Effect of long-term organic amendments on chemical and microbial properties of a luvisol

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ABSTRACT

We studied the long-term effect (about 45 years) of farmyard manure, sewage sludge and compost application in two increments on organic carbon (C_{org}), the amount (C_{mic}) and activity of the microbial biomass (soil respiration, dehydrogenase activity), total N content and N delivery of soils as compared to manuring with mineral fertilizers. The application of both increments of compost and the high sewage sludge application rate resulted in an increase in C_{org} while soils treated with both compost application rates and the high farmyard manure application rate showed a significant increase in C_{mic}. C_{mic}/C_{org} ranged between 1.7 and 3.3. Dehydrogenase activity and soil respiration were the greatest in the soil with the highest compost and farmyard manure application rates. Total soil N content was significantly higher in both compost treatments and in the treatment with the high sewage sludge application rate. This was accompanied by the highest N uptake of ryegrass.

Keywords: long-term field experiment; enzyme activity; microbial activity; N delivery; organic manure

All over the world, land application of organics produced by urban population and farmers (compost, sewage sludge, farmyard manure) is a widespread practice that allows a reuse of the increasing amounts of biosolids. They provide organic matter and therefore improve soil physical parameters such as structure and water holding capacity. Furthermore, biosolids are a source of different plant nutrients like nitrogen and phosphorus as well as micronutrients (Scherer and Sharma 2002). Therefore land application of organic wastes is an effective means of the reuse of these by-products.

Long-term application of sewage sludge, compost and farmyard manure resulted in an increase the total C and N content of the soil (Werner et al. 1988). This increase is the most pronounced in plots treated with compost and therefore the application of compost to agricultural soils should be especially recommended.

The effects of biosolids on soil mircoorganisms have also received particular attention. Following application of biosolids the size and activity of soil microbial biomass, which is widely recognized as an important agent in soil organic matter turnover, might be generally expected to increase in response to added C and N as well as other nutrients (Madsen et al. 1994). According to Werner et al. (1988) soil respiration is positively influenced by continuous sewage sludge and compost supply. Even thirteen years after a single sewage sludge application Barbarick et al. (2004) found higher microbial activity in comparison with mineral fertilizer application. However, a decrease in microbial biomass as well as its activity might be expected if biosolids contain toxic heavy metals. Chandler and Brookes (1991) found that biomass size generally decreased with increasing soil metal concentrations. These conflicting reports emphasize the need for more research on long-term effects of biosolids application on soil quality and productivity.

The effects of management practices on soil fertility criteria are best evaluated using long-term field experiments. Therefore we collected soil samples from plots of a field experiment, established in 1962, supplied with compost, sewage sludge, farmyard manure and mineral fertilizer, respectively in two increments. Our objective was to compare the effects of continuous application of different organic residues with those of mineral fertilizer on chemical soil parameters as well as on the size and activity of the soil microbial community.

MATERIAL AND METHODS

Field experiment. A long-term field experiment (randomized complete block design with four replicates) was established at the experimental farm of INRES-Plant Nutrition (50°32'42''N, 6°59'14''E), University of Bonn, Germany, on a luvisol derived from loess (17.8% clay, 76.3% silt, 5.9% sand) in 1962, following a cereal-root crop sequence. The treatments selected for the present investigations were: mineral fertilizer (MIN), 4.5 t and 9.0 t/ha FYM (FYM1 and FYM2, respectively), 1.86 t and 7.44 t/ha sewage sludge (SS1 and SS2, respectively), and 12.25 t and 49 t/ha compost from organic household waste (COM1 and COM2, respectively). These amounts (given on a dry weight basis) were applied every second year until 1997, then the amounts were changed to 5 and 20 t/ha sewage sludge for SS1 and SS2 and to 30 and 120 t/ha compost for COM1 and COM2, once in 3 years. The application rates of FYM and mineral fertilizer were kept at the same level, but they were applied every third year. From 1997 the crop sequence was cereal-cereal-root crop.

Mean amounts of carbon and nitrogen applied with organic amendments ha/year are shown in Table 1.

Soil sampling. Composite samples of 5 soil cores (3 cm diameter, 30 cm depth) were collected from each of the four field-replicate plots at the end of February 2006. Soil samples were placed in a cool box (+4°C) immediately in the field and afterwards sieved (4 mm mesh). One part was air-dried and stored at room temperature for determination of chemical properties. The other part was kept field-moist and stored sealed at –20°C for microbiological and biochemical analyses. For the determination of mineral N (N_{min}) soil samples were taken at the end of February 2006 from the layer 0–90 cm.

Analysis. Total organic C and total N values were determined by dry combustion using a Carlo-Erba NA 1500 carbon/nitrogen/sulphur analyzer.

Soil respiration. A microcentrifuge tube, containing 50 mL 0.1 mol/L NaOH was placed inside a glass jar with 50 g soil, which was then tightly capped. After a 48 h incubation at 23°C CO_2 trapped in NaOH was precipitated as $BaCO_3$ by the addition of excess $BaCl_2$. After adding a few drops of phenolphthaline as an indicator, the unreacted NaOH was titrated with 0.2 mol/L HCl.

Dehydrogenase activity. This was measured according to the method of Trevors (1984). 1 g soil was exposed to 1 mL of 0.4% INT-solution (2-*p*-iodo-3-nitrophenyl 5-phenyl tetrazolium chloride) and 50 µL of 1% glucose solution and incubated for 45 h at 23°C. The iodonitrotetrazoliumformazan (INF) was extracted with 10 mL methanol by shaking vigorously. INTF was measured in a spectrometer at 485 nm. Controls were prepared without substrate.

Soil microbial biomass. The chloroform-fumigation-incubation-assay of Jenkinson and Powlson (1976) was used to estimate the amount of microbial biomass carbon (C_{mic}) in soil samples. A k_c-factor of 0.41 was used for the estimation at 22°C.

Inorganic N. Extraction of field moist soil samples by shaking a 5:1 ratio of 2 mol/L K_2SO_4: soil for 2 h on a reciprocal shaker. Nitrate and ammonium were analyzed using an Autoanalyzer (Bran + Luebbe, Germany).

Pot experiment with *Lolium perenne*. In a completely randomized block design with 4 replications we investigated the N uptake of ryegrass (*Lolium perenne*), which was cut twice. Soil samples (1300 g air dried soil/pot) from the selected plots of the above mentioned long-term field experiment were supplied with 0.2 g K and 0.08 g P as K_2HPO_4 before planting. The water capacity was adjusted to 70% of the maximum water holding capacity and controlled every day. Plants were grown in a greenhouse and harvested at 5 and 8 weeks, respectively, after sowing.

Statistical analysis. Data were statistically treated with the SPSS 12.0 software (Oneway Anova, Tukey-HSD, $LSD_{0.05}$).

RESULTS AND DISCUSSION

In our field experiment we found a significant increase in the total N content of the soil after long-term application of the high amount of sewage sludge (SS2) as well as both compost application rates (COM1, COM2) as compared to mineral

Table 1. Mean amounts of carbon and nitrogen applied with organic amendments in the different treatments (kg/ha/year)

Treatment	Carbon	Nitrogen
FYM1	627	38
FYM2	1253	77
SS1	387	65
SS2	1549	258
COM1	2251	213
COM2	9002	854

Table 2. Influence of long-term application of organic wastes and mineral N fertilizer on soil pH, total N, C_{org}, C_{mic} and C_{mic}/C_{org} (soil samples were taken from the soil layer from 0–30 cm in spring 2006)

Treatment	pH	N_t (%)	C_{org} (%)	C_{mic} (µg/g air-dry soil)	C_{mic}/C_{org} (%)
MIN	6.1	0.109	1.238	271	2.2
FYM1	6.0	0.115	1.315	318	2.5
FYM2	6.2	0.131	1.473	504	3.3
SS1	6.0	0.113	1.306	219	1.7
SS2	6.5	0.151	1.983	364	1.8
COM1	6.7	0.145	1.745	406	2.3
COM2	7.1	0.227	2.775	513	1.9
$P < 0.05$	0.4	0.024	0.482	130	

fertilizer application (MIN) (Table 2). Depending on the proportion of ammonia in sewage sludge, up to 25% of total N is plant available in the year of application (Scherer and Steffens 1990), while in compost the amount of plant available N is closely related to the degree of maturity, being lower in mature compost (Crecchio et al. 2001). It seldom exceeds 10% of the total compost N in the year of application (Scherer et al. 1996). With the high compost application rate we applied about 850 kg/ha N each year (Table 1), while only about 80 kg/ha N was plant-available in the year of its application. Therefore N accumulation in soils is to be expected. However, it should be kept in mind that the high amounts of compost applied until 1997 were several times higher than prescribed in the compost ordinance of Germany.

Mineralization and mineral cycling of enriched organic N compounds in the soil are of considerable agronomic importance. In spring 2006 we found the lowest amount of plant available N, derived from mineralization in the treatment with mineral fertilizer (MIN) and the low farmyard manure application rate (FYM1) (both 88 kg/ha) and the highest amount in the treatment with high compost application rate (COM2) (189 kg/ha N) (Figure 1). This high N delivery potential was confirmed in an incubation experiment (Figure 2). The daily N delivery rates were 3.7 µg N/g soil/day (MIN), 5.7 µg N/g soil/day (FYM2), 4.5 µg N/g soil/day (SS2) and 7.6 µg N/g soil/day (COM2) (Figure 2). High amounts of plant available N in spring as well as the high N delivery potential may be attractive from the economic point of view of farmers. Despite the higher N delivery of the soils with organic amendments we could not find a correlation between total soil N and the N delivery rates, while in laboratory incubation trials of Bosch and Amberger (1983) nitrogen mineralisation was closely correlated with total soil N. Therefore we assume that besides total N other factors must be taken into consideration, for example the composition of the organic soil N, which depends on the kind of the organic amendments (Scherer et al. 1985).

In addition to the incubation experiment we conducted a pot experiment with ryegrass to determine the N delivery potential. Because the soil samples were supplied with all nutrients except N before planting, N taken up by plants must be derived from the available soil mineral N at the start of the experiment as well as from N mineralized throughout the growing period. However, total N uptake of the above ground material of the two harvests only ranged between 23 mg (MIN) and 57 mg N/pot (COM2) (Figure 3), being lower than the amount of plant available N at the start of the experiment. Therefore we suggest that N delivery was negligible in the short period of time of the pot experiment, a conclusion that is supported by the highly significant correlation (r = 0.96) between total N uptake of the two grass cuts and plant available N before planting. Even we could

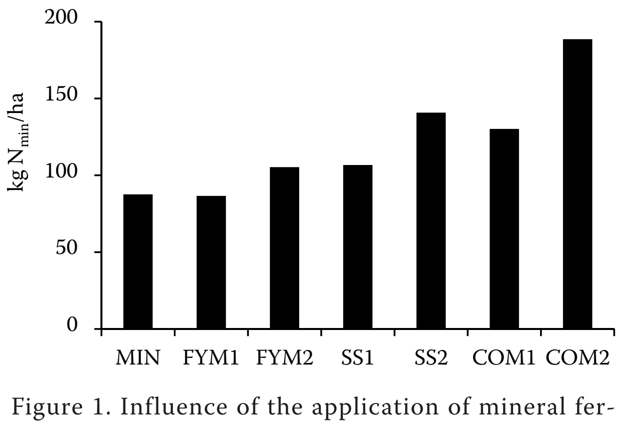

Figure 1. Influence of the application of mineral fertilizer and organic wastes on the N_{min} content in the soil layer from 0–90 cm in spring 2006

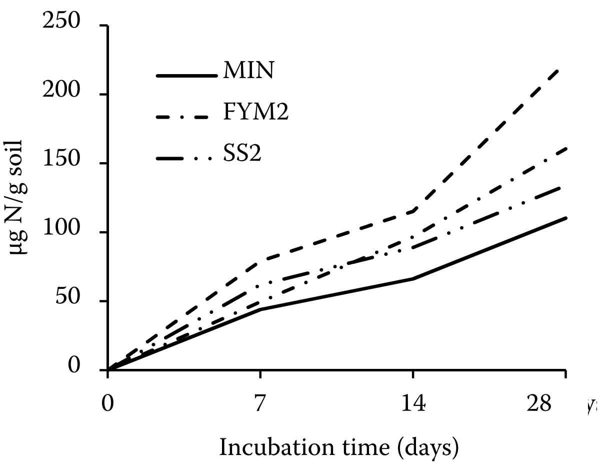

Figure 2. Influence of long-term application of mineral fertilizer and organic wastes on N delivery in an incubation experiment (soil samples were taken from the soil layer from 0–30 cm in spring 2006)

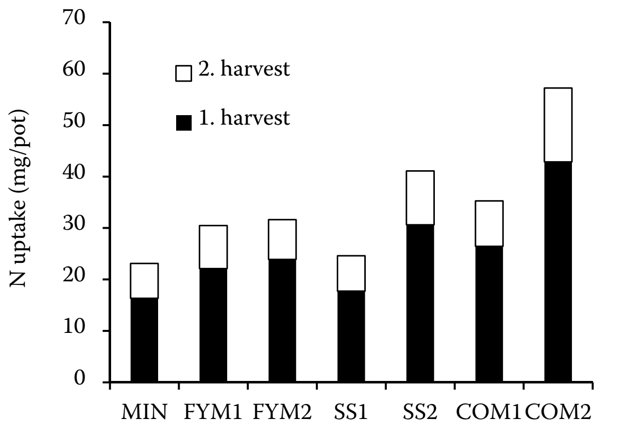

Figure 3. Influence of long-term application of mineral fertilizer and organic wastes on N uptake by *Lolium perenne* in a pot experiment ($LSD_{0.05}$ = 7) (soil samples were taken from the soil layer from 0–30 cm in spring 2006)

clearly demonstrate that there were positive effects of organic amendments on microbiological soil parameters, it may be concluded that plant growth in our experiment was inhibited by the increased heavy metal content (Werner et al. 1987). For this reason organic amendments must be viewed cautiously because of the increase of the soil content of toxic metals (Leita et al. 1999).

After about 45 years of repeated applications of farmyard manure (FYM1, FYM2) and the low rate of sewage sludge (SS1) the C_{org} content did not significantly differ from the control (MIN) (Table 2). However, repeated high application of sewage sludge increased C_{org} by 57% compared with the control, while compost applications resulted in an increase of C_{org} by 41% (low application rate) and 124% (high application rate), respectively. We propose that these differences in the C_{org} content are the result of the different amounts of C applied with the organic materials (Table 1) as well as the varying stability of the C compounds supplied with the different biosolids.

The beneficial effect of sewage sludge on microbial biomass was less pronounced (Table 2), which is in accordance with the results of Brookes and McGrath (1984), who found a 50% reduction of microbial biomass in sludged soils as compared with soils receiving farmyard manure. Smith (1991) attributed reduced biomass in sludged soils to the toxic effects of heavy metals in sludge on the soil microbial biomass. Therefore we assume that in our experiment this reduction may be caused by increased Zn, Cr, and Cd concentrations in the soil after repeated sewage sludge application (Werner 1994).

While C_{mic} typically averages 2.3 and 2.9% of C_{org} (Rasmussen and Collins 1991), we recorded C_{mic}/C_{org} ratios between 1.7 and 3.3 (Table 2). We assume that this difference in our field experiment is primarily due to the type and amounts of organic input. Further differences in available resources and their partitioning among different groups of microorganisms may also contribute to the wider range of C_{mic}/C_{org} ratios in our investigations. Insam et al. (1989), finding low correlations between these two parameters in North American soils, concluded that the relationship is dependent on factors other than C_{org}. They state that the C_{mic}/C_{org} ratio is influenced by the macroclimate, particularly the combined variables of precipitation and evaporation.

Microbial biomass alone does not provide information on microbial activity. Therefore measurements of microbial biomass turnover, such as soil respiration, which is considered to reflect the availability of carbon for microbial maintenance, are required for that assessment. In our experiment respiratory activity was the highest in the treatment with the high compost application rate (Figure 4), followed by the high farmyard manure application rate and the lowest in the treatment FYM1 followed by the treatment MIN. Confirming results of Brookes et al. (1984) we cannot detect an influence of heavy metals on soil respiration. In our investigations soil respiration was the highest in the treatment with the high compost application rate, which also contains the highest total content of the heavy metals Cu, Cd, Cr, Ni, Pb, and Zn (data not shown). Therefore we emphasize that

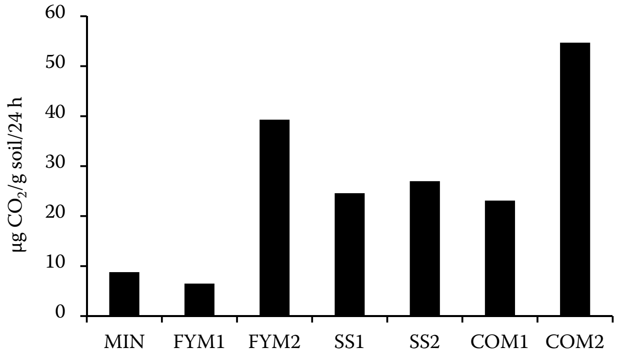

Figure 4. Influence of long-term application of mineral fertilizer and organic wastes on soil respiration ($LSD_{0.05}$ = 14) (soil samples were taken from the soil layer from 0–30 cm in spring 2006)

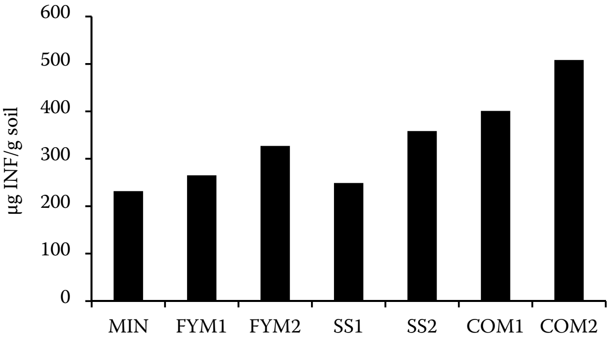

Figure 5. Influence of long-term application of mineral fertilizer and organic wastes on dehydrogenase activity ($LSD_{0.05}$ = 67) (soil samples were taken from the soil layer from 0–30 cm in spring 2006)

the toxicity of heavy metals presumably depends on their bioavailability and not on the total concentration, because when applied together with biosolids heavy metals are largely unavailable due to the formation of insoluble organo-metal complexes (Davis and Carlton-Smith 1981). This is in accordance with data obtained by Brookes and McGrath (1984) who reported that the mineralization rate of organic N is similar in soil samples from a historically sludged experimental site with high heavy metal concentrations and in a low metal soil that had only received farmyard manure. For this reason we assume that differences in soil respiration between treatments are indicative of variable amounts of organic carbon, accumulated with different organic manures, as well as a varying stability of C_{org}.

Enzymes produced by soil microorganisms catalyze biochemical processes involved in nutrient cycling in soil and may also provide an index of microbial activity (Smith 1991). However, information available on the effects of compost or sewage sludge applied to soil on enzyme activity is often contradictory and both beneficial and detrimental responses were reported.

Dehydrogenase is involved in the oxidation of soil organic matter. Its activity is stimulated by the incorporation of organic amendments to soil because the added material may contain intra- and extracellular enzymes (Liang et al. 2005). Thus, we propose that the treatments with the high organic amendments and therefore the highest soil organic C amendments may also exhibit the greatest dehydrogenase activity. Indeed, we found this to be the case (Figure 5), suggesting the availability of a high quantity of biodegradable substrates and hence an improvement of microbial activity. Confirming the results of Kowalczyk and Schröder (1988) we found a positive correlation (r = 0.73) between dehydrogenase activity and microbial biomass. However, we cannot confirm that heavy metals, which are the most important inorganic pollutants added to soils with sewage and compost (Leita et al. 1999), decrease the turnover rate of organic matter, presumably because of the inhibitory effects on microbial biomass and enzyme activity (Chandler et al. 1995).

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