

# Influence of solid vs. liquid digestate application on soil organic matter accrual and stabilization, and microbial communities evolution

Sinatra M., Giannetta B., Galluzzi G., Tondello A., Stevanato P., Squartini A., and Zaccone C.



UNIVERSITÀ di VERONA

martina.sinatra@studenti.univr.it



## 1. Introduction

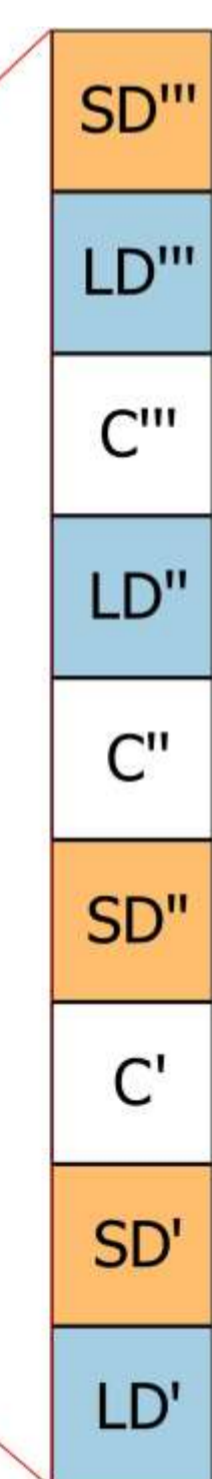
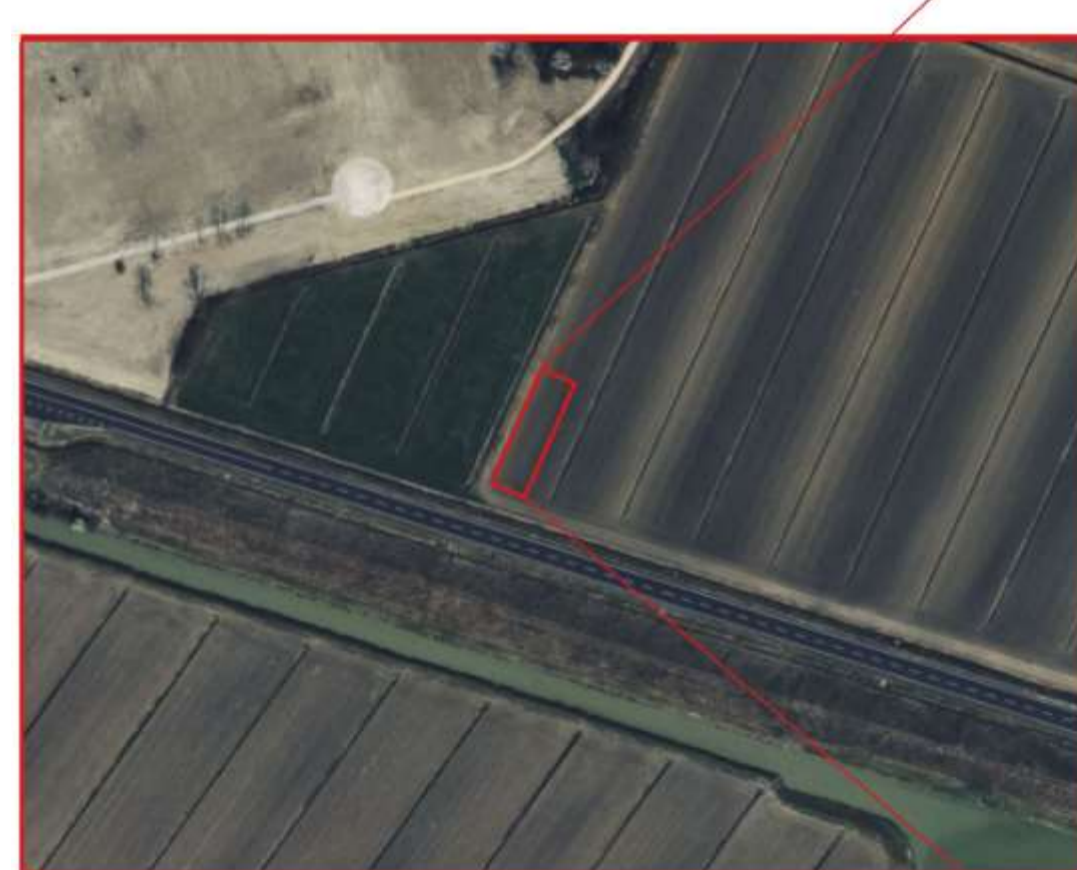
The decline of soil organic matter (SOM), as well as the depletion of micro and macronutrients, are among the most serious threats facing many agricultural soils in the world. In many Countries, soil amendments are increasingly originating from a wide range of organic wastes, as a win-win strategy to mitigate global warming while increasing soil fertility and food security. Digestate, a by-product of the anaerobic digestion, is often used as a fertilizer and/or an organic amendment. However, a safe and sustainable application of digestate in agriculture requires an in-depth understanding of its possible impacts on native SOM, on carbon (C) sequestration mechanisms, as well as on soil microbial communities.

## 2. Objective

To test the influence of solid vs. liquid digestates on both SOM accrual and the distribution of SOM in particulate organic matter (POM) and mineral associated organic matter (MAOM) pools. Moreover, the abundance of key genes related to the N cycle was assessed.

## 3. Experimental design

~210 kg N ha<sup>-1</sup> yr<sup>-1</sup> for each treatment



Depths (cm):

0-15  
15-30

Time:

t<sub>0</sub> ⇒ appl.  
t<sub>3</sub> ⇒ 3 months  
t<sub>6</sub> ⇒ 6 months

C ⇒ Control (no amend.)  
SD ⇒ soil amended with solid digestate  
LD ⇒ soil amended with liquid digestate

n = 54 bulk samples

## 4. Materials & Methods

**Bulk samples (54):**

- bulk density (BD)
- pH and EC
- texture, XRD, ICP-MS
- TG-DSC
- CHNS
- qPCR

**SOM fractions (108):**

- TG-DSC
- CHNS

## 5. Results & Discussion

Elemental and thermal characterization of digestates

	dm (%)	LOI (%)	C (%)	N (%)	C/N	TG-T <sub>50</sub> (°C)	DSC-T <sub>50</sub> (°C)	WL <sub>450-550/250-350</sub>
Solid digestate	25	85	42	1.4	30	288	352	0.079
Liquid digestate	5	57	33	3.5	9	313	374	0.392

Two-way ANOVA of SD and LD bulk samples compared to control for SOC and TN stocks.

Bulk samples			
	Treatment	Time	Treatment × Time
<b>SD:Control</b>			
SOC stock (0-15)			
SOC stock (15-30)	0.004**		
TN stock (0-15)			
TN stock (15-30)	0.001***		0.032*
<b>LD:Control</b>			
SOC stock (0-15)			
SOC stock (15-30)	0.000***		
TN stock (0-15)			
TN stock (15-30)	0.000***		0.004**

Two-way ANOVA of SD and LD samples compared to control for OC and TN stocks ratio between MAOM and POM.

MAOM/POM			
	Treatment	Time	Treatment × Time
<b>SD:Control</b>			
OC <sub>MAOM</sub> /OC <sub>POM</sub> (0-15)	0.001**		
OC <sub>MAOM</sub> /OC <sub>POM</sub> (15-30)	0.000***		0.028*
TN <sub>MAOM</sub> /TN <sub>POM</sub> (0-15)	0.003**	0.030*	
TN <sub>MAOM</sub> /TN <sub>POM</sub> (15-30)	0.009**	0.000***	0.013*
<b>LD:Control</b>			
OC <sub>MAOM</sub> /OC <sub>POM</sub> (0-15)			
OC <sub>MAOM</sub> /OC <sub>POM</sub> (15-30)			0.037*
TN <sub>MAOM</sub> /TN <sub>POM</sub> (0-15)		0.009**	
TN <sub>MAOM</sub> /TN <sub>POM</sub> (15-30)		0.015*	0.011*

Example of TG-DSC indices of POM and MAOM samples of the three treatments (Control, SD and LD) at t<sub>3</sub> and at two depths (0-15 and 15-30 cm).

	Treatment	Time	TG-T <sub>50</sub> (°C)		DSC-T <sub>50</sub> (°C)		Energy density (J mgSOM <sup>-1</sup> )		WL <sub>450-550/250-350</sub>	
			0-15 cm	15-30 cm	0-15 cm	15-30 cm	0-15 cm	15-30 cm	0-15 cm	15-30 cm
POM	Control	t <sub>3</sub>	343	342	352	351	13.4	13.6	0.42	0.42
	SD	t <sub>3</sub>	342	340	353	357	13.9	13.3	0.37	0.32
	LD	t <sub>3</sub>	344	343	353	354	13.6	13.3	0.42	0.42
MAOM	Control	t <sub>3</sub>	362	361	355	359	10.4	10.1	0.96	0.93
	SD	t <sub>3</sub>	363	363	355	360	10.8	11.4	0.94	0.94
	LD	t <sub>3</sub>	361	360	344	357	12.7	10.1	0.92	0.92

## 6. Conclusions

- The distribution of OC between POM and MAOM was exclusively affected by solid digestate application; both treatments displayed their interactive effect with time. The time factor had an evident influence after the application of both digestates on the TN<sub>MAOM</sub>/TN<sub>POM</sub> ratio.
- OM accumulated more in the POM fraction after solid digestate amendment, while the distribution between SOM pools following liquid digestate application was similar to control.
- In low-OC content soils, the interaction between SOM and mineral surfaces is the major SOM stabilization mechanism.
- Quantitative PCR showed that solid digestate application increased total microbial abundance (16S) and the quantity of genes related to the N cycle, especially at 15-30 cm and at t<sub>3</sub>.

Cycle thresholds (CT) of qPCR for the five genes investigated in the DNA extracted from soils. The conditional formatting (green through red) allows to appreciate the differences in gene abundance (lower CT mean earlier appearance and correspond to higher number of target gene copies). ND = not detectable.

Sample	Depth (cm)	Time
C <sup>0</sup>	0-15	t <sub>0</sub>
C <sup>3</sup>	0-15	t <sub>3</sub>
C <sup>6</sup>	0-15	t <sub>6</sub>
C <sup>0</sup>	15-30	t <sub>0</sub>
C <sup>3</sup>	15-30	t <sub>3</sub>
C <sup>6</sup>	15-30	t <sub>6</sub>

Sample	Depth (cm)	Time
C <sup>0</sup>	0-15	t <sub>3</sub>
C <sup>3</sup>	0-15	t <sub>3</sub>
C <sup>6</sup>	0-15	t <sub>3</sub>
C <sup>0</sup>	15-30	t <sub>3</sub>
C <sup>3</sup>	15-30	t <sub>3</sub>
C <sup>6</sup>	15-30	t <sub>3</sub>

Sample	Depth (cm)	Time
C <sup>0</sup>	0-15	t <sub>6</sub>
C <sup>3</sup>	0-15	t <sub>6</sub>
C <sup>6</sup>	0-15	t <sub>6</sub>
C <sup>0</sup>	15-30	t <sub>6</sub>
C <sup>3</sup>	15-30	t <sub>6</sub>
C <sup>6</sup>	15-30	t <sub>6</sub>

Sample	Depth (cm)	Time
SD <sup>0</sup>	0-15	t <sub>0</sub>
SD <sup>3</sup>	0-15	t <sub>3</sub>
SD <sup>6</sup>	0-15	t <sub>6</sub>
SD <sup>0</sup>	15-30	t <sub>0</sub>
SD <sup>3</sup>	15-30	t <sub>3</sub>
SD <sup>6</sup>	15-30	t <sub>6</sub>

Sample	Depth (cm)	Time
SD <sup>0</sup>	0-15	t <sub>3</sub>
SD <sup>3</sup>	0-15	t <sub>3</sub>
SD <sup>6</sup>	0-15	t <sub>3</sub>
SD <sup>0</sup>	15-30	t <sub>3</sub>
SD <sup>3</sup>	15-30	t <sub>3</sub>
SD <sup>6</sup>	15-30	t <sub>3</sub>

Sample	Depth (cm)	Time
SD <sup>0</sup>	0-15	t <sub>6</sub>
SD <sup>3</sup>	0-15	t <sub>6</sub>
SD <sup>6</sup>	0-15	t <sub>6</sub>
SD <sup>0</sup>	15-30	t <sub>6</sub>
SD <sup>3</sup>	15-30	t <sub>6</sub>
SD <sup>6</sup>	15-30	t <sub>6</sub>

Sample	Depth (cm)	Time
LD <sup>0</sup>	0-15	t <sub>0</sub>
LD <sup>3</sup>	0-15	t <sub>3</sub>
LD <sup>6</sup>	0-15	t <sub>6</sub>
LD <sup>0</sup>	15-30	t <sub>0</sub>
LD <sup>3</sup>	15-30	t <sub>3</sub>
LD <sup>6</sup>	15-30	t <sub>6</sub>

Sample	Depth (cm)	Time
LD <sup>0</sup>	0-15	t <sub>3</sub>
LD <sup>3</sup>	0-15	t <sub>3</sub>
LD <sup>6</sup>	0-15	t <sub>3</sub>
LD <sup>0</sup>	15-30	t <sub>3</sub>
LD <sup>3</sup>	15-30	t <sub>3</sub>
LD <sup>6</sup>	15-30	t <sub>3</sub>

Sample	Depth (cm)	Time
LD <sup>0</sup>	0-15	t <sub>6</sub>
LD <sup>3</sup>	0-15	t <sub>6</sub>
LD <sup>6</sup>	0-15	t <sub>6</sub>
LD <sup>0</sup>	15-30	t <sub>6</sub>
LD <sup>3</sup>	15-30	t <sub>6</sub>
LD <sup>6</sup>	15-30	t <sub>6</sub>

Sample	Depth (cm)	Time
LD <sup>0</sup>	15-30	t <sub>6</sub>
LD <sup>3</sup>	15-30	t <sub>6</sub>
LD <sup>6</sup>	15-30	t <sub>6</sub>

amoA(B)	Conditional formatting of the whole block (without 16S)				
	nirK	nosZ	amoA(A)	16S	CT (the lower the more)
32.46	29.97	36.30	30.48	20.57	
34.71	29.27	36.24	31.89	19.73	
27.80	25.67	28.61	27.05	17.74	
32.83	30.45	34.92	31.83	20.63	
31.45	29.53	35.53	30.46	19.87	
32.53	29.09	34.38	30.09	19.65	

amoA(B)	Conditional formatting by gene (by column)				
	nirK	nosZ	amoA(A)	16S	CT (the lower the more)
29.47	28.85	32.75	29.94	20.08	
31.72	30.23	34.87	31.77	20.41	
28.80	26.19	31.69	27.35	18.00	
ND	34.43	ND	ND	21.67	
33.08	30.75	36.45	33.78	20.23	
35.70	29.70	37.55	34.31	19.00	

amoA(B)	Conditional formatting by gene (by column)				
	nirK	nosZ	amoA(A)	16S	CT (the lower the more)
35.59	32.90	36.47	33.80	23.86	
33.78	30.89	34.85	33.04	21.95	
33.78	30.14	33.23	31.03	21.45	
ND	32.29	35.59	32.32	22.97	
39.65	32.09	ND	36.56	22.26	
ND	31.37	33.04	ND	23.27	

amoA(B)	Conditional formatting by gene (by column)				
	nirK	nosZ	amoA(A)	16S	CT (the lower the more)
32.07	30.10	32.03	30.27	19.65	
29.19	27.18	29.26	28.09	18.53	
28.20	25.73	28.68	27.13	17.49	
34.00	28.97	33.26	31.36	18.77	
29.88	27.24	28.24	28.40	18.31	
21.33	21.73	20.70	22.91	15.09	

amoA(B)	Conditional formatting by gene (by column)				
	nirK	nosZ	amoA(A)	16S	CT (the lower the more)
30.09	27.84	29.21	29.90	18.91	
30.72	28.62	31.78	29.64	19.03	
26.60	24.51	26.53	26.37	17.62	
26.42	24.60	25.33	26.57	17.41	
24.11	22.89	22.75	24.47	15.93	
20.60	20.50	20.10	22.44	14.32	

amoA(B)	Conditional formatting by gene (by column)				
	nirK	nosZ	amoA(A)	16S	CT (the lower the more)
32.49	30.41	30.96	31.40	21.77	
28.36	26.55	27.41	28.21	19.47	
29.97	26.82	32.81	28.71	20.18	
36.23	30.42	39.24	33.36	20.78	
29.82	28.01	33.33	28.81	20.09	
30.05	27.47	30.00	29.64	21.22	

amoA(B)	Conditional formatting by gene (by column)				
	nirK	nosZ	amoA(A)	16S	CT (the lower the more)
29.31	28.02	30.79	28.98	19.54	
35.29	29.44	35.49	34.29	20.33	
27.47	26.41	27.99	28.02	18.24	
33.67	31.14	36.10	32.24	21.77	
33.90	28.89	33.71	30.55	20.06	
35.69	30.30	35.02	33.00	20.67	

amoA(B)	Conditional formatting by gene (by column)				
	nirK	nosZ	amoA(A)	16S	CT (the lower the more)
31.18	30.09	34.46	32.34	21.33	
32.63	28.63	38.08	32.58	19.65	
31.74	28.33	36.10	29.90	19.95	
32.63	31.70	34.84	31.92	21.81	
30.33	28.71	31.11	30.11	18.71	
27.15	26.88	30.51	29.45	17.90	

amoA(B)	Conditional formatting by gene (by column)				
	nirK	nosZ	amoA(A)	16S	CT (the lower the more)
32.27	30.88	32.55	31.82	22.12	
30.29	29.08	31.35	29.37	21.01	
34.18					