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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