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Research Paper

Effect of land use and management practices on microbial biomass and enzyme activities in subtropical top-and sub-soils

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ABSTRACT

Land-use change, especially from forest to intensive agriculture, is negatively impacting soil quality and sustainability. Soil biological activities are sensitive indicators of such land-use impacts. We tested two hypotheses: i) land use and management practices affect microbial properties (microbial biomass and enzyme activities) in topsoil (0–20 cm), but have no effects in subsoil (20–100 cm); and ii) microbial properties in topsoil are highest in forest, followed by organic farming and then conventional farming.

Total organic C and N contents as well as microbial biomass were significantly higher in the organic farming topsoil compared with conventional farming and forest. Except xylanase and acid phosphatase, enzyme activities (β-glucosidase, cellobiohydrolas, chitinase, sulfatase, leucine aminopeptidase and tyrosine aminopeptidase) were also higher in organic farming soil. Crop residues and rhizodeposits support higher microbial biomass, leading to enhanced enzyme activities in organic farming soil. Incorporation of rice stubble and limitation of available phosphorus explain the higher xylanase and acid phosphatase activities, respectively, in conventional farming soil. Litter removal leads to a deficiency of labile C and N, resulting in lower enzyme activities in forest soil. Total C and N contents were higher in subsoil under organic farming. Although there was no effect of land use on microbial biomass in subsoil, activities of most enzymes were higher under organic farming.

Overall, our results indicate that land-use change significantly alters microbial properties in topsoil, with modest effects in subsoil. Microbial properties should be considered in environmental risk assessments and models as indicators of ecosystem disturbance caused by land-use and management practices.

1. Introduction

Land-use change is one of the main drivers of global environmental disturbance, greatly contributing to climate change, loss of ecosystem services and species extinctions (Turner et al., 2007; Tilman et al., 2001). The expansion of crop and pastoral land into natural ecosystems is the major form of land conversion (Lambin and Meyfroidt, 2011). Approximately 50% of the new arable land during the period of 1980–2000 came from intact forest in the tropics, while 28% came from disturbed forest. Land is becoming a scarce resource in the global context (Lambin and Meyfroidt, 2011) with the ever increasing need for agricultural land necessary to feed the growing human population.

Conversion of forest to agriculture and agricultural intensification contribute to the loss of soil organic matter (Lagomarsino et al., 2011), alter microbial biomass and its activities, and ultimately affect soil quality (Schloter et al., 2003). There is a growing global interest in the assessment of land use and management effects on physical, chemical and biological properties of soils (Nguyen et al., 1995). Microbial and biochemical characteristics of soil have been proposed as indicators of soil quality in both, natural and agricultural systems (Karlen et al., 1997; Mganga et al., 2016), due to the central role of microorganisms in C, N and nutrient cycling, and their sensitivity to alternations in soil conditions (Nannipieri et al., 2003). Extracellular enzymes, which are mainly secreted by microorganisms, play vital roles in nutrient cycling and soil organic matter (SOM)

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decomposition (Klose and Tabatabai, 2002). They can therefore be used as a 'soil fertility index' (Mganga et al., 2016). Land use and management practices have significant effects on microbial and enzyme activities as a result of fertilizer application (Allison et al., 2010; Zimmermann and Bird, 2012; van Gestel et al., 2013), tillage (Deng and Tabatabai, 1997; Balota et al., 2004) and grazing (Holt, 1997). Enzyme activities are also significantly affected by crop species and residue management practices (Bolton et al., 1985; Friedel et al., 1996). While prior studies have investigated the effects of land use and management practices on enzyme activities and microbial process in tropical soils, most analyses were limited to the topsoil (Balota et al., 2004; Acosta-Martínez et al., 2007; Tischer et al., 2014a, 2014b; Mganga et al., 2015). Thus, although the effects of management practices on soil microbial properties are much discussed, our knowledge of their vertical distribution is scant.

The study site in "Chitwan district" lies in the Terai region, a plain in southern Nepal. Known as grain house of Nepal, the Terai region covers 17% of the country's total land area. Forests, which cover 411,580 ha (20.41%) of the region's total land area (2,016,998 ha) (FRA/DFRS, 2014),are, dominated by *Shorea robusta and* possess high economic value and biological diversity. After eradication of malaria in the 1950s, a resettlement and migration scheme from the Middle Mountain region to different parts of the Terai region was induced. As the population increased, massive deforestation occurred to make way for cultivation and new settlements. The region's current population growth rate is 1.75%, the highest in Nepal, is continuously increasing pressure on forest areas (FRA/DFRS, 2014). Agricultural intensification through conventional farming practices is also being implemented to feed the growing population.

The objective of this study was to assess the effect of three land use systems, i.e. forest, organic and conventional farming, on soil microbial biomass and the activities of enzymes involved in the C-cycle (β-glucosidase, cellobiohydrolase and xylanase), N-cycle (chitinase, leucine aminopeptidase and tyrosine aminopeptidase), P-cycle (acid phosphatase) and S-cycle (sulfatase) in subtropical soil. We hypothesized that i) land use and management practices affect microbial properties (microbial biomass and enzyme activities) in topsoil, but have no effect in subsoil; and ii) microbial properties in the topsoil are higher in forest followed by organic farming and conventional farming. To test our hypotheses, we determined microbial biomass and the activities of eight enzymes involved in soil organic matter decomposition.

2. Materials and methods

2.1. Site description

The study was conducted in Chitwan district (27°35′N 84°30′E) of Nepal. Three land-use systems were selected: forest, organic, and conventional farming. Both farming sites were located in Fulbari Village Development Committee (VDC) and the forest site in Patihani VDC. The climate is subtropical with annual rainfall of 1763 mm and an average temperature of 30 °C. The soils at the study sites are Gleyic Cambisols (organic farming and forest) and Eutric Cambisol for the conventional farming site (IUSS Working Group WRB, 2015). The soil texture at all sites is sandy loam.

The organic farm site has been under organic farming practices for 15 years. The crop rotations are maize + rice + vegetables/mustard and maize + rice + wheat/lentils for the organic and conventional farms, respectively. The organic farm was under vegetable farming during soil sampling while the conventional farm was fallow with remaining rice stubbles. The broad leaf forest is dominated by *Shorea robusta* commonly known as Sal. The leaves of Sal are collected by local people for performing social and religious activities. A detailed description of land uses is given in Table 1.

2.2. Soil sampling and preparation

Soils from the three land use systems were sampled from 0 to 100 cm depth at intervals of 10 cm. The samples were kept cold (~4 °C) during transportation to the laboratory. Plant remains, debris and roots were removed using tweezers. The field-moist soil (70% of WHC) was allowed to equilibrate at room temperature for 24 h prior to analysis.

2.3. Microbial biomass carbon and nitrogen

Microbial biomass C and N was determined by the chloroform fumigation-extraction method (Vance et al., 1987), based on the difference between C or N extracted from fumigated and non-fumigated soil samples using 0.05 M K_2SO_4. A k_{EC} factor 0.45 was used to convert microbial C flush into microbial biomass C (Joergensen, 1996), while a k_{EN} of 0.54 was used for microbial biomass N (Joergensen and Mueller, 1996).

2.4. Enzyme assays

Enzyme kinetics were assayed using fluorogenically labeled substrates based on 4-methylumbelliferone (MUF) and amino-4-methyl coumarin (AMC)-, (Pritsch et al., 2004), (Table S1). The MUF and AMC substrates were dissolved in 2-methoxyethanol (Hoppe, 1983) and the dissolved substrates were further diluted with sterile water. Enzyme-saturating concentrations of fluorogenic substrates were determined in a preliminary experiment (Razavi et al., 2015). All chemicals and substrates were purchased from Sigma, Germany.

Briefly, soil (1 g) from each of the three land uses and different soil depths (0–100 cm depth at intervals of 10 cm) was suspended with 50 ml of sterile water using low-energy sonication (40 J s^{-1} output energy for 2 min). Following sonication, 50 μl of soil suspension was added to 100 μl of substrate solution and 50 μl of buffer (either MES, TRIZMA or sodium acetate, see Table S1) in a 96-well microplate and incubated for 2 h (Koch et al., 2007). Fluorescence was measured at an excitation wavelength of 355 nm and an emission wavelength of 460 nm, split width of 25 nm, with a Victor^3 1420-050 Multilabel Counter (PerkinElmer, USA). Calibration curves as well as controls for autofluorescence of the substrate

Table 1
Description of land use in the study site.

Land use	Vegetation type/Crop rotation	Management	Pesticide
Organic farming = 15years	Maize + rice + vegetables /mustard	Farmyard manure:10 ton ha^{-1} yr^{-1} Vermicomposting	No
Conventional farming	Maize + rice + wheat/lentil	Urea: 60 kg ha^{-1} yr^{-1} Potassium: 15 kg ha^{-1} yr^{-1}	Yes
Forest	Broad leaf dominated by *Shroea robusta*	Collection of litter for social and religious activities	No

were included in each series of enzyme measurements. Enzymes activities were expressed as MUF or AMC released in nmol per g dry soil per hour (nmol g^{-1} soil h^{-1}), (Razavi et al., 2015).

2.5. Elemental analysis

Oven dried subsamples of soil (60 °C) were ground and analyzed for elemental C and N with an Elementar Vario El analyzer (Elementar Analysensysteme GmbH, Germany).

2.6. Calculations and statistical analysis

The effects of land use on microbial properties were analyzed using one-way analysis of variance (ANOVA) at a significance level of $P < 0.05$ using the statistical software Statistica 12. All displayed results represent means of 3 replicates ± standard error (SE).

3. Results

3.1. Soil and microbial carbon and nitrogen

Land use had significant effects on total organic C and N contents in topsoil (Fig. 1). Total soil organic C was highest in organic farming (24 mg C g^{-1} soil) followed by conventional farming (15 mg C g^{-1} soil) and forest (9 mg C g^{-1} soil) in the topsoil layer (0–10 cm depth). Total C content declined with increasing soil depth, remaining highest in the organic farming soil al all depths tested. A similar trend was found for total N content in all three land uses (Fig. 1), with organic farming soil possessing the highest total N content in both top and subsoil.

Similarly, microbial C and N were also highest under organic farming, especially in the topsoil layer (350 and 46 μg g^{-1} soil, respectively), (Fig. 1). However, conventional farming and forest soils had similar microbial biomass content. In subsoil, there were no significant effects of land-use changes on microbial biomass C and N. Positive correlations were found for total soil C and N with microbial biomass C and N ($R^2 > 0.71$ and $R^2 = 0.32–0.77$, $P < 0.05$, respectively).

3.2. Enzyme activities

3.2.1. Carbon-cycle enzyme activities

The activities of enzymes involved in the C-cycle (β-glucosidase, cellobiohydrolase and xylanase) were significantly affected by land use, especially in topsoil (Fig. 2). The activity of β-glucosidase was higher in organic farming (199 nmol g^{-1} soil h^{-1}) followed by conventional farming (130 nmol g^{-1} soil h^{-1}) and forest soil (19 nmol g^{-1} soil h^{-1}) in the topsoil layer. The activity of cellobiohydrolase was higher in organic farming compared to forest soil, but was similar in organic and conventional farming soil. In contrast, xylanase activity was higher under conventional farming (27 nmol g^{-1} soil h^{-1}) followed by organic farming (17 nmol g^{-1} soil h^{-1}) and forest soil (12 nmol g^{-1} soil h^{-1}), (Fig. 2). Carbon-cycle-related activities were higher in organic farming subsoil, but were similar for the conventional farming and forest soils.

3.2.2. Nitrogen-cycle enzyme activities

The activities of N-cycle enzymes (chitinase, leucine amino-peptidase and tyrosine aminopeptidase) in the topsoil layer were higher under organic farming (138, 276 and 255 nmol g^{-1} soil h^{-1}, respectively) compared with other land-use systems (Fig. 3). The activities of tyrosine aminopeptidase and chitinase were also higher in subsoil under organic farming (Fig. 3).

3.2.3. Phosphorus- and sulfur-cycle enzyme activities

Acid phosphatase (P-cycle) activity in topsoil was affected by land use (Fig. 4). In contrast to C- (except xylanase) and N-cycle enzymes, the activity of acid phosphatase in the topsoil layer was higher under conventional farming (936 nmol g^{-1} soil h^{-1})

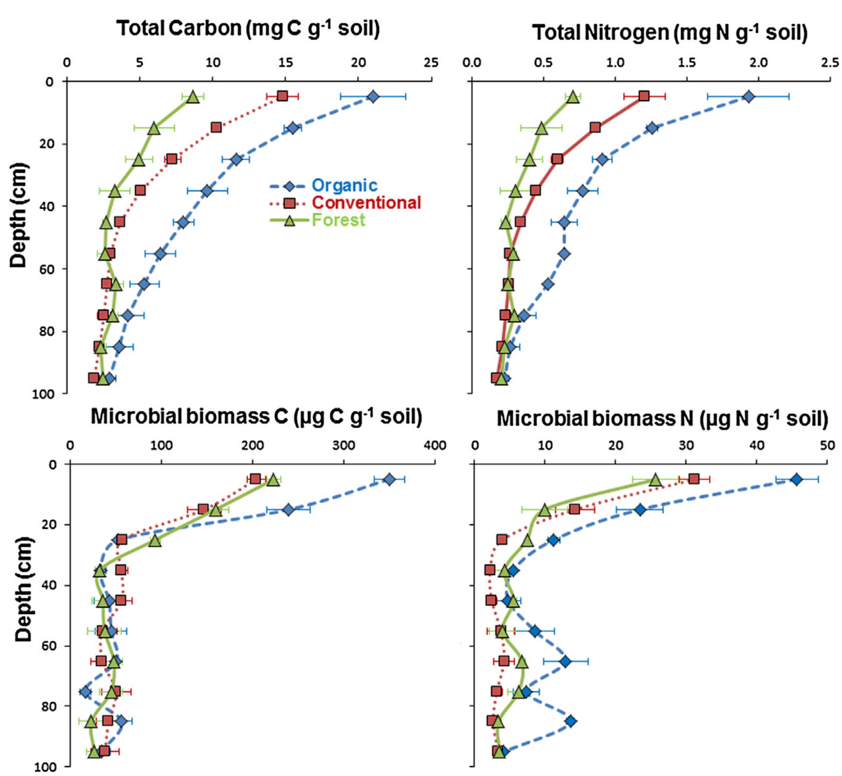

Fig. 1. Total C, N, and microbial biomass C and N depending on land use and depth. Values represent means ± SE (n = 3).

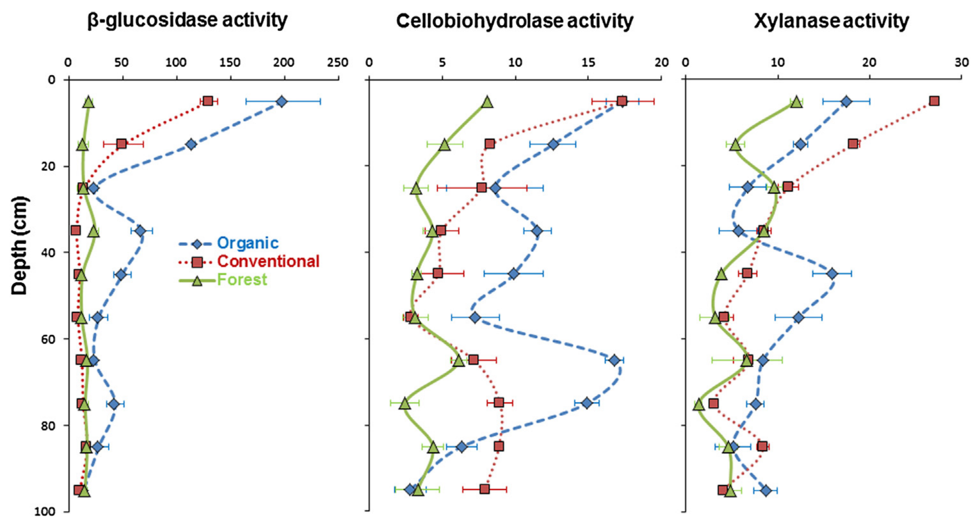

Fig. 2. Activities of C-cycle enzymes: β-glucosidase, cellobiohydrolase and xylanase depending on land use and depth. Values represent means ± SE (n = 3). Enzyme activities are expressed in nmol g^{-1} soil h^{-1}.

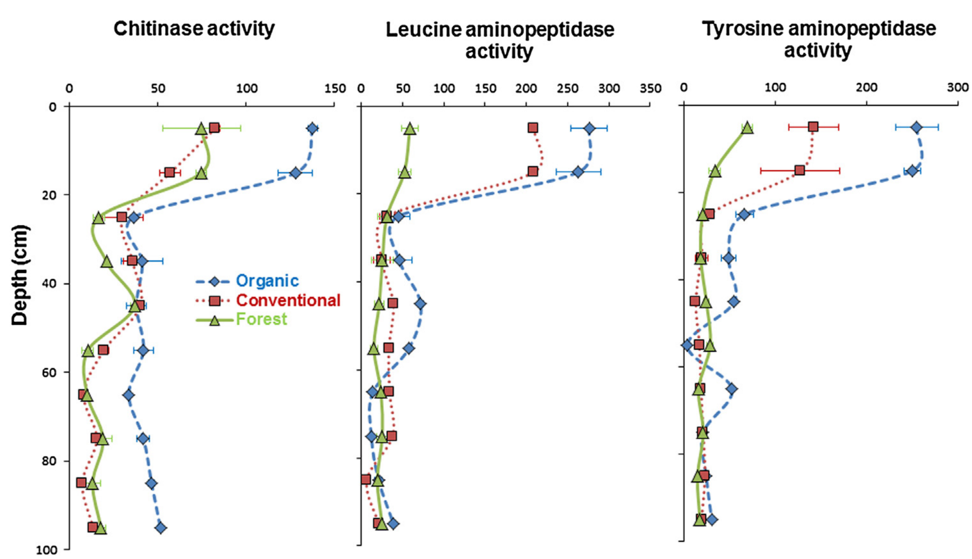

Fig. 3. Activities of N-cycle enzymes: chitinase, leucine aminopeptidase and tyrosine aminopeptidase depending on land use and depth. Values represent means ± SE (n = 3). Enzyme activities are expressed in nmol g^{-1} soil h^{-1}.

followed by forest (672 nmol g^{-1} soil h^{-1}) and organic farming soil (118 nmol g^{-1} soil h^{-1}). Under organic farming, acid phosphatase activity increased with increasing depth, while the opposite trend was noted for conventional farming and forest soil. The activity of the S-cycle enzyme, sulfatase, in the topsoil was higher in organic farming (39 nmol g^{-1} soil h^{-1}) followed by conventional farming (14 nmol g^{-1} soil h^{-1}) and forest (5 nmol g^{-1} soil h^{-1}) (Fig. 4), with similar trends identified in the subsoil.

Among C-cycle enzymes, β-glucosidase (except for forest), cellobiohydrolase and xylanase activities were positively correlated with microbial biomass C (Fig. S1). The activity of acid phosphatase showed a positive correlation with microbial biomass C in conventional farming and forest but a negative correlation in organic farming (Fig. S1). The activities of N-cycle enzymes were positively correlated with microbial biomass N (Fig. S2). There was positive correlation between sulfatase activity and microbial biomass C in both agricultural soils (Fig. S2).

4. Discussion

4.1. Soil and microbial carbon and nitrogen

Soil and microbial C and N decrease with depth due to declining C input (e.g. by plant residues) (Hu et al., 1997). However, soil C and N contents remained higher in subsoil under organic farming compared to other land uses, possibly due to effect of vermicompost application in organic management (Azarmi et al., 2008). In contrast to total C and N content, microbial biomass (C and N) in subsoil was similar among the different land use systems. This indicates that land use and management practices affected microbial biomass only in the topsoil (Liang et al., 2012), confirming our first hypothesis.

Microbial biomass C and N in topsoil followed the order: organic farming > conventional farming = forest soil which contradicts hypothesis (ii), (Fig. 1). Higher soil C and N in organic farming is mainly due to the regular application of farmyard manure and

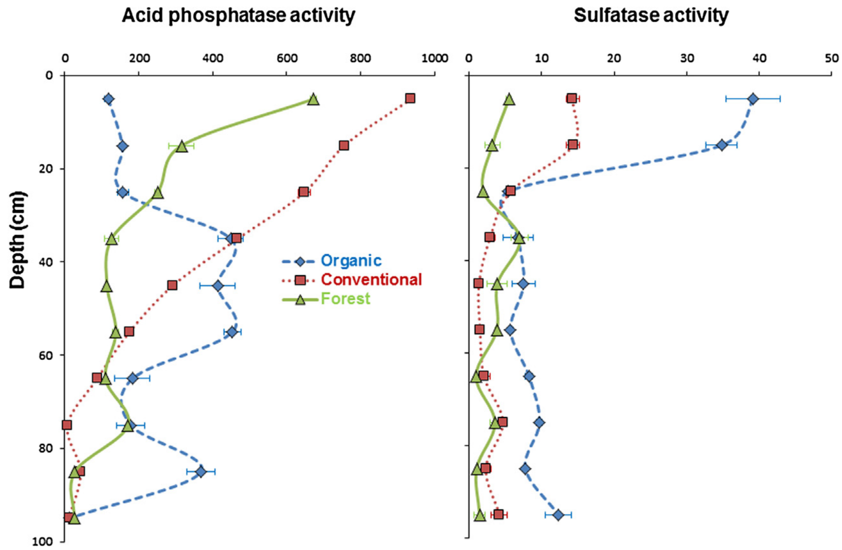

Fig. 4. Activities of P and S-cycle enzymes: acid phosphatase and sulfatase depending on land use and depth. Values represent means ± SE (n = 3). Enzyme activities are expressed in nmol g^{-1} soil h^{-1}.

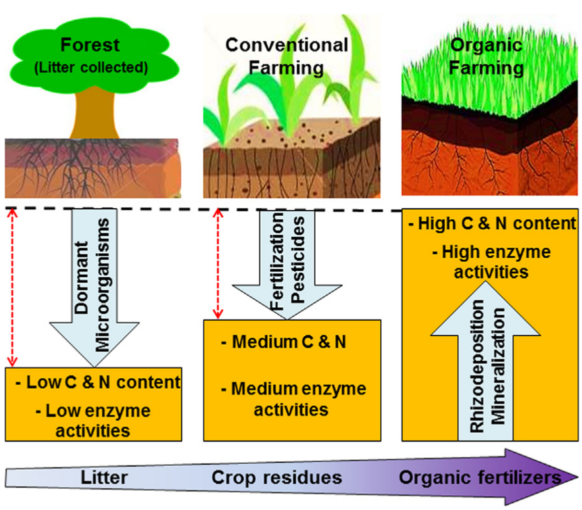

Fig. 5. Conceptual diagram representing the effect of land use on carbon and nitrogen content in soil along with enzyme activities.

vermicomposting (Table 1 and Fig. 5). Farmyard manure supplies readily available N, resulting higher plant biomass. As a result, more crop residues are incorporated through tillage, which maintains higher OM (C and N) levels in surface layers (Roldán et al., 2005). This also provides a favorable environment for microorganisms, contributing to a highly diverse and stable microbial community structure in organic farming systems (Wada and Toyota, 2007). In conventional farming, fallow periods in the crop rotation interrupt the continuous incorporation of crop residues, resulting in lower OM than for organic farming (Figs. 1 and 5). In addition, toxic effects of pesticides may reduce the microbial biomass in conventional farming (Table 1). In the forest system, regeneration and crown density is very low. Furthermore, litter is collected by villagers for performing social and religious activities (Table 1), leading to decreased C and N content relative to organic or conventional farming systems (Fig. 5). Consequently, microbial biomass was lower in forest than in the organic farming system.

The correlation of microbial biomass C and N with total organic C and N, reflects that microbial biomass is determined by the quantity and quality of OM (Kallenbach and Grandy, 2011). Thus, an increase or decrease in soil and microbial C and N content is particularly dependent on management practices.

4.2. Enzyme activities

In topsoil, enzyme activities other than xylanase and acid phosphatase followed the order: organic farming ≥ conventional farming ≥ forest soil (Figs. 2–4), which is contrary to hypothesis (ii). Higher plant growth, due to farmyard manure input, supports high microbial biomass in the organic farming system. In addition, continuous plant cover as well as varied plant species provide different qualities and quantities of crop residue and root exudates, which are substrates for microorganisms and thereby support enzyme production (Nayak et al., 2007). The continuous application of farmyard manure enhances the substrate utilization capacity of microorganisms (Wada and Toyota, 2007). Consequently, microorganisms are activated, contributing to higher enzyme activities and accelerated SOM decomposition (Fig. 5). Elevated chitinase and sulfatase activities imply that fungal biomass is high in the organic farming system (Bandick and Dick, 1999; Badiane et al., 2001), indicating a difference in microbial community resulting from the input of organic matter (Marschner et al., 2003). However, activities of xylanase and acid phosphatase were higher in the conventional farming system (Figs. 2 and 4). Incorporation of rice stubble explains the higher activity of xylanase. Hemicellulose is an insoluble substance contained in plant root detritus (Kandeler et al., 1999a; Kandeler et al., 1999b). The higher availability of these organic compounds stimulates the production of xylanase by the microbial community (Allison and Vitousek, 2005), demonstrating that enzyme activities are significantly affected by cropping and residue management practices (Kandeler et al., 1999a, 1999b; Allison and Vitousek, 2005). The higher activity of acid phosphatase is mainly due to production of this common enzyme by both plants and microorganisms (Blagodatskaya and Kuzyakov, 2008; Nannipieri et al., 2012) and also probably high demand for P (Allison and Vitousek, 2005; Frank and Groffman, 2009; Razavi et al., 2016). Weak or non-significant correlations between microbial biomass and enzyme activities (except acid phosphatase) in forest soil indicates that microorganisms are dormant due to the limited availability of labile C and N in this ecosystem (Fig. S1, S2, 5). Consequently, enzyme activities are low in the forest system (Figs. 2–4).

Concerning the subsoil, the general trend of enzyme activities demonstrated a gradual decrease with depth (except for acid phosphatase and xylanase, in organic farming). Reduced enzyme activities along with microbial biomass in subsoil is connected to decreasing C input and content (Agnelli et al., 2004; Goberna et al., 2006). According to our hypothesis (i), we expected no effect of land use on subsoil. This hypothesis is partly supported by C-, N-, P- and S-cycle enzymes in all land-use systems, with the exception of seven enzymes in organic farming (Figs. 2–4). The activity of acid phosphatase was increased in subsoil in organic farming relative to other land uses. High activity of phosphatase in top- and sub-soil indicated a high investment of microorganisms and plants for the acquisition of P (Fig. 4). By this belowground C investment, plants regulate increase the availability of organically bound P from sub-soil in the tropics. Higher activities of enzymes involved in cellulose and hemicellulose decomposition in the organic farming system are due to the favorable environment for C-degrading microorganisms in the subsoil. The higher water content following rice cultivation could also be a contributing factor. Additionally, the higher activities of enzymes are indicate for the presence of complex substrates (German et al., 2011). This explains the similar behavior of enzymes degrading C-polymers, N-polymers (chitinase and tyrosine aminopeptidase) and S-containing molecules in the organic farming system. In general, application of vermicomposts simulate activities of some enzymes (Atiyeh et al., 2001; Benitez et al., 2004) in organic farming. Positive correlations between enzyme activities and microbial biomass in conventional and organic farming systems (except for acid phosphatase) (Fig. S1, S2) reflect the microbial origin of the enzymes (Nayak et al., 2007; Wallenius et al., 2011).

5. Conclusions

Total organic C and N, soil microbial biomass and enzyme activities other than xylanase and acid phosphatase were higher in organic farming than in conventional farming and forest topsoil. Organic matter input under various management practices is the most important factor for determining C and N content and microbial properties. In the subsoil, microbial biomass was similar among land-use systems, although enzyme activities were slightly higher under organic farming. These results demonstrate that land use and management practices have significant effects on microbial properties in surface layers, with lesser effects in subsoil. Microbial response to resource limitation and substrate availability determines the production of enzymes in different land use systems. Thus, microbial properties can serve as potential biological indicators of ecological changes resulting from land-use and management practices in subtropical top-and sub-soils.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.apsoil.2017.01.008.

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