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Original article

Short-term effects of plant litter on the dynamics, amount, and stoichiometry of soil enzyme activity in agroecosystems

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ABSTRACT

Addition of plant litter can affect soil enzyme activity at three scales: dynamics, amount, and stoichiometry. In this study, we examined the dependence of soil enzyme activity at all three scales on litter quality. Soils of similar texture were collected from conventional and organic farming systems, the Center for Environmental Farming Systems, Goldsboro, North Carolina, USA. Soil samples were then amended with senescent pine needles, grass materials, and soybean residues of C:N ratio 139, 50, and 9, respectively, at 2 mg C g^{-1} soil, and the activities of soil β-glucosidase, exoglucanase, β-glucosaminidase, and phenol oxidase were measured over the course of 90-d incubation. Relationships between the dynamics of enzyme activity and litter quality appeared to be enzyme specific. Time patterns of soil β-glucosidase and β-glucosaminidase activity were independent of litter quality, with rapid increase in enzyme activity and reaching a peak several weeks after litter addition. In contrast, time patterns of polymer-degrading enzymes (exoglucanase and phenol oxidase) were dependent on litter quality. Exoglucanase activity showed a concave function with time following the addition of soybean residues or grass materials, but increased slightly following the addition of pine needles. Cumulative activities of soil enzymes were upregulated following litter addition and could be qualitatively assessed by litter C:N ratio. The activity of β-glucosaminidase was negatively related to litter C:N ratio, being greatest in soybean residues-amended soil. Litter of a low C:N ratio was generally better than litter of a high C:N ratio for increasing soil cellulase activity and vice versa for phenol oxidase. However, the stoichiometry of soil enzyme activity was decoupled with litter C:N ratio. Soybean residues and pine needles at opposite ends of the litter C:N range were more similar in the ratio of C- to N-acquiring enzyme activity. We also examined pH effects on the expression of added enzymes. Soil enzyme activities were enhanced as soil pH increased from 6 to 8. pH-associated changes in enzyme activity were generally smaller as compared to changes caused by other factors during the 42-d incubation. Our results suggest that litter effects on the dynamics, amount, and stoichiometry of soil enzyme activity were independent of soil pH. Litter C:N was a good indicator for the total amount, but not for the dynamics or stoichiometry of soil enzyme activity.

1. Introduction

Plant litter is a primary control of microbial production of extracellular enzymes [12]. Due to the induction of substrate, soil enzyme activities are often upregulated following litter addition. A number of laboratory and field studies have shown that the activity of soil hydrolases, e.g., β-glucosidase, β-glucosaminidase, acid phosphatase, and cellulase is often increased after soil is amended with organic materials [5,10,12,46]. However, degrees of upregulation may vary with litter type, because litter contains organic compounds at different levels of complexity and nutrient stoichiometry [22].

Soil microbes have been considered as cost-effective organisms in terms of acquiring C and nutrients from the environment. They may prioritize the production of enzymes that mediate the availability of growth-limiting nutrients in the environment [3,35]. As such, soil nutrient availability may regulate microbial enzyme allocation patterns, although soil C controls the total activity of soil enzymes [31,45]. During the decomposition of litter with a high C:N ratio, for example, microbes can be N limited and thus are expected to allocate resource to produce N-acquiring enzymes. Only when this N limitation is relieved, C-acquiring enzyme activity can be enhanced. As demonstrated by Refs. [15]; cellulolytic enzyme activities were increased significantly by the addition of mineral N

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during the decomposition of litter with <1.2% N. Nitrogen availability can also regulate microbial production of oxidative enzymes such as phenol oxidase. In the case of N limitation, microbes produce phenol oxidase perhaps for mining recalcitrant organic matter-bound and/or -occluded N compounds. Nonetheless, microbial enzymatic responses to C and nutrient availability in the environment imply that litter quality can considerably affect the relative abundance and activity of soil enzymes [1,6,17,36]. Indeed, litter of a low C:N ratio was found to stimulate soil hydrolase activity, whereas litter of a high C:N ratio could enhance the oxidative enzyme activity [17].

Returning crop residues to soil is a common agricultural practice that can promote soil organic matter buildup and nutrient cycling. While crop residue quality is considered as an important indicator of organic matter decomposition, the two are not always correlated [18]. Given that soil enzyme activity is a rate-liming step of decomposition and can also quickly respond to soil C and nutrient availability, understanding the effects of crop residue quality on enzyme activity may provide effective feedback. The objective of this study was to assess how litter quality affected the dynamics, amount, and stoichiometry of soil enzymes involved in C and N transformations. Because soil pH can substantially affect the expression of microbial extracellular enzymes via its controls on enzyme-organic matter/clay association as well as the availability of substrates and enzyme co-factors [24,40], we also examined pH effects on the expression of soil enzymes.

2. Materials and methods

2.1. Soil sampling

Soils were collected by coring techniques from organic (ORG) and conventional (CON) farming systems, located at the Center for Environmental Farming Systems (CEFS), Goldsboro, NC, USA where crops were rotated annually, including corn (*Zea mays* L), peanut (*Arachis hypogaea*), cotton (*Gossypium hirsutum*), soybean (*Glycine max*), wheat (*Triticum aestivum*), sweet potato (*Ipomoea batatas*), and sorghum (*Sorghum bicolor*). In CON, plant nutrients were supplied annually with synthetic N, P, K fertilizers and also herbicides, insecticides and fungicides were used. In ORG, turkey litter (i.e., a mixture of turkey excreta, bedding material, and spilled feed) was used as the fertilizer and the fertilization rate was depended on rotated crops. Gypsum was used to provide calcium at both farming systems. Each farming system consisted of three field plots, representing three replicates. Soil was classified as Wickham sandy loam (fine-loamy, mixed, semiactive, thermic Typic Hapludult) in one plot and Tarboro loamy sand (mixed, thermic Typic Udipsamment) in the other two plots. Detailed information on CON and ORG has been reported previously [42].

Twenty soil cores (2.5 cm dia. × 10 cm depth) were collected randomly from each field plot and pooled to form a composite soil sample. A total of 6 soil samples (i.e., two farming systems × three field plots) was sieved (<2 mm), adjusted for soil moisture content to 45% water holding capacity, and then stored at 4 °C prior to a series of laboratory experiments. The CON and ORG were similar in soil organic C content, soil C:N ratio, and microbial biomass C. On average, soil had 10 mg total C g^{-1} soil, C:N ratio 11.4, and 328 μg microbial biomass C g^{-1} soil. However, soil pH differed by ~0.5 units between the two farms, with pH 6.1 and 6.6 for CON and ORG, respectively. Soils in CON and ORG also differed in N mineralization and the biochemistry of water extractable organic matter [42]. Hence, by using soils from two different farming systems we were able to better assess litter effects on soil enzyme activity and determined if litter effects varied with agroecosystems.

2.2. Plant litter

Plant litter was collected from senescent vegetation ground cover at the CEFS, including long-leaf pine (*Pinus palustris*) needles, a mixture of grass materials: switchgrass (*Panicum virgatum*), eastern gamagrass (*Tripsacum dactyloides*), indiangrass (*Sorghastrum nutans*), and big bluestem (*Andropogon gerardii*), and soybean (*G. max*) residues.

Litter was dried at 80 °C for ~8 h and then ground (<0.5 mm). Carbon and N contents of litter were determined by dry combustion using a Perkin–Elmer 2400 CHN analyzer. Carbon contents were similar among different types of litter, i.e., 471, 438, and 414 mg C g^{-1} plant dry mass for pine needles, grass materials, and soybean residues, respectively. However, C:N ratios varied greatly, being 139, 50 and 9 for pine needles, grass materials, and soybean residues, respectively.

2.3. Experimental design

Effects of plant litter on microbial production of extracellular enzymes were examined in a 90-d incubation experiment with three replications. Eight treatments were made based upon a 2 × 4 factorial design, i.e., 2 farming systems and 4 types of litter addition (no litter, pine needles, grass materials, and soybean residues). Respective oven-dried litter was amended into ~50 g soil at 2 mg C g^{-1} soil and then treated soils were incubated at 25 °C in dark for three months. Soils without litter addition were used as the controls. The activities of soil phenol oxidase, exoglucanase, β-glucosidase, and β-glucosaminidase were analyzed 6 h and 14, 21, 28, 52, 73, and 90 d after the start of incubation.

Effects of pH on enzyme expression were assessed via dynamics of added commercially-available enzymes in a 42-d incubation experiment. Because in this experiment plant litter was not added to soil, microbial production of extracellular enzymes was minimized. To further minimize microbial production of enzymes during the incubation, soil microbial activity was inhibited by using cycloheximide, a fungicide at 15 mg g^{-1} soil and streptomycin sulfate, a bactericide at 3 mg g^{-1} soil. It should be noted that antibiotic inhibition is often <100% effective and also lasts for short duration, perhaps only a few days [4]. But this approach at least helped impede microbial production of enzymes and thus assess pH effects on activities of added enzymes during the early stage of incubation. Six treatments were made based upon a 2 × 3 factorial design, i.e., 2 farming systems and 3 soil pH levels. Besides initially-acidic pH, two additional pH values were made by increasing ~0.9 units (i.e., near-neutral treatment) and ~1.8 units (i.e., alkaline treatment), respectively, with 5 M KOH solution. The amount of KOH solution that brought the soils to desired pH values were calculated based upon forward and backward titration curves made with Titration Manager (model TIM 856, Radiometer Analytical, France). Enzymes were then added into soils as a mixture of horseradish peroxidase (Sigma P8250), cellulase (Sigma C9422), laccase (Sigma 53739), and tyrosinase (Sigma T3824). Stock solutions of these enzymes were added into 20 g soils to reach final concentrations of 1 unit of horseradish peroxidase g^{-1} soil, 10 units of cellulase g^{-1} soil, 5 units of laccase g^{-1} soil, and 5 units of tyrosinase g^{-1} soil. Treated soils were incubated at 25 °C in dark for 42 d and analyzed for the activities of peroxidase, exoglucanase, β-glucosidase, and phenol oxidase 6 h and 7, 25 and 42 d after the start of incubation.

2.4. Soil enzyme activities

The activities of soil enzymes, including peroxidase (EC 1.11.1.7), phenol oxidase (EC 1.10.3.2), exoglucanase (EC 3.2.1.91), β-