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Nutrient interactions and salinity effects on plant uptake of phosphorus from waste-based fertilisers

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ABSTRACT

Many organically managed farms in Europe have low levels of soil phosphorus (P). Arable farms that rely strongly on biological nitrogen fixation (BNF) have been shown to have rather low outputs and a tendency to deplete soil P and potassium (K) compared with arable farms that have a lower reliance on BNF and higher external inputs. Therefore, research focusing on providing a balanced input of nitrogen (N), P, K and sulphur (S) from alternative sources is of interest to organically managed farms in Europe.

The aim of this study was to quantify P availability from different organic wastes applied alone or in combination to improve the mixtures' N:P:K:S ratio. P availability was measured by P uptake and recovery in ryegrass grown in pots. The isotope dilution approach was used in which a non-labelled fertiliser is added to a soil that has been pre-incubated and equilibrated with labelled ^{33}P.

The P recovery of the different organic wastes varied significantly (10–20 %). Manure and anaerobically digested manure mixed with ash from straw had the lowest P recovery. All the organic waste treatments had higher plant growth and P uptake compared with the negative control, but none of them reached the values observed after application of mineral P. Mixing digested manure with ash increased soil pH at the end of the experiment, which may explain the lower P availability. The highest P recovery was found in digested products, either manure alone or mixed with municipal waste or the industrial waste product Fertigro®. However, the mixture of digested manure and Fertigro® led to lower dry matter production, whereas Fertigro® used alone resulted in high leaf P concentrations but depressed shoot and root growth, presumably due to salinity effects and a decrease in soil pH. Anaerobic digestion increased the availability of P, which may be explained by the lower immobilisation potential of the remaining organic matter in the digestate.

This study highlights the potential challenges when attempting to improve the N:P:K:S ratios of waste-based fertilisers through mixing due to material interactions. However, such effects are likely to be overexpressed in pot trials that have a limited soil volume. Field trials are therefore needed to quantify such effects in practice.

1. Introduction

In response to increasing consumer demand for organic farming products and in recognition of the services rendered to the environment, the European Commission aims to achieve the expansion of organic farming to at least 25 % of the EU's agricultural land by 2030 (EU, 2020). A large number of organically managed farms in Europe have low levels of soil P (Cooper et al., 2018). Arable farms that rely strongly on biological nitrogen fixation (BNF) are shown to have rather low outputs (Reimer et al., 2020) and a tendency to deplete soil phosphorus (P) and potassium (K) resources, whereas arable farms with lower reliance on BNF and higher reliance on external inputs of nutrients have much higher outputs and low or no soil nutrient depletion. Therefore, obtaining P but also nitrogen (N), K and sulphur (S) from alternative sources is of interest to organically managed farms in Europe. This emphasises the need for organic agriculture to increase its contribution to the evolving circular economy (Løes and Adler, 2019), posing new practical and regulatory challenges. In Denmark, organic farmers have long debated their dependency on inputs from conventional farms and in 2008 decided to advocate for a ban on the use of manure and straw from conventional farms by 2021. They subsequently had to moderate this decision to a more gradual approach due to the lack of acceptable

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alternatives. Oelofse et al. (2013) discuss the implications of phasing out the conventional nutrient supply in organic agriculture and propose that organic farmers should consider the suitability of nutrient sources available in alternative, non-farm organic waste streams for use in organic systems. A working group identified several locally abundant waste streams that would be of interest for future recycling on Danish organic farms and could potentially be acceptable under the EU legal code for fertilisation (EC no. 889/2008, Annex I).

Few studies have addressed the effect of anaerobic digestion on P speciation and fertiliser value, but there is evidence of a reduction in labile P fractions in the process through a reaction with metal ions, struvite formation, microbial immobilisation and binding to particulate solids (Güngör and Karthikeyan, 2008; Mazzini et al., 2020; Möller and Müller, 2012). However, plant growth experiments carried out with both raw and digested manure did not overall show any difference with regard to the P fertilising effect (Bachmann et al., 2014, 2011; Hupfauf et al., 2016; Zicker et al., 2020). Co-digestion may be relevant for energy-rich resources and may alter the N and P availability of the anaerobically digested manure (Li et al., 2020), whereas subsequent mixing of organic wastes with digestates may be a way to modify and potentially improve the N:P:K:S ratio of the fertilisation product.

Among the material types identified as abundant and potentially acceptable for Danish organic farmers are the source-separated organic fraction of municipal solid waste, ash from straw-fuelled combined heat and power plants, and Fertigro® – a mucosa residue from the industrial production of medical heparin (an anticoagulant, produced by Leo Pharma Ltd., Denmark). Ash from straw is known to increase the pH of soils (Schiemenz and Eichler-Löbermann, 2010), thereby potentially affecting P availability (Barrow, 2017; Penn and Camberato, 2019). The mucosa from pig guts is conditioned with sodium chloride (NaCl) in order to allow extraction of heparin molecules by ion exchange, and the remains are further stabilised with sodium bisulfite ($NaHSO_3$) before distribution as Fertigro®. It is currently used by Danish conventional farmers for barley, which is known to be tolerant of salinity stress (Ligaba and Katsuhara, 2010), but salinity issues could potentially be a limitation for other crops. It is appreciated primarily as a nitrogen (N) fertiliser with a high content of S compounds, which could make it interesting for organic farmers as, besides N, S is believed to be yield-limiting, particularly on organic farms (Eriksen, 2009; Eriksen et al., 2004). Finally, household sorted municipal organic waste was identified as a potential source of nutrients, although little is known about how it affects nutrient release after co-digestion with manure.

The aim of this study was to quantify P uptake and recovery in ryegrass from different organic wastes applied alone or in combination with other organic wastes to improve the N:P:K:S ratio of the mixed fertiliser product. Pot trials were used not only for reasons of economy, but because the isotope dilution approach was adopted. In addition, both positive and negative effects are more likely to register due to the lower soil volume, which enhances fertiliser impacts (Limpens et al., 2012). The amount of plant P derived from different organic wastes added to the soil can be determined by means of isotopic dilution principles using radioisotopes of P (Frossard et al., 2011). This method has been successfully used to quantify plant P uptake from different P sources such as rock phosphate, sewage sludge, compost and manure (Fardeau et al., 1988; Frossard et al., 1996; Oberson et al., 2010; Sinaj et al., 2002).

The hypothesis of this study was that efficiency of organic waste applied alone or combined to supply P for ryegrass growth and P uptake is affected by: 1) the pH effects of fertilisers on soil matrix reactivity, 2) salinity-mediated stress and 3) decreased P availability after digestion of manure and municipally organic waste.

2. Material and methods

2.1. Soil and organic wastes

The soil used in this experiment was a sandy loam of moderate fertility collected from the Long-Term Nutrient Depletion Trial on the University of Copenhagen's experimental farm in Taastrup, Denmark (55°40'N, 12°17'E). This soil was depleted in P and K, having received only mineral N for 30 years, but since 1996 fertilised annually with 60:10:0:25 kg ha^{-1} of mineral N:P:K:S. More information about the soil can be found in van der Bom et al. (2018). The soil was collected from the plough layer (0–25 cm), air-dried and sieved to < 4 mm. The pH_{H2O} of the soil was 7.1 and the electrical conductivity was 0.04 mS cm^{-1}, both quantified in 1:5 soil:MilliQ water extracts. The soil contained 8.3 mg Olsen P kg^{-1} and 341 mg total P kg^{-1} (data from van der Bom et al. (2018)). The amounts of plant-available nutrients in the soil were 4.43 mg $P\text{-}PO_4^{-}$ kg^{-1}, 2.58 mg $N\text{-}NO_3^{-}$ kg^{-1} and 0.60 mg $N\text{-}NH_4^{+}$ kg^{-1}.

Five different organic waste materials with potential for use as bio-based fertilisers were collected from different sources in Denmark. Fertigro® is a waste from the biotech industry marketed by HedeDanmark and an animal-based product from heparin production. It is composed of mucosa conditioned with sodium chloride, mixed with proteinase, and stabilised for storage and transport by the addition of sodium bisulfite (https://www.fertigro.dk). Ash from straw was also obtained from HedeDanmark; the ash is produced by the combustion of straw in combined heat and power plant (CHP) commonly found in rural areas in Denmark. Cattle manure, anaerobically digested cattle manure and cattle manure anaerobically co-digested with the organic fraction of municipal solid waste (OFMSW) (87.5 % manure + 12.5 % OFMSW) were supplied by Aarhus University. Manure and manure + OFMSW were digested in a continuous flow thermophilic (47–52 °C) pilot-scale digester (130 L) with on average 20 days' hydraulic retention time. The main properties of the five different organic wastes used in this study are summarised in Table 1.

Table 1
Selected properties for Fertigro® (F), cattle manure (M), digested cattle manure (DM), cattle manure co-digested with the organic fraction of municipal solid waste (DM + OFMSW) and ash from straw (ASH).

	F	M	DM	DM + OFMSW	ASH
Dry matter (%)	17.1	8.2	5.65	4.31	58.1
pH	6.25	7.27	8.26	8.26	11.9
Total N (g kg^{-1} fw)	11.4	3.77	3.60	3.09	1.01
Total N (g kg^{-1} dw)	66.3	46.2	63.7	71.6	1.73
NH_4-N (g kg^{-1} fw)	1.36	1.93	2.15	1.87	0.00
NH_4-N (g kg^{-1} dw)	7.9	23.7	38.0	43.3	0.00
NO_3-N (g kg^{-1} fw)	0.000	0.006	0.005	0.003	0.004
NO_3-N (g kg^{-1} dw)	0.00	0.070	0.088	0.076	0.007
Mineral-N (% of TN)	11.9	51.4	59.8	60.5	0.32
Total P (g kg^{-1} fw)	1.20	0.76	0.90	0.69	5.27
Total P (g kg^{-1} dw)	7.00	9.33	16.0	16.0	9.07
WEP (g kg^{-1} fw)	0.94	0.31	0.32	0.31	0.11
WEP (% of TP)	78.6	40.1	35.2	45.5	2.03
Total K (g kg^{-1} fw)	2.62	3.48	4.64	3.54	95.4
Total K (g kg^{-1} dw)	15.3	42.7	82.0	82.0	164.1
Total S (g kg^{-1} fw)	5.34	0.99	0.60	0.47	6.89
Total S (g kg^{-1} dw)	31.1	12.2	10.7	10.8	11.9
Total C (g kg^{-1} dw)	349	426	389	381	161
Total C/N-organic	5.85	18.8	15.1	13.4	96.1
Total C/N	5.27	9.24	6.09	5.31	93.6
Total C/P	49.9	45.7	24.3	23.8	17.8
Total N/P	9.47	4.95	3.98	4.48	0.19

*WEP: water-extractable P.

2.2. The pot experiment

The amount of plant P derived from the organic waste added was studied using the indirect labelling technique, where a non-labelled fertiliser is added to a soil that has been pre-incubated and equilibrated with labelled ^{33}P (Kucey and Bole, 1984; Morel and Fardeau, 1989). The soil equivalent of 1.1 kg of dry soil was weighed into a 10 L plastic bag. A P-free liquid nutrient solution was added containing (per kg soil) 150 mg N, 180 mg K, 25 mg Mg, 118 mg S, 30 mg Ca, 0.45 mg Mn, 0.3 mg Zn, 0.15 mg Cu, 0.01 mg Mo, 0.22 mg B and 2 mg Fe (added as NH_4NO_3, K_2SO_4, $MgSO_4$, $CaCl_2$, $MnSO_4$, $ZnSO_4$, $CuSO_4$, Na_2MoO_4, H_3BO_3 and $C_{10}H_{12}FeN_2NaO_8$). After two days of air-drying, the soil was thoroughly mixed to ensure the homogeneous distribution of nutrients in the soil. MilliQ water was added to each soil bag to reach 30 % water-holding capacity (WHC, which was 30 % (300 g water kg^{-1} soil)) and the soil was pre-incubated for one week. At the end of the pre-incubation period, the plant-available P pool in the soil was labelled by adding 5 ml of a carrier-free ^{33}P-orthophosphoric acid solution to achieve 2.5 MBq kg^{-1} soil. The radioactivity of the ^{33}P solution was measured by scintillation counting (Liquid Scintillation Analyzer Tri-Carb 2910 TR, PerkinElmer) using 5 ml solution and 15 ml scintillation liquid (Ultima Gold^{TM}) before labelling. Soil and solution were carefully mixed for two minutes. To reach near-equilibrium for ^{31}P and ^{33}P, the labelled soil was incubated in double plastic bags for one week in the growth chamber using the same settings as those used for the rest of the plant experiment (see below) (Nanzer et al., 2014).

On the day of sowing, the different organic wastes were applied alone or in combination to improve their N:P:K:S ratio, resulting in ten treatments that were set up in four replicates: 1) a positive control using non-labelled soil amended with ^{33}P-labelled KH_2PO_4 (C + P), 2) a negative control using labelled soil with no P added (C – P), 3) Fertigro® (F), 4) raw cattle manure (M), 5) digested cattle manure (DM), 6) digested cattle manure co-digested with the organic fraction of municipal solid waste (DM + OFMSW), 7) digested manure combined with Fertigro® (DM + F), 8) digested cattle manure co-digested with the organic fraction of municipal solid waste combined with Fertigro® (DM + OFMSW + F), 9) digested cattle manure combined with Fertigro® and ash (DM + F + ASH) and 10) digested cattle manure co-digested with the organic fraction of municipal solid waste combined with Fertigro® and ash (DM + OFMSW + F + ASH). All the treatments except the negative control were prepared by adding organic waste corresponding to 50 mg P kg^{-1} soil. Rates of N, P, K and S applied in each treatment are presented in Table 2. For treatments composed of more than one type of organic waste, the mixture was prepared by adding the same amount of P from each type of organic waste. For example, in treatment 7), 25 mg P kg^{-1} was added as digested manure, while another 25 mg P kg^{-1} was added with Fertigro®. The positive control was prepared using unlabelled soil amended with 5 ml of KH_2PO_4 solution, adding 50 mg P kg^{-1} soil labelled with a carrier-free ^{33}P-orthophosphoric acid solution with a specific activity of 50 KBq mg^{-1} P or 2.5 MBq kg^{-1} soil. Soil and organic wastes were thoroughly mixed and filled into pots with closed bottoms to reach a bulk density of approximately 1.4 g cm^{-3}. Each pot was sown with 2 g of perennial ryegrass (*Lolium perenne* var. Soriento) seeds that were covered with 30 g soil and watered up to 60 % WHC. The conditions in the growth chamber were set as follows: daylight period 16 h, temperature 20/15 °C (day/night) and photosynthetically active radiation 300/0 μmol m^{-2} s^{-1} (day/night). During the growth, the pots were completely randomly distributed and regularly rotated and watered up to 60 % WHC by weighing. The ryegrass was harvested 28, 42 and 56 days after sowing by cutting the shoots 3 cm above the soil surface in the first two cuts and at the soil surface in the third cut. After each cut, a nutrient solution containing (per kg of soil) 75 mg N, 90 mg K, 15 mg Ca and 59 mg S (added as NH_4NO_3, $CaCl_2$, K_2SO_4) was applied. At each cut, shoot biomass dry matter was determined by drying in an oven for 48 h at 50 °C. After the third cut, root biomass was also determined by weighing after carefully washing the soil from the roots and drying them in an oven for 72 h at 50 °C. To determine the P concentration, dried shoot biomass was milled and subsequently microwave-digested with 2.5 ml 70 % HNO_3 and 1 ml 15 % H_2O_2. Shoots were analysed for their content of P on a flow injection analyser (FIA star 5000, Foss Analytical, Denmark). The specific activity of the plant extracts was measured by scintillation counting (Liquid Scintillation Analyzer Tri-Carb 2910 TR, PerkinElmer) in a solution of 5 ml extract and 15 ml scintillation liquid (Ultima Gold^{TM}). The values were corrected for radioactive decay back to the day of labelling. Soil pH was analysed at a 1:5 ratio of soil with MilliQ water (w:v) on the soil samples taken from each pot at harvest time.

Table 2
Nutrients added (mg kg^{-1} soil) with the organic wastes applied singly or in mixtures.

	N	P	K	S
	mg kg^{-1} soil			
Single application				
Fertigro (F)	501	50	50	222
Raw manure (M)	250	50	229	65
Digested manure (DM)	200	50	256	33
Digested manure + organic fractions of municipal solid waste (DM + OFMSW)	224	50	256	34
Mixtures				
DM + F	350	50	153	128
DM + OFMSW + F	362	50	153	128
DM + F + ASH	237	50	404	107
DM + OFMSW + F + ASH	245	50	404	107

2.3. Seed P contribution

P derived from seeds was determined using the direct labelling approach in an additional pot experiment. The details for this pot experiment are described by Hansen et al. (submitted for publication) and follow the methodology described by Nanzer et al. (2014). Briefly, each pot was filled with 1.1 kg of acid-washed sand (particle size 0.8–1.2 mm) that was amended with the nutrient solution described above and left to dry for four days. The sand was then mixed and transferred to a pot where a solution containing carrier-free ^{33}P-orthophosphoric acid and KH_2PO_4 was added to each pot at increasing P rates (2, 3.7, 7.2, 14.5 and 26.3 mg P kg^{-1} sand) and with a specific activity (SA) of 65.1, 36.7, 18.6, 9.7 and 5.1 KBq mg^{-1} P. All treatments were replicated four times. Ryegrass seeds were sown at a rate of 2 g pot^{-1}. The growing conditions, handling of pots, harvest and plant analyses were identical to those described for the pot experiment above. Pots were regularly rotated and watered every second day by weighing and watering up to 60 % WHC at the start of the experiment, rising gradually to 90 % WHC throughout the experimental period.

2.4. Calculation of P pools

The contribution of different P pools to plant P uptake (P_{uptake}, mg P kg^{-1} soil) was calculated according to the following equation (Nanzer et al., 2014):

$$P_{uptake} = \text{Pdf seed} + \text{Pdf soil} + \text{Pdf fertiliser} \quad (1)$$

Where Pdf means P derived from seed, soil and fertilisers. To solve this equation, the principles described by Frossard et al. (2011) were used:

Pdf seed (mg P kg^{-1} soil) was calculated from the seed P contribution experiment where the ryegrass was grown in sand, therefore the equation for this experiment can be simplified to:

$$P_{uptake} = \text{Pdf seed} + \text{Pdf fertiliser} \quad (2)$$

Pdf fertiliser was calculated using equation 3:

$$\text{Pdf fertiliser}(\%) = \left(SA_{plant}/SA_{fertiliser}\right) \times 100 \quad (3)$$

where SA_{plant} is the specific activity ($^{33}P/^{31}P$, MBq g^{-1} P) in the plants and $SA_{fertiliser}$ is the specific activity of the fertiliser.

From this experiment, a function was obtained that correlated the Pdf seed with the plant P uptake for each cut:

$$\text{First cut}: y = 0.2515x + 1.6192,\ R^2 = 0.9788 \quad (4)$$

$$\text{Second cut}: y = 1.3710^*\left(1 - e^{-1.2431x}\right),\ R^2 = 0.8353 \quad (5)$$

$$\text{Third cut}: y = 0.1202x + 1.6334,\ R^2 = 0.9499 \quad (6)$$

These equations were used to calculate the Pdf seed in the experiment with soil. Pdf fertiliser was calculated using equation 7:

$$\text{Pdf fertiliser}(\%) = 100\ \times\left(1 - SA_{fertiliser}/SA_{NoP}\right) \quad (7)$$

where $SA_{fertiliser}$ is $^{33}P/^{31}P$ (MBq g^{-1} P) in the plant amended with a non-labelled fertiliser and SA_{NoP} is the specific activity of the plant with no P amendment (C – P in this study) for P uptake values corrected for the contribution from the seed. Finally, P derived from soil (Pdf soil, mg P kg^{-1} soil) was calculated by subtracting Pdf fertiliser and Pdf seed from the total P taken up by plant shoots:

$$\text{Pdf soil} = \text{P uptake} - \text{Pdf fertiliser} - \text{Pdf seed} \quad (8)$$

The fertiliser P recovery (%) in the ryegrass shoot biomass was calculated by comparing the Pdf fertiliser with the amount of P applied:

$$\text{Fertiliser P recovery} = (\text{Pdf fertiliser}/\text{total P applied}) \times 100 \quad (9)$$

Apparent fertiliser P recovery was calculated as the difference in P uptake in fertiliser treatments and the P uptake in the control treatment in proportion to the amount of P applied:

$$\text{Apparent P recovery} = (\text{P uptake fertiliser} - \text{P uptake no P})/\text{total P applied} \times 100 \quad (10)$$

2.5. Statistical analysis

All the variables studied were checked for normality of residuals and homogeneity of variance using diagnostic plots, and log-transformation was used when the data were not normally distributed. Statistical differences were tested using one-way ANOVA with treatment as a factor. The differences between fertiliser treatments were analysed using Fisher's LSD test. All differences at $p < 0.05$ were reported as significant. All statistical analyses were performed using R version 4.0.0 (R Core Team, 2017) and RStudio 1.2.5042 (RStudio Team, 2017).

3. Results

3.1. Plant growth

Ryegrass shoot growth varied among the organic wastes tested (Fig. 1a). For the first cut at 28 DAS, the highest shoot biomass was observed for the positive control (C + P), but similar growth was observed for treatments with digested manure (DM) and manure co-digested with the organic fraction of municipal solid waste (DM + OFMSW). For the rest of the organic wastes tested, shoot growth was similar to the negative control (C – P), except for treatments with Fertigro® (F) and manure co-digested with the organic fraction of municipal solid waste combined with Fertigro® (DM + OFMSW + F), where the shoot growth was significantly lower than the negative control. For the second cut, only digested manure and DM + OFMSW reached the shoot growth observed for the positive control, whereas the rest of the organic wastes showed lower shoot biomass than the former two treatments but it was higher than the negative control. For the third cut, all the treatments showed similar shoot growth to that observed for the negative control, except the positive control where shoot biomass continued to be significantly higher than in the rest of the treatments. Therefore, at the end of the experiment, the positive control resulted in higher total cumulative shoot biomass compared with the rest of the treatments. Fertilisation with organic wastes significantly increased the shoot biomass of ryegrass compared with the negative control in the following order: digested manure (DM) = manure co-digested with the organic fraction of municipal solid waste (DM + OFMSW) > digested manure combined with Fertigro® and ash (DM + F + ASH), manure co-digested with the organic fraction of municipal solid waste and Fertigro® and ash (DM + OFMSW + F + ASH). No significant differences were obtained between the rest of the organic wastes tested and the negative control treatment (Fig. 1a).

The addition of organic wastes also affected ryegrass root growth (Fig. 1a). The highest root biomass was observed for the positive control > digested manure combined with Fertigro® and ash, while the remaining treatments had similar root biomass, except the treatments with Fertigro® and manure co-digested with the organic fraction of municipal solid waste. The latter treatments had significantly reduced root growth compared with the negative control. For this reason, the root:shoot ratio was significantly lower in these two treatments (Fig. 1b). For raw manure and digested manure combined with Fertigro® and ash, the root:shoot ratio did not differ from that calculated for the positive and negative controls.

3.2. Plant shoot P concentration

The shoot P concentration of ryegrass grown in soil amended with Fertigro® was significantly higher than in the rest of the treatments at 28 DAS (Fig. 2), with values even greater than that observed for the positive control. For the other treatments, while lower than the positive control, shoot P concentrations were highest when both digested manure and manure co-digested with the organic fraction of municipal solid waste were combined with Fertigro®. Finally, the negative control showed the lowest P concentrations observed. For the second cut, the P concentration measured in the plants fertilised with Fertigro® was significantly lower than the positive control. For the rest of the treatments, shoot P concentrations in the second and the third cuts were very similar, although a significantly higher P concentration was observed when the mix included Fertigro® (DM + F and DM + OFMSW + F).

3.3. Plant shoot P uptake

The positive control had the highest total P uptake (Fig. 3). The manure treatment and treatments containing ash had the lowest uptake, exceeding only the negative control. The remaining treatments had a similar uptake. Uptake in the Fertigro® treatment increased relative to the other treatments during the experiment as the depressed plant growth during the first 28 DAS was overcome.

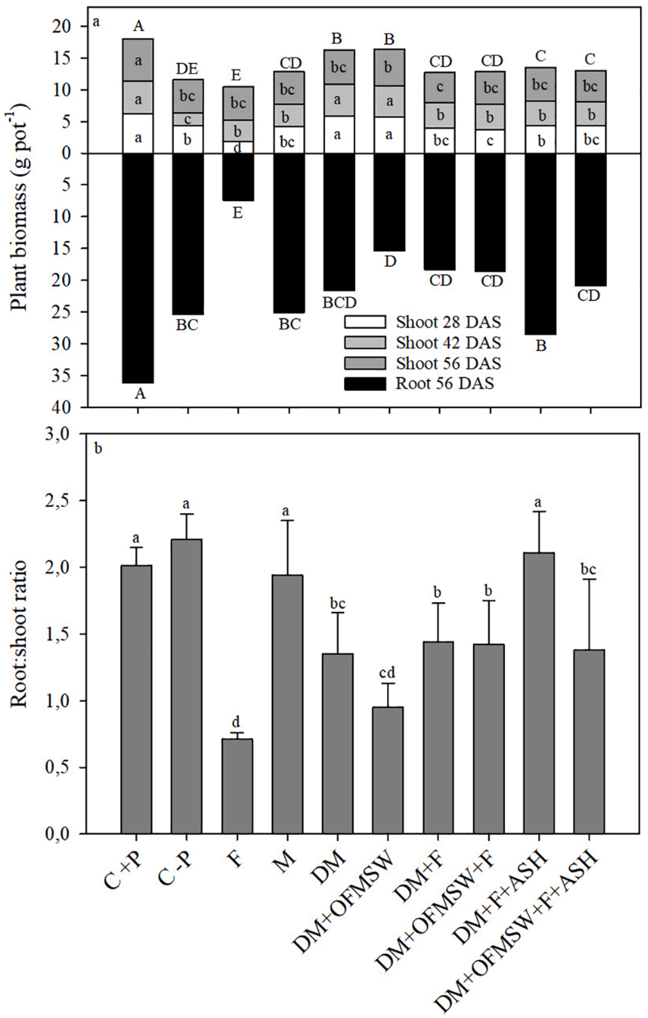

Fig. 1. Plant biomass (shoot and root) quantified after 28, 42 and 56 days of growing (a) and root:shoot ratio (b) calculated at the end of the experiment for ryegrass amended with mineral P (C + P), no P (C − P), Fertigro® (F), cattle manure (M), digested cattle manure (DM), cattle manure co-digested with the organic fraction of municipal solid waste (DM + OFMSW), and their combination between DM + F, DM + OFMSW + F, DM + F + ASH and DM + OFMSW + F + ASH. Bars are the mean of four replicates and error bars denote standard deviation. Different small letters show a significant difference between treatments in the same cut, whereas different capital letters show significant differences in the total shoot biomass between all the treatments at the end of the experiment.

3.4. P pools

Overall, the differences in P uptake derived from the soil pool (Pdf soil) observed between the organic waste treatments were similar at 28 and 42 DAS (Fig. 3a,b). At 56 DAS, there was no difference between the positive and negative controls, and no difference was observed in the Pdf soil between the organic wastes tested (Fig. 3c).

At the end of the experiment, the highest total contribution of Pdf soil was observed for the positive control with 7.3 mg P kg^{-1} soil, which was very close to the initial Olsen P measured in the soil (8.3 mg P kg^{-1}), followed by the application of digested manure alone and manure co-digested with the organic fraction of municipal solid waste, where Pdf soil was 4.2 and 4.4 mg P kg^{-1} soil respectively (Fig. 3d). For the rest of the treatments, Pdf soil ranged from 3.8 to 4.0 mg P kg^{-1} soil, with the lowest values observed when ash was included in the mixture, resulting in an uptake even lower than that of the negative control. The contribution of the P from the soil pool was in the range of 15 to 20 % of total P uptake.

Phosphorus derived from the seed provided a substantial contribution to the total P uptake and rose with increasing total uptake, mainly at 28 DAS (Fig. 3a). Thus, for the first month of growth, the P uptake derived from the seed was 5.1 mg P kg^{-1} soil for the positive control. For the digested manure (DM), manure co-digested with the organic fraction of municipal solid waste (DM + OFMSW), digested manure combined with Fertigro® (DM + F) and manure co-digested with the organic fraction of municipal solid waste combined with Fertigro® (DM + OFMSW + F) treatments, Pdf seed was on average 3.3 mg P kg^{-1} soil. For the rest of the treatments, Pdf seed was lower, with values between 2.2 and 2.8 mg P kg^{-1} soil. At 42 and 56 DAS, Pdf seed values were intermediate and the differences between treatments decreased, being on average 1.2 mg P kg^{-1} soil for the second cut and 2.1 mg P kg^{-1} soil for the third cut. At the end of the experiment, P derived from seed contributed 34 to 43 % to the total P uptake of the organic waste treatments (Fig. 3d). The different harvest protocols developed for the third cut (56 DAS) may be responsible for the high Pdf seed calculated at the last sampling time. While at 28 and 42 DAS, the shoot biomass was cut 3 cm above the soil surface to allow the regrowth of ryegrass, in the third cut shoot biomass was cut to the soil surface to quantify shoot and root biomass and their ratios. In a parallel study, the amount of shoot biomass from 0 to 3 cm and above 3 cm was quantified (Supplementary Fig. 1) and revealed that 58 % of the total ryegrass biomass was found in the fraction from 0 to 3 cm at the last cut. This substantial additional amount of plant biomass in the third cut will also affect P uptake, Pdf seed and Pdf soil for the third cut, since cutting ryegrass at the soil surface would include part of the biomass that was already present at the first and the second cuts.

P derived from added fertiliser differed in the three cuts carried out in this experiment (Fig. 3). The greatest fertiliser contribution to total P uptake was observed for the positive control in all the cuts i.e. 6.8, 5.0 and 5.1 mg P kg^{-1} soil for the first, second and third cuts respectively (Fig. 3a,b,c). Digested manure, manure co-digested with the organic

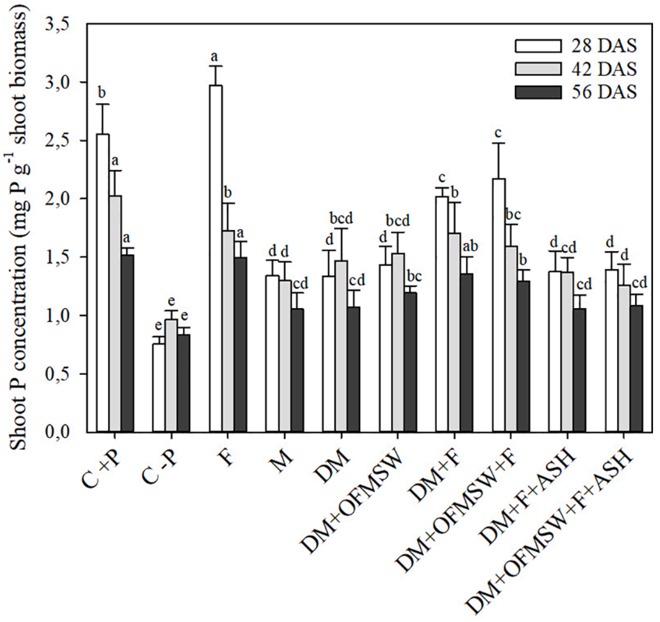

Fig. 2. Phosphorus concentration in shoots of ryegrass grown during 28, 42 and 56 days in pot amended with mineral P (C + P), no P (C – P), Fertigro® (F), cattle manure (M), digested cattle manure (DM), cattle manure co-digested with the organic fraction of municipal solid waste (DM + OFMSW), and their combination between DM + F, DM + OFMSW + F, DM + F + ASH and DM + OFMSW + F + ASH. Bars are the mean of four replicates and error bars denote standard deviation. Different small letters show a significant difference between treatments in the same cut.

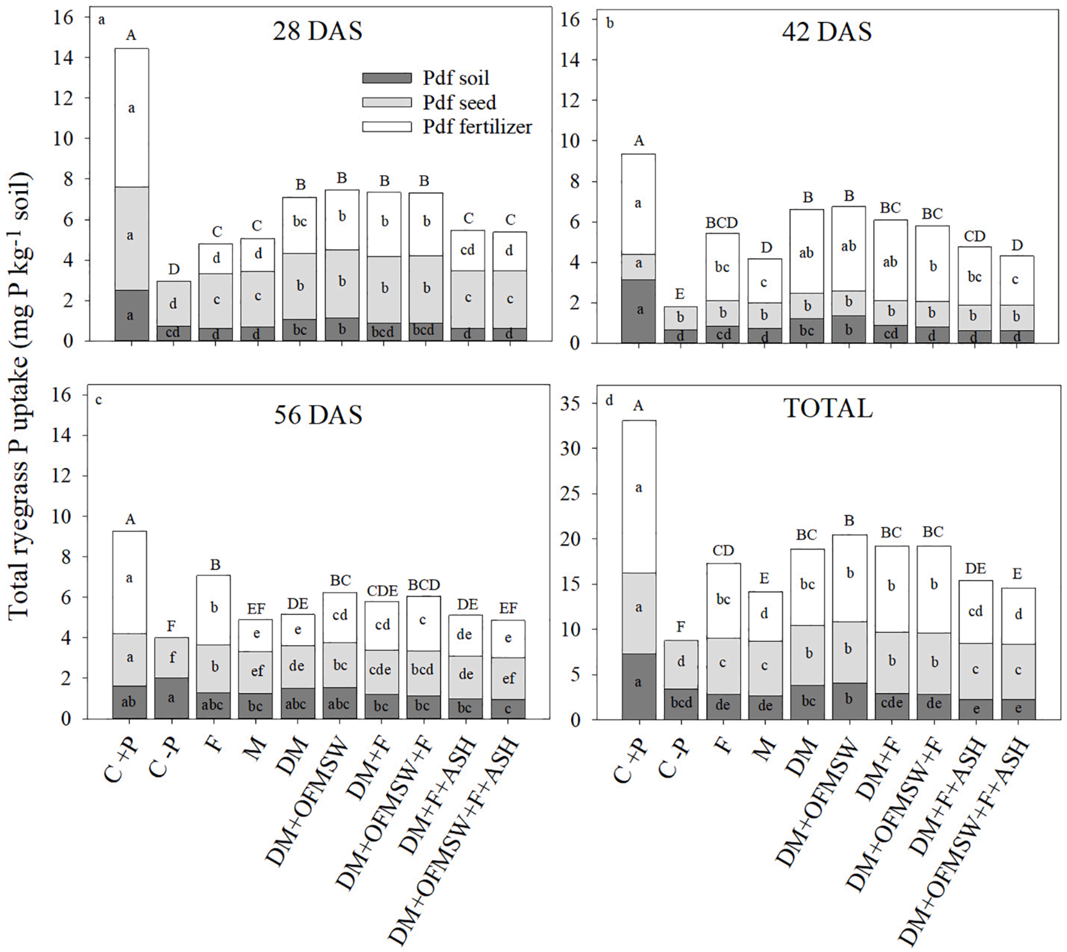

Fig. 3. Contribution of P derived from soil, seed and fertilisers to the total ryegrass P uptake in each cut and altogether for plants amended with mineral P (C + P), no P (C – P), Fertigro® (F), cattle manure (M), digested cattle manure (DM), cattle manure co-digested with the organic fraction of municipal solid waste (DM + OFMSW), and their combination between DM + F, DM + OFMSW + F, DM + F + ASH and DM + OFMSW + F + ASH. Bars are the mean of four replicates and error bars denote standard deviation. Different small letters show a significant difference between treatments in the same P pool, whereas different capital letters show significant differences in the total ryegrass P uptake between all the treatments.

fraction of municipal solid waste, digested manure combined with Fertigro® and manure co-digested with the organic fraction of municipal solid waste combined with Fertigro® treatments showed intermediate Pdf fertiliser amounts of 3.0, 4.0 and 2.3 mg P kg^{-1} soil on average for the first, second and third cuts respectively. Pdf fertiliser in the rest of the treatments (manure, and digestates with ash) was lowest. Fertilisation with Fertigro® resulted in an increase in the Pdf fertiliser over time from 1.5 to 3.4 mg P kg^{-1} soil for the first and the third cuts respectively. At the end of the experiment, around 47 % of the total P uptake by ryegrass was derived from the addition of organic wastes, except for the raw manure treatment where the Pdf fertiliser was only 38 % of the total P uptake. For the positive control (C + P), around 51 % of the total P uptake by ryegrass was derived from the mineral P added (Fig. 3d).

3.5. Fertiliser value of the different organic wastes tested

The apparent P recovery of the different organic wastes tested (calculated by the difference from the negative control) varied significantly (Fig. 4a). Treatments with manure (M) and manure co-digested with the organic fraction of municipal solid waste combined with Fertigro® and ash (DM + OFMSW + F + ASH) and digested manure combined with Fertigro® and ash (DM + F + ASH) had the lowest recovery with values of 10.8, 11.6 and 13.2 %. The apparent P recovery of Fertigro® added alone was 17.1 %. The remaining treatments showed a similar recovery to each other, reaching average values of 20 %. However, these values were substantially lower than the apparent P recovery from the positive control (48 %).

Fertiliser P recovery (Fig. 4b), calculated using the isotope dilution approach, was higher for the organic wastes compared with the positive control than the difference observed between organic wastes and mineral control in the apparent recovery, as the increased contribution from seed and especially soil in the positive control could be identified and subtracted in this measurement. However, the statistical differences between the fertilisers remained unchanged compared with those observed for the apparent P recovery, except for Fertigro®, which showed an improved recovery compared with the other treatments when calculated in this way.

3.6. Soil pH and electrical conductivity

Soil pH at harvest time varied for the different organic wastes added and the mineral P treatment (Fig. 5a). The addition of mineral P (positive control) slightly, but significantly, decreased the soil pH to 6.39 compared with the negative control, which was pH 6.5. Fertilisation with manure and ash containing digestates increased soil pH to 6.8 on average. The addition of Fertigro® resulted in a substantial decrease in soil pH to 5.5. This soil acidification was also observed when Fertigro® was combined with either digested manure or manure co-digested with the organic fraction of municipal solid waste, resulting in soil pH values of 6.0.

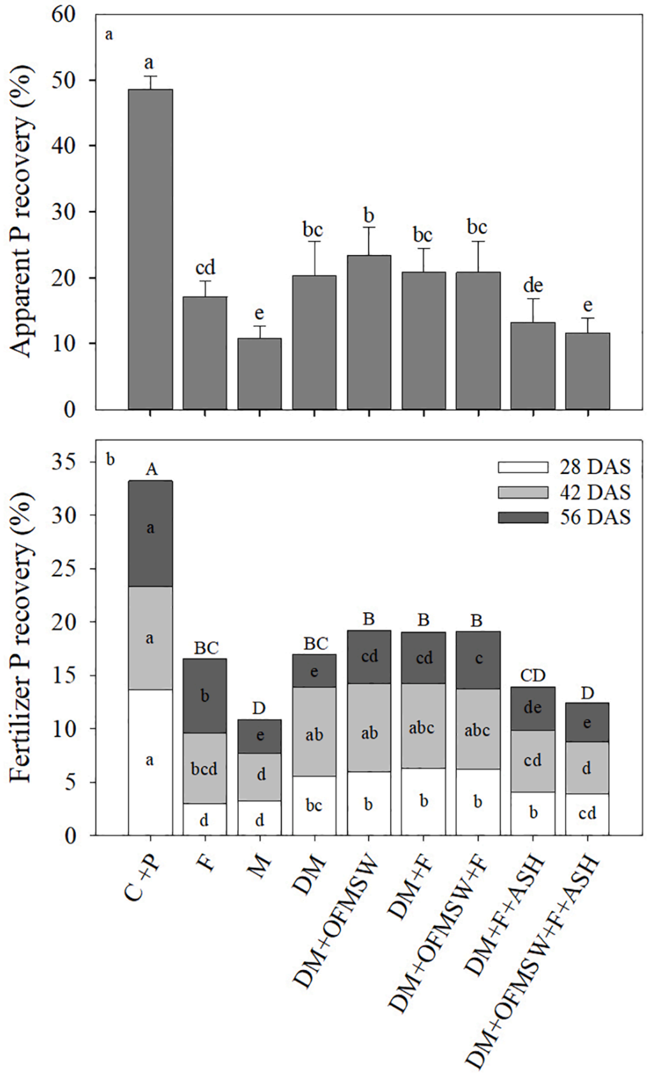

Fig. 4. Apparent P recovery (a) and fertiliser P recovery (b) for ryegrass amended with mineral P (C + P), no P (C – P), Fertigro® (F), cattle manure (M), digested cattle manure (DM), cattle manure co-digested with the organic fraction of municipal solid waste (DM + OFMSW), and their combination between DM + F, DM + OFMSW + F, DM + F + ASH and DM + OFMSW + F + ASH. Bars are the mean of four replicates and error bars denote standard deviation. Different small letters show a significant difference between treatments in the same cut, whereas different capital letters show significant differences in the total fertilizer P recovery between all the treatments at the end of the experiment.

Fertilisation with manure, digested manure or manure co-digested with the organic fraction of municipal solid wastes did not affect electrical conductivity in the soil at the end of the experiment compared with the negative and positive controls (Fig. 5b). However, fertilisation with Fertigro® significantly increased the electrical conductivity in soil up to 1.14 mS cm^{-1}. This effect of Fertigro® was also observed in the mixtures. The addition of Fertigro® to digested manure and co-digested manure with the organic fraction of municipal solid wastes (DM + OFMSW + F) resulted in 0.66 and 0.78 mS cm^{-1}, respectively, whereas the values were 0.63 and 0.54 mS cm^{-1} when these mixtures were combined with ash from straw (DM + OFMSW + F + ASH).

4. Discussion

While the waste treatments generally resulted in higher P uptake and plant growth than the negative control (C – P), the relationship between P uptake and the amount of P added was not straightforward. This complexity can be attributed to biological and chemical interactions between materials used for the mixtures, as discussed below. None of the organic wastes matched the plant growth and P uptake observed when plants were fertilised with mineral P. Jamison et al. (2021) recently studied the effect of two digestates from different feedstocks (food waste and lignocellulosic biomass) and their mixtures with mineral fertilisers on N and P uptake. Digestates alone and the mixtures showed a similar N uptake to the positive control. However, in the same study, P uptake in plants treated with the organic wastes was lower than that observed for the positive control, although larger quantities of P were applied with the organic wastes because the experiment was designed on the basis of equal N application. Low P availability has also been found in soil treated with Fertigro®. Case and Jensen (2017) report that only 24 % of the total P added with Fertigro® was quantified as WEP after 90 days of soil incubation. These findings, similar to those of the present study, confirm that although organic wastes contain significant amounts of P, their availability for plant uptake can be low, at least in the short term.

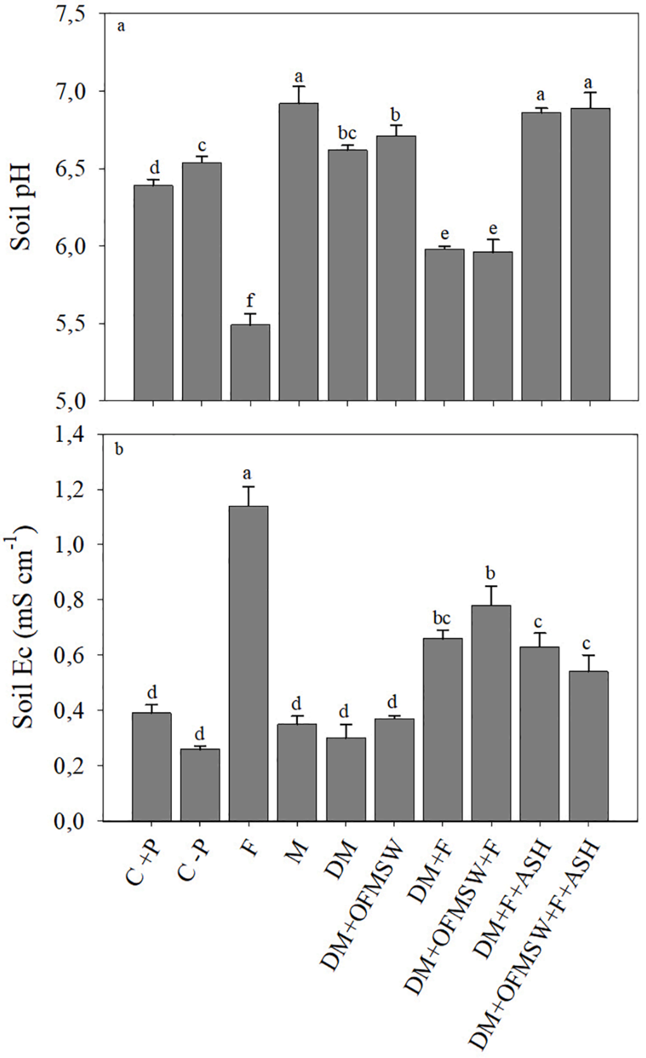

Fig. 5. Soil pH (a) and electrical conductivity (b) at the end of the experiment in soil amended with mineral P (C + P), no P (C – P), Fertigro® (F), cattle manure (M), digested cattle manure (DM), cattle manure co-digested with the organic fraction of municipal solid waste (DM + OFMSW), and their combination between DM + F, DM + OFMSW + F, DM + F + ASH and DM + OFMSW + F + ASH. Bars are the mean of four replicates and error bars denote standard deviation. Different small letters show a significant difference between treatments.

4.1. Plant P concentrations and growth

Grasses are known to be highly adaptive with regard to P availability, but tissue P concentrations below 2 mg g^{-1} are growth-limiting for many types of grass (de Bang et al., 2020), and grasses with a concentration below 1.1 mg P g^{-1} have been found to be critically deficient (Gastler and Moxon, 1944). Thus, even the P concentrations in the positive control were sub-optimal in the third cut, while those found in the negative control were clearly deficient. With the exception of the Fertigro® treatment, the organic waste treatments were P limited, with concentrations in the range of 1.2–1.5 mg P g^{-1} shoot in the first two cuts and at or near deficiency with concentrations around 1 mg P g^{-1} shoot in the third cut. Since luxury uptake of P was not observed in these treatments (except perhaps in the first cut in the Fertigro® treatment), the P uptake and shoot growth reflect the plant availability of P.

Plants respond to P deficiency by increasing the root-to-shoot ratio and changing root architecture, e.g. expressed by more secondary roots to allow a more thorough exploration of soil P resources (Gómez-Muñoz et al., 2018; Raymond et al., 2020; Richardson et al., 2009). They may further increase P uptake efficiency by increasing root hair length (Wang et al., 2016). In addition, the application of organic amendments can enhance the proliferation of natural arbuscular mycorrhizal fungi (Alguacil et al., 2009; Harinikumar et al., 1990), which are well known to improve plant access to P due to greater soil exploration, high-affinity P uptake and transport of P to the plant (Jakobsen et al., 2005). The root observations from the current experiment are limited to the actual root mass of ryegrass at the end of the experiment (day 56) and therefore do not give a detailed picture.

4.2. Effects of anaerobic digestion

Möller and Müller (2012) reviewed the effects of anaerobic digestion on digestate nutrient availability and crop growth, and found evidence that mineralisation processes during anaerobic digestion may improve plant P availability. However, they also emphasised that an increase in pH associated with digestion favours the formation of calcium (Ca) and magnesium (Mg) phosphates. However, while such precipitates (e.g. struvite) are not very water-soluble, they have been shown to be plant available (Muys et al., 2021). It is notable that soil pH increased more in the manure treatment (to 6.9) than in the digestate treatments (to 6.6–6.7), which may affect the availability of both fertiliser and soil P (see discussion below). Apart from pH effects, undigested manure is also less decomposed and has a higher content of microbially available carbon sources, which may lead to increased microbial immobilisation of P, thus limiting P uptake from the manure treatment. Similarly, in another pot experiment where organic wastes were applied according to their N content, lower N uptake was observed in raw manure compared with digested manure (Gómez-Muñoz et al., submitted for publication).

This could be one explanation for the low contribution from the soil P pool to plant P uptake in the raw manure treatment. Compared with raw manure, the digested manure had a much lower C/P ratio (24 vs 45), reflecting the decrease of easily decomposable C compounds during digestion. A similar C/P ratio (23.5) was found in manure co-digested with the organic fraction of municipal organic waste. This means that the more recalcitrant carbon remaining in digestates would cause lower P immobilisation when mixed in the soil, compared with the raw manure. The present data revealed that digested manure with or without co-digested municipal organic waste resulted in the highest yields of shoot dry matter and substantially higher P uptake as well as fertiliser P recovery compared with raw manure. Therefore, the hypothesis that decreased P availability is expected after digestion of manure and municipally sorted organic waste was rejected. Li et al. (2020) found evidence of an increased proportion of less soluble P species after anaerobic digestion of pig, chicken and dairy manure. Bachmann et al. (2014, 2011), however, found no significant differences in P availability of digested vs. undigested dairy slurry under field conditions. Significantly higher P availability from digested treatments regardless of the feedstock used (with and without the organic fraction of municipal solid wastes), as found in this study, may at least be partly due to reduced P immobilisation with digestate compared with raw manure.

4.3. Effects of ash amendment

Ash-amended digestates produced a substantial decrease in P uptake compared with the positive control in all three ryegrass cuts, although plant P availability of biomass combustion ashes has been reported to be comparable with mineral fertiliser (Li et al., 2016).

Since the ash was mixed with digestates before the amendment to soil, the formation of Ca and Mg phosphates, which according to Möller and Müller (2012) may decrease P availability, might have occurred. This is in accordance with an experiment of Moure Abelenda et al. (2021) who incubated an anaerobic digestate with wood fly ash and observed a 100-times decrease in P solubility, which they attributed to the alkalinity and high Ca content of the ash, leading to the precipitation of P. Furthermore, the soil pH in the treatment with ash at the end of this experiment increased to around 6.9, similar to the manure treatment that also showed a low P availability. Barrow (2017) and Barrow et al. (2020) argue that the conventional belief that phosphate availability is greatest at near neutral pH is incorrect and that the optimum pH is much lower. However, Penn and Camberato (2019) conclude that while real exceptions to the widely accepted assumption of maximum P availability at near neutral pH can occur, the classic textbook recommendation is generally sound.

While it cannot be ruled out that formation of insoluble P compounds may have occurred due to the admixture of ash in digestates prior to the addition of soil, there is evidence that struvite-like compounds are plant-available, particularly in soils with a pH below 6 (Hertzberger et al., 2020; Muys et al., 2021). In the absence of additional easily available carbon in ash (as in the case of manure compared with digestates), it is reasonable to think that the increase in soil pH may be a cause of the observed decrease in availability, as it affected P derived from both soil and fertiliser. This supports our first hypothesis that changes in soil pH derived from the application of organic wastes determine the amount of plant P available.

4.4. Effects of Fertigro® and Fertigro® mixtures

Some of the treatments with Fertigro® (Fertigro®, digested manure combined with Fertigro® and manure co-digested with the organic fraction of municipal solid wastes combined with Fertigro®) had higher initial plant P concentrations than the other waste treatments, while showing low or moderately low shoot growth in the first cut. The Fertigro® treatment reached even higher concentrations than the positive control while exhibiting much lower root biomass at the time of harvest. This is a clear indication of toxicity or other stress, and while the digested manure combined with Fertigro® and manure co-digested with the organic fraction of municipal solid wastes combined with Fertigro® treatments had similar yields as the manure treatment, their root:shoot ratios were significantly lower. These findings support our second hypothesis that the salinity–mediated stress due to the application of organic wastes such as Fertigro® can affect ryegrass growth and P uptake.

Fertigro® contains substantial quantities of sodium chloride (NaCl) used for conditioning the mucosa prior to extraction of heparin, and sodium bisulfite ($NaHSO_3$), which is added to avoid decay during storage of the waste product. The NaCl is likely to have caused the decrease in root growth of the Fertigro® treatment. In fact, the electrical conductivity measured in the soil at harvest (56 DAS) was extremely high for the Fertigro® treatment, followed by the mixtures of Fertigro® with digested manure and manure co-digested with the organic fraction of municipal solid wastes. However, their combination with ash showed a slightly lower electrical conductivity due to the smaller amount of

Fertigro® used in these treatments. The amount of $NaHSO_3$ remaining in Fertigro® after storage is likely to contribute to the decrease in pH recorded at harvest, as bisulfite would be oxidised to sulphate during the pot experiment. In fact, a parallel study observed how Fertigro® showed similar ryegrass growth and even higher S uptake than the application of the same amount of S as mineral fertiliser (Gómez-Muñoz et al., submitted for publication). In the same experiment, a decrease in soil pH and an increase in the electrical conductivity measured in soil at harvest was also observed when Fertigro® was applied compared to the control soil.

4.5. Field vs pot growth – Management perspectives

There is a great difference between field and pot experiments as pot experiments often show higher statistical certainty but also have substantially different growth conditions. Under field conditions, crops are sown a few weeks after the application of organic waste to favour their mineralisation, while in this pot experiment, the organic wastes were applied on the same day of sowing, therefore a low nutrient availability can be expected. In the context of this paper, it is especially relevant to emphasise the rather small soil volume provided by the pot. In a field situation, the plant roots would be able to explore a much greater soil volume, which would probably cause a greater soil contribution to P uptake. Therefore, in spite of the high availability of P in digestates, their P fertilising effect on crop yields is reported to be quite variable, ranging from no significant effect (Loria and Sawyer, 2005; Möller et al., 2008) to positive effects (Bachmann et al., 2014; Odlare, 2005) under field conditions. In contrast, in pot experiments, a positive effect of anaerobic digestates has often been found (Dahlberg et al., 1988; Kirchmann and Lundvall, 1993; Morris and Lathwell, 2004).

The stress to plant growth caused by Fertigro® would not necessarily be as prominent as was apparent from this pot experiment. In practice, Fertigro® is recommended for use mainly on barley crops, known to be tolerant towards salinity stress (Ligaba and Katsuhara, 2010), and it has not been reported to give rise to stress when used according to recommendations under Denmark's prevailing humid conditions (E. E. Olesen, personal communication). Furthermore, Fertigro® is recommended for use based on its N content, which would result in a lower application rate to the crop, than if applied on the basis of P, as it was done in the present study.

While effects on crop growth may be obscured under field conditions, the results from this experiment indicate that some caution is needed when mixing ashes into digestates in order to improve the K content (unless a reduction in P solubility is intended), and when using Fertigro® in a rotation including crops with a low salinity tolerance, even in a humid climate.

In order to assess the long-term benefits and drawbacks of nutrient sources such as Fertigro® and ash from straw as well as wood, it would be highly relevant to include such treatments in long-term experiments.

5. Conclusions

Using the indirect labelling technique in pot trials, the P recovery and dry matter production of amended ryegrass was found to vary significantly among treatments. Although none of the treatments reached the plant growth quantified in the positive control treatment, the greatest recovery was found with digested products, either manure alone or co-digested with municipal waste or mixed with Fertigro®. However, the mixture of digested manure and Fertigro® gave rise to lower dry matter production, and Fertigro® used alone decreased soil pH and increased the electrical conductivity in soil. This could have caused salinity effects, resulting in high leaf P concentrations but reduced shoot and root growth. Digestion of manure increased the availability of P in soil compared with raw manure, presumably due to the lower immobilisation potential of the digested organic matter, whereas mixing with ash probably resulted in decreased P solubility and an increase in soil pH at the end of the experiment. This highlights potential challenges when attempting to improve the N:P:K:S ratios of waste-based fertilisers due to interactions between the different fertiliser materials. Field trials are needed to validate these findings using a realistic soil volume and over the longer term.

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.2022.115939.

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