	27.77 b	25.75	21.11
ANOVA	ns	ns	ns	ns	*	ns	ns

B: control treatment; C: compost. PSBa: *Bacillus aerophilus*; PSBb: *Pseudomonas putida*. Values with different letters (same day) indicate statistical differences between treatments by Tukey test. n.s.: not significant $p > 0.05$; *, **, ***: significant at $p < 0.05$, 0.01 and 0.001, respectivel

Table 3. Effect of each factor considered (treatment, soil pH and PSB inoculation) in the CANOPY average values in the lettuce crop during the two growing cycles.

	Growing cycle 1			*Growing cycle 2*			
	15 days	30 days	45 days	15 days	30 days	45 days	60 days
Type of treatment							
B	6.06 ab	10.24 ab	16.06 a	1.31	2.16 a	2.42 a	2.83 a
C	6.18 ab	10.99 ab	26.45 bcd	1.80	4.58 d	5.73 bc	6.68 c
C-MAP	7.79 b	20.52 c	27.92 cde	1.34	2.37 a	2.64 a	2.87 a
C-TSP	7.38 b	21.42 c	28.23 cde	1.62	3.04 ab	3.14 a	3.11 a
C-RP	5.91 a	9.71 a	24.62 b	1.95	4.59 d	6.01 c	7.03 c
C-BM	6.46 ab	11.26 ab	26.12 bc	1.76	3.95 abcd	5.38 bc	6.38 bc
C-STR	6.26 ab	19.22 c	29.02 de	1.98	4.52 cd	4.97 bc	4.45 a
C-SS1	7.19 ab	17.11 bc	28.80 cde	1.79	3.89 abcd	4.10 ab	4.48 a
C-SS2	7.50 b	17.61 c	29.36 e	1.86	4.33 bcd	4.61 abc	4.91 ab
C-SS3	7.71 b	19.93 c	28.74 cde	1.54	309 abc	3.23 a	3.36 a
ANOVA	*	***	***	ns	**	***	***
Soil pH							
6.5	5.14 a	9.24 a	24.78 a	2.38 b	5.58 b	6.35 b	6.64 b
8.5	8.66 b	23.16 b	29.78 b	1.06 a	1.94 a	2.35 a	2.83 a
ANOVA	***	***	***				
PSB- inoculation							
Without PSB	6.44 a	15.73	27.49	1.53 a	3.16 a	3.73	4.22
PSBa	6.91 ab	17.16	27.11	1.87 b	4.07	4.74	5.12
PSBb	7.40 b	15.76	27.22	1.79 ab	4.11	4.65	4.93
ANOVA	*	ns	ns	*	*	ns	ns

B: control treatment; C: compost. PSBa: *Bacillus aerophilus*; PSBb: *Pseudomonas putida*. Values with different letters (same day) indicate statistical differences between treatments by Tukey test. n.s.: not significant $p > 0.05$; *, **, ***: significant at $p < 0.05$, 0.01 and 0.001, respectively.

The lettuce nutrient uptake (N, P and K) average values at each growing season was significantly affected by the type of fertilizing treatment and the soil pH, with a higher uptake with the acidic pH value, not observing a significant effect of the inoculation with the PSBs selected (Table 4). In general, the treatments C, C-RP and C-BM induced the highest N and P uptake in both growing cycles and of K only in the second growing cycle. These results also agree with the average results observed in the leaf coverage (CANOPY), indicating the highest availability of these nutrients found in the compost and in the compost + RP and compost + BM, which clearly shows that the combination with compost of those mineral fertilizers clearly improves their productivity (Valverde-Vozmediano et al., 2025).

3.2 Effect on the soil properties of the different factors (type of treatment, soil pH and PSB inoculation)

No significant differences were found in soil pH or electrical conductivity (EC) depending on the type of treatment and the PSB inoculation in both growing cycles, only observing differences depending on the factor soil pH (Table 5). These results were also reported in previous studies based on the use of different P sources for lettuce cultivation (Mancho et al., 2023; Valverde-Vozmediano et al., 2025). However, a significant effect of the type of treatment and of the soil pH was observed for soil OM and available P in the two growing cycles. In general, the treatments C-MAP and C-TSP induced the highest concentrations of soil available P, the treatments with STR, SS1 and SS3 showing a similar effect on this soil parameter. After the two growing cycles, the concentrations of available P decreased in all the soils due to the uptake of this element by the lettuce plants. However, the OM contents did not show a homogeneous trend in both growing cycles, finding the highest values in control treatment (B) and the soil amended with C-SS2.

Concerning the biological parameters studied in the soils, soil respiration, used as an indicator of soil biological activity (Bustamante et al., 2011), was significantly influenced in both growing cycles only by the type of treatment, without showing effect on the soil pH and PSB inoculation, except for this later factor in the second growing cycle (Table 6). In both lettuce seasons, all the treatments showed a higher soil respiration than the control soil without amendment (B), the treatments C, C-MAP and C-TSP showing the highest values. In the first growing cycle, the organo-mineral fertilizer C-RP showed soil respiration values statistically similar to those of the control soil (B), this effect being the complete opposite in the second growing cycle, since it induced the highest soil respiration values together the rest of treatments previously mentioned. Hernández et al. (2014) also observed in an experiment of intensive lettuce production comparing different organic and inorganic fertilizers, an improvement in the soil microbiota activity with the presence of organic fertilizers, reporting a significant influence of the properties of the organic materials.

Table 4. Effect of each factor considered (type of treatment, soil pH and PSB inoculation) on lettuce nutrient uptake at harvest in each growing cycle.

	Growing cycle 1			*Growing cycle 2*		
	N (g/pot)	P (g/pot)	K (g/pot)	N (g/pot)	P (g/pot)	K (g/pot)
Type of treatment						
B	1.66 a	0.18 abcd	9.68 cd	0.60 a	0.15 a	2.34 a
C	3.38 e	0.23 f	8.43 b	0.95 bc	0.17 ab	3.92 c
C-MAP	2.34 ab	0.21 de	7.93 ab	0.72 a	0.22 ef	2.71 a
C-TSP	2.38 bc	0.17 ab	7.39 a	0.67 a	0.20 cd	2.39 a
C-RP	3.22 de	0.22 ef	8.70 bc	0.99 c	0.23 f	4.13 c
C-BM	3.29 de	0.21 ef	8.58 bc	1.11 c	0.20 cde	3.70 bc
C-STR	2.82 cd	0.19 cd	7.20 a	0.81 ab	0.21 def	3.76 bc
C-SS1	2.59 bc	0.17 abc	8.76 bc	0.74 a	0.19 bc	3.25 b
C-SS2	2.85 cd	0.18 bcd	10.08 d	0.71 a	0.18 bc	2.58 a
C-SS3	2.43 bc	0.16 a	8.23 b	0.69 a	0.22 def	2.70 a
ANOVA	***	***	***	***	***	***
Soil pH						
6.5	3.42 b	0.20 b	9.22 b	0.94 b	0.18 a	3.49 b
8.5	2.13 a	0.19 a	7.61 a	0.69 a	0.22 b	2.92 a
ANOVA	***	*	***	***	***	***
PSB-inoculation						
Without PSB	2.67	0.19	8.43	0.75	0.19 a	2.98 a
PSBa	2.83	0.19	8.35	0.86	0.21 b	3.17 ab
PSBb	2.83	0.20	8.46	0.83	0.21 b	3.50 b
ANOVA	ns	ns	ns	ns	*	*

B: control treatment; C: compost; C-MAP: compost + monoammonium phosphate; C-TSP: compost + triple superphosphate; C-RP: compost + phosphate rock; C-BM: compost + bone meal; C-STR: compost + commercial struvite; C-SS1, C-SS2 and C-SS3: compost + recovered phosphorus from sewage sludge. PSBa: phosphorus-solubilizing bacteria (*Bacillus aerophilus*); PSBb: phosphorus-solubilizing bacteria (*Pseudomonas putida*). Values with different letters for each treatment and factor indicate statistical differences between treatments by Tukey test. n.s.: not significant $p > 0.05$; *, **, ***: significant at $p < 0.05$, 0.01 and 0.001, respectively.

Table 5. Effect of each factor considered (type of treatment, soil pH and PSB-inoculation) in physico-chemical and chemical soil parameters in each growing cycle.

	Growing cycle 1				*Growing cycle 2*			
	pH	EC (dS/m)	Pav (mg/kg)	OM (%)	pH	EC (dS/m)	Pav (mg/kg)	OM (%)
Type of treatment								
B	8.26	1.36	132 a	0.78 a	8.27	1.12	127 bcde	1.07 ef
C	8.09	1.38	131 a	0.95 bc	8.17	1.29	131 cde	0.83 bc
C-MAP	8.04	1.34	230 c	0.99 c	8.20	1.12	191 g	0.77 b
C-TSP	8.16	1.33	223 bc	0.98 c	8.19	1.22	171 fg	0.89 cd
C-RP	8.22	1.36	139 a	0.98 c	8.22	1.16	105 b	0.85 bc
C-BM	8.00	1.36	136 a	1.18 d	8.29	1.21	67 a	0.98 de
C-STR	8.11	1.33	214 bc	0.86 ab	8.15	1.16	121 bcd	0.24 a
C-SS1	7.92	1.35	205 bc	0.84 a	8.23	1.05	112 bc	0.32 a
C-SS2	8.25	1.34	195 b	0.78 a	8.22	1.28	142 de	1.18 f
C-SS3	7.97	1.33	203 bc	0.81 a	8.25	1.08	150 ef	0.77 b
ANOVA	*ns*	*ns*	***	***	ns	ns	***	***
Soil pH								
6.5	7.67 a	2.33 b	142 a	0.90	7.87 a	2.04 b	107 a	0.70 a
8.5	8.51 b	0.36 a	227 b	0.95	8.56 b	0.31 a	157 b	0.85 b
ANOVA	***	***	***	*ns*	***	***	***	**
PSB-inoculation								
Without PSB	8.06	1.34	180	0.89	8.20	1.20	138	0.77
PSBa	8.11	1.35	195	0.96	8.21	1.14	127	0.78
PSBb	8.10	1.35	179	0.92	8.23	1.17	131	0.76
ANOVA	ns	ns	ns	ns	ns	ns	ns	ns

EC: electrical conductivity; Pav: available P; OM: organic matter. B: control, C: compost. PSBa: *Bacillus aerophilus*; PSBb: *Pseudomonas putida*. Values with different letters indicate statistical differences (Tukey test). n.s.: not significant $p > 0.05$; *, **, ***: significant at $p < 0.05$, 0.01 and 0.001, respectively.

Table 6. Effect of each factor considered (type of treatment, soil pH and PSB-inoculation) in soil biological parameters.

	Growing cycle 1						*Growing cycle 2*					
	SR	AcP	AlP	β-ga	β-gb	Ur	SR	AcP	AlP	β-ga	β-gb	Ur
Type of treatment												
B	35.3 a	1.01 a	1.97 d	0.42 d	0.77 bcd	0.92 c	39.9 a	0.41 ab	2.25 abc	0.34 ab	0.40 a	0.83 bcd
C	82.5 c	1.57 c	1.46 bcd	0.28 bc	0.74 bc	0.82 a	86.7 de	0.85 d	1.96 abc	0.30 a	0.48 ab	0.80 bc
C-MAP	80.2 bc	1.46 c	1.37 abcd	0.28 b	0.70 ab	0.83 a	89.8 e	0.80 d	1.83 a	0.38 b	0.44 ab	0.81 bcd
C-TSP	80.4 bc	1.39 bc	1.29 abcd	0.29 bc	0.72 b	0.88 bc	81.9 de	0.68 c	1.76 a	0.36 b	0.49 bc	0.78 b
C-RP	51.2 a	0.97 a	1.73 cd	0.19 a	0.93 e	0.85 ab	89.7 e	0.67 c	1.88 ab	0.36 b	0.46 ab	0.73 a
C-BM	67.9 bc	1.05 a	1.25 abc	0.19 a	0.84 de	0.84 a	74.2 cd	0.68 c	2.06 abc	0.50 c	0.58 d	0.77 b
C-STR	78.7 bc	0.98 a	1.38 abcd	0.22 a	0.63 a	0.89 c	65.8 bc	0.40 a	1.89 ab	0.29 a	0.44 ab	0.78 b
C-SS1	79.0 bc	1.45 c	1.43 bcd	0.37 d	0.82 cd	0.88 bc	63.3 bc	0.43 ab	2.58 c	0.27 a	0.55 cd	0.77 b
C-SS2	66.3 b	1.17 ab	0.93 a	0.38 d	0.70 ab	0.90 c	59.2 ab	0.51 b	2.49 bc	0.33 ab	0.46 ab	0.85 d
C-SS3	71.5 bc	1.16 ab	1.17 ab	0.32 c	0.72 ab	0.90 c	63.1 bc	0.47 ab	2.48 bc	0.29 a	0.61 d	0.84 cd
ANOVA	***	***	*	***	***	***	***	***	*	***	***	***
Soil pH												
6.5	68.4	1.52 b	1.27	0.29	0.77	0.87	71.0	0.71 b	3.02 b	0.36 b	0.46 a	0.79
8.5	75.0	0.95 a	1.44	0.28	0.74	0.87	76.2	0.49 a	1.20 a	0.32 a	0.53 b	0.80
ANOVA	ns	***	ns	ns	ns	ns	ns	***	***	*	***	ns
PSB-inoculation												
Without PSB	72.52	1.24	1.34 ab	0.31 b	0.79 b	0.87	74.4 ab	0.59	2.17	0.34	0.49	0.80
PSB	73.87	1.24	1.55 b	0.26 a	0.72 a	0.86	79.9 b	0.60	2.08	0.35	0.51	0.78
NSB	68.67	1.23	1.18 a	0.27 a	0.75 ab	0.87	66.4 a	0.62	2.07	0.34	0.49	0.80
ANOVA	ns	ns	*	*	ns	ns	*	ns	ns	ns	ns	ns

SR: soil respiration (mg CO_2 kg^{-1} soil day^{-1}); AcP: acid phosphatase, AlP: alkaline phosphatase, β-ga: β-glucosidase (pH = 6.5), β-gb: β-glucosidase (pH = 9) (μmol p-nitrophenol g^{-1} h^{-1}); Ur: urease (μmol NH_4 g^{-1} h^{-1}). B: control treatment; C: compost. PSBa: phosphorus-solubilizing bacteria (*Bacillus aerophilus*); PSBb: phosphorus-solubilizing bacteria (*Pseudomonas putida*). Values with different letters indicate statistical differences by Tukey test. n.s.: not significant $p > 0.05$; *, **, ***: significant at $p < 0.05$, 0.01 and 0.001, respectively.

Regarding the soil enzymatic activities, the influence of the factors considered were quite heterogeneous, only showing a significant effect of the type of treatment on all the enzymatic activities in both growing cycles (Table 6). However, in general all the organo-mineral fertilizers increased the activity of the enzymes related to P degradation, specifically the activity of acid phosphatase during the second growing cycle. Cui et al. (2023) reported in a meta-analysis to study the effects of organic amendments on soil microbial composition and its linkage to soil organic carbon that organic amendment significantly increased enzyme activities related to N and P degradation in bulk soil. In this sense, phosphatases can constitute a good indicator of the P degradation potential of organic compounds and the biological activity of the soil. Thus, the intensity of the secretion of phosphatases by crops and microorganisms strongly depends on the crop P requirements (Ramut et al., 2025).

On the other hand, the effect of soil pH was only significant in the first growing cycle for the acid phosphatase activity, while the effect of the PSB inoculants were significant in the activities of alkaline phosphatase and β-glucosidase in acid conditions (pH 6.5). In the second growing cycle, the soil pH significantly influenced all the enzymatic activities except for the urease activity, while the PSB inoculant did not affect any of the enzymatic activities. In general, the treatments with waste-derived P sources, such as C-BM, C-STR, C-SS1, C-SS2 and C-SS3 produced higher values than the rest of treatments, specifically of the acid phosphatase, β-glucosidase in acid conditions (pH 6.5) and urease in the first growing cycle, and in alkaline phosphatase in the second cycle. The heterogeneous composition of the different organo-mineral fertilizers clearly produced different effects depending on the substrate used by each enzyme.

4. Conclusions

The results obtained have shown that the alternative organo-mineral fertilizers with struvite (STR), P recovered materials (SS1, SS2, and SS3) and bone meal from the meat industry (BM) have demonstrated comparable effects to those of the organo-mineral fertilizers with inorganic sources (triple superphosphate, TSP and monoammonium phosphate, MAP), especially in the first lettuce growing cycle, obtaining similar yield and quality parameters in the crop, such as leaf coverage (CANOPY) and chlorophyll intensity (SPAD). Furthermore, these treatments have shown similar effects on soil parameters, specifically on soil available P and enzymatic activities related to P mineralization. However, only the treatment with compost (C) and the organo-mineral fertilizers with phosphate rock (RP) and BM showed a positive residual effect on crop productivity and plant nutrient uptake. On the other hand, the soil pH values had a significant effect on the plant yield and quality, with best results in acidic conditions, but the PSB inoculation seemed not to have a clear effect across the two growing cycles in the parameters studied in the plant and in the soil.

Therefore, this study highlights the potential of the organo-mineral fertilizers with organic waste-derived P sources (STR, SS1, SS2, SS3 and BM) as alternative substitutes for traditional mineral P sources, since these materials are capable of supplying crops with essential P while promoting a more sustainable and circular nutrient use in agricultural systems, potentially lowering dependence on inorganic fertilizers.

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