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# Do Bacterial and Fungal Communities in Soils of the Bolivian Altiplano Change under Shorter Fallow Periods?

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**Abstract**

Traditional fallow periods in the Bolivian highlands are being shortened in an effort to increase short-term crop yields, with potential long-term impacts on soil microbial communities and their functions. In addition, native vegetation, such as *Parasthrephia* sp. or *Baccharis* sp. (both locally known as "thola") are often removed as a fuel for cooking. We evaluated the effects of fallow period and thola on soils in 29 farmers' fields in two municipalities in the Bolivian Altiplano (Umala and Ancoraimes). Soil fungal and bacterial community responses were characterized using 454-pyrosequencing. Soils in Ancoraimes had significantly higher levels of organic matter, nitrogen, and other macronutrients compared to Umala. Ancoraimes soils also supported more diverse fungal communities, whereas

Umala had more diverse bacterial communities. Unexpectedly, the longer fallow periods were associated with significantly lower fungal diversity in Umala and lower bacterial diversity in Ancoraimes. Fungi assigned to genera *Bionectria*, *Didymella*, and *Alternaria*, and bacteria assigned to genera *Paenibacillus*, *Segetibacter*, and *Modestobacter* decreased in frequency with longer fallow period. The presence of thola was not associated with significantly different overall soil fungal or bacterial diversity but was associated with higher frequency of some genera, such as *Fusarium* and *Bradyrhizobium*. Our results indicate that fallow period has a range of effects on soil communities, and that the removal of thola may impact the dynamics of these communities.

**Keywords:** 454-pyrosequencing, Andes, Bolivian highlands, long fallow agriculture, soil microbial communities, barley, potato, quinoa

## 1. Introduction

Vegetated fallow systems are widely used in South America, Asia, and Africa as a strategy to restore soil fertility without purchasing external inputs and are often successful in maintaining productivity in low fertility soils (Sanchez, 1999; Wezel and Haigis, 2002; Burgers et al., 2005; Couteaux et al., 2008). The term "fallow" describes resting periods in agricultural lands during which the noncrop or dormant species are allowed to reestablish by natural succession after cropping (Nair, 1993; Sanchez, 1999). Tropical fallow systems are often cropped for one to four years during which the soil fertility declines rapidly followed by a subsequent long fallow period (from four years to multiple decades) that allows soil fertility to be restored (Pestalozzi, 2000; Wezel and Haigis, 2002; Bravo-Garza and Bryan, 2005; Cabaneiro et al., 2008; Ndour et al., 2008). For example, in the Bolivian Altiplano after three years of crop production, generally potato followed by quinoa (*Chenopodium quinoa* (Willd.)) and barley (*Hordeum vulgare* L.), fields are frequently kept in fallow for up to 20 years to restore soil fertility (De Cary and Hervé, 1994; Hervé, 1994; Pacheco Fernández, 1994; Hervé et al., 2002; Couteaux et al., 2008). Such long fallow periods permit unmanaged revegetation similar to the plant communities before cropping (Masse et al., 2004; Couteaux et al., 2008). When the fields are cultivated again, the established vegetation may be ploughed into the soil as green manure (Sarmiento and Bottner, 2002; Couteaux et al., 2008).

Fallow cropping systems may have various effects on soil physical, biological, and chemical properties. However, few studies have addressed the effects of fallow on soil microbiota. Sall et al. (2006) found that soils from a 21-year fallow in Senegal had higher C, N, and total P than cultivated soils as well as higher microbial activity and diversity. Many investigations have reported that soil microbial activity depends on soil organic matter quality, quantity, and distribution as well as soil texture (Kaiser et al., 1992; Kennedy and Papendick, 1995), soil pH (Mishra and Dash, 1987), climatic conditions (Insam et al., 1989) and agricultural practices (Doran, 1980; Anderson and Domsch, 1989). Other studies have reported that soil microbial communities respond to soil management, such as crop rotation, fertilization, tillage, and manure or pesticide applications (Sigler and Turco, 2002; Crecchio et al., 2004; Spedding et al., 2004; Acosta-Martinez et al., 2010; Yin et al., 2010). Comparative studies have been conducted to evaluate the fallow period effects on soil properties and erosion control (Sarmiento and Bottner, 2002; Ndour et al., 2008; Miranda et al., 2009). In southeastern Brazil, agricultural fields after a five-year fallow had higher

macroporosity, total porosity, and saturated hydraulic conductivity than those after a two-year fallow (Miranda et al., 2009). In contrast, short-term fallows (four years) in Senegal did not increase soil organic matter or nutrient content (Masse et al., 2004). Similarly, in a study in the Bolivian highlands, there was no evidence of recovery of nutrients after a ten-year fallow (Hervé, 1994). These conflicting results illustrate the heterogeneity of farming systems and microbial responses where studies of a small number of fields reveal only part of the range of potential responses.

In recent decades, economic pressures related to the increase in human populations and the demand for additional agricultural land have led to shorter fallow duration and extent, reducing the potential beneficial effects of fallow on soil fertility and ecosystem restoration (Kang et al., 1999; Masse et al., 2004; Couteaux et al., 2008). The long-term fallows (generally 6–10 years or more) in Southwestern Nigeria, which contributed to soil fertility, have often been reduced to 3–6 years (Aweto et al., 1992). In West Africa and Latin America, shortened fallow periods (1–5 years) have led to reported soil degradation and increased need for fertilizers (Aweto et al., 1992; Kang et al., 1999; Phiri et al., 2001; Wezel and Haigis, 2002). Moreover, natural vegetation cover has often decreased, presenting a threat to maintenance of biodiversity (Breman and Kessler, 1995). In the Bolivian Altiplano, over the last two decades, economic pressures reduced the fallow length to 2–10 years, compared to the traditional length of up to 20 years (Hervé, 1994; Aguilera A., 2010). There has been little research in Bolivia to determine the effects of fallow on soil restoration. The economic pressures in the Altiplano region pose new questions about the effects that the increasing population and the competition for crop land (with dairy production and forage for livestock) can have on soil edaphic properties and microbiota.

Another important component of fallow is the reestablishing natural vegetation. In Southeast Asia, Latin America, and Africa, shrubs in the family Asteraceae have been gaining attention in fallow systems for their fast establishment, high biomass, and the high levels of nutrients they release to the soil (Roder et al., 1995; Koutika et al., 2005; Partey et al., 2011). These shrubs are collectively known as "daisy fallows" (Sanchez, 1999). For example, *Chromolaena odorata* (L) King & Robinson (Asteraceae, Eupatorieae) is considered to be "a good fallow plant" in many countries (Roder et al., 1995), beneficial to the crops as a source of organic matter (Norgrove, 2008) and exchangeable K (Kanmegne et al., 1999), and because it can adapt more readily to acidic soils than some legumes (Koutika et al., 2004). In the Bolivian Altiplano, the species *Parastrephia lepidophylla* (Wedd.) Cabrera, *Baccharis incarum* (Wedd.) Perkins (syn: *Baccharis thola* Phil.), and some close relatives in the Asteraceae are collectively known as "thola," and are considered by local farmers to be beneficial to soil quality because of their rapid colonization of bare lands (De Cary and Hervé, 1994; Stacishin de Queiroz et al., 2001), and contribution to soil organic matter (Hervé, 1994). Thola reproduces by seed and reaches its maximum height in approximately 10 years, such that farmers sometimes use thola height to estimate the length of a fallow (Hervé, 1994). Higher microbial diversity in culturable bacteria, arbuscular mycorrhizal fungi, and actinomycetes has been reported in association with thola compared to the grass *Stipa ichu* (De Cary and Hervé, 1994). Thola is also used as a fuel, so farmers face a trade-off in deciding between maintaining and harvesting thola.

Studies of soil microbial community composition have been hampered by traditional methods and the nonculturability of most soil microbes (Rondon et al., 2000; Fierer et al., 2007). Recent developments in molecular biology and biochemical assays provide new tools for microbial community analysis. Some studies have used the total rRNA from soil to quantify the abundance of Proteobacteria, Actinobacteria, Bacteria, and Eukarya under different field fertilization and tillage regimes (Buckley and Schmidt, 2001). Metagenomic and small subunit rRNA-gene sequence analyses have compared the diversity of bacteria, archaea, fungi, and viruses in soils from prairie, desert, and rainforest (Fierer et al., 2007). The use of 454-sequencing permits analysis of millions of microorganisms and bypasses culturing (Roesch et al., 2007). Additionally, the recent development of sample-specific sequence tags (DNA tagging) allows multiplexing large numbers of individual samples, making DNA sequencing and analysis more cost-efficient (Acosta-Martinez et al., 2008; Jumpponen and Jones, 2009; Lauber et al., 2009). These new techniques provide useful tools for understanding how soil communities change in response to shifts in cropping systems.

The first objective of this study was to evaluate the effects of fallow period and the presence of thola on soil physical and chemical characteristics in two municipalities, Umala and Ancoraimes, in the Bolivian Altiplano. Our hypothesis was that longer fallow period and the presence of thola would increase soil organic matter and nutrient levels in soil. The second objective was to characterize the response of soil microbial diversity (bacteria and fungi) to fallow length and to thola, using diversity indices such as Simpson's diversity. We hypothesized that fungal and bacterial diversity would increase with increasing fallow period and the presence of thola. Our third objective was to evaluate the frequency of individual fungal and bacterial taxa, and their response to fallow length and to thola. Our hypothesis was that some fungal and bacterial taxa would change in frequency with fallow period and in response to thola.

## 2. Materials and methods

### *2.1. The Bolivian Altiplano*

In Bolivia, the central highland plateau region or Altiplano (Fig. S1), is a semiarid region with temperate ecosystems and a range of elevations between 3600 and 4300 masl (Jetté et al., 2001). The Bolivian Altiplano spans about 800 km from north to south and 120–160 km from east to west, comprising 14% of the total land area of the country (Jetté et al., 2001; Valdivia et al., 2010). Compared to many other agricultural systems, the Altiplano is a challenging environment, yet 35% of Bolivians have relied on it for their livelihood (Quiroga, 1992). The Bolivian Altiplano is characterized by low annual precipitation (350 mm in the South; 550 mm in the North), frequent frost and drought during the growing season, and high diurnal temperature variations (Garcia et al., 2007; Couteaux et al., 2008).

### *2.2. Study locations*

The study was conducted at two municipalities in the northern Altiplano of Bolivia: Umala (17°19′34″S and 67°59′53″W) at approximately 3800 masl, and Ancoraimes (15°52′3″S and 68°49′16″W) at approximately 4075 masl. The semiarid Umala municipality has an average

precipitation of 350 mm $yr^{-1}$ and an average annual temperature of 11°C (Garcia et al., 2007). The relatively cool-humid Ancoraimes municipality has an average precipitation of 550 mm $yr^{-1}$ and an average annual temperature of 7–8°C (Programa Nacional de Cambios Climáticos, 2005). The soils in these two municipalities have a sandy loam texture and are classified sandy, mixed, frigid Typic Ustifluvents (United States Department of Agriculture, 1999) or locally as "Saj'e or Ch'alla" soil in Aymara (Aguilera A., 2010). Based on visual observations, soils at our sites in Ancoraimes had a higher rock content than those in Umala. Farming in these areas generally followed the pattern of a few years of crop production, typically potato followed by quinoa (*C. quinoa* (Willd.)) and barley (*Hordeum vulgare* L.), and then followed by fallow.

Seventeen fields in Umala and twelve in Ancoraimes were selected to represent a range of fallow periods (1–30 years in Umala; 0–20 years in Ancoraimes; Table S1) based on information provided by the local indigenous farming communities. Soils were sampled during the dry season in August 2008. In fields with fallow periods > 10 years, when large thola shrubs (typically *Parasthephia lepidophylla* and *Baccharis incarum*) were present, we sampled soils under thola as well as at least 1 m away from any thola. The thola in these fields was up to 40 cm tall and 60 cm in diameter, in the range previously reported (Hervé, 1994). In fields with fallow periods less than 10 years, thola was rare and smaller in size, typically 10 cm high by 15 cm in diameter.

### *2.3. Experimental design, plot layout, and soil sampling*

Each field was treated as an experimental unit. In each field, we selected a 20 m × 20 m plot at least 10 m from the field edge (Fig. S2). Subsamples were systematically collected from nine points in a 10 m × 10 m grid. At each subsample point, five soil cores (15 cm deep, 2.54 cm diam) were collected around a 1 m radius, sieved (3.35 mm mesh), and combined to represent the heterogeneity within the subsamples and the fields (Baker et al., 2009). Where thola was adequately abundant, additional soil was sampled (Fig. S3) to allow us to partition the effects of thola currently present and other factors associated with fallow length (such as absence of crop species and fertilization). In those fields, at each of the nine subsampled grid points, the closest thola was located and an additional subsample was collected under its canopy. If the thola was closer than 1 m to the grid point, the "thola-free" subsample point was relocated at least 1 m away from the nearest thola. This sampling scheme resulted in nine subsamples from fields without large thola (fields ≤ 10 yr fallow) and eighteen subsamples from fields with large thola (fields > 10 yr fallow). From each subsample, we collected ~300 g to assess physical and chemical properties and another ~0.7 g for DNA extraction. The latter subsamples were stored in MoBio bead solution tubes (Ultra Clean Soil DNA Isolation Kit; MoBio Laboratories, Carlsbad, California, USA). All soil was stored in a cooler during sample collection, then soil for DNA extraction samples was frozen at –20°C and soil for edaphic analysis was air-dried. Total DNA was extracted from each of the nine subsamples but subsamples were composited into one per field for soil moisture and physical and chemical analyses.

### *2.4. Soil physical and chemical analyses*

Soil physical and chemical properties were analyzed by the Instituto Boliviano de Ciencia y Tecnología Nuclear (IBTEN) in La Paz, Bolivia. Soil particle size was determined using the Bouyoucos hydrometer method. Soil pH was measured in water (1:5 soil:water), electrical conductivity (EC) using a 1:5 soil-to-water extract, and the of exchangeable cation concentration (i.e., calcium, magnesium, sodium, and potassium) using ammonium acetate ($NH_4OAc$) extraction (Warncke and Brown, 1998). The Effective Cation Exchange Capacity (ECEC) was calculated by adding the concentrations of the total exchangeable bases with exchangeable acidity (concentration of $H^+$ and $Al^{+3}$). The percentage soil organic matter (SOM) (Walkley-Black method), total N (Kjeldahl method), and P (Bray P1 method) were also measured.

### *2.5. DNA extraction and PCR amplification*

For each field, we extracted the soil DNA from the nine subsamples separately. We followed the Ultra Clean Soil DNA Isolation Kit protocol, except that the final elution used 100 μl of buffer S5 instead of 50 μl. All DNA templates were quantified with an ND 1000 spectrometer (NanoDrop Technologies, Wilmington, Delaware, USA) and adjusted to a final concentration of 2 ng/μl. Of the nine subsamples per field, the eight with the highest DNA content were selected to produce PCR amplicons. For fungi, we optimized the PCR reaction conditions for template and $MgCl_2$ concentrations as well as the annealing temperatures for the ITS1-F and ITS4 primers using *Saccharomyces cerevisiae* and *Agaricus bisporus*. For bacteria and archaea, the 786f and 1492-rm primers were selected based on screening 48 DNA-tagged primers with environmental DNA samples from the *Quercus macrocarpa* phyllosphere (Jumpponen and Jones, 2009) plus five DNA samples from Umala and three from Ancoraimes. The initial PCR conditions were modified from those in Roesch et al. (2007).

*Fungi*

The fungal Internal Transcribed Spacer region (ITS) was PCR-amplified in two replicate 25 μl reactions for each subsample. To accommodate direct 454 sequencing of the fungal amplicons, we synthesized primer constructs that incorporated the 454 primers (Margulies et al., 2005) with the forward primer (ITS1-F; (Gardes and Bruns, 1993)) or with the reverse primer (ITS4; (White et al., 1990)). These primer constructs combined 454-sequencing primer (A-primer) and the reverse primer (ITS4) with a five base pair (bp) DNA tag for post-sequencing sample identification in between, or the DNA capture bead anneal primer (B-primer) for the emulsion PCR (emPCR) and the forward primer (ITS1-F). This primer choice resulted in reverse sequence across the ITS2 region.

Each PCR reaction contained final concentrations or absolute amounts of reagents as follows: 100 nM of each of the forward and reverse primers, 10 ng (or 5 μl) of template DNA, 100 μM of each deoxynucleotide triphosphate, 2.5 mM $MgCl_2$, 1 unit GoTaq Hot Start DNA polymerase (Promega, Madison, Wisconsin) and 5 μl Green Go Taq Flexi PCR buffer (Promega, Madison, Wisconsin). PCR cycle parameters consisted of an initial denaturation at 94°C for 2 min, then 30 cycles of denaturation at 94°C for 1 min, annealing at

58°C for 1 min and extension at 72°C for 1 min, followed by a final extension step at 72°C for 9 min.

*Bacteria*

Similarly to fungi, bacterial variable regions V5 to V8 in the 16S rRNA gene were PCR-amplified in two replicate 25 μl reactions incorporating the 454 primers (Margulies et al., 2005) with the forward primer (786f; (Baker et al., 2003)) or with the reverse primer (1492-rm; (Roesch et al., 2007)). These two primers were chosen to increase the taxonomic range of 16S rRNA gene (Roesch et al., 2007). These primer constructs combined the 454-sequencing primer (A-primer) and the reverse primer (1492-rm) with an eight base pair (bp) DNA tag for post-sequencing sample identification in between, or the DNA capture bead anneal primer (B-primer) with the forward primer 786f.

Each PCR reaction contained final concentrations or absolute amounts of reagents as follows: 100 nM of each of the forward and reverse primers, 5 ng (or 2.5 μl) of template DNA, 100 μM of each deoxynucleotide triphosphate, 2.5 mM $MgCl_2$, 1 unit GoTaq Hot Start DNA polymerase (Promega, Madison, Wisconsin) and 5.0 μl Green GoTaq Flexi PCR buffer (Promega, Madison, Wisconsin). PCR cycle parameters consisted of an initial denaturation at 94°C for 9 min, then 30 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 1 min and extension at 72°C for 2 min. All PCR reactions for fungi and bacteria were performed in duplicates on two MasterCyclers (Eppendorf, Hamburg, Germany). Possible PCR amplification of contaminants was determined using a blank sample through the extraction protocol simultaneously with the actual samples and a negative PCR control in which the template DNA was replaced with sterile H2O. These remained free of visible amplicons.

For each of the 37 fields, 5 ml from each of the duplicate eight subsamples was pooled separately for fungi and bacteria into a final volume of 80 μl per field. The 37 pooled fungal or bacterial amplicons were cleaned using the Agencourt AMPure PCR purification system (AgenCourt Bioscience, Beverly, Massachusetts, USA) following the manufacturer's instructions. The clean fungal or bacterial PCR products were quantified with the ND1000 spectrometer and pooled equimolarly into one for fungi and one for bacteria. The pools were adjusted to a ~10 ng/μl for fungi and ~30 ng/μl for bacteria for downstream 454-pyrosequencing.

### *2.6. Pyrosequencing*

The fungal amplicons were sequenced in 5/16 of a sequencing reaction, and the bacterial amplicons in half sequencing reaction in a GS-FLX sequencer (454 Life Sciences, Branford, Connecticut, USA) at the Interdisciplinary Center for Biotechnology Research Core at the University of Florida. A total of 28,747 fungal sequences were obtained, with an average length of 242 bp. For bacteria, a total of 84,525 sequences were obtained, with an average length of 262 bp.

### *2.7. Bioinformatics and operational taxonomic unit (OTU) designation*

The fungal and bacterial sequences were analyzed using Pyro-Tagger v.1.0 (http://pyro-tagger.jgi-psf.org/; Kunin and Hugenholtz, 2009). Sequences lacking an exact match to the tag-primer sequences as well as those with low quality bases ($Q < 27$) or shorter than 200

bp were removed. The remaining reads were clustered to Operational Taxonomic Units (OTUs) at 97% sequence similarity using the "Pyroclust" algorithm (Kunin and Hugenholtz, 2009). For fungi, all OTUs were manually assigned to phylum, division, class, order, family, genera, and species based on the top-ranked BLAST matches (Zhang et al., 2000). To minimize the number of environmental "unculturable fungus" matches to our fungal queries we additionally applied an Entrez limit (Fungi [ORGANISMS] NOT environmental samples [FILTER] NOT unculturable [ALL FIELDS] NOT endophyte [ORGANISMS] NOT [root associated fungi]). For bacteria, a representative for each OTU was submitted to the Ribosomal Database Project's (RDP, Version 10) Classifier (Cole et al., 2008) for taxon assignment. To improve the reliability of our fungal taxon assignment through BLAST, we removed reads whose assignment was based on short overlap (< 80% coverage) or low sequence similarity (< 95% similarity). For bacterial taxon assignment through RDP, we removed reads with < 80% bootstrap support. PyroTagger outputs and OTU assignments were used to calculate the taxon frequencies for each sample. OTUs that occurred only once in the data set were removed before analyses.

### *2.8. Diversity indices*

Three diversity estimates were calculated for fungi and bacteria. Simpson's diversity (1-D) estimates the likelihood that two randomly chosen individuals (sequences) will be assigned to different OTUs (Simpson, 1949). It is the complement of Simpson's dominance

$$(D = \sum p_i^2)$$

where $p_i$ is the proportion of sequences assigned to the $i$th OTU. We also evaluated Shannon's diversity

$$(H' = -\sum p_i \ln p_i)$$

and Pielou's evenness (J = H′/ln(S)), where S is species richness, to estimate community evenness. Diversity indices were estimated using the vegan package in R (Oksanen et al., 2011). (These analyses and those described below are included in the R script archived at http://hdl.handle.net/2097/15198.)

### *2.9. Statistical analyses*

Each field represented an experimental unit in analyses of fallow period effects. For fields that included thola and "non-thola" samples, the thola samples were used only in paired tests of thola effects. The diversity measurements (response variables)were analyzed in linear regression against fallow years using R version 2.12.2 (R Development Core Team, 2011). The effects of fallow period and thola on frequency of specific taxa were evaluated using generalized linear models with a binomial family (R function glm) and q-value comparisons to control the false discovery rate (R package q-value, Dabney, Storey, and Warnes). Analyses of the effects of thola on soil edaphic properties were based on paired sample *t*-tests from within six fields in Umala. We evaluated the differences in community composition using nonmetric multidimensional scaling (NMDS), and compared fields in

terms of physical and chemical properties using the vegan package in R (Oksanen et al., 2011). We also evaluated the responses of OTUs assigned to 5 fungal phyla, 61 orders, and 180 genera; and OTUs assigned to 18 bacterial phyla and 73 genera.

### *2.10. Accession numbers*

Data have been deposited in the Short Read Archive of the National Center for Biotechnology Information. The accession number for both the fungal data set and the bacterial data set is SRA058560.

## 3. Results

We report the results first for the non-thola samples, partitioning the effects of thola from other factors during fallow (such as the absence of crop species and fertilization). Then we report the results for the effects of thola in the fields where enough thola was present to sample both in the presence and absence of thola.

### *3.1. Edaphic properties in the two study regions (non-thola samples)*

The soil samples collected from Umala and Ancoraimes differed in some physical and chemical properties (Table 1). Both study regions had slightly acidic soils (pH < 7), and soils in Ancoraimes were more acidic that those in Umala. Both sites were non-saline, as indicated by low EC (0.05 and 0.04 dS $m^{-1}$ for Umala and Ancoraimes, respectively). By comparison, for example, for soils affected by salts in British Columbia (Canada) the levels of EC for A and B horizons were on average 1.0 dS $m^{-1}$ (Leskiw et al., 2012), while for topmost soils in San Joaquin Valley (California, USA) the EC levels were around 2 dS $m^{-1}$ (Ibekwe et al., 2010). The average soil organic matter (SOM) content in Ancoraimes was approximately three times higher than in Umala.

**Table 1.** Soil edaphic properties at two municipalities in the Bolivian Altiplano, including soil organic matter (SOM), effective cation exchange capacity (ECEC; centimol per kg), and electrical conductivity (EC; in deci-Siemens per m).

| Municipality | Statistic | SOM (%) | Total N (%) | pH | P (mg $kg^{-1}$) | ECEC ($cmol_c$ $kg^{-1}$) | EC (dS $m^{-1}$) |
|---|---|---|---|---|---|---|---|
| Umala | Mean | 0.77 | 0.04 | 5.96 | 7.94 | 7.29 | 0.05 |
| | Max. | 1.56 | 0.07 | 6.83 | 12.81 | 13.18 | 0.10 |
| | Min. | 0.41 | 0.03 | 5.49 | 5.59 | 3.90 | 0.03 |
| | Std dev | 0.25 | 0.01 | 0.37 | 1.71 | 2.46 | 0.02 |
| Ancoraimes | Mean | 2.76 | 0.15 | 5.16 | 8.82 | 6.17 | 0.04 |
| | Max. | 4.78 | 0.24 | 5.65 | 12.00 | 11.77 | 0.19 |
| | Min. | 2.17 | 0.11 | 4.74 | 5.72 | 4.54 | 0.01 |
| | Std dev | 0.70 | 0.04 | 0.24 | 1.74 | 1.86 | 0.05 |

When fields from Umala and Ancoraimes were plotted against the NMDS axes for fungal or bacterial communities, they separated into two distinct groups (Fig. 1). Soil edaphic properties that varied significantly with NMDS axes included clay content, SOM, N, Mg,

and Al increasing in Ancoraimes fields compared to Umala, and Ca, pH, effective cation exchange capacity (ECEC), and sand, increasing in Umala fields compared to Ancoraimes.

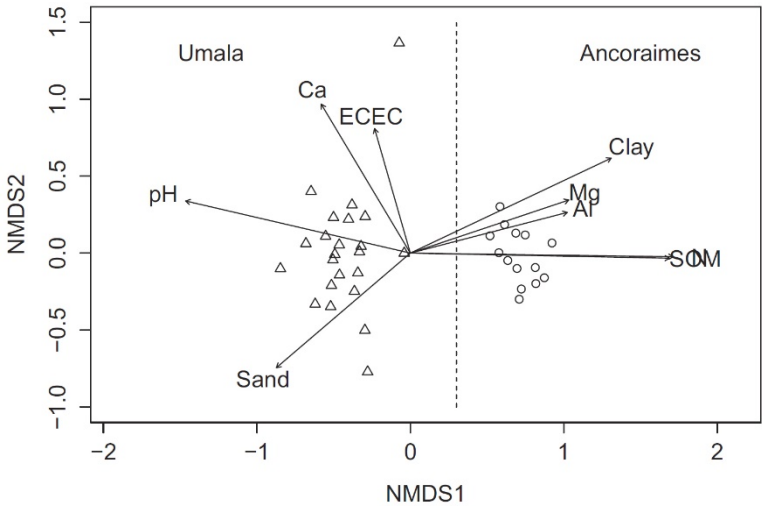


**Figure 1.** Nonmetric multidimensional scaling (NMDS) in two dimensions of fungal community composition in two municipalities (Umala and Ancoraimes) of the Bolivian Altiplano. Soil edaphic properties which varied significantly ($p < 0.1$) across the axes are indicated (ECEC = effective cation exchange capacity, SOM = soil organic matter, where SOM and N are overlapping). Results for bacterial communities were similar (not shown).

### *3.2. Fallow period effects on edaphic properties (non-thola samples)*

The relationship between fallow years and a number of edaphic properties—SOM, total N, pH, P, ECEC, and electrical conductivity (EC)—was evaluated using linear regression (Fig. S4). In Ancoraimes, SOM and total N increased significantly with longer fallow period, while in Umala SOM increased slightly over fallow years. The pH in Ancoraimes increased with increasing fallow years, compared to the lack of a trend in pH for Umala over the fallow years. Phosphorus increased with increasing fallow years in Umala, while in Ancoraimes it decreased with fallow years.

### *3.3. Thola effects on edaphic properties*

SOM and total N were significantly higher in thola samples compared to paired non-thola samples, as evaluated in six Umala fields (Fig. S5). Soil pH, P, ECEC, and EC did not differ significantly between thola and non-thola samples.

### *3.4. Microbial data characterization*

*Fungi*

After quality control and removal of 9555 reads that did not meet our minimum requirements, we retained 17,435 high-quality fungal sequences across the 37 fields. At 97% sequence similarity, these 17,435 sequences represented 2578 OTUs in all. The number of

sequences passing our quality filtering ranged from 104 (Umala-Campo L ST, 25 years of fallow) to 589 fungal sequences (Umala-Campo J ST, 12 years of fallow) per field. The average number of fungal sequences was 471 ± 86 (mean ± SD) per field (Table S1). Our data included 2171 nonsingleton OTUs and 407 singletons.

*Bacteria*

After quality control and removal of 42,856 reads that did not meet our minimum requirements, we retained 38,772 high-quality bacterial sequences across the 37 fields. At 97% sequence similarity, these 38,772 sequences represented 15,710 OTUs in all. The number of sequences passing our quality filtering ranged from 322 (Umala-Campo H ST, 9 years of fallow) to 1316 bacterial sequences (Umala-Campo L ST, 12 years of fallow) per field. The average number of bacterial sequences was 1048 ± 147 (mean ± SD) per field (Table S1). Our data represented 13,163 nonsingleton OTUs and 2547 singletons.

### *3.5. Diversity as function of fallow period*

*Fungi*

Simpson's diversity (1-D) decreased significantly with increasing fallow years in Umala but not in Ancoraimes (Fig. 2). Similar results were obtained for the other two diversity estimators Shannon's diversity (H′) and Pielou's evenness (J) (Fig. S6). Umala had lower overall fungal diversity than Ancoraimes. Inclusion or exclusion of the sample with low sequence number (25-year field in Umala) did not alter the statistical significance of the negative slope.

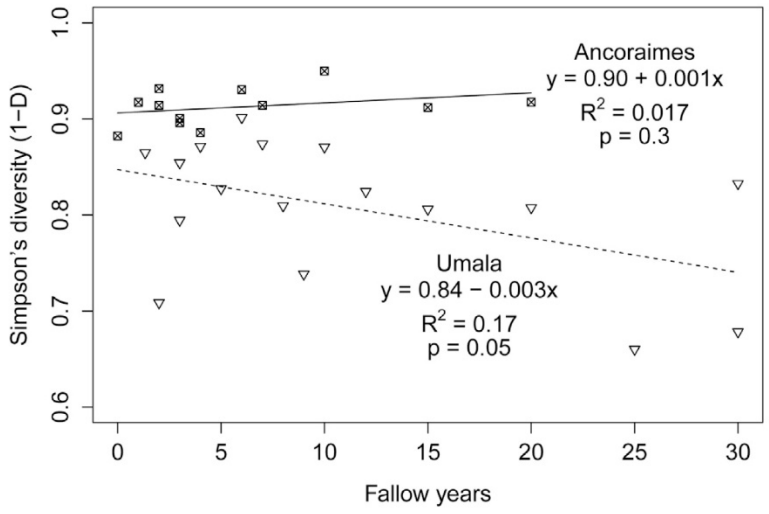


**Figure 2.** Simpson's diversity for fungi in two municipalities (Umala and Ancoraimes) of the Bolivian Altiplano across fallow periods (years) for non-thola samples, with results of a regression analysis. Taxon diversity based on 95% similarity.

*Bacteria*

Simpson's diversity decreased over fallow years in Ancoraimes, but not in Umala (Fig. 3). Results for Shannon's diversity (H′) and Pielou's evenness (J) were similar (Fig. S7). Ancoraimes had lower overall bacterial diversity than Umala.

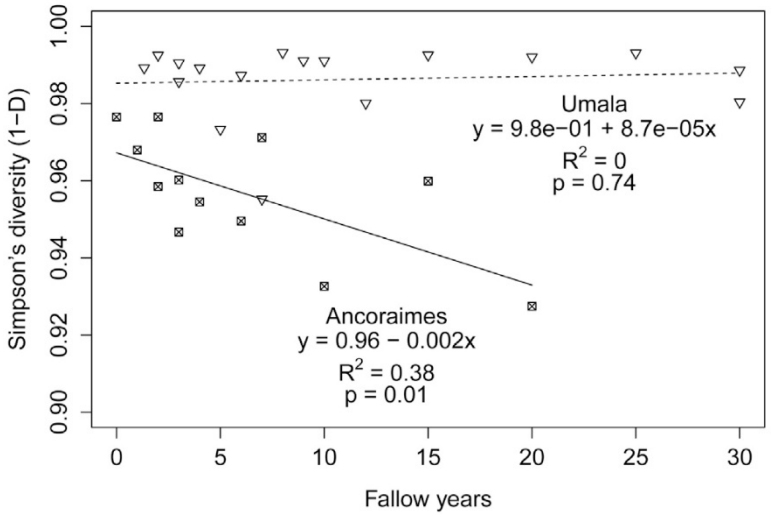


**Figure 3.** Simpson's diversity for bacteria in two municipalities (Umala and Ancoraimes) of the Bolivian Altiplano across fallow periods (years) for non-thola samples, with results of a regression analysis. Taxon diversity based on 90% bootstrapping.

### *3.6. Diversity of microbes associated with thola*

The effect of thola on the diversity estimators was evaluated in a *t*-test for fungal and bacterial communities in Umala. The samples collected under thola did not significantly differ in fungal or bacterial diversity compared to non-thola soils ($p > 0.2$).

### *3.7. Overall most frequent taxa in Umala and Ancoraimes (non-thola samples)*

*Fungi*

Averaged across all non-thola samples, the most frequent phylum, order, and genus assignments were identified (with the percentage for Umala and Ancoraimes, respectively, given in parentheses). The Ascomycota (84% and 65%) and Basidiomycota (4% and 11%) were the most frequent (Table S2). The most frequent orders were Hypocreales (45% and 38%), and Pleosporales (30% and 19%) (Table S3), both in the phylum Ascomycota. The OTUs assigned to the genera *Fusarium* (41% and 19%), and *Didymella* (28% and 16%), were the most frequent (Table S4).

*Bacteria*

Averaged across all non-thola samples in Umala, the most frequent phyla were Proteobacteria (23%) and Actinobacteria (11%) (Table S5). The OTUs assigned to the genus *Paenibacillus* (2.7%) and *Stremptomyces* (1.4%) were frequent (Table S6). In Ancoraimes, the most frequent phyla included Proteobacteria (26%) and Firmicutes (16%) (Table S5), and the OTUs assigned to genera *Pseudomonas* (3%), and *Bradyrhizobium* (2%) were the most frequent (Table S6).

### *3.8. Taxa varying with fallow period*

To examine whether the fungal composition changed with fallow period, phylum, order, and genus assignments were analyzed. No phyla increased significantly in frequency with increasing fallow period, but the orders Eurotiales and Mortierellales did increase with fallow period at both sites (Tables S7 and S8). The OTUs assigned to genera *Penicillium* and *Mortierella* significantly increased with fallow period for both sites (Table S9). The phylum that decreased in frequency with fallow period in both sites Umala and Ancoraimes was Basidiomycota (Table S10). The orders Microascales and Pleosporales decreased with fallow period in Umala, while Capnodiales, Filobasidiales, and Thelebolales decreased with fallow period in Ancoraimes (Table S11). The OTUs assigned to genera *Acremonium* and *Didymella* decreased with fallow period in Umala, while OTUs assigned to genera *Alternaria* and *Chaetomium* decreased with fallow period in Ancoraimes (Table S12).

Among the bacteria, the phyla Proteobacteria and Acidobacteria increased in frequency with increasing fallow period in Umala and Ancoraimes, respectively (Table S13). The OTUs assigned to genera *Pseudomonas* and *Sporosarcina* (Table S14) increased with fallow period in Umala. The bacterial phyla Actinobacteria (Table S15) and the OTUs assigned to the bacterial genus *Bradyrhizobium* decreased with fallow period in both sites (Table S16).

### *3.9. Taxa varying with thola (in Umala)*

*Fungi*

Although the overall diversity of fungi was not different between thola and non-thola samples, there were taxa that significantly differed in frequency (Tables S17–19). For example, Basidiomycota (Table S17), order Capnodiales (Table S18), and the OTUs assigned to the genus *Fusarium* (Table S19) were more frequent in thola samples than non-thola samples. Taxa more frequent in non-thola samples included order Mortierellales (Table S18) and the OTUs assigned to the genus *Didymella* (Table S19). Overall, 4 phyla, 24 orders, and 17 genera (which included many OTUs assigned each genera) differed significantly between thola and non-thola samples.

*Bacteria*

As for fungi, the overall bacterial diversity did not differ between thola and non-thola samples. However, there were taxa that significantly differed in frequency (Tables S20 and S21). For example, Bacteroidetes (Table S20) and the OTUs assigned to the genus *Bradyrhizobium* (Table S21) were more frequent in thola samples than non-thola samples.

Taxa more frequent in non-thola samples included Verrucomicrobia (Table S20) and the OTUs assigned to the genus *Belnapia* (Table S21). Overall 13 phyla and 55 genera differed significantly between thola and non-thola samples.

## 4. Discussion

### *4.1. Unexpected decreases in microbial diversity with increasing fallow period in Bolivian Altiplano soils*

Microbial diversity remained stable, and in some cases decreased, with increasing fallow period in these Altiplano soils. The soil physical and chemical conditions may explain some of the changes observed for OTU diversity at the level of fungal and bacterial genera. Differences in edaphic properties are often associated with differences in soil microbial communities (Lauber et al., 2008; Jenkins et al., 2009; Lauber et al., 2009; Rousk et al., 2010). In Umala soils with lower SOM and nutrient levels (Table 1), fungal OTU diversity decreased with increasing fallow years (Figs. 1 and 2). In Ancoraimes soils with relatively higher SOM and nutrient levels, bacterial OTU diversity decreased with fallow years (Figs. 1 and 3).

In addition to the differences in overall SOM and nutrient levels, sites differed in the magnitude of change in pH with increasing fallow years. In Umala, pH did not change significantly with fallow years, while in Ancoraimes it increased substantially (Fig. S4). This change in pH could explain the decrease in bacterial OTU diversity over the fallow years but is potentially less important for fungal OTU diversity. For example, Rousk et al. (2010) found that bacterial communities were more strongly influenced by pH than fungal communities across 180-m of the Hoosfield acid strip in the USA. Soil pH is often strongly associated with the composition of particular bacterial groups or the overall bacterial community composition, across land-use types for a specific location, or across continental scales (Fierer and Jackson, 2006; Lauber et al., 2008; Jenkins et al., 2009; Jones et al., 2009; Lauber et al., 2009; Rousk et al., 2010). The increase in pH with fallow years may have contributed to the changes in the composition of bacterial communities. Studies using pure cultures have also demonstrated narrow tolerances for pH in some soil bacteria (Rosso et al.,1995). In Altiplano soils, the phylum Acidobacteria in lower pH Ancoraimes soils increased in frequency with increasing fallow years (Table 1 and Table S13) although a strong negative association between relative abundance and higher pH has been observed for this phylum in a wide range of soils (Lauber et al., 2009). The combination of pH and other factors such as SOM may explain many of the changes observed in bacterial communities with increasing fallow period.

The decrease in fungal OTU diversity with increasing fallow period (in Umala but not Ancoraimes) is more difficult to interpret because there are fewer studies of soil fungal communities. The overall low levels of diversity (high dominance) can be attributed in part to the unusually high frequency of the OTUs assigned to genera *Didymella* and *Fusarium* (Table S4). These two dominant OTUs increased in frequency with increasing fallow years in Umala, as fungal diversity decreased. It would be interesting to know whether the absolute abundance of these two taxa was increasing, or whether other taxa were decreasing in abundance in this harsh environment.

### *4.2. The most frequent bacterial taxa in the Altiplano soils*

The observed frequencies of bacterial phyla in the Bolivian Altiplano soils generally agreed with other profiles (Janssen, 2006) for phyla such as Proteobacteria, Verrucomicrobia, Bacteroidetes, Chloroflexi, Planctomyces, and Gemmatimonadetes. There were also some notable differences in the frequencies for Firmicutes, Actinobacteria, and Acidobacteria.

The most frequent bacterial phylum in the Altiplano soils was the Proteobacteria (23% and 26% in Umala and Ancoraimes, respectively) (Table S5). The Proteobacteria were the most abundant soil phylum for several soils of North America based on sequencing of 16S rRNA and 16S rRNA genes, where for example this phylum represented 25% of clones from Oklahoma tallgrass prairie soils (Spain et al., 2009; Acosta-Martinez et al., 2010). Proteobacteria show extreme morphological, physiological, and metabolic diversity, participate in global C, N, and S cycling, and represent the majority of known gram-negative bacteria of medical, industrial, and agricultural significance (Kersters et al., 2006; Madigan and Martinko, 2006). Within this phylum, the OTUs assigned to bacterial genera *Pseudomonas* and *Bradyrhizobium* were relatively frequent in both Altiplano sites (Table S6).

The next most abundant phyla in the Bolivian Altiplano soils were Firmicutes (16%) and Actinobacteria (11%) (Table S5). The higher frequency of the phylum Firmicutes in the Altiplano was in sharp contrast to the low frequency (less than 5%) reported in other soil types in North and South America, or Europe (Janssen, 2006; Fierer et al., 2007). The OTUs assigned to the genus *Paenibacillus* (Firmicutes) were the most frequent in Umala (Table S6). This is a notable genus due to its role in fixing nitrogen in soil (Ma et al., 2007; Jin et al., 2011). The higher frequency of Firmicutes in the Altiplano compared to reports from other environments may be related to their ability to produce endospores which are resistant to desiccation under the harsh environmental conditions of the Bolivian Altiplano. In studies from a variety of North American, South American, and European soils, the frequency of the phylum Actinobacteria was around 13% (ranging from 0 to 34%) in bacterial communities based on 16S rRNA and 16S rRNA genes (Janssen, 2006). The OTUs assigned to the genus *Streptomyces* (Actinobacteria), which includes plant pathogens and antagonists of plant pathogens, was relatively frequent in both locations, particularly in Umala (Table S6). These filamentous bacteria can survive under low soil water content (Madigan and Martinko, 2006), a useful adaptation for the low precipitation across the Bolivian Altiplano.

### *4.3. The most frequent fungal taxa in the Altiplano soils*

Ascomycota was the most frequent phylum (84% in Umala and 65% in Ancoraimes) across all fallow period fields in Bolivian soils (Table S2), while in contrast Basidiomycota was the most frequent (54%) for tallgrass prairie soils also sampled in the cold season (Jumpponen et al., 2010). The OTUs assigned to genera *Didymella* and *Fusarium*, both belonging to the phylum Ascomycota, were the most abundant in the Altiplano, while *Omphalina*, *Pochonia*, and *Saccharomyces*, were the most abundant genera in the top 0–15 cm of the tallgrass prairie soil (Jumpponen et al., 2010). The high frequency of OTUs assigned to these two genera, *Didymella* and *Fusarium*, which both include plant pathogens and saprobes, may be because of their sturdy dormant structures that may withstand the harsh conditions of the Altiplano better than some fungal groups.

### *4.4. Microbial community composition as a function of fallow period*

For bacteria, OTUs assigned to the phyla Acidobacteria—whose members are physiologically diverse and ubiquitous, some being acidophilic and particularly abundant within soils (Madigan and Martinko, 2006)—and Proteobacteria increased in frequency with longer fallow periods in Ancoraimes and Umala, respectively (Table S13). Several OTUs assigned to fungal genera increased significantly in frequency with fallow period in both Umala and Ancoraimes. For example, the frequency of OTUs assigned to some genera including plant pathogens such as *Cladosporium* (Jacyno et al., 1993) and *Mortierella* increased with fallow period (Table S9). Interpretation of the impact of changes in the frequency of *Cladosporium* species is complicated by the many potential ecological roles that they may play, as pathogen, saprobe, or parasite of other fungi (Moran, 1998; Rivas and Thomas, 2005; Gange et al., 2012). The frequency of OTUs assigned to the genus *Clonostachys*, which has been reported to include parasites of a wide range of taxa (Baloyi et al., 2011; Rodriguez et al., 2011; Bienapfl et al., 2012), decreased with longer fallow periods (Table S12).

### *4.5. Microbial community composition as a function of thola presence or absence*

The presence of larger thola in fields with fallow periods greater than 10 years in the Bolivian Altiplano was associated with changes in the fungal and bacterial community composition. Several studies have indicated that microbial diversity is affected by plant species due to differences in root exudation that stimulate their growth in the rhizosphere (Wasaki et al., 2005; Micallef et al., 2009). For bacteria, the OTUs assigned to the genus *Bradyrhizobium* (order Proteobacteria)was more frequent under the canopy of thola, while the OTUs assigned to the genus *Paenibacillus* (order Firmicutes) was more frequent in non-thola samples (Table S21). The higher frequency of OTUs assigned to the genus *Bradyrhizobium* under thola could be associated with the ability of this symbiotic bacterium to colonize the roots of some nonlegumes (Antoun et al., 1998; Loh and Stacey, 2003). Among fungi, the OTUs assigned to the genus *Fusarium* (order Hypocreales) were more frequent in thola samples compared to non-thola samples, while the OTUs assigned to the genus *Didymella* (order Pleosporales) were less frequent in thola samples (Table S19). The sampling method allowed us to partition the effects of thola from the other effects of fallow. At a larger scale, the microbial communities of the Bolivian Altiplano would be influenced by the percentage cover of thola, as determined by plant reproduction, dispersal, and establishment as well as by patterns of thola harvest.

### *4.6. Improving fallow systems in the Bolivian Altiplano*

There are several possibilities for improving the Altiplano fallow system. One alternative farming method that may sustain soil productivity is to manage short-term fallow systems (5-years fallow period) with planted herbaceous or woody legumes ("improved fallows") to-refill soil nutrient stocks faster than plants in natural succession, decreasing the need to purchase fertilizer to maintain productivity (Kang et al., 1999; Sanchez, 1999; Phiri et al., 2001). In the Bolivian Altiplano, thola has been reported to be beneficial to the soil because of its rapid colonization of bare soils and its contributions to SOM (De Cary and Hervé, 1994; Stacishin de Queiroz et al., 2001). Our results were consistent in finding that the presence of thola in fallow fields was associated with modest increases in SOM and soil fertility,

indicating that maintaining thola in fields may improve short fallows in the Northern Altiplano of Bolivia. In fact thola has been used in the Southern Altiplano of Bolivia for controlling the desertification and degradation of natural resources caused by extensive cropping of quinoa (Jacobsen, 2011). This restoration program used thola as windbreaks between the fields at risk for desertification (Andressen et al., 2007) as a strategy to protect and restore degraded soils (Gonzales T., 2012). It may be possible to get the benefits of longer fallows when thola is planted (or at least maintained), perhaps along with other leguminous species for additional nitrogen, in shorter fallows. An economic analysis of the costs and benefits of maintaining thola in agricultural fields compared to its use as a fuel would be helpful for informed decision-making by farmers.

A number of aspects of soil microbial community interactions will need to be better understood in order to translate new information about microbial community structure into recommendations for management. While it is often assumed that higher levels of taxonomic diversity are beneficial for system productivity, this is not known. The ecological roles of any given taxon may vary widely, sometimes as a function of small changes in microbial genomes or the addition of plasmids. Until the genetic basis for these ecological functions is well understood it will be challenging to use metagenomic sequence data to characterize microbial communities in terms of function. The unexpected observation of lower microbial diversity with longer fallow in this study is another example of the current lack of understanding of microbial dynamics. In systems with more diverse unmanaged plant communities, longer fallow periods may result in a more heterogeneous environment for microbes. In contrast, the plant community in Altiplano fallows is not clearly more diverse than during the active cropping cycles. Lack of tillage during fallow may also change patterns of soil microbial diversity. It may be that there are more diverse microhabitats during Altiplano crop rotations than during fallows.

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## Appendix A

Supplemental materials are also archived at http://hdl.handle.net/2097/15198.

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# Supplemental Materials

### The *aynuqa and sayaña systems* in the Bolivian Altiplano

Two types of traditional land use predominate in the Bolivian Altiplano. The *aynuqa* system consists of extensive lands that are managed collectively; and the *sayaña* consists of field plots that are managed individually (Rivière, 1994; Hervé et al., 2002). The *aynuqa* system comprised the greatest area, and is usually divided in sectors for potato, quinoa, barley, sheep grazing, and collective fallow each year, with management decisions made by the community (Pacheco Fernández, 1994; Hervé et al., 2002). The *sayaña* are small field plots located in close proximity to the farm households (Rivière, 1994). These are private lands where the management decisions, including planting time and fallowing, are made by individual farmers (Hervé et al., 2002). The fallow periods are usually short, 1-4 years, because *sayañas* are usually cultivated permanently either for grazing or for agriculture (Pacheco Fernández, 1994; Hervé et al., 2002). Formerly, sectors within the *aynuqa* were separated by fields never cropped (*puruma* fields), but the population increase since the 1950s changed this traditional system. In a study in the Pumani area, all puruma fields were occupied, and each family began to open *sayaña* fields in *puruma* soils. This process pushed the expansion of the *sayaña* fields toward the *aynuqa* (Pacheco Fernández, 1994). Today, the *sayaña* fields are mixed with the fields in the *aynuqa*.

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# Tables

**Table S1**

Fields sampled at two locations in the Bolivian Altiplano representing a range of fallow periods. Some fields included both samples under thola (CT; 'thola samples') and samples away from thola (ST; 'non-thola samples). The total number of sequences obtained from 454-pyrosequencing, prior to removing singletons, is indicated. Puruma is a term for a field that has 'never' been planted to agricultural crops.

| Municipality | Field Name | Thola | Fallow period (years) | Total number of sequences for Fungi | Total number of sequences for Bacteria |
|---|---|---|---|---|---|
| Umala | Campo A | ST | 1 | 484 | 1005 |
| Umala | SJC 4C | ST | 2 | 472 | 1207 |
| Umala | SJC 4 | ST | 3 | 477 | 957 |
| Umala | Campo B | ST | 3 | 462 | 1001 |
| Umala | Campo C | ST | 4 | 513 | 1177 |
| Umala | Campo D | ST | 5 | 511 | 894 |
| Umala | Campo E | ST | 6 | 403 | 986 |
| Umala | Campo F | ST | 7 | 544 | 996 |
| Umala | Campo G | ST | 8 | 521 | 1041 |
| Umala | Campo H | ST | 9 | 576 | 322 |
| Umala | Campo I | ST | 10 | 586 | 1106 |
| Umala | Campo J | CT | 12 | 577 | 1042 |
| Umala | Campo J | ST | 12 | 589 | 1095 |
| Umala | Campo K | CT | 15 | 548 | 1034 |
| Umala | Campo K | ST | 15 | 506 | 1151 |
| Umala | SJC 4B | CT | 20 | 468 | 1021 |
| Umala | SJC 4B | ST | 20 | 528 | 1056 |
| Umala | Campo L | CT | 25 | 582 | 1090 |

| | | | | | |
|---|---|---|---|---|---|
| Umala | Campo L | ST | 25 | 104 | 1316 |
| Umala | SJC 13 | ST | 30 | 475 | 1008 |
| Umala | SJC 13 | CT | 30 | 492 | 1121 |
| Umala | Puruma | CT | 30 | 530 | 1109 |
| Umala | Puruma | ST | 30 | 433 | 1002 |
| Ancoraimes | Cohani 0 | ST | 0 | 464 | 1159 |
| Ancoraimes | Cohani 1 | ST | 1 | 463 | 1181 |
| Ancoraimes | Cohani 2B | ST | 2 | 439 | 1002 |
| Ancoraimes | Cohani 2C | ST | 2 | 355 | 1082 |
| Ancoraimes | Cohani 3 | ST | 3 | 495 | 959 |
| Ancoraimes | Cohani 3B | ST | 3 | 428 | 1187 |
| Ancoraimes | Cohani 4 | ST | 4 | 468 | 1059 |
| Ancoraimes | Cohani 6 | ST | 6 | 404 | 1102 |
| Ancoraimes | Cohani 7 | ST | 7 | 351 | 1049 |
| Ancoraimes | Cohani 10 | CT | 10 | 436 | 1145 |
| Ancoraimes | Cohani 10 | ST | 10 | 391 | 947 |
| Ancoraimes | Cohani 15 | CT | 15 | 496 | 1020 |
| Ancoraimes | Cohani 15 | ST | 15 | 391 | 1024 |
| Ancoraimes | Cohani 20 | ST | 20 | 473 | 1119 |

**Table S2**

The percentage of the overall most frequent fungal phyla recovered in pyrosequencing of soils ('non-thola samples') from two municipalities (Umala and Ancoraimes) in the Bolivian Altiplano.

*Note that the OTU frequencies reported in Gomez-Montano et al. 2013 were calculated as frequencies of the total high-quality sequences, while the frequencies reported here were calculated as frequencies of the total high quality sequences excluding singletons. This results in a slight difference in frequencies.*

| Phylum | Umala (%) | Ancoraimes (%) |
|---|---|---|
| Ascomycota | 84.52 | 65.21 |
| Basidiomycota | 3.81 | 10.85 |
| Chytridiomycota | 0.06 | 0.00 |
| Glomeromycota | 0.09 | 0.04 |

**Table S3**

Overall most frequent fungal orders recovered in pyrosequencing of soils (‘non-thola samples’) from two municipalities in the Bolivian Altiplano. The percentage of sequences grouped in each of the top 12 orders for Umala and Ancoraimes are given.

| Order | Umala (%) | Order | Ancoraimes (%) |
|---|---|---|---|
| Hypocreales | 44.57 | Hypocreales | 37.56 |
| Pleosporales | 30.39 | Pleosporales | 19.18 |
| Mortierellales | 3.33 | Mortierellales | 12.73 |
| Sordariales | 2.30 | mitosporic_Filobasidiales | 3.98 |
| Capnodiales | 1.68 | Tremellales | 3.53 |
| Filobasidiales | 1.56 | Sordariales | 3.23 |
| mitosporic_Filobasidiales | 1.06 | Filobasidiales | 2.25 |
| Helotiales | 0.86 | Microascales | 0.68 |
| Eurotiales | 0.82 | Capnodiales | 0.53 |
| Thelebolales | 0.80 | Chaetothyriales | 0.53 |
| Tremellales | 0.74 | Helotiales | 0.38 |
| Xylariales | 0.73 | Eurotiales | 0.23 |

**Table S4**

Overall most frequent OTUs assigned to fungal genera recovered in pyrosequencing of soils ('non-thola samples') from two municipalities in the Bolivian Altiplano. Percentage of sequences grouped in each of the top 12 OTUs assigned to fungal genera for Umala and Ancoraimes.

| Genus | Umala (%) | Genus | Ancoraimes (%) |
|---|---|---|---|
| *Fusarium* | 40.67 | *Fusarium* | 18.82 |
| *Didymella* | 27.74 | *Didymella* | 16.34 |
| *Mortierella* | 3.33 | *Mortierella* | 12.73 |
| *Cryptococcus* | 2.63 | *Verticillium* | 7.62 |
| *Cladosporium* | 1.68 | *Cryptococcus* | 6.41 |
| *Chaetomium* | 1.55 | *Chaetomium* | 2.97 |
| *Paecilomyces* | 1.28 | *Neonectria* | 2.33 |
| *Alternaria* | 1.16 | *Bionectria* | 1.75 |
| *Verticillium* | 0.99 | *Clonostachys* | 1.14 |
| *Penicillium* | 0.82 | *Paraphoma* | 0.93 |
| *Thelebolus* | 0.80 | *Acremonium* | 0.69 |
| *Microdochium* | 0.73 | *Pyrenochaeta* | 0.42 |

**Table S5**

Overall most frequent bacterial phyla recovered in pyrosequencing of soils ('non-thola samples') from two municipalities in the Bolivian Altiplano. The percentage sequences grouped in each of the top 12 bacteria phyla for Umala and Ancoraimes.

| Phylum | Umala (%) | Phylum | Ancoraimes (%) |
|---|---|---|---|
| Proteobacteria | 22.84 | Proteobacteria | 26.30 |
| Actinobacteria | 10.67 | Firmicutes | 15.79 |
| Firmicutes | 8.38 | Verrucomicrobia | 6.34 |
| Acidobacteria | 6.40 | Actinobacteria | 5.87 |
| Verrucomicrobia | 4.66 | Acidobacteria | 5.17 |
| Bacteroidetes | 2.87 | Bacteroidetes | 3.28 |
| Planctomycetes | 2.06 | Planctomycetes | 2.76 |
| Chloroflexi | 1.80 | Chloroflexi | 0.80 |
| Cyanobacteria | 0.43 | Cyanobacteria | 0.34 |
| Crenarchaeota | 0.40 | TM7 | 0.18 |
| Nitrospira | 0.22 | Nitrospira | 0.09 |
| OP10 | 0.11 | OP10 | 0.07 |

**Table S6**

Overall most frequent OTUs assigned to bacterial genera (and strains such as Gp3) recovered in pyrosequencing of soils ('non-thola samples') from two municipalities in the Bolivian Altiplano. Percentage sequences grouped in each of the top 12 OTUs assigned to bacteria genera for Umala and Ancoraimes.

| Genus | Umala (%) | Genus | Ancoraimes (%) |
|---|---|---|---|
| *Paenibacillus* | 2.76 | *Pseudomonas* | 2.93 |
| *Gp4* | 2.57 | *Bradyrhizobium* | 1.94 |
| *Streptomyces* | 1.39 | *Gp3* | 1.50 |
| *Bradyrhizobium* | 1.24 | *Singulisphaera* | 0.56 |
| *Methylobacterium* | 1.23 | *Streptomyces* | 0.43 |
| *Gp3* | 1.04 | *Segetibacter* | 0.34 |
| *Segetibacter* | 1.03 | *Modestobacter* | 0.31 |
| *Modestobacter* | 0.80 | *Dokdonella* | 0.30 |
| *Singulisphaera* | 0.36 | *Gp5* | 0.25 |
| *Cystobacter* | 0.33 | *Paenibacillus* | 0.22 |
| *Pseudomonas* | 0.30 | *Methylobacterium* | 0.22 |
| *Actinoplanes* | 0.26 | *Gemmata* | 0.20 |

**Table S7**

No fungal phyla significantly increased in frequency with increasing fallow period ('non-thola samples') in either municipality of the Bolivian Altiplano (results of a GLM).

**Table S8**

Fungal orders significantly **increasing** in frequency with increasing fallow period ('non-thola samples') in two municipalities of the Bolivian Altiplano. Results from a GLM are shown for those taxa for which $p < 0.05$ and $q < 0.05$.

| | Umala | | | | Ancoraimes | | | |
|---|---|---|---|---|---|---|---|---|
| Order | Slope p-value | Slope q-value | Slope estimate | Intercept estimate | Slope p-value | Slope q-value | Slope estimate | Intercept estimate |
| Agaricales | 0.015 | 0.009 | 0.103 | -7.088 | | | | |
| Boletales | 0.004 | 0.004 | 0.088 | -6.293 | | | | |
| Capnodiales | 7E-05 | 1E-04 | 0.101 | -6.036 | | | | |
| Eurotiales | 0.012 | 0.008 | 0.097 | -6.844 | 0.070 | 0.043 | 0.022 | -4.962 |
| Hypocreales | 4E-07 | 1E-06 | 0.024 | -0.658 | | | | |
| Mortierellales | 3E-06 | 7E-06 | 0.030 | -2.136 | 5E-39 | 3E-38 | 0.078 | -4.486 |
| Onygenales | 0.004 | 0.004 | 0.090 | -6.369 | | | | |
| Saccharomycetales | 0.011 | | 0.062 | -5.489 | | | | |
| Pleosporales | | | | | 0.051 | 0.036 | 0.005 | -0.916 |

**Table S9**

OTUs assigned to fungal genera significantly **increasing** in frequency with increasing fallow period ('non-thola samples') in two municipalities of the Bolivian Altiplano. Results from a GLM are shown for those taxa for which $p < 0.05$ and $q < 0.05$.

| | Umala | | | | Ancoraimes | | | |
|---|---|---|---|---|---|---|---|---|
| Genus | Slope p-value | Slope q-value | Slope estimate | Intercept estimate | Slope p-value | Slope q-value | Slope estimate | Intercept estimate |
| *Beauveria* | 0.039 | 0.074 | 0.050 | -5.330 | | | | |
| *Boletus* | 0.049 | 0.085 | 0.066 | -6.196 | | | | |
| *Cladosporium* | 2E-05 | 1E-04 | 0.125 | -6.551 | | | | |
| *Fusarium* | 4E-04 | 0.002 | 0.020 | -1.578 | | | | |
| *Microsporum* | 0.004 | 0.019 | 0.090 | -6.369 | | | | |
| *Mortierella* | 3E-06 | 3E-05 | 0.030 | -2.136 | 5E-39 | 4E-38 | 0.078 | -4.486 |
| *Penicillium* | 0.012 | 0.041 | 0.097 | -6.844 | 0.070 | 0.047 | 0.022 | -4.962 |
| *Preussia* | 0.019 | 0.053 | 0.070 | -6.019 | | | | |
| *Schwanniomyces* | 0.022 | 0.055 | 0.097 | -7.029 | | | | |
| *Volutella* | 1E-04 | 6E-04 | 0.052 | -4.132 | | | | |
| *Didymella* | | | | | 7E-04 | 0.001 | 0.009 | -1.096 |

**Table S10**

Fungal phyla significantly **decreasing** in frequency with increasing fallow period ('non-thola samples') in two municipalities of the Bolivian Altiplano. Results from a GLM are shown for those taxa for which $p < 0.05$.

| | Umala | | | Ancoraimes | | |
|---|---|---|---|---|---|---|
| Phylum | Slope p-value | Slope estimate | Intercept estimate | Slope p-value | Slope estimate | Intercept estimate |
| Ascomycota | | | | 0.004 | -0.009 | 1.809 |
| Basidiomycota | 4E-06 | -0.039 | -1.876 | 1E-05 | -0.034 | -2.881 |

**Table S11**

Fungal orders significantly **decreasing** in frequency with increasing fallow period ('non-thola samples') in two municipalities of the Bolivian Altiplano. Results from a GLM are shown for those taxa for which $p < 0.05$ and $q < 0.05$.

| | Umala | | | | Ancoraimes | | | |
|---|---|---|---|---|---|---|---|---|
| Order | Slope p-value | Slope q-value | Slope estimate | Intercept estimate | Slope p-value | Slope q-value | Slope estimate | Intercept estimate |
| Microascales | 0.005 | 0.005 | -0.146 | -4.359 | | | | |
| Phyllachorales | 0.007 | 0.006 | -0.635 | -4.713 | 0.008 | 0.011 | -0.053 | -4.419 |
| Pleosporales | 3E-19 | 2E-18 | -0.064 | -1.075 | | | | |
| Capnodiales | | | | | 0.015 | 0.013 | -0.027 | -3.753 |
| Filobasidiales | | | | | 0.011 | 0.012 | -0.030 | -3.817 |
| Thelebolales | | | | | 0.045 | 0.035 | -0.034 | -4.487 |

**Table S12**

OTUs assigned to fungal genera significantly **decreasing** in frequency with increasing fallow period ('non-thola samples') in two municipalities of the Bolivian Altiplano. Results from a GLM are shown for those taxa for which $p < 0.05$ and $q < 0.05$.

| | Umala | | | | Ancoraimes | | | |
|---|---|---|---|---|---|---|---|---|
| Genus | Slope p-value | Slope q-value | Slope intercept | Intercept estimate | Slope p-value | Slope q-value | Slope intercept | Intercept estimate |
| *Acremonium* | 0.037 | 0.074 | -0.082 | -4.549 | | | | |
| *Bionectria* | 0.033 | 0.074 | -0.047 | -3.832 | 0.005 | 0.006 | -0.064 | -4.502 |
| *Clonostachys* | 0.018 | 0.053 | -0.071 | -4.150 | | | | |
| *Didymella* | 1E-20 | 5E-19 | -0.074 | -1.227 | | | | |
| *Plectosphaerella* | 0.007 | 0.029 | -0.635 | -4.713 | 0.004 | 0.005 | -0.061 | -4.412 |
| *Alternaria* | | | | | 6E-06 | 1E-05 | -0.089 | -3.715 |
| *Articulospora* | | | | | 3E-08 | 1E-07 | -0.343 | -3.238 |
| *Chaetomium* | | | | | 1E-06 | 4E-06 | -0.072 | -3.477 |
| *Cladosporium* | | | | | 0.015 | 0.014 | -0.027 | -3.753 |

**Table S13**

Bacterial phyla significantly **increasing** in frequency with increasing fallow period ('non-thola samples') in two municipalities of the Bolivian Altiplano. Results from a GLM are shown for those taxa for which $p < 0.05$.

| | Umala | | | Ancoraimes | | |
|---|---|---|---|---|---|---|
| Phylum | Slope p-value | Slope estimate | Intercept estimate | Slope p-value | Slope estimate | Intercept estimate |
| Proteobacteria | 6E-10 | 0.034 | -2.945 | | | |
| Acidobacteria | | | | 0.009 | 0.010 | -3.271 |

**Table S14**

OTUs assigned to bacterial genera significantly **increasing** in frequency with increasing fallow period ('non-thola samples') in two municipalities of the Bolivian Altiplano. Results from a GLM are shown for those taxa for which $p < 0.05$, with corresponding q-value also indicated.

| | Umala | | | | Ancoraimes | | | |
|---|---|---|---|---|---|---|---|---|
| Genus | Slope p-value | Slope q-value | Slope estimate | Intercept estimate | Slope p-value | Slope q-value | Slope estimate | Intercept estimate |
| *Pseudomonas* | 2E-27 | 3E-26 | 0.078 | -4.036 | | | | |
| *Sporosarcina* | 0.007 | 0.019 | 0.139 | -8.789 | | | | |
| *Gp4* | 8E-05 | 4E-04 | 0.065 | -5.660 | 0.053 | 0.323 | 0.009 | -3.717 |

**Table S15**

Bacterial phyla significantly **decreasing** in frequency with increasing fallow period ('non-thola samples') in two municipalities of the Bolivian Altiplano. Results from a GLM are shown for those taxa for which $p < 0.05$.

| | Umala | | | Ancoraimes | | |
|---|---|---|---|---|---|---|
| Phylum | Slope p-value | Slope estimate | Intercept estimate | Slope p-value | Slope estimate | Intercept estimate |
| Acidobacteria | 0.021 | -0.020 | -3.199 | | | |
| Actinobacteria | 0.042 | -0.033 | -4.321 | 0.067 | -0.008 | -3.366 |
| Planctomycetes | 0.042 | -0.040 | -4.622 | | | |
| Verrucomicrobia | 0.002 | -0.035 | -3.608 | | | |
| Bacteroidetes | | | | 0.005 | -0.022 | -4.126 |
| Firmicutes | | | | 2E-12 | -0.042 | -3.147 |

**Table S16**

OTUs assigned to bacterial genera significantly **decreasing** in frequency with increasing fallow period ('non-thola samples') in two municipalities of the Bolivian Altiplano. Results from a GLM are shown for those taxa for which $p < 0.05$, with q-values also indicated.

| | Umala | | | | Ancoraimes | | | |
|---|---|---|---|---|---|---|---|---|
| Genus | Slope p-value | Slope q-value | Slope estimate | Intercept estimate | Slope p-value | Slope q-value | Slope estimate | Intercept estimate |
| *Bradyrhizobium* | 0.019 | 0.036 | -0.028 | -3.760 | 0.045 | 0.323 | -0.015 | -4.195 |
| *Dokdonella* | 0.010 | 0.021 | -0.111 | -5.289 | | | | |
| *Gp2* | 0.004 | 0.019 | -0.048 | -4.220 | | | | |
| *Gp3* | 0.007 | 0.019 | -0.037 | -3.984 | | | | |
| *Gp5* | 0.047 | 0.073 | -0.082 | -5.564 | | | | |
| *Modestobacter* | 0.007 | 0.019 | -0.115 | -5.227 | | | | |
| *Singulisphaera* | 0.031 | 0.054 | -0.052 | -4.879 | | | | |
| *Paenibacillus* | | | | | 2E-12 | 8E-11 | -0.042 | -3.156 |
| *Pseudomonas* | | | | | 7E-06 | 1E-04 | -0.179 | -4.619 |
| *Segetibacter* | | | | | 0.002 | 0.036 | -0.026 | -4.266 |

**Table S17**

Comparison of fungal phyla in paired samples collected under the common fallow-period shrub species (thola; Asteraceae), 'thola samples', and at least one m away from thola, 'non-thola samples'. The p-value from a generalized linear model comparing the means is given (where NA indicates that phylum was present in fewer than three samples). Bold font indicates which had higher frequency, non-thola or thola samples.

| | | Mean frequency (%) | |
|---|---|---|---|
| Phylum | p-value | Non-thola samples | Thola samples |
| Basidiomycota | **9E-06** | 1.83 | **4.06** |
| Chytridiomycota | NA | 0.03 | **0.11** |
| Glomeromycota | NA | **0.17** | 0.03 |

**Table S18**

The most frequent fungal orders in paired samples collected under the common fallow-period shrub species (thola; Asteraceae), 'thola samples', and at least one m away from thola, 'non-thola samples'. The p-value from a generalized linear model comparing the means is given (where NA indicates that an order was present in fewer than three samples), along with associated q-value. Bold font indicates which had the higher frequency, non-thola or thola samples.

| | | | Mean frequency (%) | |
|---|---|---|---|---|
| Order | p-value | q-value | Non-thola samples | Thola samples |
| Agaricales | 0.985 | 0.999 | 0.20 | **0.35** |
| Botryosphaeriales | 1.000 | 0.999 | 0.00 | **0.47** |
| Capnodiales | 1E-06 | **3E-05** | 0.49 | **2.15** |
| Chaetothyriales | 0.361 | 0.575 | **0.35** | 0.24 |

| | | | | |
|---|---|---|---|---|
| Coniochaetales | 0.670 | 0.909 | **0.10** | 0.06 |
| Cystofilobasidiales | NA | NA | 0.00 | **0.03** |
| Dothideales | 0.994 | 0.999 | **0.28** | 0.23 |
| Entylomatales | NA | NA | 0.07 | **0.14** |
| Erythrobasidiales | NA | NA | 0.00 | **0.12** |
| Eurotiales | 0.133 | 0.281 | **0.86** | 0.56 |
| Filobasidiales | 3E-06 | **3E-05** | 0.53 | **2.20** |
| Glomerales | NA | NA | **0.08** | 0.03 |
| Helotiales | 0.031 | **0.098** | 0.23 | **0.62** |
| Microascales | 0.077 | 0.210 | **0.43** | 0.17 |
| Mortierellales | 2E-05 | **1E-04** | **5.71** | 3.76 |
| Ophiostomatales | NA | NA | **0.04** | 0.03 |
| Phyllachorales | 0.387 | 0.575 | **0.54** | 0.42 |
| Sordariales | 0.003 | **0.016** | **1.87** | 0.70 |
| Spizellomycetales | NA | NA | 0.03 | **0.11** |
| Thelebolales | 0.394 | 0.575 | **0.37** | 0.24 |
| Tilletiales | NA | NA | 0.00 | **0.06** |
| Tremellales | 1.000 | 0.999 | 0.00 | **0.41** |
| Ustilaginales | NA | NA | **0.04** | 0.03 |
| Xylariales | 0.196 | 0.372 | **0.46** | 0.26 |

**Table S19**

The most frequent OTUs assigned to fungal genera in paired samples collected under the common fallow-period shrub species (thola; Asteraceae), 'thola samples', and at least one m away from thola, 'non-thola samples'. The p-value from a generalized linear model comparing the means is given, along with associated q-value. Bold font indicates which had the higher frequency, non-thola or thola samples.

| | | | Mean frequency (%) | |
|---|---|---|---|---|
| Genus | p-value | q-value | Non-thola samples | Thola samples |
| *Alternaria* | 0.12 | 0.40 | 0.20 | **0.45** |
| *Bionectria* | 0.18 | 0.40 | 0.24 | **0.44** |
| *Cercophora* | 0.03 | **0.10** | **0.58** | 0.12 |
| *Chaetomium* | 0.03 | 0.11 | **0.78** | 0.35 |
| *Cladosporium* | 4E-06 | **1E-04** | 0.46 | **1.41** |
| *Cryptococcus* | 9E-04 | **6E-03** | 1.47 | **2.77** |
| *Didymella* | 1E-03 | **6E-03** | **31.80** | 26.28 |
| *Fusarium* | 0.45 | 0.62 | 42.05 | **43.92** |
| *Microdochium* | 0.20 | 0.40 | **0.46** | 0.26 |
| *Mortierella* | 2E-05 | **3E-04** | **5.28** | 3.65 |
| *Paecilomyces* | 1E-04 | **1E-03** | **1.12** | 0.28 |
| *Paraphoma* | 0.02 | 0.10 | 0.35 | **0.89** |
| *Penicillium* | 0.13 | 0.35 | **0.48** | 0.20 |
| *Plectosphaerella* | 0.48 | 0.63 | **0.47** | 0.39 |
| *Preussia* | 0.70 | 0.84 | 0.09 | **0.10** |
| *Stagonospora* | 0.09 | 0.31 | 0.14 | **0.34** |
| *Verticillium* | 0.01 | **0.05** | **1.07** | 0.50 |

**Table S20**

The most frequent bacterial phyla in paired samples collected under the common fallow-period shrub species (thola; Asteraceae), 'thola samples', and at least one m away from thola, 'non-thola samples'. The p-value from a generalized linear model comparing the means is given (where NA indicates that a phylum was present in fewer than three samples). Bold font indicates which had the higher frequency, non-thola or thola samples.

| | | Mean frequency (%) | |
|---|---|---|---|
| Phylum | p-value | Non-thola samples | Thola samples |
| Bacteroidetes | **1E-07** | 2.10 | **3.65** |
| Chlamydiae | 0.101 | 0.05 | **0.14** |
| Chloroflexi | **0.084** | **1.90** | 1.50 |
| Crenarchaeota | 0.430 | **0.43** | 0.34 |
| Cyanobacteria | **3E-05** | **0.58** | 0.09 |
| Deinococcus-Thermus | NA | **0.03** | 0.02 |
| Euryarchaeota | NA | **0.05** | 0.02 |
| Gemmatimonadetes | NA | 0.01 | **0.03** |
| Nitrospira | 0.178 | **0.21** | 0.11 |
| OP10 | 0.471 | **0.13** | 0.09 |
| Planctomycetes | 0.203 | **2.05** | 1.77 |
| TM7 | 0.331 | 0.04 | **0.09** |
| Verrucomicrobia | **1E-05** | **4.35** | 2.90 |

**Table S21**

The most frequent OTUs assigned to bacterial genera in paired samples collected under the common fallow-period shrub species (thola; Asteraceae), 'thola samples', and at least one m away from thola, 'non-thola samples'. The p-value from a generalized linear model comparing the means is given, along with associated q-value (where NA indicates that a genus was present in fewer than three samples). Bold font indicates which had the higher frequency, non-thola or thola samples.

| | | | Mean frequency (%) | |
|---|---|---|---|---|
| Genus | p-value | q-value | Non-thola samples | Thola samples |
| *Actinoplanes* | 0.449 | 0.200 | 0.18 | **0.24** |
| *Amycolatopsis* | 0.756 | 0.247 | **0.09** | 0.08 |
| *Anaeromyxobacter* | 0.420 | 0.200 | **0.06** | 0.03 |
| *Aquicella* | NA | NA | 0.00 | **0.03** |
| *Bacillariophyta* | NA | NA | **0.06** | 0.01 |
| *Belnapia* | 0.357 | 0.195 | **0.19** | 0.12 |
| *Bradyrhizobium* | 0.078 | **0.091** | 1.08 | **1.42** |
| *Burkholderia* | NA | NA | 0.02 | 0.02 |
| *Chitinophaga* | NA | NA | 0.02 | 0.02 |
| *Chryseobacterium* | NA | NA | 0.02 | 0.02 |
| *Cystobacter* | 0.185 | 0.139 | 0.27 | **0.40** |
| *Deinococcus* | NA | NA | **0.03** | 0.02 |
| *Devosia* | NA | NA | 0.00 | **0.03** |
| *Dokdonella* | 0.666 | 0.236 | **0.05** | 0.03 |
| *Dyadobacter* | NA | NA | 0.00 | **0.03** |
| *Ferruginibacter* | NA | NA | 0.00 | **0.03** |
| *Gemmata* | 0.414 | 0.200 | 0.08 | **0.13** |
| *Gemmatimonas* | NA | NA | 0.01 | **0.03** |
| *Gp2* | NA | NA | **0.02** | 0.01 |

| | | | | |
|---|---|---|---|---|
| *Gp3* | 0.002 | **0.004** | **1.07** | 0.56 |
| *Gp4* | 0.060 | **0.084** | **3.01** | 2.46 |
| *Gp5* | 0.649 | 0.236 | **0.15** | 0.13 |
| *Gp7* | 0.992 | 0.309 | 0.38 | **0.39** |
| *Haloferula* | NA | NA | 0.01 | **0.02** |
| *Herpetosiphon* | 0.519 | 0.201 | **0.06** | 0.03 |
| *Hymenobacter* | 0.491 | 0.200 | **0.14** | 0.09 |
| *Iamia* | 0.240 | 0.161 | **0.08** | 0.03 |
| *Kineosporia* | NA | NA | 0.01 | **0.06** |
| *Lentzea* | 0.263 | 0.168 | **0.10** | 0.05 |
| *Methylobacterium* | 0.002 | **0.004** | 1.13 | **1.81** |
| *Modestobacter* | 0.095 | **0.098** | 0.80 | **1.08** |
| *Mucilaginibacter* | 0.146 | 0.117 | 0.02 | **0.08** |
| *Nitrospira* | 0.108 | **0.098** | **0.16** | 0.06 |
| *Opitutus* | 0.559 | 0.210 | **0.07** | 0.05 |
| *Paenibacillus* | 1E-12 | **1E-11** | **2.12** | 0.58 |
| *Parachlamydia* | NA | NA | **0.03** | 0.02 |
| *Phenylobacterium* | 0.716 | 0.240 | **0.21** | 0.19 |
| *Pseudomonas* | 5E-05 | **3E-04** | 0.05 | **0.54** |
| *Pseudonocardia* | 0.494 | 0.200 | **0.14** | 0.09 |
| *Ralstonia* | 0.059 | **0.084** | **0.13** | 0.03 |
| *Rhodopila* | 0.147 | 0.117 | **0.08** | 0.02 |
| *Rickettsia* | NA | NA | 0.00 | **0.02** |
| *Roseomonas* | NA | NA | **0.03** | 0.02 |
| *Rubellimicrobium* | 0.502 | 0.200 | **0.13** | 0.09 |
| *Rubrobacter* | NA | NA | 0.00 | **0.03** |
| *Sandaracinobacter* | NA | NA | 0.00 | **0.02** |
| *Segetibacter* | 0.057 | **0.084** | 0.78 | **1.09** |
| *Singulisphaera* | 0.066 | **0.084** | **0.29** | 0.14 |
| *Solirubrobacter* | 0.367 | 0.195 | **0.10** | 0.06 |

| | | | | |
|---|---|---|---|---|
| *Sorangium* | 0.714 | 0.240 | 0.22 | **0.25** |
| *Sphingobium* | 0.016 | **0.033** | 0.03 | **0.19** |
| *Sporosarcina* | 0.955 | 0.305 | 0.03 | 0.03 |
| *Stigmatella* | 0.499 | 0.200 | **0.08** | 0.05 |
| *Streptomyces* | 0.007 | **0.018** | **1.24** | 0.78 |
| *Xanthomonas* | 0.196 | 0.139 | 0.01 | **0.06** |

## Supplemental Figures

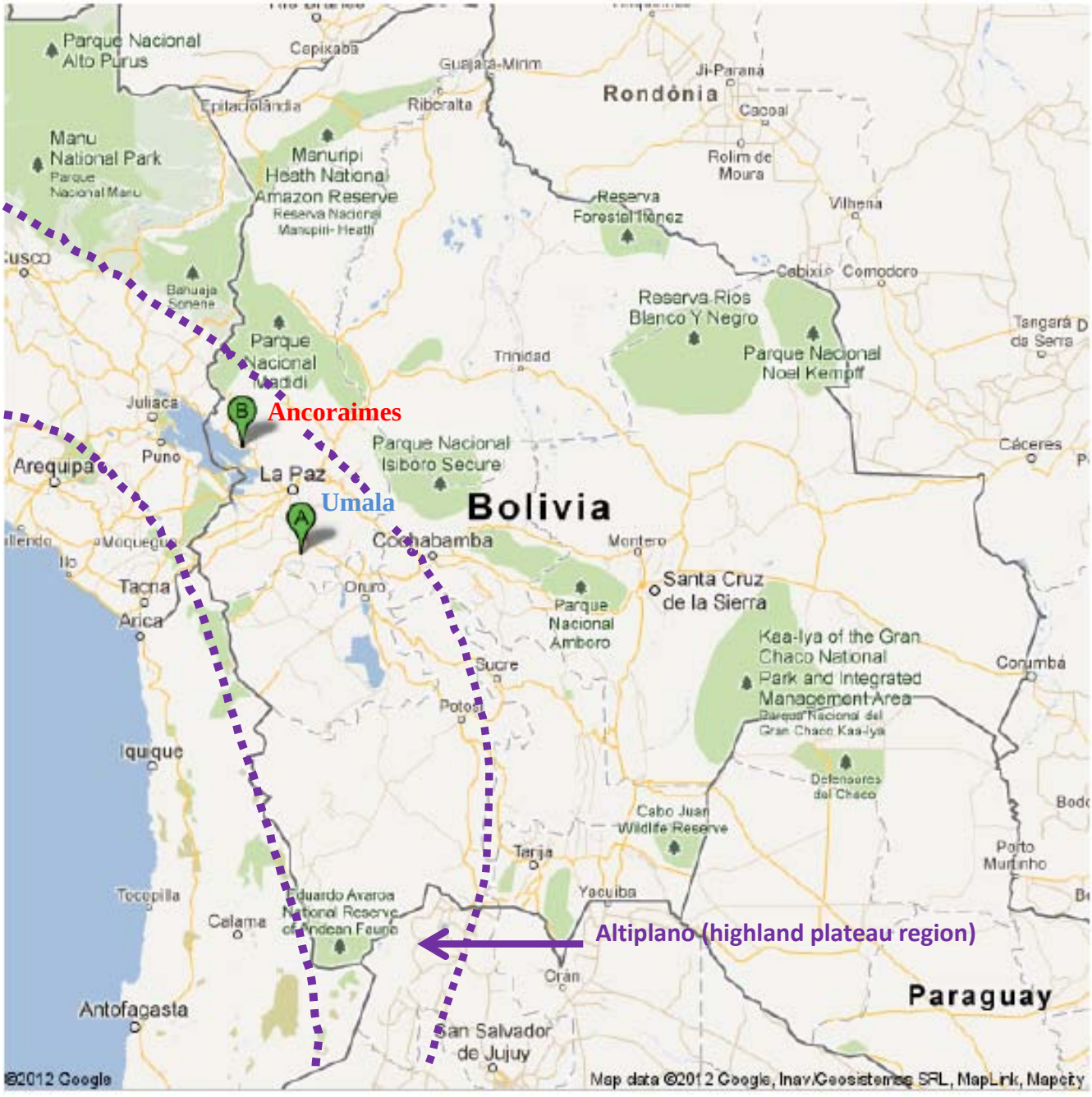



**Fig. S1.** The Bolivian Altiplano (highland-plateau) region and the location of the two municipalities Umala and Ancoraimes. Google map (Map data©2012 Google, Inav/Geosistemas SRL, MapLink, Mapcity).

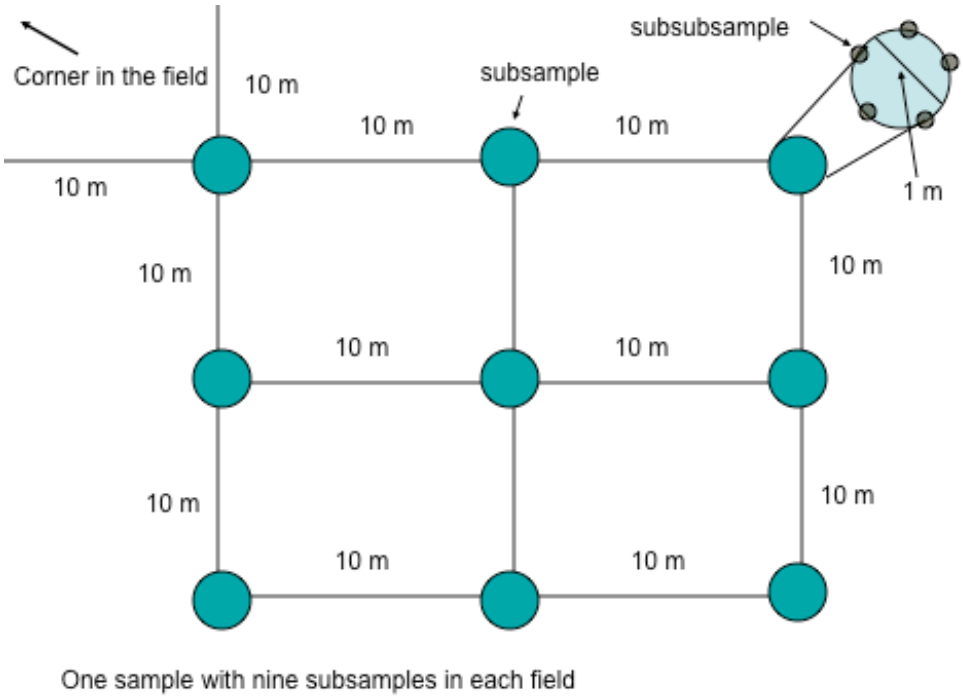



**Fig. S2**. Sampling scheme used within each field sampled at two municipalities of the Bolivian Altiplano (Umala and Ancoraimes). In each field, nine subsamples were collected where each subsample was composed of five sub-subsamples.

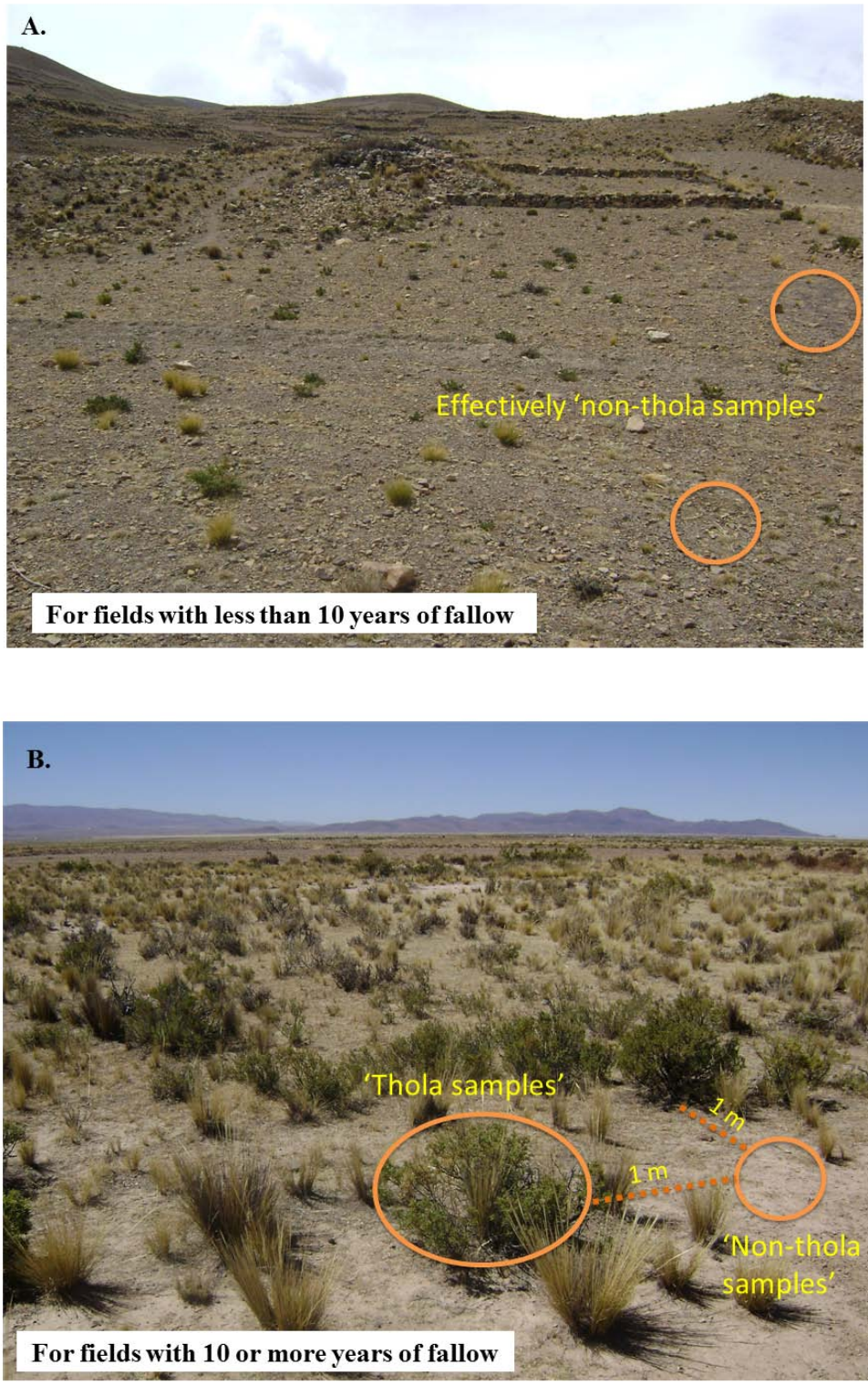
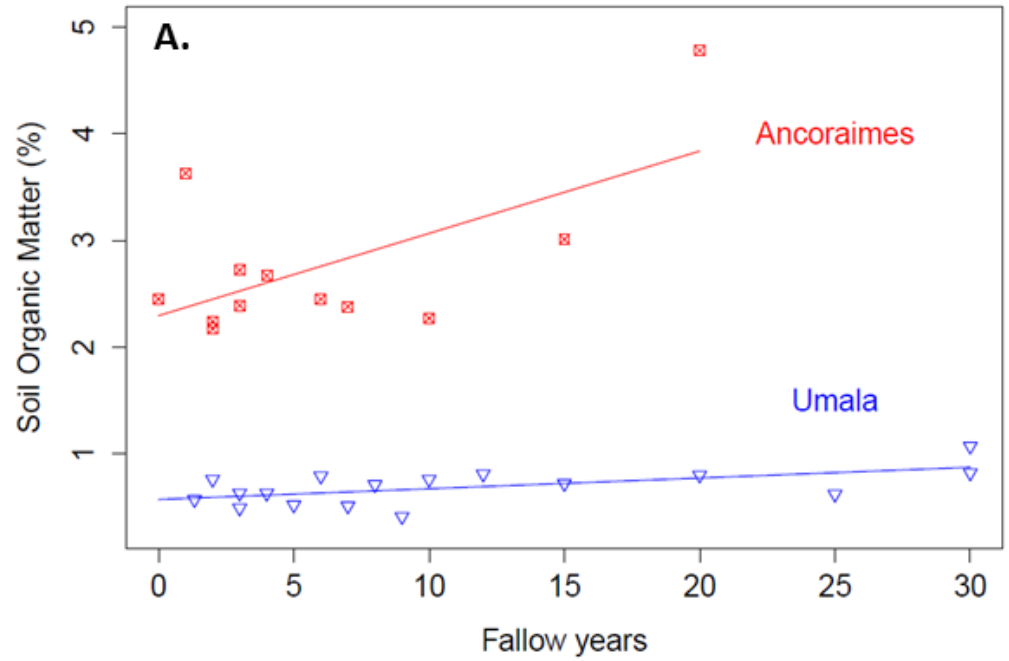
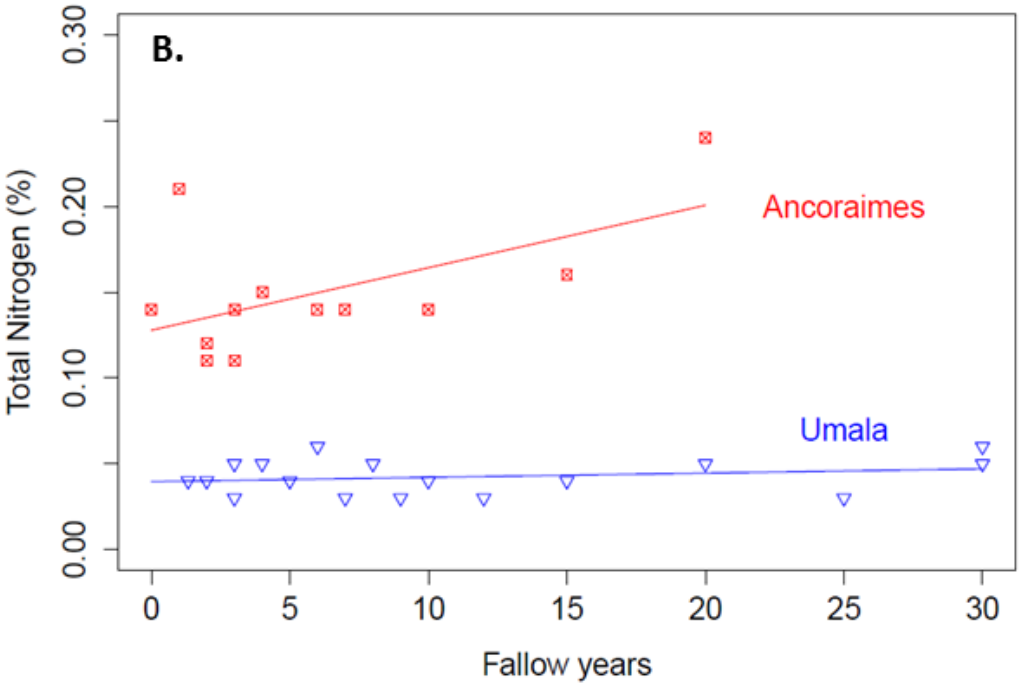
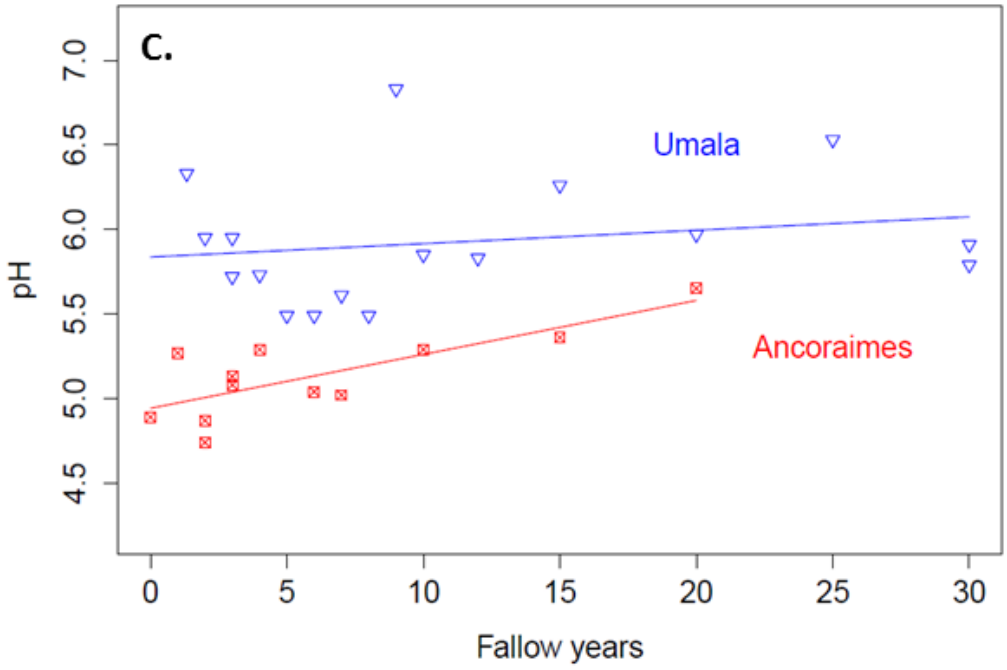


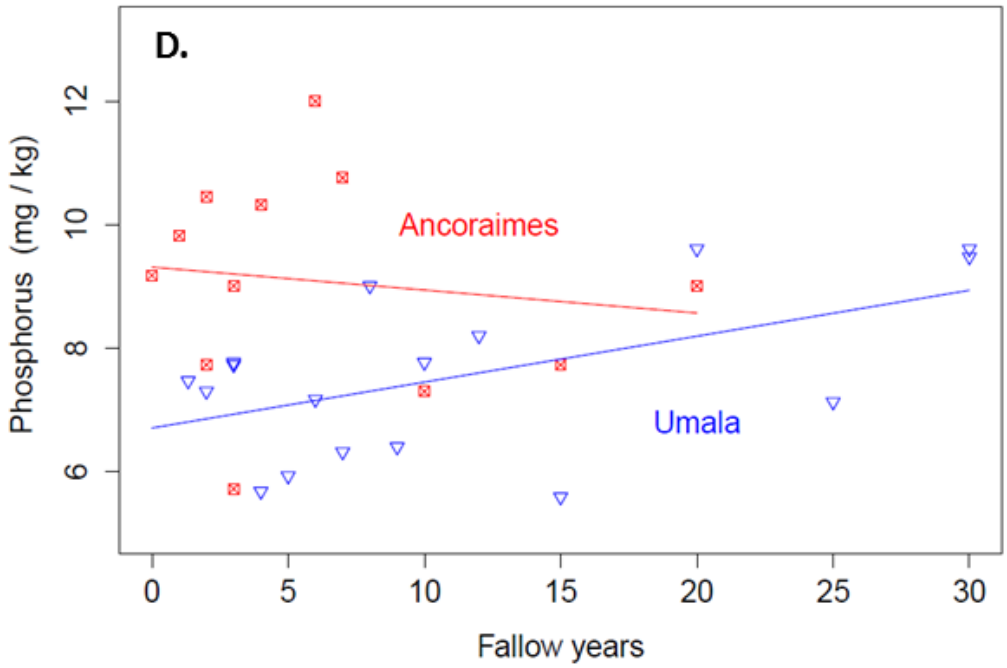

**Fig. S3**. Examples of fields in the two municipalities of the Bolivian Altiplano (Umala and Ancoraimes) illustrating the presence of the fallow period shrub species (thola: Asteraceae).

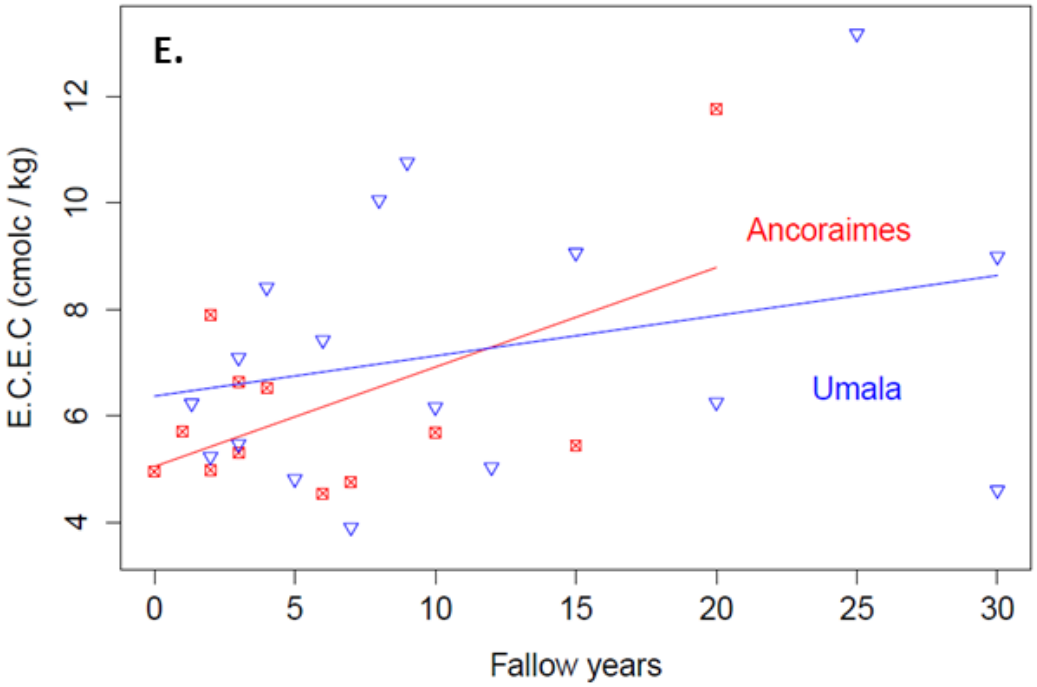
A. For fields with less than 10 years of fallow and scarce presence of thola, 9 soil subsamples were collected away from thola (non-thola samples).
B. For fields with 10 or more years of fallow and sufficient number of thola, 18 soil subsamples were collected as follows. First, at each of the nine subsample points, a subsample was collected under the canopy of the thola individual closest to the subsample point (the ‘thola subsample’). Second, the other subsample was sampled near the subsample point but at least 1 m away from the nearest thola (the ‘non-thola subsample’).


A.
Ancoraimes
Umala
Soil Organic Matter (%)
1
2
3
4
5
0
5
10
15
20
25
30
Fallow years


B.
Ancoraimes
Umala
Total Nitrogen (%)
0.00
0.10
0.20
0.30
0
5
10
15
20
25
30
Fallow years


C.
pH
7.0
6.5
6.0
5.5
5.0
4.5
Umala
Ancoraimes
0
5
10
15
20
25
30
Fallow years


D.
Phosphorus (mg / kg)
12
10
8
6
Ancoraimes
Umala
0
5
10
15
20
25
30
Fallow years


E.
E.C.E.C (cmolc / kg)
4
6
8
10
12
Ancoraimes
Umala
0
5
10
15
20
25
30
Fallow years

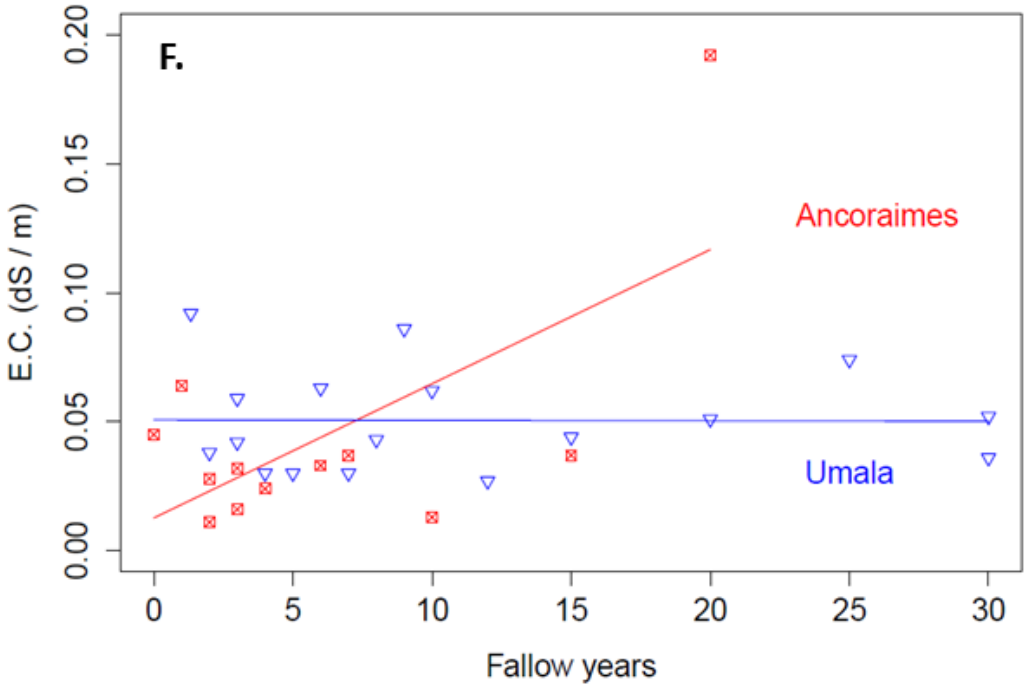

F.
E.C. (dS / m)
0.00
0.05
0.10
0.15
0.20
Ancoraimes
Umala
0
5
10
15
20
25
30
Fallow years

**Fig. S4**. Fallow period effect on edaphic properties in two municipalities of the Bolivian Altiplano (Umala and Ancoraimes) evaluated in linear regression analysis. Note that one field in Ancoraimes had particularly high leverage, in the sense of importance in determining the model fit, where animals had been grazed extensively and there was abundant manure.

(A) SOM: Ancoraimes ($p=0.03$, $R^2=0.33$), Umala ($p=0.01$, $R^2=0.31$), t-test comparing Umala and Ancoraimes ($p < 0.001$).

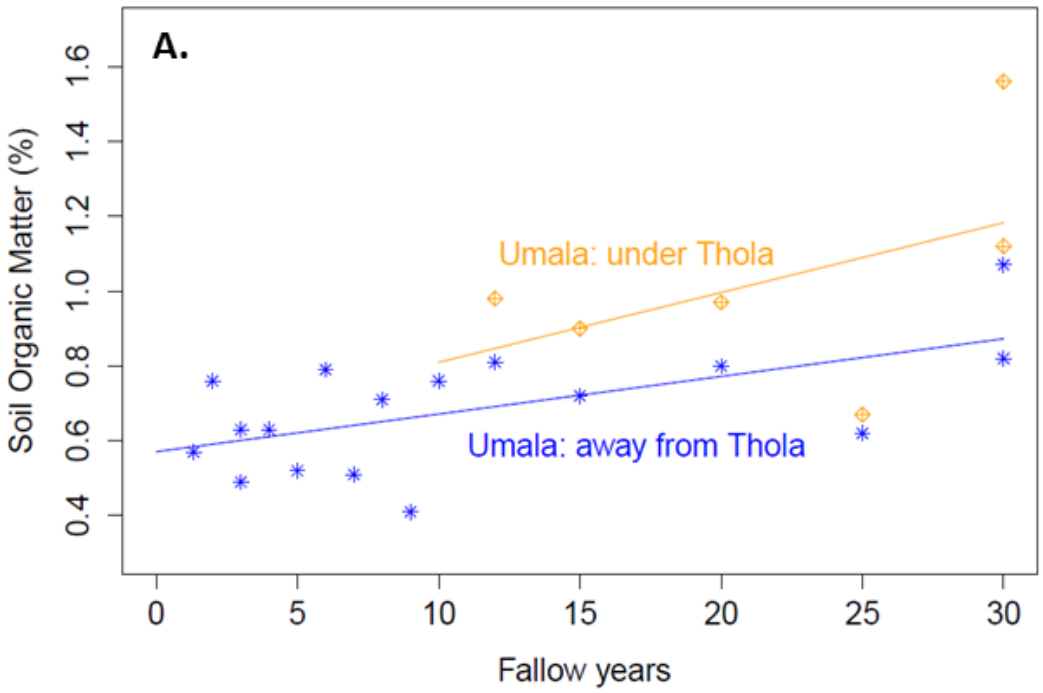
(B) Total N: Ancoraimes ($p=0.05$, $R^2=0.26$, Umala ($p=0.37$, $R^2=0$), t-test comparing Umala and Ancoraimes ($p < 0.001$).

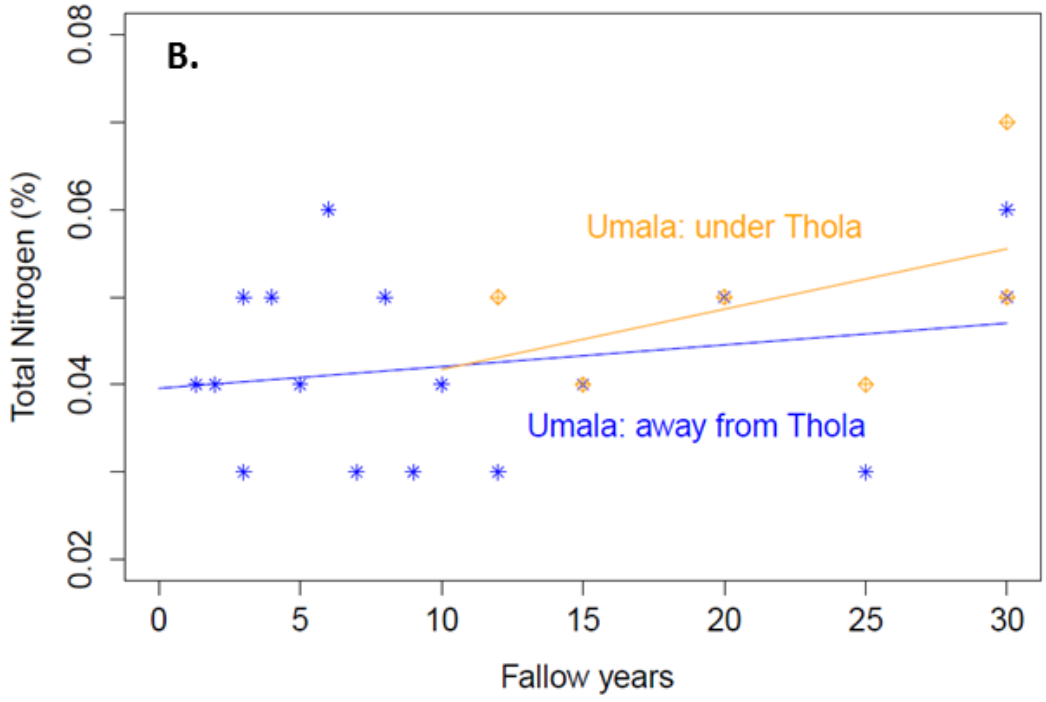
(C) pH: Ancoraimes ($p=0.003$, $R^2=0.55$), Umala ($p=0.4$, $R^2=0$), t-test comparing Umala and Ancoraimes ($p < 0.001$).

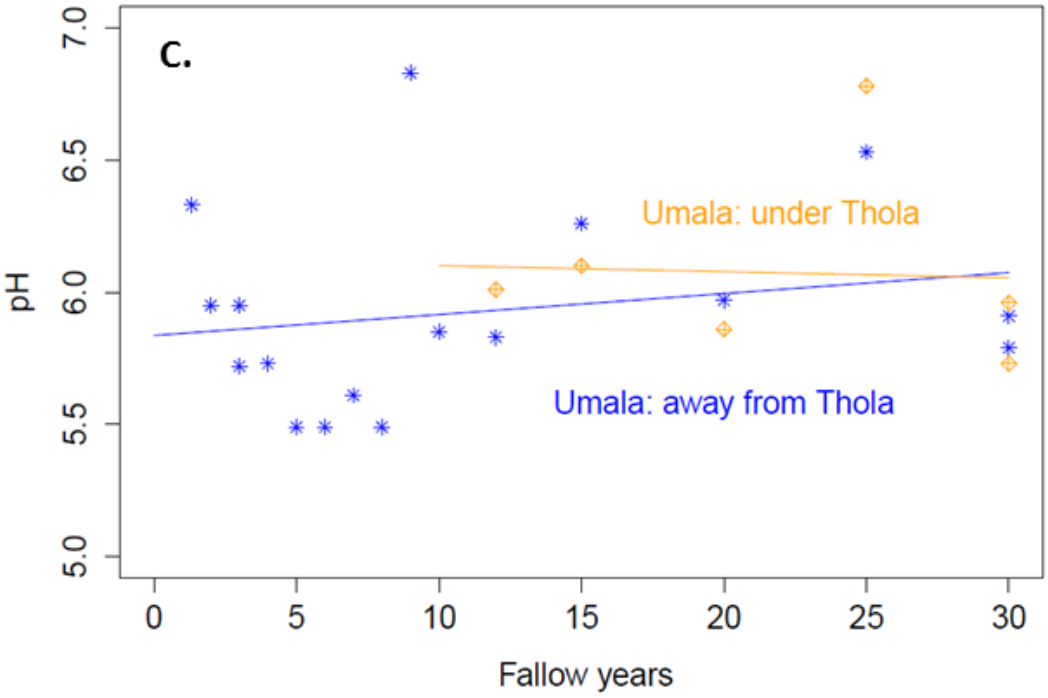
(D) P: Ancoraimes ($p=0.68$, $R^2=0$), Umala ($p=0.03$, $R^2=0.24$), t-test comparing Umala and Ancoraimes ($p < 0.05$).

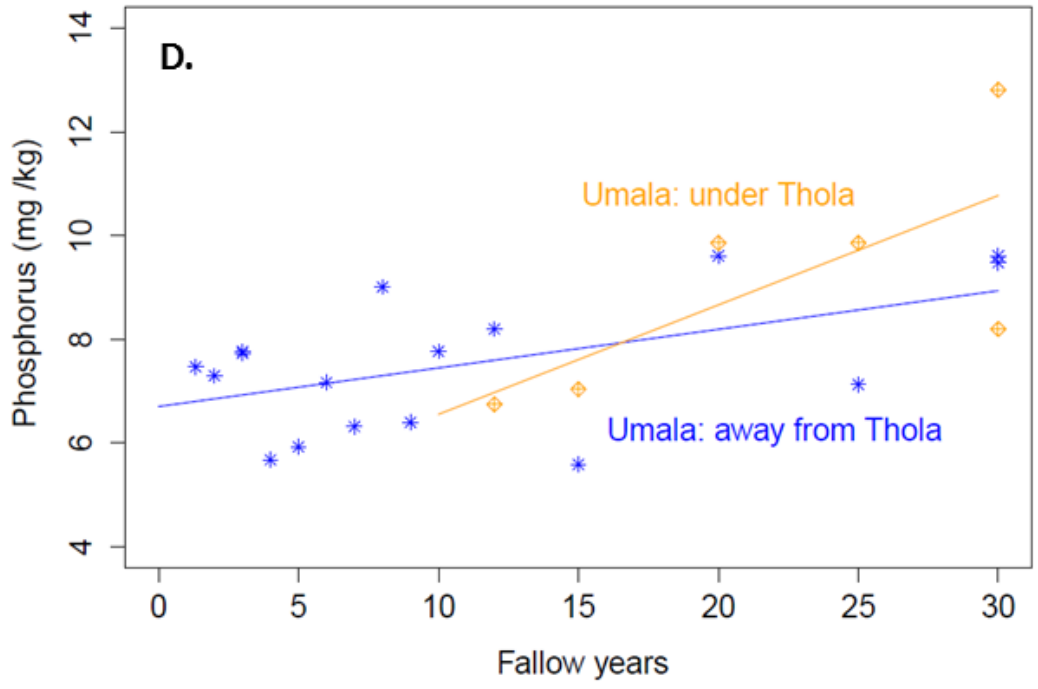
(E) ECEC: Ancoraimes ($p=0.05$, $R^2=0.26$), Umala ($p=0.26$, $R^2=0.02$), t-test comparing Umala and Ancoraimes ($p = 0.23$).

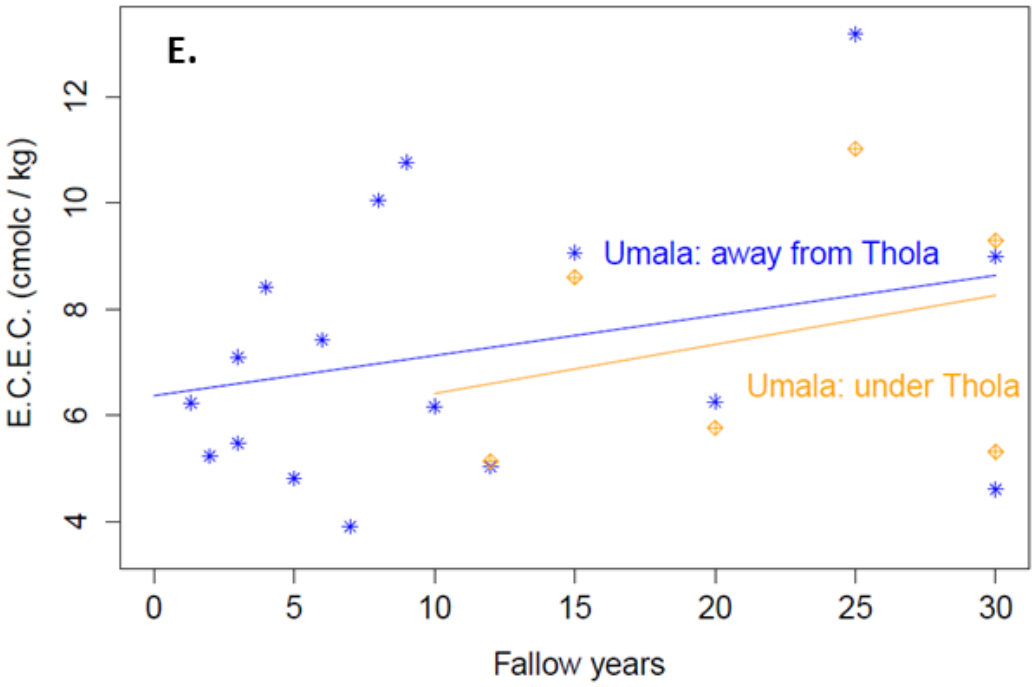
(F) EC: Ancoraimes ($p=0.02$, $R^2=0.36$), Umala ($p=0.97$, $R^2=0$), t-test comparing Umala and Ancoraimes ($p = 0.68$).


A.
Soil Organic Matter (%)
0.4
0.6
0.8
1.0
1.2
1.4
1.6
Umala: under Thola
Umala: away from Thola
0
5
10
15
20
25
30
Fallow years


B.
Total Nitrogen (%)
0.02
0.04
0.06
0.08
Umala: under Thola
Umala: away from Thola
0
5
10
15
20
25
30
Fallow years


C.
pH
7.0
6.5
6.0
5.5
5.0
0
5
10
15
20
25
30
Fallow years
Umala: under Thola
Umala: away from Thola


D.
Phosphorus (mg /kg)
14
12
10
8
6
4
0
5
10
15
20
25
30
Fallow years
Umala: under Thola
Umala: away from Thola


E.
E.C.E.C. (cmolc / kg)
4
6
8
10
12
0
5
10
15
20
25
30
Fallow years
Umala: away from Thola
Umala: under Thola

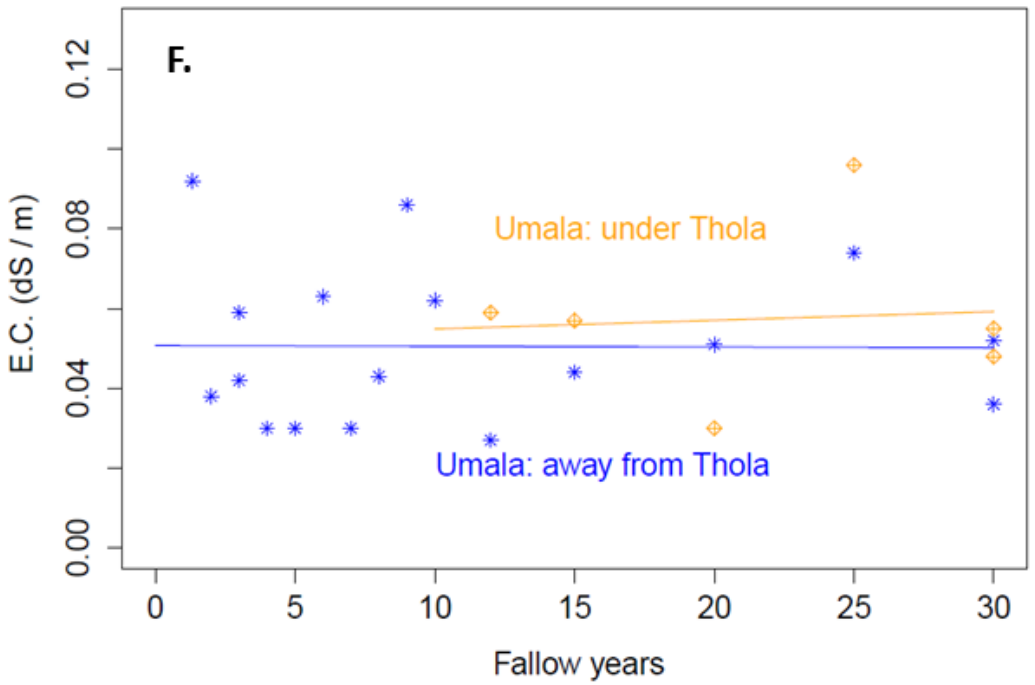

F.
E.C. (dS / m)
0.00
0.04
0.08
0.12
0
5
10
15
20
25
30
Fallow years
Umala: under Thola
Umala: away from Thola

**Fig. S5**. A comparison of the effects of fallow period duration and sampling under the common fallow-period shrub species (thola; Asteraceae), 'thola samples', versus sampling at least one m away from thola, 'non-thola samples'. Samples in Umala were evaluated using linear regression (slope p-value is given with $R^2$) and paired t-tests.

(A) SOM: non-thola (p=0.01, $R^2$=0.31), thola (p=0.34, $R^2$=0.03), Paired T-test (p = 0.01).

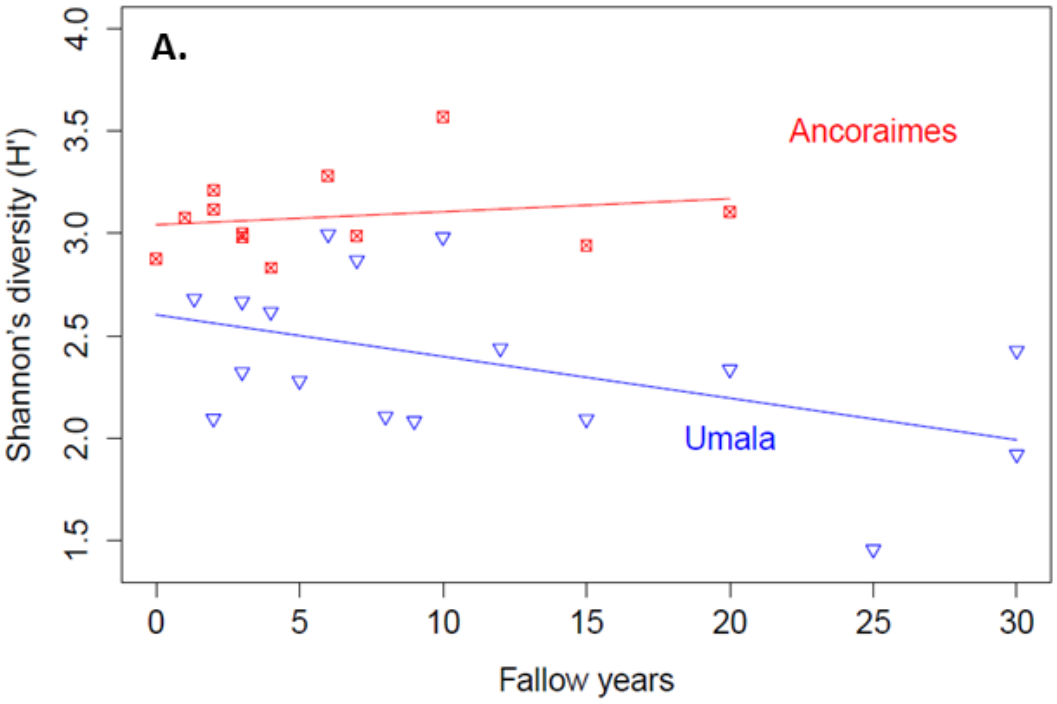
(B) Total N: non-thola (p=0.37, $R^2$=0), thola (p=0.34, $R^2$=0.04), Paired T-test (p = 0.10).

(C) pH: non-thola (p=0.4, $R^2$=0), thola (p=0.93, $R^2$=0), Paired T-test (p = 0.72).

(D) P: non-thola (p=0.03, $R^2$=0.24), thola (p=0.11, $R^2$=0.38), Paired T-test (p = 0.36).

(E) ECEC: non-thola (p= 0.26, $R^2$=0.02), thola (p=0.58, $R^2$=0), Paired T-test (p = 0.45).

(F) EC: non-thola (p=0.97, $R^2$=0), thola (p=0.88, $R^2$=0), Paired T-test (p = 0.23).


A.
Ancoraimes
Umala
Shannon's diversity (H')
1.5
2.0
2.5
3.0
3.5
4.0
0
5
10
15
20
25
30
Fallow years

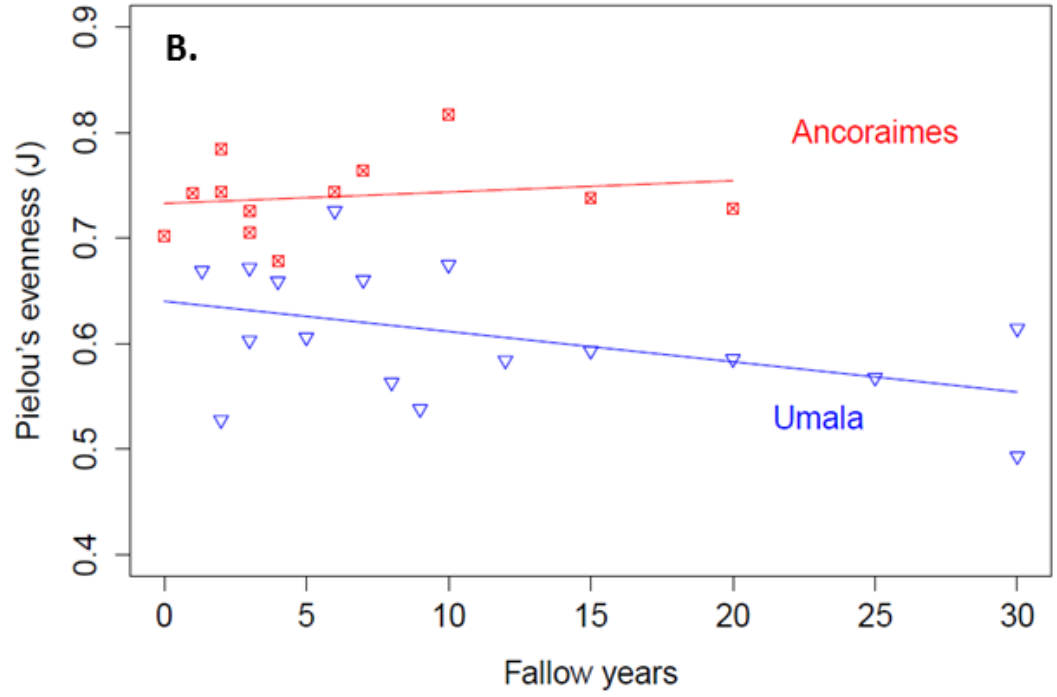
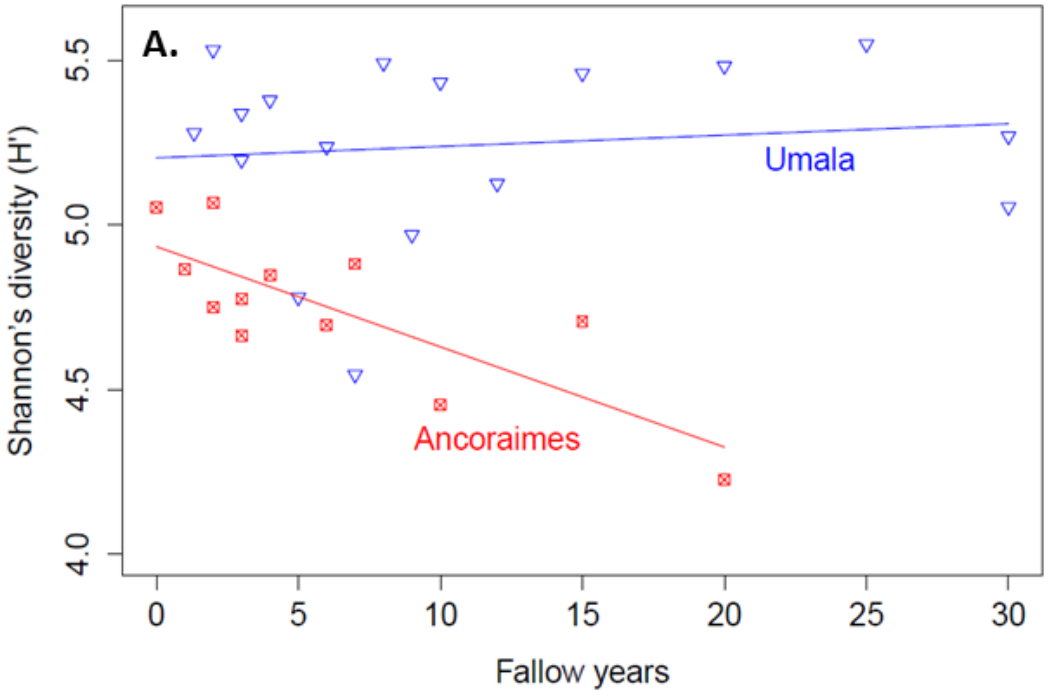

B.
Ancoraimes
Umala
Pielou's evenness (J)
0.4
0.5
0.6
0.7
0.8
0.9
0
5
10
15
20
25
30
Fallow years

**Fig. S6**. Diversity indices for **fungi** in two municipalities (Umala and Ancoraimes) of the Bolivian Altiplano across fallow periods (years) for non-thola samples. The following three estimators were evaluated in regression analyses. Taxon diversity based on 97% similarity.

(A) Shannon' s diversity (H'): Ancoraimes (slope $p=0.55$, $R^2=0$), Umala ($p=0.05$, $R^2=0.18$), t-test comparing Umala and Ancoraimes ($p < 0.001$).

(B) Pielou's evenness (J): Ancoraimes ($p=0.58$, $R^2=0$), Umala ($p=0.07$, $R^2=0.14$), t-test comparing Umala and Ancoraimes ($p < 0.001$).


A.
Shannon's diversity (H')
5.5
5.0
4.5
4.0
Umala
Ancoraimes
0
5
10
15
20
25
30
Fallow years

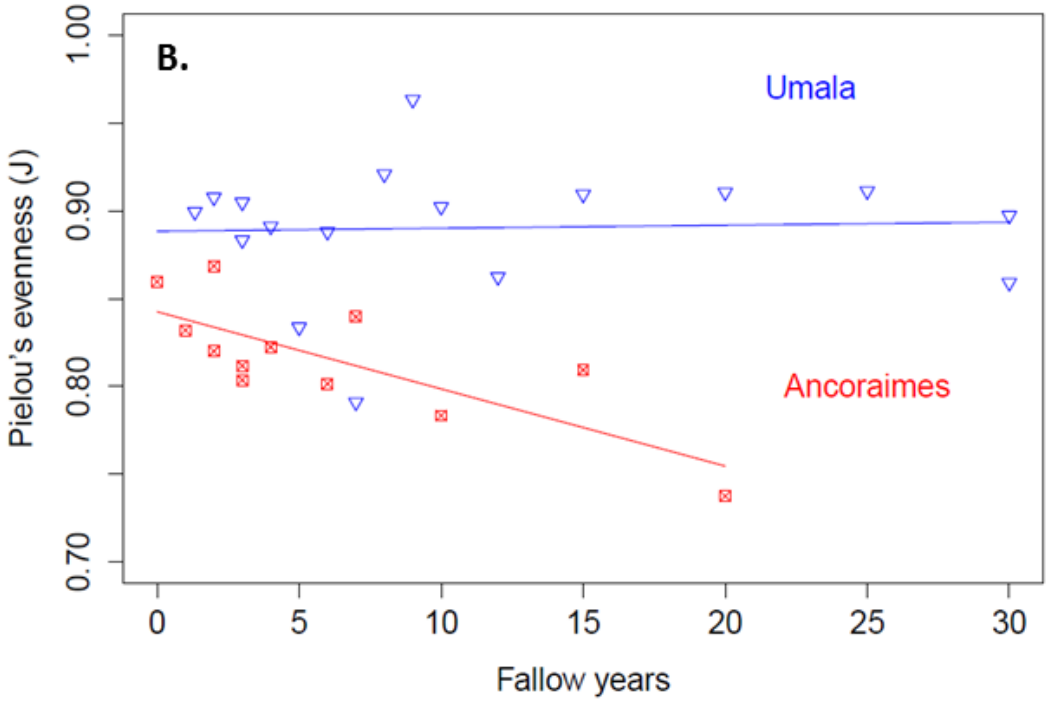

B.
Pielou's evenness (J)
1.00
0.90
0.80
0.70
Umala
Ancoraimes
0
5
10
15
20
25
30
Fallow years

**Fig. S7**. Diversity indices for **bacteria** in two municipalities (Umala and Ancoraimes) of the Bolivian Altiplano across fallow periods (years) for non-thola samples. The following three estimators were evaluated in regression analyses. Taxon diversity based on 89% similarity.

(A) Shannon's diversity (H'): Ancoraimes ($p=0.002$, $R^2=0.58$), Umala ($p=0.65$, $R^2=0$), t-test comparing Umala and Ancoraimes ($p < 0.001$).

(B) Pielou's evenness (J): Ancoraimes ($p=0.003$, $R^2=0.55$), Umala ($p=0.87$, $R^2=0$), t-test comparing Umala and Ancoraimes ( $p < 0.001$).

Do bacterial and fungal communities in soils of the Bolivian Altiplano change under shorter fallow periods?

**Casual images from project meetings and field sites**

Photos by L. Gomez-Montano

Project supported by USAID SANREM CRSP


Fig. 1. La Paz, Bolivia, and the beautiful Illimani Mountain.


Fig. 2.  La Paz, Bolivia, with Illimani.

Fig. 3. Image near a field site in the Umala municipality in the Bolivian Altiplano.

Fig. 4. Image near a field site in the Ancoraimes municipality in the Bolivian Altiplano, with Lake Titicaca in the background.


Fig. 5. Sampling team in the Umala municipality in the Bolivian Altiplano.

Fig. 6.  Bolivian women performing a dance in the Ancoraimes municipality.

Fig. 7. Sampling team in the Ancoraimes municipality in the Bolivian Altiplano.

Fig. 8.  Lunch break for the sampling team in Ancoraimes municipality in the Bolivian Altiplano.

Fig. 9. Thola, common shrubs in the Asteraceae in fallow periods of the Bolivian highlands.


Fig. 10. Group participating in R workshop in La Paz, Bolivia (supported by American Phytopathological Society Office of International Programs – APS OIP).