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***Huseyin Husnu KAYIKCIOGLU**
Orcid No: 0000-0003-0895-221X
****Nur OKUR**
Orcid No: 0000-0002-7796-1227

*Ege University Department of Soil Science and Plant Nutrition, Faculty of Agriculture (Corresponding author)

husnu.kayikcioglu@ege.edu.tr

** Ege University Department of Soil Science and Plant Nutrition, Faculty of Agriculture

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Tobacco waste, soil enzymes, cumulative CO_2, maize yield, compost, soil health

Evaluation of Soil Microbial Activity and Maize (*Zea mays* L.) Growth in Soil Amended with Composted Agroindustrial Wastes

Abstract

The present study was performed to emphasize that tobacco waste compost can be used as an organic material resource in soils under semi-arid climate conditions. We determined soil microbial activity as well as the contents of the nutrition and the biomass of maize plant to indicate which compost is the most suitable one for decomposing by microorganisms in the soil. In the greenhouse experiment, the treatments consisted of tobacco waste compost (TWC), tobacco waste+olive pomace compost (TWOPC), tobacco waste+grape pomace compost (TWGPC), tobacco waste+ farmyard manure compost (TWFYC), inorganic fertilization (NPK) and control soil. Soil respiration was higher in TWC amended soils, followed by TWOPC, TWGPC and TWFYC amended soils compared to non-amended soils. The effect of compost applications on enzyme activity of soil was significant. The activities of protease, urease and dehydrogenase were significantly higher in the soil amended with TWC as compared to those of the other composts. B-Glucosidase activity was highest (21 %) in TWOPC with respect to control soil. Aryl sulphatase activity in the soils amended with the composts increased between 20 to 26 % with respect to the control. Activity of alkaline phosphatase in soils amended with the composts was not different from the amount obtained for control soil. The maximum values of leaf N, K and biomass weight of maize plant were found in inorganic fertilizer treatment (NPK). The biomass weight increased 115 % and 83 %, respectively, in NPK and TWC treatments compared to the control soil. The results suggested that application of the composts including tobacco waste to soil at a rate of 30 t ha^{-1} increased the amounts of microbial activity, organic C, available P and K in soil. On the other hand, the amount of total N in the composts is not enough for the growth of maize plant.

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INTRODUCTION

From the first civilizations based on agriculture until now, the agriculture, which has never lost its importance in our lives, has gained increasingly a vital aspect in the 21th century. Agricultural soils have a vital importance for all living organisms. In this aspect they became more prominent with the size of arable and pasture lands are constant or even being reduced, despite the increased population. The strategic values of the soil and agricultural products have increased since the world was threatened by hunger, global warming and water scarcity. Nevertheless, deforestation, conversion from natural to agricultural ecosystems, agricultural practices, and fire events a strongly enhance the loss of organic matter as CO_2 from soils by increasing oxidative processes, besides natural disturbances (Kayikcioglu et al., 2020). An increase in soil organic matter (SOM) is also still seen, by many farmers, as a desirable objective. SOM is universally recognized to be among the most important factors responsible for soil fertility, crop production, and land protection from contamination, degradation, erosion and desertification, especially in semi-arid and arid areas (Senesi et al., 2007). On the other hand, in Mediterranean countries including Turkey, the characteristic of climate (warm, dry summers with a hard, prolonged drought, followed by strong autumn rainfall) and inadequate land management have led to a reduction in the organic matter content of soils, their structural degradation, and an eventual loss of fertility (Cala et al., 2005; Kayikcioglu et al., 2019). Applying organic wastes especially as a compost to soil, could represent a useful tool in maintaining and increasing amounts of SOM (Mondini et al., 2007; Okur et al., 2020). In Turkey, tobacco production approximately became 55 thousand tons with an increasing rate of 3.7% in the year of 2010 compared to 2009 (FAOSTAT, 2011; TurkStat, 2012). This production quantity of unmanufactured tobacco as mostly oriental type is mainly used for making cigarettes and cigars. Tobacco waste is a typical agroindustrial waste material produced during the manufacturing of tobacco. The range of waste products generated from tobacco is diverse, and mainly includes tobacco stems, tobacco dust and tobacco residue (Piotrowska-Cyplik et al., 2009; Zhong et al., 2010). During the preparation process of cigarettes a 20% waste was generated, which could not be applied directly to the production of tobacco blends, due to its physical structure (Agnieszka et al., 2009).

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Large amount of wastes including tobacco waste is generated from industrial processing of agricultural raw materials every year. Most of these wastes are used for feeding animals or burned as alternative for elimination. However, such wastes usually have a composition richness in sugars, minerals and proteins, therefore, they should not be considered "wastes" in terms of agriculture. As a result, recycling large amounts of organic residues, by-products, wastes and effluents (such as municipal sewage sludge and urban solid wastes, food industry and wood processing wastes, agricultural crop residues and animal manures) as soil organic amendments has become a very popular and efficient agricultural practice (Senesi et al., 2007). Composting, the biological degradation of organic wastes to humus, either saves natural resources or reduces the quantity of wastes, and the application of composts may promote soil microorganisms due to enhance the organic carbon status in soil, thereby contributing to a decrease in atmospheric CO_2. The agricultural and environmental consequences of compost usage are continually recognized. The application of materials with a high organic matter content and nutrients, such as composts prepared tobacco waste and sawdust/wood shavings with differed in the third ingredient, which was either cow dung, pig dung, poultry manure or cabbage waste (Adediran et al., 2004); cotton gin compost and poultry manure (Tejada et al., 2006); beet vinasse composted with a crushed cotton gin compost (Tejada et al., 2007); composts obtained from the medicinal and aromatic plants factories (oily mixed oregano wastes and oily mixed cumin wastes) (Okur et al., 2008a; Kayikcioglu, 2013); and composted and raw of tobacco waste from cigarette factory (Okur et al., 2008b; Kayikcioglu, 2009) to semi-arid soils has become a common environmental practice for soil restoration, maintaining soil organic matter, reclaiming degraded soils, and supplying plant nutrients. The present study was aimed to compare the effects of tobacco waste compost (TWC), its combination with olive pomace (TWOPC), grape pomace (TWGPC), farmyard manure (TWFYC) and commercial mineral fertilizer (NPK) on (1) the changes that may have occurred in the amended soil and (2) yield and nutrition of maize. The study was performed to emphasize that tobacco waste compost can be used as an organic material resource in soils under semi-arid climate conditions.

MATERIAL and METHODS

In the experiment, the treatments consisted of tobacco waste compost (TWC), tobacco waste+olive pomace compost (TWOPC), tobacco waste+grape pomace compost (TWGPC), tobacco waste+ farmyard manure compost (TWFYC), inorganic fertilization (NPK) and control (C). The mixing composts were prepared at a 1:1 v/v ratio. Prior to the experiment, composting was performed in cylindrical plastic bins with height 1 m and diameter 0.5 m. The moisture content of the composts was maintained at about 60 % of the water-holding capacity. Initially, turning was done twice every week and then weekly. The composting was terminated after 5 months, when the temperature of the composts declined to the ambient level. The main characteristics of the composts are given in Table 1.

Table 1. Chemical properties of TW and the composts

Parameter	TWC[b]	TWOPC[c]	TWGPC[d]	TWFYC[e]
pH[f]	9.6[a]	9.6	9.8	9.5
EC[g], dS m^{-1}	89	48	60	73
Total organic C, g kg^{-1}	331	430	432	270
Total N, g kg^{-1}	29.6	25.7	34.1	23.4
C:N	11	17	13	12
Total P, g kg^{-1}	4.2	3.2	5.8	7.6
Total K, g kg^{-1}	62.6	35	62	49

[a]: Each value is the mean of three replicates and on an oven-dry (105 °C) basis; [b]: Tobacco waste compost; [c]: Tobacco waste+olive pomace compost; [d]: Tobacco waste+grape pomace compost; [e]: Tobacco waste+ farmyard manure compost; [f]: pH of 1:10 water extract; [g]: Electrical conductivity of 1:10 water extract

The soil used in the experiment was obtained from Menemen - İzmir, Turkey and is classified as a Typic Xerofluvent (Soil Survey Staff, 2014). The soil have the following properties: 0.42% organic C; pH 7.6; total soluble salt 0.05%; $CaCO_3$ 4.58%; loamy sand texture; 0.057% total Kjeldahl N; 4.74 mg kg^{-1} Bingham available P; 4.74, 2448 and 380 mg kg^{-1} 1 N NH_4OAc (pH 7) available K, Ca and Mg, respectively. Composts were added to 10 kg soil in pots at the rate of 30 t ha^{-1} in 2007. For NPK treatment, 300 mg kg^{-1} N, 80 mg kg^{-1} P and 100 mg kg^{-1} K were applied to soil. The half of nitrogen as $(NH_4)_2SO_4$ and all of potassium and phosphorus as KH_2PO_4 were applied at the time of sowing. The other half of nitrogen as NH_4NO_3 was applied 1 month after sowing. The composts were thoroughly mixed with the soil and watered to 80% of field capacity. Treatments were allowed to equilibrate for 24 h before 10 maize seeds (*Zea mays* L. var. *Dracma*, FAO: 650) were sown to each pot at the

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room temperature. After 10 days, these were thinned to two plants per plot. Thereafter, pots were watered to approximately 80% of field capacity as and when necessary throughout the growing period. Ten weeks after sowing, the aerial parts of the plants were harvested and biomass yield was determined. The experimental layout was in a randomized parcel design with four replications per treatments. During the experiment, soil samples (0-15 cm) were collected from each pot three times. Field-moist soil was sieved (2 mm) and divided into two sub-samples. One was immediately stored at 4 °C in plastic bags until microbiological and enzymatic activity were assayed; other was air dried prior to chemical analysis (Öhlinger, 1995). Salinity, organic matter concentration and pH were determined according to McLean (1982), Nelson and Sommers (1982) and Rhoades (1982), respectively. Total N as described by Bremner (1965). After nitric and perchloric acid digestion (4:1), total K was determined by flame photometry (Kacar and İnal, 2008). Total P content in the acid digest was determined by spectrophotometer after developing the vanadomolybdo-phosphoric yellow color complex in nitric acid medium (Kacar and İnal, 2008). Alkaline phosphatases (ALKPA) activity was measured using the method of Eivazi & Tabatabai (1977). Protease (PRO)activity was determined as described by Ladd & Butler (1972). Β-Glucosidase (GLU) activity was measured using the method of Hoffmann & Dedekan (1965). Dehydrogenase (DHG) activity was determined using the modified method of Thalmann (1968). Urease (UA) activity was assayed according to the method of Kandeler and Gerber (1988). Aryl sulphatase (ArSA) activity was measured using the method of Tabatabai and Bremner (1970). C-mineralization (BSR) was determined from the quantity of CO_2-C mineralized from soil sample during 7 days incubation at 25°C (Isermeyer, 1952). Repeated measures analyses were used to compare mean values from different samples. Where significant differences were obtained, individual means were tested using Duncan's test. All statistical analyses were carried out with SPSS v.15.0 for Windows.

RESULTS and DISCUSSION

Soil chemical analysis

The addition of the composts and inorganic fertilizers to the experiment soil changes in certain soil chemical properties (Table 2). Compared with the control, C_{org}

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was higher in all the compost treated soils. Addition of the composts increased soil organic C average 24% compared to the control. This increase is particularly important in Aegean, region of our study, where the levels of organic matter in agricultural soils are generally < 2% (Eyüpoğlu, 1999). pH values in all soils decreased soil but pH range of the soils did not change. Except NPK soil, the other soils have light alkaline pH. The highest amounts of soluble salt and total N were determined in NPK soil. Amounts of available P and K were significantly higher in soils treated with TWGPC and TWC.

Table 2. Selected soil chemical properties determined in the end of experiment.

Parameter	C[b]	TWC[c]	TWOPC[d]	TWGPC[e]	TWFYC[f]	NPK[g]
pH(H_2O)	7.64 a[a]	7.48 bc	7.60 ab	7.38 c	7.45 bc	7.13 d
Salinity, %	0.047 d	0.105 b	0.069 cd	0.065 cd	0.085 bc	0.239 a
Total C_{org}, %	0.85 b	1.10 a	1.02 a	1.04 a	1.06 a	0.85 b
Total N, %	0.060 b	0.067 ab	0.061 b	0.064 b	0.064 b	0.072 a
Available P, mg kg^{-1}	4.29 d	5.36 cd	6.03 bc	8.23 a	7.04 b	4.31 d
Available K, mg kg^{-1}	200 d	563 a	375 c	536 a	445 b	222 d

[a]: Each value is the mean of four replicates of experimental soils and mean values followed by the same letter are not significantly different according to Duncan (P<0.01) between different treatments ; [b]: Non-amended soils; [c]: Tobacco waste compost; [d]: Tobacco waste+olive pomace compost; [e]: Tobacco waste+grape pomace compost; [f]: Tobacco waste+ farmyard manure compost ; [g]: Inorganic fertilization

Soil microbial analysis

BSR values of samples are shown in Fig 1. CO_2 emissions were higher in the compost-amended soils compared to the control soil. CO_2 production increase depends on the chemical composition of the organic matter applied to the soil. In this respect, soil respiration was higher in TWC amended soils, followed by TWOPC, TWGPC and TWFYC amended soils. Soil microbial respiration is a direct indicator of microbial activity, and indirectly reflects the availability of organic material (Tejada et al., 2006). The statistical analysis determined significant correlations between CO_2 production and organic C and enzymatic activities in soils (Table 3). Due to the difference in C structure in its chemical composition, 0.60 mg CO_2 24 h^{-1} g^{-1} value was obtained by applying 25 t ha^{-1} hazelnut husk compost (Irmak Yilmaz, 2020). Higher BSR values obtained by applying various composts generated with tobacco waste at the rate of 30 t ha^{-1} can be evidence that these tobacco composts are more easily decomposed by heterotrophic microorganisms than hazelnut husk compost.

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Table 3. Correlation coefficients between enzyme activities, soil respiration and total organic C (C_{org}) in soil samples

Parameter	C_{org}[c]	ALKPA[d]	ArSA[e]	GLU[f]	PRO[g]	UA[h]	DHG[i]
ALKPA	ns[b]	1					
ArSA	0.970**[a]	0.861*	1				
GLU	0.818*	ns	ns	1			
PRO	0.848*	0.943**	0.922**	ns	1		
UA	0.955**	0.870*	0.982**	ns	0.899**	1	
DHG	0.890**	0.929**	0.958**	ns	0.973**	0.914**	1
BSR[j]	0.796*	0.858*	0.843*	0.812*	0.877**	0.770*	0.938**

[a]: Correlation is significant at the 0.01 level (2-tailed), 0.05 level (1-tailed); [b]: Non-significant; [c]: Soil organic carbon value; [d]: Alkaline phosphatase; [e]: Aryl sulphatase; [f]: ß-glucosidase; [g]: Protease; [h]: Urease; [i]: Dehydrogenase; [j]: Basal soil respiration

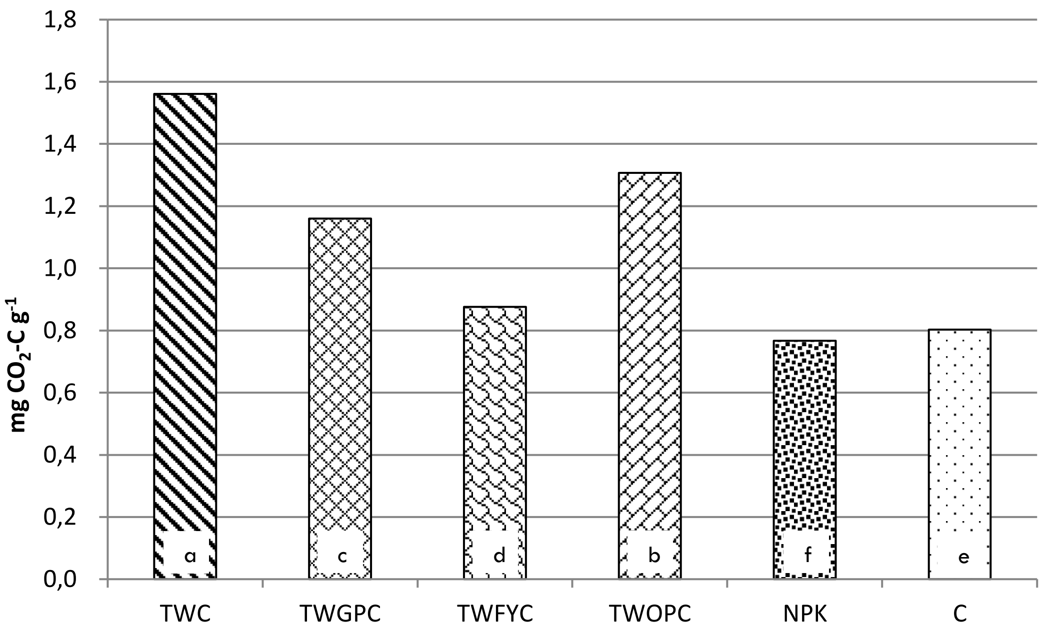

Figure 1. CO_2-produced from soils treated with the composts and NPK
*Mean values followed by the same letter are not significantly different according to Duncan (P<0.01) between different treatments. TWC: Tobacco waste compost; TWGPC: Tobacco waste+grape pomace compost; TWFYC: Tobacco waste+ farmyard manure compost; TWOPC: Tobacco waste+olive pomace compost; NPK: Inorganic fertilization; C: Non-amended soils

The effect of compost applications on enzyme activity of soil was also significant (Table 4). The activities of PRO, UA and DHG were significantly higher in the soil amended with TWC as compared to those of the other composts. PRO activity that is closely related to the N cycle and catalyzes the hydrolysis of proteins, increased 630%, with respect to the control soil. The lowest PRO activity was determined in control and NPK soils. Also, UA activity is closely related to the N cycle since it is involved in

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the hydrolysis of urea to ammonium and carbon dioxide (Tripathi et al., 2007). This activity was highest 68% in TWC compared to the control soil. Okur et al. (2008b) determined that the applications of 25% FYM+ 75% TWC and 100% TWC significantly increased urease activity in soil. Results of enzymatic activity linked to the N cycle (protease and urease) indicate that TWC represents an important source of N readily available.

Table 4. Average amounts of enzyme activities determined in the soils taken three times during growing period of maize

Parameter	C[b]	TWC[c]	TWOPC[d]	TWGPC[e]	TWFYC[f]	NPK[g]
ALKPA[h], µg p-NP g^{-1} h^{-1}	281.3 a	292.2 a	289.6 a	294.2 a	281.8 a	292.9 b
ArSA[i], µg p-NP g^{-1} h^{-1}	53.2 b	63.8 a	67.3 a	66.2 a	65.0 a	48.8 b
GLU[j], µg Saligenin g^{-1} $3h^{-1}$	67.5 bc	71.1 b	81.6 a	70.7 b	63.6 cd	60.0 d
PRO[k], µg Tyrosin g^{-1} $2h^{-1}$	347.1 d[a]	2536.5 a	475.9 b	455.8 bc	371.2 cd	288.2 d
UA[l], µg N g^{-1} $2h^{-1}$	23.2 d	38.9 a	33.7 b	32.1 b	27.4 c	19.1 e
DHG[m], µg TPF g^{-1}	66.0 d	109.5 a	101.9 ab	92.9 bc	84.5 c	58.0 d

[a]: Mean values followed by the same letter are not significantly different according to Duncan (P<0.01) between different treatments; [b]: Non-amended soils; [c]: Tobacco waste compost; [d]: Tobacco waste+olive pomace compost; [e]: Tobacco waste+grape pomace compost; [f]: Tobacco waste+ farmyard manure compost; [g]: Inorganic fertilization; [h]: Alkaline phosphatase; [i]: Aryl sulphatase; [j]: B-Glucosidase; [k]: Protease; [l]: Urease; [m]: Dehydrogenase

DHG 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). The highest amounts of dehydrogenase activity were observed in the soils amended with TWC and TWOPC. Activities of urease (P<0.01), protease and dehydrogenase (P< 0.05) were significantly related to the content of organic C in soil. Similar results were obtained by Chen et al. (2004). ß-Glucosidase is one of the key enzymes which govern carbon cycle. It catalyzes the hydrolysis of carbohydrates with ß-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). ß-Glucosidase activity was highest 21% in TWOPC compared to control soil. This could be due to the higher cellulose content of TWOPC compared to the other composts. Dick et al. (1988) determined a high ß-Glucosidase activity in composts rich in cellulose. A study was conducted to investigate the effects of different doses of hazelnut compost applications on hazelnut plantations on the seasonal basis of GLU and UA activity in the soil (Irmak Yilmaz, 2020). This study indicated that, the application that increases the GLU activity, which was determined as 14.82 µg p-NP g^{-1} h^{-1} on a seasonal average, is 100 t hazelnut husk compost ha^{-1}, whereas the application that increases the UA activity, which was determined as 30.48 µg N g^{-1} $2h^{-1}$ on average, is the 75 t hazelnut husk compost ha^{-1}. Compared to the application rates (75 and 100 t ha^{-1}) of hazelnut husk compost, it is seen that tobacco waste compost alone (TWC) and composts produced with olive (TWOPC) and grape (TWGPC) wastes (30 t ha^{-1}) showed more GLU and UA activities with the lesser application rate as a 30 t ha^{-1} compared to control and mineral fertilizer treatment (Table 4). Aryl sulphatase is the enzyme involved in the hydrolysis of arylsulphate by fission of the O-S bond (Spencer, 1958). This enzyme is believed to be involved in mineralization of ester sulphate in soils (Tabatabai, 1994). The means of activity of this enzyme in the composts ranged from 63.8 to 67.3 µg p-NP g^{-1}soil h^{-1}. Less aryl sulphatase activity was determined in NPK and control soils. The activity of this enzyme was significantly correlated with soil organic C (P<0.01).

Phosphatases are a general name used to describe a broad group of enzymes that catalyze the hydrolysis of both esters and anhydrides of phosphoric acid (Schmidt and Laskowski, 1961). Alkaline phosphatase in soils is believed to be totally derived from microorganisms because it has not been found in higher plants (Tabatabai, 1994). Amounts of this enzyme in soils amended with the composts were not different from the amount obtained for control soil. This enzyme activity was not significantly correlated with soil organic C. Phosphatase activity is generally stimulated by microbial growth and from low levels of available P and repressed by the product of the enzymatic reaction (Mondini et al., 2008). The fact that the experiment soil has a good level of available P, phosphates activity in the soil can be repressed.

Plant analysis

The effects of different soil treatments on leaf N, P, K and biomass yield were recorded (Table 5). These results indicate that all the plant parameters significantly (P<0.01) responded to the compost and inorganic fertilizer treatments on their soil application as compared to the control. The maximum values of leaf N, K and biomass weight were found in inorganic fertilizer treatment (NPK). The biomass weight

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increased 115%, 83%, 57%, 43% and 23%, respectively, in NPK, TWC, TWGPC, TWFYC and TWOPC treatments with respect to the control soil. Total leaf N increased 149%, 40%, 33%, 18% and 13%, respectively, in NPK, TWC, TWGPC, TWOPC and TWFYC treatments with respect to the control soil. These results showed that maize plant could not take from soils treated with the composts necessary N for its nutrition. However, maize plant had taken more P from the soils treated with the composts compare to NPK soil.

Table 5. Effect of the different soil treatments on leaf N, P, K and biomass weight of maize plant

Parameter	C[b]	TWC[c]	TWOPC[d]	TWGPC[e]	TWFYC[f]	NPK[g]
Total N, %	0.55 d[a]	0.77 b	0.65 c	0.73 b	0.62 c	1.37 a
Total P, %	0.21 ab	0.22 ab	0.25 a	0.25 a	0.23 ab	0.18 b
Total K, %	2.73 b	3.57 a	2.91 b	3.63 a	3.43 a	3.71 a
Biomass yield, g	79.3 f	144.9 b	97.3 e	124.3 c	113.3 d	170.8 a

[a]: Mean values followed by the same letter are not significantly different according to Duncan (P<0.01) between different treatments; [b]: Non-amended soils; [c]: Tobacco waste compost; [d]: Tobacco waste+olive pomace compost; [e]: Tobacco waste+grape pomace compost; [f]: Tobacco waste+ farmyard manure compost; [g]: Inorganic fertilization

CONCLUSIONS

These results indicated that application of the composts including tobacco waste to soil increased the amounts of microbial activity, organic C, available P and K in soil. The biological parameters in amended soils were higher than in control soil, which clearly indicates the improvement of soil biological quality brought about by the organic amendment, despite the fact that the organic matter contained in compost is more stable to degradation. The results also demonstrate that microbial biomass and soil enzyme activity is sensitive in discriminating between compost and inorganic fertilizer application on a short-term basis. In terms of agronomic value, composting grape pomace, olive pomace and farm yard manure with tobacco waste lead to a product that supplies nutrients to plants. It is important to point out that composts containing tobacco waste is efficient and free from any phytotoxic elements i.e. nicotine, since no acute effect on the development of maize plants were observed during the experiments conducted here. In the case of maize, tobacco waste compost application was the most increased plant biomass except NPK treatment. However, the amount of total N in the composts is not enough for the growth of maize plant. Therefore, there is a need for the further studies including addition of mineral N to the composts in order to obtain greater productivity. Nowadays, interest in soil management practices that will improve the biological contribution to soil fertility is steadily growing as awareness for the need of sustainable agricultural systems is increasing. Therefore, composting tobacco waste with other agricultural wastes and application at a rate of 30 t ha^{-1} to the soils can be an environment friendly solution to the disposal problem of these wastes and an adequate low-cost strategy for the recycling of agro-industrial by-products.

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