Community metatranscriptomic analysis of wheat, oat and pea rhizospheres

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Background

- Soil microbial communities are of global importance to nutrient cycling, and maintaining plant health through interactions in the rhizosphere.
- Plants influence microbial activity in the rhizosphere through deposition and exudation of carbon sources (e.g. organic acids) and secondary metabolites (e.g. hormones and antimicrobials).
- We've used high-throughput sequencing of RNA to identify the active microbes in soil and the rhizospheres of wheat (*Triticum aestivum*), oat (*Avena sativa*), and pea (*Pisum sativum*).
- This powerful comparative approach allows assessment of all domains of life, while avoiding the common biases associated with culturing or PCR amplification.

Methods

- Total RNA was extracted from rhizosphere soil of 4 week old plants and unplanted controls (3 or 4 replicates per plant).
- Samples were reverse transcribed and amplified using the Whole Transcriptome Amplification kit (Rubicon Genomics).
- Sequencing was performed using 454 GS Flx chemistry at The Genome Analysis Centre (UK). A total of **1189442** reads of mean length **≈ 230bp** were generated from 14 samples.
- Reads were queried against a clean, de-replicated small subunit rRNA databases using USEARCH (E-value ≤1E^{-7}, top 100 hits).
- Results were analysed and compared using a lowest common ancestor algorithm with MEGAN.

Results

- Proteobacteria dominated all samples. Followed by Actinobacteria, and Firmicutes, then Acidobacteria, Planctomycetaceae and Bacteroidetes (Figure 1).
- Actinomycetes and Bacilli contributed most to their respective Phyla, whereas Proteobacteria were represented by more diverse taxa, including the Rhizobiales, Burkholderiales and Myxococcales (Figure 1).
- Cyanobacteria were highly abundant in some soil samples but not in others (Figure 1). Only abundance of the Nostocales was statistically different from the rhizosphere samples (Table 1).
- Wheat had a positive effect on prokaryotes in the rhizosphere, while oat and pea exerted more negative influences (Figure 2).
- There was little overlap between selection or depletion of taxa by each plant (Figure 2).
- The rhizosphere universally enhanced eukaryotic activity (Figure 2), notably that of fungi, nematodes and bacterivorous protozoa.

Conclusion and Discussion

- Plants exerted **subtle** but **specific** effects on microbial communities in the rhizosphere.
- Oat and pea share depleted taxa in the rhizosphere, possibly reflecting similar antimicrobial compounds (i.e. saponins, which wheat does not produce).
- Taxa selected by more than one plant (general rhizosphere colonisers) possess relevant functional traits including methylotrophy and cellulose degradation (Table 1).

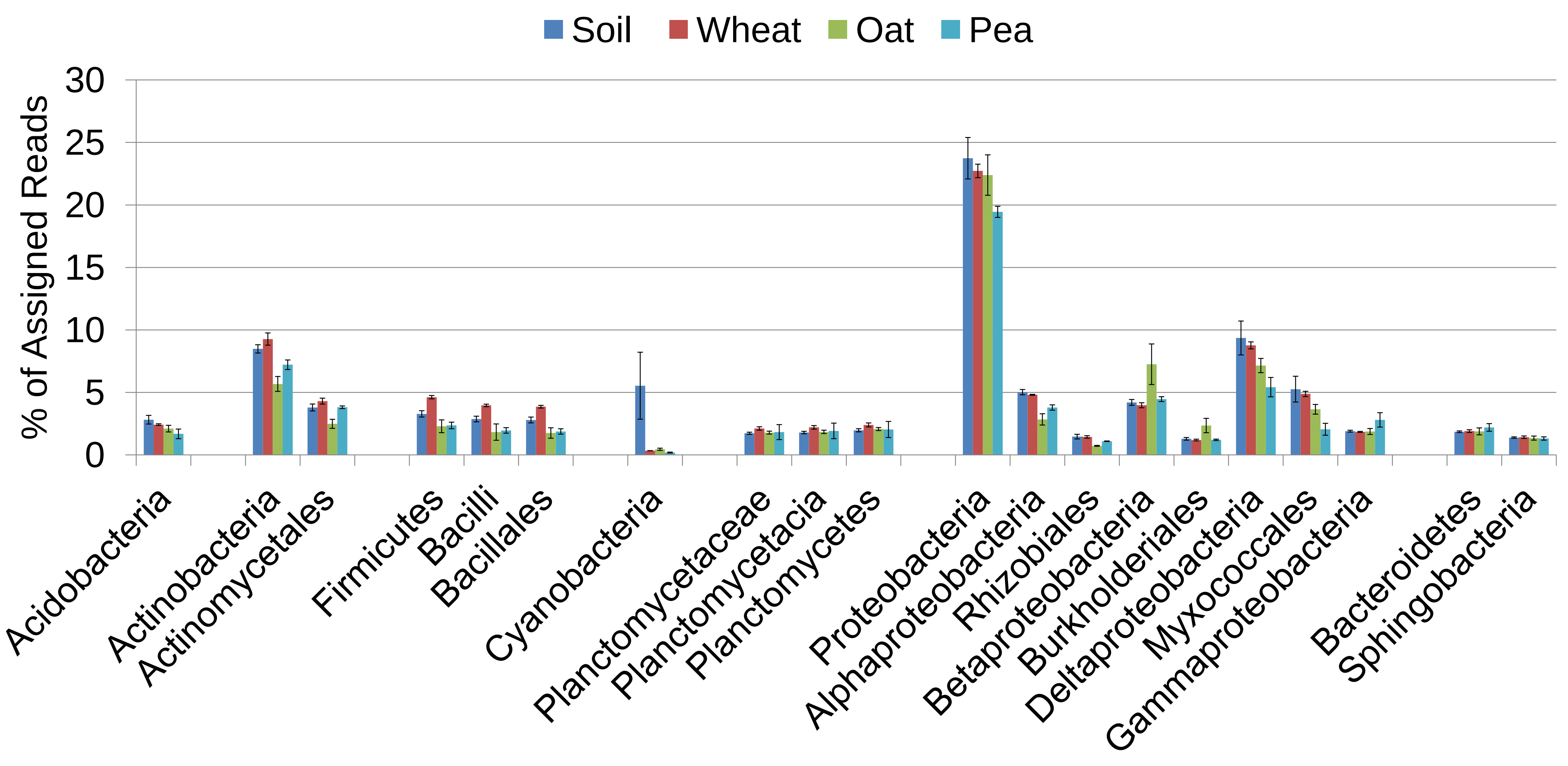

Figure 1: Twenty most abundant high-level taxa (Phyla, Orders and Classes, and Families) in the soil, wheat, oat and pea rhizospheres. Values are cumulative within their taxonomic ranking. Error bars ±1SEM, n = 4 for soil and oat, and 3 for wheat and pea. Values are expressed as a percentage of the total number of reads assigned in MEGAN. Unclassified taxa are not included.

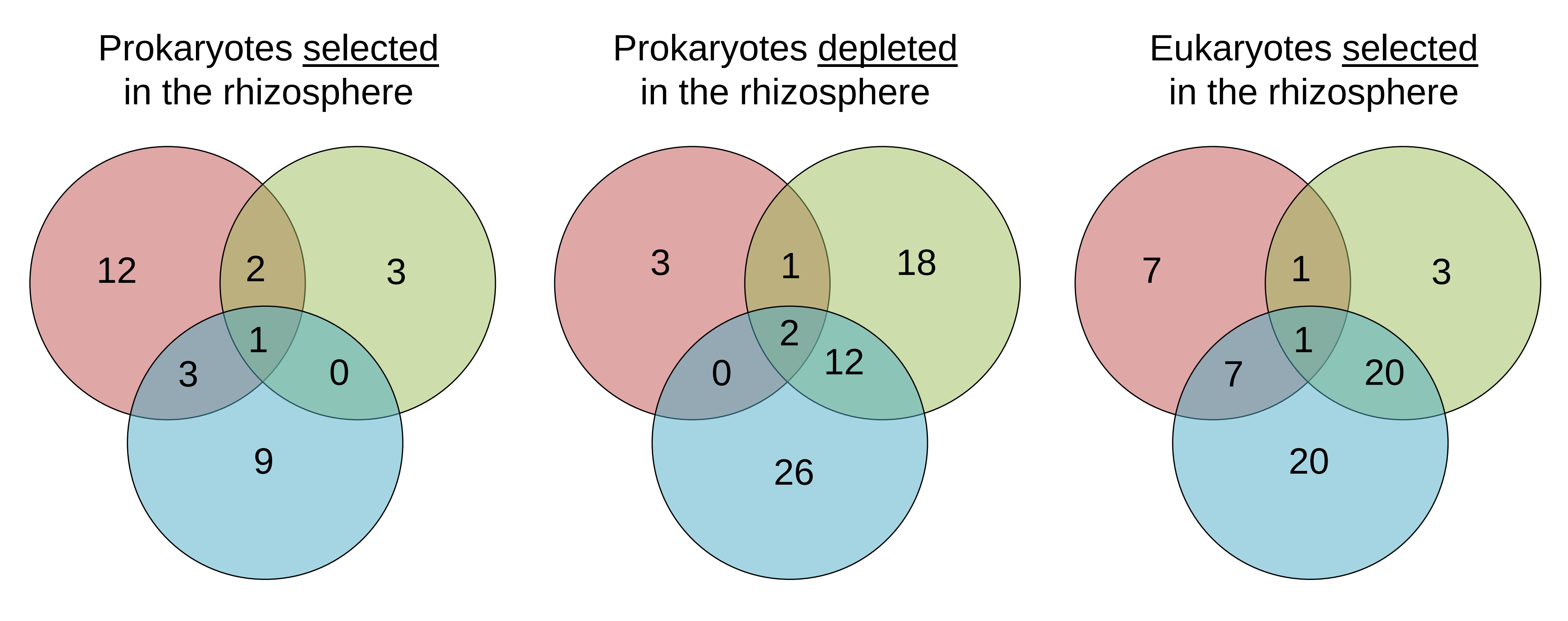

Figure 2: Number of taxa selected and depleted in the rhizosphere of wheat, oat and pea compared to soil (P≤0.05, ≥1.2 fold different, ≥0.05% reads assigned in the rhizosphere (selected) or soil (depleted). Note that no eukaryotic taxa were depleted in the rhizosphere.

Table 1: Functional traits of some taxa selected in the rhizosphere or soil (P≤0.05, ≥1.2 fold different, ≥0.05% reads assigned).

Taxa	Functional trait	Selection
Bacillus sp. IGCAR-1/07	Benzoate degradation	Wheat
Nitrospinaceae	Nitrite oxidation	Wheat
Chloroflexi	Photoheterotrophic	Oat
Streptomycetaceae	Antibiotic production	Pea
Cellvibrio	Cellulose / xylulose degradation	Wheat and Oat
unclassified Methylophilaceae	Methylotrophy	Wheat, Oat and Pea
Nostocales	Photoautotrophic, some N-fixing	Soil

Ongoing and Future Work

- Comparative metabolic mapping analyses of soil, wheat, oat and pea rhizospheres.
- Investigating the differences between *wt* oat and an avenacin deficient mutant.
- Metagenomic and metatranscriptomic analyses of modern wheat and its progenitors.

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