

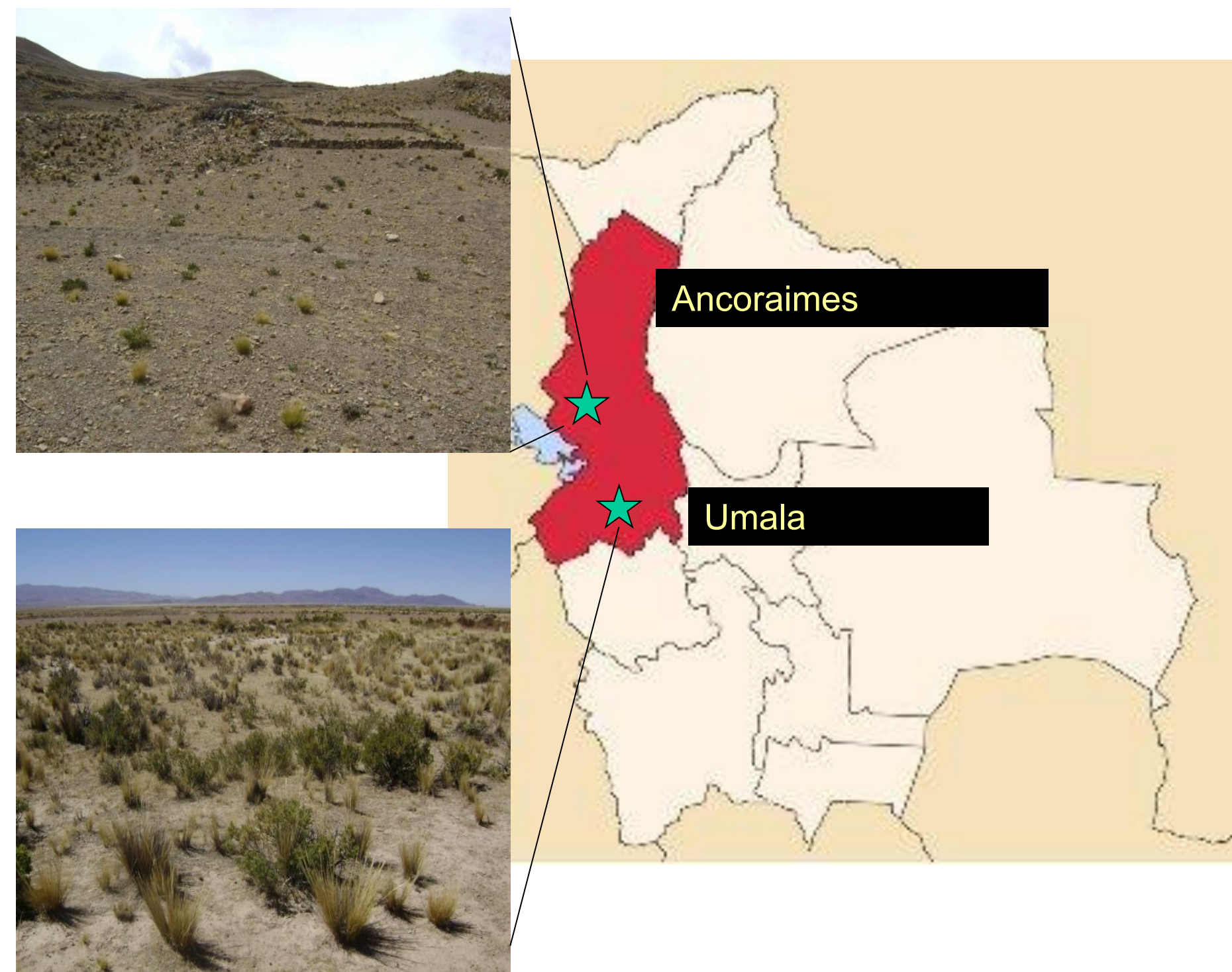
# Pyrosequencing to Determine the Influence of Fallow Period on Soil Microbial Communities in the Bolivian Highlands

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## Introduction

In the Bolivian highlands (Altiplano; approx. 4000 masl), traditional fallow periods are being shortened in an effort to increase short-term crop yields, which may be at the expense of soil quality. Using 454-pyrosequencing, we characterized the response of the microbial community to (1) the length of fallow period and (2) the presence of plants in the genera *Parastrephia* and *Baccharis* (both locally known as 'Thola'), considered beneficial to the maintenance of soil quality in this region. Our results suggest increasing fallow years were associated with an increase in dominance in both fungal and bacterial communities.



**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.

## Objectives

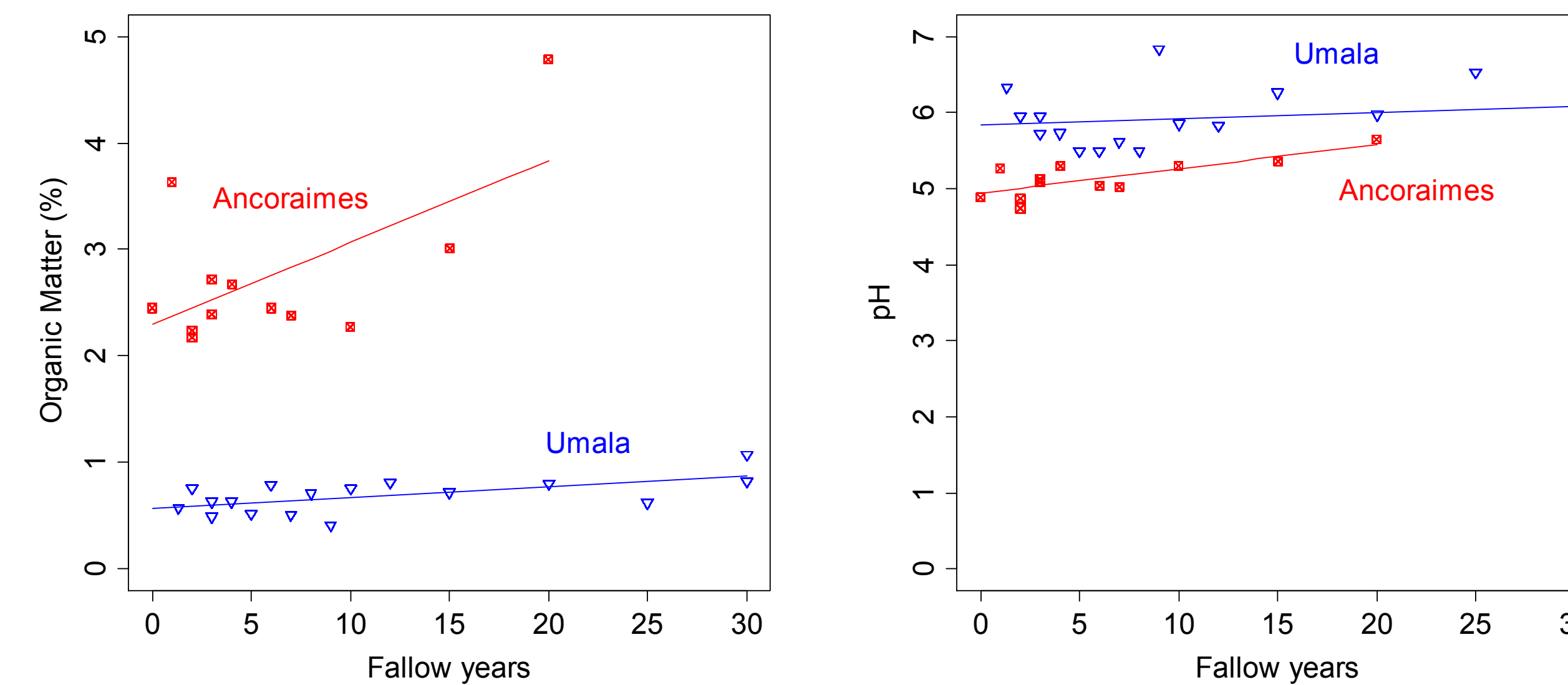
1. Compare soil characteristics in the study regions Umala and Ancoraimes.
2. Characterize soil microbial communities in fields with a range of fallow periods.
3. Determine the effect of Thola on soil microbial communities during fallow.

## Methods

- Twelve and 17 fields were sampled in Ancoraimes and Umala (Figure 1).
- DNA was extracted using Soil DNA Isolation kits, and a list of sequences was obtained using 454 pyrosequencing.
- Diversity estimators such as species richness, Simpson's, evenness were used to estimate taxon diversity based on 97% similarity. The effects of fallow period and Thola for diversity estimators were evaluated in regression analyses.
- The effects of fallow period and Thola for the frequency of specific taxa were evaluated using generalized linear models.

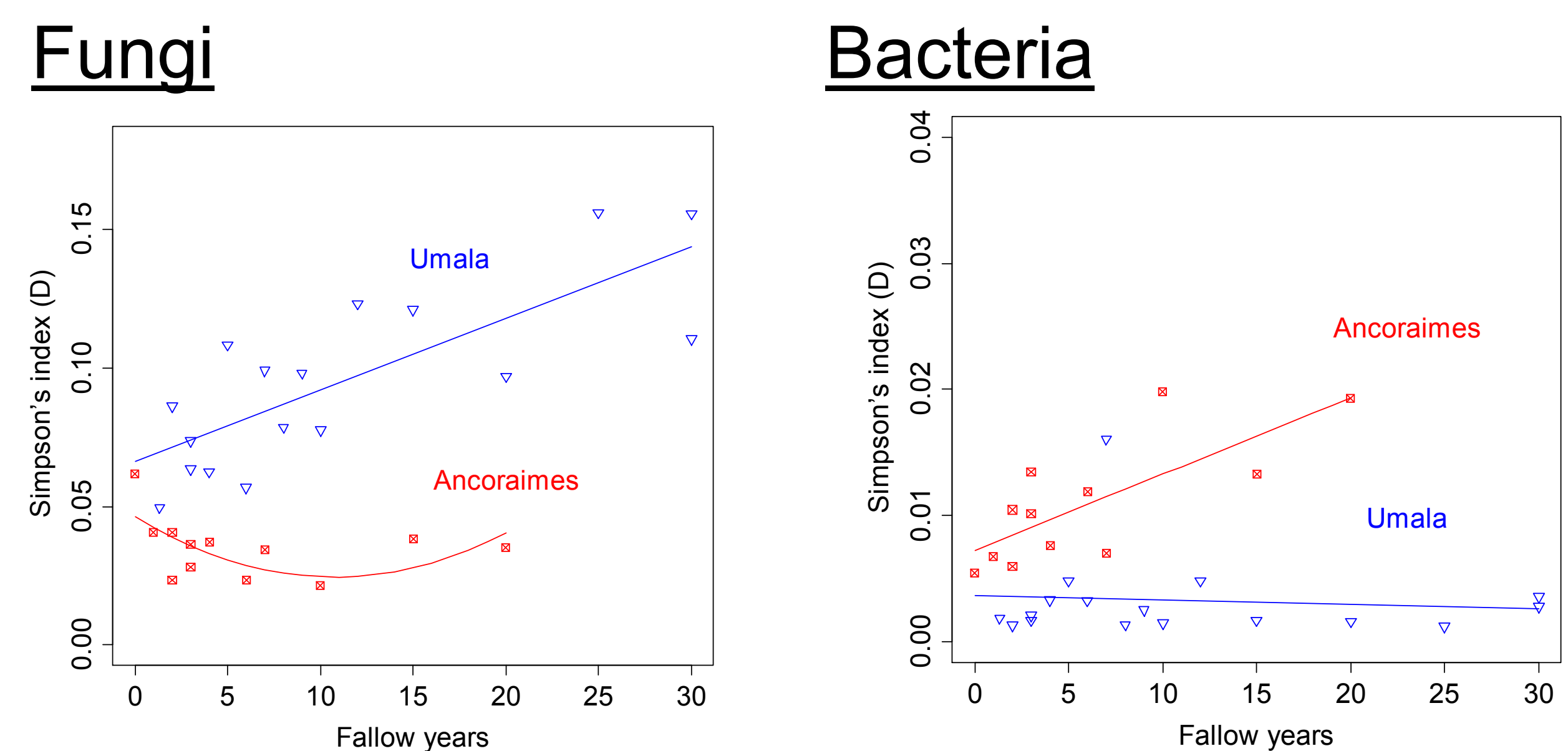
## Results

### ➤ Physico-chemical characteristics

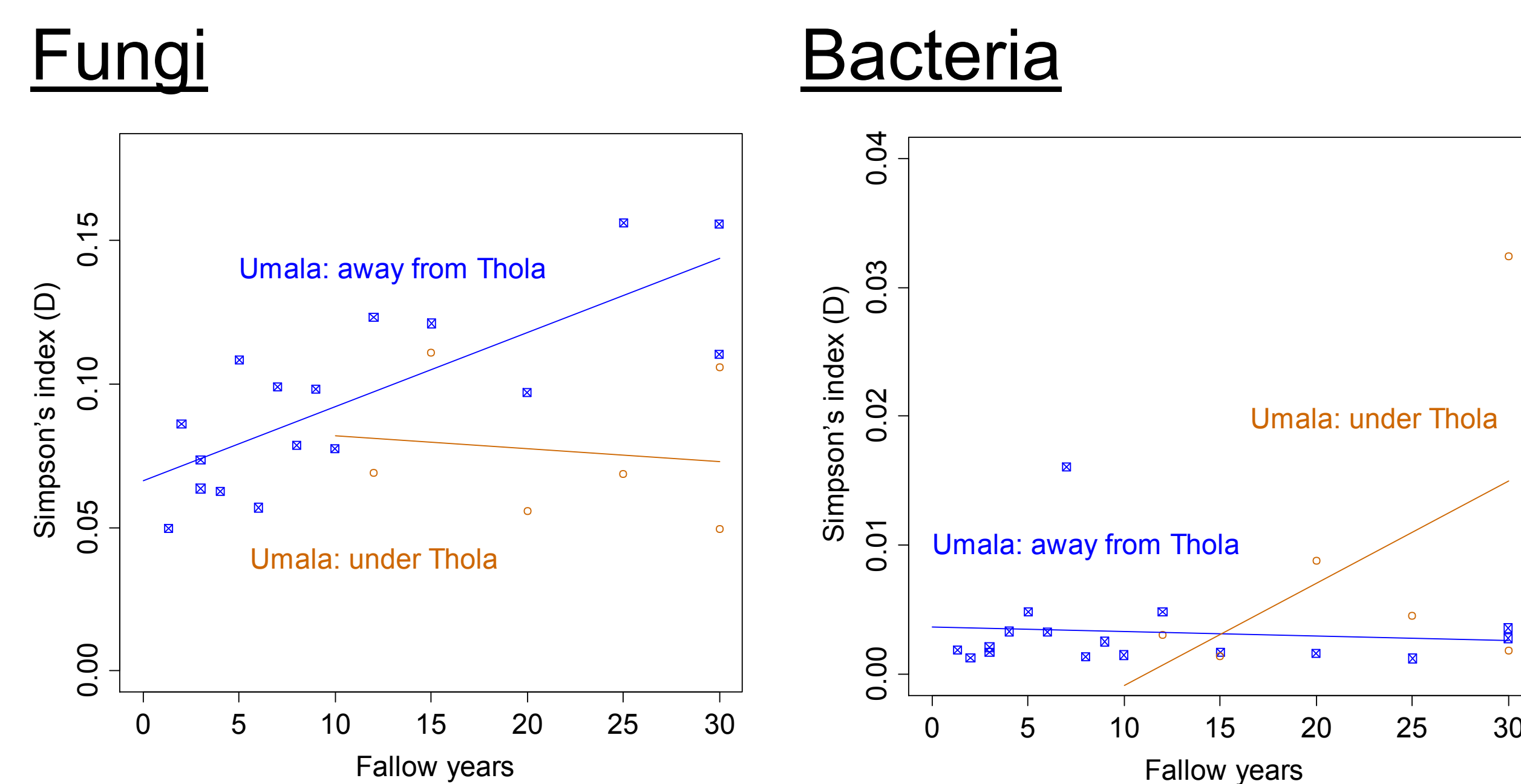


**Figure 2.** Organic matter, and pH in sampled fields in Umala and Ancoraimes.

### ➤ Diversity estimator: Simpson's dominance



**Figure 3.** Simpson's dominance for fungal and bacterial communities in Umala and Ancoraimes across fallow periods (years), sampled away from Thola. **Fungi:** The regression line for Umala (slope  $P < 0.001$ ,  $R^2 = 0.6$ ); for Ancoraimes, the linear ( $P = 0.04$ ,  $R^2 = 0.3$ ) and quadratic part ( $P = 0.05$ ); t-test comparing Umala and Ancoraimes:  $P < 0.001$ . **Bacteria:** The regression line for Umala (slope  $P = 0.7$ ,  $R^2 = 0.008$ ); for Ancoraimes ( $P = 0.004$ ,  $R^2 = 0.5$ ); t-test comparing Umala and Ancoraimes:  $P < 0.001$ .



**Figure 4.** Soil fungal and bacteria dominance as measured by Simpson's index for Umala where samples were collected under or away from Thola in the fields with longer fallow. **Fungi:** For Umala under Thola (slope  $P = 0.8$ ,  $R^2 = 0.07$ ), and for Umala away from Thola (slope  $P < 0.001$ ,  $R^2 = 0.6$ ). Paired t-test away-under:  $P = 0.02$ . **Bacteria:** For Umala near Thola (slope  $P = 0.6$ ,  $R^2 = 0.2$ ). For Umala away from Thola (slope  $P = 0.7$ ,  $R^2 = 0.008$ ). Paired t-test away-under:  $P = 0.2$ .

### ➤ Fungi: taxa associated with Thola effect

Umala: under Thola      Umala: away from Thola

Genus
<i>Capnodium</i>
<i>Cladosporium</i>
<i>Cryptococcus</i>
<i>Phaeosphaeria</i>
<i>Ulocladium</i>

Genus
<i>Chaetomidium</i>
<i>Microdochium</i>
<i>Penicillium</i>

**Table 1.** Taxa more abundant near/away from Thola for fungi in Umala. Paired t-test away-near:  $P < 0.05$ . Green font indicates a plant-associated genus.

### ➤ Bacteria: taxa associated with Thola effect

Umala: under Thola      Umala: away from Thola

Genus
<i>Balneimonas</i>
<i>Flavisolibacter</i>
<i>Methylibium</i>
<i>Rhodococcus</i>
<i>Sphingobium</i>
<i>Succinispira</i>
<i>Xiphinematobacter</i>

Genus
<i>Chloroflexus</i>
<i>Gemmatimonas</i>
<i>Humicoccus</i>
<i>Isosphaera</i>
<i>Rubrobacter</i>

**Table 2.** Taxa more abundant near/away from Thola for bacteria in Umala. Paired t-test Away-Near:  $P < 0.05$ . Green font indicates a plant-associated genus.

## Discussion

- Ancoraimes had a higher percentage of organic matter (OM) than Umala (Figure 2). This difference may be due in part to more intensive management in Ancoraimes.
- Ancoraimes had a lower fungal species dominance than Umala. However, Umala had a lower bacterial species dominance than Ancoraimes (Figure 3). Fungi in soil respond more to OM than bacteria. Bacteria not only are more abundant in soil, and have morphologies that allow them to tolerate harsh environmental conditions.
- Although the samples collected under Thola had lower levels of fungal species dominance compared with those collected away from Thola, the result observed was different for bacteria (Figure 4). Probably the presence of Thola is not making a significant contribution to the bacterial species.
- The majority of the fungal taxa are recognized plant pathogens (names colored in green -Table 1), while others such as *Capnodium* cause little harm to the plant (sooty mold group).
- A wide range of bacterial taxa were recovered. The genus *Flavisolibacter* is usually isolated from cultivated soils, while *Xiphinematobacter* is an endosymbiotic bacterium of nematodes (Table 2).

## Conclusions

- Dominance increased with fallow period for fungi in Umala and for bacteria in Ancoraimes.
- The presence of Thola decreased fungal species dominance.
- This pyrosequencing approach allowed us to identify taxa that change in frequency with fallow period.
- Our analysis of fungal and bacterial community composition in this system is ongoing.

## Acknowledgments

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