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Soil aggregate stability increase is strongly related to fungal community succession along an abandoned agricultural field chronosequence in the Bolivian Altiplano

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Summary

1. Soil aggregate stability is an important ecosystem property which deteriorates overtime due to agricultural practices. The cessation of cultivation allows the potential recovery of soil aggregate binding agents such as soil micro-organisms and biochemical properties. Consequently, an increase in soil aggregate stability is expected. However, this outcome is difficult to predict because the response of each individual soil component and its contribution to soil stability varies.

2. This study utilized a chronosequence of 12 ex-arable fields in the Bolivian Altiplano, representing six soil ages of abandonment after cessation of potato cultivation, to examine whether soil aggregate stability increases after abandonment and the extent to which changes in soil bacterial and fungal community composition and soil chemical properties are involved in stability recovery.

3. Fields with the longest time since disturbance (15 and 20 years) have a greater proportion of water-stable aggregates than more recently abandoned fields (1 and 3 years) and exhibit larger differences in bacterial and fungal composition. Total N, NH_4^+, C and organic matter also increased with time since the last intensive agricultural practice.

4. Water-stable aggregates were strongly correlated with soil fungal community composition. Analysis of covariance is also consistent with the soil fungal community being an important mediator of the recovery of aggregate stability.

5. *Synthesis and applications.* Soil aggregate stability increased by 50% over the 20 years following disturbance. This recovery was associated with shifts in soil fungal community composition, as is consistent with fungal mediation of this recovery. Land management strategies focusing on restoration of the soil fungal community may enhance soil aggregate stability, a key aspect for soil conservation, restoration, sustainability of agroecosystems and erosion prevention.

Key-words: Altiplano Bolivia pampa, arbuscular mycorrhiza, fungi, land abandoned chronosequence, soil aggregate stability, soil micro-organism

Introduction

Soil aggregate stability is an important ecosystem property as it is strongly related to soil services such as carbon storage (Balabane & Plante 2004; John *et al.* 2005), organic matter (OM) stabilization (Six *et al.* 1998), water-holding capacity (Shukla *et al.* 2003) and resistance to erosion (Teixeira & Misra 1997; Barthès & Roose 2002). The enhancement of soil stability is relevant for agroecosystems sustainability, prevention of erosion, restoration of disturbed land and global climate change.

Soil aggregate stabilization is often deteriorated by agricultural practices by directly breaking the soil particles or

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indirectly by the disruption of potential aggregate binding agents. For instance, tillage has been implicated in degradation of aggregate stability (Six, Elliott & Paustian 1999; Wright, Starr & Paltineanu 1999; Pikul *et al.* 2007), as well as particulate OM (Beare, Hendrix & Coleman 1994). Tillage has also been found to alter soil microbial community dynamics (Kabir *et al.* 1997; Jansa *et al.* 2002; Wang *et al.* 2010) and to modify arbuscular mycorrhizal fungi (AMF) density and composition (Jansa *et al.* 2002; Yuan-Ying & Liang-Dong 2007) which can enhance soil aggregate stability. Moreover, the reduction in soil aggregate stability could be a long-term effect, as it was still evident 10–20 years after cessation of tillage in North American grasslands (Duchicela *et al.* 2012).

Cessation of cultivation allows soil to recover from some of the negative effects of disturbance. Studies of abandoned agricultural fields suggest that soil OM, carbon and nitrogen increase after cessation of cultivation in both temperate (Knops & Tilman 2000; Du *et al.* 2007; Holtkamp *et al.* 2011) and lowland tropical areas (Reiners *et al.* 1994; Hughes, Kauffman & Jaramillo 1999; Toby & Compton 2003). Such responses are clearly linked with changes in soil microbial communities (Zak *et al.* 1990). Following cessation of disturbance, the soil microbial community has been found to shift towards a less bacterial- and more fungal-dominated food web (Maharning, Mills & Adl 2009), particularly dominated by AMF (Allison *et al.* 2005). However, the recovery of soil properties negatively impacted by cultivation appears to be slow (Compton *et al.* 1998; Conant, Six & Paustian 2004; Jangid *et al.* 2010), and the time of response could vary with the specific chronosequence and with the particular soil property examined (Spohn & Giani 2011). Moreover, published studies focus on a narrow range of ecosystem types and very few assess soil aggregate stability. Therefore, it is difficult to predict if recovery of a particular soil property following abandonment would result in an improvement of soil aggregate stabilization in a given ecosystem.

In this study, we focus on the recovery of aggregate stability following agricultural practice in the tropical montane ecosystem of the Andes (Páramo ecosystem). To our knowledge, no previous work has focused on recovery of aggregate stability in this ecosystem. Therefore, we asked the following questions: (i) Does soil aggregate stability increase over time in ex-arable land in the Páramo? (ii) To what extent are changes in soil aggregate stability related to abiotic and biotic soil properties?

We hypothesized that water-stable aggregates will increase over time and that this increase will be correlated with soil microbial community composition. To address these questions, we examined changes in soil aggregate stability, soil fungal and bacteria communities and soil chemical properties in 12 ex-arable fields representing six soil ages comprising a chronosequence of time since abandonment of potato cultivation in the Bolivian Altiplano.

Materials and methods

STUDY SITE

The study area is located in Bolivia, Department of La Paz, Camacho province at 3850 m.a.s.l. This area corresponds to the lowland alpine belt of the Andes and is locally known as Páramo. The area mean annual precipitation is approximately 629 mm, and the relative humidity is 60%. Daily temperature average is 7·4 °C and varies only slightly throughout the year reaching a maximum of 15·7 °C and a minimum of −2 °C. The soils are classified as vertisols and are acidic. Long-fallow agricultural systems are used for the production of potatoes *Solanum tuberosum* L. and oat *Avena sativa*.

Using the Foundation for the Promotion and Research of Andean Products (PROINPA) data base, we have been able to identify a series of 12 plots with similar land use history, geological and topographical characteristics. The plots differ only in time since abandonment, corresponding to six ages of cessation of cultivation. This allows us to explore the response of soil properties over time after abandonment from agriculture. The size of the plots ranged from 7 to 10 ha, covering a total area up to 50 ha. The average distance between plots is 672·105 m. Table 1 presents a summary of the characteristics of the sites in this study.

SOIL SAMPLING

Soil samples were collected in December 2009. The vegetation cover and litter layer were pushed aside prior to sampling. At each location, three subsamples each with a depth of 10 cm were taken by gently hammering a sterile PVC core into the ground to minimize compression during soil collection. All sampling tools were sterilized using 70% ethanol. The moist soil samples were placed into sterile Ziploc® bags which were then placed into a plastic leak-proof container for shipment for analysis in the USA.

One replicate of each sample was directed to: (i) the Department of Biology at Indiana University for soil aggregate stability analysis; (ii) the Soil and Plant Analysis Laboratory at the University of Wisconsin–Madison for determination of soil texture, pH, total OM, total organic carbon (C), total nitrogen (N), ammonium (NH_4^+), nitrate (NO_3^-), phosphorus (P) and potassium (K); (iii) and to the Department of Crop and Soil Sciences at Cornell University for molecular analysis of the soil microbial community. Samples were frozen for storage prior to molecular analyses, and replicates for chemical and soil aggregate analysis were stored at 4 °C. Care was taken throughout the sampling and shipment of the samples to the laboratory to avoid cross-contamination and disturbance of the samples that might influence the microbial community and aggregate stability.

SOIL WATER-STABLE AGGREGATES AND CHEMICAL ANALYSIS

The relative amount of soil aggregates that resist being broken by water is defined as water-stable aggregates (WSA). The proportion of WSA was estimated following the method of Kemper & Rosenau (1986) where each soil sample was separated into six aggregate groups (<0·25 mm, 0·25–0·5 mm, 0·5–1 mm, 1–2 mm, 2–4 mm and >4 mm) using a rotary sieve for a 2-min cycle. Three technical replicates of approximately 7 g of each aggregate group were separated. Roots, seeds and rocks were handpicked

Table 1. Characteristics of chronosequence fields. Field code, name, former crop, time since abandonment and location across the abandoned agricultural field chronosequence in the Bolivian Altiplano

Field code	Field name	Former crop	Time since abandonment (year)	Altitude (msnm)	Latidude (N)	Longitud (WO)
A	Pisakmankaña	Potato	1	4097	15° 31′ 11·3″	69° 03′ 11·3″
B	Pisakmankaña 2	Potato	1	4093	15° 31′ 12·8″	69° 03′ 11·9″
C	Pisakmankaña 3	Potato	1	4096	15° 31′ 10·0″	69° 03′11·2′
D	Kankauani 1	Potato-oat	3	4003	15° 31′ 35·3″	69° 03′ 01·9″
E	Kankauani 2	Potato-oat	3	4007	15° 31′ 31·6″	69° 03′ 00·7″
F	Kankauani 3	Potato-oat	3	4004	15° 31′ 35·6″	69° 06′ 00·2″
G	Sanvia Kuchu	Potato	9	3968	15° 31′ 49·0″	69° 03′ 36·5″
H	Tupu Pampa	Potato	9	3966	15° 31′ 49·6″	69° 03′ 33·6″
I	Acintajwira	Potato	10	3994	15° 31′ 40·4″	69° 03′46·8″
J	Wiscallacota Pampa	Potato-oat	15	4127	15° 30′ 38·3″	69° 02′ 55·7″
K	Haracha Pampa	Potato-oat	15	4143	15° 30′ 41·1″	69° 02′ 59·0″
L	Kuchuyu	Potato	20	4195	15° 30′ 12·2″	69° 03′ 04·7″

from each fraction, and the remaining sample was placed on a 250-mm sieve. The soil fractions were hydrated by locating the sieve above a water layer and allowing water to move through the sieve by capillary action for 24 h. After the fractions were moistened on the sieves, they were agitated in distilled water in a sieving apparatus for two cycles of 10 min each at 35 cycles min^{-1}. Materials that passed through the sieves were considered to be the water-unstable fraction. Materials that remained on the sieves were soil WSA and sand particles.

To separate the WSA from the sand, the sieves were passed through a 1 M NaOH solution at 35 cycles min^{-1} for two cycles of 10 min each. The sand retained in each sieve was rinsed with distilled water and the WSA–NaOH solution was collected in stainless steel evaporation pans. The pans and the sieves were oven-dried at 110 °C and weighed. The weights were corrected for the weight of the sodium solution fraction that could remain in the samples. The WSA fraction was calculated as the mass of aggregates that remained after wet sieving as a percentage of the initial mass of soil.

Soil pH was determined in water using a 1 : 1 soil : water ratio, and soil texture was measured by the Bouyoucos hydrometer method (Bouyoucos 1962). P and K were extracted with 0·03 N NH_4 F in 0·025 N HCl (Bray & Kurtz 1945). Extracted K was analysed via atomic absorption, and P was determined by colorimetry (Varian, Cary 50 UV-Visible spectrophotometer). Nitrate and NH_4^+ were extracted with a 2 N KCl solution and determined on a Lachat automated ion analyzer (QuikChem Method 12-107-04-1-B; Lachat Instruments). Total organic carbon and total nitrogen were determined by dry combustion using a LECO CNS-2000 analyzer. Total OM was determined by the loss-on-ignition method (Schulte & Hopkins 1996).

SOIL MICROBIAL COMMUNITY

Terminal restriction fragment length polymorphisms

Genomic DNA was extracted from 0·25 g of each of three technical replicates of each soil sample using the MoBio PowerSoil™ DNA extraction kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA) according to the manufacturer's instructions. Extracted DNA was then quantified against a calf thymus DNA standard curve in an ethidium bromide (EtBr) solution using an EC3 Imaging System (UVP Bioimaging Systems; UVP LLC, Upland, CA, USA) and Quantity One™ software (Applied Biosystems, Foster City, CA, USA). DNA extracts were diluted with nuclease-free water to approximately 3–5 ng μL^{-1} and immediately frozen at −20 °C until subsequent analysis.

Bacterial 16S rRNA genes were targeted for amplification from environmental DNA extracts by polymerase chain reaction (PCR) using the fluorescently labelled forward primer 27f (5′-[6FAM] AGA GTT TGA TCC TGG CTC AG-3′) and the unlabelled reverse primer 1492r (5′-GGT TAC CTT GTT ACG ACT T-3′) (Integrated DNA Technologies, Coralville, IA, USA) (Moeseneder *et al.* 1999). Duplicate reactions of each sample were amplified using a thermal cycler PTC 100 (MJ Research, Waltham, MA, USA) and the following programme: 5 min at 94 °C, followed by 27 cycles of 94 °C for 45 s, 56 °C for 45 s and 72 °C for 1 min, and a final extension step at 72 °C for 10 min. The final concentration of the reagents for each 50 μL reaction was as follows: 0·050 U *Taq* polymerase (Applied Biosystems), 1× PCR buffer, 2 mM $MgCl_2$, 0·2 μM deoxy-nucleotide triphosphates (dNTPs), 0·1 μg μL^{-1} bovine serum albumin (BSA), 0·1 μM of each primer, 33·0 μL nuclease-free water (Promega, Madison, WI, USA) and 5 μL of DNA template at 3–5 ng μL^{-1} DNA template.

The fungal internal transcribed spacer (ITS) region was amplified using the fluorescently labelled forward primer ITS1f (50-[6FAM] CTT GGT CAT TTA GAG GAA GTA A-30) and the unlabelled reverse primer ITS4r (50-TCC TCC GCT TAT TGA TAT GC-30) (Integrated DNA Technologies) (Bruns, White & Taylor 1991). Duplicate reactions of each sample were amplified using an PTC 100 thermal cycler and the following programme: 5 min at 94 °C; followed by 30 cycles of 94 °C for 30 s, 51 °C for 45 s and 72 °C for 45 s, and a final extension step at 72 °C for 10 min. The final concentration of the reagents for each 50 μL reaction was as follows: 0·1 U μL^{-1} *Taq* polymerase (Applied Biosystems), 1× PCR buffer, 3 mM $MgCl_2$, 0·6 mM dNTPs, 0·1 μg μL^{-1} of BSA, 0·2 μM of each primers, 27·5 μL nuclease-free water (Promega) and 5 μL of DNA template at 3–5 ng μL^{-1}.

Restriction enzyme digests of bacterial and fungal amplicons final concentration were as follows: 0·25 U mL^{-1} *Sau96I* enzyme (New England Biolabs, Ipswich, MA, USA), 1× buffer supplied with the enzyme, 0·1 μg μL^{-1} of BSA, 8·5 μL nuclease-free water and 12·5 μL of amplified sample DNA at 25 ng μL^{-1}. Restriction digestion was carried out in a PTC 200 thermal cycler at 37 °C for 4·5 h with a final step of 70 °C for 15 min to heat inactivate the enzymatic reaction. Complete digestion of the DNA was verified by inspecting digested products run on a 1·5% agarose

gel stained with EtBr and visualized using an EC3 Imaging System (UVP Bioimaging Systems; UVP LLC). Digested DNA was purified, lyophilized and resuspended in a 10 μL mix containing 9·85 μL of formamide and 0·15 μL of LIZ 500 size standard (Applied Biosystems). Terminal fragment-size analysis was performed using an Applied Biosystems Automated 3730XL DNA Analyzer (Applied Biosystems) in conjunction with the Genemapper Software (Applied Biosystems) at Cornell University's Life Sciences Core Laboratories Center, Ithaca, NY, USA.

T-RFLP DATA ANALYSIS

T-RF length profiles for soil bacteria and soil fungi were loaded into the online T-RFLP processing software *T-REX* (Culman *et al.* 2009) for noise filtering and peak alignment. In brief, *T-REX* uses the approach outlined by Abdo *et al.* (2006) to distinguish background noise from true peaks, where true peaks are identified as those whose height or area exceeds the standard deviation computed for all peaks and multiplied by the factor specified. The peak area standard deviation multiplier used in this case was 1 (the standard *T-REX* default). The peak alignment was based on the approach taken by the software program T-Align (Smith *et al.* 2005) and was utilized with a clustering threshold of 0·5. This method is often performed to account for T-RF length drift (Kaplan & Kitts 2003; Marsh 2005) and the version of the algorithm implemented here allows more than one peak from the same sample to be assigned to the same T-RF length bin. Of each sample in the study we did three technical replicates. Of 36 samples, we found a total of 301 individual and different T-RF peaks with an average of 121 T-RFs per sample. The minimum number of T-RFs in a sample was 58, and the maximum number of T-RFs in a sample was 169.

After noise filtering and peak alignment, soil microbial T-RFLP pattern discrimination was determined by nonmetric multidimensional scaling (NMS) analysis applied using the Jaccard distance measure and the Medium Autopilot mode in PC-Ord (MjM Software Design, Gleneden Beach, OR, USA). This mode specifies: (i) Maximum number of iterations = 200, (ii) Instability criterion = 0·0001, (iii) Starting number of axes = 4, (iv) Number of real runs = 15 and (v) Number of randomized runs = 30. Unlike other commonly used ordination techniques (e.g. principal components analysis), NMS does not assume a linear relationship among ecological variables (Culman *et al.* 2008). This analysis allows virtually any distance measure to be used in the construction of the similarity matrix and is currently considered the most appropriate analysis for T-RFLP data sets (Rees *et al.* 2004). We repeated the analysis five times to confirm that we obtained consistent results.

STATISTICAL ANALYSIS

Prior to analysis of the WSA, the replicate measures of the proportion of WSA of the different fractions were averaged by site and the data were logistically transformed to improve the homogeneity of variance (Zuur *et al.* 2009). The values of the NMS axes for the bacterial and fungal communities were averaged across the three technical replicates from each site.

We used a general linear model to assess the dependence of the WSA values, the textural and chemical soil analysis and the values of the NMS axes for the bacterial and fungal communities on years since disturbance (with years treated as a continuous variable). We included spatial location of the plots within the model using Proc Mixed in SAS (Littell *et al.* 1996) and found there to be no spatial autocorrelation in aggregate stability.

To identify consistency with causal mechanisms of aggregate stability, we first tested correlations of WSA with the soil measures and microbial community dimensions. For soil and microbial measures that were significantly correlated with WSA, we tested their potential to mediate the increase in WSA with time since disturbance by including the individual measures as covariates in analyses of variance of WSA. Covariates mediating the change in WSA would reduce the significance of the time effect (Sokal & Rohlf 1995). From each analysis of covariance, we then constructed *F*-tests on the reduction in sums of squared variation explained by time since disturbance. Significant *F*-tests would indicate that these covariates explained a substantial proportion of the WSA recovery with time since disturbance and therefore potentially mediate this recovery.

Results

WATER-STABLE AGGREGATES

The proportion of WSA formation after cessation of agricultural disturbance differs significantly with time ($F_{1,10} = 39{\cdot}88$, $P < 0{\cdot}001$). Sites with 20 years of cessation of agricultural disturbance had a significantly higher proportion of stable aggregates than 1 year of cessation (0·168 vs. 0·351, Fig. 1); this represents an increase of 51% of the initial WSA after 20 years of cessation of cultivation.

SOIL ABIOTIC PROPERTIES

Soil pH was positively correlated with time since abandonment ($P = 0{\cdot}007$, $r = 0{\cdot}72$). Total N and NH_4^+ showed a significant positive correlation with time since abandonment ($r = 0{\cdot}69$, $P = 0{\cdot}01$ and $r = 0{\cdot}77$, $P = 0{\cdot}003$, respectively). There was no difference in NO_3^-, P and K levels with time since last disturbance ($r = -0{\cdot}305$, $P = 0{\cdot}33$; $r = -0{\cdot}41$, $P = 0{\cdot}18$, $r = 0{\cdot}32$, $P = 0{\cdot}30$, respectively) while C : N showed modest and no significant increases with time since disturbance ($r = 0{\cdot}24$, $P = 0{\cdot}43$). Total C and OM increased with time since abandonment ($r = 0{\cdot}68$, $P = 0{\cdot}01$ and $r = 0{\cdot}66$, $P = 0{\cdot}02$, respectively,). Total OM, C and N were significantly and positively correlated with WSA ($r = 0{\cdot}58$, $P = 0{\cdot}048$; $r = 0{\cdot}59$, $P = 0{\cdot}043$; $r = 0{\cdot}52$, $P = 0{\cdot}08$, Fig 2) (see Appendix S1, Supporting Information).

SOIL MICROBIAL COMMUNITY COMPOSITION

Non-metric multidimensional scaling analysis of the soil fungal community TRFLP data revealed a linear pattern with the communities of the greatest time since abandonment (15–20 years) clustering together in the most positive region on axis 2 and all other communities clustering together in the more negative region near the bottom of the graph (Fig. 3a). The similar analysis of bacterial communities revealed distinct differences between the bacterial communities depending on time since the land was abandoned with the greatest degree of separation evident on the second axis (Fig. 3b). Those communities of the soils which had

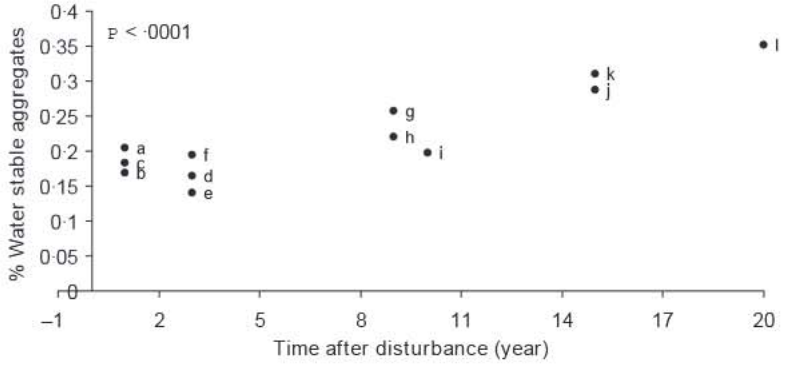

Fig. 1. Proportion of water-stable aggregates at sites along the cessation of agricultural disturbance chronosequence, Bolivia. The letters represent the fields name as shown: (a) Pisakmankaña, (b) Pisakmankaña 2, (c) Pisakmankaña 3, (d) Kankauani 1, (e) Kankauani 2, (f) Kankauani 3, (g) Sanvia Kuchu, (h) Tupu Pampa, (i) Acintajwira, (j) Wiscallacota Pampa, (k) Haracha Pampa, (l) Kuchuyu.

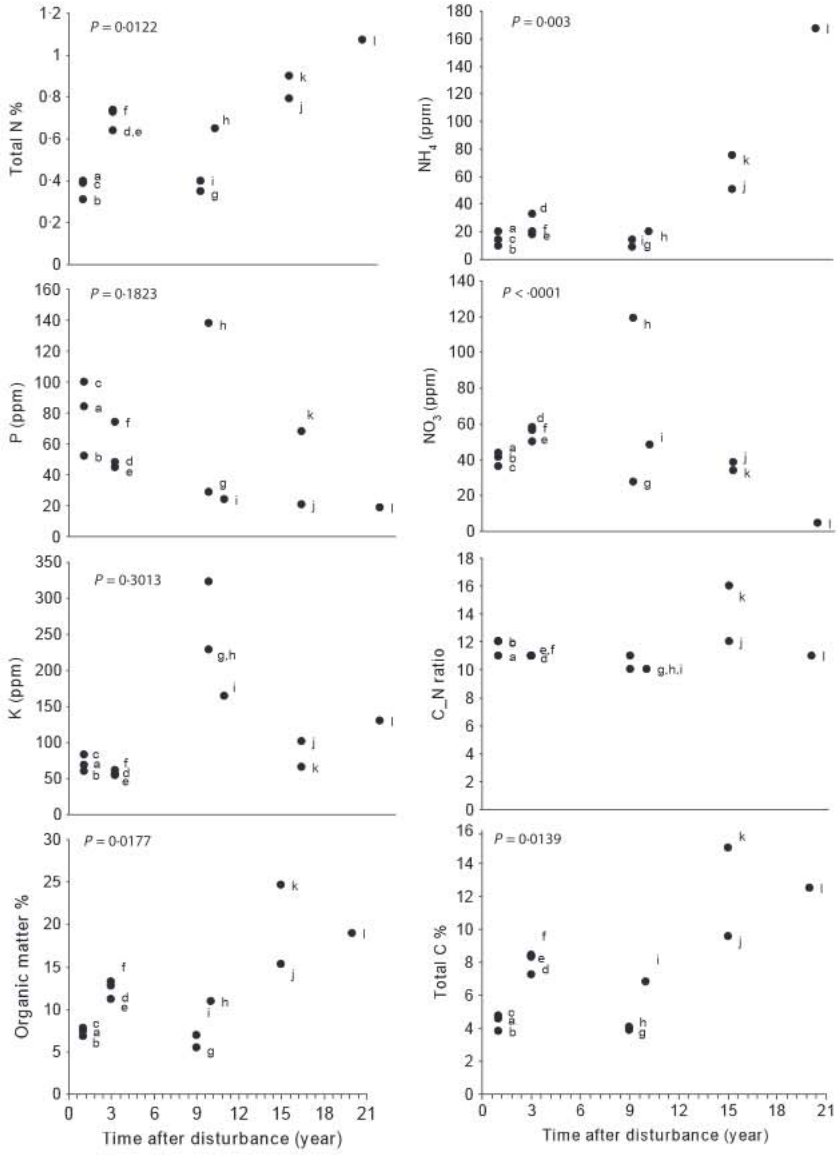

Fig. 2. Soil chemical parameters over time after disturbance in a chronosequence of agricultural land abandoned, Bolivia. The letters represent the fields name as shown: (a) Pisakmankaña, (b) Pisakmankaña 2, (c) Pisakmankaña 3, (d) Kankauani 1, (e) Kankauani 2, (f) Kankauani 1, (g) Sanvia Kuchu, (h) Tupu Pampa, (i) Acintajwira, (j) Wiscallacota Pampa, (k) Haracha Pampa, (l) Kuchuyu.

been abandoned the longest (15–20 years) clustered together in the most positive region of axis 2, while the communities of intermediate time since abandonment (9–10 years) clustered together in the most negative region at the bottom of the graph. The most recently abandoned soil communities clustered together in the central region on the graph exhibiting communities in the greatest degree of flux.

Although the plotted results of soil fungal and bacterial community compositional patterns in Fig. 3 clearly show differences in the composition of each of these soil communities depending on time since abandonment, the GLM analysis did not reveal a consistent directional change in the second axis of bacterial composition ($F_{1,10} = 1{\cdot}77$, $P = 0{\cdot}2$). The second axis of fungal compo-

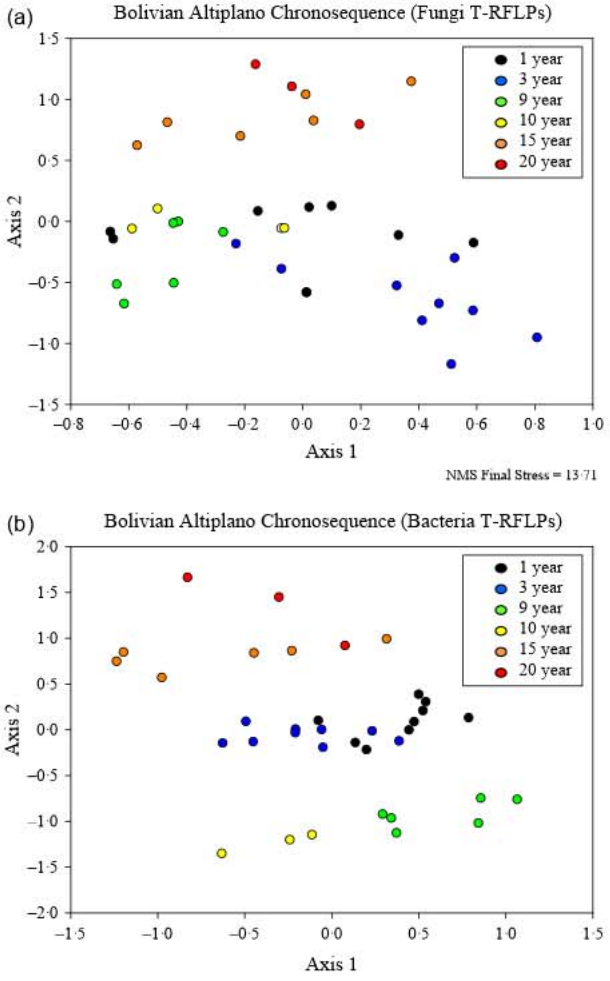

Fig. 3. Nonmetric multidimensional scaling analysis of terminal restriction fragment length polymorphisms (T-RFLPs) for the soil fungal (a) and bacterial (b) community composition within soils of the Bolivian Altiplano at various times after cessation of agricultural disturbance. The dots represent three technical replicates of the twelve sites of this study.

sition did change consistently with time since abandonment ($F_{1,10} = 17{\cdot}77$, $P = 0{\cdot}002$).

The second axis of bacterial composition was weakly correlated with WSA. However, the second axis of fungal composition showed strong positive correlation with WSA ($r = 0{\cdot}887$, $P < 0{\cdot}001$). The inclusion of the second axis of fungal composition explained a significant proportion of the sums of squared deviation previously explained by time since abandonment ($F_{1,10} = 41{\cdot}78$, $P < 0{\cdot}0001$, Table 2). This result suggests that, of the measured variables, the soil fungal community composition is most likely to mediate the change in WSA with time. In fact, analysis of covariance indicates that the second fungal axis can explain 87·5% of the recovery of WSA with time since disturbance (Table 2).

Discussion

We observed a linear improvement of WSA with time after cessation of agriculture. In this ecological area, agriculture has been shown to decrease soil aggregate stability (Cerdà 2000); to our knowledge, no study has reported changes of soil aggregate stability after cessation of cultivation. Several studies have investigated the recovery of soil aggregate stability in prairie and grassland restoration chronosequences (Jastrow 1998; Bray, Kitajima & Sylvia 2003; Guo *et al.* 2010), but very few examined this property on ex-arable land chronosequences (Li & Shao 2006; Zhu *et al.* 2010). And those studies did not distinguish a clear driver of the recovery of soil aggregate.

This study observed that several physical and biological soil properties changed with time since disturbance. Like previous findings, total N, NH_4^+, OM and total C increased with time since abandonment (Zehetner & Miller 2006; Abreu, Llambi & Sarmiento 2009), no changes in P and K were detected (Abreu, Llambi & Sarmiento 2009). Bacterial and fungal communities

Table 2. Covariance analysis of water stable aggregates (WSA) response over time

Source	Degrees of freedom	Sums of squares	Mean square	F	*P*-value	
Time after Disturbance	1	0·0380358	0·03803579	47·74	<.0001	
WSA with Covariate						
Time after Disturbance	1	0·0047438	0·00474378	9·83	0·012	
fungAxis2	1	0·0036225	0·00362247	7·5	0·0229	
Test of whether covariate fungal axis2 explains effect of time after disturbance						
Amount of variation explained by fungi axis 2						
						Proportion of variation explained
	1	0·033292	0·033292	41·78634	<.0001	87·5

changed with time after cessation of cultivation suggesting that land use is a strong factor influencing alterations in the structure of soil microbial communities.

From those factors, only OM, total carbon, total N and fungal community composition were positively correlated with soil aggregate stability (see Appendix S1, Supporting Information). These correlations are consistent with the idea that these factors influence aggregate stability during succession. The positive correlation between WSA and total C and OM are consistent with previous studies that have suggested OM as an effective binding agent for soil aggregates (Chenu, Le Bissonnais & Arrouays 2000). *A priori*, we did not expect a positive relationship between total nitrogen and WSA, and this may well be spurious correlation as nitrogen may increase with succession independently of aggregate stability (Knops & Tilman 2000; Toby & Compton 2003). In fact, analysis of covariance reveals that patterns of percentage OM, total C, total N and the axes of bacterial community composition were not consistent with their playing a role in mediating the improvement of soil aggregate stability.

Analysis of covariance revealed that the improvement of WSA over time is strongly associated with fungal community composition. Of all covariates, only inclusion of the second axis of fungal community composition explained a significant amount of the increase in soil aggregate stability. In fact, fungal community composition explained 87·5% of the recovery of WSA which is consistent with it being a primary determinant of aggregate stability. Previous findings of experimentally suppressed microbial communities show an important contribution of fungi on soil aggregate stability (Tang *et al.* 2011). Our results are also consistent with studies of prairie and desert grasslands that have found strong statistical relationships of aggregate stability with fungi, mycorrhizal fungi in particular (Jastrow 1998; Chaudhary *et al.* 2009; Wilson *et al.* 2009).

The current study reveals that soil aggregate stability increases after cessation of cultivation. Although we cannot assess what proportion of the original WSA has been recovered as comparable undisturbed sites were unavailable, our observation of improvement of stable aggregates over time suggests that fallow periods allow the recovery of soil stability. Given that this soil property reflects and is the product of the interaction of many ecosystem factors, the recovery of soil aggregate stability is relevant to overall ecosystem health. Therefore, soil stability should be a priority for conservation, restoration, erosion control and ecosystem sustainability strategies. Our results are consistent with soil microbes and soil fungi, in particular, being important drivers of the recovery of aggregate stability in postdisturbance communities. While restoration practitioners have begun to evaluate the benefits of inoculation with native microbes for re-establishing native plant communities (Bever 2003; Rowe, Brown & Paschke 2009; Middleton & Bever 2012), more work needs to be done to test the utility of native soil microbe inoculation on restoration of soil aggregate stability.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Appendix S1. Correlations between soil properties measured in the sites along the cessation of agricultural disturbance chrono sequence, Bolivia.