Fertilizing effect of a recirculated calcium phosphate – does the addition of organic acids increase efficiency?

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Fertilizing effect of a recirculated calcium phosphate – does the addition of organic acids increase efficiency?

Gödseleffekt av återcirkulerat kalciumfosfat – ökar tillsatsen av organiska syror effektiviteten?

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Abstract

Finding new ways to utilize existing phosphorus resources and increasing the recycling of fertilizer products in the European Union is important, as phosphorus is an essential nutrient for agronomic production. Previous studies have shown that organic acids, such as those exuded by plant roots, can increase phosphorus availability in soil through ligand exchange or chelation mechanisms. This study aimed to investigate how the addition of organic acids, specifically citric acid and a phenolic acid, affects phosphorus availability and plant uptake from the recycled calcium phosphate fertilizer RevoCaP. A greenhouse experiment was conducted using *Lolium perenne* L. with nine treatments in total, including RevoCaP with or without added organic acids, soft rock phosphate (RP), superphosphate (SP) (with two reduced-rate references), and an unfertilized control. The experiment was arranged in a random complete block design with four replicates. Fertilizers were applied at 15 kg P/ha, under two soil pH levels (6 and 7), over nine weeks with two harvests. Dry matter yield, phosphorus concentrations, phosphorus yield, and mineral fertilizer equivalent (MFE) were assessed. At the first harvest, no significant differences were observed among RevoCaP treatments regardless of organic acid addition or pH level. MFE values were generally low across both pH levels. At the second harvest, RevoCaP treatments showed significantly higher dry matter yield, phosphorus concentration, phosphorus yield and MFE at pH 6 compared to pH 7. However, the addition of organic acids did not significantly affect phosphorus availability or uptake within RevoCaP treatments at the same pH. In conclusion, organic acid additives did not enhance phosphorus availability in RevoCaP under the conditions of this study. Observed differences were primarily related to soil pH and the inherent phosphorus availability of the fertilizer product, with RevoCaP performing better under acidic conditions.

Keywords: phosphorus availability, organic acids, calcium phosphate, recycled fertilizer, *Lolium perenne* L., soil pH

Table of contents

List of tables

List of figures

Abbreviations

AL	Ammonium acetate-lactate
CA	Citric acid
CaP	Calcium phosphate
DM	Dry matter
EU	European Union
$H_2PO_4^-$	Dihydrogen phosphate
HCl	Hydrochloric acid
HPO_4^{2-}	Hydrogen phosphate
ICP-OES	Inductively coupled plasma-optical emission spectroscopy
LMW	Low molecular weight
MFE	Mineral fertilizer effect
PO_4^{3-}	Orthophosphate
RP	Rock phosphate
SP	Superphosphate
SSP	Single superphosphate
TSP	Triple superphosphate

1. Introduction

Phosphorus (P) fertilizers are considered highly economically important products for the European Union (EU), as P is essential for agronomic production, and no alternative element can replace its vital function in plants (European Commission, 2023; USGS, 2025). The fertilizer market is highly dependent on international trade, and currently, most P fertilizers in Europe are imported due to the geographically limited distribution of P deposits within the EU (Syers *et al.*, 2008; European Commission, 2023). Macroeconomic and geopolitical volatility strongly affects this market, including trade disruptions and uncertainties driven by sanctions, tariffs and protectionism (IFA, 2025). In the first half of 2025 alone, changes in US-China relations and the imposition of sweeping tariffs on global trade partners by the United States posed a significant risk to the stability of the global fertilizer market (IFA, 2025).

At the same time, significant amounts of P already exist within the EU as secondary resources. These include organic fertilizers such as manure and sewage sludge, as well as inorganic materials like ash. In Sweden alone, increased domestic production could theoretically meet total P demand by using organic resources combined with various resource recovery options, such as P-rich mine waste, industrial by-products, and ash (Ekman *et al.*, 2023). Such approaches would enable the recirculation of valuable nutrients into agriculture, supporting sustainability goals, and promoting better stewardship of finite resources (Ekman *et al.*, 2023). However, these alternatives often act as slow-release fertilizers, making P available slower than commercial P fertilizers (Andersson *et al.*, 2025). Therefore, increasing the plant-available P in these materials is a key challenge for closing the P cycle within the EU.

To address this, the present study examined the potential of organic acid additives as a way to improve P availability in a recycled calcium phosphate (CaP) fertilizer, with the commercial name RevoCaP. It is derived from ash from mono-incinerated sewage sludge through the patented Ash2Phos process developed by EasyMining (EM). The product is since May 2025 approved by the European Commission for use as a fertilizer in organic agriculture, in accordance with Regulation (EU) 2019/1009 and permitted under Regulation (EU) 2018/848 (EasyMining 2025a; EasyMining, 2025b).

1.1 Aims and hypotheses

The main aim of this study was to investigate how the addition of organic acids, namely citric acid (CA) and a phenolic acid (not further specified due to a nondisclosure agreement and hereinafter referred to as “Compound X”, or “CX”), affects P availability from a recycled calcium phosphate fertilizer compared to unamended fertilizer under two different pH conditions. A secondary aim was to compare the performance of the recycled calcium phosphate fertilizer with a soft rock fertilizer approved for organic production, to evaluate potential differences in P availability between products intended for organic crop production.

The following research questions and hypotheses were addressed, all referring to P uptake and plant performance, as reflected by crop dry matter (DM) yield, plant P concentrations and P yield:

1. Does the addition of organic compounds to the calcium phosphate fertilizer affect P availability?
Hypothesis 1: The addition of organic compounds increases P availability.

2. Does pH influence P uptake of the calcium phosphate fertilizer with added organic acids?
Hypothesis 2: pH affects the effectiveness of the calcium phosphate fertilizer with additives.

3. Is there an interaction between additive type and pH affecting P availability and plant performance?
Hypothesis 3: There is an interaction between additive type and pH, such that the effect of the additive depends on pH.

2. Background

2.1 Phosphorus in plants

2.1.1 Phosphorus cellular and physiological functions

Together with nitrogen (N) and potassium (K), P is among the most yield-limiting nutrients in Swedish agriculture. Although plant requirements for P are relatively low, insufficient P supply can substantially reduce productivity by reducing leaf area development and photosynthetic capacity (Wieczorek *et al*., 2022; Jiaying *et al*., 2022).

Plants primarily take up P as inorganic orthophosphate (PO_4^{3-}) by roots, mainly in the form of dihydrogen phosphate ($H_2PO_4^-$) at pH 2.1-7.2 or as hydrogen phosphate (HPO_4^{2-}) at pH 7.2-12.4. Under typical soil conditions, these two species dominate the soil solution due to their great water solubility, with $H_2PO_4^-$ generally being the predominant form (Wieczorek *et al*., 2022; Syers *et al*., 2008; Dixon *et al*., 2020; Sjöqvist, 2004). Once inside the plant, P is incorporated into organic compounds where it supports energy transfer, membrane transport and key developmental processes such as root growth, tillering, flowering, fruit formation, and ripening (Wieczorek *et al*., 2022; Andersson *et al*., 2025). Deficiency limits protein synthesis, reduces growth, and often lowers yield without obvious visual symptoms. It appears first in older leaves, as P is highly mobile within the plant and actively translocated to younger, actively growing tissues, causing older parts to wither (Andersson *et al*., 2025; Sjöqvist, 2004). In grasses and cereals, it can cause reddish lower stems, yellowing or red-brown older leaves and reduced tillering (Andersson *et al*., 2025; Yara Sweden, 2018; Eriksson *et al*., 2011). Since symptoms are nonspecific, and may result from other nutrient deficiencies, chemical analysis of total P in the plant tissue is the most reliable method for confirming deficiency (Sjöqvist, 2004). Accurate analysis requires complete digestion of the plant material, as incomplete solubilization results in an underestimation of P content (Wieczorek *et al*., 2022). Plant samples are subjected to chemical oxidation through acid digestion to decompose organic matter (Wieczorek *et al*., 2022). The resulting digest can then be analysed using inductively coupled plasma-optical emission spectroscopy (ICP-OES), a technique that quantifies elemental concentrations by exciting atoms and ions and detecting the characteristic wavelength they emitt (Wieczorek *et al*., 2022). The same analytical technique can be applied to determine elemental concentrations in soil and fertilizer samples.

2.1.2 Crop phosphorus demand

Different plant species have varying P demands, but in general, P concentrations in plants range from 0.05 to 0.5% (Wieczorek *et al.*, 2022; Dixon *et al.*, 2020). Studies on perennial ryegrass (*Lolium perenne* L.) have shown that leaf P concentrations of roughly 0.2% (2 g/kg DM) correspond to about 90% of its maximum yield (Smith *et al.*, 1985). This level is often referred to as the critical P concentration, since concentrations below this threshold results in an approximately 10% yield reduction due to P deficiency, although the exact value varies between plant species (Smith *et al.*, 1985; Dixon *et al.*, 2020).

In addition to variation between plant species, the critical P concentration in plants also depends on development stage, typically decreasing with age (Dixon *et al.*, 2020; Sjöqvist, 2004). The species-specific differences in P demand can translate into different P removal at harvest, which is why fertilization recommendations usually are based on the economically optimal P supply for the in-season production, considering crop value as well as yield, and the long-term economic benefits specific to the soil (Andersson *et al.*, 2025).

2.1.3 Root uptake of phosphorus

Crop P uptake from soil depends on several factors, including weather conditions, soil type, nutrient availability, soil pH and root system characteristics of the specific plant species (such as root size, the extent of root growth within the soil, and the efficiency with which roots absorb P) (Johnston *et al.*, 2014; Syers *et al.*, 2008). The period immediately after germination is particularly critical for P uptake, since insufficient P levels during this stage often cannot be compensated for later in plant development (Sjöqvist, 2004). As P mobility in soil is low, i.e. the ability of P compounds to move or become available within the soil system (about 0.13 mm/day for $H_2PO_4^-$), transportation to the root surface is often the primary limitation of P uptake (Sjöqvist, 2004; Syers *et al.*, 2008). Readily available P within 2-4 mm of the root surface is required for optimal uptake (Ekman *et al.*, 2023).

Plants employ various strategies to acquire P under limited conditions, including the release of root exudates, which enhance nutrient cycling and uptake by modifying pH, texture, and soil physicochemical properties of the rhizosphere (Khorassani *et al.*, 2011; Afzal *et al.*, 2025). With root exudates like low molecular weight (LMW) compounds, such as organic acids, phenolic compounds, flavonoids and other metabolites can solubilize soil P bound in weakly soluble inorganic P salts (Wu *et al.*, 2018; Khorassani *et al.*, 2011).

2.2 Phosphorus in soils

2.2.1 The soil phosphorus cycle

The soil P cycle is regulated by chemical, physical and microbiological processes that together affects the availability of P to plants. Most of the P on Earth is stored in marine and freshwater sediments in insoluble forms that are not accessible to the biota, with only a small fraction occurring in terrestrial soils (Wuenscher *et al.*, 2015; Chapin *et al.*, 2011). Although this fraction is considerable relative to crop needs, much of it is largely unavailable in the short term. Negligible amounts occur in the atmosphere, as P does not form chemically stable complexes in the gaseous phase (Wuenscher *et al.*, 2015). Since P has a low solubility and no significant gaseous phase, its transfer between major geological reservoirs (e.g. rocks, soils, sediments and oceans) is slow, with a complete geological P cycle taking more than one million years (Wuenscher *et al.*, 2015).

2.2.2 Phosphorus reactions in soil

Native P in soil derives primarily from the weathering of the primary mineral apatite and the decomposition of organic matter (Wuenscher *et al.*, 2015; Pierzynski *et al.*, 2005). Most soil P is present in more or less inaccessible forms, either as inorganic (as primary and secondary minerals or adsorbed on mineral surfaces) or organic (in soil organic matter, microbes and soil biomass) (Figure 1) (Eriksson *et al.*, 2011; Havlin *et al.*, 2014). Inorganic P in soil is distributed among different pools with varying plant availability. The most accessible P is either dissolved in the soil solution ($H_2PO_4^-$ or HPO_4^{2-}) or adsorbed on surfaces, while other pools are more strongly bound and largely inaccessible. These pools exist in a dynamic equilibrium, with P in the soil solution being replenished from surface-adsorbed and slowly desorbing pools as plants take up P (Syers *et al.*, 2008; Braun, 2020). Only a small proportion is present in the soil solution, typically ranging from near detection limits to approximately 2 mg P/L (Eriksson *et al.*, 2011).

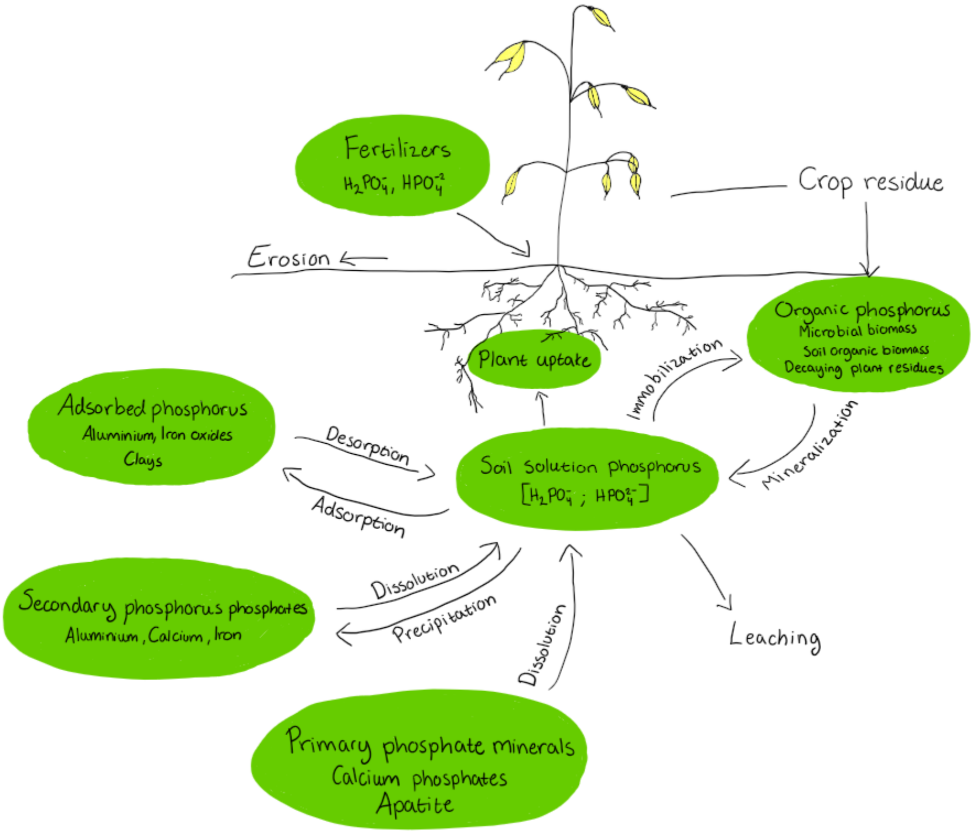

Figure 1. The soil phosphorus cycle. An overview of the microbial, physical and chemical processes controlling the availability of phosphorus to plants. Inspired by Pierzynski et al. (2005)

The availability and mobility are regulated by a range of processes, including precipitation, adsorption, mineralization, and chelation. These processes are in turn controlled by the presence of the elements iron (Fe), aluminum (Al), and calcium (Ca), as well as soil pH, mineral composition, soil surface area, and organic matter content (Dixon *et al*., 2020; Wieczorek *et al*., 2022; Eriksson *et al*., 2011).

Precipitation

Precipitation occurs when P ions react with cations like Al^{3+}, Fe^{3+} or Ca^{2+}, forming solid or semi-crystalline minerals, resulting in Al-, Fe- or Ca-phosphates (Dixon *et al*., 2020). In acidic soils ($pH <6$), precipitation primarily involves Al and Fe-oxides and may result in the formation of secondary Fe- and Al-P minerals. In neutral to alkaline soils ($pH >7$), P binds to clay minerals and Ca- or magnesium (Mg) minerals (Eriksson *et al*. 2011; Dixon *et al*., 2020; Havlin *et al*., 2014). In calcareous soils, i.e. Ca-rich soils with high pH ($pH > 8$), P can precipitate with Ca^{2+}, as octacalcium phosphate ($Ca_8H_2(PO_4)_6 \cdot 5H_2O$) or hydroxyapatite ($Ca_5(PO_4)_3OH$), both of which are sparingly soluble (Eriksson *et* *al*., 2011). These particles may aggregate, further reducing P availability (Eriksson *et al*., 2011).

Adsorption

Adsorption is the process by which P is bound to soil surface particles, reducing P concentration in the soil solution and limiting immediate plant uptake. The adsorption sites can act as a buffer, allowing adsorbed P to be released and become available to crops over time (Dixon et al., 2020). When supplied in excess, inorganic P binds strongly to available mineral sorption sites, but as sites become saturated, binding strength decreases, resulting in increased P availability in the soil solution (Sjöqvist, 2004). The most important factor controlling P sorption is the surface charge of soil particles, particularly clays and Fe- and Al-oxides, where the surface charge is highly pH-dependent (Eriksson et al., 2011). Soil pH is influenced by the balance between acid and non-acid cations on colloid surfaces and between hydrogen (H^+) and hydroxide (OH^-) ions (Brady & Weil, 2008).

At low pH, mineral surfaces are positively charged due to protonation of surface OH^- groups. As pH rises, deprotonation reduces the positive surface charge (Eriksson et al., 2011). At the same time, phosphate species become progressively more negatively charged due to successive dissociation of H^+ ions. When Fe- and Al-oxides are protonated at low pH, the electrostatic attraction between the dominate phosphate species and the mineral surface is strong, and phosphate binds through specific inner-sphere sorption to functional groups on the oxide surfaces (Blume et al., 2016). Adsorption typically occurs through ligand exchange, in which surface OH^- groups are replaced by phosphate ions, forming strongly bound and slowly exchangeable P that is difficult for plants to access (Eriksson et al., 2011).

Under alkaline conditions ($pH > 8$), protonation of Fe- and Al-oxides decreases, and instead Ca^{2+} becomes important for P sorption. At high pH, phosphate ions are more deprotonated and negatively charged, promoting electrostatic interactions with Ca^{2+}, which leads to the precipitation of insoluble calcium phosphates (CaP) and a decrease in P concentration in the soil solution (Eriksson et al., 2011).

Within the pH range 5-8, Fe- and Al-oxides are positively charged or near neutral, allowing moderate phosphate sorption without the strong fixation observed at extreme pH values. Maximum availability generally occurs in weakly acidic to neutral soils (pH 6-7), since Al^{3+} and Fe^{3+} are not soluble to a significant extent and therefore do not precipitate as insoluble phosphates (Eriksson et al., 2011).

Instead, phosphate tends to form relatively soluble CaP compounds, similar to those found in commercial fertilizers (Eriksson et al., 2011).

Mineralization and immobilization

Mineralization and immobilization are microbially mediated parallel processes central to residue turnover and nutrient cycling in soils. Immobilization refers to the incorporation of inorganic P into organic forms, whereas mineralization involves the conversion of organic P into inorganic P through the decomposition of organic material (Dixon *et al.*, 2020). The rate and extent of mineralization depend on the chemical stability of organic P fractions and microbial activity, which is influenced by soil conditions, substrate P content, and substrate availability (Sjöqvist, 2004; Hu *et al.*, 2005).

Chelation

Chelation involves multidentate ligands that donate electron pairs to metal ions, forming stable complexes (Dixon *et al.*, 2020). These ligands can be natural occurring, such as organic acids, or synthetic, and they can enhance P availability by interacting with P-binding sites in the soil (Dixon *et al.*, 2020). Both plants and microbes exudate chelating compounds that forms complexes with Fe-, Al-, or Ca-minerals, releasing phosphate ions already bound through dissolution of oxide surfaces as well as occupying sites that would otherwise fix them, thereby increasing P solubility (Sjöqvist, 2004; Eriksson *et al.*, 2011; Blume *et al.*, 2016). Considerable amounts of P can be mobilized through this process (Syers *et al.*, 2008).

2.2.3 Phosphorus analyses in soil

Soil P analyses distinguish between an easily soluble fraction, immediately available to plants, and a storage fraction, available over longer timescales (Andersson *et al.*, 2025). The results are then used to design P fertilization strategies that sustain yields and compensate for P removal through harvesting (Jordan-Meille *et al.*, 2012). Chemical extractants, however, rarely reflect the actual plant-available P over time. Instead, they indicate the amount of P available at the time of sampling.

In Sweden, the easily soluble fraction is commonly measured with P- ammonium acetate-lactate (P-AL) extraction, which reflects P potentially available to plants over a relatively short time period rather than instantly available (Jordan-Meille *et al.*, 2012; Andersson *et al.*, 2025). The storage fraction is determined using P-hydrochloric acid (P-HCl), which solubilize most inorganic forms, including apatite and Al- and Fe-oxides, making it difficult to assess the actual plant-available P (Mattsson *et al.*, 2001; Andersson *et al.*, 2025; Wuenscher *et al.*, 2015). The analysis results are compared with a classification system (class I-V) to guide fertilization (Table 1) (Mattsson *et al.*, 2001; Andersson *et al.*, 2025). For example, in P-AL Class III soils, the recommended P application rate is 10 kg P/ha for ley and grassland crops, such as *Lolium perenne* L. In Class II and Class I soils, the corresponding recommendations are 15 kg P/ha and 25 kg P/ha, respectively. Crops with higher P demand require greater application rates, e.g. 45 kg/ha for potatoes and 35 kg/ha for sugar beet in Class III soils (Andersson *et al.*, 2025).

Table 1. Classification based on the concentrations of phosphorus (P) in soil. Source: Andersson et al., 2025

	Phosphorus concentration		
	Easily soluble fraction		*Storage fraction*
Class	**P-AL (mg/100 g dry soil)**	**Class**	**P-HCl (mg/100 g dry soil)**
I	<2	I	<20
II	2.0–4.0	II	20–40
III	4.1–8.0	III	41–60
IVA	8.1–12.0	IV	61–80
IVB	12.1–16.0		
V	>16	V	>80

2.3 Phosphorus fertilizers

2.3.1 Mineral sources

Apatite is the most important primary source for mineral P fertilizer production, but in its natural form has a low solubility and limited direct plant availability (Stewart *et al.*, 2005; Hedley & McLaughlin, 2005). It can be found in rocks, natural mineral deposits and soils in silt-sized crystal forms as hydroxyapatite ($Ca_5(PO_4)_3OH$), fluorapatite ($Ca_5(PO_4)_3F$) or carbonate apatite $Ca_{10}(PO_4)_6(CO_3)(OH,F)_3$ (Blume *et al.*, 2016). Compared to fluorapatite, carbonate apatite has the highest solubility and is therefore sometimes applied directly to acidic soils as a granulate, commonly marketed as soft rock phosphate (RP). The solubility of RP in water is low (Blume *et al.*, 2016; Santos *et al.*, 2019). Therefore, it is recommended for RP to be applied as fine particles (<150 µm), as it increases the particle surface area and enhances soil factors that promote dissolution (Hedley & McLaughlin, 2005). Under acidic conditions, particularly around pH 5, the fertilizer becomes more reactive, dissolves as the carbonate component reacts with H^+ ions, gradually releasing HPO_4^{2-} (Blume *et al.*, 2016; Hedley & McLaughlin, 2005). In neutral to alkaline soils, however, P derived from RP is sparingly solubilized. Therefore, the soil-plant systems ability to modify and buffer soil solution pH is a major factor determining the extend and rate of RP dissolution (Hedley & McLaughlin, 2005).

Due to its low immediate P availability, apatite is treated with concentrated acids, typically sulfuric or nitric acid, during the production of commercial mineral P fertilizers (Ekman *et al*., 2023). Monocalcium phosphate ($Ca(H_2PO_4)_2$), the primary water-soluble component of superphosphate fertilizers (SP; e.g., single or triple superphosphate, SSP or TSP), is one commonly used fertilizer which supply plant-available P by increasing soil solution P when applied (Dixon *et al*., 2020; Havlin *et al*., 2014). It works best in neutral to slightly acidic soils, where when applied to soil dissolves and releases Ca^{2+} and $H_2PO_4^-$ to the soil solution. The dissolution creates a zone of high P concentration and slightly acidic conditions due to proton release (Eriksson *et al*., 2011; Blume *et al*., 2016). As the solution equilibrates with the surrounding soil, several processes occur depending on soil pH and composition. In neutral to alkaline soils, dissolved phosphate may precipitate with Ca^{2+} to form less soluble CaP, reducing its solubility over time (Eriksson *et al*., 2011). In acidic soils, phosphate is instead predominantly retained through adsorption to Fe- and Al-oxides. Phosphate replaces surface OH^- groups via ligand exchange, forming inner-sphere complexes (Eriksson *et al*., 2011). With decreasing pH, the positive surface charge of Fe- and Al-oxides increases, enhancing phosphate sorption. Only a very small proportion of P remains in the soil solution when water-soluble fertilizers are added, since the majority of P is distributed between less readily available pools by adsorption, increasing the risk for eutrophication (Syers *et al*., 2008).

Chemically processed fertilizers are not permitted in organic farming, where only untreated apatite and organic fertilizers are allowed (Sjöqvist, 2004). However, untreated apatite, particularly from sedimentary deposits, may contain elevated concentrations of heavy metals such as cadmium, as well as naturally occurring radionuclides. Therefore, its direct application in organic farming is generally recommended only for P-deficient soils when alternative approved fertilizers are unavailable (Sjöqvist, 2004; Ekman *et al*., 2023; Cordell *et al*., 2009).

2.3.2 Sewage sludge ash

Since 2017, the use of sewage sludge as an organic fertilizer in Sweden has increased from 35% to 60% in 2024 (Swedish Environmental Protection Agency, 2025). While the organic matter in sewage sludge can improve soil structure and biological activity, it may simultaneously introduce contaminates, since human excreta are mixed with other effluents containing metals, pathogens, and pharmaceuticals (Kirchmann *et al*., 2017). Short-term P availability from organic fertilizers is generally lower, typically 60-70% of that from mineral fertilizers, largely because a substantial proportion of P is present in poorly soluble organic forms. In sewage sludge, only about 10% of the total P is water-soluble (Delin & Engström, 2021; Andersson *et al*., 2025; Kirchmann *et al*., 2017). This limited availability results from the binding of P to precipitation chemicals such as Fe- and Al- chlorides as well as Ca-hydroxide used during wastewater treatment (Delin, 2016). Consequently, P is released more slowly, and supplementary mineral fertilization may be required during the first year after application. However, over the longer term, the availability of sludge-derived P can become comparable to that of mineral fertilizers (Delin & Engström, 2021; Delin, 2016; Andersson et al., 2025).

Through incineration, nutrients are concentrated as water and organic matter are removed, and subsequent thermo-chemical or chemical treatments may further reduce or immobilize unwanted substances. From a nutrient concentration perspective, sewage sludge ash contains around 7-13% total P, which is comparable to apatite (12-16%) (Kirchmann *et al*., 2017). Sewage sludge ash is also not regulated in the same way as the direct application of sewage sludge, where use is prohibited on soils with elevated metal concentration due to the metal content in the sludge (Andersson *et al*., 2025). Instead, ash falls under the general rules of consideration in the Environmental Code, which require farmers to justify application rates and consider heavy metal content to minimize environmental risk (Swedish Board of Agriculture, 2017).

However, a high total P concentration does not necessarily translate into high plant availability. The P mineral fertilizer equivalent (P-MFE), defined as the fraction of total P in a recycled fertilizer that is as plant-available as P applied in a reference mineral fertilizer, can be very low for untreated ash. For example, Kopp *et al.* (2023) reported a P-MFE of 4-5% for untreated mono-incinerated sewage sludge ash after six weeks in a maize trail, whereas sulfuric acid treated ash reached a P-MFE of 33-34%, compared to TSP. Similarly, in the study by Lemming *et al*. (2017), the relative fertilizer efficiency (RFE) of *Hordeum vulgare* L. var. Quench after six weeks was 27% for untreated ash and 54% for thermochemically treated ash, compared with TSP. These findings indicate that post-incineration treatment can substantially improve short-term P availability, although the treated ash still does not reach the effectiveness of highly soluble mineral fertilizers.

Within ash-based fertilizers, P-MFE values vary widely. Reported values range from 8-16% for incinerated wood ash, 13-39% for chicken manure ash and 76-99% for certain biogas ashes (Delin, 2016). Thus, the term “ash” does not refer to a uniform product, and agricultural performance depends strongly on origin and processing conditions. Although P in ash is no longer organically bound and may initially by released faster than P in untreated organic residues, its overall effect during plant growth often remains limited. After two months, the P effect of ash products (wheat straw, oat grain and rapeseed straw) corresponded to only 40-60% of that of mineral fertilizers (Delin, 2016), indicating that a substantial fraction of P remains poorly available during early growth stages.

2.3.3 Ash2Phos and RevoCaP

The patented Ash2Phos process is a wet-chemical treatment process that recovers P and other elements from ash derived from mono-incinerated sewage sludge, while simultaneously removing hazardous substances (EasyMining, 2018). Approximately 90-95% of P, 60-80% of Al, and 10-20% of Fe present in the sewage sludge ash are recovered, resulting in RevoCaP containing 15-17% of P, with a reported purity exceeding 98% (EasyMining, n.d.a; EasyMining, n.d.b). The treatment is done by first dissolving sewage sludge ash using HCl, releasing P along with Fe and Al into solution while insoluble residues are removed. Phosphorus is then purified through a two-step process involving precipitation and redissolution, allowing Fe and other heavy metals to be separated from the P-containing solution. Finally, lime is added to precipitate CaP, forming the hydroxyapatite-based calcium phosphate ($Ca_5(PO_4)_3OH$) fertilizer, with the commercial name RevoCaP (EasyMining, 2025d; Figure 2).

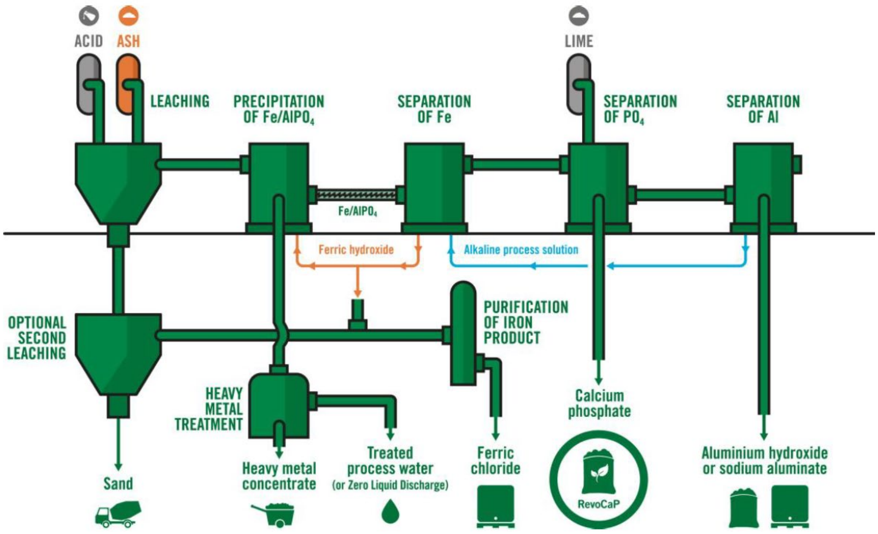

Figure 2: Schematic overview over the RevoCaP production process (EasyMining, 2025c)

Hydroxyapatite-based fertilizers exhibit limited water solubility and are generally insoluble in alkaline soils due to their inherent alkaline nature. Their dissolution is enhanced under acidic soil conditions, where proton-mediated reactions occur when both PO_4^{3-} and OH^- ions reacts with H^+ in the soil solution, increasing P-availability. Under alkaline conditions, the low availability of protons limits this reaction, thereby restricting solubility (Blume *et al.*, 2016; Ibrahim *et al.*, 2020; Noruzi *et al.*, 2023).

However, different hydroxyapatite-based CaP fertilizers vary in their physicochemical properties and crystallinity. RevoCaP contains amorphous phases, resulting in a poorly crystalline structure compared with conventional, well-crystallized hydroxyapatite materials (Cohen, personal communication, 12 February 2026). Reduced crystallinity is generally associated with increased surface area and greater exposure of reactive sites, which enhances adsorption capacity and overall reactivity (Ibrahim *et al.*, 2020). This structural characteristic likely contributes to improved P availability relative to more crystalline CaP forms.

RevoCaP has a pH around 10.5 and is classified as a slow-release P fertilizer, meaning that nutrient release occurs gradually following soil application (Liu *et al.*, 2014). It performs effectively in acidic soils (around pH 5.5) and its solubility in ammonium citrate is approximately 80%, which is a standard extraction method used to estimate plant-available P by simulating the extraction capacity of plant roots (EasyMining, n.d.b; Santos *et al.*, 2019). The availability of P from RevoCaP is influenced by soil pH and microbial activity, the latter being dependent on soil moisture and temperature. Given the pH-dependent solubility of CaP minerals, strategies that modify the chemical microenvironment of the fertilizer may further influence P release dynamics.

2.3.4 Additives to phosphorus fertilizers and their effects

The release of H^+ and organic anions, such as citrate, from plant roots and soils microorganisms accelerates key processes regulating P availability in soils, by desorption of sorbed P and dissolution of Ca phosphate (Blume *et al.*, 2016). Proton excretion lowers soil pH, thereby increasing the solubility of Ca phosphate, which are more soluble under acidic conditions. Organic anions enhance P availability by desorbing phosphate from soil surfaces or mobilizing it indirectly through complexation and chelation of Fe^{3+} and Al^{3+} (Blume *et al.*, 2016). By binding these reactive metal ions, organic acids reduce phosphate fixation and increase the concentration of P in the soil solution. Humic substances containing carboxylic (R-COOH) and phenolic (R-OH) functional groups contribute to soil acidity, and dissociate at different pH levels, releasing H^+ ions and generates negative charged sites on organic molecules (Brady & Weil, 2008). When negatively charged, the functional groups strongly attract and bind metal cation, particularly Al^{3+}, which forms stable complexes with carboxylate ($R\text{-}COO^-$) and phenolate groups on organic matter, reducing the concentration of free Al^{3+} in the soil solution (Brady & Weil, 2008). Similarly, LMW organic acids complex Al^{3+} and Fe^{3+} ions, limiting their interaction with phosphate and thereby decreasing P fixation. Organic matter can also form soluble complexes with non-acidic nutrient cations such as Ca^{3+} and Mg^{3+}, influencing their mobility and potentially facilitating their leaching (Brady & Weil, 2008). Organic anions may also directly compete with phosphate for sorption sites on soil particles. Adsorbed $H_2PO_4^-$ can be displaced by organic acids, increasing its concentration in the soil solution (Brady & Weil, 2008). Collectively, these mechanisms demonstrate that organic acids enhance P availability through acidification, complexation of metal ions, competitive desorption, and ligand-promoted mineral dissolution in soil.

Since these processes naturally increases P availability in the rhizosphere, incorporating acids into P-based fertilizers may stimulate similar mechanisms following soil application. For Ca phosphate-based ash fertilizers, acid treatment reduces the fertilizers pH, which in then in turn results in an increasing solubility of said fertilizers. In Kopp *et al*. (2023) study, a threefold increase in P-MFE occurred when sulfuric acid was used as a chemical treatment for mono-incinerated sewage sludge ash, compared with untreated ash. However, sulfuric acid is not allowed in organic production, due to it being inorganic, which makes it relevant to explore if other organic alternatives can have a similar effect.

Sutriadi *et al*. (2022) evaluated the addition of citric, humic, succinic, and oxalic acids to phosphate rock in upland acid soils, applying the acids after the phosphate rock had been incorporated into the soil, with maize as selected crop. Although the type of organic acid did not significantly affect maize yield or soil properties, increasing concentrations of acids raised soil pH toward neutrality, decreased exchangeable Al^{3+} and H^+, and increased availability of P. Among the acids tested, oxalic acid tended to be the most effective at solubilizing phosphate rock, releasing roughly twice as much total P as CA at the same concentration, followed by humic and succinic acids (Sutriadi *et al*., 2022). Similarly, Wang *et al*. (2025) found that the additions of succinic acid, luteolin and inositol significantly increased maize biomass, particularly root biomass, as well as altered the soil bacterial and fungal communities, which increased soil P bioavailability, when applied to calcareous soil. Schütze *et al*. (2020) demonstrated that CA increased P solubilization, with dissolved P concentrations rising as CA levels increased. Depending on the Fe/Al ratio and degree of hydroxide crystallization, P mobilization can increase by up to 49% in the presence of CA (Schütze *et al*., 2020).

Phenolic compounds can also influence soil pH dynamics, although their effects can be more complex. Zhou *et al*. (2025) reported that the effects of phenolic acids (salicylic acid and p-hydroxybenzoic acid) on soil nutrient availability depend on the soil type, concentration, duration of treatment, and initial soil conditions. High concentrations of phenolic acids under continuous cropping conditions decreased soil available N and P, primarily due to autotoxic compounds in root exudates that acidified the soil, reduced soil pH, and impaired physical properties such as increased compaction, bulk density and reduced water permeability. Excessive acidification also can hinder enzyme activity, further limiting nutrient availability. In contrast, moderate concentrations of phenolic acids improved nutrient availability and organic matter, likely by forming complex with metal ions that increase the solubility of otherwise insoluble nutrients (Zhou *et al*., 2025).

Hu *et al*. (2005) examined the effects of three phenolic compounds (catechol, proto-catechuic acid and caffeic acid) and CA had on P mobilization in three soils representing Ca-, Fe-, and Al-dominant soils, respectively. In terms of P mobilization, easily mobilizable P fractions increased in all soils regardless of the organic compound applied. In acidic soils, the effectiveness followed the order catechol > CA > protocatechuic acid > caffeic acid, while in calcareous soil the order was CA > catechol = protocatechuic acid > caffeic acid (Hu *et al*., 2005). Their result showed that phenolic compounds decreased soil pH, while CA either increased or had no effect on soil pH, depending on the soil type. The increase in pH under CA treatment was attributed to citrate decomposition, supported by previous studies linking CA addition to increased CO_2 production. The pH decrease following phenolic addition was due to the acidifying effects of phenolic hydroxyl and carboxyl groups, which dissociate in soil (Hu *et al*., 2005). These findings suggest that organic acids and phenols alter P fractions from stable, sparingly soluble forms to more readily soluble forms, where the magnitude depends on soil type and the organic ligands used (Hu *et al*., 2005).

Organic acids like citrate, oxalate and malate chelate Al, Fe, and Ca ions, reducing P fixation and increasing its availability (Khorassani *et al*., 2011; Wu *et al*., 2018; Sakib *et al*., 2025). For example, under P deficiency, plants often release more CA, and sugar beet specifically increases exudation of salicylic acid and citramalic acid (Schütze *et al*., 2020; Khorassani *et al*., 2011). Even at low concentrations, continuous LMW compound exudation enhances P availability via complexation, ligand-promoted mineral dissolution, and pH modification (Sakib *et al*., 2025; Zhou *et al*., 2025; Sutriadi *et al*., 2022).

3. Materials and methods

3.1 Experimental plan

The effects of P fertilization from nine different treatments (Table 2), consisting of three main fertilizer types: RevoCaP, superphosphate (SP) and soft rock phosphate (RP), were evaluated in a nine-week greenhouse pot trial from October to December in Uppsala, Sweden, using *Lolium perenne* L. grown at soil pH levels around 6 and 7. For statistical validity, each fertilizer treatment was replicated four times resulting in a total of 72 pots (36 pots with pH 7, 36 pots with pH 6) including control samples that received no P fertilization, only other macro- and micronutrients.

Table 2: Overview of the treatments tested in the study, including their pH, phosphorus concentration (P conc), and the amount of added fertilizer per pot (g/pot), calculated from the total P (%) to correspond to a field application rate of 15 kg P/ha. Aluminium (Al) and iron (Fe) concentrations in ppm are presented as well. CX stands for Compound X, CA for citric acid, RP for soft rock phosphate and lastly SP for superphosphate

Fertilizer	pH	P conc (%)	Added fertilizer (g/pot)	Al (ppm)	Fe (ppm)
RevoCaP	10.58	17.25	0.22	70.1	28.4
RevoCaP + CX 1%	9.82	16.27	0.23	59.0	27.5
RevoCaP + CX 3%	8.86	16.68	0.23	47.1	25.9
RevoCaP + CA 3%	8.51	17.33	0.22	51.2	27.9
RP	6.50	12.59	0.30	120.6	506.3
SP 100%	3.08	20.77	0.18	23.4	53.7
SP 67%	-	-	0.12		
SP 33%	-	-	0.06		
Control	-	-	-		

The RevoCaP used was a product from EM Pilot 7, derived from sewage sludge ash from Biofos (Denmark). SP was Superphosphate P20 from Yara, and RP was "Hyperkorn 26 BIO", an apatite fertilizer approved for organic production, from Timac Agro (Austria). Compound X (CX) was provided by Gyeongsang National University (South Korea).

RevoCaP treatments included pure RevoCaP, RevoCaP combined with CX at 1% (RevoCaP + CX 1%) and 3% (RevoCaP + CX 3%), as well as RevoCaP with CA at 3% (RevoCaP + CA 3%). SP was applied at three different concentrations (33%, 67% and 100%). The lower concentrations were included solely to enable linear regression analysis and were excluded from the statistical analyses. RP was applied as a single treatment.

3.2 Fertilizer preparation and analysis

RevoCaP with the respective additive was prepared at EM, where RevoCaP was combined with either 1% or 3% (w/w) of additive (CX or CA), together with deionized water to form a homogeneous mixture. The mixtures were placed in an oven at 105 °C for 24 hours and grinding again after drying. To ensure that all fertilizers, including SP and RP, had the same particle size, they were ground in an IKA MultiDrive MT 150 and subsequently sieved though a 355 μm sieve.

The pH was measured using an HI2202 Edge® Blu Bluetooth® Smart pH Electrode and Meter. A 1:10 (w/v) ratio was used, corresponding to 1 part fertilizer and 10 parts deionized water (specifically 3 g fertilizer to 30 mL deionized water). The mixtures were left to stand for 2 hours, after which the pH was measured in the liquid phase separated from the suspension.

The total P content of each fertilizer was determined after digestion with a mixture of concentrated nitric and HCl acids and then analysed using ICP-OES. Samples preparation was carried out according to the industry-standard protocol provided by Thermo Fisher Scientific (n.d.; see Appendix 1 for description). Total P content in fertilizers was determined using Equation 1, based on the sample weight (~1.00 g) and the final solution volume (0.05 L).

$$\text{Total P (mg/g)} = \frac{\text{Total P (mg/L)} \times \text{solution volume (L)}}{\text{sample weight (g)}} \tag{1}$$

The amount of fertilizer added to each pot was calculated to correspond to a field application of 15 kg P/ha, taking into account the pot surface area and the measured total P concentration of each fertilizer (Equations 2-4).

$$\text{Pot area (ha)} = \frac{\text{Pot area (m}^2\text{)}}{10\,000} \tag{2}$$

$$\text{P needed per pot (mg)} = \text{15kg P/ha} \times \text{Pot area (ha)} \times 1\,000\,000\ \text{mg/kg} \tag{3}$$

$$\text{Fertilizer needed per pot (g)} = \frac{\text{P needed per pot (mg)}}{\text{Total P (mg/g)}} \tag{4}$$

3.3 Soil properties

A commercial filter sand (particle size 0.4-0.8 mm) from Råda ridge in Lidköping, was used as the soil medium.

Nutrient concentrations in the sand were analysed by AgriLab (Uppsala, Sweden) using ICP-OES. According to the Swedish Board of Agriculture classification system, the soil had low P levels, with the easily soluble fraction classified as Class I and the storage fraction as Class II (Table 3) (Andersson et al., 2025). The soil also had low Fe and Al concentration and a neutral pH.

Table 3. pH and concentration of ammonium acetate lactate (AL) and hydrochloric acid (HCl) of extractable phosphorus (P), potassium (K), magnesium (Mg) and calcium (Ca), as well as aluminium (Al), iron (Fe) and copper (Cu) in the soil of the experiment

Properties	Value	Property	Value
pH	7.0	Cu-HCl	12.0 mg/kg
P-AL	0.5 mg/100g	P-HCl	24.4 mg/100 g
K-AL	0.7 mg/100 g	K-HCl	84.0 mg/100g
Mg-AL	1.6 mg/100g	Al-AL	2 mg/100g
Ca-AL	16 mg/100g	Fe-AL	3 mg/100g

Following the procedure described in the *Laboratory Compendium in Soil Chemistry* (Swedish University of Agricultural Sciences, 2022), soil pH was determined by mixing soil and deionized water at a 1:5 ratio (5 mL soil to 25 mL water). The mixture was shaken for 5 minutes and allowed to stand for 2 hours before measuring the pH, which was measured by using PHM 93 Reference pH meter directly in the soil aqueous suspension.

3.4 Pot trial design

3.4.1 Preparation and sowing

Approximately 2.7 kg of sand was added to each pot (Ø18 x 14 cm). To ensure a homogeneous distribution within the sand and to prevent uneven mixing, such as higher concentrations of nutrients settling at the bottom of the pot, two-thirds of the soil was transferred to a separate container and thoroughly mixed with the designated P fertilizer according to its total P concentration. In addition, solutions containing all essential nutrients except P was added in appropriate proportions to ensure that no other element would limit plant growth (Table 4). The fertilized and treated soil was then returned to the pot. To maintain nutrient levels, nutrient solutions were applied again after the first harvest.

Table 4. Composition, concentrations and volume of nutrient solutions added per pot

Solution type	Compounds included	Concentration of compounds (mg/mL)	Volume per pot (mL)
Micronutrient solution	$ZnCl_2$ $FeCl_3$ $MnSO_4$ $Cu(NO_3)_2$ NaB_4O_7 Na_2MoO_4	1.2 3.3 2.5 0.8 0.8 0.3	10
Macronutrient solution	K_2SO_4 $MgCl_2$	34.05 42.5	10
Nitrogen–calcium macronutrient solution	NH_4NO_3 $Ca(NO_3)_2$	58.06 42.84	10
Total volume	-	-	30

To test the performance of the different products at a pH ≤ 6, an aqueous citric acid-trisodium citrate buffer was applied to the soil prior to planting to reduce the pH (Table 5). The buffer was prepared by diluting 25 mL of stock solution with deionized water to a final volume of 500 mL prior to application. When the buffer was added, initial soil pH was around 5.3.

Table 5. Stock and final concentrations of the aqueous citric acid-trisodium citrate buffer applied to each pot

Component	Stock concentration (g/L)	Final concentration (mM)
Citric acid	32.4	8.4
Trisodium citrate	46.3	9.0

To avoid further increases in soil sodium (Na) concentrations, beyond the typical range for perennial ryegrass (0.2-2.4 g/kg DM; Eurofins Agro, 2024), no additional buffer was applied. Buffer-amended soils were classified as higher-pH soils (≥ 7, “C-soils”), while non-amended soils were classified as pH 6 soils (“N-soils”).

Following the addition of P fertilizer, and nutrient solution to all pots, and buffer to the C-soils, *Lolium perenne* were planted. The cultivar *Sirtaky*, harvested in 2024 was obtained from Skånefrö. Approximately 0.1 g of seeds were sown per pot. To prevent dehydration, the pots were watered three times per week with deionized water, to avoid introducing additional minerals.

3.4.2 Greenhouse environment and growing conditions

A closed watering system was employed to prevent nutrient loss. In this system, water added to the pots was collected in trays beneath them, allowing it to be reabsorbed by the soil and plants. This means that, in theory, all nutrients added to the system can be utilized by the plants (Christensen *et al*., 2010).

In the greenhouse, the photoperiod was set to 16 hours per day (04:00-20:00), to simulate spring growing conditions. Daytime temperatures were set in the beginning to 15 °C and nighttime temperatures were 10 °C. After two weeks, the daytime temperature was increased to 18 °C and nighttime to 13 °C.

To account for microclimate variation within the greenhouse, such as differences in temperature, plant position, or reflected radiation (Koller *et al*., 2016), a randomized complete block design was used, with all treatments randomly assigned within each block (Salinas Ruíz *et al*, 2024). Soil pH was used as a blocking factor, with pots of the same pH grouped within separate blocks (Figure 3). Each block included all combinations of fertilizer type (nine levels) and pH (two levels), resulting in 18 treatments. Following germination, pots were rotated weekly, and their position within each block were randomized using a Python script (see Appendix 2).

Figure 3. Greenhouse setup showing two blocks of pH treatments, each containing 36 pots arranged in 12 columns with 3 pots stacked vertically. Pots were randomly placed and rotated weekly to minimize microclimatic effects

3.4.3 Harvesting

The grass was harvested after 6 and 9 weeks of growth over two consecutive days: N-soils on the first day and C-soils on the second, since harvesting all pots on a single day was not feasible. During the first harvest, approximately 1 cm of stubble was left, whereas in the second harvest the grass was cut as close to the soil surface as possible. After harvesting, the samples were weighed, dried overnight at 50 °C to reduce moisture to approximately 5%, and then weighed again.

3.5 Calculations of dry matter and phosphorus yield

DM yield was calculated with the use of Equation 5. The P concentrations of the dried plant material were analysed by AgriLab using ICP-OES, which were then used to calculate P yield by Equation 6.

$$\text{DM yield (DM t/ha)} = \frac{\left(\frac{\text{DM (g)}}{1000}\right)}{\text{Pot area}} \times 10 \tag{5}$$

$$\text{P yield (kg P/ha)} = \text{kg DM/ha} \times \left(\frac{\text{g P/kg}}{1000}\right) \tag{6}$$

To determine the P-MFE of the fertilizers, the approach described by Delin (2014) and Shi *et al*. (2022) was used. The P yield of the mineral fertilizers (SP) treatments and the unfertilized control was plotted against the applied mineral P rates (kg P/ha), assuming mineral P to be 100% plant available. P application rate was placed on the x-axis and crop P yield on the y-axis, and a linear regression model was fitted to the data to obtain a response curve. From this regression, the MFE rate, defined as the amount of P fertilizer (kg P/ha) required to produce the same crop response as the mineral fertilizer, was determined. P-MFE (%) was then calculated by dividing the MFE (kg P/ha) by the total P applied with each fertilizer (15 kg P/ha) (Equation 7).

$$\text{P-MFE (\%)} = \frac{\text{MFE (kg P/ha)}}{\text{P applied (kg P/ha)}} \times 100 \tag{7}$$

3.6 Statistical analyses

The effects of pH, fertilizers, and their interaction on DM yield, plant P concentration, and total P yield were analysed using a two-way ANOVA (general linear model) in Minitab, with pH and fertilizer treatment as fixed factors. The significant level was set at α = 0.05. When significant effects were detected, means were compared with post hoc comparisons using Tukey HSD test.

4. Results

The results from the first and second harvest are presented in terms of DM yield, P concentrations, P yield and P-MFE. Although shown together for clarity, as the crops did not grow for the same length of time, the two harvests are not directly comparable. Interpretations are therefore made within each harvest.

4.1 Dry matter yield

Two-way ANOVA revealed significant main effects of soil pH and fertilizer treatment on DM yield (F(1, 42) = 29.97, $p < 0.001$ and F(6, 42) = 211.24, $p < 0.001$, respectively), as well as a significant interaction between pH and fertilizer (F(6, 42) = 28.37, $p < 0.001$) at the first harvest (Appendix 5, Table 16-17). This indicates that DM yield was influenced not only by soil pH and fertilizer treatment, but also that the effect of fertilizer depended on soil pH. The highest DM yields were observed under N-soil condition, however, the differences between RevoCaP treatment were small (Figure 4).

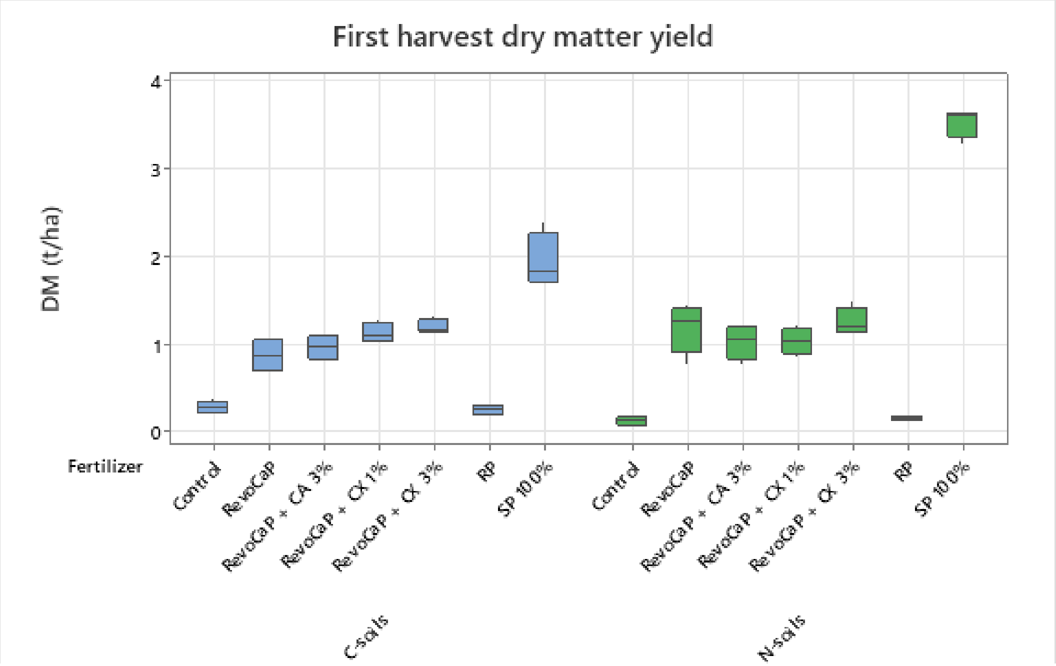

Figure 4. Boxplots showing the first harvest dry matter yield (DM yield) (t/ha)

Post hoc comparison showed no significant differences between RevoCaP treatments regardless of soil pH. The significant effect was a result of RP and control treatments producing significantly lower DM yield, and SP 100% significantly higher compared to RevoCaP treatments, independent of soil pH. Although no significant differences between samples, RevoCaP + CX 3%

produced the highest DM yield in both C-soils and N-soils (Table 6). Unamended RevoCaP had the highest DM yield in N-soils.

Table 6. Results from the first harvest showing mean values of dry matter yield (DM t/ha) and their standard deviation (SD), as well as the Tukey pairwise comparisons of the interaction between pH and fertilizer (Grouping). CX stands for Compound X and CA for citric acid. ^a^Means that do not share a letter are significantly different ($P < 0.05$)

	C-soils		N-soils	
Treatment	Mean ± SD	Grouping[a]	Mean ± SD	Grouping[a]
RevoCaP	0.872 ± 0.193	C	1.191 ± 0.286	C
RevoCaP + CX 1%	1.125 ± 0.117	C	1.030 ± 0.146	C
RevoCaP + CX 3%	1.194 ± 0.079	C	1.245 ± 0.157	C
RevoCaP + CA 3%	0.967 ± 0.137	C	1.023 ± 0.194	C
RP	0.253 ± 0.63	D	0.156 ± 0.026	D
SP 100%	1.930 ± 0.311	B	3.529 ± 0.164	A
Control	0.278 ± 0.078	D	0.129 ± 0.051	D

At the second harvest, two-way ANOVA showed significant effects of pH and fertilizer treatment on DM yield (F(1, 42) = 61.92, p < 0.001 and F(6, 42) = 318.26, p < 0.001, respectively), as well as a significant interaction between pH and fertilizers (F(6, 42) = 6.85 p < 0.001) (Appendix 6, Table 22-23). N-soils produced overall higher DM yield compared to C-soils, with the lowest yields observed in the control and RP samples across both soil pH (Figure 8).

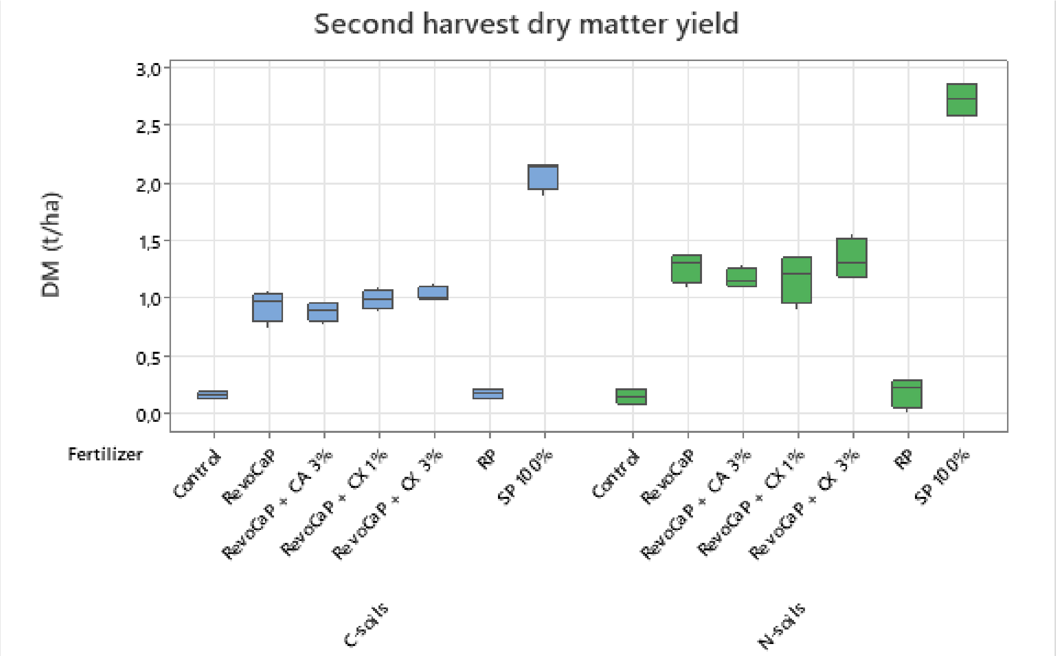

Figure 5. Boxplots showing the second harvest dry matter yield (DM yield) (t/ha)

Post hoc analysis showed no significant differences among RevoCaP treatments within the same soil pH. However, comparing the pH treatments, there were a significant difference between RevoCaP + CX 3% and unamended RevoCaP in N-soils with C-soils, with N-soil treatments producing higher P yields (Table 7). Significant differences were also observed between RevoCaP treatments and SP 100%, RP, control under both soil pH.

Table 7. Results from the second harvest showing mean values of dry matter yield (DM t/ha) and their standard deviation (SD), as well as the Tukey pairwise comparisons of the interaction between pH and fertilizer (Grouping). CX stands for Compound X and CA for citric acid. [a]Means that do not share a letter are significantly different ($P < 0.05$)

	C-soils		N-soils	
Treatment	Mean ± SD	Grouping[a]	Mean ± SD	Grouping[a]
RevoCaP	0.938 ± 0.138	E	1.274 ± 0.129	C,D
RevoCaP + CX 1%	0.990 ± 0.086	D,E	1.172 ± 0.213	C,D,E
RevoCaP + CX 3%	1.035 ± 0.064	D,E	1.340 ± 0.174	C
RevoCaP + CA 3%	0.890 ± 0.079	E	1.174 ± 0.084	C,D,E
RP	0.174 ± 0.048	F	0.191 ± 0.130	F
SP 100%	2.080 ± 0.126	B	2.724 ± 0.151	A
Control	0.158 ± 0.034	F	0.148 ± 0.072	F

4.2 Phosphorus concentrations

Two-way ANOVA showed no significant main effect of pH on P concentration ($F(1, 42) = 1.52$, $p < 0.225$) at the first harvest (Appendix 5, Table 18-19), indicating that overall concentrations were similar between C- and N-soils. In contrast, fertilizer treatment had a strong effect on P concentrations ($F(6, 42) = 80.36$, $p < 0.001$), were SP 100% had the highest concentration, and the controls the lowest. A significant interaction between soil pH and fertilizer was observed ($F(6, 42) = 3.33$, $p < 0.009$), indicating that some fertilizer treatments performed differently in C-soils compared to N-soils. Post hoc analysis revealed no significant differences among the RevoCaP treatments, regardless of soil pH. The significant differences observed were due to differences between the RevoCaP treatments and SP 100%, controls, as well as RP treatment within each soil pH. In C-soils, unamended RevoCaP had the highest mean P concentration among the RevoCaP treatments, while in N-soils, RevoCaP + CA 3% had the highest mean P concentration, although the differences were minimal (Figure 6).

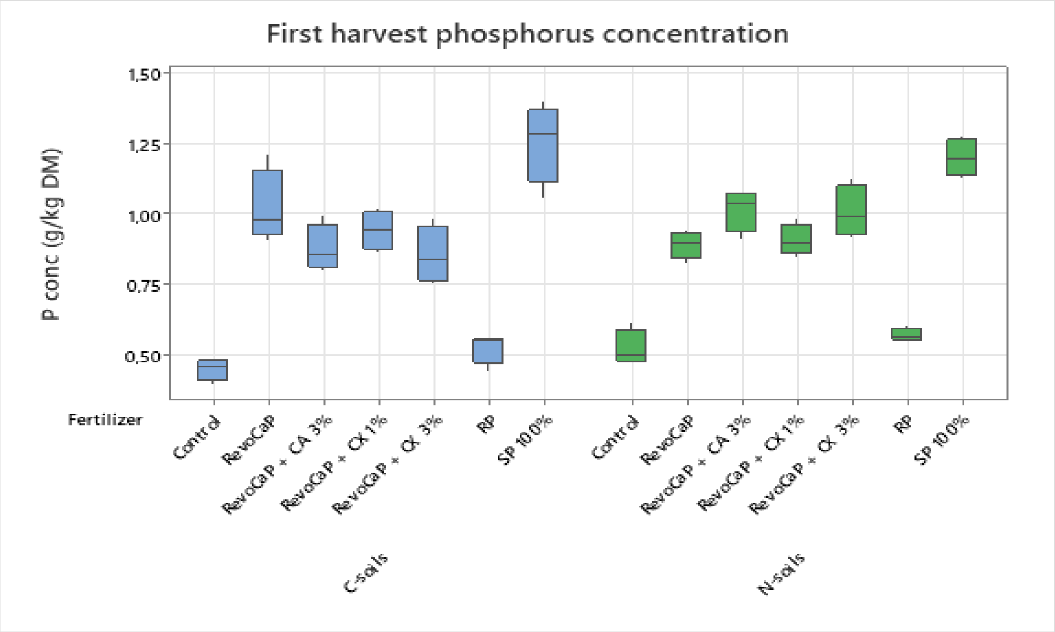

Figure 6. Boxplots showing the first harvest phosphorus concentration (P conc) (g/kg DM)

All samples were within the expected P concentration range reported by Wieczorek *et al*. (2022) and Dixon *et al*. (2020), namely 0.05-0.5%, but lower than the stated critical value of approximately 0.2% (Smith *et al.,* 1985; Table 8).

Table 8. Results from the first harvest showing mean values of P concentrations (g/kg DM) and their standard deviation (SD), as well as the Tukey pairwise comparisons of the interaction between pH and fertilizer (Grouping). CX stands for Compound X and CA for citric acid. ^{a}*Means that do not share a letter are significantly different (P < 0.05)*

	C-soils			N-soils		
Treatment	Mean ± SD	Mean (%)	Grouping^a	Mean ± SD	Mean (%)	Grouping^a
RevoCaP	1.021 ± 0.133	0.102	B,C	0.892 ± 0.048	0.089	C
RevoCaP + CX 1%	0.943 ± 0.067	0.094	C	0.908 ± 0.055	0.091	C
RevoCaP + CX 3%	0.854 ± 0.102	0.085	C	1.006 ± 0.092	0.101	B,C
RevoCaP + CA 3%	0.878 ± 0.082	0.088	C	1.017 ± 0.075	0.102	B,C
RP	0.527 ± 0.053	0.053	D	0.572 ± 0.023	0.057	D
SP 100%	1.257 ± 0.142	0.126	A	1.200 ± 0.067	0.120	A,B
Control	0.451 ± 0.038	0.045	D	0.523 ± 0.064	0.052	D

At the second harvest, two-way ANOVA showed significant effects of pH and fertilizer treatment (F(1, 42) = 38.87, p < 0.001 and F(6, 42) = 19.10, p < 0.001, respectively), as well as a significant interaction between pH and fertilizer treatment (F(6, 42) = 7.03, p < 0.001) on P concentrations, indicating that the fertilizing response varied between soil pH treatment (Appendix 6, Table 24-25). The N-soils RevoCaP and RP treatments had the highest P concentrations compared to C-soils, while SP 100% had a higher concentration in C-soils than in N-soils (Figure 7).

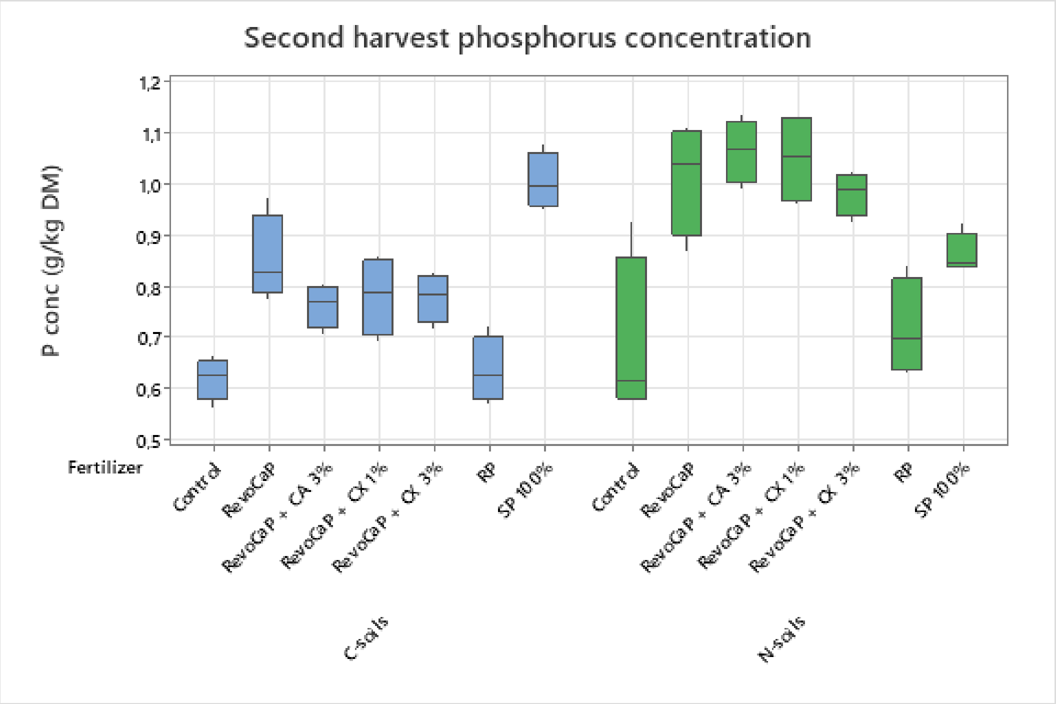

Figure 7. Boxplots showing the second harvest phosphorus concentration (P conc) (g/kg DM)

Post hoc analyses revealed no significant differences between RevoCaP treatments within the same soil type (Table 9). Across soil pH treatments, significant differences were observed for all the RevoCaP treatments with additives, with N-soil samples showing higher P concentration than C-soils, while unamended RevoCaP showed no significant difference between C-soils and N-soils. All samples were within the expected P concentration range (0.05-0.5%; Wieczorek *et al*., 2022; Dixon *et al*., 2020); however, they were below the stated critical value of 0.2%.

Table 9. Results from the second harvest showing mean values of P concentrations (g/kg DM) and their standard deviation (SD), as well as the Tukey pairwise comparisons of the interaction between pH and fertilizer (Grouping). CX stands for Compound X and CA for citric acid. [a]Means that do not share a letter are significantly different ($P < 0.05$)

	C-soils			N-soils		
Treatment	Mean ± SD	Mean (%)	Grouping[a]	Mean ± SD	Mean (%)	Grouping[a]
RevoCaP	0.851 ± 0.086	0.085	B,C,D	1.012 ± 0.108	0.101	A,B
RevoCaP + CX 1%	0.781 ± 0.079	0.078	C,D,E	1.048 ± 0.091	0.105	A,B
RevoCaP + CX 3%	0.778 ± 0.048	0.078	D,E	0.980 ± 0.043	0.098	A,B,C
RevoCaP + CA 3%	0.763 ± 0.042	0.076	D,E	1.062 ± 0.061	0.106	A
RP	0.635 ± 0.064	0.064	E	0.716 ± 0.096	0.072	D,E

SP 100%	1.004 ± 0.055	0.100	A,B	0.861 ± 0.040	0.086	A,B,C,D
Control	0.619 ± 0.041	0.062	E	0.684 ± 0.164	0.068	D,E

4.3 Phosphorus yield

Two-way ANOVA revealed significant main effects of soil pH and fertilizer treatment (F(1, 42) = 77.16, $p < 0.001$ and F(6, 42) = 504.04, $p < 0.001$, respectively), as well as a significant interaction between pH and fertilizers (F(6, 42) = 50.89, $p < 0.001$) on P yield at the first harvest (Appendix 5, Table 20-21). The interaction indicates that the effect of fertilizer depended on soil pH, however, this was primarily driven by significant differences between the RevoCaP treatments and SP 100%, RP and the control treatments. Among the RevoCaP treatments, P yield was similar, although C-soil treatments showed slightly lower yields than N-soil treatments (Figure 8).

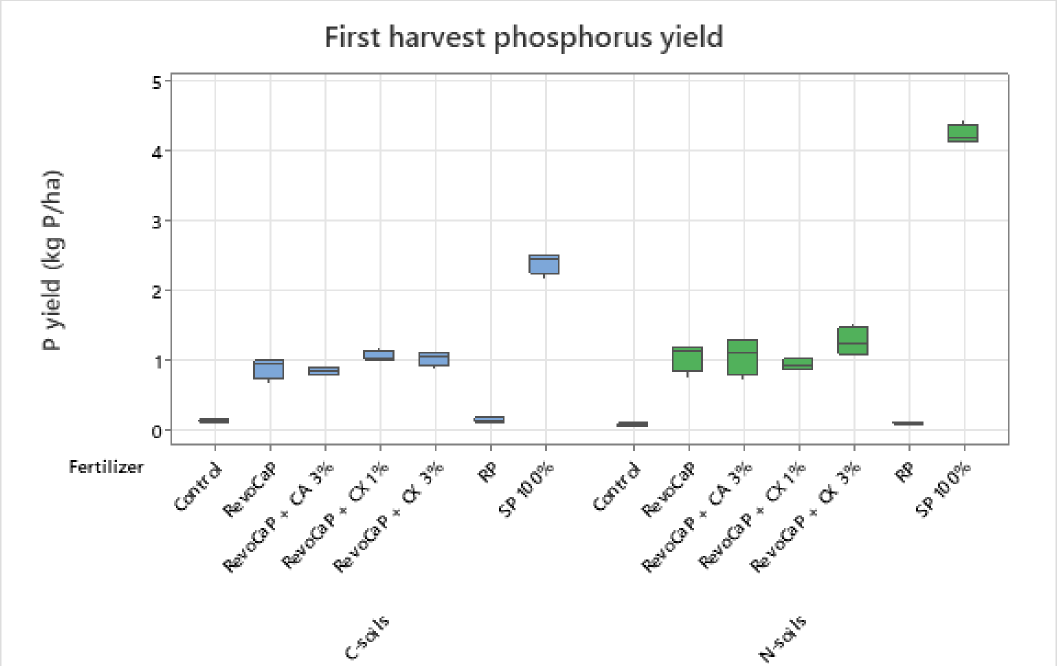

Figure 8. Boxplots showing the first harvest phosphorus yield (kg P/ha)

The highest P yield was observed for RevoCaP + CX 3% in N-soils, and for RevoCaP + 1% in C-soils (Table 10). However, post hoc analysis revealed no significant differences between the RevoCaP treatments, regardless of soil pH.

Table 10. Results from the first harvest showing mean values of P yield (kg P/ha) and their standard deviation (SD), as well as the Tukey pairwise comparisons of the interaction between pH and fertilizer (Grouping). CX stands for Compound X and CA for citric acid. [a]Means that do not share a letter are significantly different ($P < 0.05$)

	C-soils		N-soils	
Treatment	Mean ± SD	Grouping[a]	Mean ± SD	Grouping[a]
RevoCaP	0.878 ± 0.147	D	1.053 ± 0.212	C,D
RevoCaP + CX 1%	1.056 ± 0.073	C,D	0.929 ± 0.080	C,D
RevoCaP + CX 3%	1.017 ± 0.109	C,D	1.254 ± 0.205	C
RevoCaP + CA 3%	0.842 ± 0.060	D	1.051 ± 0.267	C,D
RP	0.134 ± 0.039	E	0.089 ± 0.016	E
SP 100%	2.395 ± 0.157	B	4.226 ± 0.148	A
Control	0.124 ± 0.025	E	0.067 ± 0.025	E

At the second harvest, the two-way ANOVA revealed significant main effect of pH and fertilizer treatment ($F(1, 42) = 94.49$, $p < 0001$ and $F(6, 42) = 263.79$, $p < 0.001$, respectively), as well as a significant interaction between soil pH and fertilizer ($F(6, 42) = 7.27$, $p < 0.001$) on P yield (Appendix 6, Table 26-27). The P yield was higher for all RevoCaP treatments in N-soils compared to C-soils, while SP 100%, control and RP samples showed similar yield pattern between the two soil pH treatments (Figure 9).

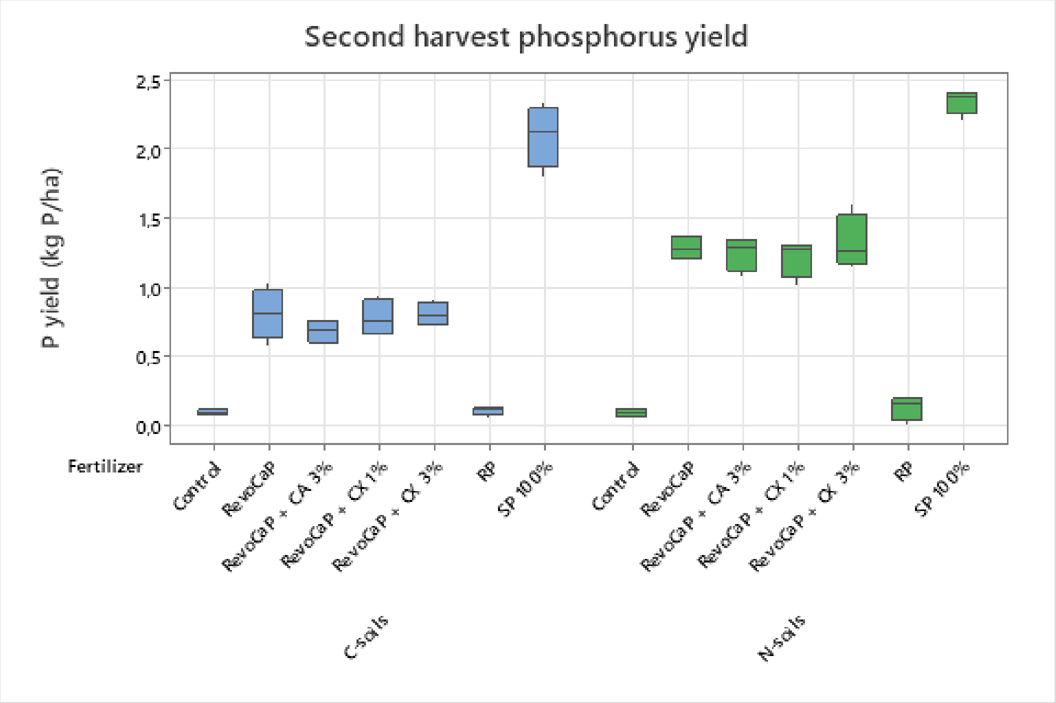

Figure 9. Boxplots showing the second harvest phosphorus yield (kg P/ha)

The N-soils had higher P yield than C-soils, particularly for RevoCaP + CX 3% and unamended RevoCaP (Table 11). Post hoc analyses indicated significant differences between the two soil pH types for the RevoCaP treatments, however, there were no significant differences among RevoCaP treatments within the same soil pH. In addition, RevoCaP treatments differed significantly from SP 100%, RP and control in both C-soils and N-soils.

Table 11. Results from the second harvest of the pot experiment, showing mean values of phosphorus yield (kg P/ha) and their standard deviation (SD), as well as the Tukey pairwise comparisons of the interaction between pH and fertilizer (Grouping). CX stands for Compound X and CA for citric acid. [a]*Means that do not share a letter are significantly different (P < 0.05)*

	C-soils		N-soils	
Treatment	Mean ± SD	Grouping[a]	Mean ± SD	Grouping[a]
RevoCaP	0.805 ± 0.186	C	1.282 ± 0.089	B
RevoCaP + CX 1%	0.777 ± 0.135	C	1.215 ± 0.135	B
RevoCaP + CX 3%	0.806 ± 0.086	C	1.315 ± 0.195	B
RevoCaP + CA 3%	0.680 ± 0.086	C	1.247 ± 0.122	B
RP	0.111 ± 0.032	D	0.129 ± 0.089	D
SP 100%	2.093 ± 0.223	A	2.342 ± 0.090	A
Control	0.098 ± 0.022	D	0.095 ± 0.033	D

4.4 Mineral fertilizer equivalent

The linear regression analysis showed a strong relationship for both pH treatments, with $R^2 = 0.9832$ for N-soils and R^2 of 0.9718 for C-soils at the first harvest (Figure 10). The resulting regression equations were then used for the calculation of MFE.

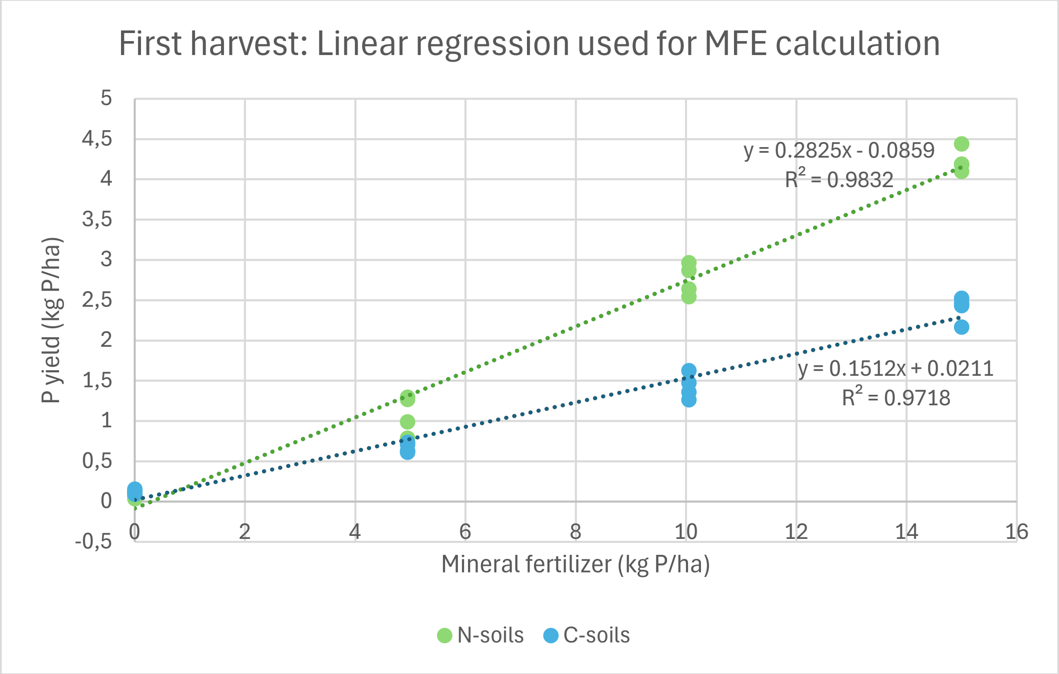

Figure 10. Linear regression analyses of mineral fertilizer equivalent for the first harvest

Among the RevoCaP treatments, the C-soil pH treatment exhibited the highest overall MFE during the first harvest, ranging between 36.2 to 45.6%, with the highest MFE observed for RevoCaP + CX 1% (Table 12). In the N-soil pH treatment, MFE values for RevoCaP treatments ranged from 24.0 to 31.6%, with RevoCaP + CX 3% again showing the highest MFE. In contrast, RP resulted in low MFE values under both pH treatments.

Table 12. Results from the first harvest showing mean values the mineral fertilizer equivalent (MFE; % of total phosphorus applied)

	C-soils	N-soils
Treatment	MFE	MFE
RevoCaP	37.8	26.9
RevoCaP + CX 1%	45.6	24.0
RevoCaP + CX 3%	43.9	31.6
RevoCaP + CA 3%	36.2	26.8
RP	5.0	4.1

At the second harvest, the linear regression analysis showed a strong relationship for both pH treatments, with $R^2 = 0.9853$ for the N-soils and $R^2 = 0.9658$ for the C-soils (Figure 11).

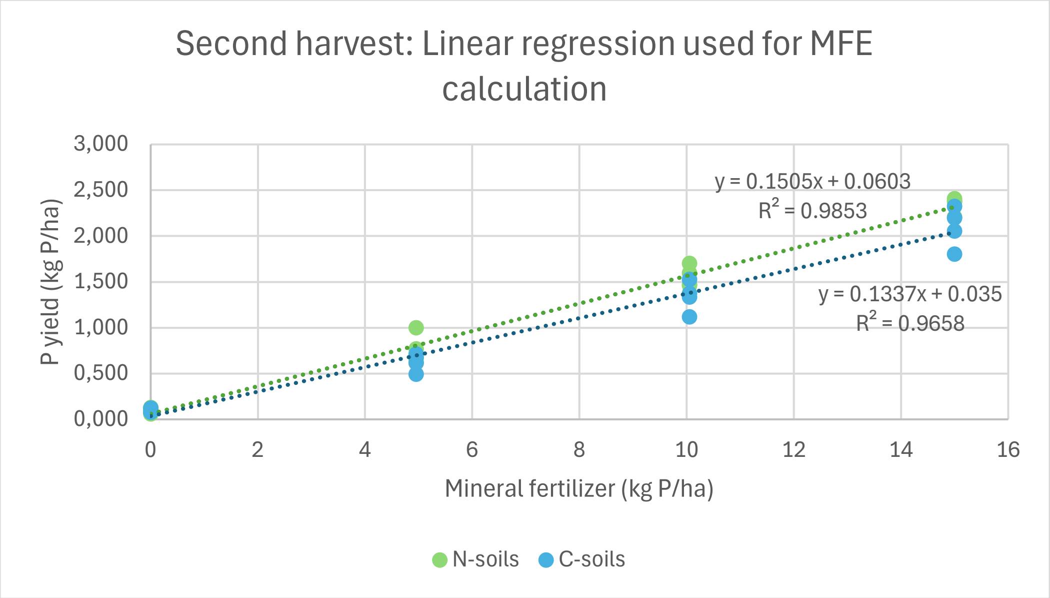

Figure 11. Linear regression analyses of mineral fertilizer equivalent for the second harvest

The RevoCaP treatments in N-soils had the highest MFE values, ranging from 51.2 to 55.6% (Table 13). In C-soils, MFE values for RevoCaP treatments ranged from 32.2 to 38.5%. RevoCaP CX 3% showed the highest MFE under both soil pH treatments, although it did not differ substantially from the second-highest treatment, unamended RevoCaP.

Table 13. Results from the second harvest of the pot experiment, showing mean values the mineral fertilizer equivalent (MFE; % of total phosphorus applied)

	C-soils	N-soils
Treatment	MFE	MFE
RevoCaP	38.4	54.1
RevoCaP + CX 1%	37.0	51.2
RevoCaP + CX 3%	38.5	55.6
RevoCaP + CA 3%	32.2	52.6
RP	3.8	3.1

4.5 Visual observations

Visual observations of plant biomass were made at the first and second harvests. At both time points, the control and RP treatments in both C- and N-soils exhibited symptoms of P deficiency, including yellowing to browning of stems, stunted growth, reduced branching and withering of older leaves.

General visual observation of the soil samples at the second harvest showed that all treated pots, except for the control and RP treatments, had well-developed root system in both N-and C-soils. In contrast, the control and RP treatments had very small root systems, or almost none. Differences in root volume were observed among treatments: the SP 100% treatments showed the largest root volume, while RevoCaP treatments had smaller. However, overall differences among the RevoCaP treatments were relatively minor.

Across the different pH treatments, all C-soils exhibited a green layer on the soil surface (Figure 12), while no such layer was observed in the N-soils.

Figure 12. Overhead photograph of a pot containing soil and grass from the second harvest, showing a green layer on the soil surface

4.6 Soil pH and salinity

During the trial, soil pH was measured at several time points to monitor changes until the first harvest, followed by a final measurement at the end of the trial. In C-soils, the initial pH was low (mean 5.3), but some samples increased sharply to 9.2 after two weeks (Control, RevoCaP + CX 3%, RevoCaP and SP 100%). In subsequential weeks, pH gradually declined, reached approximately 7.5 in the control samples. In N-soils, pH was measured at a few selected time points and remained close to expected values, around 6.

At the end of the study, C-soils control treatments had a mean pH of 6.77, while SP 100% had a mean pH of 6.20 (Figure 13). The RevoCaP treatments, as well as RP, were very similar, ranging between 6.64 to 6.68. In N-soils RevoCaP treatment, control and RP treatments were also similar, ranging between 6.17 to 6.32, while SP 100% had the lowest mean pH at 5.90.

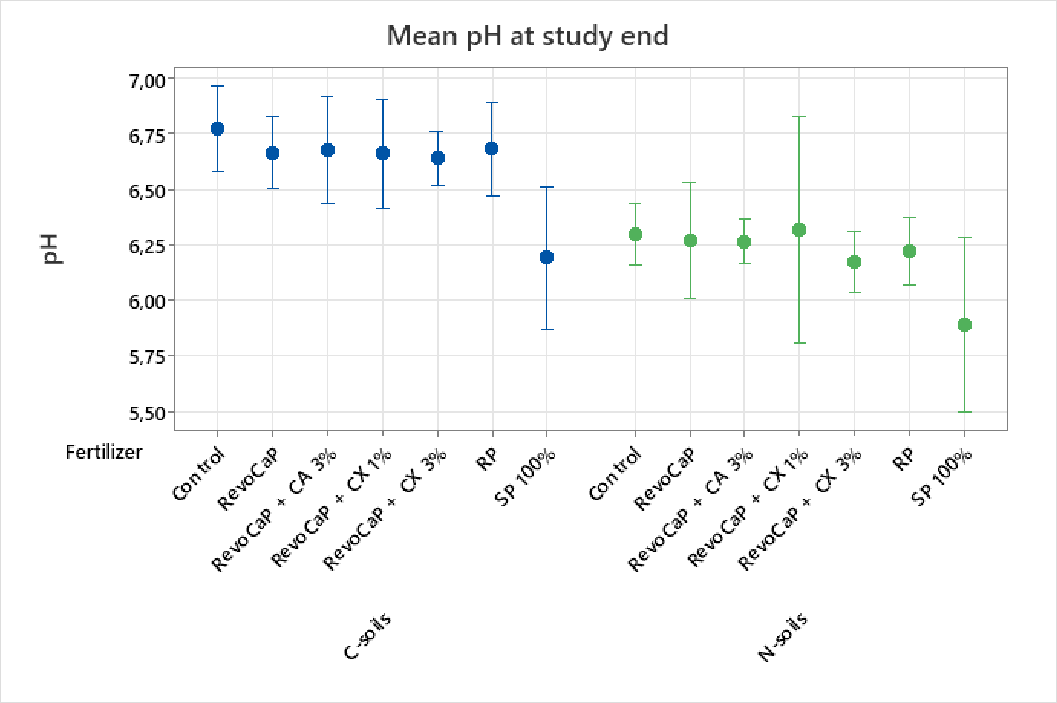

Figure 13. Interval plot of soil pH measured at the end of the experiment. Error bars represent the 95% confidence interval of the mean, calculated based on individual observations.

Regarding Na concentration, the C-soils had higher Na levels in both the first and the second harvest compared to the N-soils, which were consistently very low (<0.1-0.2 g/kg DM), and therefore not shown in the table, as they are considered negligible for further discussions (Table 14). In the first harvest, all samples except SP 100% were within the expected range (0.2-2.4 g/kg DM; Eurofins Agro, 2024). In the second harvest, all samples except RP and control samples were within the expected range.

Table 14. Results from the C-soils first and second harvest, showing mean values of sodium (Na) concentrations (g/kg DM)

	First harvest	Second harvest
Treatment	Na	Na
RevoCaP	2.1	1.4
RevoCaP + CX 1%	2.3	1.6
RevoCaP + CX 3%	2.4	1.7
RevoCaP + CA 3%	2.1	1.4
RP	2.4	4.3
SP 100%	2.6	1.6
Control	1.9	3.9

5. Discussion

Phosphorus recovery from incinerated sewage sludge represents a promising strategy to reduce the EU dependency on imported mineral fertilizers. However, untreated sewage sludge ash has been shown to exhibit very low fertilizer efficiency compared to water soluble mineral P fertilizers, whereas post-treatment of sewage sludge ash can substantially improve short-term P availability, although typically not to the level of highly soluble products such as TSP (Kopp *et al.*, 2023; Lemming *et al.*, 2017). Thus, for wet-chemically processed fertilizers, there may be potential to further enhance P availability through complementary strategies, such as the addition of organic acids. Organic acids are known to enhance P availability through acidification, complexation of Ca, Fe, and Al, and ligand-promoted dissolution of sparingly soluble phosphate minerals (Blume *et al.*, 2016; Brady & Weil, 2008; Khorassani *et al.*, 2011). Such mechanisms shift P from stable mineral phases to more labile fractions and may reduce P fixation in soil as well as in fertilizers (Schütze *et al.*, 2020; Sakib *et al.*, 2025). In this study, two different organic acids were applied to a wet-chemically processed CaP fertilizer, RevoCaP, to assess whether additional modification of the chemical microenvironment could further enhance P availability.

5.1 Effect of organic acids

No evidence was found to support the first hypothesis, that the addition of organic compounds would affect DM yield, plant P concentrations, or P yield. Under the conditions tested, the addition of organic compounds did not significantly affect P availability or improved plant performance. These results contrast with previous studies showing increased P uptake or solubilization following organic acid application, where organic acids acted as ligands or chelating agents (Sutriadi *et al.*, 2022; Schütze *et al.*, 2020). In the present study, neither the concentration nor the type of organic compound significantly affected P concentration or P yield in biomass, suggesting that these compounds did not influence P mobility under the conditions studied. Some trends were observed, for example RevoCaP + CX 3% tended to produce slightly higher DM yield in both harvests and under both soil conditions compared to the other RevoCaP treatments, but these differences were small and not statistically significant.

5.2 Effect of soil pH

Soil pH influenced RevoCaP performance, partly supporting the second hypothesis stating that soil pH affects P availability and consequently DM yield, P concentration and P yield. Under acidic conditions (N-soils), RevoCaP performance was enhanced compared to alkaline conditions (C-soils), consistent with previous observations reported by EasyMining (EasyMining, n.d.b). However, some C-soil samples reached unexpectedly high pH values (up to 9.2) when buffer was added at the beginning of the study, which complicates interpretation. In addition, the artificial filter sand used in this study likely had limited buffering capacity compared with natural soils, making the final pH difficult to interpret, and its stability over the trial duration uncertain.

Sjöqvist (2004) and Delin (2016) emphasized that soil pH can influence P solubility and may sometimes be more important than the form of P itself for plant uptake. At the beginning of the experiment, both C- and N-soils were monitored to evaluate initial pH levels. The N-soils were within the expected pH range (pH ≤ 6), with final values ranging from 5.9 to 6.5 depending on the treatment. While continuous monitoring was not performed, it is unlikely that N-soils experienced the same fluctuations as the C-soils, as no buffer were added. Since P availability is highest at pH 6 to 7 (Eriksson *et al.*, 2011), it is likely that minor pH variations in the N-soils had a limited negative effect on P uptake.

SP 100% exhibited low mean pH values for both soil treatments (5.9 for N-soils and 6.2 for C-soils) at the end of the study, reflecting the acidic nature of the fertilizer (initial pH 3.08) and its dissolution in the soil, which release Ca^{2+} and $H_2PO_4^-$ ions while slightly lowering soil pH (Eriksson *et al.*, 2011; Blume *et al.*, 2016). The other treatments, including RevoCaP, RP and control treatment, were within similar pH ranges within each soil pH type (6.64 to 6.68 for C-soils and 6.17 to 6.32 for N-soils). For both RP and RevoCaP, a pH around 5-5.5 is considered optimal for fertilizer reactivity, allowing carbonate components to react with H^+ ions, gradually releasing HPO_4^{2-} (Blume *et al.*, 2016; Hedley & McLaughlin, 2005). The intended effect of the organic acids was to increase proton concentrations, slightly lowering the fertilizers pH, and for the acids to act as chelating agents to release adsorbed HPO_4^{2-} from the fertilizer. While the additives did reduce the fertilizers pH (e.g., unamended RevoCaP = 10.58, RevoCaP + CX 1% = 9.82, RevoCaP + CX 3% = 8.86, RevoCaP + CA 3% = 8.51; Table 2), this did not result in significant differences in P availability between RevoCaP with additives and unamended RevoCaP. A stronger organic acid, such as oxalic acid, may have been more effective at lowering the fertilizer pH and could potentially have enhanced P availability, as suggested by previously studies (Wang *et al.*, 2025; Sutriadi *et al.*, 2022).

For C-soils, the initial high pH likely restricted P availability. Microbial activity most likely contributed to the pH fluctuations, as microbes initially consumed available substrates, temporarily increasing pH, a process previously observed with citrate addition (Hu *et al.*, 2005). This is supported by the observed greenish surfaces on the soils, possibly indicating microbial or algal growth, although this was not directly measured. As microbial activity declined with substrate depletion, pH decreased, resulting in final pH values between 6.2 and 6.75. These pH fluctuations may have interacted with soil chemical processes that control P availability, including mineralization, immobilization, and adsorption to soil particles, as well as fertilizer dissolution.

Under neutral to alkaline conditions, P from hydroxyapatite-based fertilizers is sparingly solubilized, and the soil-plant systems ability to buffer pH affects the extent of fertilizer dissolution (Blume *et al.*, 2016; Ibrahim *et al.*, 2020; Noruzi *et al.*, 2023; Hedley & McLaughlin, 2005). At pH values above 8, deprotonation of phosphate ions promotes electrostatic interactions with Ca^{2+}, leading to precipitation of insoluble Ca phosphate and reduced plant-available P (Eriksson *et al.*, 2011). In this study, such chemical processes, likely influenced by microbial-driven pH changes, probably contributed to limited P availability in C-soils.

It would have been interesting to monitor potential pH differences throughout the experiment and to compare levels between the first and second harvest to assess any possible effect the unexpected pH increase had on the overall results. However, due to the risk of nutrient loss from frequent pH sampling, soil pH was measured only a few times at the beginning of the trial and once at the end, leaving variation between the first and second harvest unknown. Nevertheless, in this trial the comparison is between the two soil pH treatments within the study setup and method, and the results are valid within this specific frame. Although pH fluctuations affected the study, all C-soils treatments were treated identically, allowing reasonable assumptions about relative performance compared to N-soils.

5.3 Interaction between additive type and soil pH

There were no interactions between fertilizer treatment and pH, indicating that the effect of the additives was not dependent on soil pH, and therefore did not support the third hypothesis. No significant differences were observed within pH groups between the different RevoCaP treatments, with or without additives. Observed differences in DM and P yield were mainly explained by soil pH rather than the additive type. Previous studies have shown that the effectiveness of organic acid additives can be pH dependent. Hu *et al.* (2005) reported increased P mobilization following organic acid application across Ca-, Fe-, and Al-dominated soils, with CA being more efficient in calcareous soils. In this study, the soil was not calcareous, as indicated by a Ca-AL concentration of 16 mg/100 g (Table 3), and soil pH did not exceed high levels (>7) for a prolonged period. Consequently, soil pH did not significantly modify the effectiveness of the additives, and no interactions between pH and fertilizer were observed, apart from the generally higher performance of all fertilizers in N-soils compared with C-soils.

Differences in DM yield and P yield were primarily driven by the inherent differences in P availability among the fertilizer treatments. In some cases, SP showed no significant differences compared to RevoCaP treatments in N-soils regarding P concentration. For example, SP in the first harvest compared with RevoCaP CX 3% and RevoCaP CA 3% (Table 8), and in the second harvest comparing SP with all RevoCaP treatments (Table 9). However, similar P concentrations in plant tissue do not necessarily imply similar P uptake, as treatment promoting greater biomass production can result in higher overall P uptake and P yield, even if tissue P concentration is comparable. This may explain why SP treatments produced higher DM yield and P yield overall, while tissue P concentration remained similar to some RevoCaP treatments.

When comparing P-MFE results with studies on similar fertilizers over a six-week period, RevoCaP, with or without additives, showed slightly lower values in N-soils than sulfuric acid-treated mono-incinerated sewage sludge ash reported by Kopp *et al*. (2023) (33-34% vs. 24-31.6% in the present study), but higher in C-soils (36.2-45.6%). Although, sulfuric acid is not approved for organic production. Thermochemically treated mono-incinerated sewage sludge ash showed higher P-MFE values (54%; Lemming *et al*., 2017) than RevoCaP treatments in both C- and N-soils, although this treatment is also not permitted for organic farming. In contrast, untreated ash in the same studies exhibited very low short-term P availability (4-5%, Kopp *et al*., 2023; 27%; Lemming *et al*., 2017). Compared with these values, RevoCaP performed similarly or better in N-soils and clearly better in C-soils. However, it is important to note that differences in study conditions make direct comparisons difficult. The type of chemical used for precipitation during wastewater treatment affects P availability (Delin & Engström, 2021; Delin, 2016), and subsequent the processing method of the resulting ash may also influence its plant-available P.

As noted by Delin (2016), the P-MFE of other ash products (wheat straw, oat grain and rapeseed straw), approved for organic production, is typically between 40-60% after two months and increases over time. In this study, a similar pattern was observed in the second harvest for N-soils, where nine weeks after planting, P-MFE ranged from 51.2 to 55.6%. In contrast, C-soils showed lower P-MFE at the second harvest (32.2-38.5%), reflecting that RevoCaP performs better under acidic conditions than under alkaline ones, although pH in C-soils fluctuated irregularly over time. Interestingly, P-MFE in C-soils remained within a similar range between the first and second harvest, though the reasons for this are unclear.

Compared with RP, also approved for organic production, RevoCaP showed notably higher P-MFE at both harvests, regardless of soil pH. Overall, the respective additives did not increase P-MFE in a distinct way. All RevoCaP treatments remained within a similar, expected percentage range, at least for the N-soils, and did not improve or differentiate the product compared with previous ash fertilizer studies. This relatively narrow P-MFE range could be a limitation for RevoCaP, as well as RP, as P may not be readily available during early plant growth compared with soluble mineral fertilizers like SP.

However, it should be noted that SP is not approved for organic production and is not intended as a fertilizer for practical use in this context. P-MFE is calculated relative to SP, which serves as a reference for maximum P availability under optimal conditions, allowing interpretation of how effectively RevoCaP and other treatments supply P to the plants. As a slow-release fertilizer, RevoCaP is designed to supply P over a longer period, and short-term experiments may underestimate its agronomic potential. The results therefore primarily reflect the conditions and durations of this study and the specific methodology used, and may not fully represent the performance under field conditions or over longer cropping periods. Extended experiments could potentially show higher P-MFE levels as slow-release P becomes more available over time.

5.4 Phosphorous concentrations of soil and fertilizers

By using a filter sand soil, the aim was to minimize the risks of P mobilization and immobilization that could have occurred if soil from arable land had been used. It is difficult to find an agricultural soil with no background P, which could introduced unwanted P inputs and made it harder to evaluate the fertilization effect of the added P. The soil properties showed no indication of high Al and Fe concentration that could potentially adsorb readily available P and limit P uptake, suggesting that the selected soil was appropriate for a study targeting low P-AL conditions (Table 3).

The fertilization rates used in this study (15 kg P/ha) were below the recommended 25 kg P/ha for Class I soils (1-2 mg/100 g soil with AL-extraction; Andersson *et al.*, 2025). This level was intentionally chosen to avoid over-optimal conditions that could mask potential differences between fertilization treatments. By applying a lower P rate, the likelihood of detecting differences in plant response among soils with lower P availability was increased. Although this may have imposed some degree of P limitation, the comparison between RP and RevoCaP treatments with SP and the control reduces the risk of misleading conclusions.

Apatite generally has low solubility and limited plant availability (Stewart *et al.* 2005; Hedley & McLaughlin, 2005). The RP used in this study was a form of soft or colloidal apatite used as a fertilizer in organic farming, although apatite itself is classified as a mineral fertilizer rather than an organic one. In this study, RevoCaP treatments resulted in significant higher DM yield, P concentration and P yield than RP in both the first and second harvest, suggesting that it may be a more promising option for use in organic production.

However, the comparison between the two products is not entirely straightforward. The RP used had a low total P concentration, although still within the typical range for apatite fertilizers (12-16%; Kirchmann *et al*., 2017), and considerably higher concentrations of Al and Fe, which likely reduced immediate P availability. High concentrations of Fe and Al can promote the formation of stable Fe- and Al-phosphate associations, which may further limit the release of P into the soil solution (Eriksson *et al*., 2011). In addition, the products differed in their physicochemical properties. RevoCaP contains amorphous phases and exhibits a lower degree of crystallinity than conventional hydroxyapatite-based minerals (Cohen, personal communication, 12 February 2026). Lower crystallinity is generally associated with greater surface area and higher reactivity (Ibrahim *et al*., 2020), which may enhance P dissolution. Together, these differences suggests that P in RP was likely less readily available to plants than P supplied by RevoCaP.

The physical form of the fertilizers may also have influenced the results. RevoCaP was originally supplied as a powder, whereas RP was supplied as granulate. To improve comparability between treatments, all fertilizers were ground and sieved (355 µm) prior to application. However, this procedure only controlled the upper particle size limit, and differences in particle size distribution between the materials may still have remained. It cannot therefore be excluded that RevoCaP contained a greater proportion of finer particles than RP, which could have increased the reactive surface area and potentially enhanced P dissolution and plant uptake. This potential effect of finer particles is supported by previous studies, which suggest that apatite fertilizers should have a particle size below 150 µm to increase surface area and enhance soil processes promoting dissolution (Hedley & McLaughlin, 2005).

While this approach was intended to reduce methodological bias, it does not fully represent farming conditions, where fertilizers products are typically applied in their commercial form. In practice, fertilizers differ in formulation and physical structure, and these characteristics are part of the overall product performance, where fertilizers are evaluated as complete products rather than isolated materials. Consequently, the enhanced reactivity associated with very small particle sizes may be more representative of controlled experimental conditions, such as a greenhouse studies, than of practical field situations. If RevoCaP were to be commercialized as a fertilizer product, it would likely also be granulated for practical field application. Granulation would reduce the exposed surface area compared with a powder, which could influence the effective reactivity of the material in soil.

5.5 Sources of errors and areas of improvement

Experimental design

When working with organic material, small experimental factors can influence treatment outcomes. One such factor is the placement of fertilizers in the soil at the start of the trial. In this study, precautions were taken to ensure adequate nutrient distribution by mixing two-thirds of the soil with the fertilizers in a separate container, aiming to position P within the optimal uptake zone of 2-4 mm from the root surface (Ekman *et al.*, 2023). While exact nutrient placement cannot be fully controlled in pot experiments, the observed root development across treatments suggests that P availability was not primarily limited by fertilizer positioning. Instead, the differences in plant growth are more likely related to the slow-release characteristics of RevoCaP and RP compared with SP.

Another factor that may have influenced the results was the decision to use soil pH as a blocking factor, where pots with the same pH were placed in separate blocks and randomized only within each block. As a result, C-soil treatments were positioned on one side of the greenhouse table, while N-soil treatments were positioned on the opposite side, and the samples were never fully mixed. This arrangement may have led to differences in abiotic factors such as temperature and light availability between placements, thereby posing a risk of influencing the results. However, this risk was considered low, and it was assumed that conditions across the table were similar regardless of placement.

Furthermore, the experiment was conducted as a pot trial, where soil volume, root exploration, and nutrient dynamics differ from field conditions. These factors may influence fertilizer dissolution and nutrient uptake compared with agricultural systems. Consequently, the results should primarily be interpreted within the context of this experimental setup, and further studies under field conditions and in soils with different P status would be necessary to evaluate the broader applicability of the findings.

Phosphorus deficiency and soil pH effects

In the second harvest, differences between soil pH types were primarily observed in the RevoCaP treatments with additives, where higher P concentrations were measured in the N-soils compared with the C-soils. In contrast, unamended RevoCaP showed no significant differences between soil types. This suggests that the additive may have increased the sensitivity of the fertilizers to soil pH conditions rather than improving P availability directly. The absence of significant differences between RevoCaP treatments within the same soil pH further indicates that the additives did not substantially enhance plant P uptake under the conditions of this study.

Observations of the root system suggested differences in plant capacity for P uptake, as both the control and RP treatments developed very small root systems. This, combined with the fact that plants in these treatments began to wither, indicated that P availability limited plant development. This was confirmed by the analysis of total P concentration in the DM biomass, as all samples were below the critical P concentration of 0.2% (Smith *et al.*, 1985), indicating clear P deficiency.

Although the plants clearly experienced P deficiency, the P application rate was not increased during the experiment. The aim of the study was to evaluate the performance of the fertilizers at a fixed and relatively low application rate, rather than to achieve optimal plant growth conditions. Increasing the P supply could potentially have decreased the deficiency, however, it might have also reduced the differences between fertilizer treatments. Maintaining a moderate P limitation therefore allowed potential differences in fertilizer performance to be more clearly observed. Nevertheless, the low P concentrations in plant biomass were most likely influenced by the fertilization rate used. The soil had a very low initial P concentration (0.5 mg P/100 g soil; Table 3), considerably lower than typical agricultural soils, even those classified as having low P availability. This likely contributed to the strong P limitation observed in several treatments, and it should be noted that the results obtained in this study are specific to the experimental conditions used. For future studies, a slightly higher P application rate (e.g., around 20 kg P/ha) could provide a more suitable balance, avoiding severe plant stress while still allowing differences between fertilizer treatments to be detected.

Abiotic factors and salinity of samples

Greenhouse temperature might have affected the results. At the beginning of the study, temperatures were set to 15°C daytime 10°C nighttime. After two weeks, temperatures were increased to 18°C daytime and 13°C nighttime, due to slow plant growth. This temperature adjustment may partly explain the lower DM yields, P concentrations and P yields observed at the first harvest, and the increase observed at the second.

As it was not possible to harvest all samples on the same day, harvesting was divided over two days, which could have affected the results. Although this may have introduced minor variability, the risk is considered minimal given the slow movement of P in soil (about 0.13 mm/day for $H_2PO_4^-$; Sjöqvist, 2004; Syers *et al.*, 2008).

Finally, potential Na effects from the buffer may have influenced the results, although these effects are difficult to quantify. Comparison of measured Na levels with those reported by Eurofins Agro (2024) indicated that concentrations remained within expected ranges, and no clear signs of toxicity were observed, suggesting that any impact of Na on the results was likely minor.

Experiment timeframe

The time interval between the first and second harvest was shorter than originally planned, which may have influenced the results. The first harvest occurred after six weeks of growth, whereas only three weeks passed between the first and second harvest. Initially, the plan was to harvest after five weeks and again after ten weeks. However, after five weeks the plants still appeared small, and the first harvest was therefore postponed. During week six, the control and RP treatments began to show symptoms of P deficiency, including yellowing of older leaves, reduced growth, and reduced branching (Andersson et al., 2025; Yara Sweden, 2018; Eriksson et al., 2011), which led to harvesting at that time to avoid losing biomass.

The second harvest was originally planned for week ten, but by week nine the control and RP plants again showed clear signs of withering, resulting in an earlier harvest. The shorter regrowth period may have limited biomass production and nutrient uptake in the second harvest, and may also have reduced the time available for the slow-release fertilizers RevoCaP and RP to release plant-available P. This should therefore be considered when interpreting the results from the second harvest.

Statistical validity

Statistical significance was assessed at $\alpha = 0.05$; however, the small number of replicates limits the robustness of statistical inference, particularly with respect to marginal effects. While strong statistical significance was observed in some cases, two cases of the null hypothesis could not be rejected. Potential sources of error may have influenced the results, and all interpretations are therefore based solely on the model and data presented in this study. Additional sampling dates would be required to provide sufficient data for robust model development, as a limited number of data points makes it difficult to accurately estimate the effects of SD and the overall mean.

6. Conclusions and recommendations for future research

The addition of the tested organic acids did not significantly influence plant P uptake or yield, suggesting that such additives may not provide clear agronomic benefits under the conditions of this study. The primary differences observed were instead related to soil pH, suggesting that the performance of RevoCaP may be influenced by soil pH conditions rather than by the tested additives. Future research should consider evaluating additional organic compounds not included in the present study. For example, Wang *et al.* (2025) demonstrated that compounds such as succinic acid, luteolin and inositol increased soil P bioavailability by stimulating root biomass and enhancing soil bacterial and fungal communities. Such compounds may therefore have potential to improve P release from fertilizers and warrant further investigation. Oxalic acid may also be of interest, as it has been reported to be particularly effective in dissolving P rock (Sutriadi *et al.*, 2022).

From a practical perspective, the results suggest that RevoCaP has potential as an alternative P source for organic production. Compared with RP, RevoCaP resulted in greater P availability under the experimental conditions used. RevoCaP also contained lower concentrations of Fe and Al than RP and may represent a cleaner P source with respect to impurities, although contaminants such as heavy metals were not assessed in this study. Future studies should compare RevoCaP and RP in equivalent physical forms, such as granulated fertilizers, to more accurately assess their relative performance, and perhaps also evaluate potential contaminants.

Additionally, future studies should evaluate the performance of RevoCaP, with and without additives, in natural agricultural soils with stable and contrasting pH levels, to better capture its behavior under soil conditions, rather than commercial filter sand. This would allow assessment of interactions with native microbial communities, which may differ substantially from those present in more artificial substrates and may influence P dynamics. Comparative studies involving both pot trials and field trials would also be valuable, as they would enable evaluation of treatment effects under controlled conditions as well as under more complex and variable field conditions. As demonstrated in this study, even under controlled experimental conditions, outcomes can be difficult to predict due to biological variability inherent in living systems, and results may vary between trials.

References

Andersson, E., Frostgård, G., Hjelm, E., Kvarmo, P., Stenberg, M., & Malgeryd, J. (2025). *Rekommendationer för gödsling och kalkning 2025* [*Recommendations for Fertilization and Liming 2025*]. The Swedish Board of Agriculture. https://webbutiken.jordbruksverket.se/sv/artiklar/jo219.html

Afzal, M.R., Naz, M., Yu, Y., Yan, L., Wang, P., Mohotti, J., Hao, G., Zhou, J-J., Chen, Z., Zhang, L., & Wang, Q. (2025). Root exudates: The rhizospheric frontier for advancing sustainable plant protection. *Resources, Environment and Sustainability*, 21, 100249. https://doi.org/10.1016/j.resenv.2025.100249

Blume, H.P., Brümmer, G.W., Fleige, H., Horn, R., Kandeler, E., Kögel-Knabner, I., Kretzschmar, R., Stahr, K., & Wilke, B.M. (2016). *Scheffer/Schachtschabel Soil Science*. 1st ed. Berlin & Heidelberg: Springer. https://doi.org/10.1007/978-3-642-30942-7

Brady, N.C., & Weil, R.R. (2008). *The nature and properties of soils*. Rev. 14. ed. Upper Saddle River, N.J.: Pearson Prentice Hall

Braun, S. (2020) *Long-term phosphorus supply in agricultural soils: size and dynamics of fast- and slow-desorbing phosphorus pools*. Diss. Swedish University of Agricultural Sciences. Acta Universitatis Agriculturae Sueciae. https://res.slu.se/id/publ/108625

Chapin, F. S., Matson, P. A., & Vitousek, P. M. (2011). *Principles of terrestrial ecosystem ecology* (2nd ed.). Springer.

Christensen, I., Hansson, T., & Svensson, S.-E. (2010). *Gödsling i slutet odlingssystem i växthus: underlag till utbildningsmodul* [*Fertilization in closed cultivation systems in greenhouses: basis for a training module*]. Diss. Swedish University of Agricultural Sciences. *Landskap, trädgård, jordbruk: Report series,* 2010:15. https://res.slu.se/id/publ/78960

Cordell, D., Drangert, J.-O., & White, S. (2009) The story of phosphorus: Global food security and food for thought. *Global Environmental Change*, 19(2), 292–305. https://doi.org/10.1016/j.gloenvcha.2008.10.009

Delin, S. (2016). Fertilizer value of phosphorus in different residues. *Soil Use and Management*, 32(1), 17-26. https://doi.org/10.1111/sum.12227

Delin, S., & Engström, L. (2021) *Att sprida organiska gödselmedel* [*Spreading organic fertilisers*]. Brochure JO21:2. The Swedish Board of Agriculture. https://webbutiken.jordbruksverket.se/sv/artiklar/jo212.html

Delin, S., Nyberg, A., & Sarajodin, J. (2014). *Fosforgödslingseffekt av olika restprodukter* [*The phosphorus fertilization effect of various residual products*]. Diss. Swedish University of Agricultural Sciences. Report No. 13, Department of Soil and Environment. https://res.slu.se/id/publ/52455

Dixon, M., Simonne, E., Obreza, T., & Liu, G. (2020). Crop Response to low phosphorus bioavailability with a focus on tomato. *Agronomy*. 10. 617. https://doi.org/10.3390/agronomy10050617.

EasyMining Sweden AB. (2018). *US10023464B2: Production of phosphate compounds from materials containing phosphorus and at least one of iron and aluminium.* United States Patent. https://patents.google.com/patent/US10023464B2

EasyMining Sweden AB. (2025a). EU approves recycled phosphorus for use in organic farming. EasyMining. https://newsroom.easymining.com/posts/pressreleases/eu-approves-recycled-phosphorus-for-use-in-or

EasyMining Sweden AB. (2025b). *RevoCaP – Calcium Phosphate*. EasyMining. https://www.easymining.com/products/products/revocap-calcium-phosphate/

EasyMining Sweden AB. (2025c). *Organic Fertilizer Market – Market Analysis/August 2025.* [Unpublished internal report, 15 August 2025]. EasyMining.

EasyMining Sweden AB. (2025d). *REVOCAP™ ASH2™PHOS: Data sheet*. https://www.easymining.com/products/products/revocap-calcium-phosphate/

EasyMining Sweden AB. (n.d.a). *ASH2™PHOS from sewage sludge ash to high-grade phosphates – Product handout.* EasyMining. https://www.easymining.com/technologies/ash2phos2/ash2phos/technical-details/

EasyMining Sweden AB (n.d.b). *Recycled fertilizer for organic farming – Product Handout.* EasyMining. https://www.easymining.com/products/products/revocap-calcium-phosphate/

Ekman, S., Eklöf, P., Frostgård, G., Johnsson, B., Mejersjö, E.-M., & Törnquist, M. (2023). *Gödselmedelsproduktion i Sverige: Aktuella initiativ, tekniker och förutsättningar* [*Fertiliser Production in Sweden: Current Initiatives, Technologies and Conditions*]. RA23:9. https://webbutiken.jordbruksverket.se/sv/artiklar/ra239.html

Eriksson, J., Dahlin, S., Nilsson, I., & Simonsson, M. (2011). *Marklära [Soil Science]*. Lund: Studentlitteratur. ISBN 978-91-44-06920-3.

Eurofins Agro. (2024). *Sodium (Na) – soil and crop*. https://www.eurofins-agro.com/en/sodium

European Commission, Directorate-General for Internal Market, Industry, Entrepreneurship and SMEs. (2023). *Study on the critical raw materials for the EU 2023: Final report*. Publications Office of the European Union. https://data.europa.eu/doi/10.2873/725585

Havlin, J.L., Tisdale, S.L., Nelson, W.L., & Beaton, J.D. (2014). Phosphorus. In W. Lawrensen & J.Gohn (Eds.), *Soil Fertility and Fertilizers* (8th ed., pp. 185-221). Upper Saddle River, NJ, USA: Pearson Inc. ISBN 978-0-13-503373-9

Hedley, M., & McLaughlin, M. (2005). Reactions of Phosphate Fertilizers and By-Products in Soils. In J.T Sims, A.N. Sharpley, & D.T Westermann (Eds.) *Phosphorus: Agriculture and the Environment*. https://doi.org/10.2134/agronmonogr46.c7

Hu, H., Tang, C. & Rengel, Z. (2005). Influence of phenolic acids on phosphorus mobilisation in acidic and calcareous soils. *Plant Soil* 268, 173–180. https://doi.org/10.1007/s11104-004-0280-x

Ibrahim, M., Labaki, M., Giraudon, J.-M. & Lamonier, J.-F. (2020). Hydroxyapatite, a multifunctional material for air, water and soil pollution control: A review. *Journal of Hazardous Materials*, 383, 121139. https://doi.org/10.1016/j.jhazmat.2019.121139

International Fertilizer Association (IFA). (2025). *Public Summary – Medium-Term Fertilizer Outlook 2025-2029.* https://www.ifastat.org/market-outlooks

Jiaying, M., Tingting, C., Jie, L., Weimeng, F., Baohua, F., & Li, G., & Li, H., Li, J., Zhihai, W., Longxing, T., & Fu, G. (2022). Functions of Nitrogen, Phosphorus and Potassium in Energy Status and Their Influences on Rice Growth and Development. *Rice Science*, 29. https://doi.org/10.1016/j.rsci.2022.01.005.

Johnston, A.E., Poulton, P.R., Fixen, P.E., & Curtin, D. (2014). Phosphorus: Its efficient use in agriculture. In D.L. Sparks (Eds) *Advances in Agronomy*, 123, 177-228. https://doi.org/10.1016/B978-0-12-420225-2.00005-4

Jordan-Meille, L., Rubæk, G.H., Ehlert, P.A.I., Genot, V., Hofman, G., Goulding, K., Recknagel, J., Provolo, G., & Barraclough, P. (2012). An overview of fertilizer-P recommendations in Europe: Soil testing, calibration and fertilizer recommendations. *Soil Use and Management*, 28, 419-435. https://doi.org/10.1111/j.1475-2743.2012.00453.x

Khorassani, R., Hettwer, U., Ratzinger, A., Steingrobe, B., Karlovsky, P., & Claassen, N. (2011). Citramalic acid and salicylic acid in sugar beet root exudates solubilize soil phosphorus. *BMC Plant Biology,* 11, 121. https://doi.org/10.1186/1471-2229-11-121

Kirchmann, H., Börjesson, G., Kätterer, T., & Cohen, Y. (2017). From agricultural use of sewage sludge to nutrient extraction: A soil science outlook. *Ambio* 46, 143–154. https://doi.org/10.1007/s13280-016-0816-3

Koller, M., Rayns, F., Cubison, S., & Schmutz, U. (2016). *Guidelines for Experimental Practice in Organic Greenhouse Horticulture*. BioGreenhouse COST Action FA 1105. https://doi.org/10.18174/373581

Kopp, C., Mendonça de Santis Sica, P., Lu, C., Tobler, D., Jensen, L., Müller-Stöver, D. (2023). Increasing phosphorus plant availability from P-rich ashes and biochars by acidification with sulfuric acid. *Journal of Environmental Chemical Engineering.* https://doi.org/10.1016/j.jece.2023.111489

Lemming, C., Bruun, S., Jensen, L.S., & Magid, J. (2017), Plant availability of phosphorus from dewatered sewage sludge, untreated incineration ashes, and other products recovered from a wastewater treatment system. J. Plant Nutr. Soil Sci., 180: 779-787. https://doi.org/10.1002/jpln.201700206

Liu, G., Zotarelli, L., Li, Y., Dinkins, D., Wang, Q., & Ozores-Hampton, M. (2014). Controlled-release and slow-release fertilizers as nutrient management tools:

HS1255. *EDIS*, 2014(8). Gainesville, FL. https://doi.org/10.32473/edis-hs1255-2014

Mattsson, L., Börjesson, T., Ivarsson, K., & Gustafsson, K. (2001). *Utvidgad tolkning av P-AL för mark- och skördeanpassad fosforgödsling* [*Extended interpretation of labile P for soil and yield related P fertilization*]. Report 202. Instutitionen för markvetenskap Avdelningen för växtnäringslära. https://pub.epsilon.slu.se/3226/1/Rapport_202.pdf

Noruzi, M., Hadian, P., Soleimanpour, L., Ma'mani, L. & Shahbazi, K. (2023) Hydroxyapatite nanoparticles: an alternative to conventional phosphorus fertilizers in acidic culture media. *Chem. Biol. Technol. Agric,*10, 71. https://doi.org/10.1186/s40538-023-00437-0

Pierzynski, G.M., McDowell, R.W., & Sims, J.T. (2005). Chemistry, cycling, and potential movement of inorganic phosphorus in soils. In J.T. Sims & A.N. Sharpley (Eds.), *Phosphorus: Agriculture and the Environment*. (Agronomy Monograph 46. pp. 53-86). Madison, WI: American Society of Agronomy, Crop Science Society of America & Soil Science Society of America. https://doi.org/10.2134/agronmonogr46.c3

Sakib, T.U., Nelson, N., & Hettiarachchi, G., Moorberg, C., Nippert, J., Whitaker, S. (2025). Soil phosphorus availability as affected by root exudates of cover crop species. *Scientific Reports,* 15. https://doi.org/10.1038/s41598-025-19102-7

Salinas Ruíz, J., Montesinos López, O. A., & Crossa, J. (2024). Randomized Complete Block Design. In *Introduction to Experimental Designs with PROC GLIMMIX of SAS.* Springer, Cham. https://doi.org/10.1007/978-3-031-65575-3_4

Santos, W. O., Mattiello, E. M., Barreto, M. S. C., & Cantarutti, R. B.. (2019). Acid Ammonium Citrate as P Extractor for Fertilizers of Varying Solubility. *Revista Brasileira De Ciência Do Solo*, *43*, e0180072. https://doi.org/10.1590/18069657rbcs20180072

Schütze, E., Gypser, S., & Freese, D. (2020). Kinetics of Phosphorus Release from Vivianite, Hydroxyapatite, and Bone Char Influenced by Organic and Inorganic Compounds. *Soil Systems*, 4(1), 15. https://doi.org/10.3390/soilsystems4010015

Shi, W., Healy, M.G., Ashekuzzaman, S.M., Daly, K., & Fenton, O. (2022). Mineral fertiliser equivalent value of dairy processing sludge and derived biochar using ryegrass (Lolium perenne L.) and spring wheat (Triticum aestivum). *Journal of Environmental Management*, 321, 116012. https://doi.org/10.1016/j.jenvman.2022.116012

Sjöqvist, T. (2004). *Fosfor i ekologisk lantbruk* [*Phosphorus in Organic Agriculture*]. The Swedish Board of Agriculture. Part of the course material *Ekologisk växtodling* 2004 [Organic Crop Production 2004]. https://www2.jordbruksverket.se/webdav/files/SJV/trycksaker/Pdf_ovrigt/p8_6.pdf

Smith, G.S., Cornforth, I.S., & Henderson, H.V. (1985). Critical leaf concentrations for deficiencies of nitrogen, potassium, phosphorus, Sulphur, and magnesium in perennial ryegrass. *The New phytologist*, 101(3), 393–409. https://doi.org/10.1111/j.1469-8137.1985.tb02846.x

Stewart, W.M., Hammond, L.L., & Van Kauwenbergh, S.J. (2005). Phosphorus as a Natural Resource. In J.T. Sims, A.N. Sharpley & G.M. Pierzynski (Eds.), *Phosphorus: Agriculture and the Environment*. (Agronomy Monograph 46) https://doi.org/10.2134/agronmonogr46.c1

Sutriadi, M.T., Anwar, S., Mulyanto, B., Darmawan, H., & Jaya, A. (2022*).* Improving Upland Acid Soil Properties And Increasing Maize Yield By Phosphate Rock Application With Organic Acids. *International Journal of Agronomy*, 9720632. https://doi.org/10.1155/2022/9720632

Swedish Board of Agriculture. (2017). *Användning av aska som gödselmedel på åkermark* [*Use of ash as fertilizer on arable land*]. Version 1, January 2017. https://jordbruksverket.se/download/18.1c27146717f4a95f8783ed1/1646229105759/Anvandning-av-aska-som-godselmedel-pa-akermark-tga.pdf

Swedish Environmental Protection Agency. (2025). *Avloppsslam på jordbruksmark, användning, innehåll och spridningsplatser* [*Sewage sludge on agricultural land: use, content and spread sites*]. https://www.naturvardsverket.se/data-och-statistik/avlopp/avloppsslam-pa-jordbruksmark-anvandning-innehall-och-spridningsplatser/

Swedish University of Agricultural Sciences. (2022). *Laborationskompendium i markkemi [Laboratory compendium in soil chemistry]*. Sveriges lantbruksuniversitet, Institutionen för mark & miljö [Swedish University of Agricultural Sciences, Department of Soil & Environment]. Part of the course material *Markvetenskap 2022* [*Soil Sciences 2022*].

Syers, J.K., Johnston, A.E., & Curtin, D. (2008). Efficiency of Soil and Fertilizer Phosphorus Use. Reconciling Changing Concepts of Soil Phosphorus Behaviour with Agronomic Information. *FAO Fertilizer and Plant Nutrition Bulletin 18.* Rome: Food Agricultural Organization of the United Nations (FAO).

Thermo Fisher Scientific. (n.d.). *Sample preparation techniques for AAS, ICP-OES and ICP-MS for regulated testing laboratories*. https://documents.thermofisher.com/TFS-Assets/CMD/Technical-Notes/tn-44483-aas-icp-oes-icp-ms-sample-prep-regulated-test-tn44483-en.pdf

U.S. Geological Survey (USGS). (2025). *Mineral Commodity Summaries 2025.* Reston, VA: U.S. Department of the Interior. https://www.usgs.gov/centers/national-minerals-information-center/mineral-commodity-summaries

Wang, G., Yang, Y., Yao, Y., & Wang, X. (2025). Exudate pulses throughout the entire growth period trigger the increase in maize phosphorus use efficiency by modifying soil keystone microbial taxa. *Biology and Fertility of Soils*, 61, 999-1011. https://doi.org/10.1007/s00374-025-01912-6.

Wieczorek, D., Żyszka-Haberecht, B., Kafka, A., & Lipok, J. (2022). Determination of phosphorus compounds in plant tissues: from colourimetry to advanced instrumental analytical chemistry. *Plant Methods*, 18(22). https://doi.org/10.1186/s13007-022-00854-6

Wu, L., Kobayashi, Y., Wasaki, J., & Koyama, H. (2018). Organic acid excretion from roots: a plant mechanism for enhancing phosphorus acquisition, enhancing aluminum tolerance, and recruiting beneficial rhizobacteria. *Soil Science and Plant Nutrition,* 64(6), 697-704. https://doi.org/10.1080/00380768.2018.1537093

Wuenscher, R., Unterfrauner, H., Peticzka, R., & Zehetner, F. (2015). A comparison of 14 soil phosphorus extraction methods applied to 50 agricultural soils from Central Europe. *Plant, Soil and Environment*, 61, 86-96. https://doi.org/10.17221/932/2014-PSE.

Yara Sweden. (2018). *Fosforbrist – Vall* [*Phosphorus Deficiency – Ley Crops*]. https://www.yara.se/vaxtnaring/vall/bristsymtom-vall/fosforbrist-vall/

Zhou, Y., Liu, Y., Jiang, C., El-Desouki, Z., Riaz, M., Wang, C., Zhang, X., Ding, J., Chen, Z., Liu, H., Shen, J., & Xia, H. (2025). Effects of exogenous application of phenolic acid on soil nutrient availability, enzyme activities, and microbial communities. *Agriculture*, 15(10), 1067. https://doi.org/10.3390/agriculture15101067

Popular science summary

Phosphorus is an essential nutrient for plants and is crucial for maintaining the food production that modern society depends on. Today, most phosphorus used in fertilizers is mined from phosphate rock, a limited resource found only in a few parts of the world. As a result, Europe relies heavily on imports, making the fertilizer supply vulnerable to global market and geopolitical changes. At the same time, large amounts of phosphorus already exist within Europe in the form of waste streams, such as sewage sludge. Recovering phosphorus from these sources could help reduce import dependence and contribute to a more sustainable and circular use of nutrients. One such example is calcium phosphate fertilizers made from sewage sludge ash. However, the phosphorus in recycled materials is often less available to plants and released slower into the soil compared to conventional mineral fertilizers, which limits their effectiveness.

In natural soils, plants have developed strategies to cope with low phosphorus availability. For example, roots can release organic acids that help dissolve bound phosphorus in the soil, making it easier for plants to absorb. Inspired by this mechanism, this study investigated whether adding organic acids, specifically citric acid and a phenolic acid, to a recycled calcium phosphate fertilizer (RevoCaP) could improve phosphorus availability and plant uptake.

The fertilizer was tested in a greenhouse experiment using perennial ryegrass in soils with two different pH levels: slightly acidic (pH 6) and neutral (pH 7). The recycled fertilizer was tester both with and without added organic acids, and compared with conventional fertilizers, including superphosphate (a fast-acting fertilizer) and rock phosphate (a slow-release fertilizer). Plant growth and phosphorus uptake were measured over two harvests. The results showed that adding organic acids did not improve phosphorus availability or pant uptake from the recycled fertilizer. Instead, soil pH had a much stronger effect. The fertilizer performed significantly better in slightly acidic soil than in the neutral soil, with higher plant growth and phosphorus uptake. Overall, the recycled fertilizer performed better than rock phosphate, independent of whether organic acids were added.

In conclusion, the effectiveness of this recycled phosphorus fertilizer depends more on soil conditions than on the addition of organic acids. Although the additives tested in this study did not enhance phosphorus availability, other types of additives or treatment methods may still hold potential. Further research is therefore needed to improve the performance of recycled fertilizers and support a more sustainable phosphorus cycle.

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Appendix 1

Description of how the total P concentration of each fertilizer was determined, using the industry-standard procedure of Thermo Fisher Scientific (n.d.):

Approximately 1.00 g of fertilizer was weighed into a digestion vessel that had been treated with a Zerostat 3 Milty device to eliminate static charge. To each vessel, 15 mL of concentrated nitric acid (HNO_3) and 5 mL of HCl were added and mixed gently to minimize foaming. The samples were then digested in a Multiwave GO Plus microwave system at 180 °C for 60 minutes, including a 10 min temperature ramp. After digestion, any undissolved residues were removed by vacuum filtration using a Fisherbrand filtration setup with Munktell filter paper (grade 389, Ahlstrom Munksjö) while a Lafil 300 C vacuum pump was operating. The filtrate was subsequently diluted with deionized water to a final volume of 50 mL and mixed thoroughly. The used filter papers were dried overnight at 105 °C, and their mass was subtracted from the total ICP-OES mass balance to account for undigested residues. The resulting filtrates were serially diluted (1:10, 1:100, 1:1000, and 1:10 000). For each dilution, 1 mL of sample was combined with 9 mL of ICP-grade acid to prepare solutions for ICP-OES measurement. The samples were then analyzed with Agilent 5800 ICP-OES.

Appendix 2

Python script used for randomization.

```
import random # Import Python's built-in library for randomization

# Function to shuffle pot numbers and organize them into groups of 3
def shuffle_grid(start, end):
    # Create a list of pot numbers from start to end
    numbers = list(range(start, end+1))
    # Randomly shuffle the list
    random.shuffle(numbers)
    # Group numbers in sets of 3 (3 pots stacked verti-
cally)
    grid = [numbers[i:i+3] for i in range(0, len(numbers), 3)]
    # Return the shuffled grid
    return grid

# Function to print the grid clearly with a title
def print_grid(grid, title):
    # Print the title (e.g., "Row 1 (1-36)")
    print(title)
    # Print each group of 3 pots in column format
    for row in grid:
        print(f"{row[0]:>4}{row[1]:>4}{row[2]:>4}")
    # Add an empty line after the grid
    print()

# Shuffle and print Row 1 (pots 1–36)
grid_row1 = shuffle_grid(1, 36)
print_grid(grid_row1, "Row 1 (1–36):")

# Shuffle and print Row 2 (pots 37–72)
grid_row2 = shuffle_grid(37, 72)
print_grid(grid_row2, "Row 2 (37–72):")
```

Appendix 3

Images from the first harvest illustrating the differences in growth. Image A shows all pots together, while images B-D shows the individual rows, arranged from left to right as follows: B – SP treatments (33%, 67% and 100%), C – RevoCaP with additives (CX 1%, CA 3% and CX 3% for N-soils and CA 3%, CX 1% and CX 3% for C-soils), and D – the control, RevoCaP unamended, and RP.

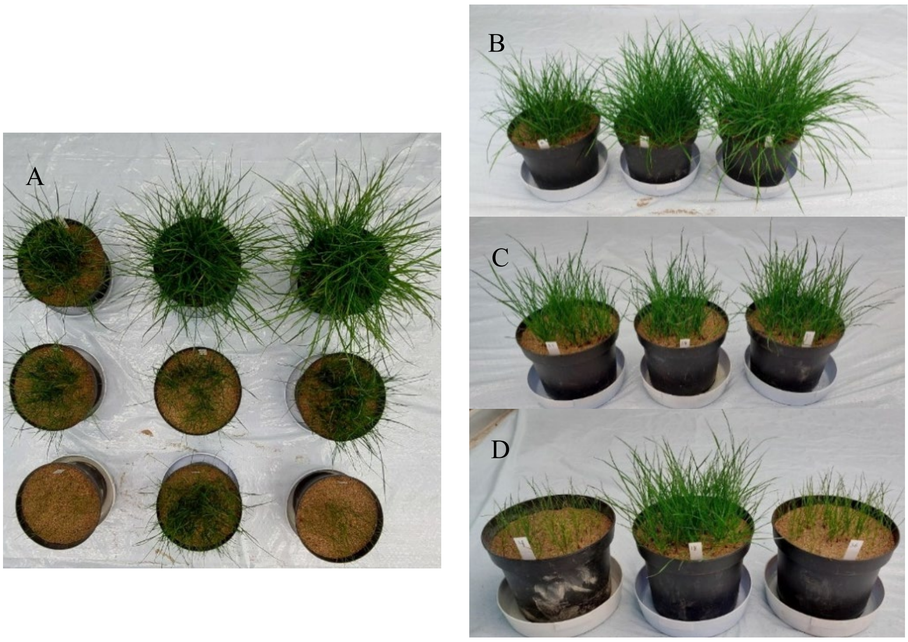

Figure 14. Accumulated biomass at the first harvest for the N-soils.

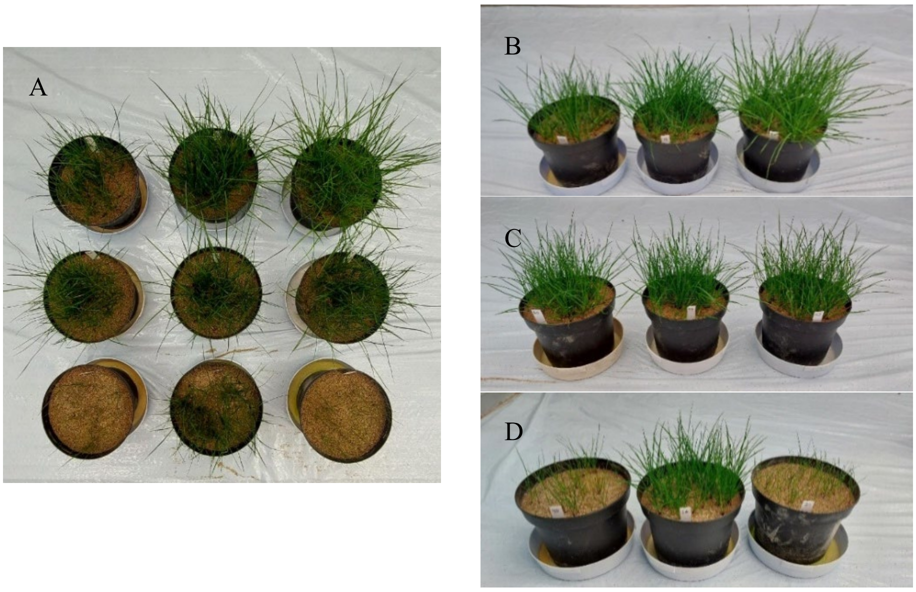

Figure 15. Accumulated biomass at the first harvest for the C-soils.

Appendix 4

Images from the second harvest illustrating the differences in growth. Image A shows all pots together, while images B-D shows the individual rows, arranged from left to right as follows: B – SP treatments (33%, 67% and 100%), C – RevoCaP with additives (CX 1%, CA 3% and CX 3% for N-soils and CA 3%, CX 1% and CX 3% for C-soils), and D – the control, RevoCaP alone, and RP.

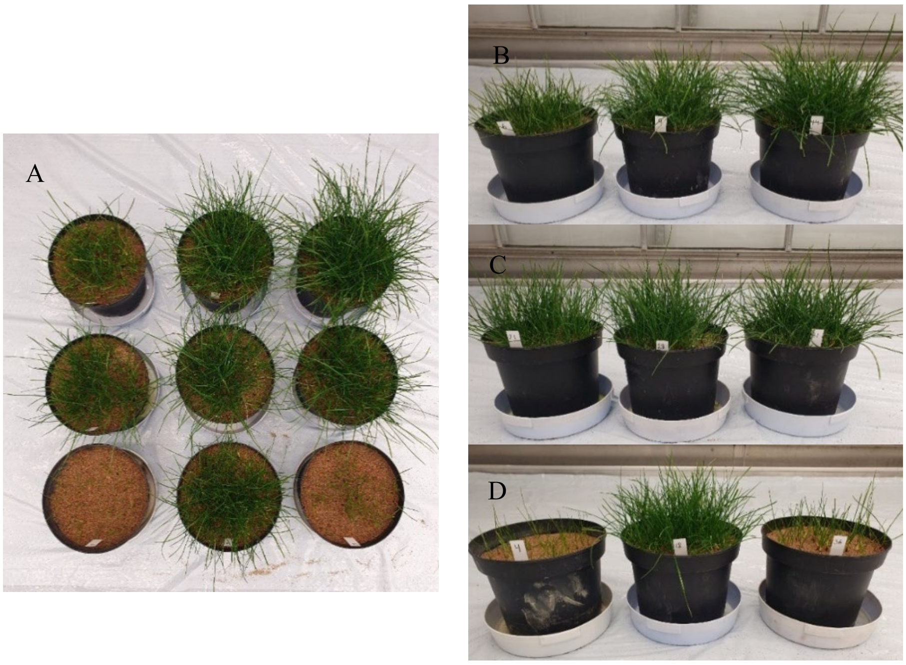

Figure 16. Accumulated biomass at the second harvest for the N-soils.

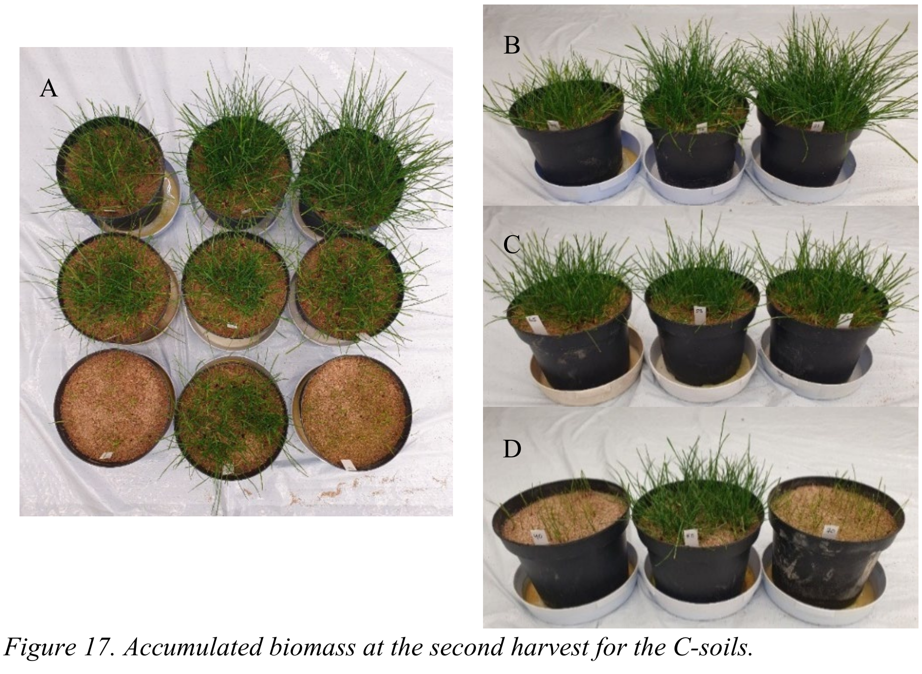

Figure 17. Accumulated biomass at the second harvest for the C-soils.

Appendix 5

Table 15. Two-way ANOVA showing significant effects of pH, fertilizer, and their interaction on dry matter yield (t/ha) at the first harvest

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
pH	1	0,8098	0,80985	29,97	0,000
Fertilizer	6	34,2497	5,70828	211,24	0,000
pH*Fertilizer	6	4,5994	0,76656	28,37	0,000
Error	42	1,1349	0,02702		
Total	55	40,7938			

Table 16. Tukey pairwise comparisons (Grouping) of the interaction between pH and fertilizer on dry matter yield at the first harvest

Grouping Information Using the Tukey Method and 95% Confidence

pH*Fertilizer	N	Mean	Grouping
N-soils SP 100%	4	3,52850	A
C-soils SP 100%	4	1,92971	B
N-soils RevoCaP + CX 3%	4	1,24511	C
C-soils RevoCaP + CX 3%	4	1,19444	C
N-soils RevoCaP	4	1,19134	C
C-soils RevoCaP + CX 1%	4	1,12515	C
N-soils RevoCaP + CX 1%	4	1,03001	C
N-soils RevoCaP + CA 3%	4	1,02277	C
C-soils RevoCaP + CA 3%	4	0,96693	C
C-soils RevoCaP	4	0,87178	C
C-soils Control	4	0,27819	D
C-soils RP	4	0,25337	D
N-soils RP	4	0,15616	D
N-soils Control	4	0,12927	D

Means that do not share a letter are significantly different.

Table 17. Two-way ANOVA significant effects of pH, fertilizer, and their interaction on phosphorus concentration (g/kg DM) at the first harvest

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
pH	1	0,01001	0,010015	1,52	0,225
Fertilizer	6	3,17788	0,529647	80,36	0,000
pH*Fertilizer	6	0,13181	0,021969	3,33	0,009
Error	42	0,27683	0,006591		
Total	55	3,59654			

Table 18. Tukey pairwise comparisons (Grouping) of the interaction between pH and fertilizer on phosphorus concentrations at the first harvest

Grouping Information Using the Tukey Method and 95% Confidence

pH*Fertilizer	N	Mean	Grouping
C-soils SP 100%	4	1,25677	A

N-soils SP 100%	4	1,19951	A B
C-soils RevoCaP	4	1,02062	B C
N-soils RevoCaP + CA 3%	4	1,01743	B C
N-soils RevoCaP + CX 3%	4	1,00633	B C
C-soils RevoCaP + CX 1%	4	0,94252	C
N-soils RevoCaP + CX 1%	4	0,90774	C
N-soils RevoCaP	4	0,89163	C
C-soils RevoCaP + CA 3%	4	0,87832	C
C-soils RevoCaP + CX 3%	4	0,85358	C
N-soils RP	4	0,57211	D
C-soils RP	4	0,52676	D
N-soils Control	4	0,52248	D
C-soils Control	4	0,45143	D

Means that do not share a letter are significantly different.

Table 19. Two-way ANOVA significant effects of pH, fertilizer, and their interaction on phosphorus yield (kg P/ha) at the first harvest

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
pH	1	1,4132	1,41321	77,16	0,000
Fertilizer	6	55,3930	9,23217	504,04	0,000
pH*Fertilizer	6	5,5927	0,93212	50,89	0,000
Error	42	0,7693	0,01832		
Total	55	63,1682			

Table 20. Tukey pairwise comparisons (Grouping) of the interaction between pH and fertilizer on phosphorus yield at the first harvest

Grouping Information Using the Tukey Method and 95% Confidence

pH*Fertilizer	N	Mean	Grouping
N-soils SP 100%	4	4,22587	A
C-soils SP 100%	4	2,39528	B
N-soils RevoCaP + CX 3%	4	1,25429	C
C-soils RevoCaP + CX 1%	4	1,05602	C D
N-soils RevoCaP	4	1,05295	C D
N-soils RevoCaP + CA 3%	4	1,05123	C D
C-soils RevoCaP + CX 3%	4	1,01717	C D
N-soils RevoCaP + CX 1%	4	0,92919	C D
C-soils RevoCaP	4	0,87817	D
C-soils RevoCaP + CA 3%	4	0,84173	D
C-soils RP	4	0,13405	E
C-soils Control	4	0,12365	E
N-soils RP	4	0,08949	E
N-soils Control	4	0,06707	E

Means that do not share a letter are significantly different.

Appendix 6

Table 21. Two-way ANOVA significant effects of pH, fertilizer, and their interaction on dry matter yield (t/ha) at the second harvest

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
pH	1	0,8828	0,88280	61,92	0,000
Fertilizer	6	27,2254	4,53757	318,26	0,000
pH*Fertilizer	6	0,5857	0,09762	6,85	0,000
Error	42	0,5988	0,01426		
Total	55	29,2927			

Table 22. Tukey pairwise comparisons (Grouping) of the interaction between pH and fertilizer on dry matter yield at the second harvest

Grouping Information Using the Tukey Method and 95% Confidence

pH*Fertilizer	N	Mean	Grouping
N-soils SP 100%	4	2,72368	A
C-soils SP 100%	4	2,08036	B
N-soils RevoCaP + CX 3%	4	1,33984	C
N-soils RevoCaP	4	1,27437	C D
N-soils RevoCaP + CA 3%	4	1,17351	C D E
N-soils RevoCaP + CX 1%	4	1,17224	C D E
C-soils RevoCaP + CX 3%	4	1,03484	D E
C-soils RevoCaP + CX 1%	4	0,99024	D E
C-soils RevoCaP	4	0,93781	E
C-soils RevoCaP + CA 3%	4	0,88959	E
N-soils RP	4	0,19064	F
C-soils RP	4	0,17391	F
C-soils Control	4	0,15762	F
N-soils Control	4	0,14787	F

Means that do not share a letter are significantly different.

Table 23. Two-way ANOVA significant effects of pH, fertilizer, and their interaction on phosphorus concentration (g/kg DM) at the second harvest

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
pH	1	0,2489	0,248876	38,87	0,000
Fertilizer	6	0,7338	0,122301	19,10	0,000
pH*Fertilizer	6	0,2699	0,044978	7,03	0,000
Error	42	0,2689	0,006402		
Total	55	1,5215			

Table 24. Tukey pairwise comparisons (Grouping) of the interaction between pH and fertilizer on phosphorus concentration at the second harvest

Grouping Information Using the Tukey Method and 95% Confidence

pH*Fertilizer	N	Mean	Grouping
N-soils RevoCaP + CA 3%	4	1,06225	A

N-soils RevoCaP + CX 1%	4	1,04799	A B
N-soils RevoCaP	4	1,01244	A B
C-soils SP 100%	4	1,00419	A B
N-soils RevoCaP + CX 3%	4	0,98040	A B C
N-soils SP 100%	4	0,86102	A B C D
C-soils RevoCaP	4	0,85058	B C D
C-soils RevoCaP + CX 1%	4	0,78107	C D E
C-soils RevoCaP + CX 3%	4	0,77788	D E
C-soils RevoCaP + CA 3%	4	0,76279	D E
N-soils RP	4	0,71572	D E
N-soils Control	4	0,68443	D E
C-soils RP	4	0,63494	E
C-soils Control	4	0,61950	E

Means that do not share a letter are significantly different.

Table 25. Two-way ANOVA significant effects of pH, fertilizer, and their interaction on P yield (kg P/ha) at the second harvest

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
pH	1	1,4538	1,45380	94,49	0,000
Fertilizer	6	24,3513	4,05855	263,79	0,000
pH*Fertilizer	6	0,6713	0,11188	7,27	0,000
Error	42	0,6462	0,01539		
Total	55	27,1226			

Table 26. Tukey pairwise comparisons (Grouping) of the interaction between pH and fertilizer on phosphorus yield at the second harvest

Grouping Information Using the Tukey Method and 95% Confidence

pH*Fertilizer	N	Mean	Grouping
N-soils SP 100%	4	2,34192	A
C-soils SP 100%	4	2,09298	A
N-soils RevoCaP + CX 3%	4	1,31475	B
N-soils RevoCaP	4	1,28161	B
N-soils RevoCaP + CA 3%	4	1,24728	B
N-soils RevoCaP + CX 1%	4	1,21510	B
C-soils RevoCaP + CX 3%	4	0,80612	C
C-soils RevoCaP	4	0,80461	C
C-soils RevoCaP + CX 1%	4	0,77691	C
C-soils RevoCaP + CA 3%	4	0,67985	C
N-soils RP	4	0,12939	D
C-soils RP	4	0,11092	D
C-soils Control	4	0,09764	D
N-soils Control	4	0,09470	D

Means that do not share a letter are significantly different.

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