Biol Fertil Soils (1999) 30:100–106

ORIGINAL PAPER

A. Saviozzi · A. Biasci · R. Riffaldi · R. Levi-Minzi

Long-term effects of farmyard manure and sewage sludge on some soil biochemical characteristics

Received: 20 October 1998

Abstract Changes in some soil biochemical properties were investigated following repeated applications of aerobically digested sewage sludge (SS) under field conditions over 12 years, and compared with those of an adjacent soil cultivated and amended with 5 t ha^{-1} $year^{-1}$ (dry weight) farmyard manure (FYM) for at least 40 years, as well as with those of an adjacent uncultivated soil, in order to ascertain changes in soil quality. A short-term aerobic incubation was used to determine the potential of the samples to mineralize the organic C supplied. Results indicated that cultivation caused a reduction in total, humified and potentially mineralizable organic C, total N, light-fraction (LF) C, total and water-soluble carbohydrates, phenolic compounds, cation-exchange capacity (CEC), microbial biomass C, specific respiration, hydrolytic and urease activities, and an increase in the heavy metal content. Total and water-soluble carbohydrates and phenolic compounds expressed as a percentage of total organic C (TOC) were similar in the differently managed plots. Of the two amendments, FYM treatments showed higher amounts of TOC and N, LF-C, total and water-soluble carbohydrates, phenolic substances, CEC, specific respiration of biomass, hydrolytic and urease activities, similar amounts and characteristics of humified organic matter and lower concentrations of Cu, Zn and Cr. Both FYM and SS were inadequate treatments for the restoration of soil organic matter lost as a consequence of cultivation.

Key words Long-term trial · Land organic waste disposal · Farmyard manure · Sewage sludge · Soil quality

---

A. Saviozzi (✉) · A. Biasci · R. Riffaldi · R. Levi-Minzi
Dipartimento di Chimica e Biotecnologie Agrarie,
Università di Pisa, via del Borghetto 80, I-56124 Pisa, Italy
e-mail: alesavio@agr.unipi.it,
Tel.: +39-50-571557, Fax: +39-50-598614

Introduction

For the long-term productivity of agroecosystems and protection of the environment it is necessary to develop and implement management strategies that maintain the quality of soil, which includes conserving the amount of organic matter present. Agricultural practices, in particular cultivation and organic amendment, regulate the soil microbial biomass; this affects C mineralization, nutrient cycling and turnover of organic matter (McGill et al. 1986), as a decline in the microbial biomass usually occurs after the introduction of arable agriculture (Saviozzi et al. 1994). Exhaustive reviews of the impacts of tillage, fertilizers, and crop residues on soil organic matter and soil biochemical and microbial parameters have been written by Rasmussen and Collins (1991) and Dick (1992).

Since supplies of farmyard manure (FYM) have diminished, the application of organic wastes such as sewage sludge (SS) to agricultural soils has received considerable attention in recent years. However, there are various opinions on the benefits of sludge amendment concerning its long-term effect on the levels and availability of soil nutrients, humus status and other chemical and biochemical soil properties, as well as on its contribution to the soil of organic pollutants, phytotoxic compounds and heavy metals.

Long-term field treatments provide a means of investigating the influences of organic amendments on soil characteristics (Collins et al. 1992; Hopkins and Shiel 1996). However, relatively limited consistent information is available concerning changes in soil chemical and biochemical properties, under long-term field conditions, in response to added complex organic amendments such as SS.

The objective of this study was to ascertain changes in soil quality in response to repeated applications of SS under field conditions over several years. Soil properties were compared with those of an adjacent soil cultivated and amended with FYM as well as with those of an adjacent uncultivated soil.

Materials and methods

Site and field treatments

The study site was located on a farm near Fidenza (province of Parma, Italy), on a uniform area consisting of a clay soil (sand 26.3%, silt 30.6%, clay 43.1%). The location has a long-term precipitation average of 900 mm year^{-1} and a mean annual temperature of 12 °C.

Long-term treatments were carried out on areas adjacent to one another and included: (1) undisturbed native grass as control (CL); (2) plots farmed conventionally for at least 40 years, receiving 5 t ha^{-1} year^{-1} (dry weight) FYM; (3) plots amended over 12 years with 5 t ha^{-1} year^{-1} (dry weight) SS. The latter site had been farmed conventionally prior to the experiment's initiation.

Starting from 1985, the sludge, collected from the municipal aerobic waste-water treatment plant in Fidenza, was mechanically incorporated in autumn into the upper 20 cm of the soil, before seed-bed preparation, with a single pass of a tractor-mounted rotary tiller.

The two amended plots were managed under a 2-year rotation consisting mainly of corn and wheat.

Soil sampling

Three 0.1-ha plots for FYM, SS and CL treatments were sampled in June 1997 at a depth of 20 cm before the yearly amendments were applied, i.e. when the organic materials incorporated in the previous autumn had already partially decomposed and the activity of the readily available substrate had ceased.

Twenty random cores were taken from each of the three plots with a 5-cm-diameter tube auger and bulked. Large pieces of organic matter were removed by hand and sub-samples of moist field soil (about 1000 g) were then brought to the laboratory, air-dried and ground to pass through a 2-mm screen.

Laboratory measurements

Granulometric composition and pH were determined according to the SISS (1995). Cation exchange capacity (CEC) was determined by Ba^{2+} saturation and subsequent complete replacement of Ba^{2+} with Mg^{2+}, according to Bascomb (1964), and total organic C (TOC) was determined after removing CO_3^{2-}–C by dry combustion (induction furnace 900CS; Eltra). The light fraction (LF) of organic matter was separated from soil samples by the method of Strickland and Sollins (1987) and weighed, and C was determined as described above. Total N was determined according to the Bremner and Mulvaney (1982) method, available P was determined spectrophotometrically on soil extracted with 0.5 N $NaHCO_3$ at pH 8.5 (Olsen et al. 1954), and exchangeable K by atomic absorption spectroscopy (AAS) on soil extracted with 1 N CH_3COONH_4 at pH 7.0 (Thomas 1982). Total carbohydrates were determined according to the method of Oades et al. (1970).

Humic substances were extracted by shaking soils with 0.1 mol l^{-1} $NaOH+Na_4P_2O_7$ (1:10, w/w) under N_2 for 24 h at room temperature. After centrifugation at 5000 *g* for 30 min, the supernatant was filtered through a 0.20-μm membrane and the raw fulvic acid (FA) and humic acid (HA) fractions separated by acidifying to pH 2 with H_2SO_4. Isolation, separation and purification of "true" FA was made by adsorption onto insoluble polyvinylpyrrolidone, as suggested by Lowe (1975). According to the standard procedure described by Sequi et al. (1986), the humification index (HI) was calculated by dividing the non-humified fraction by the HA+FA fractions. Total and humic C in any fraction was determined by dry combustion as described above for TOC.

Fluorescein diacetate hydrolytic activity was determined as absorbance at 490 nm of the filtrate from the soil suspension incubated with fluorescein diacetate at 24 °C (Schnurer and Rosswall 1982); urease activity was determined according to Tabatabai and Bremner (1972).

Total heavy metal (Co, Ni, Pb, Cd, Cu, Zn and Cr) contents were analysed by AAS on the extract obtained by treating samples with aqua regia (1:5 w/w) (Cottenie et al. 1979).

In the water-soluble fraction, obtained by refluxing samples with distilled water (1:5 w/w) for 1 h, phenolic substances were estimated by the method of Folin as modified by Kuwatsuka and Shindo (1973) and carbohydrates by the method of Dubois et al. (1956).

The moisture content was determined by heating samples at 105 °C for 24 h, and the ash content by igniting samples at 750 °C for 4 h. The following determinations were carried out on freeze-dried SS and FYM: pH, TOC, total N, humus, phenolic compounds, total and water-soluble carbohydrates by the methods previously described, with the only change regarding the values of the ratios between the material and the extractant, as reported in Table 1. Total P and K were determined after digestion with concentrated H_2SO_4; again, P was determined spectrophotometrically and K by AAS.

The results reported are the means of determinations made on three replicates. The means were compared by using least significant difference values calculated at the 5% level.

A short-term (60 days) aerobic incubation was used to determine the potential of the samples to mineralize organic C. Samples (100 g) of FYM- or SS-treated soil, as well as of the CL were moistened to 60% of maximum water-holding capacity and incubated at 22 °C in 300-ml glass containers closed with rubber stoppers. Glass vials holding 20 ml of 0.5 N NaOH were placed in the containers to trap the evolved CO_2. The excess alkali was back-titrated with standard 0.5 N HCl after precipitating the CO_3^{2-} with 1.5 M $BaCl_2$ solution. The bottles were opened daily to replenish the NaOH for CO_2 absorption so that decomposition was not inhibited by a lack of O_2. CO_2-free water was added at appropriate intervals to maintain the moisture of the soil at a constant level.

A non-linear least-squares regression analysis (Graph Pad Prism; Prism 1994) was used to calculate parameters from the cumulative C mineralization data. The comparison of model fits was evaluated by the coefficient of determination, R^2.

At the end of the incubation the amount of microbial biomass was measured by using the method of Vance et al. (1987).

The incubation experiment was performed in two replicates; the main coefficient of variation was always $<3\%$.

Reganold and Palmer (1995) suggested that gravimetric data are less ecologically relevant than volumetric data, which are thought to more accurately indicate what plant roots encounter. For this reason, data were expressed on a volume rather than mass basis.

Results and discussion

Table 1 gives the main properties of the SS and FYM. The pH, total K and P, phenolic compounds and carbohydrates were lower in the SS than in the FYM. The SS contained more total N but the same amount of organic C as the FYM, consequently, the C/N ratio was lower in the SS than in the FYM. However, the C/N ratios were <20, a value that usually reflects a satisfactory degree of compound maturity (Levi-Minzi et al. 1986). Comparisons between the two materials showed that in FYM the percentage of organic C extracted as HA was higher and characterized by a higher degree of maturity, as indicated by the greater amounts of C in the HA and FA fractions, as well as the higher humification ratio, degree of humification, and lower HI.

Table 1 Main organic and inorganic characteristics of the farmyard manure (*FYM*) and sewage sludge (*SS*) expressed on a dry matter (*DM*) basis. *TOC* total organic C, *TEC* total extracted C, C_{HA} C of humic acids (HA) expressed as percentage of TEC, C_{FA} C of fulvic acids (FA) expressed as percentage of TEC, *HD* humification degree [$100(C_{HA}+C_{FA})/\mathrm{TEC}$], *HR* humification ratio [$100(C_{HA}+C_{FA})/\mathrm{TOC}$], *HI* humification index ($\mathrm{NH}/C_{HA}+C_{FA}$), *NH* non-humified C

	DM (%)	Ash (%)	pH (1:25)	TOC (%)	Total N (%)	C/N	Total K (mg/g^{-1})	Total P (mg/g^{-1})	Phenolics (1:25) (mg/g^{-1})	Carbohydrates Total (mg/g^{-1})	Carbohydrates Water soluble (1:25) (mg/g^{-1})
FYM	17.2 a	30.5 a	7.6 a	31.7 a	3.75 a	8.4 a	43.1 a	11.0 a	10.8 a	122.6 a	10.2 a
SS	12.0 b	33.1 b	6.8 b	31.9 a	6.71 b	4.7 b	4.5 b	6.6 b	2.5 b	66.4 b	3.3 b

	TEC (%)	C_{HA} (% TEC)	C_{FA} (% TEC)	Humification parameters (1:100) HD (%)	HR (%)	HI
FYM	9.2 a	65.8 a	10.1 a	75.9 a	22.1 a	0.3 a
SS	7.5 b	39.6 b	3.5 b	43.1 b	10.1 b	1.3 b

	Total heavy metals (μg g^{-1}) Co	Ni	Pb	Cd	Cu	Zn	Cr
FYM	4 a	14 a	60 a	6 a	66 a	340 a	9 a
SS	10 a	40 b	60 a	4 a	236 b	1640 b	97 b

Means in the *same column* followed by a *different letter* are significantly different at the 5% confidence level

Table 2 Influence of repeated applications of FYM and SS on some physico-chemical properties of the soil. *CL* Control, *CEC* cation-exchange capacity, *LF-C* light-fraction C. Means in the *same column* followed by a *different letter* are significantly different at the 5% confidence level. For abbreviations, see Table 1

	Bulk density (g cm^{-3})	pH	TOC (g 100 cm^{-3})	Total N (g 100 cm^{-3})	CEC (mEq 100 ml^{-1})	C/N	LF-C (mg cm^{-3})	P available (μg cm^{-3})	K exchangeable (μg cm^{-3})
FYM	1.27 a	6.7 a	2.33 a	3.32 a	46.2 a	7.0 a	2.31 a	65 a	529 a
SS	1.28 a	6.8 a	2.00 b	2.92 b	40.4 b	6.8 a	0.45 b	92 b	360 b
CL	1.42 b	6.7 a	5.13 c	5.55 c	49.6 c	9.2 c	8.95 c	30 c	531 a

	Total carbohydrates (mg cm^{-3})	Total carbohydrate C (% TOC)	Water-soluble carbohydrates (μg cm^{-3})	Water-soluble carbohydrate C (% TOC)	Phenolic compounds (μg cm^{-3})	Phenolic compound C (% TOC)
FYM	13.6 a	23.3 a	255 a	0.44 a	20.2 a	0.06 a
SS	11.6 b	23.1 a	210 b	0.42 a	15.2 b	0.05 a
CL	25.2 c	19.7 a	543 c	0.42 a	29.0 c	0.04 a

	TEC (%)	C_{HA} (% TEC)	C_{FA} (% TEC)	HD (%)	HR	HI
FYM	0.6 a	47.2 a	12.6 a	59.7 a	15.0 a	0.67 a
SS	0.5 a	52.8 a	8.4 a	61.0 a	14.1 a	0.64 a
CL	1.2 b	51.8 a	14.7 a	66.5 a	16.0 a	0.50 a

As expected, with the exception of Co, Cd and Pb, which did not show differences the SS was richer in heavy metals than the FYM; however, the sludge met the criteria established by current Italian legislation for its application to agricultural land (law no. 99, 27 January 1992, adopted to conform to EEC directives).

Table 2 presents the effects of the application of FYM and SS on soil properties. The amended soils showed a significant reduction in bulk density, suggesting that the yearly addition of fresh organic matter facilitated soil aggregation and porosity. This result agreed with the findings of Sommerfeldt and Chang (1985), who reported that bulk density decreased over a 5-year period of cattle manure addition. Bulk density has also been shown to decrease linearly with increasing amounts of sludge (Gupta et al. 1977).

Several authors (Riffaldi et al. 1994; Reganold and Palmer 1995) have reported significant losses of organic C in cultivated soils. In this study, the amount of TOC in the control was more than double that of the cultivated sites (Table 2), confirming the negative effect of cultivation on TOC and indicating that the long-term application of both organic materials was not sufficient to maintain the same level of organic C as in the undisturbed site. This happened in spite of the low C/N ratios of the FYM and SS, which indicated that the organic matter of these materials was already humified sufficiently before incorporation into the soil, so less C should have been available for oxidation by the soil microorganisms. The explanation for the significantly higher content of TOC in plots treated with FYM compared to SS lies in the fact that, although the treatments were carried out by distributing the same amount of dry matter, and the mean TOC content in the two materials was similar, the organic C of FYM was more humified and, consequently, more resistant to mineralization.

The LF of soil organic matter represents a transitory pool between fresh residues and humified, stable organic matter, and in arable systems this fraction is sensitive to changes in management practices (Janzen 1987). In this study, LF-C, which accounted for 4.7%, 1.0% and 8.6% of TOC in FYM, SS and CL soils, respectively, was higher in the FYM- than in the SS-treated soil, probably as a result of the presence of straw in the FYM. The higher LF content of the uncultivated site was in accordance with the findings of Dalal and Mayer (1986), who observed losses of organic C from the LF following cultivation.

Total and water-soluble carbohydrates and phenolic compounds represent a very active soil organic component (Bowman et al. 1990), and a readily available source of energy for microorganisms (Chesire 1977). Table 2 shows that long-term soil cultivation resulted in a decline in these substances; this decrease was of the same magnitude as that of carbohydrates expressed as a percentage of TOC (about 50%). This result agreed with findings reported by Bowman et al. (1990) and Riffaldi et al. (1993) who found a net decrease in carbohydrates in cultivated soils, relative to undisturbed areas. Similarly, Maciak and Harms (1986) and Saviozzi et al. (1994) found the highest content of phenolic acids in undisturbed permanent grassland. The higher amount of total and water-soluble carbohydrates, as well as of phenolic compounds, in the FYM relative to the SS (Table 1) may explain the higher level of these substances in the FYM-amended soil. However, when the estimated C content of the total and water-soluble carbohydrates, as well as of phenolic compounds was expressed on the basis of TOC (Table 2), the relative abundance of the fractions was similar in the differently managed plots, indicating the same qualitative character of soil organic matter.

Generally, the addition of an organic amendment to soil is likely to increase the soil CEC, simply due to the high CEC of the amendment. For example, in a field study in which SS was applied to soil, the CEC increase was attributed to the organic matter added with the sludge (Kladivko and Nelson 1979). In our study, CEC values were lower in the treated sites (Table 2). As already noted, in spite of the application of large amounts of organic C through FYM and SS applications, the amounts of organic C in the treated plots were lower, suggesting that the decreases in organic C and CEC were related. Moreover, no decrease in pH was observed for the plots treated with the two amendments (Table 2) and, therefore, the decreasing CEC could not be attributed to any pH changes.

The patterns of total N concentrations were similar to those observed for TOC (Table 2). The higher concentration of total N found in the plots treated with FYM with respect to those amended with SS, in spite of the lower N content of the former (Table 1), could be attributed to the fact that, in FYM, humified forms of N predominate, which are less subject to hydrolysis and leaching.

The C/N ratio, lower in the treated plots, depends on different rates of loss of organic C and N, and does not indicate differently humified organic matter. Indeed, the analysis of the humification parameters did not show any significant difference between plots. Consequently, the greater amount of humified material (total extracted C; TEC) in the control reflected the higher amount of TOC in this site, rather than more stable, naturally occurring, soil organic matter. The lack of a significant difference in TEC between plots treated with FYM and SS indicated that the differences in the amount and type of humified C in the two materials (Table 1) have no implications with respect to trends of FYM and SS once added to soil.

In this research, unfortunately, a balance sheet for P and K with respect to changes in soil concentrations could be made only approximately, because information on crop yields and fertilizers applied was not complete. Values of available P were higher in the cultivated plots receiving FYM and SS than in the CL (Table 2), indicating that long-term amendment plus mineral fertilization makes the P budget highly positive under this type of soil management. The decrease in available K observed in SS-treated plots, both with respect to C and to FYM-amended soil, reflected the lower content of total K (about tenfold) in the SS as compared to the FYM (Table 1).

Table 3 reports the heavy metal contents in the three sites. With the exception of Cd, the level of which remained unchanged, cultivation and amendments enhanced the level of these metals in soil with respect to C; even after 13 years of SS application, their concentrations did not exceed the maximum levels established by the previously cited Italian legislation concerning the use of this material on agricultural land. The neutral pH and the high CEC of the soil could have led to strong adsorption of these heavy metals that decreased their solubilization, leaching and availability to plants, favouring their accumulation in the cultivated sites. Although no data are available on the annual input of heavy metals into the cultivated sites, the heavy metal content of inorganic fertilizers (Swaine 1962) and the

Table 3 Influence of repeated amendments with FYM and SS on heavy metal contents of soil. For abbreviations, see Tables 1 and 2

	Total heavy metals (μg cm^{-3})						
	Co	Ni	Pb	Cd	Cu	Zn	Cr
FYM	30 a	88 a	57 a	1.4 a	93 a	159 a	41 a
SS	33 a	90 a	52 a	1.5 a	110 b	173 b	50 b
CL	24 b	75 b	43 b	1.3 a	84 c	138 c	35 c

Table 4 Parameter estimates according to the double exponential model for C mineralization in FYM- and SS-amended soil, and in the CL. C_t Cumulative C mineralized after time t (dependent variable), t from start of incubation when $t = 0$ (days); independent variable), C_1 easily mineralizable C, C_2 slowly mineralizable C, h rate constant for C_1, k rate constant for C_2; for other abbreviations, see Tables 1 and 2

	FYM	SS	CL
C_1 (mg cm^{-3})	15.2	9.5	50.0
C_2 (mg cm^{-3})	105.5	40.7	197.4
$C_t = C_1 + C_2$ (μg cm^{-3})	120.7	50.2	247.4
h (mg day^{-1})	1.46	1.17	0.66
k (mg day^{-1})	0.07	0.08	0.05

use of insecticides or herbicides (Purves 1977) support the increase recorded in the cropped soils. Nevertheless, the situation requires monitoring.

As regards the influence of the two organic amendments, Cu, Zn and Cr accumulated in different amounts, probably as a result of the notably greater concentration of these metals in the SS (Table 1).

Five non-linear regression models were used to analyse the cumulative CO_2–C data of the mineralization process: first order (Murwira et al. 1990), double exponential (Molina et al. 1980), power function (Pal and Broadbent 1975), modified first-order (Jones 1984), and combined first-zero order (Seyfried and Rao 1988). On the basis of R^2 (data not shown), the double-exponential model, $C_t = C_1(1–e^{-ht}) + C_2(1–e^{-kt})$ (Molina et al. 1980) described the curves best. This model takes into account that the substrate can be divided into two components, an easily decomposed or labile fraction (C_1), and a more resistant fraction (C_2), both decaying exponentially at rates characterized by their own constant, h and k, respectively. As can be seen in Table 4, C_1 was highest in the CL, followed by FYM and SS treatments, confirming the results of the amounts of water-soluble carbohydrates and phenolic compounds (Table 2) which represent the most active soil organic components. For the SS and control soils, C_1 represented about 20% of the total amount of potentially mineralizable C ($C_1 + C_2$). In contrast, the FYM-treated soil showed the lowest percentage of easily mineralizable C, but the highest rate of mineralization (h), suggesting high microbial activity during the initial period of decomposition in this soil. The greater amount of C_1 plus C_2 observed in the control indicated that the untreated soil seemed to be a more effective system for supplying available organic C. This finding could indicate that the long-term application of both SS and FYM was not sufficient to maintain the same level of easily mineralizable organic C as in the undisturbed site, probably because the organic matter of the FYM and SS was already humified sufficiently before incorporation into the soil, so C was less available to the soil microorganisms for oxidation.

Measurements of biochemical properties have been used to indicate changes in soil quality due to long-term treatments and different forms of management. Long-term cultivation decreased biomass C relative to the control (Table 5), as also reported by Gupta and Germida (1988). Similar levels of biomass C were found between the two amended soils, which was consistent with other studies where the microbial biomass differed among long-term treatments of soil amendment only when the amendments varied substantially in the level of C (Collins et al. 1992).

In the cultivated and amended soils there was a higher level of biomass C as percentage of TOC than in the uncultivated soil, perhaps due to the repeated in-

Table 5 Microbial biomass C, specific respiration of biomass, fluorescein diacetate activity (*FDA*) measured as absorbance at 490 nm and urease activity of FYM- and SS-amended soil, and the CL. Means in the same row followed by different letters are significantly different at $P < 0.05$. For other abbreviations, see Tables 1 and 2

	FYM	SS	CL
Cumulative CO_2-C (μg cm^{-3})	996	436	1811
Biomass C (mg 100 cm^{-3})	2340	2282	3541
Biomass C (% TOC)	10	11	7
Specific respiration of biomass (μg CO_2-C mg^{-1} biomass C h^{-1})	6.4×10^{-3}	2.1×10^{-3}	13×10^{-3}
FDA	0.038 b	0.023 a	0.111 c
Urease activity (μg N h^{-1} cm^{-3})	224 b	162 a	410 c

corporation of carbonaceous crop residues into the cultivated soil. Similar findings were found by Riffaldi et al. (1993) in a study on soil biochemical changes after 40 years of continuous corn cropping. An increase in the biomass C/TOC ratio, caused by straw return to the soil, was also reported by Powlson et al. (1987). Generally, changes in the ratio of CO_2–C evolved to microbial biomass C reflect both inputs and outputs of organic matter into the soil, and have shown to be useful when monitoring changes in organic matter in agricultural systems (Anderson and Domsch 1990). According to the results of Riffaldi et al. (1993), the specific respiration of biomass, calculated at the steady state of respiration [CO_2–$C_{(30-60\mathrm{day})}$], was higher in undisturbed soil than in cultivated sites, suggesting a negative relationship between biomass C/soil C and specific respiration of biomass, i.e. the smaller the microbial biomass in soil, the more active the microbial population. In the cultivated soils, inorganic fertilizers could have caused a decrease in the specific respiration of biomass, as reported by Kandeler and Eder (1990), who found low biological activity in fertilized plots. FYM-treated soil showed a higher specific respiration of biomass than SS-treated soil (Table 5), probably because the presence of straw in the FYM led to a marked increase in the evolution of CO_2 (Schnurer et al. 1985). These results were confirmed by the data relative to FDA, reported in Table 5.

Riffaldi et al. (1994), in a study on the influence of long-term cultivation on soil properties, observed that urease activity was greater by a factor of about 2 in grassland compared with cultivated sites. In accordance with these findings, in the present research the urease activity was lower in cultivated than in undisturbed soil (Table 5). The soil treated with SS showed the lowest level of urease activity, which may have been due to a toxic effect of undesiderable materials, such as heavy metals, introduced with the sludge into the soil. Bonmati et al. (1985) reported that the presence of heavy metals in SS was responsible for the inhibition of soil urease activity.

On the basis of the results the following conclusions could be drawn:

1. There was a general, negative effect of cultivation on soil chemical and biochemical characteristics. The yearly addition of FYM and SS to the soil was not able to prevent the decrease in soil fertility due to cultivation; the undisturbed soil showed the highest amounts of total, humified and potentially mineralizable organic C, total N, LF-C, total and water-soluble carbohydrates, phenolic compounds, CEC, C and specific respiration of biomass, hydrolytic and urease activities, and a substantially lower level of heavy metals; the organic matter showed similar percentages of water-soluble carbohydrates and phenolic compounds.

2. With respect to SS, FYM showed higher amounts of total organic C and N, LF-C, total and water-soluble carbohydrates, phenolic substances, CEC, specific respiration of biomass, hydrolytic and urease activities, and a similar amount and characteristics of the humified organic matter.

3. Repeated applications of SS can increase the Cu, Zn and Cr concentrations, whose values in this experiment, however, never exceeded the maximum level established by Italian legislation.

It could be concluded that, at least in the investigated area with a clay soil, amounts much higher than 5 t ha^{-1} $year^{-1}$ (dry weight) FYM, and even higher levels of SS, should be added to soil to restore the fertility lost due to cultivation.

Acknowledgements The authors wish to thank Dr F. Manfredi of "REI PROGETTI" (Parma) for assistance with the field work. The research was supported by the University of Pisa.

References

Anderson TH, Domsch KH (1990) Application of eco-physiological quotients (qCO_2 and qD) to microbial biomasses from soils of different cropping histories. Soil Biol Biochem 22:251–255

Bascomb CL (1964). Rapid method for the determination of cation capacity of calcareous and non-calcareous soils. J Sci Food Agric 15:821–823

Bonmati M, Pujola M, Sana J, Soliva M, Felipo MT, Garau M, Ceccanti B, Nannipieri P (1985) Chemical properties, population of nitrite oxidizers, urease and phosphatase activities in sewage sludge-amended soils. Plant Soil 84:79–91

Bowman RA, Reeder JD, Lober RW (1990) Changes in soil properties in a central plains rangeland soil after 3, 20, and 60 years of cultivation. Soil Sci 150:851–857

Bremner JM, Mulvaney CS (1982) Nitrogen total. In: Page AL, Miller RH, Keeney DR (eds) Methods of soil analysis, part 2. Chemical and microbiological properties. American Society of Agronomy, Madison, Wis., pp 595–624

Chesire MV (1977) Origins and stability of soil polysaccharides. J Soil Sci 28:1–10

Collins HP, Rasmussen PE, Douglas, Jr CL (1992) Crop rotation and residue management effect on soil carbon and microbial dynamics. Soil Sci Soc Am J 56:783–788

Cottenie A, Verloo M, Velghe G, Kiekens L (1979) Analytical methods for plants and soils. Laboratory of Analytical and Agrochemistry, Ghent

Dalal R, Mayer RS (1986) Long-term trend in fertility of soils under continuous cultivation and cereal cropping in southern Queensland. II. Total organic carbon and its loss from the soil profile. Aust J Soil Res 24:281–292

Dick RP (1992) A review: Long-term effects of agricultural systems on soil biochemical and microbial parameters. Agric Ecosyst Environ 40:25–36

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28:350–356

Gupta SC, Dowdy RH, Larson WE (1977) Hydraulic and thermal properties of a sandy soil as influenced by incorporation of sewage sludge. Soil Sci Soc Am J 41:601–605

Gupta VVSR, Germida JJ (1988) Distribution of microbial biomass and its activity in different soil aggregate size classes as affected by cultivation. Soil Biol Biochem 20:777–786

Hopkins DW, Shiel RS (1996) Size and activity of soil microbial communities in long-term experimental grassland plots treated with manure and inorganic fertilizers. Biol Fertil Soils 22:66–70

Janzen HH (1987) Soil organic matter characteristics after long-term cropping to various spring wheat rotations. Can J Soil Sci 67:845–856

Jones CA (1984) Estimation of an active fraction of soil nitrogen. Commun Soil Sci Plant Anal 15:23–32

Kandeler E, Eder G (1990) Soil microbiological processes and aggregate stability of a 25-year permanent fallow plot under different mineral and organic fertilizer regimes. Mitt Dtsch Bodenkd Ges 62:63–66

Kladivko EJ, Nelson DW (1979) Changes in soil properties from application of anaerobic sludge. J Water Pollut Control Fed 51:325–331

Kuwatsuka S, Shindo H (1973) Behavior of phenolic substances in the decaying process of plants. I. Identification and quantitative determination of phenolic acids in the rice straw and its decayed products by gas chromatography. Soil Sci Plant Nutr 19:219–226

Levi-Minzi R, Riffaldi R, Saviozzi A (1986) Organic matter and nutrients in fresh and mature farmyard manure. Agric. Wastes 16:225–236

Lowe LE (1975) Fractionation of acid-soluble components of soil organic matter using polyvinyl pyrrolidone. Can J Soil Sci 55:119–126

Maciak F, Harms H (1986) The effect of agricultural utilization on the composition and yield of phenolic acids in low peat soils. Plant Soil 94:171–178

McGill WB, Cannon KR, Robertson JA, Cook FD (1986) Dynamics of soil microbial biomass and water-soluble organic C in Breton L after 50 years of cropping to two rotations. Can J Soil Sci 66:1–19

Molina JAE, Clapp CE, Larson WE (1980) Potentially mineralizable nitrogen in soil: the simple exponential model does not apply to the first 12 weeks of incubation. Soil Sci Soc Am J 44:442–443

Murwira HK, Kirchmann H, Swift MJ (1990) The effect of moisture on the decomposition rate of cattle manure. Plant Soil 122:197–199

Oades JM, Kirkman MA, Wagner GH (1970) The use of gas-liquid chromatography for the determination of sugars extracted from soils by sulfuric acid. Soil Sci Soc Am Proc 34:230–235

Olsen SR, Cole CV, Watanabe FS, Dean LA (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. US Dept of Agric Circular 939

Pal D, Broadbent FE (1975) Influence of moisture on rice-straw decomposition in soil. Soil Sci Soc Am Proc 39:59–63

Powlson DS, Brookes PC, Christensen BT (1987) Measurement of soil microbial biomass provides an early indication of changes in total soil organic matter due to straw incorporation. Soil Biol Biochem 19:159–164

Prism (1994) Graph Pad Prism. Graph Pad, San Diego, Calif.

Purves D (1977) Trace element contamination of the environment. Elsevier, Amsterdam

Rasmussen PE, Collins HP (1991) Long-term impacts of tillage, fertilizer, and crop residues on soil organic matter in temperate semiarid regions. Adv Agron 45:93–134

Reganold JP, Palmer AS (1995) Significance of gravimetric versus volumetric measurements of soil quality under biodynamic, conventional, and continuous grass management. J Soil Water Conserv 50:298–305

Riffaldi R, Saviozzi A, Levi-Minzi R (1993) The effect of forty years of continuous corn cropping on soil biochemical properties. Trends Agric Sci 1:47–51

Riffaldi R, Saviozzi A, Levi-Minzi R, Menchetti F (1994) Chemical characteristics of soil after 40 years of continuous maize cultivation. Agric Ecosyst Environ 49:239–245

Saviozzi A, Levi-Minzi R, Riffaldi R (1994) Forty years of continuous corn cropping on soil organic matter characteristics. Plant Soil 160:139–145

Schnurer J, Rosswall T (1982) Fluorescein diacetate hydrolysis as a measure of total microbial activity in soil and litter. Appl Environ Microbiol 43:1256–1261

Schnurer J, Clarholm M, Rosswall T (1985) Microbial biomass and activity in an agricultural soil with different organic matter contents. Soil Biol Biochem 17:611–618

Sequi P, De Nobili M, Leita L, Cercignani G (1986) A new index of humification. Agrochimica 30:175–179

Seyfried MS, Rao PSC (1988) Kinetics of nitrogen mineralization in Costa Rican soils: model evaluation and pretreatment effects. Plant Soil 106:159–169

SISS (1995) Metodi normalinati di analisi del suolo. Edagricole, Bologna

Sommerfeldt TG, Chang C (1985) Changes in soil properties under annual applications of feedlot manure and different tillage practices. Soil Sci Soc Am J 49:983–988

Strickland TC, Sollins P (1987) Improved method for separating light and heavy-fraction organic matter from soil. Soil Sci Soc Am J 51:1390–1393

Swaine DJ (1962) The trace element content of fertilizers. Commonwealth Bureau of Soils, technical communication no. 52. Commonwealth Agricultural Bureaux, Farnham Royal, Bucks

Tabatabai MA, Bremner JM (1972) Assay of urease activity in soils. Soil Biol Biochem 4:479–483

Thomas GW (1982) Exchangeable cations. In: Page AL, Miller RH, Keeney DR (eds) Methods of soil analysis, part 2. Chemical and microbiological properties. American Society of Agronomy, Madison, Wis., pp 159–165

Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass carbon. Soil Biol Biochem 19:703–707