An Approach to Analyzing Changes in Gene Expression of Non-model Plant Species Grown Under Elevated CO_2 and Soil N Levels

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ABSTRACT

The use of genetic techniques is a contemporary approach to answering ecological questions. A long-term, large-scale field study in Minnesota, USA, called BioCON, manipulates CO_2 and soil nitrogen availability. The physiological responses of several non-model plant species grown in BioCON have been previously observed; however, the gene expression responses of these species have not been studied and may reveal mechanisms that drive these physiological responses. Leaves were sampled from three native species grown in factorial combinations of atmospheric CO_2 and soil nitrogen. RNA from these leaves is currently being extracted and analyzed and will ultimately be used to assess gene expression patterns. We are specifically interested in the interactive effects of elevated CO_2 and elevated nitrogen levels on the expression of genes involved in nitrogen metabolism and photorespiration as photorespiration is directly affected by CO_2 and is linked with nitrogen assimilation and metabolism. These efforts will provide a better understanding of natural vegetation responses to changing resource availability associated with future global change.

PHENOMENA UNDER INVESTIGATION

Short-term exposure to elevated CO_2 is known to result in an increase in photosynthetic rate, which is consistent with what is expected based on biochemical models. However, studies have shown that many plant species exposed to elevated CO_2 for a longer period of time (weeks to years) acclimate, such that there is little to no increase in photosynthetic rate (Fig. 1a). These same plants grown long-term under elevated CO_2 often display a decrease in leaf nitrogen content (Fig.1b)[1]. There is evidence that explanations for these physiological responses are the result of effects on photorespiration and nitrogen metabolism[2]. A molecular approach can provide further insight into the mechanisms behind these phenomena.

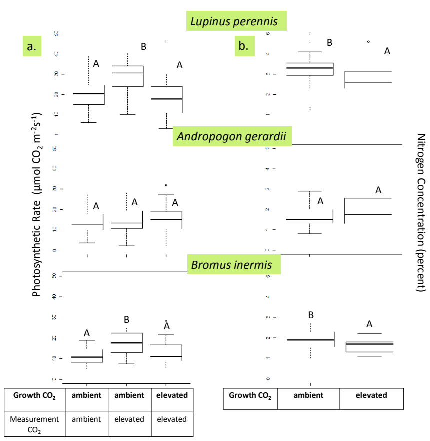
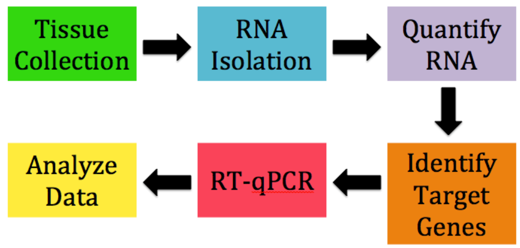


Figure 1. Boxplots of (a) photosynthetic rate (µmol CO_2 $m^{-2}s^{-1}$) and (b) nitrogen concentrations (%) for each of three species (*Lupinus perennis* n=55, *Andropogon gerardii* n=61, and *Bromus inermis* n=64) grown at ambient and elevated CO_2. Photosynthetic rates of the ambient grown plants were also measured at elevated CO_2 to represent short-term responses to CO_2. Different letters represent significant differences ($p < .05$, Tukey HSD or T-test).

METHODS



Tissue Collection

All leaf samples were collected from BioCon in Cedar Creek, Minnesota in May of 2014 (Fig. 2). Samples from three different species, *Andropogon gerardii*, *Bromus inermis*, and *Lupinus perennis*, were collected from plots with varying CO_2 and soil nitrogen levels. CO_2 levels consisted of ambient (380ppm) and elevated (580ppm). Soil nitrogen levels consisted of ambient and ambient + 4gm^{-2}y^{-1}. The uppermost, fully expanded leaf was collected from 2 plants per treatment and immediately placed in liquid nitrogen. Samples were stored at -80°C.

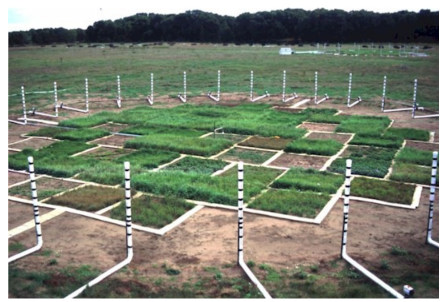


Figure 2. Plots at BioCon in Cedar Creek, Minnesota.

RNA Isolation

Total RNA was isolated from each individual leaf sample using the Qiagen RNeasy Plant Mini Kit and stored at -80°C (Fig 3).

Quantify RNA

RNA samples were subjected to a denaturation reaction to disrupt any secondary structures present in the RNA. RNA was resolved a RNA agarose denaturing gel and then stained with ethidium bromide (Fig 3).

Figure 3. Pilot agarose gel electrophoresis of RNA isolated from leaf samples. Lane 1 contains ~ 100mg of leaf frozen for 1 week. Lanes 2 and 3 contain ~ 100mg of fresh leaf.

METHODS CONTINUED

Identify Target Genes

We plan to measure expression of several critical gene products involved in photorespiration such as nitrate reductase and nitrate transporters. Exact genomic sequences from wheat will be used as reference sequences. Genes involved in these pathways will provide insight into the mechanisms underlying acclimation to elevated CO_2 (Fig. 1).

RT-qPCR

Once specific genes have been selected for further observation, the RNA from these genes will be amplified into cDNA using RT-qPCR. RT-qPCR is a commonly used technique for measuring the relative abundance of specific sequences to determine relative gene expression (Fig. 4). Fluorescent tags are used to measure amplification of sequences in real time.

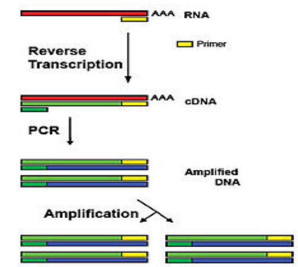


Figure 4. Diagram of the steps involved in RT-PCR. Image source: http://en.wikipedia.org/wiki/Reverse_transcription_polymerase_chain_reaction

Quantification of Gene Expression

Non-specific fluorescent dyes interchelate double-stranded DNA during the PCR cycles, resulting in fluorescence. The amount of mRNA in the sample is directly correlated to the number of cycles required before fluorescence is detected (Fig. 5). Once the relative abundance of mRNA is quantified for each leaf sample, the data will be compared between the various treatments.

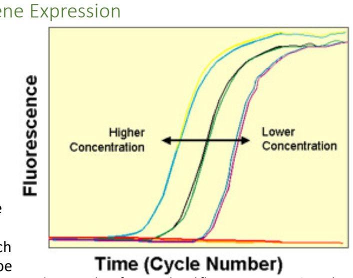


Figure 5. Plot of accumulated fluorescence vs. PCR cycle. Image source: https://dna.utah.edu/LightCycler/Top_LightCycler.html

IMPLICATIONS

Our findings will help explain the molecular mechanisms behind physiological responses to future atmospheric CO_2 levels. This will contribute to more accurate modeling of future responses of vegetation to climate change. This information may also inform genetically modified crop species more equipped to grow under future climate conditions.

ACKNOWLEDGEMENTS

Cody Fisher for initial reference sequence research. NSF LTREB DEB#1242531 (to Lee) for funding. ORSP for student-faculty collaborative research grant. Dr. Evan Weiher for assisting with statistical analysis.

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2. Rachmilevitch, S., Cousins, A. B., Bloom, A. J. (2004). Nitrate assimilation in plant shoots depends on photorespiration. PNAS, 101(31), 11506–11510.

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