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Research article

Recycling nutrient-rich hop leaves by composting with wheat straw and farmyard manure in suitable mixtures

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ABSTRACT

The harvesting of hops (*Humulus lupulus* L.) generates large amounts of nutrient-rich leaves that can be used in composting mixtures to add value to other organic resources on the farm. In this study, hop leaves were mixed with cow manure and wheat straw in several combinations with the aim of establishing guidelines on how farmers can manage the raw materials and better use these valuable organic resources. The composting process was monitored and the quality of the composts evaluated in relation to the effects on lettuce (*Lactuca sativa* L.) grown in pots over two consecutive cycles. The mixture of hop leaves with cow manure produced a stable compost after nine months of composting which may be used in horticultural crops, irrespective of the proportion of raw materials, due to their low and similar C/N ratios. However, when using mixtures of leaves and straw in proportions of less than 2:1, the composts did not mature properly, showing high C/N ratios. Their application to the soil led to a strong reduction in plant tissue N concentrations, due to biological N immobilization, which significantly reduced lettuce dry matter yield. Thus, to reduce composting time and increase the quality of the compost, the ratio leaves/straw should be as high as possible, at least 2:1. Alternatively, either the composting process should take longer, or the poorly-matured compost be applied far in advance of sowing a crop so that complementary biological processes can take place in the soil, as recorded in the second cycle of lettuce. Ash from hop stems did not benefit the composting process and proved itself not to be worth using in mixtures.

1. Introduction

Hops (*Humulus lupulus* L.) is a dioecious perennial and climbing plant which can reach nearly 7 m high, producing a large amount of biomass. In commercial fields, only female plants are grown, whose flowers are mainly used for brewing purposes (Almaguer et al., 2014). During the harvesting process, the flowers, usually known as cones, are separated from the stems and leaves which are frequently seen as waste. Leaves and stems represent a huge amount of biomass given that world hop production reached 131,173 t in 2020 (International Hop Growers' Convention, 2020). Hop cones are also a form of waste in themselves, after either extraction of α and β acids or their direct use in the brewing process. In recent years, hop waste from the brewing industry has received increasing attention. It can be composted and applied to the soil as organic amendments (Kopeć et al., 2020) or be used to obtain high added-value bioproducts (Hrnčič et al., 2019). In contrast, the leaves and stems resulting from the harvesting process continue to remain on farms and have not yet received the same attention.

The European Union (2012) has been encouraging the use of bio-waste in agriculture, since it can improve the condition of the soil and provide valuable nutrients to plants. Recycling bio-waste into organic-based fertilizers also forms part of the action plan of the European Commission (2017) to support the transition towards a carbon-neutral economy. Composting is considered one of the most effective processes to recycle organic waste, which can be applied to soils as organic amendments (Antil et al., 2014; Pergola et al., 2018). Composting is a biological process of degradation of fresh organic residues under controlled aerobic conditions with the purpose of obtaining a compost of stabilized organic matter (OM), rich in organic carbon (C) and free from pathogens and weed seeds (Cesaro et al., 2015; Pergola et al., 2018).

To ensure the quality of the final compost, several variables should be considered during the composting process. Microorganisms are the primary driving agents in the decomposition of biodegradable materials. They use organic C as a source of energy, while nitrogen (N) is used for growth and reproduction (Osman, 2013; Azim et al., 2018). Therefore, the initial C/N ratio is of great importance to favour microbial activity and to optimize the composting process (Bernal et al., 2009; Wong et al.,

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2017). Temperature during composting is a critical variable that should be monitored to ensure the reduction of pathogens and a quick decomposition and humification (Azim et al., 2018). The nutrient content of the final compost, and particularly the N content, is a quality parameter determining the value of the compost to be used in agriculture (Raviv, 2005; Wong et al., 2017).

The stability and maturity of a compost are also requirements for its safe use in agricultural fields. Compost stability is related to the level of microbial activity, while maturity depends on the level of humification and implies the absence of pathogens and phytotoxic threats to plant health (Prasad et al., 2010; Cesaro et al., 2015; Azim et al., 2018). A wide range of variables can be used to evaluate the degree of stability and maturity of a compost, such as physical properties (i.e. temperature, odour and colour), the C/N ratio, inorganic-N species (NH_4^+, NO_3^-, NH_4^+/NO_3^- ratio), content of OM and humic acids, salinity and seed germination, and plants growing in biological tests (Bernal et al., 2009; Antil et al., 2014; Azim et al., 2018).

Hop is a dioecious perennial species as mentioned above. In commercial fields only female plants are grown. This means that plants do not form seeds, which greatly reduces the remobilization of nutrients from the leaves. At harvest, hop leaves can be particularly rich in nitrogen, with values reaching 40 g/kg (Afonso et al., 2020), which makes them an excellent material for composting with farmyard manures or to add value to poorer quality materials such as cereal straw through suitable mixtures. Thus, the aim of this study was to monitor the composting process and assess the quality of the final products resulting from composting hop leaves mixed with wheat straw and farmyard manure at different rates. The quality of the composted materials was assessed by their physicochemical properties and through a biological assay, consisting of growing lettuce (*Lactuca sativa* L.) in a pot experiment over two cropping cycles in the same growing medium. It was hoped to establish guidelines for making the best use of these materials, particularly the proportions in which they should be mixed.

2. Materials and methods

2.1. Composting experiment

The leaves used in this study came from the hop harvest of 2017. All the other resouces used to prepare the mixtures for composting (cow manure, wheat straw and ash) came from the same farm, in which are raised animals and where the hop stems are usually burnt. The properties of the raw materials used in this experiment as composting materials are presented in Table 1.

Table 1
Carbon (C)/nitrogen (N) ratio, dry matter percentage and concentration of macro and micronutrients (average ± standard deviation) in the raw materials used in the composting process.

	Cow manure	Wheat straw	Hop leaves	Hop stem ash
C/N	19.95	295.7	19.21	9.63
Dry matter (%)	93.9 ± 1.14	93.1 ± 0.03	92.1 ± 0.05	99.1 ± 0.13
Carbon (g/kg)	307.2 ± 70.62	557.8 ± 3.44	472.0 ± 3.73	20.8 ± 0.28
Nitrogen (g/kg)	15.4 ± 3.22	1.9 ± 0.07	24.6 ± 0.91	2.2 ± 0.29
Potassium (g/kg)	19.8 ± 3.97	7.5 ± 0.01	15.3 ± 1.03	12.8 ± 0.25
Phosphorus (g/kg)	3.3 ± 0.43	1.0 ± 0.05	1.2 ± 0.07	5.7 ± 0.78
Calcium (g/kg)	8.7 ± 0.46	1.0 ± 0.06	46.5 ± 3.59	95.0 ± 5.38
Magnesium (g/kg)	14.3 ± 5.33	0.7 ± 0.01	4.4 ± 0.27	8.6 ± 0.37
Iron (mg/kg)	10,843 ± 6201	26.5 ± 2.12	555 ± 323.4	14,403 ± 2230
Manganese (mg/kg)	269.4 ± 47.5	37.9 ± 1.20	317.7 ± 24.97	898.6 ± 62.7
Copper (mg/kg)	49.0 ± 11.3	5.1 ± 0.35	6.5 ± 0.57	39.6 ± 1.35
Zinc (mg/kg)	87.8 ± 15.4	5.8 ± 0.10	18.8 ± 1.52	101.7 ± 2.55
Boron (mg/kg)	18.6 ± 2.96	3.0 ± 0.26	62.0 ± 1.66	56.3 ± 1.58

The raw materials were placed in the composter in thin layers according to the ratios shown in Table 2. The mixtures were turned out manually at 14, 56, 147 and 210 days after the beginning of the composting process. The temperature in the composters was monitored daily during the first 10 days and thereafter at 15, 30, 60, 120 and 240 days. At the same time, the temperature was also recorded outside the composters. The moisture of the mixtures was controlled by regular observation, and water added as required to maintain biological activity. Six months after the start (March 21st' 2018) and at the end (June 28th' 2018) of the composting process, the mixtures were sampled in triplicate for analytical determinations.

2.2. Pot experiment

A pot experiment was conducted with lettuce (cultivar Wonder of Summer) in two growing cycles (June 28th to August 21st' 2018, and April 30th to July 1st' 2019), using the composted materials as treatments and mixed with soil to create the growing medium. Additionally, the composts of higher (HS1:2) and lower (HS2:1) C/N ratios were used at single (D1) and double rates (D2). The experiment included also an untreated control (C), totalling 10 treatments and four replicates (four pots). Each pot received 3 kg of dried and sieved (2 mm) soil. The composts were used at rates equivalent to 20 (D1) and 40 (D2) t/ha dry weigh (dw), giving that the dry mass of the <2 mm soil fraction of the arable layer (0.2 m) in a hectare is 2240 t. The soil used in this experiment showed pH of 6.51, organic C of 5.2 g/kg, total N of 1.28 g/kg and extractable P and K of 47.8 mg P_2O_5/kg and 53.3 mg K_2O/kg, respectively.

Lettuce seedlings were prepared in micropots of 4 cm^3, by using a commercial germination substrate. They were transplanted at the 3rd true leaf unfolded growth stage, in June 28th and April 30th in the first and second growing cycles. Plants were harvested 54 and 60 days after transplanting in the first and second growing cycles. They were cut at ground level, oven-dried at 70 °C and ground for elemental analysis.

2.3. Analysis of soil, raw materials, composts and plant tissues

Soil was analysed for pH (H_2O) (soil: solution, 1:2.5), cation-exchange capacity (ammonium acetate, pH 7.0), organic C (wet digestion, Walkley-Black method), total N (Kjeldahl) and extractable P and K (Egner-Riehm method) (Van Reeuwijk (2002).

The electrical conductivity was determined in the composted materials from fresh samples, in a water:compost extract of 5:1 (v/v). All the other determinations were performed in dried tissue. Total organic C (TOC) was determined by dry combustion, total N by kjeldahl, B and P by colorimetry, K by flame emission spectrometry and Ca, Mg, Cu, Fe, Zn and Mn by atomic absorption spectrophotometry (Walinga et al., 1989). Ammonium (NH_4^+) and nitrate (NO_3^-) concentrations in the composts were determined by UV–Vis spectrophotometry in a 2 M KCl (3:50, compost:solution) extract.

Table 2
Proportion of raw materials and carbon (C)/nitrogen (N) ratios of the mixtures for composting.

Compostable mixtures	Ratio	Abbreviation	C/N
Hop leaves + cow manure	1:5	HM 1:5	19.97
Hop leaves + cow manure	1:3	HM 1:3	18.21
Hop leaves + cow manure	1:1	HM 1:1	14.56
Hop leaves + wheat straw	1:2	HS 1:2	41.30
Hop leaves + wheat straw	1:1	HS 1:1	29.10
Hop leaves + wheat straw	2:1	HS 2:1	21.00
Hop leaves + wheat straw + hop stem ash	1:1:0.04	HSA 1:1:0.04	29.10

2.4. Data analysis

Data was subject to analysis of variance in SPSS v. 25.0 program to check for significant differences between treatments (one-way ANOVA, according to the experimental design). When significant differences were found the means were separated by Tukey–Kramer HSD test ($\alpha = 0.05$). A principal component analysis (PCA) was applied to the results of composting experiment to evaluated the differentiation of composts groups according to their physicochemical properties.

3. Results

3.1. Temperature during the composting process

The temperatures were high during the first five days of composting, followed by a gradual decrease to the levels observed outside the composters (Fig. 1). Thereafter, the temperatures inside the composters changed in accordance with air temperature and on some dates during the autumn and winter they were lower inside than outside the composters. The highest temperature (68.5 °C) was recorded on the first day in the HM1:1 mixture. Differences between composting mixtures were only evident in the first five days with the manure-based mixtures reaching the highest values. In some straw-based mixtures, the temperature did not reach 55 °C.

3.2. Physical and chemical properties of composts

The concentration of nutrients in composted materials usually increased with time from the sixth to the ninth month of composting (Table 3). In general, the manured-based composts showed significantly higher levels of P, K, Mg, Fe, Mn, Cu and Zn than the straw-based composts. Regarding N, Ca and B, high nutrient concentrations were also found in the treatment of the highest proportion of leaves (HS2:1). Comparing the treatments containing straw, the HS2:1 treatment presented the highest concentration of the majority of nutrients due to the presence of a higher proportion of leaves. In an overall comparison of composts, the treatment containing ash (HSA1:1:0.04) showed relatively low levels of N and K and high levels of Ca, but the results of this treatment were not the highest or the lowest for any of the nutrients.

TOC and C/N ratios appeared divided into two groups, one of the mixtures containing manure and the other of the mixtures containing straw (Fig. 2). The groups of manure-based composts showed significantly lower TOC and C/N ratios than the straw-based composts. Of the manure-containing composts, the one receiving the higher proportion of leaves (HM1:1) showed significantly higher TOC and a lower C/N ratio. Between the straw-containing composts, the one receiving the higher proportion of straw (HS1:2) showed the higher average TOC. Ash seems to have contributed to reducing TOC, when comparing treatments HS1:1 with HSA1:1:0.04. Of the treatments receiving straw, the one receiving the higher proportion of leaves (HS2:1) displayed the lowest C/N ratio. During the composting process, by comparing the results of months six and nine, C/N ratios decreased more markedly than TOC.

During the composting process, from the sixth to the ninth month, EC and NO_3^- values generally increased while NH_4^+ levels and NH_4^+/NO_3^- ratios decreased (Table 4). The final composts prepared with straw presented EC values significantly and noticeably higher than the composts which included manure. The final composts containing manure presented a trend to higher NO_3^- levels in comparison to those containing straw, although the values also depended on the proportion of leaves in the mixtures. In contrast, the straw-based composts showed increased values of NH_4^+ and higher NH_4^+/NO_3^- ratios.

3.3. Principal component analysis

The results of PCA on compost physicochemical properties indicated a clear differentiation into different groups of composts (Fig. 3). The first two principal components explained 86.7% of total variance. The PC1 explained most of the variance (69.3%) and was positively affected by the levels of Cu, Fe, Mg, P, Zn, K and Mn and negatively affected by TOC, EC, NH_4^+ and C/N ratio. The manure-based composts (HM) showed higher scores in PC1 in contrast to the straw-based composts (HS), mainly due to the higher levels of Mn, K, Mg, Cu, Fe, Zn, and P but also due to the lower levels of EC, NH_4^+, TOC and C/N ratio. Within the HM group, HM1:1 diverged from the others due to the higher levels of NO_3^-. Within the HS group, HS2:1 was the most differentiated of all, displaying the higher scores in PC2, and was associated with higher levels of B, Ca and N.

3.4. Lettuce dry matter yield

A comparison of dry matter (DM) yield between the different treatments gave rise to a completely different pattern between the two lettuce cycles, with marked differences between treatments in the first growing cycle and more stable values in the second cycle (Fig. 4). In the first year, the higher average DM yield was recorded in the HS2:1D2 treatment (8.71 g/plant), which was significantly different to the DM yield recorded in the control and in several other treatments. In the first growing cycle, the manure-based composts gave, in general, significantly higher lettuce DM yields than the straw-based composts. The lower value was found in treatment HS1:2D2 (0.48 g/plant). Treatment

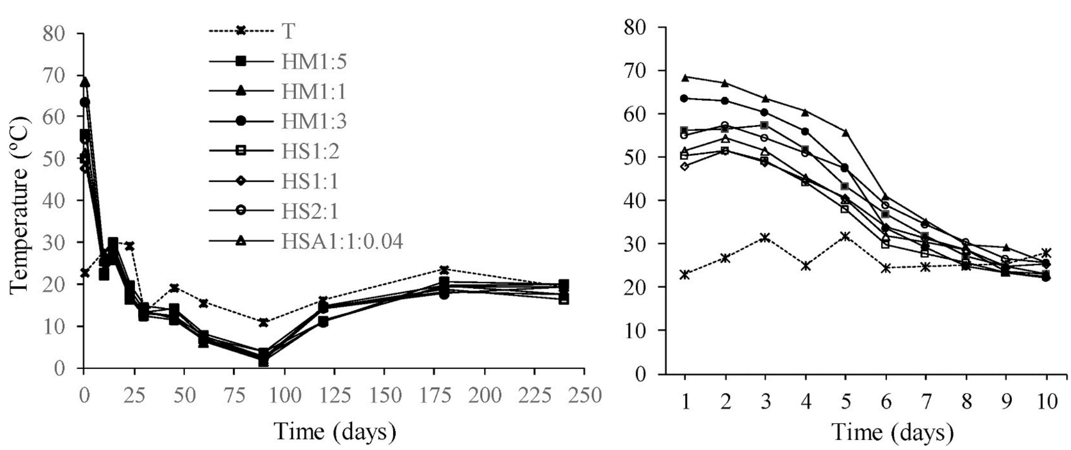

Fig. 1. Temperatures (left) on days 1 and 10 and subsequent measurements, throughout the composting process, and (right) daily for the first 10 days for more detailed observation of the beginning of the process: HM, hop leaves + cow manure at the ratios 1:5, 1:3 and 1:1; HS, hop leaves + wheat straw at the ratios 1:2, 1:1 and 2:1; and HSA, HS + ash from hop stems at the ratio 1:1:0.04); T, air temperature.

Table 3
Nutrient concentration in composted materials after six and nine months of composting (HM, hop leaves + cow manure at the ratios 1:5, 1:3 and 1:1; HS, hop leaves + wheat straw at the ratios 1:2, 1:1 and 2:1; and HSA, HS + ash from hop stems at the ratios 1:1:0.04).

Compost	N	P	K	Ca	Mg	Fe	Mn	Cu	Zn	B
	(g/kg)					(mg/kg)				
sixth month										
HM 1:5	18.3 bc	4.0 a	23.6 a	12.1 a	23.5 a	14,750 a	426.2 a	57.2 a	115.5 a	29.2 b
HM 1:3	18.1 bc	4.0 a	25.5 a	13.0 a	19.4 a	12,078 ab	416.6 a	54.8 ab	110.4 a	33.4 ab
HM 1:1	23.6 a	3.9 a	25.6 a	24.3 a	15.2 a	8959 b	453.3 a	48.4 b	91.4 a	47.9 a
HS 1:2	11.6 d	1.4 b	12.2 c	8.0 a	2.2 b	191 c	130.2 c	8.49 c	69.7 a	25.1 b
HS 1:1	12.5 d	1.6 b	15.1 bc	8.8 a	2.7 b	194 c	137.3 c	9.01 c	102.4 a	27.2 b
HS 2:1	19.5 ab	1.4 b	17.3 b	11.7 a	3.3 b	270 c	214.1 bc	12.0 c	147.3 a	42.1 ab
HSA 1:1:0.04	14.2 cd	1.8 b	15.4 bc	11.8 a	3.0 b	1493 c	280.2 b	9.85 c	70.9 a	35.4 ab
Prob. > F	<0.0001	<0.0001	<0.0001	0.1075	<0.0001	<0.0001	<0.0001	<0.0001	0.8498	0.0068
Stand. error	0.93	0.27	1.19	1.60	2.02	1328	29.37	4.89	14.31	2.08
Ninth month										
HM 1:5	20.9 d	4.5 a	26.3 ab	9.7 cd	17.0 ab	12,507 ab	495.6 ab	52.0 a	104.5 a	31.2 e
HM 1:3	20.5 d	4.3 ab	28.9 a	10.5 cd	18.3 a	13,776 a	485.1 ab	47.3 b	90.7 ab	36.4 d
HM 1:1	25.5 b	4.1 b	28.0 a	12.8 bc	15.6 b	11,226 b	544.9 a	49.3 b	81.1 b	46.5 c
HS 1:2	17.5 e	2.1 c	21.0 c	8.5 d	3.8 d	330 d	229.3 d	7.4 d	23.3 d	40.5 d
HS 1:1	21.9 c	1.8 d	21.9 c	7.9 d	3.0 d	392 d	300.3 d	7.5 d	28.4 d	56.4 b
HS 2:1	29.2 a	1.8 d	22.7 bc	16.5 a	5.1 c	521 d	412.7 c	8.9 d	35.7 cd	82.3a
HSA 1:1:0.04	16.8 f	2.1 c	20.1 c	14.2 ab	4.6 cd	3205 c	460.1 bc	14.0c	48.2 c	52.9 b
Prob. > F	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Stand. error	0.90	0.26	0.77	0.70	1.44	1290	24.05	4.48	6.84	3.53

Means followed by the same letter are not statistically different by Tukey HSD test (α = 0.05).

HS1:2D1 also gave a significantly lower value (1.21 g/plant) than the untreated control (3.67 g/plant). The higher the proportion of straw in the mixtures, the lower the productive performance of the plant. The compost containing ash in the initial mixture (HSA1:1:0.04D1) did not perform better than the treatment with a similar proportion of leaves and straw without ash (HS1:1D1). In the second year, the marked differences between the treatments containing manure or straw narrowed. Although significant differences were not usually found, the average values of lettuce DM yields were generally higher in the straw-based composts. It was also clear that the treatments producing less in the first growing cycle were those displaying the higher average DM yields in the second growing cycle. For instance, the treatment producing the lowest average value in the first growing cycle, HS1:2D2 (0.48 g/plant), gave the highest average value (11.8 g/plant) in the second growing cycle of lettuce.

3.5. Nutrient concentration in lettuce tissues

In the first growing cycle, the N concentrations in lettuce tissues showed a close relationship to lettuce productivity (Table 5). The HS1:2D2 treatment, which gave a minute productivity, showed a particularly low tissue N concentration (13.2 g/kg). Other treatments that led to very low lettuce yields, such as HS1:2D1, HSA1:1:0.04, HS1:1 and the control, showed tissue N levels below 26 g/kg, while all of the more productive treatments showed levels of N in the tissues higher than 28 g/kg. The concentrations of many other nutrients in lettuce tissues also varied significantly between treatments. However, no coherent relationship with lettuce yield was observed. The values probably varied according to the mineral composition of the mixtures, but they should not have had a relevant effect on lettuce productivity. In the 2019 growing cycle, the pattern of significant differences between treatments for some nutrients, including N, persisted, but it was not possible to establish a clear relationship between their concentrations in plant tissues and the performance of lettuce. For N, however, the concentration of the nutrient in plant tissues in the HS1:2D2 treatment increased from 13.2 g/kg in the first growing cycle to 24.6 g/kg in the second growing cycle which, in this case, was consistent with a great increase in lettuce DM yield.

4. Discussion

Temperature in the first days of composting was high, as is convenient in a composting process (Azim et al., 2018). The highest temperatures were reached in the manure-based mixtures, in particular in HM1:1 (68.5 °C). The manure had probably already started the decomposition process in the cowshed, and combined with fresh leaves, they formed an easily degradable substrate, providing conditions for the increase in temperature and rapid OM degradation (Diaz et al., 2011). In a composting process, it is important that temperatures can reach 55 °C to destroy pathogenic microorganisms, but they should not increase above 70 °C to avoid inhibiting the growth of beneficial microorganisms (Wong et al., 2017). In the straw-based mixtures, excluding HS2:1, temperatures did not reach 55 °C, indicating low microbial activity. The higher C/N ratio of these mixtures, coupled with a large particle size of straw (it was not chopped), are reasons usually given to explain low temperatures during composting and reduced rates of substrate decomposition (Bernal et al., 2009). The temperatures within the composters decreased after the first 10 days of composting to values close to outside air temperature. This rapid decrease in temperature of the mixtures being composted was probably due to the exhaustion of the easily degradable substrates but also to the poor thermal inertia of the composters whose volume was only ~0.5 m^3.

The nutrient concentrations in a compost, and particularly of N, greatly determine its quality (Bernal et al., 2009). The final composts prepared with manure presented significantly higher levels of the majority of nutrients (P, K, Mg, Fe, Mn, Cu and Zn) in comparison to the composts based on straw. This is because uncomposted manure has higher levels of those nutrients than straw. The N concentrations in the composts greatly depended on the proportion of leaves in the initial mixtures. Between manure-based and straw-based composts, the higher compost N concentrations were found in the former, if mixtures of the same rates of manure and straw were compared. Thus, the compost showing the higher N concentration was HS2:1, followed by HM1:1. In addition, the manure-based materials might have lost N during composting due to ammonia volatilization, whereas the materials with higher C content, such as straw, might have immobilized more N (Bernal et al., 2009; Lim et al., 2017; Wong et al., 2017). Thus, the C/N ratio depended on the initial mixtures, the values being higher in those containing straw. The higher proportion of leaves, however, reduced the C/N ratio of the mixtures. It is well known that the initial C/N ratio and

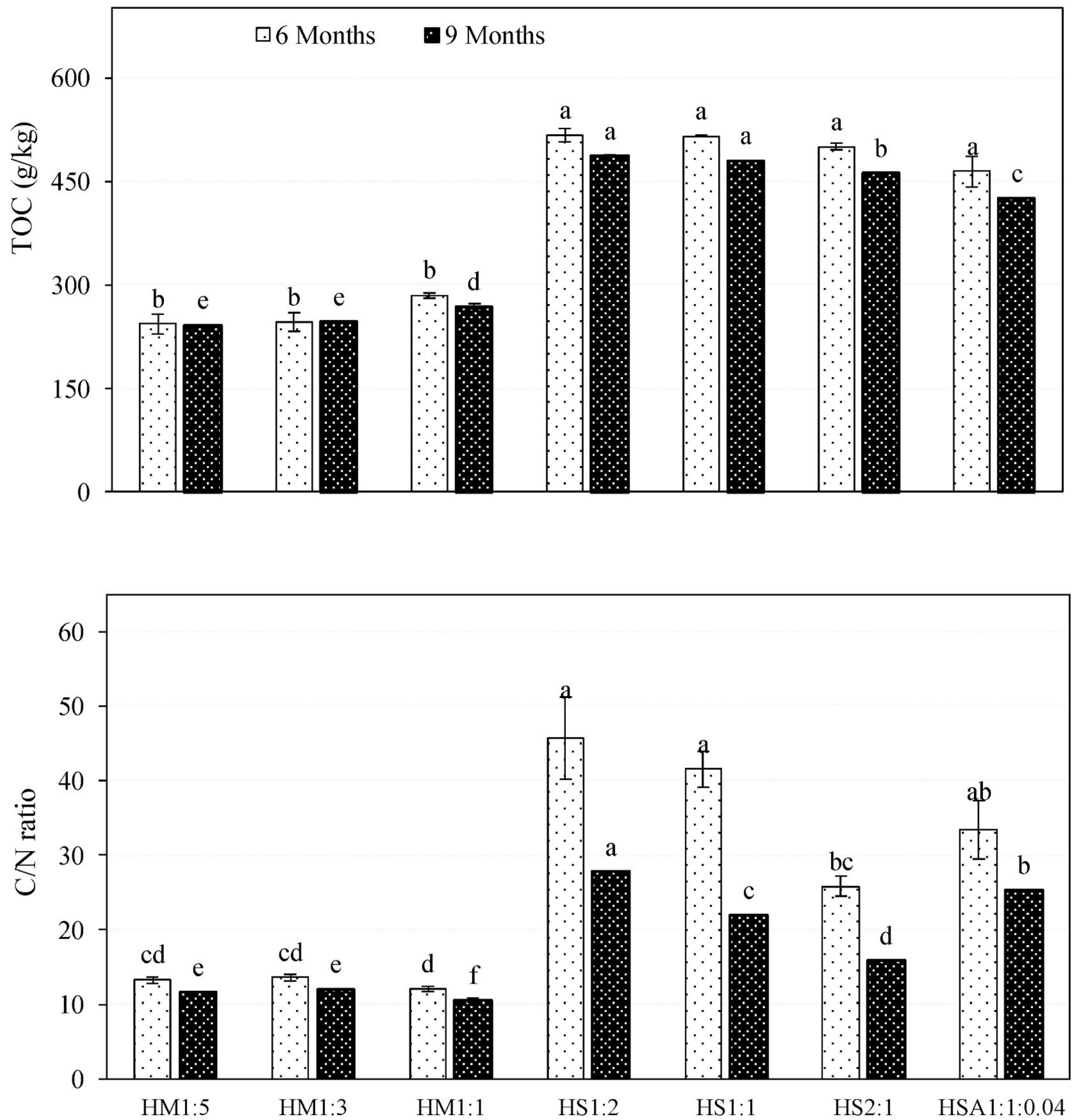

Fig. 2. Total organic carbon (TOC) and carbon (C)/nitrogen (N) ratio after six and nine months (the end) of composting (HM, hop leaves + cow manure at the ratios 1:5, 1:3 and 1:1; HS, hop leaves + wheat straw at the ratios 1:2, 1:1 and 2:1; and HSA, HS + ash from hop stems at the ratios 1:1:0.04). Within each period, means followed by the same letter are not statistically different by Tukey HSD test (α = 0.05). Error bars are the standard errors.

N content of a mixture are the main drivers of the rate and the level of decomposition, and values of a C/N ratio between 15 and 35 are considered the most adequate for a fast and effective decomposition (Lambers et al., 2008; Bernal et al., 2009; Azim et al., 2018). Organic C represents the source of energy for heterotrophic microorganisms and N is the raw material for protein synthesis (Weil and Brady, 2017). Hence, the mixtures HS2:1 and HM1:1 had an initial C/N ratio in the optimal range for composting and presented a final N concentration higher than 18 g/kg (Raviv, 2005), which should prevent net immobilization when the compost is applied to the soil. PCA results also highlighted the higher concentrations of several nutrients (P, K, Mg, Fe, Mn, Cu and Zn) in the manure-based composts and the higher concentrations of N, B and Ca in the compost prepared with a double rate of leaves (HS2:1).

The addition of ash (HSA1:1:0.04) significantly reduced the N concentration in the final compost but increased the concentrations of several other nutrients in comparison to the same mixture without ash (HSA1:1). The increase in minerals in the compost containing ash was a direct effect of their content in the ash as reported in previous studies (Bougnom et al., 2009; Juarez et al., 2015). The decrease in N concentration was probably due to the increase in the pH of the mixture, which favoured ammonia volatilization (Azim et al., 2018).

The quality of a compost depends also on its biological stability, which determines its behaviour in the soil. TOC, the C/N ratio, inorganic-N and EC are important indicators of the stability of compost (Bernal et al., 2009; Azim et al., 2018). TOC and the C/N ratio decreased during composting from the sixth to the ninth month as expected. The group of HM composts showed low C/N ratios particularly in the ninth month (10.5–12.0). The C/N ratios in the straw-based composts were higher and more dependent on the proportion of leaves in the mixtures, varying between 15.8 (HS2:1) and 27.8 (HS1:2). A well-matured compost should present a C/N ratio of below 20, and preferably below 12, and values above 25 indicate poor maturity (Bernal et al., 2009; Antil et al., 2014). The high C/N ratio of some straw-based mixtures may have slowed the process of decomposition, and nine months would not have been enough for a complete biological stabilisation. In addition, if straw had been chopped, the attack points for the microorganisms would have increased, which usually accelerates the decomposition process (Adhikari et al., 2009; Calabi-Floody et al., 2019). Following mineralization, nitrification is an important step in the composting process, reducing the levels of NH_4^+ that can be toxic to plants, mainly during the germination phase (Cáceres et al., 2018). The ratio NH_4^+/NO_3^- decreased during composting and was significantly lower in HM composts, though

Table 4
Electrical conductivity (EC), nitrate (NO_3^-) and ammonium (NH_4^+) levels and NH_4^+/NO_3^- ratio in composted materials after six and nine months of composting (HM, hop leaves + cow manure at the ratios 1:5, 1:3 and 1:1; HS, hop leaves + wheat straw at the ratios 1:2, 1:1 and 2:1; and HSA, HS + ash from hop stems at the ratios 1:1:0.04).

Compost	EC (mS/cm)	NO_3^- (mg/kg)	NH_4^+ (mg/kg)	NH_4^+/NO_3^-
Sixth month				
HM 1:5	0.0027 c	7031 b	38.0 c	0.0055 c
HM 1:3	0.0031 c	8111 ab	33.4 c	0.0042 c
HM 1:1	0.0031 c	9791 a	41.8 c	0.0043 c
HS 1:2	541 b	7763 ab	80.2 b	0.0103 b
HS 1:1	605 b	6726 b	74.0 b	0.0111 b
HS 2:1	910 a	7075 b	94.7 a	0.0134 a
HSA 1:1:0.04	708 ab	7188 b	75.1 b	0.0105 b
Prob. > F	<0.0001	0.0031	<0.0001	<0.0001
Standard error	81.42	256.62	5.07	0.0008
Ninth month				
HM 1:5	0.0038 e	11,014 b	25.2 b	0.0023 c
HM 1:3	0.0043 e	11,251 b	26.2 b	0.0023 c
HM 1:1	0.0044 e	14,310 a	29.2 b	0.0020 c
HS 1:2	909 d	7803 c	40.2 a	0.0052 a
HS 1:1	1285 b	10,401 b	43.0 a	0.0041 b
HS 2:1	1776 a	9935 b	40.3 a	0.0040 b
HSA 1:1:0.04	966 c	6316 c	38.7 a	0.0061 a
Prob. > F	<0.0001	<0.0001	<0.0001	<0.0001
Standard error	148.42	545.53	1.74	0.0003

Means followed by the same letter are not statistically different by Tukey HSD test (α = 0.05).

below 0.16 for all composts, which is a safe value for agricultural use (Bernal et al., 2009). Major differences were found in EC values between the HM and HS groups of composts. EC tends to increase during composting due to the increased concentration of soluble ions resulting from OM mineralization (Petric et al., 2009; Alavi et al., 2017). Overall, straw-based mixtures presented lower initial concentrations of most of the minerals determined. Thus, their higher EC values were probably due to the presence of non-determined ions, such as Na^+, Cl^-, CO_3^{2-} or SO_4^{2+}, which are usually responsible for an increase of EC in composted materials (Gondek et al., 2020). The PCA results also highlighted the higher maturity of the HM in comparison to the HS composts, the most stable being HM1:1. The higher levels of EC, NH_4^+, TOC and the C/N ratio, and the lower levels of NO_3^-, clearly differentiate the HM group from the HS group of composts. The addition of ash led to higher NH_4^+/NO_3^- and C/N ratios and lower EC values in the final compost in comparison to the compost resulting from the same mixture without ash. Juarez et al. (2015) reported similar results for C/N ratio and EC values with wood-ash amended compost.

In the first growing cycle of lettuce, the manure-based composts gave higher average DM yield than the straw-based composts, with the exception of the compost HS2:1D2, applied at a double rate. Within the group of straw-based composts, those treatments prepared with a higher proportion of straw, and particularly those applied at a double rate (HS1:2D2), gave the lowest DM yields of lettuce. These low producing composts showed lower maturity after nine months of composting, with higher C/N ratios and higher NH_4^+ and EC levels than the better producing composts. High levels of NH_4^+ and EC could have a detrimental effect on crop productivity (Weil and Brady, 2017). However, all the straw-based composts did not show very dissimilar values of NH_4^+ and EC, although productivity was significantly different, which may mean that those variables did not have a relevant effect on the DM yield of lettuce. HS2:1, for instance, was the compost with higher EC and also that showing the higher DM yields of lettuce among the straw-based composts. Gondek et al. (2020) also showed that composts with EC values within the range found in this study have enhanced plant growth and yield.

The less productive treatments in the first growing cycle of lettuce were those presenting higher C/N ratios, and particularly the one applied at a double rate (HS1:2D2) as above mentioned. These immature composts, as indicated by the high C/N ratios, can support intense microbial activity when applied to the soil, in the continuing decomposition process, which can have phytotoxic effects by releasing volatile organic compounds and competing for oxygen with plants (Chefetz et al., 1996; Cesaro et al., 2015). However, the treatments associated with low lettuce DM yield also showed low tissue N concentration. The HS2:1D2 treatment gave particularly low lettuce DM yield (0.48 g/plant) and showed particularly low tissue N concentrations (13.2 g/kg), whereas the highest productive treatment (HS2:1D1) gave an 18-fold higher DM yield of lettuce (8.71 g/plant) and a much higher tissue N concentration (29.6 g/kg). Thus, it seems that the real problem of the reduced growth of lettuce was N deprivation, caused by biological immobilization, which is common when organic amendments with high C/N ratios are applied to the soil (Weil and Brady. 2017).

The treatment displaying the lowest lettuce DM yield in the first growing cycle (HS1:2D2) gave the highest value of all treatments in the second cycle. This means that after a period of N immobilization (first growing cycle) a period of net mineralization occurred (second growing

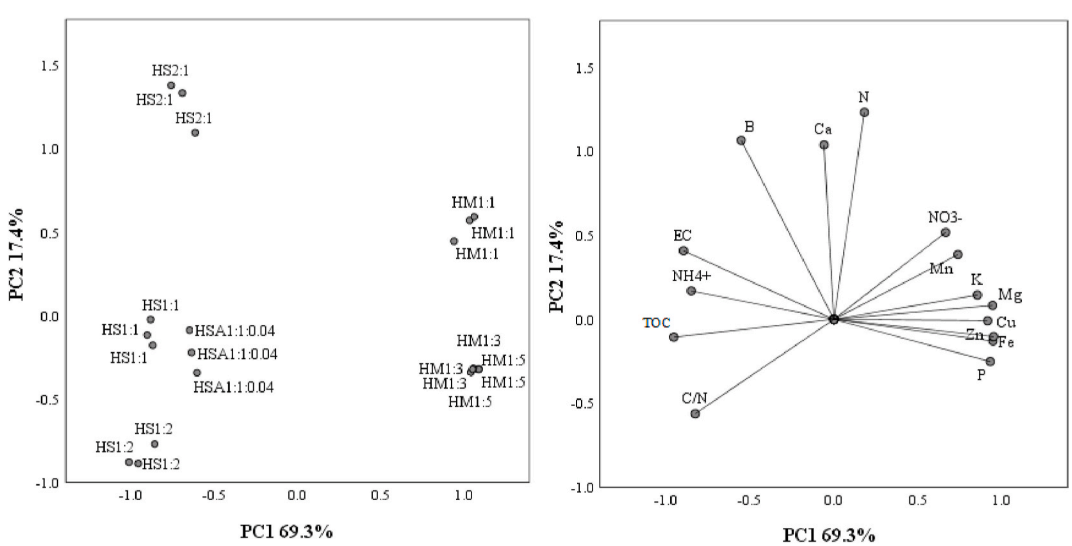

Fig. 3. PCA scores plot (left) of the final composts (HM, hop leaves + cow manure at the ratios 1:5, 1:3 and 1:1; HS, hop leaves + wheat straw at the ratios 1:2, 1:1 and 2:1; and HSA, HS + ash from hop stems at the ratios 1:1:0.04) and PCA loadings plot (right) of physicochemical properties of composts.

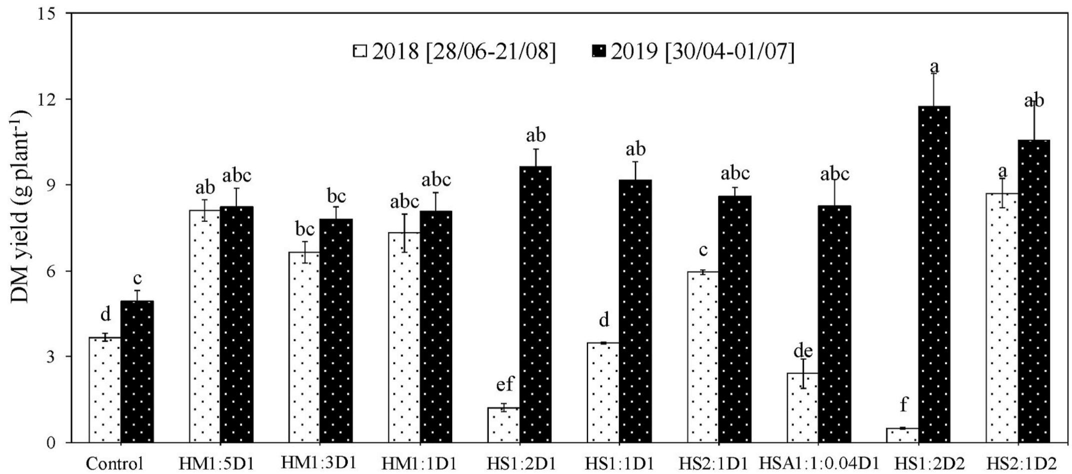

Fig. 4. Lettuce dry matter (DM) yield in 2018 and 2019 as a function of fertilizer treatments (HM, hop leaves + cow manure at the ratios 1:5, 1:3 and 1:1; HS, hop leaves + wheat straw at the ratios 1:2, 1:1 and 2:1; and HSA, HS + ash from hop stems at the ratios 1:1:0.04; D1 and D2, 20 and 40 kg dw ha^{-1}). Within each growing cycle of lettuce, means followed by the same letter are not significantly different by Tukey HSD test (α = 0.05). Error bars are the standard errors.

Table 5
Tissue nutrient concentration in the lettuces of the first and second growing cycles (HM, hop leaves + cow manure at the ratios 1:5, 1:3 and 1:1; HS, hop leaves + wheat straw at the ratios 1:2, 1:1 and 2:1; and HSA, HS + ash from hop stems at the ratios 1:1:0.04; D1 and D2, 20 and 40 kg dw ha^{-1}).

Treatment	N	P	K	Ca	Mg	Fe	Mn	Cu	Zn	B
	(g/kg)					(mg/kg)				
First growing cycle										
Control	24.7 bc	2.2 bc	101.4 a	7.1 bc	4.8 abc	1183 a	84.2 a	13.7 a	85.8 ab	43.1 ab
HM1:5D1	29.5 abc	2.6 b	93.4 ab	5.3 d	3.7 c	1013 a	82.1 a	18.2 a	82.4 ab	36.3 c
HM1:3D1	30.3 a	2.6 b	94.8 ab	5.1 d	3.6 c	772 a	84.5 a	14.7 a	146.5 ab	36.5 c
HM1:1D1	30.7 a	2.5 b	100.8 a	5.3 d	3.6 c	676 a	81.2 a	13.5 a	154.9 ab	36.6 c
HS1:2D1	24.8 bc	3.9 a	86.7 abc	8.0 ab	4.9 abc	1285 a	93.0 a	16.4 a	72.5 b	47.7 a
HS1:1D1	24.3 c	2.8 b	105.7 a	7.3 abc	4.3 bc	1369 a	96.6 a	16.4 a	57.0 b	47.5 a
HS2:1D1	28.9 abc	2.4 bc	91.5 abc	5.9 cd	4.1 bc	1297 a	102.6 a	20.5 a	172.9 a	43.3 b
HSA1:1:0.04D1	25.6 abc	2.0 bc	72.3 abc	5.2 d	4.0 bc	1639 a	100.2 a	22.2 a	125.9 ab	48.0 a
HS1:2D2	13.2 d	2.0 bc	53.3 c	8.8 a	6.3 a	1162 a	103.3 a	16.4 a	142 ab	nd
HS2:1D2	29.6 ab	1.6 c	59.4 bc	6.4 cd	5.2 ab	1251 a	96.5 a	13.8 a	91.7 ab	39.8 bc
Prob. > F	<0.0001	<0.0001	0.0004	<0.0001	<0.0001	0.4181	0.6624	0.1136	0.0026	<0.0001
Standard error	0.85	0.11	3.57	0.22	0.16	87.61	3.08	0.76	8.30	0.78
Second growing cycle										
Control	27.1 cd	2.8 ab	57.3 b	6.1 bc	5.8 a	1846 a	118.3 a	22.3 a	155.8 a	38.9 a
HM1:5D1	23.6 d	3.0 a	102.8 ab	7.8 a	4.8 b	753 b	67.2 b	11.8 b	91.7 b	40.9 a
HM1:3D1	26.9 cd	2.0 b	98.5 ab	6.0 c	4.5 b	1157 ab	93.1 ab	15.8 ab	130.4 ab	40.5 a
HM1:1D1	27.5 b cd	2.2 ab	105.7 ab	7.4 ab	4.8 b	1111 ab	91.1 ab	15.0 b	94.4 b	41.5 a
HS1:2D1	26.1 cd	1.9 b	77.9 ab	5.9 c	4.7 b	1117 ab	85.3 b	15.6 ab	110.6 ab	43.0 a
HS1:1D1	29.8 bc	2.2 ab	101.5 ab	6.3 bc	4.9 ab	1034 ab	80.7 b	17.1 ab	114.2 ab	45.3 a
HS2:1D1	32.0 ab	2.7 ab	81.5 ab	6.1 bc	5.3 ab	1104 ab	86.9 b	18.6 ab	116.3 ab	42.6 a
HSA1:1:0.04D1	26.9 cd	2.6 ab	77.4 ab	6.5 abc	4.8 b	1133 ab	90.2 ab	17.0 ab	144.1 ab	45.4 a
HS1:2D2	24.6 d	2.7 ab	115.4 a	6.41 abc	4.5 b	963 ab	91.1 ab	14.9 b	96.1 b	42.8 a
HS2:1D2	35.0 a	2.41 ab	105.7 ab	7.2 abc	5.0 ab	1040 ab	96.7 ab	16.8 ab	98.2 b	39.6 a
Prob. > F	<0.0001	0.0106	0.0093	0.0003	0.0006	0.1106	0.0013	0.0045	0.0051	0.0329
Standard error	0.58	0.07	3.93	0.13	0.08	71.68	2.62	0.58	4.71	0.52

nd, not determined (insufficient sample).
Means followed by the same letter are not significantly different by Tukey HSD test (α = 0.05).

cycle), benefiting the plants of the treatments with higher levels of total N in the soil. This is in contrast to the other treatments, in particular the manure-based ones, in which part of the N was taken up by the lettuce during the first growing cycle. This was clear from the result of the control, which showed the lowest DM yield of lettuce of all the treatments in the second growing cycle. N is a key component of proteins, nucleic acids and several enzymes and phytohormones (Hawkesford et al., 2012). Thus, in the present study, and in many others using organic amendments, increased plant growth is usually associated with increased N uptake (Woldetsadik et al., 2017; Solaiman et al., 2019; Zandvakili et al., 2019; Erdal and Ekinci, 2020).

5. Conclusions

The evaluation of the physicochemical properties of the composted materials, and the results of the biological assay with lettuce, provided interesting guidelines for farmers that are summarized below. The mixture of hop leaves with cow manure produced a stable compost after nine months of composting that can be used in horticultural crops, irrespective of the proportion of raw materials, probably due to the low and similar C/N ratios of both materials. When hop leaves were mixed with wheat straw, in proportions of less than 2:1, the compost did not mature properly in nine months, which means that the farmer is unable to use the compost in horticultural crops in the following growing season. The mixtures containing straw with less than a double rate of leaves gave high total organic carbon and C/N ratios in the composts, causing N deprivation due to biological N immobilization, and greatly reducing lettuce dry matter yield. Thus, to reduce composting time and increase the quality of the compost, the ratio leaves/straw should be as high as possible, at least 2:1. Alternatively, the poorly-matured compost must be applied far in advance of crop planting so that complementary biological processes can take place in the soil, as recorded in the second cycle of lettuce. Ash from the stems did not add any benefit to the composting process. It only increased the content of some nutrients contained within it, so it should be applied directly to agricultural soils, thereby avoiding the work of adding it to composting mixtures. Thus, as stated in the objectives for this study, farmers who follow these guidelines can take better advantage of the use of these important organic resources to fertilize their crops.

Credit author statement

Sandra Afonso: Carried out the trials; performed tissue analysis; and wrote the first draft of the manuscript; **Margarida Arrobas:** Verified and supervised the quality of laboratory results; revised the methodological aspects of the manuscript; **Ermelinda L. Pereira:** Performed tissue analysis and revised the original draft; **M. Ângelo Rodrigues:** Conceived the experiment, organized the data and prepared the final version of the manuscript.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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