Plant-Rhizosphere Interactions: the Role of Microbes in Carbon Sequestration

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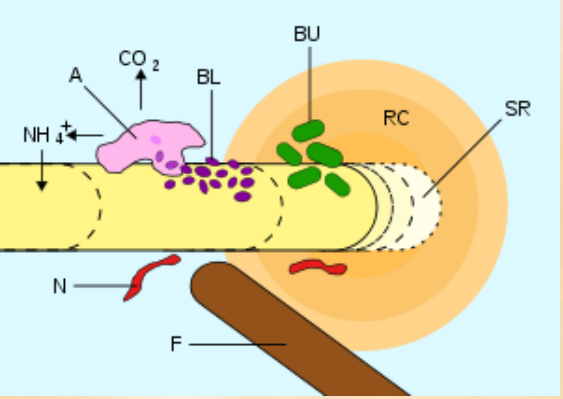
Plants Role in Carbon Cycling?

Plants uptake CO_2(g) from the atmosphere and convert/release the carbon into one of three groups of compounds

1. Tissue compounds used for above ground growth
2. Aerosolized compounds released from the leaves
3. Root material and root exudates emitted into the soil

Carbon cycling

- Aerial plant tissue material usually decomposes quickly after plant death, re-releasing CO_2
- Aerosolized carbon molecules are a minor constituent of total carbon release and may also be degraded to $C0_2$ quickly.
- Root material and root exudates have a much greater resistance to decomposition and therefore a greater potential for long term deposition.
- Greater resistance does not equal permanent deposition however as microbial communities in the soil still decompose and transform plant carbon material.
- Most microbial and plant interaction occurs in a small volume of soil called the rhizosphere.



The Rhizosphere

- The **rhizosphere** is the narrow region of soil that is directly influenced by root secretions and associated soil microrganism.[1]
- Microbial communities within the rhizosphere determine the ultimate fate of carbon particles.
- Understanding rhizosphere microbial ecology is fundamental to tracing carbon from plants through the soil and creating a global carbon budget.

- 1.http://en.wikipedia.org/wiki/Rhizosphere_(ecology

Restatement of Important: the Why

- Plants are a vital player in carbon cycling and create a terrestrial carbon sink
- Microbial communities act as an intermediary controlling what type and how much carbon can is actually removed from the atmosphere and over what time scales that carbon stay sequestered.
- Because the carbon cycle, especially CO_2, plays a large role in global climate change understanding soil micro-biology will improve understanding of climate change.

The Projects

1. Switch Grass CO_2 enrichment
2. Changes in Root Exudates
3. Soil Warming
4. Free Air Carbon Enrichment (FACE)

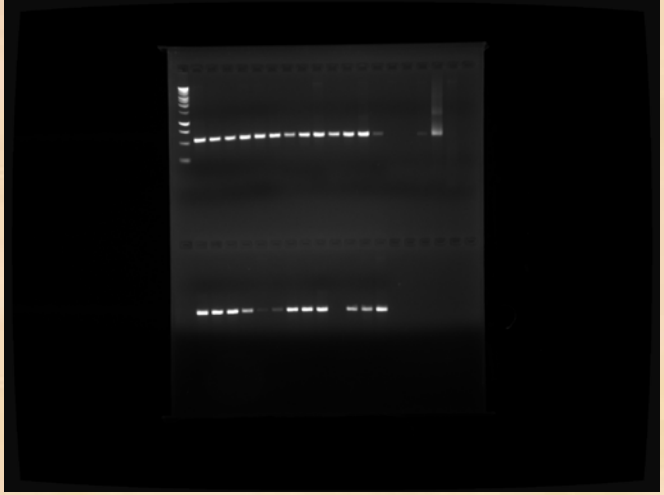
My Main Project: Switch Grass

- It is still unknown if/how atmospheric enrichment of CO_2 effects root exudates, microbial communities, and soil carbon cycling
- Switch grass was grown under normal and enriched CO_2 conditions in an attempt investigate the differences
- Switch grass was chosen for two important reasons

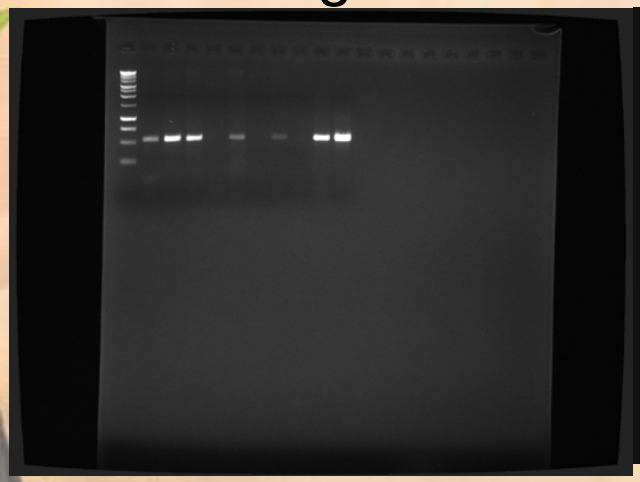
1. It grows well on non-cultivated farm land and has been suggested as a bio-remediation solution for carbon sequestration
2. Switch grass has been investigated as a potential bio-fuel and therefore could be grown in large quantities

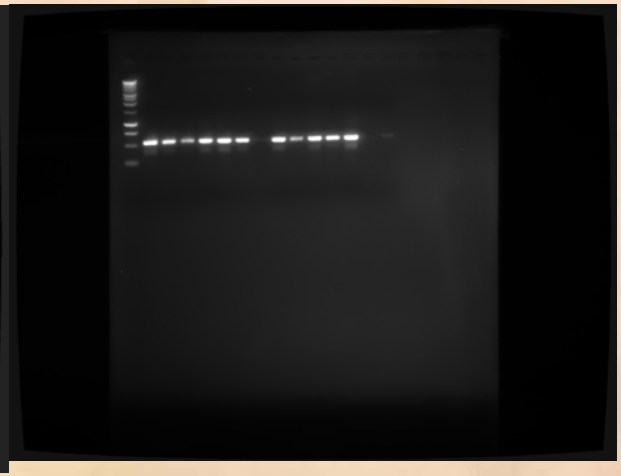
Experiment Protocol

- There are two stable isotopes of carbon C^{12} and C^{13} and the plants were grown in a chamber that only exposed them to C^{13} CO_2.
- This meant that all carbon exudates from the plant were labeled with C^{13} and any microbes utilizing the material would have their DNA C^{13} labeled
- C^{13} accounts for less than 1% of total carbon so chances are if its C^{13} it originate from our labeled CO_2.
- Theoretically the whole carbon cycle from the air through the soil could be traced by following the path of the labeled carbon.
- In order to actually trace the carbon a process known as Stable Isotope Probe (SIP) was employed
- SIP involves as complicated series of extraction, PCR, ultra-centrifugation, extraction, PCR, and cloning
- SIP was developed and proven in pure cultures but not for native soils.



- If the process had worked there would be a line in every other column in this PCR gel





- These gels should only have bars in three columns

Experiment Results

- Over two months of trial, error and refining on soil prepared to test the method failed to produce valid results
- For a variety of reasons relating to complications of using native soils rather than pure cultures we never even ran the switch grass samples.
- There appears to be little way of preventing cross contamination of C^{12} and C^{13} DNA during one especially problematic step. Even if contamination were prevented proving the samples were not contaminated presents another huge challenge.

Conclusion

- The experiment has been placed on hold until it can go back to the drawing board and a better process is created.
- While sound in theory the actual implementation of the process requires a level of human precision making it very difficult to complete without contamination and even more difficult to reproduce results.
- As a result I bounced around to a few other projects to fill the remaining time

Root Exudates: The Conflict

- Several studies have shown that increasing the amount of carbon based exudates from roots only increases microbial activity and does not enhance carbon sequestration.
- Recent studies however suggest that there is a critical threshold at which increases in carbon input from plants dramatically increases carbon sequestration.

Exudates: the Experiment

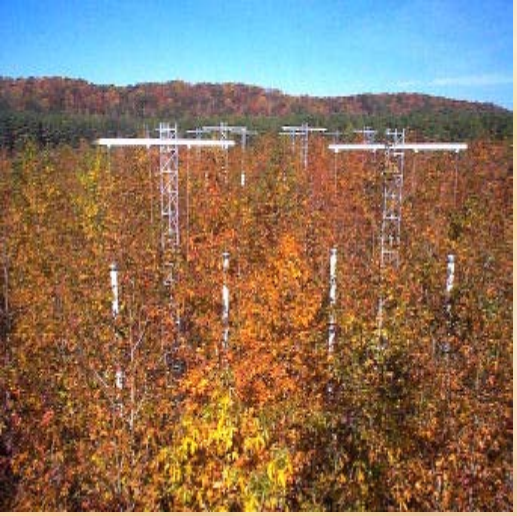
- In a modification of a previously published study realistic root exudate mixtures were created and added in incremental volumes to soil.
- Real time PCR was then used to analyze total bacterial and fungal communities and functional enzyme probes were also employed to measure enzymatic activity.
- The results showed that there is a threshold after which enzymatic activity dramatically decreases and sequestration increases
- The threshold is also correlated with a shift in the fungal to bacterial community ratios towards fungi domination

Soil Warming

- Global warming will not only warm the atmosphere but also the upper soil layers
- The effects of heat on soil microbial structures has been largely untested.
- A proof of concept model was installed in hopes that initial investigations would warrant further investment into a large scale experiment
- The protype had two chambers.
  - Control
  - Heating chamber that warmed soil up to 1m +4^{o}C above ambient temperature

Soil Warming results

- Soil from both chambers at set depths was collected and analyzed for enzymatic activity and fungal to bacterial rations
- As expected total fungal and bacterial communities decreased with soil depth in control and heating chambers
- Enzymatic activity and relationships between heating and control chambers are less clear.
- Further analysis is needed to assess the success on the experimental setup before a larger scale modulus is built.



Tall towers of ORNL FACE
http://face.ornl.gov/

FACE (Free Air Carbon Enrichment)

- FACE is in the final stages of a 21 year project where CO_2 has been pumped into a forested area and its effects on the ecosystem trapped.
- Along with above ground samples
  - Several Interns spent the summer counting 78314 leaves from trees that were cut down
- Root and soil samples were collected and are still being prepped for analysis. These samples will then be compared with similar ones from a non-enriched site.
- Along with collecting and sieving soil from pits I helped install root windows that allow for more effective and efficient collection of rhizosphere soil samples

Importance of Research

- Plants transfer carbon through root material and root exudates from the atmosphere into the soil
- The chemical and physical processes by which the carbon is cycled or sequestered is depended on microbial processes in the rhizosphere.
- A better understanding of feed backs linking the rhizosphere and climate change will allow for a better picture of global carbon cycling and the ability of the biosphere to offset rising anthropogenic increases in CO2 emissions.

What I Learned

- Always keep fundamental experimental design principles in mind.
  - If your soils may have E. Coli in them naturally don't use a cultured E. Coli spike to check for contamination.
- Verify published results before you 100% trust them
  - No matter how much Invitrogren guarantees there CYBR green is sterile ever tube may still come contaminated from the company
- Perfecting a protocol to get required data can be a tedious process especially when combining multiple new cutting edge processes. Failed trials are just part of the process.

Acknowledgments

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