Lettuce growth and nutrient uptake response to winery waste compost and biochar

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Abstract

The composting process of winery wastewater sludge with grape stalks enables these wastes to be recycled as soil organic amendments or pot substrate components. However, it is necessary to assess the potential of this compost as crop fertilizer. This assessment is also needed for the use of biochar as soil amendment because negative effects on crop growth and nutrient uptake may occur depending on application rates and biochar physical and chemical characteristics. With this aim, a randomized block design pot experiment with lettuce was set up with four treatments including: (i) 5% (w/w) of winery waste compost; (ii) 4% (w/w) biochar (commercial charcoal); (iii) 5% (w/w) winery waste compost with 4% (w/w) biochar; and (iv) a control treatment without soil amendments (T0). Lettuce shoot yield increased 13% with compost application but decreased 18% with biochar in comparison to T0 and 25% with compost and biochar compared to compost alone. Similarly, lettuce root weight increased with compost, but not with biochar. Lettuce shoot dry matter (DM), N and Ca contents clearly increased with compost application compared to lettuce grown in the treatment with biochar. The same happened for root Ca and Mg contents. The lowest P and K contents of lettuce shoots and roots were recorded in T0. However, lettuce root N content decreased with biochar compared to T0. Therefore, root and shoot growth and nutrient uptake decreased with soil application of 4% (w/w) of biochar whereas the winery waste compost showed positive effects as soil fertilizer.

Keywords: grape stalks, *Lactuca sativa* L., soil amendment, winery wastewater sludge

INTRODUCTION

The wastes from the wine industry include solid wastes (grape stalks, grape marks and wine lees) and winery sludge derived from the wastewater treatment plant when the machinery is cleaned. The grape mark and lees are usually distilled to recover ethanol and tartaric acid but can also be used to extract polyphenols that are a source of natural antioxidants (Arvanitoyannis et al., 2006). The wastewater used in irrigation, reduces the water footprint and contributes to nutrient recycling. However, it may exert phytotoxic effect if applied directly to crops and may cause potential environmental problems such as excess of organic matter, salinity, and nutrient pollution that can affect groundwater resources (Mosse et al., 2011). Composting grape stalks with the winery sludge is an effective, low-cost alternative to transform agro-industrial wastes into organic fertilizers decreasing the environmental impact (Bertran et al., 2004).

In the last decades, intensive applications of mineral fertilizers have contributed to climate change, biodiversity loss and degradation of land and fresh water (Foley et al., 2011). Organic amendments such as compost and biochar are useful tools to sustain biodiversity, decrease environmental impact and improve soil fertility. The stable and sanitized compost from winery wastewater sludge and grape stalks was recommended as a fertilizer with good physical and chemical characteristics and with suitable amount of nutrients for vineyards

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fertilization (Bertran et al., 2004).

Biochar obtained from pyrolysis of carbon-rich material, may contribute to improve soil fertility and crop nutrient availability, to sequester organic carbon and to increase soil water content (Atkinson et al., 2010; Woolf et al., 2010; Baronti et al., 2014). Moreover, crop yields and nutrient uptake may be enhanced when biochar is applied together with compost (Agegnehu et al., 2016). However, negative effects on crop growth and nutrient uptake may occur depending on biochar application rates and biochar physical and chemical properties, which differ according to feedstock, availability of oxygen and temperatures achieved during pyrolysis (Spokas et al., 2011).

There is little knowledge about the effect of biochar on lettuce growth, and no information is available on the combined effects of biochar and compost from winery wastewater sludge and grape stalks. Therefore, the aim of this study is to clarify the fertilizer effect of biochar and the effect of compost from winery wastewater sludge and grape stalks on lettuce growth and nutrient uptake to improve organic lettuce fertilization.

MATERIALS AND METHODS

A pot trial with lettuce (*Lactuca sativa* L. 'Maravilla de Verano') was conducted inside a greenhouse as a randomized block design with four treatments and five blocks. The treatments included: (i) 5% (w w^{-1}) of winery waste compost (C); (ii) 4% (w w^{-1}) biochar (B); (iii) 5% (w w^{-1}) winery waste compost with 4% (w w^{-1}) biochar (BC) and (iv) a control treatment (T0) without soil amendments. The biochar application rate of 4% (w w^{-1}) was based on previous research experiments (Lehmann et al., 2011; Kalika et al., 2014). The characteristics of the compost and biochar are shown in Table 1. The compost was produced with winery wastewater sludge and grape stalks at a mixing rate 2:1 (sludge: stalks, w w^{-1}). The compost was matured as indicated by the amount of NH_4^+-N (45.6 mg kg^{-1}) below 400 mg kg^{-1} (Zucconi and Bertoldi, 1987). The biochar used was a commercial charcoal obtained from holm oak branches with a pyrolysis temperature between 600 and 700°C (biochar piroeco provided by Piroeco Bioenergy, S.L., Spain).

Table 1. Dry matter content (DM) and chemical characteristics of winery waste compost and biochar.

Nutrients		Compost	Biochar
DM	(%)	31	37
pH		8.2	9.5
EC	(dS m^{-1})	0.39	0.83
N	(g kg^{-1})	13.0	0.1
C	(g kg^{-1})	242	780
C/N		19	7800
NH_4^+-N	(mg kg^{-1})	45.6	-
NO_3^--N	(mg kg^{-1})	26.3	-
P	(g kg^{-1})	2.8	0.06
K	(g kg^{-1})	7.4	0.83
Ca	(g kg^{-1})	4.5	6.1
Mg	(g kg^{-1})	1.5	0.24

Nutrient concentration is expressed on a dry matter basis.

Lettuce seeds were sown in polystyrene trays with 220 cells and 35 mL of volume per cell containing substrate certified for organic agriculture (Tray mix). The transplanting occurred in August, 26 days after seeding, to pots with 7 kg of sandy soil collected from 0 to 20 cm depth. The characteristics of the soil are shown in Table 2. The pots were irrigated to prevent water stress and weeding was carried out whenever necessary to avoid weed competition for nutrients. Lettuces were harvest 47 days after planting. The soil, after removed from the pots, was soaked in water to loosen the soil from the roots. Then, shoots and roots were weighted separately, and subsequently dried at 65°C in a thermoventilated oven until reaching a constant mass, to determine DM content. Dry samples were milled and used to determine total N, P, K, Ca and Mg contents.

Table 2. Soil chemical characteristics at the beginning of the pot experiment.

pH	EC ($dS\ m^{-1}$)	OM ($g\ kg^{-1}$)	N total ($g\ kg^{-1}$)	NH_4^+-N ($mg\ kg^{-1}$)	NO_3^--N ($mg\ kg^{-1}$)	P_2O_5[a] ($mg\ kg^{-1}$)	K_2O[a] ($mg\ kg^{-1}$)
5.6	0.13	46	3.6	1.2	53.7	305	315

[a]Egner-Riehm method (Egner et al.,1960).

Organic Matter and nutrient concentration are expressed on a dry matter basis.

Compost dry matter content (DM), pH and electrical conductivity (EC) were determined by standard procedures (CEN, 1999). The soil pH was measured with a pH meter in samples extracted with water at 22°C±3°C in an extraction rate of 1:5 ($v\ v^{-1}$) and the specific EC was measured in the same extract with a conductivity meter. Total C content was determined by near infrared spectroscopy, using an elemental analyzer, after combustion at 950°C. The soil OM content was calculated by multiplying C content by the factor of van Bemmelen (1.724). Total N and P were measured by molecular absorption spectrophotometry and K quantified by flame photometry after digestion with sulfuric acid. Total Ca and Mg were measured by atomic spectrophotometry following nitro-perchloric digestion. Mineral N of fresh soil and compost was extracted with KCl 1 M 1:5 solution and determined by molecular absorption spectrophotometry using a continuous flow auto-analyzer. Crop nutrient accumulation (mg pl^{-1}) was calculated multiplying plant nutrient content (mg g^{-1}) by plant dry weight (g pl^{-1}). Analysis of variance (ANOVA) of results was performed by the SPSS general linear model procedure and a probability level of $P<0.05$ was applied to determine statistical differences between treatment means.

RESULTS AND DISCUSSION

The pot experiment clearly demonstrated that the compost of winery wastewater sludge and grape stalks improved lettuce yield and nutrient uptake. On the contrary, fresh weight of lettuce shoots decreased with biochar application (Figure 1). The fresh weight of lettuce shoots was enhanced 13% with compost compared to T0. This is in agreement with lettuce yield increases reported by other authors (Manojlović et al. 2010; Montemurro, 2010; Brito et al., 2012) for matured composts. Lettuce yield increased 18% with on-farm composted horse manure (Brito et al., 2012) and 39% with olive compost (Montemurro, 2010). In contrast to compost, lettuce fresh yield decreased 18% with biochar in comparison to T0 and 25% with compost and biochar compared to compost alone.

The literature is not consistent about the relationship between biochar application rates and lettuce growth (Kalika et al., 2014; Kim et al., 2015; Trupiano et al., 2017). This is probably due to the fact that different biochars may have different effects on short-term crop growth (Tsukagoshi et al., 2010). Here, the results are in agreement with those reported by Kim et al. (2015). These authors showed that lettuce growth decreased at biochar application rates above 1% ($w\ w^{-1}$), probably because biochar, with high specific surface, may tight soil nutrients and limit their availability to plants (Lammirato et al., 2011). Trupiano et al. (2017) found no differences in lettuce growth, after compost application in combination with biochar (5% $w\ w^{-1}$) compared to compost alone. However, Kalika et al. (2014) reported that lettuce fresh weight increased with biochar rates up to 30 t ha^{-1} and then decreased probably due to the sensitivity of lettuce to salts. Carter et al. (2013) indicated that the application of high amounts of biochar (5 to 15% $w\ w^{-1}$) alone or with compost led to a positive effect on lettuce growth because biochar altered physical (bulk density) and chemical (pH and EC) soil properties.

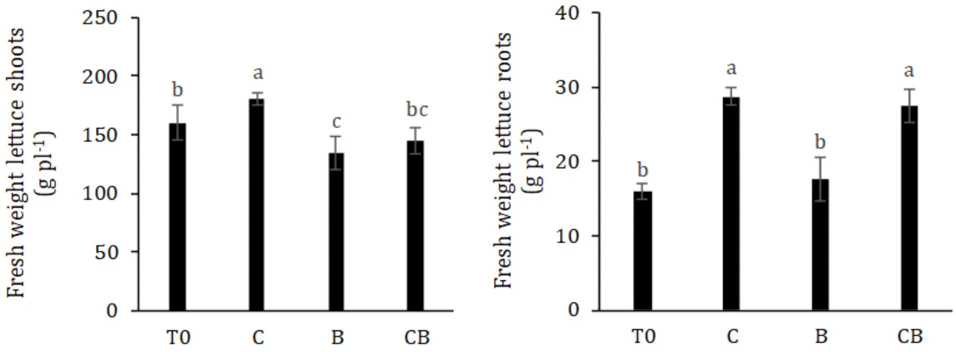

Figure 1. Fresh weight of lettuce shoots and roots in response to control treatment without fertilization (T0), 5% (w/w) compost (C), 4% (w/w) biochar (B), and 5% (w/w) compost with 4% (w/w) biochar (CB). Bars with different letters are significantly different ($P<0.05$).

Conflicting results have been reported on the relationship between biochar application and root growth (Lehmman et al., 2011; Xiang et al., 2017). Xiang et al. (2017) reported that chemical and physical characteristics of biochar such as pH, porosity and bulk density may affect the soil environment and thereby stimulate root growth. On the other hand, the releasing of inhibitory chemicals by biochar such as ethylene may reduce root growth (Spokas et al., 2012). In this experiment, no significant differences were found in lettuce root growth after biochar application compared to T0 or after compost and biochar application compared to compost alone (Figure 1). Therefore, potential improvements in the soil arising from the chemical and physical characteristics of biochar were not enough to offset possible inhibitory effects (Macdonald et al., 2014).

Lettuce N content decreased with biochar application (Table 3). The reduced N availability may be explained by the high biochar C/N ratio (7800) because of greater potential for N immobilization (Steiner et al., 2008). However, woody biochar is highly recalcitrant (DeLuca and Aplet, 2008) and the high temperature achieved during biochar pyrolysis (600 to 700°C) increased the amount of compounds resistant to microbial decomposition (DeLuca et al., 2009). Therefore, it is uncertain if biochar amendment provide sufficient available C to promote N immobilization. It is more likely that biochar adsorbed temporarily NH_4^+ and NO_3^- from the soil solution, thus reducing mineral N availability because the high temperature achieved during the pyrolysis process increases the particle surface area (DeLuca et al., 2009).

The lowest P and K contents in lettuce shoots and roots were recorded in T0. The increase in lettuce P content found for plants grown in soil amended with biochar compared to control plants was probably due to a pH increase, which reduces P precipitation with Al^{3+} and Fe^{3+} in acid soils. Biochar high specific surface area may also influence P precipitation because organic molecules involved in chelation of Al^{3+} and Fe^{3+} may be tight to biochar surface (DeLuca et al., 2009). The lettuce K content increased with biochar probably because the availability of K (water-soluble form) from biochar was enhanced by the high pyrolysis temperature (Ding et al., 2016). This potential increase in K availability after biochar application may have caused antagonistic interactions between K and Ca (Kasinath et al., 2017), decreasing Ca availability and, consequently, lettuce Ca content (Table 3). Lettuce shoot Ca content clearly increased with compost application compared to lettuce grown in the treatment with biochar. The same happened for root Ca and Mg contents.

Root nutrient content was always lower than shoot nutrient content, except for P and Mg for which the contents were similar in roots and shoots. The Ca content in the shoots doubled those of the roots but this increase was less for N and K. This indicates that nutrient distribution was held for the benefit of the leaves. The ratio N/P was similar for lettuce shoots (4.7) and compost (4.6), whereas the ratio N/K was higher for compost compared to lettuce shoots.

Table 3. Dry matter content (DM) and nutrient content in lettuce shoots and roots in response to the control treatment without fertilization (T0), and soil amended with 5% (w/w) compost (C), 4% (w/w) biochar (B), and 5% compost with 4% biochar (CB).

Treatments	DM (%)	N	P	K	Ca	Mg
		(g kg^{-1})				
Shoots						
T0	7.7	12.0	2.4	31.4	19.1	3.0
C	8.3	12.8	2.7	40.8	20.2	2.6
B	7.1	10.3	2.8	41.1	13.8	3.0
CB	7.8	11.7	2.9	42.0	13.7	2.3
LSD[a]	0.6	1.6	0.3	6.7	4.4	0.4
Roots						
T0	15.5	8.3	2.2	12.9	8.2	4.6
C	11.9	7.4	3.3	31.4	8.8	3.9
B	13.8	5.9	3.4	29.0	7.2	2.9
CB	11.5	6.9	3.1	41.7	8.5	2.7
LSD[a]	1.9	2.1	0.5	4.3	1.6	0.5

[a]LSD=least significant difference ($P<0.05$).

Nutrient concentration is expressed on a dry matter basis.

The accumulation of N, P and K increased in lettuce shoots and roots with compost incorporation (Figure 2). The increase of accumulated nitrogen in lettuces produced with compost compared to the control lettuces was greater than the mineral nitrogen in the compost. Therefore, the difference between the accumulated N in the lettuce produced with the compost and the control lettuce, minus the compost mineral N, divided by the compost organic N, suggests that the organic nitrogen mineralization rate of the compost was 2.4%, for the period of 47 days of lettuce growth. Matured composts show low N mineralization due to the formation of stable N compounds through the composting process (Larney and Hao, 2007). Ribeiro et al. (2010), for example, reported an N mineralization rate of 4.5% from on-farm compost during the period of 133 days. Nevertheless, this stabilized compost with low content of mineral N (71.9 mg N kg^{-1}) appeared to be a good fertilizer for short-term vegetable crops such as lettuce.

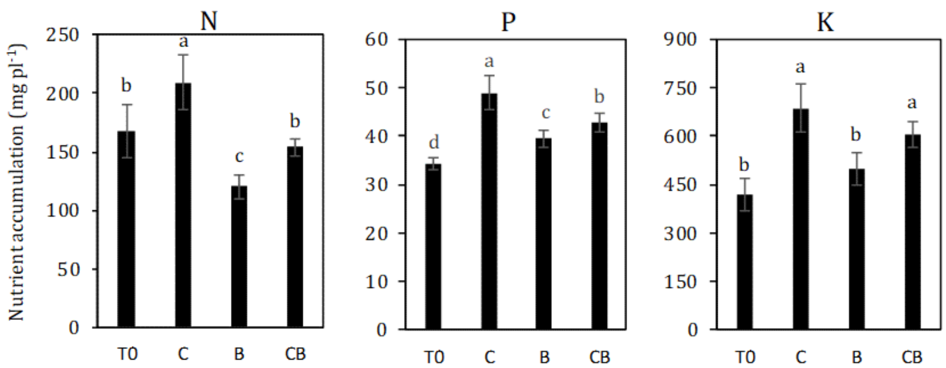

Figure 2. Nitrogen, P and K accumulation in lettuce shoots and roots (mg pl^{-1}) in response to control treatment without fertilization (T0), 5% (w/w) compost (C), 4% (w/w) biochar (B), and 5% (w/w) compost with 4% (w/w) biochar (CB). Bars of the same nutrient with different letters are significantly different ($P<0.05$).

CONCLUSIONS

The fresh weight of lettuce shoots and roots increased with compost application. Compost improved lettuce nutrient uptake and shoot dry matter content. On the contrary, biochar had a negative effect on lettuce shoot and root growth while shoot N and Ca contents also decreased after biochar application. Therefore, the composting of winery wastewater sludge with grape stalks produces a fertilizer with potential to increase nutrient uptake and support lettuce production as opposed to this biochar alone or in combination with compost that had a negative effect on lettuce yield and N accumulation.

ACKNOWLEDGEMENTS

This work was funded by the European Regional Development Fund (ERDF), through the Regional Operational Program North 2020, within the scope of Project I&D INOVINEWINE – Vineyard and Wine Innovation Platform nº Norte 01-0145-FEDER-000038, and of Project INTERACT – Integrative Research in Environment, Agro-Chains and Technology nº Norte-01-0145-FEDER-000017. The authors are grateful to Real Companhia Velha for the accomplishment of the composting in Quinta Casal da Granja and the Foundation for Science and Technology (FCT, Portugal) and FEDER under the Programme PT2020 for financial support to CIMO (UID/AGR/00690/2013).

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