Contents lists available at ScienceDirect

Soil Biology & Biochemistry

Microbial community structure and resource availability drive the catalytic efficiency of soil enzymes under land-use change conditions

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ARTICLE INFO

ABSTRACT

The turnover of nutrients bound to organic matter is largely mediated by extracellular hydrolytic enzymes (EHEs) produced by soil microorganisms. However, little is known about the environmental drivers (e.g., soil pH, C content, C:N ratio) of the catalytic properties of EHEs and their functional link to the structure of soil microbial communities. We linked catalytic properties, K_m and V_{max}, determined by Michaelis–Menten kinetics, to a set of environmental and microbial properties in the soils of a land-use sequence ranging from undisturbed natural forest to pastures of different ages and to secondary succession in the Andes of southern Ecuador. The sensitivity of the substrate affinity constant (K_m) and the maximum rate (V_{max}) of six EHEs (β-cellobiohydrolase (CBH), β-glucosidase (BG), N-acetylglucosaminidase (NAG), α-glucosidase (AG), xylanase (XYL), acid phosphomonoesterase (AP)) to changing environmental conditions was tested by fluorogenic substrates. We used the V_{max}-to-K_m ratio (K_a) as a proxy for the catalytic efficiency and the signature membrane phospholipid fatty acids as a proxy of microbial community structure.

Microbial communities adapted to environmental changes, selected for enzymes with higher substrate affinity (K_m) and catalytic efficiency (K_a) compared with pure cultures. Along the land-use sequence, catalytic efficiency increased from natural forest to young pasture, while it decreased during long-term pasture use and secondary succession. This is consistent with three to five times faster turnover of tested substrates (estimated based on Michaelis–Menten kinetic parameters) at the young pasture compared with the long-term pasture and secondary succession. Environmental drivers of the K_m were enzyme-specific (e.g., the pH for XYL, the C:N ratio for AP, and the C availability for NAG) and differed from those for V_{max}. A decoupled response of V_{max} and K_m to land-use changes observed for AG, BG, CBH, XYL, and AP, implies divers consequences for ecosystem processes mediated by these enzymes. A high abundance of Gram(−) bacteria triggered the catalytic properties (K_m and/or K_a) of enzymes decomposing cellulose, hemicellulose, starch, and monophosphoesters. The importance of climatic factors for catalytic properties of EHEs was emphasized by the K_a values extracted from the literature and demonstrated good correspondence of K_a between soils from geographically distinct experimental plots.

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1. Introduction

The cycling of major biogenic elements such as carbon (C), nitrogen (N), and phosphorus (P) in terrestrial ecosystems is susceptible to global change phenomena like increases in temperature (Allison et al., 2010) and changes in land use (Don et al., 2011). Especially in the tropics, the conversion of natural forests to arable land and pastures as well as the abandonment of degraded arable land is increasing all over the world (Bai et al., 2008). These shifts in land use are the main factors changing environmental properties such as soil pH (Ehrenfeld et al., 2005), and quantity and quality of organic compounds (e.g., C, N, P molar ratios) important for the metabolism of heterotrophic microbial communities (Cleveland and Liptzin, 2007). The co-occurrence of ecosystems differing in age after forest conversion represents a soil environmental gradient

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under similar climatic conditions. An example of a human-induced environmental gradient is the conversion of natural forests to pastures. In the study area, the south Ecuadorian Andes, burning forest biomass raises the pH (+2 pH units) and increases the nutrient availability of the topsoils of the newly established pastures. During land use, additional burning and nutrient translocation into plant biomass cause a decrease in the pH and nutrient stocks in the soil (Hamer et al., 2013). Such changes in the soil environment and nutrient availability cause substantial variation in the biomass and composition of microbial communities in the study area (Tischer et al., 2014b) as well as in other ecosystems (Lauber et al., 2008; Fierer et al., 2009). These changes affect the relative domination of organisms exhibiting various microbial life strategies (Fierer et al., 2007) that are differentiated by growth rate and substrate affinity for the enzyme systems (e.g., copiotrophs with high growth rate and low substrate affinity vs. oligotrophs with low growth rate and high substrate affinity Killham and Prosser, 2015). In turn, such changes affect the turnover and sequestration of nutrients in soil (Cusack et al., 2011; Schimel and Schaeffer, 2012).

The decomposition of organic material (OM) is largely mediated by extracellular hydrolytic enzymes (EHEs) produced by soil microorganisms (Swift et al., 1979). Due to functional redundancy, various microbial taxa produce a diverse set of enzymes (isoenzymes) that target the same substrate but differ in biochemical potential (Stres and Tiedje, 2006) and enzymatic adaptation to environmental constraints (Khalili et al., 2011). Thus, the catalytic properties of isoenzymes performing, e.g., the hydrolytic breakdown of polymers to smaller molecules, can differ significantly depending on the soil properties, nutrient availability, and quality and amount of substrate (Wallenstein et al., 2011). The substrate-dependent catalytic behavior of EHEs approximated with Michaelis–Menten kinetics (the velocity of the enzyme–substrate reaction as a function of substrate concentration) is a useful tool for determining the sensitivity of EHEs to changing environmental conditions (Marx et al., 2005; Cusack et al., 2011; German et al., 2012; Stone et al., 2012). The parameter of the Michaelis–Menten equation, the Michaelis constant (K_m), represents the substrate concentration at the half-maximal enzymatic rate. This K_m value is used as an indicator of the apparent affinity of the enzyme to the particular substrate (German et al., 2012) and characterizes the rates of enzymatic reactions at low substrate concentrations, a common situation in soil (Hobbie and Hobbie, 2012).

The second kinetic parameter of the Michaelis–Menten equation is the maximum rate of the enzyme-mediated reaction (V_{max}) at saturating substrate concentrations. In soil, this parameter represents the potential enzyme activity and depends on the overall isoenzyme concentration (Wallenstein and Weintraub, 2008). The number of studies on V_{max} focus either on the fine-scale distribution and catalytic potential of EHEs (Marx et al., 2005) or on the temperature sensitivity and effects of nutrient enrichment on the catalytic properties of EHEs (German et al., 2012; Stone et al., 2012). Less attention is paid, however, to the catalytic behavior of enzymes at substrate limitation. This is mainly due to a lack of methods for determining enzyme activities at substrate concentrations that occur in nature (Hobbie and Hobbie, 2012). Assays based on fluorogenically-labeled (4-methylumbelliferone, 4-MUF) substrates are sensitive to detect enzyme activity at substrate concentrations ranging from micromoles to nanomoles (Marx et al., 2001). Studies that link the apparent substrate affinities of EHEs to the soil microbial community structure are needed (Wang et al., 2012) in order to test how shifts in enzyme functioning are related to microbial community composition (Cusack et al., 2011). Since the parameters V_{max} and K_m are often interrelated (Kovarova-Kovar and Egli, 1998), the V_{max}-to-K_m ratio was suggested as better proxy of the catalytic efficiency (K_a) than V_{max} and K_m alone (Moscatelli et al., 2012). The K_a characterizes the inherent catalytic properties of enzymes (Moscatelli et al., 2012), associated with the competitive ability of soil microorganisms (Kovarova-Kovar and Egli, 1998). However, the sensitivity of enzyme catalytic efficiency to environmental gradients caused by land-use changes must be tested experimentally.

The incorporation of catalytic properties of EHEs in soil C models (Allison et al., 2010) requires knowledge on the environmental drivers of the parameters that affect the K_a, i.e., the V_{max} and the K_m. Different V_{max} drivers were reported for enzymes with various functions in a study of land-use change (Lauber et al., 2008; Tischer et al., 2014a). Specifically, the V_{max} of EHEs that degrade cellulose and chitin was regulated by the amount of soil microbial biomass. In contrast, the V_{max} of EHEs involved in degrading hemicelluloses and starch was mainly driven by the quantity and quality of the substrate input, and was not restricted by the abundance of soil microbes. The environmental drivers of the K_m and the K_a remain unknown.

Therefore, the present study set out to answer the question whether the apparent substrate affinities and catalytic efficiencies of six EHEs involved in C, N, and P cycling are associated with microbial community structure and with changes in soil chemical properties caused by land-use. We analyzed the kinetics of β-cellobiohydrolase (CBH), β-glucosidase (BG), β-xylanase (XYL), and α-glucosidase (AG), which are involved in depolymerizing cellulose, hemicelluloses (Wong et al., 1988), and starch (Suzuki et al., 1976). Cellulose is the most abundant carbohydrate biopolymer on Earth that is hydrolyzed by CBH and BG to low-molecular-weight substances (cellobiose, glucose). Hemicelluloses are highly abundant polymeric sugar structures in the primary cell wall of plants (up to 30%) (Barton and Northrup, 2011). Major components of hemicelluloses are xylans, which are hydrolyzed by the action of xylanases to the monomer xylose. Starch, the major carbohydrate storage component of plants, is hydrolyzed by the action of α-1,4-glucosidases to glucose (Barton and Northrup, 2011).

We also analyzed the kinetics of N-acetylglucosaminidase (NAG) that hydrolyzes N-acetylglucosamine of fungal chitin and bacterial peptidoglycan (Kögel-Knabner, 2006), and thus is linked to microbial turnover and interacts with both C and N cycles in soil (Beier and Bertilsson, 2013). Particularly in tropical soils, large proportions of P are immobilized in organic, ester-linked P forms (Doolette and Smernik, 2011). Therefore, we investigated the catalytic properties of acid phosphomonoesterase (AP) that catalyze the hydrolysis of monophosphoesters, and then release phosphate for plant and microbial uptake (Nannipieri et al., 2011).

The present study on soils in Southern Ecuador is a first attempt to establish linkages between environmental changes caused by land-use, microbial community structure and the catalytic properties of EHEs. The research questions of the study were:

How land-use change from forest to pasture will affect the patterns of enzymes' catalytic properties, i.e., K_m and K_a? Does microbial community structure matter for enzymes' catalytic properties? Is the rate of enzyme reaction related to substrate affinity or do they vary independently of each other?

In addition, we linked our data to the results of available studies (MUF assays only) in order to test whether the observed relationships to environmental drivers hold true at the large geographic scale.

2. Materials and methods

2.1. Site description

The study was conducted along a typical land-use sequence in the valley of Rio San Francisco, in the Cordillera Real, an eastern