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Potential of on-site vermicomposting of sewage sludge in soil quality improvement

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ABSTRACT

A "living mulching system" made up of sludge and earthworms (*Eisenia fetida*) were tested in a laboratory experiment with the goal of improving the biochemical and chemico-physical properties of two different agricultural soils: a clay soil (Vicarello, Italy) and a sandy-loam soil (Murcia, Spain). A sludge-earthworms mixture was layered onto the soil surface and kept moistened for 4 mo. Biochemical and chemico-physical soil properties were compared in order to assess the efficiency of living mulch as a function of soil type. The experiment showed that soil improvement starts from the activation of the microbiological processes through a gradual modification of the nutritive conditions occurring within the living mulch system due to earthworm activity. Also the physical properties of both soils were improved by increasing the total cracked area, mainly due to the formation of small (<500 μm) and small-medium cracks (500–1000 μm), which represent the typical micro-habitat for soil microorganisms. In fact, in these soils a positive correlation was found between dehydrogenase enzyme activity, total shrinkage area and small-medium size cracks. The improvement of soil structure is of great agronomic relevance in that good physical properties favour water retention, oxygen diffusion and nutrient availability, which combine to improve soil quality and fertility.

Keywords: Sewage sludge; Mulching; Earthworms; Soil shrinkage; Metabolic activity

1. Introduction

Soil erosion, nutrient runoff, and loss of organic matter are processes that can cause soil degradation, reducing its productivity and capacity to regulate the ecosystem [1]. A strategy for recovering degraded soils consists in incorporating organic matter from carbon rich materials.

The potential of organic material application in the improvement of soil structure, increasing organic substrate content and in establishing patterns of nutrient cycles similar to those observed in natural ecosystems has been widely recognized [2,3].

Recently, increasing attention has been paid to the recycling of organic wastes in soil, especially in semi-arid and degraded lands [4]. Different works show that the addition of a variety of organic waste products, such as sewage sludge, cattle manure, municipal soil waste, and other materials, can protect the soil by improving its structure [5,6,7] and by encouraging the formation of macroaggregates [8].

The surface application (mulching) of sewage sludge is also considered to be an efficient and inexpensive

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practice in order to maintain or improve soil microbial activity and growth, thus enhancing biogeochemical nutrient cycles [3,9].

It is generally assumed that differences in the stability of the organic material added influence the variation in soil carbon storage and fixation [10].

For example, composted sludge fixes more carbon in the soil than fresh sludge, since the organic matter contained in compost is more stable than that in fresh wastes due to the composting stabilization process [11].

In order to enhance the incorporation of organic matter with soil, scientific investigations have established the viability of using earthworms [12–14]. Earthworms ingest large amounts of organic matter and expel it as a partially stabilized product (cast). They accelerate the composting process, control potential environmental risks, and improve the structure and chemico-physical and biological properties of the soil [15].

In this work a "living mulching system" (on-site vermicomposting), made up of pre-conditioned sludge stratified on the soil surface with earthworms (*Eisenia fetida*), was tested in a laboratory experiment with the aim of improving the biological and chemico-physical properties of two different agricultural soils: a clay soil (Vicarello, Italy) and a sandy-loam soil (Murcia, Spain).

2. Materials

2.1. Soils

Two soils, Vicarello (Central Italy) and Murcia (South east Spain), were used to study the changes in the soils' chemico-physical and biochemical characteristics.

Each soil was sampled by taking five sub-samples (150 cm^3 soil cores) from the top layer (15 cm), randomly collected and thoroughly mixed to obtain a composite and representative sample of each area.

The Vicarello soil, with a clay texture, was a compacted soil with low oxygen flow, while the Murcia soil, with a sandy-loam texture, has been defined as a "poor soil" [16] having a low content of organic matter, nitrogen and microbial activity.

The two soils were also from different climatic zones: Vicarello is in a temperate-rainy area with average annual temperature of 13 °C and average rainfall of 700 mm, while Murcia is in a pre-desertic area with an average annual temperature of 20 °C and average rainfall of 250 mm.

The characteristics of both soils are reported in Table 1.

Table 1
Chemical and biochemical characteristics of the two agricultural soils used in the experiment

Parameters	Vicarello soil	Murcia soil
Clay (%)	46	12
Silt (%)	34	22
Sand (%)	20	66
Texture (USDA classification)	Clay	Sandy-loam
pH	7.81	8.70
E.C. (dS m^{-1})	0.205	0.225
NH_4–N (mg kg^{-1})	2.89	2.36
TN (g kg^{-1})	3.09	0.405
TOC (g kg^{-1})	11.3	5.87
C/N	3.66	14.5
NO_3–N (mg kg^{-1})	7.41	3.58
SO_4–S (mg kg^{-1})	54.7	47.9
Dehydrogenase activity (μgINTF g^{-1} h^{-1})	1.80	0.836

E.C., Electrical Conductivity; TN, Total Nitrogen; TOC, Total Organic Carbon.
Coefficient of variation of the three replicates ranged from 2 to 12%.

2.2. Sludge

The aerobic sludge was collected from an activated-sludge plant treating municipal wastewater, while the anaerobic sludge came from a digester which treats sludges obtained from municipal wastewater (10%) and paper-mill wastewaters (90%). The characteristics of both fresh aerobic and anaerobic sludges are reported in Table 2.

A preliminary study on earthworm (*Eisenia fetida*) survival, using different mixtures of aerobic-anaerobic sludge, showed that the 1:1 aerobic-anaerobic sludge mixture resulted in good worm survival and health [17]. In order to decrease the content of biotoxic compounds, before the addition of earthworms, the mixture was also daily aerated for 15 d (pre-conditioning). The characteristics of the 1:1 anaerobic-aerobic sludge (mixture) are reported in Table 2.

2.3. Soil treatment

One kilogram (dry weight) of the Murcia and Vicarello soils was placed in plastic containers (microcosms). All containers were covered with perforated lids and maintained under a controlled temperature (22 ± 2 °C) and humidity (60% of soil field capacity). 0.5 kg (as wet weight) of sludge mixture enriched with ten adult worms (*Eisenia fetida*) was spread on the soil surface, corresponding to a layer of about 3 cm. The experiments were carried out in triplicate. After four mo., the sludge was removed from the soil surface and soil samples were collected for analyses. One soil sample (made up by mixing five sub-samples) per microcosm was collected, sieved to < 2 mm, and stored at room temperature before the analyses.

Table 2
Chemical and biochemical characteristics of the pre-stabilized aerobic and anaerobic sludges and of the mixture used in the on-site vermicomposting experiment

Parameters	Aerobic sludge	Anaerobic sludge	Anaerobic-aerobic sludge mixture
pH	7.77	7.29	7.50
E.C. (dS m^{-1})	0.474	0.975	1.62
NH_4–N (mg kg^{-1})	180	209	122
TN (g kg^{-1})	41.4	8.38	19.5
TOC (g kg^{-1})	232	203	215
C/N	5.60	24.2	16.1
TP (g kg^{-1})	11.9	2.52	2.81
Dehydrogenase activity (µgINTF g^{-1} h^{-1})	650	805	768

E.C., Electrical Conductivity; TN, Total Nitrogen; TOC, Total Organic Carbon; TP, Total Phosphorus.
Coefficient of variation of the three replicates ranged from 2 to 12.

3. Methods

3.1. Chemical parameters

Electrical conductivity (EC) and pH were measured in 1:10 (w/v) aqueous solution. Water-soluble carbon (WSC) was extracted from soil with distilled water at a 1:10 solid:liquid ratio by mechanical shaking at 60 °C for 1 h. The C content of WSC was determined by dichromate oxidation using the Yeomans and Bremner (1989) method [18]. Total organic carbon (TOC) and total nitrogen (TN) contents were determined by dry combustion in a RC-412 multiphase carbon and a FP-528 protein/nitrogen determinator respectively (LECO Corporation). NH_4^+ was measured with an ammonia-selective electrode (ORION 95-12) and NO_3–N was measured with a DIONEX chromatograph.

3.2. Biological parameters

The enzyme activities were determined using 1 g of soil. BAA-hydrolysing protease activity was tested using 0.03 M N-α-benzoyl-L-argininamide (BAA) as substrate [19]. The ammonium released by the hydrolytic reaction was measured by an ammonium selective electrode (ORION 95-12).

Dehydrogenase activity was measured using 0.4% 2-ρ-iodophenyl-3 ρ-nitrophenyl-5-tetrazolium chloride (INT) as substrate; the product iodonitrotetrazolium formazan (INTF) of the reaction was measured with a spectrophotometer at 490 nm [20].

A phyto-test with *Lepidium sativum* was carried out following the method reported by Masciandaro et al. (1997) [21]. One hundred seeds were tested in a Petri-dish using 50 g each of the Vicarello and Murcia soils after four mo. from the beginning of the experiment. The germination and growth index was calculated after 4–5 d of growth, and was expressed by the following formula: GI% = P(T/C), where P is the mean percentage of seed germination, with respect to the mean value of the control assumed to be 100%; T and C are the lengths of shoot in the treatment and in the control, respectively.

3.3. Physical parameters

Particle size analysis was calculated by a pipette procedure [22]. The composition was defined by the USDA textural triangle.

Soil shrinkage was determined using the method reported by Pagliai et al. (1980) [23]; cracking measurements were carried out optically with a Quantimet 570 apparatus using an electro-optical procedure for image processing and analysis.

3.4. Statistical analysis

Differences between treated and control soils (treatment effect) were tested for both the Vicarello and Murcia soils by analysis of variance (one-way ANOVA). The means were compared by using least significant differences calculated at $P < 0.05$ (Fisher's Test) (STATISTICA 6.0 software). A correlation matrix of the data was also calculated in order to determine the relationship between the parameters. The significant levels reported ($p < 0.05$) are based on the Student's distribution.

The results, expressed on a dry weight basis, were the average of three replicates for each treatment. The Coefficient of variation (which is a measurement of the deviation of the three replicates) was calculated using the formula: CV = (D/M)*100, where D is the standard deviation and M is the estimated sample mean.

4. Results and discussion

Sludge and earthworm (*Eisenia fetida*) application, in both Vicarello and Murcia soils, brought about a significant increase ($P < 0.05$) in TOC and TN content with respect to the control soils (Table 3). This increase was particularly evident in the nutrient-poor Murcia soil (sampled in semi-arid area) compared with the Vicarello soil (sampled in a Mediterranean humid-temperate area), which had a higher content of native soil organic matter. The WSC fraction, an important pool of soil organic matter turnover in soils, because it acts as a readily decomposable substrate for soil micro-organisms and as a short-term reservoir of nutrients [24], increased in both treated soils with respect to the control soils (Table 3). This was attributable to several factors, such as the mineralization of available organic matter, leaching, the direct transport of sludge into the soil by worms and the release of their casts.

The presence of earthworms determined also a significant increase ($P<0.05$) in NO_3–N concentration, as already found in other researches [25,26]. Earthworm holes are, in fact, important pathways for oxygen, water, and solute transport in field soil, thus increasing the nitrification. The modification of the nutritive conditions occurring within the living mulch system determined the activation of the microbiological processes of the resident microbial populations. In fact, in treated soils, dehydrogenase activity, an enzyme indicating the quantity and the metabolic activity of all microbial species resulted higher than 40% with respect to the control soils (Table 4). The positive effects are presumably due to the high nutrient and organic matter content of the organic waste materials [27] and to the incorporation in the soils of an enzymatically active organic cast due to earthworm metabolism. In fact, a positive correlation was found among dehydrogenase activity, NO_3–N and WSC (Table

Table 3
Chemical parameters after four mo. from the beginning of the experiment

Parameters	Vicarello soil		Murcia soil	
	Control	Treated	Control	Treated
pH	7.81a	7.73a	8.8a	7.95b
E.C. (dS m^{-1})	0.23b	0.45a	0.20b	0.37a
NH4–N (mg kg^{-1})	3.0b	4.1a	3.5a	3.0b
TN (g kg^{-1})	2.10b	2.74a	0.25b	2.01a
TOC (g kg^{-1})	11.7b	14.0a	2.70b	7.45a
C/N	5.57	5.10	10.8	3.71
NO_3–N (mg kg^{-1})	30b	350a	45b	130a
WSC (mg kg^{-1})	180b	523a	283b	363a

E.C., Electrical Conductivity; TN, Total Nitrogen; TOC, Total Organic Carbon; WSC, Water Soluble Carbon.Coefficient of variation of the three replicates ranged from 2 to 10%. For each soil, different letters indicate statistically different values between treated and control soil ($P<0.05$).

Table 4
Biological parameters after four mo. from the beginning of the experiment

Parameters	Vicarello soil		Murcia soil	
	Control	Treated	Control	Treated
BAA-prot. (μgNH$_3$ g^{-1} h^{-1})	11.39b	14.14a	3.45b	12.36a
DH-ase (μgINTF g^{-1} h^{-1})	10.19b	16.64a	6.60b	9.67a
Metabolic potential (μg INTF mgC^{-1} h^{-1})	36.4b	39.4a	23.3b	26.6a
GI (%)	100b	823a	100b	525a

BAA-prot, protease activity; DH-ase, dehydrogenase activity; GI%, Germination and growth Index.
Coefficient of variation of the three replicates ranged from 2 to 10%. For each site, different letters indicate statistically different values between the treated and control soil ($P<0.05$).

Table 5
Correlation matrix between chemical, biochemical and physical parameters of Vicarello and Murcia soils after on-site vermicomposting experiment

	NH$_3$	TN	TOC	NO$_3$	WSC	BAA	DH-ase	GI	TC	<500	500<–1000
NH$_3$	1.00										
TN	0.16	1.00									
TOC	0.31	0.92*	1.00								
NO$_3$	0.80	0.65	0.62	1.00							
WSC	0.78	0.47	0.37	0.96*	1.00						
BAA	0.10	0.99*	0.88	0.63	0.47	1.00					
DH-ase	0.66	0.84	0.88	0.91*	0.91*	0.81	1.00				
GI	0.61	0.71	0.57	0.95*	0.95*	0.72	0.84	1.00			
TC	0.38	0.93*	0.99*	0.70	0.47	0.90*	0.93*	0.65	1.00		
<500	−0.20	−0.10	−0.47	0.07	0.32	−0.01	−0.24	0.31	−0.41	1.00	
500–1000	0.48	0.72	0.93*	0.56	0.30	0.64	0.91*	0.41	0.92*	−0.72	1.00
>1000	−0.39	0.29	0.49	−0.36	−0.62	0.24	0.05	−0.44	0.39	−0.78	0.54

TN, Total Nitrogen; TOC, Total Organic Carbon; WSC, Water Soluble Carbon; BAA, protease activity; DH-ase, dehydrogenase activity; GI, Germination and growth Index; TC, Total Cracks; <500, cracks <500 μm; 500–1000, 500 μm<cracks<1000 μm; >1000, cracks>1000 μm.
*Correlation coefficients statistically significant at $p < 0.05$.

5). The dehydrogenase activity, especially when referred to the energetic and immediately available C substrate, such as water soluble C (WSC), gives an idea of the metabolic potentiality of soil rehabilitation [28]. This metabolic potential, calculated as the ratio between the size and activity of the viable microbial community (dehydrogenase activity) and the sources of energy for microorganisms (water soluble carbon concentration), was higher in the treated living mulch than in the control soils (Table 4).

Improvement in soil metabolism involved also the N cycle, as expected by the increase in nitrate concentration. As a consequence, BAA-protease activity, an enzyme related to N turnover, showed higher values in the treated than in the control soils (Table 4). In fact, a positive correlation was found between BAA-protease activity and TN (Table 5).

Tests of plant germination and growth carried out on the soils after the treatments (Table 4) showed that no phyto-toxic substances were introduced into the soil by the earthworm movement and activity. The germination and growth index (GI%) was higher in both the treated soils with respect to the control soils, suggesting that the sludge application promoted the chemico-nutritional conditions which support plant growth and root development, as suggested by the correlation among GI, NO_3–N and WSC (Table 5). The higher GI in treated soils was also probably favoured by the modification of the soil physical structure.

The physical effect was evaluated through measurement of surface shrinkage of soils at the end of the experiment (Fig. 1).

For each site, different letters indicate statistically different values between treated and control soil ($P < 0.05$).

The physical properties of the Vicarello and Murcia soils were improved by increasing the total cracked area, mainly due to the formation of small and small-medium cracks (Fig. 1) and as also suggested by the correlation between total cracks (TC) and 500–1000 μm cracks (Table 5). The increase in small size cracks (< 500 μm) corresponding to micro-porosity, was considered important for maintaining optimum conditions of humidity for plant and microorganisms, whereas the increase in small-medium size cracks (500–1000 μm), representing the micro-habitat for soil microorganisms, was very important for microbiological and biochemical processes [23]. In fact, in these soils a positive correlation was found among dehydrogenase enzyme activity, TC and 500–1000 μm cracks and between BAA-protease activity and TC (Table 5). The improvement of the soil structure is of great agronomic relevance because good physical properties favour water retention, oxygen diffusion, and nutrient availability, which can all improve soil quality and fertility [29].

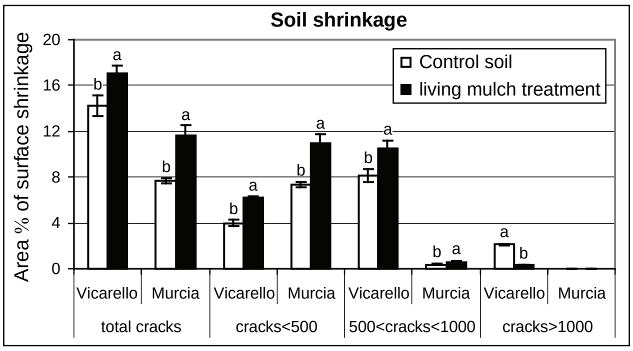

Fig. 1. Area percentage of total surface shrinkage and three size classes of cracks in Vicarello and Murcia soils after four mo. from the beginning of the experiment.

5. Conclusions

The surface application of sewage sludges and earthworms (on-site vermicomposting) to a clayey compacted soil (Vicarello soil) and to a sandy-loam nutrient-poor soil (Murcia soil), positively affected the chemico-physical and biochemical properties of both soils.

The modification of the nutritive conditions occurring within the on-site vermicomposting system determined the activation of the microbiological processes in the Vicarello and Murcia soils. The increase of metabolic potential, calculated by the ratio between dehydrogenase activity and WSC, suggested that it was possible to rehabilitate the two different agro-pedological soil ecosystems.

The direct sludge incorporation carried out by earthworm activity also brought about the improvement of the physical soil properties by increasing the small and small-medium cracked areas, which are considered important for microbiological and biochemical processes.

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