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The Impact of the Diurnal Cycle on the Microbial Transcriptome in the Rhizosphere of Barley

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Abstract While root exudation follows diurnal rhythms, little is known about the consequences for the microbiome of the rhizosphere. In this study, we used a metatranscriptomic approach to analyze the active microbial communities, before and after sunrise, in the rhizosphere of barley. We detected increased activities of many prokaryotic microbial taxa and functions at the pre-dawn stage, compared to post-dawn. *Actinomycetales*, *Planctomycetales*, *Rhizobiales*, and *Burkholderiales* were the most abundant and therefore the most active orders in the barley rhizosphere. The latter two, as well as *Xanthomonadales*, *Sphingomonadales*, and *Caulobacterales* showed a significantly higher abundance in pre-dawn samples compared to post-dawn samples. These changes in taxonomy coincide with functional changes as genes involved in both carbohydrate and amino acid metabolism were more abundant in pre-dawn samples compared to post-dawn samples. This study significantly enhances our present knowledge on how rhizospheric microbiota perceives and responds to changes in the soil during dark and light periods.

Keywords Rhizosphere · Metatranscriptome · Barley · Diurnal cycle · Microbial dynamics

The microbiome of the rhizosphere plays a crucial role in plant health, as it determines nutrient availability as well as response patterns of the plant towards abiotic and biotic stressors [1]. In turn, the microbiome of the rhizosphere benefits from plant-derived carbon as a major energy source and surfaces for colonization and formation of microbial networks [2, 3]. However, the amount and quality of carbon sources provided by the plant do not only differ over the vegetation period but undergo pronounced changes as a consequence of photosynthesis at day and respiration at night [4, 5]. So far, these short-term dynamics have mostly been ignored when composition and function of the rhizosphere microbiome have been analyzed. In this study, we investigated shifts in the metatranscriptome of the rhizosphere microbiome associated with barley (*Hordeum vulgare*, cultivar: Barke) in a greenhouse experiment. The obtained reads were compared to different databases to reveal differences in active pathways and the related microbial community composition at pre-dawn and post-dawn stages. We hypothesize that microbial activities in the rhizosphere are closely linked to the assimilation and respiration patterns of the plant and therefore differ between light and dark photoperiods.

Full details on the experimental setup, greenhouse conditions, sample processing, and bioinformatic analyses are given in the Supplementary Methods. In brief, barley seedlings were grown in an agricultural soil in a greenhouse experiment. Prior to sowing, barley seeds were surface sterilized with hypochlorite solution and germinated in sterile petri dishes at 37 °C for 2–3 days. Pots were then kept under alternate light and dark periods. Dark periods were maintained from 8:00 p.m. to 6.00 a.m., at a temperature of 18 °C, whereas light periods were maintained from 6.00 a.m. to 8.00 p.m., at a temperature of

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20 °C. Plants were grown until the tillering stage under these conditions. At that time point, rhizosphere samples were collected at 4.30 a.m. (1 h before sunrise) and 9.30 a.m. (4 h after sunrise), respectively. For each time point, root material from three independent pots was harvested and treated as true replicates for downstream analysis. Nucleic acids were extracted using the modified Griffith's protocol [6]. DNA was degraded using the MoBIO DNase Max kit, rRNA was depleted, and the remaining RNA reverse was transcribed into cDNA using the Ribo-Zero kit (Bacteria)-Low input (Epibio, Madison, USA). Sequencing of metatranscriptome libraries was performed on an Illumina MiSeq. Paired-end sequences were merged, quality filtered using AdapterRemoval v2 [7] and Deconseq [8], and the remaining ribosomal RNA sequences were removed using

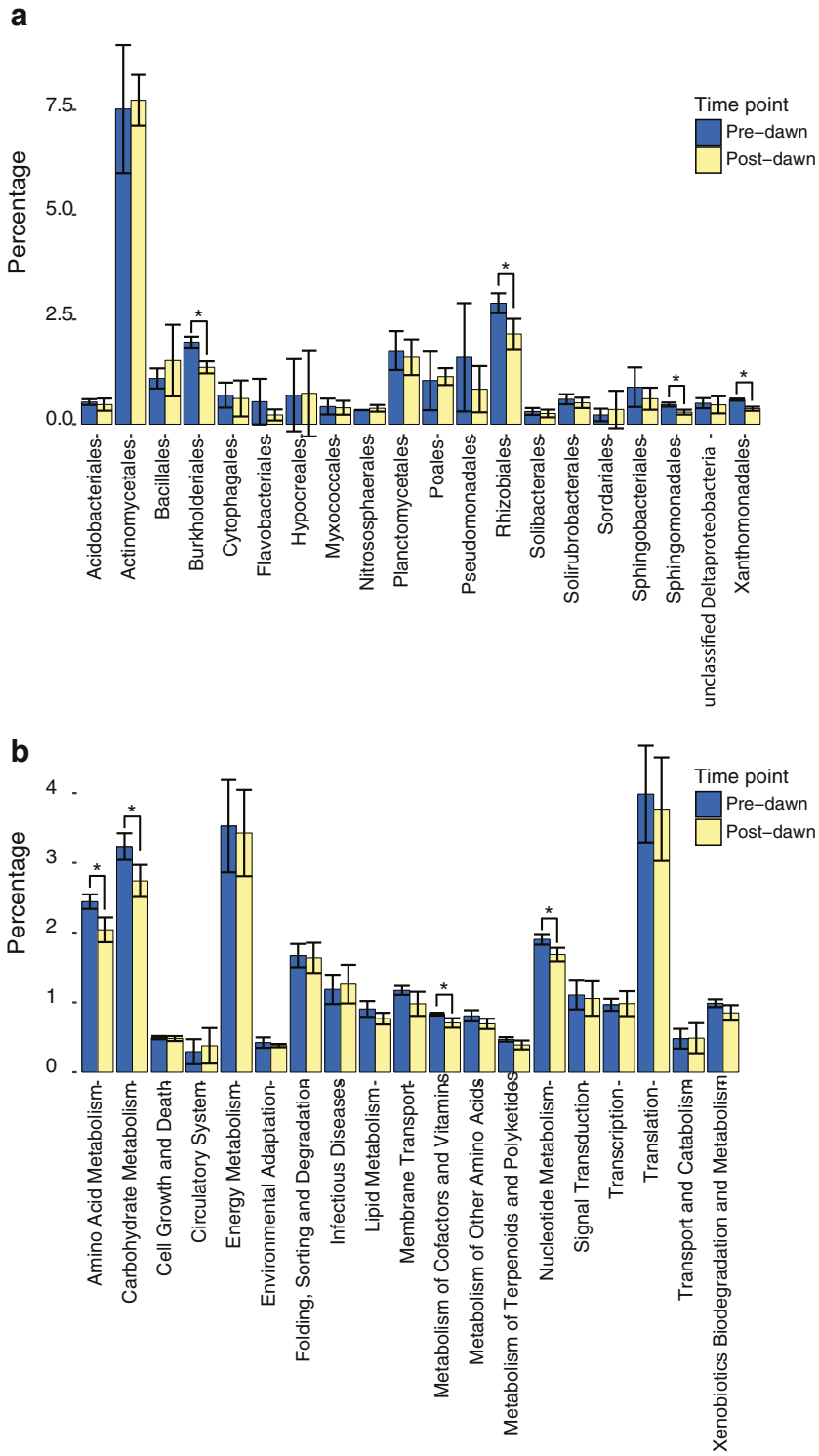

Fig. 1 Analysis of metatranscriptomic data sampled from the rhizosphere of barley at pre-dawn and post-dawn stages. **a** Taxonomic level: depicts the percentage of reads of the 20 most active orders, according to the NCBI non-redundant protein database ($n = 3$).Taxonomic ranks were assigned using MEGAN5. **b** Functional level: depicts the percentage of reads of the 19 most active KEGG pathways, according to the KEGG database ($n = 3$) and annotated using MEGAN5. Significant differences between pre-dawn and post-dawn time points were determined by unpaired *t* test statistics ($*P < 0.05$, $n = 3$)

SortMeRNA [9].Non-ribosomal RNA sequences were compared against the NCBI non-redundant protein database and KEGG and annotated using MEGAN [10]. Coverage was estimated using Nonpareil [11].Relative abundances were calculated by normalizing all data to the number of non-ribosomal reads. R was used for data visualization and statistical analysis [12] (see Supplementary Methods for more details).The sequence data was submitted to NCBI via the Sequence Read Archive (SRA) and is available under the BioProject ID: PRJNA395704.

The total raw dataset consisted of 15.5 Gbases, which was reduced to 0.73 Gbases (average length of merged reads 195 bp) after quality filtering and removal of ribosomal RNA sequences, which resulted in an average of 821.141 reads for pre-dawn and 427.918 reads for post-dawn samples. Details on the number of reads per sample before and after quality filtering and after removal of rRNA reads are provided in Supplementary Table 1. Coverage estimation showed similar coverage for all samples (Fig. S1). *Actinomycetales*, *Rhizobiales*, *Burkholderiales*, and *Planctomycetales* were the major taxa in all samples (Fig. 1a), which is in agreement with other studies on the microbiome of the barley rhizosphere [13]. Orders *Rhizobiales*, *Burkholderiales*, *Xanthomonadales*, *Sphingomonadales*, *Caulobacterales*, and *Plasmodiophorida* were significantly more abundant in pre-dawn compared to post-dawn samples (Fig. 1a and Supplementary Table 2).

At the functional level, we observed an increase of transcripts related to the carbohydrate, nucleotide, amino acid, and vitamin metabolism at pre-dawn (Fig. 1b and Supplementary Table 3). To obtain a more detailed view of the significantly different orders between pre-dawn and post-dawn time points, we investigated the functional potential of the orders, which showed a higher activity at pre-dawn by functionally annotating the sequences that were annotated to these orders using the KEGG database. We again determined significant differences between pre-dawn and post-dawn time points. Figure 2 depicts the log fold changes (log2 (pre-dawn/post-dawn)) and relative abundances of all significantly different pathways belonging to the carbohydrate metabolism, nucleotide metabolism, amino acid metabolism, and metabolism of cofactors and vitamins. At this level of single pathways, genes coding for enzymes of the citric acid cycle, pentose phosphate pathway and metabolism of galactose, starch and sucrose were significantly more abundant at pre-dawn. In addition, genes

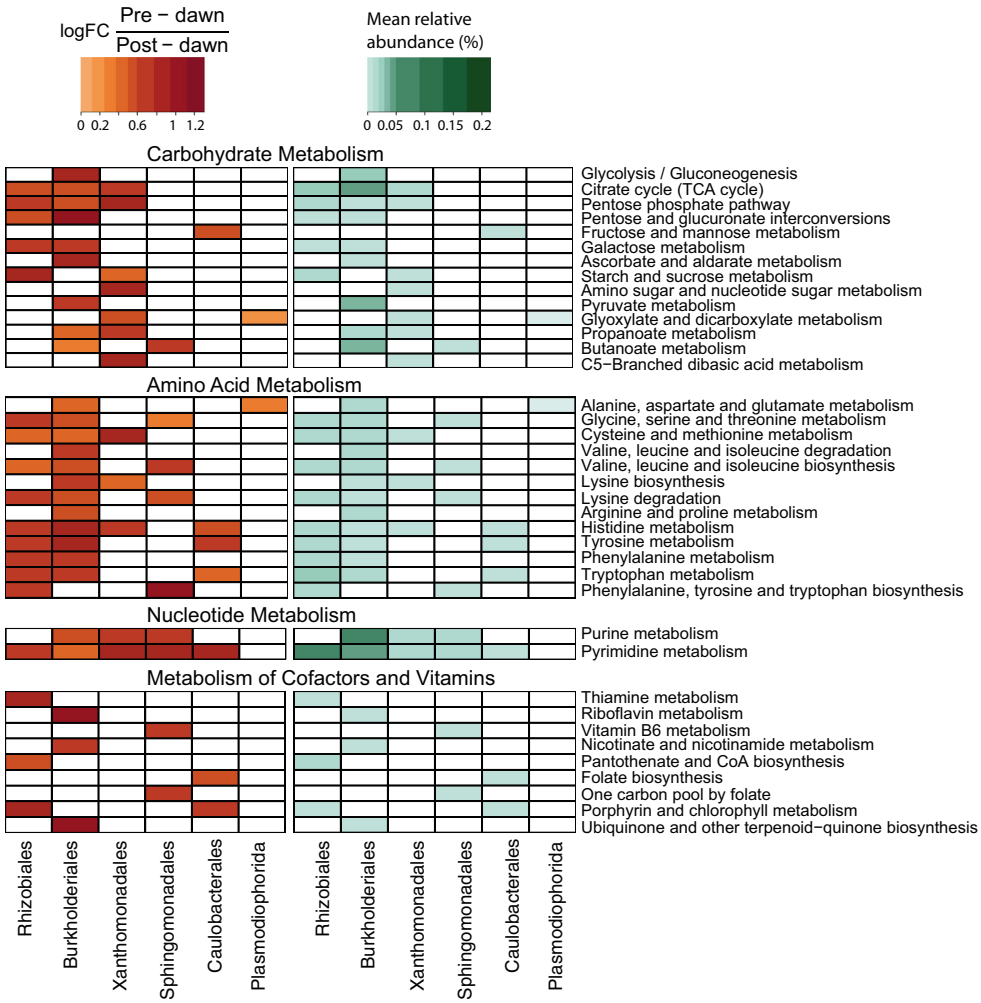

Fig. 2 Log fold changes (log2 (pre-dawn/post-dawn)) and mean relative abundances of all significantly different pathways. Depicted is the functional capacity of the six orders that were significantly increased before dawn for the following pathways: carbohydrate metabolism, nucleotide metabolism, amino acid metabolism, and metabolism of cofactors and vitamins. Relative abundances were calculated as a mean across all six samples

catalyzing pathways for several amino acids as well as for purine and pyrimidine were highly transcribed at pre-dawn compared to post-dawn. Similar observations were reported in a metatranscriptome study analyzing day and night variations in Hawaiian ocean surface waters [14]. Here, bacterioplankton housekeeping activities like amino acid and vitamin biosynthesis, membrane repair, and synthesis as well as most pathways involving C and N metabolism were expressed higher at pre-dawn compared to post-dawn. Interestingly, the highly transcribed genes at pre-drawn were mainly related to *Rhizobiales* and *Burkholderiales* (Supplementary Fig. 2). Members of these orders are able to grow on root mucilage as a sole carbon source [15], which is mostly formed during the night [16]. High activity of genes related to carbohydrate degradation from *Burkholderiales* during the night was recently also shown in a metaproteome study from a constructed wetland, where the authors could prove a stronger uptake of root exudates and reuse of the stored carbon compounds during the night by *Burkholderiales* during glyoxylate cycle [17].

The overall higher activities of genes related to C and N turnover might be linked to the increased hydraulic redistributions and transpiration rates of plants during the night [18, 19], which possibly result in increased nutrient mobility. Another interesting study proposed that higher solute and carbon flux to the rhizosphere at night facilitates microbial growth, which results in higher nutrient demands [20]. To fulfill the increased nutrient demand, microbes produce more exoenzymes at night; whereas, during day time, the diffusion of substances is enhanced in the opposite direction from the soil to the plants.

Overall, this study for the first time sheds light on the diurnal response of rhizospheric microbes to photosynthetic and non-photosynthetic phases of plants during day and night. However, the presented data reflects one time point of plant development only, and further studies are needed to show if these patterns are stable at other time points of plant growth. Furthermore, the role of natural conditions like drought periods or management options of the farmers, like fertilization regimes as modulators for the observed pattern, needs to be taken into account in future projects.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethics Approval Not applicable

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