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Effectiveness of microbial indexes in discriminating interactive effects of tillage and crop rotations in a Vertic Ustorthens

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Abstract Conservation tillage is a soil management practice able to reduce erosion, increase organic matter content and improve physical, chemical and biological soil properties. Conservation tillage reduces the incorporation of crop residues into the soil profile and minimises the exposure of humified organic matter to biotic and abiotic degradative processes. In this article the effect of conventional (ploughing at 40-cm depth coupled with harrowing at 20-cm depth) and reduced tillage (harrowing at 20-cm depth) and two rotations (vetch-oat/wheat and fallow/wheat) on biochemical and microbial properties of a Vertic Ustorthens, located in a semi-arid region of southern Italy, has been investigated. Tillage had a more pronounced effect on soil properties studied here than did rotations. By comparison to conventional tillage (CT), the reduced tillage (RT) resulted in improved soil C and microbial biomass content. Even though some of the selected enzymes showed seasonal variability and, when averaged across the sampling period, were not always able to discriminate among treatments, their sum showed a strong correlation with soil organic C and soil microbial biomass C (MBC), for all these parameters increased in RT plots.

Keywords Tillage · Rotation · Soil microbial biomass · Soil enzymes

Introduction

Most of the agricultural soils of the semi-arid climate in the Mediterranean basin are subjected to impoverishment of their physical, chemical and biological properties caused by modern agricultural practices. Current crop-

ping technologies are responsible for a reduction of organic matter content in most agricultural soils (Jenkinson 1981), and recently, Haider (1992), Wood and Edwards (1992), and Kirchner et al. (1993), highlighted the negative effect of conventional soil and crop management on global soil fertility. Harris and Bezdiek (1994) defined soil “quality/health” as “the ability of the soil, within natural limitations, to perform as a resilient and diverse soil ecosystem, support sustained production of diverse plant and animals and protect the environmental quality of the interfacing water and air”. Such a definition points out the necessity of a new approach to soil management by moving beyond the concept of soil as a simple physical support for plant growth.

Modern crop management, besides having an effect on soil quality, can lead to erosion and loss of the superficial soil layer, which plays a pivotal role in plant mineral nutrition and element recycling. Therefore, an increasing interest in reduced- or no-tillage, and in crop rotations has stimulated numerous studies aimed at comparing the impact of conventional and innovative management systems on the functioning of soil ecosystems. There is a substantial consensus for the beneficial effect of conservation tillage on soil physical, chemical and biological properties (Doran 1980; Parmelee et al. 1989; Franzluebbers et al. 1994, 1995) and on the functional and speciological diversity of soil microbial communities (Lupwayi et al. 1998). Kern and Johnson (1993) observed an increase of organic C, while Franzluebbers et al. (1995) found a significant augmentation of water-stable aggregates, C and N pools, and potential N mineralisation in the top layer of soils managed with conservation tillage systems. In addition, conservation tillage has been found to increase moisture availability (Franzluebbers et al. 1995) and improve the effective utilisation of rainfall (Wienhold and Halvorson 1999).

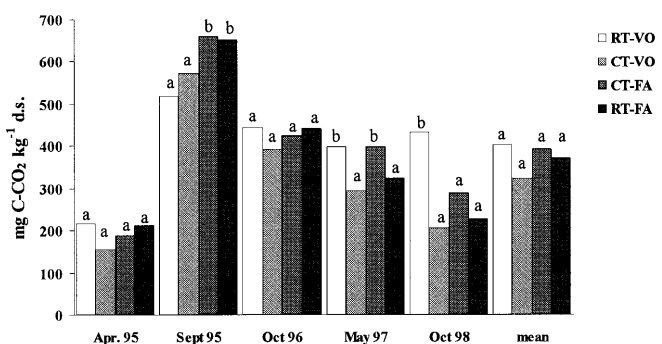
Crop rotations have been shown to be able to increase the labile C and N pools (Ross 1987; Wienhold and Halvorson 1999) and N mineralization rates (Campbell et al. 1991; Wienhold and Halvorson 1999) and allow more efficient use of soil water (Norwood 1994). All of

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Table 1 Physical and chemical characteristics of studied soil at the beginning of this study. Standard deviation in brackets (CEC cation exchange capacity)

Sand (%)	39.16 (± 4.4)
Silt (%)	21.56 (± 2.8)
Clay (%)	38.72 (± 2.2)
pH-H ₂ O	8.27 (± 0.5)
Conductivity (mS cm ⁻¹)	0.18 (± 0.04)
Org C (%)	0.93 (± 0.19)
Total N (%)	0.08 (± 0.02)
P-Olsen (mg 100 g ⁻¹)	0.63 (± 0.18)
K (mg 100 g ⁻¹)	30.30 (± 3.68)
Limestone (%)	3.33 (± 3.35)
CEC (cmol[+] kg ⁻¹)	24.12 (± 2.62)



Values in the same group marked with the same letter are not statistically different ($p < 0.05$)

Fig. 1 Climate diagram of the experimental field (mean 1970–1988)

these positive effects of conservation tillage and crop management are of paramount importance in maintaining soil fertility and reducing erosion in agricultural soils of Southern Italy, which lie under semi-arid Mediterranean climates. In this study, we compare the effect of crop fallow, crop rotation, reduce tillage and conventional tillage by measuring soil microbial biomass and selected enzyme activities as an index of soil quality (Harris and Bezdiek 1994).

Materials and methods

Studied soil and sampling procedure

The studied soil was a Vertic Ustorthens located at 725 m above sea level in a semi-arid zone of the Basilicata region, in Southern Italy. Some of its physical and chemical characteristics are reported in Table 1. In Fig. 1, the mean monthly temperature and rainfall recorded at the experimental site between 1970 and 1998 are reported.

The sampling was carried out in April and September 1995, in October 1996, in May 1997 and in October 1998. Three different samples were randomly taken from the 0- to 20-cm soil layer of each plot for each sampling data and were processed separately.

Experimental design

The experimental design (Table 2) was set as a randomised complete block with three replicates of each treatment. The conventional tillage (CT) consisted of ploughing to a 40-cm depth cou-

Table 2 Experimental design (RT reduced tillage, CT conventional tillage, VO vetch-oats, W wheat, FA fallow)

Plot	Tillage	Rotation	Culture			
			1995	1996	1997	1998
RT-VO	Reduced	VO-W	VO	W	VO	W
CT-VO	Conventional	VO-W	VO	W	VO	W
CT-FA	Conventional	FA-W	FA	W	FA	W
RT-FA	Reduced	FA-W	FA	W	FA	W

pled with harrowing to 20-cm depth, whereas the reduced tillage (RT) consisted only of harrowing to 20-cm depth. Alfalfa was grown on all plots during the 7 years preceding this experiment.

A N fertilisation (NH₄NO₃) of 30–40 kg N ha⁻¹ was applied on April 1995, April and January 1996, 1997 and 1998 in RT vetch-oat/wheat (VO-W) and CT VO-W plots.

Chemical analysis

Physical and chemical soil characteristics were determined according to McKeague (1978). Soluble organic C, soluble total N and soluble total P were measured in filtered solution obtained by shaking 40 g dry soil for 1 h in 250 ml 0.25 M K₂SO₄. The content of soluble organic C and soluble total P were measured by the method of McKeague (1978); soluble total N was estimated according to Cabrera and Beare (1993).

Biochemical analysis

The hydrolysis rate of fluorescein diacetate (FDA hydrolysis) was estimated using the method of Schnürer and Rosswall (1982) modified as described in Perucci et al. (2000). Acid and alkaline phosphatase, β -glucosidase and arylsulfatase activities were measured according to Tabatabai (1982).

Microbiological analysis

Soil respiration was monitored at 22°C for 30 days according to Dumontet and Mathur (1989). Microbial biomass-C (MBC) and microbial biomass-N (MBN) were determined using the fumigation extraction (FE) method (Sparling and West 1988). The specific respiration activities (q CO₂), expressed as the amount of CO₂-C produced per unit of biomass-C per day, were calculated.

Statistical analysis

Analysis of variance and the Duncan range test on means were computed using the SYSTAT programme.

Results

Cumulated soil respiration at 28 days of incubation was strongly influenced by sampling periods (Fig. 2). In April 1995 and October 1996 soil respiration showed no statistically significant differences among treatments, whereas in September 1995 the highest respiration values were recorded in the fallow plots and the lowest in the vetch-oat ones regardless of tillage. In May 1997 the highest values were recorded in the reduced and conventional tillage plots under vetch-oat and fallow

Fig. 2 C-CO₂ evolved from soil samples after 28 days of incubation as influenced by rotation and tillage across the sampling period (*RT* reduced tillage, *CT* conventional tillage, *VO* vetch-oat, *FA* fallow)

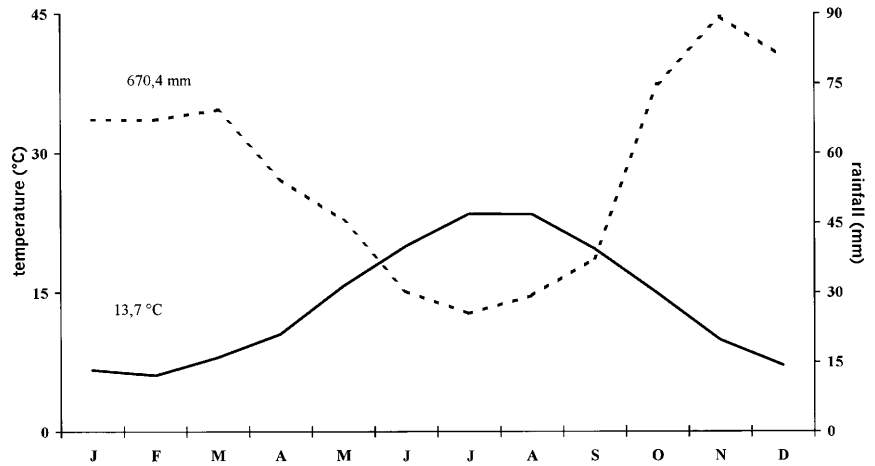


Table 3 Organic C^a, microbial biomass C (*MBC*)^b, microbial biomass N (*MBN*)^c, soluble C^b (extracted with K₂SO₄ 0.25 N), soluble N^c (extracted with K₂SO₄ 0.25 N), soluble C+MBC, soluble C/MBC ratio, soluble N/MBN ratio and respiratory quotient

(*qCO*₂) averaged across the sampling period. Values in the same column followed by the *same letter* are not statistically different ($P < 0.05$; $n=5$). (*RT* Reduced tillage, *CT* conventional tillage, *VO* vetch-oat, *FA* fallow)

Plot	Organic C	MBC	MBN	$\frac{MBC}{MBN}$	Soluble C	Soluble N	Soluble C+MBC	$\frac{SolubleC}{MBC}$	$\frac{SolubleN}{MBN}$	<i>qCO</i> ₂
RT-VO	1.24 d	441 b	82 b	5.70 a	82 a,b	51 a	523 b	0.19 a	0.64 a	0.091 a
CT-VO	1.06 b	268 a	51 a	5.22 a	70 a	39 a	338 a	0.28 a,b	0.77 a	0.125 a
CT-FA	0.97 a	274 a	53 a	5.48 a	82 a,b	37 a	356 a	0.31 b	0.69 a	0.146 a
RT-FA	1.17 c	401 b	77 b	5.30 a	94 b	42 a	495 b	0.24 a,b	0.49a	0.091 a

^a g C 100 g⁻¹d.s. ^b mg C kg⁻¹ d.s. ^c mg N kg⁻¹ d.s.

(*FA*) rotations, respectively, and in October 1998 the highest value was recorded in the reduced tillage plot under vetch-oat rotation. The other treatments were not statistically different from each other. The respiration values averaged across the sampling period showed no differences between treatments. Even though tillage caused a dilution of top soil in the conventionally laboured plots, and different rotations left soil roots residues of diverse quality and quantity, treatments did not modify the respiration rates of soil microbial biomass.

Organic C concentration averaged across sampling periods was recorded from April 1995 to October 1998 (Table 3). As expected, organic C content was significantly higher ($P < 0.05$) in plots managed with reduced tillage [1.25 mg C kg⁻¹ dry soil (d.s.)] than in plots under conventional tillage (0.97 mg C kg⁻¹ d.s.). The organic C concentration has been influenced by soil homogenisation caused by the 40-cm ploughing and the 20-cm harrowing in the conventionally laboured plots. Such a trend was not followed by soluble C.

Among the investigated microbial parameters the *MBC* and *MBN* (Table 3) were modified by tillage. The highest value was found in both *RT* plots, whereas the lowest pertained to *CT* ones. It is interesting to point out that the *MBC/MBN* ratio did not show any statistically significant difference among treatments.

Soluble C and soluble N (Table 3) displayed different behaviours. Soluble C was found to be at the lowest

level in the *CT-VO* treatment and at the highest in the *RT-FA* one. The values pertaining to *RT-VO* and *CT-FA* plots were identical and not statistically different from those of *CT-VO* and *RT-FA* plots. On the contrary, soluble N did not show any difference among treatments.

The sum of soluble C carbon and *MBC*, averaged across the sampling, showed the highest value in *RT* plots, whereas the lowest one was recorded in *CT* treatments (Table 3). Taking into account the soluble C/*MBC* ratio, treatments can be classified into two partially overlapping groups (Table 3). The highest value pertained to *RT-FA* treatment, which was not statistically different from *CT-FA* and *RT-VO* ones. The lowest value was found in the *CT-VO* treatment, which was not statistically different from *RT-VO* and *CT-FA* treatments. Soluble N/*MBC* values and the respiratory quotient (*qCO*₂) did not show any statistically significant difference among treatments (Table 3).

The studied enzymes displayed different activity patterns as a function of both tillage and rotations (Table 4). Alkaline phosphatase and β-glucosidase were affected by tillage as the highest values were recorded in *RT* treatments, whereas acid phosphatase was affected by rotations as the highest values pertained to *VO* plots, irrespective of tillage. Arylsulphatase was not influenced by both tillage and rotations.

The sum of all these enzymatic activities seems to indicate that tillage was able to exert the strongest effect on soil enzyme activity (Table 4). *FDA* hydrolysis and

Table 4 Alkaline phosphatase^a, acid phosphatase^a, β -glucosidase^a, arylsulfatase^a, sum of these enzymes^a, fluorescein diacetate (FDA)-ase, and *q*FDA averaged across the sampling period. Values in

Plot	Alkaline phosphatase	Acid phosphatase	β -Glucosidase	Arylsulfatase	Sum of enzymes	FDAase % hydrolysed	<i>q</i> FDA
RT-VO	2.79 b	1.36 b	2.73 b	1.23 a	8.11 b	57.39 a	0.150 a
CT-VO	1.37 a	1.73 b	1.60 a	0.94 a	5.64 a	60.23 a	0.217 a
CT-FA	1.84 a	0.78 a	1.59 a	0.95 a	5.16 a	62.45 a	0.218 a
RT-FA	2.41 b	0.94 a	2.48 b	1.23 a	7.06 b	79.59 b	0.219 a

^a $\mu\text{mol } p\text{-NP g}^{-1} \text{ soil h}^{-1}$

the specific hydrolytic activity (*q*FDA) did not show any statistically significant difference among treatments (Table 4).

Discussion

Organic C was found to be higher in plots managed by reduced tillage, but it also was influenced by rotations, as VO treatments were always richer in organic C than FA ones. These findings are in agreement with Franzluebbers et al. (1995), who found an increase in potentially mineralisable C in plots under no tillage compared to those subjected to conventional tillage. Such an increase was found to be strongly affected by the sampling season. Similar results have been obtained here, as the soil respiration, which can be considered as an indirect measure of bioavailable C, greatly varied with the seasons. Nevertheless, soil respiration did not show any statistically significant difference among treatments, when averaged across the sampling period, in spite of the different quality and quantity of organic residues VO and FA treatments left in the soil (Franzluebbers et al. 1995).

The highest MBC value was found in RT plots without any difference between rotations, thus contradicting what was reported by Gunapala et al. (1998), who showed that vetch was able to increase microbial abundance and activity. Franzluebbers et al. (1995) found a higher level of MBC in soil under zero tillage than in soil under conventional tillage. Such an increase in MBC can be explained as a beneficial effect of reduced disturbance on soil organic matter exerted by conventional tillage. As observed by Franzluebbers et al. (1995), the accumulation of organic matter under zero or reduced tillage regimes increased N mineralisation and stimulated soil microbial biomass. MBN behaved in the same manner as MBC, showing a strong correlation with tillage and no correlation with rotations. It is interesting to highlight that the MBC/MBN ratio was not affected by tillage or by rotations. This points out that these different microbial biomasses could have the same bacteria/fungi ratio, even though the quality and quantity of residues in the soil could vary to a large extent.

Soluble C has been questioned as a measure of C availability for soil microflora. Zsolnay and Steindl (1991) found that 85% of soluble C was biodegradable in agricultural soils, whereas Boissier and Fontveille

the same column followed by the *same letter* are not statistically different ($P < 0.05$; $n = 5$). (RT Reduced tillage, CT conventional tillage, VO vetch-oat, FA fallow)

(1991) reported that only a relatively small fraction (from 3.8% to 39.9%) of soluble C was biodegradable in forest soil leachates. On the other hand, Janzen et al. (1992) found that the labile fraction of soil organic matter was strongly correlated with total soil C and N as well as with other parameters which reflect the microbial activity in soil, such as soil respiration. These authors also underlined that the soil labile organic fraction is more sensitive to the effect of cropping systems than the total organic matter content.

In this experiment, soluble C did not follow any clear trend and it behaved in this respect as the amount of soluble C per unit of biomass (soluble C/MBC). When values of the overall labile C fraction (extractable C plus MBC) are taken into account, a reduction of this labile C was found in CT plots, as an effect of top soil dilution caused by conventional tillage. Rotations did not seem to have any effect on this parameter.

The $q\text{CO}_2$ is considered a reliable parameter for describing early modifications of microbial biomass in soil under different agricultural regimes (Anderson and Domsch 1990, 1993). The $q\text{CO}_2$ concept is based on Odum's theory of ecosystem energetics (Odum 1985), which postulated that if less organic C is channelled into energetic metabolism more C can be used to build up biomass. Ecosystems, which show lower rates of energy production per unit of biomass, can be considered mature systems. Insam and Domsch (1988) and Insam and Haselwandter (1989) had applied such a theory to soil ecosystem energetics. These authors, studying a soil sere, found a significantly higher $q\text{CO}_2$ in "young" than in "mature" sites. In this study, we have found that RT plots harboured a microbial biomass showing a more economical metabolism than the microbial population living in CT plots. Therefore, the microbial activity of plots under RT seems more conducive to increasing the MBC content. Odum (1985) also postulated that mature ecosystems are characterised by higher species diversity. Skujins and Klubek (1982) confirmed such a theory studying the physiological diversity of microbial biomass along a soil sere. These authors highlighted the fact that diversity follows the increase of soil organic matter content. In our study, tillage and rotation seem to be both responsible for a qualitative and quantitative modification of the C substrate available to soil microbial biomass. $q\text{CO}_2$ values averaged across the sampling period did not show any statistically significant differences in

response to both tillage and rotation, and failed to point out a beneficial effect of either reduced tillage or rotation on the energetic status of soil microbial biomass (Table 3).

High enzyme activities have been associated with soil conditions leading to the enhancement of microbial catalytic performances through the synthesis of both extracellular and intracellular enzymes (Bergstrom et al. 1998; Monreal et al. 1998) preceding SOM accumulation. If this were true, enzyme activities could be a useful tool in assessing early variations of soil quality under different management practices.

Alkaline phosphatase was always higher in RT plots showing a strong relationship with tillage. In contrast, acid phosphatase activity, which has been extensively used as an early indicator of soil biological quality (Nielsen and Eiland 1980; Mathur 1981; Doelmann and Haanstra 1989; Kirchner et al. 1993), showed no variation with tillage practice but seemed to point out the effect of rotations, as the VO plots showed the highest values irrespective of tillage.

β -Glucosidase, which has been reported by Hayano and Tubaki (1985) as an enzyme mainly produced by soil fungi, was found to change significantly during different seasons (Bandick and Dick 1999) and to be sensitive to soil carbon content (Miller and Dick 1995). In our case, β -glucosidase increased in RT plots, but seemed unaffected by rotations.

Arylsulphatase is responsible for organic S mineralisation in soil (Bandick and Dick 1999; Klose and Tabatabai 1999) and represents an important indirect indicator of fungi, which are the only soil microorganisms containing ester sulphate (Bandick and Dick 1999). Bandick and Dick found arylsulphatase activity positively correlated with soil organic C, as the activity of this enzyme was higher in manured plots than in those which did not receive organic amendment. The results reported here show that arylsulphatase activity, averaged across the sampling period, was unable to discriminate between tillage practices and rotations.

FDA hydrolysis was proposed as an indicator of microbial biomass by Schnürer and Rosewall (1982) and more recently by Bandick and Dick (1999) who, in a study aimed at comparing biochemical properties of continuous grass and cultivated fields, found it positively correlated with soil total C. These authors also reported that FDA hydrolytic activity was relatively stable between seasons. FDA hydrolysis averaged across the sampling period did not show any statistically significant difference and, also, q FDA, which was proposed by Perucci et al. (2000) as a new synthetic index for assessing microbial activity in reply to xenobiotic treatments, failed here to discriminate among treatments and tillage.

Even though some of the selected enzymes studied here were not always able to discriminate among treatments, the sum of them showed a strong correlation with soil organic C, MBC and MBN as all these parameters increased in RT plots.

Conclusions

Since tillage and fallow treatments strongly influence soil microbiological and biochemical parameters (Lupwayi et al. 1992; Janzen et al. 1998; Hu et al. 1999), in our study, we expected that fallow treatments would have produced more soluble C, and consequently more CO_2 from bacterial respiration, than vetch-oat ones. On the contrary, we have found that, despite the disturbance caused by top soil dilution by conventional tillage, all the labile C and N fractions (soluble C, soluble N and C- CO_2 evolved from soil respiration) failed to point to a clear tillage or rotation effect.

Combining alkaline and acid phosphatase, β -glucosidase and arylsulphatase activities allowed detection of an enhanced microbial enzymatic activity in RT plots. The use of such a synthetic enzymatic index could reduce interpretative problems arising from the natural variation over time of soil attributes (Bolton et al. 1985; Bergstrom et al. 1998; Bandick and Dick 1999) and give a reliable tool aimed at assessing the evolution of soil quality as influenced by conservation practices. This index, together with MBC, MBN and soluble C + MBC were the only parameters that clearly indicated a higher microbial activity in RT plots, but they failed to discriminate among rotations.

In conclusion, microbial and chemical parameters used in this study (soluble C and N, soluble C/MBC, soluble N/MBN, $q\text{CO}_2$, acid phosphatase, arylsulphatase, FDA hydrolysis and q FDA) were unable to point out differences due to tillage and rotation in the studied soil.

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