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Nanometer-scale structure of alkali-soluble bio-macromolecules of maize plant residues explains their recalcitrance in soil

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ABSTRACT

The quantity and quality of plant litter in the soil play an important role in the soil organic matter balance. Besides other pedo-climatic aspects, the content of recalcitrant molecules of plant residues and their chemical composition play a major role in the preservation of plant residues.

In this study, we report that intrinsically resistant alkali-soluble bio-macromolecules extracted from maize plant (plant-humic acid) (plant-HA) contribute directly to the soil organic matter (OM) by its addition and conservation in the soil. Furthermore, we also observed that a high syringyl/guaiacyl (*S*/*G*) ratio in the lignin residues comprising the plant tissue, which modifies the microscopic structure of the alkali-soluble plant biopolymers, enhances their recalcitrance because of lower accessibility of molecules to degrading enzymes.

These results are in agreement with a recent study, which showed that the humic substance of soil consists of a mixture of identifiable biopolymers obtained directly from plant tissues that are added annually by maize plant residues.

1. Introduction

Maize produces by far the world's most important crop residue at 609×10^6 Mg y^{-1} (FAO, 2001).

Addition of the corn stover to the soil results in greater increase in the soil organic carbon (SOC), than when the stover is removed (Clapp et al., 2000). Moreover, the removal of corn stover produces a negative impact on the physical, hydrological, biological, and thermal properties of the soil, as well as increases soil erosion (Blanco-Canqui et al., 2006).

In developed countries, maize residues are added to the soil, and the potential for ethanol production from cellulose using maize crop residues as the main source of raw material, has been suggested recently (Donner and Kucharik, 2007). However, in developing countries, the crop residues are not added to the soil and thus, the carbon balance becomes negative (Rasmussen et al., 1998).

The mechanism by which crop residues contribute to SOC has been reported to be through their humification (Lal, 2007), and up to 70–80% of SOC in agricultural mineral soil is composed of humic substances (HS) (Piccolo, 2002). Nevertheless, the exact description of humification and HS has been a controversial topic of recent discussion (Kelleher and Simpson, 2006).

Humification has been reported to be a complex mechanism involving the breaking of bio-macromolecules into small constituents, and their subsequent recombination to form chemically complex "geopolymers" (Myneni et al., 1999; Senesi and Loffredo, 1999). On the other hand, intrinsically resistant bio-macromolecules contribute to stable organic matter (OM) in different environments (Hatcher et al., 1983; Kögel-Knabner et al., 1992; Hedges et al., 2001) and could explain the origin of humus (Hedges and Oades, 1997). More recent findings suggested that HS are composed of a mixture of identifiable biopolymers (Kelleher and Simpson, 2006), confirming the theories that state that HS is derived from the preservation of plant and microorganism biopolymers.

The soil alkali-soluble and acid-insoluble OM fraction, i.e., humic acid (HA), along with fulvic acids, represents the most active fraction of the soil humus. It is a negatively charged colloid, relatively recalcitrant to biodegradation, and is stored in the soil, thus contributing to soil fertility (Qualls, 2004).

In this study, we have proposed a biochemical origin for the soil OM (Kelleher and Simpson, 2006; Lehmann et al., 2008) with particular reference to the alkali-soluble and acid-insoluble fraction, i.e., HAs from maize plant residues *via* CO_2 assimilation by plants, followed by plant maturation (cell wall formation and lignifica-

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tion), and the subsequent incorporation and preservation in the soil of the more recalcitrant plant molecules (plant-HA) (Fig. 1). The proposed humification pathway is consistent with our earlier observation that HA, directly isolated from maize plant residues, contributes actively to the parent soil-HA by simple preservation of bio-macromolecules in the soil because of its biologically recalcitrant properties (Adani et al., 2006a). Recalcitrance can be defined as the natural resistance of plant tissues to microbial and enzymatic degradation (Himmel et al., 2007). The nature of plant biopolymer recalcitrance is still not completely understood and further studies are necessary to elucidate the role of the chemical topography of the cell wall at a nanometer scale (Himmel et al., 2007).

Besides other chemical parameters, the cell wall architecture depends on both the lignin content and lignin *S*/*G* ratio, which plays an important role in determining the recalcitrance of the plant residue (Johnson et al., 2007). The variation in the *S*/*G* ratio depends not only on the plant species and variety, but also on the plant age (Matsui et al., 2000).

In this work, we isolated and characterized two alkali-soluble fractions (HAs), directly from the *wild-type* maize plant (*W*) (*Zea mais* L., W23 near-isogenic lines, Stock Center Resources of MaizeGDB) and its corresponding mutant *brown midrib* (*bm3*), and studied their contribution to the soil OM after one year of incubation.

2. Materials and methods

2.1. Maize plant cropping

Maize plants were grown in an open field of Haplic Luvisol soil (FAO classification) (pH 7.7, sand: 570 g kg dry matter^{-1} (dm^{-1}), silt: 379 g kg dm^{-1}, clay: 51 g kg dm^{-1}, carbon: 12.4 g kg dm^{-1}, nitrogen: 2.8 g kg dm^{-1}, $CaCO_3$: 302.4 g kg dm^{-1}, CEC: 9.46 cmol^{+} kg dm^{-1}). The maize (*Zea* mays L.) seed stocks used in this study, as a source of *bm3* mutation and *bm3* control (W23 near-isogenic lines), were provided by the Stock Center Resources of MaizeGDB (http://www.maizegdb.org/stock.php).

In early May, the soil was tilled by a disk plow and fine tilled by a harrow. On May 24, the corn was sown; the spacing between the rows was 0.70 m and approximately 0.22 m within the rows (8 plants m^{-2}). Sufficient irrigation was provided periodically as needed to supplement rainfall.

The plants were harvested without the root apparatus at senescence (plant residue at a d.m. of 850 g kg^{-1}). The samples were dried at 45 and 65 °C for 2 and 3 days, respectively, ground to 0.5 mm, and stored for subsequent incubation tests and analyses.

2.2. Macromolecule determination

Forage fiber analyses were performed for determining the neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL). Hemicelluloses, cellulose, lignin, and soluble cell materials (g kg^{-1}) were then calculated as (NDF–ADF), (ADF–ADL), (ADL), and (1000-NDF), respectively (Van Soest et al., 1991).

2.3. Elemental analysis

Elemental analysis of HA (C, H, N, S, and O) was carried out with an elemental analyzer (ECS 4010, COSTECH, Cernusco S/N, Milan, Italy).

2.4. Incubation test

The artificial soil used for the incubation tests was a sandy mineral substrate composed of 910 g kg dm^{-1} of sand (particle size, ∅ = 0.5–0.8 mm, pH 7) and 90 g kg dm^{-1} of clay (bentonite–montmorillonite-like mineral, sieved at ∅ < 1 mm; pH 7; CEC = 6.5 cmol^{+} kg^{-1}). The plant residue (plant at senescence stage) was added at the rate of 35 g kg soil dm^{-1}. An artificial medium was chosen to avoid any contamination from soil OM. In addition to the artificial medium, the soil in which the plants were cropped was both incubated and combined with plant residue at the same rate as the mineral soil (35 g kg dm^{-1}). In all these experimental steps, the incubation tests were carried out in three replicates of 3000 g of soil. The soil samples were inoculated with water–soil extract and maintained at 60% (w/w) of the maximum water-holding capacity. The water content was gravimetrically corrected every 2 days. Furthermore, the pots were incubated in a chamber, in the dark, at 20 ± 2 °C for 360 days. During the incubation tests, the soils were sampled at 0, 60, 120, 180, 240, and 360 days. 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 sub-

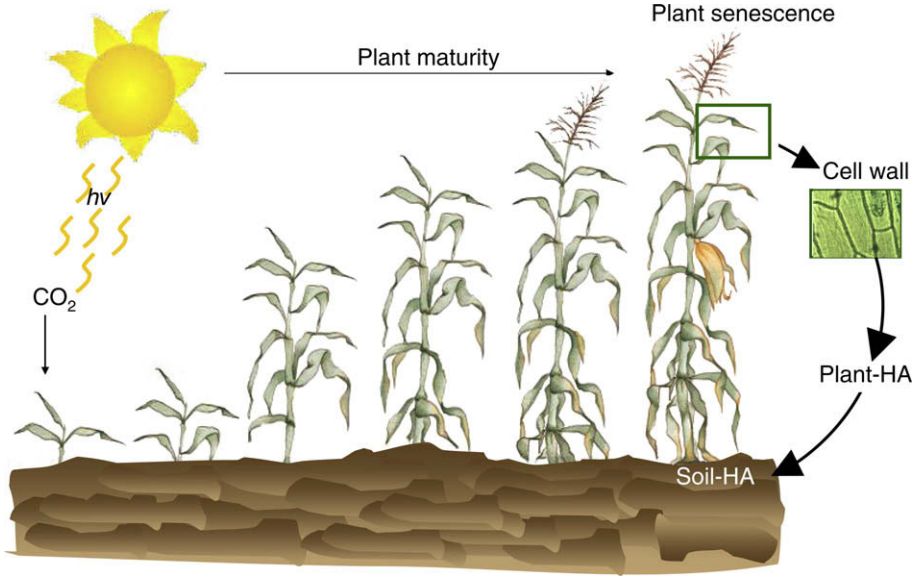

Fig. 1. Maize plant residue humification pathway proposed in this work.