Applied Soil Ecology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Maize phenology alters the distribution of enzyme activities in soil: Field estimates

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ARTICLE INFO

Keywords:
Root exudation
Vegetation stage
Microbial activation
Nutrient cycling

ABSTRACT

Microbial processes mediated by soil enzymes are crucial in soil organic matter decomposition, resulting in release of nutrients that become available for plant and microbial uptake. Therefore, it is crucial to know the sensitivity of enzyme activities (EA) along soil depths at distinct plant vegetation stages, and how the availability of mineral nitrogen (N) alters EA. We studied effects of N fertilization (0 and 160 kg N ha^{-1}), soil depth (0–35 cm), and plant-phenological stage (silking and maturity) on microbial biomass C (C_{mic}) and potential activities of C-, N- and P-acquiring enzymes in the field under *Zea mays* L.

Nitrogen fertilization increased shoot biomass by more than 80% compared to unfertilized plants. Maize roots triggered increases in C_{mic} and EA for all measured enzymes compared to bare fallow. Stimulating effect of plant roots on EA was enzyme specific and stronger at silking than maturity stage of maize. The down-regulating effect of N fertilization on EA involved in acquiring N was most pronounced on the activity of L-leucine aminopeptidase and β-1,4-*N*-acetylglucosaminidase. Soil depth was the primary determinant of EA, explaining up to 51% of the variation. Depth-dependent EA changes were stronger in rooted soil.

A pronounced biotic control on EA was demonstrated by higher EA in rooted soil than in bare fallow. This confirmed root-mediated microbial activation. Stronger effect of silking vs. maturity stage on EA indicated that actively growing roots fuel microorganisms via root-derived organics. Thus, soil depth and plant roots were major factors controlling microbial activity in arable soil.

1. Introduction

Food security will be a vital issue in meeting the demand of an increasing global population. Thus, there is renewed interest in understanding the biochemical processes in agricultural soils, and how altering these processes may be used to increase agricultural productivity. Such sustainable agricultural practices offer tremendous opportunities for maintaining or increasing soil health (i.e. fertility) (Doran and Zeiss, 2000). Sustainable agriculture refers to maintenance or enhancement of soil health with minimum disturbance and has laid the foundation for understanding soil ecological functioning (Ferrarini et al., 2017; Weiner, 2017). Soil microorganisms are central to ecological functioning (Bender et al., 2016). A better understanding of microbial functioning will help to elucidate the biogeochemical processes contributing to nutrient transformations in soils (Nannipieri et al., 1978, 2003). Decomposition and transformation of soil organic matter (SOM), nutrient mobilization/immobilization, and aggregate formation/stabilization are among the most important processes predominantly governed by microorganisms (Nsabimana et al., 2004; Six et al., 2004; Caldwell, 2005). The cycling of major nutrient elements is widely associated with enzyme activity (EA) in soil (Burns et al., 2013). EA is important in maintaining soil health, as enzymes catalyze the bottleneck steps in SOM decomposition and consequent release of nutrients for plant and microbial uptake (Aon et al., 2001). Generally, EA is

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https://doi.org/10.1016/j.apsoil.2018.02.001
Received 18 November 2017; Received in revised form 1 January 2018; Accepted 1 February 2018

dependent on various biotic and abiotic factors such as pH (Sinsabaugh, 2010), nutrients (Keuskamp et al., 2015; Olander and Vitousek, 2000), disturbance (Boerner et al., 2000), succession (Tscherko et al., 2003), microbial community structure and function (Dorodnikov et al., 2009; Tischer et al., 2015), plant species (Caravaca et al., 2005), and management practices (Shahbaz et al., 2017).

Depending on the complexity of SOM, various hydrolases and oxidoreductases are produced by microorganisms. For example, β-1,4-glucosidase (BG), cellobiohydrolases (CBH), and β-xylosidase (XYL) are a set of hydrolases produced by microorganisms to acquire C via polysaccharide decomposition. Another widely prevalent enzyme is L-leucine aminopeptidase (LAP), which is associated with the breakdown of amide-linked polypeptides, the primary form of organic N in soils (Finzi et al., 2015; Knicker, 2004). β-1,4-*N*-acetylglucosaminidase (NAG), which predominantly targets chitin and peptidoglycan breakdown, releases both C and N for microbial acquisition. Organic compounds containing ester-linked P are cleaved by phosphomonoesterase (PHO), which releases inorganic P (Finzi et al., 2015; Sinsabaugh and Follstad Shah, 2011). In rooted soils, enzyme production is triggered by root exudation, resulting in higher rates of SOM decomposition and in a consequent release of nutrients (Kuzyakov and Domanski, 2000).

Root exudates provide easily accessible substrates for microorganisms and are an ecologically important contributor to rhizosphere processes. According to the microbial activation hypothesis (Cheng and Kuzyakov, 2005; Kuzyakov et al., 2007), root exudation triggers the upregulation of metabolic activities in microbial cells. Enhanced metabolic demands lead to the production and release of enzymes. Therefore, EA are sensitive indicators of microbial activity (Nannipieri et al., 2002).

Mineral fertilizers, representing another form of easily accessible nutrients, also affect SOM decomposition by altering microbial activities. In the presence of easily accessible nutrients, microorganisms down-regulate resource allocation for enzyme synthesis and release, as they are not solely dependent on nutrient gains via SOM decomposition. However, attempts to determine the impact of N fertilization on microbial activities have been inconclusive, with studies reporting increases, decreases and even no effect on EA with fertilization (Shen et al., 2010; Ai et al., 2012; Kumar et al., 2016). It is assumed that in nutrient limited soils, microbial growth and activity are constrained due to low availability of C and nutrients and, as a result, the input of resources (via root exudation and N fertilization) will enhance microbial growth and activity (Renella et al., 2006). Increased growth will consequently increase enzyme production to mineralize more SOM to meet microbial nutrient demands. Under nutrient limitations, N addition may stimulate the production of enzymes, as N is essential for enzyme synthesis (Olander and Vitousek, 2000). In contrast, when N is not a limiting factor, microorganisms do not allocate their resources to the production of enzymes associated with N acquisition. Therefore, there is a negative feedback between supply and demand for production of enzymes. The addition of one nutrient may alter the EA of not only the enzymes involved in that particular nutrient cycle, but may also alter the activities of other enzymes involved in the cycling of other nutrients. For example, xylanase activity (involved in decomposition of hemi-cellulose) decreased in the presence of mineral N (Chen et al., 2014). Microbial activity relies not only on the availability of nutrients, but is also affected by soil depth. It has been observed that when depth increases, microbial activity decreases, as substrate inputs and gas exchange are reduced with depth (Loeppmann et al., 2016; Stone et al., 2014). The spatial distribution of roots is heterogeneous in soil and varies with the growth stage of the plant (Chimento and Amaducci, 2015), which may impact plant-mediated microbial activities at various soil depths. It has previously been demonstrated that there are distinct microbial community composition and their activities along with soil depth (Fierer et al., 2003; Jackson et al., 2009) and these changes are generally explained by substrate input varying in quality and quantity (Loeppmann et al., 2016).

Although roots and microbial activity are often linked (Kumar et al., 2017, Kuzyakov and Blagodatskaya, 2015), most of the field studies are conducted only once during a vegetation season (either at the beginning or before harvesting). However, root-mediated effects on microbial activity are taking place throughout the growing season (Bell et al., 2015). It is still unknown from direct field observations how microbial activity is influenced by roots at various plant growth stages, which are characterized by distinct morphological and physiological properties. Thus, the following research question was addressed: How sensitive is EA to the presence of plants and N fertilization (availability of mineral N) across a range of soil depths at distinct maize phenological stages? To answer this question, potential activities of six enzymes catalyzing the decomposition of organic C compounds (BG, CBH, XYL, NAG), organic N compounds (LAP and NAG), and organic P compounds (PHO) were determined with or without plants at four soil depths (0–5 cm, 5–15 cm, 15–25 cm, and 25–35 cm), at two maize phenological stages (silking and maturity), and with and without N fertilization.

2. Materials and methods

2.1. Experimental setup

The experiment was established on an agricultural research field belonging to the Georg-August-University Göttingen, Germany. The soil is characterized as a haplic Luvisol suitable for a broad range of agricultural uses with the following properties: total C content of 1.41 ± 0.04%, total N content of 0.16 ± 0.02%, pH value of 7.2 ± 0.01, and bulk density of 1.2 ± 0.2 g cm^{-3}. The experimental site is under conventional agricultural uses. Conventional tillage practices up to 30 cm of soil depth are performed twice in a year. Maize seeds (*Zea mays* L. cv. Colisee) coated with methiocarb (4(methylthio) 3,5-xylyl-*N*-methyl carbamate), a pesticide, and thiram (tetramethylthiuram sulphide), a fungicide were sown in the field. The experimental field was divided into 16 plots (5 × 5 m^2) with a 2 m wide buffer strip around each plot to exclude neighbor effects as follows: Bare fallow, bare fallow with N fertilization (Bare fallow + N), maize-planted (Planted), and maize-planted with N fertilization (Planted + N) in a completely randomized design. N fertilizer was applied as urea at the soil surface at a rate of 160 kg N ha^{-1} (Weiterer, Landhandel GmbH) 47 days after planting (DAP). Any visible weed growing in the plots was manually removed at regular time intervals throughout the experimental period.

2.2. Soil and plant sampling

Soil and plants were sampled twice during the experimental period at 72 DAP and 130 DAP, which corresponds to the silking and maturity stages of maize plants. Soils were collected from four soil depths at 0–5 cm, 5–15 cm, 15–25 cm and 25–35 cm with a corer (inner diameter 7 cm) between the maize rows in the middle of the diagonal between two plants and transported to the laboratory in cooling boxes. Soil moisture content was estimated as the difference between field moist and oven-dried soil (at 105 °C for 48 h). Afterwards, soils were passed through a 2 mm sieve and used for further analyses. For shoot biomass determination, two plants were cut at the base from each plot at randomly selected positions, oven dried at 60 °C for 5 days, and weighed. As total root biomass could not be accurately determined in the field, root biomass per unit of area (i.e. g dry weight m^{-2}) was calculated based on the measured shoot to root ratio of maize from the same field (Kumar et al., 2016). The root to shoot ratio was 0.11 and 0.14 for unfertilized and N fertilized maize at maize silking stage. These ratios were used to calculate the root biomass at maize silking stage (0.04 ± 0.01 kg m^{-2} for unfertilized maize and 0.09 ± 0.01 kg m^{-2} for N fertilized maize). At maize maturity, root to shoot ratio (0.16) and root biomass (0.22 ± 0.04 kg m^{-2}) was determined using the equation derived from Amos and Walters (2006) for unfertilized maize. At