

Diel Activity of Microbial Communities in Surface Soil Litter

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The overarching goal of this project was to quantify potential diel partitioning of metabolic functions in microbial communities of decomposing grass litter in a Mediterranean climate-grassland ecosystem. We measured litter respiration as an indicator of microbial activity overall, and used gene expression data to determine activity of specific taxa or functions. We ask 1) is there a diel signal in microbial gene expression, 2) what are the predominant abiotic drivers of short-term transcriptomic variation, 3) is there diel signal in the activity of dominant taxa, and 4) which genes are most responsive to diel cycling?

Diel fluctuations are known to be important for the functioning of photosynthesis-dominated microbial ecosystems – for example, aquatic communities, plant-associated rhizospheres, and desert crusts. In these cases, heterotrophic activity often appears to track light-dark cycles as nutrient and carbon availability fluctuates in relation to the activity of photosynthetic organisms. It is less clear whether there are prominent diel patterns in activity of soil microbes. In surface soil, including decomposing litter, microbes may be exposed to environmental variability that is diel in nature – including temperature, moisture, and UV radiation. Indeed, there is evidence that CO₂ flux from litter displays diel fluctuations in diverse ecosystems including wetlands, Mediterranean woodlands, and temperate forests. To the extent that diel variation is a predominant driver of microbial activity there is potential for specialization among soil microbes such that particular taxa may be active and/or perform different functions at different times. Our goal in this study was to quantify short-term variation in microbial activity and gene expression in litter decomposition. In particular we ask 1) is there a diel signal in microbial gene expression, 2) what are the predominant abiotic drivers of short-term variation, 3) is there diel signal in the activity of dominant taxa, and 4) which genes are most responsive to diel cycling? We addressed these questions using a metagenomic and metatranscriptomic sequencing approach in combination with litter respiration, temperature, and moisture measurements under a high-temporal resolution sampling over three continuous days. This study was performed at the Loma Ridge Global Change Experiment (LRGCE) in a Mediterranean climate, grassland ecosystem. Three plots were established within the larger experimental site, with 36 spatially randomized litterbag samples per plot, and one litterbag per plot sampled every two hours for three days. Metagenomic and metatranscriptomic sequencing was performed by the DOE Joint Genome Institute.

We observed diel fluctuations in temperature, moisture, and litter respiration over the three-day period. There was a gradient in terms of average moisture content across the three plots ($P = 0.0015$) ranging from 24% to 47%. This gradient resulted in the two plots with lower moisture content having stronger diel fluctuations in moisture content ($P < 0.01$), whereas no significant diel signal in moisture was observed in the third plot ($P = 0.12$). We also observed diel fluctuations in respiration in the two plots with lowest and highest moisture content ($P = 0.01$), with peaks in respiration coinciding with the peak in moisture content or temperature,

respectively. Community composition did not display a strong diel signal. However, there were diel shifts in the expression of specific metabolic functions correlated with moisture and temperature but varying by plot. Five of the ten most abundant taxa (identifiable to genus or family) displayed significant diel signal ($P < 0.05$) in the high-moisture plot, including the most abundant genus, *Curtobacterium*. In terms of functional genes, after correcting for multiple comparisons 101, 35, and 205 groups of KEGG orthologs displayed significant diel signal in the low-, intermediate-, and high moisture plots, respectively. Overall, our data demonstrate that gene expression of heterotrophic microbial litter decomposers responds to diel variability in the environment. Taxa appear to respond to this variation in unique ways, with some taxa responding more strongly to diel moisture variation and others responding to diel temperature variation. We further observed a diel signal in functional gene expression overall and in the expression of specific functional genes, indicating that microbes may perform particular functions in accordance with day-night cycles in environmental conditions.

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