EFFECT OF LONG TERM ADDITION OF COMPOSTS AND POULTRY MANURE ON SOIL QUALITY OF CITRUS ORCHARDS IN SOUTHERN ITALY[1].

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ABSTRACT

A six year study was conducted in an organically managed orange orchard located in Sicily (Southern Italy) to assess the effect of composts and organic fertilisers utilisation on soil quality. Adopting a randomised block experimental design with three replicates, four treatments were carried out. In treatments 1 and 2, two different composts (C1 from distillery by-products and C2 from livestock waste) were applied. The plots of treatment 3 were fertilised using dried poultry manure. The control treatment was fertilised by mineral/synthetic fertilisers. In order to verify the hypothesis that composts and organic fertilisers improve soil fertility, soil quality was evaluated by selecting dynamic soil parameters, as indicators linked to C and N cycles.

Total organic C, total N, C/N ratio, humified fraction, isoelectric focusing (IEF) of extracted organic matter, microbial biomass C, potentially mineralisable N under anaerobic conditions, potentially mineralisable C, C mineralisation quotient and metabolic quotient were determined for each sample. Moreover, the Community Level Physiological Profile (by Biolog® technique) was defined, calculating derived functional biodiversity and versatility indexes.

Parameters related to IEF and potentially mineralisable C showed significant differences among the treatments. Moreover, total C, total N and humification parameters tended to increase, while no differences were observed in biodiversity indexes. On this finding, it was concluded that composts and poultry manure weakly affected soil properties, even if it could increase soil potentially available nutritive elements to crops.

KEYWORDS: compost, poultry manure, organic fertiliser, citrus orchards, soil quality.

INTRODUCTION

In Italy, intensive citrus cultivation is typically carried out in farms, characterised by a high level of specialization. In such systems, in-farm organic residues are not available to fertilise orchards and, consequently, crop nutrition is carried out by using mainly mineral/synthetic fertilisers (Intrigliolo et al. 2003). The low input of organic materials

to soil and the intensive orchard management could reduce organic matter content, functioning and quality of soil (Canali et al. 2002 b).

Among the different strategies able to guarantee an adequate input of organic matter to soil, organic fertilisation could represent an effective solution, particularly by applying high quality composts obtained from organic wastes of different origin (i.e. agro-industries, intensive livestock, municipal solid wastes) and characterised by stabilised organic matter (Tittarelli and Canali 2002).

Long term experiments dealing with C sequestration in soil indicated different effects of organic fertilisers and amendment on soil C pools and processes, and thus on soil fertility according to climatic conditions, soil type, cropping system and characteristic of organic matter added to soils (Baldoni et al. 1996; Singh et al. 1998; Ryan et al. 2002). A number of soil descriptors tided to soil biological function were utilised by Werner (1997) in order to evaluate soil quality changes during conversion to organic management in a Californian apple orchard.

Flieβbach and Mäder (1997) and Mäder et al. (2002) found significant differences in soil quality of organically, bio-dynamically and conventionally managed soils, where different organic materials were applied for more than 20 years. Indeed, the amount and typology of organic materials affected microbial biomass, microbial activity and composition of soil microflora, being the metabolic quotient lower and functional diversity higher in organically and biodinamically managed soils. On the basis of these findings, the authors concluded that in these soils, according to Odum (1969) hypothesis, the efficiency in utilisation and conservation of organic matter and energy increased. Also Schloter et al. (2003) utilised soil biochemical and biological indicator to asses quality of agricultural soils in Central Europe.

Canali et al. (2002 a, b) demonstrated under Mediterranean conditions, nutrient elements and energy were more efficiently utilised by the crop and microflora in organic than in the conventional soils, being the potentially mineralisable C higher and the mineralization quotient lower.

However, information on the effect of long term addition of different composts on soil quality under Mediterranean conditions are scarce. Therefore, we carried out a study in an organically managed citrus orchards located in Eastern Sicily (Southern Italy), utilising an integrated approach based on the combination of chemical, biochemical and microbiological measurements of soil properties. The aim was to verify if the addition of organic fertilisers and composts over a long period of time to soil, could match the N needs of the crop and influence soil C storage, C and N processes, size and diversity of soil microflora.

MATERIALS AND METHODS

Experimental design

The study was conducted in an organically managed orange orchard (Valencia late grafted on sour orange rootstock) located in Lentini (SR), Sicily (Southern Italy), from 1996 to 2001. The orchard soil had a sandy loam texture (IUSS), sub alkaline pH value (7.8) and high C.E.C. (65 meq × $100g^{-1}$ of dry soil).

Four treatments were carried out adopting a randomised-block experimental design with three replicates. In treatments 1 and 2, two different composts, obtained respectively from distillery by-products (C1) and from livestock waste (C2), were applied. The two composts were characterised by different organic C and nutritive elements content (C1: 26% TOC, 2,0% N_{tot}; C2: 20% TOC, 1,3 N_{tot}). The plots of

treatment 3 were fertilised with dried poultry manure (PM: 30% TOC, 3,5% N_{tot}), one of the most widespread organic fertilisers in Italy. The control treatment (4) was fertilised with urea (MF). All the plots received the same amount of N (160 kg N × ha^{-1}), matching the needs of the crop, as suggested by orchard good farming practice, but different amounts of organic matter from 1995 to 2001 (Table 1). Control plots did not receive any organic C at all. Soil sampling was carried out in February 2001, 11 months after the last organic fertilisation. For each of the 12 plots, 4 sub-samples were collected, air dried and sieved (2 mm). Sub-samples of the same plot were then pooled together and the resulting sample was accurately mixed and then stored for subsequent analyses. All the laboratory tests were replicated three times.

Chemical, biochemical and microbiological parameters

Total organic C (TOC, $mg \times kg^{-1}$) was determined by wet oxidation with 2N potassium dichromate solution in acid environment for 10 minutes at 160°C, according to Springer and Klee (1954), and total N ($N_{tot.}$, $mg \times kg^{-1}$) by Kjeldahl's procedure (Bremner 1996). Humic substances were extracted by shaking 5 g of each soil sample with 100 mL of 0.1 N NaOH/0.1 N $Na_4P_2O_7$ solution, at 65°C for 48 hours, under N_2 atmosphere. Humic acids (HA) were precipitated by acidifying (with 0.1 N HCl) 25 mL of this alkaline extract to pH value lower than 2. After centrifugation at 2500 rpm, the fulvic acids (FA) were purified on a polyvinylpyrrolidone column (PVP) (Ciavatta and Govi 1993). The extracted organic C (TEC) and the C content of the humic+fulvic acids fraction (C_{HA+FA}) were determined according to Springer and Klee (1954). Humification Rate (HR%) and the Degree of Humification (DH%) were calculated according to Ciavatta et al. (1990), as follows:

$$HR\ (\%) = 100 \times C_{HA+FA} / TOC \qquad DH\ (\%) = 100 \times C_{HA+FA} / TEC$$

The humification of the extracted soil organic matter was characterised by isoelectric focusing technique (IEF). Ten millilitres of 0.1N $Na_4P_2O_7$ solution were dialyzed by 6.000-8.000 Dalton membranes and then lyophilised to obtain purified soil humic matter (Ciavatta et al. 1990). This fraction, obtained from each soil, was analysed by isoelectric focusing technique (IEF) on a polyacrylamide slab gel in a pH range 3.5-8.0 (Ciavatta and Govi 1993), for this purpose the following mixture of carrier ampholytes (Pharmacia+ Biotech) was used: 25 units of Ampholine pH 3.5-5.0, 10 units of Ampholine pH 5.0-7.0 and 5 units of Ampholine pH 6.0-8.0. A prerun (2h; 1200V; 1°C) was performed and the pH gradient formed in the slab was checked by a specific surface electrode. The electrophoretic run (2h 30'; 1200V; 1°C) was carried out loading the water-resolubilised extracts (5 mg C × 100 μL^{-1} × $sample^{-1}$). The electrophoretic bands were stained with an aqueous solution of Basic Blue 3 (30%) and then scanned by an Ultrascan-XL Densitometer, obtaining a typical IEF profile for each investigated soil. IEF peaks were then numbered and the peaks' area determined for each soil IEF profile, assuming as 100% the area under the entire IEF profiles. The sum of peaks' areas focused at pH>4.5 (corresponding to more humified organic matter) was calculated and named A_s%.

Estimation of organic C mineralisation was performed by measuring C-CO_2 production [mg(C-CO_2)×kg^{-1}_{soil} ×d^{-1}] by soil in closed environment (Isermeyer 1952), at 1, 2, 4, 7, 10, 14, 17 and 21 days. The cumulative C-CO_2 evaluation after 1 (C_1), 7 (C_7) and 21 (C_{21}) days were calculated for each soil. The kinetic study of organic C dynamic was performed by fitting the cumulative C-CO_2 *versus* time according to the first order exponential equations $C_t = C_0(1-e^{-kt})$. This elaboration allowed to calculate the potentially mineralisable C pool C_0 [mg(C)×kg^{-1}_{soil}] for each soil.

Microbial biomass C (C_{MIC}, mg $C \times kg^{-1}_{soil}$) was measured by the fumigation-extraction method (Vance et al. 1987), after a pre-incubation of air-dried soils for 10 days in open glass jars at –33 kPa water tension and at 30°C.

Basal respiration [C_B , in $(mgC_{co_2} \times kg_{soil}^{-1}) \times d^{-1}$] was defined as the value of mineralised C in a definite period of time, when steady-state condition had been reached (Odum 1969; Anderson and Domsch 1985).

Mineralisation quotient, defined as soil basal respiration in relation to the total organic C, was calculated by dividing the basal respiration per the TOC value to 1 kg of soil $[(mgC_{co_2} \times mgC^{-1}) \times d^{-1}]$. Metabolic quotient was calculated by dividing soil basal respiration values with C_{MIC} $[(mgC_{co_2} \times mgC_{mic}^{-1}) \times d^{-1}]$. The ratio C_{MIC} /TOC [%] was used as an index of microbial biomass contribution to soil organic C. Potentially mineralisable N (NPM) was estimated by calculating the NH_4^+-N $(mg \times kg^{-1})$ accumulated after 7 days of anaerobic incubation at 40°C, according to Sahrawat and Ponnamperuma (1978) and slightly modified by Canali et al. (2002 a). Sixteen grams of air-dried and sieved (<2 mm) soil, were placed in 50 mL test tubes containing 40 ml distilled water; then, the tubes were sealed, incubated at 40°C for 8 days, and shaken for a few seconds each day, in order to mix the water-soil suspension. After incubation, soil was extracted with 80 mL of 2N KCl and 40 mL of 4N KCl were added to the suspension in order to reach a soil:solution ratio of 1:5. The samples were shaken for 1 h and then filtered through paper filters. Determinations were replicated three times and the difference between the $N\text{-}NH_4^+$ after 7 days and that at 0 day represents the potentially mineralizable N (NPM).

The analysis of Community Level Physiological Profile was performed using Biolog® Inc. eco-plates for microbiology, containing different *substrata* (such as aminoacids, amines, carbohydrates, etc.). According to Torsvik (1995), each plate was inoculated with a soil extract, prepared by treating 2 g of soil with 20 mL of physiological solution (NaCl 9 g L^{-1}). The coloration of the plate during time represents the metabolic activity of the whole microbial community of the each extract (Garland and Mills 1991; Garland 1996a and b). The functional biodiversity was evaluated according to the Shannon index (Zak et al. 1994), while the *substrata* utilisation capability was defined by calculating the Gini index (Harch et al. 1997) and the catabolic versatility index (Burkhardt et al. 1993; Sharma and Insam 1996; Sharma et al. 1997).

In order to evaluate the effectiveness of the four treatments, data were elaborated by the analysis of variance (ANOVA). A purely additive model was applied, since the treatment – block interactions resulted not significant.

RESULTS AND DISCUSSION

The average value of TOC in the MF soil was not significantly lower than those of the other treatments which received different amount of organic matter (Table 2). No significant differences were also observed between the compost and poultry manure treated soils; probably, the high spatial variability of soil has increased the variability of data. Similar trend was observed for total N and, consequently, the C/N ratio did not show significant differences among the treatments (Table 2).

The TEC, HA+FA pools and the relative ratios (DH, HR), considered as sensitive indexes of humification of soil organic matter (Ciavatta et al. 1990), revealed no significant differences among the thesis (Table 2).

Isoelectric focusing (IEF) of organic matter extracted from soils is reported in figure 1. It was demonstrated that, both in natural (Trinchera et al. 1998) and agricultural (Dell'Abate et al. 2002) soils, the more humified the organic matter, the more intense the IEF peaks focused at pH values higher than 4.5. In our study, IEF peaks at pH >4.5 were higher in C1, C2 and PM than in MF soil, suggesting a positive effect of the compost and poultry manure treatments on soil humification. This hypothesis was confirmed by the A_s % values which ranged from 39.2 (C1) to 41.7 (PM) in organic fertilised samples and were higher than the MF value (32,3%) (Table 2). According to Canali et al. (2002 b) the A_s % value, calculated from IEF data, can represent a quantitative index of the humification level in soil.

The mineralisable C data of organic fertilised soils were significantly higher than values of MF soil after 7 days of the incubation (C_7) (Table 2). The highest values of potentially mineralisable C (C_0) were showed by soils amended with the two composts (C1 and C2, respectively) and the observed differences of C_0 values among the treatments were statistically significant (p-level = 0.07). These findings suggested that soils amended with composts were characterised by higher amount of easy degradable organic matter by soil microflora.

Potentially mineralisable N showed significant differences between the organically and not organically fertilised soils, with the lowest value in the MF soil, despite the amount of total N applied to the different plots was equal. The highest values were obtained in PM and C2 treatments and (Table 2), probably were related to the quality of organic materials applied to soil. Eklind and Kirchmann (2000) observed that the amount of organic C resistant to decomposition during composting process depended on the initial lignin content of the starting materials. It may be possible that

the C1 compost mainly contained a large proportion of ligno-cellulosic compounds (vegetal tissues), which were resistant to the microbial degradation activity. On the contrary, organic N added with PM and C2 materials contained more labile and easily degradable organic N compounds, because they derived from animal dejections (faeces and urines) (Nahm, 2003).

Soil microbial biomass (C_{MIC}) was the lowest in MF and the highest in C2 treated soils (Table 2). The percentage of organic C present as microbial biomass C (C_{MIC}/TOC), referred to the unit of soil, was higher in C1 and C2 (those treated with compost) than in PM and MF soils, but differences were not significant. Basal respiration was significantly higher in soils treated with organic materials than in the MF soil, while the mineralization quotients C_B/TOC and metabolic quotients C_B/C_{MIC} were non statistically different among the various treatments (Table 2). Thus, no differences in the efficiency of microflora in metabolising organic matter were observed among the different treatments. Landi et al. (2000) found that stresses as well as the increase in the bacterial/fungal ratio can increase the metabolic quotients. Since we observed that metabolic quotients were similar among the treatments we concluded that there were no changes in microbial metabolism and/or bacterial/fungal ratio. Also the indexes describing functional diversity and *substrata* utilisation capability (Shannon, Gini and Versatility indexes – Table 2) did not show significant differences among the mineral and the organic treatments, suggesting that the addition of different organic materials did not affect the microbial functioning. The lack of metabolic diversity was demonstrated by the similarity of the physiological profile at community level. Nevertheless, the K value, which represents the average degradation rate calculated for all the substrates, was higher in organically treated soils (1.35 for C1, 1.40 for C2 and

1.43 for PM) than in the MF (1.25) (p-level = 0.10) soil. This suggests that, despite similarities in functional diversity of the studied soils, the microflora of the amended soils degraded more rapidly organic compounds than the microflora of MF soil.

In conclusion, the tested biochemical and microbiological soil properties, often utilised for defining soil quality, were weakly influenced by the different treatments. Chemical parameters such as total N, total organic C, humification rate, degree of humification and IEF of extracted organic matter fraction were not statistically affected by the application of organic fertilisers and soil improvers. On the other hand, both C and N potentially mineralizable pools and basal respiration were higher in soils treated with organic matter than in soils treated with mineral fertilisers. Parameters describing functional diversity of soil microflora did not show differences among the treatments. Furthermore, the low increase of microbial C biomass seemed to be proportional to the increase of TOC, and both the mineralization and metabolic quotients did not show differences among the treatments. Probably, both the microbial composition and microbial activity were not changed by addition of organic materials. Further research is required to measure changes in the composition of soil microflora by using molecular techniques.

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Table 1. Organic matter added to soils (t/ha) corresponding to 160 kg N × ha^{-1}.

Year	C1	C2	PM
1995	0.33	4.37	2.75
1996	5.82	4.26	2.75
1997 – 2001 (every year)	4.02	4.26	2.75
Total dose	26.23	29.95	19.22

C1; compost 1 from distillery by-products; C2; compost 2 from livestock waste; PM; poultry manure

Table 2. Average values of chemical biochemical and microbial parameters of soils.

Parameter	Units	C1	C2	PM	MF	p-level
TOC	mg C × kg_{soil}^{-1}	18355	20474	19343	17302	0.35
N_{tot}	mg N × kg_{soil}^{-1}	1786	1942	1900	1646	0.51
C/N	-	10.3	10.6	10.2	10.5	0.64
TEC	mg C × kg_{soil}^{-1}	11751	14019	12877	12687	0.44
C_{HA+FA}	mg C × kg_{soil}^{-1}	8106	10110	8391	8811	0.56
HR	%	43.0	49.9	43.8	52.7	0.47
DH	%	67.7	73.2	66.5	71.1	0.93
A_s	%	39.2	41.2	41.7	32.3	0.13
C_7	mg Cco_2× kg_{soil}^{-1}	330.7	331.8	310.9	241.8	0.08
C_{21}	mg Cco_2× kg_{soil}^{-1}	499.5	507.3	460.4	355.4	0.04
C_0	mg Cco_2× kg_{soil}^{-1}	486.9	514.1	435.5	337.5	0.07
N-NPM	mg N× kg_{soil}^{-1}	154.7	174.6	176.9	138.6	0.10
C_{MIC}	mg C × kg_{soil}^{-1}	257.8	266.7	200.3	187.4	0.20
(C_{MIC} /TOC) × 100	%	1.4	1.3	1.1	1.1	0.52
C_B	(mg C_{co_2} × kg_{soil}^{-1})×d^{-1}	9.4	12.7	8.8	6.3	0.07
C_B/TOC	(mg C_{co_2}× mg C^{-1})×d^{-1}	5.0×10^{-4}	6.0×10^{-4}	5.0×10^{-4}	4.0×10^{-4}	0.21
C_B/C_{MIC}	(mg C_{co_2}× mg C_{mic}^{-1})×d^{-1}	3.7×10^{-2}	4.8×10^{-2}	4.7×10^{-2}	3.3×10^{-2}	0.36
SHANNON	-	0.343	0.355	0.352	0.355	0.79
GINI	-	0.437	0.427	0.443	0.448	0.82
Versatility	-	1.19	1.24	1.17	1.17	0.83
K	h^{-1}	1.35	1.40	1.43	1.25	0.10

TOC; total organic C; N_{tot}; total N; TEC; total extractable C; C_{HA+FA}; humic and fulvic C; HR; humification rate; DH; degree of humification; A_s; sum of areas focused at pH higher than 4.5; C_7; mineralised C after 7 days; C_{21}; mineralised C after 21 days; C_0; potentially mineralisable C; N-NPM; potentially mineralisable N in anaerobic conditions; C_{MIC}; microbial biomass C; C_{MIC} /TOC; contribution of microbial biomass carbon to soil organic carbon; C_B; basal respiration; C_B/TOC; mineralisation quotient; C_B/C_{MIC}; metabolic quotient; SHANNON Index; index of functional biodiversity; GINI Index; index of substrata utilisation capability; K; degradation rate; p-level; significant differences were observed when values were lower then 0.1 .

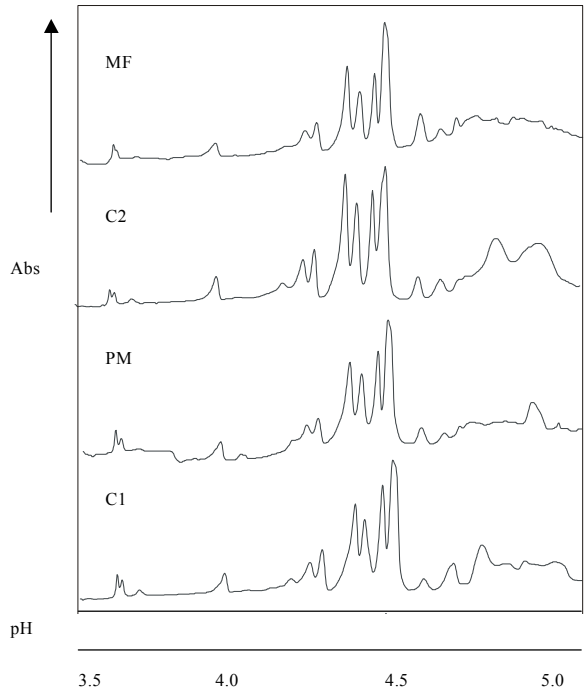

Figure 1. Isoelectric focusing profiles of organic matter extracted from C1, C2, PM and MF soils;C1, C2, PM and MF are soils treated with C1 compost, C2 compost, poultry manure and mineral fertiliser, respectively.