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RESEARCH ARTICLE

Phosphorus dynamics in a sandy soil amended with ph-modified pig slurry: insights from a rhizobox study using ^{33}P isotopes

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Abstract
Background and aims This study assessed the impact of pH modification of pig slurry on the dynamics of slurry- and soil-derived phosphorus (P) after surface application, as a potential alternative to inorganic P fertilizers.
Methods Mineral acidification (pH 5), bio-acidification (pH 5), and alkalinization (pH 9.5) were the strategies used to modify the pH of pig slurry using paper industry by-products and additive combinations. Maize seedlings were grown in rhizoboxes to monitor root and shoot traits over 23 days. The labile P pool in the sandy loam soil was labeled with ^{33}P to distinguish between soil- and fertilizer-derived P. Post-harvest analyses included acid phosphatase activity and mycorrhizal root colonization.
Results All (bio)-acidified treatments significantly increased slurry water-extractable P by 70% but spent acid by-product reduced maize root growth and total P uptake. Alkalinized slurry did not affect root growth, P uptake, or soil enzymatic activity. Root branching and length were diminished in all acidified treatments, accompanied by a 59% reduction in enzymatic activity and mycorrhizal root colonization remaining below 10% across treatments.
Conclusion While acidified slurry improved P solubility, gains in plant P uptake and biomass were

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limited, likely due to inhibitory effects on root development and symbiotic microbial functions, reflecting trade-offs between P availability and plant-soil biological interactions.

Keywords Slurry treatment · By-products · Phosphorus uptake · Mycorrhizal root colonization · Soil enzymatic activity

Introduction

Phosphorus (P) is an essential macronutrient required for key physiological and biochemical processes in plants, including energy transfer (ATP) and membrane formation (Khan et al. 2023). Most of the inorganic P fertilizers provide a readily available source of P, promoting rapid growth and high yields (Arenberg & Arai 2019), except in soils with high P content, where overapplication may lead to P leaching risk, or in soils with high sorption capacity, where P soil application presents low efficiencies. However, for their production finite P resources are exploited by the mining industry, resulting in the depletion of phosphate rock (Geissler et al. 2018). Furthermore, it is projected that the European Union (EU) will face a decrease in the available agricultural land over the next decade (European Comission 2023). This decrease will have to be compensated for by achieving higher crop yields with lower external inputs. Therefore, alternative sources of P fertilization are needed.

Crop fertilization with animal slurry is a promising bio-based alternative to mineral P fertilizers (Fangueiro et al. 2021). Despite the substantial availability of livestock manure in the EU region, there are still several barriers for an efficient adoption of bio-based fertilizers (Álvarez Salas et al. 2024). The application of untreated slurry based on the crop's nitrogen (N) demand often leads to an over-application of P, resulting in unbalanced nutrient ratios and low P availability for optimal plant uptake (Prado et al. 2022). In fact, the dominant P species in raw slurry are mainly mineral forms that can be precipitated (struvite, Ca-P) (Li et al. 2020) or more soluble forms (Jiang et al. 2024). Plants are able to take up P in the ionic orthophosphate ionic forms ($H_2PO_4^-$, HPO_4^{2-}) that are dissolved in the soil solution (Schachtman et al. 1998). As reported in the literature, untreated slurry often fails to provide adequate levels of these bioavailable P forms (He & Zhang, 2014). Hence, appropriate slurry treatment is needed to increase the plant P availability.

Slurry acidification with chemical additives—often carried out to avoid ammonia volatilization—can increase the P solubility in slurry, and consequently, improve its fertilizer value (Fangueiro et al. 2015). For instance, a significant increase in the total water extractable P (WEP) was observed in cattle slurry acidified to pH 5.5 compared to raw slurry (Pedersen et al. 2017). However, strong acids, such as sulfuric acid (H_2SO_4), are highly corrosive substances, and therefore, should be handled with caution by properly trained workers (Regueiro et al. 2022). Agro-industrial by-products can serve as alternatives to mineral acids for slurry acidification (Fangueiro et al. 2024). Within the context of circular economy, this approach is in line with the EU's long-term goal of minimizing waste disposal and promoting sustainability (Chojnacka et al. 2020). Moreover, biological acidification (bio-acidification) using fermentable C-rich substrates has been proven to reduce slurry pH to values as low as 3.9 (Regueiro et al. 2022). Previous work by Garder et al. (2023) evaluated the N fertilizer value of bio-acidified slurry in maize. However, further studies are needed to better understand the P dynamics after surface application of bio-acidified slurry.

Little attention has been paid to slurry alkalinization, a strategy that has the potential to produce a sanitized bio-based fertilizer (Chrysanthopoulos et al. 2024; Rodrigues et al. 2021). In other biowaste materials such as digestate, sewage sludge and sewage sludge ash, alkalinization with NaOH increased the apparent P recovery in soil (Sica et al. 2023), as well as plant growth and P uptake (Kopp et al. 2023).

When applying treated slurry to soil, it is essential to assess not only the plant P availability but also, its broader impact on both slurry-derived and soil-derived P pools. Evaluating this impact involves using various indicators, including enzymatic activity and arbuscular mycorrhizal fungi (AMF). Soil enzymatic activity plays a crucial role in the process of organic P mineralization into inorganic orthophosphate (PO_4^{3-}) ions, (Gómez-Muñoz et al. 2017; Rejsek et al. 2012). Acid phosphatases (ACP), which are extracellular enzymes produced by both plants and soil microorganisms (Acosta-Martínez & Waldrip, 2014), are especially relevant in studies involving organic amendments like slurry, as they serve as indicators of changes in soil P mineralization (Acosta-Martínez & Harmel, 2006). Other phosphatases, such as alkaline phosphatases primarily produced by soil microorganisms, also contribute to soil P dynamics.

Associations between plants and soil microbiota can support the mobilization of PO_4^{3-} in the soil, and consequently, P assimilation by plants (Gómez-Muñoz et al. 2017). For instance, AMF can promote plant growth by providing nutrients to the host plant, such as P, in exchange for carbohydrates and lipids (Corona Ramírez et al. 2023). While it is well documented that nutrient-rich amendments can reduce AMF root colonization and sporulation due to elevated P levels (Gryndler et al. 2006; Rouphael et al. 2015), it is crucial to investigate whether specific amendments alter soil conditions in ways that influence mycorrhizal colonization. The chemical and physical changes induced by treatments, such as shifts in soil pH, organic matter (OM) content, or nutrient availability, could affect AMF symbiosis beyond simply increasing P concentration. To our knowledge, the effect of slurry acidification and/or alkalinization on AMF has not been properly addressed.

While there are a large number of studies focusing on the effect of organic amendments on plant P uptake (Mackay et al. 2017; Sica et al. 2023), less is known about how (bio)-acidified or alkalinized slurry influences both slurry-derived and soil-derived P dynamics. Therefore, the present study aims to assess the P dynamics in the soil–plant system after surface application of pig slurry treated with different products to modify its pH. We hypothesized that:

- Slurry acidified with agro-industrial by-products (e.g., spent acids from the paper industry) or bio-acidified will enhance the solubilization of slurry-derived P to a similar extent as mineral acidification with H_2SO_4, thereby increasing its availability for plant uptake. Additionally, pH modification may influence the dynamics of soil-derived P by altering soil P fractions and interactions with plant roots.
- Maize yield will be higher after surface application of raw or treated slurry compared to the unfertilized treatment (negative control), with no significant difference expected between the acidification methods (bio, mineral-acidification).
- Soil application of raw and treated slurry will influence soil microbial activities, including phosphatase production. Acidification treatments are expected to reduce ACP activity due to increased P availability, whereas alkalinization is not expected to significantly alter ACP activity compared to raw slurry.

Materials and methods

Slurry and additives used

Pig slurry was sampled from an experimental farm located at the University of Aarhus, Denmark. To avoid excessive dilution of the slurry, collection took place prior to the routine cleaning of the pit. The pigs were at the fattening stage and were fed a mixed grain diet. The sampled slurry was immediately stored at 4 °C to minimize alternation of the physico-chemical properties.

Mineral acidification (pH 5), bio-acidification (pH 5) and alkalinization (pH 9.5) were the treatment strategies used to modify the pH of pig slurry. The target pH values were chosen based on previous studies where significant pathogen reduction (*E.coli*) was evident in pig slurry (Chrysanthopoulos et al. 2024; Rodrigues et al. 2021).

Slurry acidification was achieved through the use of concentrated sulphuric acid (H_2SO_4) solution (95–97% v/v) and spent acid (Spent.A), which is an acidic by-product generated during the bleaching process in the paper-making industry (Mendes et al. 2014). Acidifying slurry to pH 5 in this study required 11.5 mL H_2SO_4 kg^{-1} and 46 mL Spent.A kg^{-1}, which is within the range reported in previous studies; for example, Finzi et al. (2024) found that reaching pH 5.5 required 1.6–9.4 mL H_2SO_4 kg^{-1}, depending on slurry composition.

Bio-acidification of the raw slurry included the combination of H_2SO_4 with commercial sugar (H_2SO_4/Suc) as an approach to reduce the use of H_2SO_4. Similar to the work of Chrysanthopoulos et al. (2024), slurry was pre-acidified to pH 6 with H_2SO_4 (7.5 mL kg^{-1} slurry) followed by sucrose addition (22.5 g kg^{-1} slurry). A five-day incubation period (20 °C) was required to allow the slurry's native microorganisms to produce organic acids and reduce the slurry pH to 5.

For slurry alkalinization, a 10M potassium hydroxide (KOH) solution was used at a dose of 14 mL kg^{-1} slurry.

Physico-chemical analyses of the slurry

The pH of raw and treated slurry was measured directly by using a glass electrode (PHM210, Radiometer Analytical) pH meter (Table 1). The electrical conductivity (EC) was measured using a conductivity meter (CDM210, Radiometer Analytical). The dry matter (DM) content was determined by drying 20 g of a slurry sample at 105 °C for 24 h in a drying oven (Heraeus Function Line). Dried slurry subsamples of 0.5 g were incinerated (4 h, 550 °C) to obtain the OM content. The OM content of the slurry was used to calculate the OC by applying a conversion factor of 0.58, assuming that OM contains 58% OC (Rodrigues et al. 2021).

The NH_4^+-N was analyzed in 1:60 slurry:KCl (1M) suspensions that were initially agitated for 1 h, filtered (Whatman filter paper no.5) and measured on a flow injection analyzer (FIAstar 5000, Foss Analytical, Sweden). For the analysis of water extractable phosphorus (WEP), 1:100 slurry:Milli-Q water suspensions (weight per volume dry or wet weight) were prepared, followed by 1 h agitation and subsequent centrifugation (5 min, 4500 rpm). The ortho-P content was measured colormetrically using the molybdate blue method on a flow injection analyzer (FIAstar 5000, Foss Analytical, Sweden). The elemental composition of the slurries was analyzed by inductively coupled plasma—optical emission spectrometry (ICP-MS, Agilent 5100, Agilent Technologies, Manchester, UK). Sample preparation consisted of microwave-assisted acid digestion of 0.2 g of dry slurry sample with a 1:3 (v/v) mixture of nitric acid (65% (w/w)) and hydrochloric acid (37% (w/w)) at 135 °C.

Soil and ^{33}P isotope labelling

A sandy loam soil (clay 12.6%, silt 14.3%, and sand 69.8%) was used for the rhizobox experiment, sampled from the unfertilized treatment of the CRUCIAL long-term fertilization trial, located in Taastrup, Denmark. After collection, the soil was air-dried and sieved to 4 mm. The soil pH was measured to be 5.7, the organic carbon (OC) content of 13.5 g kg^{-1} and the Resin P concentration to be 10.5 mg kg^{-1}, classified as a low P soil. Quartz sand (0.4–0.8 mm) was mixed with the sieved soil on a dry weight basis to obtain a soil-sand ratio of 3:1 to improve water infiltration and facilitate maize root growth, a practice commonly used in rhizobox studies (Gómez-Muñoz et al. 2017; Bornø et al. 2023). The maximum water holding capacity (WHC) of the soil-sand mixture was determined to be 31 g of water per 100 g of soil. Plastic bags were then filled with batches of 2.4 kg of the soil-sand mixture (hereafter referred to as soil) and fertilized with nutrient solutions to provide essential macro- and micronutrients except P in sufficient amounts. The detailed rhizobox fertilization regime can be seen in Table S1 of the supplementary material.

The soil was pre-incubated for a period of 7 days after the addition of deionized water corresponding to 30% of the soil WHC. Each soil bag received 10 mL of a carrier-free ^{33}P-orthophosphate solution (Hartmann Analytic, Braunschweig, Germany)

Table 1 Physico-chemical properties of raw and treated slurry samples ± SD ($n = 3$)

Treatment	pH	EC	DM	OC	NH_4^+-N	TP	WEP
	-	mS cm^{-1}	g kg^{-1}	g kg^{-1} (FW)	g kg^{-1} (FW)	g kg^{-1} (DW)	(% total initial P)
RS	7.1 ± 0.01	20.1 ± 0.5	118.8 ± 1.7	57.7 ± 1.0	3.4 ± 0.7	13.5 ± 0.2	15.3
H_2SO_4	5.0 ± 0.01	26.9 ± 0.8	136.3 ± 2.7	57.2 ± 0.4	2.7 ± 0.3	13.6 ± 0.2	78.4
Spent.A	5.1 ± 0.03	33.4 ± 0.8	151.0 ± 4.0	60.1 ± 1.7	3.3 ± 0.3	11.7 ± 0.4	78.4
H_2SO_4/Suc	4.7 ± 0.13	24.9 ± 1.0	143.8 ± 4.6	70.8 ± 1.8	3.4 ± 0.1	13.4 ± 0.7	75.5
KOH	8.4 ± 0.05	25.6 ± 0.5	128.1 ± 5.1	68.2 ± 1.7	2.3 ± 0.4	11.9 ± 1.1	13.8

FW: fresh weight; DW: dry weight; EC: electrical conductivity; DM: dry matter; OC: organic carbon; NH_4^+-N: ammonium nitrogen; WEP: water-extractable phosphorus; RS: raw slurry; H_2SO_4: acidification with sulfuric acid; Spent.A: acidification with the by-product spent acid; H_2SO_4/Suc: pre-acidification to pH 6 with sulfuric acid followed by sucrose addition (20 g kg^{-1} slurry); KOH; alkalinization with potassium hydroxide to achieve an activity of 0.9 MBq kg^{-1} dry soil. To ensure homogeneous distribution, the soil was transferred to a plastic container and manually mixed for 2 min before being placed into plastic bags for incubation. The soil was incubated for an additional 10 days before being applied to the rhizoboxes.

Rhizobox experiment

The ^{33}P labelled soil was added in small batches to rhizoboxes (37.5 cm × 20 cm × 2.2 cm inner dimensions), to achieve a bulk density of 1.4 g cm^{-3} soil. The treatment without fertilizer application served as a negative control (CNT), while fertilization with triple superphosphate (TSP) was set up as a positive control.

Maize seeds (*Zea mays,* L. cv. 'Ambition') were pre-germinated for three days and each rhizobox received one maize seed placed in the centre 5 cm below the soil surface. Raw and treated slurry was surface applied at a rate of 40 mg P kg^{-1} soil, equivalent to 104 kg total P ha^{-1}. The TSP fertilizer was applied at the same rate and mixed well with the surface soil (upper 10 cm). Subsequently, small increments of deionized water were added to reach 60% of soil WHC. To induce root growth along the transparent side, each rhizobox was placed in supports at a 45° angle with the transparent side facing down. Furthermore, a black opaque cover was attached to the plexiglass and removed only when root photographs were taken. For more detailed information about the rhizobox setup see Lemming et al. (2016).

A total of 28 rhizoboxes with 4 replicates for each treatment were placed in a climate chamber with day and night temperatures of 23 and 15 °C, respectively. The photoperiod was set to 16 h with a daylight photosynthetic active radiation (PAR) of 600 µmol m^{-2} s^{-1}. The rhizoboxes were frequently watered to weight with deionized water to maintain the soil water content at 60% WHC. The maize growth period lasted for 23 days after sowing (DAS) and the plants were harvested at the vegetative growth stage v5.

Sequential imaging was employed to capture the dynamics of shoot and root development over the growing period of maize. A high-resolution (3984 × 2656 pixels) digital single-lens reflex camera (EOS, Cannon 4000D) manually set to an aperture of f/13 and ISO 100, was mounted on a camera dolly system and placed inside a dark chamber, containing two rows of 10 LEDs with color temperature of 4000 K. During image acquisition, the rhizobox was placed horizontally inside the dark chamber at pre-determined positions and the black cover was detached from the rhizobox. A microcontroller allowed us to trigger the camera shutter during this process. Images were taken at eight time intervals between 4 and 21 DAS.

At 23 DAS, the shoots were separated from the roots at the crown zone. After harvesting, representative soil samples were collected from the upper 10 cm of the rhizobox and homogenized. Roots were carefully washed with deionized water to remove soil particles and preserved in ethanol (70% v/v).

Post harvest analyses

Plant analyses

After harvest, the root and shoot dry weight was measured after oven-drying at 60 °C for three days. The shoots were then milled to a fine powder by using ceramic mill balls (99.5% yttria-stabilized zirconia). To quantify the total P content of the shoots, subsamples of 150 ± 15 mg were incinerated at 550 °C for one hour. The ashed samples were then placed in 50-mL falcon tubes and shaken for 16 h in an end-over-end shaker with 50 mL of 0.5 M H_2SO_4. After filtration, the ^{33}P beta emission from the shoots was analyzed by liquid scintillation counting (Tri-Carb 2910 TR, Perkin Elmer) as described in the work of Sica et al. (2024). Total P content in the extracts was determined using a flow injection analyzer (FIAstar 5000, Foss Analytical, Sweden).

Quantification of AMF in maize roots was carried out using the microscopy-based method, staining AMF structures with trypan blue dye as described by Phillips & Hayman (1970). Briefly, representative root samples were placed in scintillation vials to which 10% KOH (w/v) solution was added until the samples were completely submerged. A heat treatment followed by boiling the vials in a water bath at 90 °C for 20 min. The roots were then rinsed with deionized water and stained with trypan blue (0.05% trypan blue in lactoglycerol). Stained roots were randomly placed in Petri dishes (∅ 9 cm) with grid lines to allow the counting of intersections with intraradical mycorrhiza. For each sample, the presence or absence of fungal structures was recorded for approximately

100 intersections and the results were expressed as a percentage of the root length colonized.

Soil analyses

Soil pH was determined on a 1:5 soil:$CaCl_2$ (0.01 M) suspension by using a glass electrode pH meter (PHM210, Radiometer Analytical). The concentrations of ammonium (NH_4^+) and nitrate (NO_3^-) in fresh soil samples were determined in a 1:5 soil:KCl (1 M) suspension and analyzed as previously described for the slurry (ISO/TS 14256–1).

At the end of the rhizobox experiment, soil samples were taken from the top 10 cm of the rhizobox to assess the remaining labile P in the soil after 23 days. The resin P method was used for this analysis (Mason et al. 2013). Briefly, 2 g of air-dried soil was shaken for 16 h with 20 mL of deionised water and two 20 mm ×60 mm anion exchange membranes (VWR Chemicals, Radnor, PA, USA). After shaking, the membranes were rinsed with deionized water and eluted with 10 mL of 0.5 M HCl for 1 h. The P concentration in the eluent was determined using a flow injection analyzer (FIAstar 5000, Foss Analytical, Sweden).

The activity of ACP in the soil samples was determined by a spectrophotometric assay as proposed by Tabatabai (1994). The main principle of this method is based on quantifying the p-nitrophenol (pNP) released from the substrate *p*-nitrophenyl phosphate (pNPP) substrate under optimal conditions. For the determination of ACP, soil samples of 1 g were incubated with the substrate for 1 h at 37 °C in a modified universal buffer (MUB) solution (pH 6.5). To stop the reaction and to initiate the color development, 1 mL of 0.5 M $CaCl_2$ and 4 mL of 0.5 M NaOH, respectively were added. The absorbance was measured at 405 nm on a spectrophotometer (GENESYS 10S UV–Vis, Thermo Scientific™, USA) and results were expressed as μg pNP h^{-1} g^{-1} dry soil.

Root and shoot image analysis

Root image analysis was performed using an AI-based software called RootPainter as described in Smith et al. (2022). During the training process for the model development, pixels on each image were annotated by the user as foreground or background. The trained models were then used to automatically segment root images (Fig. S1, supplementary materials). These segmented images were fed into Rhizovision Explorer (RVE: Seethepalli & York 2020) for extraction of root traits, namely total root length (mm) and number of branching points. While only the visible part of the root system was analyzed, it serves as a proxy for the entire root structure.

The height of maize plants over time was also measured in this study. For each time interval, the shoot height was calculated by measuring the distance from the maize crown zone to the tip of the uppermost leaf in pixels. The total pixel count was then converted to centimeters (cm) by using a coin as a reference object.

Calculations and statistical analyses

The total P content of maize shoots (TotP_shoots) was calculated as the sum of three P pools namely P derived from the seed (Pdf_seed), P derived from the soil (Pdf_soil) and P derived from the fertilizer (Pdf_fertilizer). The amount of Pdf_seed was estimated by using the following equation (Eq. 1) as described in Lemming et al. (2016):

$$Pdf_{seed} = 0.15 \times \ln(TotP_{shoots}) + 0.5 \quad (1)$$

The following equation (Eq. 2) was used to calculate the Pdf_fertilizer as suggested by Lemming et al. (2016) and Sica et al. (2024):

$$Pdf_{fertilizer} = \left(P_{UptPlant+P} - Pdf_{seedPlantP} - \left(\frac{TotActPlant + P}{TotActPlant - P}\right)\right) \times \left(P_{UptPlant-P} - Pdf_{seedPlant-P}\right) \quad (2)$$

In Eq. (2), P_{Upt} denotes the P uptake by the maize shoots while TotAct represents the total activity (kBq) of the plant. The terms Plant +P and Plant-P denote whether the plants received P fertilization (+P) or no fertilizer (-P). Ultimately, the Pdf_soil was calculated as follows (Eq. 3):

$$Pdf_{soil} = TotP_{shoots} - Pdf_{seed} - Pdf_{fertilizer} \quad (3)$$

Statistical analyses and graphing were performed using R studio software. A linear mixed-effects model was applied to assess the effect of fixed (treatment ×day) and random (replicate) factors on root

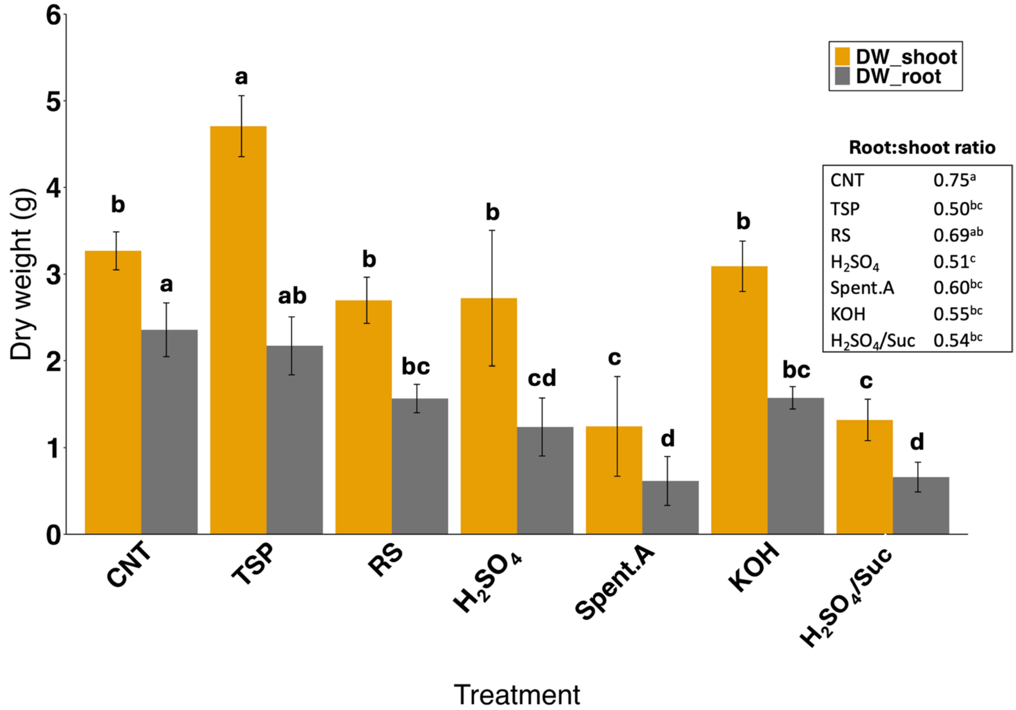

Fig. 1 Effect of slurry treatments on maize shoot and root biomass, as well as root-to-shoot ratio, at the end of growth experiment (23 DAS). Error bars represent the standard error of the means. Biomass and root-to-shoot ratio followed by the same letter are not significantly different based on Fishers LSD-test ($p > 0.05$). CNT: control; TSP: triple superphosphate; RS: raw slurry; H_2SO_4: acidification with sulfuric acid; Spent.A: acidification with the by-product spent acid; KOH; alkalinization with potassium hydroxide; H_2SO_4/Suc: pre-acidification to pH 6 with sulfuric acid followed by sucrose addition (20 g kg^{-1} slurry)

and shoot traits obtained during the maize growing period. Normality of residuals was assessed using Q-Q plots, and homogeneity of variance was tested using Levene's test. For all post-harvest statistical analyses, the non-parametric Kruskal–Wallis test by ranks was used due to deviations from normality. For pairwise comparison of the rank means, the post-hoc Fisher's least significant difference (LSD) followed at $\alpha = 0.05$.

Results

Treatment effect on maize biomass

The TSP fertilizer resulted in the highest shoot DM yield of all treatments, showing a 44% increase compared to the CNT (Fig. 1). Both the Spent.A and H_2SO_4/Suc treatments had a negative effect on maize shoot biomass, significantly reducing the DM yield compared to the CNT. A similar effect was observed on root dry weight with Spent.A and H_2SO_4/Suc resulting in the lowest below-ground biomass. In contrast, unfertilized maize plants exhibited significantly higher root biomass than those treated with RS and pH modified slurry. This is reflected in the root-to-shoot ratio, which was highest in the CNT (0.75) and lowest in the TSP treatment (0.50), with H_2SO_4 and H_2SO_4/Suc treatments also exhibiting relatively low ratios. This suggests that certain pH modification strategies may disproportionately affect below-ground biomass allocation compared to shoot growth.

Phosphorus uptake by maize shoots

Significant differences in crop P uptake were observed between the treatments (Fig. 2). Maize

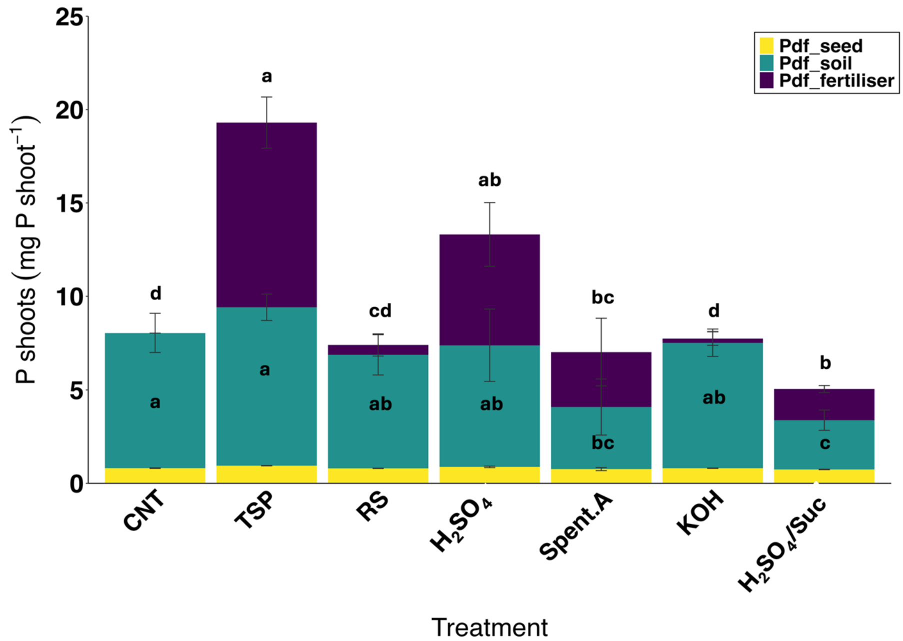

Fig. 2 Maize P uptake from the seed, the soil and the fertilizer (mean, $n = 4$). Error bars represent the standard error of the means. Pdf: phosphorus derived from; CNT: control; TSP: triple superphosphate; RS: raw slurry; H_2SO_4: acidification with sulfuric acid; Spent.A: acidification with the by-product spent acid; KOH; alkalinization with potassium hydroxide; H_2SO_4/Suc: pre-acidification to pH 6 with sulfuric acid followed by sucrose addition (20 g kg^{-1} slurry)

fertilization with TSP resulted in the highest shoot P uptake, followed by the H_2SO_4 treatment. In the TSP treatment, the P uptake was relatively balanced, with approximately 44% of the P derived from the soil and 50% from the fertilizer. In contrast, the RS treatment showed a much higher reliance on soil-derived P, with 81% of the total P uptake coming from the soil. When the slurry was acidified with either H_2SO_4 or Spent.A, the proportion of P uptake from fertilizer increased, bringing the P source distribution closer to that observed in the TSP treatment. Conversely, the alkalinization of slurry with KOH significantly reduced the fertilizer-derived P, which contributed only 3% of the total P, emphasizing a limited effectiveness of this treatment in improving fertilizer P availability to maize. It is also noteworthy that plant P uptake from the soil in the H_2SO_4/Suc treatment was significantly lower compared to RS and CNT, suggesting altered P dynamics in this treatment.

Effects of slurry treatments on maize development over time and mycorrhizal colonization

Maize root and shoot development

Image analysis revealed significant differences between treatments in maize root branching points and total root length (Fig. 3a, b). The unfertilized CNT showed a significantly higher number of branching points compared to RS and all acidified slurry treatments from Day 10 to Day 21. Conversely, slurry alkalinization with KOH appeared to have a similar effect on root branching as TSP, both showing a high number of branching points (> 900, Day 21). Neither Spent.A nor the H_2SO_4/Suc treatments exhibited a significant increase in root branching, with the development of lateral roots remaining below 300 branching points per plant.

A very similar pattern was observed for maize root length over time (Fig. 3b). Among all slurry treatments, alkalinization with KOH resulted in a total root length of over 1000 cm, which was not significantly different from the inorganic TSP treatment. In contrast, the acidified slurry had a negative effect on maize root growth, reducing the total root length by up to 81% (Spent.A) compared to the CNT.

Analyses of the aboveground images showed no significant differences in maize shoot height between CNT, TSP, RS, and KOH treatments at any of the time intervals (Fig. 3c). Although H_2SO_4 initially caused a delay in maize shoot growth (Day 7–14), the plants managed to recover and had shoot heights equivalent to the CNT and RS treatments.

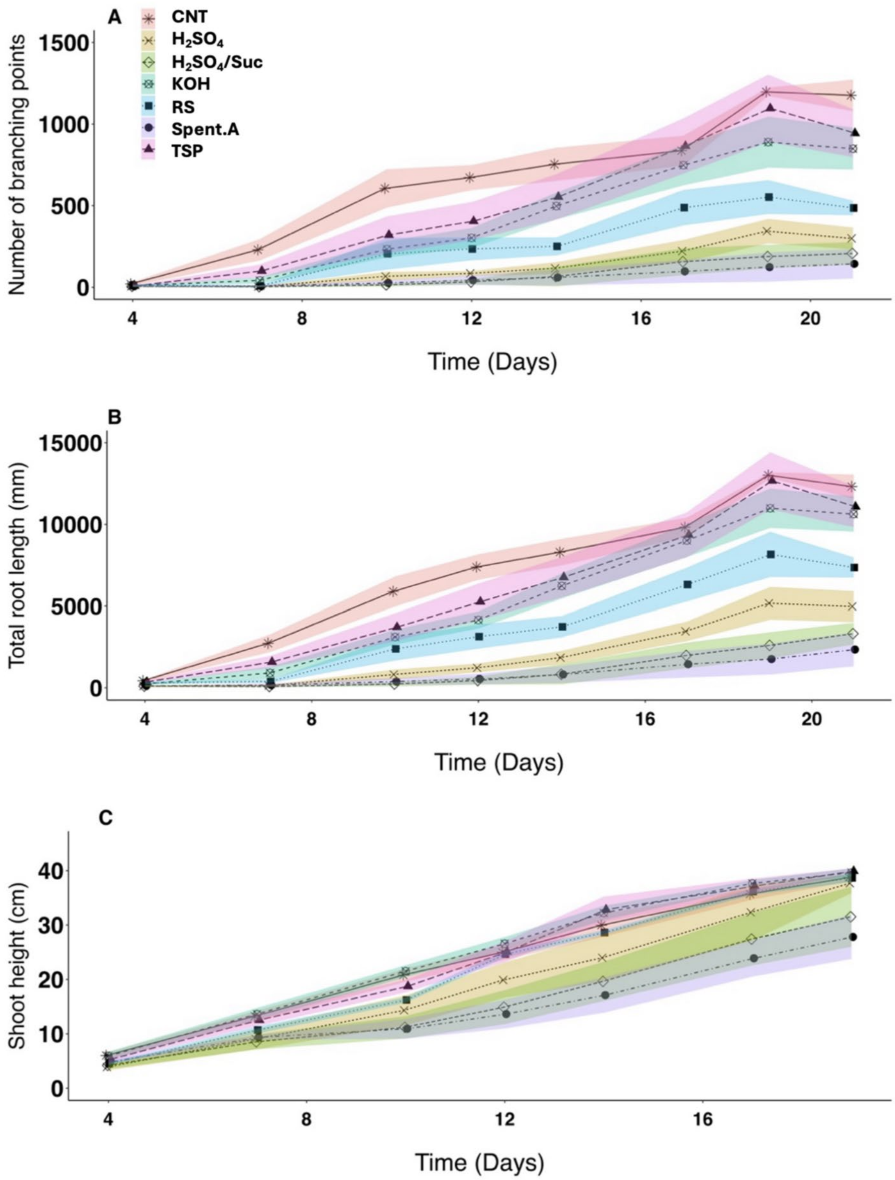

Fig. 3 Development of maize root branching points (**A**), total root length (**B**) and shoot height (**C**) over the experimental period. Coloured shaded areas represent the standard error of the means (n =4). CNT: control; TSP: triple superphosphate; RS: raw slurry; H_2SO_4: acidification with sulfuric acid; Spent.A: acidification with the by-product spent acid; KOH; alkalinization with potassium hydroxide; H_2SO_4/Suc: pre-acidification to pH 6 with sulfuric acid followed by sucrose addition (20 g kg^{-1} slurry)

However, both the Spent.A and H_2SO_4/Suc treatments had significantly lower shoot heights compared to CNT from Day 10 to Day 19.

Mycorrhizal root colonization

Mycorrhizal root colonization by AMF remained relatively low (< 17%), with the CNT showing the highest root colonization (Fig. 4). A significant decrease was observed in all treatments with slurry application, regardless of the treatment strategy. Slurry acidification with Spent.A showed the lowest root colonization rate by AMF, significantly different from the CNT and TSP treatments. The negative effect on mycorrhizal root colonization was observed not only in the treatments that involved slurry application but also with the inorganic fertilizer TSP.

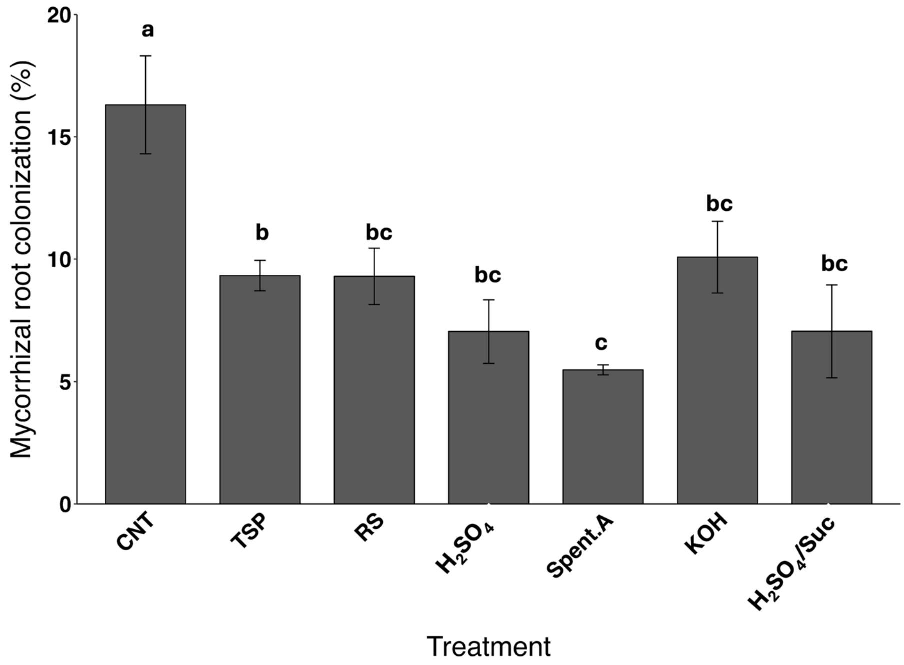

Fig. 4 Effect of raw and treated slurry on mycorrhizal root colonization by AMF (means, $n = 4$). Error bars represent the standard error of the means ($n = 4$). Means with different letters are significantly different based on Fisher's LSD test ($p > 0.05$). CNT: control; TSP: triple superphosphate; RS: raw slurry; H_2SO_4: acidification with sulfuric acid; Spent.A: acidification with the by-product spent acid; KOH; alkalinization with potassium hydroxide; H_2SO_4/Suc: pre-acidification to pH 6 with sulfuric acid followed by sucrose addition (20 g kg^{-1} slurry)

Changes in soil chemical properties and acid phosphatase activity

Effect of slurry treatment on soil physicochemical properties

While the pH in the treated slurry samples was either reduced to 5 or increased to 8.4, the soil pH after surface application remained within the range of 5.1–5.9 (Table 2). A significant reduction in soil pH was observed with the Spent.A and H_2SO_4/Suc treatments compared to the CNT.

When assessing the soil inorganic N content, large differences were found between the soil NH_4^+-N and NO_3^--N concentrations (Table 2). Except for the Spent.A treatment, the soil NH_4^+-N content remained relatively low (< 4 mg kg^{-1}). Conversely, elevated concentrations of NO_3^--N were measured in both acidified and alkalinized treatments. The KOH treatment showed the highest NO_3^--N content (158.1 mg kg^{-1}), while no significant differences were found among the acidified treatments.

The amount of phosphate extracted from the soil by ion exchange resin membranes is shown in Table 2.

Table 2 Soil chemical properties after maize harvest ($n = 4$). Values in the same column followed by the same letter are not significantly different based on Fishers LSD-test ($p > 0.05$)

Treatment	pH	NH_4^+-N	NO_3^--N	Resin-P
	-	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
CNT	5.7 ± 0.2^{ab}	2.7 ± 0.7^{ab}	72.3 ± 21.8^{cd}	21.7 ± 2.2^{c}
TSP	5.9 ± 0.1^{a}	1.6 ± 0.6^{c}	18.0 ± 15.1^{d}	55.8 ± 7.6^{a}
RS	5.7 ± 0.1^{ab}	2.6 ± 0.5^{b}	102.8 ± 14.8^{c}	20.6 ± 1.4^{c}
H_2SO_4	5.5 ± 0.5^{bc}	3.9 ± 2.3^{b}	134.7 ± 37^{b}	24.7 ± 2.5^{b}
Spent.A	5.1 ± 0.1^{c}	13.6 ± 1.5^{a}	133.6 ± 22.7^{ab}	27.6 ± 1.9^{ab}
H_2SO_4/ Suc	5.3 ± 0.1^{c}	1.2 ± 0.3^{c}	127.4 ± 11.6^{b}	24.6 ± 1.2^{b}
KOH	5.7 ± 0.1^{ab}	2.5 ± 0.6^{b}	158.1 ± 21.9^{a}	20.1 ± 1.9^{c}

NH_4^+-N: Total ammonium nitrogen; NO_3^--N: Total nitrate nitrogen; CNT: control; TSP: triple superphosphate; RS: raw slurry; H_2SO_4: acidification with sulfuric acid; Spent.A: acidification with the by-product spent acid; H_2SO_4/Suc: pre-acidification to pH 6 with sulfuric acid followed by sucrose addition (20 g kg^{-1} slurry); KOH; alkalinization with potassium hydroxide

Both raw RS and KOH treatments showed the lowest phosphate content, not significantly different from the CNT. Slurry acidification had a positive impact, significantly increasing the soil P concentration by up to 34% (Spent.A) compared to RS. However, it should be noted that the resin-P in the TSP treatment was still more than doubled after the maize harvest compared to the CNT. These results are consistent with the WEP content of the treated slurries. Acidifying the materials to pH 5 not only increased P solubility in the slurry (Table 1), but also enhanced the amount of available P in the soil.

Effect of slurry treatments on acid phosphatase activity

Significant differences in ACP activity were observed across the treatments (Fig. 5). Alkalinization with KOH and the CNT maintained the highest ACP activity, with KOH showing a positive effect compared to other treatments. In contrast, all acidified treatments significantly reduced ACP activity, with Spent.A showing the most pronounced decline. RS and TSP treatments did not significantly differ from the CNT, suggesting a neutral impact on ACP activity.

Discussion

Effect of treatments on maize biomass

In response to low soil P availability, plants can develop morphological and physiological adaptations in both above and belowground compartments. These include increased lateral root branching, secondary root growth, and physiological changes such as reduced leaf photosynthetic rate (Duque & Villordon 2019; Shu et al. 2023). Root phenotyping in climate controlled studies is a useful tool to elucidate relationships between nutrient supply and plant growth (Sica & Magid 2024). Previous rhizobox studies have investigated the effect of organic amendments or abiotic stresses on P dynamics and root morphological adaptations (Bornø et al. 2023; Sjulgård et al. 2021). According to the literature, low P availability typically results in higher lateral root density (Duque & Villordon 2019; Postma et al. 2014). In this study, the unfertilized CNT exhibited a significantly higher number of root branching points compared to the RS and acidified slurry treatments, which is consistent with the expected plant response to limited P availability. This increased root branching likely reflects an adaptive strategy to maximize soil exploration and P acquisition.

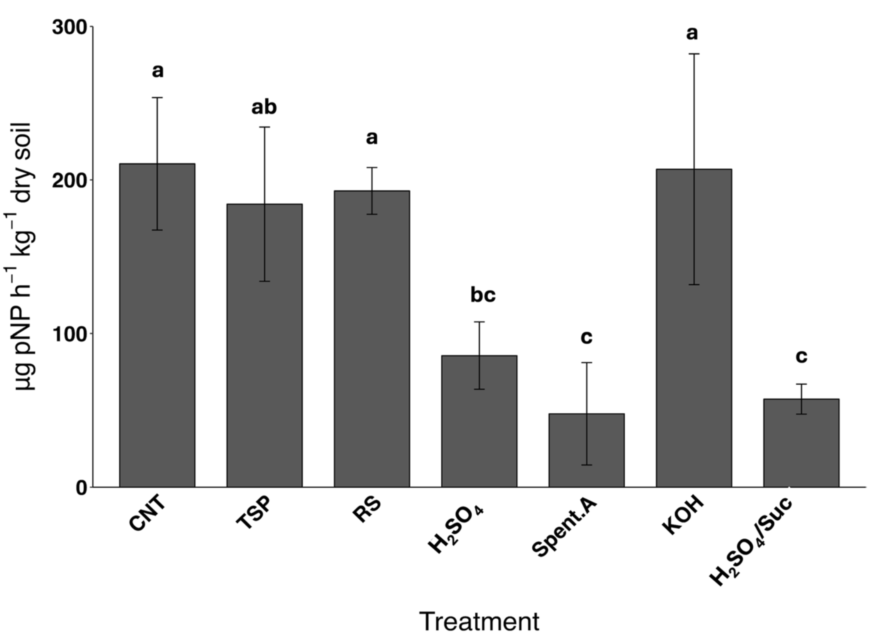

Fig. 5 Effect of raw and treated slurry on acid phosphatase activity (means, $n=3$). Error bars represent the standard error of the means ($n=3$). Means with different letters are significantly different based on Fisher's LSD test ($p > 0.05$). CNT: control; TSP: triple superphosphate; RS: raw slurry; H_2SO_4: acidification with sufuric acid; Spent.A: acidification with the by-product spent acid; KOH; alkalinization with potassium hydroxide; H_2SO_4/Suc: pre-acidification to pH 6 with sulfuric acid followed by sucrose addition (20 g kg^{-1} slurry)

The root-to-shoot ratio is commonly used as an indicator of plant nutrient status, typically increasing under nutrient-deficient conditions as plants allocate more biomass to roots to improve nutrient uptake (Lynch et al. 2012). The unfertilized maize plants (CNT) showed the highest root-to-shoot ratio while the TSP and H_2SO_4 treatments exhibited the lowest values. In the present experiment, all maize plants received equal amounts of all macro and micronutrients except for P. Under P-deficient conditions, plants tend to further develop their root system in search of nutrients in the soil (Lopez et al. 2023). Indeed, the low P content of the soil, together with the lack of P fertilization can explain the high root-to-shoot ratio observed in the CNT. In contrast, the acidified treatments not only supplied P to the maize, but also increased P solubility after the acidification process as shown in Table 1. The work of Mackay et al. (2017) corroborate our findings with TSP supply since they also observed a low root-to-shoot ratio when fertilizing with inorganic P.

The type of fertilizer application, particularly in the case of slurry, as well as its depth and placement, plays a crucial role in influencing early plant growth (Baral et al. 2021). While soil NH_4^+-N and NO_3^--N levels measured after 23 days did not show signs of ammonium accumulation, the initial impact on plant roots may have been substantial. According to Pan et al. (2016), the application of nitrogen-rich organic waste can create a localized zone of ammonium toxicity soon after application, causing visible root damage, such as burned tips, during early plant growth. This early toxicity can reduce root growth, limiting the plant's ability to access essential resources (Esteban et al. 2016), and ultimately, hindering nutrient uptake and overall plant development (Makaza & Khiari 2023). In this study, we infer that ammonium toxicity may have attributed to the impaired early development of maize, as the slurry was applied to a confined area on the rhizobox surface (2 cm × 10 cm) immediately after seedling transplantation. Although ammonium concentrations later declined, the high NO_3^--N levels observed at the end of the experiment suggest nitrification of an initially elevated ammonium pool. These early root stresses likely contributed to the reduced growth and P uptake observed in the slurry-treated groups -except for the acidified slurry (H_2SO_4), which aligned with the findings of Pedersen et al. (2017), where slurry acidification with H_2SO_4 enhanced early P availability and maize growth by mitigating ammonium toxicity.

Soil pH is an important parameter that control P speciation and thus affect P uptake by the crop (Christiansen et al. 2020; Pedersen et al. 2017). As in our study, a significant reduction of soil pH was observed after soil application of acidified slurry in other studies (Zireeni et al. 2023). The negative effects of such a pH drop, even within the narrow pH range of 5.0–6.0, can affect root development and plant growth (Goulding 2016). Specifically, maize has an optimum pH range of 6.0–6.5 but struggles to tolerate pH values below 5.5 (Goulding 2016). The prevailing acidic conditions near the rhizosphere zone in the Spent.A and H_2SO_4/Suc treatments could therefore explain the negative effect on root traits (Fig. 3a, b) and biomass (Fig. 1). In contrast, soil application of alkalinized slurry had no effect on soil pH. Although the soil pH was measured from mixed samples in the upper 10 cm, this might not fully capture the pH gradients that could have existed closer to the slurry application zones. The soil's buffer capacity and inherent pH stability, important factors that can moderate the initial effect of slurry pH modification (Pedersen et al. 2017), likely prevented any observable increase in soil pH despite the alkalinized slurry treatment.

Phosphorus uptake by maize

Maize is an arable crop that is rather sensitive to P deficiency, especially in the early development stages when rapid growth and limited root capacity can restrict P uptake, affecting final yield (Battisti et al. 2023). Our attempt to treat the pig slurry by lowering the pH to 5 had a beneficial effect on the P uptake, but only when treated with H_2SO_4. However, all acidified treatments increased the proportion of P derived from the slurry in the plants, an expected phenomenon due to the increase in slurry WEP (Table 1). Similar studies corroborate our findings, as e.g. Pedersen et al. (2017) who showed that cattle slurry acidification significantly increased the solubility of slurry inorganic P and maize P uptake.

An important consideration when assessing the soil P dynamics is the slurry WEP, which has been found to be highly correlated with the amount of P released into the soil and its subsequent availability for plant uptake (Sica et al. 2023). As seen in this study, all acidification treatments with high WEP (> 70%) also had significantly higher resin *P* values (Table 2) compared to the CNT and RS. The reason for this could be attributed to the higher P solubility in the acidified slurry, thereby improving the P release to the soil. In contrast, the alkalinization treatment (KOH) did not enhance WEP from the slurry, resulting in resin *P* values similar to those of the RS. This is likely due to P precipitation in the slurry in the form of calcium phosphates under alkaline conditions. However, in biomaterials other than slurry (e.g., sewage sludge), pre-treatment with NaOH increased soil WEP (Sica et al. 2023). Authors attributed this to the effects of alkalinity on the soil and the subsequent decreased of the soil's P sorption capacity.

The chemical characteristics of the additives used for slurry acidification may also explain the differences observed in maize P uptake between H_2SO_4 and Spent.A treatments. The by-product Spent.A is a sodium-sulphate sulphuric acid solution that is generated in paper and pulp mills (Mendes et al. 2014). Unlike H_2SO_4, Spent.A has a high concentration of Na (147 g L^{-1}) (Chrysanthopoulos et al. 2024). The total Na content of the acidified slurry with Spent.A was 27.6 mg Na kg^{-1}, seven times higher compared to the H_2SO_4 treatment (Table S2, supplementary materials). Elevated concentrations of Na^+ in the soil can decrease the osmotic potential and inhibit root development (Almeida et al. 2019), which may result in lower P uptake by maize.

On the other hand, the slurry treated with KOH did not show any significant difference compared to the RS in terms of fertilizer derived P uptake (Fig. 2) or fertilizer WEP (Table 1). This is in agreement with the literature, as the alkalinization is known to solubilize Fe- and Al-bound P species, especially at pH values above 10 (Xu et al. 2015). However, as previously discussed, these are not the predominant forms of P in pig slurry (Li et al. 2020), which explains why alkalinization had little to no effect on P solubility and availability for plant uptake in this study. This finding is consistent with Sica et al. (2023) who investigated the effects of acidification and alkalinization on various bio-based materials. Their study found that alkalinization (using NaOH) was more effective in solubilizing P from sewage sludge, where P is primarily bound to Fe and Al. Therefore, although alkalinization may have a positive effect on the sanitization of pig slurry, as demonstrated by Chrysanthopoulos et al. (2024), our results show that it has no effect on increasing its P fertilizer value.

Effect of treatments on soil chemical properties, enzymatic activity and root colonization by AMF

Mineralization of slurry organic N to NH_4^+ and subsequent nitrification during the experimental period could explain the elevated soil NO_3^--N content (Table 2). Slurry acidification to pH 5.5 or below is known to preserve the slurry NH_4^+ content (Sommer et al. 2013), as measured in this study (Table 1). In previous studies elevated NH_4^+ concentrations were observed in soils amended with acidified slurry (Zireeni et al. 2023). However, the low soil NH_4^+ content in this study after harvest suggests either (i) N uptake by the crop (not measured in this study), (ii) further conversion to NO_3^- (nitrification) or (iii) losses through NH_3 volatilization. For the acidified treatments, NH_3 volatilization after surface application should have been negligible, particularly in a soil with pH <6. The significantly higher NO_3^- content of all acidified treatments compared to RS implies no negative effect on nitrification, contrary to previous works that found inhibition of nitrification (Fangueiro et al. 2016; Owusu-Twum et al. 2017). On the other hand, increasing the pH of the slurry to 9.5 should have resulted in NH_3 losses after soil application. The residual NH_4^+ concentration and elevated NO_3^- readings after harvest support a positive effect of alkalinization on the nitrification process.

Soil application of organic residues, such as animal slurry, typically has a positive effect on ACP activity (He & Zhang, 2014). As ACP is produced by both plants and soil microorganisms, it can be challenging to directly correlate ACP with other soil parameters (Rejsek et al. 2012). However, a distinct pattern can be seen between acidified and alkalinized slurry treatments in this study. As previously discussed, acidified slurry significantly decreased the soil pH, reaching as low as pH 5.1 (Table 2). This lower pH is close to the optimal pH for ACP activity, which is 4.8 (He & Zhang, 2014). Therefore, an increase in enzymatic activity might have been expected under these conditions. However, the observed reduction in ACP activity, particularly in the acidified treatments, could be attributed to factors beyond the soil pH alone. A plausible explanation is the negative impact of acidification on root growth and the associated reduced production of plant-derived enzymes (Guo et al. 2023). Indeed, in this work, soil application of acidified slurry had a negative effect on root growth, particularly in the Spent.A and H_2SO_4/Suc treatments.

This reduction in root biomass would naturally result in lower levels of plant-derived enzymes, thereby contributing to the decreased ACP activity observed in the acidified slurry treatments, despite the pH being favorable for enzymatic function.

Soil application of pH-modified slurry is an agricultural practice that affects AMF by altering soil conditions in a way that can either support or hinder their growth (Rouphael et al. 2015). The key finding from the results obtained is that both inorganic (TSP) and organic (raw and treated slurry) fertilization negatively impacted the AMF symbiosis with maize. Although the soil pH remained within the narrow range of pH 5.0–6.0, which may have limited root mycorrhizal colonization, recent research by Tao & Liu (2024) demonstrated that AMF can withstand more extreme soil pH levels, ranging from pH 4.5 to 9.1. This suggests that other factors, such as nutrient availability, may have played a more significant role in the observed decline in AMF colonization (Nie et al. 2024). Most likely, the negative effect on AMF was due to the P supply associated with the fertilization treatments. High soluble P concentrations in the soil is known to suppress AMF activity, as it reduces the plant's reliance on mycorrhizal symbiosis for nutrient acquisition (Balota et al. 2016; Rouphael et al. 2015). This reduced reliance can lead to a reduction in mycorrhizal root colonization, as observed in this work.

Practical implications

Surface application of animal slurry has been practiced as an alternative to basal fertilization with mineral fertilizers for arable crops like maize (Ferreira et al. 2022). The high nutrient demand at early growth stages requires readily available P, which untreated slurry often fails to provide (Regueiro et al. 2020). Slurry acidification has been included in "the Best Available Techniques (BAT) reference document for intensive rearing of poultry and pigs" (European Commission, 2017). In our study, we found that agro-industrial by-products (Spent.A) can be used as alternative slurry additives to perform acidification and increase the P bioavailability of the material. Farmers may also benefit from a reduction in acidification costs. Unlike mineral acids, where the cost of slurry acidification can reach 2.4 euro L^{-1} in the case of H_2SO_4 (assuming a dose of 6 L $tonne^{-1}$ slurry and cost of 0.4 euro L^{-1}), waste acids can be applied with little to no cost (Chrysanthopoulos et al. 2024).

However, the increased P water-solubility in acidified slurry may also elevate the risk of P losses through leaching if not properly managed (Fangueiro et al. 2014), emphasizing the need for strategic application of bio-based fertilizers to improve P use efficiency and minimize environmental risks (Sica et al. 2025). This risk is particularly relevant in coarse-textured soils with high P saturation, while it is considered negligible in soils with medium to fine texture and a degree of P saturation below 25% (De Bolle et al. 2013). Avoiding application during periods of low crop uptake, such as late autumn or winter, is essential. In fine-textured soils (e.g., sandy loam), shallow injection may further reduce P leaching risk by disrupting macropore continuity (Glæsner et al. 2011).

In most open environments, the use of untreated slurry can be a source of zoonotic pathogens, especially if used for top dressing fertilization of ready-to-eat (RTE) crops. Alkalinization of slurry to pH of 9.5 has been shown to effectively sanitize (< 1000 CFU *E.coli* g^{-1} slurry) both cattle and pig slurry (Chrysanthopoulos et al. 2024; Rodrigues et al. 2021). In this study, the P fertilizer value of alkalinized slurry was not significantly higher compared to raw slurry. Nevertheless, such treatment may offer additional advantages for farmers seeking to sanitize their slurry by altering its pH, enhancing its safety for use in agricultural practices.

Conclusions

With the aim of understanding the P dynamics in soil treated with pH-modified slurry, a rhizobox study was conducted. It can be concluded that all treatments involved in the (bio)-acidification of slurry increased P solubility in the materials and in the soil, however, this was not always reflected in better P uptake by plants, due to negative effects on maize growth after surface application of the slurry. Root traits monitored during the study further support these results, suggesting potential ammonium toxicity near the maize rhizosphere. On the other hand, we also attempted to increase slurry pH to 9.5. While slurry alkalinization with KOH did not enhance P availability compared to raw slurry, it also did not negatively affect maize growth or soil enzymatic activity. Replacement of chemical additives by agro-industrial by-products (i.e. Spent.A) to perform slurry acidification was not beneficial in terms of enhancing total P uptake and maize growth. Nevertheless, to validate this observation, further studies are required with longer experimental duration, namely with field trials.

While the rhizobox experiment provided valuable insights into early-stage P availability and root development, it's small size and short duration limit the ability to fully assess long-term agronomic outcomes. To determine the broader applicability of these findings, longer-term experiments, including field trials, are needed to evaluate how pH modification of slurry influences maize growth, yield, and P uptake under real cropping conditions.

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Author contribution **Stamatis Chrysanthopoulos**: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing—original draft, Writing – review & editing. **Pietro Sica**: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing—original draft, Writing – review & editing. **Eusun Han**: Data curation, Methodology, Writing—review & editing. **Marie Louise Bornø**: Data curation, Formal analysis, Methodology, Writing—review & editing. **Amandine Germon**: Methodology, Writing—review & editing. **Luisa Brito**: Conceptualization, Formal analysis, Investigation, Methodology, Resources, Supervision, Validation, Writing—review & editing. **João Coutinho**: Conceptualization, Formal analysis, Methodology, Supervision, Writing—original draft, Writing—review & editing. **Dorette Müller-Stöver**: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing—review & editing. **David Fangueiro**: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing—review & editing.

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Data availability The datasets generated during and/or analyzed during the current study are available in the Mendeley data repository, https://data.mendeley.com/datasets/y77m2ynss6/1.

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

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