Microbial biomass and enzyme activity in vineyard soils under organic and conventional farming systems

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Abstract: Many recent studies from around the world have compared organic and conventional farming systems in terms of soil properties. Microbial biomass and enzyme activity were compared in vineyard soils managed by organic and conventional practices under Mediterranean conditions during the growing. Organic treatments were (i) green manure and farmyard manure at the rate of 30 t ha\(^{-1}\) (GM1), (ii) green manure and farmyard manure at the rate of 10 t ha\(^{-1}\), plus E2001 EM Bio-polymer gel solution (GM2), and (iii) green manure plus E2001 EM Bio-polymer gel solution (GM3). Only mineral fertilizers and pesticides were used in the conventional system (CONV). Soil organic C and soil microbial biomass C (SMBC), and protease, urease, alkaline phosphatase, and dehydrogenase activity were significantly higher in the organic system than in the conventional system. The ratio of microbial biomass C to total organic C content (C\(_{mic}/C_{org}\)) was also higher in the organic plots. Total organic C content increased in the organic system by 13%-23% in comparison to the conventional system. Application of GM1 and GM2 resulted in greater microbial biomass and activity, as compared to the other management systems. SMBC was significantly correlated with dehydrogenase, protease, urease, and alkaline phosphatase. These results indicate that organic management positively affected biochemical properties, thus improving soil quality and productivity.

Key words: Conventional management, enzyme activity, microbial biomass, organic management, vineyard soils

Organik ve konvansiyonel tarım altındaki bağ topraklarında mikrobiyal biyokütle ve enzim aktivitesi

Özet: Dünyanın farklı bölgelerinde bir çok çalışma, toprak özelliklerini açısından organik ve konvansiyonel tarım sistemlerini kıyaslamaktadır. Bu araştırmada; Akdeniz iklim koşullarında organik ve konvansiyonel sistemle yetiştiricilik yapılan bağ toprakları, mikrobiyal biyokütle ve enzim aktivitesi açısından kıyaslanmıştır. Organik sistemde uygulamalar şu şekildegeführtülmüştür: YG1 (Yeşil Gübre + Çiftlik Gübresi, 30 t ha\(^{-1}\)); YG2 (Yeşil Gübre + Çiftlik Gübresi, 10 t ha\(^{-1}\) + E2001 EM Bio-polimer jel çözeltisi); YG3 (Yeşil Gübre + E2001 EM Bio-polimer jel çözeltisi). Inorganik gübreler ve pestisidler sadece konvansiyonel sistemde (KONV) kullanılmıştır. Toprak organik C içeriği, toprak mikrobiyal biyokütle-Ç’u (TMBC), proteaz, üreaz, alkan fosfataz ve dehidrogenaz aktiviteleri konvansiyonel sisteme oranla organik sistemde

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Introduction

Land managed according to the principles of organic farming currently represents 0.4% of the total agricultural area in Turkey (Anonymous, 2003). Although organic farming and in-conversion land constitute a small proportion of the total agricultural area, this proportion is growing every year with the increasing health awareness of domestic and international markets. Major product groups are dried fruits, edible nuts, spices, and herbs, as well as fresh and processed fruits and vegetables.

Organic farming is a form of agriculture that avoids or largely excludes the use of synthetic fertilizers and pesticides, plant growth regulators, and livestock feed additives. An understanding of microbial processes is important for the management of farming systems, particularly those that rely on organic nutrient input (Melero et al., 2006). Microbial processes make a large contribution to the release and availability of nutrients required for crop growth. In organic management systems nitrogen is supplied in organic form via cover crops and manures, and large amounts of C are included in the mass of organic material required to achieve adequate amounts of N (Gunapala and Scow, 1998). Carbon additions of virtually any form to arable soils often increase the amount of microbial biomass (Böhme and Böhme, 2006) and its activity (Shannon et al., 2002; Marinari et al., 2006). Microbial biomass, rather than total organic C, has been suggested as a useful and more sensitive measure of change in organic matter status (Melero et al., 2006).

Studying the relationship between microbial biomass C (Cmic) and total organic C (Corg), and the metabolic quotient, which is the rate of CO2 per unit of biomass and time, can result in a greater understanding of the biological and chemical changes that occur with different agricultural practices (Anderson and Domsch, 1990).

Enzymes may respond to changes in soil management more quickly than other soil variables and, therefore, enzymes might be useful as early indicators of biological change (Bandick and Dick, 1999). Organic manures, such as animal manure, green manure, and crop residue, significantly increased the activity of a wide range of soil enzymes, as compared to unamended soil (Martens et al., 1992).

A number of studies have shown that organic farming leads to higher soil quality and soil biological activity than conventional farming (Carpenter-Boggs et al., 2000; Fliessbach et al., 2000; Shannon et al., 2002).

The response of soil microorganisms to organic and conventional amendments is generally studied in soils under plants in rotation, but less is known about soils used for perennial plants, such as grape. The aim of the present study was to evaluate the impact of organic production practices on soil microbial biomass and enzyme activity (alkaline phosphatases, protease, dehydrogenase, and urease) in organically and conventionally managed soils under Mediterranean conditions in western Turkey. Criteria for choosing enzyme assays were based on importance to nutrient cycling (alkaline phosphatases, protease, and urease) and oxidative capability of viable microorganisms (dehydrogenase).

Materials and methods

Study area and soil sampling

Conventional and organic-based production plots were located approximately 14 km northeast of the city of Manisa, in western Turkey. The climate of the region is typical Mediterranean—semiarid to arid—
with average temperatures of 30 °C in summer and 6 °C in winter. Most rain fall occurs between December and March, with a yearly total of 750 mm. Before 1992, both fields were managed conventionally. Later, the field was divided into 2 parts and these adjacent fields were managed organically (0.5 ha) and conventionally (0.5 ha), respectively. In both fields, round seedless grape has been growing since 1985. Organic vineyards completed the 3-year transition period necessary for organic production in 1995. Organic plots received farmyard manure at 20 t ha\(^{-1}\) (equivalent to 450 kg N ha\(^{-1}\)) every 2 years under farmer conditions between 1995 and 2000. The experiment was conducted over a 3-year period (2000-2002). In the organic field, 3 different organic treatments were used: (1) green manure (winter oats, *Avena satina*, 80 kg ha\(^{-1}\) + vetch, *Vicia sativa*, 20 kg ha\(^{-1}\) + farmyard manure 30 t ha\(^{-1}\) (GM1); (2) green manure (winter oats 80 kg ha\(^{-1}\) + vetch 20 kg ha\(^{-1}\) + farmyard manure 10 t ha\(^{-1}\) + E2001 EM Bio-polymer gel solution (GM2); (3) green manure (winter oats 80 kg ha\(^{-1}\) + vetch 20 kg ha\(^{-1}\) ) + E2001 EM Bio-polymer gel solution (GM3). Farmyard manure mixed with bedding was obtained from the farm of the Faculty of Agriculture, Ege University, İzmir. The amounts of organic C, and total N and P added to the soil by organic treatments during the study, and some chemical properties of the farmyard manure, vetch, and oat are given in Tables 1 and 2, respectively.

The organic system derived its fertility from vetch and winter oats, manure, and a commercial microbial fertilizer (E2001 EM). E2001 EM Bio-polymer gel solution, containing Azotobacter, Clostridium, and algae, was applied in order to promote microbial activity in the soil. This microbial preparation was produced by Agricultural Research Technologies, Ltd., USA and was purchased from an importer. No pesticides were used and the plots were managed according to the IFOAM Organic Guarantee System (IFOAM, 2003). Only inorganic fertilizers and pesticides were used in the conventional system (CONV). In both the organic and conventional plots, minimum tillage was used. One pass chisel plowing was used for minimum tillage. The plots were established as a randomized complete block design with 3 replications. Surface irrigation (mean: 300 mm year\(^{-1}\)) was performed according to the soil tension value and physiological growth of the plants. Data related to organic and conventional management practices are summarized in Table 3. The data

Table 1. Carbon, nitrogen, and phosphorus inputs (kg ha\(^{-1}\)) for the organic treatments.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>2000</th>
<th>2001</th>
<th>2002</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>N</td>
<td>P</td>
</tr>
<tr>
<td>GM1 (green manure + farmyard manure, 30 t ha(^{-1}))</td>
<td>6660</td>
<td>375</td>
<td>528</td>
</tr>
<tr>
<td>GM2 (green manure + farmyard manure, 10 t ha(^{-1}) + E2001 EM)</td>
<td>3420</td>
<td>125</td>
<td>192</td>
</tr>
<tr>
<td>GM3 (green manure + E2001 EM)</td>
<td>1800</td>
<td>50</td>
<td>24</td>
</tr>
</tbody>
</table>

GM1 (green manure + farmyard manure, 30 t ha\(^{-1}\))
GM2 (green manure + farmyard manure, 10 t ha\(^{-1}\) + E2001 EM)
GM3 (green manure + E2001 EM)

Table 2. Carbon, nitrogen, and phosphorus content, and C/N ratios of farmyard manure, vetch, and oat (mean values for 2000, 2001, and 2002).

<table>
<thead>
<tr>
<th>Organic fertilizers</th>
<th>Total C (%)</th>
<th>Total N (%)</th>
<th>Total P (%)</th>
<th>C/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farmyard manure</td>
<td>17.0 ± 0.5</td>
<td>0.69 ± 0.07</td>
<td>1.66 ± 0.02</td>
<td>24.6 ± 0.6</td>
</tr>
<tr>
<td>Vetch</td>
<td>24.5 ± 0.3</td>
<td>3.15 ± 0.04</td>
<td>0.30 ± 0.05</td>
<td>7.7 ± 0.4</td>
</tr>
<tr>
<td>Oat</td>
<td>20.0 ± 0.3</td>
<td>1.20 ± 0.08</td>
<td>0.25 ± 0.05</td>
<td>16.6 ± 0.6</td>
</tr>
</tbody>
</table>
reported in this study included the results from 2000 to 2002—3 growing periods.

Soil was sampled 3 times to a depth of 20 cm—in July 2000, June 2001, and September 2002. For each sampling, 10 soil cores were randomly taken from each plot to make a composite sample. Field-moist soil was sieved (2 mm) and divided into 2 sub-samples. One was immediately stored at 4 °C in plastic bags until microbiological and enzymatic activity were assayed; the other was air dried prior to chemical analysis.

General properties of soil

Some chemical and physical soil properties were determined only on the first soil sampling date (July 2000). Soil at the experimental site was sandy loam with a pH of 7.52 and 7.56 in the organic and conventional plots, respectively. Amounts of organic C and total N were similar in the 2 soils (Table 4).

Chemical, physical, and biological analyses

Soil texture analysis was made using the hydrometer method (Bouyoucos, 1962). Total salt content and pH of the soils were measured in a 1:1 soil/water extract. Organic C was measured according to Walkley and Black (1934), total nitrogen by the Kjeldahl method (Bremner and Mulvaney, 1982), and available P by the Olsen and Dean (1965) method.

Microbial biomass C was determined by the substrate-induced respiration method (Anderson and Domsch, 1978). Soil samples (100 g) were amended with glucose (400 mg) and the pattern of respiration response was recorded for 4 h. Values were converted to mg of biomass-C by means of a conversion factor; 1 mg of CO₂ 100 g⁻¹ of dm (dry matter) h⁻¹ corresponds to 20.6 mg of biomass-C 100 g⁻¹ of dm (Schinner et al., 1995).

Alkaline phosphatases activity was measured using the method of Eivazi and Tabatabai (1977). After the addition of a buffered P-nitrophenyl phosphate solution (pH 11), soil samples were incubated for 1 h at 37 °C. The P-nitrophenol released by

Table 3. Data related to cultivation practices employed in the study

<table>
<thead>
<tr>
<th>Management systems</th>
<th>GM1</th>
<th>GM2</th>
<th>GM3</th>
<th>CONV.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fertilizer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vetch-oats cover (80 + 20 kg ha⁻¹ y⁻¹) planting</td>
<td>September</td>
<td>planting</td>
<td>September</td>
<td>incorporation</td>
</tr>
<tr>
<td>FYM (30 t ha⁻¹ y⁻¹)</td>
<td>early April</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>FYM (10 t ha⁻¹ y⁻¹)</td>
<td>-</td>
<td>early April</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>E2001 EM (200 cc ha⁻¹ y⁻¹)</td>
<td>-</td>
<td>early April</td>
<td>early April</td>
<td>-</td>
</tr>
<tr>
<td>Mineral fertilizers</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>February and June</td>
</tr>
<tr>
<td>N: 150 kg ha⁻¹ y⁻¹</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P₂O₅: 50 kg ha⁻¹ y⁻¹</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>K₂O: 50 kg ha⁻¹ y⁻¹</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pesticides (3-6 kg ha⁻¹)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 4. General soil properties of organically and conventionally managed fields in January 2000

<table>
<thead>
<tr>
<th></th>
<th>Organic</th>
<th>Conventional</th>
</tr>
</thead>
<tbody>
<tr>
<td>Texture</td>
<td>Sandy loam</td>
<td>Sandy loam</td>
</tr>
<tr>
<td>Soil taxonomy</td>
<td>Typic Xerofluvent</td>
<td>Typic Xerofluvent</td>
</tr>
<tr>
<td>pH (1:1 soil/H₂O)</td>
<td>7.52</td>
<td>7.56</td>
</tr>
<tr>
<td>Total salt (%)</td>
<td>0.056</td>
<td>0.068</td>
</tr>
<tr>
<td>Organic C (%)</td>
<td>0.78</td>
<td>0.75</td>
</tr>
<tr>
<td>Total N (%)</td>
<td>0.140</td>
<td>0.130</td>
</tr>
<tr>
<td>P-available (mg kg⁻¹)</td>
<td>6.42</td>
<td>9.73</td>
</tr>
</tbody>
</table>
phosphomonoesterase activity was extracted and colored with sodium hydroxide, and was measured photometrically at 400 nm.

Protease activity was measured according to Ladd and Butler (1972). Using casein as a substrate, soil samples were incubated for 2 h at 50 °C at pH 8.1. Amino acids released during the incubation period were extracted, and the remaining substrate was precipitated after the addition of trichloroacetic acid. Aromatic amino acids react with Folin-Ciocalteu phenol reagent in an alkaline solution to form a blue complex, which was determined colorimetrically.

Urease activity was assayed according to the method of Kandeler and Gerber (1988). After the addition of a buffered urea solution, soil samples were incubated for 2 h at 37 °C. Released ammonium was extracted with potassium chloride solution and was determined by a modified Bertholet reaction.

Dehydrogenase activity was measured using the modified method of Thalmann (1968). Soil samples were suspended in a triphenyl tetrazolium chloride solution and incubated for 16 h at 25 °C. The triphenyl formazan (TPF) produced was extracted with acetone and measured photometrically at 546 nm.

### Statistical analysis
The results were analyzed by ANOVA, considering the treatment as the independent variable. All statistical analyses were carried out with SPSS v.11.0 for Windows. Significant statistical differences for all variables between the different treatments were established by Duncan's test. Correlation coefficients and associated significance were also calculated using mean values of the studied soils.

### Results

#### Organic C, soil microbial biomass C, and microbial quotient

The amounts of soil organic C and soil microbial biomass C (SMBC) were significantly higher in the plots amended with organic materials, as compared to those of the conventional plots that received only inorganic fertilizers (Table 5). Among the organic systems, GM1 and GM2 resulted in higher soil organic C and SMBC than did GM3.

The SMBC/Org. C ratio (ratio of microbial biomass C to total organic C content) of the organic soils increased considerably, as compared to the SMBC/Org. C ratio of the conventional soil (Table 5).

### Table 5. The effect of organic and conventional amendments on soil organic C and soil microbial biomass-C (SMBC).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Organic-C (%)</th>
<th>SMBC (μg g⁻¹ of soil)</th>
<th>SMBC/Org. C (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM1</td>
<td>0.95 a</td>
<td>0.97 a</td>
<td>0.98 a</td>
</tr>
<tr>
<td>GM2</td>
<td>0.93 a</td>
<td>0.94 a</td>
<td>0.96 a</td>
</tr>
<tr>
<td>GM3</td>
<td>0.88 b</td>
<td>0.88 b</td>
<td>0.90 ab</td>
</tr>
<tr>
<td>CONV.</td>
<td>0.80 c</td>
<td>0.80 c</td>
<td>0.80 b</td>
</tr>
<tr>
<td>CV (± %)</td>
<td>0.02</td>
<td>0.01</td>
<td>0.02</td>
</tr>
</tbody>
</table>

GM1 (green manure + farmyard manure, 30 t ha⁻¹)
GM2 (green manure + farmyard manure, 10 t ha⁻¹ + E2001 EM)
GM3 (green manure + E2001 EM)
CONV. (conventional)

Means followed by the same letter within each column are not significantly different according to Duncan's test (P < 0.01).

CV: Coefficient of variation
Enzyme activity

Activity of all the studied enzymes was significantly higher in the organic plots than in the conventional plot (Figure). The conventional plot had the lowest enzyme activity throughout the study period.

Protease (PA) exhibited an approximate activity level in all organic plots in July 2000 and June 2001, but the amendments containing FYM (GM1 and GM2) resulted in higher protease activity than did the GM3 amendment in September 2002. Organic treatments with FYM (GM1 and GM2) resulted in

Figure. Protease (PA), alkaline phosphatase (APA), urease (UA), and dehydrogenase (DHA) activity in soils under organic and conventional management. Organic amendments were GM1 (green manure + farmyard manure, 30 t ha⁻¹), GM2 (green manure + farmyard manure, 10 t ha⁻¹ + E2001 EM), GM3 (green manure + E2001 EM). Different letters refer to significant differences (P < 0.05) between the management systems for each year. Vertical bars are standard deviations.
higher alkaline phosphatase activity (APA) than did the GM3 treatment in June 2001 and September 2002. Urease activity (UA) was not significantly different between the organic treatments. Dehydrogenase activity (DHA) significantly increased in response to GM1 and GM2 at each sampling date. E2001 EM did not influence enzyme activity in the soils.

**Correlations between chemical and biological properties**

Correlation coefficients between chemical and biological properties are given in Table 6. TOC (total organic carbon) and SMBC contents had a high correlation coefficient with nutrient contents and enzymatic activity.

**Discussion**

**Organic C, soil microbial biomass C, and microbial quotient**

Our results show that there were significant alterations (P < 0.05) in the amount of soil microbial biomass and organic C due to FYM application. The microbial biomass contained in FYM and the addition of substrate-C could account for the increase of SMBC in the plots treated with FYM. This dual effect of organic amendments has been also reported by other researchers (Schjonning et al., 2002; Melero et al., 2007). Rampazzo and Mentler (2001) also concluded that FYM positively affects SMBC. The conventional plots had the lowest organic C content and SMBC. Generally, microbial biomass increases with increasing total organic C content (Garcia et al., 2000). Gunapala and Scow (1998) suggested that the most important factor differentiating the microbial activity in different farming systems is the amount of C entering the systems. We also observed a significant relationship between organic C and SMBC (r = 0.93**) (Table 6).

In 2000, organic matter inputs quickly increased SMBC and organic C content in the soils, but additional organic matter inputs in 2001 and 2002 did not cause significant increases in SMBC or organic C, probably because of an increased need for C by the present microbial communities and rapid mineralization under Mediterranean climate conditions.

The SMBC/Org. C ratio serves as an indicator of the quality and availability of organic substances, and of C turnover in soils. It is also useful for identifying trends over time and to compare soils (Sparling, 1997). This ratio will increase or decrease for a time as the input of organic matter in soil increases or decreases (Anderson and Domsch, 1989). According to Böhme and Böhme (2006), this ratio also increases with the decomposability of soil organic matter. The considerable SMBC/Org. C ratio values in the organic soils were probably due to the easily available C fraction of the introduced organic material.

There is considerable interest in conserving or increasing organic matter contents in agricultural soils.

**Table 6. Correlation matrix between enzyme activity, microbial biomass, total organic C, and nutrient content in soil samples.**

<table>
<thead>
<tr>
<th></th>
<th>TOC</th>
<th>Tot. N</th>
<th>Avail. P</th>
<th>SMBC</th>
<th>DHA</th>
<th>PA</th>
<th>UA</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOC</td>
<td></td>
<td>0.70*</td>
<td></td>
<td>0.95**</td>
<td>0.93**</td>
<td>0.71**</td>
<td>0.91**</td>
</tr>
<tr>
<td>Tot.N</td>
<td>0.70*</td>
<td></td>
<td>0.86**</td>
<td>0.87**</td>
<td>ns</td>
<td>0.64**</td>
<td>0.72**</td>
</tr>
<tr>
<td>Avail. P</td>
<td>0.95**</td>
<td>0.86**</td>
<td></td>
<td>0.72**</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>SMBC</td>
<td>0.93**</td>
<td>0.87**</td>
<td>0.72**</td>
<td></td>
<td>0.82**</td>
<td>0.85**</td>
<td>0.78**</td>
</tr>
<tr>
<td>DHA</td>
<td>0.71**</td>
<td>ns</td>
<td>ns</td>
<td>0.82**</td>
<td></td>
<td>0.74**</td>
<td>0.77**</td>
</tr>
<tr>
<td>PA</td>
<td>0.91**</td>
<td>0.64*</td>
<td>ns</td>
<td>0.85**</td>
<td>0.74**</td>
<td></td>
<td>0.67**</td>
</tr>
<tr>
<td>UA</td>
<td>0.73**</td>
<td>0.72**</td>
<td>ns</td>
<td>0.78**</td>
<td>0.77**</td>
<td>0.67**</td>
<td></td>
</tr>
<tr>
<td>APA</td>
<td>0.58**</td>
<td>0.89**</td>
<td>ns</td>
<td>0.73**</td>
<td>0.73**</td>
<td>ns</td>
<td>0.61*</td>
</tr>
</tbody>
</table>

*P < 0.05; **P < 0.01; n = 36; ns = non-significance.

(Paustian et al., 1995). After 10 years of transition to organic management, total organic C content increased in the organic system by 13%-23% in comparison to the conventional system. It is very difficult to increase organic matter content in irrigated soils under Mediterranean climatic conditions due to their very high rates of C mineralization. This increase is particularly important in the agricultural soils of Turkey, in which the levels of organic matter are usually low (< 2%). Scow et al. (1994) reported a smaller increase in the organic matter content (8%-15%) of organic plots after one 4-year rotation cycle in California. Although the increase in the amount of soil organic C is important, the increase in the amount of C associated with microbial biomass is more important. Microbial biomass is one of the most labile of the pools comprising organic matter. An increase in SMBC is likely to better represent changes in the nutrient-supplying capacity of organic matter than is an increase in total organic matter (Gunapala and Scow, 1998).

The addition of E2001 EM to the organic treatments did not affect the amount of microbial biomass in the soils. Similar results were also obtained by Okur et al. (2006), who studied the effect of the same biological preparation under greenhouse conditions.

**Enzyme activity**

Studies comparing conventional and organic farming have reported an increase in phosphatase (Kremer and Li, 2003), protease (Marinari et al., 2006), urease (Melero et al., 2006), and dehydrogenase activity (Masto et al., 2006) in organically managed soils. Although these organic amendments can often contain enzymes, the increase in the activity of soils amended with organic residues is likely due to the stimulation of microbial activity rather than the direct addition of enzymes from organic sources (Martens et al., 1992).

Proteases hydrolyze proteins to polypeptides, oligopeptides, and amino acids. As most N compounds in mineral soils are organically bound, these transformations are necessary to release N for plant uptake. The addition of FYM to soils increased protease activity and the highest activity in plots that received FYM was measured in September 2002. It was observed that high protease activity in plots with FYM continued until September 2002. This long-term increase in activity may have been a result of the additional protein from FYM. Soil protease activity correlated positively with soil protein content and the addition of protein significantly increased soil protease activity (Raab et al., 1999). The other reason for this high protease activity in soils treated with FYM could be the increased N demand to build up microbial biomass stimulated by organic substance amendment (Friedel, 1993). This result is also confirmed by a significant correlation between the activity of protease and Cmic (r = 0.85**) (Table 6).

Because higher plants are devoid of alkaline phosphatase, alkaline phosphatase seems to be derived totally from microorganisms (Kramer and Green, 2002). Increased activity of this enzyme in organic soils with FYM is indicative of the effects of increased Corg content in these soils on the size or metabolic activity of the soil microbial population. Böhme and Böhme (2006) similarly reported that the addition of FYM to soil served as a C source, and enhanced microbial biomass and phosphatase activity. A significant relationship was also observed between Cmic and phosphatase activity in soils (r = 0.73**), confirming that these enzymes originated from microorganisms.

All the organic amendments significantly increased urease activity in the soils. Melero et al. (2006) also reported that urease activity in soils under organic management was greater than that in soils under inorganic management. Urease activity was rather constant under both organic and inorganic fertilization during of the present study period due to the extra cellular character of this enzyme, which was adsorbed and protected by organic complexes.

Dehydrogenase is an oxidoreductase that only exists in viable cells and is considered a sensitive indicator of soil quality (Madejon et al., 2007). Dehydrogenase activity was strongly correlated with soil organic C content (Madejon et al., 2007). Higher levels of dehydrogenase activity in FYM amended-soils may have been the result of their organic C (Table 4). We also observed a significant relationship between organic C and dehydrogenase activity (r = 0.71**) (Table 6).
Conventional soils exhibited the lowest enzyme activity on each sampling date. This may have been due to inhibition of enzyme synthesis by inorganic ions. Klimanek (2000) reported that organic fertilization stimulated alkaline phosphatase activity, while P fertilizers had a negative effect. Böhme and Böhme (2006) reported that control (unfertilized) soils exhibited higher activity than NPK soils for spring barley and sugar beet. Similar results for enzymes that are involved in the N cycle were observed by Dick et al. (1988), who reported that the increasing rates of ammonia-based N fertilizer decreased amidase and urease activity. A feedback mechanism was hypothesized to suppress the production of enzymes whose reaction products were continually supplied by inorganic fertilizers (Dick, 1994). Moreover, dehydrogenase activity is sensitive to the inhibitory effects associated with the addition of large amounts of nitrogenous fertilizer (Goyal et al., 1992).

Correlations between chemical and biological properties

SMBC was significantly and positively correlated with dehydrogenase, protease, urease, and alkaline phosphatase. This indicates that enzyme activity was associated with active microorganisms in the soil that are the major source of soil enzymes. Melero et al. (2006) observed similar relationships in soils under conventional and organic management. The significant correlation between enzyme activity and organic C is likely due to higher C levels supporting greater microbial biomass and activity. Furthermore, increasing organic matter provides a better environment for stabilizing and protecting extra-cellular enzymes (Balota et al., 2004).

Conclusion

Results of the present study indicate that organic management positively affected microbial biomass and enzyme activity due to enhancements in organic matter content. Treatments including FYM (GM1 and GM2), however, had a more long-term effect on microbial activity in vineyard soils than GM without FYM (GM3). This might be a cause of the rapid mineralization rate of cover crops under Mediterranean climatic conditions. E2001 EM had no benefit to soil microbial biomass or enzyme activity in the tested soils. This could be attributed to the fact that the microorganisms introduced into the soil by this biological preparation could not compete with the indigenous microorganisms in the study soil and that they could not persist under these environmental conditions.

The increased microbial biomass and enzyme activity in organic vineyard soils expedited decomposition of organic fertilizers, nutrient cycling, and formation of organic matter and soil structure. As a result, organic management increased soil quality, which is a necessary indicator of land sustainability and management.

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References

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