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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.

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 in which the non-crop or dormant species are allowed to re-establish 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 re-vegetation 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).

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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 only reveal 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), 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 due to 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 non-culturability 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, where supplemental figures are available at http://hdl.handle.net/2097/15198), 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 120–160 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