Assessing the microbiological, biochemical, soil-physical and hydrological effects of amelioration of degraded soils

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Abstract The way of improving degraded soils fertility is to add "young" exogenous organic matter to stimulate the life of the microorganisms existing in the soil. In this study, the microbiological, biochemical, soil-physical and hydrological effects of the addition of a municipal solid waste compost to a degraded soil in El Campello, SE Spain were evaluated in a field experiment. Soil samples from experimental plots were analyzed 6 and 18 months after soil amendment. In both sampling time treated plots showed significantly higher microbial biomass carbon and dehydrogenase activity values than control, indicating that soil microbial population's development and activity were stimulated by compost addition, this effect being not ephemeral but lasting in the time. Soil urease activity was not affected by compost addition while protease hydrolysing N-α-benzoil-L-argininamide (BAA) activity was strongly stimulated by the incorporation of compost into the soils. Phosphatase and β-glucosidase activities were also stimulated by the organic amendment, this stimulation being particularly noticeable 18 months after the compost addition. Nevertheless, this increase in soil microbial populations and activity did not result in an increase in soil aggregation and hydrological parameters. This can be due to the high content of carbonates and Ca^{2+} ions in these calcareous soils, that lead to an initially high content of water-stable macroaggregates.

Key words: *degraded soil, amelioration, sewage sludge compost*

Introduction

In the Mediterranean region of SE Spain both intensive agricultural practices and the agricultural use of marginal lands, which are very susceptible to environmental degradation and unsuitable for crop production, have led to a loss of soil quality and fertility and the subsequent abandonment of the land and soil degradation. The Mediterranean region contains, therefore, large areas of low quality soils with little plant cover, which only adds to their degree of degradation. One of the key factors is indeed, the **low organic matter content of the soils in the region**, and there is no need to reiterate the close relationship that exists between this parameters and soil fertility.

In this Mediterranean region soil degradation is aggravated by the adverse environmental and climatic factors existing in the area such as:

- *Climate.* Long periods of drought interrupted by heavy, occasionally torrential, rainfall, which can be the cause of severe erosion. Summers temperatures are very high with an annual mean temperature of 17–18.5°C. Constant exposure to sun through the year favours evapotranspiration and the risk of soil salinization.
- *Relief.* The sloping landscape favours erosion, the degree of which will depend on rainfall intensity, plant cover, lithology, the nature of the soil and the use to which it is put.
- *Lithological substrate.* The most widely represented lithological substrates in SE Spain are carbonated rocks, quaternary sediments and loams. These characteristics give rise to soils which are easily eroded and which are open to others negative processes such as biological and physical degradation and/or salinization.

- *Plant cover.* The extent of soil degradation is an accurate reflection of the state of its plant cover. Degradation begins with the destruction of its plant cover and only in few parts of the Mediterranean region is the natural or potential vegetation preserved.

The way of improving the fertility of degraded soils and particularly of improving its microbial activity is to add "young" exogenous organic matter. By the latter term we mean that the amendment must contribute to providing labile organic matter in sufficient quantities to stimulate the life of the microorganisms that might exist in the soil (Ros et al. 2001, 2003). As increased total organic carbon content is, with a few exceptions, positively correlated with increased aggregate stability (Tisdall, Oades, 1982), the addition of municipal solid waste compost should improve both the retention and hydraulic characteristics of the studied soil.

The aim of this study was to assess the microbiological, biochemical, soil-physical and hydrological effects of addition of municipal solid waste compost to a degraded soil in El Campello.

Material and methods

For this study, six 2 m x 2 m plots were set out in a degraded area situated in El Campello (Alicante), South-east Spain. The degradation of this soil is due to previous land uses such as agricultural and latter abandonment. The recovery of natural vegetation is slow, mainly due to the semiarid conditions (the annual rainfall lower than 300 mm, irregularly distributed as torrential rainfall events), and soil is far from climax conditions. Three of these plots, randomly distributed, were amended with 60 t/ha (wet weight) of the sewage sludge compost in March 2004 ("treated plots"), and the other three remained non-amended ("control plots"). The soil was Ari-Anthropic Regosols (WRB, 1994) and had a clay loam texture (Soil Survey Staff, 1998). The main characteristics of the non-amended soil are shown in Table 1, and those of the compost used are shown in Table 2.

Table 1 Characteristics of the studied (non-amended) soil (dry weight)

Soil parameter	Value
Sand (%)	26.7
Silt (%)	42.0
Clay (%)	31.3
CO_3^- equivalent (%)	48.8
pH(H_2O) (1:2.5 w/v)	8.14
Electrical conductivity (μS cm^{-1})	300
Total organic carbon (%)	1.38
Available P (g kg^{-1})	0.008
Available Na (g kg^{-1})	1.4
Available K (g kg^{-1})	2.6
Available Ca (g kg^{-1})	34.9
Available Mg (g kg^{-1})	1.6

Table 2 Characteristics of the sewage sludge compost used (dry weight)

Parameter	Value
Organic matter (%)	44.9
pH	6.9
Electrical conductivity (μS cm^{-1})	4250
N-Kjeldahl (g kg^{-1})	24.9
Total Phosphorus (g kg^{-1})	17.3
Total Na (g kg^{-1})	14.5
Total K (g kg^{-1})	22.0
Total Ca (g kg^{-1})	289
Total Mg (g kg^{-1})	57
Total Fe (mg kg^{-1})	7388
Total Mn (mg kg^{-1})	303
Total Cu (mg kg^{-1})	137
Total Zn (mg kg^{-1})	435

The plots were sampled twice for analysis: first six months after soil amendment (in September 2004), and then one year after the first sampling (September 2005). The soil samples were collected from the top layer (0–15 cm) with 5–6 replicates. Taken into account the extremely low amount of rainfall, all the plots (control and treated) were irrigated every 10th day using 4 litres m^{-2} (4 mm) in June, July and August 2005.

The dilution technique (Kopčanová et al., 1990) in the 1:10000 (KTJ) ratio in three repetitions was used to determine the species diversity of the soil microscopic fungi. The microscopic fungi were cultivated in the Czapek-Dox agar, Sabourad agar, and Jensen agar, and their genera and species were determined according to macro- and micromorfological structures (Fassatiová, 1979; Tomilin, 1979; Domsch et al., 1980; Samson et al., 1981). It should be noted that fungal hyphae bind microaggregates of soil (<0.25 mm diameter) into stable macroaggregates (>0.25 mm) (Tisdall, 1991).

The microbial biomass carbon content and enzymatic activities (dehydrogenase, urease, protease, phosphatase, and β-glucosidase activity) were used as biological and biochemical indicators to assess the amelioration of the studied degraded soil with the organic amendment.

Total organic carbon (TOC) was determined by the Yeoman and Bremner (1989) method, and the microbial biomass carbon content was estimated by using the fumigation-extraction method (Vance et al., 1987).

The enzymes called dehydrogenases take part in dehydrogenation processes, by means of which the biological oxidation of organic compounds occurs. The dehydrogenase activity was estimated by a modification of the method reported by Von Mersi and Schinner (1991), (Garcia et al., 1997).

Urease catalyses the hydrolysis of urea or ureic type substrates to give carbon dioxide and ammonia as reaction products. The urease activity was estimated by the buffered method described by Kandeler & Gerber (1988).

The protease, which hydrolyses N-α-benzoil-L-argininamide (protease-BAA) is involved in hydrolysis of proteins to ammonium. The protease activity was estimated by the Nannipieri et al. (1980) method.

Phosphatases are inhibited by inorganic phosphorus, the final product of their enzymatic reaction. This is due to a feedback inhibition so that phosphatase is only synthesised if there are deficiencies in available phosphorus (Nannipieri et al., 1979). The phosphatase activity was estimated by the Tabatabai and Bremner (1969) method.

β-glucosidase is a hydrolase which intervenes in the carbon cycle, acting specifically in the hydrolysis of the β-glucosidase bonds of the long carbohydrate chains. The hydrolysis of these substrates plays an important role in the microorganisms attainment of energy from the soil (Eivazi, Zakaria, 1993). β-glucosidase was determined following the method of Eivazi and Tabatabai (1987).

Soil pH and electrical conductivity were measured by standard electrode methods. Bulk density was determined on 100 cm^3 soil samples after drying to constant weight at 105°C. Soil aggregate stability was determined by the rainfall simulator method used by Roldan et al. (1994). Dry aggregation is an important phase of structure genesis in arid and semiarid soils, as it determines the resistance of soil to wind erosion. Dry stability measures the strength of aggregates subjected to fracture and abrasion. The measurements of stability to water are generally used to estimate structural changes due to cultivation, because water is the main agent of aggregate breakdown in agricultural soils. Mean weight diameter (MWD) of stable macroaggregates was determined from content of their fractions after dry sieving and MWD of water-stable aggregates wet sieving by the Bakshajev method (Hraško et al., 1962). The sieve meshes were 3.0, 1.0, 0.5, and 0.25 mm, and calculation was based on a summation of the weight of different aggregate-size fractions compared with the total soil weight used.

Water retention capacity of the studied clay loam soil was calculated from the retention curve as the soil water content at soil moisture potential $h_w = -400$ cm (Kutilek, Nielsen, 1994). Saturated hydraulic conductivity K_s was estimated in field conditions using a standard double ring ponded (pressure head $h_0 = +2$ cm) infiltration experiment. The double-ring infiltrometer had the inner-ring diameter of 10 cm, the buffer ring diameter of 20 cm, and the length of 20 cm. Saturated hydraulic conductivity K_s was also estimated in the laboratory on 100 cm^3 samples by the falling head method (Kutilek, Nielsen, 1994).

Results and discussion

Microbiological and biochemical measurements

Few months after soil amendment spontaneous vegetation appeared in the amended soil, which was denser the second year of the experiment. Vegetation developed in the unamended soils was much scarcer than that of amended soils, particularly at the second year.

In spite of the fact that part of the organic matter added to the soil with the organic amendment must have been mineralised during the time elapsed from compost addition to the plot sampling, amended plots showed higher values of TOC than unamended plots at the two sampling times (Table 3). This fact can be due to both the non decomposable fraction of the organic matter added with the compost, and the contribution of root exudates and plant debris from the more dense vegetal cover developed in the amended plots.

MBC can be used more effectively than the total TOC content as an indicator of soil quality changes since it responds more rapidly and with a higher degree of sensitivity to any soil disturbance (Garcia et al., 2000). Six months after compost addition, treated plots showed significantly higher MBC values than control ones (Table 3), suggesting that, even under the adverse climatic conditions following the soil amendment (very dry conditions), compost addition had stimulated the development of soil microbial populations, which is of paramount importance for ecosystem functioning. Compost addition also contributes to soil MBC with the microbial populations it contains.

Eighteen months after the organic amendment, MBC content in the treated soils remained higher than in the control soil, indicating that the stimulating effect of the organic amendment on the microbial population development is durable. Differences in genera and species of soil microscopic fungi were also observed between amended and unamended soils six months after soil amendment (Table 4).

The overall metabolic microbial activity was evaluated by measuring dehydrogenase activity (Garcia et al., 1997). Dehydrogenase activity was significantly higher in treated than in control soils, suggesting that the organic amendment increased soil microbial activity (Table 3). This effect was maintained 18 months after compost addition, indicating that the microbial soil quality improvement obtained was not ephemeral but lasting in the time.

The degradation of the organic matter is largely due to hydrolitic processes. For this reason, it seemed interesting to determine some hydrolases, which, despite being specific enzymes (Nannipieri et al., 1990), may help to explain the cycle of such important elements as N (ureases and proteases), P (phosphatases) and C (β-glucosidases).

Table 3 Differences in microbiological, biochemical, soil-physical and hydrological parameters between treated and control plots in El Campello

Soil characteristics	September 2004		September 2005	
	Control plots	Treated plots	Control plots	Treated plots
Total organic carbon content (%)	1.38 ± 0.09*	1.87 ± 0.15*	1.43 ± 0.11*	2.65 ± 0.03*
Microbial biomass carbon content (mg kg^{-1} soil)	256.56 ± 25.97*	327 ± 11.93*	130.64 ± 28.99*	269.78 ± 27.9*
Dehydrogenase activity (μg INTF g $soil^{-1}$ h^{-1})	2.84 ± 0.38*	4.52 ± 0.50*	3.18 ± 0.55*	5.28 ± 0.39*
Urease activity (μmol N-NH_4^+ g $soil^{-1}$ h^{-1})	1.32 ± 0.10	1.05 ± 0.16	2.67 ± 0.32	2.83 ± 0.08
Protease activity (μmol N-NH_4^+ g $soil^{-1}$ h^{-1})	1.10 ± 0.05*	1.86 ± 0.31*	2.21 ± 0.10*	3.96 ± 0.21*
Phosphatase activity (μmol PNP g $soil^{-1}$ h^{-1})	2.06 ± 0.12*	2.34 ± 0.22*	3.71 ± 0.72*	4.44 ± 0.84*
β-Glucosidase activity activity (μmol PNP g $soil^{-1}$ h^{-1})	1.62 ± 0.18	1.61 ± 0.29	2.20 ± 0.43*	2.84 ± 0.22*
pH	8.35 ± 0.02*	8.16 ± 0.10*	8.11 ± 0.04	7.97 ± 0.13
Electrical conductivity (μS cm^{-1})	232 ± 12	271 ± 27	453 ± 82	518 ± 161
Bulk density (g cm^{-3})	0.96 ± 0.01*	0.92 ± 0.02*	0.95 ± 0.01*	0.91 ± 0.01*
Aggregate stability (%)	48 ± 2	49 ± 3	42 ± 7	43 ± 6
Water-stable macroaggregates (%)	82.8 ± 5.0	72.1 ± 4.6	84.1 ± 2.2	78.6 ± 2.7
Mean weight diameter (mm) of macroaggregates after dry sieving	1.061 ± 0.116	1.049 ± 0.057	1.609 ± 0.084	1.529 ± 0.214
Mean weight diameter (mm) of macroaggregates after wet sieving	1.243 ± 0.172	0.990 ± 0.057	1.323 ± 0.174	1.260 ± 0.201
Retention capacity of soil (cm^3 cm^{-3})	0.2384 ± 0.0332	0.2484 ± 0.0141	0.2796 ± 0.0091	0.2639 ± 0.0159
Saturated hydraulic conductivity (laboratory) (m s^{-1})	(2.77 ± 0.99) x 10^{-5}	(1.05 ± 1.09) x 10^{-5}	(1.11 ± 1.22) x 10^{-5}	(1.25 ± 0.70) x 10^{-5}
Saturated hydraulic conductivity (field) (m s^{-1})	(6.93 ± 2.09) x 10^{-5}	(6.26 ± 1.94) x 10^{-5}	(9.26 ± 1.99) x 10^{-5}	(8.64 ± 2.37) x 10^{-5}

*: significant differences at $P \leq 0.05$.

Urease hydrolyses urea-type substrates to CO_2 and ammonium, and protease BAA acts in the hydrolysis of proteins to NH_4^+ using simple peptides as substrates. Soil urease activity was not affected by compost addition (Table 3). This suggests that the added compost was poor in substrates type urea and that the formation of this substrate with time is not stimulated by compost addition. Conversely, protease BAA activity was strongly stimulated by the incorporation of compost. Differences in protease activity between the treated and control soils were significantly greater 18 months after the organic amendment, which matches with the increase in microbial activity observed in these samples, as measured by dehydrogenase activity, which can be explained by the higher amount of carbon sources entering in the system through plant remains and root exudates in the treated plots with respect to the control plots. Data from urease and protease BBA activity show that the functioning of the N cycle has been modified by the addition of the organic amendment (Nannipieri et al., 1990). Phosphatase catalyses the hydrolysis of P organic compounds to inorganic P forms available to plants. The activity of this enzyme was stimulated by the organic amendment. This stimulation was particularly noticeable 18 months after the compost addition (Table 3). β-glucosidase catalyses the hydrolysis of carbobydrate β-glucoside bonds contributing to the release of energy for soil microbial activity (Eivazi, Zakaria, 1993). Six months after soil amendment, the treated and control soils showed similar values of β-glucosidase activity. However, 18 months after the amendment the synthesis of this enzyme was stimulated in the amended plots (Table 3). The addition of compost favoured the apparition of a denser vegetal cover, but due probably to the adverse climatic conditions existing during the first six months of the experiment, differences in plant cover density were not great enough to establish differences in β-glucosidase activity stimulation. After 18 months differences in plant cover were greater and the higher amount of root exudates and plant debris existing in the treated plots contributed to stimulate the synthesis of this enzyme.

Table 4 The species of soil microscopic fungi determined in treated and control plots in El Campello in six months after soil amendment (September 2004)

Microfungi species	Control plots	Treated plots
Actinomucor elegans	+	+
Alternaria sp.	+	
Alternaria alternata		+
Alternaria tenuissima	+	
Aspergillus sp	+	+
Aspergillus flavus	+	+
Aspergillus fumigatus	+	+
Aspergillus terreus	+	
Aureobasidium pullulans	+	
Chaetomium sp.	+	+
Eurotium herbariorum		+
Fusarium graminearum		+
Fusarium culmorum	+	
Fusarium sporotrichioides		+
Humicola sp.		+
Humicola fuscoatra		+
Humicola grisea		+
Mortierella sp.	+	+
Penicillium sp.	+	+
Penicillium expansum	+	+
Penicillium frequentans	+	+
Rhizous arrhizus	+	+
Scopulariopsis brevicaulis	+	+
Scopulariopsis fusca	+	
Stachybotrys elegans	+	+
Stachybotrys charatarum		+
Mycelia sterilia	+	+

Physical and chemical parameters

Slight differences in soil pH were found at the first sampling, with lower values in treated plots (Table 3). However, no significant differences in EC were observed between amended and unamended soils, suggesting that the first spring rainfall events lixiviated the soluble salts incorporated with the compost. EC values were higher at the second than at the first sampling, probably due to salt solubilisation by the irrigation treatments applied during summer.

Bulk density decreased slightly with compost addition, which is in agreement with previous findings (Garcia-Orenes et al., 2005), indicating a slight improvement of soil structure, which can be a kind of dilution effect due to addition of OM to mineral soil. However, no statistical differences in aggregate stability were found between amended and unamended soils (Table 3). Similar results were obtained by Zaujec, Šimanský (2006).

It is well-known that organic matter enhances soil microbial activity that transforms the newly added organic matter into polysaccharides and long chain aliphatic compounds capable of binding and stabilizing aggregates (Graber et al., 2006). But in the case of the studied calcareous soil with high content of soil inorganic carbon and Ca^{2+} cations, which play dominant role in aggregation processes, the addition of organic matter had less effect on aggregation processes due to the initial high content of water-stable macroaggregates (Boix-Fayos et al., 2001; Dimoyiannis et al., 1998).

Saturated hydraulic conductivity and retention capacity of the studied soil were not affected by compost addition (Table 3). This could result from the small effect of compost addition on aggregation processes in this particular soil, due to its high initial content of water-stable macroaggregates. An increase in saturated hydraulic conductivity could be expected after formation of the surface-vented macropores by vegetation and soil animals (Kodešová et al., 2006).

Conclusions

Application of the sewage sludge compost to a degraded soil resulted in an increase in total organic carbon, microbial biomass carbon and microbial activity, but it did not bring an expected increase in the water-stable macroaggregates due to an initially high content of water-stable macroaggregates.

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