



Microbial Metatranscriptomics of the Rhizosphere

Insights into Population Structure and Metabolism

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Background

- Soil microbial communities are of global importance to nutrient cycling, and maintaining plant health through interactions in the rhizosphere.
- Diverse plant growth promoting rhizobacteria (PGPRs) aid plants by providing nutrients, defending against pathogens and modifying plant stresses.
- We've used high-throughput RNA sequencing to identify and understand the functional roles of microbes in soil and the rhizosphere of wheat (*Triticum aestivum*).

Methods

- Total RNA was extracted, and mRNA enriched using subtractive hybridisation of rRNA (MICROBExpress). Total and enriched samples were reverse transcribed and amplified using Rubicon WTA2.
- 454 sequencing was performed at The Genome Analysis Centre (UK). A total of **961388** reads were generated (mean length **260bp**) and filtered ^[1].
- Total RNA samples were searched against clean, de-replicated SILVA rRNA databases using USEARCH^[2] (E-value $\leq 1E^{-6}$, top 100 hits). Results were analysed and compared using a lowest common ancestor algorithm (MEGAN^[3]).
- Enriched mRNA samples were stripped of remaining rRNA *in silico* using USEARCH and rRNA databases. Non-rRNA reads (**18414**) were uploaded to MG-RAST^[4] to get a functional annotation.

Results

- Alpha- and Betaproteobacteria, as well as diverse Firmicutes and Actinobacteria, are abundant in soil and wheat rhizosphere (Figures 1 and 2).
- Specific taxa were selected for and against in the wheat rhizosphere (Figure 3).
- Only $\approx 13\%$ of non-rRNA reads coded for proteins, with an additional **12%** mapping to unannotated / untranslated regions of sequenced genomes.
- **Ribosomal proteins** dominate the wheat rhizosphere functional transcriptome, with **transcription related** subsystems also highly expressed (Figure 4)
- Other well represented systems are the **Serine-Glyoxylate cycle** for C1 metabolism, resistance to **oxidative stress**, and **cAMP signalling** (Figure 4).

Discussion

- Population structure is subtly different between soil and the wheat rhizosphere.
- Taxa selected for in the wheat rhizosphere include antibiotic producers and those with diverse metabolic capabilities.
- The high expression of RNA processing proteins shades most rhizosphere specific pathways from the analysis, emphasising the need for improved mRNA enrichment and deeper sequencing.
- Resistance to oxidative stress in the rhizosphere maybe a response to plant defences.
- C1 metabolism might utilise methanol derived from plant pectin.

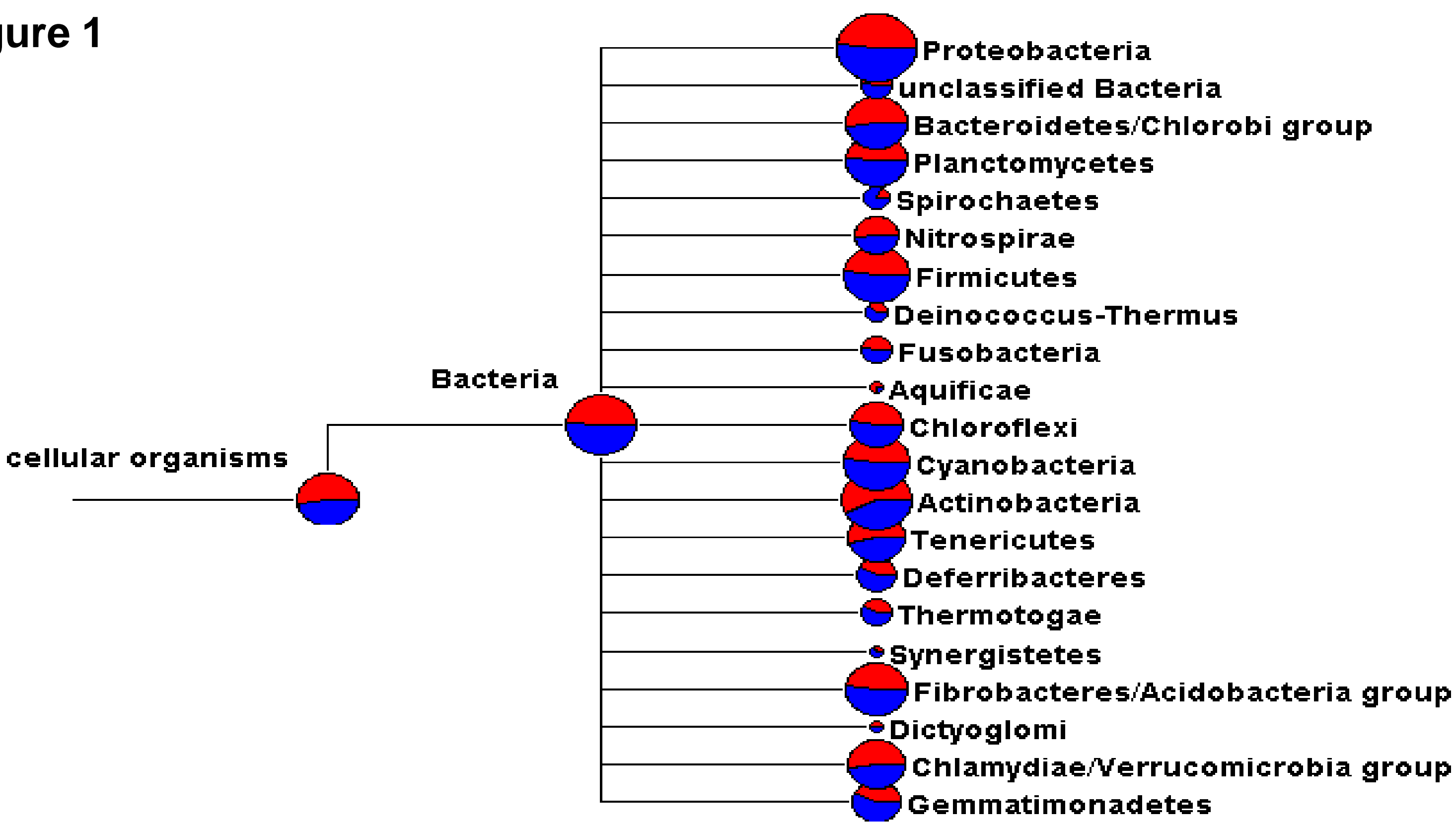
Ongoing and Future Work

- Comparative rhizosphere analyses of wheat, pea, oat (*wt* and *avenacin-* mutant).
- Optimising mRNA enrichment using sample specific rRNA capture probes^[5]
- Deeper sequencing of mRNA enriched samples using Illumina
- Quantification of sequence depth and absolute transcript number^[6]

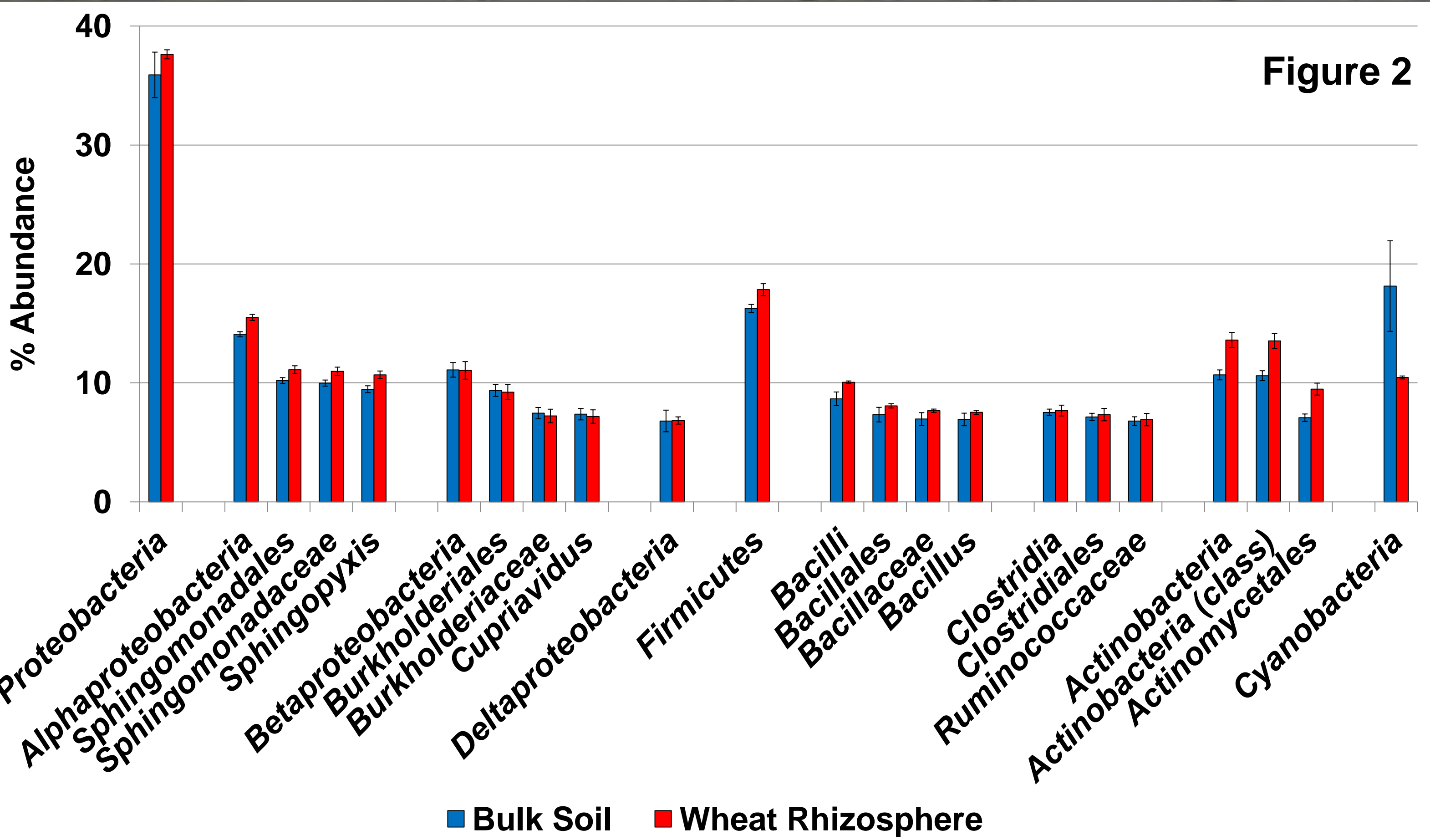
References

^[1]Gomez-Alvarez *et al.* 2009, The ISME Journal., 3: 1314-1317
^[2]Edgar, 2010, Bioinformatics 26: 2460-2461
^[3]Huson *et al.* 2007, Genome Research, 17: 377-386.
^[4]Meyer *et al.* 2008, BMC Bioinformatics, 9: 386
^[5]Stewart *et al.* 2010, The ISME Journal, 4: 896-907
^[6]Gifford *et al.* 2011, The ISME Journal, 5: 461-472

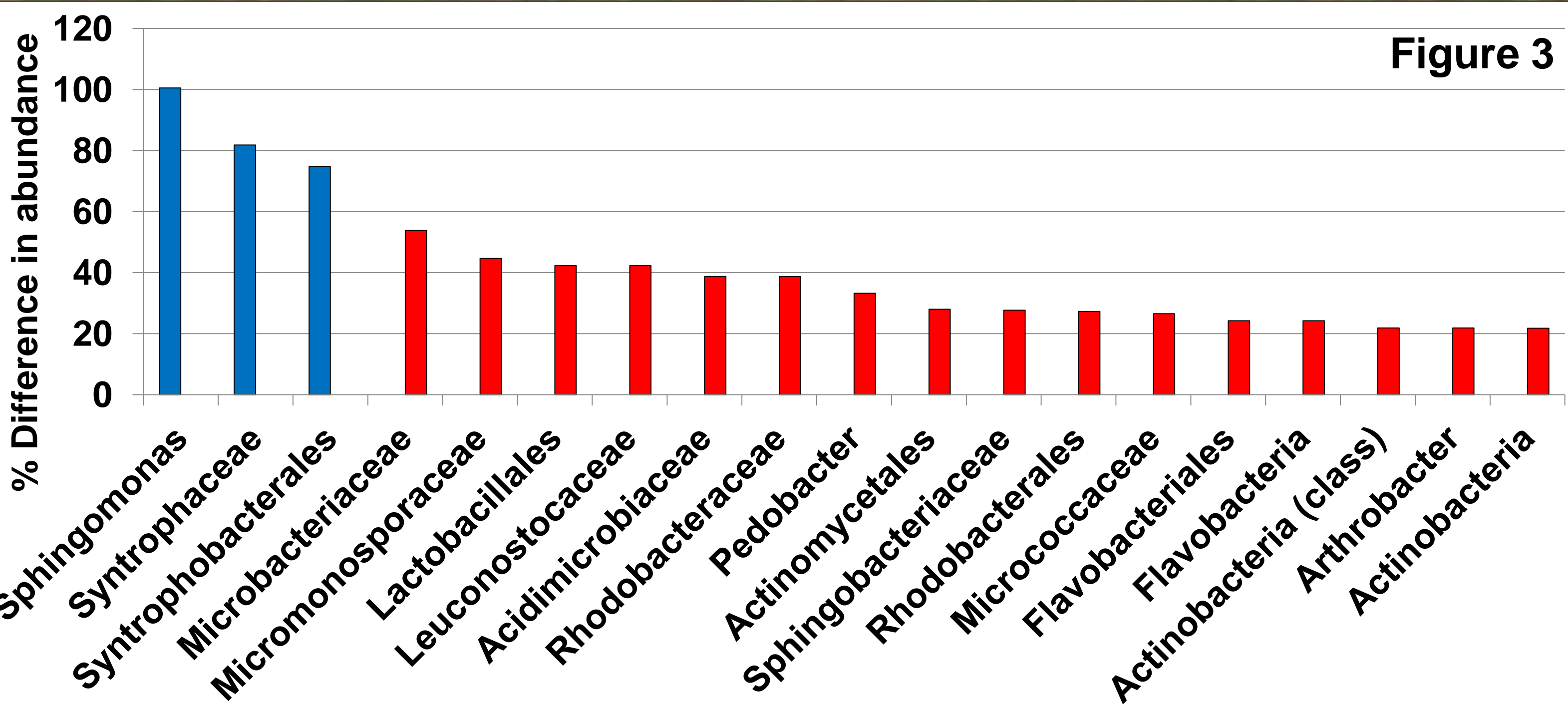
Figure 1



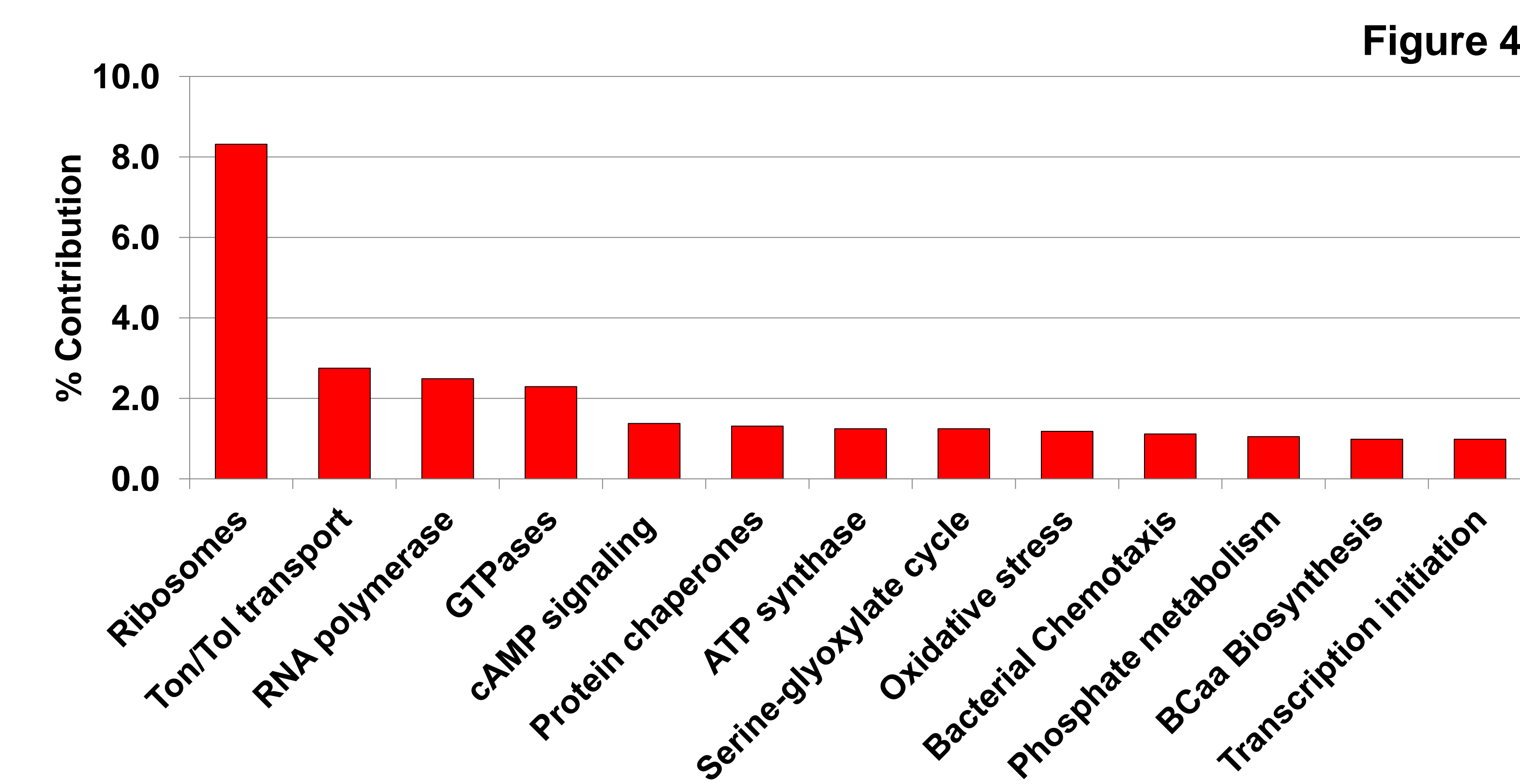
MEGAN tree of community structure in **wheat rhizosphere** and **bulk soil** based on two normalised samples.



Most abundant (at least 5%) taxa in the **wheat rhizosphere** and **bulk soil**, derived from MEGAN using normalised replicate samples. Error bars 1SEM (n=3 for wheat rhizosphere and n=4 for bulk soil).



Significant ($p \leq 0.05$) differences ($\geq 20\%$) in abundance between wheat rhizosphere and bulk soil. **Positive** and **Negative** selection show taxa more and less abundant in the wheat rhizosphere respectively.



MG-RAST functional subsystems with highest transcript numbers in the **wheat rhizosphere** (4 pooled samples). Only subsystems contributing $> 1\%$ are shown. The remaining 74% of reads hit ≈ 1340 subsystems.