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Organic Amendment Based on Tobacco Waste Compost and Farmyard Manure: Influence on Soil Biological Properties and Butter-Head Lettuce Yield

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Abstract: Agro-industrial waste presents an alternative to inorganic fertilizer. It is possible to use tobacco waste as a soil amendment due to its high organic matter and low toxic element content. Tobacco waste compost (TWC) and farmyard manure (FYM) were applied to Typic Xerofluent soil at various ratios, and butter-head lettuce (*Lactuca sativa* L. var. *Capitata* L.) was grown. The effects on soil organic C and total N content, soil microbial biomass, soil respiration, activity of 4 enzymes (dehydrogenase, urease, alkaline phosphatase, and β -glucosidase), and lettuce yield were determined. Organic materials were applied at the rate of 50 t ha⁻¹. Significantly ($P < 0.05$) higher values were observed for C_{org}, total N, soil respiration, and dehydrogenase, urease, and alkaline phosphatase activity in 25% FYM + 75% TWC and 100% TWC soils than in the control. The microbial biomass C level in the soils increased in response to all compost treatments. β -glucosidase activity values did not show statistical differences between any of the organic amendment treatments. The application of TWC and FYM resulted in a significant increase in lettuce yield when compared to the control. The results suggest that the incorporation of TWC as an alternative organic amendment might improve soil chemical and biological parameters, as well as crop yield in dryland and especially in Mediterranean soil, which are both characterized by low organic matter content.

Key Words: Tobacco waste compost, farmyard manure, lettuce, microbial and enzyme activity, organic C, total N

Tütün Atığı Kompostu ve Hayvan Gübresine Dayalı Organik Düzenleyicilerin Toprağın Biyolojik Özellikleri ve Iceberg Tipi Marul Verimi Üzerine Etkisi

Özet: Agro-endüstriyel atıklar, inorganik gübre kullanımına alternatif yollar sunmaktadır. Yüksek organik madde ve düşük toksik element içeriği nedeniyle tütün atığının, bir toprak düzenleyicisi olarak kullanımı mümkün görülmektedir. Tipik Xerofluent bir toprağa çeşitli kombinasyon oranlarında tütün atığı kompostu (TAK) ve hayvan gübresi (HG) uygulanmış ve Iceberg tipi marul (*Lactuca sativa* L. var. *Capitata* L.) yetiştirilmiştir. Daha sonra toprağın organik C ve toplam N içeriği, toprak mikrobiyal biyomasi, toprak solunumu ve dört enzim aktivitesi (dehidrogenaz, üreaz, alkalin fosfataz ve β -glukozidaz) ile marul verimi üzerine etkileri incelenmiştir. Organik materyaller 50 ton ha⁻¹ düzeyinde toprağa uygulanmıştır. Kontrolle kıyaslandığında % 25 HG + % 75 TAK ve % 100 TAK uygulamalarında; C_{org}, toplam N, toprak solunumu ve dehidrogenaz, üreaz ve alkalin fosfataz değerlerinde önemli yükselmeler saptanmıştır. Topraktaki mikrobiyal C düzeyleri, tüm kompost uygulamalarında artmıştır. β -glukozidaz aktivitesi kompost uygulamaları arasında istatistiki açıdan bir fark göstermemiştir. Tütün atığı kompostu ve hayvan gübresi uygulamaları kontrolle kıyaslandığında marul veriminde de önemli artışa neden olmuştur. Sonuçlar, tütün atığı kompostunun alternatif organik gübre olarak kullanımının organik maddece fakir özellikle kuru Akdeniz koşullarındaki topraklarda toprağın kimyasal, biyolojik parametreleri ile ürün verimi üzerine etkili olabileceğini göstermektedir.

Anahtar Sözcükler: Tütün atığı kompostu, hayvan gübresi, marul, mikrobiyal ve enzim aktivitesi, organik C, toplam N

Introduction

In Turkey, 6000-6500 t of tobacco waste per year are generated at various stages of post-harvest processing of tobacco and during the manufacture of

tobacco products. Tobacco waste has no immediate use and cigarette companies have to pay for its disposal. The majority of the waste is destroyed by burning. Tobacco solid waste is classified as agro-industrial waste. Because

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of the high organic matter and low toxic element content, it has potential use as a soil amendment. Okur et al. (1999) determined the macro and micro elements content of 4 tobacco wastes from various processing factories. Aegean tobacco content was similar to that of organic amendments, such as manure or green manure. The effects of tobacco waste on soil physical, chemical, and microbiological properties (Gök et al., 1998; Saltalı et al., 2000; Kılıç et al., 2002; Gülser and Candemir, 2004; Kara and Uygur, 2004), and on plant growth and yield (Durak and Brohi, 1986; Brohi and Karaman, 1996; Tarakçıoğlu and Erdal, 2000) have been studied by some researchers in Turkey, but tobacco waste used in these studies were applied directly to soil without composting. Palm et al. (2001) proposed that tobacco waste could be mixed with mineral fertilizers or used as a raw material in composting since it contained $< 2.5\%$ N and $> 15\%$ lignin. Adediran et al. (2003), however, showed that tobacco waste depressed microbial biomass when added to soil and speculated that the nicotine present in the waste could have caused this effect. Nicotine is an insecticide and has been reported to have toxic effects on plants and animals (Baldwin and Callahan, 1993). Direct use of tobacco waste could create an unfavorable soil environment; however, composting tobacco waste could accelerate the breakdown of nicotine and result in the production of a less toxic and more useful organic amendment (Adediran et al., 2004).

In the present study tobacco waste compost (TWC) combined with farmyard manure (FYM) at different ratios was applied to soil and the effects of these amendments on soil microbial activities and butter-head lettuce yield were studied.

Material and Methods

Experimental design

The experiment was conducted at the Research Farm of the Faculty of Agriculture, University of Ege in 2006. The soil texture was a loam, with a pH of 7.5, 1.16% organic C content, and 0.08% total soluble salt. The experiment used a randomized complete block design, with 3 replications of 3.0×2.0 -m plots. The material used for composting was tobacco waste obtained from a cigarette manufacturing processing factory in Kemalpaşa, İzmir, Turkey. Composting was performed outdoors under a roof. During the composting process, aeration was ensured by manual turning. The moisture content of the compost was maintained at about 55% by weighing the composting material regularly and adding water as necessary. The composting was terminated after 3 months, when the temperature of the compost declined to the ambient level.

TWC was mixed with FYM at various ratios. Treatments were as follows:

- Soil without fertilization (control);
- 25% FYM + 75% TWC;
- 50% FYM + 50% TWC;
- 75% FYM + 25% TWC;
- 100% FYM;
- 100% TWC.

The main characteristics of TWC and FYM are given in Table 1.

Organic materials were applied after planting at the rate of 50 t ha^{-1} . The materials were thoroughly mixed

Table 1. Chemical properties of TWC and FYM.

| Parameters | TWC ¹ | FYM ² | Parameters | TWC | FYM |
|-------------------------|------------------|------------------|-------------------------------------|-------|-------|
| pH | 9.1 | 8.7 | Available P, (mg kg ⁻¹) | 4900 | 5800 |
| EC (dSm ⁻¹) | 40 | 38.5 | Available K (mg kg ⁻¹) | 29000 | 26000 |
| Organic C (%) | 37.8 | 39.0 | Available Ca (mg kg ⁻¹) | 1850 | 1791 |
| Total nitrogen (%) | 2.18 | 2.35 | Available Mg (mg kg ⁻¹) | 2619 | 2615 |
| C:N | 17.4 | 16.5 | | | |

¹: Tobacco waste compost

²: Farmyard manure

with the soil and watered by furrow irrigation. Treatments were allowed to equilibrate for 24 h before butter-head lettuce seedlings (*Lactuca sativa* L. var. *Capitata* L.) were transplanted to each plot. Thereafter, the plots were watered by drip irrigation as necessary throughout the growing period. Data related to the cultivar and cultivation practices are summarized in Table 2.

Table 2. Data related to the cultivation practices employed in the study.

| Operation | Dates |
|------------------------------|------------|
| Incorporation of TWC and FYM | 01.09.2005 |
| Planting | 02.09.2005 |
| Harvest period | 11.11.2005 |
| Soil sampling | 07.09.2005 |

Soil Sampling

Soil samples (0-20 cm) were taken 1 week after compost application. In all, 5 randomly selected samples were taken from each plot, bulked, passed through a 4-mm sieve, and stored at 4 °C until assayed.

Chemical and physical analyses

Mechanical analysis of the soil was performed with the hydrometer method (Bouyoucos, 1962), and soluble salt content and pH were measured in saturated soil (U.S. Soil Survey Staff, 1951; Jackson, 1967). Organic C was determined according to Walkley and Black, (1934) and total N according to Bremner (1965). Macro- and microelement analyses of organic materials were determined spectrophotometrically in samples digested by a mixture of HNO₃ and HClO₄ (Kacar, 1995).

Biological analyses

All biological analyses were conducted on moist samples. Soil respiration (CO₂ production) was determined by the titration method (Isermeyer, 1952). Soil samples were incubated in a closed vessel at 25 °C. The CO₂ produced was absorbed in sodium hydroxide and quantified by titration.

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

and colored with sodium hydroxide, and then determined photometrically at 400 nm.

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 a potassium chloride solution and determined by a modified Bertholot reaction.

Dehydrogenase activity was determined 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.

β-Glucosidase activity was measured using the method of Hoffmann and Dedekan (1965). Using β-glucosido-saligenin (salicin) as a substrate, soil samples are incubated for 3 h at 37 °C. Saligenin released from the substrate was determined colorimetrically at 578 nm after coloring with 2,6-dibromchinon-4-chlorimide.

Statistical analysis

Statistical analyses were carried out using SPSS v.8.0 for Windows and the results are expressed as mean values. Significant differences between management systems were evaluated by Tukey's test at P < 0.05.

Results

Chemical parameters

The addition of compost caused changes in certain bulk soil chemical properties (Table 3). Compared with the control, C_{org} and total N were higher in all compost treated soils. Significantly (P < 0.05) higher values for C_{org} and total N were observed in 25% FYM + 75% TWC and in 100% TWC soils than in the control. The C:N ratio was significantly (P < 0.05) higher in 50% FYM + 50% TWC and in 100% TWC soils.

Biological parameters

Microbial biomass C (C_{mic}) and soil respiration values are given in Table 4. A significant (P < 0.05) increase in C_{mic} was observed in all organically amended soils, as compared to the control soil. Soil respiration was significantly higher in 100% TWC and 25% FYM + 75% TWC soils (P < 0.05) than in the control and all other organic treatment soils. The ratio of soil microbial

Table 3. Organic C (C_{org}), total N, and the C:N ratio of the study soils.

| Treatments | C_{org} (g kg ⁻¹) | Total N (g kg ⁻¹) | C/N |
|-------------------|---------------------------------|-------------------------------|-------------------------|
| Control | 14.6 (0.5) ^c | 1.29 (0.10) ^c | 11.3 (0.8) ^c |
| 25% FYM + 75% TWC | 18.7 (0.6) ^a | 1.59 (0.06) ^a | 11.7 (0.7) ^b |
| 50% FYM + 50% TWC | 17.5 (0.5) ^b | 1.43 (0.06) ^{bc} | 12.2 (0.6) ^a |
| 5% FYM + 25% TWC | 17.3 (0.7) ^b | 1.46 (0.08) ^b | 11.8 (0.5) ^b |
| 100% FYM | 17.1 (0.6) ^b | 1.46 (0.05) ^b | 11.7 (0.8) ^b |
| 100% TWC | 18.8 (0.9) ^a | 1.57 (0.10) ^a | 12.0 (0.8) ^a |

Numbers in parentheses are standard deviations ($n = 3$). Mean values followed by the same letter are not significantly different between different treatments, according to Tukey's test ($P < 0.05$).

Table 4. Microbial biomass (C_{mic}), soil respiration, and $C_{mic}:C_{org}$ ratio of the study soils.

| Treatments | C_{mic} ($\mu\text{g C g}^{-1}$ of soil) | Soil respiration ($\mu\text{g CO}_2\text{-C g}^{-1}$ of soil d^{-1}) | $C_{mic}:C_{org}$ |
|-------------------|--|--|--------------------------|
| Control | 235.6 (11.2) ^b | 28.75 (5.63) ^c | 1.61 (0.06) ^d |
| 25% FYM + 75% TWC | 481.8 (15.3) ^a | 40.71 (6.32) ^a | 2.57 (0.08) ^c |
| 50% FYM + 50% TWC | 478.2 (14.8) ^a | 38.64 (8.25) ^b | 2.73 (0.06) ^b |
| 75% FYM + 25% TWC | 469.3 (17.3) ^a | 37.32 (7.41) ^b | 2.71 (0.09) ^b |
| 100% FYM | 485.2 (15.9) ^a | 35.98 (7.55) ^b | 2.83 (0.08) ^a |
| 100% TWC | 480.04 (16.5) ^a | 41.21 (5.62) ^a | 2.55 (0.06) ^c |

Number in parentheses are standard deviations ($n = 3$). Mean values followed by the same letter are not significantly different between different treatments, according to Tukey's test ($P < 0.05$).

biomass C to that of organic C ($C_{mic}:C_{org}$) significantly ($P < 0.05$) increased with the application of 100% FYM.

The lowest soil enzymatic activity values were measured in the control soil (Table 5). The highest dehydrogenase and alkaline phosphatase activity values were observed in 25% FYM + 75% TWC soil. Additionally, the highest urease activity was observed in 25% FYM + 75% TWC and 100% TWC soils. β -glucosidase activity values did not show any statistical differences between the different compost treatments.

Organic C and total N contents demonstrated a significant correlation coefficient with enzymatic activity in the soil (Table 6).

Yield

From a practical standpoint, crop yield is the most important indicator of the success of a particular

agricultural management system. A significant ($P < 0.05$) increase in butter-head lettuce yield was obtained with each organic amendment, as compared to the control soil (Figure 1).

Discussion

Chemical parameters

Soil organic matter imparts many desirable biological, chemical, and physical properties to soil. The observed increases in C_{org} after compost application resulted in an overall benefit to soil quality. All 3 organic mixtures, as well as 100% TWC and 100% FYM resulted in higher C_{org} content than the control. The highest C_{org} value was recorded in the 25% FYM + 75% TWC and 100% TWC soils. The high organic matter content of FYM and TWC (Table 1) increased the C_{org} content of the soils. Özgüven

Table 5. Dehydrogenase (DHA), alkaline phosphatase (APA), urease (UA), and β -glucosidase (GA) activity in the study soils.

| Treatments | DHA ($\mu\text{g TPF g}^{-1}$) | APA ($\mu\text{g p-NP g}^{-1} \text{h}^{-1}$) | UA ($\mu\text{g N g}^{-1} \text{2h}^{-1}$) | GA ($\mu\text{g Saligenin g}^{-1} \text{3h}^{-1}$) |
|-------------------|-------------------------------------|--|---|---|
| Control | 143.4 (9.1) ^c | 625.3 (78.2) ^b | 50.9 (2.9) ^b | 48.8 (3.4) ^b |
| 25% FYM + 75% TWC | 230.3 (13.8) ^a | 824.2 (82.4) ^a | 71.3 (6.2) ^a | 74.8 (10.4) ^a |
| 50% FYM + 50% TWC | 176.1 (13.7) ^{bc} | 716.4 (64.4) ^{ab} | 51.1 (11.6) ^b | 72.7 (10.8) ^a |
| 75% FYM + 25% TWC | 180.1 (14.5) ^{bc} | 767.0 (62.6) ^{ab} | 58.9 (4.0) ^{ab} | 70.9 (12.9) ^a |
| 100% FYM | 166.2 (19.5) ^c | 701.2 (79.8) ^{ab} | 49.5 (7.9) ^b | 66.5 (4.1) ^a |
| 100% TWC | 208.5 (8.3) ^{ab} | 762.2 (73.9) ^{ab} | 68.2 (10.3) ^a | 76.9 (13.8) ^a |

Number in parentheses are standard deviations (n = 3). Mean values followed by the same letter are not significantly different between different treatments, according to Tukey's test (P < 0.05).

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

| | Tot.N | C _{org} | C _{mic} | SR | DHA | APA | UA | GA |
|----------|-------|------------------|------------------|---------|---------|---------|--------|---------|
| TOC | - | 0.969** | 0.928** | 0.925** | 0.947** | 0.919** | 0.816* | 0.892* |
| Tot. N | - | - | 0.871* | 0.984** | 0.908* | 0.877* | 0.856* | 0.957** |
| Avail. P | - | - | - | 0.910* | 0.916* | 0.894* | 0.876* | 0.837* |
| SMBC | - | - | - | - | 0.867* | 0.875* | ns | 0.978** |
| DHA | - | - | - | - | - | 0.938** | 0.915* | 0.865* |
| PA | - | - | - | - | - | - | 0.827* | 0.867* |
| UA | - | - | - | - | - | - | - | ns |
| APA | - | - | - | - | - | - | - | - |

*P < 0.05; **P < 0.01; n = 36; ns = not significant.

Tot. N: total N; C_{org}: total organic C; C_{mic}: soil microbial biomass C; SR: soil respiration; DHA: dehydrogenase activity; APA: alkaline phosphatase activity; UA: urease activity; GA: β -glucosidase activity.

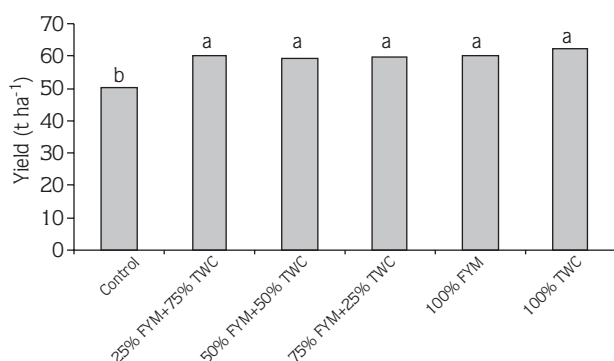


Figure 1. Butter-head lettuce yield in the study soils. The same letters above bars indicate that yields are not significantly different between different treatments, according to Tukey's test (P < 0.05).

et al. (1999) found a noticeable effect on C_{org} in plots treated with different amount of tobacco waste. According to the researchers, the increase may be

attributed to humic compounds in tobacco waste. Czekala et al. (1998) studied the quantity and quality of humic compounds that originate during the decomposition of tobacco dust and showed that organic compounds in tobacco dust were similar in structure to soil humic acids.

The increase in total N in the compost-amended soils was closely related to the high nitrogen content of the compost applied to the soil. Similar results were reported by Wang et al. (2004) in plots treated with composted dairy and swine manures. According to Ayuso et al. (1996), the increase may be attributed to a direct effect of organic N derived from the compost, which is slowly mineralized in soil after the composting process (Castellanos and Pratt, 1981). C_{org} and N_t were higher in the 25% FYM + 75% TWC and 100% TWC soils than in the other compost mixtures. These results show that TWC could serve as an alternative organic amendment instead of FYM.

Biological parameters

Microbial biomass is considered to be a better indicator of soil fertility than C_{org} , as it responds more rapidly and more sensitively to changes in soil management (Powlson et al., 1987; Garcia et al., 2000). A significant ($P < 0.05$) increase in C_{mic} in compost soils may be due to the increased availability of substrate-C, which stimulates microbial growth, but a direct effect from the microorganisms in the compost is also possible (Ros et al., 2006). Other authors have reported a similar dual effect (Garcia-Gil et al., 2000; Ros et al., 2003). In contrast to microbial biomass, soil respiration is considered to indicate the availability of C for microbial maintenance and is a measure of the basic turnover rates in soil (Insam et al., 1991). The data obtained in the present study suggest that the amendments that contained more TWC (25% FYM + 75% TWC and 100% TWC) profoundly stimulated heterotrophic microorganisms in the soil.

The ratio of biomass C to soil organic C ($C_{mic}:C_{org}$) is indicative of the contribution of microbial biomass to soil organic C (Anderson and Domsch, 1989). It also indicates substrate availability to the soil microflora or, vice versa, the fraction of recalcitrant organic matter in the soil; in fact, this ratio declines as the concentration of available organic matter decreases (Brookes, 1995). In the present study the contribution of microbial biomass to total organic C was significant in soils treated with FYM. The $C_{mic}:C_{org}$ ratio of compost treatments ranged from 2.55 to 2.83. The $C_{mic}:C_{org}$ ratios of agricultural and forest soils at neutral pH are very similar, and are in the range of 2.00-4.40, depending on nutrient status and soil management. Ratios below 2.0 could be considered critical for soil with a neutral soil pH (Anderson, 2003). In our study the $C_{mic}:C_{org}$ ratio (1.6) of the control soil indicated that substrate availability was not sufficient for the soil microflora.

The incorporation of organic amendments in soil influences soil enzymatic activity because the added material may contain intra- and extracellular enzymes, and may stimulate microbial activity in the soil (Tejeda and Gonzales, 2006; Chang et al., 2007; Melero et al., 2007). According to Garcia et al. (1994) and Pascual et al. (1998), organic amendment had a positive effect on the activity of these enzymes, particularly when the amendment was at a high concentration, probably due to the higher microbial biomass produced in response. The

development of microbial populations may also be responsible for the stimulation of dehydrogenase activity. The highest values of dehydrogenase, alkaline phosphatase, and urease activity in the present study, as well as soil respiration, were observed in the amendments containing more TWC.

Among the enzymes tested, dehydrogenase was the only endocellular enzyme, while the others were extracellular. Dehydrogenase is involved in the respiratory chain of microorganisms, and has often been used as a parameter to evaluate the overall microbial activity of soil (Nannipieri et al., 1990) and compost (Forster et al., 1993). Alkaline phosphates originate from microorganisms and are not produced by plants (Kramer and Green, 2000). Greater levels of activity of these enzymes indicated the effect of the higher C_{org} content of 25% FYM + 75% TWC and 100% TWC soils on the size of or the metabolic activity of the soil microbial population (Table 3). The present study found significant relationships between the activities of these enzymes and C_{org} content of the soil, as previously reported by Nannipieri et al. (1978) and Manna et al. (1996) (Table 6). Urease is involved in the hydrolysis of urea to carbon dioxide and ammonia, which can be assimilated by microbes and plants (Bremner and Mulvanay, 1978). Since soils contain urease-producing microorganisms, urease activity in soils can be increased by addition of organic materials that promote microbial activity. Urease was one of the enzymes that showed increased activity in response to the application of 25% FYM + 75% TWC and 100% TWC. Similar results were obtained by Özdemiş et al. (2000), who worked with different organic materials. β -Glucosidase is one of the most important glycosidases in soils because it catalyzes the hydrolysis of carbohydrates with β -D-glucosidase-bonds, such as cellobiose. As a result, these enzymes contribute to the mineralization of cellulose, the main C_{org} compound in nature (Landgraf et al., 2003). β -Glucosidase exhibited similar activity among the different treatments in the present study. A strong and positive significant correlation was found between β -glucosidase activity and the C_{org} content of soil ($r = 0.957$, $P < 0.01$). According to Landgraf and Klose (2002), β -glucosidase activity is governed by the amount of easily mineralizable organic C and soils can be classified with respect to their β -glucosidase activity (Martens, 1995). The results of FYM soil and TWC soil in the present study were classified as moderate ($50\text{-}100 \mu\text{g saligenin g}^{-1} \text{ 3 h}^{-1}$).

A significant correlation between organic C or total N, and biochemical properties indicates soil microorganism activation in response to the addition of organic residues (Masciandaro et al., 1997). Furthermore, organic matter plays an important role in the protection of enzymes in a soil humic-clay complex (Tabatabai, 1994).

Since soil enzymatic activity is responsible for important cycles, such as C, N, P, and S, butter-head lettuce yield increased significantly when FYM and TWC compost were applied to the soil.

Conclusions

The application of FYM or TWC, alone or in combination, affected bulk soil chemical and biological

parameters, and crop yield. In particular, the application of 25% FYM + 75% TWC and 100% TWC resulted in the most pronounced effects. These results demonstrate the importance of the incorporation of TWC as an alternative organic amendment for improving the quantity of organic matter in dryland and, especially, in Mediterranean soil, which are characterized by low organic matter content. In addition, there was an increase in the quantity and activity of microbial biomass, which is of great importance to organic matter turnover and nutrient availability in the studied soil.

In order to maintain and improve soil quality, further studies must be performed to confirm the positive long-term effects of TWC.

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