SSIS Training Award 2010

A Report

For the training period (Jan-Jul, 2011) at

USDA-ARS

National Laboratory for the Agriculture and the Environment

Ames, IA, USA

By

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Submitted to

Prof. Nicola Senesi

Italian Society of Soil Science, president

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Acknowledgment

I would like to express my thanks and gratitude to the Italian Society of Soil Science (SSIS) for granting me the training award 2010. My deep thanks go to my Supervisor, Prof. Teodoro Miano with University of Bari, Italy for support and help to pursue my postgraduate studies. Many thanks are extended to Dr. Dan Olk, Soil Scientist with the USDA-ARS for hosting me at his laboratory for six months.

Part I
Scientific Achievements

Summary

Laboratory schemes of two research projects were carried out during the 6-month visit. Both projects are studying the changes in soil organic carbon (SOC) after different treatments and management strategies. The research, for both project, aimed to obtain the different fractions of SOC and to characterize them by means of biochemical (amino acids, amino sugars, carbohydrates and phenols) and spectroscopic (FTIR and/or NMR) characterization. A total of 48 soil samples, 24 for each project, were fractionated by an integrated fractionation procedure. Some of the obtained fractions have been already characterized for their biochemical characteristics and the rest of the fractions will soon be characterized.

The first research project aims to identify the changes occurring in SOC, as affected by different treatments and crops, during the transition from conventional to organic farming. It included 24 soil samples collected from 2 different fields in Italy, managed organically during the transition from conventional to organic farming management. A sequential fractionation procedure was used to separate different fractions: the light fraction (LF), two size classes of the particulate organic matter (POM), the mobile humic acid (MHA) and the calcium bound humic acid (CaHA). The obtained fractions were quantified and analyzed for carbon and nitrogen contents, carbohydrates, amino acids and amino sugar contents and spectroscopic (FT-IR) characterization to determine the changes in SOC. The obtained result, so far, showed that compost application contributed to higher quantities of LF, POM and MHA compared to other amendments. The biochemical and spectroscopic analyses of the fractions will provide more information and explanation.

The second project examines the effects of long-term tillage strategies on SOC fractions. It included 24 samples collected from 3 fields in northeastern Iowa, managed under different tillage strategies (tillage, deep tillage and no-tillage). One of the investigated fields was claimed to support the highest corn production per unit area in the USA. The same fractionation methods used in the first project were utilized for separation of SOC fractions. The fractions will be analyzed for the same parameters except for spectroscopic characterization; NMR technique will instead be utilized. The obtained results showed some clear differences among the different SOC fractions with different tillage.

Scientific Background

Soil organic matter (SOM) is crucial for building soil fertility, enhancing soil quality, improving food production and reducing increased CO_2 emissions in the atmosphere. Organic agriculture is based on enriching the soil organic matter through the application of various organic amendments (compost and manure application, etc.) and strategies (cover cropping, etc.). Monitoring SOM changes is very important for site-specific evaluation of organic amendments, especially during the conversion from conventional to organic management. Total soil organic carbon is not a suitable tool to track the changes, occurring during a 3- to 4-yr transition period, in organically based soil fertility (Wander et al., 1994). Biologically active SOM-fractions that are important for nutrient cycling and supply are likely to much more sensitive to management than is total SOM. Isolating and quantifying responsive SOM fractions will show the effects of management on SOM and better provide information about fertility status. Some SOM fractions that have been shown to respond to recent crop management include POM, LF and the MHA (Marriott and Wander, 2006; Olk, 2006). But these fractions have been studied individually; no study has yet evaluated them all simultaneously, and certainly not within the context of transition to organic farming. Further, the LF and POM have not been extensively characterized for their biochemical constituents, yet the relative abundances of individual biochemical compounds (amino acids, amino sugars, carbohydrates, phenols) in active SOM fractions can help elucidate the sequestration and cycling patterns of plant and microbial tissues and of organic amendments during the transition to organic farming.

Objectives

The main goal of the first project was to monitor the changes in organic carbon fractions during the transition from conventional to organic farming management. The goal of the second project was to identify the effects of different long-term tillage strategies on SOC structure and composition in relation to carbon stability and sequestration. Both projects shared specific goals, which include:

1. Fractionation soil organic carbon and obtaining the following fractions: LF, POM, MHA and CaHA.
2. Characterization of the obtained fractions for their content of carbohydrates, amino acids and amino sugars.
3. Spectroscopic characterization, by mean of FT-IR or NMR, of the obtained fractions.

Methodologies

The SOM fractionation procedures are based on Cambardella and Elliott (1994) and Gale et al. (2000) for the POM and LF and on Mao et al. (2008) for the MHA and CaHA. Biochemical characterization of these fractions will be conducted by the methods of Olk et al. (2008) for amino acids and amino sugars and Martens and Loeffelmann (2002) for carbohydrates, both of which use anion chromatography and pulsed amperometric detection.
Phenols will be measured by the CuO oxidation method (Olk et al., 2009), and fatty acids—if time permits--will be measured by gas chromatography (Frostegård et al., 1993; Mendez-Millan et al., 2010). Carbon and nitrogen contents of the SOM fractions will be measured through automated combustion analysis (Fisons NA 1500 NC Series 2).

Research Description

- SOC study during transition into organic farming

Soil samples were collected form 2 field experiments in 2 different experimental stations located in the South of Italy. Each experiment included 2 crops (rotation of wheat and lentil) and four different treatments, with three replicates, of organic amendments (compost, compost enriched with microbial stimulator, fertilizer and fertilizers enriched with microbial stimulator). A 0-25 cm soil sample was taken from each plot after the harvest of each crop for SOC study and then composited by treatment. An initial sample, T0, was taken from each field at the start of the project.

- SOC study under different management strategies

Soil samples were collected from 3 different fields, near Ames, IA, under different management systems (tillage, deep tillage and no till). Samples were taken from 2 different locations from each field. Four depths (0-5, 5-20, 20-40 and 40-60 cm) were sampled at each location. Soils were all cultivated with corn, grown in rotation with soybean.

SOC Sequential Extraction Procedures

Soil samples from both research projects were extracted following the same integrated physical–chemical procedure. For each samples, in sequence, the LF, two size fractions (500- and 53-µm) of POM, and two NaOH-extractable humic acid fractions, MHA and CaHA, were extracted.

The integrated procedure for fractionation is a time consuming procedure, it takes one month (timetable is shown below), to obtain all the 5 fractions. The extraction procedure is briefly described here below.

The light fraction was extracted by floating 35 g soil in 150 mL of 1.6 g cm−3 sodium polytungstate (SPT), per lab replicate, after 10 min shaking in 500-ml bottle. LF was collected by vacuum suction on a 20-µm nylon filter, water-washed and oven dried at 58°C.
Prior to POM extraction, SPT was removed by centrifuging at 9800 rpm (16,200 × g) for 14 min and soil washing with 250 mL of deionized water and 3 g of Na2SO4. The wash process was repeated twice using 1.5 and 1 g of Na2SO4 respectively. In each step a 10 min shaking on a reciprocal shaker and centrifuging at 9800 rpm was carried out and the supernatant was decanted.

For extraction of the POM fraction, soil was dispersed by adding 120 mL of sodium metaphosphate (5 g L−1) to each bottle and then was shaken overnight. Then, the bottle's content was poured into stacked 500- and 53-µm sieves overlying a Pyrex pan to collect the silt and clay fractions. Silt and clay factions were dried in oven at 58°C. POM fractions, remaining on the sieve, were well-washed and transferred to drying tins. Directly afterwards, the content of each tin was transferred into vacuum suction system to eliminate water as much as possible. To separate organic material from inorganic materials, POM fractions were transferred into beakers and floated in 140 and 100 ml of 2.0 g cm−3 SPT for 53-µm and 500-µm POM fractions, respectively. Beakers content were stirred and allowed to settle for 6 h. Then floating materials were collected by vacuum suction on a 20-µm nylon filter, and beakers content were restirred and allowed to settle for another 6 h. Then floating materials were collected by suction and added to the previously collected materials. The stirring and vacuum suction processes were repeated a third time after an overnight settling. Collected materials were washed with excess of water, transferred into preweighed tins and oven dried at 58°C.

The MHA and CaHA were extracted according to Mao et al. (2008). Silt and clay material were divided evenly, based on their weight, into 2 or 3 500-ml bottles. Bottle contents were extracted by a 10:1 (v:w) volume of 0.25 mol L−1 NaOH for 20-h under an N2 atmosphere. Afterwards, bottled were centrifuged and supernatants were decanted and

acidified by addition of 2 mol L^{-1} HCl, to pH 1.95 – 2.0. The acidified supernatant was transferred to 1000-mL cylinders and allowed to settle over night. The extraction process was repeated at least 2 more times and the collected MHAs were combined.

To extract CaHA, calcium was removed from the silt and clay materials by shaking the bottle contents, for 10 min with 300 mL of 0.2 mol L^{-1} HCl. Bottles were centrifuged and the supernatant pH was measured. The HCl washing was repeated until the supernatant pH decreased to <1.0. Then, excess HCl was removed with 300 mL of double-deionized water/ bottle followed again by shaking, centrifuging, and decanting the supernatant., This cycle was repeated until the supernatant pH was greater than 2.0. Then, the bottle content was extracted as described previously for the MHA.

Collected MHA and CaHA were cleaned of soil by resolubilization in a KOH-KCl solution under an N_2 Atmosphere. This process was repeated twice when the collected humic material was too large to permit complete solubilization. MHA and CaHA were re-precipitated with HCl, then shook overnight in 0.2% HCl–HF to eliminate any remaining contamination of soil. Afterwards, the MHA and CaHA were dialyzed for 3 days in successively weaker HCl solutions and at the end against water. Finally, the fractions were freeze-dried and stored for subsequent analysis.

Extraction and determination of Carbohydrates

This extraction was carried out for whole soil and the extracted fractions. Sample mass used for carbohydrates extraction varied based on fraction type. Carbohydrates were extracted using a 2-step extraction, first a weak acid extraction for hemi-celluloses followed by a strong acid extraction for cellulosic glucose. For the weak acid extraction, samples were weighed into culture tubes then 800 µL of 6 mol L-1 H2SO4 were added. After 30 min, samples were diluted to 1 mol L-1 H2SO4. Samples were autoclaved for 30 min at 121°C, allowed to cool, then centrifuged and the supernatant was decanted and adjusted to pH 5.5 – 6.5 using NaOH. The supernatant was centrifuged and frozen.

The sample residue from the weak acid extraction was oven dried at 58°C overnight. Then 300 µL of 18 mol L-1 H2SO4 were added, allowed to sit for 30 min, and then diluted to 1.5 mol L-1 H2SO4. Subsequent steps (centrifugation, dilution, pH adjustment) of the samples were the same as for the weak acid extraction described above.

Then samples were thawed and diluted and carbohydrates were measured by a Dionex anion chromatograph. Detected carbohydrates were mainly fucose, arabinose, rhamnose, galactose, glucose, xylose, mannose and ribose.

Extraction and determination of Amno acids

Amino acids and amino sugarswere extracted, in culture tube, by 4 mol L^{-1} methanesulfuric acid. Tubes were autoclaved for 16 h at 121°C, allowed to cool down, centrifuged and the supernatant was decanted its pH adjusted to 4.0 – 6.0. Samples were, then centrifuged and frozen. Amino acids and amino sugars were detected by a Dionex anion chromatograph

Discussion

Some of the extracted fractions showed a response to the different treatments applied during the study. The integrated physical - chemical fractionation method seems to be very efficient in extracting the SOC fractions that are responsive to changes in tillage, crop, fertilization and management. This suggests the usefulness of the methods for tracking changes in SOC for short-term studies. Still the reproducibility of the methods, with various soils, is of crucial concern. CaHA extraction, using this method, was not clearly effective when extracted from calcareous soils. Also, the resolubilization of humic fractions (MHA and CaHA) in KOH-KCl, to remove soil, was incomplete with soil with very high content of organic matter.

Knowledge Development and Exchange

I acquired very good knowledge of organic matter extraction and fractionation especially the physical fractionation. I practiced the extract procedures with 3 different types of soil and I got so familiar with the procedures.

It was the first time for me, to extract carbohydrates and amino acids from soil and SOC extracted fractions. The knowledge, I gained, will be well employed at my university, Bari, Italy.

I contributed with some technical enhancements to the extraction procedure. I suggested not drying SPT between extraction cycles as drying contributed to loss of SPT, through grinding, resolubilization and filtration. I, also, suggested using the vacuum suction, instead of drying, to eliminate water of the two size classes of the POM before floating them. The vacuum suction eliminated the loss of POM through grinding and transfer of dry POM fractions and saved more time.

Future collaboration

There is a planned visit in the next fall (Sep – Dec) to the same host institution to carry out the extraction procedures on the next set of samples coming one year later from the project of transition into organic farming management, in Italy.

During this visit, a set of samples will be extracted for the different fractions and then these fractions will be characterized for their biochemical and spectroscopic properties

Projected Publication

At least 2 scientific papers will be published out of the work carried out during this visit. The first paper will focus on explaining the sequential fractionation procedure and its importance for tracking the changes in SOC for short-term studies. The other paper will focus on the effect of soil tillage on SOC characteristics. Another possible paper would be developed during the next visit. ;It will address the 2-year changes in the SOC fractions during the transition from conventional to organic farming.

Letter of Confirmation by the tutor of the host institute at the successful execution of the mission:

United States Department of Agriculture

Research, Education and Economics
Agricultural Research Service

01 July 2011

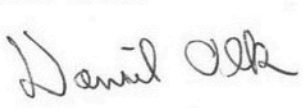
To whom it may concern:

Mr. Hamada Abdelrahman, Ph.D. student in Agricultural Chemistry at the Universitá degli Studi di Bari, in Bari, Italy, has been performing research in my laboratory since his arrival on January 19, 2011. He has satisfactorily completed all assignments for both of his projects here. As he now leaves my group and returns to Bari, I certify that Mr. Abdelrahman has successfully concluded his work planned for this six-month visit in my laboratory.

I have read and approved his report to you that describes his activities in my lab. Specifically, he used both physical and chemical means to extract soil organic matter fractions from two sets of field experiments—(i) two sites in southern Italy that study soil changes during the transition from conventional to organic farming, and (ii) three farmers' fields in Iowa that collectively study soil changes resulting from deep incorporation of crop residues through tillage. These fractions are now undergoing biochemical analyses, in part through Mr. Abdelrahman's efforts.

Sincerely,

Daniel C. Olk



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