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Kinetic parameters of dehydrogenase in the assessment of the response of soil to vermicompost and inorganic fertilisers

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Abstract Kinetic parameters (V_{max} and K_m) of dehydrogenase activity were determined in order to assess the metabolic response of a soil about 1 year after organic and mineral treatments. The soil was planted with maize (*Zea mays*) and treated with the following fertilisers: organic (vermicompost; VC), mineral (ammonium phosphate and urea), and an organo-mineral mixture. V_{max}, which represents a measurement of the quantity of enzyme, markedly increased in organic and organo-mineral treatments, indicating that the addition of organic matter caused an increase in dehydrogenase in the active microbial biomass. K_m, representing enzyme-substrate affinity and/or different sources of the enzymes, was similar in VC-treated soil and control soil, while it doubled in organo-mineral and mineral treatments. These results suggest that the use of VC did not alter the enzyme-substrate affinity, while mineral fertiliser reduced this affinity or changed the composition and activity of soil microbiota. A positive correlation was found between V_{max}, the metabolic index (dehydrogenase/water-soluble carbon ratio), and the soil organic matter content. The kinetic constants of dehydrogenase activity and the metabolic index may be considered valid parameters to monitor the evolution of microbiological activity in soil.

Keywords Vermicompost · Mineral fertiliser · Dehydrogenase kinetics · Soil fertility

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Introduction

Microbial activity plays an important role in regulating soil fertility. Indeed, the microbiological processes taking place in soil are at the centre of many ecological functions (Nannipieri et al. 1990), since microbiological activity is related to soil structure, soil fertility, and the transformation of organic matter (Ladd et al. 1996). Microbiological processes in soil have been measured using several parameters, such as microbial biomass, respiration, and enzymatic activities (Nannipieri et al. 1990; Garcia et al. 1994). Dehydrogenase is considered an indicator of overall microbial activity because it occurs intracellularly in all living microbial cells, and it is linked with microbial respiratory processes (Bolton et al. 1985). Therefore, the use of dehydrogenase activity as an index of microbial activity has been suggested (Skujins 1976; Benefield et al. 1977; Trevors 1982; Nannipieri et al. 1990; Tabatabai 1994). In fact, numerous studies have revealed correlations of soil dehydrogenase activity with metabolic parameters, such as the number of microbial cells, soil respiration, ATP concentration, carbon and nitrogen turnover, and organic matter content (Nannipieri et al. 1990; Malkomes 1991). Determination of dehydrogenase activity in soils is based on the use of soluble tetrazolium salts [2,3,5-triphenyltetrazolium chloride (TTC) or 2-*p*-iodophenyl-3-*p*-nitrophenyl-5-phenyltetrazolium chloride (INT)] as artificial electron acceptors, which are reduced to red-coloured formazans, extracted, and then determined colorimetrically (Trevors et al. 1982; Trevors 1984). Benefield et al. (1977) and Trevors et al. (1982) found INT to be more suitable than TTC for dehydrogenase activity measurement, since INT has a higher electron affinity than TTC and competes more effectively with oxygen for free electrons. Even if the present methods have been criticised with respect to the determination of dehydrogenase activity and the interpretation of these measurements, we have used kinetic constants of dehydrogenase activity to compare the effect of different amendments (organic and mineral) after a relatively long period (1 year) on microbiological activity of soil. According to Pascual et al. (1997, 1998) there is a rapid flush of microbial activity in the first stages of organic fertiliser application, followed by a rapid decrease; the real contribution of fertiliser treatments to soil microbial metabolism should be evaluated when the equilibrium is established. We have hypothesised that V_{max} and K_m might be useful markers to assess changes in microbial activity of soil, since they represent the "quantity" and the affinity of these enzymes, respectively.

Materials and methods

Field experiments were carried out on a sandy-clay soil (located in Castelvecchio di Compito, Capannori, Lucca, Tuscany) in plots of 100 m^2 (10×10 m) with a space between the plots of 3 m. The characteristics of the soil are reported in Table 1. The design of the experiment consisted of a complete randomised block with three replicates per treatment. The entire soil experimental area was planted with maize (*Zea mays*). The following fertilisation treatments were carried out: (1) non-amended soil (control); (2) vermicompost (VC) from sewage sludges (obtained as reported in Masciandaro et al. 1997); (3) mineral fertiliser (urea and ammonium phosphate; F); and (4) a mixture of VC and F (VC+F).

The VC stabilised for 8 months, and with a water content of about 50% (Table 1), was used as organic fertiliser. The VC was applied on a fresh basis at a rate of 8000 kg ha^{-1} for the VC treatment, giving 1240 kg carbon ha^{-1} and 120 kg nitrogen ha^{-1} dry weight, respectively. Ammonium phosphate contained 46% phosphorus and 18% nitrogen, while urea contained 46% nitrogen; 4 kg of ammonium phosphate and urea (1:1) was added to the appropriate plots, giving 128 kg nitrogen ha^{-1} and 92 kg phosphorus ha^{-1}, respectively. The VC+F mixture was made using 4000 kg ha^{-1} of fresh VC and 2 kg mineral fertilisers (ammonium phosphate:urea 1:1), so as to reach a rate of 620 kg carbon ha^{-1} and 124 kg nitrogen ha^{-1} (on the basis of the total carbon and nitrogen concentration per dry weight of VC). Organic fertiliser was incorporated into the top 15 cm of soil by a mechanical rotary tiller 4 weeks before planting. F was applied to the soil surface 4 weeks after sowing; maize seeds were sown at a depth of 5 cm. Soil samples were collected 32 weeks after maize harvesting, i.e. on average, 1 year after the treatments were applied (corresponding to exactly 1 year after sowing). Three sub-samples were taken from the surface layer (0–15 cm) diagonally in each plot. These sub-samples were mixed (to have a single sample for each plot), air-dried at 22±2 °C, sieved (<2 mm), and stored at 4 °C before chemical and biochemical analyses.

Dehydrogenase was assayed in triplicate by adding 0.2 ml water g^{-1} soil according to the method reported by Garcia et al. (1993), which is a combination of two previously reported methods (Benefield et al. 1977; Trevors 1984). Soil was mixed with 0.2 ml of 0.4% (7.91 mM) INT solution (in distilled water, w/v). The control was the soil treated with distilled water (0.2 ml), instead of INT. Soils were incubated for 20 h at 22 °C in darkness; pH values did not vary during the incubation time. The iodonitrotetrazolium formazan (INTF) formed by the reduction of INT was extracted by adding 10 ml of a mixture of 1:1.5 ethylene chloride and acetone; then the soil mixture was vigorously shaken by hand for 1 min and finally filtered through a Whatman no. 5 filter paper. The INTF concentration was measured spectrophotometrically at 490 nm, and the results were expressed as nmol INTF g^{-1} soil h^{-1}.

Kinetic parameters were determined by using five different concentrations of the substrate, INT, varying from unsaturated to saturated conditions: 1.97, 3.95, 7.91, 15.8, 23.7 mM (corresponding to 0.1%, 0.2%, 0.4%, 0.8%, and 1.2%, w/v) each at different incubation times (0, 1, 2 4, 8, 14, 16, 24, 32, 45 h). Progress curves (*P* versus *t*, where *P* is the reaction product, INTF, and *t* is the incubation time), at each INT concentration were plotted and used for calculating values of the initial velocity (v_o). K_m and V_{max} were estimated by plotting the experimental data according to the Lineweaver-Burk equation derived from the Michaelis-Menten equation (Tabatabai 1994). Since the progress curves are biphasic in some cases, the second portion (8–45 h) of the plots was used to derive the V_{max} and K_m values.

Total organic carbon and water-soluble carbon (WSC, determined in water extracts 1:10 w/v, Garcia et al. 1990) were determined by dichromate oxidation (Yeomans and Bremner 1988). Total nitrogen was determined by the Kjeldhal method (Jackson 1960) and nitrate analysis was performed in a DIONEX ion chromatograph, model 2000i, equipped with a Dionex AS4A 4-mm analytical column according to the methodology of the handbook instructions.

Total concentrations of heavy metals (cadmium, nickel, zinc, and copper) were determined using a Perkin-Elmer model 3030 atomic absorption spectrophotometer.

All results reported in the text are the means of three replicates and were tested for treatment effects by ANOVA with calculation of the least significant difference (LSD) at $P<0.05$.

Table 1 Characteristics of soil and vermicompost (*VC*)

Parameter Texture	Units	Soil Sandy-clay	VC
pH		6.5	7.2
Total organic carbon	%	0.95	31
Total nitrogen	%	0.085	3.5
Cadmium	mg kg^{-1}	0.6	4.2
Nickel	mg kg^{-1}	14	46
Copper	mg kg^{-1}	33.5	575
Zinc	mg kg^{-1}	40.3	1830

Results and discussion

Figure 1 shows the variations in dehydrogenase activity with incubation time (progress curve) at different concentrations of the electron acceptor INT. The progress curves for all treatments, including the control soil, showed kinetics characterised by a lag phase from 0 to 8 h of incubation; this was less pronounced in the VC-treated soil. The lag phase may be interpreted as a period of metabolic adaptation following the addition of INT and water to soil. This adaptation might involve: (1) temporary enzyme repression by INT, and/or (2) a reversible adsorption of INT on soil colloids making it not immediately available as an electron acceptor. It is probable that the lag phase in the control depended on the lack of fresh substrate in the soil, whereas after drying and rewetting there is a flush of metabolism due to survivors that feed on killed biomass (Ciardi 1998). The amendments with VC probably reduced the lag period due to the addition of easily degradable organic matter which supported the activity and growth of soil microbiota (Table 3).

As reported in the Introduction, V_{max} and K_m of an enzyme express the quantity of enzyme and substrate affinity, respectively. Enzymes catalysing the same reactions can have different sources in soil, and thus,

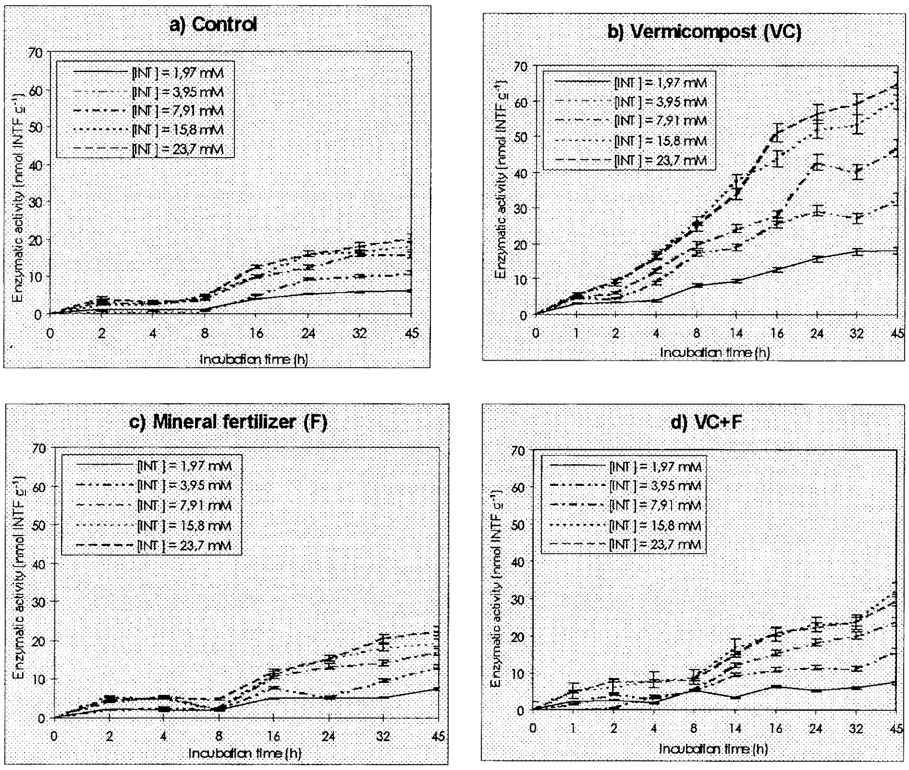

Fig. 1 Changes in dehydrogenase activity during incubation at different concentration of substrate, for untreated soil (**a**), and soil treated with vermicompost (**b**), mineral fertiliser (**c**), or a mixture vermicompost-mineral fertiliser (**d**). *Error bars* represent 95% confidence interval for the mean determined on three replicates

different K_m values (Nannipieri et al. 1990). In addition, different management systems can influence K_m values (Nannipieri and Gianfreda 1998). This illustrates the difficulty of interpreting enzyme assays in the heterogeneous and complex soil system.

The highest V_{max} value was observed in the soil treated with VC alone (Table 2). According to the Michaelis-Menten theory, this means a higher concentration of dehydrogenase in soil, which may be the result of a higher microbial biomass (Nannipieri and Gianfreda 1998). Thus, the addition of organic matter in the VC treatment may have increased microbial activity and microbial biomass and, consequently, the concentration of dehydrogenase (Cooper and Warman 1997). K_m is independent of enzyme concentration and kinetically reflects the apparent affinity of the enzyme for the substrate: the smaller the K_m value, the greater the affinity. K_m values were similar for the VC-treated soil and untreated, control soil, suggesting that enzyme affinity for the substrate was not affected by the addition of VC. A twofold increase in K_m values were instead measured in soils treated with F, even when F was added in the mixture with VC (Table 2). Thus, the addition of F apparently reduced the enzyme-substrate affinity, probably due to a change in the composition of soil microbiota with a change in the "community" of dehydrogenase.

The metabolic index, defined as the ratio between dehydrogenase activity and WSC (DH-ase/WSC), has been suggested to represent the metabolic activity of soil (Masciandaro et al. 1998). This index was markedly increased by adding VC to soil (Table 3). The high positive correlation (Table 4) between the metabolic index DH-ase/WSC, the "quantity" of enzymes (V_{max}), and the organic matter content indicated that soil responses to treatments may be evaluated even 1 year after treatments have been applied. However, ecological implications related to changes in soil metabolic activity need to be considered. Metabolic products, such as WSC and nitrate concentrations, which better express the evolution of carbon and net nitrogen mineralisation/nitrification, respectively, were positively correlated (Table 4), indicating the close relationship between carbon and nitrogen turnover. Both WSC and nitrate concentrations were consistently higher in the fertiliser treatments than in the control soil (Table 3); the highest values were observed in treatment F. This result may be hypothesised to be due to a sort of "priming effect" in the latter treatment, due to the degrada-

Table 2 Kinetic parameters (K_m and V_{max}) of dehydrogenase activity for soil 1 year after the organic and mineral treatments. For each parameter, values followed by the *same letter* are not significantly different at $P<0.05$. *F* Mineral fertiliser

Treatments	K_m (mM)	V_{max} (nmol g^{-1} h^{-1})
Control	3.95 b	1.06 c
VC	4.35 b	2.65 a
VC+F	8.70 a	1.63 b
F	7.90 a	0.76 c

Table 3 Chemical and biochemical characteristics of soils 1 year after the VC, F or VC+F treatments were applied. For each parameter, values followed by the *same letter* are not significantly different at $P<0.05$. *EC* Electrical conductivity, *OM* organic matter, *WSC* water-soluble carbon, *Ntot* total nitrogen, *DH-ase* dehydrogenase activity (determined at 20 h incubation and 7.91 mM 2-*p*-iodophenyl-3-*p*-nitrophenyl-5-phenyltetrazolium chloride concentration), *DH-ase/WSC* metabolic potential index, *INTF* iodo-nitrotetrazolium formazan; for other abbreviations, see Table 2

Treatments	pH	EC (μS cm^{-1})	OM (%)	WSC (mg kg^{-1})	Ntot (mg kg^{-1})	Nitrate (mg kg^{-1})	DH-ase (nmol INTF g^{-1} h^{-1})	DHase/ WSC
Control	7.53 a	28.0 b	1.64 c	1861 c	900 c	39.9 c	0.373 c	0.094 c
VC	7.61 a	46.7 a	2.00 a	2857 b	1040 c	114 b	1.251 a	0.206 a
VC+F	7.57 a	41.7 a	1.87 b	2620 b	1240 b	145 a	0.840 b	0.151 b
F	7.60 a	29.3 b	1.69 c	3622 a	1403 a	158 a	0.708 b	0.092 c

Table 4 Correlation matrix between chemical, biochemical and kinetic parameters. The correlation values are significant at $P<0.05$ (but see footnote). For abbreviations, see Table 3

	OM	WSC	Ntot	Nitrate	DH-ase	DH/WSC	K_m	V_{max}
OM	1.000							
WSC		1.000						
Ntot		0.871	1.000					
Nitrate		0.871	0.925	1.000				
DH-ase	0.948			0.500[a]	1.000			
DH/WSC	0.986				0.916	1.000		
K_m		0.561[a]	0.884	0.830			1.000	
V_{max}	0.943				0.861	0.986		1.000

[a] $0.05<P<0,1$

tion of soil organic matter (Jenkinson et al. 1985; Marinari et al. 2000).

In conclusion, each type of treatment (i.e. organic, mineral, organo-mineral) affected the metabolic response of the soil, as determined by the metabolic potential index (DH-ase/WSC), the WSC and nitrate concentrations, or by the kinetic parameters of dehydrogenase; these effects were detectable even 1 year after the treatments were applied. The changes in the kinetic parameters of dehydrogenase activity seem to indicate a change in the composition and activity of soil microbiota. Both the metabolic potential index and the kinetic constants of dehydrogenase activity may be used to monitor the evolution of soil fertility during cropping or during soil amelioration practices.

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