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Organic amendments as phosphorus fertilisers: chemical analyses, biological processes and plant P uptake

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Abstract

As phosphorus (P) fertilisers become increasingly expensive there is a need to find innovative ways to supply crops with P. Organic amendments (OA) can contain high concentrations of total P, although the P is present in various forms. We aimed to determine the forms of P and carbon (C) in a range of OA and the effect of these OA on soil microbial biomass, P release, arbuscular mycorrhizal (AM) colonisation, and plant P uptake. Four OA were investigated: two chicken litters (CHK-STR and CHK-SD, one with straw bedding and one with sawdust bedding), a pig litter (PIG-STR) and a municipal waste compost (COMP). An incubation experiment and a plant growth experiment were conducted in which OA and INORG-P were supplied at 15 mg P kg^{-1} soil and a zero P control was included. All OA had high P concentrations and did not result in an increase in the soil microbial biomass C. There were few temporal changes in available P throughout the incubation experiment suggesting that solubilisation and/or mineralisation of P occurred at a similar rate as conversion of P to unusable forms. Of the OA, PIG-STR had the largest proportion of orthophosphate P and bicarbonate extractable P, and it provided the most P to plants. While CHK-STR had a higher proportion of orthophosphate P and bicarbonate

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extractable P than CHK-SD, both CHK-STR and CHK-SD provided plants with similar amounts of P. This could be because CHK-SD had a higher proportion of phytate, which can be rapidly mineralised to orthophosphate, and/or because plants in the CHK-SD had higher rates of arbuscular mycorrhizal (AM) colonisation compared with CHK-STR. This study provides new insights into plant and microbial responses to OA which could help in the development of sustainable food production systems.
Keywords: Phosphorus, Organic amendments, Diffusive gradients in thin films (DGT), Microbial biomass carbon, Nuclear magnetic resonance spectroscopy, Wheat

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1. Introduction

Phosphorus (P) is an important nutrient for plant growth; however, in many regions of the world soils are low in plant-available P (Holford, 1997). Therefore, P fertilisers play an important role in agriculture. Most P fertilisers in current use are derived from phosphate rock; however, easily mined deposits of phosphate rock are becoming scarce (Cordell and White, 2011). It is therefore imperative that alternative sources of fertiliser P are found to ensure the sustainability of food production systems.

Organic amendments (OA) such as manures, composts, and plant residues have long been used to provide nutrients to crops (Quilty and Cattle, 2011). Moreover, utilising OA can recycle large amounts of P that could otherwise cause environmental problems. However, OA can vary considerably in the amount of P they contain (Sharpley and Moyer, 2000). While many studies have found that OA treatments provide plants with more P than unfertilised controls (e.g. Waldrip et al., 2011; Requejo and Eichler-Löbermann, 2014; Duong et al., 2012), most studies do not have appropriate comparisons among OA or with mineral fertilisers. For example, studies often include P over-application or application of P and N at varying rates, which make it difficult to interpret P results. Moreover, studies rarely examined the chemical nature of the P in OA (with Peirce et al., 2013 as one

good exception), which is necessary to better understand plant P uptake from OA. Studies which link plant P uptake from OA with chemical properties of OA and soil chemical and biological processes are needed if OA are to be accurately used in agriculture.

The carbon (C):P ratio of OA is often used as an indicator of fertiliser quality (Takeda et al., 2009) and therefore plant P uptake from OA. If C:P is high, more C is added to soil to reach the same level of P addition than for OA with a low C:P. Carbon is often the factor limiting microbial biomass in soil. Therefore, if large amounts of C are added with OA there is a rapid increase in microbial biomass and microbial demand for P (Malik et al., 2013; Ros et al., 2006; Takeda et al., 2009). Soil microorganisms immobilise P when C:P ratios of OA are higher than 20 (Malik et al., 2013; Takeda et al., 2009), reducing the amount of P available for plant uptake. However, this has not been well investigated for OA with a C:P below 20. Moreover, the chemical nature of the C in OA might also be important. For example, if OA contain stable C forms that cannot be readily decomposed, then OA might not stimulate microbial growth. Moreover, if immobilisation does not occur, then the forms of P present in the OA may be a better indicator of fertiliser quality.

Different species of P behave in different ways when added to soil, some contributing more than others to the plant-available P pool. More than 60% of the P in OA is commonly found to be orthophosphate (Sharpley and Moyer, 2000). If this orthophosphate is soluble it can readily leach out of amendments and become available to plants or be sorbed to soil particles (Alamgir and Marschner, 2013; Malik et al., 2013). Conversely, insoluble orthophosphate needs to be solubilised before it is available to plants. Both soil microbes and plant root exudates can play a large role in solubilising P in soil (Richardson et al., 2011). In addition to orthophosphate, OA contain organic P species. Phospholipids and nucleic acids can be rapidly mineralised by soil microbes (Harrison, 1982; Islam and Ahmed, 1973) and therefore can quickly contribute to the plant-available pool of P. Phytate, which is present in seeds (Nelson et al., 1968; Noack et al., 2012) and therefore in

44 manures of livestock fed on grains (Toor et al., 2005), has been considered to be stable in
45 soils (He et al., 2006; Celi et al., 1999). However, there is emerging evidence that suggests
46 otherwise (Doolette et al., 2010; Hill and Richardson, 2007; Peirce et al., 2013). When
47 added to a calcareous soil, Doolette et al. (2010) report the disappearance of phytate cou-
48 pled with an increase in orthophosphate P and α- and β-glycerophosphate over 13 weeks,
49 suggesting microbial degradation of phytate. Therefore, soil microbial activity can have a
50 large influence on soil P transformations and plant P uptake.

51 Plant uptake of P from OA could also be affected by arbuscular mycorrhizal fungi
52 (AMF). Approximately 80% of terrestrial plant species form symbiotic associations with
53 AMF (Smith and Read, 2010) which can enhance plant P uptake (Facelli and Facelli, 2002;
54 Pearson and Jakobsen, 1993; Tibbett, 2000). It is well established that at high levels of in-
55 organic P fertiliser addition the percentage of roots colonised by AMF decreases (Abbott
56 et al., 1984; Bolan et al., 1984; Treseder, 2004). However, it is unclear whether or not
57 addition of P in the form of OA has a similar effect. Cavagnaro (2015) found that compost
58 generally has a neutral or positive effect on arbuscular mycorrhizal (AM) colonisation;
59 however, as compost application increases AM colonisation may decrease (Cavagnaro,
60 2014). If OA provide a more sustained release of plant-available P rather than the im-
61 mediate increase provided by soluble fertilisers they could supply P to crops throughout
62 the growing season without adversely affecting the formation of AM. This could be ben-
63 eficial to crops such as wheat which require P for the entire growing season (Römer and
64 Schilling, 1986).

65 The results of a study investigating the use of a range of OA as P amendments are
66 reported here. There were five main research questions addressed in this study:

67 1. What forms of P and C are present in the OA?
68 2. Does addition of OA lead to an increase in microbial biomass?

3. When is the P in the OA released over the short-term?
4. Do OA affect AMF colonisation?
5. How much P in the OA is available to plants?

To answer these questions an incubation experiment and a plant growth experiment were conducted. We hypothesised that:

1. Forms of P and C would vary among the different OA, with most OA containing high levels of orthophosphate.
2. OA with higher C:P ratios would lead to increased microbial biomass.
3. P in OA would be released slowly over time.
4. OA would have a neutral or positive effect on AMF colonisation.
5. The amount of P in OA which is taken up by plants will be related to the forms of P and C, and the C:P ratio of the OA.

2. Methods

2.1. Soil and organic amendments: sources and analysis

The soil used in the experiments was collected from the top 10 cm layer of a P deficient sandy loam from a block of un-farmed land near Black Point on the Yorke Peninsula in South Australia (S34°36.776', E137°48.599'). The soil in this location is a Calcarosol according to the Australian Soils Classification (Isbell, 2002). Soil was air-dried, passed through a 2 mm sieve to remove any stones and large invertebrates, and stored at room temperature prior to use. Electrical conductivity (EC) and pH were measured in a 1:5 soil:water suspension. Calcium carbonate content was measured according to Martin and Reeve (1955). Total P was determined by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) following aqua regia digestion of soil samples (Zarcinas et al., 1996). The availability of P in the soil was estimated using two methods: the Colwell P

93 method (Colwell, 1963) and the diffusive gradients in thin-films (DGT) P method (Mason
94 et al., 2010).

95 Four organic amendments (OA) were used in this study. Two chicken litters, one with
96 straw bedding (CHK-STR) and one with sawdust bedding (CHK-SD), were obtained from
97 a waste collection/redistribution company in South Australia. Pig litter with straw bedding
98 (PIG-STR) was collected from the piggeries at the Roseworthy Campus of the University
99 of Adelaide. A commercially available compost made from municipal waste was also
100 obtained (COMP). The OA were dried at 40 °C and were passed through a 5 mm sieve
101 before being stored at room temperature.

102 Organic amendments were analysed for a number of different properties. Three sam-
103 ples of each OA were ground to a fine powder in a grinding mill (IKA® MK Basic 10
104 Grinder). The concentration of total P, along with a variety of other elements, was deter-
105 mined by inductively coupled plasma-mass spectrometry (ICP-MS) following digestion
106 of the materials in aqua regia (Zarcinas et al., 1996). Bicarbonate extractable P, as an
107 indicator of potentially plant-available P, was determined following the method for Col-
108 well P extraction of soils (Colwell, 1963). Total C and total nitrogen (N) were measured
109 by dry combustion using a LECO CNS200 Analyser. Electrical conductivity and pH of
110 the OA were measured in a 1:5 OA:water suspension. Moisture content was determined
111 gravimetrically by drying samples at 105°C.

112 The P species in the OA were analysed using solution ^{31}P nuclear magnetic resonance
113 (NMR) spectroscopy. Samples (2 g) were extracted with 40 ml of a solution containing
114 0.25 M NaOH and 0.05 M Na_2EDTA. Extracts were filtered through Whatman no.42 filter
115 paper and a 30 ml aliquot was immediately frozen and freeze-dried. After freeze-drying, a
116 500 mg sub-sample was ground and redissolved in 5 ml of deionised water. This was then
117 centrifuged at 1300 × *g* for 20 min and a 3.5 ml aliquot of the supernatant was placed in a
118 10 mm NMR tube with 0.3 ml of deuterium oxide D_2O and 0.1 ml of a 6 g L^{-1} methylene

119 diphosphonic acid (MDP) solution. Solution ^{31}P NMR spectra were acquired at 24°C on
120 a Varian INOVA400 NMR spectrometer (Varian, Palo Alto, CA) at a ^{31}P frequency of
121 161.9 MHz. Recovery delays ranged from 13 to 26 s and were set to at least five times the
122 T_1 value of the orthophosphate resonance determined in preliminary inversion-recovery
123 experiments for each extract (data not presented). A 90° pulse of 23 μs was used with an
124 acquisition time of 1.0 s and broadband ^{1}H decoupling. Between 360 and 4500 scans were
125 acquired for each sample, depending on the P concentration of the freeze-dried extract.
126 The spectra presented have a line broadening of 2 Hz.

127 The relative concentrations of P species in the NaOH-EDTA extracts were determined
128 from ^{31}P NMR spectra using integration. The spectra were divided into five different re-
129 gions representing separate P types: inorganic orthophosphate, phytate, other monoester
130 P, diester P and pyrophosphate. The absolute concentration of each P species was calcu-
131 lated using a spin counting approach, in which the added P compound (MDP) was used
132 as an intensity standard. The total P concentration detected by NMR spectroscopy for
133 each sample was calculated by integrating all signals from the extract against that of the
134 MDP standard. A minor correction was needed in the determination of orthophosphate
135 and phytate concentrations due to the overlap of the phytate C-2 peak with the orthophos-
136 phate peak. Total phytate was calculated as 6/5 times the total concentration of the three
137 observable phytate resonances. A 1/5 proportion of this value was then subtracted from
138 the total orthophosphate concentration.

139 The C species in the OA samples were analysed using solid-state ^{13}C NMR spec-
140 troscopy. Solid-state ^{13}C cross polarization (CP) NMR spectra were acquired with magic
141 angle spinning (MAS) at a ^{13}C frequency of 50.33 MHz on a Bruker 200 Avance spec-
142 trometer. Samples were packed in a 7 mm diameter cylindrical zirconia rotor with Kel-F
143 end-caps, and spun at 5 kHz. Spectra were acquired using a ramped-amplitude cross po-
144 larization (CP-ramp) pulse sequence, in which the ^{1}H spin lock power was varied linearly

145 during the contact time. A 1-ms contact time and a 1-s recycle delay were used and 30,000
146 transients were collected for each spectrum.

147 All spectra were processed with a 50 Hz Lorentzian line broadening. Chemical shifts
148 were externally referenced to the methyl resonance of hexamethylbenzene at 17.36 ppm.
149 All spectral processing was completed using Bruker TopSpin 3 software. Empty rotor
150 background signals were subtracted and the resultant spectra were integrated across the
151 following chemical shift limits to provide estimates of broad carbon types: 0-45 ppm (alkyl
152 C), 45-60 ppm (N-alkyl C), 60-110 ppm (O-alkyl C), 110-145 ppm (aryl C), 145-165 ppm
153 (O-aryl-C), and 165-215 ppm (carbonyl C). Signal intensity found in spinning side bands
154 was allocated back to their parent resonances according to the calculations presented by
155 Baldock and Smernik (2002).

156 *2.2. Soil incubation experiment and plant growth experiment*

157 Two experiments were conducted, an incubation experiment and a plant growth exper-
158 iment. Organic amendments were added to soil at a rate of 20 kg P ha^{-1} (15 mg P kg^{-1}
159 soil) for both experiments. Additionally, an inorganic P fertiliser (phosphoric acid) at a
160 rate of 20 kg P ha^{-1} (INORG-P), and a zero P control (CONT), were also included. To
161 allow realistic P release rates the OA were not ground before use.

162 For the incubation experiment, 200 g of soil was added to plastic, non-draining, 300
163 ml pots. Reverse osmosis (RO) water was added to soil to 55% of water holding capacity,
164 which has been found to be the optimal level for microbial activity in soils with similar
165 clay content (Setia et al., 2011). The soil was then pre-incubated for five days to avoid
166 the flush of microbial activity upon re-wetting of dry soil (Fierer and Schimel, 2003) and
167 to re-establish the soil microbiota. Macro- and micro-nutrients, other than P, were added
168 to the soil to ensure adequate concentrations of nutrients for wheat growth: 75 mg kg^{-1}
169 potassium sulphate, 75 mg kg^{-1} calcium chloride, 50 mg kg^{-1} ammonium nitrate, 45 mg

kg^{-1} magnesium sulphate, 2.1 mg kg^{-1} copper sulphate, 5.4 mg kg^{-1} zinc sulphate, 6.4 mg kg^{-1} manganese sulphate, 0.33 mg kg^{-1} cobalt chloride, 0.18 mg kg^{-1} sodium molybdate, 0.3 mg kg^{-1} boric acid, 0.4 mg kg^{-1} iron EDTA. Then, OA or phosphoric acid was added at 15 mg P kg^{-1} and mixed in thoroughly. Phosphoric acid was first diluted in RO water to make a solution with pH 1.62. Soil was then packed into pots to a bulk density of 1.3 g cm^{-3} (similar to the bulk density of this soil in the field). Soil in control pots received no additional P but were mixed in the same way as the other treatments. Pots were then watered to 70% of water holding capacity (optimal for plant growth) and moved to a naturally lit greenhouse (mean minimum temperature: 20 °C; mean maximum temperature: 25 °C). Water content in pots was maintained every 2-3 days. The incubation experiment was conducted in September, 2014. Soil was harvested at five sampling times over a period of 30 days (on days: 0, 2, 9, 16, and 30). At each sampling time three replicates of each treatment were destructively harvested.

For the plant growth experiment, 1 kg of soil was used per pot and there were four replicates per treatment. Pots were plastic, non-draining, 1 l pots. Pre-incubation of soil and addition of nutrient solutions (other than P) and P treatments (OA, INORG or CONT) were conducted as described in the incubation experiment. Additionally, 100 g of AMF inoculum (*Rhizophagus irregularis*) was mixed into the soil at the same time as OA were added to ensure adequate AMF levels for effective colonisation. Pots were kept in the same greenhouse with the same watering schedule as for the incubation experiment, but the plant growth experiment was conducted in October, 2014. Four days after moving the pots into the greenhouse, four pre-germinated wheat (var. Axe) seeds were planted per pot. Three days after planting, the seedlings were thinned to three seedlings of a similar size per pot. After 14 d of growth, an additional 50 mg of ammonium nitrate was added to each pot to ensure plants did not become nitrogen deficient. After 30 days, plants and soil were destructively harvested.

2.3. Soil sampling and plant analysis

For the incubation experiment, at each sampling time all soil was removed from pots and mixed in plastic bags before soils were subsampled for analysis. For the plant growth experiment, sampling was conducted as follows. Shoots were separated from roots and weighed. Roots were hand-picked from soil, after which soil was mixed and subsampled for analysis. Roots were then washed free of remaining soil, weighed and subsampled for determination of AM colonisation. Shoots and remaining roots were oven-dried at 50°C until a constant mass was obtained and then weighed. Dried root and shoot biomass samples were then ground to a fine powder using a ball mill. Ground material was analysed for total P concentration using inductively coupled plasma-optical emission spectrometry (ICP-OES) and total N concentration using complete combustion gas chromatography. The subsample of roots for determination of AM colonisation was cleared in potassium hydroxide (KOH, 10% W/V) then stained with ink and vinegar using a modification of the method of Vierheilig et al. (1998). AM colonisation was determined using the gridline intersect technique (Giovannetti and Mosse, 1980).

2.4. Soil analyses

Microbial biomass C (MBC) was determined for the first three sampling times (day 0, 2, and 9) in the incubation experiment and at the end of the plant growth experiment. Microbial biomass C was determined in duplicate by the fumigation-extraction method (Vance et al., 1987). Fresh soil samples were split into two subsamples, one of which was fumigated in a chamber with chloroform. Extraction of both fumigated and non-fumigated samples was conducted using 0.5 M potassium sulphate (K_2SO_4). The K_2SO_4 extracts were acidified and analysed using a Shimadzu TC analyser. Microbial biomass C was determined by subtracting the C content of the non-fumigated sample from the C content of the fumigated sample. Often, conversion factors are used in the calculation of

221 MBC; however, due to uncertainty with these conversion factors they were not used here.
222 Therefore, it is likely that MBC was underestimated in these experiments, but can still
223 be used in a comparative manner among treatments. Soil samples from the incubation
224 experiment were also analysed for plant-available P using two different methods: the Col-
225 well method (Colwell, 1963) and the DGT method (Mason et al., 2010). The phosphorus
226 buffering index (PBI) was determined following Moody (2007). A single addition of phos-
227 phorus (KH_2PO_4 in 0.1 M $CaCl_2$) at 1000 mg P kg^{-1} was added to the soil at a 1:10 soil to
228 solution ratio. The soil solution was shaken for 17 h and the P remaining in solution after
229 this time was determined and compared to the amount added. The PBI index calculation
230 was performed as described by Moody (2007) using Equation 1, where Ps is the P sorbed
231 (mg P kg^{-1} soil) and c is the final solution P concentration (mg P l^{-1}). The pH of soil
232 samples collected on day 0 and day 9 of the incubation experiment were determined in 1:5
233 soil:water suspensions.

$$\mathrm{PBI} = (\mathrm{Ps} + \mathrm{initial\,Colwell} - \mathrm{P})/\mathrm{c}^{0.41} \quad (1)$$

234 *2.5. P budget analysis*

235 As the bicarbonate-extractable P method (for analysing OA) and the Colwell P method
236 (for analysing soil) are essentially the same method, the behaviour of this pool of P can
237 be investigated following addition to soil. While each treatment in the incubation experi-
238 ment received the same amount of total P, they received different amounts of bicarbonate-
239 extractable P. The total amount of bicarbonate-extractable P added (a) was calculated for
240 each treatment (Equation 2).

$$a = \text{bicarbonate-extractable P (g kg}^{-1}\text{) in OA} \times \text{amount of OA added} \quad (2)$$

241 All of the P in the INORG-P is assumed to be bicarbonate extractable. The hypothe-
242 sised Colwell P of amended soil (b) was calculated using Equation 3:

$$b = \text{initial Colwell P of soil} + a \tag{3}$$

243 Then, the difference between the hypothesised Colwell P of the soil (b) and the actual
244 Colwell P of the soil in the incubation experiment at day 0 (c) was determined (Equation
245 4). The average Colwell P of the PIG-STR treatment at day 0 was greatly affected by
246 one replicate having more than a two-fold higher Colwell P than the other two replicates.
247 Therefore, this replicate was excluded from the dataset for this analysis.

$$c = \text{Colwell P of soil at day 0} - b \tag{4}$$

248 The percentage of a which contributed to c was calculated (d), that is, the percent of
249 bicarbonate extractable P added that contributed to an increase in the Colwell P of the soil.

$$d = \frac{c}{a} \times 100 \tag{5}$$

250 *2.6. Statistical anlaysis*

251 For the incubation experiment, the effects of 'treatment' and 'time' on DGT P and
252 Colwell P were explored using generalised linear models (GLM). As there was an in-
253 teraction between 'treatment' and 'time' in the DGT P model, multiple Tukey's honest
254 significant difference (HSD) tests (with appropriate p adjustments) were used to explore
255 the differences among treatments at day 30, and to explore the differences over time within
256 each treatment. For the Colwell P model there was no interaction between 'treatment' and
257 'time'. The differences among treatments were explored using a Tukey's HSD test. The
258 effect of 'treatment' and 'time' on MBC was explored using a two-way ANOVA. As there

259 was an interaction between 'treatment' and 'time', multiple Tukey's HSD tests (with ap-
260 propriate p adjustments) were used to explore significant differences among treatments at
261 each sampling time.

262 For the plant growth experiment, ANOVAs were used to explore the effect of 'treat-
263 ment' on shoot and root biomass, shoot and root P uptake, AM colonisation (%), infected
264 root length, and MBC. When ANOVAs were significant, Tukey's HSD tests were used to
265 identify specific differences. All statistics were performed in R (version 3.2.3) with α level
266 0.05 unless specified otherwise.

267 3. Results

268 *3.1. Soil and organic amendment analyses*

269 The soil had a low EC (0.07 dS m^{-1}), was slightly alkaline (pH 8.5) and had a water
270 holding capacity of 22.2%. Calcium carbonate content was negligible (< 0.2%). The total
271 P concentration in the soil was very low (48 mg kg^{-1}) as was the Colwell P (3 mg kg^{-1})
272 and the DGT P (4 μg l^{-1}).

273 The four OA analysed varied in their chemical compositions, with COMP differing
274 the most from the other OA having lower total C, N, and P concentrations, a lower EC
275 and a higher pH. The chicken litters were most similar in composition, differing from
276 the PIG-STR largely due to higher total P and N concentrations, higher EC and lower
277 bicarbonate-extractable P (as a proportion of total P).

278 Solid-state ^{13}C NMR spectra of the OA are shown in Figure S1. The spectra indi-
279 cate the CHK-STR sample and the CHK-SD sample were very similar in composition.
280 The PIG-STR sample was also similar to the CHK-STR and CHK-SD samples, while the
281 COMP sample was very different in composition compared to the other three OA. The
282 signal to noise ratio of the spectrum for the COMP sample was poorer than for the other

283 OA. This in part reflects the lower C content of this sample (Table 1). However, it also
284 reflects a lower observability for this sample (40.8%, Table 2) than for the other three sam-
285 ples (81.9-101.2%, Table 2). This is consistent with the relatively high iron (Fe) content of
286 the COMP sample (8.9 g kg^{-1}, Table S1). Iron and other paramagnetic metals are known
287 to interfere with detection of NMR signals (Oades et al., 1987). A quantitative analysis
288 of the spectra was carried out by integrating the spectra across broad chemical shift re-
289 gions assigned to broad C types. Table 2 confirms the similarity of the CHK-STR and
290 CHK-SD samples in their composition of C species, with only small differences attributed
291 to alkyl:O-alkyl ratios and aryl concentrations. The chicken litters differed from the PIG-
292 STR largely due to higher alkyl concentrations and lower aryl and O-aryl concentrations.
293 The COMP sample was very different in composition from other OA; in particular, COMP
294 had lower O-alkyl and higher aryl concentrations.

295 Solution ^{31}P NMR spectra of the OA are shown in Figure S2. The spectra indicate
296 that all litters contained high amounts of orthophosphate. The CHK-STR and CHK-SD
297 samples also contained high amounts of phytate. The signal to noise ratio of the spec-
298 trum for the COMP sample was poorer than that for the other OA. This can be attributed
299 to the lower P content of this sample (2.5 g kg-1, Table 2), the lower percentage of total
300 P extracted from this sample (63.9%), and interference from the high Fe content of this
301 sample (8.9 g kg-1, Table S1). A quantitative analysis of the spectra was carried out by
302 integrating the spectra across broad chemical shift regions assigned to broad P types. Due
303 to the poor signal, P types in the COMP sample could not be determined by deconvolution.
304 The quantitative analysis confirms that all litters contained a large amount of orthophos-
305 phate, with the PIG-STR sample containing the most (77.3%, Table 3). The CHK-STR
306 and CHK-SD samples also contained a high amount of phytate (23.1-31.1%) compared to
307 the PIG-STR sample, for which only 6.4% of the detectable P was phytate (Table 3). The
308 largest differences in P composition lay between the CHK-SD and the PIG-STR due to

phytate, orthophosphate, and phospholipid concentrations.

3.2. Incubation experiment

There were no significant differences in MBC among treatments at any of the sampling times. Microbial biomass C was highest for all treatments at day 2 (129.8-163.6 mg kg^{-1}). On day 9, MBC for all treatments was similar to those on day 0 (Table 4).

There was a large amount of variability within some treatments for both DGT P and Colwell P (Figure 1). There was a significant ($p < 0.05$) interaction between 'treatment' and 'time' for DGT P. At day 30 there were significant differences in DGT-P between treatments, where INORG > CHK-STR, CHK-SD, PIG-STR > COMP, CONT. The INORG-P treatment demonstrated a significant decline in DGT-P over time, while the OA treatments did not. There were no significant differences in Colwell P among times; however, there were some significant differences among treatments. While PIG-STR did not differ from INORG-P, it had a greater Colwell P than CHK-STR and CHK-SD. There was no difference in Colwell P between COMP and CONT. The PBI was similar across all treatments and times, ranging from 55.6 to 71.7 (data not shown). The pH of the soil at day 0 was similar among treatments (7.9-8.2, data not shown).

3.3. Plant growth experiment

Colonisation of roots by AMF was affected by treatment (Figure 3). Percent colonisation of roots by AM was significantly lower in the INORG-P treatment than all other treatments (Figure 3a). While none of the litter treatments differed significantly from the CONT treatment, the COMP treatment had significantly greater percent colonisation than the CONT treatment. When AM colonisation was measured as infected root length, CHK-SD had the longest infected root length and INORG-P the shortest (Figure 3b). The CHK-SD, PIG-STR and COMP treatments all had significantly longer infected root lengths than the control.

At the end of the 30 day plant growth experiment there were significant differences in plant biomass (Figures 2a and 2c). Plants from the INORG-P treatment had significantly greater shoot biomass than any other treatment, with almost double the shoot biomass of PIG-STR. The litter treatments all had significantly greater shoot biomass than the CONT treatment (74-133% greater), but the COMP treatment did not differ significantly from the CONT treatment in shoot biomass. Plants from the PIG-STR treatment had the greatest root biomass, which was significantly greater than plants of the other OA treatments and the CONT treatment (26-120% greater). Plants from the CHK-STR and CHK-SD treatments had similar root biomass to those from the INORG-P treatment. Root biomass of plants from the COMP treatment did not differ significantly from those of the CONT treatment. Plants from the INORG-P treatment had more shoot biomass than root biomass (root biomass:shoot biomass ratio = 0.91), while plants from all other treatments had more root biomass than shoot biomass (with root biomass:shoot biomass ratios ranging from 1.91 to 2.30).

Treatments also differed in plant P uptake at the end of the plant growth experiment (Figures 2c and 2d). Plants from the INORG-P treatment had significantly greater shoot P content than any other treatment (123-1005% greater). The litter treatments all had significantly greater shoot P content than the CONT treatment (248-397% greater), but the COMP treatment did not differ significantly from the CONT treatment in shoot P content. Plants from the PIG-STR treatment had significantly greater root P content than plants from all other treatments (36-947% greater). Both the CHK-STR and CHK-SD also had significantly greater root P content than the INORG-P, COMP and CONT treatments. The COMP treatment did not differ significantly from the CONT treatment in regards to root P content. Whereas none of the litter treatments had significantly greater root biomass than the INORG-P treatment, they all had significantly greater root P content than the INORG-P treatment. Plants from the INORG-P treatment had more P in their shoots than in their

360 roots, while plants in CHK-STR, CHK-SD and PIG-STR all had more P in their roots than
361 in their shoots.

362 Microbial biomass C was significantly larger in the INORG-P, CHK-STR, CHK-SD
363 and PIG-STR treatments than in the COMP and CONT treatments (Table 4).

364 *3.4. P budget analysis*

365 The data collected here permitted the development of a P budget analysis. While OA
366 were added at the same rate of total P (15 mg kg^{-1}), the amount of bicarbonate-extractable
367 P added varied among treatments from 3.5-9.0 mg kg^{-1}. The predicted Colwell P of
368 soil did not match the actual Colwell P of the soil for any treatment. The percentage
369 of bicarbonate-extractable P added which contributed to an increase in the Colwell P of
370 the soil was highest for INORG-P (61.3%), followed by PIG-STR (57.7%), then CHK-SD
371 (54.5%), then COMP (51.4%) then CHK-STR (36.4%).

372 **4. Discussion**

373 OA with high C:P ratios stimulate the microbial biomass, which immobilise P (Malik
374 et al., 2013; Takeda et al., 2009). However, while the C:P ratio of the PIG-STR was about
375 three-fold larger than that of the CHK-STR and CHK-SD, the PIG-STR resulted in greater
376 plant P uptake. This suggests that P immobilisation was not a dominant mechanism in
377 this plant growth study, and is further supported by our incubation results demonstrating
378 no effect of OA on the size of the microbial community (MBC). It has been suggested
379 that the critical P concentration needed in an amendment to avoid the negative effects of P
380 immobilisation is between 2-3 g P kg^{-1} (Nziguheba et al., 1998; Six et al., 2014). In our
381 study, apart from the COMP (2.5 g P kg^{-1}), all OA had total P concentrations much higher
382 than this (Table 1). However, in addition to the P concentration, the forms of C in OA can
383 influence microbial biomass and hence immobilisation of P.

The OA varied in their proportions of different C species as detected by NMR spectroscopy. The CHK-STR and CHK-SD samples were quite similar to each other, despite having different bedding materials, indicating that straw and sawdust are chemically similar in terms of C speciation. This has been found previously, with both straw and sawdust containing similar amounts of cellulose (70-75% of weight) and lignin (15-20% of weight; Lv et al. 2010). The COMP sample had the highest alkyl C:O-alkyl C ratio, indicating that much of the C present in the sample had already been degraded by microbes in the composting process (Baldock et al., 1997), which is why the C in a compost is often more stable than in its feedstock (Bernal et al., 1998). Skene et al. (1996) reported the alkyl:O-alkyl ratio of a straw based feedstock increasing from 0.2 to 0.4 after 168 days of composting. In our case, the compost had an alkyl:o-alkyl ratio of 0.6. This could explain why, while COMP had the lowest P concentration, and hence amendment with COMP induced the greatest addition of C, it did not result in a higher MBC in the incubation experiment compared with the other OA. The PIG-STR sample had a lower alkyl C:O-alkyl C ratio compared to the CHK-STR and CHK-SD samples, indicating that PIG-STR had been less degraded by microbes (Baldock et al., 1997). This is likely as the PIG-STR was collected directly from the farm whereas the CHK-STR and CHK-SD were collected from a waste collection/redistribution centre and therefore are likely to be older and more decomposed. This would suggest that the C in the PIG-STR would be more readily available to soil microbial biomass compared with the C in CHK-STR or CHK-SD. Therefore, the lack of an increase in MBC in the PIG-STR treatment in the incubation experiment (compared to the chicken litters) cannot be explained by the forms of C in the PIG-STR and is likely because the quantity of C added in all of the litter treatments was relatively small.

As plants can only take up P in an inorganic orthophosphate form, it was expected that the proportion of orthophosphate P in the OA would be a good indication of the availability of the P in the OA. While the P speciation of the COMP sample could not be

410 determined by deconvolution due to the broadness of the NMR signal for this sample, we
411 found that all three litters in this study were high in orthophosphate, which is normal for
412 animal manures (Sharpley and Moyer, 2000). The PIG-STR sample contained the highest
413 proportion of orthophosphate which could explain why PIG-STR resulted in a greater
414 plant P uptake than CHK-STR and CHK-SD. Conversely, CHK-STR contained a higher
415 proportion of orthophosphate compared to CHK-SD. However, plant P uptake was similar
416 for both chicken litter treatments. Therefore, the proportion of orthophosphate in the OA
417 alone is not enough to determine the availability of P in OA.

418 After orthophosphate, the second greatest pool of P in the OA was phytate. The CHK-
419 SD contained a higher proportion of phytate than CHK-STR, and both chicken litters con-
420 tained higher proportions of phytate than PIG-STR. Chicken manures often contain a high
421 proportion of phytate (e.g. 11-37%; Peirce et al., 2013) because of the high amount of
422 seeds in their diet (Nelson et al., 1968) and the inability of chickens to efficiently digest
423 phytate (Toor et al., 2005). While phytate was once thought to be stable in soils (He et al.,
424 2006; Celi et al., 1999), there is now evidence that phytate can be rapidly mineralised to
425 orthophosphate by a range of soil microbes (Doolette et al., 2010). Moreover, mineral-
426 isation of phytate may replenish the soluble orthophosphate pool faster than the rate of
427 orthophosphate stabilisation by soil, providing a more constant pool of plant-available P.
428 This could explain why CHK-SD resulted in similar plant P uptake to CHK-STR, despite
429 being lower in orthophosphate P.

430 The proportion of P in OA present as bicarbonate-extractable P may give a better indi-
431 cation of P availability than P species. The bicarbonate extractable method was developed
432 for soil (as the Colwell P method), but it has been used for biochar (Hossain et al., 2010;
433 Chan et al., 2008) and could also be used to determine plant-available P in litters and
434 composts. However, the amount of bicarbonate-extractable P added with a treatment did
435 not always correspond with the increase observed in Colwell P of the soil, with less than

436 58% of the of the bicarbonate-extractable P in OA contributing to an increase in Colwell
437 P, compared with the 61.3% of P in the INORG-P which contributed to an increase in soil
438 Colwell P. This could be driven by differences in P buffering capacity between the OA
439 and the soil. While the PBI of the soil was very low (55.6 - 71.7), indicating that small
440 additions of P should have a large effect on plant-available P, it is possible that the PBI
441 of the OA (not measured) was even lower. While PBI of OA is not generally determined,
442 there is evidence that chicken litter can stabilise P in a similar way that soil does, but over
443 a longer time-frame than soil (months compared with days; Peirce et al., 2013). There-
444 fore, it is likely that upon addition to soil, the bicarbonate-extractable P in the OA was
445 quickly sorbed to soil particles and was no longer extractable by the Colwell P method.
446 Moreover, there were differences among treatments in the increase observed in Colwell P.
447 While 51.4-57.7% of the bicarbonate-extractable P in the PIG-STR, COMP and CHK-SD,
448 contributed to an increase in Colwell P, only 36% of the bicarbonate-extractable P in the
449 CHK-STR contributed to an increase in Colwell P. This corresponds with the finding that
450 CHK-STR provided plants with similar amounts of P as CHK-SD even though it had a
451 higher proportion of orthophosphate P and bicarbonate-extractable P.

452 While Colwell P has been commonly used to determine plant-available P for many
453 years, DGT P is developing into a promising technique which has been proven successful
454 in fields fertilised with inorganic P (Mason et al., 2010), as well as more recently in pot
455 experiments with OA (Six et al., 2014). When P is removed from the soil solution, e.g.
456 via plant P uptake, insoluble P then often moves into solution. The DGT method seeks
457 to mimic this process by measuring the movement of P from the soil solution into the
458 DGT device. The Colwell P method, on the other hand, measures the pool of P that
459 can be extracted with bicarbonate. This is an estimation of the P pool that can become
460 available to plants, although it is not always accurate as the processes that affect soil P
461 pools are complex. This study indicated that the DGT P method may be preferable over

462 the Colwell P method in systems where P is applied as OA. This is for two reasons: i)
463 DGT P had a larger range than Colwell P, potentially indicating a greater sensitivity of
464 the DGT method to detect differences in plant-available P compared with the Colwell
465 method; and ii) sample sizes for DGT method are greater than for the Colwell method,
466 which can eliminate some of the variability in the results (as seen for Colwell P, Figure
467 1b). This variability is likely driven by the fact that the OA were coarse and therefore
468 could not be mixed homogeneously throughout the soil. However, it is likely that neither
469 method correctly identifies the actual plant-available pool of P, given the range of complex
470 processes involved in plant P uptake. While alternative P extractions exist, such as the
471 sequential P extraction of Hedley et al. (1982), there is little evidence that these provide
472 better results than more common soil analyses (Motavalli and Miles, 2002).

473 Many processes can lead to changes in soil P pools over time. Plant-available P (when
474 measured as DGT P) decreased over time for the INORG-P treatment (Figure 1a). Or-
475 thophosphate can be stabilised with cations such as calcium to produce insoluble phos-
476 phates, reducing plant-available P (Bünemann et al., 2006; Holford, 1997; Bolland and
477 Gilkes, 1998). Furthermore, it is possible that the addition of phosphoric acid (the ma-
478 terial used in the INORG-P treatment) may have led to solubilisation of residual P in the
479 soil. Changes in soil pH lead to carbonate dissolution and P solubilisation (Jalali and Zinli,
480 2011; Alt et al., 2013). However, in this experiment only a small amount of phosphoric
481 acid was added to soil and it did not result in changes to the overall soil pH. Despite this,
482 it is possible that some localised, transient changes in pH occurred. However, there is no
483 research that suggests application of such small quantities of acid to a large amount of soil
484 (i.e. 5 ml of acid solution to 1 kg soil) will have any significant effect on soil P availability.
485 Moreover, given that the soil used in this experiment had a low calcium carbonate con-
486 tent ($< 0.2\%$) we believe that any effect will be negligible. While there was a decrease in
487 plant-available P over time for the INORG-P treatment, this was not observed in the litter

treatments. This could indicate that mineralisation and solubilisation were occurring at a similar rate to stabilisation in these treatments. The soil microbial biomass plays an important role in solubilisation and mineralisation of P (Hinsinger, 2001; Richardson, 2001) resulting in more P available to plants. While there were no differences in MBC among treatments in the incubation experiment, differences were observed in the plant growth experiment, with greater MBC values in treatments with a larger root biomass. Plants alter the soil microbial community around them which can result in a higher proportion of microbes that release P to the soil solution (Li et al., 2014). Plants with greater root biomass can supply more C to soil microbes from root turnover and root exudates. Additionally, particular groups of soil microbes, such as AMF, may have a disproportionate influence on plant P uptake.

Arbuscular mycorrhizal fungi colonisation generally decreases with increasing available P in soil (Bolan et al., 1984; Treseder, 2004; Cavagnaro, 2014). Similarly, in the current study, colonisation (measured as % colonisation and as infected root length) was lowest in the INORG-P treatment. At this level of plant-available P colonisation was clearly suppressed by the plant. Colonisation can also be low when there are very low levels of plant-available P in soil (Bolan et al., 1984) as was observed in the CONT treatment. However, the COMP treatment had a significantly greater percent colonisation compared with the CONT treatment. This is unexpected, as the COMP treatment contained similar plant-available P as the CONT treatment, as determined by soil analysis in the incubation experiment (Colwell P and DGT P). While it is possible that the compost contained AMF spores, it is unlikely that this is the sole cause of the high level of colonisation, given the small amount of compost added to each pot. Similarly, Duong et al. (2012) found that addition of compost increased AM colonisation compared with a control. Despite the high colonisation rate in the COMP treatment this did not seem to benefit the plant in terms of P uptake, with plant P uptake in the COMP treatment similar to the CONT treatment.

514 When colonisation was measured as infected root length, all of the OA treatments had
515 more infected root length compared with the CONT treatment, with the CHK-SD treat-
516 ment having significantly more infected root length compared with all other treatments
517 except the PIG-STR treatment. The CHK-SD had a lower proportion of orthophosphate P
518 and bicarbonate-extractable P compared to CHK-STR and PIG-STR, which could account
519 for the higher rates of root length colonised. The higher infected root length in CHK-
520 SD compared with CHK-STR could explain how plants in this treatment took up similar
521 amounts of P than those in the CHK-STR treatment despite the evidence that CHK-STR
522 should have higher P availability.

523 It is common for plants that are deficient in P to invest in their roots in order to max-
524 imise P uptake from the soil. Here, plants grown in soil amended with OA had a higher
525 root biomass:shoot biomass ratio compared with plants in the INORG-P treatment. Addi-
526 tionally, greater allocation of C to roots has been observed when plants have higher rates
527 of colonisation by AMF (Grønlund et al., 2013; Koch and Johnson, 1984; Yano et al.,
528 1996), which, as discussed above, was highest in OA treatments. The plants from all lit-
529 ter treatments also had significantly more P in their roots than plants from the INORG-P
530 treatment. This could be further evidence that these plants were investing strongly in their
531 roots in order to maximise P uptake. However, there is limited research investigating root
532 P contents at low levels of soil plant-available P, with most research on plant P uptake ei-
533 ther focusing on older plant roots (e.g. after 60-70 days growth; Tarafdar and Marschner,
534 1994; Akhtar et al., 2011) or on shoots (e.g. McBeath et al., 2012).

535 **5. Conclusion**

536 While previously the C:P ratio of OA was thought to be an important determinant of
537 plant P uptake from OA (e.g. Takeda et al., 2009) we found that this was not the case.
538 Alternatively, the proportion of P in OA that was orthophosphate gave a reasonable indi-

cation of the availability of P in the OA; however, it could not explain differences between the chicken litters. While the CHK-STR had a higher proportion of orthophosphate P and a higher proportion of bicarbonate extractable P compared with CHK-SD, both chicken litters provided plants with similar amounts of P. This could possibly be explained by the higher proportion of phytate in CHK-SD and the higher colonisation of roots in the CHK-SD by AMF compared with CHK-STR. This study provides valuable insights into the interactions between soil chemistry and biology and shows that chicken and pig litters contain P that can be utilised by plants. Further work should investigate OA application to varying soils in longer pot trials and, subsequently, field trials. Additionally, the effect of AM on P uptake from this materials needs to be quantified. This research could lead to more precise usage of OA for P fertilisation and ultimately more sustainable agricultural systems.

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Table 1: Physicochemical properties of four organic amendments: chicken litter with straw bedding (CHK-STR); chicken litter with sawdust bedding (CHK-SD); pig litter with straw bedding (PIG-STR) and compost (COMP).

Physicochemical Property	CHK-STR	CHK-SD	PIG-STR	COMP
Total P (g kg^{-1})	16.5	14.3	5.9	2.5
Bicarbonate-extractable P (% of total P)	37.0	29.4	59.3	24.0
Total C (g kg^{-1})	384.0	369.2	386.5	220.8
Total C:total P	23.4	25.8	65.4	88.2
Total N (g kg^{-1})	38.7	32.5	18.2	13.6
C:N	9.9	11.4	21.2	16.2
pH*	6.9	7.6	7.5	8.2
EC (dS m^{-1})*	10.6	6.4	12.7	3.8
Gravimetric moisture content (%)	16.4	20.1	12.2	27.0

*pH and EC measured in a 1:5 soil:water suspension.

Table 2: Total amount of C which was observable (%) in NMR spectroscopy analysis of a range of OA and the amount of each C species detected using NMR spectroscopy as a percentage of the total observable C, as well as the Alkyl:O-Alkyl ratio. OA were: chicken litter with straw bedding (CHK-STR); chicken litter with sawdust bedding (CHK-SD); pig litter with straw bedding (PIG-STR) and a compost (COMP).

C pool (%)	CHK-STR	CHK-SD	PIG-STR	COMP
Total Observable C	89.7	81.9	101.2	40.8
Alkyl	18.3	15.2	8.4	19.5
N-Alkyl/Methoxyl	7.0	7.9	5.5	8.7
O-Alkyl	48.3	50.1	51.2	28.9
Di-O-Alkyl	10.6	10.3	12.3	8.3
Aryl	5.2	7.2	10.0	18.0
O-Aryl	3.0	3.1	5.2	7.5
Amide/Carboxyl	7.2	6.1	6.5	7.0
Ketone	0.3	0.1	1.0	2.1
Alkyl:O-Alkyl*	0.4	0.3	0.2	0.6

* Values are ratios, not percentages.

Table 3: Total amount of P which was extracted (%) for NMR spectroscopy analysis of a range of OA and the amount of each P species detected using NMR spectroscopy as a percentage of the total extractable P. OA were: chicken litter with straw bedding (CHK-STR); chicken litter with sawdust bedding (CHK-SD); and pig litter with straw bedding (PIG-STR)

P pool (%)	CHK-STR	CHK-SD	PIG-STR
Total Extractable P	67.3	70.2	80.3
Orthophosphate	65.2	54.4	77.3
Phospholipid	2.6	3.4	6.4
Phytate	23.1	31.1	6.4
Other Monoester	4.4	5.7	4.6
Scyllo-inositol phosphate	0.4	0.6	0.0
Diester	3.9	4.7	4.1
Pyrophosphate	0.5	0.1	1.3

Table 4: MBC results from an incubation experiment (Days 0, 2 and 9) and a plant growth experiment (Day 30) in which P was applied at the same rate as either an inorganic P fertiliser (INORG-P), an organic amendment (chicken litter with straw bedding (CHK-STR), chicken litter with sawdust bedding (CHK-SD), pig litter with straw bedding (PIG-STR), or compost (COMP)), or no P was added (CONT). Letters after values indicate significant differences within sampling times (Days) among treatments ($p < 0.5$). Values are means ± standard errors. n = 3 for incubation experiment. n = 4 for plant growth experiment.

	MBC (mg kg^{-1})			
	Incubation			Plant growth
Treatment	Day 0	Day 2	Day 9	Day 30
INORG-P	113.4 ± 21.5	142.6 ± 7.4	59.5 ± 3.8	128.5 ± 5.3 a
CHK-STR	82.2 ± 6.7	143.6 ± 4.6	75.8 ± 9.7	122.4 ± 5.9 a
CHK-SD	109.9 ± 5.5	142.4 ± 13.5	75.1 ± 8.9	109.9 ± 5.0 a
PIG-STR	103.8 ± 6.8	163.6 ± 30.4	72.4 ± 12.6	114.2 ± 2.8 a
COMP	94.9 ± 8.1	137.8 ± 6.0	93.2 ± 16.3	87.5 ± 2.9 b
CONT	87.4 ± 4.2	129.8 ± 3.4	101.3 ± 7.8	79.9 ± 4.7 b

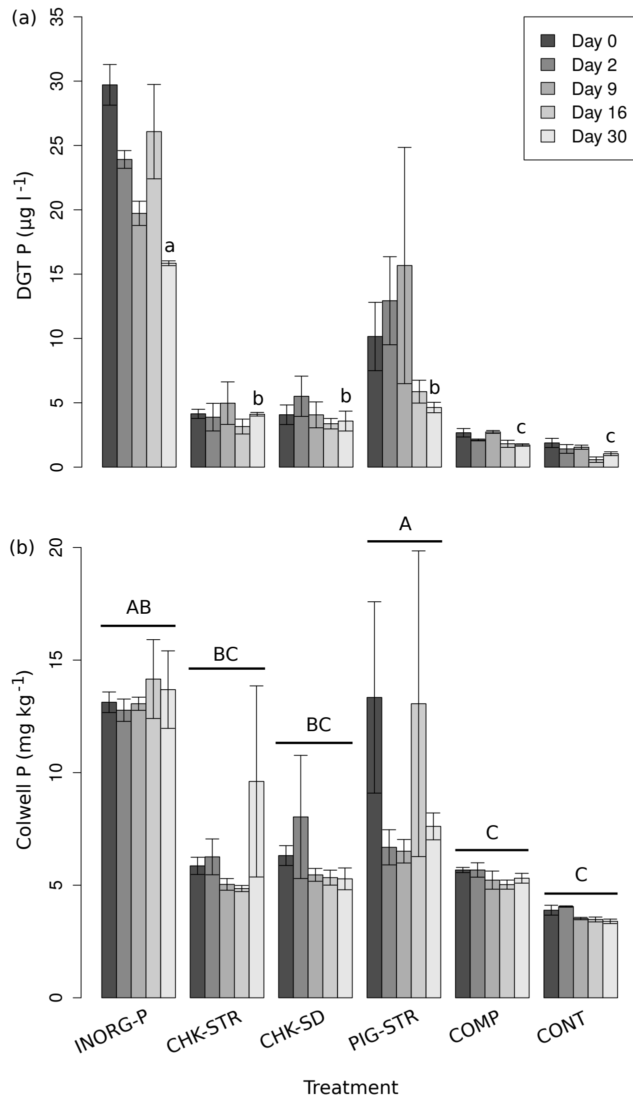

Figure 1: DGT P (a) and Colwell P (b) of soil at different time points in an incubation experiment in which P was applied at the same rate as either an inorganic P fertiliser (INORG-P), an organic amendment (chicken litter with straw bedding (CHK-STR), chicken litter with sawdust bedding (CHK-SD), pig litter with straw bedding (PIG-STR), or compost (COMP)), or no P was added (CONT). Letters above bars indicate significant differences ($p < 0.05$). Values are means ± standard errors. n = 3.

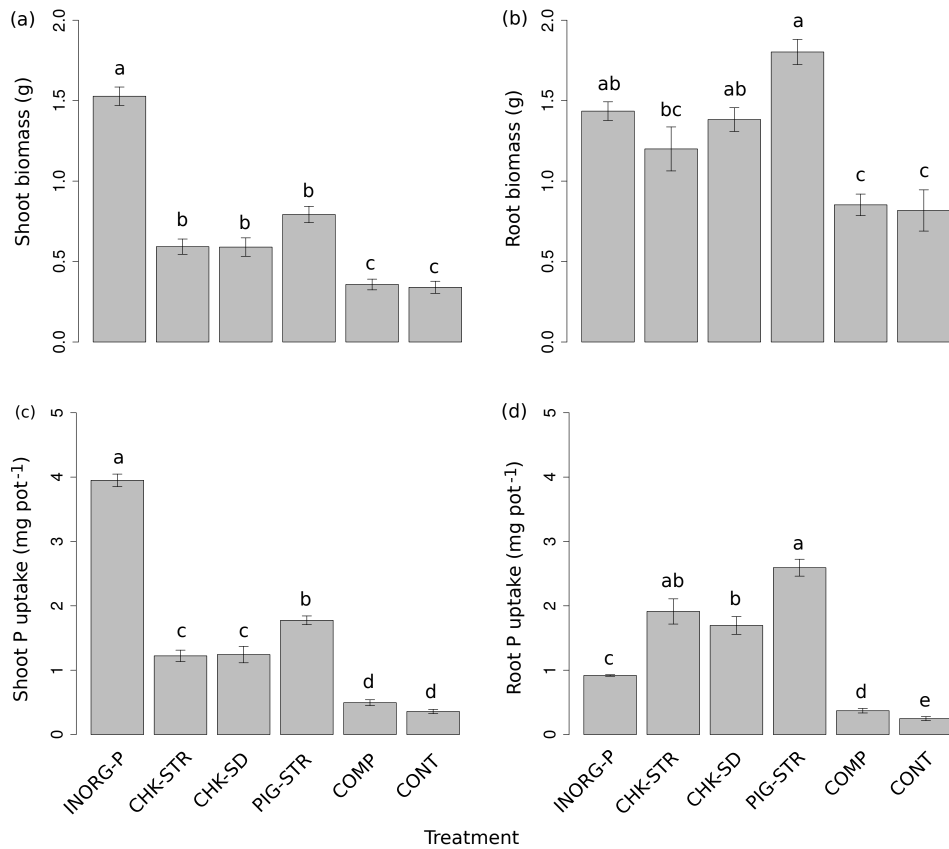

Figure 2: Dry mass of shoots (a) and roots (c) and plant P content (uptake) per pot of shoots (b) and roots (d) of plants grown in a plant growth experiment in which P was applied at the same rate as either an inorganic P fertiliser (INORG-P), an organic amendment (chicken litter with straw bedding (CHK-STR), chicken litter with sawdust bedding (CHK-SD), pig litter with straw bedding (PIG-STR), or compost (COMP)), or no P was added (CONT). Letters indicate significant differences ($p < 0.05$). Error bars indicate standard error. n = 4.

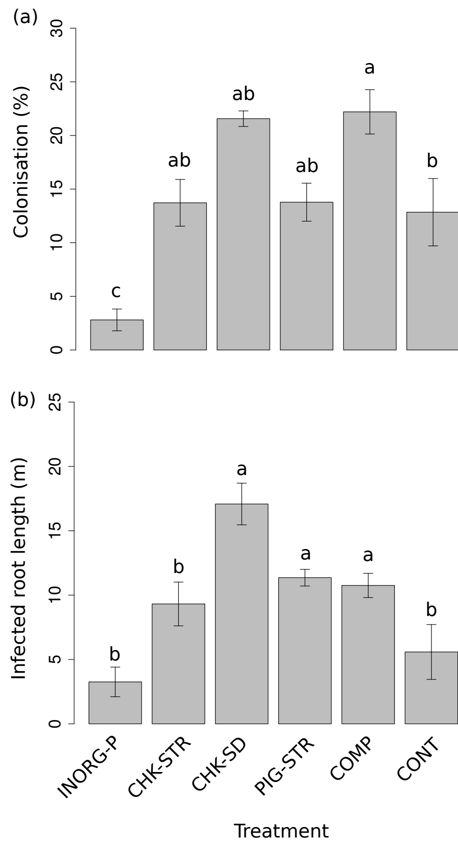

Figure 3: Arbuscular mycorrhizal colonisation of roots measured as (a) % colonisation and (b) infected root length of plants grown in a plant growth experiment in which P was applied at the same rate as either an inorganic P fertiliser (INORG-P), an organic amendment (chicken litter with straw bedding (CHK-STR), chicken litter with sawdust bedding (CHK-SD), pig litter with straw bedding (PIG-STR), or compost (COMP)), or no P was added (CONT). Letters indicate significant differences ($p < 0.05$). Error bars indicate standard error. n = 4.

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