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Enzyme activity and microbial biomass in a field soil amended with municipal refuse

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Summary. Changes in enzyme activity levels, in biomass-C content, and in the rate of fluorescein diacetate hydrolysis were measured in a loamy soil to which solid municipal refuse had been applied as compost over a 3-year period at two different rates. Addition of the compost caused significant increases in the activity of all enzymes tested. The increases were much higher at 90 t ha^{-1} $year^{-1}$ than at 30 t ha^{-1} $year^{-1}$. Significant increases were also observed in the biomass-C content and in the rate of fluorescein diacetate hydrolysis. Significant correlations among changes in biomass-C content and the rate of fluorescein diacetate hydrolysis and the changes in all enzymes tested were found.

Two activity indices were calculated; a biological index of fertility and an enzyme activity number. No correlations were found between the biological index of fertility and the changes in the various enzyme activities. However, significant correlations were found either between enzyme activity number and most of the changes in enzyme activity, or between the enzyme activity number index and the biomass-C content ($r = 0.850$). The use of a new activity index, the hydrolysis coefficient, is proposed. This coefficient was significantly correlated with biomass-C content ($r = 0.925$) and with the enzyme activity number index ($r = 0.780$).

Key words – Biological index of fertility – Biomass-C content – Enzyme activity – FDA hydrolase – Soil fertility

The gradual decrease in the organic matter content of cultivated soils, which can lead to loss of soil fertility, is particularly worrying. To counteract this depletion, any management practice that maintains or increases soil fertility, thus optimizing crop yields, should be encouraged.

In recent years, the addition of municipal refuse to agricultural land as compost has been recognized as a cost-effective method of waste disposal, a useful source of organic material, and a means of improving soil physical properties (Gallardo-Lara and Nogales 1987). Unfortunately, the observations reported by numerous studies have produced conflicting and confusing results (De Bertoldi et al. 1987; Perucci 1990), because these composts generally contain potentially undesirable materials, such as glass, plastic, and particularly heavy metals, accumulations of which can create serious problems for crops. High concentrations of heavy metals in soil have harmful effects on soil microorganisms (Brookes et al. 1986), especially in soils where the organic matter content is low or has declined (McGrath et al. 1988; Giller et al. 1989). In contrast, Perucci (1990), in analysing the changes in some biochemical properties in a soil amended with municipal refuse and maintained under laboratory conditions for 1 year, reported that the use of the compost improved many parameters related to soil microbiological activity. In view of these results further information on the changes in microbiological activity levels in a municipal refuse-amended soil may contribute to a greater understanding of the factors that influence soil fertility, whether positively or negatively, since many processes related to soil fertility are mediated by microorganisms.

Soil enzyme activity is a key feature of plant nutrient and cycling processes, and therefore measurements of specific enzyme activities may be useful in determining soil biological activity, which might be used as an index of soil fertility. At the moment, the determination of specific enzyme activities (e.g. phosphatases, urease, amylase, etc.) or other parameters (e.g. ATP content, respiration, adelylate energy charge, etc.) together with the use of general soil parameters seems to be the best approach for evaluating the state of soil microbial activity and for understanding its response to compost amendments, cultivation practices, and environmental factors (Nannipieri et al. 1990).

The main aim of the present research was to investigate the behaviour of some parameters related to soil fertility in a field soil to which solid municipal refuse had been applied as compost over a 3-year period at two different rates. To achieve this objective, the specific activity of some enzymes (amylase, arylsulfatase, catalase,

deaminase, dehydrogenase, phosphatases, and protease), the biomass-C content and the rate of fluorescein diacetate hydrolysis were measured. In addition, an attempt was made to use a new activity index to rapidly determine either the changes in metabolic activity following a particular management practices or the metabolic activity status of a soil.

Materials and methods

Chemicals and apparatus

Sodium p-nitrophenyl phosphate, sodium *p*-nitrophenyl sulphate, sodium bis-*p*-nitrophenyl phosphate, 2,3,5-triphenyltetrazolium chloride and 1,3,5-triphenylformazan were obtained from Sigma (St. Louis, Missouri, USA), and 30% H_2O_2 was obtained from BDH (Dorset, England). Casein, 1,2-diaminonitrobenzene, tyrosine, *p*-nitrophenol and starch were purchased from Carlo Erba (Milan, Italy). Fluorescein diacetate (3',6'-diacetylfluorescein) was obtained from Aldrich-Chemie (Steinheim, Germany).

A Varian model Cary 210 double-beam grating spectrophotometer was used to determine the release of *p*-nitrophenol and tyrosine, and the 1,2-diaminonitrobenzene residue.

Soil and compost

The experiment was performed in a loamy soil classified as a Fluventic Xerochrept (pH 8.3; 30.0, 41.7, and 28.3% sand, silt, and clay, respectively) located in Umbria, a region in Central Italy.

Solid municipal refuse, composted under aerobic conditions by a fast fermentation method (25 days), was supplied by Gesenu SpA (Perugia, Italy).

Chemical properties of the soil and the compost are shown in Table 1. All determinations were made according to recommendations by the Italian Soil Science Society (Società Italiana Scienza del Suolo 1985).

Experimental field

The experimental field was planned according to a random block scheme (Le Clerg et al. 1962) with four replications, each with a surface area of 56.25 m^2. The compost was dug into the soil in spring, starting from March 1988, to a depth of 10–15 cm depth, at 30 and 90 t ha^{-1} $year^{-1}$. The experiment was carried out for 3 years.

Table 1. Some chemical properties of soil and composted solid municipal refuse

	Soil	Compost
pH	8.30	7.60
Organic C (%)	0.82	27.43
Total N (%)	0.09	1.91
Total P (%)	0.06	0.92
Total K (%)	0.73	1.10
C:N	9.1	14.4
Total Cu (ppm)	34	240
Total Zn (ppm)	68	647
Total Cr (ppm)	31	81
Total Ni (ppm)	64	52
Total Pb (ppm)	81	750
Total Cd (ppm)	<0.02[a]	5

All data are expressed on a dry weight basis (105 °C), and represent the mean of three determinations which do not differ from ±5%. The compost data are means for 3 years of experiment.

[a] Limit of sensitivity of the method used

Simultaneously, an analogous experiment, in which corn was planted, was carried out.

One and seven months after the compost amendment, samples of the soil control (0 t ha^{-1}) and the amended soils were collected from the surface layer (0–20 cm), partly air-dried at room temperature, passed through a 0.5-mm sieve, stored in the dark in plastic boxes at 4 °C, and analysed.

Enzyme activity assays

The assays of various enzyme activities were based on the release and quantitative determination of the product in a reaction mixture, the soil samples being incubated with a suitable substrate and a suitable buffer solution.

Assays were performed to determine the activity of amylase (EC 3.2.1; Sparling 1981), arylsulphatase (EC 3.1.6.1; Tabatabai and Bremner 1970), catalase (EC 1.11.1.6; Roberge 1978), "deaminase", a term used to describe an unclassified enzyme capable of hydrolysing 1,2-diaminonitrobenzene in soil (Killham and Rashid 1986), dehydrogenase (Casida et al. 1964), phosphomonoesterase alkaline (EC 3.1.3.2; Eivazi and Tabatabai 1977), phosphodiesterase (EC 3.1.4; Eivazi and Tabatabai 1977) and protease (casein-hydrolysing proteases; Ladd and Butler 1972).

Microbial biomass

A direct extraction method was used to estimate soil microbial biomass. Duplicate samples (20 g) of the control and amended soils were fumigated with ethanol-free $CHCl_3$. After removal of $CHCl_3$, soil moisture content was adjusted to 60% water-holding capacity. Fumigated and unfumigated soil samples were then extracted with 0.5 *M* K_2SO_4 as described by Sparling and West (1988). Biomass C was estimated as the difference between C extracted from the fumigated and non-fumigated treatments, multiplied by 264 (Vance et al. 1987).

Fluorescein diacetate hydrolase

The rate of fluorescein diacetate hydrolysis was estimated according to the Swisher and Carroll method (1980). Fluorescein diacetate was dissolved in acetone (2 mg ml^{-1}) and stored as a stock solution at −20 °C. To 5 g of soil in 100 ml phosphate buffer (60 m*M*; pH 7.6), 0.5 ml fluorescein diacetate solution was added, and the mixture was incubated at 25 °C on a rotary shaker. Hydrolysis of the fluorescein diacetate was stopped after 2 h by the addition of acetone (final concentration 50% v:v), and the concentration of hydrolysed fluorescein diacetate was determined at 490 nm after removal of the soil by centrifugation and filtration.

Results and discussion

Enzyme activities

Recently, Perucci (1990) in an experiment carried out for 1 year under laboratory conditions, observed that a soil amended with composted solid municipal refuse reached a maximum biomass level 1 month after the amendment. The same study further showed that enzyme activity was also highest 1 month after the amendment and then slowly decreased to reach a plateau 6 months later. Therefore, in the present experiment, soil samples were collected 1 and 6 months after addition of the compost.

All the measured enzyme activities increased after addition of the compost (Fig. 1), and the values measured at 90 t ha^{-1} were significantly different from those at 30 t ha^{-1}. In particular, deaminase and protease activity tended to increase over the 3 years of the experiment. The other enzymes reached maximal activity levels after each addition of compost, with slightly lower values following

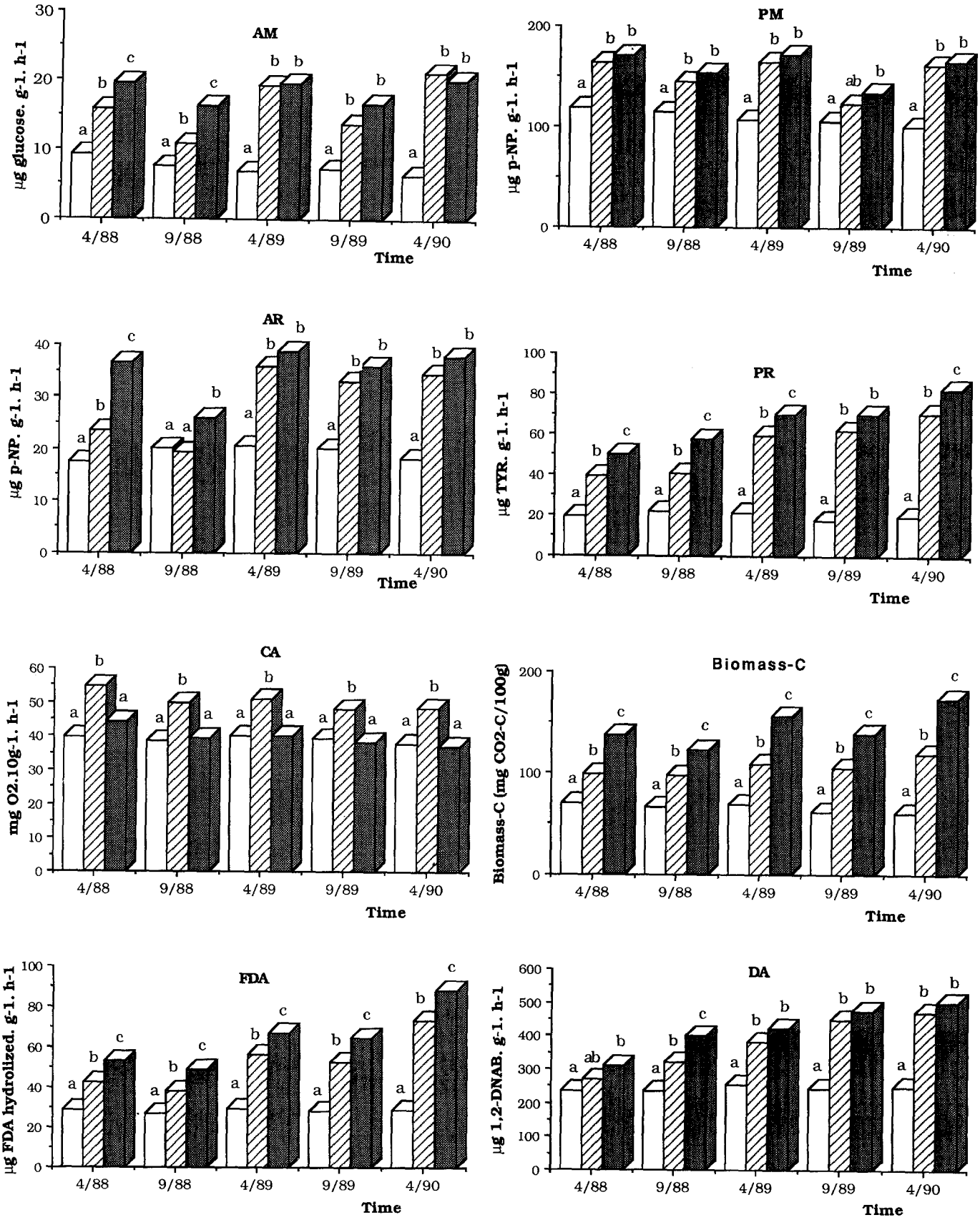

Fig. 1. Changes in enzyme activity values, biomass C, and in fluorescein diacetate (*FDA*) hydrolase during a 3-year experiment in control soils (□) and soils amended with composted solid municipal waste at 30 (▨) and 90 t ha^{-1} $year^{-1}$ (▩). Each value is the mean of four replicates. Means associated with the same letter for the same time are not significantly different ($P<0.05$, Duncan's multiple range test). *AM*, amylase; *AR*, arylsulphatase; *CA*, catalase; *DA*, deaminase; *DH*, dehydrogenase; *PD*, phosphodiesterase; *PM*, phosphomonoesterase; *PR*, protease; *p-NP*, *p*-nitrophenol; *1,2-DNAB*, 1,2-diaminonitrobenzene; *TPF*, 1,3,5-triphenylformazan; *TYR*, tyrosine

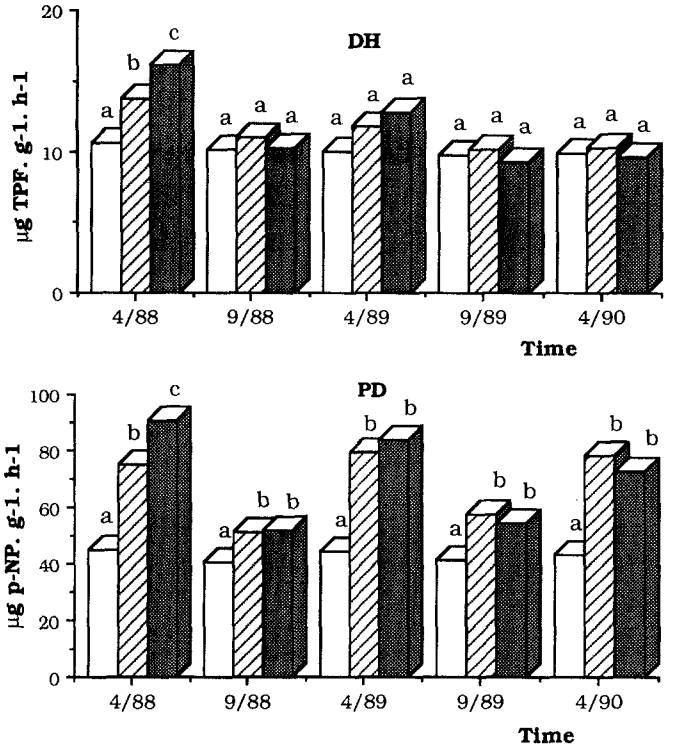

Fig. 1 (continued)

successive applications. In general, the increases in activity may be ascribed either to the easily biodegradable organic matter included with the compost, which stimulating the growth of soil microorganisms, increasing the activity of each enzyme, or to the fact that, along with the increase in organic matter an addition of exogenous microorganisms, grown during the composting process, also occurred. The decreases may be attributed to a decrease in the level of microorganisms, in particular of that fraction brought with the compost, which was less competitive than the endogenous microorganism fraction, or to exhaustion of the easily biodegradable organic matter, or to a toxic effect of undesiderable materials introduced into the soil with the compost. In addition to these general considerations, the strong relationship observed between phosphodiesterase and phosphomonoesterase activities and the soil P concentration, 6 months after the compost amendment, must not be disregarded. It is known that an increase in available soil P may be accompanied by a decrease in soil phosphatase activity (Juma and Tabatabai 1978; Perucci and Scarponi 1985).

Dehydrogenase activity

Soil dehydrogenase activity is thought to reflect the total range of oxidative activity of soil microflora, and consequently may be a good indicator of microbiological activity (Skujins 1973), it has been shown to increase with the addition of nutrients to the soil (Ladd and Paul 1973). In the present experiment, a significant increase in dehydrogenase activity occurred after the first application of compost, but a slow and progressive decrease in activity followed, at both rates. These findings can be attributed to the toxic effect exerted by the high heavy metal concentrations in this compost, especially of Pb (Doelman and Haanstra 1979; Reddy and Faza 1989). Among oxidoreductase enzyme systems, also for catalase and the various dehydrogenases, there is an intracellular enzyme that has weak activity in soil since it is adsorbed on clay and humic substances when it is outside microbial cells (Stotzky 1974; Peres-Mateos et al. 1988). The addition of composted solid municipal refuste appeared to have same effect on dehydrogenase activity.

However, although many authors have found positive correlations between the activities of these enzymes and the soil organic matter content, the present study showed no correlation between catalase or dehydrogenase activity and the activity of other enzymes, nor with biomass-C levels or fluorescein diacetate hydrolysis. These two enzymes were probably very sensitive to the great amounts of heavy metals introduced into the soil with the amendments, which were likely to have exerted a strong inhibiting effect that masked the positive effect of the organic matter addition. These findings make it difficult to consider dehydrogenase activity as an effective indicator of overall microbial activity.

Soil biomass

Biomass measurements have been used as an early indication of the response by the organic matter cycle to management changes in soils. In addition, measurements of microbial biomass show a more rapid response than those of organic C to changes in organic matter or to the rate of decomposition (Nannipieri 1984). In the present research, the biomass-C content was significantly increased

Table 2. Correlation coefficients

	AM	AR	CA	DA	DH	PD	PM	PR	BIF	Biomass C	EAN	FDA
AM	–	0.897**	0.322	0.787*	0.381	0.982**	0.925**	0.905**	0.352	0.889**	0.930**	0.985**
AR		–	0.110	0.837**	0.286	0.801**	0.728**	0.906**	0.114	0.880**	0.767**	0.989**
CA			–	0.055	0.386	0.458	0.435	0.152	0.950**	0.007	0.406	0.071
DA				–	0.161	0.500	0.575*	0.965**	0.095	0.853*	0.663**	0.925**
DH					–	0.658**	0.553*	0.071	0.429	0.733**	0.866**	0.707**
PD						–	0.903**	0.682**	0.418	0.288	0.512	0.045
PM							–	0.758**	0.473	0.819**	0.981**	0.743**
PR								–	0.192	0.928**	0.822**	0.965**

* $P<0.05$, ** $P<0.01$. 13 degrees of freedom. AM, amylase; AR, arylsulphatase; CA, catalase; DA, deaminase; DH, dehydrogenase; PD, phosphodiesterase; PM, phosphomonoesterase; PR, protease; BIF, biological index of fertility; EAN, enzyme activity number index; FDA, fluorescein diacetate hydrol

by the compost amendment, and the increases were dependent on rates of application. In particular, for both rates, the maximum biomass-C content always occurred 1 month after each amendment, and successively, small but non-significant decreases were observed 6 months after an amendment (Fig. 1). This finding is not surprising, because these decreases may be ascribed to C mineralization.

Fluorescein diacetate hydrolysis

The rate of hydrolysis of fluorescein diacetate by soil has been considered a suitable index of overall enzyme activity, because this hydrolysis is carried out by active cells with a variety of enzymes, including lipases, proteases, and esterases (Schnurer and Rosswall 1982). The rate of fluorescein diacetate hydrolysis was significantly increased by the compost, at both rates, with a gradual increase to maximum values at the end of the experiment (Fig. 1). These findings are in accord with a positive effect on soil microbial activity following the amendment, as observed for the biomass-C content.

Activity index

In order to evaluate the metabolic activity of soil, some authors determine specific enzyme activities (phosphatase, urease, etc.), but often, the measure of specific enzyme activities, though useful, does not seem a sufficient evaluation of the potential microbial activity in soil, since different experimental conditions can produce conflicting results. Other authors, to abviate this disadvantage, have proposed general criteria for measuring microbiological activity in soil, e.g., respiration, ATP, AEC, (Nannpieri et al. 1990).

Therefore, to verify that in this type of experiment, besides specific enzyme activities, the parameters mentioned above (dehydrogenase activity, biomass C, and fluorescein diacetate hydrolysis) can also be used to evaluate microbiological activity in soil, an analysis of variance was performed. Table 2 shows the correlation coefficients for various enzyme activities and various soil parameters used as indicators of soil metabolic activity. Biomass C and fluorescein diacetate hydrolysis were correlated with most enzyme activities, but dehydrogenase activity was not correlated with any specific enzyme activity tested.

In recent years, some authors have criticized the use of enzyme activity values as indicators of overall microbiological activity in soil. Stefanic et al. (1984) and Beck (1984) proposed the use of empirical indices, a biological index of fertility and the enzyme activity number index. The biological index of fertility (BIF) is calculated from dehydrogenase (DH) and catalase (CA) activity values according to the formula proposed by Stefanic et al. (1984): $BIF = (DH + kCA)/2$; where k is a proportionality coefficient. The analysis of variance (Table 2) showed no significant correlations, either between this index and the various enzyme activities, or with biomass C ($r = 0.004$). A correlation was found only with catalase activity, but this was not significant because, with the proposed formula, there is a direct correspondence between the biological index value and catalase activity. In contrast, the enzyme activity number (EAN) index, calculated from the formula proposed by Beck (1984); EAN = 0.2(mg TPF + catalase number/10 + μg phenol/40 + mg amino-N/2 + amylase number/20), where TPF is 1,3,5-triphenylformazan, was significantly correlated with most enzyme activities. In addition, as in numerous other experiments, a significant correlation ($r = 0.841$) was observed between the enzyme activity number index and biomass C (Fig. 2). Therefore, this index seems to be better than the biological index of fertility as an indicator of microbiological activity. However, the enzyme activity number index has serious limitations as an index of metabolic activity because it can be used only with neutral or alkaline soils, and to obtain an activity number value, it is necessary to determine five enzyme activities (amylase, catalase, dehy-

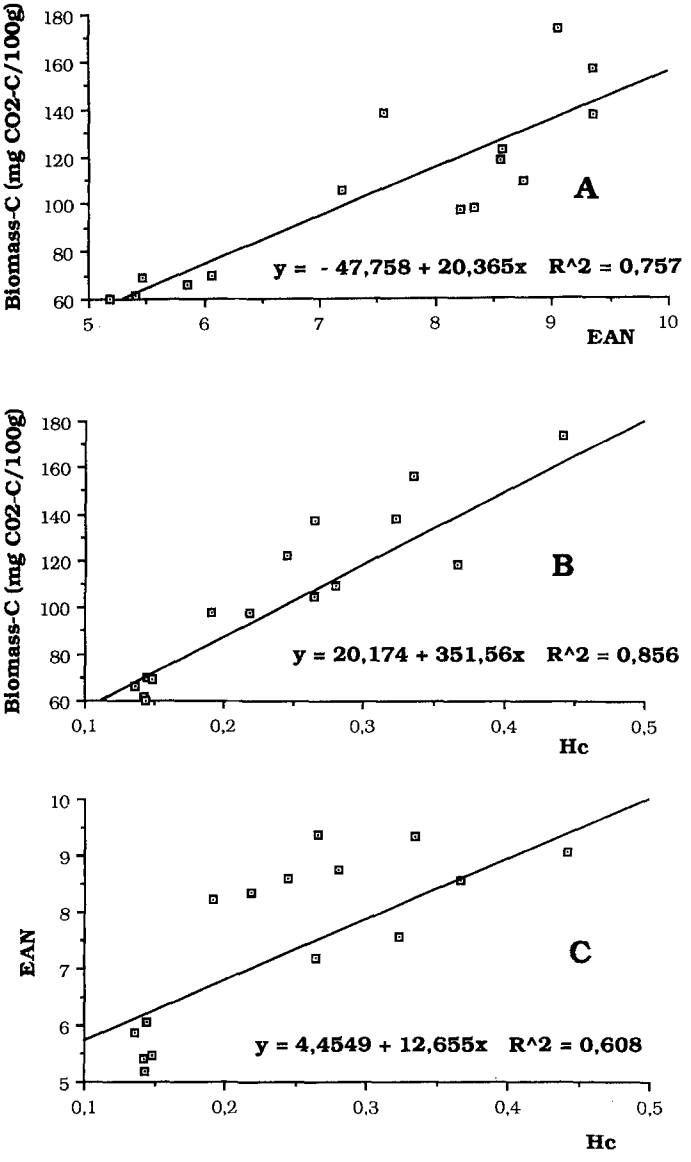

Fig. 2. Correlation between (**A**) the enzyme activity number index (*EAN*) and biomass C; (**B**) the hydrolysing coefficient (*Hc*) and biomass C; and (**C**) the hydrolysing coefficient and the enzyme activity number index

drogenase, phosphomonoesterase alkaline and protease) according to Beck's formula (1984). Often, a rapid method is needed to compare the microbial activity of different soils, especially in their response to management practices.

In view of the difficulties with these two indices and since fluorescein diacetate hydrolysis can be rapidly detected and, in the present experiment, was significantly correlated with most of the measured enzyme activities, a new index is proposed to compare the overall microbiological activity in soil, the hydrolysing coefficient. This coefficient represents the ratio between μmol of fluorescein diacetate hydrolysed and μmol of total fluorescein diacetate before hydrolysis, calculated from an analysis procedure based on Swisher and Carroll's method (1980). The values range from 0 to 1; with a value close to 1 the hydrolysing capacity of the soil is much higher, and since the soil hydrolysing capacity is related to the concentration of plant-available nutrients, the hydrolysing coefficient can be considered to represent the soil fertility status.

In the present experiment, in comparison with a hydrolysing coefficient mean value of 0.143 calculated for the soil control, hydrolysing coefficients ranging from 0.218 to 0.367 and from 0.245 to 0.442 were calculated for soils amended with 30 and 90 t ha^{-1} $year^{-1}$, respectively. Significant correlations between the hydrolysing coefficient and biomass C ($r = 0.925$) and between this coefficient and the enzyme activity number index ($r = 0.780$) were found. These findings indicate a greater hydrolysing capacity in the amended soils, and consequently a greater concentration of plant-available nutrients and an increase in soil fertility. To confirm this assumption, an analogous experiment was carried out with corn, and a consistent increase in corn productivity compared with the control was observed during the first 2 years of compost application, i.e., 46 and 65% for 30 and 90 t ha^{-1}, respectively. These results represent 50% of the maximum productivity obtained with a normal application of mineral fertilizer in the Umbrian region (Businelli et al. 1990; Giusquiani et al. 1991), confirming that soil fertility was higher in the amended soils than in the control soil.

These findings suggest that a hydrolysing coefficient of about 0.3–0.4 can be used as an index of good soil fertility. However, to verify the possibility of using this coefficient as an activity index, further field experiments are required.

Conclusions

In conclusion, besides the enzyme activity level of a series of reductases and hydrolases, the biomass-C content and the rate of fluorescein diacetate hydrolysis are very good indicators of the intensity of soil life and soil microbal activity. Since significant correlations were found for all activity indices, except the biological index of fertility, measurement of the hydrolysing coefficient may be used as a convenient, inexpensive and relatively fast routine evaluation of changes in soil fertility and, in particular, for reliable detection of changes occuring in biological activity in amended soils during a field experiment.

However, it is clear that the type of the compost used, the type of soil, and the environmental conditions of the experiment can produce a unique set of conditions for which certain determinations may fail to respond while others may be effective. Therefore, before a hydrolysing coefficient of 0.3–0.4 can be used as an index of good soil fertility, much more additional information is necessary.

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