Soil Biology & Biochemistry 115 (2017) 11–20

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

A key role for arbuscular mycorrhiza in plant acquisition of P from sewage sludge recycled to soil

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ARTICLE INFO

Article history:
Received 29 May 2017
Received in revised form
29 July 2017
Accepted 3 August 2017

Keywords:
Arbuscular mycorrhizal fungi (AMF)
Incineration
Isotopic technique
Phosphorus
Sewage sludge
Wheat

ABSTRACT

Dried and incinerated sewage sludge (SS) have the potential to be used as phosphorus (P) fertilisers. Arbuscular mycorrhizal fungi (AMF) contribute to plant P uptake; however, their role in P uptake from SS has yet to be fully explored. A compartmented pot system with an isotope pool dilution approach was used to investigate wheat (*Triticum aestivum* L.) P uptake from soluble P, dried SS and incinerated SS, via roots and/or AMF hyphae. Wheat was sown into an inner compartment containing a ^{33}P label with/without AMF (*Rhizophagus irregularis*) inoculum. An outer soil compartment contained the P source. Compartments were separated by mesh of different sizes to allow root and/or AMF hyphal access. Plants obtained P from dried SS via AMF but there was limited evidence that plants obtained P from incinerated SS via AMF. Phosphorus uptake was significantly greater when both roots and hyphae could access dried SS than when only roots could access dried SS. We discuss the results in terms of availability of P to roots and hyphae. We conclude that AMF play an important role in wheat P acquisition from dried SS and therefore can assist in the recycling of P in waste.

1. Introduction

Sewage sludge (SS) contains large amounts of phosphorus (P) (Metson and Bennett, 2015) and therefore could be used as an alternative to fertilisers produced from phosphate rock, which is a finite resource (Scholz and Wellmer, 2013). Re-use of SS can help close the anthropogenic P cycle (Metson and Bennett, 2015). Moreover, SS can be converted to ash through incineration, and this ash could be used as a P fertiliser (Wang et al., 2014). Incineration of SS destroys organic pollutants present in SS (Harrison et al., 2006; Krüger and Adam, 2015) and the resulting ash has a higher P concentration than raw SS (Mackay et al., 2017). While there are some risks associated with the use of dried and incinerated SS due to a possible high content of heavy metals, a major limitation to the use of incinerated SS as a P fertiliser may be the availability of P to crop (Krüger and Adam, 2015).

Dried and incinerated SS contain a range of P species which vary in their availability to plants (Wang et al., 2014). In a previous study, we found that while dried SS contained more P in easily-extractable (water- and sodium bicarbonate-extractable) pools than incinerated SS, both dried and incinerated SS provided the same amount of P to wheat in a low pH soil (Mackay et al., 2017). However, the P in both the dried and incinerated SS became more available to plants over time, indicating that the P needed to be mineralised or solubilised before it could be taken up by plants.

Plants have multiple strategies to help them obtain P, including release of root exudates which can solubilise P. Moreover, plant root exudates and root turnover can have an indirect effect on P forms in soil by leading to changes in the soil microbial community (Olsson et al., 1996) which could favour P cycling microorganisms (Li et al., 2014). Additionally, plants can increase their P uptake potential, for example by maximising uptake surface areas, such as fine roots and root hairs, and by forming associations with arbuscular mycorrhizal fungi (AMF). This increased surface area for P uptake may be particularly important in systems where P first needs to be mineralised and/or solubilised and where there might be high competition between plants and soil microorganisms for available

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http://dx.doi.org/10.1016/j.soilbio.2017.08.004
0038-0717/

P (Deubel and Merbach, 2005).

Approximately 80% of terrestrial plant species form arbuscular mycorrhizas (AM), which are symbiotic associations with AMF (Smith and Read, 2008). These associations can benefit plant nutrition, particularly plant P uptake (Pearson and Jakobsen, 1993; Tibbett, 2000). Arbuscular mycorrhizal plants can obtain P through their roots (direct pathway) or via AMF hyphae (AM pathway) (Smith et al., 2003). In addition to increasing P uptake surface area, AMF hyphae may mineralise and solubilise P, either directly, through exudation (Koide and Kabir, 2000; Yao et al., 2001), or indirectly, through modification of the soil microbial community (Joner and Jakobsen, 1995; Marschner et al., 2001; Zhang et al., 2016). It is therefore possible that, when P is applied in complex forms, AMF hyphae are better able to acquire P compared with plant roots. For example, greater P uptake from bone char was observed by maize with AM than by non-mycorrhizal (NM) maize (Zwetsloot et al., 2016). However, this study did not determine whether the difference in P uptake was due to more efficient P uptake by AMF hyphae compared to the plant roots, or more efficient uptake of P by AM plants because of an additive effect of root and hyphal P uptake. Moreover, P uptake from the bone char was not differentiated from P uptake from the soil.

Radioactive isotopic techniques can be used to distinguish between uptake of P from amendments and from the soil. Amendments can either be labelled directly or, in cases where this is not possible, the soil P can be labelled and the dilution in radioactivity caused by uptake of unlabelled P in the amendments is measured (Bertrand et al., 2006; Peirce et al., 2013). Comparisons of P uptake from amendments by AM and NM plants can provide insights into the importance of the AM pathway in P uptake; however, they do not allow determination of P uptake via AMF hyphae alone. Quantification of plant P uptake via AMF hyphae has typically been done by placing labelled material in a compartment which only AMF hyphae can access (Pearson and Jakobsen, 1993; Smith et al., 2003). Similar compartments could be incorporated into an isotope pool dilution approach to determine P uptake via AMF hyphae from materials which cannot be directly labelled.

We used a compartmented pot system in an isotope pool dilution experiment to examine the role of AMF in plant P uptake from dried and incinerated SS and four main research questions were addressed:

1. Can wheat acquire P from dried and incinerated SS via the AM pathway?
2. Are AMF hyphae more effective at acquiring P from dried and incinerated SS than wheat roots?
3. Do wheat roots and AMF hyphae together take up more P from dried and incinerated SS than roots alone? (i.e. are AM plants better at acquiring P from dried and incinerated sewage sludge than NM plants?)
4. If AM plants are better at acquiring P from dried and incinerated SS, does this lead to greater biomass in AM plants?

2. Materials and methods

2.1. Pot system, soil preparation and experimental design

A compartmented pot system, which contained an inner compartment and an outer compartment, was used (Fig. 1). In these pots, plants were grown in the inner compartment. The inner compartments had two large windows, and a perforated base and their inside was lined with a mesh bag made of either coarse (700 μm) or fine nylon mesh (25 μm). The inner compartment was filled with 0.8 kg of soil which had been mixed with carrier-free

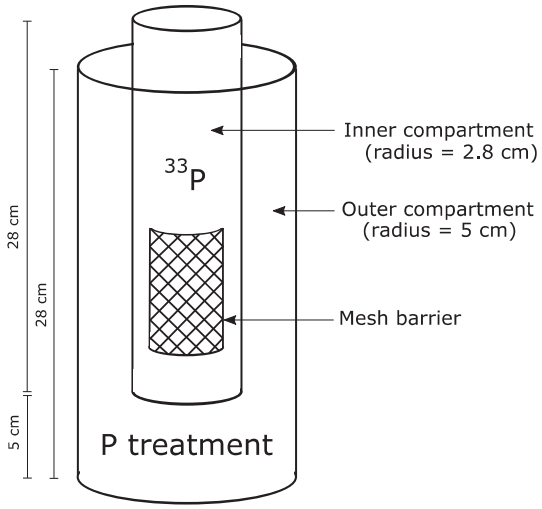

Fig. 1. Diagram of the compartmented pots used in the experiment. The inner compartment received ^{33}P and either AMF inoculum or washing from AMF inoculum. The outer compartment received the P treatment. Plants were sown in the inner compartment. The compartments were separated by a mesh barrier which was either a coarse mesh (which both roots and AMF hyphae could pass through, 700 μm) or a fine mesh (which only AMF hyphae could pass through, 25 μm).

^{33}P-labelled orthophosphate at 0.63 kBq g soil^{-1}. This soil had also either been mixed with inoculum of the AMF *Rhizophagus irregularis* BEG87 (9:1, soil:inoculum) or was left un-inoculated. The outer compartment contained 1.6 kg soil which had been amended with P either as soluble P (KH_2PO_4), dried SS, incinerated SS, or was left un-amended. Phosphorus amendments were added to soil at the same rate of total P so that outer compartments received 75 mg P kg^{-1} soil.

The dried and incinerated SS were the same as those described in detail in Mackay et al. (2017). Briefly, sewage sludge from the wastewater treatment facility Bjergmarken Renseanlæg in Roskilde, Denmark was divided into two batches. One batch was dried and granulated, resulting in the dried SS. The other batch was incinerated at 850 °C. The P concentration was 38.3 mg g^{-1} in the dried SS and was 88.1 mg g^{-1} in the incinerated SS. The easily extractable P (H_2O-extractable P pool plus $NaHCO_3$-extractable P pool) constituted 6.84% and 0.54% of the P in the dried SS and incinerated SS, respectively. The carbon (C) to P ratio (C:P) was 7.0 in the dried SS but only 0.1 in the incinerated SS. In a previous experiment, the dried and incinerated SS were found to have no significant effect on soil pH when added at a rate of 50 mg P kg-1 (Mackay et al., 2017).

The soil used was taken in 2014 from the plough layer (20 cm) of a nutrient depletion trial at the University of Copenhagen's experimental research farm in Tåstrup, Denmark, from a treatment receiving neither P nor potassium (K) for 50 years. This soil was a sandy loam (16.5% clay) low in plant-available P (Olsen P: 10.5 mg kg^{-1}; water-extractable P: 2.31 mg kg^{-1}) with a pH of 5.3 (measured in 1:2.5, soil:0.01M $CaCl_2$ suspension). The soil was air dried, passed through a 5 mm sieve, gamma irradiated (15 kGy) and stored in a dark, cool room before use. The soil was mixed with oven-dried quartz sand (1:1, w:w). This mix (hereafter referred to as soil) had a water holding capacity (WHC) of 18% gravimetric water content. The soil was amended with basal nutrients (other than P) at a rate sufficient for plant growth (as in Mackay et al., 2017).

To minimise for the potential of wider changes in the soil microbial community resulting from the AMF inoculation, washings from the inoculum were added to the inner compartments of the

non-inoculated treatment. These AMF washings were prepared by suspending 800 g of inoculum in 1.5 l H_2O then sieving the suspension through fine mesh (25 μm). 100 ml of this filtrate was applied to soil in inner compartments of un-inoculated treatments and 100 ml of H_2O was applied to soil in inner compartments of inoculated treatments. Additionally, washings from non-irradiated field soil were added to the outer compartment for each treatment. These were added in order to re-establish part of the soil microbial community, while keeping the soil free of AMF. The soil used to obtain the washings was collected from the same field as the irradiated soil used in the experiment, except that it was not irradiated. Soil washings were prepared by suspending 320 g soil in 1 l H_2O then sieving the suspension through the fine mesh. 50 ml of this filtrate (derived from 16 g of soil corresponding to 1% of the soil mass in each outer compartment) was applied to soil in each outer compartment.

The combination of ± inoculum and mesh size barriers provided an experimental design with three levels of access to the outer soil compartment: i) inoculum and fine mesh allowed hyphal access only (HYPHAE); ii) inoculum and coarse mesh allowed both root and hyphal access (ROOTS + HYPHAE); and iii) no inoculum and a coarse mesh allowed root access only (ROOTS). The three access treatments and the four P treatments (soluble P, dried SS, incinerated SS and no P) were used in a fully factorial design, so that there were 12 main treatments. In addition to these 12 treatments, there were three extra control treatments. The first control (AM-disturbed) used inoculated soil and fine mesh and the second control (NM-disturbed) used un-inoculated soil and fine mesh. In these treatments, the inner compartments were rotated every 2–3 days to break any hyphae growing into the outer compartment and, therefore, plants could only obtain nutrients from the inner compartment. These treatments were used to determine the amount of P which AM and NM plants obtained from the inner compartment, respectively. The third control had plants growing in inoculated soil in fine mesh bags. However, in this treatment, instead of labelling the soil in the inner compartment with ^{33}P, the soil in the outer compartment was labelled with ^{33}P. In this treatment, we measured the radioactivity of the plants as a real-time indicator of growth of hyphae into the outer compartment.

2.2. Plant growth conditions

After packing soil into pots, the AMF washings and soil washings were pipetted onto the soil and the soil was watered to 70% WHC. The pots were then incubated in a controlled growth chamber for five days before seeds were sown. The growth chamber had a day:night regime of 14:10 h with corresponding temperatures set at 25 °C:18 °C. Two pre-germinated spring wheat (*Triticum aestivum* L. var. Axe) seeds were sown in each inner compartment. After four days the seedlings were thinned to one seedling per pot. Pots were watered to 70% WHC 3–4 times per week. When watering pots, approx. 60% of the water applied was added to the inner compartment, as this is where the bulk of the roots were likely to be. Plants were grown for 42 days in the growth chamber. Radioactivity was observed using a hand-held device in the third control (with ^{33}P in the outer compartment) 18 days after sowing.

2.3. Shoot and root harvest and analysis

At harvest, shoot material was collected, oven-dried and dry masses were determined. Ground-up shoot samples were digested in nitric acid and hydrogen peroxide in an Ultrawave (Milestone Inc.). Mixtures (1:3) of digested samples and scintillation fluid (Ultima Gold XR) were analysed for ^{33}P activity with a PerkinElmer Liquid Scintillation Analyzer Tri-Carb® 2910TR. Another sample of the digestate was used to determine total P concentration by ICP-OES.

Two soil samples were taken from each outer compartment to determine hyphal length (Jakobsen et al., 1992). The inner compartment was removed and the roots were quantitatively sampled from each compartment separately. Subsamples of the roots from each compartment were used to determine total and colonized root length (Newman, 1966) after staining with trypan blue.

The specific activity of shoots (SA) was calculated as follows:

$$SA\left(kBq\ mg\ P^{-1}\right) = \frac{shoot\ activity\left(kBq\ ^{33}P\ pot^{-1}\right)}{shoot\ P\ uptake\left(mg\ P\ pot^{-1}\right)}$$

The SA of the disturbed controls was used as an indicator of the specific activity of the labile P pool in the inner compartment (Zapata and Axmann, 1995). From this, the shoot P derived from the inner compartment was calculated as:

$$Shoot\ P\ from\ inner\ compartment\left(mg\ pot^{-1}\right) = \frac{Shoot\ activity\left(kBq\ ^{33}P\ pot^{-1}\right)}{SA\ of\ disturbed\ control\ \left(kBq\ mg\ P^{-1}\right)}$$

where the AM-disturbed control was used for pots which received inoculum (ROOTS + HYPHAE and HYPHAE), and the NM-disturbed control was used for pots which did not receive inoculum (ROOTS). However, in the ROOTS + HYPHAE treatment with soluble P, root length colonised was low and therefore the NM-disturbed control was used in the calculations for this treatment. The shoot P uptake from outer compartments was determined as the difference between total shoot P uptake and shoot P uptake from inner compartments.

The mycorrhizal P response (mg pot^{-1}) was calculated based on Cavagnaro et al. (2003) as follows:

$$Mycorrhizal\ P\ response\left(mg\ pot^{-1}\right) = Shoot\ P\ uptake_{outer}\ RH\left(mg\ pot^{-1}\right) - mean\ Shoot\ P\ uptake_{outer}\ R\left(mg\ pot^{-1}\right)$$

where $P\ uptake_{outer}\ RH$ refers to P uptake from the outer compartment in ROOTS + HYPHAE treatments and $P\ uptake_{outer}\ R$ refers to P uptake from the outer compartment in ROOTS treatments. It is important to note that this is not an estimate of the proportion of P taken up via the AM pathway, but the difference in P uptake as a result of being AM compared to being NM.

The shoot P uptake per unit root length (RL), that is, RL specific shoot P uptake was calculated based on Cavagnaro et al. (2003) as follows:

$$RL\ specific\ shoot\ P\ uptake\left(\mu g\ P\ pot^{-1}\ m^{-1}\right) = \frac{Shoot\ P\ uptake_{outer}\ \left(\mu g\ pot^{-1}\right)}{Root\ length_{outer}(m)}$$

where $Root\ length_{outer}$ refers to the total root length in the outer compartment. RL specific shoot P uptake was only calculated for treatments in which there were roots in the outer compartment.

2.4. Statistical analysis

Generalised linear models (GLMs, family = Gaussian) were used to determine the effect of P treatment, access treatment, and the

interaction on root length, root length colonised, percent root colonised, hyphal length plant P uptake and plant biomass. In the HYPHAE access treatment, there was no root growth in the outer compartment, and so this treatment was omitted from the analysis for root length, colonised root length and percent root colonised. In the ROOTS access treatment, there was little or no colonisation of roots, and so this treatment was omitted from the analysis for root length colonised and percent root colonised. Where there was a significant interaction, the effect of access treatment was investigated within all levels of P treatment, and the effect of P treatment was investigated within all levels of access treatment. A GLM was also used to determine differences among P treatments in mycorrhizal shoot P response. When GLMs were significant, they were followed up with a Tukey's HSD test. Individual T-tests were used to determine differences in RL specific shoot P uptake between ROOTS + HYPHAE and ROOTS treatments for each P treatment, and also to further investigate plant P uptake in some specific treatments. All statistical analyses were conducted in R (version 3.2.3) with α level of 0.05.

3. Results

3.1. Root growth from planting compartments into P amended soil was restricted by fine mesh, but not coarse mesh

The absence of roots in the outer compartments of pots with fine mesh, and their presence in pots with coarse mesh confirmed that the fine mesh prevented roots from spreading to the outer compartment (Fig. 2). There was a significant effect of access treatment on root length density in inner compartments ($F = 25.3$, $P < 0.001$, Table 1), with root length density being significantly greater when roots were restricted to the inner compartment (HYPHAE mean = 13.1 cm g^{-1}) compared to when roots could grow into the outer compartment (ROOTS mean = 8.2 cm g^{-1}; ROOTS + HYPHAE mean = 7.9 cm g^{-1}; Fig. 2). Root length density was similar in the AM-disturbed and NM-disturbed pots, at 7.8 and 6.4 respectively (Table 2).

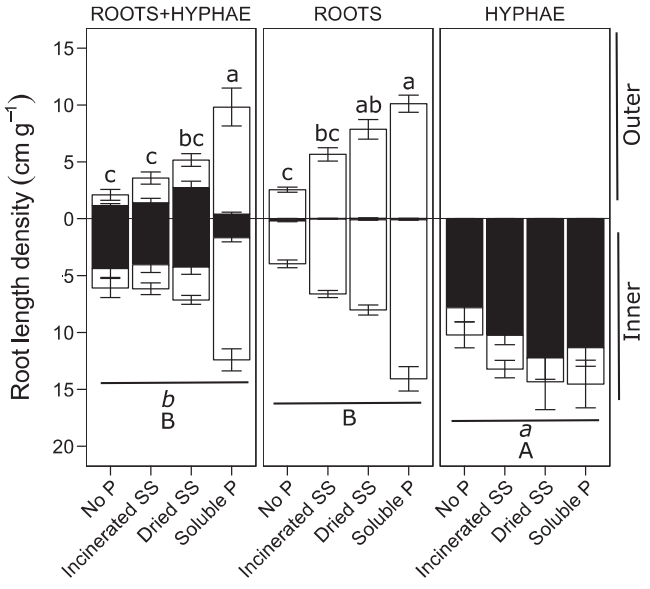

Fig. 2. Root length density (m g soil^{-1}) of wheat grown in compartmented pots. Outer compartments either received no P, soluble P, or P in the form of incinerated or dried sewage sludge (SS). Either both roots and hyphae, only roots, or only hyphae of AMF could access the outer compartment. Root length density in inner compartments is shown below the x-axis and in outer compartments above the x-axis. Dark grey bars indicate colonised root length density. Values are mean ± standard error of colonised and total root length density. $N = 4$. Lower case letters indicate significant effects of P treatments and access treatments on root length density in outer compartments. Lower case italicised letters indicate significant main effects of access treatments on colonised root length density in inner compartments. Upper case letters indicate significant main effects of access treatments on total root length density in inner compartments.

3.2. Colonisation of roots by AMF varied among treatments

Both the total colonised root length density and the percentage of root length colonised by AMF in the inner compartments was high for all treatments receiving AMF inoculum (4.0–12.2 cm g^{-1}; 59–87%), except for when roots could access the outer compartment with soluble P (1.7 cm g^{-1}; 14%, Fig. 2, Tables S1 and S2). There was a significant effect of access treatment on colonised root length density in the inner compartments ($F = 132.1$, $P < 0.001$, Table 1). HYPHAE treatments had significantly greater colonised root length density than ROOTS + HYPHAE treatments (Fig. 2). Colonised root length density was lower in the outer compartments than the inner compartments in ROOTS + HYPHAE treatments (Fig. 2). The colonised root length density in the inner compartment of the AM-disturbed treatment was 5.9 cm g^{-1}, significantly greater than the NM-disturbed treatment which was 0.3 cm g^{-1} (Table 2).

3.3. Hyphae grew from planting compartment into the P amended soil

Background hyphal length density in the outer compartments of treatments which had not received AMF inoculum (ROOTS treatment) averaged 4.7 m g^{-1} (Table 3), which is likely to be dead hyphae as there was no colonisation to support hyphal growth. There was definite growth of hyphae into the outer compartment for all inoculated treatments, with hyphal length densities 4.5–9.42 m g^{-1} greater than the un-inoculated treatment, except for when roots could access the outer compartment with soluble P, where hyphal length density was similar to un-inoculated treatments (Table 3). The hyphal length density in outer compartments was slightly higher in disturbed pots compared with un-disturbed pots, with similar hyphal length densities for both the AM- and the NM-disturbed treatments, which were 7.9 and 7.6 m g^{-1} respectively (Table 2).

3.4. Shoot P uptake from planting compartments varied among treatments

Shoot P uptake from inner compartments was consistently approx. three times greater in treatments receiving AMF inoculum than in those without AMF inoculum, except for in pots where both roots and hyphae could access the outer compartment with soluble P; in that case shoot P uptake from the inner compartment was similar to uptake in the treatment without AMF inoculum (Table S3). Shoot P uptake in the AM-disturbed treatment was 3.64 mg pot^{-1} which was significantly greater than in the NM-disturbed treatment at 1.21 mg pot^{-1} (Table 2).

3.5. Shoot biomass was limited by P uptake from P sources

There was a strong positive correlation between shoot biomass and shoot P uptake from the outer compartment (Fig. 3), indicating that P was the limiting nutrient to plant growth in this experiment. Shoot P uptake from the outer compartment, and hence shoot biomass, varied considerably among treatments. There was a significant interaction between access treatment and P treatment on shoot P uptake from the outer compartments ($F = 42.5$, $P < 0.001$,

Table 1
ANOVA table of GLMs investigating the effect of P treatment, access treatment and the interaction (P treatment x access treatment) on a number of variables.

	P treatment	Access treatment	P × Access
Total root length density (inner)	<**0.001**	<**0.001**	0.065
Total root length density (outer)[a]	<**0.001**	**0.025**	0.381
Colonised root length density (inner)[b]	0.266	<**0.001**	0.060
Colonised root length density (outer)[c]	0.087	n/a	n/a
Percent root colonised (inner) [b]	<**0.001**	<**0.001**	<**0.001**
Percent root colonised (outer)[c]	0.313	n/a	n/a
Hyphal length density (outer)	**0.020**	<**0.001**	0.134
Shoot P uptake (inner)	<**0.001**	<**0.01**	<**0.001**
Shoot P uptake (outer)	<**0.001**	<**0.001**	<**0.001**
Shoot P uptake (total)	<**0.001**	<**0.001**	<**0.001**
Shoot biomass	<**0.001**	<**0.001**	<**0.001**

Significant *P*-values are indicated in bold text.
[a] HYPHAE access treatment excluded from analysis.
[b] ROOTS access treatment excluded from analysis.
[c] ROOTS and HYPHAE treatments excluded from analysis.

Table 2
Plant and fungal variables in the AM-disturbed and NM-disturbed treatments from an experiment in which wheat was grown in compartmented pots.

	AM-disturbed	NM-disturbed
Root length density (inner) (cm g^{-1})	7.79 ± 0.74	6.36 ± 0.57
Colonised root length density (inner) (cm g^{-1})	5.94 ± 0.58***	0.29 ± 0.13
Hyphal length density (outer) (m g^{-1})	7.87 ± 1.06	7.63 ± 1.50
Shoot biomass (g pot^{-1})	2.87 ± 0.17***	1.29 ± 0.11
Shoot P uptake (mg pot^{-1})	3.64 ± 0.13***	1.21 ± 0.12

Growth in these treatments was restricted to the inner compartment by regular disturbance (twisting) of the inner compartment. In the AM-disturbed treatment, irradiated soil was inoculated with AMF, in the NM-disturbed treatment irradiated soil was left un-inoculated. Values are means ± standard error. $N = 4$. Significant differences in variables between the two treatments are indicated with stars (*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$).

Table 3
Hyphal length density (m g $soil^{-1}$) of AMF in the outer compartment from an experiment in which wheat was grown in compartmented pots.

P treatment	Access treatment		
	ROOTS + HYPHAE	ROOTS	HYPHAE
No P	12.3 ± 1.3	5.1 ± 0.9	14.1 ± 2.9
Incinerated SS	12.3 ± 2.1	4.8 ± 0.4	12.0 ± 1.9
Dried SS	12.4 ± 1.6	4.6 ± 0.6	9.2 ± 1.2
Soluble P	5.8 ± 0.4	4.2 ± 0.2	10.1 ± 1.4
Mean	10.7 a	4.7 b	11.3 a

Values are means ± standard error. $N = 4$. Letters indicate significant differences among access treatments.

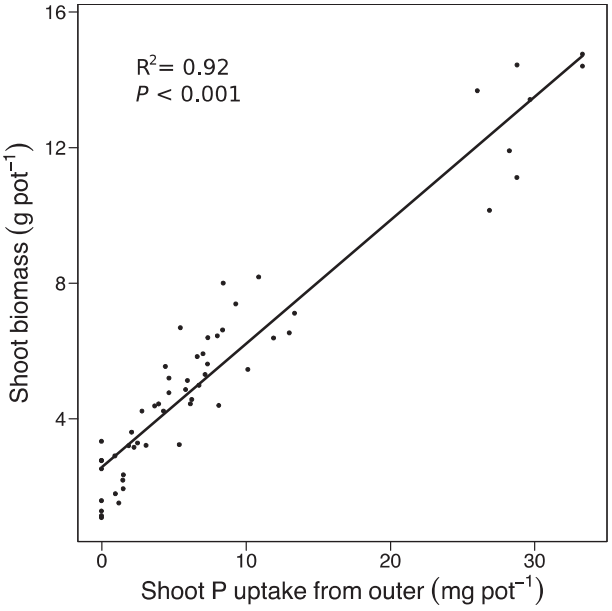

Fig. 3. Plot showing the correlation between shoot P uptake from outer compartments and shoot biomass of wheat. The black line indicates a linear model.

Table 1) and shoot biomass ($F = 12.4$, $P < 0.001$, Table 1), so we investigated both the effect of P treatment within all levels of access treatment, and the effect of each access treatment within all levels of P treatment.

3.6. Shoot P uptake from P sources and shoot biomass depended on P treatments

Generally, shoot P uptake from outer compartments and shoot biomass was greatest when soluble P was added to outer compartments and lowest when no P was added; however, there were some differences in the effects of P treatment depending on the access treatment (Figs. 4 and 5). Phosphorus uptake from outer compartments with soluble P was greater than from compartments with no P regardless of access treatment (Fig. 4), which resulted in greater biomass in these treatments (Fig. 5). Evidence that both plant roots and AMF could obtain P from dried SS was provided as P uptake from outer compartments was significantly greater with dried SS than with no P added for all access treatments (Fig. 4). Shoot P uptake from outer compartments with dried SS was significantly lower than from outer compartments with soluble P when roots alone and roots with hyphae could access the P source, but was not significantly different when hyphae could access the P source. The addition of dried SS resulted in greater biomass than obtained in the no P treatment when roots alone or hyphae alone could access the dried SS, this was not the case when both roots and hyphae could access the dried SS (Fig. 5). Phosphorus uptake from outer compartments with incinerated SS was not significantly different from outer compartments with no P when either both roots and hyphae, or just hyphae could access the outer compartment (Fig. 4). Still, an individual T-test provides some evidence that hyphae could access P in incinerated SS as P uptake via hyphae from outer compartments with incinerated SS was found to be significantly greater than from outer compartments with no P ($t = 2.56$, $df = 6$, $P = 0.043$). Further, clear evidence that roots could access P in incinerated SS was provided as P uptake from compartments with incinerated SS was greater than from compartments with no P when only roots could access the P source (Fig. 4). Addition of

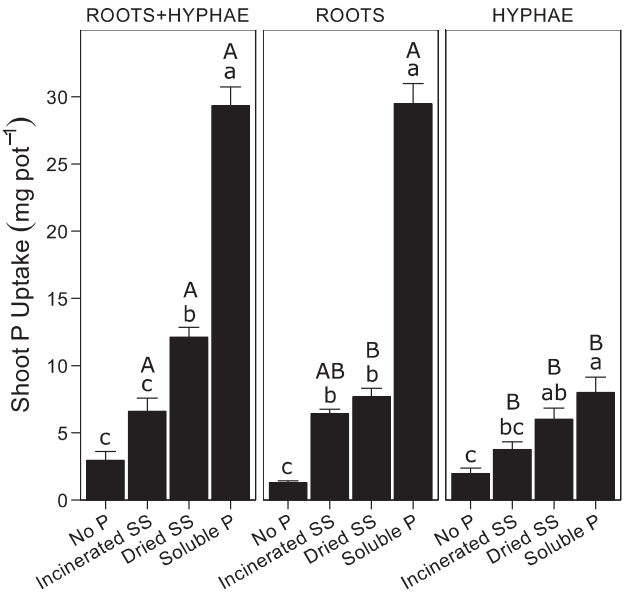

Fig. 4. Shoot P uptake (mg pot^{-1}) of wheat from outer compartments. Outer compartments either received no P, soluble P, or P in the form of incinerated or dried sewage sludge (SS). Either both roots and hyphae, only roots, or only hyphae of AMF could access the outer compartment. Values are means ± standard error. $N = 4$. Lower case letters indicate significant differences within access treatments. Upper case letters indicate significant differences within P treatments.

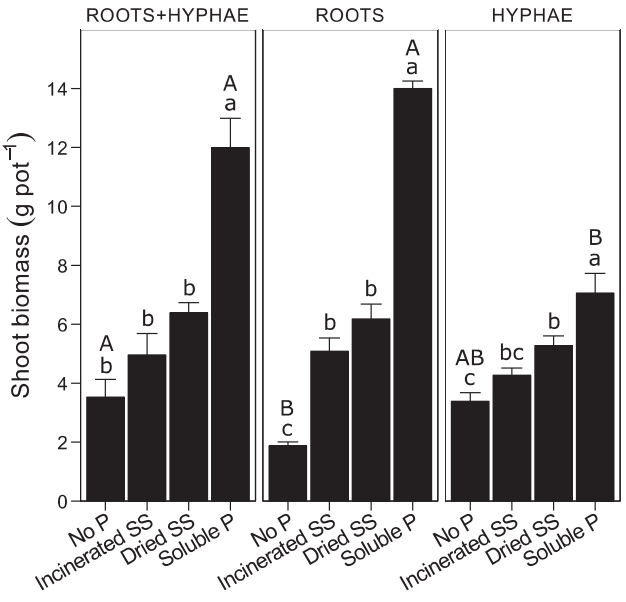

Fig. 5. Biomass (g pot^{-1}) of wheat grown in compartmented pots. Either both roots and hyphae, only roots, or only hyphae of AMF could access the outer compartment. Outer compartments either received no P, soluble P, or P in the form of incinerated or dried sewage sludge (SS). Values are means ± standard error. $N = 4$. Lower case letters indicate significant differences within access treatments. Upper case letters indicate significant differences within P treatments.

incinerated SS resulted in significantly greater biomass than no P when roots alone could access the P source (Fig. 5), but not when hyphae alone or both roots and hyphae could access the P source.

3.7. Shoot P uptake from P sources and shoot biomass depended on access treatment

Generally, shoot P uptake from outer compartments and shoot biomass were greater when both roots and hyphae could access P sources, compared with when either roots alone or hyphae alone could access P sources; however, there were slight differences in the effect of access treatment depending on the P source (Figs. 4 and 5). All access treatments had similar shoot P uptake from outer compartments with no P, with P uptake ranging from 1.29 to 2.95 mg pot^{-1} (Fig. 4). However, biomass was significantly greater when roots and hyphae could access outer compartments with no P compared to when only roots could access outer compartments with no P (Fig. 5). When dried SS was added to outer compartments, P uptake was significantly greater when both roots and hyphae could access the P source, at 12.12 mg pot^{-1}, than when only hyphae or only roots could access the P source, which took up 6.01 and 7.69 mg pot^{-1} respectively (Fig. 4). However, there were no significant differences in biomass among access treatments when dried SS was added (Fig. 5). When incinerated SS was added to outer compartments, P uptake was greater when both roots and hyphae could access the P source, at 6.59 mg pot^{-1}, than when hyphae alone could access the P source, at 3.76 mg pot^{-1} (Fig. 4). However, P uptake when only roots could access incinerated SS did not differ significantly from when either hyphae alone or both roots and hyphae could access incinerated SS. There were no significant differences in biomass among access treatments when incinerated SS was added (Fig. 5). When soluble P was added to outer compartments, both shoot P uptake and shoot biomass were significantly greater when either both roots and hyphae or roots alone could access the P source than when hyphae along could access the P source (Figs. 4 and 5).

3.8. AM enhanced P uptake in wheat when P was added as dried SS, but not when P was added as incinerated SS

The mycorrhizal shoot P response was significantly greater when dried SS was added than when incinerated SS or no P were added (Fig. 6). RL specific shoot P uptake was generally greater in AM wheat than NM wheat and this was significant when pots had received either no P or dried SS (Fig. 7).

4. Discussion

4.1. Evaluation of the experimental system

This experiment successfully employed the isotope pool dilution technique in a compartmented pot system to compare P uptake from different P sources by plant roots and AMF hyphae. A positive correlation between shoot P uptake and shoot biomass (Fig. 3) confirmed that P was the limiting nutrient for plant growth. Different mesh sizes successfully allowed or restricted root access to outer compartments, and where roots accessed outer compartments, root length densities were representative for values in the field (Barraclough and Leigh, 1984). Growth of AMF hyphae from colonised roots and into the outer compartment occurred as expected, except when roots had also access to soil fertilised with soluble P (Fig. 2, Table 3). The low colonisation and hyphal abundance in roots and soil of this treatment could be explained by higher shoot P uptake (Breuillin et al., 2010). Phosphorus uptake from inner compartments was greater in treatments receiving inoculum than those without inoculum (Table S2), likely due to greater exploration of the soil by AMF (Jakobsen et al., 1992; Tibbett, 2000). Hyphal length densities in outer compartments of disturbed treatments were higher than un-disturbed treatments;

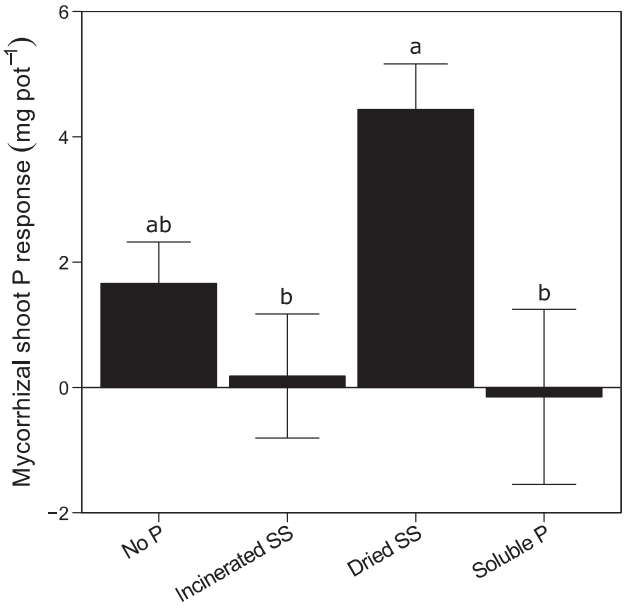

Fig. 6. Mycorrhizal shoot P response (mg pot^{-1}) determined from shoot P uptake of wheat from the outer compartment of a pot which had two compartments. Outer compartments either received no P, soluble P, or P in the form of incinerated or dried sewage sludge (SS). Inner compartments received either AMF inoculum or washings from AMF inoculum. Values are means ± standard error. $N = 4$. Letters indicate significant differences.

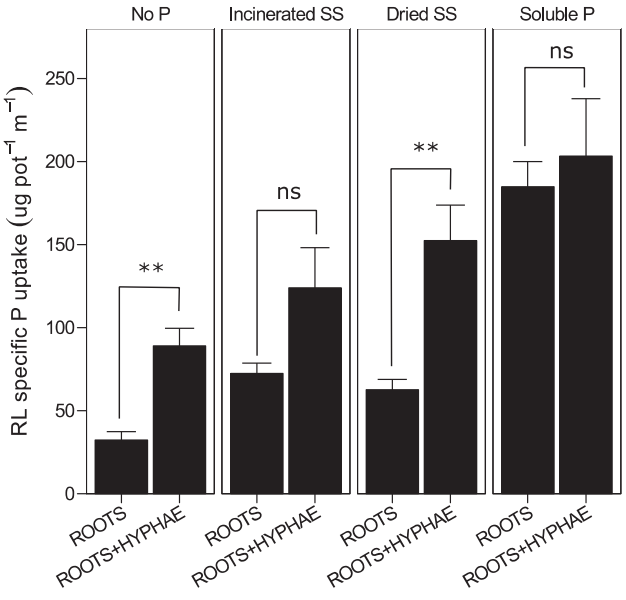

Fig. 7. Root length (RL) specific shoot P uptake (μg pot^{-1} m^{-1}) of wheat from outer compartments when wheat was grown in compartmented pots. Outer compartments either received no P, soluble P, or P in the form of incinerated or dried sewage sludge (SS). Either both roots and hyphae of AMF had access to the outer compartment or only roots had access to the outer compartment. Values are means ± standard error. $N = 4$. Significant differences within P treatments are shown.

however, as densities were similar in both the AM and NM disturbed treatments, we do not believe that the higher densities were due to a failure of twisting to prevent hyphal growth. Instead, perhaps the background hyphal length density contained a significant non-AM component that was stimulated by the soil disturbance.

4.2. Availability of P in SS materials to AMF hyphae

Plants often acquire a large proportion of their P via the AM pathway, with plants colonised by *Rhizophagus irregularis* often receiving P exclusively via the AM pathway (Smith et al., 2003). Similar to plant roots, AMF hyphae can only take up inorganic P in solution (Smith and Read, 2008). Therefore, when P is added to soils in complex forms, such as dried and incinerated SS, much of this P will not be immediately available to AMF. We found strong evidence that wheat plants obtained P from dried SS via the AM pathway, and weak evidence that they obtained P from incinerated SS via the AM pathway (Fig. 4). The difference between dried and incinerated SS could be related to the P speciation in these materials. The dried SS contained a larger proportion of easily-available P than the incinerated SS, which should be readily available to AMF hyphae. Furthermore, the dried SS likely contained some organic P, given that 20% of the P in SS can be organic (Medeiros et al., 2005). AMF are known to be able to take up N from organic sources, probably by contributing to mineralisation of N (Nuccio et al., 2013), via priming of native soil organic matter (Paterson et al., 2016). It is possible that they can also take up P from organic sources due to their contribution to mineralisation of P. There is evidence that AMF can alter soil microbial communities (Marschner et al., 2001; Wamberg et al., 2003) which can lead to communities more capable of P mineralisation (Ye et al., 2015; Zhang et al., 2016). Moreover, there is some evidence that AMF extraradical hyphae can directly hydrolyse organic P in a petri dish (Koide and Kabir, 2000); however, this has yet to be confirmed in soil. Therefore, the organic P in the dried SS may have been available to AMF hyphae in this experiment. On the other hand, the incinerated SS contained negligible amounts of C and therefore all the P would be inorganic. Previously, we found that 91% of the P in the incinerated SS was only extractable with hydrochloric acid (Mackay et al., 2017). Therefore, the P in the incinerated SS would need to undergo solubilisation before it is available. Changes in soil microbial communities driven by AMF could also lead to greater P solubilisation potential. For example, phosphorus-solubilising bacteria have been shown to grow along AMF hyphae (Ordoñez et al., 2016), and a synergistic effect of inoculation with a P solubilising bacteria and AMF on P solubilisation has been observed (Barea et al., 2002). However, our results suggest that AMF could not contribute greatly to the solubilisation of P in the incinerated SS in the timeframe of this study.

4.3. Comparison of the uptake of P in SS materials by AMF hyphae and plant roots

The AM pathway is often considered more effective than the direct pathway because AMF hyphae are able to access microsites not accessible to roots and can extend beyond the rhizosphere depletion zone (Jakobsen et al., 1992; Tibbett, 2000). We hypothesised that the ability of AMF hyphae to grow over large surface areas would be particularly beneficial in systems where P was applied as dried or incinerated SS, and would first have to be mineralised or solubilised before becoming available. In these systems, AMF hyphae might be better at acquiring P at the point of solubilisation/mineralisation than plant roots. However, we found that AMF hyphae were not better at acquiring P from dried and incinerated SS than the wheat roots themselves. This could be because wheat is known to grow many fine roots that are very efficient at P uptake. Results may differ for different plants, and this

could be worth investigating further. Moreover, although the hyphal length density in the outer compartments was more than 100 times the root length density, it is possible that hyphae did not explore the entire soil volume.

The maximum spread of this AMF in soil is estimated to be 1.5 mm day^{-1} (I. Jakobsen, unpublished). Given that radioactivity was observed in the third control (with ^{33}P in the outer compartment) 18 days after sowing, the approximate maximum spread in the outer compartment would have been 35 mm. At this spread, the hyphae would have reached the wall of the outer compartment directly opposite the mesh barrier; however, hyphae would not have reached the bottom of the outer compartment. In contrast, roots would have likely explored the whole outer soil volume as the growth rate of wheat root tips is in the range 8–35 mm day^{-1} (Watt et al., 2006). Therefore, P uptake by hyphae compared to roots could have been limited by growth of hyphae in the outer compartment. However, P uptake by hyphae from the outer compartment with soluble P was only 27% of the P uptake by roots. This large difference is unlikely to be completely caused by geometry considerations. AMF species differ in their ability to take up P (Smith et al., 2004) and it is possible that the AMF used here is not as effective as others at acquiring soluble P. Alternatively, the differences in uptake from P sources could be due to fluxes in available P, or the effect that plant roots have on soil compared to AMF hyphae.

The pool of available P in soil is constantly in flux, depending on the processes occurring. After soluble P is added to soil, the available P pool decreases due to sorption of P to colloids and precipitation with Fe, Al, Mg or Ca. While most adsorption of newly added P occurs very quickly, it can also continue to happen for years after the addition of P to soil (Bramley and Barrow, 1992; Javid and Rowell, 2002). Therefore, it is possible that fast growing plant roots were better able to utilise P while it was available, compared to hyphae which might not have been able to explore the soil volume before a large proportion of P was adsorbed. On the other hand, when dried or incinerated SS is added to soil, the available P pool increases with time as P is solubilised/mineralised, and therefore the slower growth of hyphae compared with roots may not have had the same disadvantage in treatments receiving SS.

Both plant roots and AMF influence the soil around them, for example due to exudation of C, and the zone around roots and hyphae have been termed rhizosphere and hyphosphere respectively, with the zone influenced by both roots and AMF known as the mycorrhizosphere (Priyadharsini et al., 2016). As discussed above, there is some evidence that AMF hyphae can modify the soil microbial community in favour of P cycling organisms. There is also strong evidence of plant roots being capable of such modification of the soil microbial community; however, much of this evidence comes from comparisons of AM roots with bulk soil (Li et al., 2014). Still, it is likely that the hyphosphere effect is not as strong or significant as the rhizosphere effect, which could explain why plants acquired similar, or less, P when only hyphae could access P compared with when only roots could access P.

4.4. Comparison of the uptake of P in SS materials by AM roots and NM roots

While plants are known to obtain a large proportion of their P via the AM pathway, this does not necessarily mean that AM plants acquire more P than NM plants (Cavagnaro et al., 2008; Smith et al., 2003). We found that both wheat roots and AMF hyphae together could take up more P from dried SS than roots alone. Moreover, shoot P uptake per root length was more efficient in AM plants than NM plants when P was applied as dried SS. This shows that AM can benefit plants when P is applied as dried SS. However, when P was applied as incinerated SS, plant P uptake by roots and hyphae together was no different than by roots alone. While it is probable that hyphae did contribute to plant P uptake in this treatment, there was clearly no additive effect of being able to take up P from both hyphae and roots. This lack of a difference could have been because uptake by hyphae was limited by hyphal growth in the outer compartment. As mentioned above, in treatments where only hyphae could grow into outer compartments, it is unlikely that hyphae would have explored the whole soil volume. Conversely, in the treatment where both roots and hyphae could grow into the outer compartment, AMF colonised roots in the outer compartment as well as roots in the inner compartment. This could have led to greater exploration of the outer compartment by hyphae. However, colonisation of roots in the outer compartment was lower than in the inner compartment. This is likely because AMF inoculum was only added to the soil in the inner compartment. Inoculating soil in outer compartments might be necessary to see larger differences between AM and NM plants in all P treatments.

4.5. Translation of P uptake into plant growth

While there was a positive mycorrhizal response for shoot P uptake when P was added as dried SS, being mycorrhizal did not benefit wheat shoot biomass. Growth depressions have been observed in AM plants compared with NM plants under various conditions (Buwalda and Goh, 1982; Graham and Abbott, 2000; Peng et al., 1993), which has been attributed to host-fungus competition for C, although other possible mechanisms have been described (Johnson et al., 1997; Li et al., 2008). However, the lack of a mycorrhizal benefit to plant biomass in this experiment could be due to the timing of when P in the dried SS became available. Previously, we showed that the P in this dried SS became more available over time (Mackay et al., 2017). While in the current experiment we showed that P was more available to AM wheat plants than NM wheat plants over the course of the experiment, further investigation is needed to determine the timing of P availability. If the P only became available later in the experiment, there may not have been enough P available for early plant growth, which could result in lower biomass.

4.6. Conclusions

We used a compartmented pot system with an isotope pool dilution approach to investigate wheat P uptake from three different P sources (soluble P, dried SS and incinerated SS) via different structures (roots and AMF hyphae). Importantly, P uptake from dried SS was similar by AMF hyphae and by un-colonized roots, but P uptake was significantly greater by AM colonized roots and their external hyphae. In contrast, P uptake from incinerated SS was lower and was not enhanced by the presence of mycorrhizas, possibly because solubilisation of the P in incinerated SS may have been limited within the time-frame of this study. Therefore, maintaining high levels of AMF colonisation in wheat crops could be a useful way to ensure sufficient P uptake when P is applied as dried SS, but may not be important when P is applied as incinerated SS. Our results suggest that SS can be recycled, helping close the anthropogenic P cycle, and that AMF play an important role in this process.

Acknowledgements

The authors wish to acknowledge Lena Asta Byrgesen and Morten Læssøe Stephensen for technical assistance, and F. Andrew Smith and Ronald Smernik for advice on data interpretation. We acknowledge funding from the Grains Research and Development

Corporation via a Grains Industry Research Scholarship (GRS10686) to J.E.M., and from the Innovation Fund Denmark (1308-00016B). Additionally, J.E.M. acknowledges support through an Australian Government Research Training Program Scholarship and travel grants to undertake this research in Denmark from the Plant Nutrition Trust and the University of Adelaide.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2017.08.004.

References

Barea, J.M., Toro, M., Orozco, M.O., Campos, E., Azcón, R., 2002. The application of isotopic (32P and 15N) dilution techniques to evaluate the interactive effect of phosphate-solubilizing rhizobacteria, mycorrhizal fungi and Rhizobium to improve the agronomic efficiency of rock phosphate for legume crops. Nutrient Cycling in Agroecosystems 63, 35–42. http://dx.doi.org/10.1023/A:1020589732436.

Barraclough, P.B., Leigh, R.A., 1984. The growth and activity of winter wheat roots in the field: the effect of sowing date and soil type on root growth of high-yielding crops. The Journal of Agricultural Science 103, 59–74.

Bertrand, I., McLaughlin, M.J., Holloway, R.E., Armstrong, R.D., McBeath, T., 2006. Changes in P bioavailability induced by the application of liquid and powder sources of P, N and Zn fertilizers in alkaline soils. Nutrient Cycling in Agroecosystems 74, 27–40. http://dx.doi.org/10.1007/s10705-005-4404-3.

Bramley, R.G.V., Barrow, N.J., 1992. The reaction between phosphate and dry soil. II. The effect of time, temperature and moisture status during incubation on the amount of plant available P. Journal of Soil Science 43, 759–766. http://dx.doi.org/10.1111/j.1365-2389.1992.tb00175.x.

Breuillin, F., Schramm, J., Hajirezaei, M., Ahkami, A., Favre, P., Druege, U., Hause, B., Bucher, M., Kretzschmar, T., Bossolini, E., Kuhlemeier, C., Martinoia, E., Franken, P., Scholz, U., Reinhardt, D., 2010. Phosphate systemically inhibits development of arbuscular mycorrhiza in *Petunia hybrida* and represses genes involved in mycorrhizal functioning. The Plant Journal 64, 1002–1017. http://dx.doi.org/10.1111/j.1365-313X.2010.04385.x.

Buwalda, J.G., Goh, K.M., 1982. Host-fungus competition for carbon as a cause of growth depressions in vesicular-arbuscular mycorrhizal ryegrass. Soil Biology and Biochemistry 14, 103–106. http://dx.doi.org/10.1016/0038-0717(82)90052-9.

Cavagnaro, T.R., Langley, A.J., Jackson, L.E., Smukler, S.M., Koch, G.W., 2008. Growth, nutrition, and soil respiration of a mycorrhiza-defective tomato mutant and its mycorrhizal wild-type progenitor. Functional Plant Biology 35, 228–235. http://dx.doi.org/10.1071/FP07281.

Cavagnaro, T.R., Smith, F.A., Ayling, S.M., Smith, S.E., 2003. Growth and phosphorus nutrition of a Paris-type arbuscular mycorrhizal symbiosis. New Phytologist 157, 127–134. http://dx.doi.org/10.1046/j.1469-8137.2003.00654.x.

Deubel, A., Merbach, W., 2005. Influence of microorganisms on phosphorus bioavailability in soils. In: Varma, P.D.A., Buscot, P.F. (Eds.), Microorganisms in Soils: Roles in Genesis and Functions, Soil Biology. Springer Berlin Heidelberg, pp. 177–191. http://dx.doi.org/10.1007/3-540-26609-7_9.

Graham, J.H., Abbott, L.K., 2000. Wheat responses to aggressive and non-aggressive arbuscular mycorrhizal fungi. Plant and Soil 220, 207–218. http://dx.doi.org/10.1023/A:1004709209009.

Harrison, E.Z., Oakes, S.R., Hysell, M., Hay, A., 2006. Organic chemicals in sewage sludges. Science of the Total Environment 367, 481–497. http://dx.doi.org/10.1016/j.scitotenv.2006.04.002.

Jakobsen, I., Abbott, L.K., Robson, A.D., 1992. External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. New Phytologist 120, 509–516. http://dx.doi.org/10.1111/j.1469-8137.1992.tb01800.x.

Javid, S., Rowell, D. l, 2002. A laboratory study of the effect of time and temperature on the decline in Olsen P following phosphate addition to calcareous soils. Soil Use and Management 18, 127–134. http://dx.doi.org/10.1111/j.1475-2743.2002.tb00230.x.

Johnson, N.C., Graham, J.-H., Smith, F.A., 1997. Functioning of mycorrhizal associations along the mutualism–parasitism continuum. New Phytologist 135, 575–585. http://dx.doi.org/10.1046/j.1469-8137.1997.00729.x.

Joner, E.J., Jakobsen, I., 1995. Growth and extracellular phosphatase activity of arbuscular mycorrhizal hyphae as influenced by soil organic matter. Soil Biology and Biochemistry 27, 1153–1159. http://dx.doi.org/10.1016/0038-0717(95)00047-I.

Koide, R., Kabir, Z., 2000. Extraradical hyphae of the mycorrhizal fungus *Glomus intraradices* can hydrolyse organic phosphate. New Phytologist 148, 511–517.

Krüger, O., Adam, C., 2015. Recovery potential of German sewage sludge ash. Waste Management Urban Mining 45, 400–406. http://dx.doi.org/10.1016/j.wasman.2015.01.025.

Li, H., Smith, F.A., Dickson, S., Holloway, R.E., Smith, S.E., 2008. Plant growth depressions in arbuscular mycorrhizal symbioses: not just caused by carbon drain? New Phytologist 178, 852–862. http://dx.doi.org/10.1111/j.1469-8137.2008.02410.x.

Li, X., Rui, J., Xiong, J., Li, J., He, Z., Zhou, J., Yannarell, A.C., Mackie, R.I., 2014. Functional potential of soil microbial communities in the maize rhizosphere. PLoS One 9, e112609. http://dx.doi.org/10.1371/journal.pone.0112609.

Mackay, J.E., Cavagnaro, T.R., Jakobsen, I., Macdonald, L.M., Grønlund, M., Thomsen, T.P., Müller-Stöver, D.S., 2017. Evaluation of phosphorus in thermally converted sewage sludge: P pools and availability to wheat. Plant and Soil 1–11. http://dx.doi.org/10.1007/s11104-017-3298-6.

Marschner, P., Crowley, D., Lieberei, R., 2001. Arbuscular mycorrhizal infection changes the bacterial 16 S rDNA community composition in the rhizosphere of maize. Mycorrhiza 11, 297–302. http://dx.doi.org/10.1007/s00572-001-0136-7.

Medeiros, J.J.G., Cid, B.P., Gómez, E.F., 2005. Analytical phosphorus fractionation in sewage sludge and sediment samples. Analytical and Bioanalytical Chemistry 381, 873–878. http://dx.doi.org/10.1007/s00216-004-2989-z.

Metson, G.S., Bennett, E.M., 2015. Phosphorus cycling in Montreal's food and urban agriculture systems. PLoS One 10, e0120726. http://dx.doi.org/10.1371/journal.pone.0120726.

Newman, E.I., 1966. A method of estimating the total length of root in a sample. Journal of Applied Ecology 3, 139–145. http://dx.doi.org/10.2307/2401670.

Nuccio, E.E., Hodge, A., Pett-Ridge, J., Herman, D.J., Weber, P.K., Firestone, M.K., 2013. An arbuscular mycorrhizal fungus significantly modifies the soil bacterial community and nitrogen cycling during litter decomposition. Environmental Microbiology 15, 1870–1881.

Olsson, P.A., Bååth, E., Jakobsen, I., Söderström, B., 1996. Soil bacteria respond to presence of roots but not to mycelium of arbuscular mycorrhizal fungi. Soil Biology and Biochemistry 28, 463–470. http://dx.doi.org/10.1016/0038-0717(96)00011-9.

Ordoñez, Y.M., Fernandez, B.R., Lara, L.S., Rodriguez, A., Uribe-Vélez, D., Sanders, I.R., 2016. Bacteria with phosphate solubilizing capacity alter mycorrhizal fungal growth both inside and outside the root and in the presence of native microbial communities. PLOS One 11, e0154438. http://dx.doi.org/10.1371/journal.pone.0154438.

Paterson, E., Sim, A., Davidson, J., Daniell, T.J., 2016. Arbuscular mycorrhizal hyphae promote priming of native soil organic matter mineralisation. Plant and Soil 408, 243–254. http://dx.doi.org/10.1007/s11104-016-2928-8.

Pearson, J.N., Jakobsen, I., 1993. The relative contribution of hyphae and roots to phosphorus uptake by arbuscular mycorrhizal plants, measured by dual labeling with P-32 and P-33. New Phytologist 124, 489–494.

Peirce, C. a. E., Smernik, R.J., McBeath, T.M., 2013. Phosphorus availability in chicken manure is lower with increased stockpiling period, despite a larger orthophosphate content. Plant and Soil 373, 359–372. http://dx.doi.org/10.1007/s11104-013-1807-9.

Peng, S., Eissenstat, D.M., Graham, J.H., Williams, K., Hodge, N.C., 1993. Growth depression in mycorrhizal citrus at high-phosphorus supply (analysis of carbon costs). Plant Physiology 101, 1063–1071. http://dx.doi.org/10.1104/pp.101.3.1063.

Priyadharsini, P., Rojamala, K., Ravi, R.K., Muthuraja, R., Nagaraj, K., Muthukumar, T., 2016. Mycorrhizosphere: the extended rhizosphere and its significance. In: Choudhary, D.K., Varma, A., Tuteja, N. (Eds.), Plant-Microbe Interaction: an Approach to Sustainable Agriculture. Springer Singapore, pp. 97–124. http://dx.doi.org/10.1007/978-981-10-2854-0_5.

Scholz, R.W., Wellmer, F.-W., 2013. Approaching a dynamic view on the availability of mineral resources: what we may learn from the case of phosphorus? Global Environmental Change 23, 11–27. http://dx.doi.org/10.1016/j.gloenvcha.2012.10.013.

Smith, S.E., Read, D.J., 2008. Mycorrhizal Symbiosis. Academic press.

Smith, S.E., Smith, F.A., Jakobsen, I., 2004. Functional diversity in arbuscular mycorrhizal (AM) symbioses: the contribution of the mycorrhizal P uptake pathway is not correlated with mycorrhizal responses in growth or total P uptake. New Phytologist 162, 511–524. http://dx.doi.org/10.1111/j.1469-8137.2004.01039.x.

Smith, S.E., Smith, F.A., Jakobsen, I., 2003. Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. Plant Physiology 133, 16–20. http://dx.doi.org/10.1104/pp.103.024380.

Tibbett, M., 2000. Roots, foraging and the exploitation of soil nutrient patches: the role of mycorrhizal symbiosis. Functional Ecology 14, 397–399.

Wamberg, C., Christensen, S., Jakobsen, I., Müller, A.K., Sørensen, S.J., 2003. The mycorrhizal fungus (*Glomus intraradices*) affects microbial activity in the rhizosphere of pea plants (*Pisum sativum*). Soil Biology and Biochemistry 35, 1349–1357. http://dx.doi.org/10.1016/S0038-0717(03)00214-1.

Wang, T., Camps-Arbestain, M., Hedley, M., 2014. The fate of phosphorus of ash-rich biochars in a soil-plant system. Plant and Soil 375, 61–74.

Watt, M., Silk, W.K., Passioura, J.B., 2006. Rates of root and organism growth, soil conditions, and temporal and spatial development of the rhizosphere. Annals of Botany 97, 839–855. http://dx.doi.org/10.1093/aob/mcl028.

Yao, Q., Li, X., Feng, G., Christie, P., 2001. Mobilization of sparingly soluble inorganic phosphates by the external mycelium of an arbuscular mycorrhizal fungus. Plant and Soil 230, 279–285. http://dx.doi.org/10.1023/A:1010367501363.

Ye, S., Yang, Y., Xin, G., Wang, Y., Ruan, L., Ye, G., 2015. Studies of the Italian ryegrass–rice rotation system in southern China: arbuscular mycorrhizal symbiosis affects soil microorganisms and enzyme activities in the *Lolium mutiflorum* L. rhizosphere. Applied Soil Ecology 90, 26–34. http://dx.doi.org/10.1016/j.apsoil.2015.01.017.

Zapata, F., Axmann, H., 1995. 32P isotopic techniques for evaluating the agronomic effectiveness of rock phosphate materials. Fertilizer Research 41, 189–195.

http://dx.doi.org/10.1007/BF00748308.
Zhang, L., Xu, M., Liu, Y., Zhang, F., Hodge, A., Feng, G., 2016. Carbon and phosphorus exchange may enable cooperation between an arbuscular mycorrhizal fungus and a phosphate-solubilizing bacterium. New Phytologist 210, 1022–1032. http://dx.doi.org/10.1111/nph.13838.
Zwetsloot, M.J., Lehmann, J., Bauerle, T., Vanek, S., Hestrin, R., Nigussie, A., 2016. Phosphorus availability from bone char in a P-fixing soil influenced by root-mycorrhizae-biochar interactions. Plant and Soil 408, 95–105. http://dx.doi.org/10.1007/s11104-016-2905-2.