***New Phytologist* Supporting Information**

Article title: **Species-specific Root Microbiota Dynamics in Response to Plant-Available Phosphorus**

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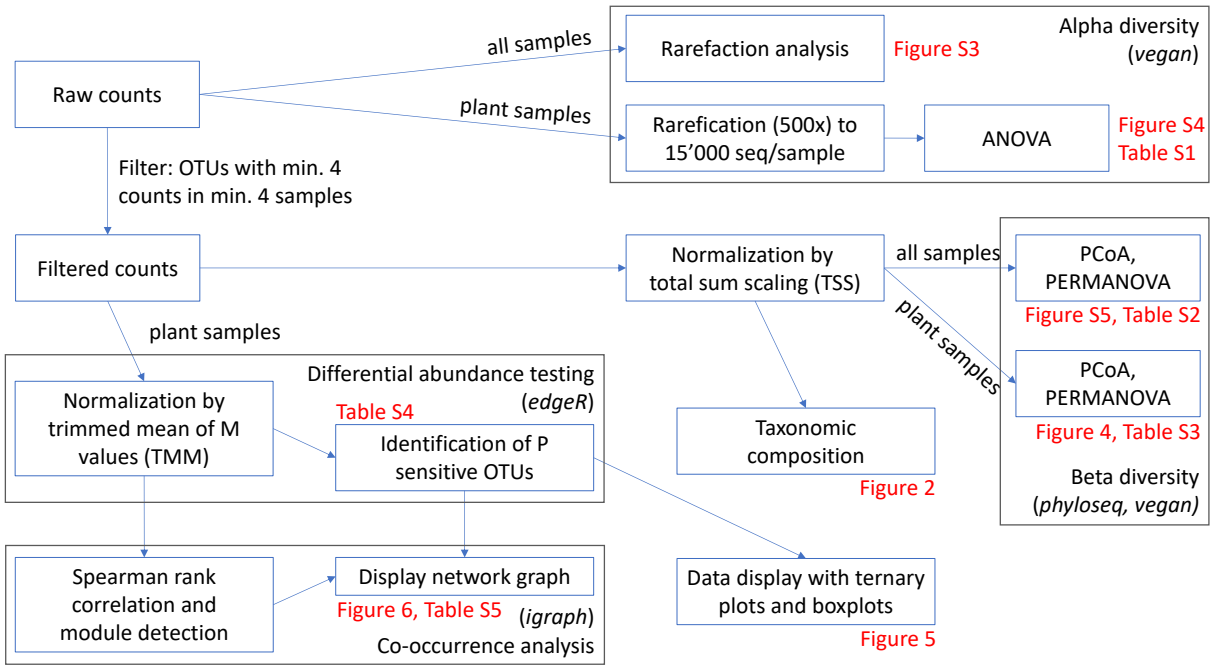


**Figure S1 | Analysis steps**

The schematic flow diagram illustrates the steps of the analysis in R. Individual types of normalization steps, analyses or statistical tests are indicated with the blue boxes. Larger grey boxes segment the analysis and indicate the major R-packages that were used in alpha- and beta diversity analyses, differential abundance testing and network analysis. Analysis outputs (Figures and Tables) are indicated in red at their respective analysis steps.

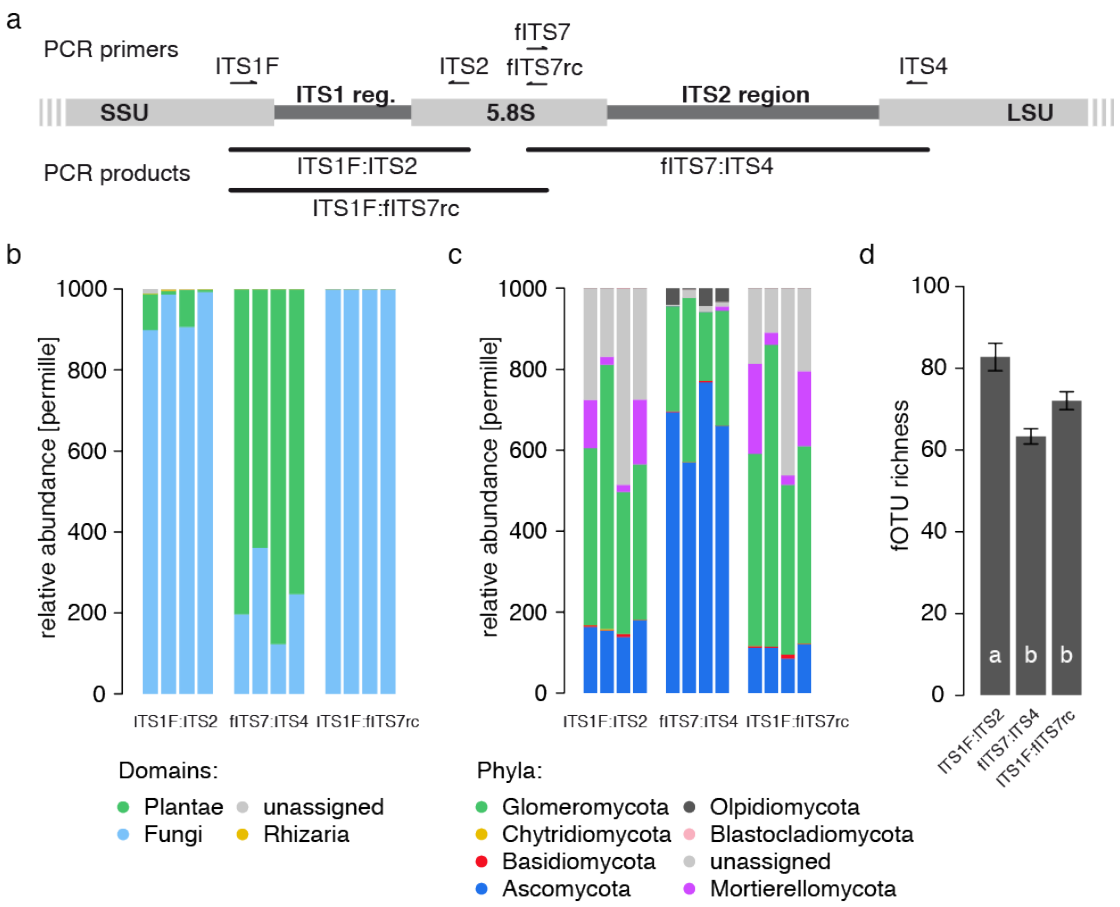


**Figure S2 | Comparison of ITS PCR approaches for plant root samples**

To profile the root-associated fungal communities of Petunia, we first evaluated three ITS PCR approaches to test whether they avoid co-amplification of plant ITS sequences and whether they permit a reliable quantification of Glomeromycotina fungi. Four root DNA extracts from Petunia growing under low P conditions (=heavily colonized by AMF) were amplified with ITS1F and ITS2 by McGuire et al. (2013), fITS7 and ITS4 by Ihrmark et al. (2012) and the ITS1F with the reverse complement of fITS7. (**a**) The illustration depicts the positions of the PCR primers amplifying the internal transcribed spacer (ITS) regions between the small- and large ribosomal sub-units (SSU/LSU) of the ribosomal operon. Separate community profiles were produced and inspected for the proportions of plant and AMF sequences as well as for fungal diversity. Taxonomic composition of the 4 replicate extracts is reported at the level of Domain (**b**) and within the fungi at the level of detected Phyla (**c**). (**d**) The diversity captured by the PCR approaches was determined by rarefying the fungi data and recording OTU richness. Bars represent means (n = 100; ± s.e.m.) and letters indicate groups differing significantly at $P < 0.05$ (Tukey's HSD).

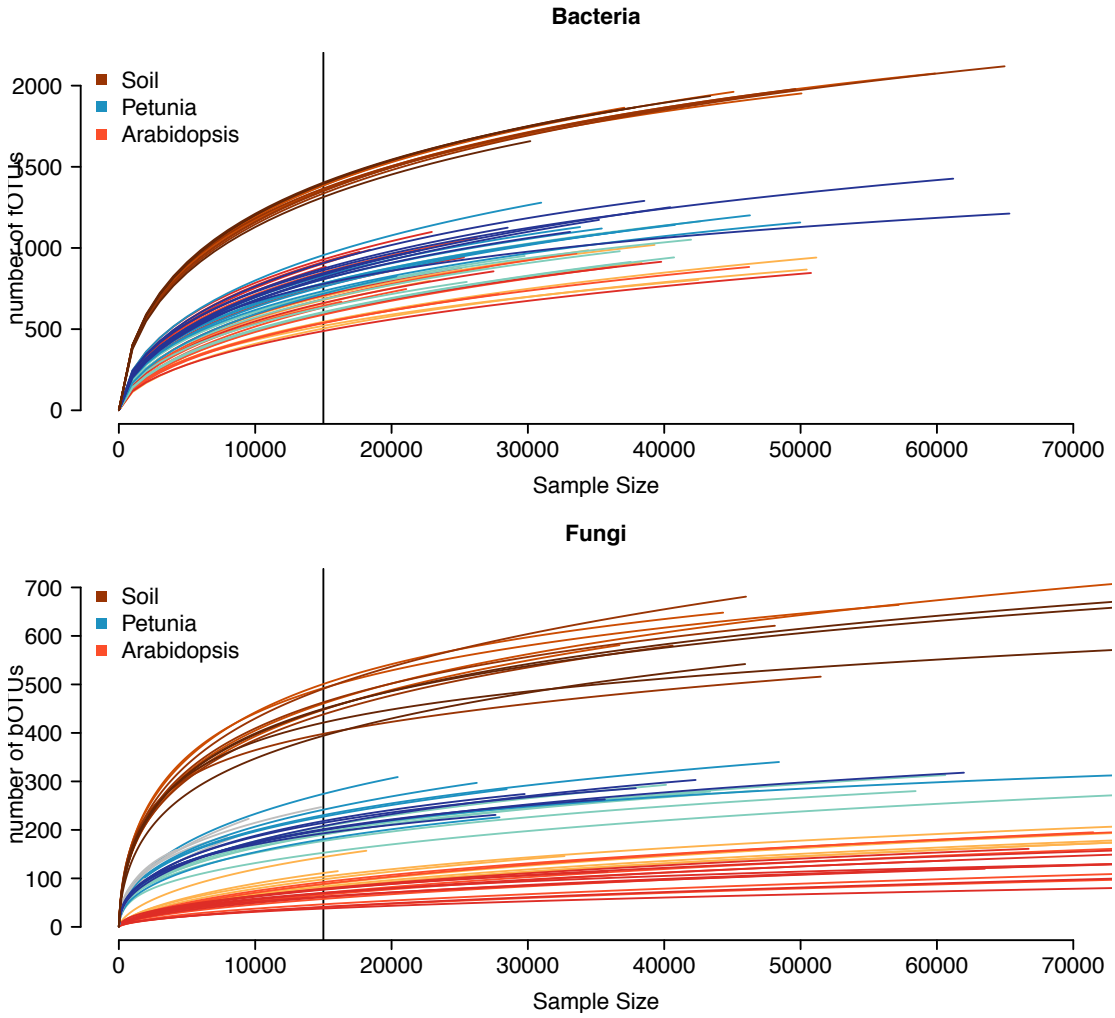


**Figure S3 | Rarefaction curves for bacterial and fungal OTU richness**

We conducted a sampling intensity analysis for bacteria (**a**) and fungi (**b**) with all samples (Arabidopsis and Petunia with reddish and blueish colors, respectively, and the increasing P-levels (low, medium to high) are marked with increasing hue. Random sub-samplings were conducted for sequencing depths in steps of 100 sequences with 1000 iterations per sequencing depth. The average number of detected OTUs is reported for each sampling depth. The black vertical line indicates the selected rarefaction depth (15,000 sequences) used statistical analysis of alpha diversity (**Fig. S4**).

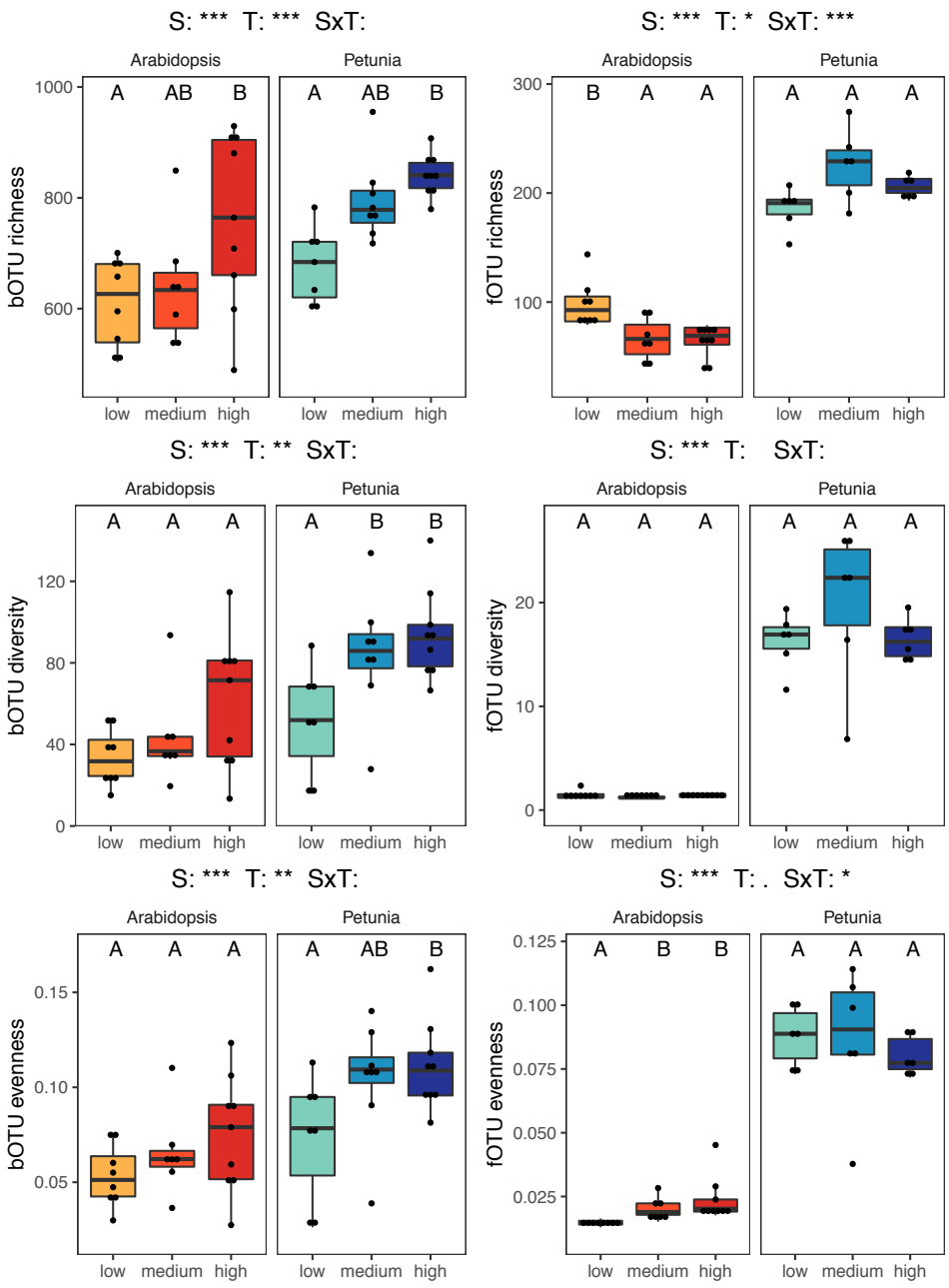


**Figure S4 | Effects of plant species and P-levels on microbial richness, diversity and evenness**
Alpha diversity was assessed based on OTU richness, Shannon diversity and Sheldon evenness on data with a common sequencing depth of 15'000 sequences per sample. ANOVA was used to test for species- (S), treatment- (T) or their interaction (SxT) effects and their level of significance is indicated above plots ($P < 0.001$ ***; $P < 0.01$ **; $P < 0.05$ *; **Table S1** contains the details of this ANOVA). Different letters indicate significant pairwise differences between different levels of P availability ($P < 0.05$, Tukey HSD).

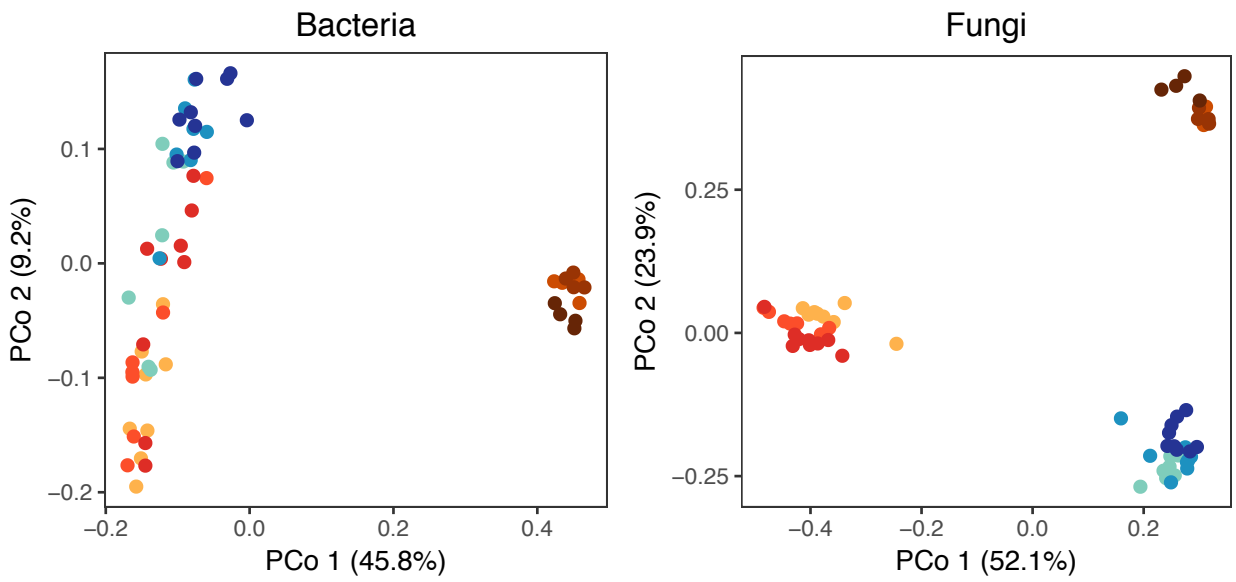


**Figure S5 | Beta-diversity analysis including the soil samples**

Principal coordinate analysis using Bray-Curtis dissimilarities were performed to investigate effects of plant species and P-levels on community composition. (**a**) Bacterial and (**b**) fungal communities associated with Arabidopsis roots (reddish colors), with Petunia roots (blueish colors) and found in unplanted soil (brownish colors), all sampled from varying levels of P availability (low, medium to high, marked with increasing hue).

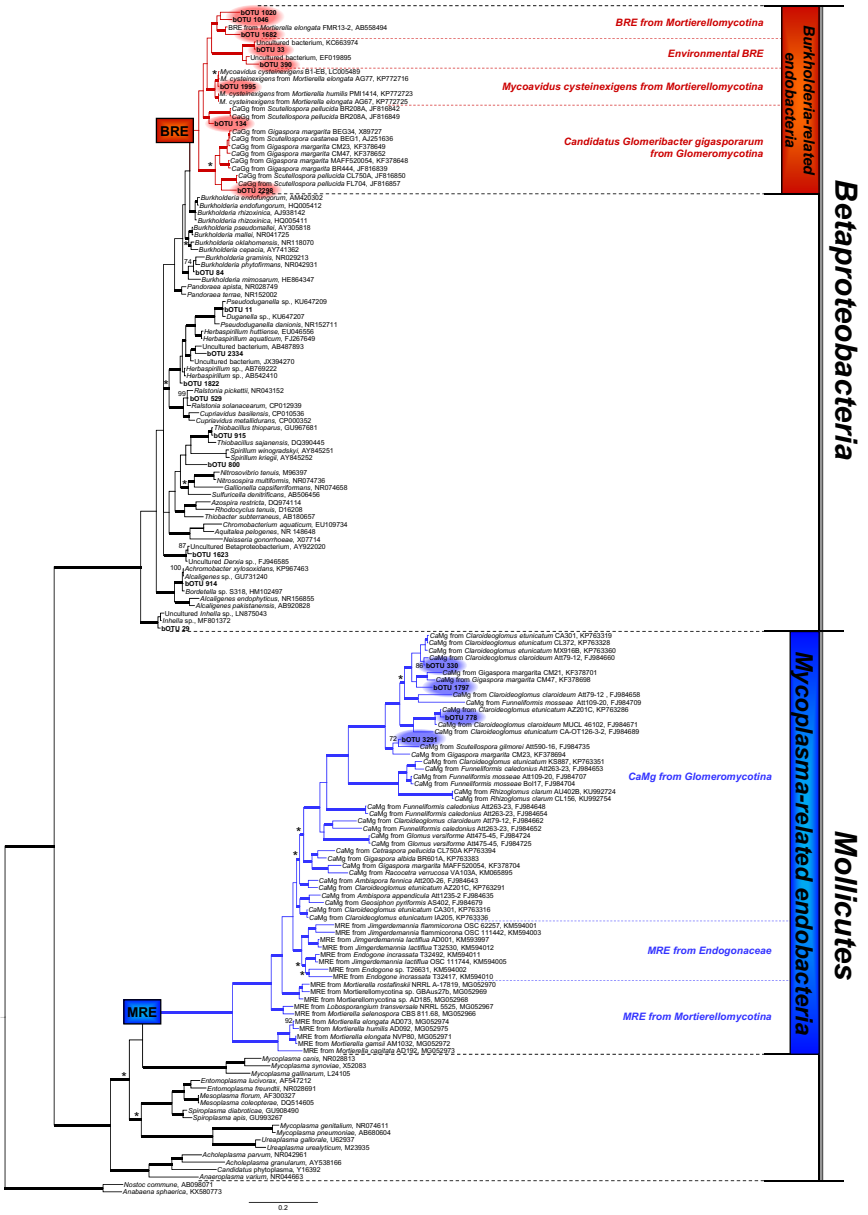


**Figure S6 | Phylogenetic placement of the 22 candidate endobacteria OTUs as identified by the clustering approach**

Twelve OTUs out of 22 turn out to be phylogenetically close to *Burkholderia*-related endobacteria (BRE) or *Mycoplasma*-related endobacteria (MRE). In detail, eight and four candidate endobacteria OTUs cluster within the BRE (in red) and MRE (in blue) clade, respectively. The remaining ten candidate endobacteria OTUs cluster with non-endobacteria Betaproteobacteria taxa. Further details are in Figure 7. The tree shows the topology obtained with the Bayesian method. Branches with Bayesian posterior probabilities (BPP) ≥0.95 and ML bootstrap support values ≥70 are thickened; asterisks (*) indicate branches with BPP ≥0.95 but ML bootstrap support values <70; ML bootstrap support values ≥70 are shown for branches having BPP <0.95. Sequences generated in this study are in bold.

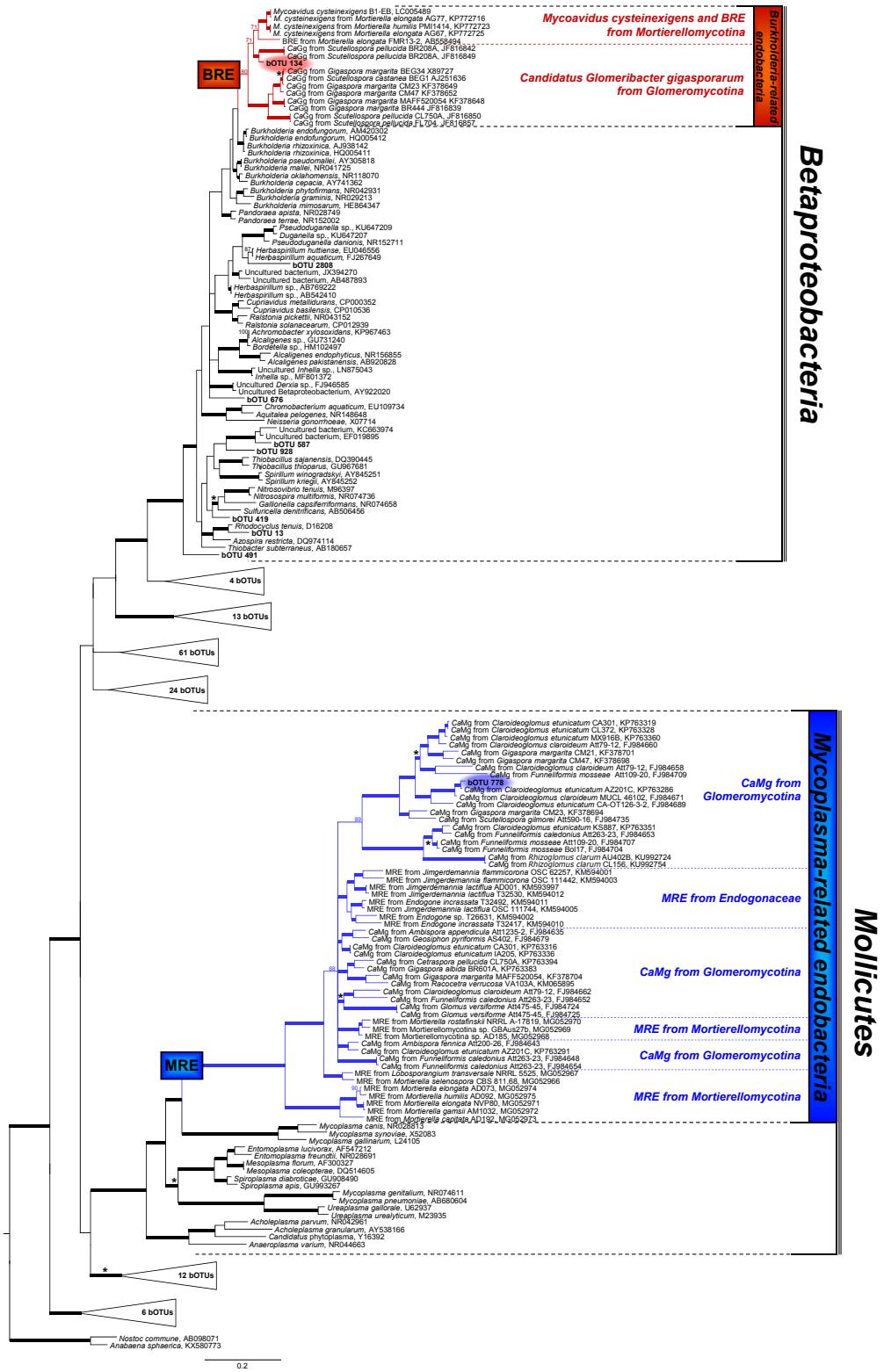


**Figure S7 | Phylogenetic placement of the 129 candidate endobacteria OTUs as identified by the network analysis approach**

Fig. S7 ff: Two OTUs out of 129 turn out to be phylogenetically close to *Burkholderia*-related endobacteria (BRE) or *Mycoplasma*-related endobacteria (MRE). In detail, bOTU 134 clusters within the BRE clade (in red), as sister to a clade encompassing *Candidatus* Glomeribacter gigasporarum (*Ca*Gg) sequences retrieved from *Scutellospora pellucida* (Glomeromycotina), whereas bOTU 778 clusters within the MRE clade (in blue), together with *Candidatus* Moeniiplasma glomeromycotorum (*Ca*Mg) sequences retrieved from different strains of *Claroideoglomus* spp. (Glomeromycotina). Seven candidate endobacteria OTUs out of 129 cluster with non-BRE Betaproteobacteria taxa. The remaining bOTUs (120) are not related to Betaproteobacteria or Mollicutes: clades encompassing those bOTUs are drawn as collapsed (triangles) and the number of bOTUs clustering within these clades is given. The tree shows the topology obtained with the Bayesian method. Branches with Bayesian posterior probabilities (BPP) ≥0.95 and ML bootstrap support values ≥70 are thickened; asterisks (*) indicate branches with BPP ≥0.95 but ML bootstrap support values <70; ML bootstrap support values ≥70 are shown for branches having BPP <0.95. Sequences generated in this study are in bold.

**Table S1 | Effects of plant species and P treatment on alpha diversity (ANOVA)**
Statistic testing for differences in α-diversity between Arabidopsis and Petunia root bacterial and fungal root microbiota in varying levels of P availability was performed using analysis of variance (ANOVA). ANOVA was used to test for species- (S), treatment- (T) or their interaction (SxT) effects on OTU richness, Shannon diversity and Sheldon evenness. Alpha diversity metrics were determined on data with a common sequencing depth of 15'000 sequences per sample. Significant *F*-tests are indicated in bold.

| | | **species** | | **treatment** | | **interaction** | |
|---|---|---|---|---|---|---|---|
| | Metric | F | *P* | F | *P* | F | *P* |
| Bacteria | richness | **14.826** | **0.000396** | **9.565** | **0.000378** | 0.822 | 0.446615 |
| | diversity | **18.255** | **0.000108** | **7.432** | **0.001724** | 0.749 | 0.479160 |
| | evenness | **18.233** | **0.000109** | **5.203** | **0.009581** | 0.540 | 0.586555 |
| Fungi | richness | **245.820** | **<2e16** | **3.704** | **0.03446** | **8.496** | **0.00095** |
| | diversity | **1078.151** | **<2e16** | 0.004 | 0.996 | 1.000 | 0.378 |
| | evenness | **407.092** | **<2e16** | 3.107 | 0.0569 | **4.540** | **0.0175** |

**Table S2 | Effects of plant species and P treatment on community composition (PERMANOVA)**
Statistic testing for differences in beta-diversity between Arabidopsis and Petunia root bacterial and fungal root microbiota in varying levels of P availability was performed using permutational analysis of variance (PERMANOVA). PERMANOVA was used to test for species- (S), treatment- (T) or their interaction (SxT) effects on community composition based on Bray-Curtis dissimilarities. Significance is indicated in bold.

| | **species** | | **treatment** | | **interaction** | |
|---|---|---|---|---|---|---|
| | R2 | *P* | R2 | *P* | R2 | *P* |
| Bacteria | **0.1412** | **0.001** | **0.07188** | **0.004** | **0.05336** | **0.023** |
| Fungi | **0.5306** | **0.001** | 0.03846 | 0.066 | 0.03748 | 0.069 |

**Table S3 | Effects P treatment on species-specific community compositions (PERMANOVA)**
Statistic testing for differences in beta-diversity as a function of varying levels of P availability was performed separately for Arabidopsis and Petunia root microbiota using permutational analysis of variance (PERMANOVA). PERMANOVA was used to test for treatment effects on community composition based on Bray-Curtis dissimilarities. Significance is indicated in bold.

| | **Arabidopsis** | | **Petunia** | |
|---|---|---|---|---|
| | R2 | *P* | R2 | *P* |
| Bacteria | **0.15022** | **0.001** | **0.14124** | **0.003** |
| Fungi | **0.21702** | **0.002** | 0.132266 | 0.057 |

**Table S4 | Statistics from identifying phosphate sensitive microbes**
This additional file (separate XLSX table) reports statistic results from the edgeR analyses of P sensitive bOTUs and fOTUs in Arabidopsis and Petunia. All OTUs with FDR < 0.05 are listed with their taxonomy assignments, log fold-change (FC), log counts per million (CPM), the likelihood ratio (LR) test and probability (P) values and false-discovery rate (FDR) corrected P values. In addition, the logCPM abundances of each OTU in Arabidopsis, Petunia and soil in low, medium and high P conditions are given.

**Table S5 | Network characteristics**
This additional file (separate XLSX table) reports characteristics from the co-occurrence network analyses presented in Figure 7. All OTUs of the networks are listed with their taxonomy assignments, module assignments and whether the present keystone OTUs in Arabidopsis and Petunia.

**Methods S1 | Microbiota profiling and analysis**

DNA extraction and PCR

DNA was extracted from the root and soil samples using the NucleoSpin Soil kit (Macherey-Nagel, Düren, Germany). Roots were lyophilized, placed in 2 ml centrifuge tubes, to which one metal bead was added. Samples were ground to a fine powder for 2 min at 25 Hz using a Retsch TissueLyser (Retsch, Haan, Germany). Buffer SL1 and enhancer solution SX was used. DNA was quantified with Picogreen and diluted to 1 ng/μl for soil samples and 10 ng/μl for root samples.

We first evaluated several PCR approaches to compare the levels of co-amplified plant sequences, abundance of AMF and general fungal diversity in Petunia roots: 1) ITS1F (Gardes & Bruns, 1993) and ITS2 (White *et al.*, 1990), 2) fITS7 (Ihrmark *et al.*, 2012) and ITS4 (White *et al.*, 1990) and 3) ITS1F with the reverse complement of fITS7. The **Notes S1** contain the bioinformatic script, barcode-to-sample assignments, input data, analysis script and markdown report for the comparison of the PCR approaches.

Based on this analysis, the PCR primers ITS1F and ITS2 were chosen to study the fungal community. PCR primers 799F (Chelius & Triplett, 2001) and 1193R (Bodenhausen *et al.*, 2013) were used to amplify hypervariable regions V5, V6 and V7 of the 16S rRNA gene for the bacterial community. To confirm the fungal community results from Illumina sequencing, we prepared an additional library for SMRT sequencing where the entire ITS region was amplified with the PCR primers ITS1F and ITS4. The barcode-to-sample assignment can be taken from the sample table included in **Notes S3**.

PCR reactions for each library were prepared in similar way. The reaction volume was 20 μl, and contained 1x 5Prime Hot Master mix 200 nM of each primer and 0.3% BSA. Cycling programs consisted of an initial denaturation at 94°C for 2 minutes (16S) or 3 minutes (ITS, SMRT), followed by 30 cycles of denaturation at 94°C for 30 seconds (16S), 45 seconds (ITS, SMRT), annealing at 55°C (16S, SMRT) or 50°C (ITS) for 30 seconds (16S) or 1 minute (ITS, SMRT), and elongation at 65°C (16S) or 72°C (ITS, SMRT) for 30 seconds (16S) or 1 minute (SMRT) or 90 second (ITS). PCR were run in triplicate with a negative control for each primer mix and verified on a 1% agarose gel.

Triplicate PCR products were pooled, cleaned with PCR clean-up kit (Macherey-Nagel, Düren, Germany), quantified using a Quant-iT Picogreen dsDNA Assay Kit (Invitrogen, Eugene, OR USA) on a Varian Cary Eclipse fluorescence spectrometer (Agilent Technologies, Santa Clara, CA USA). Equimolar amount of each PCR product were combined. For 16S library preparation, the smaller band which corresponds to 16S rRNA gene was selected with the gel extraction kit (Macherey-Nagel, Düren, Germany). Pooled PCR products were concentrated with Agencourt AMPure XP kit (Beckman Coulter, Brea, CA, USA) and quantified with Qubit dsDNA HS assay on a Qubit 2.0 fluorometer (Invitrogen, Eugene, OR USA) and combined with other libraries before sequencing.

Sequencing and Bioinformatics

The MiSeq libraries were prepared at the Functional Genomics Center Zurich (www.fgcz.ch) with the NEBNext DNA library Ultra kit (New England Biolabs, Ipswich, MA, USA). After end-repairing and polyadenylating the amplicons, NEBNext Adaptor were ligated. The ligated samples were run on a 2% agarose gel and the desired fragment length were excised (50bp +/- the target fragment length). DNA from the gel was purified with MinElute Gel Extraction Kit (Qiagen, Hilden, Germany). Fragments containing Nebnext adapters on both ends were selectively enriched with PCR using 4 cycles. Quality and quantity of the enriched libraries were validated using Qubit® (1.0) Fluorometer and Tapestation (Agilent Technologies, Santa Clara, CA USA). The libraries were normalized to 4nM in Tris-Cl 10 mM, pH 8.5 with 0.1% Tween 20. The libraries were sequenced at FGCZ on the Illumina MiSeq Personal Sequencer (Illumina, San Diego, CA, USA) using a 600 cycle v3 Sequencing kit (Cat n° MS-102-3003), in paired-end 2x 300 bp mode.

The PacBio SMRT bell library was prepared at the FGCZ using the DNA Template Prep Kit 1.0 (Pacific Biosciences p/n 100-259-100), following the manufacturer's instructions. After DNA quantification with a Qubit Fluorometer dsDNA Broad Range assay (Life Technologies p/n 32850), the fragment size distribution was assessed with a Bioanalyzer 2100 12K DNA Chip assay (Agilent p/n 5067-1508). 500-750ng of amplicon DNA was DNA-damage repaired and end-repaired using polishing enzymes. A blunt end ligation reaction followed by exonuclease treatment was performed to create the SMRT bell template. The library was quality inspected and quantified on

the Bioanalyzer 12Kb DNA Chip and on a Qubit Fluorometer (Life technologies) respectively. A ready to sequence SMRT bell-Polymerase Complex was created using the Sequel binding kit 2.0 (Pacific Biosciences p/n 100-862-200) according to the manufacturer instructions. The Pacific Biosciences Sequel instrument was programmed to sequence the sample on 1 Sequel™ SMRT® Cell 1M v2 (Pacific Biosciences p/n 101-008-000), taking 1 movie of 10 hours using the Sequel Sequencing Kit 2.0 (Pacific Biosciences p/n 101-310-400). After the run, the quality of the sequencing data was checked using the "run QC module" of the PacBio SMRT Link software.

DNA sequence analysis were performed at the Scientific Compute Cluster Euler, at ETH, Zurich. The MiSeq data was processed similar to the workflow described in Hartman *et al*. (Hartman *et al.*, 2017). Briefly, to improve merging, read ends were trimmed by run if needed (seqtk v.1.2-r94, https://github.com/lh3/seqtk.git) and subsequently merged (FLASH v.1.2.11; (Magoč & Salzberg, 2011) into amplicons. In a next step, CUTADAPT v1.4.2 (Martin, 2011) was used to trim off barcode and primer sequence and demultiplex amplicons based on barcode information. Demultiplexed reads were subsequently quality filtered using prinseq-lite v0.20.4 (Schmieder & Edwards, 2011). The quality filtered sequences were clustered into operational taxonomic units (OTUs, ≥97% sequence similarity) using usearch v10.0.240 (Edgar, 2013). SINTAX (http://dx.doi.org/10.1101/074161) was used for taxonomic assignments using either SILVA 16S v128 (Quast *et al.*, 2013) for the bacterial community or UNITE v7.2 (Abarenkov *et al.*, 2010) database for the fungal community.

The SMRT sequencing data was processed following Schlaeppi *et al*. (2016). In brief, ≥5-pass 'reads of insert' (ROI; also, circular consensus sequences CCS) were extracted from the raw data 8) using default parameters. The software mothur v.1.34.4 (Schloss *et al.*, 2009) and flexbar (Dodt *et al.*, 2012) were employed for quality filtering and demultiplexing, respectively. Some raw reads were affected by multi-primer artefacts (Tedersoo *et al.*, 2018)and we employed Usearch (v10.0.240, (Edgar, 2013) to detect and discarded these reads containing primer sequences within the read. OTU clustering and taxonomic annotation were conducted using the same tools as for the MiSeq data.

Bioinformatics scripts and report files are available as **Notes S2**. The raw sequencing data of the two MiSeq runs and the SMRT sequencing are available from the European Nucleotide Archive under the study accession PRJEB27162.

Identification of endobacteria OTUs by phylogenetic placement

To identify endobacteria OTUs, we pre-selected candidates in the microbiome dataset using two approaches and then validated their representative sequences by fine mapping to a reference tree of known endobacteria sequences. For the latter we created a custom database with curated endobacteria 16S rDNA references containing published sequences of *Burkholderia*-related endobacteria (BRE), such as *Candidatus* Glomeribacter gigasporarum and *Mycoavidus cysteinexigens*, and *Mycoplasma*-related endobacteria (MRE) retrieved from Glomeromycotina, such as *Candidatus* Moeniiplasma glomeromycotorum, Endogonaceae (Mucoromycotina) and Mortierellomycotina. We pursued two alternative strategies to identify candidate endobacteria OTUs. The first approach was based on sequence clustering and secondly, we employed co-occurrence characteristics from network analysis (e.g., high degree of co-occurrence between fungal and bacterial OTUs) and we explored if this type of information would be useful to identify candidate endobacteria OTUs.

For the first strategy, we employed usearch (v8) to map the curated endobacteria sequences to the representative bacteria OTUs (bOTU) sequences of the microbiome dataset. We allowed up to 10% sequence divergence to account for the high variability among 16S rDNA endobacteria sequences, in particular from MRE, known to display high level of sequence diversity (Desirò *et al.*, 2018). This approach yielded 22 candidate endobacteria OTUs, which we then placed into the reference tree. For the second strategy, we searched the co-occurrence characteristics from network analysis as follows: we first identified all fungal OTUs (fOTUs) assigned to Glomeromycotina and Mortierellomycotina (we did not identify fOTUs assigned to Endogonaceae; may be linked to the use of universal ITS primers, which tend not to capture this family, (Tedersoo *et al.*, 2016), the fungal lineages known to harbor BRE and/or MRE, and then selected all bOTUs that significantly co-occur with them (Spearman's rho > 0.7 and $P < 0.001$).

This approach yielded 129 candidate endobacteria OTUs, which we then placed into the reference tree.

Placement to a common phylogenetic tree was achieved by aligning candidate endobacteria OTUs (from clustering or network approaches) to an endobacteria reference dataset. Sequences were aligned with MAFFT (Katoh & Standley, 2013) Phylogenetic analyses were carried out with MrBayes v.3.2.6 (Ronquist *et al.*, 2012) and RAxML v.8.2.4 (Stamatakis, 2014). Prior to Bayesian phylogenetic reconstructions, best-fit nucleotide substitution models were estimated with jModelTest v.2.1.9 (Darriba *et al.*, 2012). Bayesian analyses were performed running the Markov chain Monte Carlo for 10 million generations under the TrN+I+G nucleotide substitution model. Maximum likelihood analyses were conducted with the automatic 'bootstrapping' option under the GTRCAT nucleotide substitution model. For tree inference, Betaproteobacteria and *Mollicutes* reference sequences were included together with outgroup sequences belonging to Cyanobacteria. Candidate endobacteria OTUs were considered as BRE or MRE OTUs if they clustered within an endobacteria clade in the reference phylogenetic tree.

Command line and analysis code in R (including markdown report) as well as the database with curated endobacteria 16S rDNA reference sequences are available as **Notes S4**.

**Notes S1 | Comparison of PCR approaches**
This additional zip archive comprises the bioinformatic command line code, input data and R script for the comparison of the PCR approaches. It also contains the R markdown output as a detailed PDF-report of ITS PCR approaches.

**Notes S2 | Bioinformatic scripts**
This additional zip archive comprises all bioinformatic command line scripts including all individual parameters and support files that were utilized to process the raw sequencing data from the MiSeq- and SMRT-sequencing data.

**Notes S3 | Data analysis in R**
This additional zip archive comprises the R scripts, functions and support files that document and permit to reproduce the main data analysis in R. The main data analysis covers the steps illustrated in **Fig. S1**.

**Notes S4 | Mapping endobacteria**
This additional zip archive comprises the command line and analysis code in R as well as the database with curated endobacteria 16S rDNA reference sequences. In addition, it contains the R markdown output as a detailed PDF-report of mapping the OTUs to the endobacteria database.

**Notes S5 | Comparison of ITS profiling approaches**
This additional zip archive comprises the R code (and its markdown report), which was used to compare the MiSeq- versus the SMRT-sequencing based profiling of fungal communities. It contains the R markdown output as a detailed PDF-report for the comparison of ITS profiling approaches.

**References:**


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