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SOIL MICROBIAL BIOMASS AND OXY-HYDROXIDES CONTRIBUTE TO AGGREGATE STABILITY AND SIZE DISTRIBUTION UNDER DIFFERENT LAND USES IN THE CENTRAL ANDES

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ABSTRACT

Context. Agricultural intensification leads to land use changes with potential consequences for soil aggregate stability and size distribution, affecting nutrient and water retention capacity, aeration, sequestration of soil organic carbon, and biogeochemical cycling. ***Aims***. This study evaluated soil aggregate stability and size distribution under potato, fallow and *Eucalyptus globulus* L. land uses in Cambisols of the eastern branch of the Central Andes, Bolivia. We also investigated the relation between aggregates and total C, extractable C, oxy-hydroxides, microbial biomass and activity. ***Methods***. Aggregate stability, size distribution and oxy-hydroxides were measured in soil samples from eight plots of each land use. ***Key results***. Compared to fields cultivated with potato (*Solanum tuberosum* L.), *Eucalyptus* increased aggregate stability, megaaggregate content, and C and N in the free silt + clay fraction. Fallow did not lead to significant changes in soil structure. Soil aggregate stability was related to both microbial biomass and oxy-hydroxides. Microbial biomass

C, microbial activity and dithionite extractable Fe were positively related to megaaggregates and aggregate stability. Oxalate extractable Fe and Mn were related to microaggregates. ***Conclusions***. The plantation of *Eucalyptus* is suitable for soil structural amelioration and C sequestration, but its introduction to cultivated areas should be carefully evaluated due to its effects on soil chemistry and microbiology. Short-term fallowing did not contribute to the maintenance of soil structure. ***Implications***. In a context of land uses change, modifications of microbial biomass and activity would affect megaaggregate formation and stability. Alternative management practices are required to maintain soil structure and optimize sustainable land use of cultivated and fallow fields.

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Introduction

Soil structure, the grouping of soil particles (sand, silt, clay, organic matter) into aggregates, determines soil nutrient and water retention capacity, aeration, the sequestration of soil organic carbon and ultimately biogeochemical cycling. A change in soil structure may thus influence plant growth and biomass production by altering the capacity of a soil to provide water and nutrients to plants (Emadi *et al.* 2009). Soil aggregates result from the interaction between organic molecules, minerals and microorganisms (Oades and Waters 1991; Bronick and Lal 2005). In their model of hierarchical organisation, Tisdall and Oades (1982) suggest a specific contribution of different binding agents to the formation and stability of aggregates. Organic binding agents that are easily degradable, such as polysaccharides, of plant and microbial origin, initiate the formation of megaaggregates (>2000 µm) and macroaggregates (250-2000 µm) around fresh plant material. Labile organic carbon also promotes the activity of soil microorganisms, which in turn produce extracellular polymeric substances that act as binding agents and further contribute to mega- and macroaggregate formation and stability (Tang *et al.* 2011; Costa *et al.* 2018; Robertson *et al.* 2019). Higher microbial biomass and activity would thus be linked to the mega- and macroaggregate fractions. This has been supported by experimental studies showing that the decomposition of labile organic compounds (i.e. glucose) by microorganisms triggered the formation of mega- and macroaggregates, and that the binding agent derived primarily from microbial sources (Krause *et al.* 2019; Rabbi *et al.* 2020). The link between soil microbial biomass and soil aggregates also supports this mechanism, as microbial biomass may reflect organic matter decomposition and the production of bonding compounds (Degens 1997). Within the mega- and macroaggregates, the organic binding agents are then transformed into smaller and complex molecules that interact with oxy-hydroxides, silt and clay minerals to form the stable occluded microaggregate (53-250 µm); and the occluded silt and clay (<53 µm; Tisdall and Oades 1982; Six *et al.* 2000) fractions. Finally, the mega- and macroaggregates release the stable free microaggregate and stable free silt and clay fractions. Electrostatic interactions between soil oxy-hydroxides and organic compounds are the main binding agents for these occluded and free fractions (Shanmuganathan and Oades 1982).

While the hierarchical model was confirmed in several studies (Six *et al.* 2000; Park *et al.* 2012), indicating that soil C (as driver of microbial activity) and microbial biomass were the main contributors to soil aggregate formation, the degree of correlation between microbial biomass and activity and mega- and macroaggregate content varied (Capriel *et al.* 1990; Angers *et al.* 1993). This discrepancy may be due to the influence of soil oxy-hydroxides, such as iron, manganese and aluminium (Duiker *et al.* 2003; Zhao *et al.* 2017). In fact, oxide-rich soils may not follow the proposed hierarchy model (Oades and Waters 1991) because oxy-hydroxides could play a more important role in aggregate formation and stability compared to soil organic C or microbial-derived binding agents. Commonly, the relevance of soil C (including total, water extractable and microbial C) or soil (Fe, Mn and Al) oxy-hydroxides in determining soil structure has been determined by assessing their association with aggregate size distribution and stability. However, the joint contribution of soil C, microbial biomass and soil oxy-hydroxides is poorly understood, and recent studies suggest that the interacting effect of soil C and oxy-hydroxides also plays a key role in determining aggregate stability and size distribution (Zhao *et al.* 2017).

Understanding the link between soil C, microorganisms and soil aggregates under different land uses is essential to shed light on the consequences of modifications in soil structure for soil organic matter dynamics and the potential resulting effects on nutrient availability and biogeochemical cycles. Indeed, increased relative abundance of mega- and macroaggregates indicate higher labile carbon content, soil porosity, oxygen diffusion, water infiltration, root penetration and decreased risk of erosion (Whalen and Chang 2002; Pagliai *et al.* 2004; Tobiašová *et al.* 2016). Higher abundance of mega- and macroaggregates is thus generally linked to increased rates of biological processes and higher nutrient availability (Cai *et al.* 2016). In contrast, as occluded and free microaggregate fractions are considered to contain a more stable C fraction, they are generally linked to soil C sequestration. Also, their increased abundance is linked to a higher erosion risk (Pagliai *et al.* 2004; Wei *et al.* 2006), but not to microbial activity. Different land uses strongly influence both soil organic C and microbial activity (Li *et al.* 2020; Vazquez *et al.* 2020). For example, in agricultural land uses, tillage may lead to the breakdown of macro- and megaaggregates (Whalen and Chang 2002; Tobiašová *et al.* 2016), and increased decomposition, leading to a depletion of labile C with a subsequent decrease in microbial biomass and microbial activity (e.g. C respiration and N mineralisation). Organic amendments increase labile C availability, potentially promoting microbial activity and the formation of macro- and megaaggregates (Caravaca *et al.* 2002; Chakraborty *et al.* 2011). Other land uses, such as fallow soils or forest plantations may lead to soil organic matter availability, with subsequent increases in microbial biomass, activity (Coca-Salazar *et al.* 2021*a*), and macro- and megaaggregate formation (Caravaca *et al.* 2002; Nielsen and Calderon 2011). However, long fallow periods of 10 years or more may be required to induce significant changes in soil structure (Duchicela *et al.* 2013), while changes in forest plantations have been reported after 3 years (Gupta *et al.* 2009). Within the context of land use changes, modifications in microbial biomass and activity might be more relevant in explaining aggregate formation than soil mineral components, due to the faster responses of soil microorganisms to changes. For the soils investigated in this study, we measured higher microbial biomass and activity under *Eucalyptus* plantations (Coca-Salazar *et al.* 2021*a*), potentially leading to higher macro- and megaaggregate formation.

In the mountainous areas of the Central Andes of Bolivia, the growing population and food demand led to intensification of land used for cropping over the last decades. The traditional practise of leaving arable soil under fallow for long periods (>10 years) was thus shortened to 2-6 years. Moreover, *Eucalyptus globulus* L. plantations were introduced as an alternative economic income to farmers, and replaced previously cultivated areas. Currently, the landscapes are dominated by three types of land use: (1) agricultural fields; (2) fallow fields; and (3) *Eucalyptus* plantations. The current soil management may influence soil structure and lead to long-term negative consequences for agriculture in these areas where potato (*Solanum tuberosum* L.) production is essential for providing potatoes and seeds to the country, as well as being the main income for these rural populations. Indeed, increased risk of soil erosion and nutrient depletion have been linked to the intensification of these agricultural ecosystems (Ellis-Jones and Mason 1999; Aalto *et al.* 2006), with negative consequences for potato production.

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Understanding the role of the different soil constituents in explaining changes in soil aggregate stability and size distribution is essential to guide management practises aiming the maintenance of soil structure of cultivated fields and to sustain long term agricultural production.

In this study, we investigated the effects of three land uses (agricultural fields cultivated with potatoes, fallow fields and *Eucalyptus* plantations) on soil aggregate stability and aggregate size distribution. We hypothesised that *Eucalyptus* plantations and fallow land uses would lead to an improvement in soil structure compared to potato crops. Further, we aimed to understand which soil constituents (total organic, extractable, microbial biomass, and soil oxy-hydroxides) contributed to aggregate stability and size distribution under the three land uses. Finally, we aimed at determining the link between microbial activity and aggregate size distribution. We hypothesised that higher microbial biomass, respiration potential and N mineralisation rates would be related to higher amounts of larger aggregates and increased aggregate stability.

Materials and methods

STUDY AREA AND SOIL SAMPLING

The study was conducted in an agricultural community (a group of ca. 70 families with an organisational structure for strategic decision-making concerning potato production) of Cochabamba-Bolivia located in the eastern branch of the Central Andes range (17°32'30"-17°33'30"S, 065°20'08"- 065°21'36"W; 3100-3400 m altitude). Climate is characterised by a rainy summer season (November-March) and a dry winter season (April-October), with a mean annual rainfall of 500.7 mm, and a mean annual temperature of 17.9°C (Navarro and Maldonado 2002; SENAMHI 2016).

Potato crops, fallow fields and *Eucalyptus* plantations were interspersed within the landscape in areas of ca. 0.5-1 ha, defined here as 'plots'. The selected plots were located on soils classified as Cambisols (Ministerio de Medio Ambiente y Agua 2014), with a pH ranging from 4.0 to 4.3 without differences between land uses, though higher exchangeable Al indicated soil acidification under *Eucalyptus* (Coca-Salazar *et al.* 2021a). Fields cultivated with potato were ploughed (20 cm depth) before cultivation, and fertilisers (chicken manure and industrial N, P, K) were added at planting according to farmers' personal judgement. Potato harvesting was conducted manually. Crop residues of the last cultivation cycle (secondary crops *Vicia faba* L., faba beans; *Hordeum vulgare* L., barley) were mixed into the soils before fallowing, and fields were then left unmanaged. *Eucalyptus* plantations were not managed after seedling plantations. Before conversion to fallow or to *Eucalyptus* plantations, all plots had been cultivated according to the traditional cultivation cycle (potato-faba beans-barley).

Eight plots of each land use type were selected within an area of ~4 km^2 based on the following criteria: agricultural plots in which potato had been grown during the last two cropping cycles, fallow plots (2-6 years old) in which the spontaneously grown vegetation (grass-shrubland of semiarid high Andes; Navarro and Maldonado 2002) fully covered the soil, and *Eucalyptus* plantations, 5-20 years old. Fallowing duration of 2-6 years, represented the real field conditions commonly found in agricultural communities in the Andes of Bolivia. A minimum of 5 years after *Eucalyptus* plantation was selected, since the effects of *Eucalyptus* on soil characteristics can be detected after 3 years of cultivation (Bai *et al.* 2015). As each farmer decides individually on the duration of their fields under cultivation, fallow or tree plantation, according to the general guidelines established within the community, it was not possible to select plots with the exact same duration under each land use. At the end of the rainy season (February 2017), soils of the 24 plots under the three land uses were sampled. One composite soil sample (one central sample and four samples 2 m around) was taken in each plot with a shovel from 0 to 20 cm depth at a randomly selected point. In order to compare mineral soils for each studied site, the thin forest floor layer (<0.5 cm) of the *Eucalyptus* plots was discarded. All samples were analysed for soil aggregate size classes, chemical parameters and microbial processes. Soils were sieved (5 mm) and air-dried for aggregate size fractionation. All other analyses were performed on fresh, 2 mm sieved soils stored at 4°C. For microbial activities (respiration potential and net N mineralisation), the fresh soils were adjusted to 60% water holding capacity.

SOIL AGGREGATE SIZE CLASSES AND QUANTIFICATION OF OXY-HYDROXIDES

Soil aggregate size fractionation was performed through the wet sieving method (Elliott 1986; Six *et al.* 1998). Briefly, 60 g of soil were placed on a 2000 µm sieve submerged in distilled water for 5 min at room temperature. Wet sieving was conducted by moving the sieve out of the water and immersing it again 50 times during 2 min (Elliott 1986). The water and particles that passed through the 2000 µm sieve were used to repeat the process with the 250 µm and 53µm sieves. The particles retained by the sieves were recovered, oven-dried at 50°C and weighted. This allowed the isolation of the >2000 µm (megaaggregates), 250-2000 µm (macroaggregates), and 53-250 µm (free microaggregates) size classes. The remaining water was oven-dried at 50°C to recover the <53 µm (free silt + clay) size class.

Occluded microaggregates (53-250 µm, microaggregates contained within macroaggregates) were mechanically separated from the macroaggregates (Six *et al.* 2000). Briefly, 10 g of macroaggregates were suspended in 50 mL distilled water and left overnight. The solution was poured on top of a 250 µm sieve and shaken with 60 glass beads (3 mm diameter) for 15-20 min at 250 rpm under a continuous flow of distilled water. The solution was then passed through a 53 µm sieve at the outlet of the system to recover the occluded microaggregates that were oven-dried at 50°C and weighted. The occluded silt + clay (<53 µm particles contained within macroaggregates) size class was calculated by subtracting the weight of the occluded microaggregates and the weight of the particles retained in the 250 µm mesh from the total weight of macroaggregates used for isolation of occluded microaggregates.

Given that there is little or no binding of organic matter with sand particles (Elliott *et al.* 1991), the weight of mega-, macro- and microaggregates was corrected by subtracting the weight of sand contained in the respective size class. The sand contents of mega- and microaggregates were determined by dispersing 3-5 g of each size class in 15 mL 5% sodium hexametaphosphate solution. After overnight agitation, the solutions were passed through 2000 and 53 µm sieves, respectively, and the particles remaining in the sieves were dried and weighted. The weight of the sand particles retained in the 250 µm mesh during occluded microaggregate isolation was used to calculate the sand content on macroaggregates.

The percentage of total water-stable aggregates (WSA) and the mean weight diameter (MWD) were used as indicators of aggregate stability (Kemper and Rosenau 1986; Yin *et al.* 2016). WSA is the percentage of aggregates retained in the sieves >53 µm relative to the total weight of the soil sample sieved. The mean weight diameter (MWD) was calculated as:

$$MWD = \sum_{i=0}^{n} X_i \, x \, W_i \tag{1}$$

where n is the number of aggregate size classes (n = 4, with the classes being mega, macro, microaggregates and silt + clay), X_i is the mean diameter of the isolated aggregate size class i (with the diameters being 3.5, 1.125, 0.1515, 0.053 mm for the mega-, macro-, microaggregates and silt + clay size classes, respectively; Yin *et al.* 2016), and W_i is the weight of the aggregate size class i.

The content of total organic carbon (TOC) and total nitrogen (TN) in the mega-, macro-, microaggregates, occluded microaggregates and free silt + clay size classes (g kg^{-1} aggregate size) were measured by flash-dry combustion (TruMac CN analyser, LECO), and the data was used to calculate the C:N ratios of each aggregate size class. Carbon and nitrogen contents of each aggregate size class per kilogram of bulk soil were calculated using the TOC and TN contents and the amount of each aggregate size class in the soil. We assumed that the TOC and TN contents in the free silt + clay class was the same as the contents in the occluded silt + clay class for the C and N content calculation according to de Tombeur *et al.* 2018). Particulate organic matter (POM) was separated during the sieving process, however, we did not quantify this C pool in our study due to the fundamental biochemical differences between aggregate fractions and POM in terms of their formation, persistence, and functioning (Lavallee *et al.* 2020) and as the amounts of POM recovered were too low to allow quantification. In this study, we thus focused on the soil aggregates only, without quantifying POM.

The free (crystalline and amorphous) Fe, Al, Mn oxy-hydroxides were measured in bulk soil using the dithionite- citrate sodium bicarbonate (DCB) method (Mehra and Jackson 1958), and the amorphous forms of oxy-hydroxides were extracted with the ammonium oxalate method (Schwertmann 1964). The DCB-extractable Fe, Al and Mn were quantified from 1 g soil mixed with 40 mL sodium citrate 0.3 M and sodium bicarbonate 0.25 M solution on a water bath at 75°C and mixed with 3 g of sodium dithionite. The solution was centrifuged (10 min, 676g), poured into a 250 mL Erlenmeyer flask, and the remaining soil was rinsed with 25 mL NaCl until the red-brown colour disappeared. The oxalate-extractable Fe, Al and Mn were extracted from 1 g soil agitated with 100 mL of an oxalic acid-ammonium oxalate solution for 4 h in the dark and filtered with Whatman 602 h ½. The DCB (Fe_{DCB}, Al_{DCB}, and Mn_{DCB}) and oxalate (Fe_{Ox}, Al_{Ox}, and Mn_{Ox}) extractable elements were measured by atomic absorption spectrometry (Varian).

BULK SOIL C FRACTIONS AND BIOLOGICAL CHARACTERISATION

TOC, TN, water soluble carbon (WSC), hot water extractable carbon (HWC), microbial biomass C (MBC), and the respiration potential and net N mineralisation were measured in bulk soil. TOC and TN were measured with flash dry combustion on 0.5 g soil. WSC and HWC were determined by measuring TOC in cold and hot water extracts according to Ghani *et al.* (2003). MBC was determined as the difference of K_2SO_4 extractable C between chloroform fumigated and non-fumigated soil samples (Vance *et al.* 1987). Respiration potential was calculated as the CO_2-C increase over a 3-h incubation of 20 g fresh soil (Robertson *et al.* 1999). Net N mineralisation is the increase in the total mineral N after a 28 day incubation period (20°C and constant humidity; Hart *et al.* 1994). Detailed methods and land use effects on WSC, HWC, MBC and microbial processes can be found in Coca-Salazar *et al.* (2021a).

STATISTICAL ANALYSES

Within each land use, differences between the amounts of mega-, macro-, and microaggregates, their TOC, TN contents were assessed with one-way ANOVA and post hoc Tukey tests. Between land uses, differences in the amounts of aggregates in the size classes, their TOC, TN contents, stocks and Fe, Al, Mn oxy-hydroxides were assessed with one-way ANOVA and post hoc Tukey tests. When heterogeneity of variances were detected, Welch ANOVA and Games-Howell *post hoc* tests were used (Mangiafico 2015; Faria *et al.* 2018).

Redundancy analysis (RDA) was used to explore the relationships between MWD, WSA and amounts of aggregates within size classes (dependent variables) with the contents of soil TOC, WSC, HWC, MBC, and oxy-hydroxide (explanatory variables), according to Borcard *et al.* (2018). Data was centred by subtracting the mean from every value of each variable. A full model including all dependent and explanatory variables was constructed, and an optimal model was selected through backward selection process by removing explanatory variables with variance inflation factors (VIF) higher than 10, and by comparing the Akaike information criteria (AIC) of the models. The significance of the optimal model and the variables were tested through permutation tests (significance level of 0.05). The variance explained by the explanatory variables of the optimal model was also calculated (variance partitioning of the RDA model). To corroborate the association between the variables of the optimal RDA model, we performed least square linear regressions between the amounts of soil aggregates within size factions, MWD and WSA as response variables, and MBC and Fe and Mn extracts as explanatory variables.

The covariation between changes in the amount of aggregates of different sizes and soil processes (respiration potential, net N mineralisation) was assessed through multivariate co-inertia analyses (Dray *et al.* 2003). The co-inertia analyses (COIA) was conducted with the following datasets: (1) amounts of aggregates within size classes; and (2) their TOC and (3) TN contents with (4) microbial processes (respiration potential and net N mineralisation). First, we performed principal component analyses (PCA) on the above mentioned centred datasets to calculate the total inertia for each (Dray *et al.* 2003; Li *et al.* 2009), then COIA were computed between pairs of PCAs. Monte Carlo permutation tests were conducted on the sum of eigenvalues of each COIA, to test the significance of the correlation coefficients between the two data sets used (RV coefficient). To corroborate the association of the variables that co-varied, we performed least square linear regression analyses between amount of soil aggregates within size classes, their C and N contents as response variables, and microbial processes as explanatory variables.

Statistical analyses and graphs were performed with the R software (R Core Team 2019) using the packages 'multcomp' (Hothorn *et al.* 2008), 'multcompView' (Graves *et al.* 2015), 'ade4' (Dray *et al.* 2018), and 'adegraphics' (Siberchicot *et al.* 2017), 'factoextra' (Kassambara and Mundt 2019), 'vegan' (Oksanen *et al.* 2019).

Results

SOIL AGGREGATE SIZE CLASSES AND OXY-HYDROXIDES

The recovery of soil aggregates from wet sieving ranged from 98 to 100% of the total amount of soil used. Under *Eucalyptus*, aggregate size distribution was dominated by mega- and macroaggregates, with significantly higher amounts ($P < 0.05$) compared to the other size classes (Table 1), in the following order: megaaggregates (37.7%), macroaggregates (32.6%), occluded silt + clay class (16.1%), and the other size classes (<8%). Under potato and fallow land uses, macroaggregates were most abundant (46.5 and 49.6%, respectively, $P < 0.05$), followed by the occluded silt + clay (23.7% and 28.0%, respectively), the free silt + clay (17.5% and 15.5%, respectively), and remaining aggregate sizes (<10%). Average TOC recovery (g C kg^{-1} bulk soil) was 86, 89 and 97%, while average TN recovery was 92, 94 and 96% for *Eucalyptus*, fallow and potato land uses, respectively. Within each land use, TOC content per bulk soil was not significantly different between the aggregate size classes (Table 2). TN contents under fallow and *Eucalyptus* land uses were highest in the free silt + clay fraction compared to the other aggregate size classes. Under potato land use, TN contents within aggregate size classes were not significantly different. For all land uses, C:N ratios in the free silt + clay fraction were lower compared to the other aggregate size classes.

Land use change led to clear differences in aggregate size distribution and stability (Table 1). Total amounts of aggregates were significantly higher under *Eucalyptus* (763 g kg^{-1}), compared to potato and fallow land uses (708-740 g kg^{-1}). *Eucalyptus* land use led to significantly higher MWD, WSA and megaaggregate content and significantly lower amounts of macroaggregates, occluded microaggregates, free and occluded silt + clay in comparison to potato and fallow land uses ($P < 0.05$). The amount of free microaggregates was lowest under *Eucalyptus*, followed by fallow, and potato with significant differences between the three land uses ($P < 0.05$).

TOC and TN contents (per kg of aggregate) were significantly higher in the occluded silt + clay class under *Eucalyptus* compared to potato and fallow land uses, while no differences between land uses were shown for other aggregate size classes (Table 2). C:N ratios in the macroaggregates, free microaggregates and occluded microaggregates were significantly higher for *Eucalyptus* compared to potato and fallow land uses.

TOC and TN contents in aggregates (per kg bulk soil) showed significant differences between land uses for most size classes (Fig. 1). Patterns were similar for C and N, with, on average, 4.7 and 3.1 times C and 4.1 and 2.5 times N significantly higher amounts in the megaaggregates under *Eucalyptus* compared to potato and fallow. For the free and occluded microaggregates, as well as the free silt + clay fraction, values were significantly lower under *Eucalyptus* compared to potato and fallow land uses. The macroaggregate and occluded silt + clay did not show significant differences between land uses for both their amounts of C and N.

Fe_{DCB} ranged from 2.3 to 24.7 mg g^{-1}, with significantly higher values under *Eucalyptus* and fallow fields compared to potato fields (Table 3). Al_{DCB} oxy-hydroxides ranged from 0.5 to 5.1 mg g^{-1} without significant differences between land uses, whereas the Mn_{DCB} ranged from 0.2 to 1.2 mg g^{-1} with significant lower values under *Eucalyptus* compared to fallow soils. The Fe_{Ox} ranged between 1.5 and 5.1 mg g^{-1} and the Al_{Ox} ranged between 0.1 and 3.8 mg g^{-1}, both without differences between land uses. The Mn_{Ox} ranged between 0.2 and 1.2 mg g^{-1} with significantly higher values in fallow compared with *Eucalyptus* plots, while potato had intermediate values.

RELATION BETWEEN SOIL AGGREGATE SIZE CLASSES AND STABILITY WITH ORGANIC AND MINERAL BINDING AGENTS

The selected RDA model explained 58% of the variation in aggregate stability and size distribution. MBC, Fe_{DCB}, Fe_{Ox} and Mn_{Ox} were the most important soil constituents explaining the variation of the aggregate stability and size distribution. Variance partitioning showed that MBC explained 21%, the soil oxy-hydroxides Fe_{DCB}, Fe_{Ox} and Mn_{Ox} explained 16%, and the interaction of MBC with Fe_{DCB}, Fe_{Ox} and Mn_{Ox} accounted for 21% of the variation (Fig. 2a). Multivariate ordination of the RDA model showed a clear separation of *Eucalyptus* from potato and fallow soils (Fig 2b). The estimated data points for *Eucalyptus* were associated with higher values of MWD, WSA, megaaggregates, MBC and Fe_{DCB}, while the estimated data points for potato and fallow soils overlapped and were associated with higher values of macroaggregates, microaggregates, silt + clay and the Mn_{Ox} and Fe_{Ox} oxy-hydroxides. Regression analyses of individual variables corroborated these results, with MBC and Fe_{DCB} showing positive correlations with the amount of megaaggregates, MWD and WSA, and the Fe_{Ox} and Mn_{Ox} showing correlations with <2000 µm aggregates (see Supplementary Table S1).

RELATION BETWEEN MICROBIAL PROCESSES WITH AGGREGATE SIZE DISTRIBUTION AND C, N CONTENTS

COIA between the soil processes respiration potential and net N mineralisation and the amount of aggregates in size classes and their TOC and TN content relative to bulk soil indicated significant co-variation between datasets. The coefficients of correlation (RV coefficient) were 0.53 (Fig. 3a), 0.51 (Fig. 3c), and 0.60 (Fig. 3e), for the amount of aggregates, the TOC and TN contents ($P < 0.05$), respectively. Multivariate ordination discriminated *Eucalyptus* from potato and fallow land uses along the main projected axis in the three COIA, which accounted for 74-99% of the total projected co-inertias (Fig. 3). Estimated data points for potato and fallow overlapped.

The association of individual variables showed positive co-variation of respiration potential with the amount of megaaggregates and their TOC and TN contents (Fig. 3b, d, f), which was corroborated by the regression analyses (R^2 = 0.75, 0.77 and 0.89, respectively, $P < 0.01$; Supplementary Tables S2, S3). COIA results indicated co-variation of net N mineralisation with the amount of occluded silt + clay and free microaggregates, but regression analyses indicated a weak correlation with free microaggregates only ($R^2 = 0.17$, $P = 0.03$; Table S2). Net nitrogen mineralisation also co-varied with TOC and TN contents of macroaggregates and free microaggregates (Fig. 3d, 3f). Regression analyses corroborated these results, with free microaggregate TOC and TN contents showing the strongest associations (R^2 = 0.42 and 0.43, respectively, $P < 0.05$; Table S2), relative to macroaggregate TOC and TN contents (R^2 = 0.14 and 0.16, respectively, $P < 0.05$; S3 of Supplementary Material). Regression analyses also indicated positive relationships of net N mineralisation with the free silt + clay, occluded microaggregates and occluded silt + clay size classes (R^2 = 0.15, 0.24 and 0.13, respectively, $P < 0.05$; Table S2) despite the fact that COIA did not show co-variation of these variables.

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Table 1*. Mean weight diameter (MWD), water stable aggregates (WSA), and aggregate size distribution in potato, fallow and Eucalyptus land uses (mean ± s.d.).*

	Potato	**Fallow**	***Eucalyptus***
MWD (mm)	0.83 ± 0.01b	0.96 ± 0.16b	1.36 ± 0.01a
WSA (%)	80.08 ± 5.79b	82.49 ± 7.34b	91.35 ± 2.44a
Megaaggregates (g kg^{-1})	88.20 ± 28.66aD	142.30 ± 55.39aC	377.17 ± 81.87bA
Macroaggregates (g kg^{-1})	464.72 ± 44.69aA	496.10 ± 67.42aA	325.68 ± 61.13bA
Free microaggregates (g kg^{-1})	154.86 ± 26.50aDB	101.95 ± 37.62bC	60.13 ± 14.48cC
Free silt + clay (g kg^{-1})	175.14 ± 49.27aBC	155.39 ± 61.37aC	72.41 ± 22.52bC
Occluded microaggregates (g kg^{-1})	134.38 ± 36.81aDB	119.99 ± 22.72aC	62.75 ± 22.33bC
Occluded silt + clay (g kg^{-1})	236.63 ± 68.36aC	279.87 ± 63.85aB	160.85 ± 41.38bB

Different lowercase letters indicate significant differences between land uses, and different uppercase letters indicate significant differences between the amounts of aggregates in the different size classes within each land use (*P* ≤ 0.05, ANOVA and Tukey tests, *n* = 8).

Table 2*. Total organic carbon (TOC), total nitrogen (TN) content and C:N ratios of bulk soil and sand-free aggregates of different size classes under potato, fallow and Eucalyptus land uses (mean ± s.d.).*

Aggregate size classes	**TOC (g C kg^{-1} aggregate)**			**TN (g N kg^{-1} aggregate)**			**C:N ratio**		
	Potato	**Fallow**	***Eucalyptus***	**Potato**	**Fallow**	***Eucalyptus***	**Potato**	**Fallow**	***Eucalyptus***
Bulk soil	23.57 ± 6.95a	22.90 ± 5.40a	28.55 ± 12.47a	2.16 ± 0.55a	2.10 ± 0.35a	2.20 ± 0.84 a	10.80 ± 0.97b	10.80 ± 1.09b	12.72 ± 1.04a
Megaaggregates	27.68 ± 3.29aA	26.14 ± 5.38aA	28.78 ± 15.83aA	2.42 ± 0.33aA	2.40 ± 0.37aAB	2.21 ± 0.92aB	11.49 ± 0.75aA	10.98 ± 1.00aA	12.45 ± 2.60aA
Macroaggregates	26.55 ± 6.91aA	22.63 ± 5.58aA	29.59 ± 13.70aA	2.34 ± 0.55aA	2.16 ± 0.38aAB	2.37 ± 0.93aB	11.32 ± 1.39abA	10.67 ± 0.89bAB	12.12 ± 1.49aA
Free microaggregates	20.08 ± 5.57aA	18.47 ± 5.10aA	24.22 ± 12.52aA	1.92 ± 0.45aA	1.75 ± 0.38aB	1.95 ± 0.93aB	10.33 ± 0.81bA	10.42 ± 1.12bAB	12.19 ± 0.92aA
Free silt + clay	23.06 ± 7.61aA	22.98 ± 5.65aA	33.78 ± 11.06bA	2.69 ± 0.76aA	2.50 ± 0.42aA	3.81 ± 1.10bA	8.49 ± 0.69aB	9.09 ± 1.07aB	8.69 ± 1.09aB
Occluded microaggregates	22.03 ± 8.45aA	22.93 ± 6.2aA	21.90 ± 11.29aA	2.16 ± 0.67aA	2.20 ± 0.56aAB	1.67 ± 0.76aB	10.13 ± 1.64bA	10.40 ± 0.85bAB	12.79 ± 1.21aA

Different lowercase letters indicate significant differences between land uses and different uppercase letters indicate significant differences between aggregates in the different size classes within each land use (*P* ≤ 0.05, ANOVA and Tukey tests, *n* = 8).

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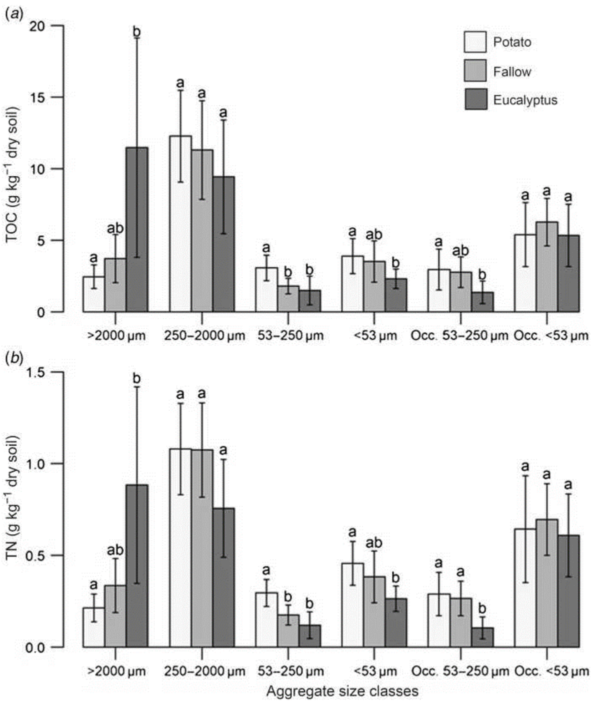
Figure 1.



Total organic carbon (TOC) and total nitrogen (TN) contribution of each aggregate class to total soil mass under potato, fallow and Eucalyptus land uses (mean ± s.d.). Megaaggregates (>2000 µm), macroaggregates (250-2000 µm), free microaggregates (53-250 µm), free silt + clay fraction (<53 µm), occluded microaggregates (Occ. 53-250 µm), and occluded silt + clay fraction (Occ. <53 µm). Different letters indicate significant differences between land uses (P ≤ 0.05, ANOVA and Tukey tests, *n* = 8).

Table 3. *DCB and oxalate extractable Fe, Al and Mn oxy-hydroxides under potato, fallow and Eucalyptus land uses (mean ±s.d.).*

		Potato	**Fallow**	***Eucalyptus***
Fe (mg kg^{-1})	DCB	3.66 ± 0.98b	20.72 ± 3.11a	18.09 ± 4.08a
	Oxalate	3.14 ± 1.01a	3.04 ± 0.48a	2.48 ± 0.79a
Al (mg kg^{-1})	DCB	3.35 ± 1.32a	2.27 ± 1.00a	2.68 ± 1.36a
	Oxalate	1.23 ± 0.76a	0.81 ± 0.65a	1.51 ± 1.07a
Mn (mg kg^{-1})	DCB	0.78 ± 0.23ab	0.90 ± 0.28b	0.47 ± 0.31a
	Oxalate	0.68 ± 0.18ab	0.77 ± 0.24b	0.41 ± 0.32a

Different letters indicate significant differences between land uses (P ≤ 0.05, ANOVA and Tukey tests, *n* = 8).

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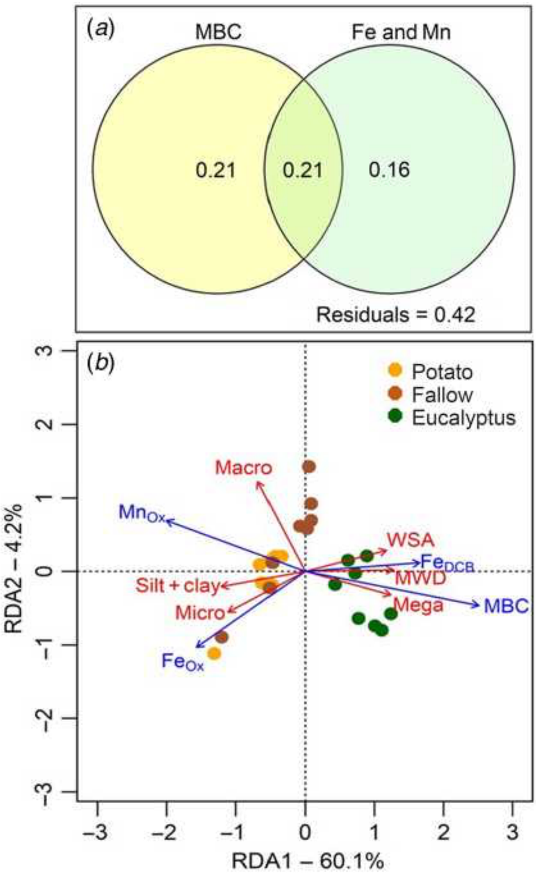
Figure 2.



Redundancy analysis between mineral binding agents with aggregate size distribution and stability under potato, fallow and Eucalyptus land uses. (*a*) Variance in the aggregate variables explained by microbial biomass carbon (MBC), and oxy-hydroxides (Fe_{DCB}, Fe_{Ox} and Mn_{Ox}). (*b*) Ordination plot of the redundancy analysis conducted on the aggregate variables mean weight diameter (MWD), water stable aggregates (WSA), megaaggregates, macroaggregates, microaggregates and silt + clay fractions, with MBC, Fe_{DCB}, Fe_{Ox} and Mn_{Ox} oxy-hydroxides.

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Discussion

LAND USE EFFECTS ON SOIL AGGREGATES, TOC, TN CONCENTRATIONS AND CONTENTS

In the present study, *Eucalyptus* plantations led to higher total amounts of aggregates, to an increase in the formation of megaaggregates and lower contents of macroaggregates, microaggregates and silt + clay fractions, compared to soils under potato and fallow land uses. This is likely due to the higher amount of organic matter entering to the soil (litter and root exudates), promoting the formation of mega- and macroaggregates around fresh plant material through microbial activity (Costa *et al.* 2018). In the studied plots, cold water-extractable C (55-69 mg C kg^{-1}), HWC (750-1367 mg C kg^{-1}), microbial biomass C (333- 600 mg C kg^{-1}), and respiration potential (0.15-1.07 CO_2-C h^{-1} g^{-1}) were significantly higher under *Eucalyptus* compared to fallow and potato land uses (Coca-Salazar *et al.* 2021a), indicating higher microbial abundance and activity. These increases in HWC, microbial biomass and respiration potential were correlated to the soil organic matter content, which was 37% higher under *Eucalyptus* compared to potato and fallow land uses (Coca-Salazar *et al.* 2021a). Moreover, the absence of soil physical perturbation (*Eucalyptus* soils are not managed after seedling plantation) could also favour the formation of new aggregates and the binding of the already existing free macroaggregates, microaggregates and silt + clay (Tisdall and Oades 1982; Tobiašová *et al.* 2016). The observed higher soil aggregate stability in water under *Eucalyptus* is also generally explained by higher soil C content (Wei *et al.* 2006) and a more active microbial community, which is considered to be the consequence of successful afforestation (Caravaca *et al.* 2002). Concomitantly, the higher TOC and TN contents (g kg^{-1} aggregate) in the free silt + clay fraction under *Eucalyptus* compared to the same fraction in soils under potato and fallow land uses (Table 2)isin accordance with previous studies suggesting that higher C and N contents in this aggregate size fraction are indicative of physical protection and stabilisation of soil organic matter (Six *et al.* 2000; Del Galdo *et al.* 2003; Wei *et al.* 2006; Tobiašová *et al.* 2016). Stabilisation under *Eucalyptus* would be the result of slower aggregate turnover allowing the formation of organo-mineral associations (Six *et al.* 1998, 2000; Del Galdo *et al.* 2003; Denef *et al.* 2004), increasing the amount of less accessible organic carbon for decomposition by soil microorganisms (Six *et al.* 1999; Tobiašová *et al.* 2016). Despite the positive effect of *Eucalyptus* plantation on soil structure and C stabilisation, its introduction to this agricultural ecosystem decreased microbial activity (Coca-Salazar *et al.* 2021*a*, 2021*b*). Plantation of *Eucalyptus* in the Andean region may thus be a suitable alternative for soil structural amelioration and C sequestration, but its introduction should be carefully considered in agricultural areas due to the potential effects on soil chemistry and microbiology.

Under potato crops, soil management practises such as tillage and potato harvesting lead to reduced aggregate stability and increased megaaggregate breakdown, resulting in an increased amount of macroaggregates, microaggregates and silt + clay. In particular, megaaggregates were 4.3 times less abundant under potato compared to *Eucalyptus*, while macroaggregates were 1.4 times more abundant. These results are in accordance with previous studies reporting reduced stability of megaaggregates due to soil mechanical disruption (Chan *et al.* 2002; Wei *et al.* 2006). Increased amounts of macroaggregates, microaggregates, and silt + clay fractions in cultivated soils may be also linked to the dispersive effects of fertilisers on soil aggregates (Yin *et al.* 2016). Particularly, Na^+, K^+ and NH_4^+ are known to promote the dispersion of soil colloids and to contribute to the breakdown of large aggregates (Whalen and Chang 2002). In contrast, the addition of chicken manure (the main organic fertiliser in this agricultural community) could contribute to aggregate stability (Pare *et al.* 1999; Whalen and Chang 2002), and counter the disrupting effects of tillage and potato harvesting. The observed increase in macroaggregates could increase the risk of soil erosion in cropped plots (Pagliai *et al.* 2004; Wei *et al.* 2006), as well as decrease in soil porosity, oxygen diffusion, water infiltration and root penetration (Whalen and Chang 2002; Pagliai *et al.* 2004; Tobiašová *et al.* 2016). Given the characteristic mountainous topography, negative consequences of reduced aggregate stability, such as increased soil erosion, the runoff of soil particles and associated nutrients are highly probable, as reported in similar potato agricultural systems in the north Andean region (Otero *et al.* 2011).

The effect of potato cropping on aggregate size distribution seemed to persist after fallowing plots for 2-6 years, since no differences were observed between soils under fallow and potato. The proportion of water stable aggregates increased after 10 years of fallowing agricultural soils in the Altiplano region of the country (Duchicela *et al.* 2013), likely due to the accumulation of organic matter in the presence of shrubs (Duchicela *et al.* 2013; Gomez-Montano *et al.* 2013). Thus, the current short fallow periods (<6 years) would not allow an improvement of soil structure. However, fallowing soils may still contribute to reducing the risk of soil and nutrient losses by runoff due to the protective effects of vegetation colonising the fallow fields (Sims *et al.* 1999; Otero *et al.* 2011).

CONTRIBUTION OF SOIL CONSTITUENTS TO SOIL AGGREGATION

Our results suggest that the measured soil properties contribute differently to the aggregate stability and size distribution. MBC and Fe_{DCB} would mainly contribute to megaaggregate formation and stability, while the oxalate- extractable Fe and Mn would be more important for the macroaggregates, microaggregates, and silt + clay. This is in accordance with the hierarchical model of Tisdall and Oades (1982).

MBC explained most of the variation of megaaggregate content and aggregates stability, compared to total and water extractable C. Soil microorganisms contribute to aggregate formation through the binding effect of extracellular polymeric substances and the bonding effect of hyphae (Degens 1997; Six *et al.* 2006; Tang *et al.* 2011). Particularly, soil fungi have been recognised as important drivers of aggregate stability in fallow soils in the Bolivian Altiplano (Duchicela *et al.* 2013; Gomez-Montano *et al.* 2013). Management practises contributing to build-up of the MBC in the upper soil layers (e.g. reduced or no-tillage cultivation; Meyer *et al.* 1997; Mathew *et al.* 2012) could contribute to improvement of soil structure and reduce the risk of erosion.

Dithionite extractable Fe was related to megaaggregates content and aggregate stability. Similar links have been reported in the arable layer of agricultural soils (Sanden *et al.* 2017), and were attributed to the formation of oxy- hydroxides-organic matter-clay associations (Oades and Waters 1991; Duiker *et al.* 2003). Additionally microaggregates resulting from the encapsulation of organic compounds in the core of Fe nodules act as nucleus for the formation of bigger aggregates (Eusterhues *et al.* 2005; Xue *et al.* 2019). However, the observed Fe_{DCB}-megaaggregate correlation could also be due to the encapsulation of soil C in megaaggregates and not contribute to soil aggregation (Li *et al.* 2017).

The positive correlation of Fe_{Ox} with the amount of free silt + clay fraction in bulk soil, and of Mn_{Ox} with the amount of free and occluded silt + clay in bulk soil stresses their role in the formation of microaggregates (Doetterl *et al.* 2015). Previous studies reported that the Fe_{Ox} are the main inorganic binding agents of microaggregates (Pinheiro- Dick and Schwertmann 1996). The interaction between these amorphous Fe and Mn oxy-hydroxides and soil organic matter forms very stable aggregates through cohesion (Barral *et al.* 1998; Xue *et al.* 2019), due to their large and reactive surface area and their high affinity with anions (Pinheiro-Dick and Schwertmann 1996). Despite the fact that Al oxy-hydroxides have been also reported to contribute to soil aggregate formation (Barral *et al.* 1998; Rampazzo *et al.* 1999), we did not find evidence that Al forms play a major role in aggregate stability and size distribution of these soils.

The interaction between MBC, Fe and Mn oxy-hydroxides explained 21% of the variation in aggregate stability and size distribution. This may be due to microaggregate formation through the accumulation of organic compounds and microorganisms on abiotic aggregates of soil oxy-hydroxides (e.g. montmorillonite and goethite) (Krause *et al.* 2019). Moreover, the adsorption affinity of bacterial cells with minerals, such as crystalline goethite would lead to the formation of clay-sized mineral hutches around bacterial cells, which would act as nucleus for microaggregate formation (Lunsdorf *et al.* 2000; Krause *et al.* 2019). Given that soil microorganisms are more sensitive to environmental changes than soil mineralogical characteristics, changes in MBC are more likely to take place as a result of changes in management practises.

CONTRIBUTION OF SOIL MICROBIAL PROCESSES TO AGGREGATE FORMATION

Most recent studies on soil aggregates and microbial processes measure soil microbial properties and processes within the aggregates separated through the wet sieving method (Xiao *et al.* 2017; Tian *et al.* 2019; Han *et al.* 2020; Li *et al.* 2020; Vazquez *et al.* 2020). This technique simulates aggregate stability and size distribution caused by re-wetting events and slacking that take place after rain or irrigation (Elliott 1986; Kemper and Rosenau 1986; Cambardella and Elliott 1993). However, chemical (pH, nutrient removal; Seech and Beauchamp 1988; Sainju 2006) and microbial characteristics (Nishio and Furusaka 1970) of the isolated aggregates are modified through this procedure, which may result in misleading links between aggregates and processes. In this study, we addressed the co-variation between soil aggregate size classes and soil microbial processes measured in bulk soil. This allowed us to evaluate whether changes in soil microbial activity in the bulk soil were linked to changes in soil structural properties, although we cannot localise soil microbial processes within specific soil aggregate size classes.

Results showed strong co-variation of the quantity of megaaggregates and their TOC and TN contents with the respiration potential, suggesting that higher availability of C and N to microorganisms would lead to increased microbial respiration, promoting aggregate formation. Recent studies show that microbial processing of C substrates initiates the formation of >250 µm aggregates (Rabbi *et al.* 2020). Therefore, the factors affecting microbial activity (e.g. soil pH, exchangeable cations, substrate quality, plant-microbe interactions, and environmental variables) would indirectly influence the formation of megaaggregates by altering the assimilation and allocation of plant-derived organic matter into different microbial products. This is in accordance with recent hypothesis indicating that metabolism and substrate use efficiency play central roles in the formation of organo-mineral associations involved in organic matter stabilisation (Cotrufo *et al.* 2013; Liang *et al.* 2017).

Net N mineralisation was positively related to the amount of free microaggregates and the TN content of macro-, microaggregates and silt + clay fraction. This microbial process may thus potentially depend on their N content, and at the same time contribute to their formation. N mineralisation is mediated by extracellular enzymes, such as β-glucosaminidase and arylamidase, which produce amino sugars (Ekenler and Tabatabai 2004) that may accumulate in macroaggregates and enhance the formation and stabilisation of occluded microaggregates by promoting microbial activity (Simpson *et al.* 2004). Favourable conditions for N mineralisation in macroaggregates could also contribute to the production of organic binding agents that contribute to aggregates formation. For example, Muruganandam *et al.* (2009) found higher potential activity of enzymes associated to N mineralisation in 250-1000 µm aggregates. Although no single aggregate size is responsible for N mineralisation, the association of TN content of macro- and microaggregates with net N mineralisation suggests that they may contain a larger proportion of readily available organic N (Cai *et al.* 2016).

Conclusions

The plantation of *Eucalyptus* on fields previously cultivated with potatoes improved soil structure through increased soil aggregate stability and megaaggregate formation, and led to higher stable C and N contents in the free silt + clay fraction. Fallowing soils for 2-6 years did not lead to significant changes in soil structure and aggregate stability compared to fields cultivated with potatoes. Results were in agreement with the hierarchical model of organisation, as megaaggregate formation and aggregate stability were mainly related to microbial biomass and activity, while macro-, microaggregates and silt + clay fractions were related to Fe and Mn oxy-hydroxides. The plantation of *Eucalyptus* is suitable for soil structural amelioration and C sequestration, but its introduction to cultivated areas should be carefully evaluated due to its effects on soil chemistry and microbiology. As short-term fallowing did not contribute to the maintenance of soil structure, alternative management practises limiting the risk of erosion and optimising sustainable land use are needed.

Supplementary material

Supplementary material is available online.

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Data availability. The data that support this study will be shared upon reasonable request to the corresponding author.

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