Metagenomic insights into the structure and functional potential of Switchgrass soil microbial communities under preindustrial and future CO_2 treatment

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INTRODUCTION

Switchgrass (*Panicum virgatum* L) is a perennial C4 grass and also an important biomass crop for biofuel production. Although effects of rising atmospheric CO_2 concentration on C3 grasses have been extensively studied, little is known about the complex interactions between CO_2 enrichment, soil type and legacy effects (CO_2 x soil x year) on the structure and functional potential of soil microbes associated with C4 plants.

Here, we utilized shotgun metagenome sequencing to elucidate the changes in community structure and functional gene abundance linked to carbon (C), nitrogen (N) and phosphorus (P) cycling. This method also allowed us to identify the taxonomic identification and contribution of key microbial groups involved in C, N, and P cycling.

We expected that CO_2 enrichment would have significant influence on microbial community structure as well as on the abundance of functional genes linked to carbohydrate degradation, nitrogen cycling processes and phosphate metabolism. However, we hypothesized that the effect would be rather indirect through changes in soil nutrient dynamics under preindustrial and future CO_2 treatment scenario.

MATERIAL AND METHODS

LYCOG Experimental design

- LYCOG consists of two longitudinal chambers where the CO_2 gradient was maintained since 2005 until 2015 in super-ambient and sub-ambient sections.
- 2015 CO_2 levels were considered in this study to assess the long term CO_2 enrichment effect in the last year (2015) of CO_2 treatment and its short term legacy effects in 2016, which was the year following the cessation of LYCOG experiment.

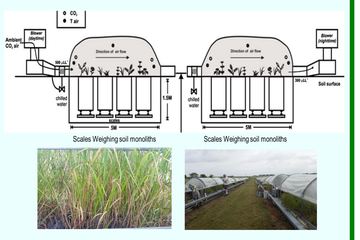

Figure 1. Experimental setup diagram (*Fay et al., 2009)* and pictures from Lysimeter CO_2 gradient (LYCOG) facility in USDA-ARS at Temple, TX

Soil sampling

- Three soil cores from top 0-5 cm of each plot was collected in a centrifuge tube and stored at -80°C freezer until further analysis . A total of 20 soil monoliths were collected during August 2015 (n=10) and 2016 (n=10) growing season.
- We considered the sub-ambient and super-ambient CO_2 treatment levels as discrete rather than continuous variables because we had a limited number of switchgrass monocultures (n=5) each year from each of the two soil types (a clay-rich Vertisol and a Silty clay Mollisol).
- Soil samples were also analyzed for soil C/N ratio, NO_3^--N, NH_4^+-N and PO_4^{3-} .

Library preparation and Metagenome sequencing

- Community DNA from individual soil samples were extracted using MO bio kit and sent to mrDNA (Shallowater, TX) for linear amplification and library preparation.
- Whole community (shotgun) metagenome sequencing was performed on individual soil samples using Illumina HiSeq 2500 (2x150 bp paired end reads). Paired-end reads were aligned and filtered using the join-fastq algorithm from eautils .

Data processing and analysis

DIAMOND — Alignment against NCBI NR database (.daa files) → **MEGAN6** — Taxonomic and functional mapping (generate output files) → **SEED** — Functional annotation of individual genes at level 4 → **Statistics** — R-Packages: vegan, ggplot2, tidyR

RESULTS

Microbial community structure

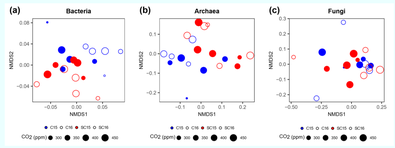

Figure 2. Non-metric multidimensional scaling (NMDS) ordinations with Bray-Curtis distance based on taxonomic community structure of (a) Bacteria, (b) Archaea and (c) Fungi at species level.

C15 = Clay 2015
C16 = Clay 2016
SC15 = Silty Clay 2015
SC16 = Silty Clay 2016

Carbohydrate degradation gene abundance

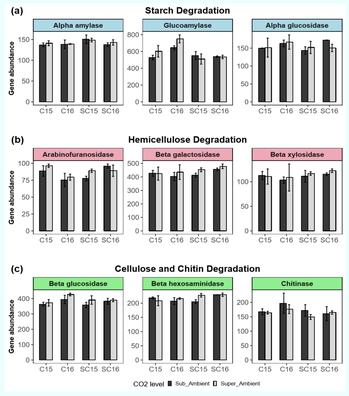

Figure 3. Bar plots showing absolute gene counts for different carbohydrate substrate categories.

Sub-ambient
CO_2 level= 250-360 ppm

Super-ambient
CO_2 level = 420-500 ppm

Nitrogen cycle gene abundance

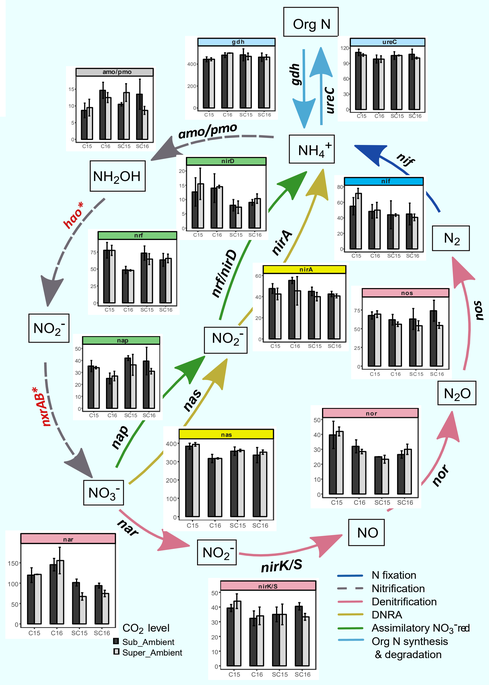

Figure 4. Bar plots showing the absolute gene counts (y-axis values) for individual genes involved in Nitrogen cycling processes. Genes with asterisk labels (*) were either rarely detected or absent in SEED level 4 Nitrogen metabolism category.

Taxonomic contribution to functional gene groups

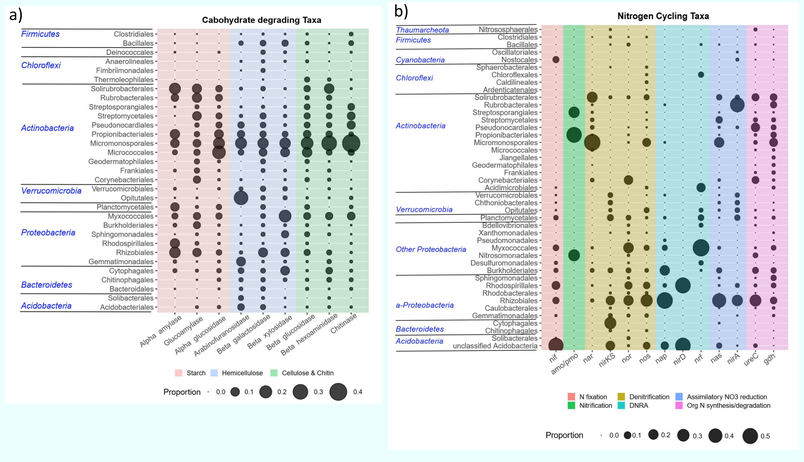

Figure 5. Bubble plots showing the relative proportion of microbial populations involved in **a)** carbohydrate degradation and **b)** nitrogen cycling processes per gene category . The taxa identification is represented at phylum and order level.

Phosphate (PO_4^{-3}) genes

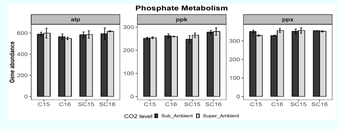

Figure 6. Bar plots showing absolute gene counts for gene counts for alkaline phosphatase (alp), polyphosphate kinase (ppk), and exopolyphosphatase (ppx).

Soil Nutrient

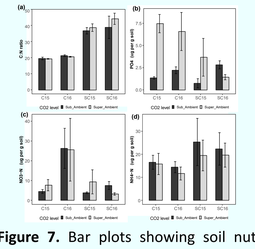

Figure 7. Bar plots showing soil nutrient concentrations.

CONCLUSIONS

- CO_2 alone did not have a significant effect on microbial community structure (Figure2) perhaps due to stronger soil effect as illustrated in prior study (Raut et al, 2018)
- Most genes involved in carbohydrate degradation, nitrogen cycling pathway and phosphate (PO_4^{-3}) metabolism remained largely unaffected by CO_2 enrichment (Figure 3, 4 and 6) but the legacy effects in 2016 persisted with an exception of a few genes.
- The interactive effects of soil × CO_2 on the abundance of genes including glucoamylase, nitrate reductase (*nar)* and polyphosphate kinase (*ppk)* were significant but CO_2 alone did not have a strong influence.
- At taxonomic level, members of *α-Proteobacteria* and phylum *Actinobacteria* were dominant taxa involved in C degradation (Figure 5a) and N cycling (Figure 5b).
- Switchgrass plants in LYCOG system were particularly well-watered (Fay et.al 2012) and CO_2-induced nutrient limitation was not evident (Figure 7) or directly correlated to functional gene abundance.
- These findings expand our current understanding of C, N and P cycling processes, specifically in switchgrass soil microbes exposed to preindustrial and future CO_2 treatment scenario.

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