CSIRO PUBLISHING
Functional Plant Biology, 2021, **48**, 871–888
https://doi.org/10.1071/FP20351

The microbiomes on the roots of wheat (*Triticum aestivum* L.) and rice (*Oryza sativa* L.) exhibit significant differences in structure between root types and along root axes

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Abstract. There is increasing interest in understanding how the microbial communities on roots can be manipulated to improve plant productivity. Root systems are not homogeneous organs but are comprised of different root types of various ages and anatomies that perform different functions. Relatively little is known about how this variation influences the distribution and abundance of microorganisms on roots and in the rhizosphere. Such information is important for understanding how root–microbe interactions might affect root function and prevent diseases. This study tested specific hypotheses related to the spatial variation of bacterial and fungal communities on wheat (*Triticum aestivum* L.) and rice (*Oryza sativa* L.) roots grown in contrasting soils. We demonstrate that microbial communities differed significantly between soil type, between host species, between root types, and with position along the root axes. The magnitude of variation between different root types and along individual roots was comparable with the variation detected between different plant species. We discuss the general patterns that emerged in this variation and identify bacterial and fungal taxa that were consistently more abundant on specific regions of the root system. We argue that these patterns should be measured more routinely so that localised root–microbe interactions can be better linked with root system design, plant health and performance.

Keywords: rhizosphere, root–microbe interactions, soil microorganisms, *Triticum aestivum* L., *Oryza sativa* L., bacterial colonies, fungal colonies, root type, root axes.

Received 12 November 2020, accepted 22 March 2021, published online 3 May 2021

Introduction

The biology of the microbial communities on and around roots has attracted increasing interest because of their potential to affect plant growth and productivity (Lareen *et al*. 2016; Mitter *et al*. 2019). While most soil microorganisms are relatively benign to plants, some reduce root growth by causing diseases or competing for resources (Raaijmakers *et al*. 2009). A small number of microorganisms actually benefit plants by suppressing pathogens, activating plant defences to biotic and abiotic stresses or by releasing compounds that stimulate root growth or improve the acquisition of resources (Zamioudis *et al*. 2013; Pieterse *et al*. 2014; Tian *et al*. 2017; de Vries *et al*. 2020; Gupta *et al*. 2020).

Vertical transmission of endophytes through the seed can contribute to the root microbiome but most bacteria and fungi on roots are recruited from the surrounding soil (Berg and Smalla 2009). Root exudates and other rhizodeposits are major determinants of the microbiome community structure because

they provide a rich source of organic carbon. Nevertheless, the environment around roots suits some microorganisms better than others so while the total microbial biomass near roots is usually greater than the bulk soil, the composition of the communities can be different and less diverse compared with the bulk soil (Dennis *et al*. 2008; Bakker *et al*. 2013; Reinhold-Hurek *et al*. 2015; Sasse *et al*. 2018; Richardson *et al*. 2021). The root microbiome can be compartmented into the communities living in the external periphery of roots (ectorhizosphere), those colonising the outer surface of roots and perhaps forming biofilms (rhizoplane), and those organisms spending part of their life cycle within the root tissues (endorhizosphere). These fractions can be separated and examined individually by a series of washing, sonication and lysozyme treatments (Bulgarelli *et al*. 2012; Lakshmanan *et al*. 2017; Chaluvadi and Bennetzen 2018).

Root microbiomes have been characterised for many model species and crop plants including *Arabidopsis thaliana* L. (Bulgarelli *et al*. 2012; Lundberg *et al*. 2012), barley (*Hordeum vulgare L.*; Bulgarelli *et al*. 2015), soybean (*Glycine max* L.; Rascovan *et al*. 2016), canola (*Brassica napus* L.; Lay *et al*. 2018), rice (*Oryza sativa* L.; Edwards *et al*. 2015), *Brachypodium distachyon* L.(Kawasaki *et al*. 2016) and wheat (*Triticum aestivum* L.; Donn *et al*. 2015). These types of studies have demonstrated that the distribution of individual microorganisms or the structure of whole-root microbiomes can differ between plant species and change with plant health, development, nutritional status and environmental factors (Yang and Crowley 2000; Watt *et al*. 2003; İnceoğlu *et al*. 2010; Chaparro *et al*. 2014; Chaluvadi and Bennetzen 2018). Fewer studies have investigated the spatial variation occurring within root systems with the same degree of detail and those that have mostly targeted a small number of taxa. The reason for this is that the methods commonly used to sample roots rarely account for the heterogeneity of the microbiome communities within root systems. For example, whether plants are grown in the field or in pots, the entire root system is often analysed from small plants or random sub-samples of the root system from larger plants. Descriptions are seldom provided for the proportion of different root types collected or the depth in the soil they were collected from (Wagner *et al*. 2016; Lakshmanan *et al*. 2017; Simmons *et al*. 2018; Chen *et al*. 2019).

We know from previous reports that the density of individual microorganisms and composition of communities can vary within the root system (Liljeroth *et al*. 1991; Chin-A-Woeng *et al*. 1997; Yang and Crowley 2000; Watt *et al*. 2006*a*; Dennis *et al*. 2008; DeAngelis *et al*. 2009). For example, an early report found that seminal roots of wheat supported significantly larger populations of bacteria and fungi than nodal roots (Sivasithamparam *et al*. 1979*b*). Kawasaki *et al*. (2016) detected significant differences in the bacterial and fungal communities on the seminal and nodal roots of *Brachypodium*. Similarly, the abundance of certain fungal taxa differed between the lateral roots and axile roots of maize (*Zea mays* L.; Yu *et al*. 2018). This local variation is important for understanding the progression of diseases or ability of plants to acquire resources. For instance, Donn *et al*. (2017) identified small but significant differences in the colonisation of arbuscular mycorrhizal fungi on the different root types of *Brachypodium*, and Sivasithamparam and Parker (1978) and Gilligan (1980) showed that take-all fungus (*Gaeumannomyces graminis*) was more detrimental to wheat when the nodal roots were infected than when the seminal roots were infected. That difference was caused by the greater abundance of fluorescent pseudomonads on the seminal roots, which were natural antagonists to take-all fungus (Sivasithamparam *et al*. 1979*a*).

The heterogeneity in microbiome structure and composition within a root system is not unexpected because roots are not homogeneous organs (Hochholdinger and Zimmermann 2018). There are different types of roots, all of which vary in age from the root tip to the base. Grasses develop the seed-borne seminal roots, the shoot-borne nodal roots as well as primary and secondary lateral roots. These different roots emerge at different times, develop different anatomies (Watt *et al*. 2009) and contribute to anchorage and resource acquisition in different ways (Kuhlmann and Barraclough 1987; Volkmar 1997; Wiengweera and Greenway 2004; Gamuyao *et al*. 2012; Ahmed *et al*. 2016, 2018; Sun *et al*. 2018; Liu *et al*. 2020). Seminal and nodal roots have distinct transcriptomes (Tai *et al*. 2016) and proteomes (Liu *et al*. 2020), which means they are likely to release different compounds into the rhizosphere.

Previous studies investigating the spatial variation in root microbiomes mostly focussed on a small number of organisms or used techniques that were unable to fully describe community complexity. The present study used amplicon sequencing to test a series of hypotheses concerning the spatial variation in the root microbiome colonising the root systems of wheat and rice.

Materials and methods

Plant materials

Dehusked seeds of wheat (*Triticum aestivum* L. cv. EGA-Burke) and rice (*Oryza sativa* L. cv. Nipponbare) were surface sterilised for 20 min in 20% household bleach and germinated on a moist filter paper on a Petri dish for 2 days before being transferred to soil.

Soils

The two soils used in this study were a yellow Chromosol collected from Ginninderra Experiment Station, Canberra, ACT, Australia (35°10′30″S, 149°02′33.4″E) and a Ferrosol from Robertson, NSW, Australia (34°37′37.9″S, 150°28′53.7″E). The Chromosol was a sandy loam with 48 g kg^{-1} organic C and the Ferrosol was a highly P-fixing clay loam with 164 g kg^{-1} organic C. The soils were collected from the 10–20 cm layer of pasture paddocks. They were air-dried, sieved (5 mm mesh) and stored at room temperature. The pH ($CaCl_2$) of both soils was 4.3. The Chromosol was not aluminium toxic and was unamended. The Ferrosol contained toxic levels of aluminium and was amended with KH_2PO_4 (250 mg P kg^{-1} dry soil) to raise plant-available P and with $CaSO_4$ (5 g kg^{-1} dry soil) to remove the aluminium toxicity without changing the pH. The aluminium toxicities of the soils were confirmed in

preliminary experiments by comparing the root growth of Al-sensitive and tolerant wheat lines (data not shown). Moisture content of the soils was adjusted to 80% and 90% of field capacity for the Chromosol and Ferrosol, respectively. Soils were packed into pots made from polyvinylchoride (PVC) tubes (~10 cm diameter × 20 cm height) lined with plastic bags (for easy removal of the root system) at the density of ~1 g cm^{-3}.

Plant growth condition, and the root and soil sampling

Two experiments were performed in this study. In Experiment 1, we investigated the bacterial communities on different locations along the root (tip and base) in wheat and rice. Pre-germinated wheat (cv. EGA-Burke) and rice (cv. Nipponbare) seedlings were transferred into pots with the Chromosol soil (three seedlings per pot for wheat and 10 seedlings per pot for rice) and white plastic pellets (~2 mm diameter) were spread on top to prevent evaporation. The pots were weighed and placed in a growth cabinet (Conviron, Canada) with a 16 h day/8 h night cycle (24°C/20°C) and 600 µmol photon m^{-2} s^{-1}. The pots were watered daily to their starting weights. Similar pots without plants were prepared the same way for the collection of bulk soil samples. At 8 days, wheat seedlings only developed seminal roots (~5 roots $plant^{-1}$) while rice seedlings developed a single seminal root and multiple nodal roots; these were combined for analysis. The intact root system was washed from the pots and each root was excised from the seed and carefully detangled in a large shallow tray containing sterile 0.2 mM $CaCl_2$ solution. Since soil adhering to the roots was washed off, the microorganisms remaining were those tightly associated with the root surface (rhizoplane) and endophytes, which in this study is referred to as the 'root microbiome'. From each of the five replicate pots, 10–15 root tips (apical 1 cm) and 5–6 root bases (2 cm segments between 1 and 3 cm from the base) (Fig. 1) were excised with a scalpel, rinsed with sterile 0.2 mM $CaCl_2$ in a tube and stored at −80°C for DNA extraction. The basal root segments included lateral roots if present. Bulk soil samples were collected from the middle of the separate (unplanted) pots.

Experiment 2 compared both the bacterial and fungal communities on different root types (seminal and nodal) and different root locations (tip and base) of wheat plants grown in the Ferrosol soil. Seminal and nodal roots could not be sampled at the same time from the same plant because seminal roots emerge first from the seed while nodal roots begin to emerge ~14 days later from the crown (base of the stem and tillers) (Fig. 1). By the time the nodal roots would have been ready to sample, the seminal roots would be at the bottom of the pots, which could have affected the microbiome. Therefore, two sets of six pots were prepared so that seminal roots could be destructively sampled after 8 days and nodal roots sampled after 31 days. Each pot contained three wheat seedlings. Pots were placed in a glasshouse with natural light and their position on the bench changed every 2 days. The roots were washed and sorted as described for Experiment 1 and different root types identified and grouped together. The tips and bases of seminal and nodal roots were sampled and stored as described above for Experiment 1. Bulk soil samples were collected at both times from separate replicated pots and from three depths: (1) top, 1 cm below the surface; (2) middle, centre of pot; and (3) bottom, 1 cm from bottom of the pot.

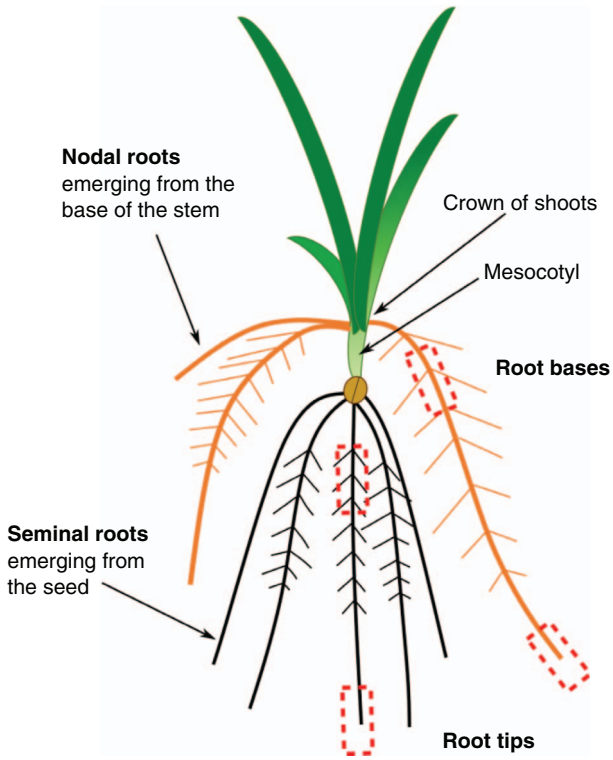

Fig. 1. A schematic drawing of a typical root system of cereal species. The root system comprises the seed-borne seminal roots (one in rice and approximately five in wheat) and the nodal roots that emerge from the crown at the base of the shoots (>100 in mature rice and wheat). Red boxes show the tissues sampled from the root tips (apical 1 cm) and the root bases (2 cm segments between 1 and 3 cm from the base) for microbiome analysis. Lateral roots were included in the samples if present in the root segments.

DNA extraction and microbial community analysis

DNA extraction: Root samples were lyophilised and then homogenised with two stainless steel balls (3 and 5 mm diameter) in a 2 mL Safe-Lock microcentrifuge tube (Eppendorf). Homogenisation was carried out with a TissueLyser LT bead mill (QIAGEN) at 50 s^{-1} oscillation for 5 min. DNA was isolated from soil and homogenised root samples using a DNeasy PowerSoil Kit (QIAGEN) according to the guidelines, except that the bead beating step used the TissueLyser LT bead mill at 50 s^{-1} oscillation for 1 min.

PCR primers and PNA PCR blocker for plant DNA: For amplification of bacterial 16S rRNA (rRNA) genes, 799F (5′-AACMGGATTAGATACCCKG-3′) and 1391R (5′-GACGGG CGGTGWGTRCA-3′) primer set, targeting V5-V7 regions was chosen as it was previously shown that these primers did not amplify non-target DNA such as plant chloroplast 16S rRNA gene (Beckers *et al*. 2016). However, preliminary tests with the primer set using wheat root DNA showed that these primers

co-amplify the wheat mitochondrial 18S rRNA gene, identified by its amplicon size. Moreover, little or no bacterial amplicons were detected in the PCR of the root tip samples, perhaps because of the much greater abundance of plant DNA in those samples Therefore, a peptide nucleic acid (PNA) PCR blocker was designed to inhibit co-amplification of plant derived DNA (Kawasaki and Ryan 2021). Wheat and rice mitochondrial 18S rRNA gene sequences were aligned with several bacterial 16S rRNA gene sequences from different clades using Vector NTI software (Invitrogen). Regions unique to the plant mitochondrial 18S rRNA gene sequences were identified within the amplicon region (799F-1391R) and a 16-mer PNA sequence named TaMtPNA1-F (5′-GCCCCGCTCCGA AACA-3′) was designed to enable it to bind to plant mitochondrial DNA but not bacterial DNA. Inclusion of the PNA in the PCR inhibited or minimised amplification of plant derived DNA with the 799F-1391R primers. The specificity of the PNA sequence to the plant was tested with the Ribosomal Database Project (RDP) Probe Match program (https://rdp.cme.msu.edu/probematch/search.jsp) (Cole *et al*. 2014) to ensure that no bacterial sequences matched the PNA sequence. The sequence specificity was also tested with PCR using a DNA primer with the same sequence as the PNA (PNA primer), in combination with either 799F or 1391R primers, using sterile plant DNA (positive control) and soil DNA (negative control) as the templates. The PNA PCR blocker was synthesised (PANAGENE Inc., South Korea) and resuspended in water to working stock concentrations of 50 µM.

For amplification of fungal internal transcribed spacer (ITS) region, a semi-nested PCR approach was used to avoid co-amplification of plant derived sequences. First round PCR was performed with ITS1F_KYO1 (5′-CTHGGTCATTTAGAGGAASTAA-3′) (Toju *et al*. 2012) and ITS4-R (5′-TCCTCCGCTTATTGATATGC-3′) (White *et al*. 1990) primer set, and the second round PCR was performed with gITS7-F (5′-GTGARTCATCGARTCTTTG-3′) (Ihrmark *et al*. 2012) and ITS4-R primer set.

The bacterial 16S and the fungal ITS primers for the final amplification were modified on the 5′ end to contain the Illumina overhang adaptor for compatibility with Illumina's Nextera XT index adapters.

Bacterial and fungal community sequencing: Bacterial 16S rRNA genes were amplified in a 25 µL PCR with MyFi DNA Polymerase (Bioline) according to the manufacturer's recommendation. Typically, the template was 1 µL of the undiluted DNA preparations (root tip samples) or 10-fold diluted (root base and bulk soil samples) extracted DNA, and the PNA blocker was added to a final concentration of 10 µM (wheat root samples) or 20 µM (rice root samples). The PCR cycle consisted of initial denaturation at 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 72°C for 30 s (PNA annealing), 55°C for 30 s and 72°C for 30 s, and lastly, with a final elongation step at 72°C for 5 min. For wheat root samples, addition of PNA blocker did not eliminate the plant amplicon (~1000 bp) completely, but bacterial amplicons (~700 bp) were only detected after the addition of the PNA blocker (especially in the root tip samples). The PCR products were separated on 1.5% agarose gel and the bacterial bands were excised. Some wheat root tip samples showed low amplification; in this case, multiple PCRs were prepared and the products were pooled. The newly designed TaMtPNA1-F blocker also bound to the rice mitochondrial 18S rRNA gene sequence. However, since the rice mitochondrial amplicon was similar in size with the bacterial 16S amplicon, it was not possible to separate the two products on an agarose gel. Therefore, for rice roots samples, a final concentration of 20 µM of the PNA was added to the PCR, to minimise the amplification of the plant DNA.

Fungal ITS region was initially amplified in a 25 µL PCR with MyFi DNA Polymerase. The reaction typically used 1 µL of undiluted (root tip samples) or 10-fold diluted (soil samples and other root samples) DNA extracts as template (first round PCR). The PCR cycle consisted of initial denaturation at 95°C for 5 min, followed by 15 cycles of 95°C for 30 s, 56°C for 30 s and 72°C for 30 s, and lastly, with a final elongation step at 72°C for 5 min. The PCR products were purified with SureClean Plus (Bioline), and re-dissolved in 25 µL of water. The purified PCR product (1 µL) was used as the template for the second round PCR to amplify fungal ITS2 region with gITS7-F and ITS4-R primer set (with the Illumina adapters). The second round PCR was carried out in the same condition, but with 25 cycles of amplifications.

All PCRs were carried out in a Bio-Rad C1000 Touch Thermal Cycler (Bio-Rad). The final PCR products, either from the excised gel (wheat root 16S samples) or direct product (rice root and bulk soil 16S, and all ITS samples), were purified with ISOLATE II PCR and Gel Kit (Bioline), and the purified PCR products were quantified using Qubit dsDNA HS Assay Kit (ThermoFisher Scientific) with Qubit fluorometer (ThermoFisher Scientific). Equal concentrations of each sample were pooled and sequenced on an Illumina MiSeq platform at The University of Queensland's Institute for Molecular Biosciences (UQ, IMB) using 30% PhiX Control v3 (Illumina) and a MiSeq Reagent Kit v3 (600 cycle; Illumina) according to the manufacturer's instructions.

The bacterial 16S and the fungal ITS sequence data were processed essentially as described previously (Forstner *et al*. 2019). For both datasets, a modified UPARSE pipeline (Edgar 2013) was used to analyse the sequences and analyses were performed using the forward reads only. For 16S reads, primer sequences were removed and the remaining sequences were trimmed to 250 bp. Chimeras were removed and USEARCH (ver. 10.0.240) (Edgar 2010) was used to filter and cluster the 16S sequences into operational taxonomic units (OTUs). For ITS reads, ITSx (ver. 1.0.11) (Bengtsson-Palme *et al*. 2013) was used to identify and extract ITS2 sequences. Chimeric ITS2 sequences were removed and the remaining sequences were clustered into OTUs with USEARCH and OTU table was generated. Taxonomy was assigned to the bacterial and fungal OTU with SILVA SSU (ver. 128) (Quast *et al*. 2013) and UNITE (ver. 7.2–2017.10.10) (Nilsson *et al*. 2019) databases respectively using BLASTN (ver. 2.3.0+) (Zhang *et al*. 2000) within QIIME2 (Bolyen *et al*. 2019). Non-bacterial OTUs were removed from the 16S OTU table using BIOM tool suite (McDonald *et al*. 2012). An equal number of sequences (minimum library size) were rarefied from each

experimental dataset. From Experiment 1, 9950 sequences were rarefied from each sample (16S sequencing only). From Experiment 2, a total of 1150 and 2050 sequences were rarefied from each sample for the 16S and ITS sequencing data, respectively. The number of observed (Sobs) and predicted (Chao1) OTUs and the Shannon diversity indices were calculated using QIIME2, and the OTU tables were then used for subsequence statistical analyses.

Statistical analyses

Statistical analyses were performed using R ver. 3.5.3 (R Core Team 2019). Differences in α diversity (Sobs, Chao1 and Shannon indices) between sample groups were assessed using one-way ANOVA. To visualise the differences in the composition of microbial communities associated with each sample, Hellinger transformation was applied to the OTU tables (Legendre and Gallagher 2001) and detrended correspondence analysis (DCA) was performed using *vegan* package (Oksanen *et al*. 2019). Differences in the structure of microbial communities between the sample groups were assessed with multivariate generalised linear models (GLM) using a negative binomial distribution, as implemented in the *mvabund* package (Wang *et al*. 2012). OTUs showing significant differences in abundance between sample groups were identified with *DESeq2* package (Love *et al*. 2014) by converting the OTU counts, taxonomies and the sample metadata with *phyloseq* package (McMurdie and Holmes 2013) as previously described (McMurdie and Holmes 2014). Negative binomial GLMs were fit for each OTU and the logarithmic fold changes in the OTU abundance between the two groups being compared were calculated. Significance was tested using the Wald test with a threshold of Benjamini-Hochberg adjusted $P < 0.05$.

Results

Bacterial communities along the roots of wheat and rice seedlings

Experiment 1 measured the bacterial communities along the roots of wheat and rice seedlings grown in a Chromosol. The null hypotheses tested were: (1) no differences exist between the microbiomes in the bulk soil and on the roots; (2) no differences exist between the microbiome communities on wheat and rice roots; and (3) no differences exist between the microbiome communities at the root tips and the root bases.

The count of unique OTUs (Sobs), as well as the Chao1 and Shannon indices for α diversity, tended to be lower on the root samples compared with the bulk soil, but the differences were significant for wheat only (Table 1). Bacterial community structures were presented after detrended correspondence analysis (DCA) (Fig. 2*a*) and pairwise comparisons were made using a multivariate GLM. The results showed that bacterial communities collected from the root tips and bases of wheat and rice were all significantly different from each other and different from the bulk soil ($P < 0.005$). Specifically, the root communities on wheat were significantly different from rice and the communities at the root tips were significantly different from those at the root bases. Therefore, all three null hypotheses were rejected. Overall, members of the Actinobacteria and Bacilli were enriched at the roots compared with the bulk soil while Acidobacteria, Alphaproteobacteria and Gemmatimonadetes were more abundant in the bulk soil (Fig. 2*b*). Members of the Thermoleophilia, Bacilli and Alphaproteobacteria were relatively more abundant on rice roots compared with wheat roots whereas Betaproteobacteria were more abundant on wheat than rice ($P < 0.05$) (Fig. 2*b*).

Table 1. Alpha diversity indices of the bacterial communities from Experiment 1

Observed number of OTUs (Sobs), Chao1 and Shannon indices of the bacterial community in the tips and bases of wheat and rice roots, and bulk soil samples. Data are means ± s.e. (*n*, 5). Different lowercase letters next to the values indicate significant differences between the samples (ANOVA, $P < 0.05$)

	Sobs	Chao 1	Shannon
Bulk soil	566.8 ± 6.8^{a}	719.7 ± 15.2^{a}	6.6 ± 0.1^{a}
Rice_Tip	490.4 ± 17.3^{a}	596.9 ± 27.0^{ac}	5.9 ± 0.2^{ac}
Rice_Base	523.0 ± 9.6^{a}	680.2 ± 28.6^{ac}	6.7 ± 0.1^{a}
Wheat_Tip	349.4 ± 36.1^{b}	449.8 ± 35.6^{b}	4.2 ± 0.4^{b}
Wheat_Base	371.0 ± 14.6^{b}	569.7 ± 34.5^{bc}	5.0 ± 0.2^{bc}

We identified the individual bacterial OTUs that were significantly more abundant on specific root tissues using *DESeq2* analysis by comparing the root tips and root bases in rice and wheat (Fig. 3; see Table S1). Several OTUs showed similar patterns of enrichment on the roots of both wheat and rice. For instance, four OTUs from the Class Actinobacteria (Family: Propionibacteriales; Genera: *Nocardioides* and *Marmoricola*) were more abundant on the root tips of both wheat and rice, and 13 OTUs were significantly more abundant on the root bases of both cereals (from Families: Frankiaceae, Micromonosporaceae, Pseudonocardiaceae, Chitinophagaceae, Caulobacteraceae, Bradyrhizobiaceae, Rhizobiaceae, Burkholderiaceae, Comamonadaceae, Sphingomonadaceae and Xanthomonadaceae) (Fig. 3). For further taxonomic classifications, see Table S1.

DESeq2 analysis also identified the bacterial OTUs that differed in abundance between wheat and rice roots. A total of 29 OTUs (mostly class Actinobacteria) showed significant differences in abundance between the root tips of wheat and rice, and 83 OTUs (largely Actinobacteria, Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Thermoleophilia and Bacilli) were significantly different between the root bases of wheat and rice (Fig. 4; see Table S2). A subset of 16 OTUs (Families: Microbacteriaceae, Nocardioidaceae, Pseudonocardiaceae, Streptomycetaceae, Burkholderiaceae and Xanthomonadaceae) were significantly more abundant on both the root tips and root bases of wheat roots compared with rice (Fig. 4; see Table S2).

Spatial variation of the microbiome on different root types of wheat

Experiment 2 compared the bacterial and fungal communities at different positions along the seminal and nodal roots of wheat plants grown in a Ferrosol. The null hypotheses tested

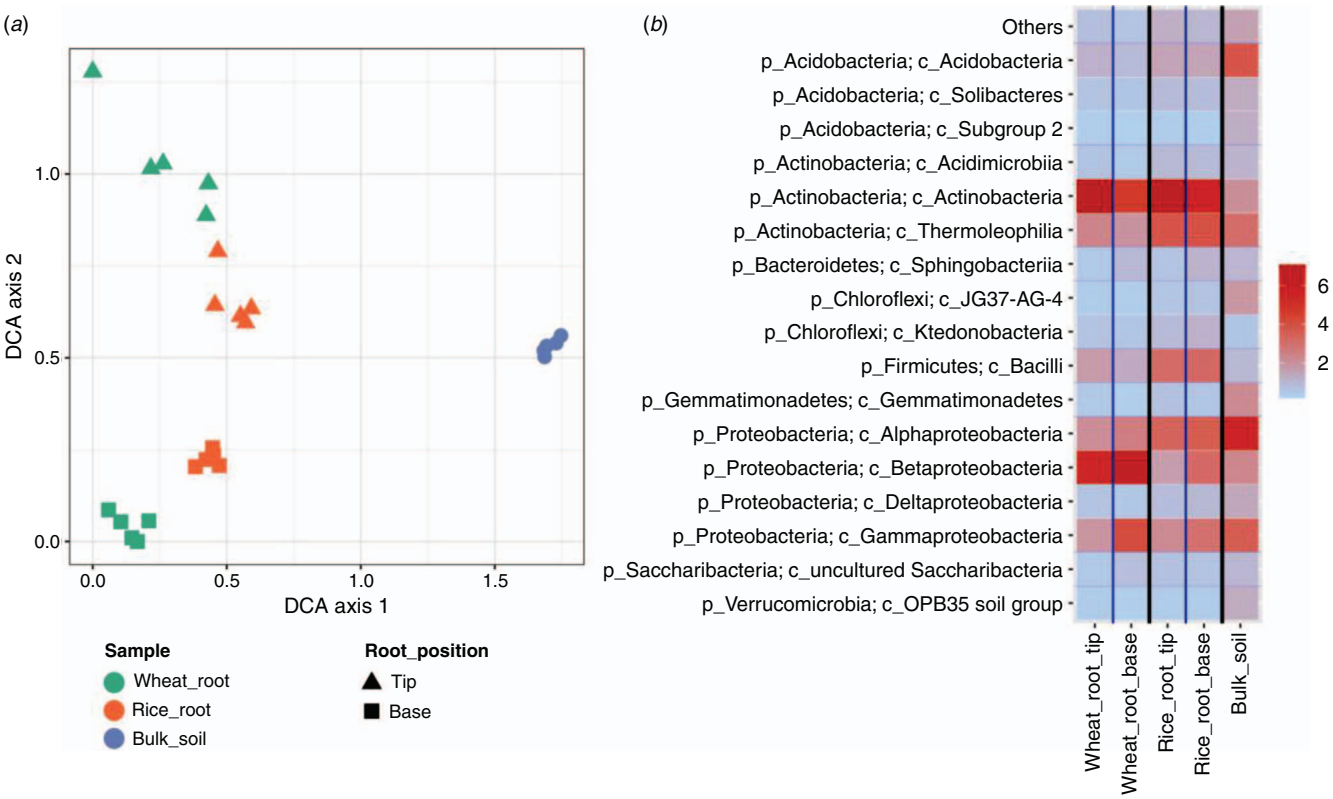

Fig. 2. Bacterial community structures on the roots and bulk soil samples from Experiment 1. (*a*) Detrended correspondence analysis (DCA) shows differences in the bacterial community structures between the wheat roots (tips and bases), rice roots (tips and bases) and bulk soil samples. (*b*) Heatmap shows the relative abundance of the major bacterial phyla (p__) and classes (c__) found on wheat roots, rice roots and bulk soil. Minor bacterial classes (<0.5% in the abundance in any single sample) were compiled into 'Others'. Data are means (n = 5) and the colour scale represents the square root of relative abundance.

here included: (1) no differences exist between the bulk soil microbiome and the root microbiome; (2) no differences exist between the bacterial and fungal communities on the root tips and the root bases; and (3) no differences exist between the bacterial and fungal communities on seminal roots and nodal roots.

The Chao1 indices (number of estimated OTUs) indicated that the bacterial and fungal richness in most root samples was lower than that in the bulk soil (P < 0.05) as found in Experiment 1. The one exception was the bacterial community on the tips of nodal roots, which was not significantly different from the bulk soil (Table 2). By contrast, the Sobs (count of unique OTUs) and Shannon indices (which reflects community richness and evenness) indicated that the bacterial diversities on the root tips (both seminal and nodal) were not significantly different from the bulk soil, but the diversities on the root bases were significantly lower than in the bulk soil (Table 2). For fungal communities, all three indices showed that the diversity in all root samples was significantly lower than in the bulk soil (Table 2).

DCA ordination plots and the multivariate GLM pairwise comparisons indicated that the bacterial and fungal populations colonising the bulk soil were significantly different from all the root samples (P < 0.005 for all pairwise comparisons) (Figs 5*a*, 6*a*). Since the seminal and nodal roots were sampled 23 days apart and from different depths in the pot (root tip and root base), we assessed whether the differences between root types and root locations could be attributed to changes in the adjacent bulk soil communities. When analysed alone, the bulk soil samples did show significant differences with depth in the pot (top, middle and bottom layers) (bacteria P = 0.01; fungi P = 0.01) and incubation times (8 days and 31 days) for the bacterial populations (P = 0.01) but not the fungi (P = 0.36). However, the magnitude of these differences was much smaller than the differences between the soil and root samples and between the different root samples (Figs 5*a*, 6*a*). We conclude that the small differences in soil microbiome associated with depth in the pot or with sampling time cannot account for the large differences detected between the root tips and bases, and between seminal and nodal roots. Therefore, all the null hypotheses were rejected because significant differences were detected for the bacterial and fungal communities between the bulk soil and roots, between the root tips and root bases and between the seminal roots and nodal roots.

Broad patterns emerged for some of the bacterial taxa among all the samples. For example, the Actinobacteria

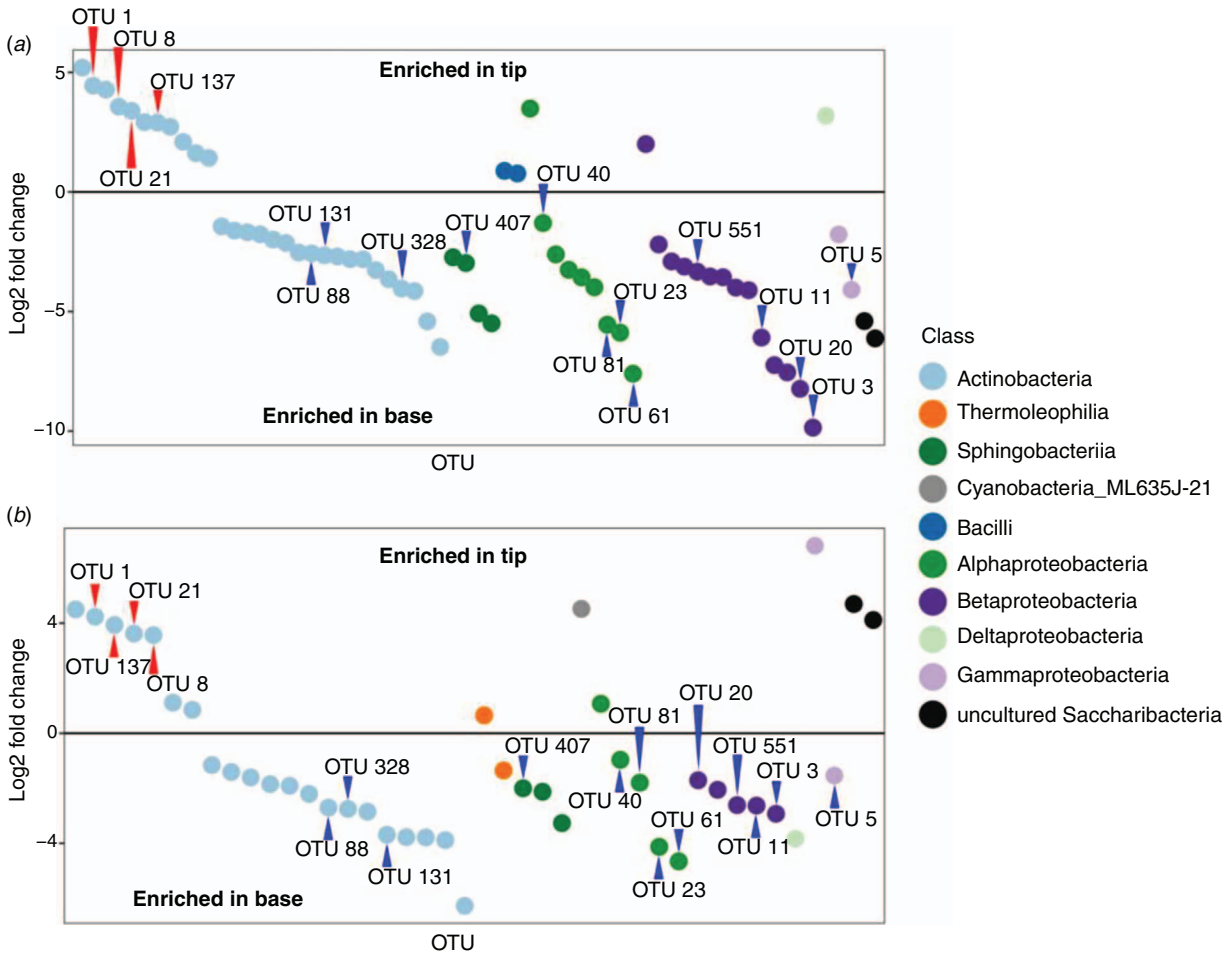

Fig. 3. *DESeq2* analysis of the bacterial OTUs from Experiment 1 showing different abundances on the root tips and root bases of plants. Data show only the OTUs that were significantly more abundant on the tip or base of (*a*) wheat and (*b*) rice roots (Benjamini-Hochberg adjusted $P < 0.05$). The OTUs that were significantly more abundant on the root tips of both wheat and rice are labelled with red arrows, and OTUs that were significantly more abundant on the root bases of both wheat and rice are labelled with blue arrows. For a full taxonomic list of OTUs, see Table S1.

were generally enriched in the root samples compared with the bulk soil, while others were more abundant in the bulk soil (Classes Alphaproteobacteria, Sphingobacteriia, Gemmatimonadetes, Gammaproteobacteria and Acidobacteria) (Fig. 5*b*). Members of the phyla Acidobacteria, Chloroflexi and Firmicutes were more abundant in the root tips than root bases (regardless of root type), while members of Classes Sphingobacteriia and Betaproteobacteria were more abundant in the nodal roots compared with seminal roots regardless of location on the root ($P < 0.05$) (Fig. 5*b*). A total of 94 bacterial OTUs showed significant differences in abundance between the root tips and root bases of seminal roots, and 70 OTUs showed differences between the root tips and root bases of nodal roots. The majority of these differences reflected a greater abundance at the root tips (Fig. 7). For further taxonomic classifications of these, see Table S3. A subset of 40 OTUs was enriched on the root tips of both the seminal and nodal roots compared with the root bases with most of these belonging to the Classes Actinobacteria, Thermoleophilia, Chloroflexi JG37-AG-4, Ktedonobacteria, Bacilli, Clostridia, Alphaproteobacteria and Gammaproteobacteria (Fig. 7; see Table S3). Six OTUs were more abundant on root bases of both seminal and nodal roots than on the root tips, and these belonged to the Actinobacteria and Betaproteobacteria (Families: Streptomycetaceae, Alcaligenaceae and Burkholderiaceae) (Fig. 7; see Table S3). The Actinobacteria OTUs enriched on root tips mostly belonged to Orders Corynebacteriales, Frankiales, Propionibacteriales and Pseudonocardiales, while those enriched on the root bases belonged to Orders Micrococcales and Streptomycetales (see Table S3). We identified 17 bacterial OTUs that showed significant differences in abundance between the seminal and nodal roots at the tips, and 17 OTUs that showed differences between the seminal and nodal roots at the bases (Fig. 8; see Table S4). Six Bacilli OTUs (five of them belonging to the Family Planococcaceae) were exclusively enriched at the seminal root tips compared with the nodal root tips, while

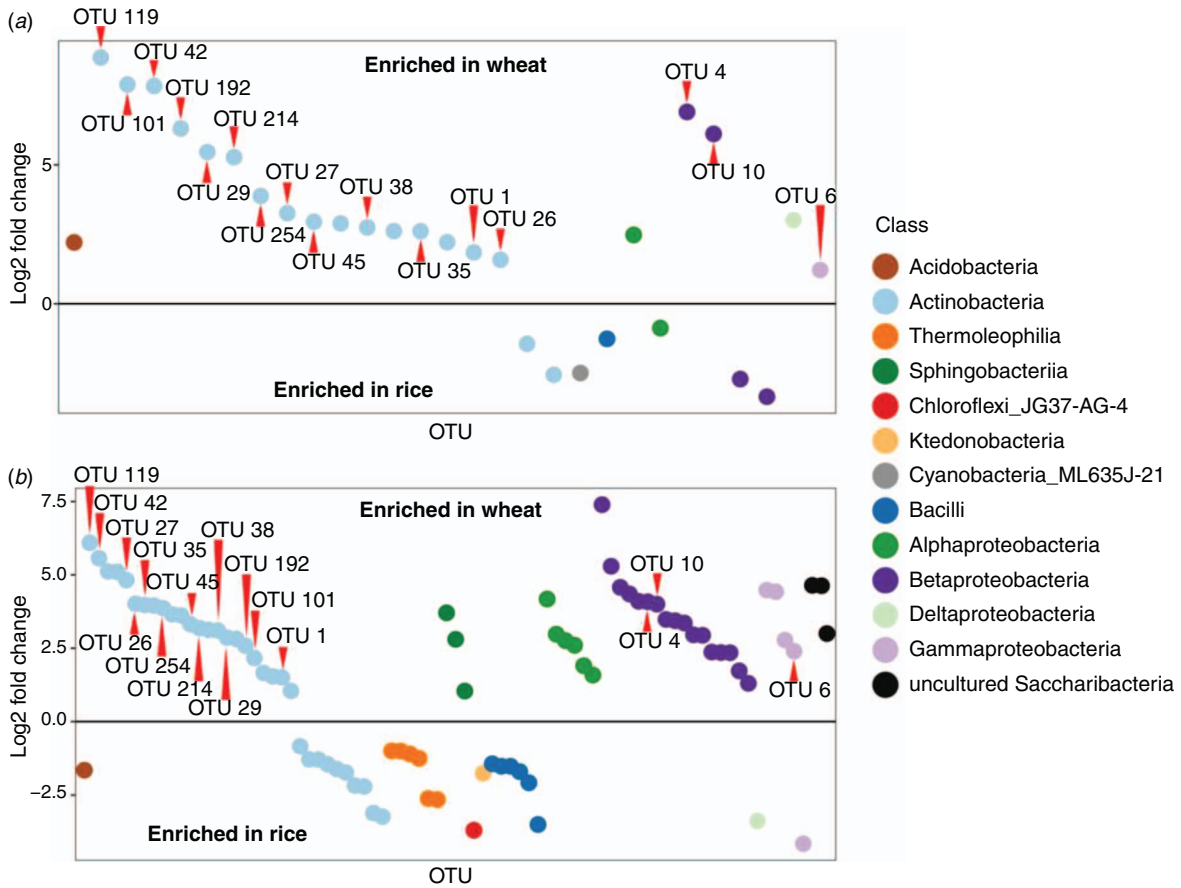

Fig. 4. *DESeq2* analysis of the bacterial OTUs from Experiment 1 showing different abundances on wheat and rice roots. Data show only the OTUs that were significantly more abundant on wheat or rice roots at the (*a*) root tips or the (*b*) root bases (Benjamini-Hochberg adjusted $P < 0.05$). The OTUs that were significantly more abundant in wheat roots than rice roots at the tips and bases are labelled with red arrows. No OTUs were significantly more abundant in rice roots than wheat roots at both root locations. For a full taxonomic list of OTUs, see Table S2.

Table 2. Alpha diversity indices of the bacterial and fungal communities from Experiment 2

Observed number of OTUs (Sobs), Chao1 and Shannon indices of the microbial communities in the tips and bases of seminal and nodal roots of wheat, and the bulk soil samples. Bulk soil data are a combination of samples collected from the top, middle and bottom layers of the pots. Data are means ± s.e. (*n*, 6–18). Different lowercase letters next to the values indicate significant differences between the samples (ANOVA, $P < 0.05$)

	Bacteria			Fungi		
	Sobs	Chao 1	Shannon	Sobs	Chao 1	Shannon
Bulk soil_8d	242.9 ± 2.8^{a}	389.3 ± 6.9^{a}	6.2 ± 0.1^{a}	137.6 ± 2.0^{a}	209.2 ± 4.0^{a}	4.7 ± 0.1^{a}
Bulk soil_31d	232.6 ± 3.4^{a}	379.8 ± 10.5^{a}	6.0 ± 0.1^{a}	155.4 ± 4.9^{b}	212.5 ± 7.0^{a}	4.8 ± 0.2^{a}
Seminal_Tip	215.3 ± 10.4^{a}	278.8 ± 19.5^{b}	6.3 ± 0.1^{a}	46.3 ± 4.6^{c}	71.8 ± 8.1^{b}	3.3 ± 0.3^{bd}
Seminal_Base	91.2 ± 4.0^{b}	216.3 ± 27.2^{b}	3.6 ± 0.1^{b}	62.5 ± 3.5^{cd}	99.8 ± 8.5^{bc}	2.7 ± 0.1^{bc}
Nodal_Tip	224.0 ± 20.3^{a}	367.0 ± 30.4^{a}	5.9 ± 0.5^{a}	51.2 ± 2.7^{c}	70.2 ± 6.0^{b}	2.1 ± 0.2^{c}
Nodal_Base	131.0 ± 11.2^{c}	257.2 ± 30.5^{b}	4.7 ± 0.3^{c}	78.3 ± 2.9^{d}	109.4 ± 7.3^{c}	3.9 ± 0.1^{d}

four Betaproteobacteria OTUs (Families Burkholderiaceae and Alcaligenaceae) were more abundant on nodal roots than seminal roots, regardless of position along the root (Fig. 8; see Table S4).

Among the fungi, members of the Class Sordariomycetes dominated most samples and fewer higher order taxa showed differences in abundance between the samples compared with the bacteria. (Fig. 6*b*). Members of the Class Eurotiomycetes were enriched on the seminal roots compared with nodal roots and were generally more abundant on the root bases. The Dothideomycetes and Mortierellomycetes were more abundant at the tips of seminal roots compared with the tips of nodal roots (Fig. 6*b*). For the seminal roots, 34 fungal OTUs (mostly classes Dothideomycetes, Eurotiomycetes, Sordariomycetes

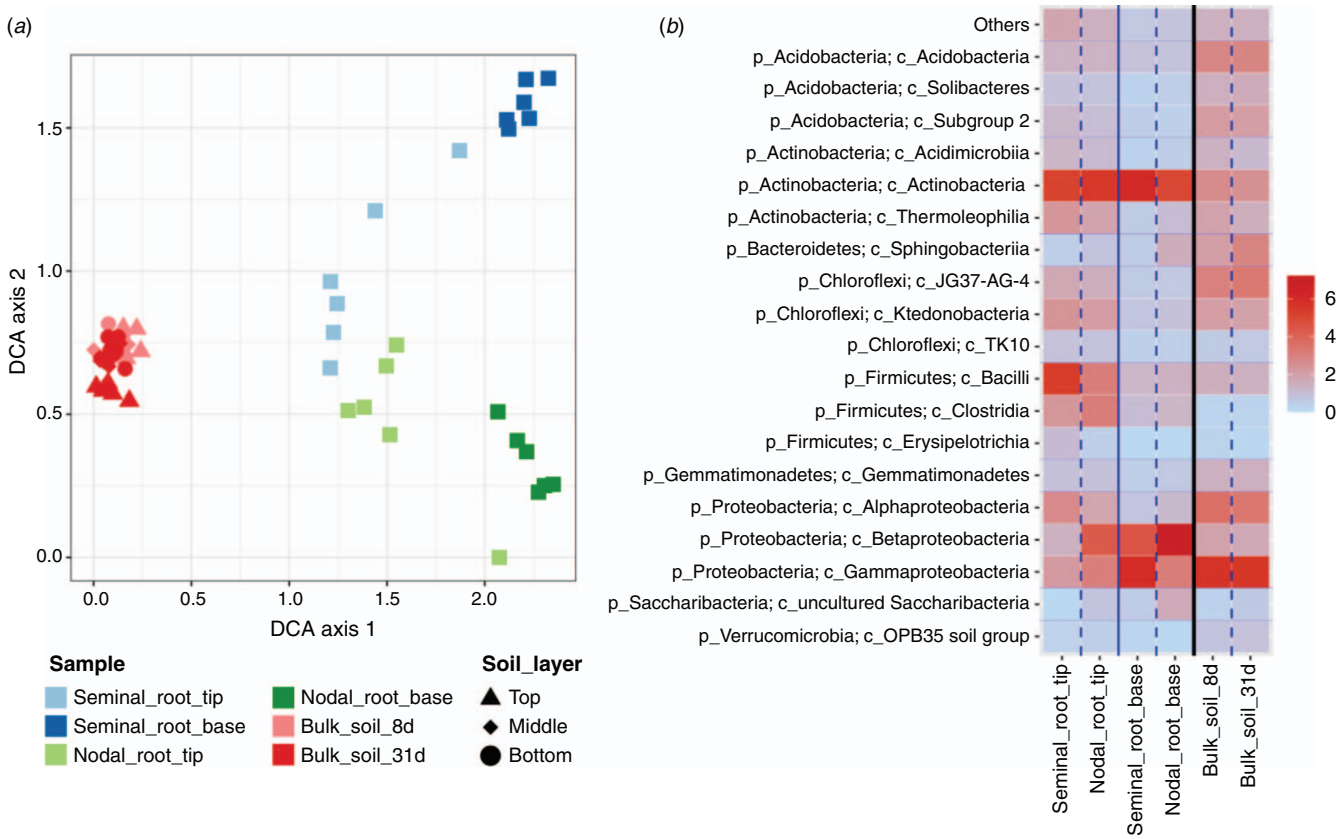

Fig. 5. Bacterial community structures in the root and bulk soil samples from Experiment 2. (*a*) Detrended correspondence analysis (DCA) illustrates the differences in the community structure between the wheat seminal and wheat nodal roots (tips and bases), and the bulk soil samples collected from the top, middle and bottom layers of pots at 8 days and 31 days (*n*, 6). (*b*) Heatmap shows the relative abundance of the major bacterial phyla (p__) and classes (c__) found on the wheat roots and in the bulk soil samples. For the bulk soil samples, data from the top, middle and the bottom layers were combined. Minor bacterial classes (<0.5% in the abundance in any single sample) were compiled into 'Others'. Data are means (*n*, 6–18) and the colour scale represents the square root of abundance.

and Mortierellomycetes) were significantly more abundant at the root tips than root bases of seminal roots, and 11 OTUs were more abundant at the bases than tips (mostly Classes Eurotiomycetes and Sordariomycetes) (Fig. 9*a*; see Table S5). For nodal roots, only four OTUs from the Orders Capnodiales, Eurotiales and Hypocreales were significantly more abundant at the tips than bases while 24 OTUs were more abundant at the basal root tissue than root tips (mostly classes Dothideomycetes, Eurotiomycetes, Leotiomycetes, Orbiliomycetes and Sordariomycetes) (Fig. 9*b*; see Table S5). A single *Cladosporium* sp. (OTU #44) was more abundant in the tips of both seminal and nodal roots and seven OTUs (Genera: *Ophiosphaerella*, OTU #23; *Penicillium*, OTU #4 and #10; *Trichoderma*, OTU #49; unidentified Chaetothyriales, OTU #8; unidentified GS33, OTU #17; unidentified Glomerellales, OTU #31) were more abundant at the bases of both seminal and nodal roots (Fig. 9; see Table S5).

We also identified fungal OTUs that were significantly different in abundance between root types. Twenty eight fungal OTUs showed significant differences in abundance between the seminal or nodal roots at the tips (Fig. 10*a*) and 30 OTUs showed different abundances between the seminal and nodal roots at the bases (Fig. 10*b*; see Table S6). Three OTUs that were enriched at the tips and bases of seminal roots compared with nodal roots were from the *Mortierella* (OTU #21), *Penicillium* (OTU #62) and unidentified Chaetomiaceae (OTU #19). Only two OTUs of *Fusarium* sp. (OTU #3 and #7) showed an inconsistent enrichment with location because they were more abundant at the root tip in nodal roots but more abundant at the root base in seminal roots (Fig. 10; see Table S6).

Discussion

This study tested specific hypotheses concerning the spatial variation in the root microbiome of wheat and rice. We demonstrated that the root tips had significantly different bacterial and fungal communities from the root bases, the seminal roots were different from the nodal roots and all root communities were different from those in the bulk soil. The significant differences between the seminal and nodal roots cannot be attributed to the different sampling times for the following reasons. Root growth occurs from the tips which

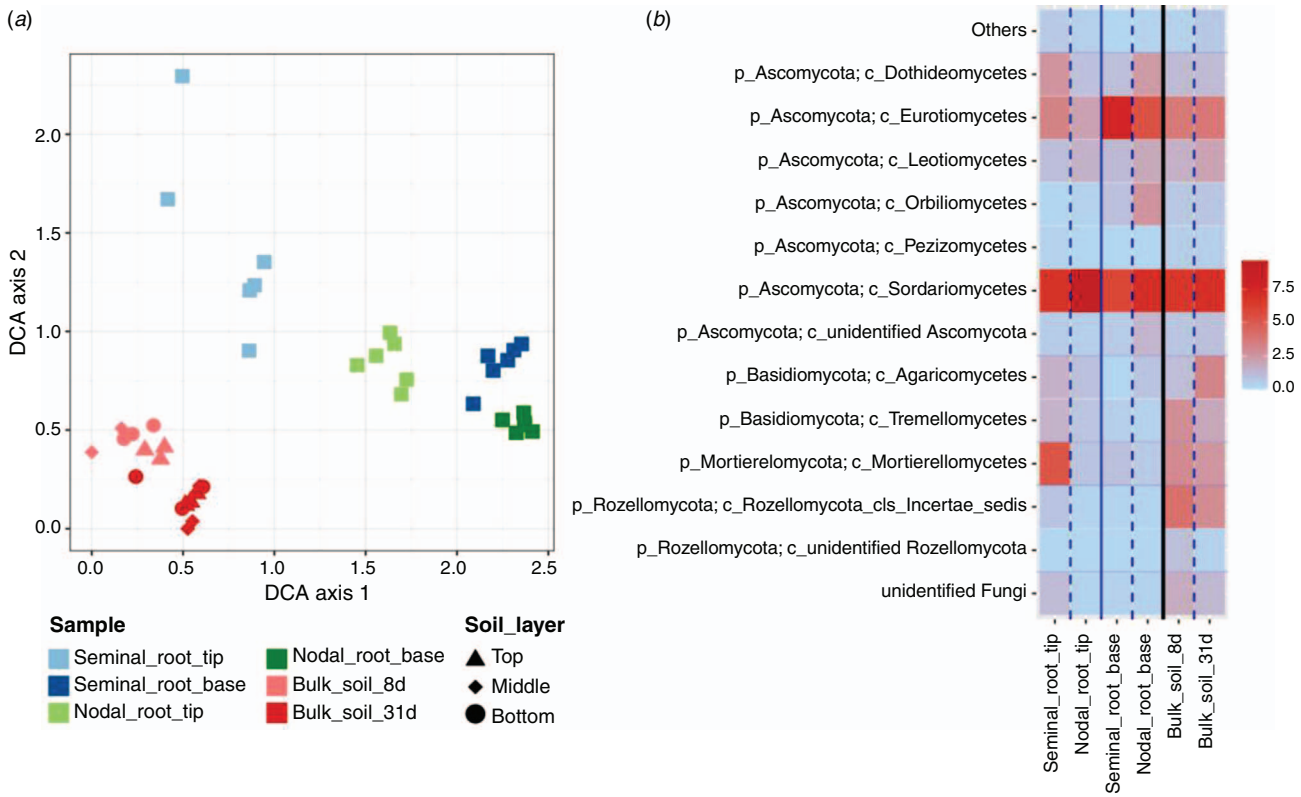

Fig. 6. Fungal community structures on the roots and bulk soil samples from Experiment 2. (*a*) Detrended correspondence analysis (DCA) shows differences in the fungal community structures between the wheat seminal and nodal roots (tip and base), and the bulk soil samples (from the top, middle and bottom layers of pots at 8 days and 31 days incubation periods) (*n*, 3–6). (*b*) Heatmap shows the relative abundance of the major fungal phyla (p__) and classes (c__) found on the wheat roots and in the bulk soil samples. Bulk soil data are a combination of the samples from the top, middle and the bottom layers. Minor fungal classes (<0.5% in the abundance in any single sample) were compiled into 'Others'. Data are means (*n*, 6–9) and the colour scale represents the square root of abundance.

means the cells and tissues in that apical region will have similar ages regardless of when the roots first emerged (assuming similar growth rates). Additionally, for the basal tissues, our sampling protocol minimised the age differences between the root types. New nodal roots emerge continuously throughout tillering, which means a proportion of the shorter nodal roots will be of a similar age as the seminal roots when sampled.

The differences between the root tips and root bases were larger than the differences between seminal and nodal roots, within each experimental soil type. This was especially true for bacteria where almost five-fold more OTUs differed in abundance between the root tips and roots bases (Fig. 7) than between seminal roots and nodal roots (Fig. 8). More importantly, the differences in microbiome structure along individual roots were comparable to the differences detected between the two species of wheat and rice. This reinforces the importance of trying to capture this variation more routinely.

The ordination plots from Experiment 2 (Figs 5*a*, 6*a*) revealed another trend. A transition emerged in bacterial and fungal communities starting from the bulk soil to the root tips and then to the root bases. In other words, the largest differences in microbiome structure occurred between the bulk soil and the root bases with the root tip communities tending to fall in-between. The same trend appeared in the estimates of α diversity. For the bacterial communities in wheat, α diversity on the root bases was consistently lower than the bulk soil while diversity on the root tips was more variable, sometimes similar to the bulk soil and sometimes lower (Tables 1, 2). Dennis *et al*. (2008) found a similar trend by mapping microbial diversities along roots at an even finer scale. Watt *et al*. (2003) concluded that the rate of root growth is a contributing factor to the structure of microorganism communities along roots. As the growing root tips move through the soil, they allow less time for microorganisms to be recruited and influenced by that local environment (Watt *et al*. 2003; Dennis *et al*. 2008). The influence of growth rate on colonisation patterns was modelled by Zelenev *et al*. (2000) and subsequently used in kinematic studies by Watt *et al*. (2006*a*) and Dupuy and Silk (2016). Assuming a typical growth rate for wheat roots growing in pots of 1.0 mm h^{-1} (Watt *et al*. 2006*b*), then the oldest cells in the 1.0 cm long samples from the root tip would be ~10 h old. *Bacillus subtilis* was unable to form biofilms on the root tips of *Arabidopsis* in that time in gnotobiotic conditions (Massalha *et al*. 2017). Maloney *et al*. (1997) also noted that the microbial populations

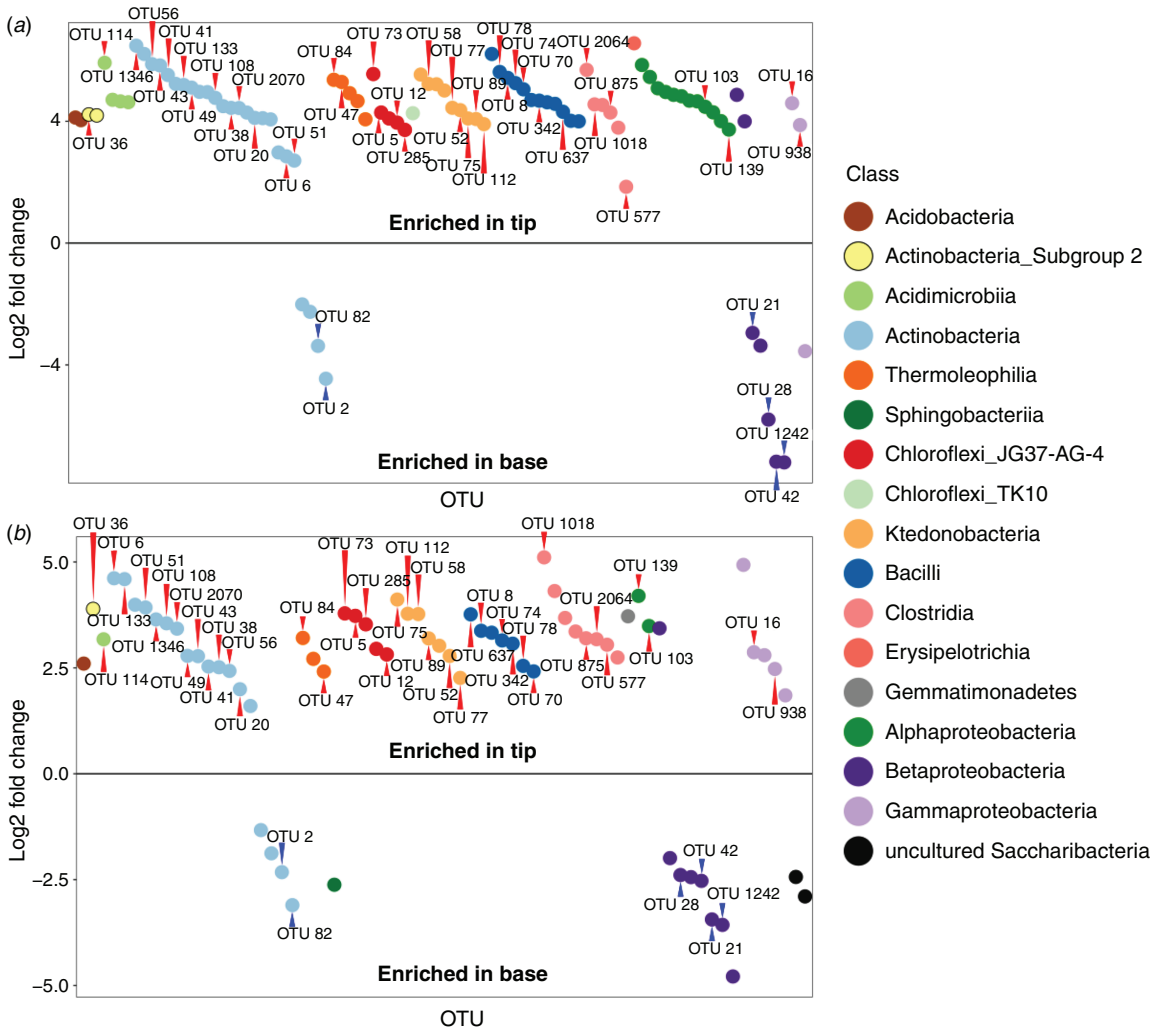

Fig. 7. *DESeq2* analysis of the bacterial OTUs in Experiment 2 showing different abundances along the length of different root types. Data show only the bacterial OTUs that were significantly more abundant on the tips or the bases of (*a*) seminal and (*b*) nodal roots of wheat (Benjamini-Hochberg adjusted $P < 0.05$). The OTUs that were significantly more abundant on the tips of both seminal and nodal roots are labelled with red arrows, and OTUs that were significantly more abundant on the bases of both seminal and nodal roots are labelled with blue arrows. For a full taxonomic list of these OTUs, see Table S3.

colonising the tip might not necessarily reflect the exudates occurring from that region because as the root grows through the soil, the exudates and rhizodeposits from the tips are left behind and linked with more mature tissues. Resolving the role of root growth rate on microbial colonisation is challenging since it would require nearly simultaneous measurements of the root tip position with spatial information on the microbial communities and root exudates.

Rhizodeposits, including root exudates, represent a rich source of nutrients which explains why copiotrophic organisms tend to proliferate around roots (Paterson *et al*. 2007). Therefore, the variation in microbiome structure between different root types and at different locations will be partly explained by differences in the volume and composition of compounds released from the various tissues (Dennis *et al*. 2010). A large proportion of rhizodeposition occurs near the root tips in the form of organic compounds, mucilage, and sloughed-off cells (Farrar *et al*. 2003; Nguyen 2003). Indeed, some exudates are exclusively released from the root tips including phytosiderophores for iron uptake (Marschner *et al*. 1987) and organic anions for detoxifying aluminium (Delhaise *et al*. 1993; Ryan *et al*. 2009). By contrast, more of the organic carbon available around root bases is derived from cortical shedding, root lysates and microbial activity (Dennis *et al*. 2010).

In this study, Betaproteobacteria, Actinobacteria and Bacilli were generally enriched on and within the roots compared with the bulk soil (Figs 2*b*, 5*b*). Betaproteobacteria are well known to be copiotrophic and Actinobacteria can be copiotrophic in some environments (Fierer *et al*. 2007; Ho *et al*. 2017). The enrichment of these two taxa on roots has been previously reported in several other species (Chelius and Triplett 2001; DeAngelis *et al*. 2009; Bulgarelli *et al*. 2012; Lundberg *et al*. 2012; Donn

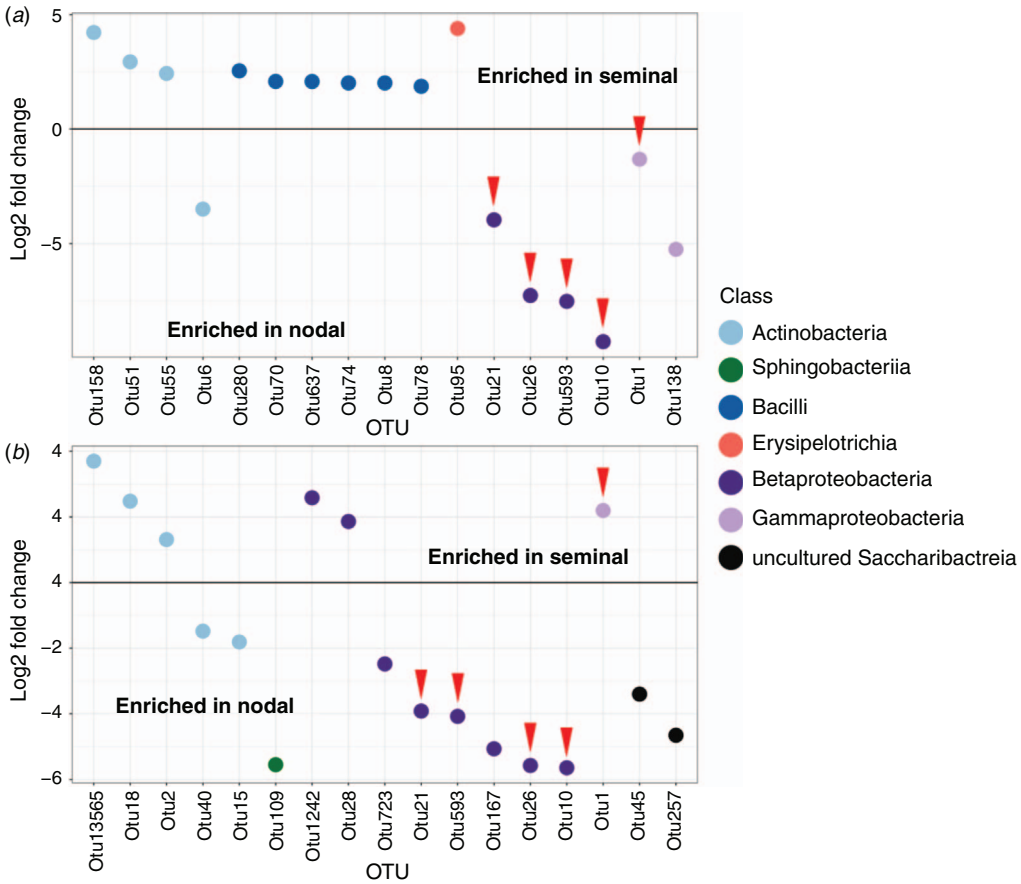

Fig. 8. *DESeq2* analysis of the bacterial OTUs in Experiment 2 showing different abundances between the seminal and nodal roots at different positions. Data show only the bacterial OTUs that were significantly more abundant on the seminal or the nodal at the (*a*) root tips and (*b*) root bases of wheat (Benjamini-Hochberg adjusted $P < 0.05$). The OTUs that showed differential abundance between seminal and nodal roots at both tips and bases are labelled with red arrows. For a full taxonomic list of these OTUs, see Table S4.

et al. 2015; Edwards *et al*. 2015). Actinobacteria colonisation can change during periods of stresses (Naylor *et al*. 2017) and some taxa are disease-suppressive (Mendes *et al*. 2011; Palaniyandi *et al*. 2013). Classes of bacteria that remained more abundant in the bulk soil than the roots included members of the Acidobacteria, Chloroflexi (JG37-AG-4), Alphaproteobacteria, and Gemmatimonadetes, which is consistent with many members of these taxa being soil oligotrophs (Fierer *et al*. 2007; Peiffer *et al*. 2013; Ho *et al*. 2017).

Distinct patterns were detected in the relative abundance of certain bacterial and fungal OTUs at the root tips compared with the root bases, and on the seminal roots compared with nodal roots. Maloney *et al*. (1997) noted that the ratio of copiotrophic to oligotrophic bacteria along roots varied with plant species because copiotrophic species were more abundant at the root bases than root tips of tomato (*Solanum lycopersicum* L.), but the reverse was found for lettuce (*Lactuca sativa* L.). The present study indicates that this ratio might also vary with soil type. In the Chromosol, the bacteria enriched on the base of wheat roots included likely oligotrophs (Sphingobacteriia, Alphaproteobacteria) and copiotrophs (Orders: Burkholderiales, Catenulisporales, Micromonosporales, Pseudonocardiales and Streptomycetales) whereas in the Ferrosol, only likely copiotrophs were enriched on the root bases (Orders: Micrococcales, Burkholderiales and Streptomycetales). The enrichment of Burkholderiales on roots has been reported in other plant species (Peiffer *et al*. 2013; Kawasaki *et al*. 2016; Aguirre-von-Wobeser *et al*. 2018); however, this report is the first to demonstrate that the abundance of some members is consistently greater at the root bases than the root tips. The Burkholderiales can be endophytic and also form endosymbiotic relationships with arbuscular mycorrhizae and have both beneficial and pathogenic effects on the host (Bianciotto *et al*. 1996; Coenye and Vandamme 2003; Schlaeppi *et al*. 2014). The Streptomycetales are common soil bacteria (especially genus *Streptomyces*) that can also become endophytic. Some members have growth-promoting properties, perhaps by releasing antibiotic compounds that suppress the pathogenicity of other organisms (Schrey and Tarkka 2008; Vurukonda *et al*. 2018; Suárez-Moreno *et al*. 2019). The present study did not differentiate between endophytes and the organisms colonising the root surfaces, so

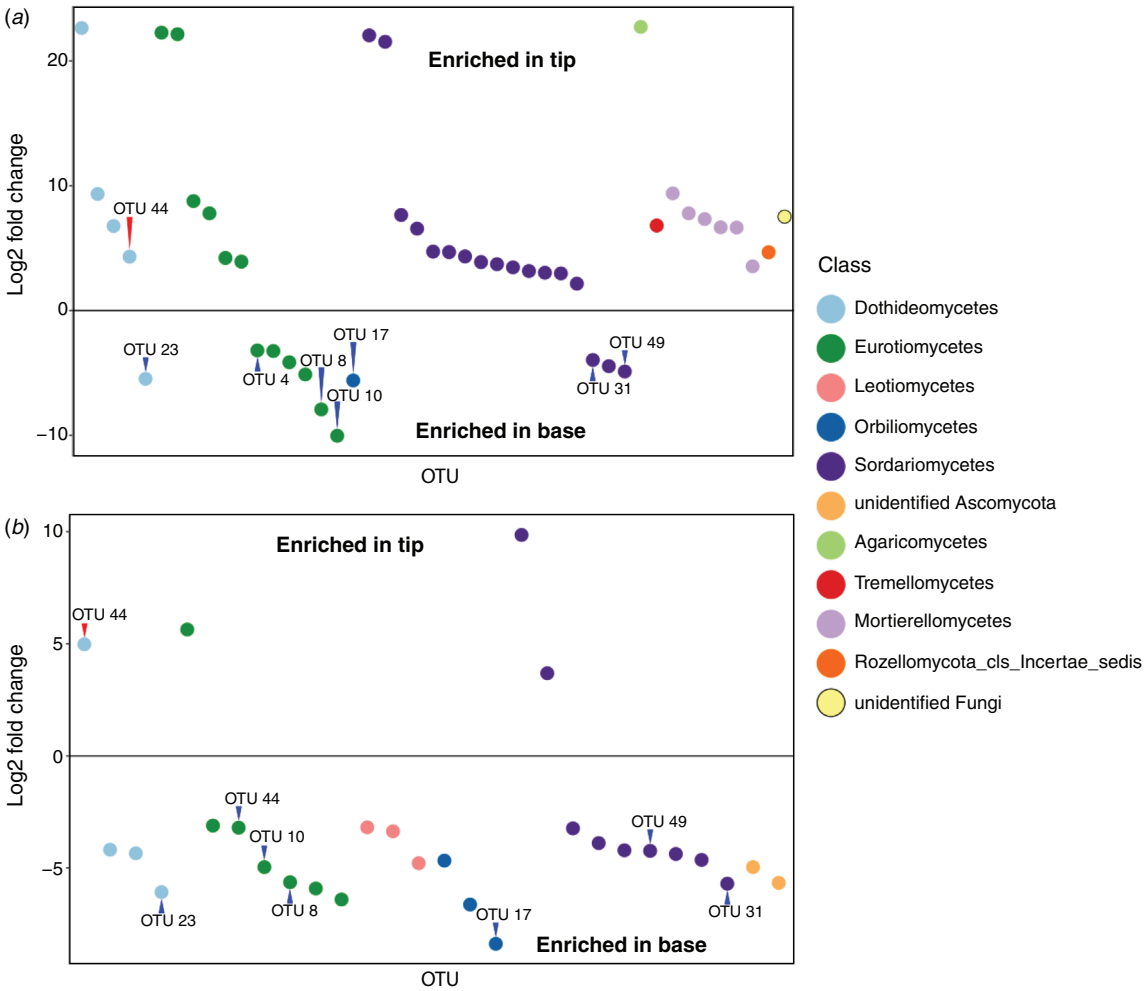

Fig. 9. *DESeq2* analysis of the fungal OTUs from Experiment 2 showing different abundance along the length of different root types. Data show only the OTUs that were significantly more abundant on the root tips or the root bases of (*a*) seminal and (*b*) nodal roots of wheat (Benjamini-Hochberg adjusted $P < 0.05$). The OTUs that were significantly more abundant in the tips of both seminal and nodal roots are labelled with red arrows, and OTUs that were significantly more abundant at the bases of both seminal and nodal roots are labelled with blue arrows. For a full taxonomic list of these OTUs, see Table S5.

it is possible that some of the bacterial OTUs are endophytic. Endophytes are more likely to occur in the basal part of the root than the root tips because the cells are fully expanded and there is more time for the organisms to establish a relationship with older plant cells. Furthermore, root hairs and cracks that form when lateral roots emerge from mature tissues can be major entry points for endophytic microorganisms (Kandel *et al*. 2017). A practical difference between the tissues sampled from the root tip and root base is that the basal root samples included lateral roots. The presence of lateral roots will tend to reduce the differences between the root tips and bases to some extent (Dennis *et al*. 2010).

OTUs from the Genera *Nocardioides* and *Marmoricola* (Order Propionibacteriales) were consistently more abundant at the root tips than the root bases in wheat and rice. The members of Propionibacteriales have been associated with roots previously (Lee *et al*. 2011; Bouam *et al*. 2018), but this is the first study showing their preference for younger growing tissues. Some OTUs were exclusively enriched at the tips of seminal roots compared with the tips of nodal roots (Family Planococcaceae), and others from the Family Burkholderiaceae were more enriched on nodal roots compared with seminal roots regardless of the position. These robust differences between root types most likely reflect differences in rhizodeposits from these tissues the details of which remain unknown.

Among the fungi, Sordariomycetes dominated all samples, which is likely explained by the Ferrosol being collected from a grazed pasture paddock because many members of the Sordariomycetes proliferate on animal dung (Maharachchikumbura *et al*. 2016). OTUs of Class Eurotiomycetes were consistently more abundant on seminal roots than nodal roots and those from Eurotiomycetes and Leotiomycetes were more abundant at the root bases compared with tips, which could be related to their tendency to be endophytic (Wang *et al*. 2006; Bei *et al*. 2019; Fernández-

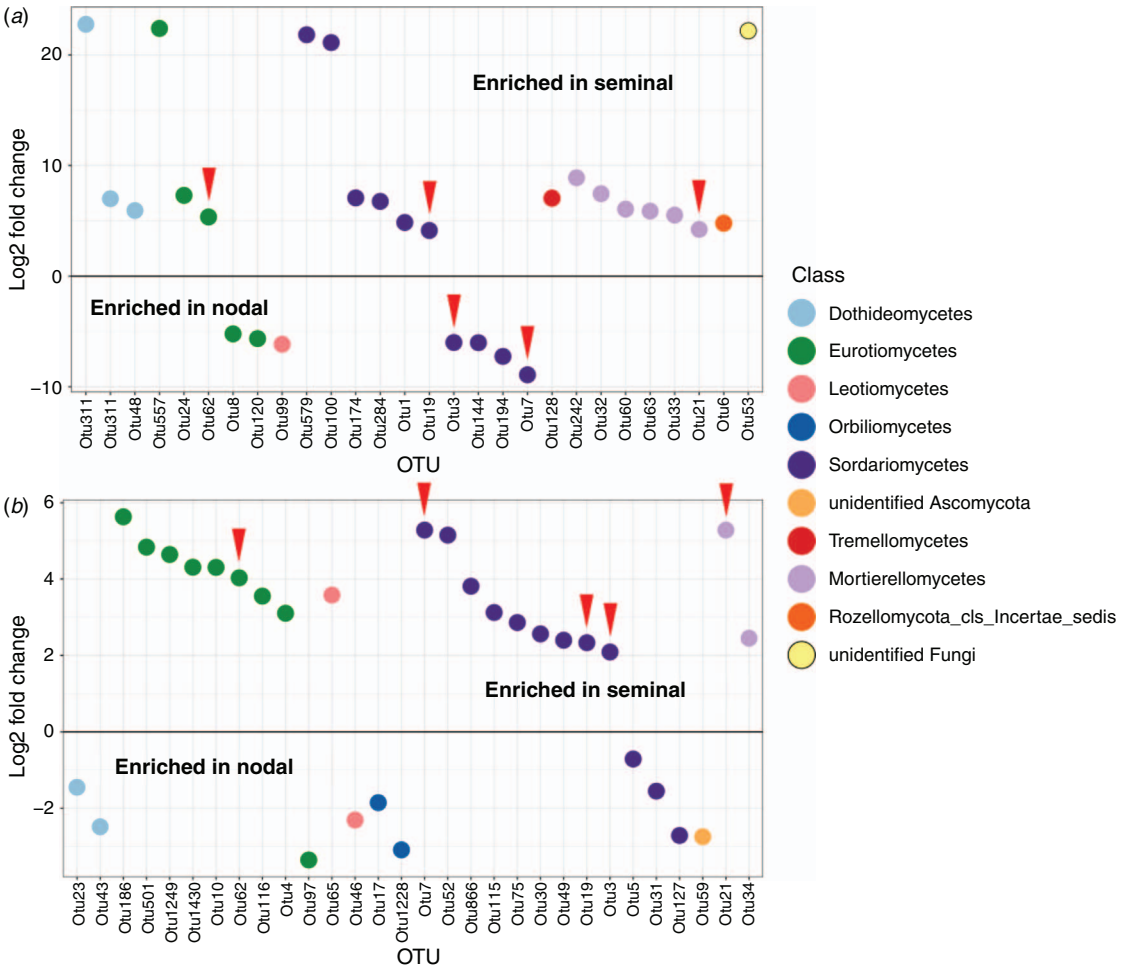

Fig. 10. *DESeq2* analysis of the fungal OTUs from Experiment 2 showing different abundance between the seminal and nodal roots at different locations. Data show only the fungal OTUs that were significantly more abundant on the seminal or the nodal at the (*a*) root tips and (*b*) root bases of wheat (Benjamini-Hochberg adjusted $P < 0.05$). The OTUs that showed differential abundance between seminal and nodal roots at both tips and bases are labelled with red arrows. For a full taxonomic list of these OTUs, see Table S6.

González *et al*. 2019). Many fungal endophytes grow through plant tissues intercellularly by hyphal extension (Rodriguez *et al*. 2009) and this might result in different patterns of microsite occupation compared with the biofilms generated by bacteria. OTUs from the Genus *Mortierella* were singularly most abundant on the tips of seminal roots. This group is rarely endophytic (Xu *et al*. 2012; Gkarmiri *et al*. 2017) and some species are known to increase phosphate solubilisation in the rhizosphere (Zhang *et al*. 2011). A single fungal OTU from the Dothideomycetes was enriched in the root tips of both seminal roots and nodal roots, whereas seven OTUs from various Classes were enriched in the bases of both seminal and nodal roots.

Bacterial abundance along roots is not solely determined by local organic carbon concentrations (Semenov *et al*. 1999). Indeed, Dennis *et al*. (2008) argues that the influence of exudates could be restricted to the root tips whereas rhizodeposits and even the variation in physical environment could affect microbiomes more in other regions. For example, pH changes along the length of roots and differences of up to one unit can occur at locations only 1.0 mm apart. This large variation over a small distance will have a significant impact on microbiome composition (Dennis *et al*. 2009). Soil strength can also influence the recruitment of microorganisms by affecting root growth (Watt *et al*. 2006*b*).

Conclusions

Most investigations of the root microbiome begin by sampling whole root systems or random sub-samples of it. While these descriptions have proved very useful for demonstrating gross differences in microbiome structure, they inevitably represent an averaging of all the communities present on the tissue. We demonstrated that the variation in community structure along the length of roots and between different types of roots can be comparable to the differences between plant species. Indeed, there is evidence that the spatial and temporal variation of the microbiome at finer scales is likely to be even more complex than those described in this study (Dennis *et al*. 2008). Just as the horizontal stratification of root microbiomes into the

rhizosphere soil, rhizoplane and endorhizosphere has received much interest, we propose that more attention should be paid to the substantial variation within the root system. While this poses extra challenges to an already challenging area, potentially important interactions will be missed if microbiome descriptions continue to be based on large, random sub-samples of the root system. Our understanding of the biological and physico-chemical interactions that drive this spatial variation needs to be improved so they can be better linked with root function and plant health.

We propose that standardised protocols for collecting roots from monocotyledons and eudicots need to be developed so that more consideration can be given to the tissues sampled. For instance, single intact roots could be excised from the seed or crown and divided into different sections or more details recorded on the approximate ratios of seminal to nodal roots, axile to lateral roots, root age or at least younger roots to older roots. Such information could also reduce the variation between replicates and reveal more subtle patterns. This is important because the local proliferation of beneficial or pathogenic microorganisms can have large effects on plant growth and health. For example, Chin-A-Woeng *et al*. (1997) concluded that localised, high-density colonies of the biocontrol strain *Pseudomonas fluorescens* WCS365 were ideal for the efficient accumulation of *N*-acyl-L-homoserine lactones and subsequent antibiotic production. By paying closer attention to the spatial variation in the root microbiome, important patterns may emerge across species and environments that reflect fundamental plant–microbe interactions related to resource acquisition or pathogen defence. Identifying these patterns and understanding the interactions occurring at finer scales could also expedite ongoing attempts to engineer the rhizosphere to benefit plant growth. Once important microhabitats are identified, they could be targeted for manipulation using biotechnological techniques to either enhance their function or to protect them from competition or other inhibitory factors.

Conflicts of interest

The authors declare no conflicts of interest.

Declaration of funding

This study was supported by a CSIRO OCE postdoctoral fellowship to AK. MW is supported by the University of Melbourne Botany Foundation.

Acknowledgements

The authors acknowledge many helpful comments from Vadakattu VSR Gupta.

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Handling Editor: Thomas Roberts