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Unraveling the long-term stabilization mechanisms of organic materials in soils by physical fractionation and NMR spectroscopy

Denis Courtier-Murias[a,1], André J. Simpson[a], Claudio Marzadori[b], Guido Baldoni[b], Claudio Ciavatta[b], José M. Fernández[c], Esther G. López-de-Sá[c], César Plaza[c,*]

[a] Department of Chemistry, University of Toronto, Scarborough Campus, 1265 Military Trail, Toronto, ON M1C 1A4, Canada
[b] Department of Agroenvironmental Sciences and Technologies, Alma Mater Studiorum – University of Bologna, Viale Fanin 44, 40127 Bologna, Italy
[c] Instituto de Ciencias Agrarias, Consejo Superior de Investigaciones Científicas, Serrano 115 bis, 28006 Madrid, Spain

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ABSTRACT

The fundamental mechanisms whereby organic inputs stabilize in soil are poorly resolved, which limits our current capacity to predict the dynamics of soil organic matter (OM) turnover and its influence on soil quality and functioning. Here we fractionated soil OM from long-term experimental field plots either unamended or amended with two organic materials of different quality (i.e., solid cattle manure and crop residues) for 44 years into five measurable and meaningful pools directly related to conceptual preservation mechanisms: dissolved OM, mineral-free particulate OM located outside aggregates (unprotected from decomposition), OM occluded within both macroaggregates and microaggregates (weakly and strongly protected by physical mechanisms, respectively), and OM intimately associated with soil mineral particles (protected by chemical mechanisms). Compared to the unamended soil, the application of cattle manure and crop residues increased total organic C content by 35 and 10%, respectively. Most of these increases (up to 60 and 72% for cattle manure and crop residues, respectively) were explained by the mineral-associated OM pool, followed by the intra-microaggregate OM fraction. In general, the distribution and dynamics of N content paralleled those of C content. As determined by a range of modern nuclear magnetic resonance (NMR) techniques, including ^{13}C cross polarization magic angle spinning (MAS), ^{1}H high resolution (HR)-MAS, and ^{1}H—^{13}C heteronuclear single quantum coherence HR-MAS NMR, the mineral-associated OM fraction was found to be predominately of microbial origin, unlike free and intra-aggregate OM pools, which were dominated by plant structures at different stages of decomposition. As a whole, our results indicate that the main mechanism by which organic inputs are stabilized and OM accrues in soils is not the physical and chemical protection of undecayed or partially degraded organic structures, but the adsorption on mineral surfaces of microbial biomass and microbial by-products resulting from microbial growth, transformation, and degradation processes. It is possible that organic amendments increase more than previously thought the microbial populations of the soil, which live, thrive, and die in close association with the mineral surfaces. This mechanism appears to be enhanced with the addition of stable organic materials.

1. Introduction

The addition of organic amendments, such as animal manures and crop residues, is universally known to improve soil fertility (Watson et al., 2002; Diacono and Montemurro, 2011). This practice has the benefit of providing plant nutrients and especially organic matter (OM), which is essential for soil quality and functioning (Johnston et al., 2009). Besides improving soil physical, chemical, and biological conditions, increasing the levels of soil OM through organic material additions has the potential to contribute to climate change mitigation by sequestering C from the atmosphere, especially if applied to degraded arable lands depleted in soil OM, albeit this potential depends on the alternative fate of the organic material (Powlson et al., 2011).

Understanding the underlying processes of soil OM stabilization is pivotal for agricultural and environmental considerations. Leaving aside climatic and environmental factors that govern soil OM persistence (Schmidt et al., 2011), the fundamental mechanisms that protect organic compounds entering the soil against decomposition include (a) selective preservation and synthesis of resistant molecular structures, arrangements, and associations (biochemical mechanisms), (b) physical disconnection from decomposers,

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[*] Corresponding author. Tel.: +34 91 745 2500x950191.
E-mail address: c.plaza@ica.csic.es (C. Plaza).
[1] Present address: Université Paris-Est, Laboratoire Navier (ENPC-IFSTTAR-CNRS), 2 allée Kepler, 77420 Champs sur Marne, France.

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enzymes, O_2, etc. by occlusion within aggregates (physical mechanisms), and (c) chemical inaccessibility by intimate association (sorption) with mineral surfaces (chemical mechanisms) (Piccolo, 2001; Six et al., 2002a; Lützow et al., 2006; Schnitzer and Monreal, 2011).

Chemical extraction and fractionation of soil OM to quantify and characterize nonhumic and humic constituents has been by far the dominant approach to investigate transformation and stabilization processes of soil OM in organically amended soils (Francioso et al., 2000; Senesi and Plaza, 2007; Senesi et al., 2007). More recently, driven by growing evidence that the stability of soil OM does not depend on its molecular structure alone (Schmidt et al., 2011), physical fractionation techniques have been increasingly used to capture the influence of the soil mineral matrix on soil OM dynamics (Olk and Gregorich, 2006). However, physically fractionated OM pools still need to be better resolved especially at the molecular level to achieve a more complete understanding of the biochemical, physical, and chemical mechanisms that together control the dynamics of soil OM turnover. Nuclear magnetic resonance (NMR) spectroscopy is arguably one of the most powerful tools to unravel the highly complex structural composition of soil OM (Simpson et al., 2011). In particular, advanced solid-state cross polarization magic angle spinning (CP-MAS) and high resolution (HR)-MAS NMR methods can be used complementarily to extract valuable information. While solid-state CP-MAS NMR spectroscopy provides semi-quantitative information on the types and distribution of C present in soil OM, HR-MAS NMR provides increased spectral resolution and structural information as to the soluble and swellable OM components.

The main objective of this research was to obtain new molecular-level information about the mechanisms and processes that control the stabilization of OM in agricultural soils amended with organic materials. To achieve this objective, soil OM from long-term experimental field plots unamended and amended with solid cattle manure and crop residues were fractionated by a recent physical methodology into five pools meaningfully related to the abovementioned conceptual protection mechanisms: dissolved OM, mineral-free particulate OM located outside aggregates (unprotected), OM occluded within both macroaggregates and microaggregates (weakly and strongly protected by physical mechanisms, respectively), and OM intimately associated with soil mineral particles (protected by chemical mechanisms). The whole soils and OM fractions were analyzed for organic C and N content, and the molecular composition was investigated using CP-MAS and HR-MAS NMR methods.

2. Materials and methods

2.1. Long-term field experiment and soil samples

The long-term field plot experiment used in this work started in 1966 at the research and outreach farm of the Agricultural Faculty of the University of Bologna, located in Cadriano, northern Italy (44°33′ N, 11°24′ E; 31 m above sea level). The climate is humid subtropical with an average annual precipitation of 714 mm and an average annual temperature of 13.0 °C. The soil is a Haplic Calcisol (IUSS Working Group WRB, 2006). The topsoil (0–20 cm) is sandy clay loam (560 $g\,kg^{-1}$ sand, 160 $g\,kg^{-1}$ silt, and 280 $g\,kg^{-1}$ clay). In 1966, bulk density was 1.16 $g\,mL^{-3}$; pH, 6.9; total organic C content, 7.7 $g\,kg^{-1}$; total N content, 1.1 $g\,kg^{-1}$; and C/N ratio, 7.0.

The experiment was laid out in a randomized split-plot design with three replicates, several types of organic amendments on the main plots, and different rates of mineral N fertilizer at the split subplot level. Each subplot was 3 m wide and 11 m long, and all of them were in a corn (*Zea mays* L.)-winter wheat (*Triticum aestivum* L.) rotation. For this study, we selected three treatments with no mineral N application: a control without any amendment, amendment with cattle manure, and amendment with crop residues (wheat straw and corn stalk alternately). The organic amendments were applied every year in early October at rates of either 7.5 $t\,ha^{-1}$ after corn crops or 6.0 $t\,ha^{-1}$ after wheat crops, and immediately buried by moldboard plowing. Average organic C content of the cattle manure, wheat straw, and corn stalk applied was 331, 328, and 326 $g\,kg^{-1}$, average total N content was 26.8, 4.7, and 7.0 $g\,kg^{-1}$, and average C/N ratio was 12.4, 69.8, and 46.6, respectively. Further experimental details and data were reported in Triberti et al. (2008).

Soil samples were taken from each plot after wheat harvest in 2010. Ten soil cores were taken per plot with a probe to a depth of 20 cm and composited. The samples were air-dried, gently crushed, and passed through a 2-mm sieve prior to OM fractionation. For organic C and N analysis, a representative aliquot of each soil sample was ground with a ball mill. For NMR analysis, composite samples of ground soils were prepared for each treatment and repeatedly treated with 10% HF to improve the signal-to-noise ratio (Schmidt et al., 1997).

2.2. Soil organic matter fractionation

Soil OM fractionation into dissolved, free, intra-macroaggregate, intra-microaggregate, and mineral-associated OM was accomplished using the method of Plaza et al. (2012a) modified by Plaza et al. (2013). Briefly, 80 mL of sodium polytungstate (SPT) solution at a density of 1.85 $g\,mL^{-1}$ was added to 20 g of soil and shaken on an overhead shaker (1 revolution s^{-1}, 30 s). After centrifugation (2500 × *g*, 30 min), the floating light fraction (free OM) was separated from the heavy fraction by suction and filtration through a glass fiber filter and washed thoroughly with deionized water. The heavy fraction was transferred to a device designed by Six et al. (2000, 2002b) to break up macroaggregates into stable microaggregates (<250 μm), oven-dried at 70 °C, and gently transferred into a centrifuge tube together with the filtrate from the first step (SPT solution). The tube was rotated (1 revolution s^{-1}, 30 s) and centrifuged (2500 × *g*, 45 min). The floating light particles (intra-macroaggregate OM) were separated from the heavy fraction by suction and filtration and washed with deionized water. Finally, the heavy fraction was dispersed in the SPT solution by sonication (1500 $J\,g^{-1}$) and the floating light fraction (intra-microaggregate OM) was separated from the heavy fraction (mineral-associated OM) by centrifugation (2500 × *g*, 60 min), suction, and filtration and washed with deionized water.

The free, intra-macroaggregate, intra-microaggregate, and mineral-associated OM fractions recovered after fractionation were oven-dried at 70 °C, weighed, and ground with a ball mill. Free and intra-aggregate fractions were ground with the glass fiber filters. For NMR analysis, composite samples of OM fractions were prepared by mixing equal amounts of the replicates. To improve the signal-to-noise ratio, the mineral-associated OM fractions were treated with 10% HF (Schmidt et al., 1997).

2.3. Organic C and N analysis

Organic C and total N content of the whole soil samples and solid OM fractions were determined by dry combustion using a Thermo Flash 2000 NC Soil Analyzer. Dissolved organic C in the SPT solution after fractionation (dissolved OM fraction) was determined using a Shimadzu TOC 5000A analyzer. We detected no C in the SPT powder used to prepare the solution for density separations, but relatively high levels of N (0.665 $g\,kg^{-1}$). Since some N inevitably remained in the mineral-associated OM pool after the fractionation procedure, the N content in the mineral-associated pool was calculated