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Comparative Evaluation of Organic and Synthetic Fertilizers on Lettuce Yield and Metabolomic Profiles

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Abstract

The excessive use of synthetic fertilizers in agriculture has raised environmental concerns, prompting the search for sustainable alternatives, such as organic amendments. This study evaluated the agronomic performance, nutrient use efficiency and metabolomic profiles of lettuce (*Lactuca sativa* L. var. baby leaf) cultivated using synthetic and organic (olive mill waste-based compost pellets and sewage sludge) in a controlled pot experiment. The treatments included three doses of inorganic fertilizer and two organic fertilizers applied at equivalent nitrogen (N) rates, alongside an unfertilized control. Soil physicochemical properties, plant biomass, nutrient uptake and metabolite profiles, including amino acids, sugars and organic acids, were analyzed. Inorganic fertilization rapidly increased soil mineral N and phosphorus (P), enhancing leaf chlorophyll, canopy development and fresh biomass, and promoting the accumulation of reducing sugars ($p < 0.05$). However, it reduced amino acid and phenolic levels, indicating a metabolic shift towards growth at the expense of stress and antioxidant compounds. Sewage sludge increased soil organic matter and amino acid and sucrose accumulation, but also induced stress-related metabolites. Pelletized compost maintained an intermediate level of nutrient availability, preserved phenolic compounds and improved phosphorus use efficiency. This surpassed the results achieved with sewage sludge in terms of dry matter yield, despite limited short-term growth stimulation. These findings highlight the potential of integrating moderate mineral fertilization with pelletized compost to balance immediate productivity, nutrient efficiency and long-term soil and metabolic quality in lettuce cultivation.

Keywords: organic waste; olive mill waste pelletized; fertilizers; *Lactuca sativa* L.; nutrient use efficiency

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1. Introduction

Modern agriculture has become increasingly dependent on synthetic fertilizers due to their high nutrient content and effectiveness in increasing crop yield. However, excessive N fertilization has given rise to significant environmental issues, such as nitrate leaching, soil acidification and increased greenhouse gas emissions, particularly nitrous oxide (N_2O) [1]. Organic fertilizers such as compost and sewage sludge (biosolids) are becoming increasingly being promoted as an alternative. These amendments provide essential nutrients that are

released more slowly, thereby improving soil structure, microbial activity and nutrient cycling. Using compost, vermicompost or biochar, for example, has been shown to increase lettuce yield and reduce nitrate accumulation compared to mineral fertilizer when specific irrigation regimes are employed [2].

Lettuce (*Lactuca sativa* L.) is a fast-growing leafy vegetable with high nutrient demands and is therefore a suitable model for evaluating fertilization strategies [3]. Organic amendments have been shown to increase yield and influence quality parameters, such as bioactive compounds [4,5]. However, although synthetic N fertilizers effective promote foliar biomass, they often lead to excessive nitrate accumulation and reduced phenolic content, thereby compromising the nutritional and functional quality of lettuce [6]. Furthermore, the form and availability of N significantly impact shoot-to-root ratios and regulate metabolic pathways associated with secondary metabolite biosynthesis [4].

From a sustainability perspective, integrating organic fertilizers into cropping systems enhances agroecological resilience, reduces dependence on synthetic inputs and promotes nutrient recycling. Such practices align with the European Union's Circular Economy Action Plan [7], which emphasizes sustainable product design and waste reduction, and as well as with Spain's Law 7/2022 on waste and contaminated soils for a circular economy [8].

Metabolomic approaches provide further insight into how plants respond to different fertilization regimes. For instance, Matamoros et al. [5] demonstrated that the presence of N rather than microcontaminants was primarily responsible for changes in the metabolites of lettuce cultivated using sewage sludge, compost, swine manure, and chemical fertilizers. Lettuce is not only a key leafy vegetable, but also an outstanding metabolomics model thanks to its high sensitivity to changes in nutrients, rapid metabolite turnover, and well-understood secondary metabolic pathways. Gao et al. [4] demonstrated that N and phosphorus deficiencies induce distinct metabolic changes, with compounds such as inositol, citrate, and succinate acting as potential biomarkers of nutrient stress.

Despite the growing popularity of organic fertilizers, previous studies have mostly focused on agronomic traits or specific metabolic responses to individual amendments, rather than combining the two approaches. However, direct comparisons of agronomic performance and metabolomic profiles under different fertilization regimes remain underexplored. This study therefore aims to compare the agronomic performance, nutritional quality, and metabolomic profiles of lettuce cultivated using organic fertilizers (pelletized compost and sewage sludge) and a synthetic fertilizer. We hypothesize that organic fertilizers, particularly those derived from composted materials, will improve the nutritional quality of lettuce and produce distinctive metabolomic signatures compared with synthetic fertilization. By integrating agronomic and metabolomic perspectives, this research seeks to provide evidence in support of sustainable fertilization practices within the framework of regenerative agriculture and the circular economy.

2. Materials and Methods

2.1. Pot Experimental Design

A pot experiment was carried out with lettuce (*Lactuca sativa* L. baby leaf variety) under controlled greenhouse conditions at FertiLab-EPSO UMH (Orihuela, Spain). The environment was maintained at a temperature of 21–25 °C with a relative humidity of 50–60%, and a photoperiod of 12 h light (1700 μmol s^{-1}) and 12 h of darkness was provided by RX600, Solray® 385 artificial lamps (Helsinki, Finland) (Supplementary Material Figure S1). The trial was design using a completely randomized approach, incorporating five fertilization treatments, each replicated three times, as well as an unfertilized control. This resulted in a total of 18 experimental units (n = 18). The fertilization treatments consisted of: (a) three rates of a commercial synthetic NPK fertilizer (ComplexIN, 15 N-15 P_2O_5-15 K_2O),

corresponding to 100 (IN100), 200 (IN200) and 300 (IN300) kg NPK ha^{-1}; (b) two organic fertilizers: a pelletized compost (OCP) and a sewage sludge-based amendment (SS) (Table 1). All treatments received supplemental potassium nitrate (KNO_3, 13-0-46) to meet the N requirements of the lettuce plants. The organic fertilizers were applied at standardized rate of 200 kg N ha^{-1} and 120 kg P ha^{-1}.

Table 1. Fertilization treatments applied to lettuce in the pot experiment.

Treatments	Fertilizer Type	Acronym	Nutrient Dose (kg N ha^{-1})
Control without fertilizer	Control	C	
Complex (15-15-15)	Synthetic	IN100	100
Complex (15-15-15)	Synthetic	IN200	200
Complex (15-15-15)	Synthetic	IN300	300
Pelletized compost	Organic	OCP	200
Sewage Sludge	Organic	SS	200

Plastic pots, with a diameter of 11 cm and a volume of 1200 cm^3 were filled with 1.5 kg of soil. They were arranged randomly on a growth table and periodically rotated to minimize positional effects. The soil was prepared according to OECD 207(1984) guideline [9] for soil testing and consisted of a mixture of natural loam (from a depth of 0–20 cm depth, air-dried and sieved to 5 mm) from the EPSO-UMH experimental farm (38°4′9.066″ N, 0°59′6.148″ W) and fine and coarse sand (in a 50:25:25% w:w:w ratio), resulting in a sandy loam texture. The soil was not sterilized in order to preserve native microbial activity relevant to nutrient mineralization. This artificial soil contained 66% sand, 12% silt and 22% clay, giving it a bulk density of 1.43 kg m^{-3}, an electrical conductivity (EC) of 3.75 mS cm^{-1}, an organic matter (OM) of 0.59%, a total N (TN) of 0.86 g kg^{-1}, an ammonium N ($N\text{-}NH_4^+$) of 1.62 mg kg^{-1}, a nitrate N ($N\text{-}NO_3^-$) of 26.2 mg kg^{-1}, an extractable P (P_{ext}) content of 15.6 mg kg^{-1}, and a pH adjusted to 6.5 using ferric sulphate ($FeSO_4$).

The pelletized compost used in this study was produced through large-scale windrow composting at CompoLab EPSO-UMH (Orihuela, Spain) [10]. It was made from a mixture of 60% olive mill waste, 20% poultry manure and 20% olive leaf waste. The organic fertilizers used in this study were sewage sludge (SS) and organic compost pellets (OCP). The physicochemical and chemical properties of both fertilizers (Supplementary Material Table S1) were examined using the methods described by Paredes et al. [11]. Total macro-, meso-, and micronutrients were determined using a multi-elemental analysis spectrophotometer (ICP-OES), while total organic carbon (OC) and total nitrogen (TN) were analyzed using an automatic elemental microanalyzer (EuroVector, Milan, Italy). The SS pellets exhibited a pH of 6.56 and an OC to TN ratio of 6.3, indicating well-stabilized material derived from anaerobically digested sludge with a dry matter content of around 90%. The OCP showed a higher pH (9.05) and a C/N ratio of 15.2, which is consistent with mature compost. The moisture content of both pelletized fertilizers ranged from 10 to 15%, ensuring adequate handling and homogeneous application during the experiment. The pellets were produced at CompoLab EPSO-UMH (Orihuela, Spain) by extruding the mixtures using a small-scale, low-power (4 kW) pelletizer with a capacity of 100 kg h^{-1} and two rotating rollers (78 mm), operating on a fixed, flat die with 5 mm openings and a diameter of 119 mm. To simulate field-like conditions, fertilizers were lightly incorporated (to a depth of 0.5 cm) near the seed zone. After the application of the fertilizers lettuce seedlings at 2–3 true leaf stage were transplanted and irrigated with 200 mL of deionized water. An additional 75 mL was the applied after infiltration to reach 60% of field capacity. Throughout the experiment, manual irrigation was performed to maintain the soil moisture at field capacity, as verified gravimetrically, in order to compensate for evapotranspiration losses.

2.2. Sampling and Analytical Methods

2.2.1. Soil Analysis

At harvest (45 days after transplanting), three soil subsamples were collected from each pot. The soil was then air-dried at 60 °C and sieved (<2 mm), with the roots removed first. Soil pH and EC were measured in 1:2.5 and 1:5 (*w*/*v*) soil/water (*w*/*v*), respectively [12]. Total organic carbon (TOC) [13], total nitrogen Kjeldahl (TN) [14] and extractable P (P_{ext}) [15] were quantified using standard methods. Nitrate ($N\text{-}NO_3^-$) and ammonium ($N\text{-}NH_4^+$) were extracted using 2 M KCl 1:5 (*w*/*v*) and analyzed using MgO-devarda alloy distillation method [16].

2.2.2. Plant Analysis

Non-destructive measurements included the chlorophyll content (Chl) using a SPAD-502 (Konica Minolta®, Tokio, Japan) meter and calculating the leaf area index (LAI) using the Canopy® mobile application [17] at 15, 30 and 45 days after transplanting. On day 45, the plants were harvested to determine the fresh shoot biomass dry weight (including shoots and roots) and yield. The fresh mass (g. pot^{-1}) was recorded and then the samples were oven-dried at 60 °C for 48 h to obtain the dry biomass [18]. The dried material was milled to a size less than <1 mm for physicochemical analysis.

Total tissue C and N total were determined using an elemental microanalyzer and the concentration of macro- and micronutrients (Ca, S, Mg, Cu, Fe, Mn, Zn) was quantified using ICP-OES after digestion in a nitric perchloric acid mixture ($HNO_3/HClO_4$. 2:1 *v*/*v*) in a microwave system [19]. Total phosphorus (TP) was measured spectrophotometrically and potassium (K) and sodium (Na) were assessed with a flame photometer. Chlorophyll quantification was quantified after extraction in 80% acetone followed and measured using a microplate reader [20]. The nitrogen use efficiency (NUE), phosphorus use efficiency (PUE) and potassium use efficiency (KUE) were calculated as the ratio of nutrient uptake to the amount supplied via fertilizer [21].

Non-targeted metabolomic profiling identified 29 compounds, which were classified as follows: amino acids (glutamate, glutamine, alanine, arginine, GABA, leucine, valine, isoleucine, asparagine, aspartate, phenylalanine, proline and threonine); organic acids (formate, malate, succinate, citrate, fumarate, acetate, ascorbate, 2-oxoglutarate, tartrate); and sugars (glucose, fructose, sucrose, myo-inositol). The samples were extracted and analyzed using a H–NMR (Ascend 500 MHz AVANCE III HD, Weinheim, Germany) [22]. Spectral data were processed using Chenomx NMR Suite v8.3 [23].

2.3. Statistical Analysis

All statistical analyses were conducted using R software (v4.3.1) [24]. Data normality and homogeneity of variance were verified using the Shapiro-Wilks and Levene tests, respectively ($p > 0.05$). Differences among treatments were evaluated via one-way ANOVA within a generalized linear mixed model (GLMM) framework, with fertilizer treatments and sampling dates considered as fixed effects and pot identity as a random effect. Mean separation was performed using Fisher's LSD test ($\alpha = 0.05$).

The variables were categorized as soil chemistry, yield and quality attributes. Pearson correlation matrices were produced for each group using the corrplot package (v.0.95). We then employed principal component analyses (PCA) to explore the multivariate patterns associated with the treatments. To improve the robustness of the PCA weakly correlated variables were excluded when the number of variables exceeded the number of observations. All analyses were conducted using the stats package in R (v.4.3.1).

3. Results

3.1. Soil Parameters

Most soil parameters showed highly significant differences between treatments with the exception of pH and EC (Figure 1). Overall, soil pH remained slightly alkaline across all treatments, with the highest value observed in pots amended with SS. Although EC values did not differ significantly, the control exhibited the highest EC, while soils treated with SS had the lowest.

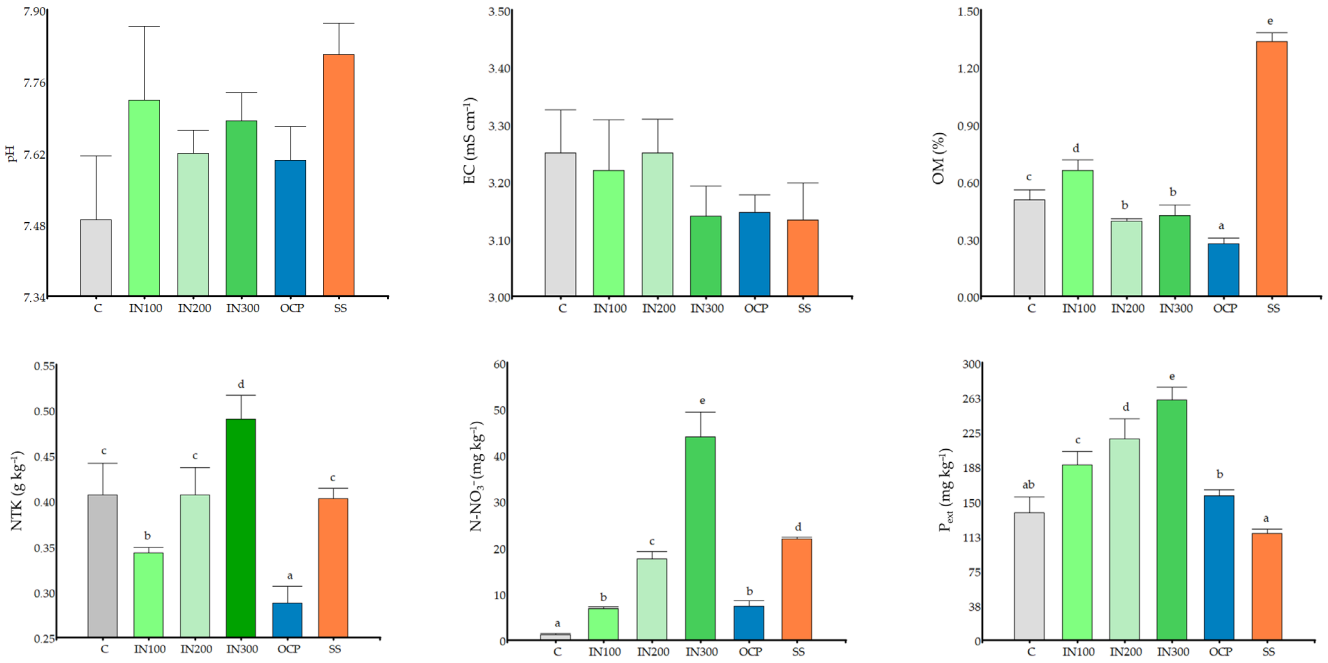


Figure 1. Soil physical-chemical properties at harvest. EC: electrical conductivity; OM: organic matter; NTK: total nitrogen Kjeldahl; NO_3^--N: nitrate. P_{ext}: extractable phosphorus. Different letters above the bars indicate significant differences between the treatments ($p < 0.05$). Values indicate mean (n = 3). See Table 1 for acronyms.

The content of OM differed significantly between all fertilized treatments and the control (Figure 1). The highest OM concentrations were found in IN100 and SS soils, while the largest decline OM was observed in OCP amended soil.

No significant differences were detected in the NTK levels between the control and the IN200 or SS treatments (Figure 1). The IN300 treatment had the highest NTK levels, reflecting the great contribution of mineral nitrogen from the 15-15-15 fertilizer. The lowest NTK levels were measured in the OCP treatment. None of the soils exceeded the initial baseline N level. Significantly higher concentration of $N\text{-}NH_4^+$ were found in all fertilized soils than in the control, with the highest concentration produced by synthetic fertilizers, followed by OCP and SS. $N\text{-}NO_3^-$ was the dominant form of inorganic N in all soils. Only IN300 significantly increased $N\text{-}NO_3^-$ content, with decreasing values in the order IN300 > SS > IN200 > OCP = C.

The IN300 and IN200 treatments markedly enhanced P_{ext}, followed by IN100 and OCP (Figure 1). Soils amended with SS contained less extractable phosphorus than the control. However, none of the reduced the initial soil P content.

3.2. Crop Response to Fertilizer

3.2.1. Biophysical Parameters and Biomass at Harvest

Table 2 summarizes the biophysical characteristics of the lettuce at harvest. Significant differences were detected between the control group and those receiving inorganic or

organic treatments. Regarding the SPAD index, lettuce plants that were fertilized with organic treatments (OCP and SS) had lower readings than the control group. IN200 had the highest chlorophyll value. Among the inorganic treatments, there was no significant difference in canopy coverage between IN100, IN200 and IN300. However, IN200 produced the lowest coverage. A similar trend was observed for total chlorophyll concentration. The greatest fresh biomass was produced under IN300 fertilization, although there were no significant differences among the inorganic treatments. Among the organic treatments, OCP plants yielded slightly more biomass than SS plants, but performed comparably to the control group.

Table 2. Lettuce biophysical parameters and fresh biomass and at harvest.

Treatments	SPAD	Canopy (%)	Chl (mg g^{-1})	Biomass (g pot^{-1})
C	5.61 b	2.68 a	0.43 a	15.85 a
IN100	10.29 c	10.01 c	0.64 c	54.52 b
IN200	16.03 e	11.67 c	0.80 d	57.06 b
IN300	12.99 d	10.75 c	0.78 d	60.42 b
OCP	4.96 ab	3.14 ab	0.53 b	22.35 a
SS	4.39 a	4.48 b	0.58 bc	19.30 a
F-ANOVA	***	***	***	***

Chl: chlorophyll. ***: significant difference between treatments at $p < 0.0001$. Different letters within a column indicate significant differences between treatments ($p < 0.05$). Values indicate the mean (n = 3). For acronyms see Table 1.

3.2.2. Nutrient Uptake

Significant differences in N, P and K uptake were detected among treatments ($p < 0.0001$) (Table 3). Lettuce grown with inorganic fertilizers (IN100, IN200 and IN300) accumulated the highest N concentrations. The IN300 and IN200 treatments produced significantly greater N uptake than all the other treatments. The lowest uptake was observed in plants fertilized with organic treatments, although no significant differences were found between OCP, SS and IN100. All fertilized plants exceeded the N uptake of the unfertilized plants ($p < 0.0001$).

Table 3. Nutrient uptake and nutrient use efficiency in lettuce at harvest.

Treatments	N Uptake (g N pot^{-1})	NUE (%)	P Uptake (g P pot^{-1})	PUE (%)	K Uptake (g K pot^{-1})	KUE (%)	Yield (g pot^{-1})
C	0.05 a	-	0.01 a	-	0.01 a	-	2.47 a
IN100	0.12 b	71.43 d	0.03 c	24.90 c	0.03 b	24.40 c	6.81 d
IN200	0.18 c	62.72 c	0.05 d	23.90 c	0.05 d	25.60 c	6.07 d
IN300	0.20 e	52.54 b	0.05 e	18.90 b	0.04 c	11.06 b	5.98 c
OC P	0.07 b	14.48 a	0.02 b	23.93 c	0.01 a	0.00 a	3.33 b
SS	0.07 b	11.72 a	0.01 a	8.23 a	0.01 a	0.00 a	2.61 a
F-ANOVA	***	***	***	***	***	***	***

***: significant difference between treatments at $p < 0.0001$. Different letters within a column indicate significant differences between treatments ($p < 0.05$). Values indicate mean (n = 3). For acronyms see Table 1.

P uptake followed a similar pattern to N. IN300 and IN200 yielded the highest P absorption, followed by IN100 and OCP. SS-treated plants displayed values comparable to the control. K uptake was greatest under inorganic fertilization, with significant differences observed between the mineral treatments. The control and organic amendments exhibited the lowest K uptake and no statical differences were detected between them.

3.2.3. Yield and Nutrient Use Efficiency

A significant difference in dry biomass was observed among the treatments at harvest (Table 3). The highest yields were obtained from pots that received mineral fertilization (IN100, IN200 and IN300), with plants that received OCP amendments producing intermediate values. Lettuce treated with SS did not differ significantly from the control.

Nutrient use efficiency (NUE, PUE and KUE) also differed markedly ($p < 0.0001$). The inorganic treatments (IN100, IN200 and IN300) recorded the greatest NUE, followed by OCP and SS with no significant difference between the two organic sources. For P use efficiency, IN100, IN200 and OCP achieved the highest efficiencies, which were not statistically different. The greatest KUE was observed under IN200, followed closely by IN100. IN300 showed intermediate performance, while both organic treatments exhibited negligible KUE values.

3.2.4. Nutrient Concentration Analysis

The metabolomics data obtained provides an insight into the metabolic state of the plants at harvest time. The levels of key metabolites involved in energy metabolism, such as glycolysis and the TCA cycle, were examined. The greatest accumulation of fructose (15–17 g kg^{-1}) and glucose (11–12 g kg^{-1}) was observed with medium- and high-dose inorganic fertilization (IN200 and IN300) (Supplementary Material Table S2), whereas compost values were comparable to the control. In contrast, the application of sewage sludge markedly increased sucrose levels (6.38 g kg^{-1}), reaching values similar to those observed with high inorganic fertilization. Overall, intensive inorganic fertilization promoted the accumulation of reducing sugars, while organic fertilization with sludge was primarily associated with increased sucrose levels.

Significant differences in amino acid concentrations were observed among the fertilization treatments (Supplementary Material Table S2). GABA levels were markedly higher under low inorganic fertilization (IN100), while compost showed the lowest levels. Key intermediates in N metabolism, glutamine and glutamate, were strongly enhanced by SS, while remaining low under control and compost. Similarly, arginine and asparagine (major N storage amino acids) reached their highest levels under SS, contrasting with the sharp declines observed under medium and high inorganic fertilization (IN200 and IN300). Compared with inorganic treatments, sewage sludge also promoted higher concentrations of aspartate, valine, phenylalanine and threonine, whereas compost maintained intermediate values. In contrast, medium–high inorganic fertilization generally suppressed amino acid accumulation, except for consistently low levels of leucine across all treatments. Overall, organic fertilization, particularly with sewage sludge, favoured the accumulation of amino acids associated with N storage and stress responses, while inorganic fertilization shifted metabolism away from N compounds. The concentration of organic acids in lettuce leaves varied significantly according to fertilization type, reflecting differences in the plant's energetic response (Supplementary Material Table S2). Low inorganic fertilization (IN100) stimulated the accumulation of Krebs cycle intermediates, such as malate, succinate and tartrate. This suggests enhanced respiratory activity and osmotic adjustment. Conversely, medium and high doses (IN200 and IN300) markedly reduced citrate levels, indicating a metabolic shift towards rapid growth and reduced storage of energy intermediates. Compost, however,-maintained citrate, succinate. and malate levels similar to those of the unfertilized control, highlighting a more balanced and stable metabolic profile linked to the gradual release of nutrients.

Regarding the concentration of secondary metabolites, these also varied significantly depending on the type of fertilization (Supplementary Material Table S2). Chlorogenate reached its highest value in the unfertilized control and decreased under inorganic fer-

tilization, reaching a minimum at IN300; meanwhile, compost and sludge maintained intermediate levels. Choline showed the opposite trend, increasing under low inorganic fertilization (IN100) and sludge compared to lower values at IN200–IN300. Although trigonelline was present at low absolute levels, it was slightly higher under sludge treatment and remaining stable across the other treatments.

4. Discussion

4.1. Effects of Fertilization on Soil Properties

Soil pH remained slightly alkaline across all treatments, with no significant differences observed. This stability indicates the strong buffering capacity of both the compost and the sludge, which is consistent with the results of other studies [25]. Electrical conductivity (EC) also remained stable. Neither the compost nor the sludge increased salinity, with values comparable to or lower than those observed with mineral fertilization. This aligns with the findings of Bello et al. [26], who reported that careful management of organic amendments can prevent secondary salinization in Mediterranean soils.

Soil organic matter (OM) content varied significantly between treatments. The unfertilized control exhibited 0.51% OM, while the lowest value (0.28%) was recorded for olive mill waste pelletized compost (OCP). This decline may indicate that the compost stimulated microbial activity, accelerating the mineralization of native soil carbon via a priming effect [27]. Similar short-term decreases in OM have been reported in vegetable production systems following organic inputs [28]. Inorganic fertilization (IN100–IN300) maintained OM levels close to those of the control (0.39–0.42%), suggesting that mineral inputs supplied nutrients without increasing soil carbon content [29,30]. In contrast, sewage sludge (SS) resulted in the highest OM content (1.34%), reflecting its higher proportion of stable organic matter, which can accumulate in soil over short cultivation periods. Recent field studies have confirmed that sludge increases soil carbon and N stocks more effectively than compost in Mediterranean conditions [31].

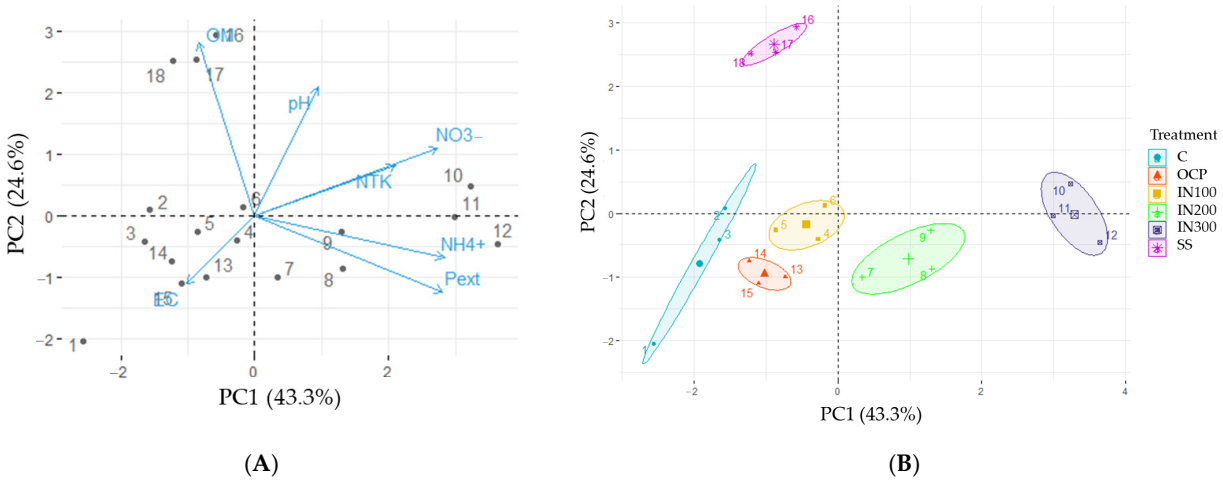
The N dynamics highlighted the contrast between mineral and organic sources. Total Kjeldahl N (NTK) was higher under mineral fertilization, reflecting the immediate availability of N in chemical fertilizers (such as ammonium nitrate), which can be readily detected in soil analyses. In contrast, the N supplied by organic amendments was in complex forms that mineralize slowly, and was not fully expressed within the 45-day lettuce cycle [28,32]. Compost may also have triggered priming effects, enhancing microbial turnover and increasing N losses through volatilization or leaching [32]. No significant differences in NTK were found among the control, IN200, and SS treatments, although these occurred through different mechanisms. Only the highest mineral input (IN300) exceeded crop demand and soil buffering capacity, resulting in detectable N accumulation. Similar non-linear dose–response effects have been reported in Mediterranean soils under short-cycle crops [28,30,33].

The highest nitrate (NO_3^--N) levels were found in mineral treatments, confirming the rapid release of synthetic fertilizers. Intermediate values were yielded by compost and sludge, indicating a gradual nutrient supply driven by microbial decomposition [28]. This slower release reduces the risk of leaching and improves the synchronization of nutrient with crop uptake [34]. Ammonium nitrogen (NH_4^+-N) exhibited a complementary pattern. Mineral treatments provided the highest concentrations shortly after application, but these decreased rapidly due to nitrification or plant uptake. By contrast, the levels of NH_4^+-N in the compost and sludge remained moderate throughout the crop cycle, reflecting ongoing mineralization [28].

The extractable phosphorus (P_{ext}) content also varied across the different treatments. The highest values were observed under mineral fertilization. particularly in IN300,

reflecting the immediate solubility of phosphorus in the 15-15-15 formulation. In contrast, compost and sludge exhibited lower or intermediate P_{ext} levels, which is consistent with their slower nutrient release and the partial immobilization of P in organic complexes [32,34]. This gradual release may reduce the risk of leaching and improve long-term phosphorus availability.

Overall, the results demonstrate a dual behaviour: while mineral fertilization ensures immediate nutrient availability, it does not contribute to the build-up of soil organic matter (Figure 2A). Olive mill waste pelletized compost acts as a biological activator, stimulating microbial activity rather than increasing soil carbon in the short term. Sewage sludge provided the greatest short-term improvement in organic matter while maintaining stable pH and EC levels. The patterns observed for P_{ext} and NH_4^+-N confirm that organic amendments release nutrients gradually, reducing environmental risks, but requiring longer timeframes to match the effectiveness of mineral fertilizers (Figure 2B). These findings support the integration of mineral and organic inputs to optimize soil fertility and balance productivity with long-term sustainability [35,36].



(**A**) (**B**)

Figure 2. (**A**) Principal component analysis (PCA) of soil physicochemical parameters. The soil variables include: pH, electrical conductivity (EC), organic matter (OM) and nutrients (NTK, NO_3^--N, NH_4^+-N, P_{ext}). (**B**) PCA highlights the main axes of variation among the different treatments. Coloured ellipses represent 95% confidence intervals, highlighting the grouping of individuals within each treatment. For acronyms see Table 1.

4.2. Effects of Fertilization on Crop Response

4.2.1. Effects of Fertilization on Yield and Morphological Parameters

In terms of leaf status, SPAD and chlorophyll values reflected N availability. IN200 recorded the highest SPAD value (16.03) and the highest chlorophyll values (0.80 mg g^{-1}), followed by IN300 (12.99; 0.78) and IN100 (10.29; 0.64) (Figure 3). In contrast, the OCP, SS and control values remained low (SPAD = 4.39–5.61; chlorophyll = 0.43–0.58). These results confirm the central role of mineral N in chlorophyll synthesis and leaf greenness [37,38]. Similar responses to mineral N in lettuce have been reported in other short-cycle vegetables [39].

Clear differences in canopy and fresh biomass values were observed between mineral and organic fertilization. The IN100, IN200 and IN300 groups produced the largest canopies (10.01–11.67%) and the highest fresh biomass (54.5–60.4 g). By contrast, the OCP, SS and control groups produced much smaller canopies (2.68–4.48%) and biomass (15.8–22.3 g), clustering together. The lack of significant differences within each group highlights two contrasting fertilization scenarios: a rapid nutrient supply with mineral inputs versus limited short-term availability from organic sources. Similar patterns have been reported in lettuce and other leafy crops, where composts and sludges did not improve growth

compared to the control in the first crop cycle [40]. The slow mineralization of organic N and P in OCP and SS, combined with lettuce's rapid nutrient demand, explains their weak short-term performance [33].



Figure 3. Heatmap shows the mean values of physiological traits and nutrient uptake in plants subjected to different treatments The colour scale shows the magnitude: green indicates higher values and red indicates lower values.

The highest dry yield was achieved under IN100 (6.81 g) and IN200 (6.07 g). IN300 produced a slightly lower yield of 5.98 g. Notably, OCP outperformed SS in terms of dry yield (3.33 g vs. 2.61 g), despite producing similar amounts of fresh biomass. This suggests that, despite being limited in immediate nutrient release, OCP, supported better structural biomass formation than sludge. Similar findings have been reported for Mediterranean vegetables, where compost promoted more efficient dry matter accumulation than sludge [41,42]. OCP's superiority to SS in terms of dry yield reinforces its agronomic value, even in short cycles.

A slight increase in the absorption of P relative to K could be observed in the plant tissue. In our experimental conditions, this outcome may have been caused by several factors: the pH was adjusted to 6.5, which increased the availability and mobility of P in the nutrient solution compared to K; young lettuce plants often require high levels of P to support rapid root and leaf development, which can result in disproportionately high accumulation of P; and K uptake can be sensitive to competition with other cations (Ca^{2+} and Mg^{2+}), which may have limited its absorption.

Regarding nitrogen use efficiency (NUE), it decreased as mineral input increased: IN100 = 71.43%, IN200 = 62.72%, IN300 = 52.54%. Moderate N rates matched crop demand most efficiently, while higher inputs reduced efficiency through diminishing returns and potential N losses [43]. NUE was very low in both OCP (14.48%) and SS (11.72%), confirming limited mineralization within 45 days. Phosphorus use efficiency (PUE) produced an important result. OCP reached 23.93%, which was statistically equivalent to IN100 (24.90%) and IN200 (23.90%), while IN300 was lower at 18.90%. SS performed the worst at 8.23%. Despite poor performance in terms of SPAD, canopy and yield, OCP promoted efficient P use per unit applied. This is likely due to organic acid complexation and microbial mineralization, which improve P availability [34,44]. This highlights a key agronomic advantage of OCP. However, K use efficiency (KUE) was highest in IN100–IN200 (24.4–25.6%), lower in IN300 (11.06%), and zero in OCP and SS. In the short term, organic inputs contributed negligible amounts of plant-available K in the short term, while excessive mineral input reduced efficiency at the highest rate [38].

Overall, there was no improvement in lettuce SPAD, canopy, chlorophyll or fresh biomass compared to the control group when using OCP, which confirms its slow nutrient release in short-cycle crops. However, OCP surpassed SS in terms of dry yield and equalled mineral fertilization in terms of PUE, demonstrating its specific advantages over sludge and even over high mineral inputs. These results are consistent with evidence that olive mill waste pelletized compost improves P efficiency, soil organic matter, microbial biomass and enzymatic activity in the long term [33]. Therefore, although OCP alone is ineffective in maximizing short-term lettuce productivity, combining it with moderate mineral fertilization could provide immediate yield increases alongside improved P efficiency and long-term soil health.

4.2.2. Effects of Fertilization on Metabolites

Inorganic fertilization promoted the strongest accumulation of reducing sugars. Specifically, IN200–IN300 increased fructose (15–17 g kg^{-1}) and glucose (11–12 g kg^{-1}), confirming the stimulatory role of mineral NPK on carbohydrate metabolism and growth [39,45] (Figure 4). In contrast, compost-maintained values similar to the unfertilized control, reflecting slower nutrient release and more conservative carbohydrate allocation. However, sewage sludge (SS) induced a distinct pattern, with a marked increase in sucrose (6.38 g kg^{-1}), comparable to that observed with high mineral fertilization. This is consistent with previous findings that link sludge to osmotic adjustment and stress-related sucrose accumulation in leafy crops [46]. Therefore, while mineral fertilization favoured fast growth by reducing sugars, sludge shifted metabolism towards sucrose storage (Figure 4).

In addition, amino acid profiles differed significantly between the different treatments. Low mineral fertilization (IN100) increased the level of γ-aminobutyric acid (GABA), which is related to stress and signalling. In contrast, medium and high mineral inputs (IN200–IN300) suppressed most amino acids. This pattern is consistent with evidence that excessive nitrogen fertilization promotes rapid biomass accumulation, but reduces amino acid levels [47]. By comparison, compost produced the lowest GABA levels, supporting the view that its slow nutrient release minimizes transient metabolic stress. Conversely, SS triggered significant increases in the levels of glutamine. glutamate. arginine and asparagine, which are major N assimilation and storage amino acids, as well as higher levels of aspartate, valine, phenylalanine and threonine. This accumulation is consistent with previous reports that sludge enhances amino acid synthesis due to its higher labile N content and possible stress signalling from heavy metals or salinity [40,48]. Thus, compost maintained intermediate amino acid concentrations, reinforcing its role as a moderate input with a stable metabolic footprint.

Furthermore, differences were evident in the levels of organic acids linked to the TCA cycle. IN100 promoted higher levels of malate, succinate and tartrate, which suggests enhanced respiration and osmotic regulation in the presence of moderate nutrients. Conversely, IN200–IN300 reduced citrate levels, indicating a metabolic shift towards growth and reduced storage of organic acid intermediates. These shifts are similar to those observed in previous metabolomic studies of lettuce, which showed N-driven alterations to TCA intermediates [49]. In contrast, the compost resembled the control again, maintaining citrate, malate and succinate at baseline levels.

Furthermore, the levels of secondary metabolites also varied with fertilization. Chlorogenate, a key phenolic compound with antioxidant properties, peaked in the unfertilized control sample (1.12), declining under mineral fertilization to reach its lowest level at IN300 (0.43). Compost (0.98) and sludge (0.95) maintained intermediate levels of chlorogenate, thus preserving their antioxidant potential compared to mineral treatments. This is consistent with studies indicating that organic amendments can maintain or enhance pheno-

lic content compared to intensive mineral fertilization [50]. In contrast, choline exhibited an opposite trend, increasing under IN100 (1.6) and SS (1.5), both of which are linked to osmotic and membrane stabilization functions [51]. Similarly, although minor, trigonelline was slightly higher under SS (0.04), consistent with sludge-induced stress responses. Notably, compost consistently produced stable intermediate values for all secondary metabolites, avoiding the sharp reductions observed under intensive mineral fertilization.

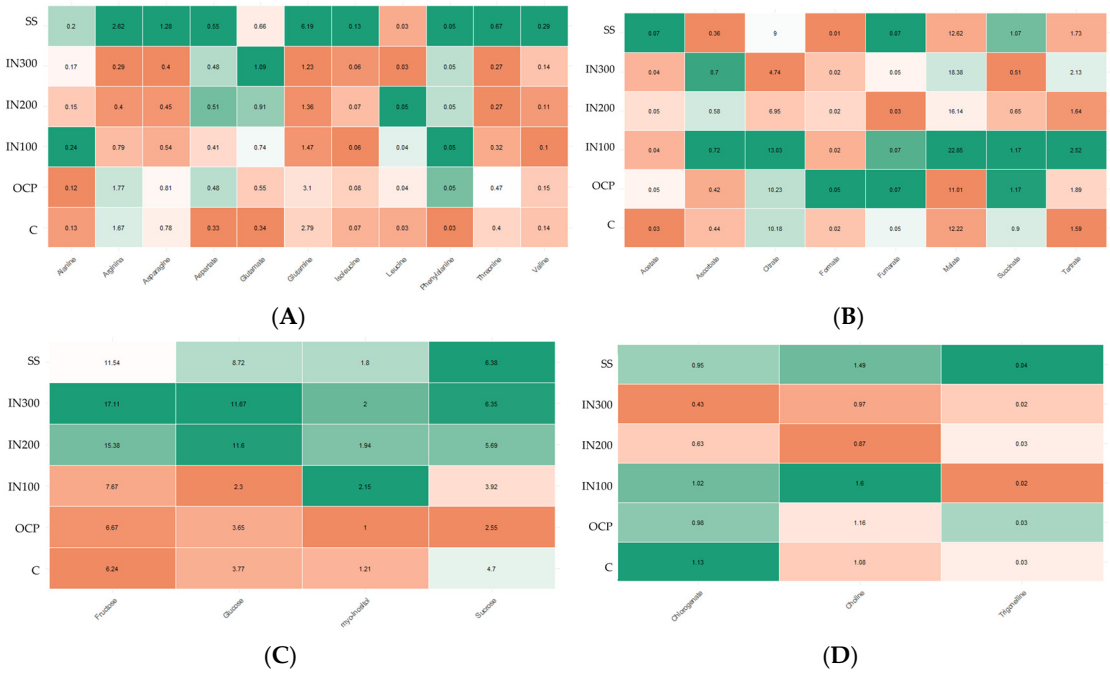


Figure 4. The heatmaps shows the abundance of metabolites in plants subjected to different treatments. (**A**) Amino acids: alanine, asparagine, aspartate, glutamate, glutamine, isoleucine, leucine, phenylalanine, threonine and valine; (**B**) Organic acids and sugars: arabinose, arabinitol, citrate, fumarate, formate, malate, quinate and tartrate; (**C**) Major sugars: fructose, glucose, myo-inositol and sucrose; (**D**) Secondary metabolites: chlorogenic acid, choline and trigonelline. Values represent means (n = 3). Statistical differences among treatments were determined by ANOVA ($p < 0.05$). Colour gradients indicate relative abundance, with green representing higher values and red representing lower values. For acronyms see Table 1.

Overall, the metabolic profile of olive mill waste compost was more stable and conservative than that of mineral fertilization or sludge. It maintained sugars and TCA intermediates at control levels, minimized stress-associated GABA accumulation, and preserved antioxidant phenolics compounds such as chlorogenate. In contrast, mineral fertilization maximized reducing sugars and growth, but depleted amino acid and phenolic reserves. Meanwhile, sludge enhanced amino acid storage and sucrose, albeit at the expense of stress-related metabolic activation. Overall, the intermediate performance of the compost is consistent with previous reports in which olive mill by-product composts were shown to maintain metabolic balance while improving long-term soil quality [33]. Thus, while the compost did not match the immediate metabolic stimulation of mineral fertilization, it outperformed sludge by supporting dry matter yield and maintaining antioxidant compounds.

5. Conclusions

This study demonstrates that the type of fertilization has a significant effect on soil fertility, crop performance and lettuce metabolism. Mineral fertilization was found to rapidly increase the mineral content of soil N and P; enhance SPAD, canopy development

and yield; and promote the accumulation of reducing sugars. However, it depleted amino acid and phenolic reserves, suggesting a metabolic shift towards growth at the expense of stress-related and antioxidant compounds.

By contrast, organic fertilization produced more gradual and differentiated effects. Sewage sludge markedly increased soil organic matter markedly and favoured the accumulation of amino acids and sucrose. However, it also induced stress-associated metabolites.

Overall, olive mill waste-based compost pellets exhibited a more stable and conservative metabolic profile than mineral fertilization or sludge. Although it cannot replace synthetic fertilizers for short-term production of lettuce, it offers specific benefits by enhancing P efficiency, maintaining antioxidant metabolism and improving soil quality in the long term. These results support integrated fertilization strategies that combine moderate mineral inputs with olive mill waste-based compost pellets to balance short-term productivity with long-term sustainability.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae11121421/s1, Figure S1: experimental setup at FertiLab-UMH greenhouse. Table S1: Treatment characterization; Table S2: Metabolite profile of lettuce at harvest.

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