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Micropore surface area of alkali-soluble plant macromolecules (humic acids) drives their decomposition rates in soil

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ABSTRACT

Previous studies suggested that micropore surface area (MSA) of alkali-soluble bio-macromolecules of aerial plant residues of maize constitutes an important factor that explains their humification in soil, that is, preservation against biological degradation. On the other hand, root plant residue contributes to the soil humus balance, as well.

Following the experimental design used in a previous paper published in this journal, this study shows that the biochemical recalcitrance of the alkali-soluble acid-insoluble fraction of the root plant material, contributed to the root maize humification of both *Wild-type* maize plants and its corresponding mutant *brown midrib* (*bm3*), this latter characterized by reduced lignin content.

Humic acids (HAs) existed in root (root-HAs) were less degraded in soil than corresponding HAs existed in shoot (shoot-HAs): shoot-HAs *bm3* (48%) > shoot-HAs *Wild-type* (37%) > root-HAs *Wild-type* (33%) > root-HAs *bm3* (22%) (degradability shown in parenthesis).

These differences were related to the MSA of HAs, that is, root-HAs having a higher MSA than shoot-HAs: shoot-HAs *bm3* (41.43 ± 1.2 $m^2\ g^{-1}$) < shoot-HAs *Wild-type* (43.43 ± 1.7 $m^2\ g^{-1}$) < root-HAs *Wild-type* (51.7 ± 3.6 $m^2\ g^{-1}$) < root-HAs *bm3* (54.08 ± 3.9 $m^2\ g^{-1}$).

Taking into account both the previous data obtained for maize shoots and the results of this study, it was possible to find a very good correlation between degradability of HAs and HA-MSAs ($r = -0.88$, $P < 0.08$, $n = 4$), confirming that MSA was able to explain bio-macromolecules recalcitrance in soil.

1. Introduction

The maintenance of soil quality is one of the main current challenges and a significant worldwide issue of the last two decades. Given that conservation and improvement of soil organic carbon (SOC) levels are crucial to preserving soil quality and fertility (Herrick and Wander, 1997; Lal, 2005), there is still a need to thoroughly study the global biogeochemical carbon cycle dynamics and to understand the key factors that determine transfer of carbon into the soil organic matter (SOM) fraction, which is more resistant to decomposition (Johnson et al., 2007).

It has been shown that this fraction, traditionally called humus, contributes vital properties to soil (Piccolo, 1996; Sutton and Sposito, 2005) and has an important role from the agronomical point of view. Humus has an essential influence on the quality of soil and it is involved in many of the physical, chemical, and biological processes determining the capacity of a soil to support plant growth (Stevenson, 1994). The growth-promoting effect of humic materials has been observed by many investigators; in particular, humus is known to affect soil aggregation (Piccolo, 1997), compactability (Soane, 1990), water retention properties (Piccolo, 1996), and the cation exchange capacity (Stevenson, 1994).

Humic acids (HAs) are a major component of humic substances (HS) (Stevenson, 1994) representing the most active fraction of soil humus.

The transformation of plant detritus into recalcitrant HS that would then form the slow carbon pool has been known for a long time as an important mechanism for SOM stabilization (Stevenson, 1994). Nevertheless, Balesdent (1996) clearly demonstrated that the conventional SOM fractionation in humic acid, fulvic acid and humin have no explaining power in terms of the residence time of carbon in soil. Therefore, these kinds of SOM pools cannot explain carbon turnover rates in soil (Gleixner et al., 2002; Helfrich et al., 2006; von Lützow et al., 2006). Recent findings indicate that mechanisms that contribute at the same time to SOM protection against decomposition in soil are biochemical recalcitrance, chemical association and physical sequestration (Marschner et al., 2008; von Lützow et al., 2006). Marschner et al. (2008) specifies that, if biological recalcitrance allows plant molecules to be preserved in soil, long-term stabilization of organic carbon, implies more complex mechanisms such as chemical association and physical sequestration with the mineral components of soil that are not yet understood.

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As for all operationally defined fractionation procedures, HS contain a wide range of molecules that could be preserved in soil not only because of their biochemical recalcitrance but also because of physical protection (Marschner et al., 2008).

The characterization and formation of HS have been a subject of debate for decades, yet, a general agreement among scientists has not been reached. Recent articles suggest a need to probe the biogeochemical pathways of HS (Sutton and Sposito, 2005) and HAs formation (Adani et al., 2006a; Kelleher and Simpson, 2006), thus redefining the concept of humification processes. It has been shown, that HAs are a very complex mixture of biopolymers (Kelleher and Simpson, 2006), confirming the theories that humification can be identified as preservation and conservation of bio-macromolecules in soil.

Our experimental results (Adani et al., 2006b) showed that plants represent the first step of the humification process through the preservation of recalcitrant alkali-soluble acid-insoluble biomacromolecules existed in the plant. The term 'recalcitrance' is used to describe the phenomenon by which plant tissues exhibit the natural resistance against microbial and enzymatic deconstruction (Himmel et al., 2007).

Despite recent progress, the nature of plant biopolymer recalcitrance remains unclear and new methodological approaches such as analysis, for example, at the nanometer scale, may be promising tools to identify the ultrastructure and the chemical topography of plant cell walls (Himmel et al., 2007).

In a previous our article (Adani et al., 2009), we showed that HAs contained in the aerial part of maize plant, *Wild-type* and *brown midrib* (*bm3*) genotypes, were recalcitrant to the degradation because of the physical structure at nanometer scale, i.e. high micro-porosity makes plant tissues less accessible to enzyme (Carpita and McCann, 2000).

Less attention has been given to the root apparatus with belowground processes than the shoots, and the contribution of root to the soil carbon pool has been overlooked. Nevertheless, the few available experimental data on this subject (Balesdent and Balabane, 1996; Gale and Cambardella, 2000; Wilts et al., 2004; Hooker et al., 2005), have shown that the contribution of plant roots to SOM stock is larger than that of plant shoots.

Therefore, the objective of this study was to investigate the contribution of the alkali-soluble acid-insoluble root plant biomolecules (root-HAs), to the HA soil pool after short-term incubation (one year), thus completing our previous research published in this journal (Adani et al., 2009).

2. Materials and methods

2.1. Soil and maize roots

Maize root were obtained from *Wild-type* and mutant *bm3* maize plants, collected previously. Details are reported in a previous publication (Adani et al., 2009). Plants were harvested at senescence stage, soil particles were then removed and roots were washed with deionized water. Samples were then dried at 45° C for 2 d and 65 °C for 3 d, respectively, and stored for subsequent incubation tests and analyses.

2.2. Chemical analyses and humic acid extraction and characterization

Acid-detergent fiber (ADF) and acid-detergent lignin (ADL) on maize roots were determined following the Van Soest procedure (Van Soest et al., 1991). The total carbon, hydrogen, nitrogen, sulfur, oxygen of the roots and root-HAs were measured by elemental analysis (ECS 4010, COSTECH, Cernusco S/N, Milan, Italy). HAs extraction from both soil and root materials was performed by using 0.1 M NaOH plus 0.1 M $Na_4P_2O_7$ and successive precipitation of the suspension to $pH < 1.5$. HAs extracted were washed till neutral pH, dried (Adani and Ricca, 2004) and stored for successive nuclear magnetic resonance (NMR) and micropore analyses. Humic acids existed in plant root and plant shoots were named, respectively, root-humic acids (root-HAs) and shoot-humic acids (shoot-HAs).

All analytical procedures are described in Adani and Ricca (2004). All the HAs extracted were quantified by organic carbon determination revealed by the Springer–Klee method using $K_2Cr_2O_7$ as oxidant (Ciavatta et al., 1989).

2.3. Incubation test

The artificial soil used for the incubation tests was a sandy mineral substrate. Details are reported in a previous article (Adani et al., 2009). Both the artificial medium and the soil on which plants were cropped (in 2005) and sampled 1 year later (in 2006) (pH 7.2, sand: 559 g kg dm^{-1}, silt: 292 g kg dm^{-1}, clay: 149 g kg dm^{-1}, carbon: 10.75 g kg dm^{-1}, nitrogen: 1.15 g kg dm^{-1}, $CaCO_3$: 32.4 g kg dm^{-1}, CEC: 9.37 $cmol^+$ kg dm^{-1}) were incubated as they were and, also, by adding plant residue at the rate of 35 g kg dm^{-1} (Adani et al., 2009). Incubation tests were carried out in three replicates of 3000 g of soil. The soils were inoculated with water soil extract and maintained at 60% (w/w) of the maximum water holding capacity. Water content was gravimetrically corrected every 2 d. Furthermore, the pots were incubated in a chamber, in the dark, at 20 ± 2 °C for 360 d. During the incubation tests, the soils were sampled at 0, 120, 240, and 360 d. Each sample, formed from subsamples taken from each replicate, weighed about 300 g. After taking the sample, the soils were dried at 65 °C under vacuum and then used for HA extraction and analytical determination that consisted in the HAs quantification by organic carbon determination (Adani and Ricca, 2004). The extracted HAs were washed until neutral pH, dried (Adani and Ricca, 2004) and stored for successive NMR and micropore analyses.

2.4. CP MAS ^{13}C NMR spectroscopy

Cross-polarization magic-angle spinning ^{13}C nuclear magnetic resonance (CP MAS ^{13}C NMR) spectra on solid samples were acquired at 10 MHz on a Bruker AMX 600 spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany). Further NMR details are reported in Adani et al. (2009).

The spectra obtained were subdivided into four regions: alkyl-C (0–50 ppm) (lipids, aliphatic polymers, and fatty acid; O-alkyl-C (50–110 ppm) (polysaccharides and proteins); aromatic-C (110–162 ppm) (lignin); carbonyl C (162–190 ppm) (carboxyl groups and amide carbonyls).

2.5. Micropore analyses

Micropore surface area (MSA) (half pore diameter of 0.22–0.72 nm) were determined by gas adsorption analysis of dried samples (0.5 g) using a porosimeter (NOVA 2200e, Quantachrome, Boynton Beach, FL, USA). Analyses were carried out by using CO_2 (273 K) and were preceded by a degassing procedure performed at 80 °C for 16 h. For calculation of micropore distribution, the nonlocal density functional theory method was applied to measure the CO_2 adsorption isotherms.

2.6. Statistical analysis

All statistical analyses were carried out using SPSS statistical software (SPSS, Chicago, IL).