Pyrosequencing to Determine the Influence of Fallow Period on Soil Microbial Communities in the Bolivian Highlands

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Introduction

The Bolivian highlands (altiplano) are experiencing changes in agricultural practices due in part to climate change and economic pressures. Traditional fallow periods are being shortened in an effort to increase short-term yield in crops such as quinoa and potato, but this may be at the expense of soil quality. A common component of fallow vegetation in this region is the evergreen plants *Parastrephia* sp. and *Braccharis* sp., locally known as "Thola". It is widely believed that Thola improves soil quality. Using pyrosequencing methods, we are characterizing the responses of microbial communities in these systems to fallow period and the presence of Thola. In the first phase of the project, we have analyzed soil fungal diversity, as reported here. In the next phases of the project we will analyze both fungal and bacterial community composition in more detail in the Bolivian altiplano and in Zambian agricultural systems, synthesizing microbial community data with socioeconomic and soil physico-chemical data.

Objectives

1. Compare soil characteristics in the study regions Umala and Ancoraimes

2. Characterize soil microbial communities in fields with a range of fallow periods, beginning with characterization of fungi

3. Determine the effect of Thola on soil microbial diversity during fallow

Methods

• Twelve and 17 fields were sampled in Ancoraimes and Umala (Figure 1), respectively (eight subsamples per field)
• DNA was extracted using Soil DNA Isolation kits, and a list of sequences was obtained using 454 pyrosequencing
• Physico-chemical characteristic were evaluated for each field.
• Diversity estimators such as Chao1, Simpson, Alpha were used to estimate taxon diversity based on 97% similarity.
• The effects of fallow period were analyzed in a regression analysis for samples collected away from Thola, and the effects of Thola were analyzed for longer fallow fields of Umala in a paired t-test.
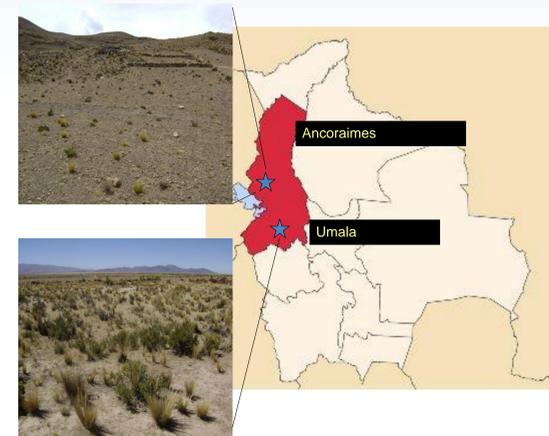

Figure 1. Location of the 29 study fields in Ancoraimes and Umala, Bolivia. Photos illustrate fields after two years of fallow during the winter season.
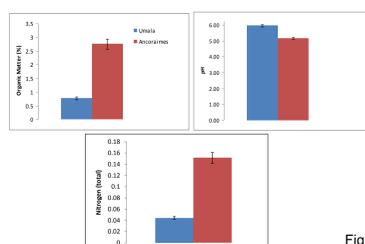
Results and Discussion

❖ Ancoraimes had a higher percentage of Organic Matter (OM) and higher level of total Nitrogen (N) than Umala (Figure 2). This difference may be due in part to different patterns of OM management in the two regions.
❖ Management to increase OM in soil often leads to an increase in species richness and overall density in the microbial community (Brussaard et al., 2007).
❖ pH is an important criterion for predicting the capacity of soils to support microbial reactions (Paul and Clark, 1989). The pH in soils in Umala is near 6, while soils in Ancoraimes are more acidic.

Figure 2. Organic matter, N and pH in sampled fields in Umala and Ancoraimes.

❖ Fungal diversity in subsamples without Thola was significantly higher in Ancoraimes than Umala when measured using Simpson's index (Fig. 3; P < 0.001) and Alpha (P = 0.002), but not the Chao1 estimator (P= 0.18).
❖ A peak in the diversity around 10 years of fallow might be explained by the increasing presence of Thola in the fields over time.
❖ A decrease in fungal taxon diversity could be related to the removal of Thola from fields for use as a fuel source by farmers.

Figure 4. "Thola" in a Bolivian field. Thola refers to two genera common in Bolivian fallow: *Baccharis* and *Parastrephia*.
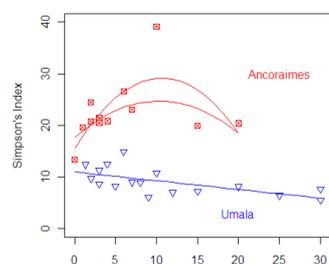
Figure 3. Simpson's index for fungal diversity in Umala and Ancoraimes across fallow periods (years), sampled away from Thola. The regression line for Umala has a significant negative slope (P=0.007). For Ancoraimes, there are significant linear (P=0.02) and quadratic (P=0.02) parts to the model, even when the unusually high value is removed (P=0.05 for both linear and quadratic parts, lower red line).

❖ In **Umala** the samples collected near Thola had significantly higher levels of diversity for soil fungal populations compared with those collected far from Thola (Simpson's P=0.08, Alpha P=0.03, Chao1 P=0.07).
❖ In **Ancoraimes** the difference between Simpson's index for samples near and far from Thola was not significant, at least in part because only two fields were available for the comparison (P=0.7).
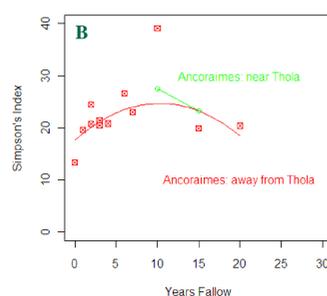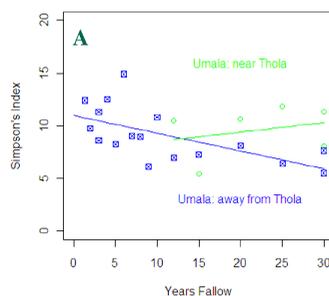
Figure 5. Soil fungal diversity as measured by Simpson's index in two communities in Bolivia where samples were collected near/ far from "Thola" in the fields with longer fallow. A) 17 fields in Umala B) 12 fields in Ancoraimes

Conclusions

In the first phase of our research, we found that the presence of "Thola" after ten years of fallow may have a positive effect on the diversity of the fungal populations in the soil, and the removal of Thola may also impact the microbial diversity. Increasing fallow period did not increase fungal diversity in a straightforward fashion as we had anticipated.

In the next phase of our research we will evaluate fungal and bacterial community composition in this system in more detail.

References

• Brussaard L, De Ruiter PC, Brown GG. 2007. Soil biodiversity for agricultural sustainability. Journal Agriculture Ecosystems and Environment 121: 233- 244.
• Paul EA, and Clark FE. 1989. Soil microbiology and biochemistry. Academic Press, Inc., San Diego, California. 265 pp.

Acknowledgments