

# **Phosphorus solubility changes following additions of bioenergy wastes to an agricultural soil: implications for crop availability and environmental mobility**

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## Abstract

Pathways for replacing chemical fertilisers (reliant on rock P resources) with alternative P-bearing materials require assessment of soil processes, crop nutrient acquisition and potential pollution consequences. We examined bioenergy waste materials, individually and as combined ash and anaerobic digestate in terms of plant P availability and mobility. We compared initial effects on mixing of amendments with a test soil and effects after 6-weeks pot trials, with and without wheat growth, against soil and chemical fertiliser controls. Chemical extractions,  $^{31}\text{P}$  NMR spectroscopic determination of P forms and phytase-labile P assays examined processes of P release.  $^{31}\text{P}$  NMR analysis revealed that ash comprised dominantly inorganic orthophosphate P with inherent low P solubility. Initial ash mixing with soils increased solution pH, soluble P in water (pure ash alone) and in citrate (ash alone and in blends). Digestate comprised a diverse array of orthophosphate and organically-complexed P forms, similar to the test soil P compositions, with limited P solubility on initial mixing. Following no plant incubations high water-soluble P with ash additions remained but all effects on citrate-soluble P were normalised. Incubations with plants increased water-soluble P in digestate only and blended amendment treatments relative to initial mixing. When comparing to chemical fertiliser the digestate plus ash blends led to smaller water-soluble P, but equal P in above-ground biomass after incubation. The ash-digestate C:N:P ratios and P form diversity appeared to promote microbial regulation of plant P availability versus potential leaching. The results suggest that the initial days-months are important periods for amendment interactions with soils during plant establishment and the lag before strong growth when system P mobility may induce polluted runoff. Biogeochemical P solubility controls require further study across differing soils and timescales to inform management of bioenergy wastes as fertilisers, particularly in terms of trade-offs such as crop nutrition versus system P losses.

**Key words:** Bioenergy waste; soil P biogeochemistry; anaerobic digestate; ash.

## 1. Introduction

Phosphorus (P) is a key nutrient required for crop production and global food security. Yet, reserves of rock phosphate driving agricultural production are declining in quality and may be insecure geopolitically (Dawson and Hilton, 2011; Heckenmüller et al., 2014). Meanwhile, inefficient use of P in agriculture leads to off-site impacts on aquatic ecosystems (Smith et al., 2007; Cordell et al., 2009). This and other sustainability issues present challenges and opportunities for utilising alternative P fertiliser materials. Crop uptake requires P to be in soluble, available forms. Fertilisers are used in an agricultural context to replenish the soil P pool and increase P available to crops in soils solution

(Vuuren and Beusen, 2010). Attention is increasingly given to the potential P resources recoverable from waste materials as a way to sustainably supplement or replace rock-phosphate-based fertilisers (Lee et al., 2006) and improve soil condition for improved crop yields if other criteria (e.g. water pollution) can be met (Stutter, 2015). As with other fertilising materials, there is a balance between positive aspects for utilisation/uptake of P by a crop and pollution arising from P mobility and loss from the soil, which likely differs across different crop growth stages and durations following application.

Bioenergy wastes are increasingly available due to renewable energy policies, but knowledge is lacking on effects for nutrient cycling following application of by-products to soils. Wood ash, as a by-product of commercial wood burning, paper industry or power generation, has been shown to have potential to complement inorganic fertilisers for nutrients and soil amendment properties (Pitman, 2006). Ash can be categorised into either fly or bottom ash; the former is fine and light ( $\sim 200 \mu\text{m}$ ) (Sear, 2001); while the latter contains coarser components and settles at the bottom of the incinerator. Wood ash can act as P source fertiliser and also as soil conditioner (e.g. liming agent), potentially improving plant nutrient uptake in degraded acidic soils (Ukwattage et al., 2013).

Anaerobic digestate and biogas are by-products of the anaerobic digestion, a biological process which can be sustained with organic waste inputs (manure, animal processing materials, plants or food) (Vanden Nest et al., 2015). Up to 80% of the biomass inputs can be transformed into nutrient rich digestate, typically disposed as waste or applied inefficiently to land (WRAP, 2019). Whilst its potential to replace inorganic N-fertilisers is significant (approximately 80% of total nitrogen is readily available to plants), its application can also induce ammonia emissions and nitrate leaching to watercourses (Smith et al., 2007). The use of the digestate as bio-fertiliser requires careful management because of its potential to release chemical and microbial pollutants (Al Seadi and Lukehurst, 2012; WRAP, 2019). Whilst its use is currently banned in some European countries, in the UK quality protocol frameworks have been established which enable its use as a fertiliser (Environment Agency, 2014).

Ash and digestate, contain appreciable amounts of N, P and K (and C in digestate) that can be recycled back to soils for crop growth (Lee et al., 2006; Al Seadi and Lukehurst, 2012). Numerous studies have focused on soil amendments of individual recycled waste materials, including ash (Pitman, 2006; Augusto et al., 2008; Ukwattage et al., 2013; Pugliese et al., 2014; Hannam et al., 2018) and anaerobic digestate (Arthurson, 2009; Tambone et al., 2009; Albuquerque et al., 2012; Nkoa, 2015; Gómez-Brandón et al., 2016; Koch et al., 2019; Thomas et al., 2019). The effect of combining ash and digestate in a fertiliser blend is less well examined. Whilst some studies exist investigating blended wastes as feedstocks (Thomas et al., 2019; Brod et al., 2018), there are currently no studies which consider P supply associated with ash-digestate blends.

Understanding effects of such blends on nutrient processes in soils is critical for informing guidance around usage and for optimising blend ratios. This requires balancing the matrix conditions of the amendment (organic, inorganic complexation and pH) for biogeochemical processes governing nutrient availability alongside reducing potential for soil pollution (Smith et al., 2007; Möller and Müller, 2012; Ukwattage et al., 2013) and potential leaching of nutrients (Stutter, 2015). The controls on release and cycling of P following material additions to soils can involve changes in the P added with the amendment and the native soil P. The original form of the P is important as is the environment of the amended soil in terms of ability to induce geochemical or biological effects. P may be readily soluble (e.g. a soluble compound like a phosphate fertiliser) or found in large organic molecules which are subject to the rates of microbial organic matter decomposition. Potentially available inorganic or organic P complexes can also strongly adsorb to di- and tri-valent cations on surfaces of the amendment or inherent in soils (Stutter et al., 2015; Celi and Barbaris, 2005). Resulting pulses or progressive P release from surfaces are strongly governed by geochemical and plant-microbial interactions, most notably by changes in pH, acid anions competing with exchange surfaces and enzymes, in turn affected by soil conditions (temperature, moisture, element stoichiometry).

On the one hand research shows certain P fractions applied to soils, or biogeochemically-cycled within soils, are less plant-available and can therefore accumulate in soil (Stutter et al., 2015). Alternatively, materials such as digestate may contain highly soluble and mobile forms of P during short-term mixing that may lead to environmental losses in the absence of plant uptake (Stutter, 2015). Hence, waste materials for potential nutrient replacement must be examined for P solubility versus accumulation of recalcitrant P in soils that require specific agronomic strategies (Stutter et al., 2012; Blackburn et al., 2018).

This study characterised phosphorus forms and solubility in soil-amendment mixtures before and after a pot trial (planted and unplanted conditions for six weeks) using chemical extractions, enzyme-availability assays and spectroscopic ( $^{31}\text{P}$  NMR) techniques. The experimental duration of six weeks provides an insight into the biogeochemical controls for an important period of early crop growth following amendment applications when P mobility may induce leaching and crop health is established. The specific hypothesis was that addition of P via ash and digestate, separately and as blends, to soil leads to different chemical and biological processes affecting P solubility, which in turn impact on P mobility (leaching) and availability to crop uptake. The specific aims were to: 1) characterise the P forms in the materials and test soil, 2) assess initial chemical responses on mixing versus six-week responses following pot growth trials with and without a plant against chemical fertiliser and soil controls.

## 2. Materials and Methods

### 2.1 Ash, digestate, control soil and soil-amendment blends

Two ash fractions sourced from a UK energy site of untreated timber combustion comprised wood fly ash (A1) and wood bottom ash (A2). Two anaerobic digestates derived from the anaerobic digestion of food (D1) and crop (D2) wastes were also sourced from the UK. An agricultural circumneutral pH, loamy soil was taken from grassland (no chemical fertiliser for 5 years) at Myerscough Agricultural College (latitude 53.853214, longitude -2.7693987) from a depth of 5-15 cm (beneath the main root mat and litter). The fresh soil was sieved to <2 mm and stored field-moist, in the dark at 4°C until use for soil-blend mixtures and the control soil. The chemical analyses of the bioenergy materials were carried out by an external accredited laboratory (NRM laboratories). Plant available N was determined by 1:5 w/v extractions in 1M KCl, plant available P by Olsen extraction (data not shown) and colorimetric analyses, pH at 1:2.5 w/v in water, conductivity at 1:5 w/v in water and water soluble metals according to BS EN 13652 with ICP-OES analysis following filtration.

A six-week glasshouse experiment was carried out at Lancaster University with two factors: fertiliser material (13 levels; n=5) x winter wheat plant presence (planted [n=8] and unplanted [n=5]). From the thirteen fertilising treatments, a subset of ten (disregarding those focussed on N mechanisms) was used in this study that focused on P form availability and solubility (Table 2). These comprised a combination of soil and bioenergy waste materials, focusing on the effect observed on digestate/ash blends. Unamended soil (negative), inorganic fertiliser (urea + superphosphate; positive) served as controls and treatments using individual bioenergy wastes accounted for individual materials effects. A fixed total N - P target was set as optimum, based on recommended agronomical practices (RB209 Fertilisation manual, DEFRA; with final additions in Table S4). The P addition levels (via total P contents) were approximately consistent for the chemical fertiliser, ash and the blended amendments of digestate plus ash. However, the overall project sought to balance N as well as P inputs and a pragmatic decision was taken to add the digestates according to balanced N inputs. Hence the P addition levels shown in Table 2 highlight some lack of balance in P addition levels and the results here are presented and discussed with respect to this.

Soil P form solubility and availability were assessed across all amendment treatments before (response to initial mixing, referred to as time-zero  $t_0$ ) and after (following six-week mesocosm incubations) the glasshouse trial. The total soil mass required was weighted and soil moisture was maintained by spraying (initially, 60% of water was added in batches of 40-60 ml). The soil was mixed thoroughly after each water addition. Meanwhile, fertilising materials were weighed, distributed evenly over the moist soil surface and thoroughly mixed. This prepared soil was used to fill the pots

as described in Table S4. Both planted (winter wheat) and unplanted conditions were incubated at 18-22°C, 14/10 hours light/dark and each pot (1070 g soil DM) was maintained at a target 50% water holding capacity ( $0.272 \text{ g H}_2\text{O g}^{-1} \text{ soil}$ ) using tap water. Winter wheat seeds were germinated under controlled conditions ( $20 \pm 1^\circ\text{C}$ ; dark) and once germinated, 6 seedlings were transferred to each pot and sowed to a depth of 2-4 cm, thinning down to two plants per pot after one week. When wheat plants had reached a stage just following head emergence soil samples were taken from the planted (referred to as  $\text{Pl-t}_{\text{end}}$ ) and unplanted ( $\text{Unpl-t}_{\text{end}}$ ) mesocosms. For the investigations described here 20 g of each of the soil/amendments and controls were retained fresh from  $t_0$ ,  $\text{Unpl-t}_{\text{end}}$  and  $\text{Pl-t}_{\text{end}}$  treatments from randomly assigned triplicates of each treatment combination. Shortly after setting up the experiment,  $t_0$  samples were non-destructively sampled, while  $\text{Unpl-t}_{\text{end}}$  and  $\text{Pl-t}_{\text{end}}$  were destructively sampled from the whole pots (soil was gently shaken from the roots back into the bulk soil and soils were representatively subsampled). All samples were stored in the dark at  $4^\circ\text{C}$  until processing.

## 2.2 Nutrients determination and various batch equilibrium experiments

The control soil was air-dried at  $30^\circ\text{C}$ , and a fraction of the soil was milled and used for oxalate extractable P, iron (Fe) and aluminium (Al) determination using acid ammonium oxalate following the method in Farmer et al. (1983). Oxalate extractable  $\text{P}_{\text{ox}}$ ,  $\text{Al}_{\text{ox}}$  and  $\text{Fe}_{\text{ox}}$  were quantified by inductively coupled plasma mass spectroscopy (ICP-MS, Agilent 7500i, Shield-Torch System, Agilent Technologies, Tokyo, Japan). The P saturation index was calculated as  $\text{P}_{\text{sat}} = [\text{P}_{\text{ox}}] / ([\text{Al}_{\text{ox}}] + [\text{Fe}_{\text{ox}}])$ , where  $[\ ]$  denotes the element's molar concentration.

Batch equilibrations utilising different extractants were conducted in triplicate on all amendment for  $t_0$ ,  $\text{Pl-t}_{\text{end}}$  and  $\text{Unpl-t}_{\text{end}}$  treatment combinations and controls. Water extractable P, nitrogen (N) forms and dissolved organic carbon (DOC) were conducted using fresh soil equivalent to 5 gDM in Millipore water at 1:5 w/v, at room temperature, shaken for 1h on an orbital shaker at 100 rpm in the dark. The supernatant was centrifuged (3500 g, 15 mins) and filtered  $<0.45 \mu\text{m}$  (Millipore syringes). Water extract filtrates were analysed colorimetry, for P species: total dissolved P (TDP) and molybdate reactive P (hereby termed  $\text{PO}_4\text{-P}$ ) by automated colorimetry (San++ analyser, Skalar, Breda, Netherlands). Dissolved organic P (DOP) was calculated as  $\text{DOP} = \text{TDP} - \text{PO}_4\text{-P}$ . For N species: total dissolved N (TDN), ammonium N ( $\text{NH}_4\text{-N}$ ), nitrate ( $\text{NO}_3\text{-N}$ ) and DOC were detected by automated colorimetry (San++ analyser, Skalar, Breda, Netherlands). The pH of the filtrates was also determined using Hanna pH 210 meter. In the results the subscript  $X_{\text{water}}$  denotes these determinants (X) in the water extracts.

To assess the forms of P that may be made available to plants in of the presence of organic acids in rhizosphere soil solutions, equivalent comparative equilibrations were undertaken but using 50 mM citrate (pH 5.5) at 1:5 w/v and shaken for 1h. Following equivalent centrifuging and filtering citrate extractable P was determined by analysis of P fractions colorimetrically as above (termed here  $TDP_{\text{citrate}}$  and  $PO_4\text{-}P_{\text{citrate}}$ ). The dissolved organic component of P ( $DOP_{\text{citrate}}$ ) was calculated as the difference between the  $TDP_{\text{citrate}}$  and  $PO_4\text{-}P_{\text{citrate}}$  concentrations in the citrate extracts.

### 2.3 Enzyme-labile P extraction

A phytase-specific enzyme assay was used to determine the contributions of monoester P forms (a major component of stored organic P in soils; Stutter et al., 2015) to organically complexed forms of P before and after growth. Natuphos enzyme (3-phytase, EC 3.1.3.8: BASF SE, Ludwigshafen, Germany) was added to the citrate extracts in excess of 10 nkat/ml as described by George et al. (2007). The enzyme assay was incubated at 37°C for 2 hours before the enzyme reaction was stopped by adding chilled trichloroacetic acid (10% w/v). The 2h incubation reaction was optimised in preliminary investigations. Soluble reactive  $P_{\text{enzyme}}$  was determined colorimetrically as above and stated as  $PO_4\text{-}P$ . The amount of plant available P released by the enzyme reaction (denoted by  $PO_4\text{-}P_{\text{enz-release}}$ ) was calculated as the difference between  $PO_4\text{-}P$  at the end of enzyme reaction ( $PO_4\text{-}P_{\text{enz-end}}$ ) and  $PO_4\text{-}P$  at the start of enzyme reaction ( $PO_4\text{-}P_{\text{enz-start}}$ ).

### 2.4 P mass balance using P extraction and $^{31}\text{P}$ NMR

To support the biogeochemical evidence of soil P extractions on the changes in P availability in the soil amendment mixtures pre- and post- plant growth trials, we examined the changes in the soil P speciation using  $^{31}\text{P}$  NMR spectroscopy. This gives characterisation of the amounts and forms of P species present in the amendments and the background soil at time zero ( $t_0$ ) and insight into the P availability of different P forms and changes in these forms post- plant growth to relate to the water, citrate, enzyme-labile P extraction data.

Nine samples, comprising two digestate, two ash, control soil and four blend-soil mixtures post-pot experiments were prepared for  $^{31}\text{P}$  NMR. All materials were air-dried (30°C) and extracted on an end-over-end shaker (20 °C, 16h) with 0.25 M NaOH and 0.05 M EDTA at a 1:20 w/v ratio as per the method in Bowman and Moir (1993) and in Turner et al. (2003 and 2009). The extract was subsampled for the determination of TP, Fe and Mn concentration by ICP-OES (Agilent 7500ce, Tokyo, Japan), and the remainder of the extract was freeze-dried. The extraction efficiencies of the NaOH-EDTA were derived relative to total P from aqua-regia analyses and showed recoveries of 105, 88, 31, 43 and 100% for D1, D2, A1, A2 and control soil, respectively (Supplementary Information Table S1). Extraction efficiencies for ash <100% are expected to represent acid soluble P predominantly in a mineral matrix (Turner et

al., 2007) referred to here as ‘alkali insoluble’ P (Table 3). Due to the cost of  $^{31}\text{P}$  NMR analysis, these analyses were not replicated so that P forms were determined on one sample each of D1, D2, A1, A2 and the control soil to establish  $P_{\text{initial}}$  and on one sample each of D1A1, D1A2, D2A1 and D2A2 pots to establish  $P_{\text{final}}$ . These blend-soil mixtures were from planted pots, whereas  $P_{\text{final}}$  forms were not determined on the unplanted pots.

For NMR analyses and immediately prior to placing the sample in the spectrometer, 150 mg of the freeze-dried material was dissolved in 1.5 ml of 1M NaOH containing 10% v:v  $\text{D}_2\text{O}$  for frequency locking and an internal standard of 0.2 mM methylene diphosphonic acid (MDP). The solution was centrifuged (to remove particles that would contribute to line broadening) and 1 ml transferred to the NMR tube. Spectra were acquired using an Avance 500 II instrument (Bruker, Germany) operating with proton decoupling at 203 MHz with a 5 mm probe. We used a  $90^\circ$  pulse angle with acquisition time 1.6 s, operating at  $21^\circ\text{C}$  and collected 1000 scans. Delay times were calculated by preliminary run time experiments optimising relaxation times (initial  $T_1$  inversion recovery experiments) targeting the ortho- $\text{P}_i$  peak ( $n=8$  time point curves). Pre-observed  $T_1$  values were 2.4, 10.1 and 9.3 s for digestate 1, ash 2 and control soil 1 (Table S1) and a delay time of 50 s was used consistently (approximating to  $5 \times T_1$ ).

Sample peak positions were set relative to that of the internal standard MDP shift at 16.6 ppm. In previous work with common extraction and instrumentation (Stutter et al., 2015) we found the chemical shifts for the very distinctive peaks of phosphonates at 20.6 (and 16.5) ppm, MDP 16.8 ppm, orthophosphate P 6.1 to 6.2 ppm and pyrophosphate -4.2 to -4.4 ppm. In the current analyses these ‘anchor point’ positions were found to be  $\sim 24.3$ , 16.6 to 16.7, 5.33 to 5.44 and -5.1 to -5.2 ppm, respectively and consistent between soil, digestate and ash. In the absence of spiking experiments no fine structure was evaluated instead following groups were assigned to regions: 3.59 to 4.41 ppm for monesters,  $\sim 1.0$  to  $-0.7$  ppm for phospholipids,  $\sim -0.9$  to  $-1.7$  ppm for DNA and a sharp peak in one sample at  $-2.6$  ppm as other diester (Table 3) on the basis relative positions in literature reference library tables (Cade-Menun, 2015) and spectra (Cade-Menun et al., 2010; McLaren et al., 2015). Processing of NMR spectra was carried out using MestReNova v8.1. Quantitative NMR signal recovery was assessed as the P mass from summed peak areas scaled by the internal MDP signal relative to P mass introduced into the instrument with each sample. The mean ( $\pm$  coefficient of variation) for quantification was  $109 \pm 15\%$ . Detection limits (not formally determined) were taken as  $< 0.1\%$ .

A P mass balance calculation assumed that the P species available to plant-microbial-chemical transformations in the pot experiments (involving only the four blends) as:

$$P_{\text{initial}} \sum_{m=1}^{m=n} \text{for a given blend} = P \sum_{m=1}^{m=n} \text{soil} + P \sum_{m=1}^{m=n} \text{ash} + P \sum_{m=1}^{m=n} \text{digestate}$$



Where P is the P mass sum of individual P forms determined by <sup>31</sup>P NMR being m<sub>1</sub>, m<sub>2</sub>...m<sub>n</sub>. The P<sub>initial</sub> is the P mass sum of the mixing of the soil + ash + digestate determined at time zero (Table 2). The change in P mass was assessed for P<sub>final</sub> (on direct analysis of planted soils post-incubations) as:

$$\Delta P_{soil} = P_{final} \sum_{m=1}^{m=n} \text{directly measured on a pot soil} - P_{initial} \sum_{m=1}^{m=n} \text{for a given blend}$$

This allowed a mass balance check on P removed during the plant growth period compared with TP uptake from analysis of above ground plant biomass (excluding root P uptake; methods already described):

$$\Delta P = zero = P_{final} \sum_{m=1}^{m=n} \text{pot soil} - P_{initial} \sum_{m=1}^{m=n} \text{for a given blend} + P_{plant}$$

## 2.5 Statistical analysis

Nutrient concentration data were subjected to descriptive statistical analysis using GenStat 16 and Minitab 16. Samples were tested for normality using Anderson-Darling testing ( $p < 0.05$ ) and  $\log_{10}$  transformed where necessary. The structured allowed testing of differences between start and end of pot trials using ANOVA, where first the assumption of equal variance was tested by Levene's test ( $p < 0.05$ ) between t<sub>0</sub>, unpl-t<sub>end</sub> and pl-t<sub>end</sub> groups using pooled data across all treatments. One-way analysis of variance (ANOVA) was performed ( $p < 0.05$ ) to examine the significance of concentration differences resulting from amendment additions, with separate analyses for treatment groups t<sub>0</sub>, Pl-t<sub>end</sub> and Unpl-t<sub>end</sub>. A Tukey test was used to compare mean values and to assess the significant differences between treatment means. The results are discussed relative to the P addition levels highlighted in Table 2.

## 3. Results

### 3.1. Control soil and individual amendment compositions

The control soil and amendment individual compositions are compared in Table 1 as fresh weight (FW) compositions. The control soil had a near-neutral pH, low organic C, N and moderate total P contents (15, 2.2 and 0.9 g/kgFW, respectively; with molar C:N:P of 43:5:1). The two ash materials were consistently alkaline (pH 12.6-12.7), of high dry matter (89-100%), similar in N content (0.7-1.0 mgN/kgFW) but varied in organic C (13-89 gC/kgFW) and total P (11-25 gP/kgFW). In contrast, the digestate materials were of low dry matter (3-4%) and consistent in compositions of organic C, N and total P (ranges 15-18, 3-6 and 0.4-0.5 g/kgFW, respectively). The molar C:N:P of ash ranged between 1-19C: <0.1-0.2N: 1P and for digestate between 93-97C: 14-30N: 1P. The acid ammonium oxalate extractable P, Fe and Al content of the control soil was 0.94, 17.42 and 2.08 g/kg dry matter, respectively, with a P<sub>sat</sub> (molar P/(Fe+Al) oxalate ratio) of 0.08.

Characterisation of the P forms by  $^{31}\text{P}$  NMR on the NaOH-EDTA extracts of individual amendments (Table 3) showed that the TP comprised dominantly inorganic orthophosphate in the digestate (77-91 % by mass) and the control soil (88% orthophosphate). Digestate also showed a diversity of P forms relative to the other samples: monoester P comprised 5-7% of P mass and phospholipids (1-2%), polyphosphate (~0.5%) and for D1 other diesters (0.1%) were present when these forms were below detection limits for all other samples. The soil P mass distribution was 88% inorganic orthophosphate and 12% monoester P. In contrast, the ash materials comprised 31-43% inorganic orthophosphate, a dominant P pool within alkaline-insoluble forms (57-69%), with other P forms below detection limits.

### 3.2 Soil, individual and blended amendment P release at $t_0$

Due to the experimental design regarding P addition levels (Table 2) it is possible to directly compare the extract P concentrations of A1, A2, all four blends and the conventional fertiliser control, since these had approximately standardised addition levels. However, the P addition levels of D1 and D2 alone were 23% and 46% of that in the conventional fertiliser control and results are discussed relative to this.

Batch equilibrium results for time zero ( $t_0$ ) samples showed that the control soil had low concentration of extractable P in water ( $\text{PO}_4\text{-P}_{\text{water}}$ ; 0.28 mg/kg) and greater in citrate ( $\text{PO}_4\text{-P}_{\text{citrate}}$ ; 15.6 mg/kg), 23% of citrate-extracted organic P ( $\text{DOP}_{\text{citrate}}$ ) converted by phytase to the molybdate-reactive form of dissolved P (Table S2). When conventional fertiliser (urea + P; the positive control) was added to soil the dissolved P release increased to  $\text{PO}_4\text{-P}_{\text{water}}$  0.4 mg/kg and  $\text{PO}_4\text{-P}_{\text{citrate}}$  20.4 mg/kg, being significantly greater than the negative control ( $p < 0.05$ ). More than 40% of  $\text{DOP}_{\text{citrate}}$  in the fertiliser treatment was converted by phytase to the molybdate-reactive  $\text{PO}_4$  form at  $t_0$ .

The addition of A1 to the soil increased  $\text{PO}_4\text{-P}_{\text{water}}$  and  $\text{PO}_4\text{-P}_{\text{citrate}}$  at  $t_0$  ( $p < 0.05$ ) compared to both the control soil and the conventional fertiliser addition, however, only 18% of  $\text{DOP}_{\text{citrate}}$  in A1 treatment was converted by phytase to the reactive form of P (Table S2). The addition of A2 only to the soil also increased  $\text{PO}_4\text{-P}_{\text{citrate}}$  at  $t_0$  compared to the control soil, however, these increases were less than in A1 addition and were comparable to the conventional fertiliser P release. Approximately, 50% of  $\text{DOP}_{\text{citrate}}$  in A2 treatment was converted by phytase to the reactive form of P at  $t_0$ .

Soil amendment with D1 alone showed no difference in  $\text{PO}_4\text{-P}_{\text{water}}$ ,  $\text{PO}_4\text{-P}_{\text{citrate}}$  or  $\text{DOP}_{\text{citrate}}$  compared to the control soil at  $t_0$  (Figure 1). More than 30% of  $\text{DOP}_{\text{citrate}}$  of D1 treatment was converted to available P by phytase, which was less than the conventional fertiliser. Soil amendment with D2, similarly to D1, showed limited differences to the control soil in  $\text{PO}_4\text{-P}_{\text{water}}$ ,  $\text{PO}_4\text{-P}_{\text{citrate}}$  and  $\text{DOP}_{\text{citrate}}$  compared to the control soil at  $t_0$ . Approximately, 31% of  $\text{PO}_4\text{-P}_{\text{citrate}}$  in D2 treatment was converted by enzymatic

activity to soluble  $\text{PO}_4\text{-P}$ . It should be remembered that D1 and D2 had smaller P addition levels (23% and 46%, respectively, of that for the conventional fertiliser control).

The amendment of soils with the four blend combinations showed no statistically significant change in the concentrations of  $\text{PO}_4\text{-P}_{\text{water}}$  at  $t_0$  when compared to the controls or the individual amendments of D1, D2 or A2; instead, it reduced the significant increase seen in  $\text{PO}_4\text{-P}_{\text{water}}$  with A1 addition to soil. However, the differences between the blended amendments became manifested in the citrate extracted P forms (Table S6). For  $\text{PO}_4\text{-P}_{\text{citrate}}$  the individual amendments D1, D2 had shown no concentration differences against positive or negative controls, with A1, A2 leading to significantly greater concentrations. For the blends  $\text{PO}_4\text{-P}_{\text{citrate}}$  was only significantly greater than controls for the D1A1 blend whereas the blending of ash with other digestate combinations had removed the  $\text{PO}_4\text{-P}_{\text{citrate}}$  concentration increase seen before with both ash amendments. The extract concentrations of  $\text{DOP}_{\text{citrate}}$  did not differ significantly between treatments at  $t_0$  and the blends had 22 to 33% of  $\text{DOP}_{\text{citrate}}$  made labile with the phytase enzyme.

### **3.3. pH, N and DOC at the start of the experiment ( $t_0$ )**

The level of pH at  $t_0$  ranged from 5.5-6.7. Nitrate concentrations ( $\text{NO}_3\text{-N}_{\text{water}}$ ; Figure 2a) significantly differed between treatments; all treatments except A1 and A2 exceeded concentrations of the control soil (Figure 2). Table 2 shows that additions of N with the ash alone treatments were of a negligible increase beyond that native to the control soil. The individual D1, D2 treatments, four blends and the fertiliser all had identical total N mass additions in the different N forms. But the D1A1 and D1A2 blends had greater  $\text{NO}_3\text{-N}_{\text{water}}$  concentrations than the fertiliser, whereas D2 alone or in a blend reduced  $\text{NO}_3\text{-N}_{\text{water}}$  release at  $t_0$  (Figure 2). Concentrations of DOC (Figure 2c) significantly differed ( $p < 0.05$ ) from the control soil only in the D1 individual amendment, D1A2 blend and the fertiliser treatment.

### **3.4. P form concentrations in amended unplanted (Unpl- $t_{\text{end}}$ ) pots compared to $t_0$**

Significant effects of soil amendment treatments on P forms at the end of the unplanted period were only observed for responses of water extractable  $\text{PO}_4\text{-P}$  and  $\text{DOP}_{\text{citrate}}$  (Figure 1). Concentrations of water extractable  $\text{PO}_4\text{-P}$  remained higher in the A1 treatment than most other treatments (except A2). Concentrations of TDP were greater only in A1 than D2A2 treatments (Table S2). Concentrations of  $\text{DOP}_{\text{citrate}}$  showed that A2 was significantly greater than the control or D1A2. Statistically significant P form changes between  $t_0$  to unpl- $t_{\text{end}}$  samples (Table 4) were limited to a decrease in enzyme-labile P in D2A1 only.

### **3.5. pH, N and DOC levels in unplanted (Unpl- $t_{\text{end}}$ ) pots compared to $t_0$**

The water extract pH at the end of the unplanted period ranged from 5.7-6.6 (Figure 1a). Concentrations of nitrate (Figure 2a) were similarly low in the control, A1, A2 treatment whilst all other treatments showed significantly greater concentration ( $p < 0.05$ ) compared to the control. This is related to the low N addition levels for ash alone compared with all the other amendments and conventional fertiliser control (Table 2). Concentrations of DOC (Figure 2c) did not differ significantly between amendment treatments at unpl- $t_{end}$ . Statistically significant changes between  $t_0$  to unpl- $t_{end}$  samples (Table 4; Figure 2a) were limited to a decrease in  $NO_3$  concentration for D2 only.

### **3.6. P form concentrations in planted (Pl- $t_{end}$ ) pots compared to $t_0$ and Unpl- $t_{end}$**

Considering firstly effects only within the group defined by pl- $t_{end}$ , significant effects of soil amendment treatments on P forms at the end of the planted period were only observed for water extractable  $PO_4$ -P (Figure 1), where D1 had a smaller concentration than either A1 or the conventional fertiliser. As shown (Table 2) D1 had the smallest of the P addition levels.

Considering next the changes between pl- $t_{end}$  group and unplanted soils, there were abundant statistically significant P form changes between  $t_0$  to pl- $t_{end}$  samples and P concentrations increases from  $t_0$  to pl- $t_{end}$  (Table 4). This commonly involved both water extractable  $PO_4$ -P and TDP (for the control soil, D2, D1A1, D2A2 and the conventional fertiliser, however, D1 increased for water-extractable TDP but not  $PO_4$ -P. There are no changes in citrate extractable P forms or that made labile by the enzyme.

### **3.7. pH, N and DOC levels in planted (Pl- $t_{end}$ ) pots compared to $t_0$ and Unpl- $t_{end}$**

The pH in pl- $t_{end}$  pots ranged from 6.2-6.8. Within the pl- $t_{end}$  treatment the concentrations of nitrate (Figure 2a) only differed with amendment treatments in D1 and D1A2 being greater than the controls ( $p < 0.05$ ). Concentrations of DOC (Figure 2b) did not differ significantly between amendment treatments at pl- $t_{end}$ .

Statistically significant changes between  $t_0$  to pl- $t_{end}$  samples (Table 4; Figure 2a) showed a substantial decrease in  $NO_3$  concentrations by the end of the pot trial across all treatments. In contrast, DOC showed significant concentration increases ( $p < 0.05$ ) during plant growth relative to  $t_0$  in D2, D1A1, D2A2 and the conventional fertiliser (except D2A1).

### **3.8. Determination of P mass balance using $^{31}P$ NMR**

The  $^{31}P$  NMR species for the individual components are presented (Table 3) alongside the compositions of the soils following the 6-week growth period used to examine final P compositions and assess change. These  $^{31}P$  NMR data was used to compare P form contributions to total P mass between  $t_0$  ( $P_{initial}$ ; calculated from the mixing quantities of the analysed raw materials) and at the end

of the growth period ( $P_{\text{final}}$ ; directly analysed) for the four soil blends (Figure 3). Full mass balance data are given in Table S3.

For blends utilising D1 the contributions of TP for the D1A1 and D1A2 were 1.0% from digestate, 3.2-3.6% from ash and 95.4-95.7% from soil (Table S4). For blends utilising D2 this was 2.3% from digestate, 1.8-1.9% from ash and 95.7-95.9% from soil (Table S4). The dominant components of  $P_{\text{initial}}$  were inorganic ortho-P > monoester P > alkali-insoluble P (Figure 3) that mirrored P composition of the soil (as dominant contributor in the mixture) but with alkali-insoluble P derived from the high component present in ash. In all cases  $P_{\text{initial}}$  contributions of the trace labile P forms contributed exclusively from the 1-2% digestate P had small maximum concentrations in initial mixtures (phosphonate 0.12 mgP; phospholipids 0.50 mgP; DNA P 0.25 mgP; other diesters 0.03 mgP; polyphosphate 0.09 mgP). Hence, what is often considered readily labile organic P was <1 mgP. Monoester P had an appreciable contribution of 101-102 mgP (linking to investigations with the phytase-specific enzyme P solubility).

The  $P_{\text{final}}$  mass shows two pots with increased mass (up to 57 mgP) and two with decreased mass (to -197 mgP) (Figure 3). In theory, since pot watering never exceeded saturation the P mass should reduce in line with plant P uptake. The range of  $\Delta P$  from positive to negative is likely resulted from accumulation of errors in multiple calculation stages. The mean  $\Delta P$  of -30 mgP can be compared to P uptake into plant above ground biomass of 7.7 to 9.6 mgP/pot for the blend treatments. In terms of the P form changes from  $P_{\text{initial}}$  to  $P_{\text{final}}$  the trace levels of phosphonates, phospholipids, DNA P, other diesters and polyphosphates were below detection in the final soils and considered removed or converted. Consistently the 11-21 mg of alkaline-insoluble P present on initial mixing was removed from final soils. Otherwise, variously the major components of inorganic ortho-P and monoester P showed increases or decreases without clear pattern (Figure 3, Table S3).

#### 4. Discussion

This study was a subset of experiments in a wider project on the use of individual and blended bioenergy waste materials as potential chemical fertiliser replacement for soils. This current work sought to provide a complimentary description of P solubility mechanisms contributing to available P to crop growth or to potential leaching. The methods here sought to evaluate water-soluble P forms, citrate extraction and phytase-specific P release as analogues of soil biogeochemical processes (Menezes-Blackburn et al., 2016; Giles et al., 2017), supported by spectroscopic P analysis. Often crop growth trials are considered resource intensive and therefore, chemical analysis analogues are sought (Katz et al., 2019); here we combined these approaches to complement the plant growth trial.

#### 4.1. Amendment P compositions and rapid physico-chemical effects induced by initial mixing

Oxalate extractable Fe and Al values for the control soil (17.4 and 2.1 g/kgDM, respectively) with P/(Fe+Al) oxalate ratio of 0.08 showed the soil as strongly P fixing (exceeding values for 32 UK agricultural soils in Stutter et al. (2015) but of low P saturation. Hence, the test soil was P-depleted and presents a strong P fixing context analogous to some important global soils for agriculture (e.g. Ferrasols in Brazil and elsewhere).

Ash was alkaline and low in N, while digestate was moderately alkaline and N-enriched, consistent with Case et al. (2017) and Nkoa (2015). Nutrient content on a fresh mass is a consideration to users handling materials and for this total P contents were similar between the two digestates but greater in one ash. The nutrient stoichiometry (Table 1; molar C:N:P of ash 1-19C: <0.1-0.2N: 1P and digestate 93-97C: 14-30N: 1P) showed that ash had high potential P relative to C or N, while digestates were C- and N-rich (Stutter, 2015; Risberg et al., 2017; Fernandez-Bayo et al., 2017; Sharifi et al., 2019), suggesting that blending helps achieve more ideal ratios for soil microbial biomass cycling (60:7:1; Cleveland and Liptzin, 2007).

Plants take up P dominantly as orthophosphate supplied directly soluble from chemical P fertiliser followed by the conversion of P in the soil and waste material. The continued plant-availability depends on interactions between the P-bearing amendment and the soil surfaces, P form and transformations by soil biota. The <sup>31</sup>P NMR P speciation (Table 3) showed that digestate and the control soil contained appreciable monoester P that may be considered less available in P fixing soils in the absence of biological enzymes. Additionally, the digestate had a wider diversity of diester and other organically-complexed P considered to be highly labile and rapidly cycled (Stutter, 2015), whereas ash was dominantly inorganic orthophosphate but with 57-69% of P termed 'alkaline insoluble'. Brod et al. (2015a, b) noted that wood ash P was in the form of Na-Mg-Ca phosphate complexes with total P having zero solubility to water, compared to 6% in digestate, where both had similarly ~70% of total P soluble in neutral ammonium citrate. Hence, metal-P complexation in ash suppresses P solubility even in the presence of EDTA in the extractant for NMR analysis. Ash is enriched in major elements such as K, Na, Zn, Ca, Mg and Fe (Table S5) with potential for yields benefits for crops (Lee et al., 2006; Ukwattage et al., 2013; Pugliese et al., 2014; Hannam et al., 2018). However, this may point to long residence times of ash-origin metal-P complexes in soils until conditions occur that leach metals from the soil (potentially co-leaching P).

Short term P effects on immediate mixing ( $t_0$ ) were likely rapid chemical effects induced by pH and metal cations. At the start of the growth trials ( $t_0$ ) significant increases on PO<sub>4</sub> availability to water relative to the control soil only occurred for A1 (Figure 1a). Considering the argument above regarding

low solubility of ash-origin P it may instead be that pH-related ash matrix effects causing native soil P solubilisation, since water extract solution pH was greatest with A1 soil mixtures. While complexation with Fe and Al limits solubility at soil solution pH<5.5, and complexation where Ca is present is increased at pH>8 within this window P solubility is raised with pH increase (Smithson, 1999; Ch'ng et al., 2014; Penn and Camberato, 2019). Hong et al. (2018) used diffusive gradient in thin films (DGT) to show that fly ash soil amendments increased P availability in a range of soils through modification of soil pH as Al-P and Ca-P increased relative to Fe-P complexes decreasing P fixation and increasing diffusion flux rates.

Citrate extraction was used to simulate the rhizosphere process of exudation of low molecular weight organic anions as a plant strategy of P acquisition (P complexed by bridging cations or bound to soil Fe or Al oxyhydroxide surface exchange complexes), (Menezes-Blackburn et al., 2016). The citrate extractant at  $t_0$  brought up to 50× increase in extracted  $PO_4$  relative to water and showed elevated P availability relative to the control soil for A1, A2 and D1A1 blend.

The rationale for examining enzyme labile P was that recycled organic amendments may have a wide range of P forms that require specific microbial and plant strategies to acquire P in forms available for root uptake (Stutter, 2015; George et al., 2018). Phytase is a specific enzyme acting on phytate monoester P known to accumulate in soils (Stutter et al., 2015; Menezes-Blackburn et al., 2018) and not normally available to plants or microbes (Celi and Barberis, 2005; Turner, 2007). The phytate (monoester IHP) organic P fraction becomes available through phytase enzymes from rhizosphere microbes and roots (Zhu et al., 2017). In the  $t_0$  soils, no difference was found in citrate-extractable organically complexed P (Figure 1c) and limited patterns were apparent in P made soluble to phytase and links to P speciation. The  $^{31}P$  NMR results for P speciation (Table 3) showed that the NaOH-EDTA extractable P for ash was solely inorganic orthophosphate, although a dominant amount associated with A1 and A2 was not extracted by the preparative steps for NMR analysis, and described as 'alkali-insoluble'. Hence, the phytase-labile P for the ash amendments (18% and 50% for A1 and A2, respectively) may have either been an interactive effect of the ash matrix with indigenous phytate forms (myo-, scyllo- IHP; Table 3) in the soil, or the alkaline insoluble P with ash was phytate complexed with ash metals.

Elevated water extractable DOC (Figure 2b) at  $t_0$  for the A1 amendment is likely to be an effect of enhanced DOC solubility from soil organic matter with greater pH, since A1 had low OC content. However, relative to the control soil the smaller DOC concentration for D1 alone, D1A2 blend and the fertiliser control may likely be a rapid DOC depletion following incorporation of available N and P in microbial biomass. The presence of acid anions of DOC may affect P solubility via competitive sorption

on soil exchange sites, which may have exacerbated P solubility in the presence of ash and may contribute to leaching in a field context.

#### **4.2. Additional biological interactions affecting P solubility induced by incubations**

Comparing effects on initial mixing ( $t_0$ ) with those following six-week incubations allows assessment of biological controls (microbial only for unplanted, plant-microbial for planted pots) superimposed on physico-chemical effects. At the end of the incubation, unpl- $t_{end}$  soils had elevated  $PO_4$  and TDP in water extracts in A1 treatment, with no further treatment differences relative to the control soil, even for the fertiliser. No significant treatment effects were seen for  $PO_4$  in the citrate extract (Table S6). At the end of the planted period (pl- $t_{end}$ ) the treatments D1, D1A2, D2A1 and D2A2 had lower water extractable  $PO_4$  concentrations than the fertiliser and were not elevated above levels of the control soil-only (Figure 1a). This reduced availability of soluble P was accompanied by the greater above ground biomass P (data not shown) for all four blends and D2 alone (despite the reduced P input of D2; Table 2); this is evidence for benefits of the amendment blending of ash and digestate. In summary, the plant was equally able to effectively mine the P from the bioenergy waste amendment as from conventional fertiliser (Table 5), but the former had less water soluble P in excess of crop requirements after six-weeks. In a field context this could limit potential for P leaching. Since the P biomass accumulation of these blends was equal to that of the fertiliser-grown wheat plants it can be summarised that the blended amendment reduced potential P leaching with no impact on crop P-acquisition relative to conventional fertiliser (Table 5).

In contrast to these results, Brod et al. (2018) examined the blending of organic wastes in pelleted compound recycled fertilisers that sought to improve crop nutrient uptake and yields. The authors found that the best incorporation of P (expressed as %P incorporation into biomass relative to conventional fertiliser) was for digestate (115%) whereas the blended fertilisers with ash were smaller (34-50%). This was attributed to available P in digestate whereas the blends with ash P contained more stable Ca-phosphates, despite that ash addition did not raise soil pH >6. Ash alone did raise pH in the current study but when ash and digestate were blended the pH remained in a similar range to the control soil. Additionally, the current comparisons must be judged in terms of the unbalanced P inputs of the amendments where the P levels of digestate alone were considerably smaller than for the blends (Table 2). Other studies have highlighted the considerable risk of digestate leaching reactive P both in laboratory trials (Stutter, 2015) and in field trials (Koch et al., 2019).

The comparisons between nutrient parameters at  $t_0$  vs end of the incubations show few significant changes with no plants present (Table 4). Conversely, with the plant present increased  $PO_4$ -P, TDP, DOC and decreased  $NO_3$ -N was common to all amendment treatments including controls. By the end



of the pot trials, differences in DOC concentrations became non-significant between amendment treatments or the controls. Increased DOC between  $t_0$  to  $pl-t_{end}$  may indicate soil organic matter decomposition to supply microbial C. However, the increased presence of labile OC to microbes does not always favour crop P-acquisition. Thomas et al. (2019) observed microbial P competition reduced that available to plants when straw was added over 5-years to field plots of digestate to balance amendment C/N.

The data suggest that short-term chemical effects, likely pH-driven, mean that labile dissolved P becomes soluble when ash (particularly A1) was amended to a soil that would be considered strongly P-fixing. This increased solubility to water was not apparent when ash was blended with digestate. When a stronger P extractant (citrate, mimicking plant strategies to overcome soil surface P sorption) the immediate solubilising effects of the ash matrix remained. During the six-weeks incubation, with microbial activity and no plant, the increased P solubility to water effects of A1 remained. In contrast, when incubated in the presence of wheat, when the plant-microbial biological effects were combined, the effect of the ash matrix on water soluble P was lost.

Under the stronger extract of citrate all differences in P solubility between amendment treatments that were apparent immediately following initial mixing were subsequently removed following the incubation with or without the plant. The  $^{31}P$  NMR mass balance evaluated the difference between final and initial  $t_0$  conditions for the four amendment blends during the wheat growth trial period (Figure 3; Table S3) noted uncertainties in summing P species mass balances and hence these are indicative P form changes. However, the analyses of above-ground biomass indicate that 4 to 11 mgP were accumulated in plant tissue (data not shown) from the P sources in the pots. The general patterns in Figure 3 suggest that a small contribution of diester and diverse labile organically-complexed P was removed during crop growth, an appreciable pool of alkaline-insoluble P associated with the ash matrix was altered during the incubation and removed potentially supporting crop growth. The accumulated calculation errors and small sample size made changes in the dominant pools of monoester P and inorganic ortho-P inconclusive.

The methods employed here combined assessment of abiotic and biotic processes for amendments for soils and soil indicators for agronomic and environmental performance with reference to P sustainability. The duration of the experiments aimed towards early stages of initial mixing of waste materials with soils and crop establishment; an important period for overall crop health and risks of environmental P losses. Equally longer periods of material transformation in soils are important for future work. For example, in the UK agronomic guidance assumptions are made based on earlier farmyard manures research, estimating 50-60% of P being mineralised from waste materials to the following years crop (RB209, 2020).

The laboratory batch tests and pot incubation methods employed here did not induce leaching as in column transport experiments or pot and field experiments that induced runoff. Clearly, the field context of initiation of water transport (soil porosity and infiltration, slope, rainfall intensity, duration, antecedent conditions and irrigation management) affect the residence time of the solubilised P in the root zone for either uptake or export. However, our research gives a process understanding of solubility controls onto which more realistic field transport factors can be added, alongside climatic factors (moisture and temperature) affecting plant and microbial activities. Our results suggest strong chemical influences of matrix effects are modified by biotic processes, but this can be tested in future by modifying biological drivers such as temperature. Furthermore, our results must be considered based on some imbalances in P addition levels that make direct comparison of single amendment soils against those of blended amendments difficult. This arose as the pot experiments favoured treatment N balances in some amendments.

An encouraging effect of equivalent P uptake into above ground biomass was seen for some blended amendments equal to conventional fertiliser whilst in cases of two amendments (D1A2 and D2A1) the reduced water soluble P (Table S2) indicates potential to manage more of the added P into the plant and less to damaging environmental impacts of P leaching. As the test soil here had strong P sorption, future work should use a range of soils differing in P-fixation (and hence P-leaching risk and soil matrix effects on crop P-availability).

## **5. Conclusion**

A novel combination of chemical tests for P composition and solubility, supported by enzyme and <sup>31</sup>P NMR spectroscopy was applied to trials of bioenergy waste amended soils. This work has highlighted interactions between chemical and biological processes of P solubility. Findings suggest that geochemical matrix (e.g. pH, ionic strength, metals) effects in the presence of ash induce solubility increases rapidly after initial mixing with soil, that are stronger than initial digestate P solubility. However, incubations involving digestate result in favourable C:N:P stoichiometry promoting microbial transformations affecting solubility. An important finding was that a blend of ash and digestate increased available P for crop growth but limited excess P solubility when compared to conventional chemical fertiliser up to the six-weeks experimental duration. The P solubility was affected by time since mixing, incubation with and without a plant and use of individual versus blended waste materials. Hence, material types, blends and ratios, timescale and crop growth stage are key factors requiring process knowledge informing management for maximising crop P uptake against trade-offs of P mobility due to enhanced solubility that, under field conditions may induce P leaching.

The work highlights dominant P cycling processes as a basis to develop further studies to inform different management stages of utilising bioenergy wastes. Additionally, to support effective replacement of bioenergy waste combinations for conventional fertilisers. This preliminary work needs to be further developed across emerging waste material types and inherent processing variabilities. Future work needs to examine soils differing in risk factors (P sorption, leaching, pH buffering) and microbial activity, with an extended experimental duration suitable to assess longer-term mineralisation rates and environmental factors of climate and runoff.

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**Table 1:** Characteristics of the control soil, recycled waste material ash and anaerobic digestates as fresh weight, used for the pot experiments. Ash 1 is fly ash, ash 2 is bottom ash, digestate 1 is generated from food waste and digestate 2 is generated from crop waste.

Material	pH	EC mS/cm	LOI %	Dry matter %	Total C g/kg FW	Total N g/kg FW	Total P g/kg FW	Soluble P <sub>water</sub> g/kg FW	Molar C:N:P
Ash 1 (A1)	12.7	50.3	1	99.9	13.0	0.7	25.2	≤0.10	1.3 : 0.1 : 1
Ash 2 (A2)	12.6	25.8	14	89.1	81.1	1.0	11.0	≤0.10	18.9 : 0.2 : 1
Digestate 1 (D1)	9.0	9.6	66	3.2	15	5.5	0.4	0.14	96.7 : 30.4 : 1
Digestate 2 (D2)	8.5	3.9	76	3.6	18	3.2	0.5	0.10	92.8 : 14.2 : 1
Control soil	6.5	0.04	22	78	15	2.2	0.9	<0.10	43.0 : 5.4 : 1

EC, electrical conductivity; LOI, Loss On Ignition; FW, Fresh weight



**Table 2:** Phosphorus amendments treatment structure with two ash, two digestate materials, four blend combinations, positive (conventional fertiliser) and negative (soil only) controls. Target P or N values denote the addition level for each of the mixes with the soils in mesocosm experiments.

Treatment	ID	Material Source	Material N:P mass ratio	Added N (gN/kg soil DM) <sup>1</sup>	Added P (gP/kg soil DM) <sup>2</sup>	Intended element to balance		
						N	P	N and P
Ash 1	A1	Wood fly ash	0.03	0.001	0.038		yes	
Ash 2	A2	Wood bottom ash	0.09	0.003	0.038		yes	
Digestate 1	D1	Food wastes	13.75	0.120	0.009	yes		
Digestate 2	D2	Crop wastes	6.40	0.120	0.017	yes		
Blend 1	D1A1	Blended		0.120	0.038			yes
Blend 2	D1A2	Blended		0.120	0.038			yes
Blend 3	D2A1	Blended		0.120	0.035			yes
Blend 4	D2A2	Blended		0.120	0.035			yes
Conventional fertiliser	Urea + P	Urea + Superphosphate		0.092	0.038			yes
Soil control	Control	Myerscough soil	2.55					

<sup>1</sup>The intention was to maximise the treatments where N was balanced against the conventional fertiliser control. 0.120 gN/kg soil was chosen based on an expected 30% volatilisation of ammonia from the digestate; <sup>2</sup>The intended P mass of 0.038 gP/kg converts to 0.086 g/kg P<sub>2</sub>O<sub>5</sub>. The intention was to maximise the treatments where P was also balanced against the conventional fertiliser control. However, an analytical correction led to blends 3, 4 having 0.035 in instead of 0.038 gP/kg soil.

**Table 3:** <sup>31</sup>P NMR characteristics of the soils and amendments following NaOH-EDTA extractions. Data represent % distributions expressed relative to total P measured in solid extracts by aqua-regia extractions.

Source material P species	Ash 1 (A1)	Ash 2 (A2)	Digestate 1 (D1)	Digestate 2 (D2)	D1A1 PI-t <sub>end</sub>	D1A2 PI-t <sub>end</sub>	D2A1 PI-t <sub>end</sub>	D2A2 PI-t <sub>end</sub>	Control soil
~24.3 ppm, phosphonates	<0.1	<0.1	1.2	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
5.33 to 5.44 ppm, inorganic PO <sub>4</sub>	31.3	43.3	90.9	77.4	89.4	86.4	89.8	91.1	87.8
3.59 to 4.41 ppm, monoester region	<0.1	<0.1	5.4	6.6	10.6	13.6	10.2	8.9	12.2
~1.0 to -0.7 ppm, intact phospholipids	<0.1	<0.1	0.6	2.3	<0.1	<0.1	<0.1	<0.1	<0.1
~-0.9 to -1.7 ppm, DNA	<0.1	<0.1	1.3	1.2	<0.1	<0.1	<0.1	<0.1	<0.1
~2.6 ppm, other diesters	<0.1	<0.1	<0.1	0.1	<0.1	<0.1	<0.1	<0.1	<0.1
~-5.12 ppm, polyphosphate	<0.1	<0.1	0.5	0.4	<0.1	<0.1	<0.1	<0.1	<0.1
Alkaline insoluble P <sup>1</sup>	68.7	56.7	<0.1	11.9	<0.1	<0.1	<0.1	<0.1	<0.1

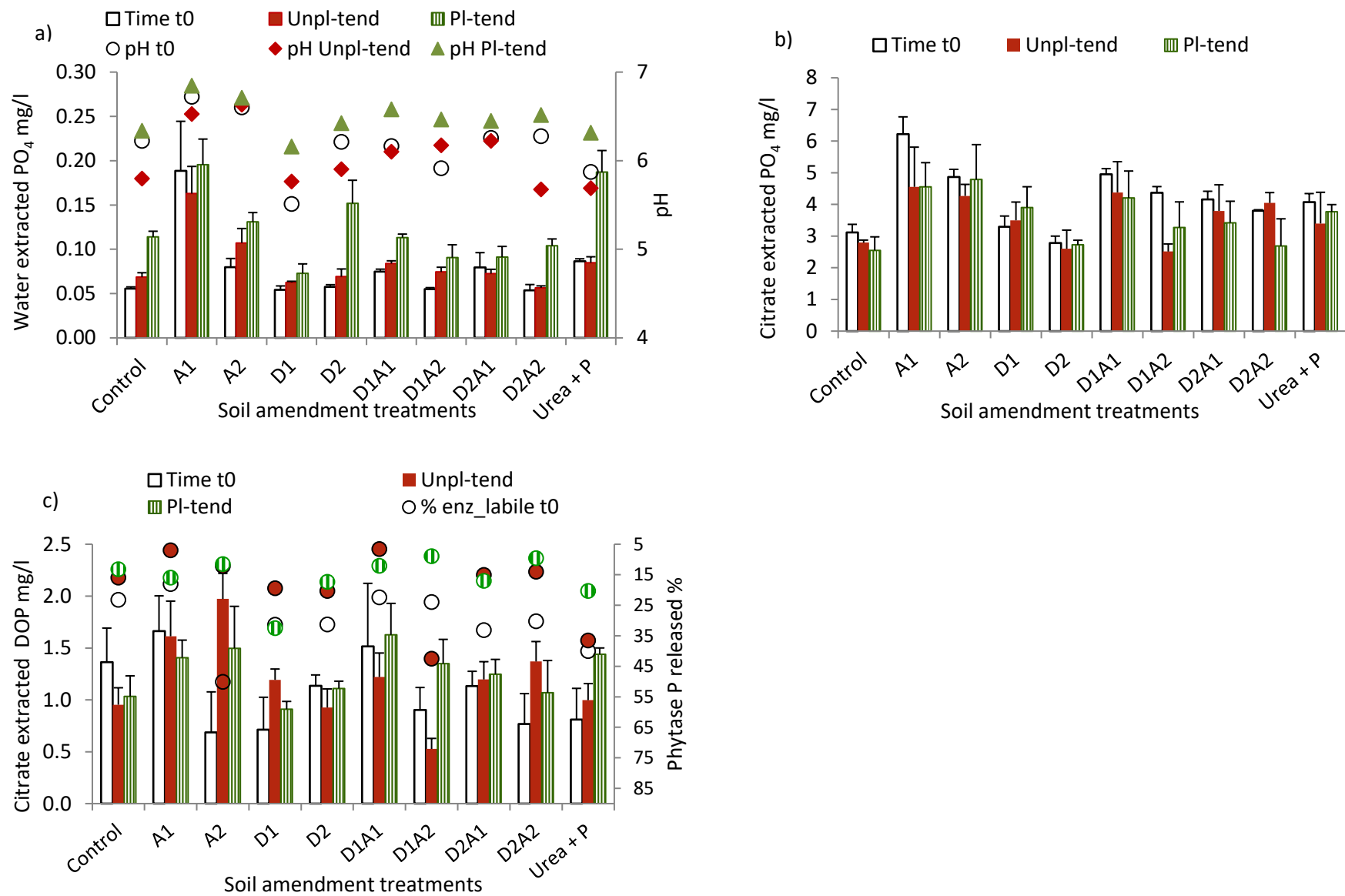
<sup>1</sup>The alkaline insoluble P equals the difference between total dissolved P of the NaOH-EDTA extracts prepared for NMR analyses and the total P analysis of the original solid material.

**Table 4:** Changes in extractable nutrients between time zero ( $t_0$ ) to experiment end for pots that were either unplanted ( $unpl_{end}$ ) or planted ( $pl_{end}$ ) with winter wheat within each level of amendment treatment. Directions of change are significant increases ( $\uparrow$ ), decreases ( $\downarrow$ ) or no significant change ( $\leftrightarrow$ ) according to Tukey testing ( $p < 0.05$ ).

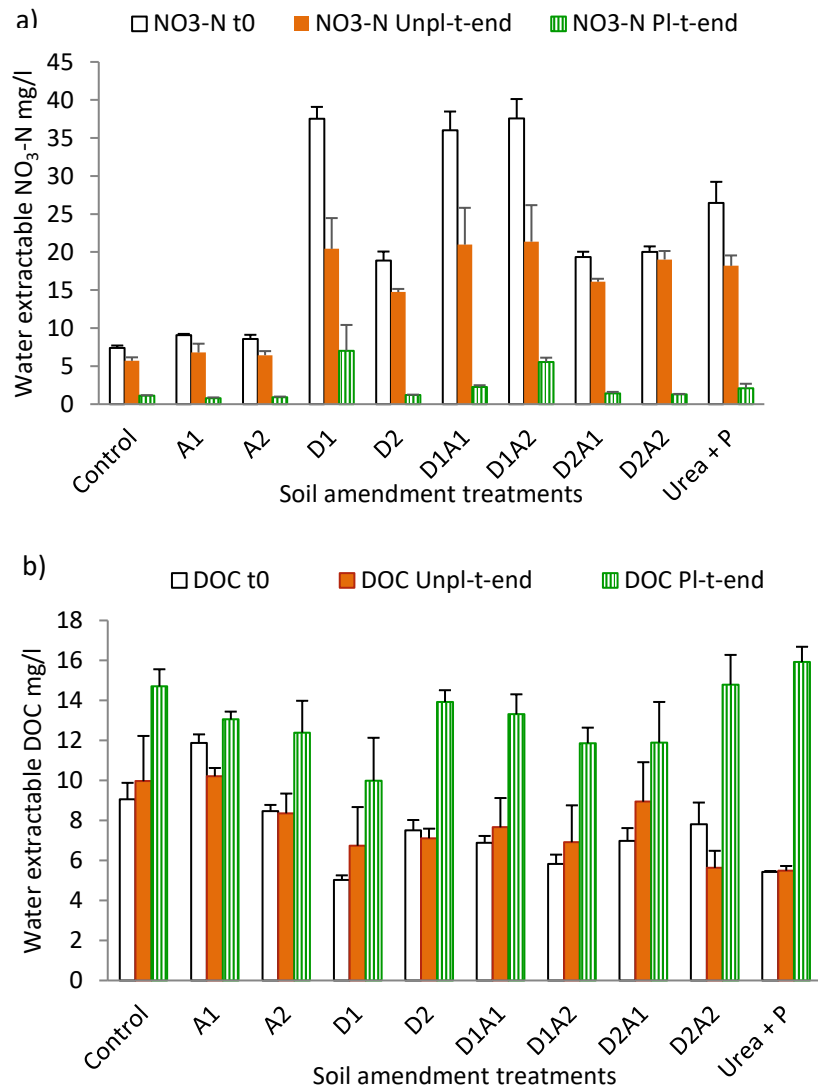
		water PO <sub>4</sub>	water TDP	DOC	NO <sub>3</sub>	citrate PO <sub>4</sub>	citrate TDP	Enzyme PO <sub>4</sub>
Control soil	$t_0 \rightarrow unpl_{end}$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$
	$t_0 \rightarrow pl_{end}$	$\uparrow$	$\uparrow$	$\leftrightarrow$	$\downarrow$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$
A1	$t_0 \rightarrow unpl_{end}$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$
	$t_0 \rightarrow pl_{end}$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$	$\downarrow$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$
A2	$t_0 \rightarrow unpl_{end}$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$
	$t_0 \rightarrow pl_{end}$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$	$\downarrow$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$
D1	$t_0 \rightarrow unpl_{end}$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$
	$t_0 \rightarrow pl_{end}$	$\leftrightarrow$	$\uparrow$	$\leftrightarrow$	$\downarrow$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$
D2	$t_0 \rightarrow unpl_{end}$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$	$\downarrow$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$
	$t_0 \rightarrow pl_{end}$	$\uparrow$	$\uparrow$	$\uparrow$	$\downarrow$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$
D1A1	$t_0 \rightarrow unpl_{end}$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$
	$t_0 \rightarrow pl_{end}$	$\uparrow$	$\uparrow$	$\uparrow$	$\downarrow$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$
D1A2	$t_0 \rightarrow unpl_{end}$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$
	$t_0 \rightarrow pl_{end}$	$\leftrightarrow$	$\leftrightarrow$	$\uparrow$	$\downarrow$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$
D2A1	$t_0 \rightarrow unpl_{end}$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$	$\downarrow$
	$t_0 \rightarrow pl_{end}$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$	$\downarrow$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$
D2A2	$t_0 \rightarrow unpl_{end}$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$
	$t_0 \rightarrow pl_{end}$	$\uparrow$	$\uparrow$	$\uparrow$	$\downarrow$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$
Conv fertiliser	$t_0 \rightarrow unpl_{end}$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$
	$t_0 \rightarrow pl_{end}$	$\uparrow$	$\uparrow$	$\uparrow$	$\downarrow$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$

**Table 5:** Mean and the first standard error (1SE) of P uptake mgP/pot for the controls and soil amendments.

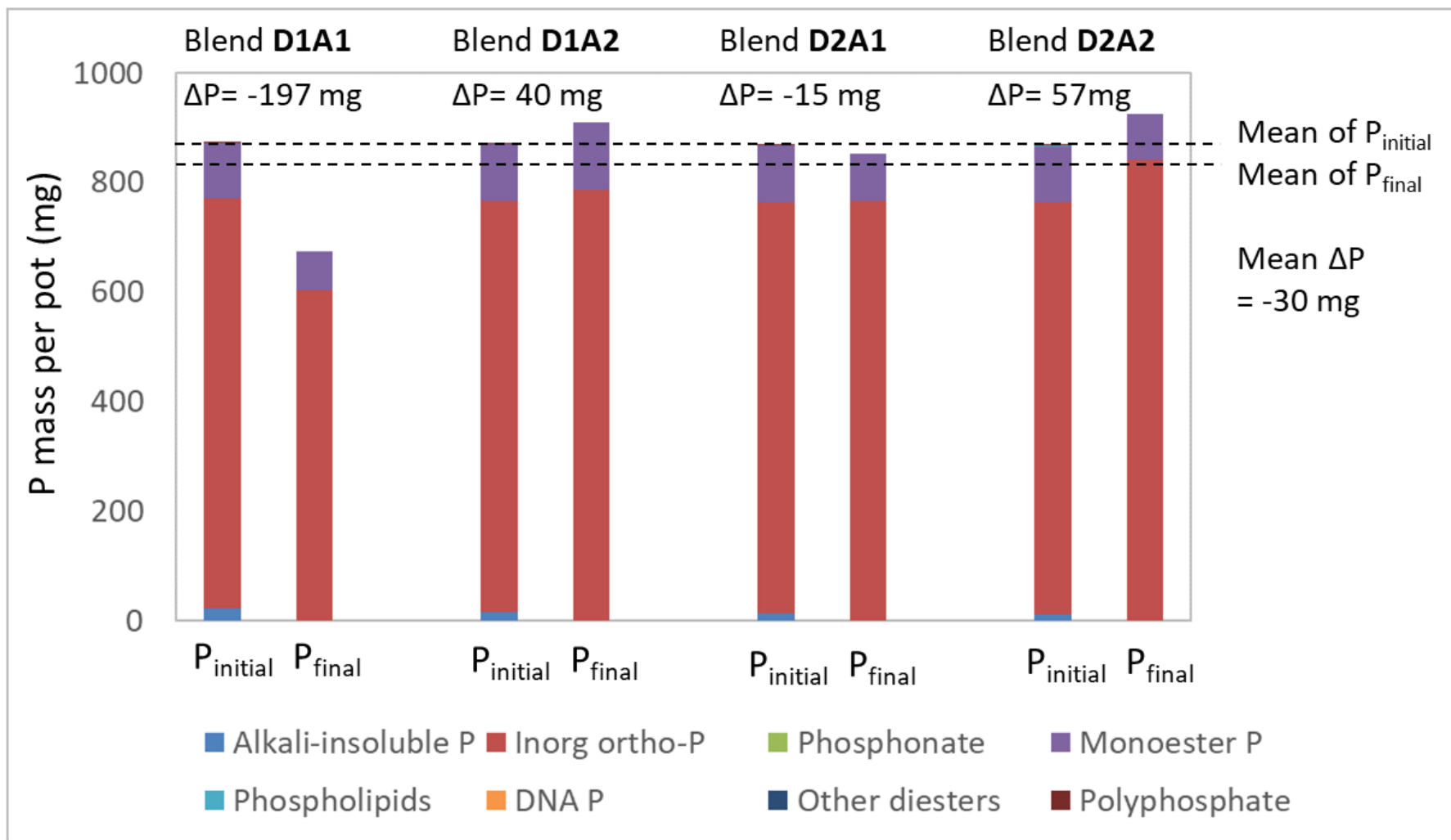
Treatment	P uptake (mgP/pot)	±1SE
Control soil	3.62	0.1
ABaF (A1)	4.48	0.4
ABaB (A2)	3.66	0.4
DB4 (D1)	7.54	1.0
DbA (D2)	7.88	0.5
D1A1	9.58	1.1
D1A2	7.74	0.6
D2A1	8.16	0.6
D2A2	7.86	0.6
Urea + P	10.56	0.8



**Figure 1:** Phosphate concentration (mg/l  $\pm$ 1S.E.) and pH in soil amendment pot experiment at the start of the experiment (Time t<sub>0</sub>), at the end of the growth period for unplanted (Unpl-t<sub>end</sub>) and planted (PI-t<sub>end</sub>) pots, illustrating: a) water extracted reactive phosphate, b) citrated extracted phosphate, c) citrate extracted dissolved organic P and the % of enzyme labile P converted to available P.



**Figure 2:** a) Water extractable soluble nitrate (NO<sub>3</sub>-N mg/l ±1S.E.), b) dissolved organic carbon (DOC mg/l ±1S.E.) for the control soil, ash, digestate and blends at the start of the experiment (t<sub>0</sub>), unplanted pots at the end of the experiment and planted pots at the end of the experiment for all treatments.



**Figure 3:** Changes between the initial P (time zero constituents; calculated from analyses of constituent component combinations) and final P (following growth trials; directly measured) form distributions from <sup>31</sup>P NMR extractions for the four soil and amendment blend treatments. Values are the sum of P form masses scaled per pot. Only inorganic ortho-P (brown), monoester P (purple) and alkali-insoluble P (mid blue) are apparent as other forms were trace quantities or below detection. The individual treatment ΔP should theoretically be in a negative direction (due to plant uptake) but shows the effect of cumulative errors for individual pots whilst the overall mean is a decrease of 30 mgP per pot.





