Metatranscriptomic response of the wheat holobiont to decreasing soil water content

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Abstract

It is admitted that multicellular hosts and their microbiota form an indissociable unit, the holobiont. Even though it is posited that this unit would react in a coordinated fashion to environmental stress, the role of each of the partners is ill-defined. Here, we subjected wheat to a gradient of water availability in the field using rainout shelters that let through 100%, 75%, 50% and 25% of the precipitation. At the height of the growing season, we sampled plant roots and rhizosphere, and extracted and sequenced their RNA. We more specifically compared the 100% to the 25% treatments using differential abundance analysis. In the roots, the majority of the differentially abundant (DA) transcripts belonged to the fungi, and most were overexpressed in the 25% precipitation treatment. Approximately 10% of the DA transcripts belonged to the plant, and most were underexpressed in the 25% precipitation treatment. In the rhizosphere, most of the DA transcripts belonged to the bacteria and were mostly overexpressed in the 25% precipitation treatment. Taken together, our results are showing that the response of the wheat holobiont to decreasing precipitation levels is mostly carried out by fungi and bacteria, with very little response from the plant itself.

Introduction

Drought is one of the most significant threats to global agriculture and will become more frequent and intense as climate change strengthens [1]. In Canada, this will particularly affect the Prairies. Wheat is the most important crop grown in the Prairies and drought stress can diminish wheat yield by 50% or more [2, 3] and recent shifts in the climate are linked to a dramatic decrease in global wheat production [4]. Simultaneously, the worldwide demand for cereal is increasing by roughly 1.5% annually, while genetic improvement of wheat increases yield by approximately 1%, leaving a shortfall of around 3.3 million metric tons of wheat per year (FAOSTAT 2010 http://faostat.fao.org/) [5]. There is a need for alternative approaches to improve wheat yields under sub-optimal conditions. Although there are numerous reports on the genetic, molecular and physiological bases of how plants respond to drought stress [6], the nature of plant adaptation to high stress remains unresolved. One possible avenue would be to manipulate plant-associated microbial communities.

Many specific bacteria of the phylum *Actinobacteria* and *Proteobacteria* have been shown to improve plant tolerance to drought- or salinity-related stresses [7-10]. Fungal endophytes have also shown the potential to improve plant performance under abiotic stress [11-13]. Mycorrhizal fungi can improve water use efficiency and reduce drought stress in wheat [14], oat [15], and corn [16]. Interestingly, endophytic and rhizospheric microorganisms isolated from environments prone to drought tend to confer plants with a better resistance to drought [13, 17]. Many mechanisms have been shown to be involved in the enhancement of plant drought tolerance by microbes. These include modulation of plant drought stress genes [18], reduction of the stress hormone ethylene levels through degradation of its precursor 1-aminocyclopropane-1-carboxylic acid (ACC) by the bacterial enzyme ACC deaminase [7, 8], stimulation of the

expression of plant genes related to osmolytes and osmoprotectants by bacterial volatile organic compounds [19] and modulation of the plant epigenetics response to drought [20]. Even though some microbial taxa and functions do appear to help plants adapt to drought stress, the relative importance of plant vs. microbiota for the adaptation of holobionts remains unresolved.

An interesting framework for the modification of host-associated microbial communities is the hologenome theory of evolution [21], which considers the host and its microbiota (the holobiont) as one evolutionary unit. It implies that there are rapid, microbial-driven means by which holobionts can adapt to new environmental conditions [22, 23]. A holobiont can rapidly change through 1) recruitment of new microbial partners from external sources, 2) amplification or reduction of the microbial partners already in place, and 3) horizontal gene transfer (HGT) from the external communities to the microbial partners already in place. These are coherent with the mechanisms of ecological community change put forward in the theory of ecological communities [24] and as applied to plant microbial communities [25], namely 1) migration, 2) selection, 3) speciation and 4) drift. However, there is still a lack of empirical evidence to ascertain if the holobiont response to environmental stresses really relies on the microbial partners.

We have previously showed that when willows were subjected to contaminant stress, the most differentially expressed genes in the roots were from the fungi whereas bacteria expressed genes related to the degradation of contaminants and plants did not show a strong response [26]. We further showed that plant gene expression in the roots was more variable across plant genotypes than between contaminated and non-contaminated soils, in contrast to the strong response of bacteria in contaminated soils [27]. The transcriptomic response of the microbiota can stem from two mechanisms: 1) a change in the composition of the microbial community and

2) a change in the gene expression of the member of the community. Although these two mechanisms are difficult to disentangle in metatranscriptomic datasets, the result will be the same: a change in the complement of gene expressed within the holobiont.

We hypothesized that the microbiota-centered response observed by the willow holobionts to contaminant stress will also be seen for other plant holobionts facing other stresses, because the microbial component of the holobiont can respond faster to environmental changes. To test this hypothesis, we subjected wheat plants to a gradient of precipitation using rainout shelters that let through 25, 50, 75 or 100% of the natural precipitation. Plant roots and rhizosphere were sampled, their RNA extracted and sequenced.

Materials and methods

Experimental design and sampling

Four rainfall manipulation treatments were set-up in 2016 at the Armand-Frappier Sante Biotechnologie Centre (Laval, Québec, Canada) using 2m x 2m rain-out shelters that passively let through 25%, 50%, 75% and 100% of the natural precipitation. The rainfall exclusion treatments were performed using rain-out shelters, which were covered with various amount of transparent plastic sheeting. The rain was intercepted by the plastic sheeting and guided in a gutter and downspout and collected in 20L buckets that were manually emptied following significant rainfall events. Two wheat genotypes were seeded under these shelters (drought sensitive, *Triticum aestivum* cv. AC Nass and drought tolerant, *Triticum turgidum spp. durum* cv. Strongfield), and the experiment was replicated over 6 fully randomized blocks, resulting in 48 plots (4 treatments x 2 genotypes x 6 blocks). Seeds harvested from each of the plots were re-seeded in the exact same plot the following year. In 2017, each plot was seeded on May 23, at a density of 500 seeds per m^2. For the current manuscript, only the Strongfield cultivar was used, from which rhizosphere soil and root samples were taken on July 26. For rhizosphere sampling, a plant was randomly selected (avoiding the edge of the plots), uprooted and shaken vigorously to remove the loosely attached soil. Soil tightly adhering to roots after shaking was considered as rhizosphere soil and was collected in sterile 1.5 ml microcentrifuge tubes. After collecting the rhizosphere soil, roots were washed with distilled water, separated from the plant and stored in sterile 15 ml Falcon tubes. Collected rhizosphere soil and root samples were flash frozen in liquid nitrogen within a span of 2 minutes after uprooting the plant to maintain the RNA integrity. Tubes were stored at -80°C until the samples were processed for RNA extraction. At sampling, we also collected a bulk soil sample from the center of each plot for soil water content

measurement. We measured soil water content by weighing soils before and after drying overnight at 105°C.

RNA extraction and sequencing

Total RNA was extracted from 2 g of rhizosphere soil using the RNeasy PowerSoil Total RNA Kit (QIAGEN, Canada) and 0.5 g roots using RNeasy Plant Mini Kit (QIAGEN, Canada). Extracted RNA was treated with DNAse (ThermoFisher, Canada) to remove the DNA prior to sequencing. The absence of DNA was confirmed by the lack of PCR amplification using 16S rRNA gene specific primers. Total RNA was sent for Illumina HiSeq4000 2 x 100 bp pair end sequencing at the Centre d'Expertise et de Services Génome Québec (Montréal, Québec). Libraries for rhizosphere samples were created using a microbial ribosome subtraction approach to capture all microbial transcripts, whereas libraries for root samples were created using a poly-dT reverse transcription approach to focus on the plant transcripts. The raw data produced in this study was deposited in the NCBI under Bioproject accession PRJNA880647.

Bioinformatics

The metatranscriptome sequencing of the 24 root and 24 rhizosphere samples resulted in 2,639M reads resulting in 264 giga bases which were processed together through our metatranscriptomics bioinformatics pipeline [28]. Briefly, sequencing adapters were removed from each read and bases at the end of reads having a quality score less than 30 were cut off (Trimmomatic v0.32) [29] and scanned for sequencing adapters contaminants reads using DUK (http://duk.sourceforge.net/) to generate quality controlled (QC) reads. QC-passed reads from

each sample were co-assembled using Megahit v1.1.2 [30] with iterative kmer sizes of 31,41,51,61,71,81 and 91 bases. Gene prediction was performed by calling genes on each assembled contig using Prodigal v2.6.2 [31]. Genes were annotated following the JGI's guidelines [32] including the assignment of KEGG orthologs (KO). QC-passed reads were mapped (BWA mem v0.7.15) (unpublished - http://bio-bwa.sourceforge.net) against contigs to assess quality of metagenome assembly and to obtain contig abundance profiles. Alignment files in bam format were sorted by read coordinates using samtools v1.2 [33] and only properly aligned read pairs were kept for downstream steps. Each bam file (containing properly aligned paired-reads only) was analyzed for coverage of called genes and contigs using bedtools (v2.17.0) [34] using a custom bed file representing gene coordinates on each contig. Only paired reads both overlapping their contig or gene were considered for gene counts. Coverage profiles of each sample were merged to generate an abundance matrix (rows = contig, columns = samples) for which a corresponding CPM (Counts Per Million – normalized using the TMM method) (edgeR v3.10.2) [35]. Each contig was blasted (BLASTn v2.6.0+) against NCBI's nt database (version downloaded from NCBI's server on January 9th 2019) and the best hit's taxonomic identifier was used to assign a taxonomic lineage to the contig. Taxonomic summaries were performed using MicrobiomeUtils v0.9 (github.com/microbiomeutils). The metatranscriptome co-assembly, gene abundance, read count summaries and mapping statistics and other results generated by our bioinformatic workflow are provided in the companion online Zenodo archive (https://doi.org/10.5281/zenodo.7121038).

Statistical analyses

All statistical analyses were performed in R version 4.1.0. [36]. Transcript differential abundance analyses between the 100% and 25% precipitation treatments were carried out using the EBTest function of the EBSeq library with a false discovery rate (FDR) of 0.05. Paired t-test were performed using the t.test function of the stats library. Anovas were performed using the aov function of the stats package. All graphs were generated using ggplot2. The R project folder containing the R code used for data manipulation, statistical analyses, and tables and figure generation is available on our lab GitHub repository (https://github.com/le-labo-yergeau/MT_Holobiont_Wheat). The associated transcript abundance and annotation tables, the metadata, and the soil water content files used with the R code are available on Zenodo: https://doi.org/10.5281/zenodo.7096909.

Results

Soil water content (SWC)

There was a significant difference (p = 0.000367) between the mean SWC across the four treatments. The water content was highest in plots exposed to 100% of the natural precipitation and gradually decreased in plots receiving 75%, 50% and 25% of the natural precipitation (Fig. 1a). The SWC was of 11% at its lowest (in the 25% precipitation treatment) and of 23% at its highest (in the 100% treatment). The rest of our analyses focus on the two most extreme conditions, the 25% and 100% precipitation treatments.

Responses of the holobiont partners

We retrieved 1,069,108,624 clean sequencing reads (per sample, mean: 22,746,992, max: 43,912,775, min: 13,943,395) that were assembled in a total of 1,269,055 transcripts, among which 1,193,501 and 1,136,587 transcripts were found in the rhizosphere soil and wheat roots, respectively. Among the wheat root transcripts, 12,792 (1.1%) belonged to the Archaea, 365,435 (32.2%) to Bacteria, 200,233 (17.6%) to Fungi, 200,823 (17.7%) to plants, 132,313 (11.6%) to other Eukaryotes, 3,660 (0.3%) to viruses and 221,331 (19.5%) were not identified at the kingdom level (Fig 1b). Among the rhizosphere soil transcripts, 14,943 (1.3%) belonged to the archaea, 430,984 (36.1%) to the bacteria, 186,745 (15.6%) to the fungi, 169,788 to the plants (14.2%), 132,042 (11.1%) to other eukaryotes, 4,255 (0.4%) to viruses, and 254,744 (21.3%) were not classified at the kingdom level (Fig. 1b).

In the roots, among the 1,136,587 transcripts, 42,001 (3.70%) were differentially abundant (DA) at a false discovery rate (FDR) of 0.05. Among these DA transcripts, 2,309

belonged to the bacteria (5.50%), 23,274 to the fungi (55.41%), 4,357 to the plants (10.37%), 5,303 were not classified at the kingdom level (12.63%) and 6,758 belonged to other taxa (16.09%) (Fig. 1c and 2a). For bacteria and fungi, most of the DA transcripts were more abundant in the 25% treatment as compared to the 100% treatment (23,042 and 2,231 more abundant vs. 232 and 78 less abundant for fungi and bacteria, respectively), whereas it was the inverse for plant (1,295 genes more abundant vs. 3,061 less abundant) (Fig. 1c and 2a).

In the rhizosphere, among the 1,193,501 transcripts, 21,765 (1.82%) were differentially abundant at an FDR of 0.05. Among these DA transcripts, 14,178 belonged to the bacteria (65.14%), 159 to the archaea (0.73%), 402 to the fungi (1.85%), 219 to the plants (1.01%), 5,224 were not classified at the kingdom level (24.00%) and 1,583 belonged to other taxa (7.27%) (Fig. 1c and 2b). For bacteria, most DA transcripts were more abundant in the 25% treatment as compared to the 100% treatment (7,938 more abundant vs. 6,240 less abundant), whereas it was the inverse for plant (41 more abundant vs. 178 less abundant) and fungi (149 more abundant vs. 253 less abundant) (Fig. 1c and 2b).

Taxonomy and function of the DA transcripts: roots

We compared the taxonomic affiliation and COG categories for the whole dataset vs. the one for the more and less abundant transcripts using paired t-tests (Fig. 3a and b). For the root transcripts, we focused on the fungal classes and found that as compared to their prevalence among all transcripts, the *Dothideomycetes* were overrepresented among the more abundant DA transcripts (P=0.00054) and underrepresented among the less abundant DA transcripts (P=0.00049) (Fig. 3a). In contrast, the *Eurotiomycetes* were underrepresented among the more abundant DA transcripts (P=0.0011) and overrepresented among the less abundant DA

transcripts (P=0.0077) (Fig. 3a). For the transcripts affiliated to the *Agaricomycetes* and the *Sordariomycetes*, there were no significant differences between their prevalence among all transcripts and among the DA transcripts (Fig. 3a).

As for COG categories found among the fungal DA transcripts significantly more abundant in the 25% precipitation treatment (Fig. 4a), there was an overrepresentation of the "Carbohydrate transport and metabolism" "DNA replication, recombination and repair", "Inorganic ion transport and metabolism", "Signal transduction mechanisms", "Transcription" categories (paired t-tests, P=0.022, P=0.00028, P=0.00014, P=0.0066, P=9.40 x 10^{-5}, respectively) as compared to their prevalence among all transcripts, whereas the "Amino acid transport and metabolism", "Cell envelope biogenesis, outer membrane", "Cell motility and secretion", "Energy production and conversion", "Posttranslational modification, protein turnover, chaperones", and "Translation, ribosomal structure and biogenesis" categories were significantly underrepresented (paired t-tests, P=0.015, P=7.21 x 10^{-5}, P=0.010, P=0.026, P=1.89 x 10^{-6}, 4.76 x 10^{-7}, respectively) as compared to their prevalence among all transcripts. In contrast, among the fungal DA transcripts that were significantly less abundant in the 25% precipitation treatment (Fig. 4a), there was a significant overrepresentation of the "Translation, ribosomal structure and biogenesis" category (paired t-test, P=0.0085) as compared to their prevalence among all transcripts, whereas the categories "Carbohydrate transport and metabolism", "Energy production and conversion", "Lipid metabolism", "Posttranslational modification, protein turnover and chaperones", "Transcription" were significantly underrepresented (paired t-tests, P=2.38 x 10^{-6}, P=4.34 x 10^{-7}, P=9.77 x 10^{-5}, P=0.00023, P=0.0041, respectively).

Taxonomy and function of the DA transcripts: rhizosphere

For the rhizosphere transcripts, we focused on bacterial phyla for the taxonomical comparisons and for bacterial DA transcripts for COG categories (Fig. 3b, 4b). Among the transcripts significantly more abundant in the 25% treatment rhizospheres, the *Actinobacteria* were overrepresented (paired t-tests, $P=5.45 \times 10^{-9}$), whereas the *Acidobacteria*, the Candidatus *Rokubacteria*, the *Chloroflexi*, the *Gemmatimonadetes*, the *Nitrospirae*, the *Planctomycetes*, the *Proteobacteria*, and the *Verrocumicrobia* were underrepresented as compared to their relative abundance across all transcripts (paired t-tests, $P=1.58 \times 10^{-7}$, $P=1.97 \times 10^{-10}$, $P=1.73 \times 10^{-9}$, $P=1.71 \times 10^{-5}$, $P=2.23 \times 10^{-8}$, $P=8.69 \times 10^{-8}$, $P=2.82 \times 10^{-3}$, $P=1.88 \times 10^{-6}$, respectively) (Fig. 3b). Among the transcripts significantly less abundant in the 25% treatment rhizospheres, the *Acidobacteria*, the *Bacteroidetes*, the *Nitrospirae*, the *Planctomycetes*, and the *Proteobacteria* were overrepresented (paired t-tests, P=0.019, P=0.014, $P=2.55 \times 10^{-5}$, $P=5.13 \times 10^{-7}$, $P=1.82 \times 10^{-10}$, respectively), whereas the *Actinobacteria*, the Candidatus *Rokubacteria*, the *Chloroflexi*, the *Cyanobacteria*, the *Gemmatimonadetes* were significantly underrepresented as compared to their relative abundance among all transcripts ($P=2.20 \times 10^{-7}$, $P=8.32 \times 10^{-8}$, $P=4.62 \times 10^{-9}$, P=0.0056, P=0.00094, respectively) (Fig. 3b).

For COG categories, among the transcripts significantly more abundant in the 25% treatment rhizospheres (Fig. 4b), the “Carbohydrate transport and metabolism”, “Coenzyme metabolism”, “Inorganic ion transport and metabolism”, “Lipid metabolism”, “Posttranslational modification, protein turnover, chaperones”, “Transcription”, and “Translation, ribosomal structure and biogenesis” categories were significantly overrepresented as compared to their occurrence across all transcripts (paired t-tests, $P=5.28 \times 10^{-6}$, P=0.012, P=0.029, P=0.012, $P=7.89 \times 10^{-6}$, $P=1.23 \times 10^{-6}$, P=0.00011, respectively), whereas the “Amino acid transport and

metabolism", "Cell envelope biogenesis and membrane", "Intracellular trafficking and secretion", and "Signal transduction mechanisms" COG categories were significantly underrepresented as compared to their occurrence across all transcripts (paired t-tests, P=0.00084, P=2.67 x 10^{-6}, P=3.91 x 10^{-10}, P=0.0075, respectively). Among the transcripts that were significantly less abundant in the 25% treatment rhizospheres (Fig. 4b), the, "Cell motility and secretion", "DNA replication, recombination and repair", "Intracellular trafficking and secretion", and "Translation, ribosomal structure and biogenesis" COG categories were significantly overrepresented as compared to their occurrence across all transcripts (paired t-tests, P=8.25 x 10^{-6}, P=0.023, P=1.68 x 10^{-10}, P=1.13 x 10^{-5}, respectively), whereas the "Amino acid transport and metabolism", "Carbohydrate transport and metabolism", "Coenzyme metabolism", "Inorganic ion transport and metabolism", "Lipid metabolism", "Posttranslational modification, protein turnover, chaperones" and the "Signal transduction mechanisms" COG categories were underrepresented as compared to their occurrence across all transcripts (paired t-tests, P=0.00027, P=2.54 x 10^{-6}, P=8.36 x 10^{-6}, P=3.72 x 10^{-5}, P=5.36 x 10^{-6}, P=9.39 x 10^{-10}, P=1.07 x 10^{-7}, respectively).

Most differentially abundant genes

For DA analyses of the root samples, there were many transcripts that had a P-value=0, so we sorted them by mean abundance and are showing the top 50 transcripts in Table 1. Twenty-seven transcripts among the top 50 transcripts belonged to the *Agaricomycetes*, mostly *Coprinopsis cinerea*, and were almost all more abundant in the 25% precipitation treatment (Table 1). Seven transcripts could be related to the wheat tribe (*Triticum aestivum* or *Aegilops tauschii*), all of which were less abundant in the 25% precipitation treatment (Table 1). Many of the most

significantly more abundant transcripts in the 25% precipitation treatment were related to amino acid and carbohydrate transport and metabolism, with transcripts such as “Amino acid transporters”, “Glycerol uptake facilitator and related permeases”, “Beta-glucanase/Beta-glucan synthetase”, “Dipeptide/tripeptide permease”, “Fucose permease”, “Neutral trehalase” and “Hexokinase” (Table 1). In contrast, many of the most significantly less abundant transcripts in the 25% precipitation treatments were linked to the COG categories “Posttranslational modification, protein turnover, chaperones” and “Secondary metabolites biosynthesis, transport and catabolism” (Table 1).

For the rhizosphere, as not that many DA transcripts had a P-value=0, we are presenting the 50 lowest P-values observed in Table 2. Half the DA transcripts with the lowest P-values belonged to the *Proteobacteria*, mainly the *Alpha*- and *Delta*- classes (Table 2). Many of the most significantly less abundant transcripts in the 25% precipitation treatment were linked to the COG categories “Cell motility and secretion” and “Intracellular trafficking and secretion”, with functions related to pilus, flagella and type II and VI secretion systems (Table 2). Similar to what we observed in the roots, the fungal transcripts more abundant in the 25% precipitation treatment belonged to the *Agaricomycetes* and were related to carbohydrate and amino acid transport and metabolism (e.g. “monoamine oxidase”, “hexokinase”) (Table 2).

Discussion

We wanted to know how the wheat holobiont would respond to change in soil water availability at the transcriptomic level, and which of the partners would be more responsive. We were successful in reducing soil water content in a field experiment using rainout shelters, and found that, when comparing the two most contrasting treatments, most of the differentially abundant (DA) genes were linked to the fungi in the roots and to the bacteria in the rhizosphere. In the roots, most of the fungal genes were more abundant, whereas it was more balanced for bacterial transcripts in the rhizosphere. These DA genes belonged to specific taxa and many of them could be related to genes known to help plants and microorganisms cope with water stress.

The microbial component of the holobiont partnership is much more dynamic and plastic than the host part. Indeed, the microbiota can be modified rapidly by changing the relative abundance of the community members, by recruiting new members from the environment or through mechanisms such as horizontal gene transfer (HGT) [37]. For sessile hosts, like plants, the possible response is limited to modulating gene expression, with cascading effects on hormone levels, physiology, etc. Our results are highly coherent with a previous study from our group that showed that the fungal component of willow holobionts subjected to contaminant stress showed the strongest response in the roots [26]. Bacteria also responded, mainly by expressing genes related to the degradation of the pollutants, whereas plants did not show large transcriptomic responses [26]. It should be kept in mind that the water depletion caused by our rainout shelters did not result in extremely low soil water content (around 12% soil water content at the lowest), which did not result in any visible stress on wheat. The wheat variety used is also known to be water stress resistant, and this could explain why the plant did not show a strong transcriptomic response to the lower soil water concentrations. It would be interesting to measure

the transcriptomic response of plant holobionts when exposed to much more extreme stress levels.

With the method used here, it is difficult to disentangle the metatranscriptomic response due to shifts in the composition of the microbial community and in the gene expression within the same community. Therefore, we refer to our differential expression analysis as a transcript differential abundance analysis. The *Actinobacteria* well exemplify this. There was an overrepresentation of transcripts belonging to the *Actinobacteria* among the ones that were overexpressed in the rhizosphere. This phylum was shown at multiple occasions to increase in relative abundance when soils get drier [38-42]. Inversely, transcripts belonging to the *Proteobacteria* and *Acidobacteria* were overrepresented among the significantly less abundant transcripts and underrepresented among the significantly more abundant transcripts, which is coherent with their heightened sensitivity to water stress [42, 43]. Other taxa, such as the *Chloroflexi* and the *Gemmatimonadetes*, could be categorized as non-responsive, as they were found to be underrepresented among both significantly more and less abundant transcripts. Looking only at high-level functional categories, like in Figure 4, could partly solve this problem, as general trends in gene expression at this level is less likely to be influenced by shifts in community composition. Nevertheless, we argue that whatever the underlying mechanisms are, variation in the rhizosphere and root metatranscriptome complement will have functional consequences on the holobiont adaptation to stress.

Among the most significantly overexpressed genes in the roots under 25% precipitation regime, many could be related to amino acid and carbohydrate transport and metabolism. Amino acids, such as proline, glutamine, and glycine, betaine, and carbohydrates, such as trehalose and ectoine can be used as osmolytes [44] to maintain cellular turgor and protect macromolecular

structures [45]. Gram-negative bacteria produce osmolytes purely as a drought-inducible response, whereas Gram-positive bacteria tend to produce osmolytes, at least partially, on a constitutive basis (Schimel et al., 2007), which could explain some of the differences in the transcriptomic response of different taxa observed here. It would be interesting to know how much this overexpression is beneficial to the microbes vs. the host plant. There is some evidence that microbial endophytes and rhizobacteria can increase plant osmolyte concentration [46, 47], including proline [48], and some studies have reported that microbes can exude these compounds in the plant environment [49, 50], enabling them to directly contribute to the plant osmolyte concentration during water stress. For instance, *Coprinopsis* were often reported as endophytes of plants, including *Arabidopsis* [51] and were found here among the root fungi that showed the strongest response to decreasing soil water content, with many of their overexpressed genes related to carbohydrate or amino acid transport and metabolism.

Other genes were affected by the precipitation treatments. Among the rhizosphere bacteria, many genes related to pilus and flagella formation were downregulated with decreasing soil water content, which might be indicative of a switch from a free-living to a sessile lifestyle. Indeed, biofilm formation is a well-known mechanism that bacteria use to cope with environmental stresses [52]. The expression of genes coding for heat shock proteins were also upregulated in the rhizosphere and the roots, which is coherent with previous reports about their important roles in the response to drought stress for both microbes and plants [53-55]. In both root and rhizosphere, there was a significant overrepresentation of genes related to translation among the downregulated genes. Interestingly, a similar down-regulation of the protein biosynthesis machinery was observed in a recent metatranscriptomic study of the response of soil microorganisms to short, medium and long-term warming [56]. The author suggested that the

increased enzymatic activity and overall metabolism caused by warming could call for a lower energy investment in ribosomes, thus optimizing resource allocation [56]. It is unclear how this explanation would apply in the context of decreasing soil water content. It was shown that during soil drying, *Acidobacteria* and *Verrocumicrobia* reduced their ribosomal content, whereas the *Actinobacteria* increased it [57]. It could be that the shifts observed here in community composition had a net result of decreased investment in protein synthesis. This common translation level response to different stress by plant and soil microbial communities would warrant further investigations.

Taken together, our results are pointing out that the microbial component of the wheat holobiont is the strongest responder to decreasing soil water content. These transcriptomic shifts were clearly not at random, being mostly related to genes and organisms that are known to respond to water stress. This suggests that the microbiome of crops, which is often overlooked in breeding efforts, should be a prime target for adapting crops to water stress.

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Figure legends

Figure 1: (a) mean soil water content at the time of sampling for the four different precipitation manipulation treatments, (b) kingdom-level taxonomic affiliation of the sequencing reads retrieved for all roots and rhizosphere samples, (c) kingdom-level affiliation of the reads linked to differentially abundant (DA) transcripts together with information if they were more or less abundant in the 25% treatment as compared to the 100% treatment.

Figure 2: Volcano plot of transcripts log2 fold change vs. mean relative abundance, with significantly differently abundant (DA) transcripts highlighted by colors corresponding to their kingdom-level taxonomy for (a) roots and (b) rhizosphere soil. Blue: fungi, green: plant, red: bacteria, yellow: others, purple: unclassified, pink: archaea.

Figure 3: Stack bar chart comparing the taxonomical affiliations of transcripts associated to fungi in roots (a) and to bacteria in the rhizosphere (b) across all samples vs. among transcripts significantly more or less abundant in differential abundance analyses.

Figure 4: Stack bar chart comparing the functional affiliations of transcripts associated to fungi in roots (a) and to bacteria in the rhizosphere (b) across all samples vs. among transcripts significantly more or less abundant in differential abundance analyses.

Table 1: Mean abundance, log2 fold change (25% vs 100% precipitation), COG function and category and taxonomic affiliation for the top 50 most abundant genes with a P-value=0 in the roots.

Gene id	mean	log2	P-value	COG function	COG category	Class	Species
751566	805.00	9.57	0	Neutral trehalase	Carbohydrate transport and metabolism	*Agaricomycetes*	*Coprinopsis cinerea*
148591	759.45	8.04	0	Dipeptide/tripeptide permease	Amino acid transport and metabolism	*Agaricomycetes*	*Coprinopsis cinerea*
130818	659.18	8.17	0	Molecular chaperone, HSP90 family	Posttranslational modification, protein turnover, chaperones	*Agaricomycetes*	*Coprinopsis cinerea*
945131	441.27	8.04	0	NAD-dependent aldehyde dehydrogenases	Energy production and conversion	*Agaricomycetes*	*Hebeloma cylindrosporum*
560	359.64	7.75	0	Uncharacterized conserved protein	Function unknown	*Agaricomycetes*	*Coprinopsis cinerea*
934948	310.09	8.19	0	Hexokinase	Carbohydrate transport and metabolism	*Agaricomycetes*	*Gymnopilus dilepis*
1189377	300.36	8.14	0	Phosphatidylserine synthase	Lipid metabolism	*Agaricomycetes*	*Coprinopsis cinerea*
956836	243.82	7.64	0	Nucleoside permease	Nucleotide transport and metabolism	*Agaricomycetes*	*Coprinopsis cinerea*
121608	241.82	7.83	0	Amidases related to nicotinamidase	Secondary metabolites biosynthesis, transport, and catabolism	*Agaricomycetes*	*Coprinopsis cinerea*
92085	224.27	8.94	0	6-phosphogluconate dehydrogenase	Carbohydrate transport and metabolism	*Agaricomycetes*	*Coprinopsis cinerea*
86792	213.27	8.87	0	Phosphatidylserine/phosphatidylglycerophosphate/cardiolipin synthases and related enzymes	Lipid metabolism	*Agaricomycetes*	*Coprinopsis cinerea*
1107703	211.18	7.98	0	Polyketide synthase modules and related proteins	Secondary metabolites biosynthesis, transport, and catabolism	*Sordariomycetes*	*Stachybotrys chartarum*
2979	194.00	9.32	0	Thiamine pyrophosphate-requiring enzymes [acetolactate synthase, pyruvate dehydrogenase (cytochrome), glyoxylate carboligase, phosphonopyruvate decarboxylase]	Amino acid transport and metabolism / Coenzyme metabolism	*Agaricomycetes*	*Coprinopsis cinerea*
183197	190.18	7.70	0	Glycerol uptake facilitator and related permeases (Major Intrinsic Protein Family)	Carbohydrate transport and metabolism	*Agaricomycetes*	*Mycena chlorophos*
14009	178.73	8.61	0	NAD-dependent aldehyde dehydrogenases	Energy production and conversion	*Agaricomycetes*	*Coprinopsis cinerea*
636334	165.64	10.09	0	Predicted aminopeptidases	General function prediction only	*Agaricomycetes*	*Panaeolus cyanescens*
196702	159.00	9.03	0	Nucleoside-diphosphate-sugar epimerases	Cell envelope biogenesis, outer membrane / Carbohydrate transport and metabolism	*Agaricomycetes*	*Coprinopsis cinerea*
47528	150.45	8.37	0	Ca2+-binding actin-bundling protein fimbrin/plastin (EF-Hand superfamily)	Cytoskeleton	*Agaricomycetes*	*Coprinopsis cinerea*
657208	147.45	8.34	0	Amino acid transporters	Amino acid transport and metabolism	*Agaricomycetes*	*Dichomitus squalens*
166571	140.73	8.86	0	Predicted hydrolases or acyltransferases (alpha/beta hydrolase superfamily)	General function prediction only	*Sordariomycetes*	*Sodiomyces alkalinus*
488208	136.36	8.81	0	RNA-binding proteins (RRM domain)	General function prediction only	*Agaricomycetes*	*Coprinopsis cinerea*
222991	134.27	9.79	0	Nicotinic acid mononucleotide adenylyltransferase	Coenzyme metabolism	*Agaricomycetes*	*Coprinopsis cinerea*
165517	107.55	-Inf	0	Ca2+-binding protein (EF-Hand superfamily)	Signal transduction mechanisms / Cytoskeleton / Cell division and chromosome partitioning / General function prediction only	*Liliopsida*	*Aegilops tauschii*

210176	102.55	8.40	0	O-Glycosyl hydrolase	Cell envelope biogenesis, outer membrane	*Sordariomycetes*	*Sodiomyces alkalinus*
202276	99.73	9.36	0	Cytochrome P450	Secondary metabolites biosynthesis, transport, and catabolism	*Agaricomycetes*	*Coprinopsis cinerea*
414450	98.55	9.34	0	NAD-dependent aldehyde dehydrogenases	Energy production and conversion	*Agaricomycetes*	*Laccaria amethystina*
660987	96.09	8.30	0	Asp-tRNAAsn/Glu-tRNAGln amidotransferase A subunit and related amidases	Translation, ribosomal structure and biogenesis	*Agaricomycetes*	*Coprinopsis cinerea*
1184958	79.64	9.03	0	Beta-glucanase/Beta-glucan synthetase	Carbohydrate transport and metabolism	*Agaricomycetes*	*Coprinopsis cinerea*
572300	78.27	-Inf	0	Predicted membrane protein	Function unknown	*Agaricomycetes*	*Panaeolus cyanescens*
210781	76.73	-Inf	0	Ribosomal protein S3	Translation, ribosomal structure and biogenesis	*NULL*	*Populus trichocarpa*
720478	74.91	-Inf	0	FKBP-type peptidyl-prolyl cis-trans isomerases 1	Posttranslational modification, protein turnover, chaperones	*NULL*	*Populus trichocarpa*
547100	64.45	-Inf	0	Chromosome segregation ATPases	Cell division and chromosome partitioning	*Clitellata*	*Hirudo medicinalis*
466738	61.55	-Inf	0	Dehydrogenases with different specificities (related to short-chain alcohol dehydrogenases)	Secondary metabolites biosynthesis, transport, and catabolism / General function prediction only	*Liliopsida*	*Aegilops tauschii*
1041684	60.91	Inf	0	Fucose permease	Carbohydrate transport and metabolism	*Dothideomycetes*	*Ascochyta rabiei*
1022565	60.55	-Inf	0	Putative multicopper oxidases	Secondary metabolites biosynthesis, transport, and catabolism	*NULL*	*Populus trichocarpa*
82978	60.27	-Inf	0	Ribosomal protein S19E (S16A)	Translation, ribosomal structure and biogenesis	*NULL*	*Populus trichocarpa*
140966	58.09	Inf	0	Uncharacterized stress protein (general stress protein 26)	General function prediction only	*Sordariomycetes*	*Stachybotrys chartarum*
539196	57.45	-Inf	0	Beta-fructosidases (levanase/invertase)	Carbohydrate transport and metabolism	*Liliopsida*	*Aegilops tauschii*
665062	55.18	-Inf	0	Cytochrome P450	Secondary metabolites biosynthesis, transport, and catabolism	*Liliopsida*	*Triticum aestivum*
200364	55.00	-Inf	0	Ubiquitin-protein ligase	Posttranslational modification, protein turnover, chaperones	*Liliopsida*	*Triticum aestivum*
690880	54.64	Inf	0	Peptidyl-prolyl cis-trans isomerase (rotamase) - cyclophilin family	Posttranslational modification, protein turnover, chaperones	*Insecta*	*Onthophagus taurus*
222916	54.55	-Inf	0	Secreted trypsin-like serine protease	Posttranslational modification, protein turnover, chaperones	*Arachnida*	*AcarusAcarus siro*
1044721	52.00	-Inf	0	FOG: WD40 repeat	General function prediction only	*Liliopsida*	*Aegilops tauschii*
715083	49.45	-Inf	0	Zn-dependent oligopeptidases	Amino acid transport and metabolism	*Liliopsida*	*Aegilops tauschii*
1245890	48.91	Inf	0	Deoxyribodipyrimidine photolyase	DNA replication, recombination, and repair	*Agaricomycetes*	*Leucoagaricus sp. SymC.cos*
247073	48.91	-Inf	0	Ca2+-binding protein (EF-Hand superfamily)	Signal transduction mechanisms / Cytoskeleton / Cell division and chromosome partitioning / General function prediction only	*NA*	*NA*
113358	48.82	Inf	0	Probable taurine catabolism dioxygenase	Secondary metabolites biosynthesis, transport, and catabolism	*Agaricomycetes*	*Coprinopsis cinerea*
258573	47.82	-Inf	0	Secreted trypsin-like serine protease	Posttranslational modification, protein turnover, chaperones	*Insecta*	*Aethina tumida*
1203022	47.00	-Inf	0	Uncharacterized conserved protein	Function unknown	*Liliopsida*	*Aegilops tauschii*
1010199	46.18	-Inf	0	Ribosomal protein HS6-type (S12/L30/L7a)	Translation, ribosomal structure and biogenesis	*NA*	*Populus trichocarpa*

Table 2: Mean abundance, log2 fold change (25% vs 100% precipitation), COG function and category and taxonomic affiliation for the top 50 microbial genes with the lowest P-values in the rhizosphere.

Gene id	Mean	Log2	PPEE	COG function	COG category	Class	Species
1150899	22.42	Inf	0	Monoamine oxidase	Amino acid transport and metabolism	*Agaricomycetes*	*Leucoagaricus sp. SymC.cos*
25017	12.17	-Inf	0	Zn-dependent protease with chaperone function	Posttranslational modification, protein turnover, chaperones	*Gammaproteobacteria*	*Buchnera aphidicola*
252300	20.67	-2.34	0	Cold shock proteins	Transcription	*Deltaproteobacteria*	*Minicystis rosea*
25926	15.83	Inf	0	Hexokinase	Carbohydrate transport and metabolism	*Agaricomycetes*	*Gymnopilus dilepis*
685694	25.67	-1.87	0	Phage tail sheath protein FI	General function prediction only	*Deltaproteobacteria*	*Haliangium ochraceum*
90220	31.67	-2.15	0	Actin and related proteins	Cytoskeleton	*Dictyostelia*	*Dictyostelium discoideum*
125923	554.00	-7.44	6.66E-16	Putative intracellular protease/amidase	General function prediction only	*Microbotryomycetes*	*Rhodotorula graminis*
625425	18.00	-2.05	1.89E-15	Uncharacterized protein conserved in bacteria	Function unknown	*Deltaproteobacteria*	*Sorangium cellulosum*
900820	41.58	-1.77	3.55E-15	Predicted permease	General function prediction only	*Verrucomicrobia*	*Verrucomicrobia bacterium*
988040	16.50	-3.51	5.00E-15	Translation elongation factor EF-1alpha (GTPase)	Translation, ribosomal structure and biogenesis	*Stramenopile*	*Stramenopile sp. ex Nuclearia delicatula CCAP1552/1*
438624	22.83	-1.49	1.17E-14	Outer membrane protein (porin)	Cell envelope biogenesis, outer membrane	*Betaproteobacteria*	*Betaproteobacteria bacterium*
1155936	36.50	-1.21	1.35E-14	Serine/threonine protein kinase	General function prediction only / Signal transduction mechanisms / Transcription / DNA replication, recombination, and repair	*Deltaproteobacteria*	*Minicystis rosea*
843326	19.50	-2.46	1.83E-14	Response regulator containing CheY-like receiver, AAA-type ATPase, and DNA-binding domains	Signal transduction mechanisms	*Nitrososphaeria*	*Nitrosopumilales archaeon*
963336	20.17	-2.61	7.27E-14	ABC-type sugar transport system, periplasmic component	Carbohydrate transport and metabolism	*Actinobacteria*	*Streptomyces sp. NEAU-SSA 1*
920879	10.00	Inf	1.98E-13	Predicted integral membrane protein	Function unknown	*Sphingobacteriia*	*Mucilaginibacter gotjawali*
1143951	141.75	1.51	2.50E-13	Molecular chaperone (small heat shock protein)	Posttranslational modification, protein turnover, chaperones	*Alphaproteobacteria*	*Skermanella aerolata*
666947	19.08	-2.73	3.36E-13	Tfp pilus assembly protein PilE	Cell motility and secretion / Intracellular trafficking and secretion	*Latescibacteria*	*Candidatus Latescibacteria bacterium 4484_7*
998558	36.75	-2.31	4.82E-13	Type II secretory pathway, pseudopilin PulG	Cell motility and secretion / Intracellular trafficking and secretion	*Spartobacteria*	*Chthoniobacter flavus*
308612	18.92	-1.54	5.51E-13	Carbon dioxide concentrating mechanism/carboxysome shell protein	Secondary metabolites biosynthesis, transport, and catabolism / Energy production and conversion	*Deltaproteobacteria*	*Haliangium ochraceum*
5291	7.42	-3.34	1.34E-12	Predicted component of the type VI protein secretion system	Intracellular trafficking, secretion, and vesicular transport	*Deltaproteobacteria*	*Minicystis rosea*
91601	17.67	-2.29	2.07E-12	Type II secretory pathway, pseudopilin PulG	Cell motility and secretion / Intracellular trafficking and secretion	*Acidobacteria*	*Acidobacteria bacterium*
993945	45.58	-1.31	2.20E-12	Uncharacterized conserved protein	Function unknown	*Caldilineae*	*Caldilineae bacterium*
731684	22.67	1.82	4.13E-12	Putative intracellular protease/amidase	General function prediction only	*Actinobacteria*	*Micromonospora auratinigra*

920153	13.50	-1.91	4.87E-12	DNA-directed RNA polymerase, alpha subunit/40 kD subunit	Transcription	*Deltaproteobacteria*	*Haliangium sp. UPWRP_2*
389139	16.67	-1.66	7.06E-12	Flagellin and related hook-associated proteins	Cell motility and secretion	*Alphaproteobacteria*	*Asticcacaulis taihuensis*
647044	11.50	-2.74	9.44E-12	Glutamine synthetase	Amino acid transport and metabolism	*Deltaproteobacteria*	*Geobacter sp. DSM 9736*
1215469	48.33	-1.06	9.81E-12	Heme/copper-type cytochrome/quinol oxidases, subunit 1	Energy production and conversion	*Deltaproteobacteria*	*Sorangiineae bacterium NIC37A_2*
758119	14.33	-1.92	1.03E-11	Nitrogen regulatory protein PII	Amino acid transport and metabolism	*Deltaproteobacteria*	*Anaeromyxobacter dehalogenans*
372651	28.00	-1.24	1.32E-11	S-adenosylmethionine synthetase	Coenzyme metabolism	*Deltaproteobacteria*	*Labilithrix luteola*
377149	14.92	-2.15	1.43E-11	Biopolymer transport proteins	Intracellular trafficking and secretion	*Deltaproteobacteria*	*Deltaproteobacteria bacterium*
1018589	19.17	-1.44	2.74E-11	F0F1-type ATP synthase, alpha subunit	Energy production and conversion	*Clostridia*	*Butyricicoccus pullicaecorum*
303242	13.33	-2.98	2.76E-11	Competence protein ComGC	Intracellular trafficking and secretion	*Acidobacteria*	*Acidobacteria bacterium SCN 69-37*
1232742	49.17	-2.26	2.90E-11	Type II secretory pathway, pseudopilin PulG	Cell motility and secretion / Intracellular trafficking and secretion	*Acidobacteria*	*Acidobacteria bacterium*
1041075	20.58	-1.77	3.42E-11	Cytochrome c peroxidase	Inorganic ion transport and metabolism	*Planctomycetes*	*Planctomycetes bacterium*
720588	9.50	-2.61	4.18E-11	DNA-directed RNA polymerase, beta subunit/140 kD subunit	Transcription	*Deltaproteobacteria*	*Haliangium ochraceum*
754729	12.83	-2.58	6.88E-11	Type VI protein secretion system component Hcp (secreted cytotoxin)	Intracellular trafficking, secretion, and vesicular transport	*Deltaproteobacteria*	*Chondromyces crocatus*
1082904	17.33	-2.68	7.54E-11	Uncharacterized conserved protein	Function unknown	*Sordariomycetes*	*Sporothrix schenckii*
136368	51.50	-1.91	7.75E-11	Type II secretory pathway, component PulD	Cell motility and secretion / Intracellular trafficking and secretion	*Armatimonadetes*	*Armatimonadetes bacterium 55-13*
609055	9.50	-2.23	7.95E-11	Response regulators consisting of a CheY-like receiver domain and a winged-helix DNA-binding domain	Signal transduction mechanisms / Transcription	*Deltaproteobacteria*	*Sorangium cellulosum*
336735	35.50	-1.28	9.77E-11	Uncharacterized protein conserved in bacteria	Function unknown	*Bacilli*	*Anaerobacillus isosaccharinicus*
36391	17.17	-1.53	1.05E-10	GTPases - translation elongation factors	Translation, ribosomal structure and biogenesis	*Deltaproteobacteria*	*Sorangium cellulosum*
440660	24.83	-1.16	1.56E-10	Transcriptional regulators, similar to M. xanthus CarD	Transcription	*Deltaproteobacteria*	*Labilithrix luteola*
173453	22.00	2.53	1.58E-10	Ribosomal protein S18	Translation, ribosomal structure and biogenesis	*Actinobacteria*	*Streptomyces venezuelae*
245926	34.83	-1.91	1.72E-10	Glucose dehydrogenase	Carbohydrate transport and metabolism	*Betaproteobacteria*	*Betaproteobacteria bacterium TMED22*
644030	7.33	-3.77	1.81E-10	Ribosomal protein L10	Translation, ribosomal structure and biogenesis	*Flavobacteriia*	*Flavobacterium gilvum*
962644	11.58	-2.05	2.18E-10	Type II secretory pathway, pseudopilin PulG	Cell motility and secretion / Intracellular trafficking and secretion	*Acidobacteria*	*Acidobacteria bacterium*
368884	35.25	1.49	2.39E-10	Predicted membrane protein	Function unknown	*Gammaproteobacteria*	*Lysobacter enzymogenes*
529782	10.00	3.17	2.44E-10	Uncharacterized protein conserved in bacteria	Function unknown	*Alphaproteobacteria*	*Methylobacterium sp. 4-46*
459033	39.67	-2.61	3.25E-10	Type VI protein secretion system component Hcp (secreted cytotoxin)	Intracellular trafficking, secretion, and vesicular transport	*Deltaproteobacteria*	*Chondromyces crocatus*
936599	13.42	-3.92	3.57E-10	Actin and related proteins	Cytoskeleton	*Cercozoa*	*Lecythium sp. 3678_261K*

560	956036	17.58	-2.38	3.82E-10	Signal transduction histidine kinase	Signal transduction mechanisms	*Nitrososphaeria*	*Nitrosopumilales* archaeon

Figure 1.

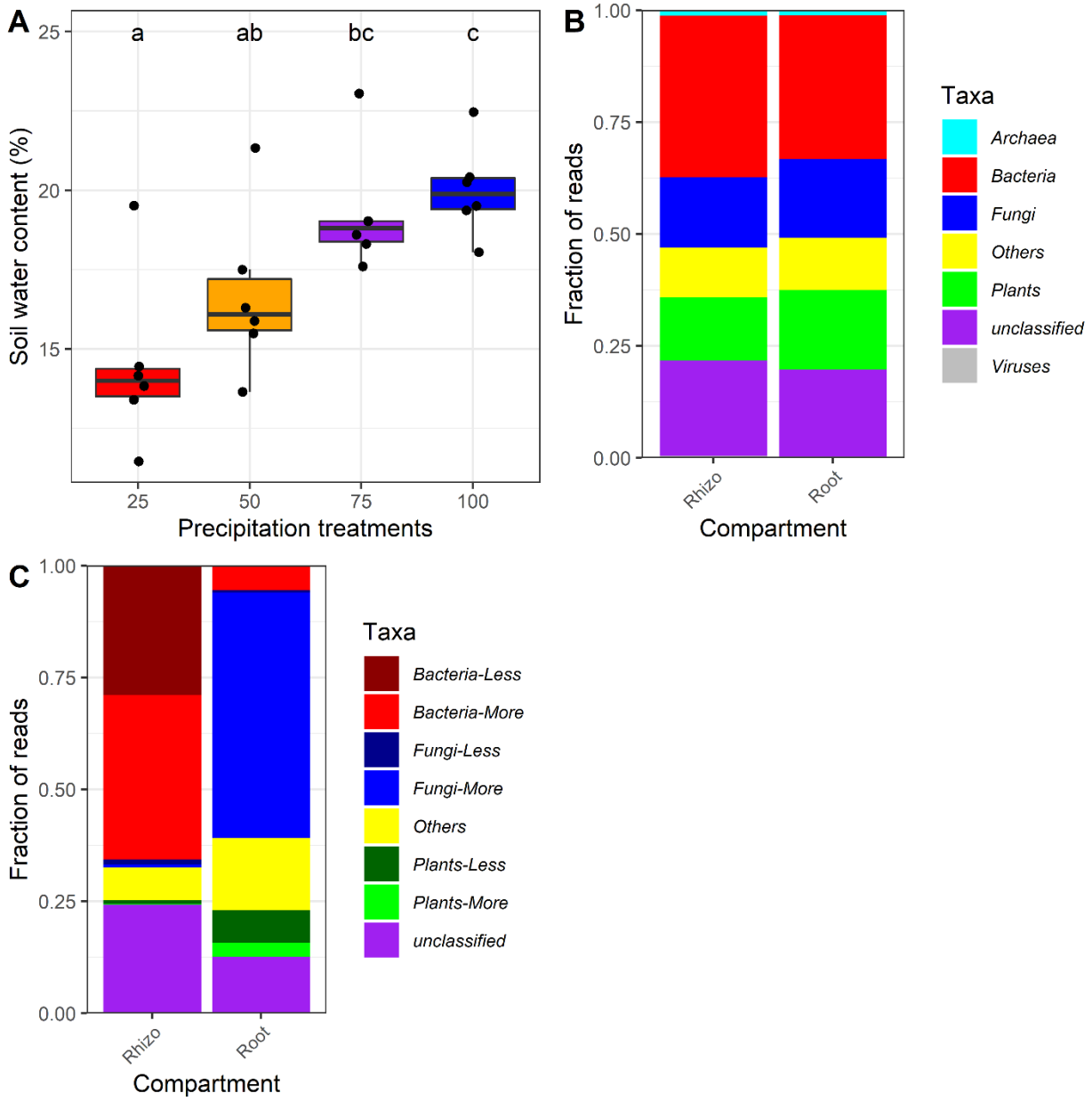

A
a
ab
bc
c
Soil water content (%)
25
20
15
25
50
75
100
Precipitation treatments
B
Fraction of reads
1.00
0.75
0.50
0.25
0.00
Rhizo
Root
Compartment
Taxa
Archaea
Bacteria
Fungi
Others
Plants
unclassified
Viruses
C
Fraction of reads
1.00
0.75
0.50
0.25
0.00
Rhizo
Root
Compartment
Taxa
Bacteria-Less
Bacteria-More
Fungi-Less
Fungi-More
Others
Plants-Less
Plants-More
unclassified

Figure 2.

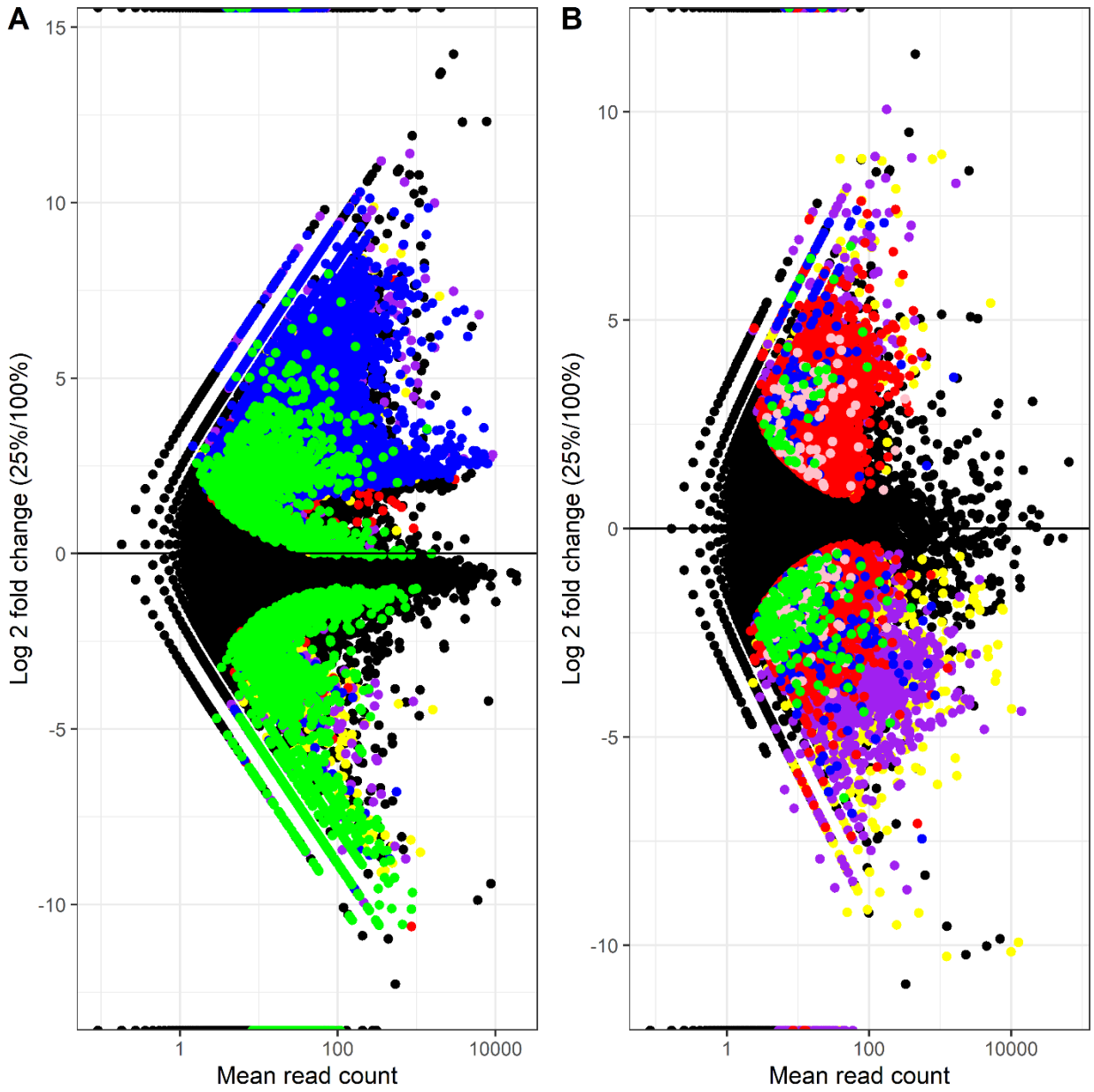

A
B
Log 2 fold change (25%/100%)
Mean read count
15
10
5
0
-5
-10
1
100
10000

Figure 3.

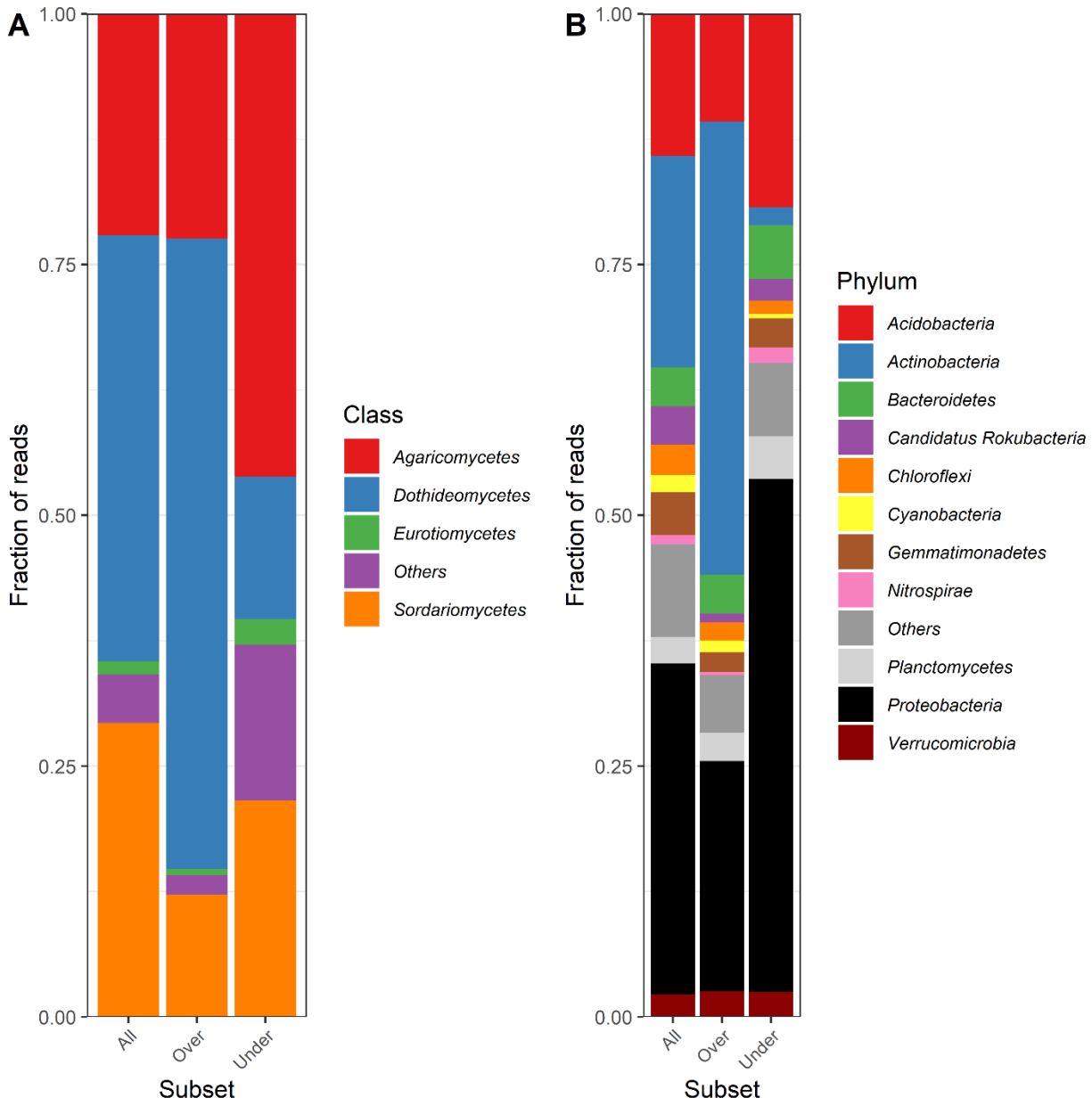
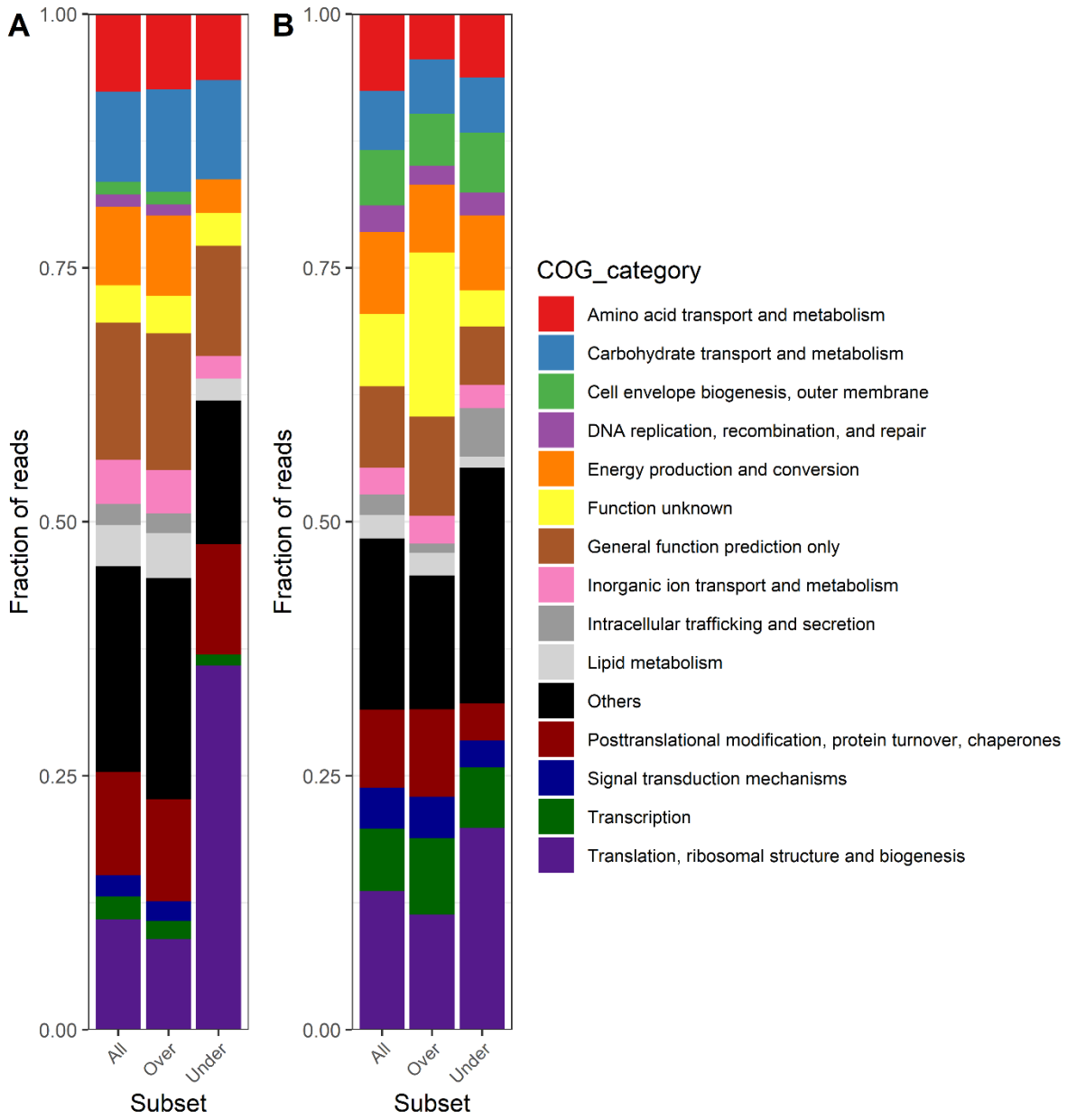

A
B
1.00
0.75
0.50
0.25
0.00
Fraction of reads
All
Over
Under
Subset
Class
Agaricomycetes
Dothideomycetes
Eurotiomycetes
Others
Sordariomycetes
Phylum
Acidobacteria
Actinobacteria
Bacteroidetes
Candidatus Rokubacteria
Chloroflexi
Cyanobacteria
Gemmatimonadetes
Nitrospirae
Others
Planctomycetes
Proteobacteria
Verrucomicrobia

Figure 4.


A
B
1.00
0.75
0.50
0.25
0.00
Fraction of reads
All
Over
Under
Subset
COG_category
Amino acid transport and metabolism
Carbohydrate transport and metabolism
Cell envelope biogenesis, outer membrane
DNA replication, recombination, and repair
Energy production and conversion
Function unknown
General function prediction only
Inorganic ion transport and metabolism
Intracellular trafficking and secretion
Lipid metabolism
Others
Posttranslational modification, protein turnover, chaperones
Signal transduction mechanisms
Transcription
Translation, ribosomal structure and biogenesis