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Soil biodegradation of maize root residues: Interaction between chemical characteristics and the presence of colonizing micro-organisms

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ABSTRACT

Due to their direct contact with the soil, roots are exposed to colonizing micro-organisms that persist after the plant has died. These micro-organisms may affect intrinsic root-chemical quality and the kinetics of root residue decomposition in soil, or interact with soil micro-organisms during the decomposition process. The aims in this work were i) to determine the interactions between the presence of root-colonizing micro-organisms and root-chemical quality and ii) to quantify the effect of these micro-organisms on root decomposition. Roots were selected from six maize genotypes cultivated in the field and harvested at physiological maturity. The roots of two genotypes (F2 and F2bm1) had a higher N content, lower neutral sugars content and higher Klason lignin content than the other genotypes (F292, F292bm3, Mexxal, Colombus). Location of the root residue micro-organisms by scanning electron microscopy and transmission electron microscopy revealed that F2 and F2bm1 roots were more colonized than roots of the other genotypes. Electron Dispersive X-Ray microanalyses of *in situ* N confirmed a higher N content in the colonizing micro-organisms than in the root cell walls. Residues of F2 and F2bm1 roots decomposed more slowly and to a lesser extent than those of the other genotypes during incubation in a silty loam soil under controlled conditions (15 °C, −80 kPa). After 49 days, 40.6% of the total C from F292 was mineralized but only 20.7% of from F2bm1. These results suggest that residue-colonizing micro-organisms decompose the cell-wall sugars to varying extents before soil decomposition thereby modifying the chemical quality of the residues and their mineralization pattern in soil. Due to their high N content, colonizing micro-organisms also impact on the total N content of root residues, reducing their C to N ratio. Gamma sterilized root residues were incubated under the same conditions as non-sterilized residues to see if micro-organisms colonizing root residues could modify the action of soil micro-organisms during decomposition. Similar C mineralization rates were observed for both non-sterilized and sterilized residues, indicating that the residue micro-organisms did not quantitatively affect the activity of soil micro-organisms.

1. Introduction

Most roots remain in the soil after harvest and are known to greatly contribute, possibly more than aerial plant parts, to building up soil organic matter (Puget and Drinkwater, 2001; Rasse et al., 2005). Frequently, roots contain more cell walls and are more lignified than aerial plant parts (Bertrand et al., 2006; Carrera et al., 2008). These intrinsic characteristics help to explain the low rate of root decomposition in soil but the impact of colonizing micro-organisms on root residue quality and decomposition remains unclear.

The fact that most plant residues contain populations of bacteria and fungi has often been overlooked (Parr and Papendick, 1978). These micro-organisms, some of which may be plant pathogens, are known to colonize the crop tissues before and after harvest (Cook et al., 1978). Species of *Alternaria*, *Cladosporium* and *Fusarium* (field fungi) are frequently observed in field infections of cereal seeds (Clarke and Hill, 1981; Sauer et al., 1982). Many field fungi are pathogenic on senescent, weakened, or damaged plants and may persist into a saprophytic growth phase after the plant has died (Miller, 1983). These micro-organisms are able to modify the chemical composition of above and underground parts of the living plant that may persist after harvest. Lignin, for instance, is formed

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in response to mechanical damage or wounding and many plants respond to microbial attack by depositing lignin and other wall-bound phenolic materials at the point of attack (Boudet et al., 1995; De Ascensao and Dubery, 2003). The cellulose and hemicellulose in plant tissues may be degraded during intensive colonization by micro-organisms such as rot-fungi so that the residue becomes enriched in microbial organic nitrogen (Watteau et al., 2002; Karroum et al., 2005). It was previously shown that wheat straw was populated with micro-organisms able to decompose readily available substrates in the straw during the first stage of decomposition, whereas the final stage of straw decomposition seemed to be accelerated by soil micro-organisms (Tester, 1988). However, to our knowledge, few results have been published concerning the quantitative impact of residue-colonizing micro-organisms on the dynamics of residue decomposition in soils.

Roots are probably the plant parts most exposed to micro-organisms due to their direct contact with the soil matrix in the rhizosphere. This root-soil contact can lead to beneficial (mycorrhizas) or damaging (pathogenous) associations for the plant, all of which can affect the chemical composition and cell wall structure of the root residues. Maize roots taken up just before harvest, i.e. from a living plant, were shown to be colonized by Gram-negative bacteria that altered root cell wall anatomy and probably the pattern of root biodegradation (Watteau et al., 2006).

The aims in this study were to investigate interactions between root-colonizing micro-organisms and root-chemical quality and to quantify their impact on root decomposition in soil. As part of a larger program to assess the relationships between the chemical characteristics of crop roots and their decomposition in soil (Machinet et al., 2009), roots were selected from several maize genotypes with the same tissue architecture but different chemical characteristics and organic N contents. The location and nature (bacteria or fungi) of the residue micro-organisms were first examined by scanning electron microscopy (SEM), and then identified by transmission electron microscopy (TEM). Their effects on the nitrogen content of the maize roots were determined at an ultrastructural level by EDX micro-analysis (TEM/EDX). The impact of micro-organisms on root residue decomposition in soil was quantified in incubation experiments under sterile and non-sterile conditions.

2. Materials and methods

2.1. Soil and maize roots

Soil was collected from 5 to 30 cm depth at the INRA Experimental Station in Estrées-Mons, France. The soil had a silty loam texture (17.8% clay, 77.3% silt, 3.8% sand), with 0.95% organic C, and a pH (H_2O) of 7.6. The soil was air-dried to a moisture content of 120 mg g^{-1} dry soil for two days, and then immediately sieved to 2–3.15 mm. All visible organic residues were removed by hand after sieving. The soil was stored at 15 °C for a week prior to incubation.

Four maize (*Zea mays* L.) lines (F2, F2bm1, F292, F292bm3), and two maize hybrids (Mexxal and Colombus) were studied. All genotypes were grown in experimental fields at the INRA Experimental Station in Lusignan and harvested at physiological maturity. Only the roots were kept for experiments. These roots were washed with a 50 g l^{-1} sodium metaphosphate solution for 24 h, rinsed with deionised water to remove soil particles, and then dried for one week at 30 °C. Calibrated roots of 2–3 mm diameter were selected for the study and represented nearly half of the maize root biomass sampled.

For incubations in sterile conditions, root and soil samples in hermetically sealed plastic bags were sterilized by 45 kGy gamma irradiation from a ^{60}Co source (Ionisos, Dagneux, France). After sterilization, the soil was stored at 4 °C for eight weeks until the beginning of the experiment to limit the action of active enzymes in the soil samples after irradiation (Lensi et al., 1991). Sterile soil was designated as SS and non-sterile soil as NSS.

2.2. Incubation experiment

Soil samples (SS and NSS) were mixed with 5 mm long pieces of maize roots (sterilized (SR) or not (R)) at a rate equivalent to 3 g C kg^{-1} dry soil, and incubated for 49 days at 15 °C. Potassium nitrate was added to obtain a final concentration of 65 mg N kg^{-1} soil in the SS and NSS treatments so that decomposition would not be limited by N availability (Recous et al., 1995). The concentration of the added N solution was calculated to bring the soil moisture content to 200 g H_2O kg^{-1} soil, equivalent to −80 kPa. Soil moisture was maintained throughout the incubation period by weighing weekly and readjusting with deionised water when necessary. Control treatments (no C added) with sterilized and non-sterilized soils were also included. A glucose solution was added to SS at the rate of 3 g C kg^{-1} dry soil (SS-Glu) to check that the soil remained sterile throughout the incubation period (49 days). All the treatments, including sterilized soils or residues, were manipulated under sterile conditions to avoid contamination.

Carbon mineralization was measured in triplicate for each residue-amended, control and glucose treatment, in soil samples (equivalent to 50 g dry soil) incubated in 250 ml glass jars in the presence of a CO_2 trap (10 ml 1 M NaOH). Carbon mineralization was measured 3, 8, 14, 21, 28, 38 and 49 days after the beginning of incubation.

The concentrations of CO_2 trapped in the NaOH solutions were measured by continuous flow colorimetry (Chaussod et al., 1986) using an auto-analyzer (TRAACS 2000, Bran & Luebbe, Norderstedt, Germany). The mineral N in the soil and residue extracts was analyzed by continuous flow colorimetry (TRAACS 2000, Bran & Luebbe, Norderstedt, Germany). Concentrations of NO_3^- and NO_2^- were determined using an adaptation of the method proposed by Kamphake et al. (1967). Ammonium ions were determined following the method described by Krom (1980).

2.3. Chemical analysis of maize roots

The chemical characteristics of the roots were determined on two sample replicates before soil incubation. The total C and N contents of the roots were measured by elemental analysis (NA 2000, Fisons Instruments, Milan, Italy).

The initial root residues were subjected to a cell wall preparation process, which consisted of extracting the neutral detergent fiber (NDF) fraction as described by Goering and Van Soest (1970). Briefly, the soluble fraction was removed by boiling 1.5 g of roots (about 5 mm long/2 mm diameter) in deionised water at 100 °C for 30 min then extracting with a neutral detergent solution at 100 °C for 60 min to remove cytoplasmic components and obtain the NDF fraction. This fraction was designated the cell wall residues. All residues from cell wall preparations were dried for one week at 30 °C and ground to 80 μm.

The neutral sugar content of the cell wall residues was determined using the method described by Blakeney et al. (1983). Ten mg of sample were swollen in 125 μl 12 M H_2SO_4 for 2 h at 20 °C followed by acid hydrolysis with 1 M H_2SO_4 for 2 h at 100 °C. The monosaccharides released by the acid were separated by high performance anion-exchange chromatography (HPAEC) on a CarboPac PA-1 column (4 × 250 mm, Dionex) as described by Beaugrand et al. (2004). Monosaccharide composition was analyzed and quantified using 2-deoxy-D-ribose as internal standard and standard solutions of neutral carbohydrates (L-arabinose, D-glucose, D-xylose, D-galactose, D-rhamnose, D-mannose and L-fucose).