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Original article

Relationships between the density and activity of microbial communities possessing arylsulfatase activity and soil sulfate dynamics during the decomposition of plant residues in soil

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ABSTRACT

A laboratory incubation experiment was performed to examine the changes of soil microbial communities possessing arylsulfatase (ARS) activity. Different plant residues were incorporated into soils, and the resulting changes in the density and ARS activity of these communities relative to the sulfate content were studied. Mustard, fescue and wheat plant residues with different biochemical compositions and carbon (C):sulfur (S) ratios ranging from 60 to 486 were tested. Soil lacking residues was included as a control. Among the tested residues, mustard was the most labile and the most rapidly mineralized, leading to a significant 67% increase in the soil microbial biomass C (SMBC) compared with the control. The incorporation of mustard into soil induced a significant shift in the balance of microbes possessing ARS activity (ie. ratio bacteria:fungi) with an increase in the bacterial component producing ARS (ARS-B) but no change in ARS activity. A principal component analysis revealed clear differences between the soil samples according to both the nature of the residues incorporated and the duration of incubation. The correlation coefficients between the different variables showed that the ARS-B:ARS-F ratio was strongly correlated with the soil sulfate content ($r = 0.81$, $p < 0.05$). However, ARS activity was more closely correlated with ARS-F density than with ARS-B density ($r = 0.63$, $p < 0.05$ and $r = 0.29$, $p < 0.1$, respectively). The relative stability of the soil ARS activity, regardless of the nature of the residues incorporated was likely due to non-limiting S conditions for microbial growth in the soil used in this study.

1. Introduction

Sulfur (S) plays vital roles in plant metabolism and development [1], and S deficiencies can affect both the yield and quality of harvested products [2]. Organic S is the main S fraction in soils, contributing up to 95% of the total S in top layers [3]. Two groups of organic S compounds have been identified based on their reactivity to reducing agents [4], a result that was further confirmed using X-ray absorption near-edge spectroscopy (XANES) ([5–7]. These two groups are i) carbon (C)-bound S (C–S linkage), represented by amino acids, sulfonates and heterocyclic S and ii) organic S not directly bound to C, primarily represented by sulfate esters (C–O–S linkage) and sulfamates (C–N–S linkage) [8]. The second group represents 27–78% of the total S in the surface horizons of soil [9] and is considered the most labile fraction of organic S in agricultural soils [4]. The mineralization of sulfate esters in organic soil matter and organic residues into forms available to plants (i.e., sulfates) is tightly controlled by microbiological enzymatic

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processes that could be of central importance to the liberation of sulfates for plant nutrition [10,11].

Among the microbial enzymes implicated in S cycling, arylsulfatase (ARS) is believed to be involved in mineralization of ester sulfate in soils [12,13]. Different bacterial and fungal strains isolated from diverse environments have been reported to possess the capacity to hydrolyze sulfate esters *via* ARS activity [14–16]. *In vitro*, ARS is positively regulated at the transcriptional level in culture media containing limited amounts of the optimal sources of S for microbial growth (i.e., sulfates or S-containing amino acids) [17–20]. This finding suggests that ARS synthesis could be induced to allow the liberation of sulfates from alternative S sources, such as sulfate esters, particularly in soils containing sulfate esters as a readily available S source for microorganisms. However, in soils, there is no clear evidence of ARS induction resulting from sulfate-S limitations. Several studies have shown significant correlations between ARS activity and organic C content [21–23], suggesting that C availability is a key factor governing S transformations in soil [24]. For example, the addition of glucose was found to strongly increase soil ARS activity at a rate of 1000 mg glucose-C kg^{-1} soil [25]. In agricultural soils, crop residues constitute an important source of labile C. The net mineralization of S was found to be strongly related to the C/S ratio of plant residues, with a lower net mineralization of sulfates in soil amended with high C/S residues compared with low C/S residues [26–29]. Thus, the chemical composition of plant residues can be considered as a predictor of the changes of sulfate availability during plant residue decomposition in soils [30].

To date, there have been no studies of the relationships between net S mineralization and the ARS activity and density of ARS-producing microbial communities (bacteria and fungi) following the incorporation of plant residues with contrasting chemical compositions into soil. Due to increasing evidence that bacteria and fungi act together to decompose plant residues [31], both bacterial (ARS-B) and fungal communities producing ARS activity (ARS-F) must now be considered in the studies of plant residue decomposition. Although molecular primers have facilitated the detection and functional characterization of ARS in some soil bacteria [17], and even if GenBank contains a lot of sequences for the *ats*A gene, which encodes the key enzyme that catalyzes the hydrolysis of sulfate esters, it is difficult to design conserved primers to allow the development of molecular approaches with sufficient sensitivity to directly target ARS-producing microbial communities in soil. As a consequence, a cultivation-based approach involving plate counting was employed to estimate the abundance of microbial communities possessing ARS activity [14,16]. Therefore, the objectives of this study were to evaluate the impact of incorporating residues with varying C:S ratios and different biochemical compositions on the abundance and ARS activity of culturable microbial communities possessing ARS activity and to relate these changes to soil sulfate-S dynamics. We hypothesized that the incorporation of C derived from residues would increase the heterotrophic microbial biomass (estimated as the soil microbial biomass C [SMBC]), thus increasing ARS-producing microbial communities, whose abundance and ARS activity depend on the presence of C and the availability of S.

2. Materials and methods

2.1. Soil

The soil used in this study was a silt loam (Orthic Luvisoil) sampled from the 0–30 cm layer at the INRA experimental station at Estrées-Mons in northern France (50°27′N, 03°56′E). A field-moist soil sample was air-dried, and 2-mm sieved soil was analyzed for physicochemical properties. The percentages of clay, silt and sand were 16.7%, 76.4% and 6.4%, respectively. The soil organic C content was 9.2 g kg^{-1}, and the C/N and C/S ratios were 9.2 and 32.9, respectively. The soil pH (in water) was 8.

2.2. Incubation

Three types of residues were added to the soil: whole mustard (*Sinapis arvensis*) plants in the vegetative phase, tall fescue (*Festuca arundinacea*) and wheat (*Triticum aestivum*) stems without nodes or leaves. These residues differed markedly in their composition and biochemical fractions [30], and the C/S ratios were 60, 155 and 486 for mustard, fescue and wheat, respectively. The residues were dried in an oven (72 h at 60 °C), and cut into 3–5 mm pieces with scissors. The residues were homogeneously mixed into the soil samples (40 g equivalent dry soil) at a rate of 1.5 g of C kg^{-1} of dry soil equivalent. Control soils (40 g equivalent dry soil) consisted in homogenized sieved soil without residues incorporated. For all samples (control and amended soils), the soil was moistened to 80% of its water-holding capacity (WHC), and the samples were incubated in tightly sealed jars for 56 days at 20 ± 1 °C. The subsequent experiment consisted of 4 treatments (control, mustard, fescue and wheat), with three replicates of each treatment and five time points of destructive sampling (0, 7, 14, 28 and 56 days after the start of the experiment). C mineralization was measured using CO_2 traps (20 ml of 0.25 M NaOH) placed in tight jars. The CO_2 trapped in the NaOH solutions was precipitated with a 0.1 M $BaCl_2$ solution and was back titrated with 0.25 M HCl. The amount of C mineralization from the residues (the "apparent mineralization") was calculated as the difference in the amount of mineralization between the samples with residues and the controls.

2.3. Soil microbial biomass C (SMBC)

The SMBC was estimated using the fumigation-extraction method [32]. Two subsamples of 10 g of moist soil were sampled from each jar, and one subsample from each pair was fumigated with alcohol-free chloroform for 24 h. Then, both subsamples were separately shaken with 40 ml of 0.03 M K_2SO_4 for 30 min [33], followed by centrifugation at 5800 g for 20 min. The soluble C concentration in each extract was measured with an auto-analyzer (1010, O.I. analytical) using oxidation at 100 °C in a persulfate medium, and the CO_2 produced was measured using infrared spectrometry. The microbial biomass C was calculated as the difference between the soluble C in fumigated and non fumigated soils divided by a coefficient $K_{EC} = 0.38$ [32].

2.4. Densities of ARS-B and ARS-F

The densities of ARS-B and ARS-F were estimated using a culture-dependent method. Briefly, diluted soil suspensions were spread onto modified media containing an ARS chromogenic substrate, 5-bromo-4-chloro-3-indolyl sulfate (X-Sulf, Sigma, France) as the sole source of S; a modified M9 medium [14] was used for ARS-B, and a modified Czapek mineral-based medium was used for ARS-F [16]. After incubation on plates at 28 °C for 21 or 28 days for ARS-B and ARS-F, respectively, the number of microorganisms producing ARS activity was determined and was expressed as the log-transformed CFU g^{-1} of dry soil. The ratio of ARS-B to ARS-F was then calculated.

2.5. ARS activity

The ARS activity in the soil samples was determined using a method proposed in the following study [12]. Fresh soil (1 g) was