Nutrient interactions and salinity effects on plant uptake of P from waste-based fertilizers

Gómez-Muñoz, B., Müller-Stöver D., Hansen, V., Jensen, L.S., and Magid, J.

Abstract

A large number of European organically managed farms have low levels of soil P and it has been shown that arable farms that rely strongly on biological nitrogen fixation have rather low outputs, and tend to deplete soil phosphorus and potassium resources, whereas arable farms with lower reliance on BNF and higher reliance on external inputs have much higher outputs and low or no soil resource depletion. Therefore, research focusing on providing P, as well as N, K and S from alternative sources, is of interest to organically managed farms not only in Denmark but in Europe as a whole.

The aim of this study was to quantify P uptake in ryegrass grown in pots from different organic wastes applied alone or in combination with other organic wastes to improve the N:P:K:S ratio. We used the indirect labelling technique, where a non-labelled fertilizer is added into a soil that has been preincubated and equilibrated with labelled ^{33}P.

The apparent P recovery of the different organic wastes tested varied significantly, with the lowest recovery from manure and digested products admixed with ash from straw. The highest recovery was found in digested products, either manure alone or mixed with municipal waste or Fertigro. However, the mixture of digested manure and Fertigro gave rise to lower dry matter production, and Fertigro used alone gave rise to depressed shoot and root growth. The evidence points to a suite of effects. The Fertigro gave rise to salinity effects and a decrease in pH resulting in high leaf P concentrations but reduced shoot and root growth.

Digestion increased the availability of P, presumably due to the lower immobilization potential of the added organic matter. Mixing digested manure with ash increased resulted in an increase of the soil pH at the end of experiment, which may explain the lower P availability. This points to potential challenges when attempting to improve the N:P:K:S ratios of waste-based fertilizers, due to nutrient interactions. Such effects are presumably overexpressed in pot trials that have very limited soil volume, why field trials are needed to quantify such effects in practise.

Keywords: organic wastes, P availability, ^{33}P labelling technique, nutrient interactions, salinity effects

1. Introduction

In response to the increasing consumer demand for organic farming products, and in recognition of the services rendered to the environment, the European Commission will promote the expansion of organic farming to at least 25% of the EU's agricultural land by 2030 (EU 2020). This emphasizes the need for organic agriculture to increasingly take part in the evolving circular economy (Løes and Adler 2019), posing new challenges both practically and regulatory. Danish organic farmers have long debated the unsustainable 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. Subsequently they had to moderate this decision, due to the lack of acceptable alternatives, in favour of a more gradual approach to replenishing fertility from alternative sources. (Oelofse et al. 2013) discussed the implications of phasing out conventional nutrient supply in organic agriculture and proposed that organic farmers consider the suitability of nutrient sources available in alternative, non-farm organic waste streams for use in organic systems. A working group was established to do so and identified several locally abundant waste streams that would be of interest for future recycling to Danish organic farms and could potentially be acceptable under the EU legal code for fertilization (EC no 889/2008, Annex I). In this paper, we examine some of these resources. We assess how they may be utilized to mix into anaerobically digested manure or co-digested with manure, focusing on the plant uptake of P from such composite materials. Co-digestion may be relevant for energy rich resources, whereas subsequent mixing with digestates may be a way to modify and potentially improve the N:P:K:S ratios of the fertilization product.

A large number of European organically managed farms have low levels of soil P (Cooper et al. 2018). It has been shown that arable farms that rely strongly on biological nitrogen fixation (BNF) have rather low outputs (Reimer et al. 2020), and tend to deplete soil phosphorus and potassium resources, whereas arable farms with lower reliance on BNF and higher reliance on external inputs have much higher outputs and low or no soil resource depletion. Therefore, research focusing on providing P, as well as N, K and S from alternative sources, is of interest to organically managed farms not only in Denmark but in Europe as a whole.

Among the material types identified as abundant and potentially acceptable for Danish organic farmers were sorted municipal organic household waste, ashes from straw-fired combined heat- and power plants, and Fertigro® - a mucosa residue from the production of Heparin. Ashes from straw are known to modify the pH of soils (Schiemenz and Eichler-Löbermann 2010) thereby potentially affecting phosphorus availability (Barrow 2017; Penn and Camberato 2019). The mucosa from pig guts is conditioned with NaCl in order to allow extraction of Heparin molecules by ion exchange, and the remains are further stabilized with sodium bisulfite before distribution as Fertigro®. It is currently used by Danish conventional farmers for barley, known to be tolerant towards salinity stress (Ligaba and Katsuhara 2010), but salinity issues could potentially be a limitation for other crops. It is appreciated primarily as a Nitrogen fertilizer with high content of Sulphur compounds, which could make it interesting for organic farmers, as besides Nitrogen, Sulphur is believed to be yield limiting particularly in organic farms (Eriksen et al. 2004; Eriksen 2009). Finally, sorted municipal organic waste was identified as a potential source of nutrients, albeit little is known on how it affects the nutrient release after co-digestion with manure.

The aim of this study was to quantify P uptake in ryegrass from different organic wastes applied alone or in combination with other organic wastes to improve the N:P:K:S ratio. We used pot trials, not only for reasons of economy, but also because positive as well as negative effects are more likely to register due to the lower soil volume which enhances fertilizer impacts. 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 2011). We use the indirect labelling technique, where a non-labelled fertilizer is added into a soil that has been preincubated and equilibrated with labelled (e.g. ^{33}P) fertilizer (Kucey and Bole 1984; Morel and Fardeau 1989). This method has been successfully used to quantify plant P uptake from different P sources such as rock phosphate, sewage sludge, compost or manure (Fardeau et al. 1988; Frossard et al. 1996; Sinaj et al. 2002; Oberson et al. 2010).

We hypothesized that, assessing ryegrass growth and P uptake from soil and fertilizer, the efficiency of treatments relative to triple superphosphate will be determined by: 1) pH effects of fertilizers on soil matrix reactivity, 2) salinity mediated stress, 3) effects of digestion on manure and municipally sorted organic waste.

2. Material and Methods

2.1. Soil and organic wastes

The soil used in this experiment was a sandy loam soil of moderate fertility collected from the Long-Term Nutrient Depletion Trial at the University of Copenhagen's experimental farm in Taastrup, Denmark (55°40′N, 12°17′E). This soil was depleted in P and K, receiving only mineral N for 30 years, but since 1996 fertilized 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 7.13 and the electrical conductivity 0.04 mS cm^{-1}, both quantified in 1:5 soil:MiliQ 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 amount of plant available nutrients in soil was 4.43 µg $P\text{-}PO_4^-$ g^{-1}, 2.58 µg $N\text{-}NO_3^-$ g^{-1} and 0.60 µg $N\text{-}NH_4^+$ g^{-1}.

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

2.2. The pot experiment

Soil equivalent to 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 2 days of air-drying the soil was thoroughly mixed to ensure the homogeneous distribution of nutrients in soil. MilliQ water was added to each soil bag to reach 30% water holding capacity (WHC) 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 labeled by adding 5 ml of a carrier-free ^{33}P-orthophospate solution to achieve 2.5 MBq kg soil $^{-1}$. Soil and solution were carefully mixed for 2 minutes. To reach near-equilibrium for ^{31}P and ^{33}P, the labeled soil was incubated in double plastic bags for one week in the growth chamber using the same settings that were used for the rest of the plant experiment (see below) (Nanzer et al. 2014).

At the day of sowing, the following treatments were set up in four replicates: 1) a positive control amended with ^{33}P-labeled KH_2PO_4 (C+P), 2) a negative control with no P added (C–P), 3) Fertigro (F), 4) raw cattle manure (M), 5) digested cattle manure (DM), 6) digested cattle manure with the organic fraction of municipal solid waste (DM+OFMSW), 7) digested manure combined with Fertigro (DM+F), 8) digested cattle manure 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 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 to add 50 mg P kg^{-1} soil. For those treatments composed by more than one organic waste, the mixture was prepared by adding the same amount of P from each organic waste. For example, in the treatment 7): 25 mg P kg^{-1} was added as digested manure, whereas another 25 mg P kg^{-1} was added with Fertigro. The positive control was prepared using unlabeled soil that was amended with 5 ml of KH_2PO_4 solution, adding 50 mg P kg^{-1} soil labeled with carrier-free ^{33}P-orthophospate with a specific activity of 50 KBq mg P^{-1} or 2.5 MBq kg^{-1} soil. Soil and organic wastes were thoroughly mixed and filled into pots with closed bottom to a bulk density of approx. 1.4 g cm^{-3}. Each pot was sown with 2 g of perennial ryegrass (*Lolium perenne* ver. 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 hours, 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 regularly randomly distributed and 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 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 harvest, shoot biomass dry matter was determined by drying in an oven for 48 hours 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 hours 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™). The values were corrected for radioactive decay back to the day of labeling. 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.

2.3. Seed P contribution

P derived from seeds was determined using the direct labeling approach in an additional pot experiment. The details for this pot experiment are described by Hansen et al., (in preparation) 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) which was amended with the nutrient solution described above and left to dry for 4 days. After that, the sand was mixed and transferred to a pot where a solution containing carrier-free ^{33}P-orthophsophate 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 sand^{-1}) and with a specific activity (SA) of 65.1, 36.7, 18.6, 9.7 and 5.1 KBq mg P^{-1}. All treatments were replicated four times. Ryegrass seeds were sown at a rate of 2 g pot^{-1}. Growing conditions, handling of pots, harvest and plant analyses were identical to those described above for the pot experiment. 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 pot^{-1} soil) was calculated according to the following equation (Nanzer et al. 2014):

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

To solve this equation, the principles described by Frossard et al. (2011) were used:

Pdf seed (mg P pot^{-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:

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

Pdf fertilizer was calculated using equation 3:

$$\text{Pdf fertilizer (\%)} = 100 \text{ x } (1 - SA_{plant} / SA_{fertilizer}) \quad (3)$$

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

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

First cut: $y = 0.2515x + 1.6192$, $R^2 = 0.9788$ (4)

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

Third cut: $y = 0.1202x + 1.6334$, $R^2 = 0.9499$ (6)

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

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

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

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

The fertilizer P recovery (%) in the ryegrass shoot biomass was calculated by comparing the Pdf fertilizer to the amount of P applied.

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

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

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

2.5. Statistical analysis

All variables studied were checked for normality of residuals and homogeneity of variance using diagnostic plots, and log-transformation was used when data were not normal distributed. Statistical differences were tested using one-way ANOVA with treatment as factor. The differences between fertilizer treatments were analyzed using Tukey's HSD 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 the RStudio 1.2.5042 (RStudio Team 2017).

3. Results

3.1. Plant growth

Ryegrass shoot growth varied among the organic wastes tested (Figure 1). For the first cut, after 4 weeks of growing, the highest shoot biomass was observed for the positive control as expected, but similar growth was also observed for treatments with digested manure and the digested manure with the organic fraction of municipal solid waste. For the rest of the organic wastes tested, the shoot growth was similar to the negative control, except for treatments with Fertigro and digested manure with the organic fraction of municipal solid waste combined with Fertigro, where the shoot growth was significantly lower than the negative control. For the second cut, only digested manure reached the shoot growth observed for the positive control, whereas the rest of organic wastes showed lower shoot biomass than the former two treatments but higher than the negative control. After 56 days of growing, 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 the rest of the treatments. Therefore, at the end of the experiment, the positive control resulted in higher total shoot biomass compared to the rest of the treatments. Fertilization significantly increased the shoot biomass of ryegrass compared to the negative control, by the following order: digested manure = digested manure with the organic fraction of municipal solid waste > digested manure with Fertigro and ash, digested manure with the organic fraction of municipal solid waste and Fertigro and ash. No significant differences were obtained for the rest of the organic wastes tested and the negative control treatment.

The addition of organic wastes also affected ryegrass root growth (Figure 1). 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 treatments with Fertigro and digested manure with the organic fraction of municipal solid waste, that significantly reduced root growth compared to the negative control. For this reason, the root:shoot ratio was significantly lower in these two treatments (Figure 1). For raw manure and digested manure combined with Fertigro and ash the root:shoot ratio was not different to those calculated for the positive and negative controls.

3.2. Plant P concentration

Shoot P concentration of ryegrass grown in soil amended with Fertigro was significantly higher than the rest of the treatments for 28 DAS (Figure 2), with values even greater than 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 digested manure 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, P concentration measured in the plants fertilized with Fertigro was significantly lower than the positive control. For the rest of the treatments, shoot P concentrations in the second and the third cut were very similar, although a significantly higher P concentration was observed when the mix included Fertigro (digested manure combined with Fertigro and digested manure with the organic fraction of municipal solid waste combined with Fertigro).

3.3. Plant P uptake

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

3.4. P pools

Overall, the differences for the P uptake derived from the soil pool observed between the organic waste treatments were similar at 28 and 42 DAS (Figure 3), whereas 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.

At the end of the experiment, the highest contribution of P derived from soil was observed for the positive control with 8.0 mg P pot^{-1}, followed by the application of digested manure alone and digested manure with the organic fraction of municipal solid waste, where Pdf soil was 4.2 and 4.4 mg P pot^{-1}, respectively. For the rest of the treatments, Pdf soil ranged from 2.4 to 3.8 mg P pot^{-1} with the lowest values observed when ash was included in the mixture resulting in an uptake even lower than the negative control. The contribution of the P from the soil pool was in the range of 15 to 20% of the total P uptake.

Phosphorus derived from the seed provided a substantial contribution to the total P uptake and increased with increasing total uptake, mainly at 28 DAS (Figure 3). Thus, for the first month of growing, the P uptake derived from the seed was 5.6 mg P kg^{-1} for the positive control. For the digested manure, digested manure with the organic fraction of municipal solid waste, digested manure combined with Fertigro and digested manure with the organic fraction of municipal solid waste combined with Fertigro treatments, Pdf seed was on average 3.6 mg P pot^{-1}, whereas, for the rest of treatments, Pdf seed was lower with values between

2.4 to 3.1 mg P pot^{-1}. For the second and the third cut, Pdf seed was intermediate and the differences between treatments decreased, being on average 1.3 mg P pot^{-1} for the second cut and 2.4 mg P pot^{-1} for the third cut. At the end of the experiment, P derived from seed contributed with 34 to 43 % to the total P uptake of the organic waste treatments. The increase in Pdf seed in the third cut was not in accordance with results from other studies, where a decrease in seed P contribution over time was observed (refs). The different harvest protocol developed in the third cut may be responsible for the high Pdf seed calculated at the last sampling time. Whereas in the first and the second cuts, 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, we quantified the amount of shoot biomass from 0-3 cm and above 3 cm, showing that 58% of the total ryegrass biomass was found in the fraction from 0 to 3 cm. 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 the ryegrass at the soil surface would include part of the biomass that was already present at the first and the second cut.

P derived from added fertilizer differed in the three cuts carried out in this experiment (Figure 3). The highest fertilizer contribution to the total P uptake was observed for the positive control in all the cuts, with 7.5, 5.3, and 5.4 mg P pot^{-1} for the first, second and third cut, respectively. Digested manure, digested manure with the organic fraction of municipal solid waste, digested manure combined with Fertigro and digested manure with the organic fraction of municipal solid waste combined with Fertigro treatments showed intermediate Pdf fertilizer amounts with 3.3, 4.4 and 2.6 mg P pot^{-1} on average for the first, second and third cut, respectively. Pdf fertilizer in the rest of the treatments (manure, and digestates with ash) was lowest. Fertilization with Fertigro resulted in an increase in the Pdf fertilizer over time from 1.6 to 3.8 mg P pot^{-1} for the first and the third cut, 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 fertilizer was only 38 % of the total P uptake.

3.5. Fertilizer value of the different organic wastes tested

The apparent P recovery of the different organic wastes tested varied significantly (Figure 4a). Treatments with manure and digestates with ash had the lowest recovery with values of 10.8 and 11.6 % respectively. Slightly, but significantly higher recovery (13.2 %) was found when Fertigro was added to the ash containing manure digestate, while the remaining treatments showed similar recovery reaching average values of 20 %. However, these values were substantially lower than apparent P recovery from the positive control (48%).

Fertilizer P recovery (Figure 4b) calculated using the isotope dilution approach for the organic wastes tested compared somewhat better to the positive control than the apparent recovery, as the increased contribution from seed and especially soil in the positive control can be identified and subtracted in this measure.

3.6. Soil pH

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

4. Discussion

While the waste treatments generally resulted in higher P uptake and plant growth than the unamended control, their effects were complex, and far from showing a straightforward relationship with the amount of P added. This can be attributed to biological and chemical interactions across treatments, as will be discussed below.

4.1 P concentrations and growth.

Grasses are known to be highly adaptive with regard to P concentrations, but levels below 2 mg g^{-1} are growth limiting for many grasses (de Bang et al. 2020), and range grasses with a concentration below 1.1 mg P g^{-1} were found to be critically deficient (Gastler and Moxon 1944). Thus, even the P concentrations in the positive control were sub-optimal, 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 declining towards 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 of Fertigro), 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 change root architecture by expressing more secondary roots to allow a more thorough exploration of soil P resources (Tansley review + (Richardson et al. 2009) +(Gomez-Munoz et al. 2018). They may further increase the P uptake efficiency by increasing root hair length (Wang et al. 2016). The root observations from the current experiment are limited to the actual root mass of ryegrass at the end of experiment (day 56), and therefore do not give a detailed picture. The positive control attained the highest P uptake and the highest root biomass but was least affected by P deficiency, and may therefore have relied more on thicker and heavier primary roots. Among the remaining treatments there is a negative relationship between the root mass and the total P uptake at day 56 (please calculate R2, p>0.xx), when excluding the F treatment that showed signs of salinity related root growth stress.

4.2 Effects of anaerobic digestion

Compared to 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 municipal organic waste. This entails that the more recalcitrant carbon remaining in digestates would cause lower P immobilization when mixed in soil, compared to the raw manure. (Möller and Müller 2012) reviewed the effects of anaerobic digestion on digestate nutrient availability and crop growth and found evidence that degradation processes during anaerobic digestion may improve phosphorus (P) plant availability. By contrast, (Möller and Müller 2012) emphasized that increase in pH associated with digestion favours the formation of calcium (Ca)- or magnesium (Mg)-phosphate, thus potentially decreasing the solubility of P and micronutrients. 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 fertilizer and soil P, but see discussion below. Our data shows that digested manure with or without co-digested municipal organic waste gave the highest yields of shoot dry matter, substantially higher P uptake, as well as fertilizer P recovery, comparing favourably to raw manure. Apart from pH effects, microbial immobilization from less decomposed and less recalcitrant carbon sources may be important for limiting P uptake from the manure treatment.

4.3 Effects of ash amendment

Ash amended digestates gave rise to a substantial decrease of P uptake in all three ryegrass cuts. Since the ash was mixed with digestates before amendment to soil, formation of Ca and Mg phosphates that may decrease P availability according to (Möller and Müller 2012) could have occurred. Furthermore it is notable that the soil pH at the end of the experiment had increased to around 6.9, similar to the manure treatment that also showed a low P availability. (Barrow et al. 2020) have vigorously argued that the conventional belief that phosphate availability is greatest near neutral pH is wrong, and that the optimum pH is much lower (Barrow 2017). On the other hand (Penn and Camberato 2019) conclude that while real exceptions to the rule of thumb of maximum P availability at near neutral pH can occur, the classic textbook recommendation is generally sound. While we cannot exclude that formation of insoluble P compounds may have occurred due to admixture of ash in digestates prior to addition of soil, there is evidence that struvite-like compounds are plant available (Muys et al. 2021)(refs). Thus, in absence of additional easily available carbon in ash (as in the case of manure compared to digestates) it is reasonable to think that the increase in soil pH may be a cause for the observed decrease in availability, as it affected both P derived from soil and fertilizer.

4.4. Effects of Fertigro and Fertigro amendment.

Some of the treatments with Fertigro (F, DM+F and DM+OFMSW+F) had high initial P concentrations compared to the other waste treatments, while having low or moderately low shoot growth in the first cut. The Fertigro treatment reached even higher concentrations than the positive control (Fig. 2), while exhibiting a much lower root biomass at the time of harvest (Fig. 1). This is a clear indication of toxicity or other stress, and while the DM+F and DM+OFMSW+F treatments had similar yields as the M treatment, their root:shoot ratios were significantly lower.

Fertigro contains substantial quantities of sodium chloride (NaCl) used for conditioning the mucosa prior to extraction of heparin, and sodiumbisulfite ($NaHSO_3$) 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, while $NaHSO_3$ remaining after storage is likely to contribute to the decrease in pH recorded at harvest, as bisulfite would be oxidized to sulphate during the pot experiment.

Field vs pot growth – management perspectives

There is a great difference between field and pot experiments, that often show higher statistical certainty, but also have substantially different growth conditions. In the context of this paper, it is especially relevant to emphasize the rather small soil volume constrained by the pot. In a field situation the plant roots would be able to explore a much greater soil volume which would likely cause a greater soil contribution of P to uptake. Therefore, in spite of the high availability of P in digestates, the P fertilizing effect of anaerobic digestates on crop yields is reported to be quite variable from no significant effect (Loria and Sawyer 2005; Möller et al. 2008) to positive effects (Odlare 2005) under field conditions. By 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 is apparent from this pot experiment. In practise, Fertigro is recommended to be used 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 the prevailing humid conditions in Denmark (E.E. Olesen, personal communication). Furthermore, Fertigro is recommended to be used based on its nitrogen content, which would result in a lower input to the crop, than if applied on the basis of P.

While effects on crop growth may be obscured under field conditions, the results from this experiment indicate that some caution should be taken when mixing ashes into digestates in order to improve the K content, and there is further need for caution 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 challenging sources such as Fertigro and ashes from straw as well as wood, it would be highly relevant to include such treatments in long-term experiments.

4. Conclusions

Using the indirect labelling technique in pot trials, we found that the apparent P recovery and the dry matter production of amended ryegrass varied significantly among treatments. The highest recovery was found in digested products, either manure alone or mixed with municipal waste or Fertigro. However, the mixture of manure and Fertigro gave rise to lower dry matter production, and Fertigro used alone gave rise to depressed shoot and root growth. The evidence points to a suite of effects. The Fertigro gave rise to salinity effects and a decrease in pH resulting in high leaf P concentrations but reduced shoot and root growth. Digestion increased the availability of P, presumably due to the lower immobilization potential of the added organic matter, whereas mixing with ash increased resulted in an increase of the soil pH at the end of experiment. This points to potential challenges when attempting to improve the N:P:K:S ratios of waste-based fertilizers, due to nutrient interactions. Such effects are presumably overexpressed in pot trials that have very limited soil volume, why field trials are needed to quantify such effects in practise.

ACKNOWLEDGEMENT

We acknowledge support from the Green Development and Demonstration Program (GUDP: NutHY project) coordinated by the International Centre for Research in Organic Food Systems (ICROFS), and support from the RELACS project (Replacement of Contentious Inputs in organic farming Systems), which has received funding from the European Union's Horizon 2020 research and innovation programme under the European Union's Horizon 2020 research and innovation programme grant agreement No 773431. This publication reflects the views only of the authors, and the funding bodies cannot be held responsible for any use, which may be made of the information contained therein.

References

Barrow NJ (2017) The effects of pH on phosphate uptake from the soil. Plant Soil 410:401–410. https://doi.org/10.1007/s11104-016-3008-9

Barrow NJ, Debnath A, Sen A (2020) Measurement of the effects of pH on phosphate availability. Plant Soil 454:217–224. https://doi.org/10.1007/s11104-020-04647-5

Brookes PC (1982) Correction for seed-phosphorus effects in L-value determinations. J Sci Food Agric 33:329–335. https://doi.org/10.1002/jsfa.2740330405

Christiansen NH, Sørensen P, Labouriau R, et al (2020) Characterizing phosphorus availability in waste products by chemical extractions and plant uptake. J Plant Nutr Soil Sci 1–13. https://doi.org/10.1002/jpln.202000015

Cooper J, Reed EY, Hortenhuber S, et al (2018) Phosphorus availability on many organically managed farms in Europe. Nutr Cycl AGROECOSYSTEMS 110:227–239. https://doi.org/10.1007/s10705-017-9894-2

Cruz-Paredes C, López-García Á, Rubæk GH, et al (2017) Risk assessment of replacing conventional P fertilizers with biomass ash: Residual effects on plant yield, nutrition, cadmium accumulation and mycorrhizal status. Sci Total Environ 575:. https://doi.org/10.1016/j.scitotenv.2016.09.194

Dahlberg SP, Lindley JA, Giles JF (1988) Effect of Anaerobic Digestion of Nutrient Availability From Dairy Manure. Trans Am Soc Agric Eng 31:1211–1216. https://doi.org/10.13031/2013.30846

de Bang TC, Husted S, Laursen KH, et al (2020) The molecular–physiological functions of mineral macronutrients and their consequences for deficiency symptoms in plants. New Phytol 2446–2469. https://doi.org/10.1111/nph.17074

Eriksen J (2009) Chapter 2 Soil Sulfur Cycling in Temperate Agricultural Systems. Adv Agron 102:55–89. https://doi.org/10.1016/S0065-2113(09)01002-5

Eriksen J, Thorup-Kristensen K, Askegaard M (2004) Plant availability of catch crop sulfur following spring incorporation. J Plant Nutr Soil Sci 167:609–615. https://doi.org/10.1002/jpln.200420415

EU (2020) Farm to Fork Strategy. DG SANTE/Unit 'Food Inf Compos food waste''' 23

Fardeau JC, Morel C, Jahiel M (1988) Does long contact with the soil improve the efficiency of rock phosphate? Results of isotopic studies. Fertil Res 17:3–19. https://doi.org/10.1007/BF01050453

Frossard E (2011) The Use of Tracers to Investigate Phosphate Cycling in Soil–Plant Systems. In: Bünemann E, Oberson A, Frossard E (eds) Phosphorus in Action, Soil Biolo

Frossard E, Achat DL, Bernasconi SM, et al (2011) The Use of Tracers to Investigate Phosphate Cycling in Soil–Plant Systems BT - Phosphorus in Action: Biological Processes in Soil Phosphorus Cycling. In: Bünemann E, Oberson A, Frossard E (eds). Springer Berlin Heidelberg, Berlin, Heidelberg, pp 59–91

Frossard E, Sinaj S, Zhang LM, Morel JL (1996) The fate of sludge phosphorus in soil-plant systems. SOIL Sci Soc Am J 60:1248–1253. https://doi.org/10.2136/sssaj1996.03615995006000040041x

Gastler GF, Moxon AL (1944) Calcium and Phosphorus Content of Grasses at Different Stages of Growth

Gomez-Munoz B, Jensen LS, de Neergaard A, et al (2018) Effects of Penicillium bilaii on maize growth are mediated by available phosphorus. Plant Soil 431:159–173. https://doi.org/10.1007/s11104-018-3756-9

Grigatti M, Boanini E, Mancarella S, et al (2017) Phosphorous extractability and ryegrass availability from bio-waste composts in a calcareous soil. Chemosphere 174:722–731. https://doi.org/10.1016/j.chemosphere.2017.02.039

Kirchmann H, Lundvall A (1993) Relationship between N immobilization and volatile fatty acids in soil after application of pig and cattle slurry. Biol Fertil Soils 15:161–164. https://doi.org/10.1007/BF00361605

Kratz S, Vogel C, Adam C (2019) Agronomic performance of P recycling fertilizers and methods to predict it: a review. Springer Netherlands

Kucey RMN, Bole JB (1984) Availability of phosphorus from 17 rock phosphates in moderately and weakly acidic soils as determined by 32p dilution, a value, and total p uptake methods. Soil Sci 138:

Li X, Rubæk GH, Sørensen P (2016) High plant availability of phosphorus and low availability of cadmium in four biomass combustion ashes. Sci Total Environ 557–558:851–860. https://doi.org/10.1016/j.scitotenv.2016.03.077

Ligaba A, Katsuhara M (2010) Insights into the salt tolerance mechanism in barley (Hordeum vulgare) from comparisons of cultivars that differ in salt sensitivity. J Plant Res 123:105–118. https://doi.org/10.1007/s10265-009-0272-2

Løes A-K, Adler S (2019) Increased utilisation of renewable resources: dilemmas for organic agriculture. Org Agric. https://doi.org/10.1007/s13165-018-00242-2

Loria ER, Sawyer JE (2005) Extractable soil phosphorus and inorganic nitrogen following application of raw and anaerobically digested swine manure. Agron J 97:879–885. https://doi.org/10.2134/agronj2004.0249

Möller K, Müller T (2012) Effects of anaerobic digestion on digestate nutrient availability and crop growth: A review. Eng Life Sci 12:242–257. https://doi.org/10.1002/elsc.201100085

Möller K, Oberson A, Bünemann EK, et al (2018) Improved Phosphorus Recycling in Organic Farming: Navigating Between Constraints. Adv Agron 147:159–237. https://doi.org/10.1016/bs.agron.2017.10.004

Möller K, Stinner W, Deuker A, Leithold G (2008) Effects of different manuring systems with and without biogas digestion on nitrogen cycle and crop yield in mixed organic dairy farming systems. Nutr Cycl Agroecosystems 82:209–232. https://doi.org/10.1007/s10705-008-9196-9

Morel C, Fardeau JC (1989) Native soil and fresh fertilizer phosphorus uptake as affected by rate of application and P fertilizers. Plant Soil 115:123–128. https://doi.org/10.1007/BF02220702

Morris DR, Lathwell DJ (2004) Anaerobically digested dairy manure as fertilizer for maize in acid and alkaline Soils. Commun Soil Sci Plant Anal 35:1757–1771. https://doi.org/10.1081/CSS-120038567

Müller-Stöver D, Ahrenfeldt J, Holm JK, et al (2012) Soil application of ash produced by low-temperature fluidized bed gasification: Effects on soil nutrient dynamics and crop response. Nutr Cycl Agroecosystems 94:193–207. https://doi.org/10.1007/s10705-012-9533-x

Müller-Stöver DS, Jakobsen I, Grønlund M, et al (2018) Phosphorus bioavailability in ash from straw and sewage sludge processed by low-temperature biomass gasification. Soil Use Manag 34:9–17. https://doi.org/10.1111/sum.12399

Muys M, Phukan R, Brader G, et al (2021) A systematic comparison of commercially produced struvite: Quantities, qualities and soil-maize phosphorus availability. Sci Total Environ 756:143726. https://doi.org/10.1016/j.scitotenv.2020.143726

Nanzer S, Oberson A, Berger L, et al (2014) The plant availability of phosphorus from thermo-chemically treated sewage sludge ashes as studied by P-33 labeling techniques. Plant Soil 377:439–456. https://doi.org/10.1007/s11104-013-1968-6

Oberson A, Tagmann HU, Langmeier M, et al (2010) Fresh and residual phosphorus uptake by ryegrass from soils with different fertilization histories. Plant Soil 334:391–407. https://doi.org/10.1007/s11104-010-0390-6

Odlare M (2005) A Resource for Arable Soils

Oelofse M, Jensen LS, Magid J, et al (2013) The implications of phasing out conventional nutrient supply in organic agriculture: Denmark as a case. Org Agric 3:41–55. https://doi.org/10.1007/s13165-013-0045-z

Ohno T, Zibilske LM (1991) DETERMINATION OF LOW CONCENTRATIONS OF PHOSPHORUS IN SOIL EXTRACTS USING MALACHITE GREEN. Soil Sci Soc

Am J 55:892–895

Penn CJ, Camberato JJ (2019) A critical review on soil chemical processes that control how soil ph affects phosphorus availability to plants. Agric 9:. https://doi.org/10.3390/agriculture9060120

Pypers P, Van Loon L, Diels J, et al (2006) Plant-available P for maize and cowpea in P-deficient soils from the Nigerian Northern Guinea savanna - Comparison of E- and L-values. Plant Soil 283:251–264. https://doi.org/10.1007/s11104-006-0016-1

R Core Team (2017) R: a Language and Environment for Statistical Computing. R foundation for Statistical Computing, Vienna, Austria

Reimer M, Hartmann TE, Oelofse M, et al (2020) Reliance on Biological Nitrogen Fixation Depletes Soil Phosphorus and Potassium Reserves. Nutr Cycl Agroecosystems. https://doi.org/10.1007/s10705-020-10101-w

Richardson AE, Hocking PJ, Simpson RJ, George TS (2009) Plant mechanisms to optimise access to soil phosphorus. Crop Pasture Sci 60:124–143. https://doi.org/10.1071/CP07125

RStudio Team (2017) RStudio: Integreated Development for R. RStudio, Inc., Boston, MA.

Schiemenz K, Eichler-Löbermann B (2010) Biomass ashes and their phosphorus fertilizing effect on different crops. Nutr Cycl Agroecosystems 87:471–482. https://doi.org/10.1007/s10705-010-9353-9

Sinaj S, Traore O, Frossard E (2002) Effect of compost and soil properties on the availability of compost phosphate for white clover (Trifolium repens L.). Nutr Cycl Agroecosystems 62:89–102. https://doi.org/10.1023/A:1015128610158

Truong B, Pichot J (1976) Influence du phosphore des graines de la plante test sur la determination du phosphore isotopiquement diluable (Valeur L). Agron Trop 31:379–385

van der Bom F, Nunes I, Raymond NS, et al (2018) Long-term fertilisation form, level and duration affect the diversity, structure and functioning of soil microbial communities in the field. Soil Biol Biochem 122:91–103. https://doi.org/10.1016/j.soilbio.2018.04.003

Wang YS, Jensen LS, Magid J, et al (2016) Differential responses of root and root hair traits of spring wheat genotypes to phosphorus deficiency in solution culture. Plant, Soil Environ 62:540–546. https://doi.org/10.17221/485/2016-pse

Table 1. Selected properties of the organic wastes used in this work.

	Fertigro (F)	**Raw manure (M)**	**Digested manure (DM)**	**Digested + organic fractions of municipal solid waste (DM+OFMSW)**	**Ash straw (ASH)**
Dry matter (%)	17.1±0.16	8.16±0.23	5.65±0.10	4.31±0.21	58.1±5.85
pH	6.25±0.01	7.27±0.04	8.26±0.02	8.26±0.01	11.9±0.02
NH_4-N (g kg^{-1} fw)	1.36±0.05	1.93±0.12	2.15±0.03	1.87±0.05	0.00±0.00
NO_3-N (g kg^{-1} fw)	0.00±0.0	0.01±0.0	0.00±0.0	0.00±0.0	0.00±0.0
TN (g kg^{-1} fw)	10.5±0.32	1.88±0.01	1.46±0.17	1.23±0.07	0.00±0.00
TN+NH_4^+(g kg^{-1} fw)	11.8±0.32	3.81±0.01	3.61±0.17	3.09±0.07	0.00±0.00
WEP (g kg^{-1} fw)	0.94±0.05	0.31±0.01	0.32±0.02	0.31±0.00	0.11±0.00
TP (g kg^{-1} fw)	1.20	0.76	0.90	0.69	5.27
TK (g kg^{-1} fw)	1.20	3.48	4.64	3.54	95.40
TS (g kg^{-1} fw)	5.34±0.04	0.99±0.00	0.60±0.07	0.47±0.02	6.89±1.08
TN (% dw)	6.11±0.19	2.30±0.01	2.59±0.30	2.84±0.17	0.00±0.00
TC (% dw)	34.5±0.9	42.2±0.5	38.5±4.8	37.7±1.7	16.2±4.4
TP (% dw)	0.70	0.93	1.60	1.60	0.91
TK (% dw)	0.70	4.27	8.20	8.20	16.41
TS (% dw)	3.11±0.03	1.22±0.01	1.07±0.12	1.08±0.05	1.19±0.19
C/N	5.7	18.4	14.9	13.3	0.0
C/P	49.3	45.2	24.1	23.5	17.8
N/P	8.73	2.46	1.62	1.78	0.00

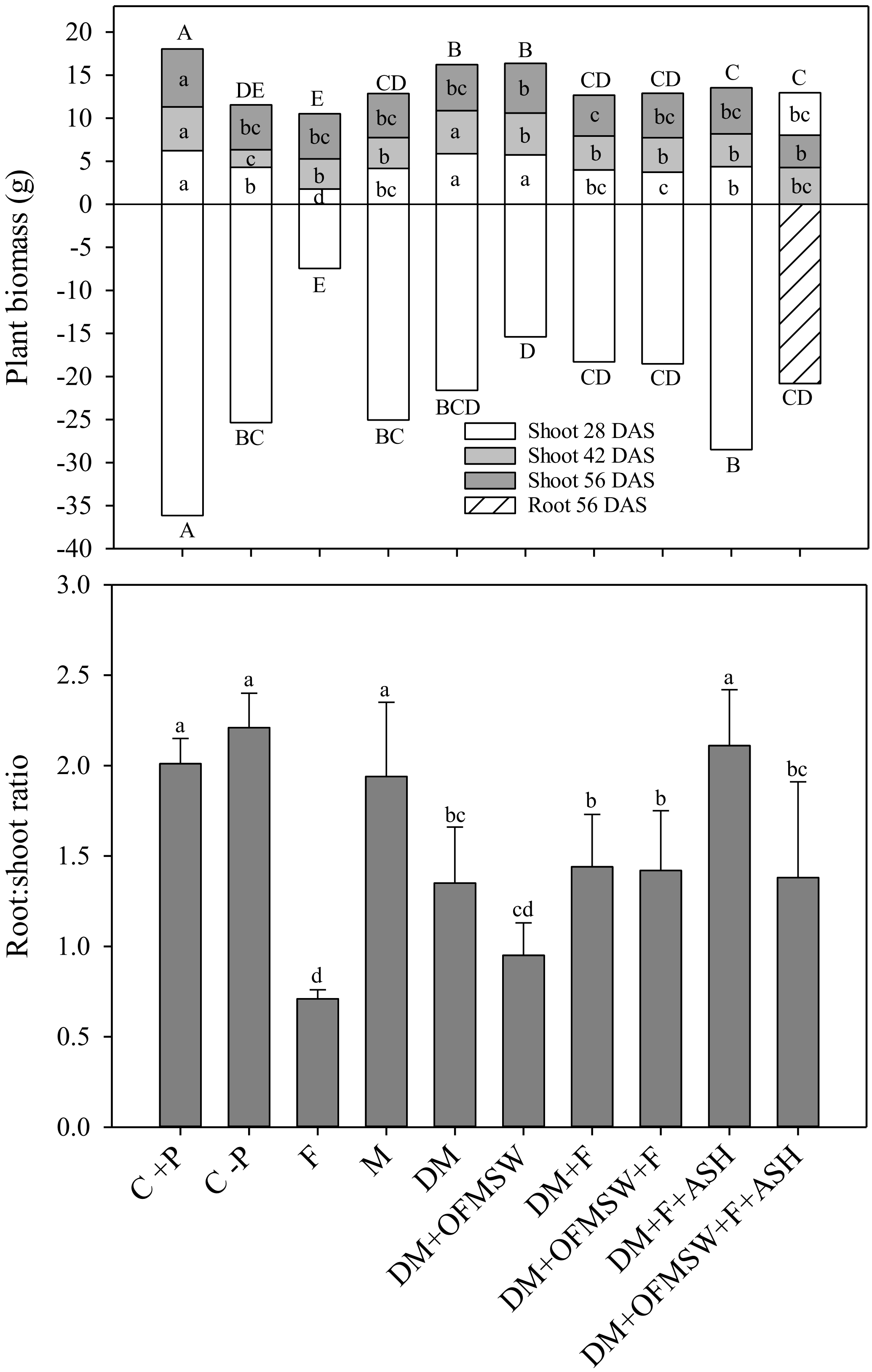

Figure 1. Plant biomass (Shoot and root) quantified after 28, 42 and 56 days of growing and root:shoot ratio calculated at the end of the experiment for ryegrass amended with mineral P (C +P), no P (C –P), Fertigro (F), cattle manure (M), digested cattle manure (DM), digested cattle manure with the organic 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 mean of four replicated and errors bars denote standard deviation. Different small letters show significant difference between treatments in the same cut, whereas different capital letters show significant differences of the total shoot biomass between all the treatments at the end of the experiment.

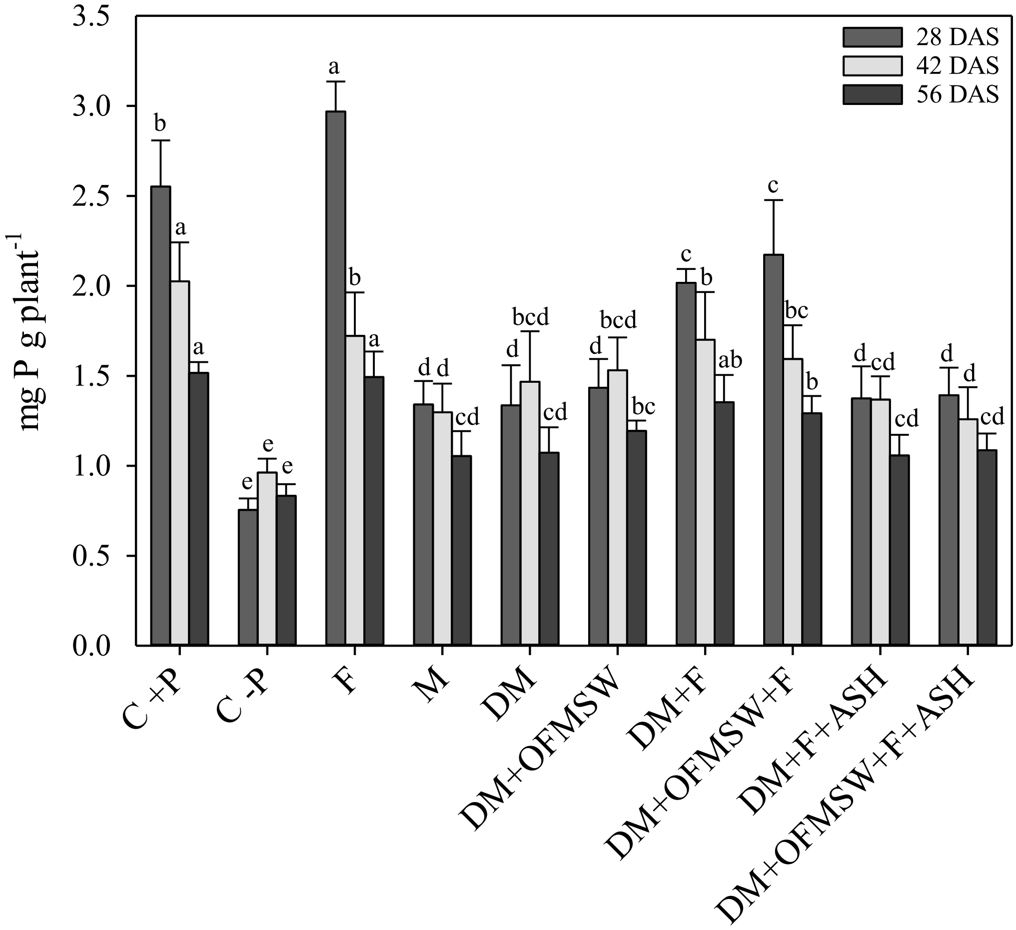

Figure 2. Phosphorus concentration in shoots of ryegrass grown during 28, 42 and 56 days in pot amended with mineral P (C +P), no P (C –P), Fertigro (F), cattle manure (M), digested cattle manure (DM), digested cattle manure with the organic fraction of municipal solid waste (DM+OFMSW), and their combination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

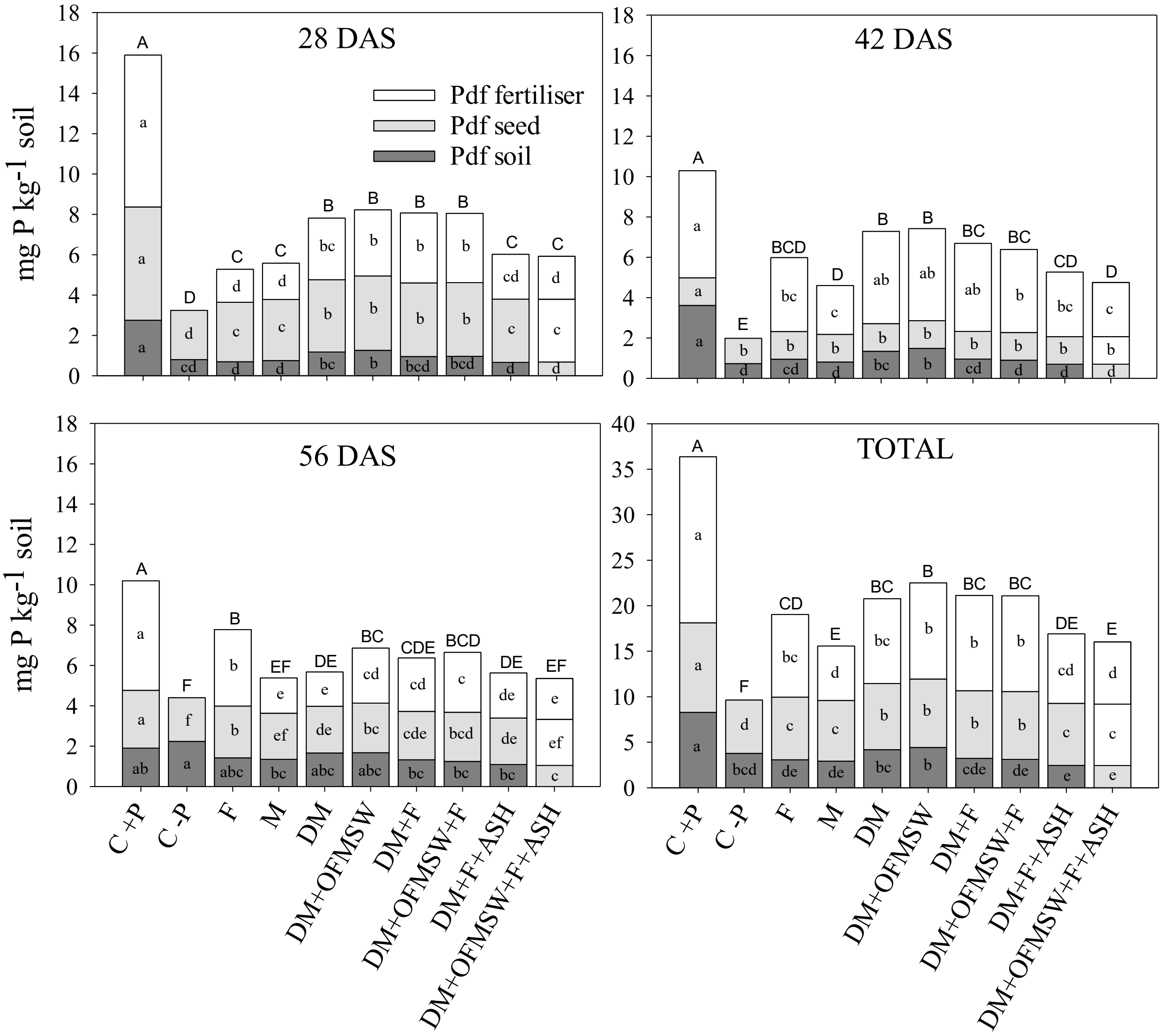

Figure 3. Contribution of P derived from soil, seed and fertilizers to the total ryegrass P uptake in each cut and all together for plants amended with mineral P (C +P), no P (C –P), Fertigro (F), cattle manure (M), digested cattle manure (DM), digested cattle manure with the organic fraction of municipal solid waste (DM+OFMSW), and their combination between DM+F, DM+OFMSW+F, DM+F+ASH and DM+OFMSW+F+ASH. Bars are mean of four replicated and errors bars denote standard deviation. Different small letter show significant difference between treatments in the same cut, whereas different capital letters show significant differences of the total shoot biomass between all the treatments at the end of the experiment.

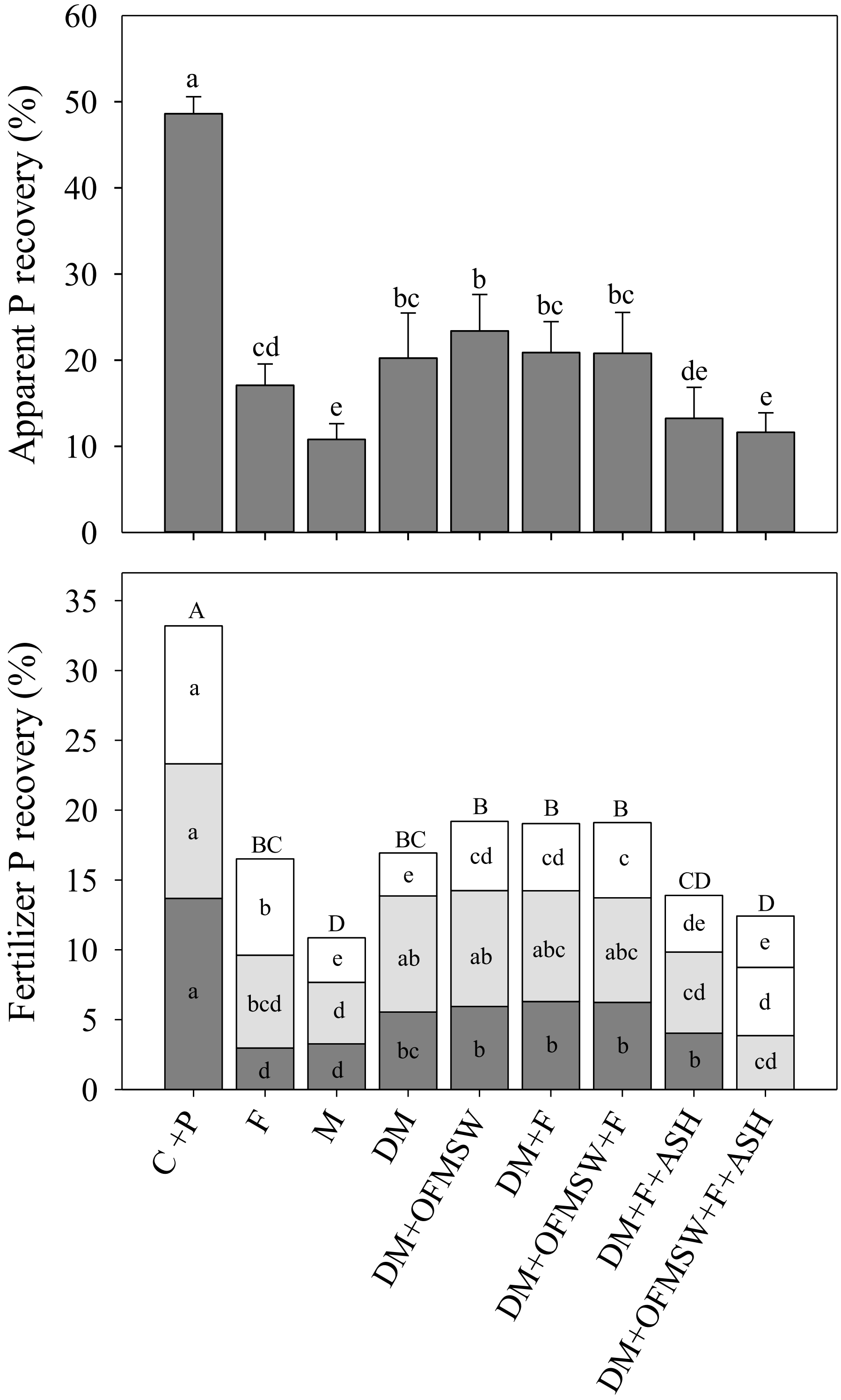

Figure 4. Phosphorus relative efficiency (PRE) and fertilizer P recovery for ryegrass amended with mineral P (C +P), no P (C –P), Fertigro (F), cattle manure (M), digested cattle manure (DM), digested cattle manure with the organic fraction of municipal solid waste (DM+OFMSW), and their combination between DM+F, DM+OFMSW+F, DM+F+ASH and DM+OFMSW+F+ASH. Bars are mean of four replicated and errors bars denote standard deviation. Different small letter show significant difference between treatments in the same cut.

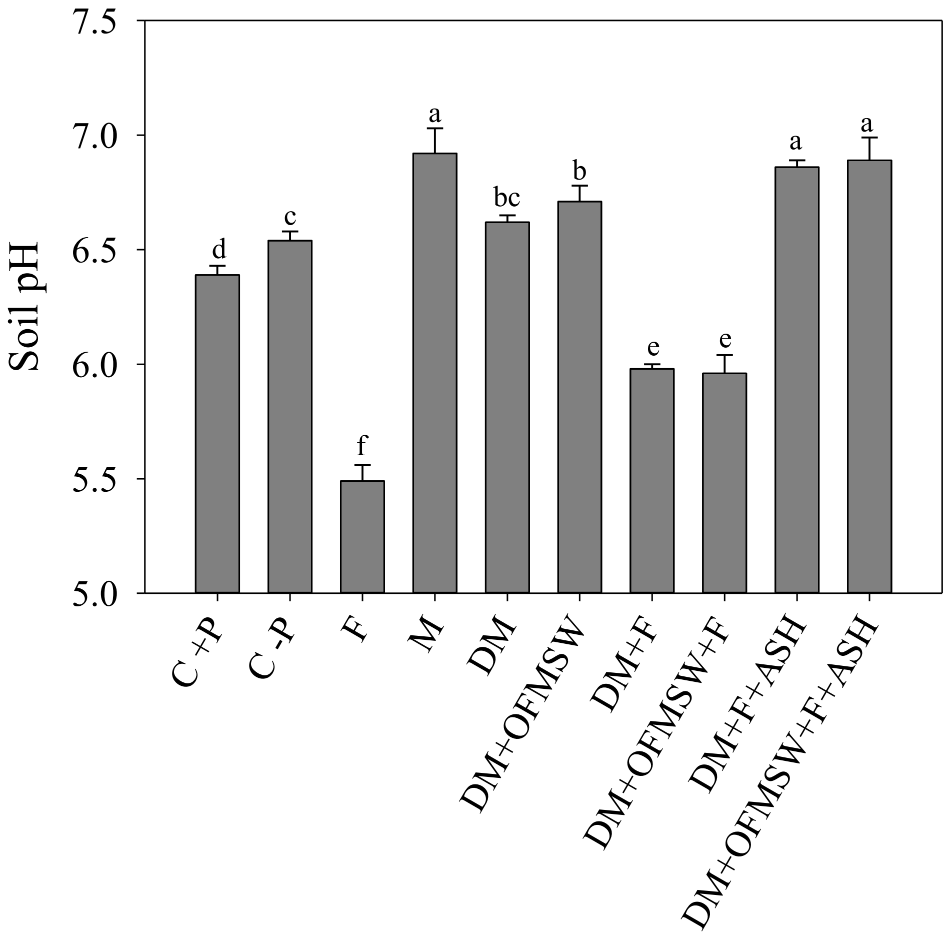

Figure 5. Soil pH at the end of the experiment in soil amended with mineral P (C +P), no P (C –P), Fertigro (F), cattle manure (M), digested cattle manure (DM), digested cattle manure with the organic fraction of municipal solid waste (DM+OFMSW), and their combination between DM+F, DM+OFMSW+F, DM+F+ASH and DM+OFMSW+F+ASH. Bars are mean of four replicated and errors bars denote standard deviation. Different small letter show significant difference between treatments in the same cut.

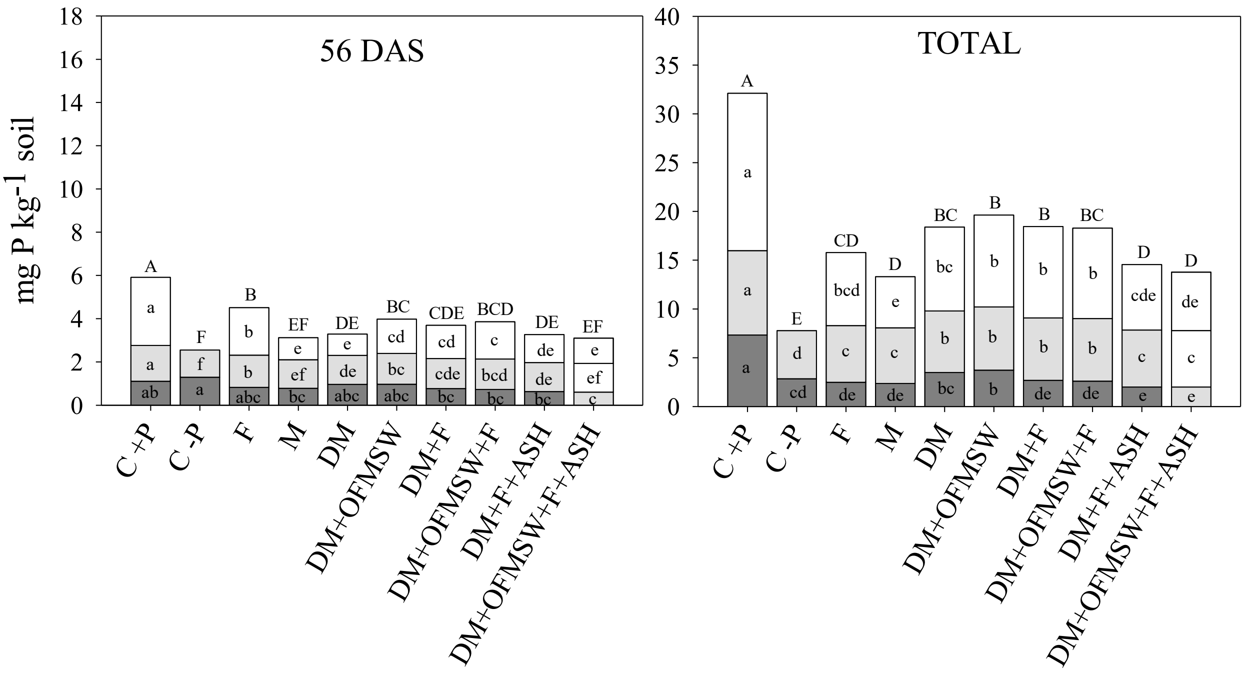

Figure 6. Contribution of P derived from soil, seed and fertilizers corrected by the ryegrass biomass corresponding from 0 to 3 cm for the third cut (56 DAS) and the total for plants amended with mineral P (C +P), no P (C –P), Fertigro (F), cattle manure (M), digested cattle manure (DM), digested cattle manure 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 mean of four replicated and errors bars denote standard deviation. Different small letter show significant difference between treatments in the same cut, whereas different capital letters show significant differences of the total shoot biomass between all the treatments at the end of the experiment.