Supplemental Material

Materials and Methods

RNA and DNA extraction

Residual DNA from the extracted RNA, was removed using the RTS DNase kit (MO BIO). Rhizosphere and bulk soil DNA was extracted using the same kit (and samples), but incorporating at step 16 the RNA Power Soil DNA Elution Accessory Kit (MO BIO).

For root RNA and DNA extractions, 50 mg of freeze-dried roots were homogenized in sterile 2 ml screw cap tubes containing stainless steel beads (2.38 mm diameter, MOBIO laboratories, CA, USA) using a Precellys 24 tissue homogenizer (Bertin Technologies, France) at 5000 r.p.m. for 2 x 30s with a 3 min gap to cool down the samples on ice. DNA and RNA were extracted from the lysed root material using the Qiagen plant DNeasy mini kit but without adding RNase (Qiagen, Germany) so that both nucleic acids were extracted. For RNA, DNase treatment was performed using the same DNase kit as above. Twelve biological replicates were used for all extractions.

PCR amplification parameters

Each 25 µl bacterial reaction volume contained the following concentrations of reagents: 200 µM dNTPs (ThermoFisher Scientific, Germany), 0.4 µM of each primer, 0.5 U Polymerase, 5x Phusion GC buffer and 1 µl template. The thermocycling program for bacterial amplifications included an initial denaturation step at 98°C for 30 s, followed by 30 cycles at 98^{o}C 10 s, 50^{o}C 30 s, 72^{o}C 30 s and a final extension step at 72^{o}C for 7 min. The fungal reactions were the same, with the only difference that the primer concentrations were 0.5 µM of forward primer and 0.3 µM of reverse primer. The thermocycling program for fungal amplifications included an initial denaturation step at 98°C for 30 s, followed by 35 cycles at 98^{o}C 10 s, 57^{o}C 30 s, 72^{o}C 30 s and a final extension step at 72^{o}C for 7 min.

Microbial Community Analysis

The sequences obtained from the pyrosequencing runs were analyzed using QIIME (1) (MacQiime version 1.9.0). Both bacterial and fungal reads were demultiplexed based on the barcode sequences and forward and reverse reads were combined. The split_libraries.py command was run in order for the reads to be binned according to the sample and for initial filtering to be performed, allowing maximum 2 primer-mismatches, in order to increase the yield output of the reads. For bacterial data, denoising was performed for both normal and reverse complement using initially the denoiser_preprocess.py script followed by the denoiser.py script implemented in QIIME. The results from the two independent denoising runs were inflated. The sequences were then clustered into operational taxonomic units (OTUs) by UCLUST (2) based on 97% pairwise identity using QIIME's (1) open reference OTU picking strategy which used the Greengenes 16S rRNA database (13_8 release) as a reference

(3). Taxonomic classification of the representative sequence for each OTU was done using QIIME's version of the Ribosomal Database Project's classifier (4) against the Greengenes 16S rRNA database (13_8 release) using default parameters. The representative sequences for each OTU were aligned using PyNAST (3).

References

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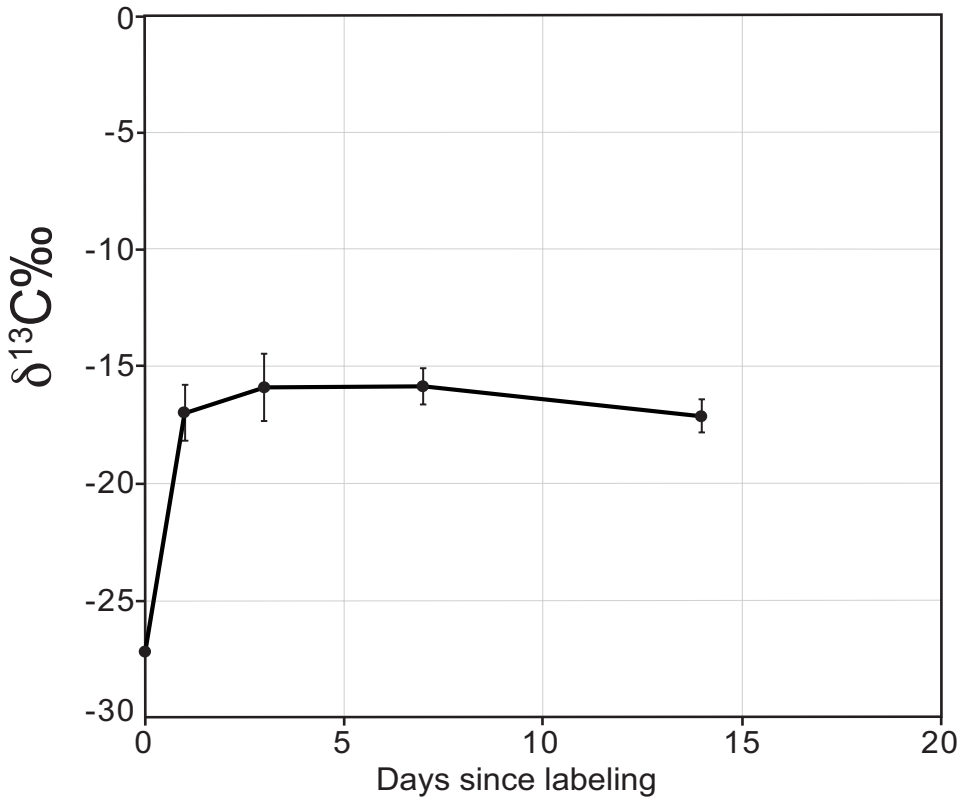
Figure Legends

Figure S1. $\delta^{13}C$ values (‰) of rhizosphere soil of oilseed rape plants following pulse labeling with $^{13}CO_2$. Vertical bars indicate ± standard error (n=12).

Figure S2. Venn diagrams illustrating unique and shared numbers of **(A)** bacterial and **(B)** fungal OTU's for i) bulk soil DNA, rhizosphere soil DNA and root DNA; ii) rhizosphere soil RNA and rhizosphere soil DNA; iii) root RNA and root DNA; iv) rhizosphere soil RNA and root RNA.



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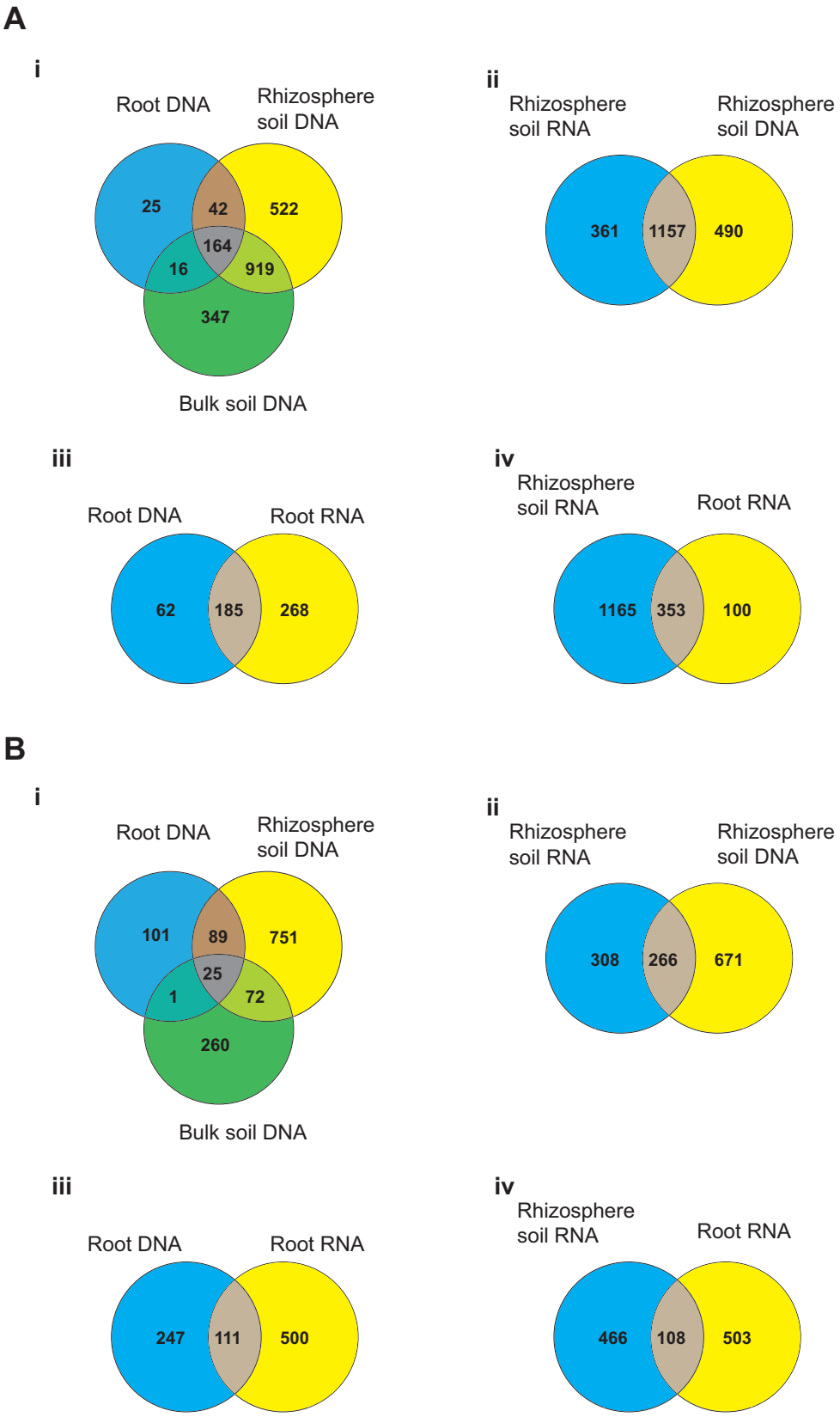

Figure S2. Venn diagrams illustrating unique and shared numbers of **(A)** bacterial and **(B)** fungal OTU's for i) bulk soil DNA, rhizosphere soil DNA and root DNA; ii) rhizosphere soil RNA and rhizosphere soil DNA; iii) root RNA and root DNA; iv) rhizosphere soil RNA and root RNA.