Work package A2.1

# Response of the rhizosphere microbiomes to $eCO_2$ in grassland

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## Introduction / Background

The concentration of atmospheric $CO_2$ is still increasing, largely due to human activities, and it is predicted to reach 700 ppm by the end of this century (IPCC 2007). While grassland plant communities have been widely studied for their response to $eCO_2$, little is known about the effects on rhizosphere microbiomes. Here, we applied rRNA-centered metatranscriptomics to assess the effects of $eCO_2$ on the rhizosphere microbiome in semi-natural grassland. Our approach allowed us to analyze such effects simultaneously for the three domains of life, including *Archaea*, *Bacteria*, and *Eukarya*. Study site was the Gi-FACE.

## Current Results

Our results revealed domain-level changes in the rhizosphere microbiomes. In summer, $eCO_2$ induced a significant increase in the abundance of bacteria relative to the eukaryotes. This $eCO_2$ effect was confirmed by analysis of both SSU rRNA and mRNA. Among the eukaryotes, the abundance of the *Glomeromycota* (arbuscular mycorrhiza fungi), however, was significantly increased relative to the other fungal groups, in particular relative to the *Basidiomycota*. Among the *Bacteria*, the relative abundance of the class *Thermoleophilia* (*Actinobacteria*) was significantly increased under $eCO_2$. No effects on the community composition were observed between $aCO_2$ and $eCO_2$ for the winter samples.

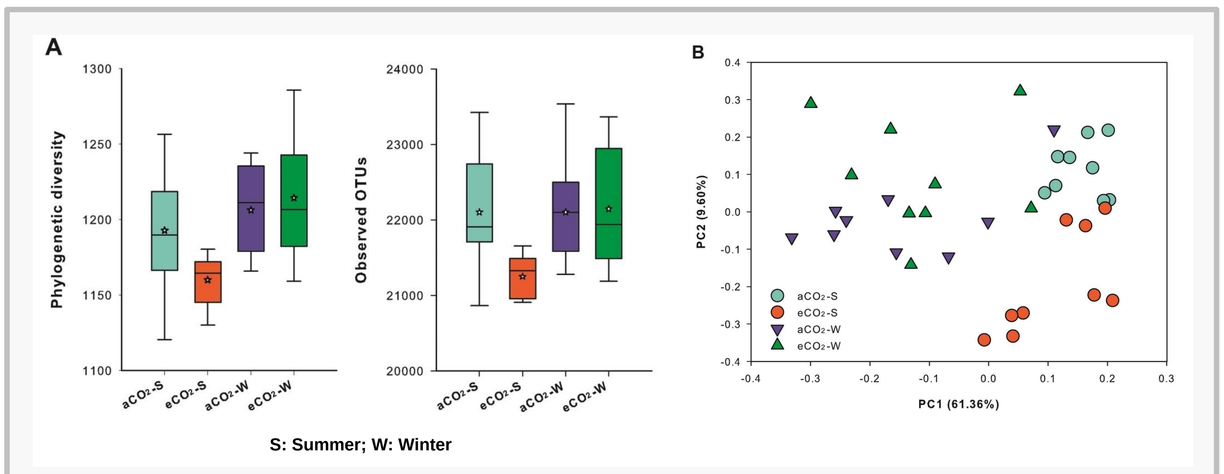


**Figure 1** Alpha- and beta-diversity comparisons of rhizosphere microbiomes inferred from the SSU rRNA sequence data of four treatments. (A) Alpha diversity comparisons based on phylogenetic (Faith's PD) and non-phylogenetic richness (Observed OTUs). (B) PCoA analysis of weighted UniFrac distances.

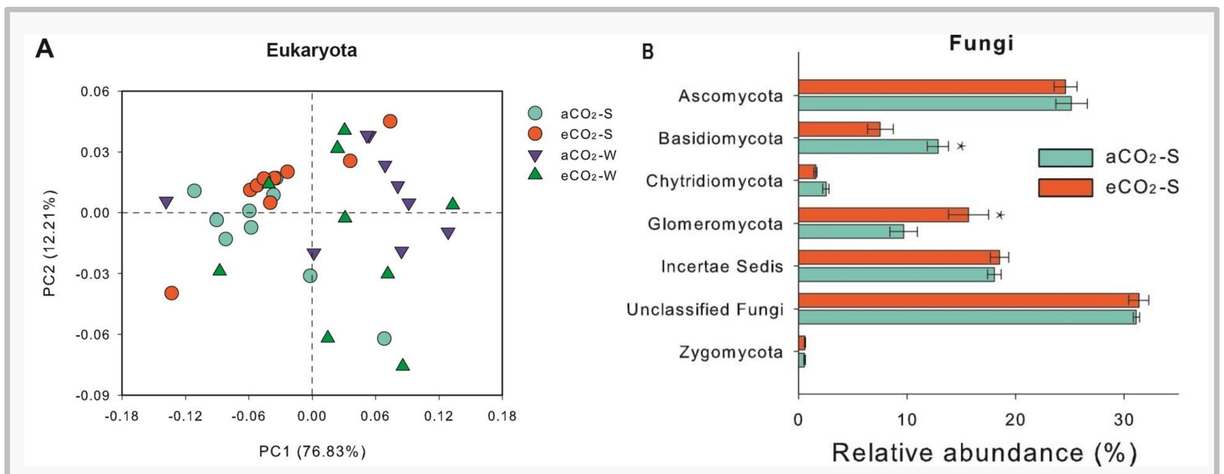


**Figure 2** PCA analysis of eukaryotes in the rhizosphere microbiomes inferred from SSU rRNA sequence data (A). Taxonomic profiles of the rhizosphere fungi in summer 2015 inferred from SSU rRNA sequence data (B).

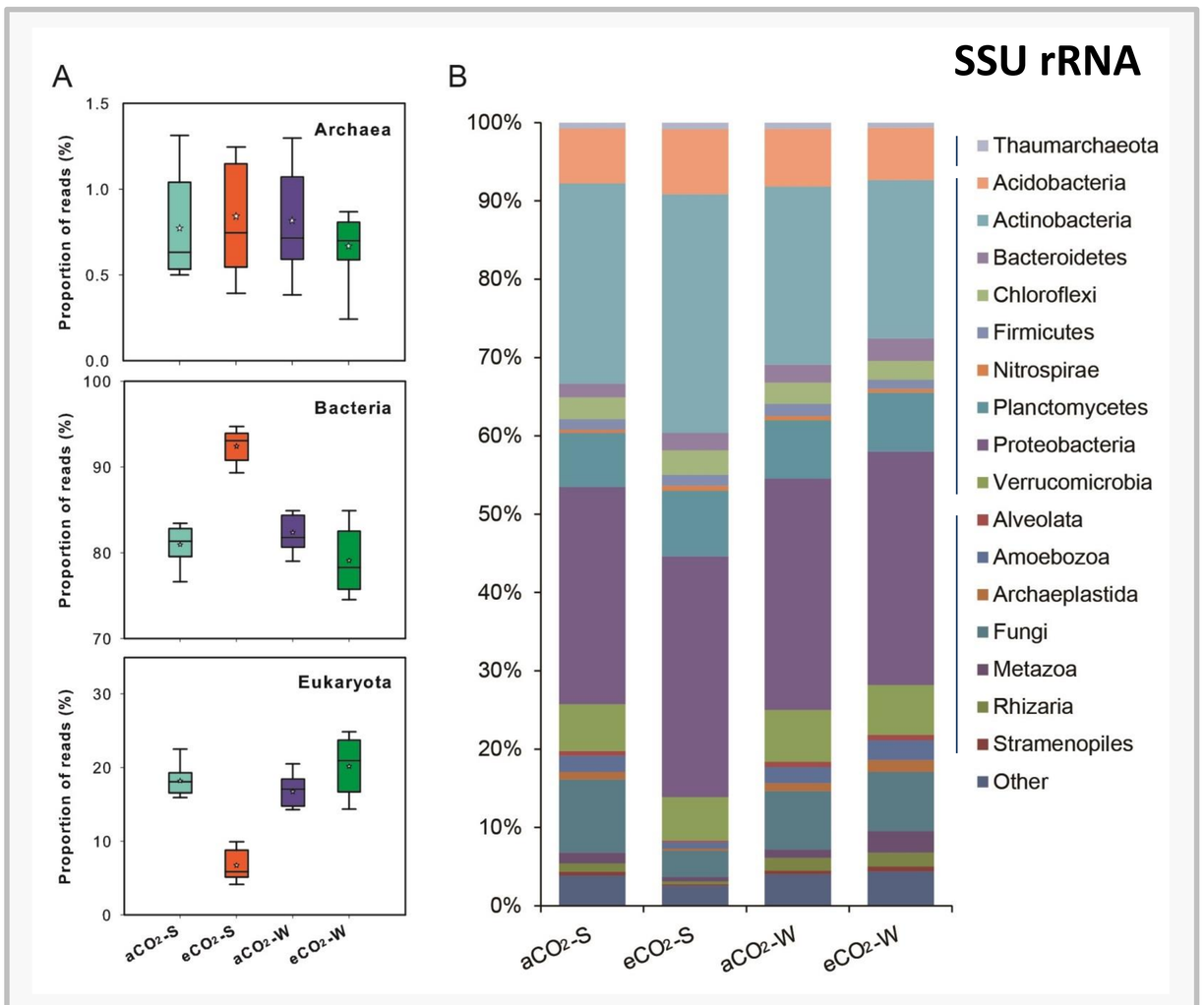


**Figure 3** Proportion of archaea, bacteria and eukaryotes in the rhizosphere microbiomes inferred from SSU rRNA sequence data (A). Relative abundance (%) of major taxonomic groups (B).

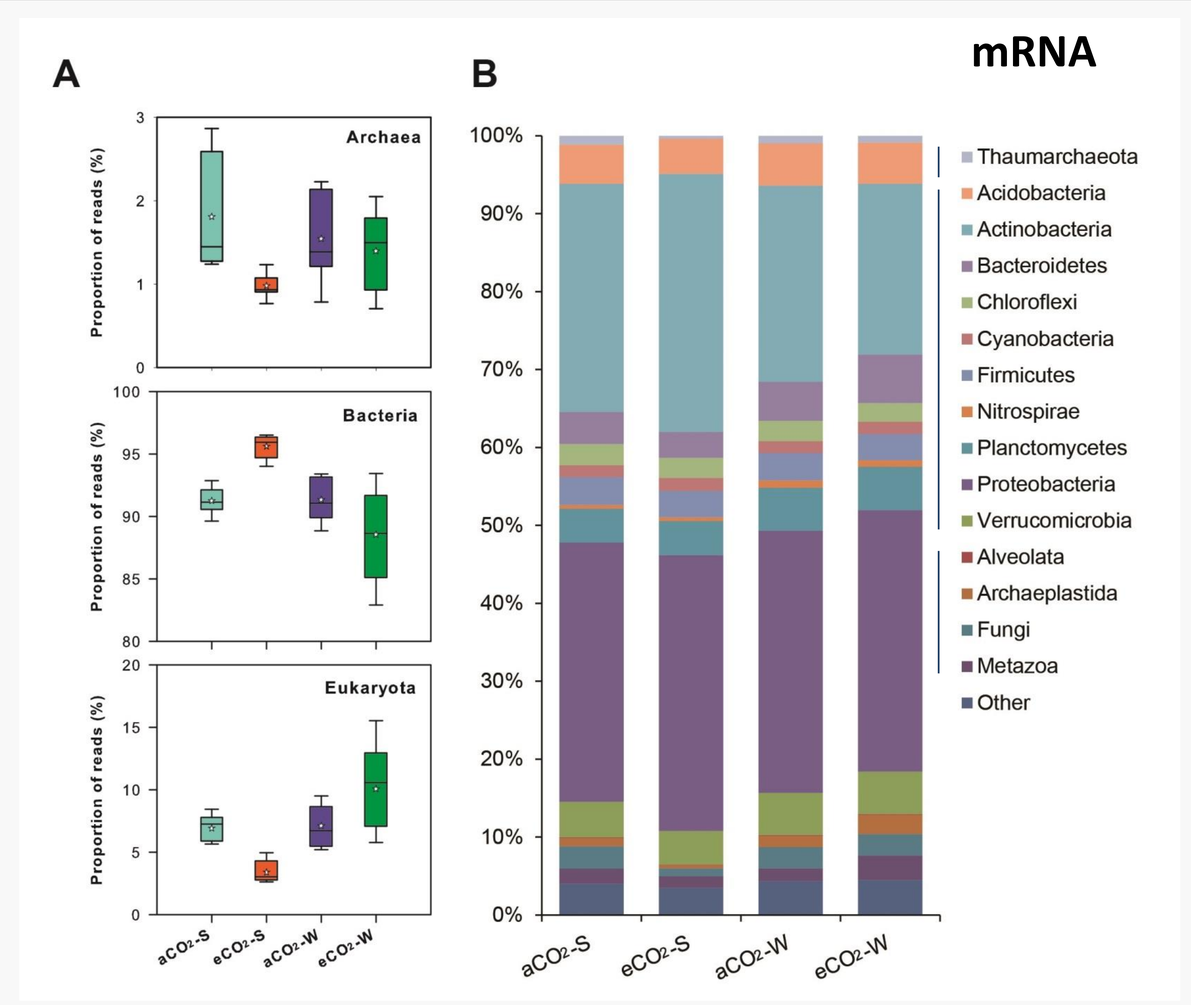


**Figure 4** Proportion of archaea, bacteria and eukaryotes in the rhizosphere microbiomes inferred from mRNA sequence data (A). Relative abundance (%) of major taxonomic groups (B).

## Materials & Methods

Study site was the Free-Air $CO_2$ Enrichment experiment near Gießen (Gi-FACE), where atmospheric or ambient $CO_2$ ($aCO_2$) is moderately enriched to 450 ppm (+20% above $aCO_2$) as predicted to occur within the next few decades. Soil cores (diameter: 3.5 cm) were taken from the upper soil layer (8 - 10 cm) of each FACE ring in summer (August 2015) and winter (February 2016). The extraction of total soil rhizospheric RNA was done as described previously (Mettel *et al*., 2010). Total RNA was reverse transcribed using random-primed hexamers. The cDNA libraries were mixed in equal molar ratio and sequenced at the Max Planck Genome Centre Cologne using Illumina HiSeq 2500. Sequence data analysis involved small-subunit ribosomal RNA (SSU rRNA) and messenger RNA (mRNA).

## Perspectives

Our study provides the first analysis on the impact of $eCO_2$ on rhizosphere microbial communities across all three domains of life (archaea, bacteria, and eukaryotes). Most notably is the different trend in the relative abundances of SSU rRNA and mRNA between bacteria and eukaryotes (in particular fungi) under $eCO_2$ during the extremely dry summer in 2015. Overall, our study confirms the power of metatranscriptomics to reveal shifts in relative activity among different microbial groups, even on domain level. In our future studies (samplings in 2016 and in 2017), the focus shall be placed on functional metatranscriptomics and plant-associated (rhizoplane) microbial communities.

**References:**

Mettel, Carsten, *et al.* "Extraction of mRNA from soil." *Applied and Environmental Microbiology* 76 (2010): 5995-6000.