Effect of arbuscular mycorrhizal colonisation on the growth and phosphorus nutrition of *Populus euramericana* c.v. Ghoy

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Abstract

Arbuscular mycorrhizal (AM) fungi are known to associate with a range of *Populus* species particularly in the early stages of development yet there is little information on their potential role in sustainable biomass production. Moreover, while the AM association may enhance plant establishment, growth and nutrition, different AM fungi (AMF) vary in their ability to confer such benefits. In this study we investigated the impact of four different AMF on growth and phosphorus nutrition of *Populus euramericana* c.v. Ghoy. Plant biomass, particularly root biomass, was generally reduced by AMF colonisation and the extent of this reduction varied with the AMF species that had colonised the roots. This growth reduction was not simply due to differences in root colonisation, which ranged from 59 to 71% by the end of the experiment at 105-d and did not vary among the AMF screened. Phosphorus content was also lower in some AMF treatments than in non-AMF controls after growth for 105 d although P concentration in aboveground tissues (stems and leaves) was higher. The possible interaction between AMF and poplar in converted arable systems is discussed.

1. Introduction

Biomass crop production has largely been driven by the ability of fast-growing members of the Salicaceae, such as *Populus* (poplar) and *Salix* (willow), to regenerate vegetatively following coppicing, which typically takes place every 4-5 years [1] in short-rotation coppice (SRC) plantations. SRC plantations promote agricultural sustainability, as they often require minimal nutrient input once established, without further need for tillage or intensive land management. The contribution of biomass-derived energy to the global renewable energy market is significant (~50% in 2004), but two major concerns exist: (1) the sustainability of high biomass yield and (2) the diversion of quality agricultural land away from food production. Mineral fertilisers are often added in SRC forestry to maintain yield but could potentially be replaced by the exploitation of processes carried out by essential soil microorganisms, such as mycorrhizal fungi and symbiotic bacteria, to facilitate/enable sustainable (minimal fertilisation, minimal pesticide application, minimal tillage) production of biomass-derived energy for the future [2]. Understanding such plant-soil relationships may also offer opportunities to exploit the ecosystem services that mycorrhizas can provide to their associated poplar host including increased resistance to drought [3], and phytoremediation of marginal or waste land [3,4], rather than targeting valuable agricultural land for biomass plantations.

Arbuscular mycorrhizal fungi (AMF) form symbiotic associations with the majority of land plants. Although AMF, unlike many of the fungi involved in the ectomycorrhizal (ECM) association [5,6], have no known saprotrophic capability (see [7,8]) they play a major role in nutrient acquisition for their associated host plant. This includes the acquisition of the key nutrients phosphorus [9,10] and nitrogen [11-13], making them a fundamental component of the soil microbial community. AMF obtain photosynthetically derived carbon from their host plants, and in return offer both enhanced nutrient uptake and a range of other benefits, such as protection against pathogens [14,15] and soil stability [16]. Carbon from the plant is also used to construct extensive extraradical hyphal networks throughout the soil that act as an interface between plant roots and the soil environment and for other activities associated with completion of the fungal life history, such as spore production [17,18].

Mycorrhizas are associated with a range of *Populus* species [19-21], yet there is little information on their potential role in sustainable biomass production. Members of the *Populus* genus can form both AM and ECM associations, sometimes with both present in the same root system [22,23]. Many studies focus on ECM associations of *Populus* species. For example, Quoreshi and Khasa [21] suggest that inoculation of poplar seedlings with appropriate ECM fungi and selected bacteria in commercial nursery systems may enhance poplar establishment at plantation sites. However, there is also evidence that AM colonisation is more important in young poplar seedlings with ECM associations dominant only later in more established trees [21,24]. Evidence from other studies using a number of different plant and AM fungal species suggests host plant performance can vary depending on the AMF species present [25], even when the plants and fungi co-exist [26]. Despite evidence that diverse AM fungi associate with poplar [27], the functional significance to the plant of the AM symbiosis remains unclear. Hooker et al. [28] found that colonisation of poplar by three AMF species (*Scutellispora calospora*, *Glomus* sp E3 and *Glomus caledonium*) did not significantly affect overall plant growth, although colonisation rates were low, and effects on nutrition were not investigated.

Despite the near ubiquity of AMF and the importance of poplar as a biomass crop there are few studies of the impacts of AMF on poplar growth and nutrition. Investigation of the colonising potential of AMF species in the early growth stages of poplar offers the potential for future manipulation of biomass crop species prior to establishment on plantation sites, with possible favourable consequences for nutrient cycling and crop nutrition and minimal inputs. In particular, the ability of AMF to acquire phosphorus for their associated host [5] is likely to become increasingly important in low input bioenergy systems in which fertiliser inputs are reduced. There may also be other benefits such as increased carbon flow belowground and incorporation into the soil organic matter pool [29] but, as AM colonised roots often have increased respiration rates [30,31], this would only be beneficial if incorporation exceeded C loss. Thus, the development of sustainable, productive agricultural and forestry systems must consider the important roles of soil microbes and soil processes in soil nutrient management [15,32] and carbon sequestration [33].

Understanding the relationship between mycorrhizas and biomass crop species and how mycorrhizas affect the growth and nutrition of such species, may contribute to more sustainable practices in the future. We therefore investigated the effects of four different AMF species on poplar growth and phosphorus nutrition. Phosphorus acquisition was targeted, as exhaustion of global supplies of rock phosphate, a non-renewable resource used in the production of high quality fertilisers, is predicted within the next century [34,35]. Our specific objectives in this study were to determine (i) whether all AM fungi colonised poplar cuttings equally and (ii) whether the consequences of colonisation were dependent on the AMF. We determined the latter by following the impact on the host poplar plant via biomass production and phosphorus nutrition and the extent of the external mycelium produced by the AMF.

2. Materials and methods

Plant material and arbuscular mycorrhizal inocula

*Populus euramericana* (a hybrid of *P. nigra* x *P. deltoides*) c.v. Ghoycuttings (obtained from Bowhayes Trees, Devon, UK) of 6-8 cm in length (to include a node) were washed for 15 s in 70% (v/v) ethanol, rinsed in sterile deionised water at least three times and placed immediately in the experimental growth medium (see below). AMF inocula were *Glomus mosseae* (Nicol. & Gerd.) Gerdemann & Trappe (isolate BEG 12; Biorhize, Dijon, France), *Glomus intraradices* (isolate BB-E; Biorhize, Dijon, France), *Glomus hoi* (University of York (UY) isolate number UY110) and *Gigaspora rosea* (Biorhize, Dijon, France). Cultures of the above fungi (with the exception of *Gi. rosea*) were established at least c. 3 months before use in pots with *Plantago lanceolata* L. (Emorsgate Wild Seeds, Nottingham, UK) in a sand and Terragreen® (a calcinated attapulgite clay soil conditioner; Oil-Dri, Cambridge, UK) mixed (1:1, v/v) with 0.25 g l-1 of bone meal. The AM inocula consisted of 60 g fresh weight (FW) of culture (including root pieces and the sand:Terragreen® growth medium), with the exception of *Gi. rosea* inoculum which consisted of 60 g dry weight (DW) of the pot culture. Non-AM controls received 60 g of the combined AM fungal inoculum which had been autoclaved (121oC; 30 min). As bacteria can also be present in the AM inoculum, the non-AM controls each received 20-ml of the combined AM inoculum washing solution following filtration to remove AM propagules and prior to the inoculum being autoclaved [36].

The AMF species used in this experiment were selected for the following reasons. *G. hoi* (UY 110) was originally isolated from a woodland site in North Yorkshire, UK where it associates almost exclusively with another woody host, sycamore (*Acer pseudoplatanus*) [37]. *G. intraradices* is widely used in AM research as it occurs in a large number of ecosystems [7], it readily colonises a wide range of plant species (including poplar and many agriculturally important crops) and, because of its association with poplar, was selected as the first AMF for genome sequencing [38]. *G. mosseae* is frequently detected as among the dominant AMF in arable soils [37] thus, if arable land is converted to the production of bioenergy crops *G. mosseae* would be expected to be present. Finally, *Gi rosea* was selected as it is a different genus from the other (*Glomus*) AMF screened [7].

Experimental system

Washed poplar cuttings were planted in 2.5-l pots (30 cm height, 10.2 cm diameter) in sterilised sand:Terragreen® (1:1; v/v) mix with the AMF inocula (as above) and 0.5 g l-1 bone meal. Sand:Terragreen® growth medium is commonly used in AM experimental systems as it is low in nutrients, permits good AM establishment and facilitates AMF hyphal extraction [13,36]. The base of each pot was covered by a 700-µm, metal mesh (Plastok® Birkenhead, UK). Four replicates of each AMF type were prepared and grown for up to 105 d in controlled glasshouse conditions from August-November 2007 with a 16-h photoperiod and a mean temperature of 18.6 ± 0.1oC. Maximum and minimum temperatures over the experimental period were 24.2 and 17.0oC respectively. Plants were fed twice weekly with 50-ml Rorison’s nutrient solution but containing reduced (i.e. 0.1-mM) phosphate.

Harvest and sample analysis

After growth in the glasshouse for 77 and 105 d, pots were destructively harvested and FW of leaves, stems and roots, stem length and the number of leaves were determined. Samples of root and growth medium were taken for mycorrhizal assessment. Leaf area was determined using a Li-300A area meter (LiCor Biosciences, Nebraska, USA), and leaf material was then dried at 700C for calculation of specific leaf area (SLA m2 kg-1) [39].

Subsamples of fresh root material were cleared with 10% KOH, acidified with 1% HCl and stained with acid fuchsin ([40], but omitting phenol). Percentage total root length colonisation (%RLC; the percentage of total root intercepts with AMF structures) and arbuscule and vesicle presence were assessed at x250 magnification using the gridline intercept technique for a minimum of 100 root intersections for each sample [41]. AMF extraradical mycelium (ERM) was extracted from two 5-g (FW) samples of the sand:Terragreen® growth medium using a modified membrane filter technique [42] and a minimum of 50 fields of view, counted at x125 magnification using the gridline intercept technique [36]. AMF hyphal length was then converted to hyphal length density g-1 DW. The FW of the remainder of the root sample was determined and the samples then dried at 700C in an oven to constant dry weight (5 d). Dried plant material was separated into roots, stems and leaves and milled to a fine powder and phosphate was determined after triple digestion using the molybdenum blue method [43].

Data analysis

Where possible, data were analysed by a two way analysis of variance (ANOVA) with AMF treatment and harvest time as the main factors. All data were checked for normality and were transformed, if necessary, to meet the requirements of homoscedasticity. In cases where data showed a non-parametric distribution, even after transformation, when both harvest points were included, data were analysed by a one-way ANOVA at each time point separately with AMF treatment as the factor. The Bonferroni mean comparison *post-hoc* test was applied for comparisons among AMF treatments. If homoscedasticity was still not possible a Mann-Whitney *U* test was used to compare non-AMF and AMF treatments. At the first harvest (77 d) there was insufficient stem material for determination of phosphorus content in two replicates from the *G. hoi* treatment and one from the *G.* *intraradices* treatment. The entire *G. hoi* treatment was therefore excluded from both the stem and total phosphorus content analyses at this time, and *n* = 3 for the *G.* *intraradices* treatment. In all cases, a randomised block design was used. All results were analysed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

In this study we followed the impact of one-to-one interactions between different AM fungi and *P. euramericana* cuttings compared to a non-AM control. These conditions are rather artificial as the ‘non-AM control’ in the natural environment would not exist and the poplar roots would be colonised, often by more than one AMF. However, such conditions, widely used in mycorrhizal research [13,30,36], enable the impact of individual fungi to be analysed without the complicating factors of multiple colonisation events and confounding impacts of differing environmental conditions that would occur in the field. Thus, they are a first stage in understanding how different AMF may impact upon poplar growth and P nutrition.

At the end of the experimental period (105-d), AM colonisation in general had a large negative impact on root and total DW production of *P. euramericana* cuttings, although stem length (with the exception of cuttings colonised by *G. hoi*) and leaf number were largely unaffected (Table 1). AMF colonisation has previously been demonstrated to have positive, neutral or negative impacts on plant biomass depending on the host plant-fungal combination and the experimental conditions used [44-46]. The reduction in plant growth observed in this study (Table 1) was likely due to the added burden for the poplar cutting associated with mycorrhizal growth. The proportion of total plant carbon partitioned to mycorrhizal symbionts differs widely (e.g. 4-20%), although values of c. 10% are common [47]. In the field such costs are generally less, as developing seedlings can link into already established common mycorrhizal hyphal networks [48] rather than establishing the fungus *de novo*, as in the present study. However, certain agricultural practises, such as high fertiliser input and ploughing, severely disrupt the mycelial network and necessitate repeated establishment. Thus, our results suggest that conversion of land from intensive crop production to production of bioenergy crops may reduce growth in the early phases, when mycorrhizas are establishing. However, reduction in early stage biomass production is not necessarily reflected in biomass during later developmental stages (reviewed by [10]) and must also be viewed in the context of other potential benefits including enhanced soil stability [16] and increased carbon storage [29].

Hooker et al. [28] using three different AMF (i.e. *S. calospora*, *Glomus* sp E3 and *G. caledonium*) found no effect on leaf area of 115 d old poplar seedlings. In contrast, in this study specific leaf area (SLA, m2 kg-1) was higher in the AMF treatments, except for plants colonised by *G. intraradices* and *G. mosseae*, which did not differ from non-AMF controls (Table 1). The difference in SLA was presumably due to significantly higher leaf DW in the non-AMF plants at both the 77 d (Mann-Whitney *U* test, *P* = 0.012) and 105 d harvests (*P* < 0.001; 4.61 ± 0.30 g non-AMF vs 1.78 ± 0.27 g across all AMF treatments). Leaf DW produced by the AMF fungal treatments at 105 d did not differ significantly, as determined by a Bonferroni *post-hoc* test. Stem DW, a key parameter in the production of bioenergy crops, did not differ (*F*4,19 = 2.80; *P* = 0.064) among the various treatments (mean across all treatments = 0.59 ± 0.13 g) at 77 d. By 105 d, however, stem DW in the non-AMF controls was higher (*F*4,19 = 12.35; *P* < 0.001) than in all the AMF treatments which did not differ (2.46 ± 0.31g non-AMF vs 0.74 ± 0.13 g across all AMF treatments). Harvest time was significant for root DW, total DW and SLA but not for either stem length or leaf number. The interaction between harvest time and AMF treatment was not significant in any case.

Phosphorus content at 77 d in the non-AMF roots was significantly higher than in those colonised by *G. hoi* or *G. mosseae*. Leaf and stem P content did not differ (Fig 1A), although *G. hoi* was excluded from the stem analysis at 77 d due to insufficient material. By 105 d overall P content had increased but there was still no difference in leaf P content among treatments (*H*1,4 = 9.14, *P* = 0.058; Fig. 1B). Stem, root and total P contents in the non-AMF cuttings were significantly higher than in cuttings colonised by either *G. hoi* or *Gi. rosea* but not *G*. *intraradices* or *G. mosseae* (Fig. 1B). In addition, root and total P contents of both *G. mosseae* and *G. intraradices* colonised seedlings at 105 d were higher than that of *G. hoi* but not different to *Gi. rosea* (Fig. 1B). The negative impact of colonisation by *G. hoi* on poplar was particularly surprising. This isolate, originally obtained from a woodland site in North Yorkshire, UK [26], has previously been shown consistently to out-perform other AMF species screened under controlled conditions (similar to those used in this study) and improved both growth and P acquisition for 4 out of 5 woodland plant species tested [26]. Moreover, it was the only AMF species of the 4 screened to colonise and improve P acquisition for *A.* *pseudoplatanus*, the only woody tree species examined [26]. Thus, we expected this fungus to benefit the host *P. euramericana*, but our results demonstrate that *G. hoi* consistently had a negative impact on both growth and total P nutrition of its host plant.

AM uptake of P is complex. Acquisition of P via the symbiotic pathway can down-regulate direct plant P uptake, even in the absence of a growth response [9,10], but the extent of this phenomenon depends on the AM fungi present [44,49]. At the end of the experimental period (105 d) P concentration of roots and leaves did not differ among the various treatments (stem and total P concentrations were not normally distributed, even after transformation, and were therefore not tested). However, when the AMF treatments were tested as one group against the non-AM treatment by a non-parametric Mann-Whitney *U* test, the AM treatments contained significantly (*P* < 0.05) higher P concentrations in their leaves (8.26 ± 0.61 mg P g-1 AM vs 5.42 ± 0.20 mg P g-1 non-AM) and stems (8.28 ± 1.01 mg P g-1 AM vs 4.94 ± 0.19 mg P g-1 non-AM) than the non-AM controls. In contrast, root P concentration did not differ. Thus, the AM plants contained more P per unit D.W. in aboveground tissues than the non-AM controls. The difference in aboveground but not belowground tissues is important because it suggests that enhanced P capture by the AMF was passed to the plant rather than potentially still being held in AMF tissue [see 50].

Total %RLC did not vary significantly among the AMF treatments but increased from 46% (± 2%) at 77 d to 66% (± 2%) at 105 d (Fig. 2). This value is similar to the 69% colonisation of 6-month old hybrid (*Populus* X *euramericana* cv. 1-214) poplar cuttings inoculated with *G. mosseae* reported by Lopez-Aguillon and Garbaye [24] and at the upper range of the 20-50% colonisation found in 5-year old poplar clone stands in Alberta, Canada [20]. These studies did not report the frequency of arbuscules, the diagnostic structure of a functioning AM symbiosis, or the presence of vesicles, believed to be fungal storage structures [7]. Arbuscule turnover can be relatively rapid but depends on the plant species, (being more rapid in fast growing plant species) and the type (e.g. *Arum*- or *Paris*-type) of arbuscule structure formed [51,52]. Environmental factors can also influence arbuscule development and turnover [52,53]. At the end of the present study, arbuscules were less frequent when roots were colonised by *G. hoi* than by *Gi. rosea* (Fig. 2). Arbuscule frequency also increased with time (c. 20% at 77 d to c. 26% at 105 d). Vesicles, were rarely observed except in roots colonised by *G. intraradices*, where vesicle frequency was significantly higher than in all the other AMF treatments (at 105 d 11.7 ± 1.5% vesicles in *G. intraradices* roots vs 2.4 ± 0.6% across the other AMF treatments). No AMF structures were observed in the non-AM control roots at either harvest.

There was a strong relationship between plant P content and %RLC even after the effect of time was eliminated (Fig. 3) indicating that the relationship was not simply due to both parameters increasing with time. Although arbuscules are the site of P transfer from the fungus to the plant [7,54], as noted above, their turnover can be rapid, and arbuscule frequency recorded at harvest may not necessarily reflect P transfer to the plant over the duration of the experimental period. In contrast, hyphae internal to the roots, which dominate %RLC, are much longer lived [7], which may explain why the relationship was still apparent for total %RLC and plant P content. Colonisation by AMF may down-regulate root P acquisition [10], in some cases to such a degree that the fungus acquires all of the P [55]. The significant overall relationship seen in plants colonised by the AMF does suggest that these fungi did play an active role in P capture for their associated poplar host in this study.

While the presence of arbuscules is important in determining if the symbiosis is mutual, extraradical mycelium development is important for nutrient acquisition from the soil environment [56,57] and as a conduit for carbon flow below-ground [58,59]. Although low levels of aseptate hyphae were observed in the non-AMF controls at 77 d (0.03 ± 0.01 m g-1 DW) and 105-d (0.02 ± 0.005 m g-1 DW), hyphal length density in the AMF treatments was an order of magnitude higher at 77 d (mean across AMF treatments 0.67 ± 0.15 m g-1 DW) and two orders of magnitude higher at 105 d (1.02 ± 0.08 m g-1 DW). Although *G. intraradices* produced 4.5 x the hyphal lengths of *G. hoi* at 77 d, potential differences among treatments at either harvest may have been obscured by considerable variation among replicates (Fig. 4). However, the data in Fig. 4 demonstrate that reductions in poplar growth as a result of different AMF colonisation (Table 1) are not simply explained by the extent of ERM development

4. Conclusions

AMF colonisation had a large negative impact on root growth, although importantly for a biomass crop, stem weight was also reduced. Plant P content was, however, related to %RLC in the AMF treatments and overall P concentration in the aboveground structures (stems and leaves) of AMF colonised plants was higher than in non-AM controls. Strikingly, there was little difference among the four AMF screened on their impact on poplar growth and host plant nutrition, although there was some evidence that *G. hoi* had the largest negative impact (Table 1). This was particularly surprising given that this AMF was originally isolated from a woodland site where it associates almost exclusively with the woody *A. pseudoplatanus*. By the end of the experiment, *G*. *intraradices* and *G. mosseae* arguably had the least negative impact on poplar P nutrition. *G*. *intraradices* is often present in commercially available inocula because it both colonises roots rapidly and, unlike many other AMF, establishes well under *in vitro* conditions [8]. While, *G. mosseae* is often detected as either the dominant, or among the dominant, AM fungi in arable soils [37,60,61], it sporulates abundantly and readily colonises host plant roots from spores. These characteristics may enable this species to tolerate high disturbance events such as tilling, crop removal and so forth, all common features in arable situations. Thus, arable systems converted to bioenergy crops may already harbour *G. mosseae* species, but if the cuttings are treated with inocula, *G. intraradices* will likely be present and both these AMF had the least negative impact on poplar total P nutrition in this study.

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Figure legends:

Fig. 1. Phosphorus content of stems (grey bars), leaves (white bars) and roots (black bars) of *P. euramericana* cuttingsafter 77 d (A) and 105 d (B). Different letters in lower case indicate significant differences among treatments within the plant parts analysed while different letters in upper case indicate significant differences in total plant phosphorus. At 77 d there was insufficient stem material for phosphorus analysis in the plants colonised by *G. hoi* and stem and total phosphorus contents from this treatment were excluded from statistical analysis. Data are means ± S.E. (*n* = 4, except at 77 d for stems of plants colonised by *G. intraradices* where *n* = 3). Note the difference in the scale on the Y-axis between the two harvest points.

Fig. 2. Percentage total root length colonised (%RLC; white bars) and percentage arbuscules (black bars) in the AMF treatments at 105 d. Data are means ± S.E (*n* = 4). Different letters represent significant differences among the treatments as determined by a Bonferroni *post hoc* test.

Fig. 3. Relationship between percentage root length colonisation (%RLC) and total plant P content (mg) for *G. intraradices* (filled squares), *G. hoi* (squares), *G. mosseae* (filled circles) and *Gi rosea* (circles). The overall relationship for all the fungal treatments was fitted by a significant regression (Plant P content (mg) = (-) 3.36 + 9.50 %RLC.; *P* < 0.001, *F*1,27 = 22.74, *R*2 = 45.7%). Phosphorus contents were square-root transformed and %RLC were arcsin transformed prior to statistical analysis. Data are from both harvests (77 and 105 d) and the relationship was confirmed by a partial correlation eliminating the effect of time (*r* = 0.379, *P* < 0.001), *n* = 29 (note: for the individual AMF species *n* = 8, except for *G. hoi* (*n* = 6) and *G. intraradices* (*n* = 7) as there was insufficient stem material at the first (77 d) harvest to conduct phosphorus analysis).

Fig. 4. The influence of AMF inoculation on external hyphal length density (m g-1 DW) after 77 d (white bars) and 105 d (black bars). Data are means ± S.E (*n* = 4). Different lower case letters represent significant differences among the treatments at 77 d while upper case letters indicate significant differences after 105 d as determined by a Bonferroni *post hoc* test.

Table 1. The impact of different arbuscular mycorrhizal fungi (AMF) on plant growth compared to non-mycorrhizal controls (non-AMF) at the last harvest (105 d). Data are means ± standard errors (*n* = 4). Data were analysed by a one-way ANOVA. Different letters within a column denote significant (*P* < 0.05) differences as determined by a Bonferroni *post-hoc* test. SLA is specific leaf area (m2 kg-1). In a two-way ANOVA the interaction term between harvest time and AMF treatment was never significant for the parameters shown below with the exception of leaf DW and stem DW which were not normally distributed and thus excluded from the two-way ANOVA analysis. This indicates that differences due to AMF did not differ between the two sampling points.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | Root DW (g) | SLA(m2 kg-1) | StemDW (g) | Stem Length (cm) | LeafDW (g) | Leaf Number | Total DW (g) |
| non-AMF | 16.8 ± 4.31**b** | 12.1 ± 0.8**a** | 2.46 ±0.31**b** | 59.0 ± 5.6**b** | 4.61 ±0.30**b**  | 30 ± 8**a** | 23.9 ± 4.64**b** |
|  |  |  |  |  |  |  |  |
| *G. hoi* | 1.37 ± 0.48**a** | 19.6 ± 1.8**b** | 0.33 ±0.25**a**  | 17.0 ± 8.9**a** | 0.93 ±0.72**a** | 14 ±4**a** | 2.63 ± 1.34**a** |
|  |  |  |  |  |  |  |  |
| *G. intraradices* | 4.25 ± 0.71**a** | 14.0 ± 1.4**ab** | 0.89 ±0.10**a** | 34.8 ± 2.7**ab** | 2.54 ±0.18**a** | 19 ± 1**a** | 7.67 ± 0.93**a** |
|  |  |  |  |  |  |  |  |
| *G. mosseae* | 4.60 ± 1.09**a** | 16.6 ± 2.5**ab** | 1.18 ±0.33**a** | 35.5 ± 9.6**ab** | 2.34 ±0.50**a** | 20 ±4**a** | 8.12 ± 1.69**a** |
|  |  |  |  |  |  |  |  |
| *Gi. rosea* | 2.49 ± 0.50**a** | 20.1 ± 0.2**b** | 0.55 ±0.05**a** | 48.3 ± 3.8**b** | 1.32 ±0.08**a** | 21 ±2**a** | 4.36 ± 0.42**a** |

A

B

Fig. 1. Phosphorus content of stems (grey bars), leaves (white bars) and roots (black bars) of *P. euramericana* cuttingsafter 77 d (A) and 105 d (B). Different letters in lower case indicate significant differences among treatments within the plant parts analysed while different letters in upper case indicate significant differences in total plant phosphorus. At 77-d there was insufficient stem material for phosphorus analysis in the plants colonised by *G. hoi* and stem and total phosphorus contents from this treatment were excluded from statistical analysis. Data are means ± S.E. (*n* = 4, except at 77 d for stems of plants colonised by *G. intraradices* where *n* = 3). Note the difference in the scale on the Y-axis between the two harvest points.

Fig 2



Fig. 2. Percentage total root length colonised (%RLC; white bars) and percentage arbuscules (black bars) in the AMF treatments at 105 d. Data are means ± S.E (*n* = 4). Different letters represent significant differences among the treatments as determined by a Bonferroni *post hoc* test.

Fig. 3.

Fig. 3. Relationship between percentage root length colonisation (%RLC) and total plant P content (mg) for *G. intraradices* (filled squares), *G. hoi* (squares), *G. mosseae* (filled circles) and *Gi rosea* (circles). The overall relationship for all the fungal treatments was fitted by a significant regression (Plant P content (mg) = (-) 3.36 + 9.50 %RLC.; *P* < 0.001, *F*1,27 = 22.74, *R*2 = 45.7%). Phosphorus contents were square-root transformed and %RLC were arcsin transformed prior to statistical analysis. Data are from both harvests (77 and 105 d) and the relationship was confirmed by a partial correlation eliminating the effect of time (*r* = 0.379, *P* < 0.001), *n* = 29 (note: for the individual AMF species *n* = 8, except for *G. hoi* (*n* = 6) and *G. intraradices* (*n* = 7) as there was insufficient stem material at the first (77 d) harvest to conduct phosphorus analysis).

Fig 4

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