DO MICROBIAL COMMUNITIES IN SOILS OF THE BOLIVIAN ALTIPLANO CHANGE UNDER ECONOMIC PRESSURES FOR SHORTER FALLOW PERIODS?

by

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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 communities. Using 454-pyrosequencing, we characterized fungal and bacterial community responses to (1) the length of fallow period and (2) the presence of the plants *Parasthrephia* sp. or *Baccharis* sp. (both locally known as 'thola'). Thola is widely considered by farmers as beneficial to soil health, although it is also frequently harvested as a source of fuel by farmers. Soils in one study area, Ancoraimes, had higher levels of organic matter, nitrogen and other macronutrients compared to the other study area, Umala. In our analyses, Ancoraimes soils supported more diverse fungal communities, whereas Umala had more diverse bacterial communities. Unexpectedly, the longer fallow periods were associated with lower fungal diversity in Umala and lower bacterial diversity in Ancoraimes. Fungi assigned to genera *Verticillium, Didymella,* and *Alternaria*, and bacteria assigned to genera *Paenibacillus, Segetibacter, and Bacillariophyta* decreased in abundance with longer fallow period. The presence of thola did not significantly affect overall soil fungal or bacterial diversity, but did increase the frequency of some genera such as *Fusarium* and *Bradyrhizobium*. Our results suggest that fallow period has a range of effects on microbial communities, and that the removal of thola from the fields impacts the dynamics of the soil microbial communities.

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Dedication

To God who is my guide and my strength. To my beloved husband, German A. Vargas, for his loving support, continuous encouragement, and for being a marvelous colleague. To my parents, Jose Fernando Gomez and Adda Montaño, for their constant love and support in each step of my life; to my sisters Liliana and Luz Angela, and to my parents in-law, Honorio Vargas and Martha Orozco for sending me all their loving energy and emotional support across the distance.

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 a successful practice for utilizing soil with low nutrient content (Sanchez, 1999; Wezel and Haigis, 2002; Burgers et al., 2005; Couteaux et al., 2008). The term 'fallow' is commonly used to describe resting periods in agricultural lands in which the non-crop or dormant species are allowed to reestablish themselves by natural succession after a cropping period (Nair, 1993; Sanchez, 1999). In semiarid areas of North America, fallow is used for recharging subsoil water (Brady and Weil, 2002). Often tropical fallow systems are characterized by a cropping period of one to four years during which the soil fertility declines rapidly, and a subsequent fallow period that can be very long (from four years to multiple decades) allowing 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 instance, in the Bolivian Altiplano after three consecutive cropping years, in which potato is the main crop followed by quinoa (*Chenopodium quinoa* (Willd.)) and barley (*Hordeum vulgare* L.) the next two years, the lands are frequently left under extended fallows (up to 20 years) for soil fertility restoration (De Cary and Hervé, 1994; Hervé, 1994; Pacheco Fernández, 1994; Hervé et al., 2002; Couteaux et al., 2008). These long fallow periods can lead to a return to unmanaged vegetation similar to that before the cropping period (Masse et al., 2004; Couteaux et al., 2008). When the fields are cultivated again, the successional vegetation may be ploughed into the soil as green manure (Sarmiento and Bottner, 2002; Couteaux et al., 2008).

Incorporation of a fallow period in a cropping system has an effect on soil physical and chemical properties, as well as an influence on microbial communities. Nevertheless, little research has been conducted in studying the effects of fallow periods on soil microorganisms. Sall et al. (2006) found that soil samples taken from a 21-year fallow in Senegal had higher C, N, and total P than cultivated soils, as well as higher microbial activity and microbial diversity. Only a few studies have been conducted to investigate this type of effect, in contrast with the great number of studies that had addressed the effect of different edaphic properties or agricultural practices on soil microorganisms. Many investigations have reported that the activity of soil microbial populations 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 community structure changed under different soil management such as crop rotation, fertilization, tillage, and manure or pesticide applications (Sigler and Turco, 2003; Spedding et al., 2004; Crecchio et al., 2004; Acosta-Martinez et al., 2010; Yin et al., 2010). Comparative studies have been conducted to evaluate the effect of different fallow periods on the improvement of soil properties and the control of soil erosion (Sarmiento and Bottner, 2002; Ndour et al., 2008; Miranda et al., 2009). In southeastern Brazil, after a five-year fallow, fields recovered higher macroporosity, total porosity, and saturated hydraulic conductivity compared to a two-year fallow (Miranda et al., 2009). Nevertheless, short-term fallows (four years) in Senegal did not significantly increase soil organic matter or nutrient content (Masse et al., 2004), and in the Bolivian highlands, there was no evidence of recovery of nutrient elements after ten years of fallow (Hervé, 1994). These differences illustrate the heterogeneity of farming systems and microbial responses in different regions.

In recent decades, economic pressures related with the increase in human population and the need for additional land in production in agricultural areas 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). For example, the long-term fallow (generally 6-10 years or more) in Southwestern Nigeria, which contributed to soil fertility, has often been reduced to 3-6 years (Aweto et al., 1992). In West Africa and Latin America, shorter fallow periods (1-5 years) have led to soil degradation and increased need of fertilizers (Aweto et al., 1992; Kang et al., 1999; Phiri et al., 2001; Wezel and Haigis, 2002). Moreover, natural vegetation cover has often decreased significantly (Breman and Kessler, 1995). In the Bolivian Altiplano, over the last two decades, these economic pressures reduced the length of fallow period (now 2-10 years), which is shorter compared to the traditional term of up to 20 years of fallow in this region (Hervé, 1994; Aguilera A, 2010). There has been little research conducted in Bolivia to determine the effects of fallow on soil restoration and soil characteristics, and the economic pressures in the Altiplano region pose new questions about the effect that the increase in population and the competition for crop land (with dairy production

and forage for livestock) can have on the edaphic properties and on soil microorganisms in the Altiplano region.

Another important component of fallow periods is the vegetation found in this type of field. 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 high level of nutrients released 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 crop as a source of organic matter (Norgrove, 2008), exchangeable K concentration (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 related species, also members of the Asteraceae and collectively known as 'thola', are considered by local farmers to be beneficial to the maintenance of soil quality due to their fast 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 can reach its maximum height in approximately 10 years, such that farmers sometimes use thola height to estimate the length of a field's fallow period (Hervé, 1994). A higher microbial diversity (especially, bacteria, arbuscular mycorrhizal fungi and actinomycetes) has also been reported in association with thola compared to the grass *Stipa ichu* (De Cary and Hervé, 1994).

Soil microorganisms are critical to the maintenance of good soil health because they are involved in many important soil functions such as carbon and nitrogen cycling, uptake of nutrients for plant growth, maintenance of soil structure, degradation of pollutants/agrochemicals, and disease suppressiveness (Pankhurst et al., 1996; van Bruggen and Semenov, 2000; van der Heijden et al., 2008). Therefore, microbial diversity supports the health of soil (Jain et al., 2005). However, despite the importance of soil microorganisms, very little is known about their diversity and community structure (Fierer et al., 2007).

Estimation of the taxonomic diversity of soil microbial communities has been limited by traditional methods and the non-culturability of the majority of the microbial species present in soil (Fierer et al., 2007; Rondon et al., 2000). Recent developments in molecular biology and

biochemical assays provide new tools for the analysis of soil microbial communities. Some studies have used extraction of 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 analysis techniques have been used to compare the diversity of bacteria, archaea, fungi and viruses in soils collected from prairie, desert and rainforest (Fierer et al., 2007). The use of 454-sequencing techniques allows analysis of millions of microorganisms, bypassing culturing (Roesch et al., 2007), and the recent development of sample-specific sequence tags (DNA tagging) for this technique allows multiplexing large numbers of individual samples, making DNA sequencing and analysis more efficient (Acosta-Martinez et al., 2008; Jumpponen and Jones, 2009; Lauber et al., 2009). These new techniques are useful for understanding how soil communities change in response to shifts in cropping systems.

In the Bolivian Altiplano, land use is shifting. Two types of land use predominate. 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 comprises 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.* Use of shorter fallow periods and changes to the proportion of *aynuqa* and *sayaña* systems has the potential to reduce ecosystem services that have previously been provided (Cheatham et al., 2009).

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 increasing fallow period and the presence of thola will increase soil organic matter and nutrient levels in the soil. The second objective was to characterize the response of soil microbial diversity (bacterial and fungal) to fallow period length and to thola, hypothesizing that fungal and bacterial diversity would increase with increasing fallow period or the presence of thola. We used 454-pyrosequencing to evaluate fungal and bacterial communities. Soils in one study area, Ancoraimes, had higher levels of organic matter, nitrogen and other macronutrients compared to the other study area, Umala. In our analyses, higher nutrient (Ancoraimes) soils supported more diverse fungal communities, whereas lower nutrient (Umala) soils had more diverse bacterial communities. Unexpectedly, longer fallow periods were associated with lower fungal and bacterial diversity. Our third objective was to evaluate the frequency of fungal and bacterial taxa overall, and their response to fallow period length and to thola. Our hypothesis was that the frequency of some fungal and bacterial taxa will change with fallow period and in response to thola. Diversity did not change significantly between thola and non-thola samples, but there were several significant changes in the frequency of particular microbial taxa.

Materials and methods

The Bolivian Altiplano

In Bolivia, the central highland plateau region called the Altiplano (Fig.5, Supplemental material) is a semi-arid 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 to 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 agricultural systems, the Altiplano has a challenging environment for agriculture, yet 35% of Bolivians rely on it for their livelihood (Quiroga, 1992). The harsh climatic conditions of the Bolivian Altiplano are characterized by low precipitation during the growing season (annual precipitation ranging from 350 mm in the South to 550 mm in the North), frequent frost and drought during the cultivation

period, and high diurnal temperature variations (Garcia et al., 2007; Couteaux et al., 2008). There is only one harvest per year (Garcia et al., 2007).

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 Umala municipality is a semi-arid region with average precipitation 350 mm/year, and average temperature 11°C (PROINPA, 2005). The Ancoraimes municipality is a relatively cool-humid region with average precipitation 550 mm/year, and average temperature of 7 to 8 °C (Programa Nacional de Cambios Climáticos Bolivia, 2005). The soils in these two municipalities are commonly sandy loam textural class which were classified as a sandy, mixed, frigid Typic Ustifluvents using the U.S. Soil Taxonomy (1999), and are locally classified as 'Saj'e or Ch'alla' soil in Aymara (Aguilera, 2010). At our sample sites, soils in Ancoraimes were strikingly rocky compared to the soils in Umala.

Seventeen fields in Umala, and twelve fields in Ancoraimes were selected to represent a range of different fallow period lengths. Soil samples were collected during the dry season in August, 2008. Fields sampled had fallow periods ranging from 1 to 30 years in Umala, and 0 to 20 years in Ancoraimes (Table 3), representing the full range of fallow periods in those regions. The fields were selected in collaboration with the local indigenous farming communities who could provide information about the fallow period. Where larger thola individuals (typically *Parasthephia lepidophylla* and *Baccharis incarum*) were present, generally fields with fallow periods greater than 10 years, we sampled soils under larger thola plants and at least 1 m away from thola plants, as described below. Larger thola plants in these fields were approximately 40 cm high and 60 cm in diameter, the larger size of the plant reported by Hervé (1994). In fields with fallow periods less than 10 years, thola was rare and smaller in size, generally corresponding to the 10 cm height by 15 cm diameter reported by Hervé (1994) for smaller thola. We often observed grasses associated with thola.

Experimental design, plot layout, and soil sampling

Each field was treated as an experimental unit with an associated vegetated fallow period. In each field, we selected a 20 m x 20 m area at least 10 m from the field edge for sampling (Fig.6, Supplemental material). Nine subsamples were collected from points at least 10 m apart. At each point, five sub-subsamples of soil were collected around the perimeter of a 1 m radius circle, sieved, and mixed together in a bucket to better represent the potentially variable microbial communities and the heterogeneity of the field (Baker et al., 2009). One subsample was then drawn from each bucket, resulting in a total of nine subsamples from each field where thola was absent or not adequately frequent. In fields where thola was present in sufficient numbers, 18 soil subsamples were collected as follows (Fig.7, Supplemental material). 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'). All samples from fields with fallow periods shorter than 10 years were effectively 'non-thola', and when we later discuss general results without reference to thola, these will be for samples collected away from thola.

We collected the top 15 cm of soil for each sub-subsample using 2.54 cm diameter soil corers. The five sub-subsamples of soil were passed through a 6 mesh per 2.54 cm in sieve, collected in a bucket, and mixed to produce a subsample. From each subsample, we placed approximately 300 g into a small Ziploc bag for measurement of physical and chemical properties, and approximately 0.7 g into a MoBio bead solution tube from the Ultra Clean Soil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA), which was shaken well. All samples were stored in a cooler for transportation to the laboratory where samples for DNA extraction were frozen in -20°C and samples for chemical analyses were air-dried. DNA was extracted individually from each of the nine subsamples. For gravimetric moisture and physical and chemical analyses, subsamples were combined into one composite sample per field.

Soil physical and chemical analyses

We determined the percentage of sand, silt and clay (Bouyoucos hydrometer method), pH in water (1:5 soil:water method), electrical conductivity (EC) (1:5 soil-to-water method), and

concentration of cations calcium, magnesium, sodium, potassium (using the ammonium acetate (NH_4OAc) extraction method) (Warncke and Brown, 1998), and aluminum. The CEC (Cation Exchange Capacity) and total exchangeable bases were also measured (using summation of K, Ca, Na, Mg for CEC, and for total exchangeable bases using summation of the previous bases plus Al). In addition, the percentage of soil organic matter (SOM) (Walkley-Black method), total N (Kjeldahl method), and P were determined (Bray and Kurtz test). These analyses were performed by the Instituto Boliviano de Ciencia y Tecnología Nuclear (IBTEN) in La Paz, Bolivia.

DNA extraction and PCR amplification

For each field, we extracted the soil DNA from the nine subsamples separately. We followed the manufacturer's protocol except we eluted the total DNA in 100 μl of buffer S5. 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 an ascomycete (*Saccharomyces cerevisiae*) and a basidiomycete (*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 *Quercus macrocarpa* phyllosphere (Jumpponen and Jones, 2009), five DNA samples from Umala and three from Ancoraimes. The preliminary PCR conditions were modified from those used in Roesch et al. (2007). All DNA templates were quantified with an ND 1000 spectrometer (NanoDrop Technologies, Wilmington, DE, USA) and adjusted to a final concentration of 2 ng/μl. Of the nine subsamples from each field, the eight with the highest DNA content were used to produce the PCR amplicons.

Fungi. The fungal ITS region was PCR-amplified in two replicate 25 μl reactions for each sample. To accommodate direct 454 sequencing of the fungal internal transcribed spacers 1 and 2 (ITS1-ITS2) 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).

The resulting primer sequences were as follows: ITS4 5'-GCCTCCCTCGCGCCATCAGNNNNN**TCCTCCGCTTATTGATATGC-** 3', and ITS1- F 5'-GCCTTGCCAGCCCGCTCAG**CTTGGTCATTTAGAGGAAGTAA-** 3' where the underlined sequences are the 454 primers A and B, respectively, and the bold letters denote the fungus specific primers ITS4 and ITS1-F for internal transcribed spacer (ITS) of the nuclear DNA (nr DNA). The 5 bp barcode within primer ITS4 is denoted by 5 Ns. 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 Go Taq Hot Start DNA polymerase (Promega, Madison, Wisconsin) and 5μl 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. Bacterial variable regions V5 to V8 in the 16S rRNA was PCR amplified in two replicate 25 μl reactions to account for heterogeneous amplification from the environmental template. For direct 454 sequencing of the bacterial small subunit of the ribosome (16S rRNA) amplicons, we incorporated 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)) as described for fungi. 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.

The resulting primer sequences were as follow: 1492-rm 5'-GCCTCCCTCGCGCCATCAGNNNNNNNN**GNTACCTTGTTACGACTT-** 3', and 786f 5'-GCCTTGCCAGCCCGCTCAG**GATTAGATACCCTGGTAG-** 3 where the underlined sequences are the 454 primers A and B, respectively, and the bold letters denote the small subunit of the ribosomal RNA (SSU rRNA) primers 1492-rm and 786f. The 8 bp barcode within primer 1492-rm is denoted by 8 Ns. These two primers, modified from Roesch et al. (2007), were chosen to increase the taxonomic range of 16S rRNA gene.

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 Go Taq Hot Start DNA polymerase (Promega, Madison, Wisconsin) and 5.0 µl Green Go Taq 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, without final extension.

All PCR reactions for fungi and bacteria were performed in duplicates on two MasterCyclers (Eppendorf, Hamburg, Germany). Possible PCR amplification of contaminants, for fungi and bacteria, 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 H_2O. These remained free of visible amplicons.

For each of the 37 fields, 5µl from each of the duplicate eight subsamples was pooled into a final volume of 80µl per field, where samples were processed separately for fungi and bacteria. The 37 pooled fungal or bacterial amplicons were cleaned using the Agencourt AMPure PCR purification system (AgenCourt Bioscience, Beverly, MA, USA) following the manufacturer's instructions. This clean-up system was selected because it discriminates against fragments of less than 100 bp in size, removes salts, enzymes and effectively eliminates dimers of the fusion primer constructs that exceed 40 bp in size. 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 final concentration of ~10 ng/µl for fungi and ~ 30 ng/µl for bacteria for downstream emulsion PCR and 454-pyrosequencing.

Pyrosequencing

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

Bioinformatics and operational taxonomic unit (OTU) designation

The sequences generated for both fungi and bacteria were submitted to the computational pipeline PyroTagger v.1.0 (http://pyrotagger.jgi-psf.org/; Kunin and Hugenholtz (2009)). Sequences from the fasta and quality files that had an exact match with the tag-primer sequences were retained for quality filtering. Sequences with low quality bases (Q < 27) or shorter than 200 bp were removed. The acceptable 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 non-redundant, representative sequence for each OTU were submitted to the Ribosomal Database Project's (RDP, Version 10) Classifier (Cole et al., 2008) for taxonomical 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 (<90% similarity) among the query and match sequences. For bacterial taxon assignment through RDP, we removed reads with <80% bootstrap support (confidence threshold 80%). The PyroTagger output and OTU assignments were used to calculate the taxon frequencies for each sample.

Diversity indices

Three diversity measurements were calculated for fungal and bacterial genera. Simpson's diversity (1-D), which is the complement of Simpson's dominance ($D=\sum p_i^2$), estimates the likelihood that two randomly chosen individuals will be different OTUs (Simpson, 1949). We also used Shannon's diversity ($H'= -\sum p_i \ln p_i$) and Pielou's evenness ($J= H' / \ln (S)$) to estimate community evenness. Diversity indices were estimated using a SAS script (SAS Institute Inc., Cary, NC).

Statistical analyses

We considered each field to represent 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 (treated as response variables) were analyzed with 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 function qvalue). Analyses of the effects of thola were based on paired samples from within six fields in Umala. Using the assigned OTUs to taxa for fungi, we evaluated 5 phyla, 59 orders, and 182 genera; and using the assigned OTUs to taxa for bacteria, we evaluated 18 phyla and 90 genera.

Results

Edaphic properties in the two study regions (non-thola samples)

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

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, cation exchange capacity (C.E.C) and electrical conductivy (E.C) – was evaluated using linear regression (Fig.1,Table 2). In Ancoraimes, SOM and total N, increased significantly with longer fallow period, while in Umala SOM increased slightly over fallow years. The pH for 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.

Thola effects on edaphic properties

SOM and total N were significantly higher in thola compared to paired non-thola samples, as evaluated in six Umala fields (Fig. 2, Table 2). pH, P, C.E.C, and E.C did not differ significantly between thola and non-thola samples.

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 803 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 3).

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 4880 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 3).

Diversity as function of fallow period

Fungi. The three diversity estimators (Simpson's diversity (1-D), Shannon's diversity (H'), and Pielou's evenness (J)) decreased with increasing fallow years in Umala but not in Ancoraimes (Fig. 3). Umala had lower overall fungal diversity than Ancoraimes.

Bacteria. Simpson's diversity, Shannon's diversity (H') and Pielou's evenness (J) decreased over fallow years in Ancoraimes, but not in Umala. Ancoraimes had lower overall bacterial diversity than Umala (Fig.4).

Diversity of microbes associated with thola

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

Overall most frequent taxa in Umala and Ancoraimes (non-thola samples)

Fungi. Averaged across all non-thola samples, the most frequent phyla, orders and OTUs assigned to genera were identified (with the frequency in percentage for Umala and Ancoraimes, respectively, given in parentheses). The Ascomycota (85% and 66%) and Basidiomycota (4% and 11%) were the most frequent (Table 4). At the order level, the most frequent were Hypocreales (45% and 38%), and Pleosporales (30% and 19%) (Table 5), both in the phylum Ascomycota. The OTUs assigned to genera *Fusarium* (40% and 19%), and *Didymella* (28% and 16%), were the most frequent (Table 6).

Bacteria. Averaged across all non-thola samples in Umala, the most frequent phyla were Proteobacteria (24%), and Actinobacteria (11%) (Table 7). The OTUs assigned to genera *Paenibacillus* (2.7%), and *Gp 4* (2.6%) were the most frequent (Table 8). In Ancoraimes, the most frequent phyla included Proteobacteria (27%), and Firmicutes (16%) (Table 7). The OTUs assigned to genera *Pseudomonas* (3%), and *Bradyrhizobium* (2%) were the most frequent (Table 8).

Taxa varying with fallow period

To examine whether the microbial composition changed with fallow period, phyla, orders, and OTUs assigned to genera were analyzed. For example, the phylum Basidiomycota increased in frequency with increasing fallow period in Umala, but not in Ancoraimes (Table 9). The orders Capnodiales and Mortierellales increased with fallow period at both sites (Table 10). The OTUs assigned to genera *Cladosporium* and *Mortierella* significantly increased with fallow period for both sites (Table 11). The only phylum that decreased in frequency with fallow period was Basidiomycota in Ancoraimes (Table 12). The orders Hypocreales and Thelebolales

decreased with fallow period in Umala, while Microascales, Pleosporales, Sordariales and Tremellales decreased with fallow period in Ancoraimes (Table. 13). The OTUs assigned to genera *Alternaria* and *Fusarium* decreased with fallow period in Umala, while OTUs assigned to genera *Acremonium* and *Bionectria* decreased with fallow period in Ancoraimes (Table 14).

Among the bacteria, the phyla Chloroflexi and Proteobacteria (Table 15) and the OTUs assigned to genera *Actinoplanes* and *Sorangium* (Table 16) increased with fallow period at both sites. The bacterial phyla Firmicutes (Table 17) and the OTUs assigned to the bacterial genus *Paenibacillus* decreased with fallow period in both sites (Table 18).

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 19, 20, 21). For example, Basidiomycota (Table 19), order Capnodiales (Table 20), and the OTUs assigned to the genus *Fusarium* (Table 21) were more frequent in thola samples than non-thola samples. Taxa more frequent in non-thola samples included order Mortierellales (Table 20), and the OTUs assigned to the genus *Didymella* (Table 21). Overall 4 phyla, 35 orders, and 17 genera (which included many OTUs assigned each genera) differed significantly between thola and non-thola samples.

Bacteria. Similarly to fungi, the overall bacterial diversity did not differ between thola and non-thola samples. However, there were taxa that significantly differed in frequency (Tables 22, 23). For example, Bacteroidetes (Table 22), and the OTUs assigned to the genus *Bradyrhizobium* (Table 23) were more frequent in thola samples than non-thola samples. Taxa more frequent in non-thola samples included Verrucomicrobia (Table 22), and the OTUs assigned to the genus *Belnapia* (Table 23). Overall 13 phyla, and 24 genera (which included many OTUs assigned to each one of these genera) differed significantly between thola and non-thola samples.

Discussion

Why did microbial diversity decrease 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 OTUs diversity assigned to fungal and bacterial genera. Differences in edaphic properties are often associated with differences in soil microbial communities (Lauber et al., 2008; Lauber et al., 2009; Jenkins et al., 2010; Rousk et al., 2010). In Umala soils with lower SOM and nutrient levels (Table 1), fungal OTU diversity decreased with increasing fallow years (Fig. 1). In Ancoraimes soils with relatively higher SOM and nutrient levels, bacterial OTU diversity decreased with fallow years (Fig. 4).

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 showed a flat trend with fallow years, while in Ancoraimes it increased substantially (Table 2, Fig. 1). 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. Investigating the direct influence of pH on the composition of fungal and bacterial communities across 180-m of the Hoosfield acid strip in the USA, Rousk et al. (2010) found that bacterial communities were more strongly influenced by pH than fungal communities. Additionally, 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). We hypothesized that the increase in pH with fallow years contributed to the changes in the frequency of OTUs assigned to bacterial genera. Studies using pure cultures have also demonstrated narrow tolerances for pH in some soil bacteria (Rosso et al., 1995). In Altiplano soils, the phyla Bacteroidetes in Ancoraimes and Actinobacteria in Umala increased in frequency as both fallow years and pH increased (Table 15 and Fig. 1 for Ancoraimes). For both these phyla, a strong positive association between relative abundance and higher pH has been observed for 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 explain because there are few 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 6). These two dominant OTUs assigned to fungal genera 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.

The most frequent bacterial taxa in the Altiplano soils

Our observed frequencies of bacterial phyla in the Bolivian Altiplano soils generally agreed with other profiles published by 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 (24% and 27% in Umala and Ancoraimes, respectively) (Table 7). 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, Paenibacillus, Bradyrhizobium,* and *Streptomyces* were frequent in both Altiplano sites (Table 8).

The next most abundant phyla in the Bolivian Altiplano soils were Firmicutes (16%) and Actinobacteria (11%) (Table 7). The higher frequency of the phylum Firmicutes in the Altiplano was in sharp contrast to the low frequency reported (less than 5%) 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 8). 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.

The frequency of the phylum Actinobacteria was around 13% (ranging from 0 to 34%) in bacterial communities in 16S rRNA and 16S rRNA genes based studies from a variety of North American, South American, and European soils (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 8). These filamentous bacteria can survive under low water potentials (Madigan and Martinko, 2006), a useful adaptation for the low precipitation across the Bolivian Altiplano.

The most frequent fungal taxa in the Altiplano soils

Ascomycota was the most frequent phylum (85% in Umala and 66% in Ancoraimes) across all fallow period fields in Bolivian soils (Table 4), 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 which may withstand the harsh conditions of the Altiplano better than some fungal groups.

Microbial community composition as a function of fallow period

For bacteria, the phyla Chloroflexi (made up of thermophilic and anoxygenic phototrophs (Madigan and Martinko, 2006)) and Proteobacteria were taxonomic groups that increased in frequency with longer fallow periods in both sites (Table 15). 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 genera *Cladosporium,* which includes plant pathogens (Jacyno et al., 1993), and *Mortierella* both increased with fallow period (Table 11). Interpretation of the impact of changes in the frequency of the airborne fungi *Cladosporium* is

complicated by the many potential ecological roles that it 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 *Thelebolus*, which often is isolated from very cold environments (psychrophiles) (De Hoog et al., 2005), decreased with longer fallow periods (Table 14).

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. For bacteria, the OTUs assigned to the genus *Bradyrhizobium* was more frequent under the canopy of thola, while the OTUs assigned to the genus *Belnapia* was more frequent in non-thola samples (Table 23). This high frequency of OTUs assigned to the genus *Bradyrhizobium*, (Proteobacteria) under thola, could be associated with the ability of this symbiotic bacteria to colonize the roots of some non-legumes (Antoun et al., 1998; Loh and Stacey, 2003). Among fungi, the OTUs assigned to the genus *Fusarium* (order Hypocreales) was more frequent in thola samples compared to non-thola samples, and the OTUs assigned to the genus *Didymella* was less frequent in thola samples (Table 21). Plants have important effects on microbial communities, and 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).

Future Perspectives

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, while at the same time avoiding the use of chemical inputs (Kang et al., 1999; Sanchez, 1999; Phiri et al., 2001). In the Bolivian Altiplano, the shrub thola (including the species *Parasthrephia lepidophylla* and *Baccharis incarum*) has been previously reported to be beneficial to the soil, due to its fast colonization of

bare lands and to contribute to SOM (De Cary and Hervé, 1994; Stachshin de Queiroz et al., 2001). Our results also showed that the presence of thola in fallow fields was associated with modest increases in SOM and soil fertility, and we suggest that this shrub may improve short fallows in the Northern Altiplano of Bolivia.

This alternative has been implemented by using thola 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 with indicators of desertification (Andressen et al., 2007) as a strategy to protect and restore degraded soils (Gonzales T, in http://www.dry-et.org/index.php?page=3&successstoryId=32&Language=en). It may be possible to get the benefits of longer fallows when thola is planted (or at least maintained), perhaps along with other plant species, in shorter fallows.

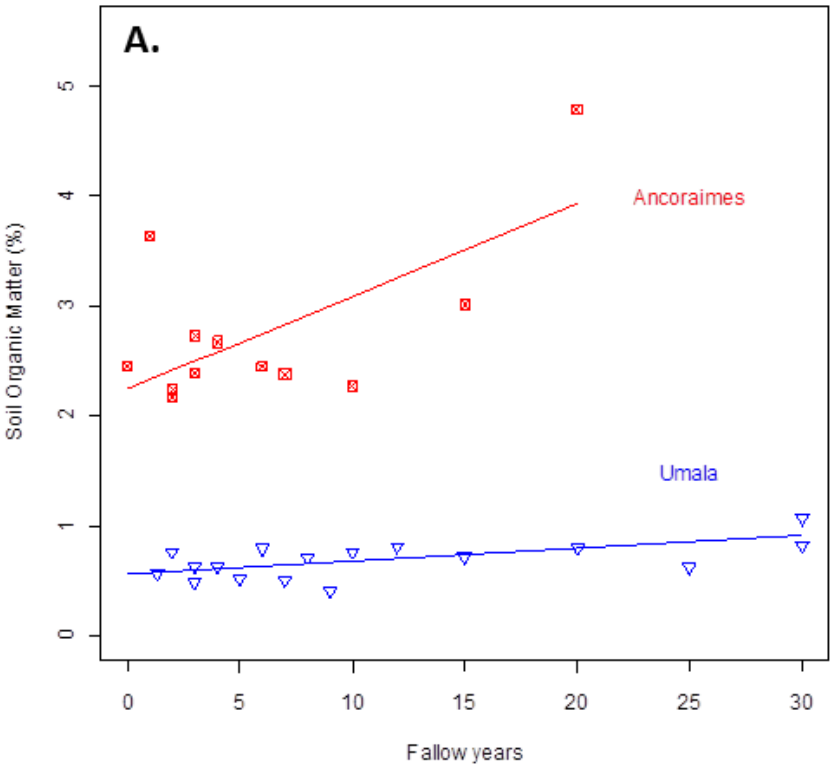
Another possibility is to plant herbaceous legumes along with a non-leguminous shrub such as thola in short fallows. Leguminous plants have been used in agricultural production for centuries for supplying nitrogen to many cropping systems.

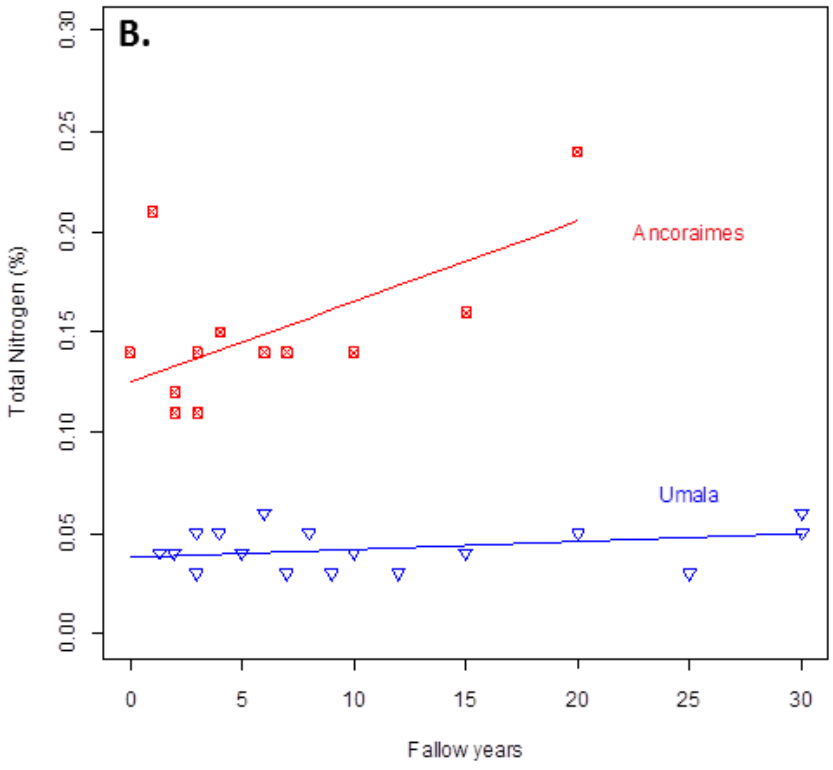
A number of aspects of soil microbial community interactions will need to be understood better in order to translate new information about microbial community structure into recommendations for management. While it is often assumed that higher levels of taxonomic diversity are beneficial for system productivity, 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 surprising 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. It may be that there are more diverse microhabitats during Altiplano crop rotations than during fallows.

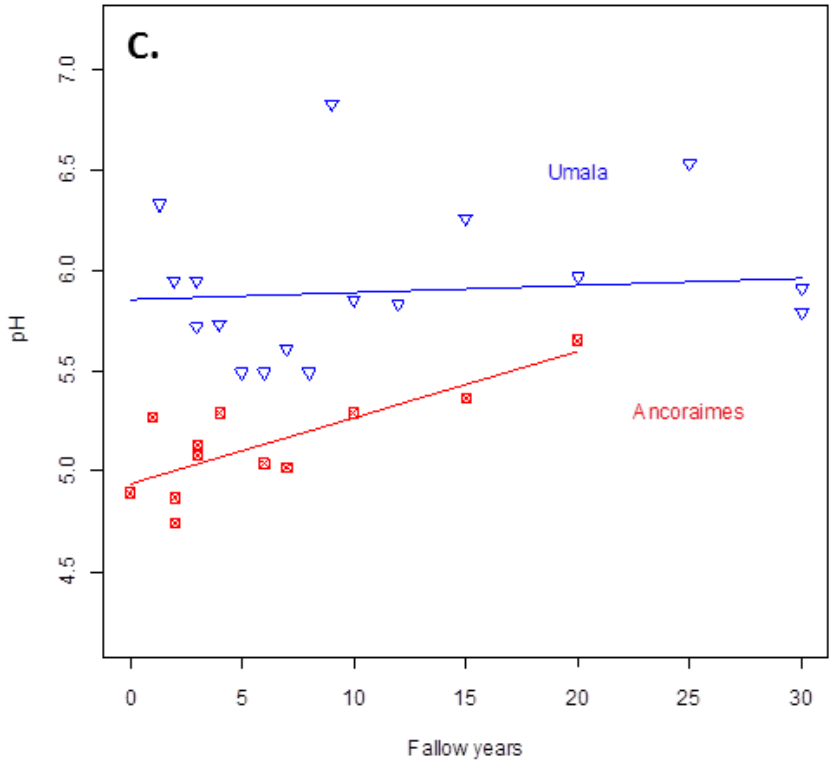
Figures and Tables

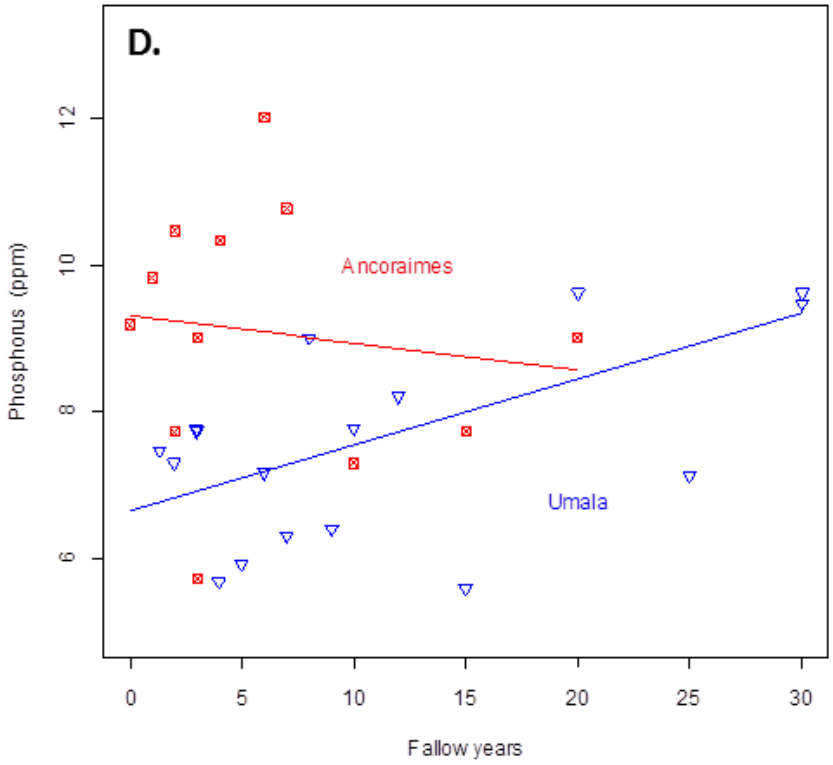
Figure 1. Fallow period effect on edaphic properties in two municipalities of the Bolivian Altiplano (Umala and Ancoraimes) evaluated in linear regression analysis.

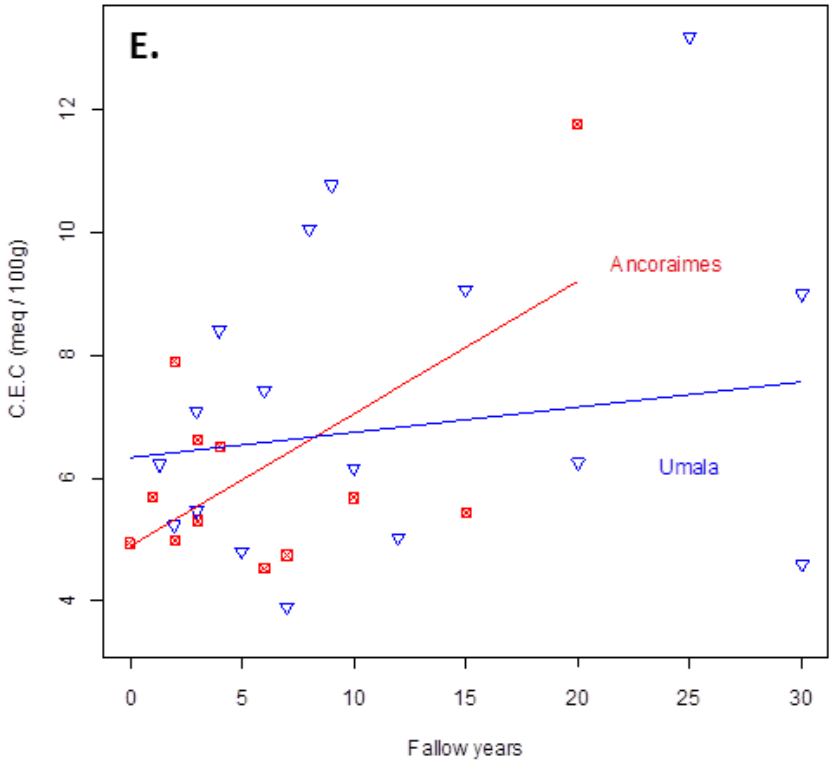
(**A**) SOM: Ancoraimes ($p=0.03$, $R^2=0.33$), Umala ($p=0.01$, $R^2=0.31$), t-test comparing Umala and Ancoraimes ($p < 0.001$). (**B**) Total N: Ancoraimes ($p=0.05$, $R^2=0.26$, Umala ($p=0.37$, $R^2=0$), t-test comparing Umala and Ancoraimes ($p < 0.001$). (**C**) pH: Ancoraimes ($p=0.003$, $R^2=0.55$), 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**) CEC: 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 (%)
Fallow years
Ancoraimes
Umala
0
1
2
3
4
5
0
5
10
15
20
25
30


B.
Total Nitrogen (%)
Fallow years
Ancoraimes
Umala
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0
5
10
15
20
25
30


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


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


E.
C.E.C (meq / 100g)
Fallow years
Ancoraimes
Umala
4
6
8
10
12
0
5
10
15
20
25
30

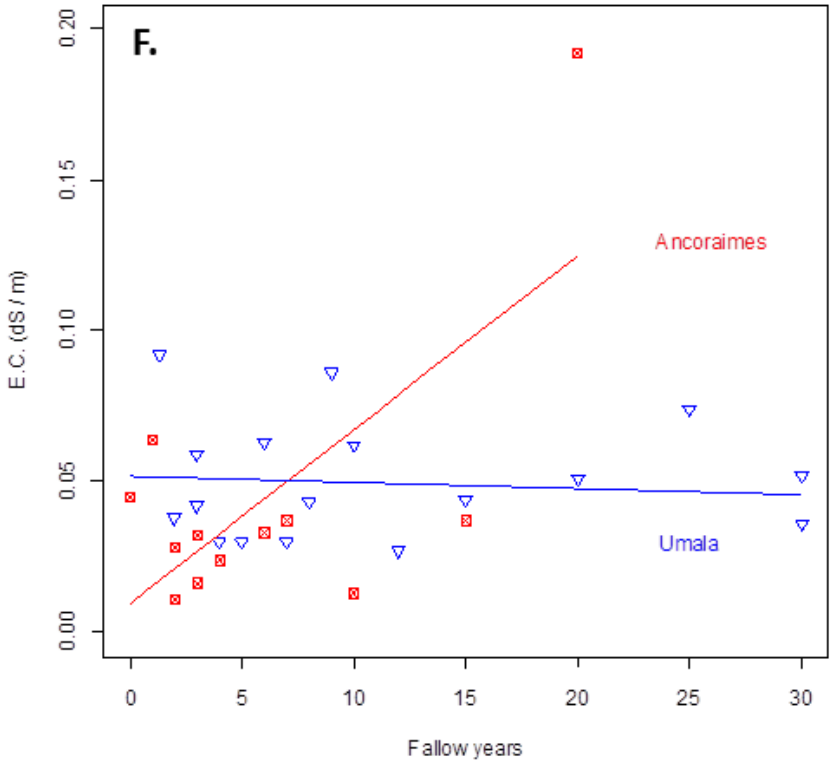
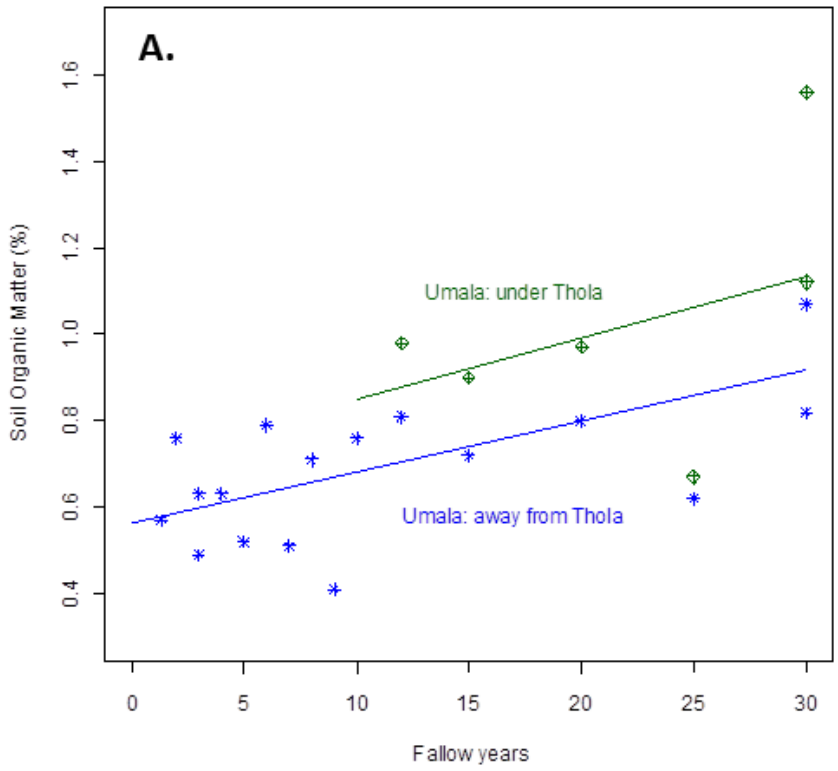
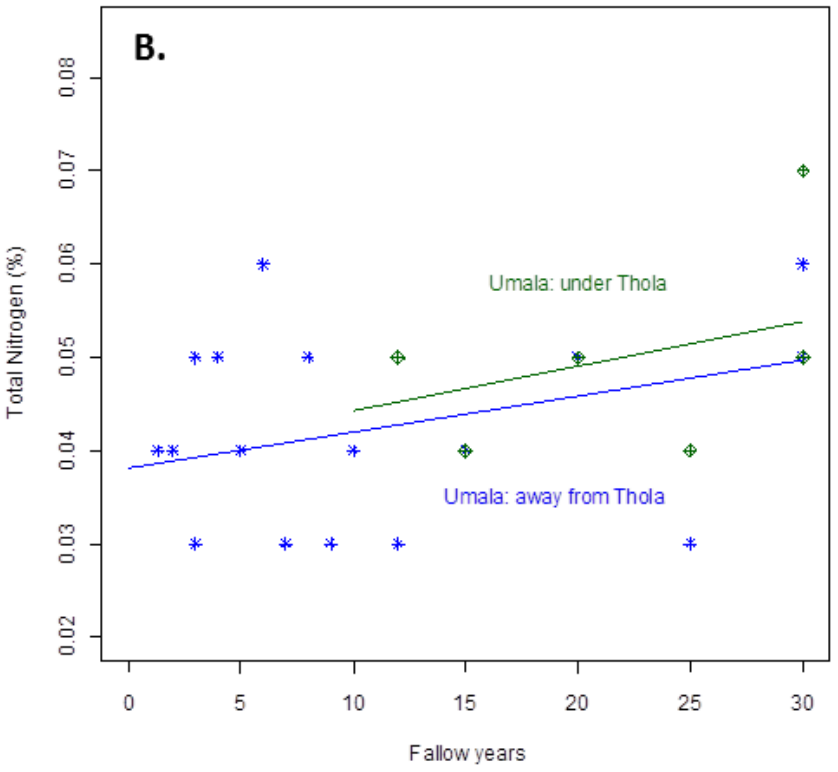
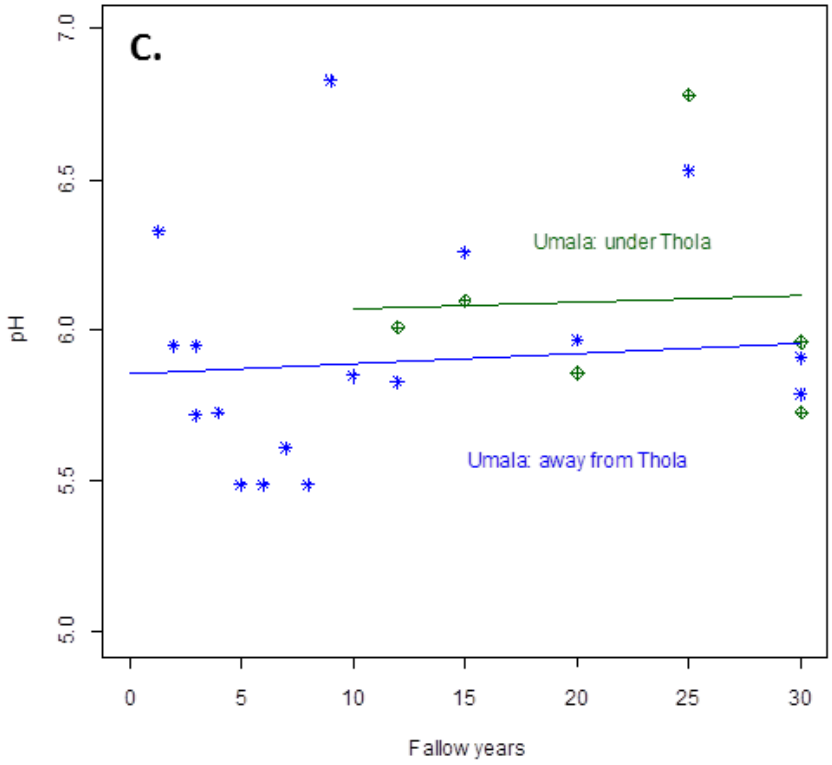

F.
E.C. (dS / m)
Fallow years
Ancoraimes
Umala
0.00
0.05
0.10
0.15
0.20
0
5
10
15
20
25
30

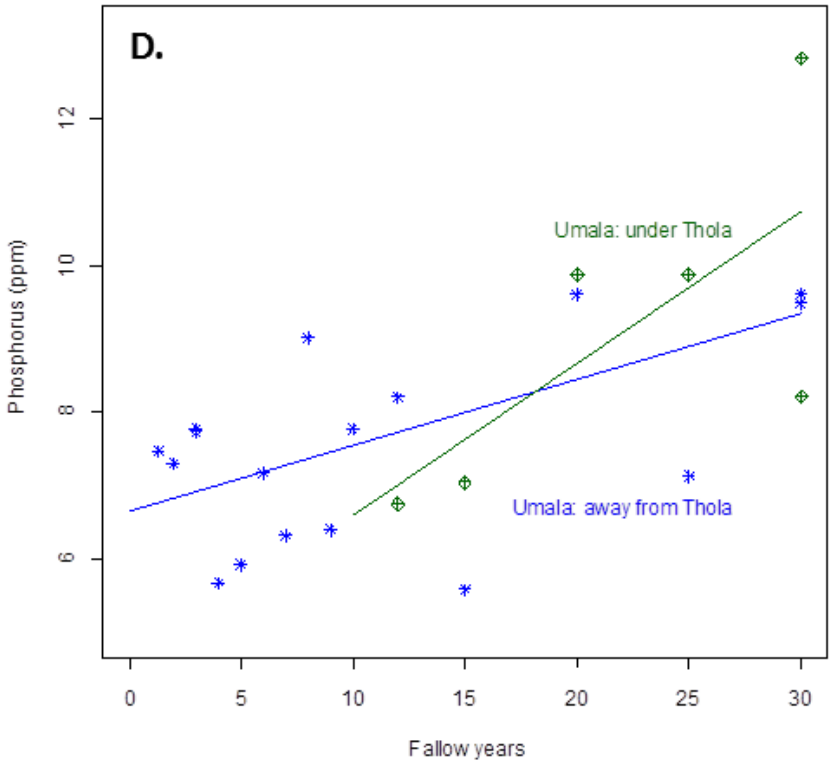
Figure 2. A comparison of the effects of fallow period duration and sampling under the common fallow-period shrub species (thola; Asteraceae), 'thola samples', versus sampling at least one m away from thola, 'non-thola samples'.

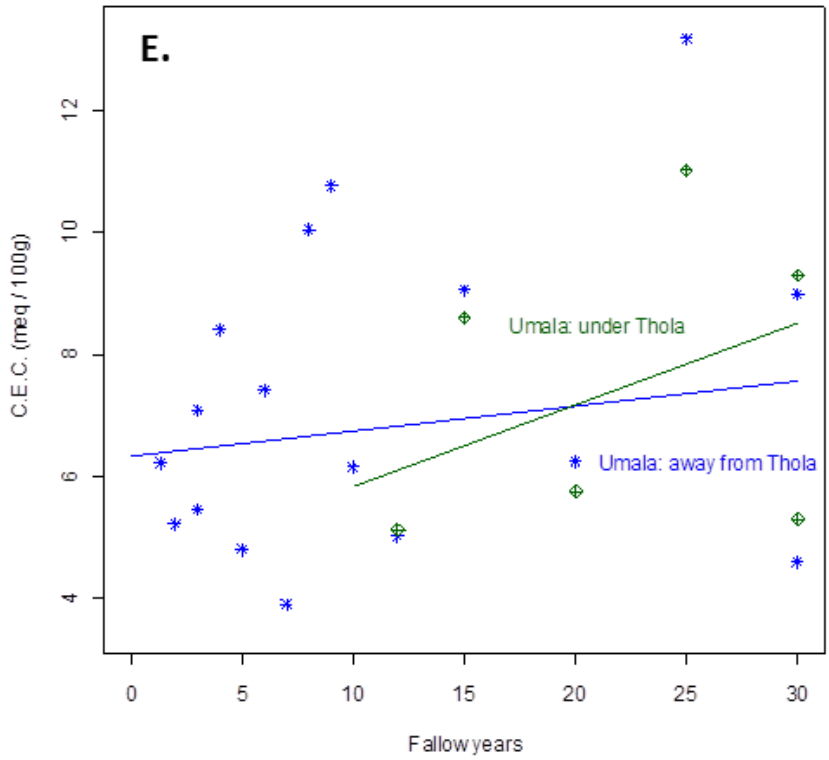
Samples in Umala were evaluated using linear regression (slope p-value is given with R^2) and paired t-tests. (**A**) SOM: non-thola (p=0.01, R^2=0.31), thola (p=0.34, R^2=0.03), Paired T-test (p = 0.01). (**B**) Total N: non-thola (p=0.37, R^2=0), thola (p=0.34, R^2=0.04), Paired T-test (p = 0.10). (**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**) CEC: 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.
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
0.03
0.04
0.05
0.06
0.07
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 (ppm)
12
10
8
6
0
5
10
15
20
25
30
Fallow years
Umala: under Thola
Umala: away from Thola


E.
C.E.C. (meq / 100g)
Fallow years
Umala: under Thola
Umala: away from Thola

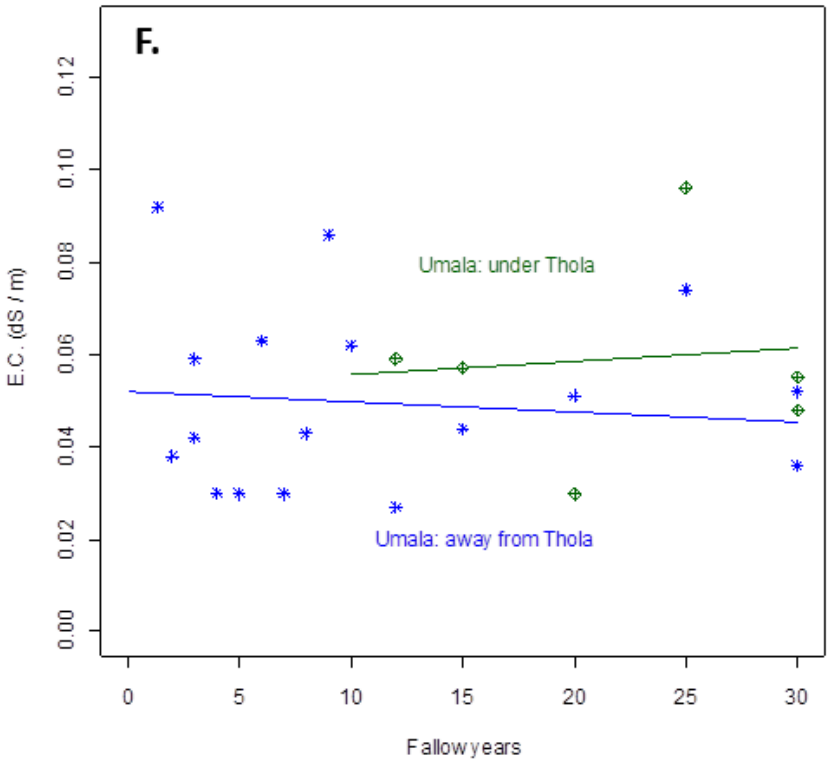
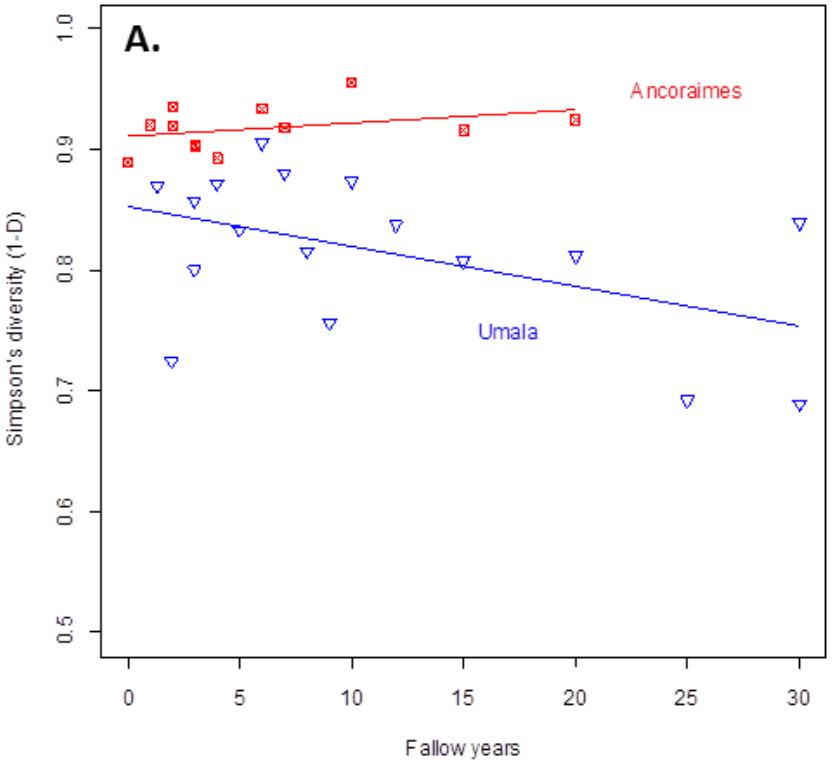
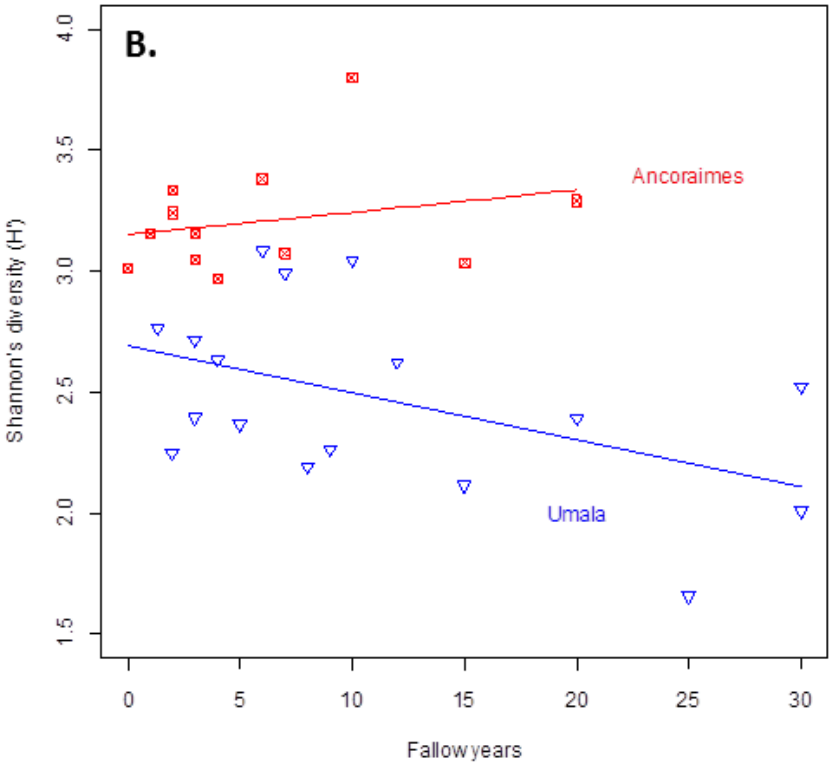

F.
E.C. (dS / m)
Fallow years
Umala: under Thola
Umala: away from Thola

Figure 3. 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**) Simpson's diversity (1-D): Ancoraimes (slope p=0.26, R^2=0.03), Umala (slope p=0.05, R^2=0.17), t-test comparing Umala and Ancoraimes ($p < 0.001$). (**B**) Shannon' s diversity (H'): Ancoraimes (slope p=0.44, R^2=0), Umala (p=0.05, R^2=0.18), t-test comparing Umala and Ancoraimes ($p < 0.001$). (**C**) Pielou's evenness (J): Ancoraimes (p=0.56, R^2=0), Umala (p =0.08, R^2=0.13), t-test comparing Umala and Ancoraimes ($p < 0.001$).


A.
Ancoraimes
Umala
Simpson's diversity (1-D)
Fallow years
0.5
0.6
0.7
0.8
0.9
1.0
0
5
10
15
20
25
30


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

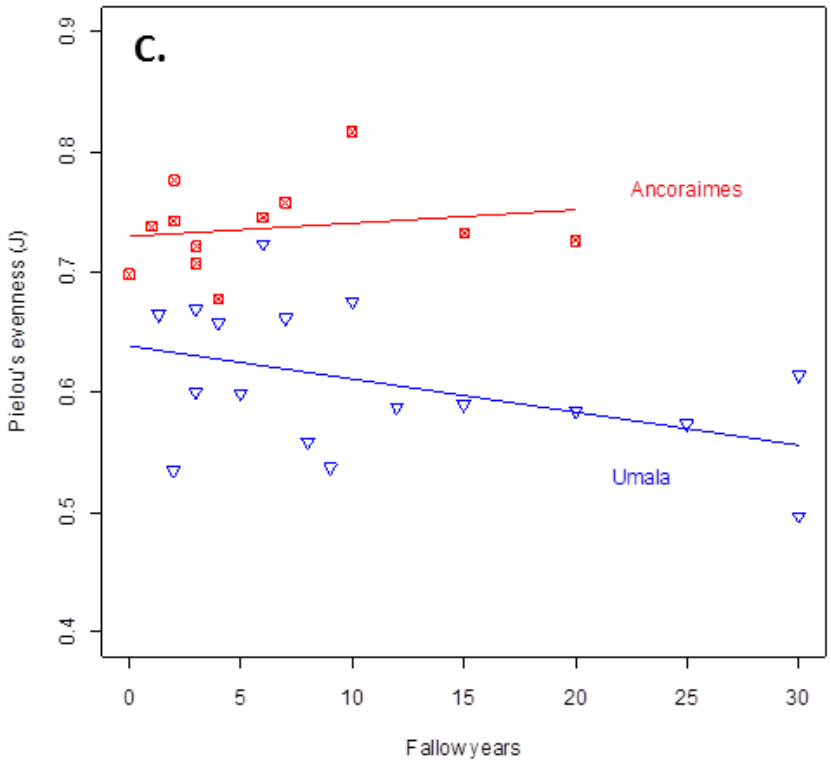
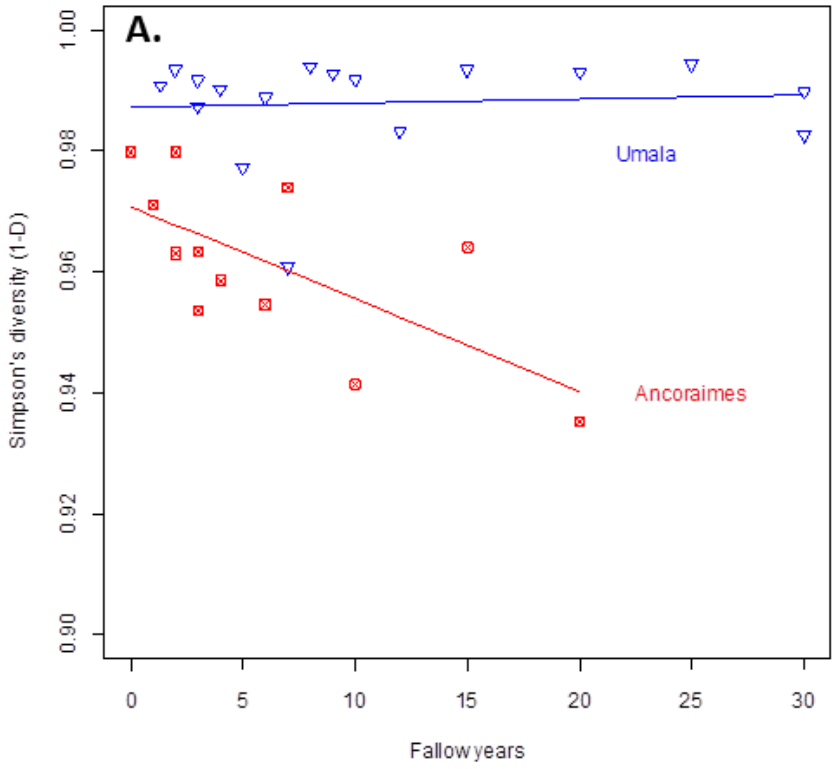
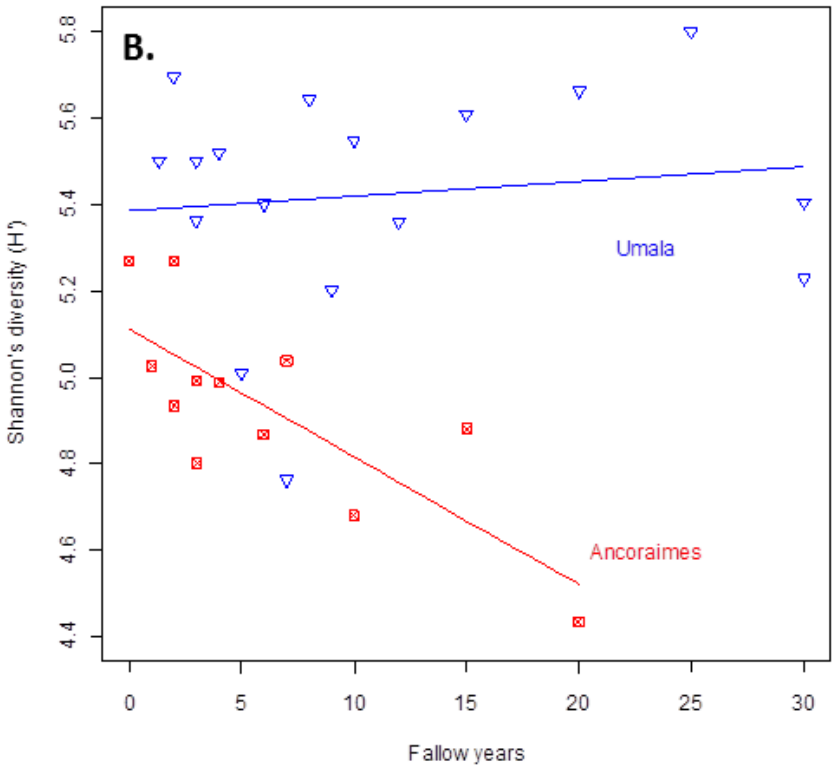

C.
Ancoraimes
Umala
Pielou's evenness (J)
Fallow years
0.4
0.5
0.6
0.7
0.8
0.9
0
5
10
15
20
25
30

Figure 4. 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**) Simpson's diversity (1-D): Ancoraimes ($p=0.01$, $R^2=0.39$), Umala ($p=0.75$,$R^2=0$), t-test comparing Umala and Ancoraimes ($p < 0.001$). (**B**) Shannon's diversity (H'): Ancoraimes ($p=0.002$, $R^2=0.56$), Umala ($p=0.63$, $R^2=0$), t-test comparing Umala and Ancoraimes ($p < 0.001$). (**C**) Pielou's evenness (J): Ancoraimes ($p=0.003$, $R^2=0.55$), Umala ($p=0.88$, $R^2=0$), t-test comparing Umala and Ancoraimes ($p < 0.001$).


A.
Simpson's diversity (1-D)
1.00
0.98
0.96
0.94
0.92
0.90
Umala
Ancoraimes
0
5
10
15
20
25
30
Fallow years


B.
Shannon's diversity (H')
5.8
5.6
5.4
5.2
5.0
4.8
4.6
4.4
Umala
Ancoraimes
0
5
10
15
20
25
30
Fallow years

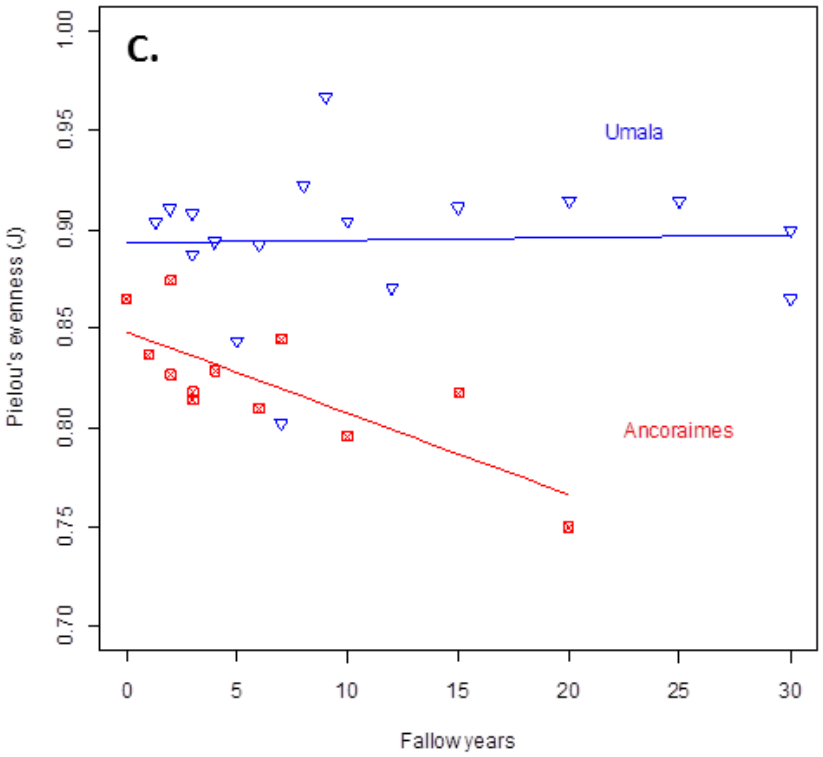

C.
Umala
Ancoraimes
Pielou's evenness (J)
Fallow years
1.00
0.95
0.90
0.85
0.80
0.75
0.70
0
5
10
15
20
25
30

Table 1. Soil edaphic properties at two municipalities in the Bolivian Altiplano, including soil organic matter (SOM), cation exchange capacity (CEC; milliequivalents per 100 g of soil), and electrical conductivity (EC; in deci-Siemens per m).

Municipality	Estimate	SOM (%)	Total N (%)	pH	P (ppm)	CEC (meq/100g)	EC (dS/m)
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	0.096
	Min.	0.41	0.03	5.49	5.59	3.9	0.027
Ancoraimes	Mean	2.76	0.15	5.16	8.82	6.17	0.04
	Max.	4.78	0.24	5.65	12	11.77	0.192
	Min.	2.17	0.11	4.74	5.72	4.54	0.011

Table 2. Statistics from analyses of the effects of fallow period length and the presence or absence of common fallow period shrub species (thola; Asteraceae) on soil edaphic properties at two municipalities in the Bolivian Altiplano.

The effect of fallow period length was evaluated using linear regression. The effect of the presence or absence of thola was evaluated using a paired t-test. Soil edaphic properties included soil organic matter (SOM), cation exchange capacity (CEC; milliequivalents per 100 g of soil), and electrical conductivity (EC; in deci-Siemens per meter).

Municipality	Effects	Statistics	SOM (%)	Total N (%)	pH	P (ppm)	CEC (meq/100g)	EC (dS/m)
Umala	Fallow (non-thola samples)	slope p-value	0.01	0.37	0.4	0.03	0.26	0.97
		R^2	0.31	0	0	0.24	0.02	0
	Fallow (thola samples)	slope p-value	0.34	0.34	0.93	0.11	0.58	0.88
		R^2	0.03	0.04	0	0.38	0	0
Ancoraimes	Fallow (non-thola samples)	slope p-value	0.03	0.05	0.003	0.68	0.05	0.02
		R^2	0.33	0.26	0.55	0	0.26	0.36
Both	Comparing Umala and Ancoraimes (non-thola samples)	Paired t-test (p-value)	<0.001	<0.001	<0.001	<0.05	0.23	0.68
Umala	Comparing paired thola and non-thola samples	Paired t-test (p-value)	0.01	0.10	0.72	0.36	0.45	0.23

Table 3. 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 is indicated.

Municipality	Field	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 4. Overall most frequent fungal phyla recovered in pyrosequencing of soils ('non-thola samples') from two municipalities in the Bolivian Altiplano.

The percentage of sequences grouped in each phylum for Umala and Ancoraimes.

Phylum	Umala (%)	Ancoraimes (%)
Ascomycota	84.96	66.03
Fungi_incertae_sedis	3.36	12.78
Basidiomycota	4.06	11.15
Chytridiomycota	0.10	0.10
Glomeromycota	0.09	0.06

Table 5. 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	44.62	Hypocreales	37.75
Pleosporales	30.51	Pleosporales	19.25
Mortierellales	3.36	Mortierellales	12.76
Sordariales	2.33	mitosporic_Filobasidiales	3.98
Capnodiales	1.70	Sordariales	3.29
Filobasidiales	1.58	Filobasidiales	2.25
mitosporic_Filobasidiales	1.06	Microascales	0.72
Helotiales	0.87	Capnodiales	0.59
Xylariales	0.75	Chaetothyriales	0.58
Phyllachorales	0.72	Helotiales	0.42
Microascales	0.51	Agaricales	0.38
Agaricales	0.30	Eurotiales	0.25

Table 6. 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	40.70	*Fusarium*	18.92
Didymella	27.74	*Didymella*	16.34
Mortierella	3.36	*Mortierella*	12.74
Cryptococcus	2.65	*Verticillium*	7.64
Cladosporium	1.68	*Cryptococcus*	6.41
Chaetomium	1.55	*Bionectria*	1.75
Paecilomyces	1.28	*Paraphoma*	0.93
Alternaria	1.17	*Preussia*	0.43
Verticillium	1.00	*Pyrenochaeta*	0.42
Penicillium	0.83	*Cladosporium*	0.41
Microdochium	0.75	*Penicillium*	0.23
Plectosphaerella	0.68	*Plectosphaerella*	0.18

Table 7. Overall most frequent bacteria 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	23.90	Proteobacteria	27.11
Actinobacteria	11.12	Firmicutes	15.94
Firmicutes	8.51	Verrucomicrobia	6.43
Acidobacteria	6.47	Actinobacteria	6.14
Verrucomicrobia	4.78	Acidobacteria	5.24
Bacteroidetes	3.05	Bacteroidetes	3.42
Planctomycetes	2.53	Planctomycetes	3.14
Chloroflexi	1.96	Chloroflexi	0.83
Cyanobacteria	0.48	Cyanobacteria	0.38
Crenarchaeota	0.40	TM7	0.24
Nitrospira	0.22	Nitrospira	0.07
OP10	0.11	OP10	0.07

Table 8. Overall most frequent OTUs assigned to bacterial genera (and the strain Gp4) 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	2.58	*Bradyrhizobium*	1.94
Streptomyces	1.39	*Gp4*	0.57
Bradyrhizobium	1.25	*Singulisphaera*	0.56
Methylobacterium	1.23	*Streptomyces*	0.43
Segetibacter	1.04	*Segetibacter*	0.34
Modestobacter	0.80	*Modestobacter*	0.31
Singulisphaera	0.36	*Paenibacillus*	0.22
Cystobacter	0.33	*Methylobacterium*	0.22
Pseudomonas	0.30	*Gemmata*	0.21
Actinoplanes	0.26	*Phenylobacterium*	0.15
Hymenobacter	0.20	*Actinoplanes*	0.12

Table 9. Fungal 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 $p < 0.05$ and $q < 0.05$.

	Umala			Ancoraimes		
Phylum	Slope p-value	Slope estimate	Intercept estimate	Slope p-value	Slope estimate	Intercept estimate
Basidiomycota	0.000	0.093	-3.848			
Fungi incertae sedis	0.002	0.088	-4.059	0.001	0.065	-2.484

Table 10. 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 estimate	Intercept estimate	Slope p-value	Slope estimate	Intercept estimate
Capnodiales	2E-15	0.285	-6.193	0.001	0.217	-6.578
Chaetothyriales	0.025	0.284	-8.787			
Filobasidiales	9E-12	0.253	-6.017			
Mortierellales	0.002	0.088	-4.059	0.001	0.063	-2.475
Hypocreales				0.008	0.035	-1.194

Table 11. 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 estimate	Intercept estimate	Slope p-value	Slope estimate	Intercept estimate
Cladosporium	2E-16	0.294	-6.261	3E-06	0.335	-7.856
Cryptococcus	8E-10	0.180	-4.907			
Didymella	0.008	0.030	-1.207			
Exophiala	0.016	0.388	-10.141			
Mortierella	0.001	0.090	-4.040	0.001	0.062	-2.466
Paecilomyces	0.014	0.102	-5.011			
Beauveria				0.000	0.221	-6.484
Cylindrocarpon				0.005	0.223	-7.013
Preussia				0.001	0.236	-7.021
Verticillium				0.019	0.057	-2.934
Volutella				2E-13	0.245	-5.461

Table 12. 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 $p < 0.05$ and $q < 0.05$. (No phyla significantly decreased in Umala).

	Ancoraimes		
Phylum	Slope p-value	Slope estimate	Intercept estimate
Basidiomycota	9E-06	-0.124	-1.434

Table 13. 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 intercept	Intercept estimate	Slope p-value	Slope intercept	Intercept estimate
Hypocreales	0.034	-0.020	-0.655			
Thelebolales	0.000	-0.363	-2.561			
Microascales				0.018	-0.435	-2.450
Pleosporales				0.005	-0.054	-1.299
Sordariales				0.009	-0.137	-2.620
Tremellales				0.000	-0.383	-1.108

Table 14. 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 intercept	Intercept estimate	Slope p-value	Slope intercept	Intercept estimate
Alternaria	0.014	-0.129	-3.565			
Fusarium	0.001	-0.035	-0.108			
Paraphoma	0.050	-0.150	-4.164			
Plectosphaerella	0.043	-0.137	-4.019			
Thelebolus	0.000	-0.366	-2.536			
Verticillium	0.004	-0.170	-3.456			
Bionectria				0.004	-0.270	-2.522
Chaetomidium				0.004	-0.167	-2.551
Cryptococcus				0.010	-0.088	-2.213
Didymella				0.000	-0.075	-1.337
Trichosporon				0.000	-0.357	-1.289

Table 15. 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 $p < 0.05$ and $q < 0.05$.

	Umala			Ancoraimes		
Phylum	Slope p-value	Slope estimate	Intercept estimate	Slope p-value	Slope estimate	Intercept estimate
Actinobacteria	8E-10	0.057	-2.500			
Chloroflexi	0.015	0.052	-4.327	0.001	0.135	-5.672
Euryarchaeota	0.001	0.332	-10.164			
OP10	0.003	0.217	-8.446			
Proteobacteria	5E-16	0.056	-1.561	4E-24	0.087	-1.859
Bacteroidetes				0.037	0.048	-3.672

Table 16. 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 $p < 0.05$ and $q < 0.05$.

	Umala			Ancoraimes		
Genus	Slope p-value	Slope estimate	Intercept estimate	Slope p-value	Slope estimate	Intercept estimate
Actinoplanes	0.024	0.126	-7.003	0.036	0.197	-8.011
Cystobacter	0.049	0.093	-6.361			
Herpetosiphon	0.027	0.305	-10.505			
Iamia	0.000	0.329	-9.893			
Phenylobacterium	0.001	0.238	-8.499			
Rubellimicrobium	0.000	0.320	-9.352			
Sorangium	0.005	0.172	-7.634	0.045	0.308	-10.024
Streptomyces	0.000	0.131	-5.229			
Parachlamydia				0.026	0.311	-9.871
Pseudomonas				0.000	0.272	-5.367
Pseudonocardia				0.003	0.334	-9.650
Xanthomonas				0.028	0.260	-9.054

Table 17. 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 $p < 0.05$ and $q < 0.05$.

	Umala			Ancoraimes		
Phylum	Slope p-value	Slope estimate	Intercept estimate	Slope p-value	Slope estimate	Intercept estimate
Bacteroidetes	3E-07	-0.104	-2.757			
Chlamydiae	0.012	-0.436	-4.273			
Crenarchaeota	0.000	-0.224	-4.015			
Firmicutes	2E-89	-0.324	-0.282	0.000	-0.073	-1.403
Verrucomicrobia	0.044	-0.029	-2.762	0.004	-0.060	-2.389
Planctomycetes				0.018	-0.070	-3.028

Table 18. 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 $p < 0.05$ and $q < 0.05$.

	Umala			Ancoraimes		
Genus	Slope p-value	Slope estimate	Intercept estimate	Slope p-value	Slope estimate	Intercept estimate
Bacillariophyta	0.025	-0.352	-4.746			
Gp4	0.002	-0.062	-3.166			
Gp5	0.049	-0.250	-5.177			
Paenibacillus	0.000	-0.290	-1.694	0.038	-0.407	-3.765
Segetibacter	0.000	-0.166	-3.419			
Dokdonella				0.002	-0.712	-1.821
Gp2				0.000	-0.245	-3.021
Gp3				0.022	-0.104	-3.571
Streptomyces				0.027	-0.243	-4.028

Table 19. The most frequent 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. Bold font indicates which had higher frequency, non-thola or thola samples.

		Mean frequency (%)	
Phylum	p-value	Non-thola samples	Thola samples
Basidiomycota	1.12E-05	2.06	**4.40**
Chytridiomycota	NA	0.03	**0.18**
Fungi incertae sedis	2.6E-04	**5.74**	3.76
Glomeromycota	NA	**0.17**	0.03

Table 20. 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. Bold font indicates which had the higher frequency, non-thola or thola samples.

		Mean frequency (%)	
Order	p-value	Non-thola samples	Thola samples
Agaricales	0.991	0.40	**0.54**
Botryosphaeriales	NA	0.00	**0.47**
Cantharellales	NA	0.00	**0.04**
Capnodiales	0.000	0.49	**2.22**
Chaetothyriales	0.119	**0.35**	0.33
Coniochaetales	0.781	**0.10**	0.09
Cystofilobasidiales	NA	0.00	**0.03**
Dothideales	0.995	**0.28**	0.23
Dothideomycetes	NA	0.00	**0.03**
Entylomatales	NA	0.07	**0.14**
Erythrobasidiales	NA	0.00	**0.18**
Eurotiales	0.117	**0.89**	0.59
Filobasidiales	0.000	0.53	**2.20**
Glomerales	NA	**0.08**	0.03
Helotiales	0.053	0.25	**0.62**
Microascales	0.079	**0.43**	0.17
mitosporic_Ascomycota	NA	**0.04**	0.03
mitosporic_Filobasidiales	0.100	**1.00**	0.58
Mortierellales	0.000	**5.74**	3.76
Onygenales	NA	0.00	**0.03**
Ophiostomatales	NA	**0.04**	0.03

Phyllachorales	0.389	**0.54**	0.42
Rhizophydiales	NA	0.00	**0.03**
Saccharomycetales	NA	0.00	**0.03**
Sordariales	0.003	**1.90**	0.70
Spizellomycetales	NA	0.03	**0.15**
Thelebolales	NA	**0.37**	0.24
Tilletiales	NA	0.00	**0.06**
Tremellales	NA	0.00	**0.43**
Ustilaginales	NA	**0.04**	0.03
Xylariales	0.199	**0.46**	0.26

Table 21. 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. Bold font indicates which had the higher frequency, non-thola or thola samples.

		Mean frequency (%)	
Genus	p-value	Non-thola samples	Thola samples
Alternaria	0.12	0.20	**0.45**
Bionectria	0.18	0.24	**0.44**
Cercophora	0.03	**0.58**	0.12
Chaetomium	0.03	**0.78**	0.35
Cladosporium	0.00	0.46	**1.41**
Cryptococcus	0.06	1.47	**2.77**
Didymella	0.02	**31.80**	26.28
Fusarium	2E-01	42.05	**43.92**
Microdochium	0.20	**0.46**	0.26
Mortierella	0.00	**5.28**	3.65
Paecilomyces	0.00	**1.12**	0.28
Paraphoma	0.03	0.35	**0.89**
Penicillium	0.07	**0.48**	0.20
Plectosphaerella	0.48	**0.47**	0.39
Preussia	0.99	0.09	**0.10**
Stagonospora	0.10	0.14	**0.34**
Verticillium	0.01	**1.07**	0.50

Table 22. 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. Bold font indicates which had the higher frequency, non-thola or thola samples.

		Mean frequency (%)	
Phylum	p-value	Non-thola samples	Thola samples
Bacteroidetes	2E-07	2.28	**3.85**
Chlamydiae	0.139	0.08	**0.17**
Chloroflexi	0.031	**2.10**	1.58
Crenarchaeota	0.430	**0.43**	0.34
Cyanobacteria	1E-05	**0.66**	0.12
Deinococcus-Thermus	NA	**0.06**	0.02
Euryarchaeota	NA	**0.07**	0.02
Gemmatimonadetes	NA	0.01	**0.03**
Nitrospira	0.178	**0.21**	0.11
OP10	0.471	**0.13**	0.09
Planctomycetes	0.391	**2.62**	2.40
TM7	0.150	0.09	**0.18**
Verrucomicrobia	8E-05	**4.46**	3.12

Table 23. 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. Bold font indicates which had the higher frequency, non-thola or thola samples.

		Mean frequency (%)	
Genus	p-value	Non-thola samples	Thola samples
Actinoplanes	0.449	0.18	**0.24**
Amycolatopsis	0.756	**0.09**	0.08
Anaeromyxobacter	NA	**0.06**	0.03
Aquicella	NA	0.00	**0.03**
Bacillariophyta	NA	**0.06**	0.01
Belnapia	0.357	**0.19**	0.12
Bradyrhizobium	0.095	1.10	**1.42**
Burkholderia	NA	0.02	0.02
Chitinophaga	NA	0.02	0.02
Chryseobacterium	NA	0.02	0.02
Cystobacter	0.185	0.27	**0.40**
Deinococcus	NA	**0.06**	0.02
Devosia	NA	0.00	**0.03**
Dokdonella	NA	**0.05**	0.03
Dyadobacter	NA	0.00	**0.03**
Ferruginibacter	NA	0.00	**0.03**
Gemmata	0.836	**0.16**	0.14
Gemmatimonas	NA	0.01	**0.03**
Haloferula	NA	0.01	**0.02**
Halomonas	NA	0.00	**0.01**
Herpetosiphon	NA	**0.06**	0.03

Hymenobacter	0.491	**0.14**	0.09
Iamia	NA	**0.09**	0.03
Kineosporia	NA	0.01	**0.06**

Supplemental Material

Figure 5. The Bolivian Altiplano (highland-plateau) region and the location of the two municipalities Umala and Ancoraimes.

Figure 6. 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.

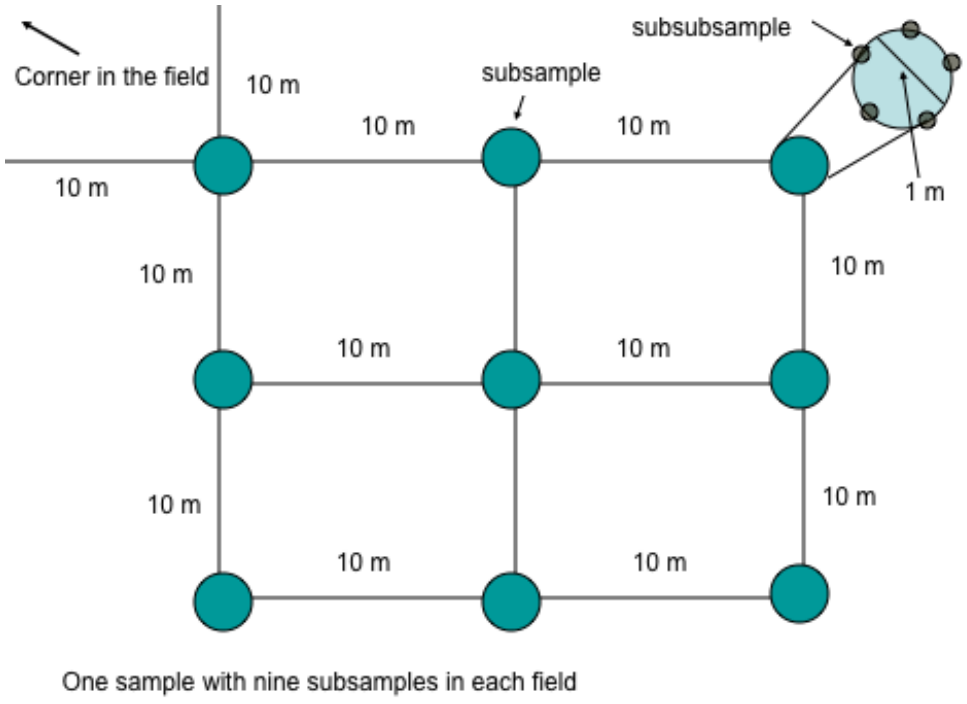
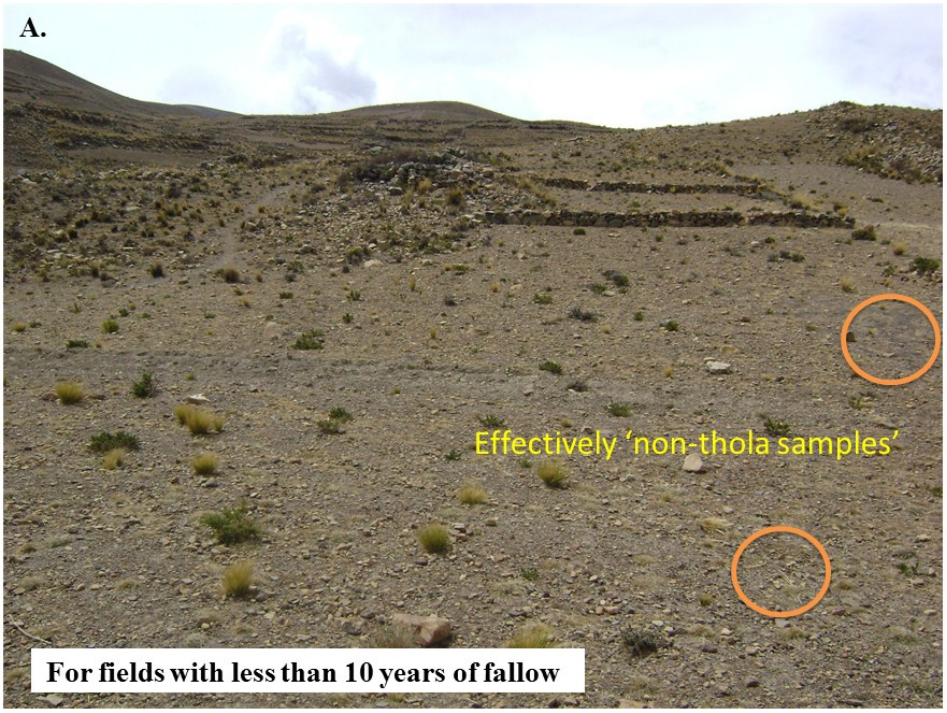
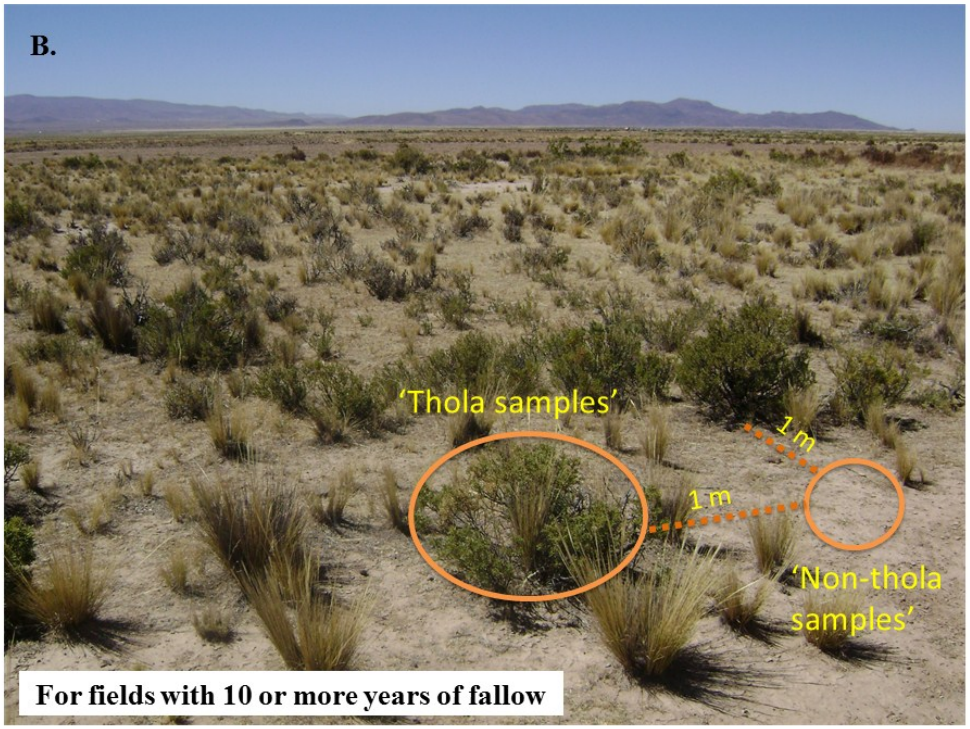


Figure 7. Sampling fields in the two municipalities of the Bolivian Altiplano (Umala and Ancoraimes) considering the presence of the fallow period shrub species (thola: Asteraceae).

A. For fields with less than 10 years of fallow and scarce presence of thola, 9 soil subsamples were effectively 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.
Effectively 'non-thola samples'
For fields with less than 10 years of fallow


B.
'Thola samples'
1 m
1 m
'Non-thola samples'
For fields with 10 or more years of fallow

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