Dynamics and fate of natural and waste organic material in soils: the role of the soil organic matter (SOM) recalcitrance in SOM turnover

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Abstract
Previous studies suggested that micropore surface area (MiS) of bio-macromolecules of biomass constitutes an important factor that explains their preservation in soil, that is, the preservation against biological degradation. On the other hand it has been reported that biochemical catalysis are limited in theirs action by the very complex macroscopic and microscopic structure of cell walls that limit mass transportation. Results of this work indicated the structure (nanostructure) of the cell wall playing a main role in the preservation of the organic matter in soil. Total microporosity and pore dimension correlated with biomass degradation. On the other hand chemical composition determined by CPMAS 13- NMR does not play a role in the definition of biomass recalcitrance.

Key Words
Biomasses, microporosity, organic wastes, recalcitrance, soil organic matter, surface gas adsorption

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 (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 The transformation of plant detritus and organic wastes 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. Nevertheless, Authors 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 (Helfrich *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). Marschner *et al.* (2008) specifies that, if biological recalcitrance allows plant molecules and organic molecules from biomasses 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.

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). Cell wall structure has e natural recalcitrance that limited enzymes activity (Himmel *et al.* 2007). It has been reported that biochemical catalysis are limited in theirs action by the very complex macroscopic and microscopic structure of cell wall that limit mass transportation (Himmel *et al.* 2007). In this study, the preservation of biomass organic matter has been investigated pointed out the role that nano-scale structure of the biomass plays in the preservation of the OM in soil and so the role that the organic matter of organic wastes play in the soil OM balance. Here we proposed preliminary results of a more large study under construction.

Methods

Biomasses
Different biomasses were selected for this work: plant residues, crop energy plants and lingo-carbohydrates complexes isolated after acid hydrolysis (Table 1). Other biomasses and organic wastes are under study. Biomasses were incubated in soil for long time (3 months) dosing an amount of 20 g C/kg soil dry matter. CO_2 evolution was detected by using NaOH trap and data reported as cumulative results.

CPMAS ^{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). 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).

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.

Result
Biomasses studied differed for both chemical composition and physical characteristics (Table 1 and 2). All biomasses were characterized by the high presence of microporosity surface (MiS) (pores of 0.3-1.5 nm of diameter). Data indicated that the recalcitrance, i.e. the natural resistance against microbial and enzymatic deconstruction was associated to the MiS. LCC complexes as expected, were less degraded in soil during incubation trials, and they were characterized by a high MiS. On the other hand biomasses characterized by young tissues, such as plant residues were largely degraded in soil and showed low MiS values.

Table 1. Microporosity surface and volume measured for biomasses studied.

	$S_{\mu p}$ (m^2/g) 0.3-1 nm	$S_{\mu p}$ (m^2/g) 1-1.5 nm	$S_{\mu p}$ tot (m^2/g)	$V_{\mu p}$ (cm^3/g)
Giant Cane[a]	39.93±1.08	1.59±1.16	41.52±0.08	0.014±0.00
Miscanthus [a]	48.11±2.01	3.98±2.62	52.10±4.63	0.018±0.002
Proso millet[a]	52.12±1.67	1.68±0.13	53.80±1.80	0.017±0.001
Sorgum[a]	36.46±3.46	1.76±0.66	38.22±2.79	0.013±0.001
Rivet wheat[a]	46.2±0.90	0.67±0.73	46.87±1.63	0.016±0.001
Wheat[a]	37.84±2.45	0.00±0.00	37.84±2.45	0.013±0.001
Wheat[b]	87.07±1.3	8.25±0.8	95.32±1.2	0.033±0.01
AlfaAlfa[a]	11.69±1.32	0.00±0.00	11.69±1.32	0.004±0.000
AlfaAlfa[b]	54.09±1.29	7.72±1.93	61.81±0.65	0.022±0.001
Pine needles[a]	35.16±5.18	0.25±0.36	35.41±5.53	0.0115±0.002
Pine needles[b]	82.47±2.46	7.73±0.93	90.20±3.39	0.0285±0.002
Leaves of beech[a]	51.91±0.74	7.32±5.11	59.23±5.85	0.0215±0.003
Leaves of beech[b]	82.35±4.36	10.83±0.67	93.18±3.68	0.0315±0.000
Wood of beech[a]	70.69±1.91	0.65±0.73	71.34±1.17	0.022±0.000
Straw mix	80.19±0.83	3.59±5.07	83.78±5.90	0.03±0.00

[a]Untreated plant.
[b]After acid hydrolysis with H_2SO_4 13.50 mol/L at 4°C for 24 h.

Degradability, measured as CO_2 (data not showed) produced during incubation tests, was not influenced by chemical composition. On the other hand MiS well correlated with degradability ($r = -0.87$, $p<0.01$). Results obtained can be discussed and interpreted taking into consideration that microporosity makes the enzyme inaccessible to organic molecules having a size larger than pores (Carpita and McCann, 2000; Adani *et al.* 2006; Adani *et al.* 2009; Papa *et al.* 2010). Therefore, the presence of a higher proportion in the biomass of fractions not accessible to the enzyme, i.e. higher MiS, could explain the lower degradability of biomass.

Conclusion
Results indicated that biomass is formed by a ultra-microporosity (pores below 0.8 nm) that limiting the enzyme activity preserved the OM from degradation. Therefore it can be concluded that the first step of OM preservation (humification ?) in soil consist in the preservation of the organic molecules
Nevertheless, literature suggest that most likely chemical recalcitrance is not the only mechanism in the preservation of OM and that, more complex processes such as physical protection or interactions with mineral surface should be considered and could play a main role in OMN preservation.

Table 2. Area of CP MAS ^{13}C NMR bands.

	Aliphatic C bonded to other aliphatic chain or to H	O-CH_3 or N-alkyl-C O-alkyl-C di-O-alkyl-C	Aromatic-C phenol-C or phenyl ether-C	Carboxyl C + keto C
	0-50	50-115	115-160	160-210
		Band range (ppm)		
Giant Cane[a]	10.77	76.16	8.50	4.58
Miscanthus[a]	8.22	75.73	10.73	5.32
Proso Millet[a]	8.94	78.97	8.16	3.94
Sorghum[a]	11.31	75.71	8.00	4.98
Rivet Wheat[a]*(straw)*	10.26	73.10	10.09	6.55
Wheat[a] *(straw)*	10.02	78.01	7.70	4.27
Wheat[b]*(straw)*	20.56	42.46	29.40	7.59
Alfa Alfa[a]	27.14	56.95	6.17	9.75
Alfa Alfa[b]	44.36	30.93	13.01	11.70
Pine needles[a]	19.01	64.31	11.27	5.40
Pine needle[b]	40.47	29.50	23.24	6.78
Leaves of beech[a]	19.27	61.36	12.58	6.79
Leaves of beech[b]	34.24	32.85	25.28	7.62
Wood of beech[a]	5.86	80.60	9.09	4.44
Straw mix[a]	6.75	79.71	9.49	4.05

[a]Untreated plant.
[b]After acid hydrolysis with H_2SO_4 13.50 mol/L at 4°C for 24 h.

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