UNIVERSITY OF NAPOLI "FEDERICO II" AGRICULTURAL FACULTY D.I.S.S.P.A.P.A.

Soil chemistry department

Soil Organic Matter dynamics

¹³C labelled organic material

- TMAH thermochemolysis

Dr. Riccardo Spaccini

one of the pressing problems in agro-ecosystems is represented by the continuous loss of soil organic matter content

a part the immediate effect on plant productivity the lowering of SOM level lead to a long term decrease of overall soil chemical and biochemical fertility and soil aggregate stability (soil erosion)

all these effects have as a feed back consequence, the further increase of SOC losses: agricultural activities have nowadays a significant contribute to green-house gas emissions in the earth atmosphere

in 1997 the estimation for the current loss rate from virgin soils converted to intensive agricoltural exploitation was around $0.9 \ge 10^{15} \ge C \ yr^{-1}$

different practices are therefore currently applied in order to reduce, in the short/medium terms, the decrease of SOC content and to produce a long term restoring or improvement of SOM level

- integration or replacement of mineral fertilizers with organic materials such as green manure, animal manure, pig slurry, recycled organic biomasses etc.
 - among SOM pools an important component are represented by humic materials; this colloidal organic fraction largely affect all the various aspect of long term soil fertility, moreover acting as a long term sink of soil organic matter
 - humic substances, such as humic acids and humin represent in fact the most persistent pool for SOC accumulation, through the selective preservation of recalcitrant material and the close interaction with finest soil fractions
 - humic material is believed to play a fundamental role in the stable incorporation of more labile organic compounds released from root exudates, plant litter and microbial activity, thereby increasing SOM sequestration and reducing the OC mineralization

"Organic carbon sequestration through protection by humic substances"

Objective

Study the effect of humified organic matter on the mineralization of labile organic compound in soil

a short chain alcohol (2 decanol) was labelled with ¹³C isotope and used for soil incubation to study the OC mineralization

the ¹³C labelled alcohol was added to soil either alone or in mixture with two humic acids extracted from lignite and from recycled organic biomass

the mineralization of labelled alcohol was followed for 6 months in all soil treatments either in bulk soils and in soil-size fractions, determining also the final incorporation of labelled compounds in soil humic substances

Why using ¹³C labelled compound

the use of organic material labelled with ¹³C isotope is a useful tool for the analysis of SOM dynamics and of OC mineralization

the enzymatic systems involved during photosynthesis in plant C fixation, discriminate against the ¹³C isotope, reducing the ¹³C content in plant tissues; the amount of ¹³C is indicated as δ and it is determined (with IR-MS) comparing the ratio 13C/12C of the sample and that of a reference standard

$$\delta = \frac{(13C/12C)_{sample} - (13C/12C)_{reference}}{(13C/12C)_{reference}} \times 1000$$

the ¹³C content in the various plant species change with different photosynthetic pathway:

-C3 plants (wheat) (*Calvin* cycle) have an average ¹³C content $\delta = -27\%_0$

- -C4 plants (corn) (Hatch & Sluck cycle) have average ¹³C content $\delta = -14\%$
- -CAM plants have an average ¹³C content $\delta \cong C4$ plants (facultative CAM have a ¹³C content $\delta = -14\% \div -27\%$)

since there is not a net or significative microbial discrimination of ¹³C during microbial degradation and microbial synthesis the ¹³C abundance of SOM reflect the composition of the plant species cultivated

by adding to soil an organic material with different ¹³C content it is therefore possible to label the new entering SOC and differentiate between old and new organic material, thereby following both processes of OC accumulation and mineralization



in long term field experiment the shift between old and recent SOM is obtained by "natural labelling" replacing the previous cultivated plant species with another crop with different δ e.g. wheat (C₃ plant) replaced with corn (C₄ plant)

in short term laboratory incubation experiment, synthesized labelled organic compound, with a significant different δ , are likely used in order to have a rapid shift between old and recent SOM



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<u>13C-labelling of 2-decanol</u>

¹³C labelled 2-decanol was synthesized in our laboratory by a Grignard reaction between nonanoic aldehyde and a ¹³C-labelled methyliodide with a yield of 76.3% as reported in the following scheme:





CPMAS NMR spectra of Humic acids used for soil incubation:



incubation experiment:



soil samples from surface horizon (0-20 cm) were collected from agricultural soil (FAO cl. Dystric Cambisol) air dried and sieved through a 2 mm sieve : pH (KCl)= 5.6; sand/silt/clay (%) =48/43/9; OM (%)= 3.1



soil samples were incubated at constant moisture for 6 months 4 replicates for each treatments

Sampling times = **t0** 1week; **t1** 12 weeks; **t2** 25 weeks

in order to follow the distribution of labelled ¹³C-decanol also in soil fractions, the soil samples at each sampling date were physically separated in particle-size fractions through the application of low energy sonication method

low energy sonication (170 Jg⁻¹) was used in order to reproduce on incubated samples the disturbing action of agricultural practices (ploughing), raindrop and drying-wetting cycles, which cause natural breakdown of soil macro-aggregates (> 250 μ)

the applied low energy thus preserve the integrity of microaggregates (< 250 μ) thereby avoiding the artificial redistribution of organic matter among soil fractions.

Particle size fractions were obtained in the following ranges associated to classical textural classes:

2000-200μm "*coarse sand*", 200-63 μm "*fine sand*", 63-2 μm "*silt*", < 2μm"*clay*", < 0.1 μm "*fine clay*" (DOM)



Results



Coarse s.		Contro	ol	1	³ C2−d	ec	¹³ C	-HAC		$^{13}\mathrm{C}$	HAL	
	t0	t1	t2	tO	t1	t2	t0	t1	t2	t0	t1	t2
OC g kg ⁻¹	3.8	3.6	3.6	3.8	3.5	3.0	4.0	3.8	3.8	3.6	3.6	4.4
$\delta^{13}\mathrm{C}(\%)$.	-26.3	-26.3	-26.3	-22.3	-23.6	-24.6	-21.1	-21.6	-24.5	-19.5	-20.5	-22.5
¹³ C (µg)	/	/	/	241	150	91	270	280	110	440	380	300
¹³ C (%)	/	/	/	2.4	2.5	3.3	2.6	3.1	3.4	4.1	3.7	4.9
¹³ C E.F.	/	/	/	0.32	0.35	0.36	0.33	0.32	0.38	0.46	0.49	0.54
Fine sa	and						 					
Fine sa OC g kg ⁻¹	and 7.9	6.5	6.0	7.4	6.1	5.0	6.7	7.5	6.9	7.7	7.4	7.0
Fine so $OC \ g \ kg^{-1}$ $\delta^{13}C(\%)$	and 7.9 -26.8	6.5 -26.6	6.0 -26.7	7.4 -21.7	6.1 -23.1	5.0 -24.4	6.7 -21.1	7.5 -21.3	6.9 -22.9	7.7 -18.9	7.4 -19.9	7.0 -21.7
Fine so $OC \ g \ kg^{-1}$ $\delta^{13}C(\%)$ - $^{13}C \ (\mu g)$	and 7.9 -26.8 /	6.5 -26.6 /	6.0 -26.7 /	7.4 -21.7 302	6.1 -23.1 176	5.0 -24.4 85	6.7 -21.1 270	7.5 -21.3 260	6.9 -22.9 150	7.7 -18.9 400	7.4 -19.9 350	7.0 -21.7 300
Fine so $OC g kg^{-1}$ $\delta^{13}C(\%)$ $^{13}C (\mu g)$ $^{13}C (\%)$	and 7.9 -26.8 /	6.5 -26.6 /	6.0 -26.7 /	7.4 -21.7 302 3.0	6.1 -23.1 176 3.0	5.0 -24.4 85 3.0	6.7 -21.1 270 2.6	7.5 -21.3 260 2.9	6.9 -22.9 150 3.0	7.7 -18.9 400 3.5	7.4 -19.9 350 4.3	7.0 -21.7 300 4.3
Fine sa OC g kg ⁻¹ $\delta^{13}C(\%)$ - $^{13}C (\mu g)$ $^{13}C (\%)$ $^{13}C E.F.$	and 7.9 -26.8 /	6.5 -26.6 /	6.0 -26.7 /	7.4 -21.7 302 3.0 0.35	6.1 -23.1 176 3.0 0.37	5.0 -24.4 85 3.0 0.39	6.7 -21.1 270 2.6 0.44	7.5 -21.3 260 2.9 0.46	6.9 -22.9 150 3.0 0.52	7.7 -18.9 400 3.5 0.52	7.4 -19.9 350 4.3 0.63	7.0 -21.7 300 4.3 0.67

¹³C E.F. ¹³C Enrichment factor = $\frac{g^{13}C \times 100 \text{ g}^{-1} \text{ TOC size fraction}}{g^{13}C \times 100 \text{ g}^{-1} \text{ TOC bulk soil}}$

~ 11		Contr	col	1	³ C2-d	lec	_ ¹³ C	-HAC	r -	¹³ C	-HAL	
Silt	t0	t1	t2	t0	t1	t2	t0	t1	t2	t0	t1	t2
OC g kg ⁻¹	¹ 17	15.9	22	16.4	16.1	19.0	17.3	17.6	22	16.2	16.6	26
$\delta^{13}C(\infty)$	-26.3	-26.5	-26.3	-21.6	-23.2	-24.8	-20.3	-20.7	-22.0	-19.9	-20.0	-20.7
¹³ C (mg)	/	/	/	1.45	1.00	0.89	1.8	1.76	1.64	2.0	2.1	2.8
¹³ C (%)	/	/	/	14.4	17.1	26.8	17.3	19.5	35.7	19.0	20.4	45.2
¹³ C E.F.	/	/	/	0.39	0.43	0.41	0.48	0.5	0.65	0.51	0.51	0.75
Clay	50.2	(2)	550	(1 0)	(\mathbf{c})	520	$c_0 \epsilon$	(= 0	(\mathbf{c})	60 1	62.5	620
OC g kg ⁻¹	59.5	62.2	55.0	61.0	62.6	52.0	60.5	65.8	62	00.4	03.3	02.0
$\delta^{13}C(\%)$ -	-25.8-	-26.5	-26.8	-18.5	-12.9	-20.0	-18.3	-17.2	-13.7	-19.3	-17.5	-11.0
$^{13}C (mg)$	/	/	/	1.14	2.2	0.81	1.05	1.34	1.94	1.26	2.3	2.6
¹³ C (%)	/	/	/	11.3	37.7	32.0	10.2	14.9	42.3	11.7 2	22.1	42.2
¹³ C E.F.	/	/	/	0.60	1.77	1.40	0.58	0.77	2.0	0.67	1.04	2.1
DOM					·							
OC g kg ⁻¹	305	240	216	360	263	244	364	337	155	347	335	156
$\delta^{13}C(\%)$	-25.0	-25.1	-25.2	+49			+52			+41		
$^{13}C (mg)$	/	/	/	 			 			1 1 1 1		
¹³ C (%)	/	/	/	69			67.3			60.8		
¹³ C E.F.	/	/	/	, , , , ,								

Results

C % tot / /
% tot / /
/
/
30.8
59.5
65
2.6
.0.6
0.4
9.2)
$\overline{0.2}$
6.0

~ 11	Control		$^{13}C2$ -dec			¹³ C-HAC			¹³ C-HAL			
Silt	tO	t1	t2	tO	t1	t2	tO	t1	t2	t0	t1	t2
OC g kg ⁻	¹ 17	15.9	22	16.4	16.1	19.0	17.3	17.6	22	16.2	16.6	26
$\delta^{13}C(\%)$	-26.3	-26.5	-26.3	-21.6	-23.2	-24.8	-20.3	-20.7	-22.0	-19.9	-20.0	-20.7
¹³ C (mg)	/	/	/	1.45	1.00	0.89	1.8	1.76	1.64	2.0	2.1	2.8
¹³ C (%)	/	/	/	14.4	17.1	26.8	17.3	19.5	35.7	19.0	20.4	45.2
¹³ C E.F.	/	/	/	0.39	0.43	0.41	0.48	0.5	0.65	0.51	0.51	0.75
Clay											< 7 7	60 0
OC g kg ⁻¹	59.3	62.2	55.0	61.0	62.6	52.0	60.5	65.8	62	60.4	63.5	62.0
$\delta^{13}C(\%)$	-25.8-	-26.1 -	-26.8	-18.5	-17.9	-20.0	-18.3	-17.2	-13.7	-19.3	-17.5	-11.0
¹³ C (mg)	/	/	/	1.14	1.2	0.81	1.05	1.34	1.94	1.26	2.3	2.6
¹³ C (%)	/	/	/	11.3	37.7	32.0	10.2	14.9	42.3	11.7	22.1	42.2
13 C E.F.	/	/	/	0.60	1.77	1.40	0.58	0.77	2.0	0.67	1.04	2.1
DOM					-							
OC g kg ⁻¹	¹ 305	240	216	360	263	244	364	337	155	347	335	156
$\delta^{13}C(\%)$	-25.0	-25.1	-25.2	+49	+14	+9	+52	+49	-16.4	+41	+39 -	-18.2
¹³ C (mg)	/	/	/	6.94	2.4	0.9	7.0	5.3	0.3	6.6	5.2	0.2
¹³ C (%)	/	/	/	69	39.8	35	67	59	9.5	61	50.3	3.4
¹³ C E.F.	/	/	/	5.7	5.1	7.0	5.8	6.2	1.5	5.3	5.1	0.9

Results

			bulk s	oil		Sum of fractions (2000<µ>0.1				
		TOC		¹³ C-C	C	TC	DC	¹³ C-OC		
treatments		%	δ‰	% TC	OC mg	%	% tot	mg	% tot	
Control	t0	1.8	-26.2	/	/	1.6	88.9	/	/	
	t1	1.7	-26.2	/	/	1.6	94.1	/	/	
	t2	1.5	-26.3	/	/	1.4	93.3	/	/	
¹³ C2-dec	t0	1.8	-13.9	0.47	10.2	1.6	88.9	3.14	30.8	
	t1	1.7	-18.5	0.29	6.0	1.6	94.1	3.60	59.5	
	t2	1.4	-22.3	0.19	2.9	1.3	92.8	1.90	65	
	t0	1.9	-13.4	0.46	10.4	1.6	84.2	3.40	32.6	
¹³ C-HAC	t1	1.8	-14.4	0.42	9.1	1.6	88.9	3.70	40.6	
	t2	1.7	-19.4	0.29	4.2	1.7	100	3.80	90.4	
	t0	1.8	-13.5	0.50	10.8	1.6	88.9	4.21	39.2	
¹³ C-HAL	t1	1.7	-13.9	0.49	10.2	1.7	100	5.12	50.2	
	t2	1.7	-18.9	0.30	6.1	1.7	100	5.94	96.0	

Residual (%) content of ¹³C-OC respect to initial added amount



 13 C-2dec. 13 C-HAC 13 C-HAL





¹³C-OC content in soil humic substances extracted at the start (t0) and the end (t2) of soil incubation

treatments		Humi	ic acids	Fulvic acids		
		δ ¹³ C‰	δ^{13} C‰ ¹³ C-OC mg		¹³ C-OC mg	
Control	t0	-26.2	/	-25.8	/	
	t2	-26.0	/	-25.7	/	
13 C 2 days	t0	-25.7	0.12	-25.1	0.1	
C2-ucc	t2	-21.5	1.2	-23.4	0.25	
¹³ C-HAC	t0	-22.0	0.9	-23.2	0.25	
	t2	-15.7	2.8	-22.0	0.4	
¹³ C-HAL	t0	-25.6	0.14	-23.7	0.2	
	t2	-15.6	3.7	-22.4	0.4	



¹³C-OC content in soil humic acids respect to bulk soil



Conclusions

- in a short term incubation experiment the utilization of labelled ¹³C organic compound represent a useful and effective tool to study the organic carbon behaviour and distribution in soil and soil size fractions
- in this laboratory incubation experiment, addition of humified organic materials to soil significantly increased the sequestration of organic carbon in bulk soil, soil size fractions and soil humic acids thereby reducing the mineralization of the relatively labile aliphatic alcohol such as ¹³C-2-decanol.
- a considerable amount of ¹³C-OC was incorporated in the stable portion of soil OM represented by HS for all treatments. This finding confirm the importance of soil humic materials for the stable incorporation of labile organic compounds.

- the distribution of residual ¹³C-OC among soil particle-size fractions indicated in all treatments the incorporation of labile organic compound was most effective in the soil sized fractions (*silt*- and *clay*-) usually associated with stable SOM pool. This result confirms the importance of associations between fine textural fractions and recalcitrant OM
- the different protection of 13C-OC shown by the two exogenous HA in the finer fractions may be related to the different chemical nature of their hydrophobic components:
 the hydrophobicity of HAC (HI

¹/₄ 1.20; ArI ¹/₄ 5%) is mostly due to its large alkyl-C content that may have promoted, for chemical affinity, a preferential interaction with the predominantly aliphatic native humic matter present in the claysized fractions.

conversely, the larger hydrophobicity of HAL (HI ¼ 1.40; ArI ¼ 25%), due to a larger content of both alkyl- and aromatic-C groups, may have favored a progressively larger incorporation of 13C-OC not only into the clay- but also into the silt-sized fractions that is generally associated with more aromatic humic matter

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application of OM to soil increase soil fertility and plant nutrition and may also reduce mineralization of biolabile compounds, thereby enhancing the role of soil organic matter (SOM) as sink of OC these effects are achieved by improving

both quantity and quality of SOM with an increased accumulation of various classes of organic compounds.

both mid- and long-term organic matter accumulation are, in fact, related to the nature and composition of OM whose effectiveness arise from the stable incorporation of exogenous organic compounds into native bulk SOM.

an increasing interest is therefore given to a deeper molecular characterization of both bulk SOM and added material, as well as to their transformed products, in order to understand the processes of effective incorporation and stabilization of specific exogenous organic molecules in soil. Thermochemolysis of Soil Organic Matter in soil amended with recycled organic biomass (compost)

Objective

The main objective of this work was to characterize the organic matter components in cultivated soil amended with recycled organic biomass.

This objective was accomplished applying the Off-Line TMAH-thermochemolysis GC-MS technique on organic material and on soil samples in order to evaluate the incorporation of organic material in SOM and the transformation after one year of compost additions. Soil treatments were as following:

- Control soil with inorganic fertilizer
- Soil + compost (30 and 60 t ha⁻¹)

after one year of compost addition about 1kg of soil from the ploughed horizon (25 cm⁻¹) for each treatment was randomly sampled, dried and sieved at 2mm.

soil aggregates were destroyed with ultrasonication and soil and compost samples were powdered with electrical agate ball mill and finally sieved at 500 μ m.

Soil and compost samples were analyzed by TMAH thermochemolysis GC-MS – quantitative analysis were based on calibration curves built by mixing the following standards: tridecanoic acid, octadecanol, 16-hydroxy hexadecanoic acid, docosandioic acid, beta-sitosterol, and cinnamic acid







from the data of lignin derivatives it is possible to determine a structural index related to the degradation stage of lignis materials



μg g ⁻¹ soil d.w. Conyferil	Initial control soil 120	Lignin	index > 2 prevalence of oxydisized structures (high degradation)
$\frac{\text{Ad/Al}_{G}}{\Gamma_{G}}$	4.9 6.1		Ad=acidic formstructuralAl=aldehydic form
Sinapyl (S) Ad/Al _S Γ _s	5.3 5.9		indexes $\Gamma = \frac{\text{acidic form}}{\text{undegraded structures}}$
FAME 415 C_{14} - C_{28} alkanes 37 C_{15} - C_{27}		Alkyl	intermediate values indicate the variable source origin of alkanes (bacterial, fungi, plant)
• • • •	CPI=2.3 ACL=23.5		nlant markers
mid chain wOH acids	$\begin{array}{c} 69 \ C_{16}, \ C_{18} \\ 87 \ C_{16} \ -C_{22} \\ \hline \end{array}$		cutin and suberin components
dioic acids sterols resin acids	59 C ₁₆ ,C _{18:1} / /		angiosperm gimnosperm



µg g-1	Initial		Compost
soil d.w.	control soil	lignin	
Conyferil	120		3460
Ad/Al _G	4.9	structural indexes	1.7
Γ _G	6.1	index $\cong 2$	2.2
Sinapyl (S)	125	presence of fresh litter	3730
Ad/Al _s	5.3	(IOW degradation)	2.1
Γ _s	5.9	11 1	1.6
FAME	415 C ₁₄ -C ₂₈		10250 C ₁₂₋ C ₃₂
alkanes	37 C ₁₅ -C ₂₇ CPI=2.3 ACL=23.5	high values indicate the prevalent plant origin of	4850 CPI=4.2 ACL=29.3
mid chain OH acids	69 C ₁₆ , C ₁₈	alkanes	5500 C ₁₆ C ₁₈
ωOH acids	87 C ₁₆ -C ₂₂		6300 C ₁₆ -C ₂₆
dioic acids	59 C ₁₆ ,C _{18:1}		4900 C ₁₆ -C ₂₄
sterols resin acids		plant markers	- 2780 1360







µg g-1	Initial	Final	Soil+ Com	Soil+ Com	Compost
soil d.w.	control soil	control soil	30 t ha ⁻¹	60 t ha ⁻¹	
Conyferil	120	110 ^{lig}	nin 159	186	3460
Ad/Al _G	4.9	4.7	4.0	3.8	1.7
$\Gamma_{\mathbf{G}}$	6.1	6.2	4.9	5.0	2.2
Sinapyl (S)	125	120	157	225	3730
Ad/Al _s	5.3	5.5	4.3	4.1	2.1
Γ_{s}	5.9	6.1	4.4	3.9	1.6
FAME	415 C ₁₄ -C ₂₈	$\begin{array}{c} \text{al} \\ 380 \text{ C}_{14}\text{-}\text{C}_{28} \end{array}$	kyl 492 C ₁₂ -C ₃₀	595 C ₁₂ -C ₃₂	10250 C ₁₂₋ C ₃₂
alkanes	37 C ₁₅ -C ₂₇ CPI=2.3 ACL=23.5	40 C ₁₅ -C ₂₇ CPI=2.4 ACL=24.4	123 C ₁₅ -C ₃₃ CPI=3.8 ACL=27.7	172 C ₁₅ -C ₃₃ CPI=3.9 ACL=28.4	4850 CPI=4.2 ACL=29.3
mid chain OH acids	69 C ₁₆ , C ₁₈	40 C ₁₆ , C ₁₈	98 C ₁₆ , C ₁₈	166C ₁₆ , C ₁₈	5500 C ₁₆ C ₁₈
ωOH acids	87 C ₁₆ -C ₂₂	62 C ₁₆ -C ₂₂	121 C ₁₆ -C ₂₆	200 C ₁₆ -C ₂₆	6300 C ₁₆ -C ₂₆
dioic acids	59 C ₁₆ , C _{18:1}	55 C ₁₆ ,C _{18:1}	126C ₁₆ ,C ₂₄	186C ₁₆ ,C ₂₄	4900 C ₁₆ -C ₂₄
sterols resin acids	/	/	32 16	66 32	2780 1360

- thermochemolysis analysis provided a detailed qualitative and quantitative characterization of SOM revealing an effective incorporation of exogenous organic compounds in soil amended with compost.
- plant biopolymers such as lignin, waxes, and aliphatic polyesters were recognized as the main sources of organic components in both soil and compost. the identified derivatives allowed the evaluation of related structural indexes associated with the overall origin and biochemical stability of SOM components.
 - these results indicate that the TMAH thermochemolysis technique is a rapid and effective method for molecular characterization of SOM; such method enables the identification of a large range of lignin, alkyl and cyclic lipid biomarkers, which are useful to trace both origin and transformation of organic matter in soil .