Assessing the Active Bacterial Community Composition in Response to Drying and Rewetting Stresses in a California Grassland Soil

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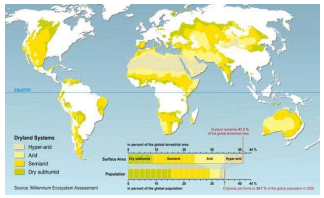
Introduction

- Terrestrial ecosystems are exposed to more droughts and bigger rainfall events

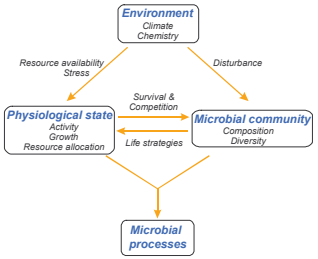
⇒ **What are the impacts on ecosystem functioning?**



Intergovernmental Panel on Climate Change, 2007

- Large scale biogeochemistry is strongly mediated by microbial communities

⇒ **How will microbial processes respond to environmental changes?**



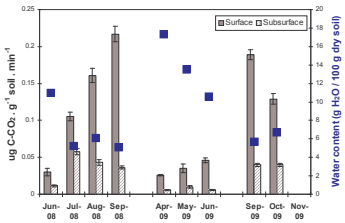
Schimel et al., Ecology (88) 2007

- Microorganisms acclimate to survive: physiological response and population dynamics

⇒ **Who are the active bacteria with potentially important ecological roles?**

Background

- Bioavailable C accumulate in dry soils
- Microorganisms respond immediately to a rewetting event, even after 3 months of drought

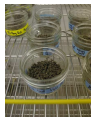


C mineralization rate 1h after rewetting the soil

Methods

➢ Soil sampling at UCSB Sedgwick Reserve located in the Santa Ynez valley of Central California

➢ Rewetting to 47% WHC with BrdU (thymidine analog)

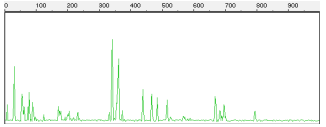


➢ Incubation at 22°C for 12h, 24h or 48h

➢ Genomic DNA extraction
➢ BrdU-labeled DNA immunocapture*

➢ PCR of 16S rDNA (8F and 1389R primers)

➢ T-RFLP analysis (*HhaI* and *MspI*)

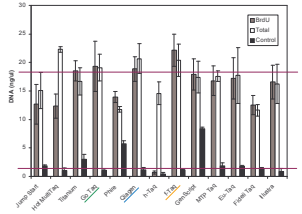


*Protocol adapted from Borneman, 1999; Yin et al., 2004; Allison et al., 2005

Results

✓ Pushing the limits for 16S rDNA amplification on BrdU-labeled DNA

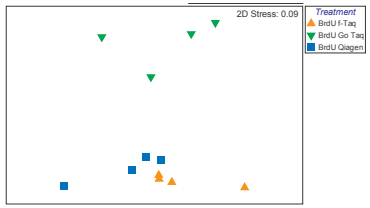
DNA concentration from purified PCR products



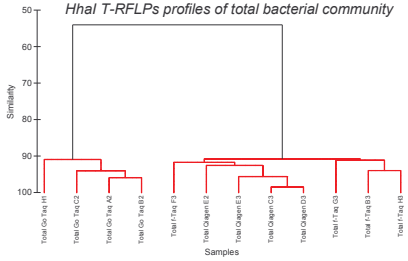
Among the 13 different Taq polymerases we tested, only 3 provided adequate yield and minimal contamination

Composition of the dominant bacterial community

MDS plots of the genetic structure generated from HhaI T-RFLPs profiles of actively growing community

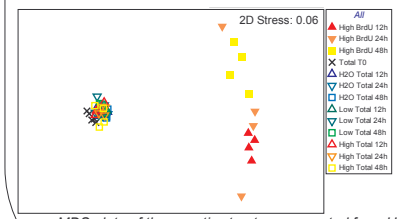


Cluster Analysis of the genetic structure generated from HhaI T-RFLPs profiles of total bacterial community



Amplification with Go Taq generated a different community structure: 15% dissimilarity for BrdU-labeled DNA and 46% for total DNA compared with Qiagen or f-Taq (less than 9% dissimilarity)

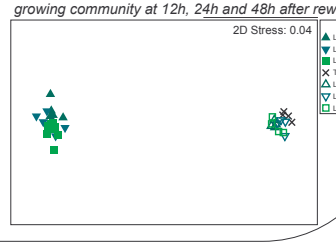
✓ Rapid response of actively growing bacteria under a drying – wetting cycle



MDS plots of the genetic structure generated from HhaI T-RFLPs profiles for total community and actively growing community at 12h, 24h and 48h after rewetting

- BrdU incubation did not affect total bacterial community composition
- Actively growing bacteria can be separated from the total bacterial community (about 70% of dissimilarity)
- Total community structure did not change during 48h following the rewetting event whereas active community evolved
- High BrdU concentration (10 µmol / g soil) illustrated population dynamics better than low BrdU concentration (2 µmol / g soil)

MDS plots of the genetic structure generated from HhaI T-RFLPs profiles for total community and actively growing community at 12h, 24h and 48h after rewetting



Conclusions

❖ The main issue when working with difficult and/or low amount templates DNA is to find a threshold between PCR yield and specificity. Success in amplification depends of several factors, but mostly on Taq polymerase

❖ An optimized BrdU technique is useful for characterizing actively growing bacteria and for revealing population dynamics after rewetting dry soils

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