Separating Soil Respiration into Plant, Fungal, and Bacterial Components using Molecular Targets and Assays

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Human activity alters carbon cycling

http://earthobservatory.nasa.gov/Library/CarbonCycle/Images/carbon_cycle_diagram.jpg
Soil Respiration is a critical component of carbon cycling

http://earthobservatory.nasa.gov/Library/CarbonCycle/Images/carbon_cycle_diagram.jpg
Develop plant, fungal, and bacterial primers for enzymes relevant to respiration

Construct & sequence environmental gene libraries for soil community

Determine relative proportions of bacterial, fungal, and plant respiration in field experiment

Compare results with stable isotope results
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Winners:
Citrate synthase (TCA pathway)
Enolase (Glycolysis pathway)
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Soil DNA isolation followed by rtPCR for the isolation of enolase genes. PCR cloning into a vector, transforming the mixture into E. coli. Sequencing and phylogenetic analysis were then performed to study the enolase fragment.
• All three primers (bacterial, fungal, and plant) magnified bacterial enolase genes

• Phylogeny shows diversity of bacterial enolase genes

• More specific primers need to be developed
- Hurt, et al.
- Brodie
- TruRNA mini kit
- MoBio PowerSoil
Hurt, et al.

Brodie

TruRNA mini kit

MoBio PowerSoil

DNA

23s rRNA

16s rRNA
Next step: clean up DNA contamination
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Inhibitive respiration experiment

- Autoclaved soil
- Soil
- Soil + antibacterial
- Soil + antifungal
- Soil + Antibacterial + antifungal
Substrate optimization

- Autoclaved soil
- Soil + 0.5% glucose
- Soil + 1% glucose
Glucose changes ratios of soil organisms
Old Field Community Climate and Atmospheric Manipulation

- Oldfield ecosystem:
  - Ribgrass
  - Meadow fescue
  - Orchardgrass
  - Broomsedge
  - Goldenrod
  - Lespedeza
  - Red clover

- Treatments:
  - $T=\text{ambient or ambient } + 3.5^\circ C$
  - $[\text{CO}_2]=\text{ambient or ambient } + 300 \text{ ppm}$
  - Precipitation=$2 \text{ mm/wk or 25 mm/wk}$
Future Directions

• Integrate what I’ve learned at soyFACE and OCCAM
• Ecosystem-level research
• Agricultural system
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