Deliverable 2.1. Composting process performance and compost characteristics

30/09/2024

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Co-funded by the European Union

101113948-LIFE22-CCM-ES-CLIMAWIN

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1. SUMMARY

ENGLISH: This deliverable aims to outline the composting experiments conducted at Bosque de Matasnos, highlighting the agronomic and environmental advantages of incorporating biochar during the composting of manure. The experiments demonstrated that adding biochar accelerates the composting process, as evidenced by quicker stabilization in temperature, improved germination index, and enhanced nitrification activity. Additionally, the biochar-treated pile exhibited lower nitrogen losses and higher concentrations of recalcitrant carbon and total nitrogen at the end of the process. Calculations indicate a potential mitigation of 61.488 kg CO_2 eq of methane (CH_4) emissions per year, based on recent findings by Harrison et al. (2022) and the amount of manure used to fertilize the vineyards annually.

SPANISH: Este documento tiene como objetivo describir los experimentos de compostaje realizados en Bosque de Matasnos, destacando las ventajas agronómicas y ambientales de incorporar biochar durante el compostaje de estiércol. Los experimentos demostraron que la adición de biochar acelera el proceso de compostaje, evidenciado por una estabilización más rápida de la temperatura, una mejora más rápida en el índice de germinación y una mayor actividad de nitrificación en la fase mesófila. Además, la pila co-compostada con biochar mostró menores pérdidas de nitrógeno y mayores concentraciones de carbono recalcitrante y nitrógeno total al final del proceso. Los cálculos indican un potencial de mitigación de 61.488 kg eq CO_2 de emisiones de metano (CH_4) por año, basados en hallazgos recientes de Harrison et al. (2022) y en la cantidad de estiércol utilizado anualmente para fertilizar los viñedos.

2. Background

Bosque de Matasnos has taken significant steps in becoming an environmentally sustainable Estate Winery. The Winery is deeply committed to organic farming practices and adheres strictly to regulations by using only approved organic materials as fertilizers for its vineyards. A cornerstone of their sustainability efforts is the application of manure as an organic fertilizer, with **around 1000 tons of sheep manure** used annually to nourish their soil.

Currently, the manure is mixed with wine-making residues (grape stalks and pomace) and stored in piles until it is needed for application in the fields. While this practice aligns with organic standards, there is considerable room for improvement in how these organic residues are managed.

This deliverable is aimed at describing the composting experiments performed at Bosque de Matasnos to **demonstrate the agronomical and environmental benefits of integrating composting with biochar** as an agricultural practice in terms of enhancing the quality of the mature compost and minimizing CH_4 emissions**.**

2.1. Why composting?

Bosque de Matasnos could significantly enhance the efficiency of nutrient recycling, improve soil C storage, and reduce greenhouse gas emissions by introducing composting or co-composting techniques, particularly in combination with biochar (Sánchez-Monedero et al., 2018). This approach would not only optimize the use of organic materials but also further set the winery's commitment to sustainability and environmental stewardship in the viticulture industry. Compost is the product resulting from aerobic decomposition of organic matter by bacteria, fungi, and often micro and macro-fauna under controlled conditions. The activity of microorganisms (and often larger organisms) is necessary for the composting process (Epstein, 1997).

A compost pile requires adequate moisture levels, ideally between 40% and 60% water, to support microbial activity. It should also have a structure that allows for proper aeration, facilitating oxygen flow throughout the pile. Additionally, maintaining an appropriate carbon-to-nitrogen (C/N) ratio of approximately 30:1 is essential for optimal decomposition. This balance ensures that microorganisms have sufficient energy (from carbon) and nutrients (from nitrogen) to thrive, promoting the heating of the pile and an efficient composting process.

The most common method for achieving a proper aeration in composting piles **is by windrow turning**. This technique facilitates the homogenization of materials, prevents compaction, and ensures adequate oxygen supply throughout the compost pile.

Typically, a composting pile undergoes four stages (Figure 1): (i) an initial mesophilic stage, during which microorganisms begin to colonize the raw organic

residues. This stage is characterized by moderate temperatures and the breakdown of easily degradable organic compounds. (ii) The thermophilic stage follows, where temperatures rise significantly due to the rapid activity of thermophilic bacteria, leading to the decomposition of more complex organic matter. During this stage, temperatures as high as 50-70 °C are reached. (iii) The cooling stage occurs as microbial activity decreases, allowing mesophilic organisms to re-colonize the pile. Finally, (iv) the maturation stage involves the stabilization of the compost, where remaining organic matter further decomposes, resulting in a mature, stabilized and odorless product rich in nutrients.

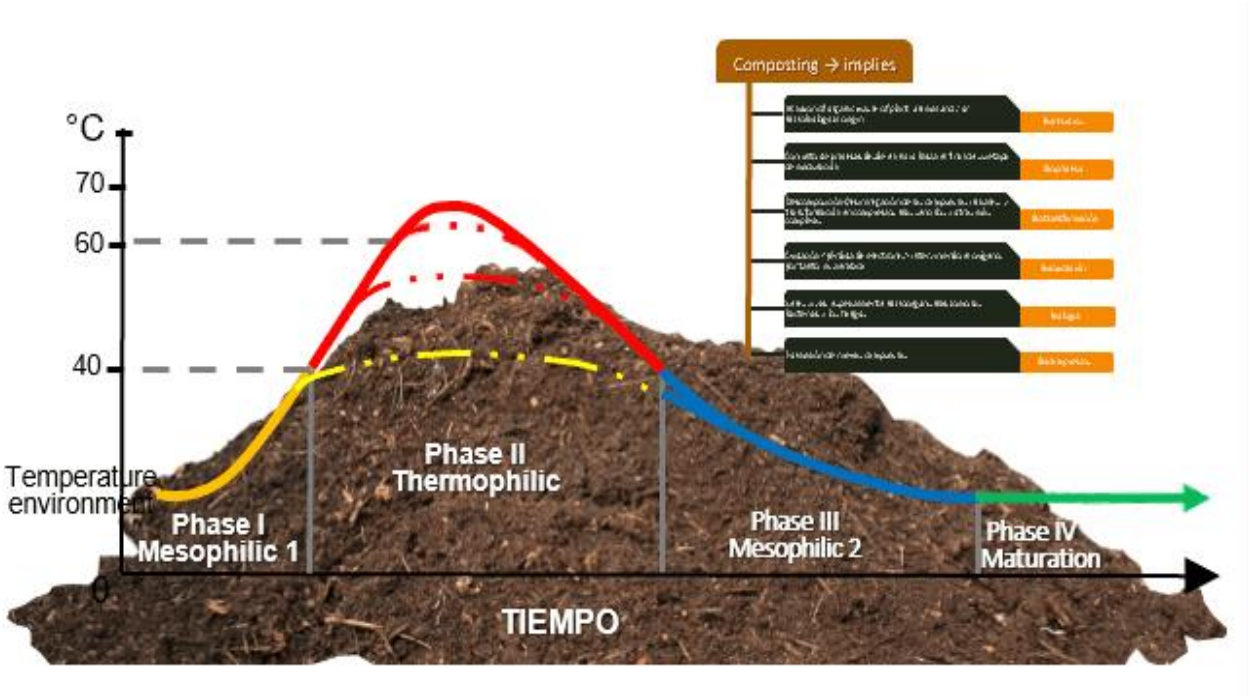

Figure 1. Different stages of the composting process (Paez, 2021).

Some of the main advantages of **composting compared to stockpiling manures** include:

-**Reduced odors, toxic compounds and pests**: when properly managed, composting minimizes bad odors. By reaching high temperatures for prolonged periods of time during the thermophilic stage, it can suppress relevant human pathogens (such as *E. coli or Salmonella*). Weed seeds often found in manure have been found to or relevant pathogens, such as antibiotic resistant bacteria

-Lower Environmental Impact: composting reduces the risk of nutrient runoff and leaching into water sources. It also decreases methane (CH_4) emissions helping to protect local ecosystems and water quality.

-**Enhanced Nutrient Availability**: composting accelerates the breakdown of organic materials, making nutrients more readily available to plants compared to stockpiling.

-Reduction in the volume of material to be managed. Mature compost is easier to handle than fresh manure. It acts as a more concentrated fertilizer, with more stable carbon and nutrients, making it less likely to cause nutrient imbalances or leaching.

-Microbial Activity Promotion: The composting process fosters beneficial microbial activity that can enhance soil health and fertility, which is often lacking in stock-piled manure.

2.2. Co-composting with Biochar

Biochar is a carbon-rich material produced by heating biomass, such as wood, or crop residues, in a low-oxygen environment through a process called **pyrolysis**. Biochar is primarily used as a soil amendment to improve soil fertility, increase water retention and sequester organic carbon, helping to mitigate climate change (Joseph et al., 2021).

Several studies have analyzed the impact of adding biochar to the composting process, revealing a wide range of benefits:

-Faster Composting: Introducing biochar in the composting piles (even at low proportions, e.g 5%) accelerates the composting process, achieving higher temperatures sooner due to enhanced microbial activity (Sánchez-García et al., 2015).

-Reduced Nutrient Losses: Biochar contributes to higher nitrogen and carbon retention in compost, decreasing nutrient losses through improved nitrification and reduced anaerobic processes (López-del Cano et al., 2016).

-Increased Nitrification: Studies indicate that nitrification is more effective in biochar-enhanced compost, resulting in higher NO3-N concentrations and greater stabilization of volatile ammonium (Camps & Tomlinson, 2015).

-**Lower Greenhouse Gas Emissions**: Biochar produced in the range of 400-700 °C enhances aeration due to its increased surface area, and pore volume. This reduces anaerobic reactions that generate methane, resulting in lower methane emissions from compost. Additionally, biochar application may reduce nitrous oxide emissions (Camps & Tomlinson, 2015).

3. Methodology

3.1. Composting process and sampling.

The composting experiment began on **November 16, 2023** and ended on **April 16, 2024.** The composting piles were prepared using compositions similar to those described by Harrison et al. (2022). The initial material used to prepare the composting piles comprised **83% fresh sheep manure** and bedding, along with **17% grape pomace and stalks** sourced from the winery.

Two composting piles were prepared with the following composition:

1. **COMPOST**: One compost pile did not contain biochar, but only the initial material (the mixture of sheep manure and winery residues).
2. **COMPOST with BIOCHAR**. This pile was prepared by mixing the initial material with biochar at 20% by volume. Considering that Biochar has a much lower moisture and bulk density than manure, the approximate proportion in dry weight was 83:17 (see calculations in section 4.2).

Figure 2. Emptying the biochar sacks (A) and mixing manure with biochar (B) in Bosque de Matasnos (November 2023).

Figure 3. View of composting piles with Biochar (left side) and without (right side) in Bosque de Matasnos (November 2023)

The **biochar used in this study** was sourced from a local supplier and was a woody biochar made from forest clippings by traditional charcoal making (slow pyrolysis with higher treatment temperatures above 600 °C). The main properties of the biochar are as follows: bulk density: 330 g/l, pH 8,1; Electrical conductivity 0,65 mS/cm; TOC 73,3 %; TN 1,0%.

Both compost piles were similar in size and weighed approximately 15 tons. The original mixture and the biochar were thoroughly mixed with the help of a front-end loaded tractor and the piles were left in the open (Figures 2 and 3). During the thermophilic phase the piles were turned biweekly with a front-end loaded tractor. This stage lasted approximately 60 days. From that time the maturation stage started, which lasted other 65 days. Figure 4 shows the evolution of temperature during the thermophilic phase.

Three composting samples were taken to monitor de evolution of the composting process. The first sample was taken immediately after the set-up of the experiment, i.e. at the beginning of the composting process (November, 16th, 2023), the second was taken after 12 weeks (February 1st, 2024) and the third at the end of the maturation process (April 16th, 2024), after 23 weeks. In order to take a representative sample, five subsamples were taken from different locations of each composting pile and subsequently mixed to get two representative samples. Samples were refrigerated until arrival at the laboratory. Upon arrival, they were homogenized, air-dried, and ground to a particle size of less than 1 mm, then stored at room temperature until analysis.

3.2. Compost monitoring and analyses

3.2.1. Temperature monitoring.

Composting temperature was measured at three locations within each pile using a temperature probe. Monitoring was discontinued once the pile no longer showed a temperature increase after turning, which occurred after the third turn. From that point, the pile's temperature remained consistent with ambient conditions.

3.2.2. Moisture and organic matter (OM) concentration.

Moisture was determined by the weight difference between moist and dry samples. The samples were dried in an oven at 105 °C for 24 hours, and the dried sample mass was subtracted from the initial weight to calculate the water content. The organic matter concentration (OM) was analyzed by loss on ignition in an oven at 550 °C for 6 hours.

3.2.3. pH and electric conductivity (EC).

To determine the pH and electric conductivity of the sample, a 1:10 water extract is required. The sample should be shaked for 1.5 hours to create a homogenized extract. After shaking, the samples should be placed in a centrifuge for 15 minutes at 8500 rpm. The extract should then be filtered through a cellulose membrane with a pore diameter of 0.45 µm. Finally, two electrodes should be placed in the extract to measure pH and electrical conductivity.

3.2.4. Germination index (GI).

Phytotoxicity, expressed as germination index (GI), was assayed by the Lepidium sativum test proposed by Zucconi et al. (1981).

A water extract of the compost samples (1:10 dry weight) was shaken for 1 hour and centrifuged for 15 minutes at 8500 rpm. This extract was filtered through a cellulose membrane with a pore diameter of 0,45 µm. Eight seeds of *Lepidium sativum* were placed in 10 cm diameter Petri dishes covered with filter paper. Two ml of the aqueous extract was added. The test was replicated 10 times. The seeds were incubated for 48 hours at 27 °C in the dark. After this time, the number of germinated seeds and the length of the roots per plate were counted. The results were expressed as a germination index, which is obtained by multiplying the percentage of germination (%G) by the percentage of root growth (%R) relative to the control and dividing by one hundred. The formula used is:

$$\%_{GI} = \frac{\%_G * \%_R}{100}$$

3.2.5. Ammonium, nitrate and phosphate concentrations.

Ammonium (NH_4^+) was extracted with 2.0 M KCl at 1:10 (weight:volume dry weight) and determined by a colorimetric method based on Berthelot's reaction (Sommer et al., 1992).Nitrate (NO_3^-) and phosphate (PO_4^{3-}) ions were determined in

water extracts (1:10 weight:volume dry weight) by High Performance Liquid Chromatography (HPLC).

3.2.6. Total organic carbon (TOC) and nitrogen (TN).

Total nitrogen (TN) and total organic carbon (TOC) were analyzed by automatic elemental microanalysis (LECO CHNS-932, USA).

4. Results

4.1. Process monitoring and compost quality

4.1.1. Temperature.

During the composting process, several general parameters were monitored to assess its progress. One of the most fundamental and important parameters is the temperature of the pile. Both piles (with and without biochar) reached high temperatures rapidly, indicating a well-structured material with adequate moisture and appropriate C/N ratios. Temperatures around 65 °C ensure effective sanitization of the pile, as this level is sufficient to eliminate pathogens and weed seeds. The pile was regularly turned to reactivate the composting process. After the third turn, the temperature no longer increased, indicating the completion of the thermophilic phase. Notably, the pile with biochar stabilized more quickly, as it reached a temperature decline earlier than the pile without biochar.

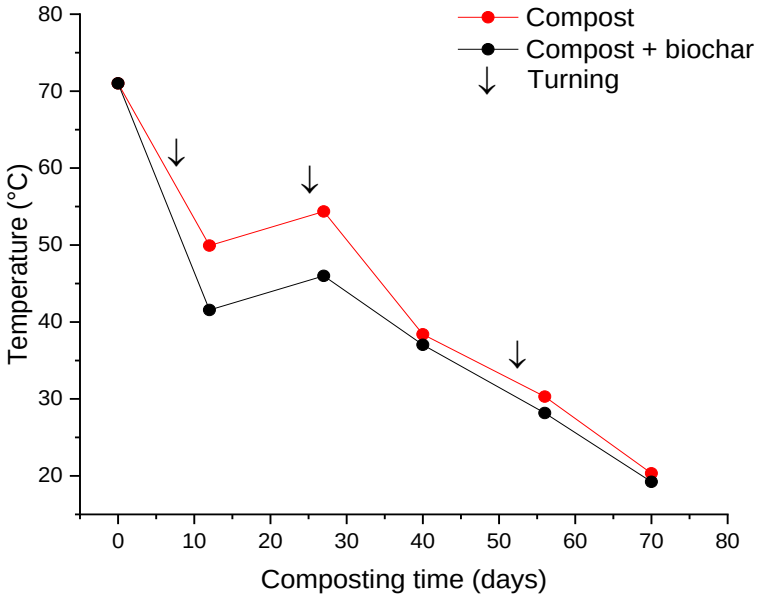

Figure 4. Temperature of the composting piles during the first 70 days. After this period, the compost piles reached ambient temperature.

4.1.2. Moisture, pH and electrical conductivity.

Figure 5 shows the progression of moisture (A), pH (B) and EC (C) during composting in both piles. At the beginning of the process, both piles exhibited moisture levels within the optimal range for composting (between 40% and 60%). The pile containing biochar had a slightly lower moisture content due to the inherent dryness of the biochar. As composting progressed, moisture levels decreased to around 40%, which is a typical evolution as high temperatures promote drying. At this stage, both piles had similar moisture levels.

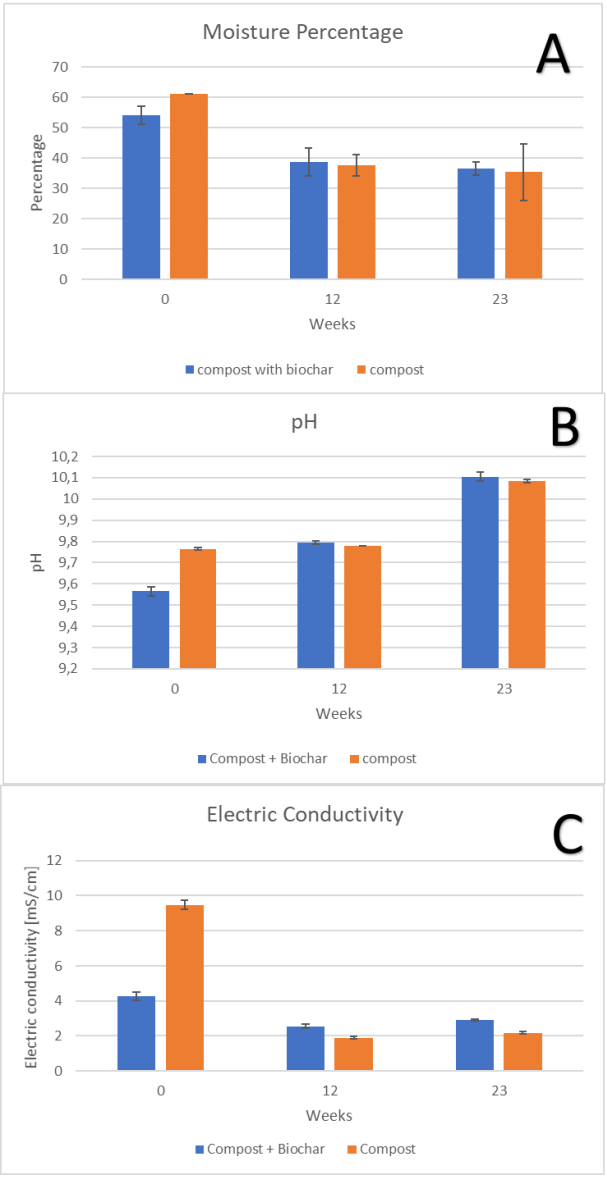

Figure 5. Evolution of moisture (A), pH (B) and electrical conductivity (C) during composting.

By the end of the composting process, moisture remained elevated, largely due to the open exposure of the compost piles during a particularly rainy spring in Bosque de Matasnos. Notably, the moisture in the biochar pile was more homogeneous throughout, as indicated by its lower standard error.

The pH followed the typical trend during the process, showing a slight increase over time. Initially, the biochar pile had a slightly lower pH, likely due to its slightly lower pH (compared to the mixture manure/winery residues). However, as composting progressed, both piles exhibited an increase in pH, ultimately reaching 10.1 in each. This increase in pH during the active phases of composting reflects a normal progression in the composting process and is associated with the mineralization of proteins and the decarboxylation of organic anions during the aerobic decomposition of organic matter (Cayuela et al., 2008).

Electrical conductivity (EC) levels were initially high (around 10 mS/cm) in the pile without biochar. This is usual and has been related to the high concentration of water-soluble organic compounds in fresh manure and elevated levels of NH_4^+. The addition of

biochar decreased the initial EC probably through two mechanisms—by diluting the concentration of soluble compounds and by absorbing organic molecules on its surface.

EC decreased as the composting process progressed, eventually reaching values typical for this type of compost and with similar values in both piles.

4.1.3. Evolution of organic matter and Carbon fractions.

Figure 6 illustrates the evolution of organic matter concentration (A), C/N ratio (B), percentage of organic carbon (C), and water-soluble carbon (D). The percentages of organic matter and organic carbon are closely related, often allowing one to be calculated from the other by multiplying by a specific factor. We observed the typical trend during the composting process: a decrease in both organic matter and organic carbon due to the mineralization of organic matter. However**, a significant difference emerged between the compost with and without biochar**. Although both piles initially showed similar values for organic matter and carbon, **the pile with biochar retained significantly more organic carbon by the end of the process**. This is also reflected in the C/N ratio, which remained higher in the biochar pile throughout the entire composting process.

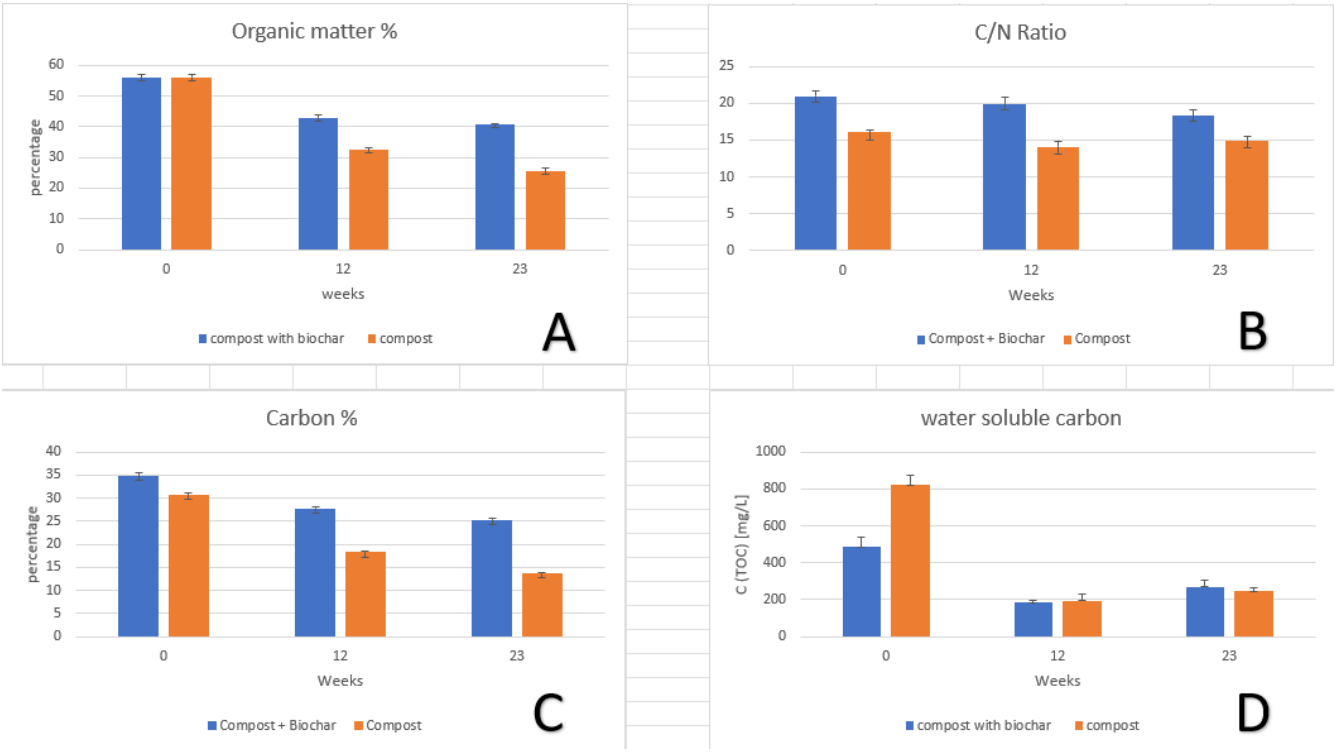

Figure 6. Evolution of organic matter (A), C/N ratio (B), concentration of carbon (C) and water soluble carbon (D) during the composting of manure with and without biochar.

Biochar is known to contain forms of carbon that are highly recalcitrant and resistant to microbial degradation, resulting in a much slower mineralization process compared to other organic materials. This slow decomposition contributes to the long-

term stability of carbon in soils, making biochar an effective tool for carbon sequestration and enhancing soil fertility over extended periods.

The evolution of water-soluble carbon aligns with the trend observed in electrical conductivity (EC), supporting the hypothesis that the initial high EC levels were primarily due to low molecular weight soluble organic compounds. These molecules were efficiently adsorbed by the biochar early in the composting process.

4.1.4. Evolution of Nitrogen fractions.

Figure 7 depicts the evolution of total N concentration (A), water soluble N (B) and mineral N, ammonium (C) and nitrate (D). The initial mixture of manure and winery residues had a relatively low total nitrogen concentration (< 2%), likely due to the high proportion of bedding material in the manure, which significantly affects nitrogen content. **Nitrogen losses occurred throughout the composting process**, which is usual due to the high temperatures and elevated pH levels that promote ammonia (NH_3) volatilization. **However, nitrogen losses were significantly lower in the pile with biochar.**

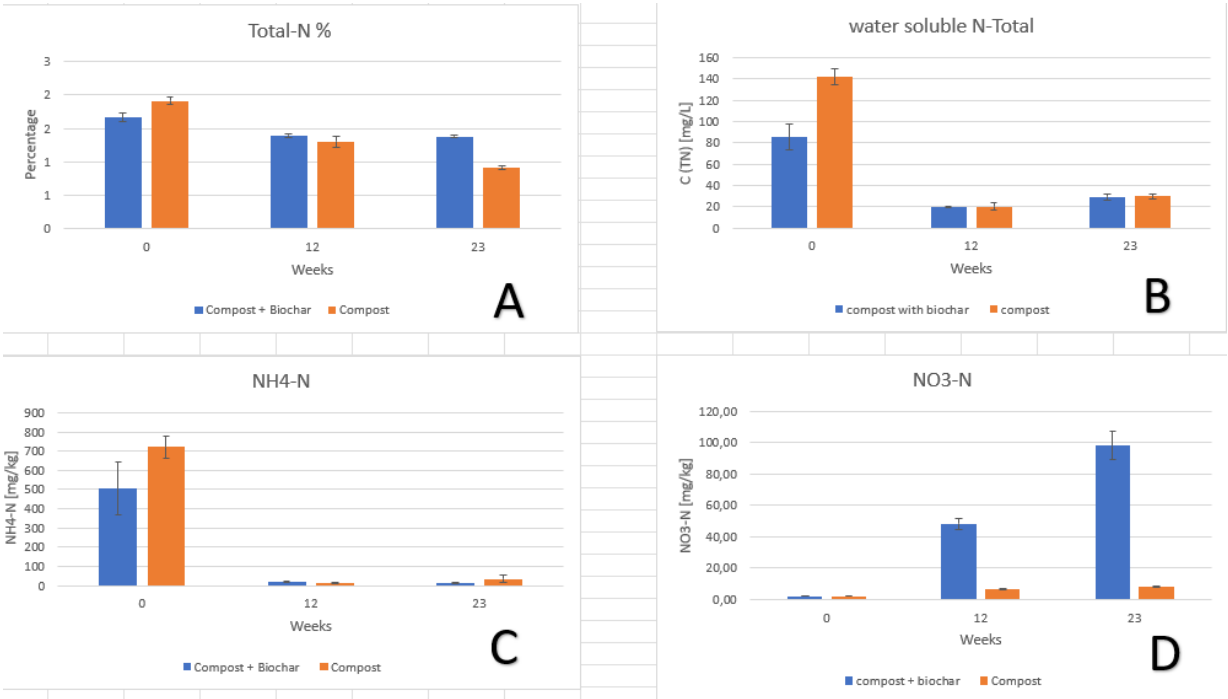

Figure 7. Evolution of total N concentration (A), water soluble N (B) and mineral N: ammonium (C) and nitrate (D). during the composting of manure with and without biochar.

This may be attributed to the lower concentration of NH_4^+ in the biochar-treated pile, the lower initial pH when temperatures were high, and the faster cooling of the pile. Additionally, biochar may adsorb nitrogen-containing soluble compounds, slowing their decomposition and reducing nitrogen losses (Sánchez-Monedero et al., 2018).

The trend in water-soluble nitrogen mirrored that of water-soluble carbon, following the expected pattern during composting. Ammonium (NH_4^+) concentrations

were highest at the start of the process and decreased substantially, reaching near-zero levels by the end. The nitrate concentrations were particularly noteworthy, as they revealed a marked difference between the piles with and without biochar. **Biochar significantly enhanced the nitrification process**, resulting in a higher concentration of nitrate in the biochar-treated compost.

4.1.5. Maturation Indexes.

Several indices are commonly used to assess the degree of compost stabilization, maturity, and readiness for use. From all these indices, the germination index (GI), which evaluates the compost phytotoxicity, was selected as one of the most widely used. A GI above 50% indicates that the compost is mature and safe for use. Initially, both mixtures had GI close to zero, reflecting their high phytotoxicity. **After 12 weeks, the biochar-treated pile achieved a higher GI** than the control pile, aligning with other parameters that indicate **faster composting in the presence of biochar**. Ultimately, both composts reached values above 50%, making them safe for soil application.

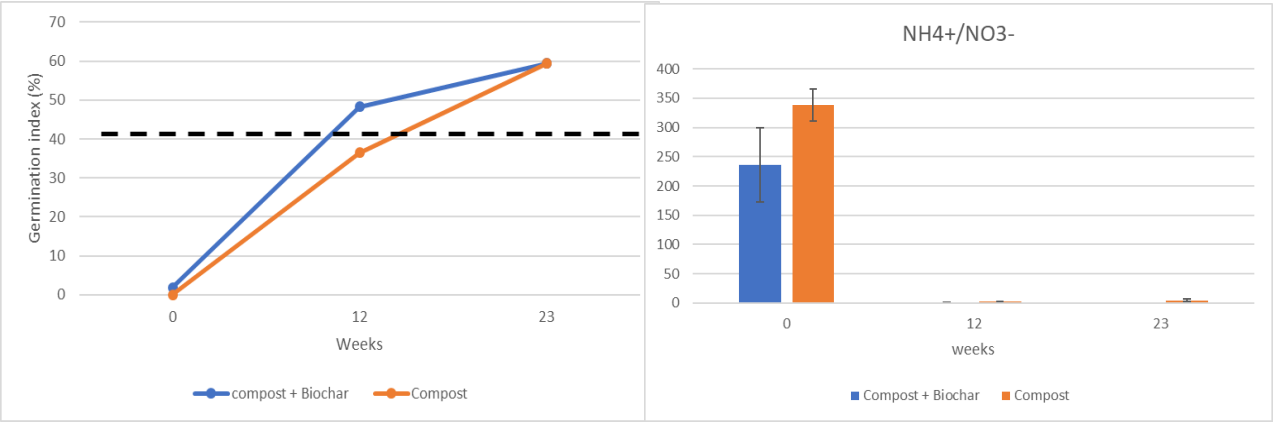

Figure 8. Evolution of the germination index (left panel) and the NH_4^+/NO_3^- ratio during the composting of manure with and without biochar.

Finally, another commonly used maturation index is the NH_4^+/NO_3^- ratio. Early in the process, especially during the thermophilic phase, ammonium is the predominant form of mineral N because of the breakdown of organic nitrogen into NH_4^+. This leads to a high NH_4^+/NO_3^- ratio, as shown during the first stage in Figure 8. As composting progresses and the temperature decreases, nitrifying bacteria become active, transforming NH_4^+ into NO_3^- through nitrification. By the end of the composting process, the NH_4^+/NO_3^- ratio usually decreases significantly, with nitrate becoming the prevalent mineral form.

4.1.6. Compost quality.

Table 1 shows macronutrients and other quality parameters in both composts, which exhibited similar pH, electrical conductivity (EC), and concentrations of nutrients. Notably, the compost with biochar had almost double the carbon content compared to the control without biochar. It also showed significantly higher nitrogen concentrations, indicating reduced nitrogen losses during the process. Additionally, the biochar compost had slightly higher concentrations of potassium (K).

TABLE 1. Main chemical characteristics of the composts produced in Bosque de Matasnos.

	Compost	SD	Compost + Biochar	SD
pH	10,09	± 0,01	10,11	± 0,02
EC (mS/cm)	2,18	± 0,06	2,91	± 0,04
TOC (%)	13,7	± 0,31	25,3	± 0,51
TN (%)	0,92	± 0,01	1,38	± 0,03
K (g/kg)	23,46	±1,06	27,51	±0,73
P (g/kg)	2,97	± 0,08	3,96	±0,04
NH4+-N (mg/kg)	35,1	± 18,9	16,0	± 1,5
NO3—N (mg/kg)	8,0	± 0,4	98,2	± 8,8
PO43- (mg/kg)	36,8	± 1,6	31,0	± 4,3
GI (%)	59,4	± 3,4	59,4	± 3,8

EC: electrical conductivity; TOC: total organic C; TN: total N; GI: germination index.

4.2. Methane (CH_4) mitigation via biochar composting: calculations based on Harrison et al. (2022)

In this study, we estimate the potential reduction of methane (CH_4) emissions from composting with biochar. Livestock is a major source of anthropogenic CH_4 emissions, with manure management contributing significantly. While most CH_4 reduction efforts target liquid manure, stockpiled solids also emit large amounts of CH_4. By incorporating biochar into the composting process, Harrison et al (2022) have shown a reduction of CH_4 emissions by up to 79% compared to conventional composting. We used these findings to calculate the potential CH_4 mitigation from biochar addition during composting in Bosque de Matasnos.

For this experiment, we built piles with similar proportions of manure and biochar as in Harrison et al (2022) since changing the proportion of biochar added has been found to affect the outcomes.

Composition of composting piles

Pile 1: Fresh Manure mixed with grape stalks (83 % sheep manure)

Pile 2: Fresh Manure mixed with grape stalks (83% sheep manure) + Biochar (20% volume)

Bulk density (Fresh manure) = 1000g/l Fresh manure moisture= 61%.	Bulk density (Biochar) = 330 g/l Biochar moisture= 6%

Considering the moisture and bulk density of the composting materials, we can calculate the dry weight of each pile:

<u>Pile 1</u>: 13627 l of fresh manure = 5300 kg of dry manure

<u>Pile 2:</u> 4250 kg of dry manure + 850 kg biochar (83:17 dry weight) = 5100 kg of dry manure + biochar

We used 900kg of Biochar=2727 l of Biochar

We used 10900 l of fresh manure mixed with grape stalks =10.900 kg of fresh manure

According to Harrison et al (2022) these are the ammounts of CH4 emitted per kg of dry compost:

2,43 g CH4/kg dry manure compost
0,51 g CH4/kg dry manure+ Biochar compost

CH_4 emissions during composting experiment in Bosque Matasnos in 2024

Pile 1 CH_4 emissions= 2,43 *5300= 12,9 kg CH_4

Pile 2 CH_4 emissions= 0,51* 5100 = 2,6kg CH_4

Considering that Bosque de Matasnos uses around 1000 tons of fresh manure per year, this would imply around 400 tons of dry manure. There are no limitations regarding biochar feedstock, as both pruning and forestry residues are abundantly available each year.

Scenario 1: Composting:2,43 *400.000= 972 kg CH_4/year

Scenario 2: Composting with biochar: 0,51 * 400.000= 240 kg CH_4/year

A 20-year global warming potential (GWP) for methane (CH_4) is included, as noted in Harrison et al. (2022), because CH_4 has a high GWP over its short lifespan of 12 years. This consideration is particularly relevant for informing effective CH_4 reduction policies.

Taking into account that 1 kg of CH_4 has a GWP of 84 kg of CO_2 over a 20-year timeframe.

Scenario 1: Composting: 81.648 kg CO_2-eq /year

Scenario 2: Composting with biochar: 20.160 kg CO_2-eq/year

This would imply that biochar addition during composting would save **61.488 kg CO_2-eq /year**. Considering that Bosque Matasnos produces around 200.000 bottles of wine per year, this implies that each bottle would save 307 g CO_2 eq/bottle.

Considering an average emission factor for cars of 122 g of CO_2 /km (https://www.europarl.europa.eu/topics/en/article/20190313STO31218/co2-emissions-from-cars-facts-and-figures-infographics), the addition of biochar to the composting process could lead to significant methane reductions, equivalent to the **emissions savings from driving 2,5 kilometers for each bottle of wine produced.** This highlights the environmental benefits of incorporating biochar in composting, making it a viable strategy for mitigating methane emissions.

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