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Phosphorus fertilization of grasses with dairy processing waste materials and contributions of soil microorganisms towards phosphorus uptake

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ABSTRACT

Dairy processing waste (DPW) is one of the largest agro-industrial residues globally and has the potential of reducing the dependency on mineral phosphorus (P) fertilizers in grasslands. However, the agronomical value and the effects of DPW and the secondary materials produced from it on grass yields and soil microorganisms remains largely unexplored. In a 112-day greenhouse experiment, we evaluated the biomass production and P uptake of two grass species, grown on a loamy topsoil, with contrasting growth strategies (*Lolium perenne* L. fast-grower vs *Dactylis glomerata* L. slow-grower) and the effects on soil microorganisms under the fertilization of raw DPW sludge, and its secondary materials (ash and hydrochar). We compared the fertilized pots with a positive control with triple-superphosphate (TSP) and a negative control without P application. Our results showed that Olsen P and plant P concentrations were highest in the treatment with TSP after one month of application for both grass species. However, biomass production, P uptake and phosphorus use efficiency (PUE) were the greatest for sludge at day 112 for both species (56.39 ± 3.34 g pot⁻¹, $0.20 \text{ g} \pm 0.02$ P and 56.18 ± 12.44 %, $n = 8$). Ash and hydrochar resulted in lower biomass production than TSP and sludge and their PUE was low (< 20 %). Fungi and arbuscular mycorrhiza fungi PLFA markers were among the most important variables in explaining P uptake. The results of our study suggest that application of DPW sludge and soil's fungal biomass can reduce the dependency on mineral P fertilizers in grassland.

1. Introduction

Phosphorus (P) mineral fertilizers are among the most used fertilizers in agriculture. Yet, P reserves are not evenly distributed across the globe and the production costs of P fertilizers are expected to increase (Penuelas et al., 2023). As a result of this, P, in some areas of the world such as the European Union, has been listed as a critical raw material (Bertrand et al., 2016). Following up on this decision it has been proposed that dependency on mineral P sources in agriculture can be reduced through the recycling of P from organic residues and the transition towards a more circular economy (Chojnacka et al., 2020).

Waste from the dairy industry (Dairy Processing Waste, DPW) has the potential to reduce the dependency from mineral P fertilizers. The dairy sector is among the largest agro-industries globally, generating

approximately 2.5 million tons of waste yearly in the European Union alone (Hu et al., 2021). Waste from DPW is rich in P, with concentrations that can exceed 5 % of dry weight (Ashekuzzaman et al., 2019). DPW is a promising raw material for the production of secondary materials with a higher concentration of P such as precipitated P salts (e.g. struvite), thermal oxidation materials (e.g. ash) and/or pyrolysis and gasification materials (e.g. char) (Delgado Sancho et al., 2019).

The effects of DPW and its secondary materials on crop yields and P uptake remain poorly understood. For instance, the mineral fertilizer equivalent value of raw DPW sludge has been suggested to range between -80 % to $+110$ % depending on the plant species, sludge's pre-treatment, soil P status and fertilization rate (Ashekuzzaman et al., 2021; Shi et al., 2022). For DPW secondary materials such as biochar, studies showed varied results on crop yields, ranging from positive

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yields (Arutselvy et al., 2021), to low effects (Shi et al., 2022) or even decreasing plant available P (Khomenko et al., 2023a). The lower biomass production of recycled P fertilizers compared to mineral ones can be associated with a more complex chemical composition. While P in mineral fertilizers is mostly found in water-soluble calcium phosphates, such as monocalcium phosphate ($\text{Ca}(\text{H}_2\text{PO}_4)_2$), the forms of P are more diverse in recycled fertilizers, and include poorly bio-available forms such as hydroxyapatite or iron and aluminium phosphates (Kratz et al., 2019; Velasco-Sánchez et al., 2024).

Soil microorganisms could potentially enhance the fertilizer value of DPW through a range of mechanisms, that contribute to increase the availability of poorly soluble forms of P to plants (Richardson and Simpson, 2011; Velasco-Sánchez et al., 2024). Mechanisms to solubilize poorly available P are diverse and include the production of enzymes, chelates, organic acids and symbiotic associations with plants (Richardson and Simpson, 2011). In grasslands, soil microorganisms play an important role in P nutrition (Mander et al., 2012) and their role is likely to become more important in scenarios of reduced mineral P fertilization (Simpson et al., 2014). However, the effects of DPW or its secondary materials on the composition and activities of the soil microbial community remain largely unknown, and DPW might contain different pollutants that could impact negatively soil microorganisms (López-Mosquera et al., 2000). Some of the few articles that focused directly on the effects of DPW sludge on microbial communities have shown both increases and decreases in enzymatic activities and microbial community functioning (Frac et al., 2012; Gryta et al., 2014; Oszust et al., 2015). Most of these studies were however short-term studies or were restricted to a few geographical areas. It is crucial to understand the impacts of the application of DPW on soil microorganisms to study their potential in enhancing DPW's fertilizer value.

In this experiment, we therefore aimed to study the fertilizer value of DPW and two of its secondary materials, ash and hydrochar, and their impacts on soil microorganisms. In particular, our research objectives were: (i) to study the effects of DPW and its secondary materials on the biomass production and P uptake of two commonly cultivated grass species (*Lolium perenne* L., english-ryegrass and *Dactylis glomerata* L., cock's-foot), and (ii) to evaluate the impacts of DPW materials on soil microbial communities and their contribution towards P uptake. Our hypotheses were that (i) DPW or its secondary materials present high contents of available P for grass growth, that (ii) the importance of soil microorganisms towards P nutrition is more important for DPW materials than for mineral fertilizers because of their lower P availability for plants and that (iii) different grass species result in different P use efficiencies and different associations with soil microorganisms.

2. Materials and methods

2.1. Soil sampling and preparation

Soil was collected from a Cambisol (WRB, World Reference Base) from the top 0.2 m at Haudricourt, Normandy, France (49°42'16.6" N, 1°41'10.9" E) in the summer of 2021. This soil is classified as loamy, it has a neutral pH-H₂O of 7.1, low available P (Olsen P) of 12 mg P kg⁻¹, cation exchange capacity (CEC) 9.15 cmol kg⁻¹ and a soil organic matter content of 3 % (weight-based).

After soil collection, soil was passed through a 5 mm sieve and homogenised. Then soil was subdivided and placed in 80 cylindrical pots of 0.2 m diameter by 0.26 m height (Soparco, Sablons-sur-Huisne, France). The pots were filled up to 30 mm below their top opening. Soil moisture was adjusted to 70 % water holding capacity (WHC). The final soil weight of each pot, when adjusted to the target WHC, was 4.3 kg pot⁻¹. Water holding capacity was estimated by placing dry soil in a steel cylinder and then rewetting it by capillarity and immersion. Soil was then drained by placing the cylinder over a dry sand bed for 2 h. The gravimetric water content at this point represents the soils 100 % water holding capacity. Plants were grown in a glasshouse in the facilities of

Institute Polytechnique Unilasalle (Mont-Saint-Aignan, France), with an automatic drip irrigation system to maintain soil water content close to 70 % WHC by regular water addition, light cycle of 15 h, temperature of 21 °C and air humidity of 60 %. Pots were monitored on a weekly basis to correct their WHC, adding additional water when necessary. Soil was amended with nitrogen (N) and potassium (K) fertilizers at a rate of 0.31 g N pot⁻¹ and 0.37 g K pot⁻¹ (100 kg N ha⁻¹ and 120 kg K ha⁻¹, respectively) to ensure no N and K limitation during the experiment and promote seed germination. These fertilizers were added after solubilizing N fertilizer (27 % N concentration) (YaraBela AXAN) and K fertilizer (potassium chloride, KCl) in water.

2.2. Experimental design

The experiment was divided into two phases: an initial germination phase of 28 days during which the plants were allowed to establish; and a second phase of 112 days during which the pots were fertilized with the different P fertilizers. The experiment consisted of 4 replicates for each grass species (*Lolium perenne* L. (Lp)), a fast-grower grass species and *Dactylis glomerata* L. (Dg) a slow-grower grass species), as well as 3 different P fertilizer treatments (DPW's raw sludge; its secondary materials ash and hydrochar. Pots fertilized with mineral P fertilizer (TSP) and with no P application (control) were included in the study. Pots were destructively sampled twice during the experiment. In total the experiment resulted in 80 pots (5 treatments × 2 grass species × 2 destructive samplings × 4 replicates = 80) in a completely randomized design. Detailed information about the experimental design can be seen in Fig. S1.

At the beginning of the germination phase, we applied 2 g of seeds (Semences de France, La Chapelle-d'Armentières, France) to each pot ensuring proper colonization of the soil surface (Ros et al., 2018). The seeds were buried at 10–20 mm depth and the pots were covered with a plastic film to stimulate germination. The plastic film was removed once the grass seeds sprouted.

At the end of the germination phase, grass was well established and was cut 40 mm above the soil surface. Additional N fertilizer was applied (0.09 g N pot⁻¹ or 30 kg N ha⁻¹). DPW materials and TSP were then spread over the soil surface at a P fertilizers rate of 0.14 g P pot⁻¹ (45 kg P ha⁻¹), as recommended by the seed commercial provided (Semences de France), based on the total P content of the DPW materials.

After the first harvest of leaf biomass and the application of the P fertilisers, we performed 4 harvests, each after a 28-day growth period. Two of these harvests were destructive to collect root biomass and soils for biological and chemical analyses (days 28 and 112, n = 40) and the other 2 were non-destructive and consisted only of harvesting leaf biomass (days 56 and 84) (Fig. S1). After each harvest, a maintenance dose of N fertilizer was added to prevent N limitation (0.09 g N pot⁻¹).

2.3. Dairy processing materials

The DPW materials used in this study were raw DPW sludge treated with iron (Fe) salts, and its secondary materials ash and hydrochar. Characterization of the DPW materials can be found in Table 1.

Hydrochar and ash were produced from the same raw DPW sludge. Hydrochar was produced by adjusting the dry matter of the raw DPW sludge to 15 %, a pH of 5 and heating to 225 °C for 1 h. Ash was produced by burning the raw DPW at 250 °C for 2 h then 550 °C for 2–3 h. Elemental concentrations (Al, Ca, Fe, K, Mg, Na, Cd, Co, Cr, Mn, Ni, Pb and Zn) in DPW fertilizers were determined after calcination of 0.2 g of material at 450 °C for 3 h, followed by an extraction with 3.5 M HCl for 16 h. Elements concentration was measured in ICP-MS. Determination of pH and EC was done at a ratio of 1:5 v:v after shaking for 1 h in demineralized water (ISO 10390:2005). EC and pH were measured in the same extract with a Mettler Toledo instrument. The concentration of C, N and H in the samples was examined using an elemental analyzer ThermoScientific Flashsmart. Total P was determined after a microwave

Table 1

Dairy processing waste (DPW) materials characterization. Values indicate means $\pm$ standard deviation, $n = 3$. Units are expressed in relation to dry weight. DM = Dry weight.

Element	Hydrochar	Ash	Sludge	Unit
Al	3.7 $\pm$ 0.3	7.6 $\pm$ 0.8	1.2 $\pm$ 0.2	g kg ⁻¹
Ca	68.1 $\pm$ 9.7	137.2 $\pm$ 14.7	46.8 $\pm$ 6.9	g kg ⁻¹
Fe	133.4 $\pm$ 10.7	262.6 $\pm$ 30.8	97.4 $\pm$ 14.7	g kg ⁻¹
K	05.1 $\pm$ 0.5	31.9 $\pm$ 3.6	10.7 $\pm$ 0.8	g kg ⁻¹
Mg	8.3 $\pm$ 0.6	18.6 $\pm$ 1.9	5.9 $\pm$ 0.9	g kg ⁻¹
Na	15.1 $\pm$ 8.0	20.0 $\pm$ 3.6	17.2 $\pm$ 4.0	g kg ⁻¹
Cd	9.7 $\pm$ 0.1	9.4 $\pm$ 0.4	—	mg kg ⁻¹
Co	—	—	—	mg kg ⁻¹
Cr	35.6 $\pm$ 6.0	40.6 $\pm$ 3.6	15.7 $\pm$ 5.3	mg kg ⁻¹
Mn	362.0 $\pm$ 28.3	807.0 $\pm$ 115.9	204.5 $\pm$ 36.0	mg kg ⁻¹
Ni	—	—	—	mg kg ⁻¹
Pb	895.4 $\pm$ 73.6	1748.7 $\pm$ 142.7	645.7 $\pm$ 135.5	mg kg ⁻¹
Zn	214.0 $\pm$ 86.7	495.6 $\pm$ 104.7	169.2 $\pm$ 114.3	mg kg ⁻¹
pH	5.1 $\pm$ 0.1	5.9 $\pm$ 0.1	8.6 $\pm$ 0.1	—
EC	5.5 $\pm$ 0.2	4.3 $\pm$ 0.1	4.6 $\pm$ 0.2	mS/cm
C	384.4 $\pm$ 25.6	6.7 $\pm$ 1.7	325.3 $\pm$ 14.6	g kg ⁻¹
N	62.6 $\pm$ 9.4	1.6 $\pm$ 0.4	58.1 $\pm$ 1.2	g kg ⁻¹
H	50.3 $\pm$ 1.5	0.7 $\pm$ 0.3	52.7 $\pm$ 3.6	g kg ⁻¹
P-total	48.8 $\pm$ 3.3	56.9 $\pm$ 0.6	30.5 $\pm$ 0.2	g kg ⁻¹
P-H ₂ O	1.4 $\pm$ 0.5	10.9 $\pm$ 7.0	2.4 $\pm$ 0.8	g kg ⁻¹

assisted digestion with concentrated HNO₃. P was then measured colorimetrically following the Murphy and Riley blue coloration method (Murphy and Riley, 1962). Water soluble P was determined after an extraction with de-ionized water (H₂O) during 16 h in an orbital shaker. P in H₂O was measured using an ICP-MS. All DPW materials were characterized using triplicates.

2.4. Plant analyses

2.4.1. Biomass sampling

Aboveground biomass (leaves) was collected 4 times over the course of the experiment (days 28, 56, 84 and 112) by clipping the leaves 40 mm above the soil surface. Leaves were dried at 40 °C for one week and weighed on a precision scale (Mettler Toledo, Greifensee, Switzerland) to calculate leaf biomass production. At the destructive harvests (days 28 and 112) roots and basal stem (aboveground biomass between 0 to 40 mm) were also collected. This was done by clipping the basal stems and by washing the soil out of the pot over a 50 μ m mesh size.

A number of plant biomass variables were calculated with leaf biomass. Cumulative leaf biomass was calculated as the sum of the leaf biomass production of days 28, 56, 84 and 112. Cumulative total biomass was calculated by adding cumulative leaf biomass and basal stem and root biomass at day 112.

2.4.2. Biomass elemental composition

Carbon (C) and nitrogen (N) content in plant material were measured in an elemental CHNS analyser (Shimadzu, Kyoto, Japan). P content in 0.2 g of plant biomass was determined after microwave assisted digestion with concentrated nitric acid, HNO₃ (16.24 M), and measured colorimetrically using the molybdate blue method (Murphy and Riley, 1962). The digestion was performed in a MARS-6 instrument (CEM, Matthews, USA) using a program with a ramp and hold time of 15 min, a target temperature of 200 °C, pressure of 800 psi and 900 – 1050 W of power. The complete digestion, including cooling lasted approximately 1 h. Phosphorus use efficiency (PUE) was calculated as the difference in P uptake between fertilized and unfertilized control (Equation 1) (Syers et al., 2008):

$$PUE = \frac{P_{uptake_{fertilized}} - P_{uptake_{control}}}{P_{added}} \times 100 \quad (1)$$

PUE was evaluated on leaf biomass, cumulatively on leaves (cumulative leaf PUE) by considering the cumulative uptake of P by leaves in

the 4 different harvests. Similarly, cumulative total PUE was calculated considering as well root and basal stem P uptake at day 112.

2.5. Soil analyses

2.5.1. Soil sampling

Soil samples were collected on the destructive harvests (days 28 and 112). This was done by taking 3 soil cores through the whole profile of the pots with a 20 mm diameter auger. The 3 subsamples were then pooled and sieved at 4 mm. The cores were taken following a line, crossing the centre of the pot and sampling the whole depth of the pot. Afterwards, soil was either dried at 40 °C for soil chemical analyses or frozen at – 80 °C for soil biological analyses.

2.5.2. Soil chemical analyses

Content of total C and N in the soil was measured with a CHNS elemental analyser (Shimadzu, Kyoto, Japan). Moreover, N-NO₃ and N-NH₄ contents in the soil were measured colorimetrically using a Gallery instrument (Thermo Fisher Scientific, Waltham, USA), after 1 h extraction with 1 M KCl. Plant available P was estimated using the Olsen P method, where P was extracted from 5 g of soil with 100 mL of a 0.5 M solution of sodium bicarbonate (NaHCO₃) at pH of 8.5 for 30 min. P was then measured colorimetrically following the molybdate blue method in a spectrophotometer at 882 nm wavelength (Varian Cary 50 Scan UV-Visible Spectrophotometer) (Agilent Technologies, Santa Clara, USA) (Murphy and Riley, 1962). Total P in soil was determined after microwave assisted digestion with concentrated HNO₃ and measured colorimetrically with the molybdate blue method (Murphy and Riley, 1962). Soil pH was determined in a 1:5 v:v water extract using a glass electrode (Mettler Toledo, Greifensee, Switzerland) after being shaken for 1 h.

2.5.3. Soil biological variables

2.5.3.1. Phospholipid fatty acid characterization. Microbial biomass and composition were assessed by measuring and characterizing phospholipid fatty acids (PLFA) and neutral fatty acids (NLFA) (Bligh and Dyer, 1959; Frostegård et al., 1991). PLFAs were divided into Actinobacteria, Gram +, Gram –, Anaerobe, Eukaryote and saprophytic Fungi microbial groups and AMF from NLFAs were also quantified using the MIDI Sherlock Software v.6.3B with PLFA package v.2.00 (Shimadzu, Kyoto, Japan) (Buyer & Sasser, 2012). The different microbial groups were evaluated in absolute and in relative abundances. We also calculated the total amount of bacteria and fungi of the PLFA profile (Saprophytic fungi, Actinobacteria, Gram +, Gram –, Anaerobe), the ratio Fungi Bacteria⁻¹ in the PLFA (Saprophytic fungi / (Actinobacteria, Gram +, Gram –, Anaerobe)), the total fungi peaks (NLFA-AMF, Saprophytic fungi) and the total bacteria peaks (Actinobacteria, Gram +, Gram –, Anaerobe). Specific fatty acids assigned to the different microbial groups can be found in Table S1.

2.5.3.2. Enzymatic activities. Enzymatic activities (β -Glucosidase (E.C. 3.2.1.21) (Bglu), arylamidase (E.C. 3.4.11.2) (ARYLN) and acid and alkaline phosphatase (E.C. 3.1.4.1) (PAC) and (PAK), respectively, were measured colorimetrically using a microplate technique (Cheviron et al., 2021). In short, we used 25 mL of ultrapure water for Bglu, ARYLN and of Trizma buffer (50 mM) adjusted to pH 5.5 for PAC and 11 for PAK to create a soil suspension with 4 g of soil. After centrifugation for 10 min at 250 rpm, 125 μ L of soil suspension were placed in 96-well microplates and mixed with their respective substrate solutions: 4-nitrophenyl β -D-glucopyranoside (CAS N°: 2492-87-7) for Bglu, l-leucine β -naphthylamide hydrochloride (CAS N°: 893-36-7) for ARYLN and 4-nitrophenylphosphate disodium salt hexahydrate (CAS N°: 333338-18-4) for PAC and PAK, respectively. The concentrations of the substrate solutions were 0.05, 0.008 and 0.05 mol L⁻¹, respectively. After mixing,

microplates were incubated at 37 °C for 1 h for Bglu, 2 h for ARYLN and 30 min for PAC and PAK. The reaction was stopped with 25 μ L 0.5 M of calcium chloride (CaCl_2) and 100 mM 100 μ L Trizma at pH 12 for Bglu, PAC and PAK and microplates were centrifuged for 5 min at 1500 g. In the case of ARYLN, the reaction was stopped with ethanol 96 % and coloration was developed with 100 μ L of acidified ethanol and 100 μ L of DMCA. ARYLN's plates were incubated in the dark for 20 min at room temperature before measurement. The measurements for all enzyme activities were performed in a Varioskan Flash-Thermo microplate reader (Thermo Fisher Scientific, Waltham, USA). Absorbance was measured at 405 nm for Bglu, PAC and PAK and 540 nm for ARYLN. Results of enzymatic activities were expressed in nmol PNP (para-nitrophenol) $\text{min}^{-1} \text{g}^{-1}$ of dry soil for Bglu, PAC and PAK activities and in β -naphthylamine $\text{min}^{-1} \text{g}^{-1}$ of dry soil for ARYLN activities.

2.6. Statistical analyses

All statistical analyses were performed using RStudio version 1.4.1717 (RStudio Team, 2021). Figures were made using the package “ggplot2” in the RStudio environment (Wickham, 2016) and further edited in PowerPoint (Microsoft, Redmond, USA).

To test of the effect of P fertiliser application over time and for the two grass species on biomass production, nutrient uptake, chemical properties, soil microbial composition and biomass and enzymatic activities, we performed three-way analysis of variance (ANOVA) using the “aov” function. Each observation of chemical properties, soil microbial composition and biomass and enzymatic activities was treated as a sample and the interactive effects of fertiliser, harvest date and grass species included as factors was tested. In the case of leaf biomass or leaf nutrient uptake, samples were obtained at 4 different dates (at 28, 56, 84 and 112 days after fertiliser application), resulting in a violation of the assumption of independence and in an unbalanced design (80 observations at day 28 and 40 observations at days 56, 84 and 112). For this reason, we performed an ANOVA type III with fertilizers, grass species and harvest time as fixed factors and the pot's ID as a random factor to account for the lack of independence associated with repeated observations performed on the same location (Shaw and Mitchell-Olds, 1993). The mixed effect ANOVA type III model was performed using the “lmer” and “anova” functions of the “lme4” package (Bates et al., 2015) and “lmerTest” package (Kuznetsova et al., 2017), respectively. When the effects of one of the three fixed factors or the interactions of the factors were found significant, we performed Tukey's post-hoc test using the “emmeans” package (Lenth, 2023). In case of significant interactions, Tukey's post hoc were conducted on single main effects after estimating marginal means using the R package “emmeans”. This allows to test the effect of a factor while accounting for the interaction effect. Moreover, we tested the effects of grass species and fertilizer on cumulative biomass production or cumulative nutrient uptake by performing a two-way ANOVA. For all models, the assumptions of homogeneity, normality and heteroscedasticity were assessed by analysing the residuals.

To identify the potential biological and chemical soil indicators that contribute most to P uptake by grass leaves, we conducted a multiple regression analysis following Moinet et al. (2016). The aim of this exercise was to determine the relative importance of chemical vs biological variables in explaining P uptake, as well as which biological variables, if any, would most strongly explain it. A set of candidate models with all possible combinations of chemical soil variables and root biomass that could explain P uptake (root biomass, pH, soil C, soil C:N and Olsen P) were first computed. To this set of models, different soil biological variables (PAC, PAK, Bglu, ARYLN, Gram + biomass, Gram - biomass, saprophytic fungal biomass (PLFA peak), AMF biomass (NLFA peak), total PLFA biomass, Fungi Bacteria⁻¹ ratio or total fungal biomass) were added sequentially, one at a time to form all possible combinations of models with chemical variables. Then, the interaction of soil biological, root biomass and soil chemical variables with the fertilizer or with the grass species was added to give all possible combinations. We performed

the analysis separately for day 28 and 112 as we found strong differences in most variables between harvest dates. This approach led to a set of 6428 different candidate models for each date that included models with or without biological variables. The models were ranked using the AIC to determine the Kullback–Leibler (KL) best model which is based on bias-corrected, maximized log-likelihood (LogLik) of the fitted model with a penalty for the number of parameters used (Burnham and Anderson, 2004). The model with the lowest AIC (AICcmin) is the best explanatory model of P uptake. The ΔAICc value is calculated for each model as $\Delta\text{AICc} = \text{AICc} - \text{AICcmin}$. Differences of $\Delta\text{AICc} < 2$ indicate no significant improvement by the next ranked model, whereas $\Delta\text{AICc} > 2$ indicate a considerably better explanation of P uptake (Anderson, 2007). A measure of the strength of support is described by the model probability (Akaike weights, Awc), which estimates the probability that a given model is the KL best model, given the dataset and candidate set of models. The sum of Awc in a candidate set of models equates to 1. Models were inspected for collinearity by calculating their variance inflation factor (VIF). VIF values higher than 5 suggest high collinearity (Kim, 2019). Lastly, we also calculated the root mean square error (RMSE) for the best ranked models.

3. Results

3.1. Grass biomass production

The application of the different P fertilizers had significant effects ($p < 0.05$) on biomass production. Particularly, fertilizer had a significant effect on leaf and root biomass, cumulative leaf biomass and cumulative total biomass production (including leaf, basal stem and root biomass) (Table 2). The effect of the fertilizer on leaf biomass was influenced by the time of harvest as shown by the significant interaction term (Table 2), grass species had a significant effect on root biomass ($p < 0.001$) and this effect changed over time, as indicated by the significant interaction. This was also the case for leaf biomass (Table 2). Cumulative leaf biomass and cumulative total biomass production were not affected by grass species.

Sludge produced on average the highest leaf biomass (Fig. 1 A), and was significantly higher than the control at days 28 and 56 after application, while no significant differences were observed for days 84 and 112. Sludge resulted in the highest cumulative total biomass, significantly higher than the control and hydrochar treatments (Fig. 1 B). In addition, sludge also showed the highest cumulative leaf biomass. The mean $\pm$ standard error, se, ($n = 8$) cumulative leaf biomass production in the sludge treated pots, for both species, was $30.85 \pm 0.58 \text{ g pot}^{-1}$ and cumulative total biomass production was $56.39 \pm 3.34 \text{ g pot}^{-1}$, both values being significantly higher than the control of both species. Root biomass was affected by the fertiliser treatments ($p = 0.013$), but no significant differences were found between groups after performing the post-hoc test. Mean $\pm$ se values for leaf, root and basal stem biomass,

Table 2

P-values of main factors and interactions from three-way anovas on leaf and root biomass production and two-way anovas for cumulative leaf and cumulative total biomass. values under 0.05 denote a significant effect and are highlighted in bold. not applicable = n.a.

Factor	Leaf biomass	Root biomass	Cumulative leaf biomass	Cumulative total biomass
Fertilizer	<0.001	0.013	<0.001	0.008
Grass species	0.897	<0.001	0.934	0.148
Time	<0.001	0.018	n.a.	n.a.
Fertilizer x Grass species	0.642	0.256	0.734	0.313
Fertilizer x Time	<0.001	0.848	n.a.	n.a.
Grass species x Time	<0.001	<0.001	n.a.	n.a.
Fertilizer x Grass species x Time	0.073	0.778	n.a.	n.a.

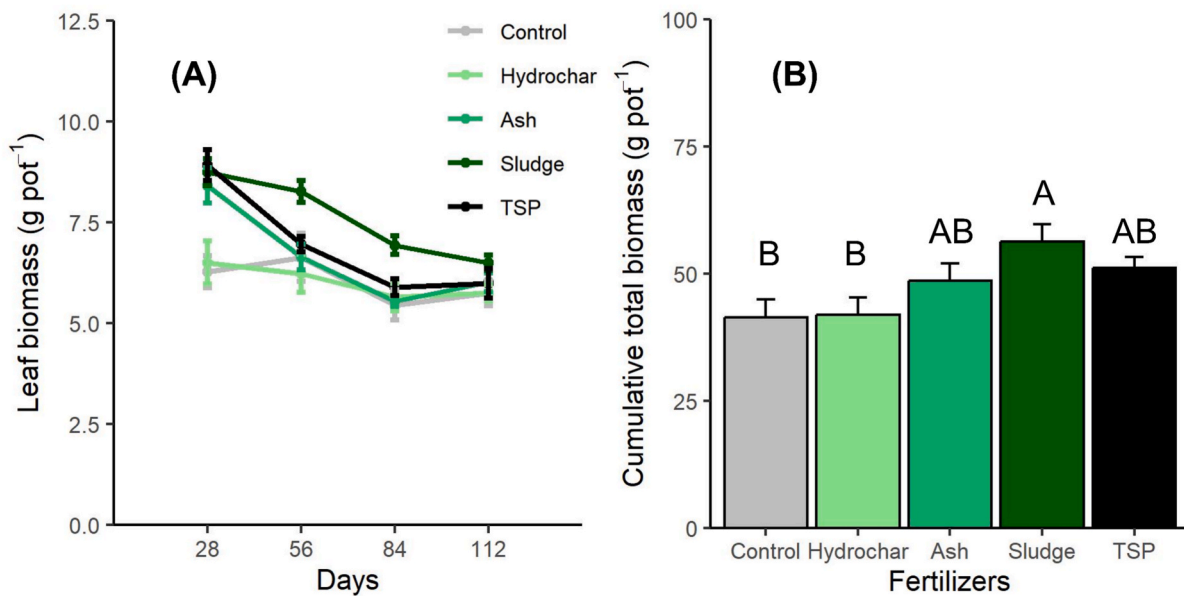


Fig. 1. Mean values of both grass species for (A) leaf biomass as affected by the fertilizers and time, and (B) cumulative total biomass (cumulative sum of root, basal stem and leaf biomass) for each fertilizer. Whiskers indicate standard errors, $n = 16$ at day 28 and $n = 8$ for rest of days and panel B. Letters denote statistical differences after performing Tukey's post hoc test.

cumulative leaf biomass and cumulative total biomass for the different P treatments, grass species and harvest dates, as well as summary output of ANOVA models can be found in Table S2.

Regarding the species effect, the biomass production dynamics of both Lp and Dg were affected by the time of harvest. Lp produced significantly more biomass than Dg at day 28, yet this was reverted by day 56, and at day 112 Dg produced significantly more leaf biomass than Lp (Fig. 2 A). At days day 56 and at day 84, leaf biomass was similar for both species. Root biomass increased over time for Dg plants and remained constant for Lp (Table 2). Root biomass was significantly higher in Lp pots than Dg at both dates, 22.29 ± 1.99 vs 5.81 ± 0.62 and 20.73 ± 1.79 vs 14.46 ± 1.26 g pot⁻¹ respectively. At the end of the experiment, no statistical differences were found between grass species

on cumulative leaf biomass or cumulative total biomass (Fig. 2 B). The average cumulative total biomass production was 49.99 ± 2.57 and 45.86 ± 1.97 g pot⁻¹ (mean $\pm$ se, $n = 20$) for Lp and Dg, respectively.

3.2. Nutrient uptake

P concentration, P uptake and PUE were affected significantly by the different fertilizers (Table 3). However, the effect of the fertilizer was dependent on harvest time for P uptake and PUE as showed by the significant interactions. Grass species only significantly affected leaf P concentration ($p = 0.013$), showing a higher P concentration in Lp plants compared to that in Dg, with 3.93 ± 0.13 g kg⁻¹ and 3.55 ± 0.13 g kg⁻¹ (mean $\pm$ se, $n = 20$) for Lp and Dg, respectively. Mean values and

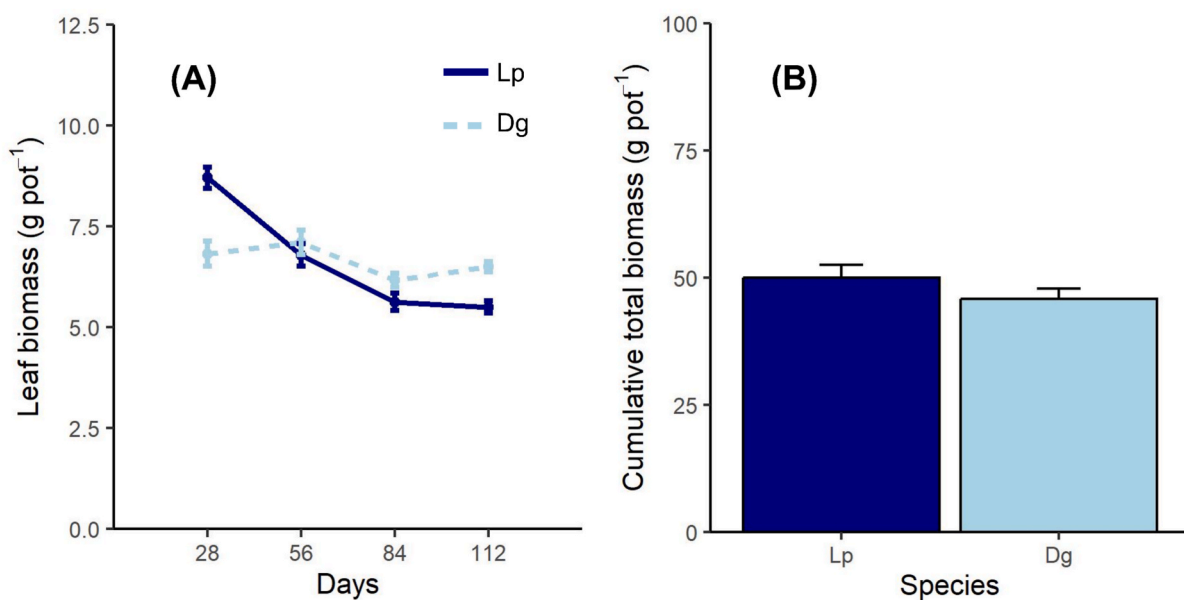


Fig. 2. Biomass production of grass species. (A) displays the differences in leaf biomass production between *L. perenne* (Lp, solid) and *D. glomerata* (Dg, dotted) and (B) shows cumulative total biomass production (root, basal stem and cumulative leaf biomass) for grass species. Whiskers indicate standard errors, $n = 40$ at day 28 and $n = 20$ for rest of days and panel B.

Table 3

Results of three-way ANOVA tests on leaf P concentration, uptake and PUE and two-way ANOVA for cumulative total P uptake and PUE (p-values). Values under 0.05 denote a significant effect and are highlighted in bold. Cumulative total P uptake and PUE include leaf, basal stem and root biomass. Not applicable = n.a.

Factor	Leaf P concentration	Leaf P uptake	Cumulative total P uptake	Leaf PUE	Cumulative total PUE
Fertilizer	<0.001	<0.001	<0.001	<0.001	<0.001
Grass species	0.013	0.134	0.088	0.098	0.077
Time	<0.001	0.001	n.a.	<0.001	n.a.
Fertilizer x Grass species	0.973	0.922	0.855	0.954	0.914
Fertilizer x Time	0.131	<0.001	n.a.	<0.001	n.a.
Grass species x Time	0.121	0.136	n.a.	0.562	n.a.
Fertilizer x Grass species x Time	0.462	0.452	n.a.	0.856	n.a.

standard errors for each treatment, plant species and harvest time as well as ANOVA summaries can be found in [Tables S3, S4 and S5](#).

Triple superphosphate application increased significantly P concentration in leaves ([Fig. 3 A](#)). The average P concentration in leaves ranged from 4.87 ± 0.22 for TSP to 3.26 ± 0.16 g kg⁻¹ (mean $\pm$ se, n = 8) for the control. None of the DPW fertilizers resulted in a significant increase in mean leaf P concentration compared to the control. Leaf P concentration increased over time until day 112 when P concentration started to decrease ([Fig. 3 A](#)). The maximum P concentration was reached on day 84 for the control, hydrochar, sludge and TSP treatments, reaching peak concentrations of 3.99 ± 0.33 , 4.52 ± 0.30 , 4.68 ± 0.23 and 5.24 ± 0.39 g kg⁻¹ (n = 8) respectively. The concentration of P was highest in the ash treatment on day 56 with an average P concentration of 4.53 ± 0.44 g kg⁻¹, n = 8. Leaf P concentration was higher in Lp than in Dg plants, 3.93 ± 0.29 vs 3.55 ± 0.29 g P kg⁻¹, n = 20. Leaf P uptake was affected by harvesting time and the different fertilizer treatments as shown by their significant interaction ([Table 3](#)). Plants fertilized with

TSP showed a higher P uptake compared to the other treatments only at days 28 and 56 ([Fig. 3 B](#)). The leaf uptake of P was constant for all the fertilizers with the exception of TSP, which decreased over time ([Fig. 3 B](#)). Leaf P uptake for TSP was 43.05 ± 3.45 and 24.60 ± 2.00 mg pot⁻¹, n = 8, for days 28 and 112 respectively. Information on root and basal stem P concentration and uptake can be found in [Table S5](#).

Cumulative total P uptake (including leaf, basal stem and root biomass) was the highest in the sludge and TSP treatments, hydrochar and ash treatments did not increase significantly cumulative P uptake in relation to the control ([Fig. 4 A](#)). The two grass species showed no significant differences in cumulative total P uptake ([Table 3](#)). Leaf PUE from the different fertilizer depended on the harvest time, as shown by the significant interaction ([Table 3](#)). Post hoc analyses on the interaction of harvest time and fertilizer treatment showed no differences at day 28, TSP produced the highest, on average, PUE in leaves at day 56, significantly higher than the control. On days 84 and 112, sludge also showed significantly higher leaf PUE than the control ([Table S3](#)). Detailed information on leaf PUE can be found in [Table S3](#). Cumulative total PUE (including leaf, basal stem and root biomass) was the highest in the pots (of both grass species) fertilized with sludge (56.18 ± 12.44 %, n = 8) and TSP (48.77 ± 4.66 %, n = 8), followed by ash (27.49 ± 7.26 %, n = 8) and hydrochar (5.99 ± 6.19 %, n = 8) ([Fig. 4 B](#)). Grass species had no significant effect on cumulative total PUE ([Table 3](#)).

The application of the different fertilizers had some minor impacts on N concentration, uptake and nutrient ratios ([Table S4](#)). For instance, only on day 28, N concentration in leaves was significantly highest in the hydrochar fertilized Dg pots and lowest in the TSP, sludge and ash fertilized ones. Cumulative total N uptake (including leaf, basal stem and root biomass) was the highest in the sludge fertilized pots, significantly higher than the control, TSP and hydrochar (0.71 ± 0.01 g N pot⁻¹, n = 8). C:N ratio in plants was significantly higher in Lp at day 28 (24.26 ± 0.85 for Lp vs 17.89 ± 0.81 for Dg, n = 20), and significantly higher in Dg at day 112 (26.29 ± 0.73 for Dg vs 23.45 ± 0.66 for Lp, n = 20). Detailed information and ANOVA summaries on N nutrition and nutrient ratios can be found in [Tables S4 and S5](#).

3.3. Soil chemical variables

Olsen P values were significantly affected by fertilizer addition, grass species and harvest time. However, the effect of the fertilizer depended

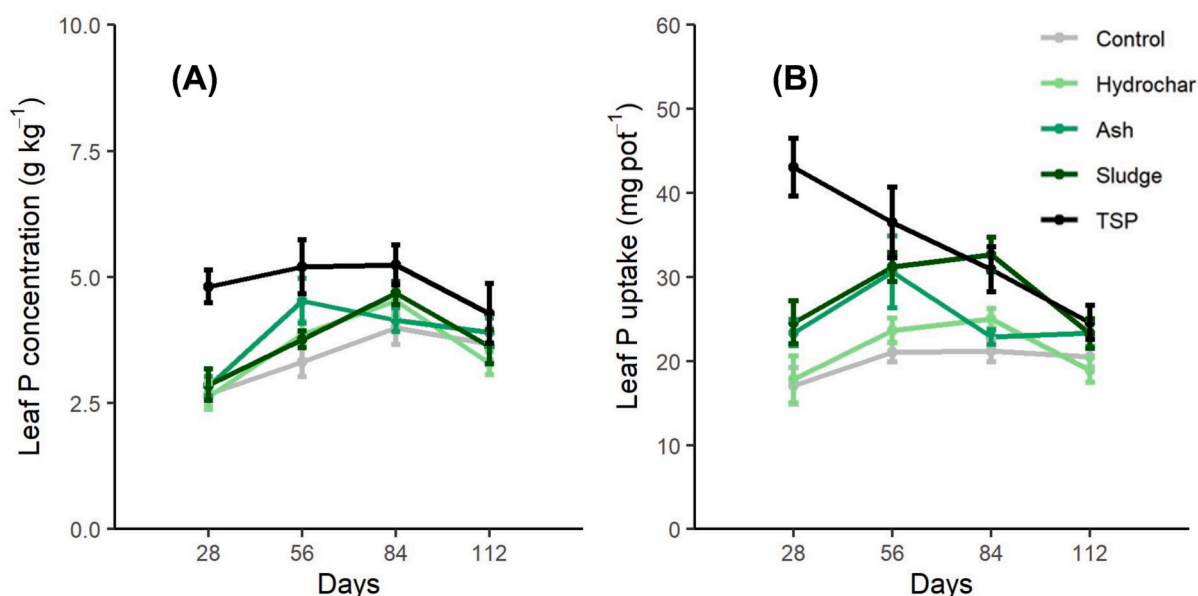


Fig. 3. Mean leaf phosphorus (P) concentration (A) and uptake (B) after fertilization for *Lolium perenne* and *Dactylis glomerata*. Whiskers indicate standard errors, n = 16 at day 28 and n = 8 for the rest of days for both A and B panels.

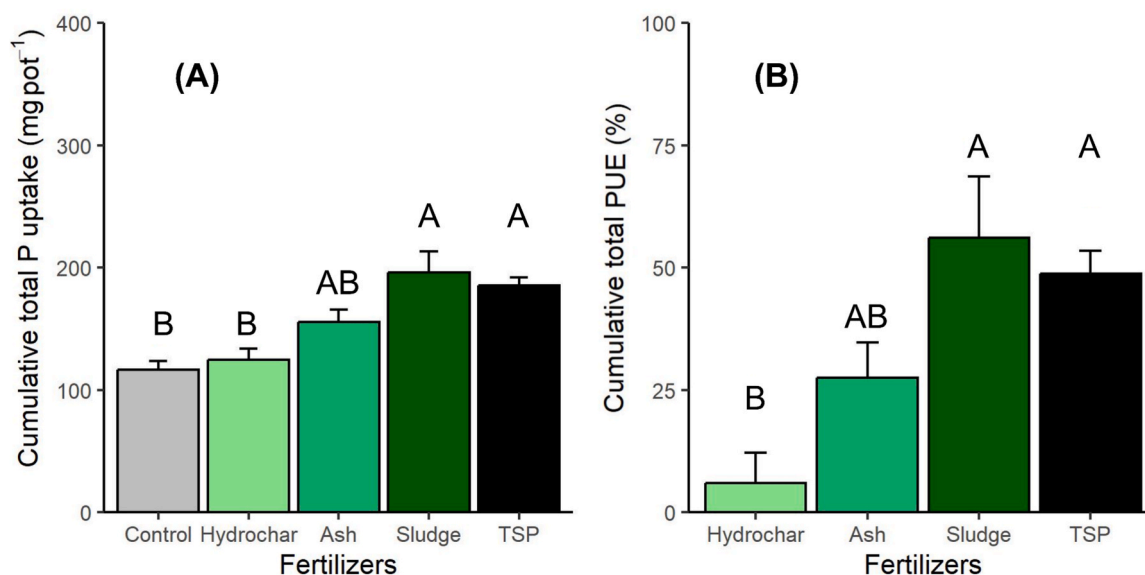


Fig. 4. Average cumulative total (including leaf, basal stem and root biomass) P concentration (A) and cumulative total phosphorus use efficiency (PUE) (including roots, basal stem and leaves) (B) after fertilization for the two studied grass species, *Lolium perenne* and *Dactylis glomerata*. Whiskers indicate standard errors, $n = 8$. Letters denote statistical differences after performing Tukey's post hoc test.

on the sampling time, as shown by the significant interaction between these two factors (Table 4). Olsen P values were significantly higher in the pots amended with TSP at day 28, yet no differences between fertilizers were found at the end of the experiment (Fig. 5). Moreover, Olsen P decreased significantly over time in the pots fertilized with hydrochar and particularly in the ones fertilized with TSP (Fig. 5) and was significantly higher in Dg pots compared to Lp, 15.60 ± 0.72 vs 13.32 ± 0.53 mg P kg⁻¹. Total P over the two grass species was significantly affected by fertilizer type, showing that hydrochar and ash were the only fertilizers that increased P content compared to the control, with values of 795.48 ± 37.44 , 1057.23 ± 47.37 , 1012.35 ± 88.93 , 867.98 ± 45.41 and 967.75 ± 43.09 mg P kg⁻¹ (mean $\pm$ se, $n = 8$), for the control, hydrochar, ash, sludge and TSP respectively.

N-NH₄ content of the soil was affected by the time of the harvest, fertilizer and grass species, as shown by the significant triple interaction. By exploring the triple interaction, we observed that sludge application, in average, increased N-NH₄ in the Dg pots at day 28 and in the Lp pots at day 112, yet only statistically different from the hydrochar fertilized pots (Table S6). Soil C was affected by harvest time, showing significantly higher values at day 28 than at 112, 2.71 ± 0.07 vs 2.37 ± 0.05 g kg⁻¹ respectively. All mean values $\pm$ se and ANOVA summaries for each plant species, plant and treatment can be found in Table S6.

3.4. Soil biological variables

The application of P fertilizers had no significant effects on soil microbial communities with the exception of NLFA-AMF and anaerobe biomass. The effect of fertilizer addition on NLFA-AMF biomass was dependent on grass species ($p = 0.022$). Tukey's post-hoc analyses

showed that NLFA-AMF biomass was significantly higher than the control in the Lp pots fertilized with sludge and ash. No differences were found in the Dg pots. Total NLFA peaks, PLFA-AMF and NLFA-AMF biomass significantly increased over time. Mean values $\pm$ se and ANOVA summaries of PLFA, NLFA microbial groups and ratios for plant species and fertilizer treatments can be found in Table S7 and Table S8.

The effects of the different P fertilizers on enzymatic activities were also limited. Only Bglu activities were affected by P fertilization, as shown by the significant interaction of fertilizer and grass species ($p = 0.034$). Bglu mean activities, in Lp plants, were on average lowest in soils fertilized with sludge (24.83 ± 1.11 PNP min⁻¹ g⁻¹, mean $\pm$ se, $n = 8$), yet only statistically different from the TSP (28.65 ± 0.65 PNP min⁻¹ g⁻¹), which was the treatment that had the highest Bglu mean activities. The actual means $\pm$ se of enzymatic activities and ANOVA summaries, for each fertilizer, grass species and sampling time can be found in Table S9.

3.5. Models of P uptake

P uptake by leaves at days 28 and 112 was modelled by conducting a multiple regression analysis on over 6428 different models (Table 5). In table 5, the first 8 models ranked based on their AICc at days 28 and 112 are displayed. The variables that related most to P uptake were root biomass, Olsen P, pH and Soil C or Soil C:N. Moreover, soil microbial variables contributed significantly to improve the models. At day 28, we observed that the microbial variables that contributed most were PLFA-Fungi biomass and total fungal biomass (PLFA-Fungi + NLFA-AMF) and at day 112 the microbial variables that contributed the most were NLFA-AMF and total fungal biomass.

Table 4

Results of three-way ANOVA tests on soil chemical properties (p-values). Values under 0.05 denote a significant effect and are highlighted in bold.

Factor	Olsen P	Total P	N-NO ₃	N-NH ₄	Total N	Total C	pH
Fertilizer	<0.001	0.003	0.264	0.005	0.091	0.200	0.634
Grass species	<0.001	0.536	0.157	0.193	0.485	0.248	0.095
Time	<0.001	0.134	0.080	0.868	0.038	<0.001	<0.001
Fertilizer x Grass species	0.355	0.975	0.636	0.186	0.280	0.339	0.049
Fertilizer x Time	0.004	0.205	0.284	0.645	0.211	0.901	0.964
Grass species x Time	0.132	0.131	0.146	0.058	0.984	0.583	0.118
Fertilizer x Grass species x Time	0.312	0.152	0.666	0.003	0.520	0.410	0.297

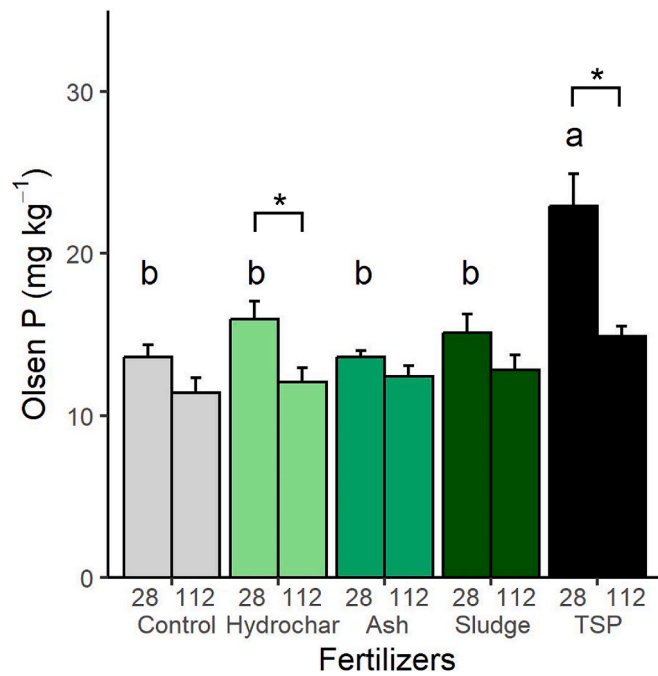


Fig. 5. Plant available soil P (Olsen P) 28 days after the application of fertilizers and at 112 days after the application of fertilizers. Whiskers indicate standard errors, $n = 8$. Letters denote statistical differences after performing Tukey's post hoc test at each harvest time. Asterisks and brackets indicate significant differences over time for a given treatment.

At day 28, models 1–4 explained equally good P uptake. Cumulatively, there is a 67 % chance that one of these models is the best in explaining P uptake. All these 4 models included root biomass, Olsen P and soil pH and different combinations of PLFA-Fungi (saprophytic fungi) and fungal biomass (PLFA-Fungi + NLFA-AMF) or soil C and soil C:N as explanatory factors. At day 112, models 1 – 7 were equally good in explaining P uptake. In this case, the cumulative likelihood of one of these models being the best at explaining P uptake is 74 %. Root biomass and Olsen P were included as explanatory variables in all 7 best ranked

models. Soil pH was an important explanatory variable in models 1 – 4. Combinations of either fungal biomass and NLFA-AMF or soil C and soil C:N were important explanatory variables for all the 7 first ranked models.

The best explanatory model at day 28 was “Root biomass + Olsen P + pH + Soil C:N + PLFA-Fungi” ($p < 0.001$; R^2 of 0.77; Table 5). The coefficients in the model were 0.67, 2.03, -3.57 , -0.12 and 0.01 for root biomass, Olsen P, pH, Soil C:N and PLFA-Fungi respectively. At day 112, the best model to predict P uptake is “Root biomass + Olsen P + pH + Soil C:N + Fungal biomass”, with a considerably lower R^2 than the best model at day 28 ($R^2 = 0.37$, $p = 0.026$). The estimates for this model were 0.34, 1.00, 2.04, -0.08 and -0.01 for root biomass, Olsen P, pH, Soil C:N and fungal biomass respectively. VIF values for all the best ranked models were < 1.5 , indicating no collinearity issues.

4. Discussion

4.1. Fertilizer value of dairy processing waste materials

Among all the materials studied, we observed that sludge was the material that, on average, increased biomass production, P uptake and PUE the most as compared to the control (Figs. 1 and 4). We assumed that the higher biomass production in pots fertilized with sludge is related to a higher availability of nutrients. TSP significantly increased P concentration and P uptake more than sludge, but only at the first harvest (Fig. 3). After this date, P concentrations and uptake in the sludge treatment equalled with that in the TSP treatment. TSP application increased Olsen P in the soil, yet P from TSP are known to be rapidly sorbed into the soil mineral phase or leached out from the pot (Jalali and Jalali, 2020; Teles et al., 2020), decreasing plant available P and uptake over time in this treatment. This was corroborated by the decrease over time in Olsen P in the TSP fertilized pots (Fig. 5). On the other hand, the forms of P in sludge may have contained organic forms of P (Yu et al., 2021), which could have performed as slow releasing sources of P for grass and soil microorganisms. It has been demonstrated that available P from TSP decreases sharply over time whereas organic forms of P over time are potentially mobilizable by soil microorganisms over a longer period of time and by a re-equilibrium with the mineral phase (Khomenko et al., 2023a; Velasco-Sánchez et al., 2024).

Table 5

Model ranking based on Akaike's information criterion explaining P uptake. Models displayed show models within 0 – 2 $\Delta AICc$ range and the following best model. $AICc$ = Akaike's Information Criterion, $\Delta AICc$ = $AICc - AICc_{min}$ for best model, Awc = Akaike's weight, $Awcc$ = Awc cumulative and $LogLik$ = Log likelihood ratio, $RMSE$ = Root mean square error. ^a = PLFA-Fungi + NLFA-AMF.

		Models	AICc	$\Delta AICc$	Awc	Awcc	LogLik	RMSE
P uptake (day 28)	1	Root biomass + Olsen P + pH + Soil C:N + PLFA-Fungi	262.96	0.00	0.23	0.23	−122.48	7.27
	2	Root biomass + Olsen P + pH + Soil C + PLFA-Fungi	263.15	0.19	0.20	0.43	−122.58	7.29
	3	Root biomass + Olsen P + pH + Soil C:N + Fungal biomass ^a	264.05	1.09	0.13	0.56	−123.02	7.38
	4	Root biomass + Olsen P + pH + Soil C + Fungal biomass ^a	264.42	1.47	0.11	0.67	−123.21	7.42
	5	Root biomass + Olsen P + Soil C:N + PLFA-Fungi	266.00	3.05	0.05	0.72	−125.60	7.21
	6	Root biomass + Olsen P + Soil C:N + Soil C + pH + PLFA-Fungi	266.26	3.30	0.04	0.76	−122.46	7.26
	7	Root biomass + Olsen P + Soil C + PLFA-Fungi	266.35	3.39	0.04	0.80	−125.77	7.25
P uptake (day 112)	8	Root biomass + Olsen P + Soil C:N + Fungal biomass ^a	266.98	4.02	0.03	0.83	−126.09	7.31
	1	Root biomass + Olsen P + pH + Soil C:N + Fungal biomass ^a	201.36	0.00	0.16	0.16	−91.35	11.56
	2	Root biomass + Olsen P + pH + Soil C:N + NLFA-AMF	201.50	0.14	0.15	0.31	−91.41	11.52
	3	Root biomass + Olsen P + pH + Soil C + Fungal biomass ^a	201.73	0.37	0.13	0.44	−91.53	11.56
	4	Root biomass + Olsen P + pH + Soil C + NLFA-AMF	202.21	0.85	0.10	0.54	−91.77	11.60
	5	Root biomass + Olsen P + Soil C:N + Fungal biomass ^a	203.14	1.77	0.07	0.61	−93.95	11.40
	6	Root biomass + Olsen P + Soil C:N + NLFA-AMF	203.15	1.79	0.07	0.68	−93.96	11.36
	7	Root biomass + Olsen P + Soil C + Fungal biomass ^a	203.22	1.86	0.06	0.74	−94.00	11.41
	8	Root biomass + Olsen P + Soil C + NLFA-AMF	203.59	2.23	0.05	0.79	−94.18	11.44

The application of sludge not only increased cumulative P uptake but also increased total N uptake and in some conditions soil N-NH₄ compared to the control (Table 4, Table S4 and Table S6), regardless of the additions of N fertilizer that, we applied after each cut. This suggests that sludge improved both P and N uptake and that the forms of P and N in sludge are readily available for plants over time. Our results also showed a higher limitation of N for the grasses grown in TSP fertilized at day 28, as shown by the significantly lower N:P ratios, and higher P limitation for the plants fertilized with hydrochar (Table S4). DPW's sludge has also been proposed, in previous research, as a material with a great replacement value of mineral N fertilizers (Shi et al., 2022).

Hydrochar showed lower P uptake and PUE than sludge or TSP (Fig. 4). Ash was not statistically different from the other fertilizers but its cumulative total PUE was, on average, roughly 50 % lower than those from the sludge and TSP treatments. For ash, we observed similar dynamics as TSP: an increase of P uptake at the beginning of the experiment which decreased at the later stages of the experiment (Fig. 3 B). This can potentially be explained by a quick sorption of the plant available fractions of P in ashes to the mineral phase, or leached out from the system. By the end of the experiment, the mean PUE of ashes was approximately 25 %, nearly half of those from TSP or sludge (Fig. 4 B). Hydrochar was the DPW material that performed worst with values of PUE that were not higher than the control (Fig. 4 B). We assumed that the P in hydrochar was recalcitrant (Schneider and Haderlein, 2016). Our results match the observations from other greenhouse and field experiments in which sludge from DPW resulted in higher or equal biomass production or P uptake as TSP and hydrochar resulted in poor biomass production or even negative effects on crops (Schimmelpfennig et al., 2015; Shi et al., 2022; Khomenko et al., 2023b; Hu et al., 2024).

4.2. Impact of DPW materials on soil microorganisms and their contribution towards P uptake

The second research question of this study was to evaluate the impacts of DPW materials on soil microbial communities and their contribution to P uptake. Soil microorganisms have been typically regarded as key players in P nutrition by crops (Richardson and Simpson, 2011). Recently, this view has also been contested, indicating that most of the experiments addressing P nutrition and microbial activities were conducted in artificial conditions (Raymond et al., 2021; O'Callaghan et al., 2022). The results of our experiment showed that the addition of DPW materials or TSP had no major significant effects on most of the soil microbial variables studied, while, regardless of the plant species or P fertilizer treatment, fungal biomass played an important role in P uptake.

Sludge and ash application only affected AMF and anaerobe communities in the Lp pots (Table S7) and sludge resulted in a decrease in Bglu activities compared to TSP (Table S9), probably because of the introduction of labile C sources (de Almeida et al., 2015). It is generally assumed that enzyme production is only triggered when the substrates are in limitation (Allison et al., 2010). Our results contrast with other studies in which the application of DPW sludge resulted in changes on the microbial composition and functions (Frąc et al., 2012; Gryta et al., 2014). The responses of microbial communities to the addition of ashes and hydrochar from DPW remain poorly studied. However, other studies which tested materials from other sources, such as sewage sludge, indicated significant effects on the soil microbial community. These included increased soil respiration after ash application (Pichtel, 1990) or significant alteration in the soil microbial composition after application of hydrochar (Zhu et al., 2023). The lack of significant differences found in our study could be associated with the length of our experiment (López-Mosquera et al., 2000) and the chemical composition of DPW materials applied, which tend to be highly heterogeneous (Ashkuzzaman et al., 2019).

Yet, in our study, we observed that regardless of the plant species or P fertilizer treatment, fungal biomass played a key role in P uptake (Table 5). Fungal communities were important variables to explain P uptake in at least the first ranked 8 models at dates 28 and 112 (Table 5). Our results also suggest that the effects of fungi were superior than those of bacteria. The effects of fungi, however, cannot solely explain P uptake by the different grasses as the association of Olsen P, pH, root biomass and soil C:N were equally important variables in the most explanatory models (Table 5). The effects of the fungal community on P uptake were dependent on the sampling date and soil C. Particularly, PLFA-Fungi biomass, associated with saprophytic fungi, was included in the best ranked models based on their AIC at day 28 (Table 5). Saprophytic fungal groups such as *Aspergillum* spp., *Penicillium* spp. or *Trichoderma* spp. have been shown to be important in P solubilization (Daynes et al., 2008; Bononi et al., 2020; Nascimento et al., 2021). These saprophytic fungi were possibly important at the earlier stages of the experiment when more C was present (Table 4) (Almonacid et al., 2015). It has been demonstrated that C is a very important requirement for the soil microbial community to solubilize recalcitrant forms of P (Brucker et al., 2020; Velasco-Sánchez et al., 2024). By the end of the experiment, when the C content of the soil had decreased, the relevance of saprophytic fungi was less pronounced. At this date, AMF was the biological variable that stood up in our best models (Table 5). The estimate for AMF in the best ranked model was negative, suggesting that AMF colonization was more important in those plants grown under more limiting P conditions (Fornara et al., 2020; Mitra et al., 2023). AMF have been widely associated with improved P nutrition in grasslands (Fornara et al., 2020) and our results tend to confirm this observation.

4.3. Grass species and P utilization

In our experiment, we also tested the effects of DPW on two grass species with contrasting growth strategies: Lp as a fast-grower and Dg as a slow-grower. As such, we expected that the different growth strategies would result in different PUEs and microbial associations. We observed numerous significant plant effects and/or significant interactions of grass species with fertilizer application or with harvest time. For instance, Olsen P (Table 4) values were lower in Lp pots and P uptake and concentration was higher in Lp pots (Table 3), suggesting a higher utilization of the Olsen P pool by Lp plants. By the end of the experiment, however, no differences were found in cumulative biomass production or PUE (Tables 2 and 3 and Fig. 2B), suggesting a more efficient use of P by Dg plants. Dg has already been suggested to have a high use efficiency of P and N (Ryser et al., 1997). The differences in grass biomass production over time (Fig. 2), as well as the decrease in biomass production in Lp and the constant biomass production in Dg, contrasted with the availability of P (Olsen P), which decreased over time. This contrast should be considered, since different grass species would benefit from different P application timings (Simpson et al., 2014).

Lp and Dg showed also different associations with soil microorganisms. We observed that the effects of P fertilizers on AMF and anaerobe communities and Bglu were only significant on Lp plants (Tables S7 and S9). Moreover, Lp had significantly higher PAC activities than Dg (Table S9). Production of enzymes by microorganisms in the rhizosphere of Lp plants has shown to be an important factor to improve P uptake in P limiting conditions (Scott and Condron, 2003; Sanguin et al., 2016).

Finally, we noted that root biomass was one of the most important variables for explaining P uptake, as shown by the AIC model rankings (Table 5). For both dates (days 28 and 112), the coefficient of root biomass for the best ranked models was positive, suggesting a positive relationship between root biomass production and P uptake. Our results show the importance of species selection for a more efficient P utilization in grasslands. Selecting grass species, such as Lp, that make use of the more available forms shortly after fertilization would result in a

higher PUE, whereas using slow growers like Dg would potentially reduce the required frequencies of P applications. Combinations of grass species with contrasting root traits and growth strategies should be further explored to reduce the dependency on mineral P fertilization (Simpson et al., 2014; de Oliveira et al., 2018; Ros et al., 2018).

4.4. Outlook

The results of our experiment present also challenges for nutrient recovery from DPW. Although our results show that raw sludge is the best fertilizer for the studied grass species, the moisture content of DPW sludge can easily reach values of 90 % (Ashkuzzaman et al., 2019; Hu et al., 2021) and this contribute to economical costs due to drying or transportation. The farmers perception towards the use of recycled P fertilizers should be taken into consideration, they must be convinced about the PUE when advocating for recycling of DPW sludge in agriculture (Behjat et al., 2022; Garmendia-Lemus et al., 2024). Moreover, in our study, we used an iron-chelated sludge. The results could have been different if the studied sludge was treated with aluminium chelates or if limestone was added to the sludge (Shi et al., 2022). The lack of widespread regulation makes even more complicated to predict the potential of P fertilization from DPW sludge (Kellis et al., 2013; Colli-vignarelli et al., 2019). In our experiment, we tested the secondary materials from technologies that aim to concentrate P by reducing the moisture content of sludge. Thermal oxidation and ashing resulted in PUE that were low (Fig. 4). These materials have the potential to be used as raw material for the production of precipitated P salts such as struvite (Darwish et al., 2017; Becker et al., 2019), or as soil amendments (Azharul et al., 2021; Ma and Rosen, 2021), however their direct use as P fertilizers needs further experiments and confirmation.

Further experiments, on a wider variety of soils and depths, under field conditions and longer term (multiple seasons) should be performed to validate our findings. Moreover, experiments that focus on the combination of grass species with contrasting P acquisition strategies (e.g. different root traits, associations with soil microorganisms, etc.) under fertilization with DPW materials should be performed. Likewise, more experiments should focus on the toxicology aspect of dairy processing waste application and the long-term effects on the environment (greenhouse gas emissions, heavy metals and nutrient leaching).

5. Conclusion

Our results revealed that, whereas DPW sludge has the potential to replace mineral P fertilizers, secondary materials such as ash or hydrochar are not good candidates to reduce mineral P dependency. Ash and hydrochar could be used as soil amendments or as intermediary materials for the production of precipitated P salts, such as struvite. Our results also highlight the pivotal role of fungal communities in P uptake and nutrition in grasses.

CRediT authorship contribution statement

Á. Velasco-Sánchez: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Y. Hu:** Writing – review & editing, Investigation. **S.G. Sommers:** Writing – review & editing, Supervision, Funding acquisition. **G.Y.K. Moinet:** Writing – review & editing. **J.W. Van Groenigen:** Writing – review & editing, Supervision. **I. Trinsoutrot-Gattin:** Writing – review & editing, Supervision, Funding acquisition. **N. Bennegadi-Laurent:** Writing – review & editing, Supervision, Methodology, Conceptualization.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.geoderma.2025.117406>.

Data availability

Data stored on zenodo: 10.5281/zenodo.10625465

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