Title: Root type is not an important driver of mycorrhizal colonisation in *Brachypodium distachyon*

Authors: Suzanne Donn, Akitomo Kawasaki, Brendan Delroy, Vincent Chochois, Michelle Watt, Jeff R. Powell

PII: S0031-4056(17)30079-3
DOI: http://dx.doi.org/doi:10.1016/j.pedobi.2017.08.001
Reference: PEDOBI 50509

To appear in:

Received date: 31-3-2017
Revised date: 4-8-2017
Accepted date: 4-8-2017

Please cite this article as: Donn, Suzanne, Kawasaki, Akitomo, Delroy, Brendan, Chochois, Vincent, Watt, Michelle, Powell, Jeff R., Root type is not an important driver of mycorrhizal colonisation in Brachypodium distachyon.Pedobiologia - International Journal of Soil Biology http://dx.doi.org/10.1016/j.pedobi.2017.08.001

Root type is not an important driver of mycorrhizal colonisation in *Brachypodium distachyon*.

Suzanne Donn[1], Akitomo Kawasaki[2], Brendan Delroy[1], Vincent Chochois[3], Michelle Watt[4], Jeff R. Powell[1]

[1]Hawkesbury Institute for the Environment, Western Sydney University, Richmond, NSW, Australia

[2]CSIRO Agriculture and Food, Canberra, ACT, Australia

[3]ANU, Research School of Biology, 134 Linnaeus Way, Canberra, ACT, Australia

[4]Institute of Bio and Geosciences (IBG 2), Plant Sciences, Forschungszentrum Jülich GmbH, Jülich, Germany

Highlights

- Plant effects on mycorrhizal community composition were minor compared to soil effects.
- Extent of colonisation varied between *B.distachyon* accessions but not between different root types.
- Specific root length was not related to AM fungal colonisation.
- AM fungal abundance determined by qPCR correlated to percent of root with arbuscules present.
- *B. distachyon* is a useful model for studying mechanisms underlying cereal interactions with AM fungi.

Abstract

Breeding for favourable root traits in food and energy crops should be considered in the context of interactions with soil biota, notably those that can increase the nutrient use efficiency of crops. Arbuscular mycorrhizal (AM) fungi can provide services to plants but a better understanding of the interactions between root traits and AM fungi is required to maximise these benefits.

One source of intraspecific variation in root architecture is the allocation of resources to different root types. We hypothesised that different root types would have different traits and be colonised differently by AM fungi, either in the amount of colonisation or identity of colonisers. We studied communities colonising the seminal, coleoptile nodal and leaf nodal roots of seven *Brachypodium distachyon* accessions grown in three crop or pasture soils.

Leaf nodal roots had low specific root length compared to coleoptile nodal and seminal roots, yet all three root types harboured similar AM fungal communities and levels of colonisation. Most of the variation in the AM fungal communities was explained by soil; significant proportions were explained by plant accession and root type but these effects were weak.

Differential allocation of resources between root types is not a trait that could be selected to maximise beneficial interactions with AM fungi and we found no link between a root phenotypic trait (specific root length) and AM fungal colonisation. Accessions did vary in extent of colonisation by arbuscules, meaning *B. distachyon* may be a useful model to study mechanisms underlying the symbiotic interface and mycorrhizal growth response of cereals.

Keywords: seminal root; nodal root; AMF; microbial community; root architecture

1. Introduction

Increasing global food demand requires improvements to crop yields while environmental, economic and supply pressures require us to achieve these gains while also reducing fertiliser applications. There is increasing interest in targeting root traits to improve nutrient and water uptake in crops (Richards *et al.*, 2007; Lynch & Brown, 2012; Rose *et al.*, 2013), yet breeding for root traits is still usually considered in isolation from the surrounding soil biology. Given that plants can show species-specific (Burns *et al.*, 2015) and even genotype-specific (Schweitzer *et al.*, 2008; Mwafulirwa *et al.*, 2016) selection for their microbial communities, these communities may also be considered as a heritable trait of the plant and one that can feed back to other root traits (Sukumar *et al.*, 2013).

Arbuscular mycorrhizal (AM) fungi colonise the root cortex and can perform a range of services to the plant including nutrient uptake or pathogen protection (Sikes *et al.*, 2010). Intensive agricultural practices including fertilizer inputs and disruption of hyphal networks by tillage reduce both abundance and diversity of AM fungi (Oehl *et al.*, 2003; Verbruggen *et al.*, 2013). However, diverse AM fungal communities still exist in agricultural soils and have the potential to support plant growth, particularly in low-input rotation-based systems (Hijri *et al.*, 2006). While the emphasis for exploiting AM fungi in agriculture is currently placed on applying commercial AM fungal inocula (Rodriguez & Sanders, 2015), the ability of introduced microorganisms to compete and establish in the resident community is a challenge that comes with the risk of introducing exotic species or alleles to these native populations (Verbruggen *et al.*, 2013; Rodriguez & Sanders, 2015). Responsiveness of crop species to AM fungi varies between species; for example, it is typically high in maize but typically low in wheat and barley (Smith & Smith, 2011). Furthermore, within a crop species, genotypes may vary in mycorrhizal responsiveness (Boyetchko & Tewari, 1995; Singh *et al.*, 2012). By considering how the plant interacts with native AM fungal communities, we aim to identify root traits with existing variation that could be selected to best exploit the symbiosis in order to increase crop productivity.

Historically, root traits associated with dependence on, or responsiveness to, mycorrhizae include short roots or low specific root length, little branching, few or short root hairs and slow growth (Brundrett, 1991; Smith & Read, 2010). However, in a meta-analysis, Maherali (2014) demonstrated no significant relationship between mycorrhizal growth response and root : shoot ratio, root diameter, specific root length, root hair length and root hair density. Much of the recent work concerning root trait and fungal interactions have focussed on trees (Comas *et al.*, 2014; Eissenstat *et al.*, 2015; Chen *et al.*, 2016; Valverde-Barrantes *et al.*, 2016) ; we aim to determine whether root traits (such as diameter and specific root length) historically associated with mycorrhizae in the literature affect the colonisation of a grass by AM fungi.

A member of the Poaceae, *Brachypodium distachyon* is a C3 grass increasingly used as a model for closely related species of importance in food and feedstock production (Catalan *et al.*, 2014). As a model for microbial interactions, *Brachypodium* has been studied in association with both pathogenic (Fitzgerald *et al.*, 2015) and potentially beneficial (Hong *et al.*, 2012) fungi, while the associated rhizosphere bacterial community was found to be similar to that of wheat (Kawasaki *et al.*, 2016). With respect to AM fungi, mycorrhizal colonisation of *B. distachyon* was found to vary depending on AM species, ranging from 25% to 75% of root length colonised in a low phosphorus substrate (Hong *et al.*, 2012). Jakobsen *et al.* (2016) found declining colonisation (from a maximum of 60%) by *R. irregularis* with increasing phosphorus. Similarly, growth response of *B. distachyon* to mycorrhizal colonisation can be positive, neutral or negative, depending on the species of AM fungi inoculated (Hong *et al.* 2012) and the nutrient conditions (Jakobsen *et al.*, 2016).

Brachypodium distachyon shares root characteristics of economically important crops (Watt *et al.*, 2009). A single seminal root emerges from the seed (hereafter primary seminal root, PSR). The seminal root system supports the initial growth of the plant and but also persists to maturity (Hardtke & Pacheco-Villalobos, 2016). In wheat, seminal roots had a higher rate of absorption of water than nodal roots while also absorbing a more concentrated nutrient solution (Krassovsky, 1926). Approximately one week later, at the three leaf stage, a variable number of nodal roots, usually from zero to two, emerge from the coleoptile (coleoptile nodal roots, CNR) and finally a week later (at the leaf 5 stage) leaf nodal roots (LNR) emerge from the leaf base (Watt *et al.*, 2009); thus the emergence of different root types is separated in time and space. Leaf nodal roots are associated with tillers and ultimately form the bulk of the root system. In *B. distachyon* LNRs are reported to be more responsive to the environment, for example, being suppressed under drought (Chochois *et al.*, 2015) or nutrient limitation (Poire *et al.*, 2014) and increasing in number in response to a pathogen (Schneebeli *et al.*, 2015). Both seminal and nodal root systems of *B. distachyon* form functional symbioses with AM fungi (Hong *et al.*, 2012). Recently Kawasaki *et al.* (2016) reported different bacterial and, to a lesser extent (more broadly than AM) fungal communities are associated with seminal and leaf nodal roots of *B. distachyon*.

Natural variation in root systems exists in collected accessions of *B. distachyon* (Ingram *et al.*, 2012; Pacheco-Villalobos & Hardtke, 2012; Chochois *et al.*, 2015) providing an opportunity to assess how traits of mature plants correlate to colonisation by complex AM fungal communities. In particular, the allocation of resources to the three root types has been shown to vary between accessions (Chochois *et al*. 2015). Transcriptional profiles of primary, seminal and nodal (crown) roots of maize suggest these root types occupy different functional niches (Tai *et al.*, 2016), while changes in the transcriptional profile of rice roots in response to AM fungal colonisation changes with branching order (Gutjahr *et al.*, 2015). We hypothesized that observed differences in morphological and anatomical characteristics and potential differences in function of the three *B. distachyon* root types would result in different levels of mycorrhizal colonisation or in colonisation by different AM fungi. Furthermore, different allocation of resources to these root types by accessions would result in variation in colonisation between accessions. Soil factors may be as or more important than plant-associated factors to the construction of rhizosphere microbial communities (Nunan *et al.*, 2005; Rousk *et al.*, 2010; Edwards *et al.*, 2015). Thus, including different soils in our experimental design provides an opportunity to estimate the scale of root type and accession effects relative to other, ecologically important drivers. Root age is a confounding factor in the study of microbes associated with different root types, since roots emerge at different times (Watt *et al.*, 2009) and AM fungal taxa vary in the time it takes to achieve colonisation (Hart & Reader, 2002). We address this by comparing communities in different root types of equivalent age.

First, we characterised traits of the three root types present in two accessions of *B. distachyon*. We predicted that there would be trait differences between the three *B. distachyon* root types, and differences between the two accessions. In a second experiment, we grew seven accessions of *B. distachyon* in three soils and profiled the colonising AM fungal community to determine whether there were differences between soils, root types and accessions. Plant measures were recorded to test for correlations between plant traits and AM fungal communities. Finally, to separate effects of root age from root type, we performed a third experiment where seminal and nodal roots were harvested at equivalent ages and the AM fungal communities compared.

2. Material and methods

2.1 Soil and plant material

Soils were collected from the top 20cm of the soil profiles at three field sites: a sandy loam from the Hawkesbury Institute for the Environment in Richmond, NSW (referred to here as the 'Hawkesbury' soil); a loam soil from the Ginninderra Experiment Station near Canberra, ACT ('Ginninderra' soil); and a loam from near the Australian Cotton Research Institute in Narrabri, NSW ('Narrabri' soil). Ginninderra and Hawkesbury soils were collected in May 2014, just prior to the experiments. Narrabri soil had been collected in December 2013, transported to the Western Sydney University Hawkesbury campus, and stored in bags under an outdoor shelter. All soils were air-dried and passed through a 4 mm sieve to remove stones and large aggregates. Soil analyses were performed by Environment Analysis Laboratory, Southern Cross University, Lismore, NSW; soil characteristics are presented in the Supplementary materials (Table S1).

Seven *B. distachyon* accessions were selected. Bd21 is the standard accession sequenced first and Bd21-3 is the parent of a large set of transfer-DNA lines. Additional accessions were selected for their variable allocation of resources to root types (Chochois *et al.*, 2015): Bd3-1, TR2b, TR11i, TR13c and Kah1. The small stature and the accompanying small root system of *B. distachyon*, compared to its economically important relatives such as wheat and sugar cane, make root system analyses on mature plants feasible. Seeds were dehusked, surface-sterilised in 10% bleach for 5 minutes and rinsed with sterile water prior to sowing.

For Experiments 1 and 2, plants were grown in pots 9 cm (inner diameter) by 50 cm (height), made from PVC pipe. For Experiment 3 plants were grown in similar pots of 25 cm height.

2.2 Experiments

Experiment 1: Trait differences between root types in AM fungi-free soil

We first aimed to quantify differences between the primary seminal, coleoptile nodal and leaf nodal roots. *Brachypodium distachyon* accessions Bd21 and Kah1 were grown in soil free of AM fungi to quantify differences in root architecture. These two accessions were chosen because they were previously shown to allocate relatively large proportions of their root system to leaf nodal and coleoptile nodal roots respectively (Chochois *et al.*, 2015). Pots were lined with plastic bags that had drainage holes cut in the base. Gamma irradiated (50 kGy) Hawkesbury soil, supplemented with a bacterial wash from live soil, was lightly packed to a bulk density of 1.2 g cm^{-3} and gradually watered to 80% field capacity. Seven replicate pots were planted with sterilised seeds of accessions Kah1 and Bd21 and grown for 6 weeks in a glasshouse during summer with day time temperature at 24^{o}C and nights at 18^{o}C.

After four weeks photosynthesis was measured (section 2.3) and at harvest roots were carefully washed from the soil and separated by root type. Root traits were measured as described in section 2.3.

Experiment 2: Root type, accession and soil as drivers of AM fungal community

Seven *B. distachyon* accessions were grown in three field soils to assess differences in AM fungal community composition associated with accessions and root types. Preliminary experiments had shown poor growth of *B. distachyon* in Ginninderra and Narrabri soils was improved by mixing with sand. Sand was twice autoclaved for one hour at 121^{o}C, with 24 h between autoclave cycles, and each of the three soils were mixed with autoclaved sand to achieve a 50:50 dry weight / weight mix.

Pots were lightly packed with the soil sand mixes to bulk density of approximately 1.0 g cm^{-3} for Narrabri and 1.2 g cm^{-3} for Ginninderra and Hawkesbury and gradually watered over four days to 80%

of field capacity (7.4, 9.4 and 17.1% soil moisture for Hawkesbury, Ginninderra and Narrabri soil-sand mixes, respectively).

Seeds were planted at 2 cm depth, with Ginninderra, Narrabri and Hawkesbury pots seeded on consecutive days. Two seeds were sown per pot and one seedling randomly removed after emergence. Six replicates of each accession and soil treatment were included, making a total of 126 pots.

Pots were arranged in three blocks, one for each soil, on glasshouse benches; accessions were completely randomised within each soil block and pots were re-randomised weekly. Soil treatment was included as a way to establish a gradient of AM fungal communities in order to estimate the relative importance of the factors of interest (accession, root type). We do not aim to find specific soil drivers of community effects that would need to be independent of location effects in the glasshouse here. Plants were grown for seven weeks during spring, supplemented with growth lights to achieve 12 hour days at 24^{o}C and 12 hour nights at 18^{o}C. Light levels varied between 300 and 1600 µmol m^{-2} s^{-1}. Pots were watered with tap water three times per week, watering to starting weight at least once per week.

Photosynthesis was measured before harvest which was approximately seven weeks after planting. The harvest took place over the course of ten days, with pots harvested in the same order as they were planted. This resulted in plants grown in the Hawkesbury soil and harvested last were grown for one week longer than the first plants harvested from the Ginninderra soil. Pots were saturated, the soil pushed out intact and roots were carefully washed from the soil. Plant measurements are described in section 2.3 and AM fungal analyses in section 2.4.

Experiment 3: Disentangling root type and age effects

A single accession, Bd21-3, was grown in 50 Ginninderra soil : 50 sand w/w mix. Details of growth conditions, and of the bacterial and fungal communities of these plants, are described in Kawasaki et al. (2016). Briefly, 18 pots were sown with four seeds and thinned to two seedlings after emergence. Due to the timing of root emergence, leaf nodal roots harvested 44 days after sowing were equivalent in age to seminal roots harvested at 30 days. Nine replicates were harvested at each of these time points. The PSR and longest LNR were sampled, but CNRs were absent in these plants. For axile roots, 4 cm at both the base and at the tip were sampled with any attached branch roots. This yielded 72 root samples for analysis. AM fungal T-RFLP was performed for all samples as described in section 2.4.2. Tip samples from both PSR and LNR at day 30 failed to amplify with AM fungal specific primers therefore all tip samples were excluded from further analyses.

2.3 Plant trait measurements

For Experiments 1 and 2, photosynthetic rate was measured using a LI-6400 Portable Photosynthesis System (LI-COR, NE, USA), with the chamber temperature set to 25^{o}C and light at 1000 µmol m^{-2} s^{-1}. Measurements were taken on the youngest fully unfurled leaf on the main stem and adjusted for leaf area. Transpiration efficiency was calculated as photosynthetic rate / stomatal conductance and photosynthetic water use efficiency as photosynthetic rate / transpiration (Birhane *et al.*, 2012).

To assess trait differences between root types in Experiment 1, the number of roots and length of the main axes were recorded and each root type scanned separately in WinRhizo (Regent Instruments Inc., QC, Canada) to measure total root length, diameter, surface area and volume. Roots were then freeze-dried and weighed. Specific root length was calculated as root length (m) / root dry weight (g); specific surface area as surface area (cm^2) / root dry weight (g) and tissue density as root dry weight

(g) / root volume (cm^3). An estimate of the degree of branching was made by calculating the root length including laterals / main axis length for each root type.

For Experiment 2, shoots were removed, dried at 70^oC and weighed. Roots were separated into three root types (PSR, CNR and LNR), and each was divided into the roots from the top 25 cm, measured from the crown, and the next 25 cm. We expected to find different AM communities colonising the younger roots at the bottom of the root system and focus on the top section samples since these had more time to develop their AM fungal community. Remaining roots which were in contact with the bottom of the pot were dried and weighed but no AM fungal community analysis was performed on them. These root samples were freeze-dried prior to recording dry weight. Due to the large size of root systems at harvest, scanning for root traits was not feasible, however, a subset of three plants per accession grown in Hawkesbury soil were scanned for total root length and root length density was estimated from these.

2.4 AM fungal colonisation and community analyses

Freeze dried roots from Experiment 2 were cut into 30–50 mm lengths and mixed well. Subsamples of root were used for scoring presence of fungal structures and DNA extraction. While there is some debate on the appropriateness of freeze drying for sample preservation (Bainard *et al.*, 2010; Janoušková *et al.*, 2015), recent studies have reported little effect of this sample preservation method on community analyses of fungi (Castaño *et al.*, 2016) including AM fungi (Weißbecker *et al.*, 2017). Freeze drying allowed roots to be processed quickly at harvest and the consistent handling of samples allows for comparison between them.

2.4.1 Microscopy

Percentage colonization of roots in Experiments 1 and 2 was assessed by ink-vinegar staining (Vierheilig *et al.*, 1998). Briefly, roots were rehydrated in water overnight and then cleared in 10% KOH for 10 minutes in a 90^{o}C water bath, washed in acidified water and stained in 10% ink in 10% acetic acid for 10 minutes at 90^{o}C. Roots were de-stained in lactoglycerol overnight before being mounted on slides. Ten root segments of approximately 25 mm were arranged on a slide and 50 points on the slide scored at x200 for the presence of arbuscules, vesicles, spores, AM hyphae, and hyphae belonging to other fungi. For Experiment 1 LNRs were scored for all pots to check for contamination by AM fungi. For Experiment 2 scoring was performed for LNRs collected from the top 25 cm of all root systems; in addition, CNRs and PSRs of accession Kah1 were scored for each of the three soils. Colonisation was not observed to vary between root types of Kah1, therefore only LNRs were scored for the remaining accessions. This was the dominant root type across soils and accessions. A subset of roots from the bottom 25 cm of the pot was scored for 20 samples; there was no significant difference in colonisation from the top section and so for the remaining roots, only the top section was scored for colonisation.

2.4.2 Terminal restriction fragment length polymorphism (T-RFLP) analysis of AM fungal community composition

For Experiment 2, DNA was extracted from root subsamples taken from each of the three root types (PSR, CNR and LNR) at the top 25 cm of each root system, including axial roots and laterals. A subset of 20 samples from the next 25 cm root length across the three soils was also extracted. A 10 mg subsample of freeze-dried root was first milled with a 4mm steel ball in a tissue lyser (Qiagen, Hilden, Germany) for 90 seconds.

For Experiment 3, samples were ground in liquid nitrogen with a mortar and pestle before DNA extraction.

DNA was extracted using a Powersoil DNA extract kit (MOBio, CA, USA) and T-RFLP performed for all root samples. A 10 ng subsample of DNA was used as template to first selectively amplify AM fungal sequences, and then amplify the ITS1 region for analysis (Horn *et al.*, 2017). This method requires three rounds of PCR and such a large number of amplification cycles could potentially distort the perceived fungal community composition; however, we selected the AM fungal primers to achieve the greatest possible coverage of the phylum while maintaining species level resolution (Krüger *et al.*, 2009) and base our subsequent community analyses on the presence or absence of taxa, rather than relative abundance for this reason. A nested reaction was performed with 400 nM of primers SSUmAf and SSUmAr for round 1 and SSUmCf and SSUmBr for round 2 (Krüger *et al.*, 2009). Reactions of 10 µl were performed with MyTaq (Bioline, NSW, Australia) supplemented with 400 ng µl^{-1} BSA. First round PCR conditions were: 1 min at 95^{o}C; 40 cycles of 30 s denaturation at 95^{o}C, 30 s annealing at 60^{o}C and 1 min extension at 72^{o}C. First round products were diluted to 1 in 100 and 1 µl used as template in the second PCR. For the nested primers, only 30 cycles were performed at 58^{o}C annealing.

Second round products were used as template in a third round of PCR to amplify the ribosomal ITS region with a FAM labelled forward primer (ITS1F)(Gardes & Bruns, 1993) and reverse primer (ITS4)(White *et al.*, 1990). Where faint agarose gel bands were detected, second round products were used undiluted and products yielding strong bands were diluted to 1 in 20 before amplification. PCR conditions were as above but with 30 cycles at 55^{o}C annealing.

FAM-labelled ITS products were purified using the AMPure PCR purification system (Beckman Coulter, CA, USA) and digested for 3 hours with *Taq*I (New England Biolabs, MA, USA), after which 20 ng of

digested DNA was separated on a 3500 Genetic Analyzer (Applied Biosystems, CA, USA) alongside LIZ600 size standard. Raw data was exported from Genemapper (Applied Biosystems, CA, USA), thresholded, peaks aligned and the percentage composition of operational taxonomic units (OTUs) by sample calculated as described in Bissett (2010). There was no significant difference between communities profiled from roots from the top and bottom halves of the pots; therefore, results are reported from samples taken from the top 25 cm only.

The peak area over a two base pair region of the T-RFLP profiles accounted for more than 80 % of the total peak area in all Narrabri samples; in order to identify this dominant AM fungal taxa, and those colonising *B. distachyon* roots grown in the other soils, we performed sequencing on a subset of samples. From each soil, we selected samples from each root type that covered the range of OTUs found in the TRFLP profiles. DNA from three samples from each root type per soil was mixed (9 mixed DNA samples in total), AM fungal sequences were amplified using the nested primers as above and the ITS2 region sequenced by Illumina MiSeq at the Ramaciotti Centre for Genomics, NSW, Australia using primers fITS7 and ITS4. The aim of sequencing was not to perform in depth community analyses, but to identify the taxa colonising *B. distachyon* in these field soils.

2.4.3 Relative abundance of AM fungi by qPCR

Due to the large number of samples generated by dividing root systems into three root types and studying several accessions, qPCR provided an efficient alternative to microscopy for comparing colonisation levels. Sequencing revealed that roots grown in Narrabri soil were dominated by *Funneliformis mosseae*, which accounted for over 90% of the sequences in all but one sample. To test whether the abundance of *F. mosseae* differed between root types and accessions in the Narrabri soil, we performed qPCR using primers and probe specific for *F. mosseae* (Thonar *et al.*, 2012). Reactions were run in triplicate for each root DNA sample on a Cfx 96 cycler (Bio-Rad, CA, USA) along with a standard curve and no template controls on each plate. Reactions (15 µl volume) contained IQ mix (Bio-Rad, CA, USA), 0.45 nM primers, 0.175 nM probe, 400 ng μl^{-1} BSA and 2 µl DNA extract. Standard curves were a 10 fold dilution series of purified PCR products from $1x10^8$ copies to $1x10^2$ copies and efficiency ranged from 81.5% to 89.4% between runs with R^2 of 0.998 or 0.999. Due to the difference between template for the standard curve (PCR product) and samples (genomic DNA), absolute quantification of *F. mosseae* is not accurate and abundances are used only to compare between root types and accessions. Copy number in samples was normalised by DNA concentration to copies per ng root DNA.

2.5 Statistical analyses

Analyses were conducted in R version 3.3.1 (R Core Team, 2016) unless stated otherwise. For Experiment 1, root traits were analysed by two-way analysis of variance (ANOVA) using the 'Anova' function from the 'car' package (Fox & Weisberg, 2011), with accession (Bd21, Kah1) and root type (PSR, CNR, LNR) as factors. Significant differences between factor levels were determined by Tukey's HSD.

For Experiment 2, plant traits (dry weight shoot, dry weight PSR, dry weight CNR, dry weight LNR, root shoot ratio, proportion of roots PSR, proportion of roots CNR, proportion of roots LNR, photosynthetic rate, stomatal conductance, transpiration efficiency and photosynthetic water use efficiency) were analysed in a Euclidean distance matrix and visualised by MDS in PRIMER version 6 (Clarke & Gorley, 2006). Root weight data were first log-transformed. Homogeneity of dispersions was tested using the PERMDISP routine in PERMANOVA+ (Anderson *et al.*, 2008). Significant differences in plant traits between factors (accession, soil, soil x accession) were tested by permutational multivariate ANOVA (PERMANOVA) in PERMANOVA+ with Type I sums of squares. AM fungal community T-RFLP data were analysed in a Jaccard distance matrix (analysing presence or absence of OTUs) and visualised by MDS. Main effects of root type, accession and soil on fungal community were assessed by PERMANOVA using the "adonis" function in the 'vegan' package (Oksanen *et al.*, 2016). Pairwise distances between samples belonging to the same or different root types, accessions and soils were visualised in violin plots constructed using the 'ggplot2' package (Wickham, 2009).

Colonisation by AM structures of the three root types in Kah1 plants grown in Hawkesbury soil was compared by ANOVA, as described above. Colonisation of LNRs of all plants was compared by two-way ANOVA with soil and accession as factors. Significance of relationships between qPCR and microscopy data were tested after calculating Pearson correlations. Within soils, Pearson correlations between plant traits (dry weight shoot, dry weight root, photosynthetic rate, stomatal conductance) and percent colonisation by active AM fungal structures (arbuscules, vesicles and hyphae combined), arbuscules only or no colonisation were calculated and *P* values adjusted for multiple testing using the Benjamini-Hochberg false discovery rate.

In Experiment 3, pairwise Jaccard distances between samples within and between factors were visualised in violin plots and comparisons made by linear modelling using the 'lm' function in R.

3. Results

3.1 Experiment 1: Trait differences between root types in AM fungi-free soil

Six weeks after sowing, the longest LNRs of both Bd21 and Kah1 plants were beginning to reach the bottom of the pots. Bd21 plants had 1 seminal root, 1 CNR and 18-27 LNRs. Kah1 plants had 1 seminal root, 0-2 CNRs, and 13-27 LNRs.

A large proportion of the root length at harvest was allocated to LNRs (Table 1, Supplementary material Fig. S1) but there was no significant difference in the mean root length allocated to PSR and CNR, though CNRs were absent from some plants. Total root length was significantly higher in Bd21 compared to Kah1, due to increased root length of LNRs (Supplementary material Fig. S1). None of the other root traits measured differed significantly between accessions.

All three root types were extensively branched with approximately ten times the main axis length in branch roots (Table 1). The average LNR diameter tended to be greater than that for PSR and CNR, but this was marginally nonsignificant (*P*=0.10). Both SRL and SSA were significantly lower in LNRs compared to CNR and PSR, while tissue density was greater in LNRs.

Low levels of AM fungal contamination were observed in 4 of the 14 pots with between 1 and 5 % of the root length colonised in these pots; all other roots were free of hyphae.

3.2 Experiment 2: Root type, accession and soil as drivers of AM fungal community

Germination of Bd21, Bd21-3 and Bd3-1 was poor in the Hawkesbury soil with a single plant of accessions Bd21 and Bd21-3 surviving to harvest and only 3 plants of Bd3-1. Germination of Bd21 was also poor in the Narrabri soil (1 plant harvested). Accession TR11i grew poorly in Ginninderra soil with only 3 of 6 plants harvested and one of these was very small.

Bd21, Bd21-3 and Bd3-1 plants had begun flowering in all 3 soils at the time of harvest; with the exception of one TR11i (Narrabri soil), two TR13c (Hawkesbury soil) and one TR2b (Hawkesbury soil) all other plants were growing vegetatively at harvest.

3.2.1 Plant traits

Plant traits varied significantly with the soil they were grown in (PERMANOVA Pseudo-$F_{2,93}$ = 24.13, *P*=0.001, Fig. 1a). Dry weight of roots, root shoot ratio and photosynthetic rate were higher in plants grown in Hawkesbury soil (Fig. 1a). Plants grown in Hawkesbury soil show relatively little variation in the measured traits as compared to those in the other two soils (PERMDISP $F_{2, 91}$ = 12.35 *P*=0.001 Fig. 1a), these plants were characterized by large LNR systems and lower photosynthetic water use efficiency.

Plant traits differed significantly between accessions (PERMANOVA Pseudo-$F_{6,93}$ = 3.55, *P*=0.001) with this accession effect also depending on interactions with soil (PERMANOVA Pseudo-$F_{11,93}$ = 2.34, *P*=0.007, Supplementary material Fig. S2).

A subset of root systems grown in Hawkesbury soil were scanned for total root length in order to estimate root length density. RLD for Bd3-1 was lower than for other accessions with an average of 2.4 cm cm^{-3} (+/- 1.5), the average RLD across all other accessions was 6.6 cm cm^{-3} (+/- 1.9).

3.2.2 AM fungal community

AM fungal communities determined by T-RFLP were not significantly different between roots sampled from the top 25cm and 25-50 cm depth. The remaining analyses focus only on samples from the top 25 cm of the root systems. Communities were significantly different between plants grown in the three soils (PERMANOVA $F_{2, 161}$ = 61.412, *P*=0.001, explaining 42 % of the variation in AM fungal community Fig. 1(b)). Communities associated with roots grown in different soils also differed in dispersion, with communities in the Hawkesbury soil more similar to each other than those within Narrabri and Ginninderra soils (PERMDISP $F_{2, 163}$ = 20.622 P=0.001, Fig. 1 (b)). Roots grown in Narrabri soil were dominated by *Funneliformis* spp., Hawkesbury soil by *Paraglomus* spp. and Ginninderra by unidentified species of Paraglomerales and *Rhizophagus* (Table S3).

Accession (Supplementary material Fig. S3) and root type effects on AM fungal communities were significant but minor compared to the soil effect (accession: $F_{6,161}$ = 2.35, *P* = 0.002, explaining 4.8 % of the variation; root type $F_{2,161}$ = 2.81, *P* = 0.008, explaining 1.9% of the variation). Communities were only observed to be appreciably more different between root types than within root types when comparisons are made within an individual pot (*i.e.*, on the same plant; Fig. 2(a)). No difference was observed when comparing communities associated with the same or different accessions of *B. distachyon* (Fig. 2(b i) compared to 2(b iii)), but comparing communities associated with roots grown in different soils resulted in greater differences than when comparing those grown in the same soil (Fig. 2(b i) compared to 2(b iv)).

We noticed there was some clustering of AM fungal communities based on the geographic origin of the accession with which they were associated, with communities of accessions from Iraq (Bd21, Bd21-3 and Bd3-1), western Turkey (TR2b, TR11i and TR13c) and eastern Turkey (Kah1) clustering (Supplementary material Fig. S3).

3.2.3 Quantification of AM fungi in different root types

All three root types of Kah1 plants grown in Hawkesbury soil were assessed by microscopy for AM fungal colonisation. There was no significant difference in the colonisation of the different root types by any AM fungal structures (ANOVA $F_{2,12}$ = 0.39, Fig. 3a). For the remaining accessions and soils colonisation was only quantified in the LNRs (Fig. 3b).

Active AM structures (including arbuscules, vesicles and hyphae) were most abundant in LNRs grown in Hawkesbury soil (ANOVA $F_{2,74}$ = 51.88 P<0.001, Fig. 3b). We observed a significant soil by accession interaction for both the percentage root length colonised by combined active AM structures ($F_{12,74}$ = 2.41 P = 0.01) and by arbuscules ($F_{12,74}$ = 2.41 P = 0.01). This was mainly driven by relatively low colonisation of accession TR2b in the Hawkesbury and Narrabri soils (Supplementary material Fig. S4). Spores were present in roots grown in Ginninderra and Narrabri soils but were absent from Hawkesbury roots (Fig. 3b).

rDNA copy number of the most abundant species sequenced from roots grown in Narrabri soil, *F. mosseae*, was assessed by qPCR. Abundance of *F. mosseae* rDNA gene copies in the PSR was correlated to that in nodal roots (both coleoptile and leaf; Fig. 4(a)). The percentage colonization of the LNRs by arbuscular structures (confirmed by microscopy) was positively correlated with the *F. mosseae* rDNA copy number (Fig. 4(b)). We did not observe a correlation between hyphae and copy number (Fig. 4(c)), but we observed a negative correlation between copy number and the percentage of points scored as spores (Fig. 4(d)). In the Narrabri soil, vesicles were only observed in one sample. We did not observe a significant effect of accession on the abundance of *F. mosseae* rDNA number in LNR (one-way ANOVA $F_{6,28}$ = 0.92).

3.2.4 Correlation of plant and fungal data

We did not observe a significant correlation between plant traits and the relative abundance of AM fungal structures in LNRs grown in Ginninderra or Hawkesbury soil (Supplementary material Table S2). In Narrabri soil, dry weight of shoots was positively correlated with both the percentage of points scored as arbuscules (r = 0.51, P = 0.046) and *F. mosseae* rDNA copy number (r = 0.44, P = 0.063).

3.3 Experiment 3: Disentangling root type and age effects

To further investigate the small but significant effect of root type on AM fungal community composition, we analysed communities sampled from the base of the PSR and LNR at 30 days and 44 days after planting. At day 30, the PSR is approximately 21 days old and the LNR is approximately 7 days old, while at day 44 the PSR is 35 days old and the LNR 21 days old. Thus, the PSR sampled at day 30 and the LNR at day 44 are comparable in terms of root age. Communities sampled from the PSR of the same age were no more similar than those of different ages (Fig. 5a) and the same was true of LNR (Fig. 5b). PSR and LNR communities from the same pot (sampled when the roots were different ages) were more similar to each other than communities sampled from PSR and LNRs from different pots (Fig. 5c). Different root types sampled from the same pot were also more similar than roots of the same type and same age sampled from different pots (Fig. 5c compared to 5a and 5b).

In these younger roots there was no significant effect of root type on AM fungal community, as previously observed in Experiment 2. During the two weeks between sampling events, there was no significant change in AM community in the PSR or LNR.

4. Discussion

We had expected that different form and function of root types of *B. distachyon* would result in different colonisation by AM fungi, either in species composition, or extent of colonisation. Root type did explain a significant proportion of the variation in AM fungal community composition (1.9%) but this was small compared to soil (42%) and accession (4.8%). Contrary to our prediction, there was no difference in the extent of colonisation of different root types.

Previous studies have reported variation of AM fungal colonisation within a single root system depending on root age (Hepper, 1985; Amijee *et al.*, 1993) and branching order (Yano *et al.*, 1996; Gutjahr *et al.*, 2009; Valverde-Barrantes *et al.*, 2016). Colonisation of different branching orders varies between plant taxa; for legumes (Yano *et al.*, 1996) and rice (Gutjahr *et al.*, 2009) relatively low colonisation is reported in the finest orders of branch roots, while in trees the converse was true (Valverde-Barrantes *et al.*, 2016). Within a single branching order, roots of AM trees with different morphological traits and proposed functions vary in colonisation (Zadworny & Eissenstat, 2011). Therefore, the consistent mycorrhizal colonisation we found, in terms of both abundance and composition, across root types was surprising given the differences we observed between root types in tissue density and specific root length, hypothesized different roles that root types play in terms of nutrient and water uptake and transport and the transcriptional differences seen between different root types of species related to *Brachypodium*. Watt *et al.* (2009) identified 7 types of branch root ranging from one to seven vascular xylem tracheary elements surrounded by layers of cortical cells, all of which occur across the three root types and orders of branching in *B. distachyon*. Both Gutjahr *et al.* (2009) and Valverde–Barrantes *et al.* (2016) cite the cortical cel l space as the trait most important to the degree of colonisation. The conserved cellular structure across *B. distachyon* root types and branching orders is in contrast to plant species in previous studies (Gutjahr *et al.*, 2009; Zadworny & Eissenstat, 2011) and may account for the lack of differences in AM fungal colonisation we observed. In addition, the high root length density in Experiment 2 likely would have led to substantial overlapping in the rhizosphere zones of the different root types, contributing to horizontal transfer of AM fungi between root types.

Despite the conserved structure across *B. distachyon* root types we found differences in the traits of the leaf nodal roots compared to the coleoptile nodal and seminal roots. In particular, LNRs had relatively low specific root length and high tissue density. The high specific root length in the earlier emerging PSR and CNR may allow roots to maximise water and nutrient uptake in the early growth stages. Although low specific root length is a trait that has been linked with increased mycorrhizal dependence, LNRs did not support higher colonisation by AM fungi. This result is in line with recent work regarding the association of root traits and AM fungi. In a meta-analysis Maherali (2014) demonstrated a lack of relationship between several root traits and mycorrhizal growth response; while specific root length tended to be negatively related to response in trees (though not significant), the single study measuring specific root length in grass showed no significant correlation with mycorrhizal growth response. Similarly Koziol and Bever (2015) found mean root tips per unit mass had no significant effect on mycorrhizal growth response and Stanescu and Maherali (2017) found root diameter was not related to mycorrhizal growth response. In contrast Cortois *et al.* (2016) found that specific root length was significantly correlated to percent colonisation by AM fungi, with increased colonisation resulting in positive plant growth response.

One limitation of our study was that root systems were large and complex by harvest, limiting our ability to get good root trait data from the same plants that we analysed communities from in Experiment 2. There is a tradeoff when analysing fibrous root systems of grasses between studying associations beyond the seedling stage and having a manageable root system for analysis of traits. The time that different AM fungal species take to colonise roots, ranging from 1 to 8 weeks (Hart & Reader, 2002) necessitates a relatively long study time when considering field populations. Nevertheless, plants grown under similar greenhouse conditions (experiment 1) exhibited clear differences in specific root length between different root classes which was not reflected in the colonisation by AM fungi.

A small amount of the variation across AM fungal communities was explained by plant accession, with AM fungal communities clustering by geographic origin of the accession they were associated with. Although it was not possible to determine the basis of these differences, it is notable that flowering times for these groupings vary with Iraq lines flowering relatively quickly (50-60 days), W. Turkey lines in 70-90 days and the E. Turkey accession in 98 days (Schwartz *et al.*, 2010). Therefore, subtle differences in the AM community composition could potentially be linked to plant development.

There was also a significant interaction between soil and accession for the percentage of root colonised by arbuscules. Notably accession Bd3-1 was relatively highly colonised in both Narrabri and Hawkesbury soils, with arbuscule abundance of TR2b relatively low in both these soils. In focussing on how plant traits affect fungal colonisation, we did not quantify mycorrhizal benefits by comparing plant growth to an uncolonised control. Whether the differences in colonisation rate we observed are reflected in mycorrhizal growth responses is yet to be determined. A recently reported genome wide association study of 94 wheat genotypes and AM root colonisation highlighted orthologous *Brachypodium* genes with functions including sugar accumulation and defence (Lehnert et al. 2017). These are potential targets for resolving the differential colonisation extent of *B. distachyon* accessions that we observed.

We used qPCR to increase the throughput of assessing colonisation of roots which sequencing revealed were primarily colonised by a single species. The high number of PCR cycles we used in sequencing the AM fungal community may have distorted the true community composition, thus the apparent dominance of one species may be an artefact. In addition, Janoušková *et al*. (2015) note that dried root may yield unreliable results for quantification of AM fungi by qPCR. Nevertheless, arbuscule abundance and *F. mosseae* copy number were positively correlated though we found no correlation between abundance by qPCR and the scoring of hyphae or of total percent root length colonised by AM fungi. In this study, arbuscules in *B. distachyon* roots tended to occur in high density and nuclei have been observed in both the large and fine branches of arbuscules as well as in intercellular hyphae (Bianciotto *et al.*, 1995) possibly explaining the correlation. Gamper *et al.* (2008) previously found abundance by qPCR did not reflect extraradical hyphal length determined by microscopy and although they found a positive correlation between spore count and qPCR copy number abundance, we did not. A possible explanation is that the DNA extraction methods used here did not adequately disrupt spores. The ability to use qPCR for quantification of colonisation by AM fungi could increase sample throughput and reduce the subjective nature of scoring. Homogenisation of whole root samples prior to DNA extraction would also reduce sampling error associated with scoring a relatively small proportion of the root system. The method requires further calibration and validation against visually examined roots, and careful consideration of sample processing (Janoušková *et al.*, 2015), but our results are promising in well colonised roots with abundant arbuscules.

5. Conclusions

The approach taken in previous studies correlating root traits to mycorrhizal colonisation or growth response has been to compare different plant species with conflicting results (Sikes *et al.*, 2009; Koziol & Bever, 2015; Cortois *et al.*, 2016; Unger *et al.*, 2016; Stanescu & Maherali, 2017; Unger *et al.*, 2017). Plant species differ in many ways in addition to their root architecture, for example, molecular signalling between plant and microbe, nutritional requirements and life history strategies. These effects are difficult to disentangle and the use of multiple accessions of the same species allows much closer comparison, but there are still differences in addition to root phentoypic traits. Here we compared colonisation of roots with different traits within an individual plant, revealing roots with different specific root lengths are colonised to a similar extent by AM fungi and by largely similar communities. While not a comprehensive analysis of root traits, since many traits were not significantly different between the root classes analysed, we have demonstrated that specific root length was not an important determinant of AM colonisation in *B. distachyon*. Despite the lack of root type effects, differences in the level of AM fungal colonisation of *B. distachyon* accessions mean this may be a useful model to study mechanistic aspects of the cereal – AM fungal symbiosis.

Acknowledgements

This study was funded by an Australian Research Council grant DP140103936 to JRP. The authors are grateful to Bethanie Coleman, Christian Dransfield and Rhiannon Wright for assistance in maintaining and harvesting the experiments and to Alyssa Magallanes for assistance in the molecular laboratory. We thank the Hawkesbury Institute for the Environment fungi lab group and anonymous reviewers for comments that improved this manuscript.

Appendix A. Supplementary data

References

Amijee BF, Stribley DP, Lane PW. 1993. The susceptibility of roots to infection by an arbuscular mycorrhizal fungus in relation to age and phosphorus supply. *New Phytologist* **125**(3): 581-586.

Anderson M, Gorley R, Clarke K. 2008. *PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods.* Plymouth: PRIMER-E.

Bainard LD, Klironomos JN, Hart MM. 2010. Differential effect of sample preservation methods on plant and arbuscular mycorrhizal fungal DNA. *Journal of Microbiological Methods* **82**(2): 124-130.

Bianciotto V, Barbiero G, Bonfante P. 1995. Analysis of the cell cycle in an arbuscular mycorrhizal fungus by flow cytometry and bromodeoxyuridine labelling. *Protoplasma* **188**(3): 161-169.

Birhane E, Sterck FJ, Fetene M, Bongers F, Kuyper TW. 2012. Arbuscular mycorrhizal fungi enhance photosynthesis, water use efficiency, and growth of frankincense seedlings under pulsed water availability conditions. *Oecologia* **169**(4): 895-904.

Bissett A, Richardson AE, Baker G, Wakelin S, Thrall PH. 2010. Life history determines biogeographical patterns of soil bacterial communities over multiple spatial scales. *Molecular Ecology* **19**(19): 4315-4327.

Boyetchko SM, Tewari JP. 1995. Susceptibility of barley cultivars to vesicular-arbuscular mycorrhizal fungi. *Canadian Journal of Plant Science* **75**(1): 269-275.

Brundrett M. 1991. Mycorrhizas in natural ecosystems. In: Macfayden; A, Begon; M, Fitter A eds. *Advances in Ecological Research*. London: Academic Press, 171-313.

Burns JH, Anacker BL, Strauss SY, Burke DJ. 2015. Soil microbial community variation correlates most strongly with plant species identity, followed by soil chemistry, spatial location and plant genus. *AoB PLANTS* **7**: plv030.

Castaño C, Parladé J, Pera J, Martinez de Aragón J, Alday JG, Bonet JA. 2016. Soil drying procedure affects the DNA quantification of Lactarius vinosus but does not change the fungal community composition. *Mycorrhiza* **26**(8): 799-808.

Catalan P, Chalhoub B, Chochois V, Garvin DF, Hasterok R, Manzaneda AJ, Mur LAJ, Pecchioni N, Rasmussen SK, Vogel JP, et al. 2014. Update on the genomics and basic biology of Brachypodium. International Brachypodium Initiative (IBI). *Trends in Plant Science* **19**(7): 414-418.

Chen WL, Koide RT, Adams TS, DeForest JL, Cheng L, Eissenstat DM. 2016. Root morphology and mycorrhizal symbioses together shape nutrient foraging strategies of temperate trees. *Proceedings of the National Academy of Sciences of the United States of America* **113**(31): 8741-8746.

Chochois V, Vogel JP, Rebetzke GJ, Watt M. 2015. Variation in Adult Plant Phenotypes and Partitioning among Seed and Stem-Borne Roots across *Brachypodium distachyon* Accessions to Exploit in Breeding Cereals for Well-Watered and Drought Environments. *Plant Physiology* **168**(3): 953-967.

Clarke K, Gorley R. 2006. *PRIMER v6: User Manual.* Plymouth: PRIMER-E.

Comas LH, Callahan HS, Midford PE. 2014. Patterns in root traits of woody species hosting arbuscular and ectomycorrhizas: implications for the evolution of belowground strategies. *Ecology and Evolution* **4**(15): 2979-2990.

Cortois R, Schroder-Georgi T, Weigelt A, van der Putten WH, De Deyn GB. 2016. Plant-soil feedbacks: role of plant functional group and plant traits. *Journal of Ecology* **104**(6): 1608-1617.

Edwards J, Johnson C, Santos-Medellín C, Lurie E, Podishetty NK, Bhatnagar S, Eisen JA, Sundaresan V. 2015. Structure, variation, and assembly of the root-associated microbiomes of rice. *Proceedings of the National Academy of Sciences* **112**(8): E911-E920.

Eissenstat DM, Kucharski JM, Zadworny M, Adams TS, Koide RT. 2015. Linking root traits to nutrient foraging in arbuscular mycorrhizal trees in a temperate forest. *New Phytologist* **208**(1): 114-124.

Fitzgerald TL, Powell JJ, Schneebeli K, Hsia MM, Gardiner DM, Bragg JN, McIntyre CL, Manners JM, Ayliffe M, Watt M, et al. 2015. Brachypodium as an emerging model for cereal-pathogen interactions. *Annals of Botany* **115**(5): 717-731.

Fox J, Weisberg S. 2011. An R Companion to Applied Regression. Thousand Oaks, CA: Sage.

Gamper HA, Young JPW, Jones DL, Hodge A. 2008. Real-time PCR and microscopy: Are the two methods measuring the same unit of arbuscular mycorrhizal fungal abundance? *Fungal Genetics and Biology* **45**(5): 581-596.

Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. *Molecular Ecology* **2**(2): 113-118.

Gutjahr C, Casieri L, Paszkowski U. 2009. Glomus intraradices induces changes in root system architecture of rice independently of common symbiosis signaling. *New Phytologist* **182**(4): 829-837.

Gutjahr C, Sawers RJH, Marti G, Andres-Hernandez L, Yang SY, Casieri L, Angliker H, Oakeley EJ, Wolfender JL, Abreu-Goodger C, et al. 2015. Transcriptome diversity among rice root types during asymbiosis and interaction with arbuscular mycorrhizal fungi. *Proceedings of the National Academy of Sciences of the United States of America* **112**(21): 6754-6759.

Hardtke CS, Pacheco-Villalobos D. 2016. The *Brachypodium distachyon* Root System: A Tractable Model to Investigate Grass Roots. In: Vogel JP ed. *Genetics and Genomics of Brachypodium*. Cham: Springer International Publishing, 245-258.

Hart MM, Reader RJ. 2002. Taxonomic basis for variation in the colonization strategy of arbuscular mycorrhizal fungi. *New Phytologist* **153**(2): 335-344.

Hepper CM. 1985. INFLUENCE OF AGE OF ROOTS ON THE PATTERN OF VESICULAR-ARBUSCULAR MYCORRHIZAL INFECTION IN LEEK AND CLOVER. *New Phytologist* **101**(4): 685-693.

Hijri I, Sykorova Z, Oehl F, Ineichen K, Mader P, Wiemken A, Redecker D. 2006. Communities of arbuscular mycorrhizal fungi in arable soils are not necessarily low in diversity. *Molecular Ecology* **15**(8): 2277-2289.

Hong JJ, Park Y-S, Bravo A, Bhattarai KK, Daniels DA, Harrison MJ. 2012. Diversity of morphology and function in arbuscular mycorrhizal symbioses in Brachypodium distachyon. *Planta* **236**(3): 851-865.

Horn S, Hempel S, Verbruggen E, Rillig MC, Caruso T. 2017. Linking the community structure of arbuscular mycorrhizal fungi and plants: a story of interdependence[quest]. *ISME J* **11**(6): 1400-1411.

Ingram PA, Zhu JM, Shariff A, Davis IW, Benfey PN, Elich T. 2012. High-throughput imaging and analysis of root system architecture in Brachypodium distachyon under differential nutrient availability. *Philosophical Transactions of the Royal Society B-Biological Sciences* **367**(1595): 1559-1569.

Jakobsen I, Smith SE, Smith FA, Watts-Williams SJ, Clausen SS, Gronlund M. 2016. Plant growth responses to elevated atmospheric CO2 are increased by phosphorus sufficiency but not by arbuscular mycorrhizas. *Journal of Experimental Botany* **67**(21): 6173-6186.

Janoušková M, Püschel D, Hujslová M, Slavíková R, Jansa J. 2015. Quantification of arbuscular mycorrhizal fungal DNA in roots: how important is material preservation? *Mycorrhiza* **25**(3): 205-214.

Kawasaki A, Donn S, Ryan PR, Mathesius U, Devilla R, Jones A, Watt M. 2016. Microbiome and Exudates of the Root and Rhizosphere of *Brachypodium distachyon*, a Model for Wheat. *Plos One* **11**(10): e0164533.

Koziol L, Bever JD. 2015. Mycorrhizal response trades off with plant growth rate and increases with plant successional status. *Ecology* **96**(7): 1768-1774.

Krassovsky I. 1926. Physiological activity of the seminal and nodal roots of crop plants. *Soil Science* **21**(4): 307-325.

Krüger M, Stockinger H, Kruger C, Schussler A. 2009. DNA-based species level detection of Glomeromycota: one PCR primer set for all arbuscular mycorrhizal fungi. *New Phytologist* **183**(1): 212-223.

Lynch JP, Brown KM. 2012. New roots for agriculture: exploiting the root phenome. *Philosophical Transactions of the Royal Society B* **367**(1595): 1598-1604.

Maherali H. 2014. Is there an association between root architecture and mycorrhizal growth response? *New Phytologist* **204**(1): 192-200.

Mwafulirwa L, Baggs EM, Russell J, George T, Morley N, Sim A, Canto CD, Paterson E. 2016. Barley genotype influences stabilization of rhizodeposition-derived C and soil organic matter mineralization. *Soil Biology & Biochemistry* **95**: 60-69.

Nunan N, Daniell TJ, Singh BK, Papert A, McNicol JW, Prosser JI. 2005. Links between plant and rhizoplane bacterial communities in grassland soils, characterized using molecular techniques. *Applied and Environmental Microbiology* **71**(11): 6784-6792.

Oehl F, Sieverding E, Ineichen K, Mader P, Boller T, Wiemken A. 2003. Impact of land use intensity on the species diversity of arbuscular mycorrhizal fungi in agroecosystems of Central Europe. *Applied and Environmental Microbiology* **69**(5): 2816-2824.

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, et al. 2016. vegan: Community Ecology Package. R package version 2.4-1.

Pacheco-Villalobos D, Hardtke CS. 2012. Natural genetic variation of root system architecture from Arabidopsis to Brachypodium: towards adaptive value. *Philosophical Transactions of the Royal Society B-Biological Sciences* **367**(1595): 1552-1558.

Poire R, Chochois V, Sirault XRR, Vogel JP, Watt M, Furbank RT. 2014. Digital imaging approaches for phenotyping whole plant nitrogen and phosphorus response in *Brachypodium distachyon*. *Journal of Integrative Plant Biology* **56**(8): 781-796.

R Core Team. 2016. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Richards RA, Watt M, Rebetzke GJ. 2007. Physiological traits and cereal germplasm for sustainable agricultural systems. *Euphytica* **154**(3): 409-425.

Rodriguez A, Sanders IR. 2015. The role of community and population ecology in applying mycorrhizal fungi for improved food security. *The ISME Journal* **9**(5): 1053-1061.

Rose TJ, Impa SM, Rose MT, Pariasca-Tanaka J, Mori A, Heuer S, Johnson-Beebout SE, Wissuwa M. 2013. Enhancing phosphorus and zinc acquisition efficiency in rice: a critical review of root traits and their potential utility in rice breeding. *Annals of Botany* **112**(2): 331-345.

Rousk J, Baath E, Brookes PC, Lauber CL, Lozupone C, Caporaso JG, Knight R, Fierer N. 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. *The ISME Journal* **4**(10): 1340-1351.

Schneebeli K, Mathesius U, Watt M. 2015. *Brachypodium distachyo*n is a pathosystem model for the study of the wheat disease rhizoctonia root rot. *Plant Pathology* **64**(1): 91-100.

Schwartz CJ, Doyle MR, Manzaneda AJ, Rey PJ, Mitchell-Olds T, Amasino RM. 2010. Natural Variation of Flowering Time and Vernalization Responsiveness in *Brachypodium distachyon*. *Bioenergy Research* **3**(1): 38-46.

Schweitzer JA, Bailey JK, Fischer DG, Leroy CJ, Lonsdorf EV, Whitham TG, Hart SC. 2008. Plant-soil-microorganism interactions: Heritable relationship between plant genotype and associated soil microorganisms. *Ecology* **89**(3): 773-781.

Sikes BA, Cottenie K, Klironomos JN. 2009. Plant and fungal identity determines pathogen protection of plant roots by arbuscular mycorrhizas. *Journal of Ecology* **97**(6): 1274-1280.

Sikes BA, Powell JR, Rillig MC. 2010. Deciphering the relative contributions of multiple functions within plant-microbe symbioses. *Ecology* **91**(6): 1591-1597.

Singh AK, Hamel C, DePauw RM, Knox RE. 2012. Genetic variability in arbuscular mycorrhizal fungi compatibility supports the selection of durum wheat genotypes for enhancing soil ecological services and cropping systems in Canada. *Canadian Journal of Microbiology* **58**(3): 293-302.

Smith FA, Smith SE. 2011. What is the significance of the arbuscular mycorrhizal colonisation of many economically important crop plants? *Plant and Soil* **348**(1-2): 63-79.

Smith S, Read D. 2010. *Mycorrhizal Symbiosis*: Elsevier Science.

Stanescu S, Maherali H. 2017. Mycorrhizal feedback is not associated with the outcome of competition in old-field perennial plants. *Oikos* **126**(2): 248-258.

Sukumar P, Legue V, Vayssieres A, Martin F, Tuskan GA, Kalluri UC. 2013. Involvement of auxin pathways in modulating root architecture during beneficial plantmicroorganism interactions. *Plant Cell and Environment* **36**(5): 909-919.

Tai HH, Lu X, Opitz N, Marcon C, Paschold A, Lithio A, Nettleton D, Hochholdinger F. 2016. Transcriptomic and anatomical complexity of primary, seminal, and crown roots highlight root type-specific functional diversity in maize (*Zea mays* L.). *Journal of Experimental Botany* **67**(4): 1123-1135.

Thonar C, Erb A, Jansa J. 2012. Real-time PCR to quantify composition of arbuscular mycorrhizal fungal communitiesumarker design, verification, calibration and field validation. *Molecular Ecology Resources* **12**(2): 219-232.

Unger S, Friede M, Hundacker J, Volkmar K, Beyschlag W. 2016. Allocation trade-off between root and mycorrhizal surface defines nitrogen and phosphorus relations in 13 grassland species. *Plant and Soil* **407**(1-2): 279-292.

Unger S, Friede M, Volkmar K, Hundacker J, Beyschlag W. 2017. Relationship between mycorrhizal responsiveness and root traits in European sand dune species. *Rhizosphere* **3**: 160-169.

Valverde-Barrantes OJ, Horning AL, Smemo KA, Blackwood CB. 2016. Phylogenetically structured traits in root systems influence arbuscular mycorrhizal colonization in woody angiosperms. *Plant and Soil* **404**(1-2): 1-12.

Verbruggen E, van der Heijden MGA, Rillig MC, Kiers ET. 2013. Mycorrhizal fungal establishment in agricultural soils: factors determining inoculation success. *New Phytologist* **197**(4): 1104-1109.

Vierheilig H, Coughlan AP, Wyss U, Piche Y. 1998. Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. *Applied and Environmental Microbiology* **64**(12): 5004-5007.

Watt M, Schneebeli K, Dong P, Wilson IW. 2009. The shoot and root growth of Brachypodium and its potential as a model for wheat and other cereal crops. *Functional Plant Biology* **36**(10-11): 960-969.

Weißbecker C, Buscot F, Wubet T. 2017. Preservation of nucleic acids by freeze-drying for next generation sequencing analyses of soil microbial communities. *Journal of Plant Ecology* **10**(1): 81-90.

White TJ, Bruns T, Lee S. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M, Gelfand D, Sninsky JJ, White TJ eds. *PCR Protocols: A Guide to Methods and Applications*. New York: Academic Press Inc.

Wickham H. 2009. *ggplot2: Elegant Graphics for Data Analysis*. New York: Springer-Verlag.

Yano K, Yamauchi A, Kono Y. 1996. Localized alteration in lateral root development in roots colonized by an arbuscular mycorrhizal fungus. *Mycorrhiza* **6**(5): 409-415.

Zadworny M, Eissenstat DM. 2011. Contrasting the morphology, anatomy and fungal colonization of new pioneer and fibrous roots. *New Phytologist* **190**(1): 213-221.

Figure Caption

Figr-1

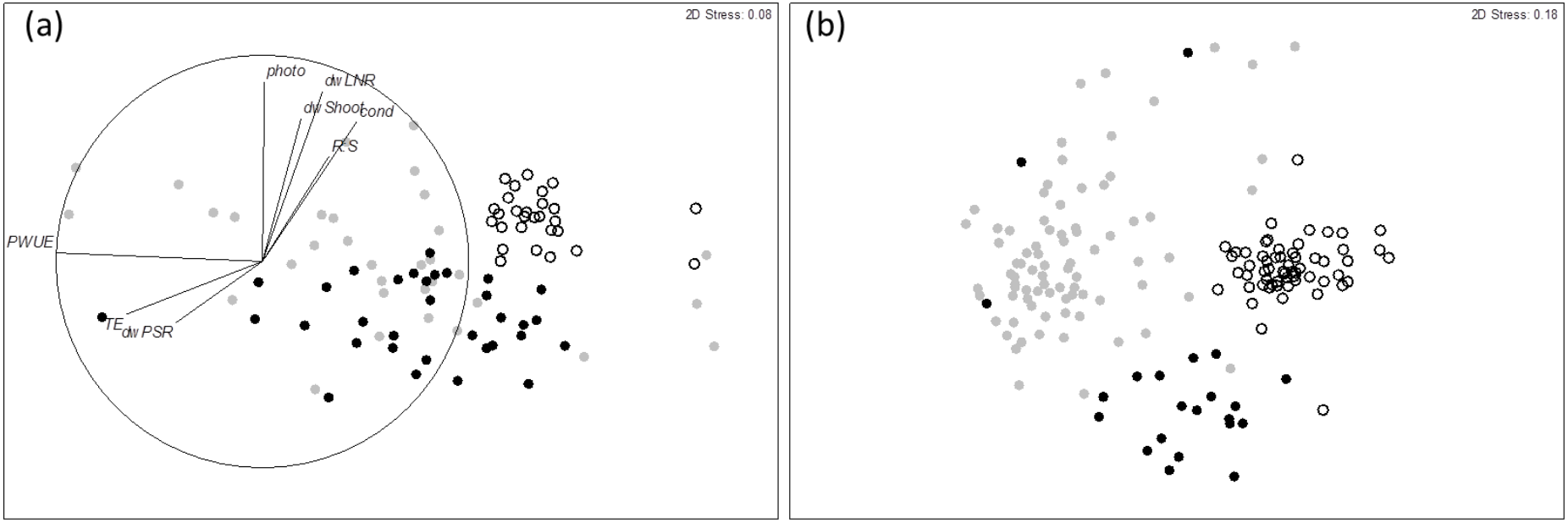


Figure one. MDS ordination of (a) plant trait data based on Euclidean distance. Each circle represents a *Brachypodium distachyon* plant grown in one of three soils where black is Ginninderra, grey is Narrabri and white is Hawkesbury. Vectors show variables with Pearson correlations > 0.5; photosynthetic rate (photo), dry weight of shoot (dwShoot), log dry weight of leaf nodal roots (dwLNR), stomatal conductivity (cond), root shoot ratio (R:S), log dry weight of primary seminal root (dwPSR), transpiration efficiency (TE) and photosynthetic water use efficiency (PWUE) (b) Arbuscular mycorrhizal fungal community data based on Jaccard similarity of molecular data. Each circle represents a community profiled from *B. distachyon* roots grown in soils coloured as in plot (a).

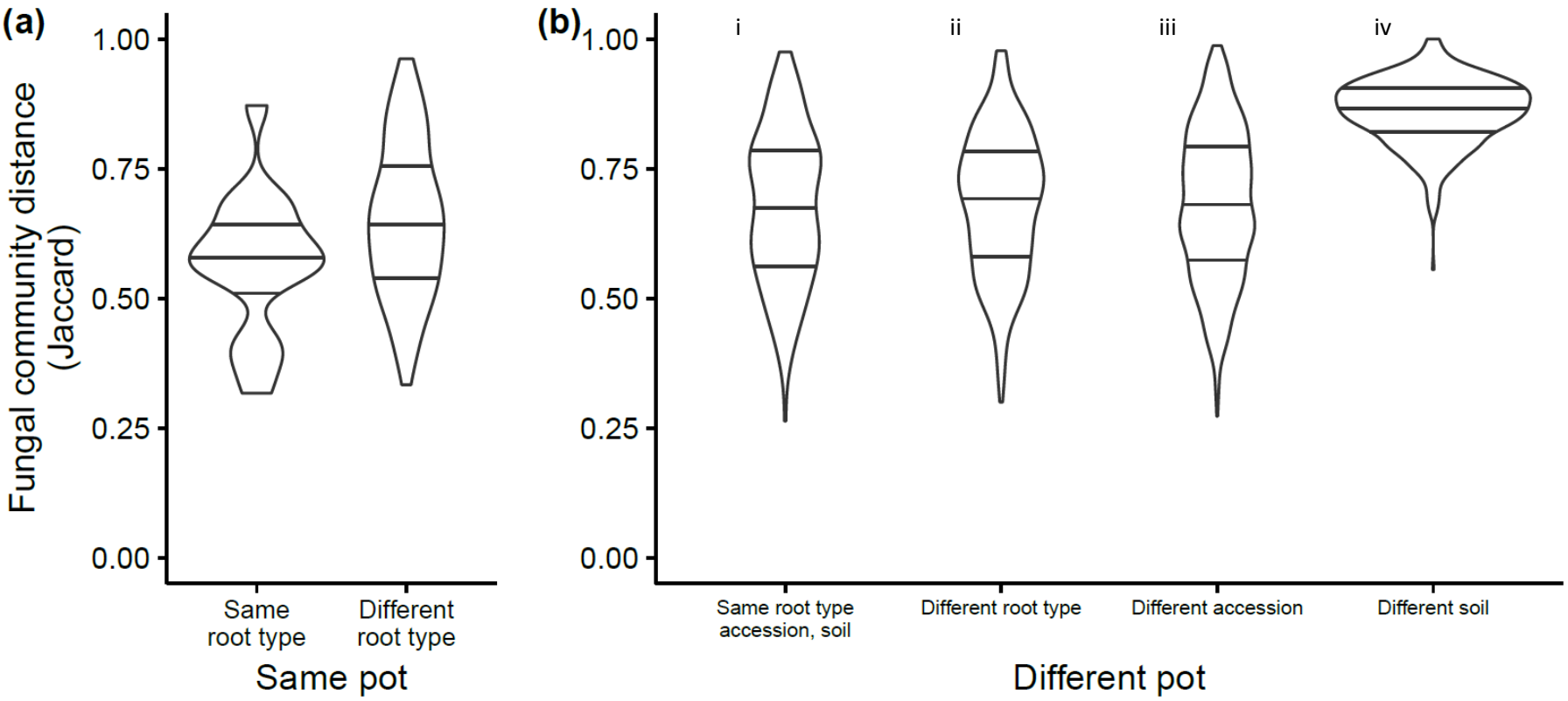
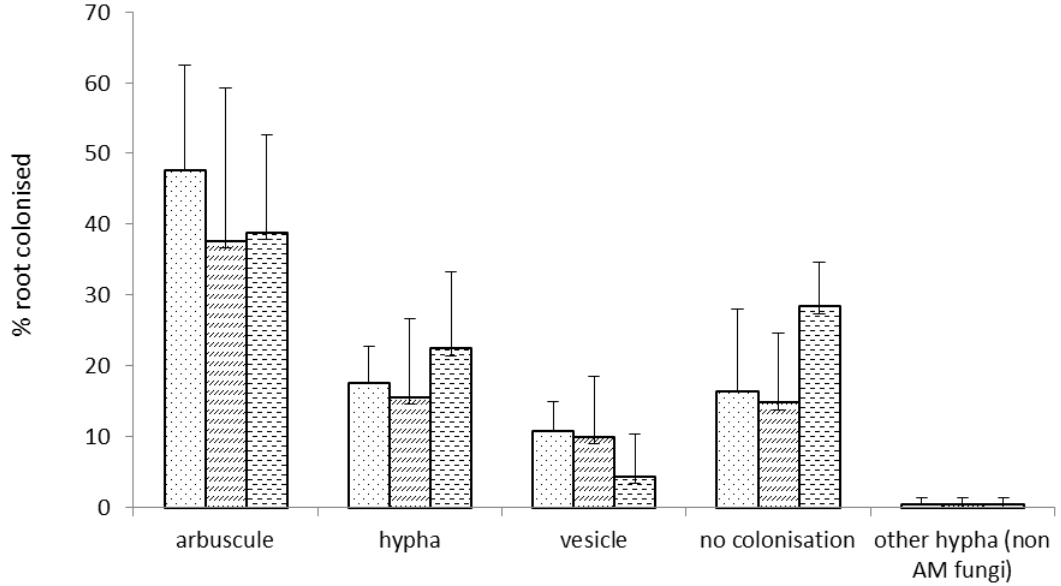


Figure two. Violin plots demonstrating minimal effects of *B. distachyon* root type (primary seminal, coleoptile nodal or leaf nodal roots) and accession and the relatively large effects of soil origin on AM fungal community composition, calculated based on pairwise Jaccard dissimilarity. The width of the plot represents the distribution of dissimilarity between pairs of AM fungal communities within each group (points), and shows the probability density of the data at different measured dissimilarities. The horizontal lines represent the quartiles of the measured dissimilarities. (a) Comparisons among communities observed within and between different root types are made within individual pots. (b) Comparisons of communities between pots but within (i) the same root type, accession and soil; and (ii-iv) varying one of these three factors.



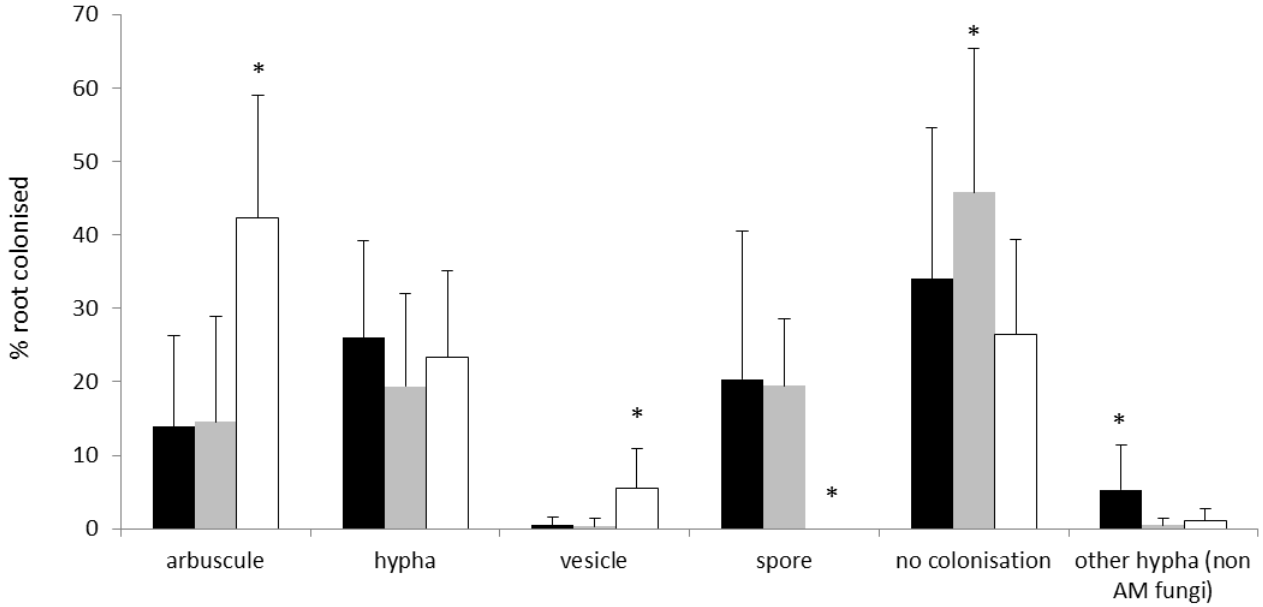


Figure three. Arbuscular mycorrhizal (AM) fungal colonisation of (a) *Brachypodium distachyon* accession Kah1 roots grown in Hawkesbury soil, where bars filled with dots represent primary seminal roots (PSR), diagonal lines coleoptile nodal roots (CNRs) and horizontal lines leaf nodal roots (LNRs). Means (n=5) plus one standard deviation are shown. There was no significant difference in the abundance of mycorrhizal structures between root types; (b) LNRs grown in three field soils: Ginnindera (black bars), Narrabri (grey bars) and Hawkesbury (white bars). Means across seven accessions are shown plus one standard deviation.*, significant differences ($P < 0.05$) between soils in the abundance of mycorrhizal structures.

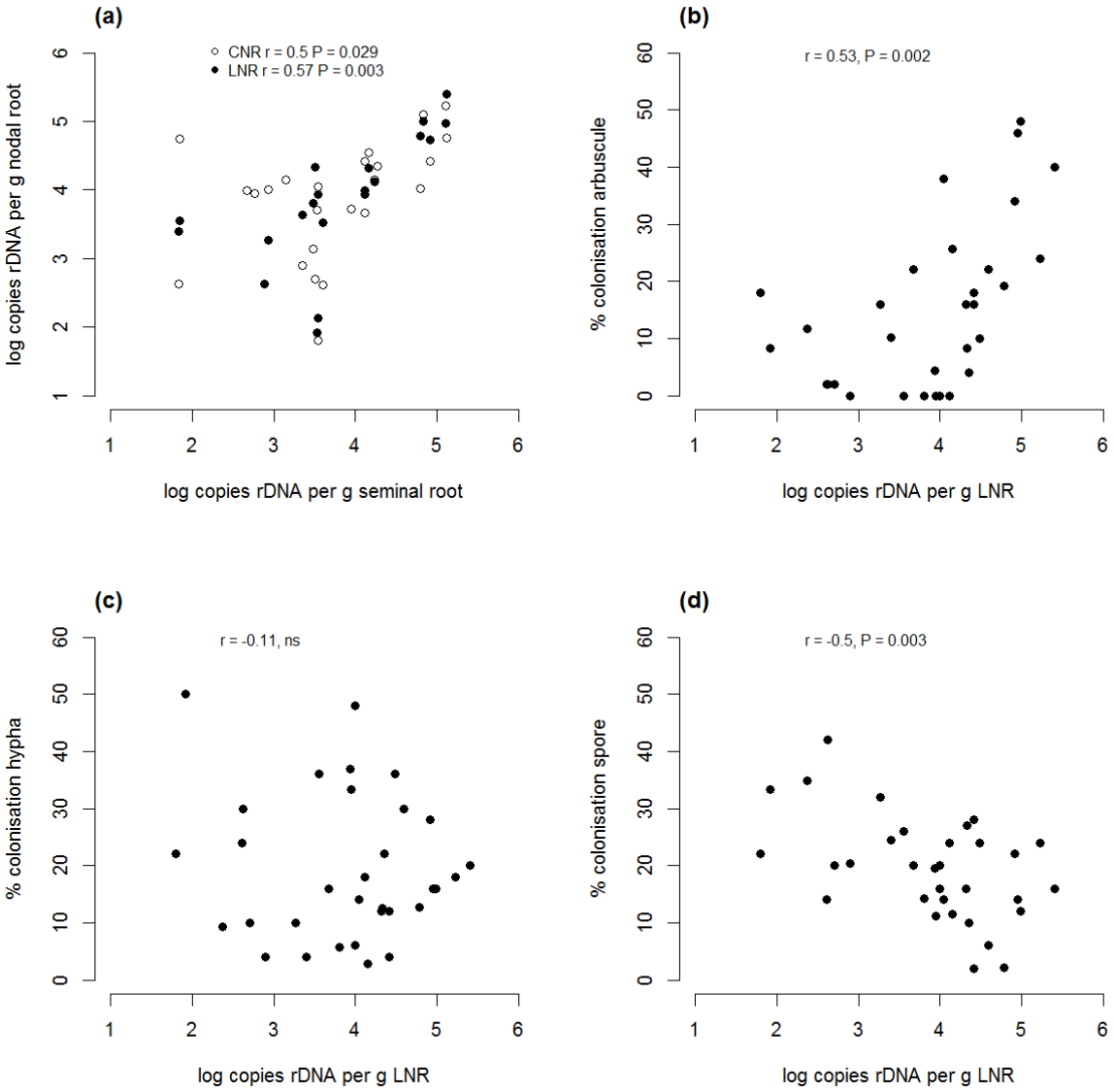


Figure four. Quantification of *Funneliformis mosseae* in *Brachypodium distachyon* roots grown in Narrabri soil measured by quantitative PCR and microscopy. (a) Abundance of *F. mosseae* ribosomal DNA (rDNA) gene copies per gram dry weight of primary seminal root is plotted against that in the coleoptile nodal root (CNR) and leaf nodal root (LNR) of the same plant with Pearson correlation coefficients (r). (b-d) Abundance of *F. mosseae* rDNA gene copies in the LNR is plotted against the percentage of points scored as AM fungal structures following ink-vinegar staining of roots of the same plant.

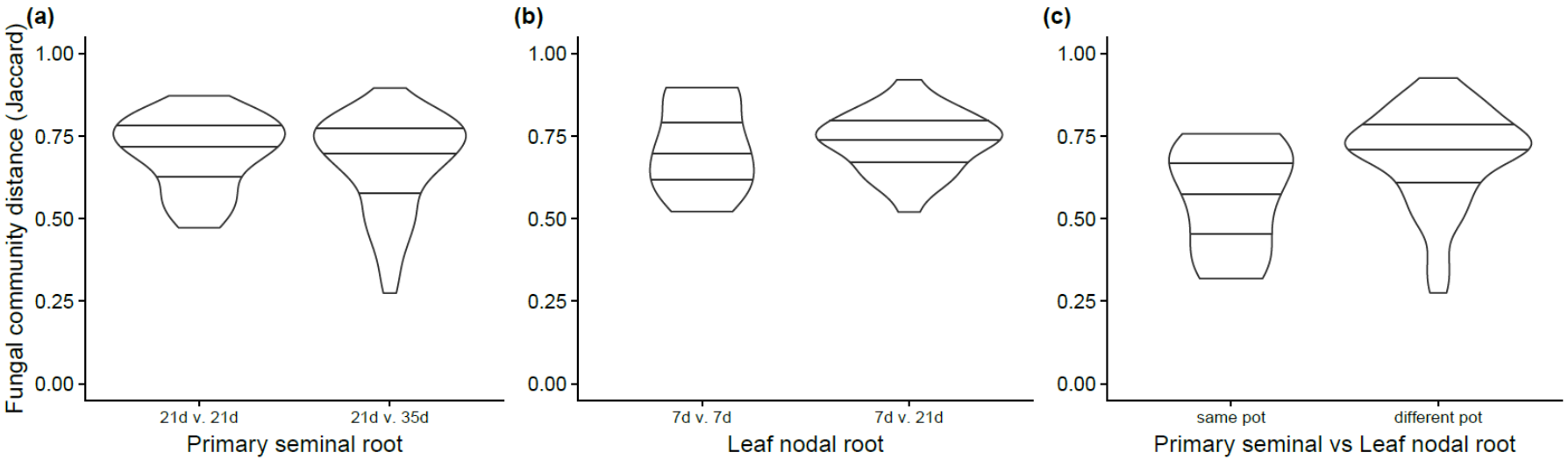


Figure five. Violin plots demonstrating minimal effects of *B. distachyon* root type and age and the relatively large effects of pot on AM fungal community composition, calculated based on pairwise Jaccard dissimilarity. The width of the plot represents the distribution of dissimilarity between pairs of AM fungal communities within each group (points), and shows the probability density of the data at different measured dissimilarities. The horizontal lines represent the quartiles of the measured dissimilarities. Comparisons among communities sampled on roots of different ages, where "d" indicates age of the root in days after emergence. AM fungal communities are compared within root types (a) primary seminal root (PSR) and (b) leaf nodal root (LNR) and between root types (c) sampled from the same pot or different pots.

Table one. Traits of different root types (primary seminal root PSR, coleoptile nodal root CNR and leaf nodal root LNR) of two *B. distachyon* accessions, Bd21 and Kah1, grown in pots containing HIE pasture soil. Differences significant at $P < 0.05$ are highlighted in bold in the ANOVA table and $P < 0.1$ in italics. SRL specific root length, SSA specific surface area.

	Mean ± SE					
	Bd21			Kah1		
Trait	PSR	CNR	LNR	PSR	CNR	LNR
SRL (m g^{-1})	710 ± 81	713 ± 59	313 ± 6	856 ± 87	696 ± 59	306 ± 11
Branching (cm lateral cm $axis^{-1}$)	11.7 ± 1.9	8.2 ± 1.2	11.0 ± 1.3	10.8 ± 1.8	10.1 ± 2.0	8.7 ± 0.5
SSA (m^2 g^{-1})	1.06 ± 0.1	1.03 ± 0.1	0.47 ± 0	1.3 ± 0.2	1.03 ± 0.1	0.47 ± 0
average diameter (mm)	0.48 ± 0.01	0.46 ± 0.01	0.49 ± 0.01	0.48 ± 0.02	0.47 ± 0.01	0.50 ± 0.01
tissue density (mm^3 g^{-1})	8.6 ± 1.2	8.6 ± 0.5	17.8 ± 0.4	7.1 ± 0.9	8.5 ± 0.8	17.2 ± 0.6
length	280 ± 44	222 ± 54	5046 ± 436	204 ± 33	409 ± 119	3888 ± 219

	ANOVA					
	root type		accession		root type x accession	
	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
SRL	37.33	**<0.001**	0.79	0.38	1.18	0.32
Branching	1.14	0.33	0.2	0.65	0.97	0.39
SSA	33.99	**<0.001**	1.25	0.27	1.09	0.35
Average diameter	2.45	*0.10*	0.601	0.44	0.15	0.86
Tissue density	91.65	**<0.001**	1.19	0.28	0.39	0.68
Length	260.26	**<0.001**	4.63	**0.04**	5.45	**0.009**