

## Article

# Evaluation of Olive Mill Waste Compost as a Sustainable Alternative to Conventional Fertilizers in Wheat Cultivation

Ana García-Ránde, Silvia Sánchez Méndez , Luciano Orden , Francisco Javier Andreu-Rodríguez , Miguel Ángel Mira-Urios , José A. Sáez-Tovar , Encarnación Martínez-Sabater , María Ángeles Bustamante , María Dolores Pérez-Murcia and Raúl Moral

Centro de Investigación e Innovación Agroalimentaria y Agroambiental (CIAGRO-UMH), Universidad Miguel Hernández, Carretera de Beniel Km 3.2, Orihuela, 03312 Alicante, Spain; ana.garcia@umh.es (A.G.-R.); s.sanchezm@umh.es (S.S.M.); jandreu@umh.es (F.J.A.-R.); miguel.mirau@umh.es (M.Á.M.-U.); jose.saezt@umh.es (J.A.S.-T.); e.martinezs@umh.es (E.M.-S.); marian.bustamante@umh.es (M.Á.B.); perez.murcia@umh.es (M.D.P.-M.); raul.moral@umh.es (R.M.)

\* Correspondence: l.orden@umh.es

## Abstract

This study evaluates the agronomic and environmental performance of pelletized compost derived from olive mill waste as a sustainable alternative to mineral fertilizers for cultivating wheat (*Triticum turgidum* L.) under conventional tillage methods. A field experiment was conducted in semi-arid Spain, employing three fertilization strategies: inorganic (MAP + Urea), sewage sludge (SS), and organic compost pellets (OCP), each providing 150 kg N ha<sup>-1</sup>. The parameters analyzed included wheat yield, grain quality, soil properties, and greenhouse gas (GHG) emissions. Inorganic fertilization yielded the highest productivity and nutrient uptake. However, the OCP treatment reduced grain yield by only 15%, while improving soil microbial activity and enzymatic responses. The SS and OCP treatments showed increased CO<sub>2</sub> and N<sub>2</sub>O emissions compared to the control and inorganic plots. However, the OCP treatment also acted as a CH<sub>4</sub> sink. Nutrient use efficiency was greatest under mineral fertilization, though the OCP treatment outperformed the SS treatment. These results highlight the potential of OCP as a circular bio-based fertilizer that can enhance soil function and partially replace mineral inputs. Optimizing application timing is critical to aligning nutrient release with crop demand. Further long-term trials are necessary to evaluate their impact on the soil and improve environmental outcomes.



Academic Editor: Nguyen V. Hue

Received: 30 May 2025

Revised: 15 July 2025

Accepted: 16 July 2025

Published: 17 July 2025

**Citation:** García-Ránde, A.; Sánchez Méndez, S.; Orden, L.; Andreu-Rodríguez, F.J.; Mira-Urios, M.Á.; Sáez-Tovar, J.A.; Martínez-Sabater, E.; Bustamante, M.Á.; Pérez-Murcia, M.D.; Moral, R. Evaluation of Olive Mill Waste Compost as a Sustainable Alternative to Conventional Fertilizers in Wheat Cultivation. *Agriculture* **2025**, *15*, 1543. <https://doi.org/10.3390/agriculture15141543>

**Copyright:** © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

**Keywords:** olive mill waste compost; pelletized fertilizer; wheat yield; nitrogen use efficiency; greenhouse gas emissions

## 1. Introduction

In 2022, the production of fertilizers in Europe reached 17.3 million tonnes, with nitrogen (N) fertilizers, both simple and compound, accounting for around 72% of total chemical fertilizer consumption. During the 2021/22 growing season, 26% of N fertilizer use in Europe was allocated to cultivating wheat (*Triticum aestivum* L.), highlighting the crop's high N demand [1]. Nitrogen is a key macronutrient that is essential for optimizing wheat yield and grain quality. Efficient N management enhances productivity and minimizes environmental and economic costs [2]. Wheat performance is the result of a complex interplay between genetic traits and environmental conditions during development [3]. Among agronomic practices, fertilization significantly influences yield and grain composition. Advances in plant metabolomics now enable the detailed characterization of physiological

responses to nutrient regimes, providing valuable insights into stress assessment and crop phenotyping [4]. Empirical studies have demonstrated that high N inputs increase grain protein content and improve starch composition and baking quality [5]. For instance, one study [6] reported elevated levels of amino acids such as aspartate, asparagine, and tryptophan under high N conditions, while the levels of compounds such as fructose and succinate were reduced.

Despite these agronomic benefits, excessive N input often results in poor nitrogen use efficiency (NUE) and substantial environmental costs. N fertilizer losses contribute to ammonia (NH<sub>3</sub>) volatilization and the greenhouse gas (GHG) emissions, particularly nitrous oxide (N<sub>2</sub>O) and methane (CH<sub>4</sub>), which have global warming potentials (GWP) 265 and 28 times greater than CO<sub>2</sub>, respectively [7]. Furthermore, the production of synthetic fertilizers relies on non-renewable resources, raising long-term sustainability concerns for intensive agricultural systems [8]. In response, the European Union has adopted the Green Deal [9], which targets a 55% reduction in net GHG emissions by 2030 (compared to 1990 levels), while also promoting more sustainable fertilization practices. Key initiatives, such as the Farm to Fork Strategy and the Circular Economy Action Plan, advocate the recovering of nutrients from organic waste streams in order to close nutrient cycles and reduce reliance on synthetic inputs [10].

Olive oil production is one sector with significant potential for implementing circular nutrient strategies. Global output has tripled over the last six decades, reaching three million tonnes in the 2020/21 season [11] (IOC, 2021). The two-phase extraction process generates substantial amounts of olive mill waste (OMW), also known as olive pomace or *alperujo*. This phenolic-rich by-product has phytotoxic and antimicrobial properties that pose environmental risks [12–14]. Composting and vermicomposting have proven effective in detoxifying OMW and converting it into valuable organic amendments [15,16]. These composted materials contribute to restoring soil fertility, enhancing microbial reactivation and sequestering carbon through the stabilizing organic matter [17]. Notably, the application of composted olive pomace has demonstrated the potential to replace mineral fertilizers while improving soil quality and reducing environmental impacts [18]. However, its use in agriculture is hindered by logistical limitations, such as low bulk density, handling difficulties, and the release of dust and volatile organic compounds [19]. Pelletization, a mechanical densification process, offers a viable solution by improving storage, transport, and application efficiency [20,21]. Unlike prior studies using raw olive mill waste, our pelletization process enhances nutrient homogeneity, reduces phytotoxicity, and improves field applicability.

In addition to compost, the use of other organic materials, such as sewage sludge (SS), as an alternative fertilizer is becoming increasingly popular, particularly in arid and semi-arid regions where synthetic inputs are expensive [22]. SS is rich in organic matter, as well as the macronutrients (NPK) and micronutrients. When applied at the correct dosage, it has been shown to increase wheat yields and soil fertility [23], while also contributing to soil carbon stocks and mitigating climate change [24]. Given the trade-offs between mineral and organic fertilization, evaluating their combined use in conventional tillage systems is becoming increasingly important [25]. A deeper understanding of how different fertilizer types and application rates affect crop productivity, soil properties and environmental outcomes is essential for guiding sustainable management.

This study aims to evaluate the agronomic and environmental effects of mineral and organic fertilization strategies on wheat (*Triticum aestivum* L.) cultivated using conventional tillage methods. Specifically, the research will focus on the application of pelletized compost derived from olive pomace, assessing its impact on wheat yield, grain quality, metabolomic profiles, soil physicochemical properties and greenhouse gas emissions. Unlike prior

studies using raw olive mill waste, our pelletization process enhances nutrient homogeneity, reduces phytotoxicity, and improves field applicability. This study will contribute to the development of circular nutrient management practices by promoting the use of agro-industrial residues in sustainable agriculture.

## 2. Materials and Methods

### 2.1. Site and Experimental Design

The field experiment was conducted at the Aula Dei Experimental Station (EEAD-CSIC) in Zaragoza, Spain (41°43'15.2" N, 0°48'51.8" W) during the 2022/23 growing season. The site is located in the semi-arid Ebro River valley, which is characterized by high potential evapotranspiration and variable annual precipitation [26]. During the study period, the mean air temperature was 14.5 °C and total precipitation reached 390 mm, whereas annual potential evapotranspiration typically exceeds 1200 mm.

The soil at the site is classified as a fine loam, mixed, thermic Xerollic Calciorthid (USDA taxonomy) with a loam texture [27]. In the topsoil layer (0–20 cm), the soil properties were 1.67% organic matter, had a pH of 8.43 (at a soil–water ratio of 1:2.5), and an electrical conductivity of 236  $\mu\text{S m}^{-1}$ . Meteorological data for the 210-day experimental period were obtained from a nearby automated weather station belonging to the SIAR network (Spanish Ministry of Agriculture) (Supplementary Materials Figure S1).

The experiment was laid out in a randomized complete block design with three replicates. Each 8 m × 3 m plot was sown with durum wheat (*Triticum turgidum* L. subsp. *durum*, cv. SCULPTUR) at a rate of 250 kg ha<sup>−1</sup>, with a row spacing of 17.5 cm. Three fertilization treatments were applied, each providing 150 kg N ha<sup>−1</sup> via manual surface application (Table 1). The three treatments, (a) pelletized compost (OCP), (b) sewage sludge (SS) and (c) inorganic MAP (11-52-00), were applied at sowing on 18 November (Zadoks 0.0). Treatment (c) was supplemented with urea (IN, 46-0-0) at the tillering stage on 16 February (Zadoks 2.3).

**Table 1.** Fertilizing treatments applied in each wheat field experiment.

| Treatment                  |     | Nutrient Rate<br>(kg ha <sup>−1</sup> ) |     | Fertilizer Rate<br>(kg ha <sup>−1</sup> ) |     |
|----------------------------|-----|-----------------------------------------|-----|-------------------------------------------|-----|
|                            |     | P                                       | N   | P                                         | N   |
| Control <sup>1</sup>       | C   | 0                                       | 0   | 0                                         | 0   |
| Inorganic (MAP + Urea)     | IN  | 50                                      | 150 | 188                                       | 326 |
| Sewage Sludge              | SS  | 60                                      | 150 | 7500                                      |     |
| Organic Pelletized Compost | OCP | 40                                      | 150 | 6000                                      |     |

<sup>1</sup> Control (without fertilization). The number indicates the nutrient rate in kg ha<sup>−1</sup>, “N” and “P” indicate nitrogen and phosphorus.

The OCP comprised composted olive mill waste, poultry manure, and olive leaves in a 60:20:20 ratio [17], processed into 5 mm pellets using a 4 HP pelletizer. Conventional tillage was employed, and flood irrigation was applied on 24 March, 20 April, and 12 May. Weed and pest control were conducted uniformly across treatments.

The physicochemical properties of the organic fertilizers were determined using the methodology outlined in reference [28] (Table 2). The total concentrations of macro-, meso-, and micronutrients were measured using inductively coupled plasma optical emission spectrometry (ICP-OES) [29]. The total organic carbon (TOC) and total nitrogen (TN) content was analyzed using an automatic elemental analyzer (EuroVector, Milan, Italy).

**Table 2.** Composition of the organic fertilizers used on the trial.

| Nutrient <sup>a</sup>                                  | SS          | OCP         |
|--------------------------------------------------------|-------------|-------------|
| pH                                                     | 6.56 ± 0.43 | 9.05 ± 1.61 |
| EC (dS m <sup>-1</sup> )                               | 5.68 ± 0.87 | 3.51 ± 2.34 |
| OM (%)                                                 | 52.4 ± 0.01 | 79.6 ± 1.17 |
| TOC (g kg <sup>-1</sup> )                              | 292 ± 1.02  | 386 ± 0.79  |
| TN (g kg <sup>-1</sup> )                               | 46.2 ± 0.96 | 25.4 ± 0.49 |
| NH <sub>4</sub> <sup>+</sup> -N (mg kg <sup>-1</sup> ) | 26.3 ± 2.22 | 54.7 ± 0.09 |
| NO <sub>3</sub> <sup>-</sup> -N (mg kg <sup>-1</sup> ) | 8.20 ± 0.05 | 25.5 ± 10.1 |
| P (g kg <sup>-1</sup> )                                | 8.03 ± 10.9 | 6.56 ± 0.28 |
| K (g kg <sup>-1</sup> )                                | 2.51 ± 1.02 | 26.9 ± 1.49 |
| Na (g kg <sup>-1</sup> )                               | 1.20 ± 5.57 | 1.12 ± 0.65 |
| S (g kg <sup>-1</sup> )                                | 14.0 ± 2.3  | 3.68 ± 0.01 |
| Ca (g kg <sup>-1</sup> )                               | 44.6 ± 7.8  | 38.2 ± 0.6  |
| Mg (g kg <sup>-1</sup> )                               | 5.84 ± 4.23 | 4.40 ± 1.20 |
| Fe (g kg <sup>-1</sup> )                               | 12062 ± 12  | 2212 ± 0    |
| Mn (mg kg <sup>-1</sup> )                              | 240 ± 2     | 294 ± 2     |
| Cu (mg kg <sup>-1</sup> )                              | 166 ± 3     | 96.9 ± 0.5  |
| Zn (mg kg <sup>-1</sup> )                              | 743 ± 2     | 235 ± 0     |

<sup>a</sup> Values on a dry matter basis. EC: Electric Conductivity, TOC: Organic Carbon. Values indicate mean ± SE (*n* = 3). For acronyms see Table 1.

## 2.2. Soil Analysis

Topsoil samples (from a depth of 0–20 cm) were collected using a stainless steel probe at two time points: sowing (Ti: 0 days) and harvest (Tf: 210 days). This was done in order to evaluate the effects of fertilization on the soil's physicochemical, chemical and microbiological parameters. For each treatment, three composite samples were obtained by homogenizing five subsamples per plot. The samples were air-dried and sieved to <2 mm, and all analyses were performed in triplicate.

Soil pH and electrical conductivity (EC) were measured in 1:2.5 and 1:5 soil–water extracts, respectively [30]. Oxidizable organic carbon (COT) was determined [31] and total nitrogen (TN) was measured using the Kjeldahl method [32]. The mineral N forms nitrate (NO<sub>3</sub><sup>-</sup>-N) and ammonium (NH<sub>4</sub><sup>+</sup>-N), which were extracted with 0.2 M KCl (1:5 *w/v*) and quantified [33]. Available phosphorus was analyzed using the Olsen method [34]. Heavy metals were determined by ICP-OES at harvest.

Water-filled pore space (WFPS) was monitored during the growing season by measuring the gravimetric water content of samples that were oven-dried at 105 °C for 48 h [35]. Soil enzymatic activity was assessed via spectrophotometric assays: urease and acid phosphatase activities were measured [36]. Microbial biomass carbon was estimated by substrate-induced respiration (SIR) with the addition of glucose (3 mg g<sup>-1</sup> soil) [37]. Basal respiration was evaluated by incubating soil at 60% water-holding capacity. In both assays, CO<sub>2</sub> evolution was measured using an automated impedance meter (BacTrac 4200, Sylab, Austria) which is based on the changes in impedance caused by the absorption of CO<sub>2</sub> in a 2% KOH solution [38].

## 2.3. Plant Analysis

Samples of crop straw were taken at physiological maturity (Z9.0) by harvesting plants by hand from a 1 m<sup>2</sup> area within each plot. The spikes were then removed by hand, after which the remaining straw was oven-dried at 60 °C for 48 h prior to weighing. The dry matter yield was expressed in kg ha<sup>-1</sup>. The number of spikes (spikes ha<sup>-1</sup>) and the number of grains per 20 spikes were recorded to estimate the grain count per hectare (grains ha<sup>-1</sup>). The 1000-grain weight was determined from representative subsamples. Grain yield was

obtained by harvesting wheat spikes mechanically from the central 8 m<sup>2</sup> of each plot, weighing them in the field and correcting for a standard moisture content of 10%.

Tissue mineral composition was determined following nitric-perchloric acid digestion [29]. Total C and N contents were measured using an automatic elemental analyzer (EuroVector, Milan, Italy). Hectoliter weight (HW, kg hL<sup>-1</sup>) was assessed as a measure of grain bulk density and grain protein content (%) was estimated using a nitrogen-to-protein conversion factor of 5.75 [39,40]. Macro- and micronutrients, including phosphorus (P) and potassium (K), were quantified using ICP-OES. Nutrient uptake (kg ha<sup>-1</sup>) was calculated as the product of concentration and yield and was used to evaluate the impact of the treatment.

Nutrient use efficiencies (NUE, PUE and KUE) were calculated as the ratio of nutrient uptake to the amount of nutrient supplied via fertilization [41].

Non-targeted metabolomic analysis was performed on freeze-dried wheat grains sampled at the late milk stage (Zadoks 7.5). Metabolite extraction followed the protocol of [42]. Proton nuclear magnetic resonance (<sup>1</sup>H-NMR) spectra were acquired using a Bruker Ascend 500 MHz AVANCE III HD spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany). Metabolite identification and quantification were carried out using Chenomx NMR Suite v8.3 [43].

#### 2.4. Greenhouse Gas Measurements

Greenhouse gas (GHG) emissions, including nitrous oxide (N<sub>2</sub>O), methane (CH<sub>4</sub>) and carbon dioxide (CO<sub>2</sub>), were monitored at days 0, 20, 30, 50, 65, 80, 90, 115, and 140 of the wheat growing season using a static closed-chamber method. The 15 cm, 20 cm high chambers had a headspace volume of 3.53 dm<sup>3</sup> and were made of non-corrosive steel. They had inlet and outlet ports connected to a multi-gas photoacoustic infrared spectrometer (Gasera One, Gasera Ltd., Turku, Finland).

Permanent steel bases were installed 15 cm into the soil at sowing time and remained in place throughout the experiment. Vegetation was manually removed inside and around each chamber before each sampling event. During measurements, the chambers were sealed with rubber gaskets and secured with clamps to ensure airtight conditions.

Gas concentrations were recorded continuously at one-minute intervals over a six-minute period. The first and last readings were excluded to ensure linearity ( $R^2 > 0.9$ ) for flux calculation. The instrument was calibrated using certified multi-point gas standards (Linde Gas España S.A.U. (Barcelona, Spain)). The calibration curves showed an  $R^2$  value of 0.99 across the ranges of 0.3–2 ppm for N<sub>2</sub>O, 1–10 ppm for CH<sub>4</sub> and 200–1500 ppm for CO<sub>2</sub>.

Fluxes (μg m<sup>-2</sup> h<sup>-1</sup>) were calculated based on the rate of change in concentration over time, and were corrected for chamber volume, temperature, and atmospheric pressure, in accordance with the methods described in [44]. Cumulative seasonal emissions were estimated using trapezoidal integration between sampling intervals, as described by Menéndez et al. (2006) [45]. These were then converted to CO<sub>2</sub>-equivalents (CO<sub>2</sub>-eq) using global warming potential (GWP) factors of 273 for CH<sub>4</sub> and 27.2 for N<sub>2</sub>O [46].

The N<sub>2</sub>O emission factor (EF<sub>N<sub>2</sub>O</sub> %) was calculated by dividing the total N<sub>2</sub>O-N emissions by the amount of N applied to each treatment. Yield-scaled emissions (g N<sub>2</sub>O-N Mg<sup>-1</sup> grain) were calculated to assess environmental efficiency [47,48].

#### 2.5. Statistical Analysis

The Infostat statistical software package (version 2020), which is connected to the R language (version 4.3.1), was used to conduct the statistical analyses [49]. The Shapiro–Wilk test was used to check for normality, and the Levene test ( $p > 0.05$ ) was used to evaluate and confirm the homogeneity of variance. An analysis of variance (ANOVA) test was

performed on all of the experimental variables at  $p < 0.05$  using a generalized linear mixed model, where each plot was a random component and fertilizer treatments and sampling dates were fixed factors. The LSD Fisher test ( $\alpha = 0.05$ ) was then used to compare the means.

Correlations were analyzed using linear regressions between soil and crop yield variables. To evaluate the main soil chemical variables associated with changes in wheat productivity and quality, the Mantel test was performed using the Vegan community ecology package [50]. Pearson's correlation was also calculated for variables associated with soil characteristics.

### 3. Results

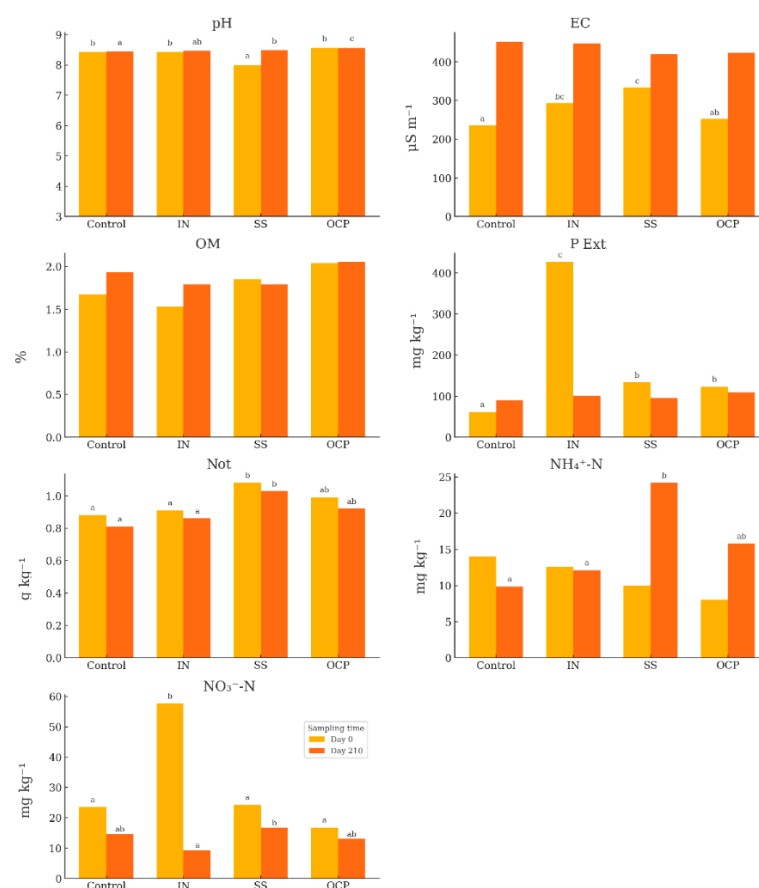
#### 3.1. Soil Parameters

When the treatments were applied at sowing (Ti: 0 day), the pelletized compost was found to increase the soil pH slightly compared to the unfertilized control. However, this difference was not statistically significant (Figure 1). In contrast, the SS treatment was found to significantly decrease soil pH relative to both the unfertilized control and the inorganic fertilizer IN (MAP+U), resulting in the lowest pH value observed. EC ( $\text{dS m}^{-1}$ ) was higher in plots treated with the inorganic fertilizer (MAP+U) and the SS at Ti compared with the control and the pelletized compost (OCP). No significant differences in OM (%) were observed between the treatments and the control. TN was significantly higher in the SS treatment plots at the first sampling (Ti). At sowing, plots fertilized with IN (MAP+U) showed the highest  $\text{NO}_3^-$  content in the soil, followed by the SS, the control, and finally the pelletized compost (OCP). No significant differences were found between treatments in relation to  $\text{NH}_4^+$ . However, the content of available phosphorus (P) was significantly higher in soils treated with the inorganic fertilizer (MAP+U) than in soils receiving the organic amendments (SS and OCP).

By the end of the experiment (Tf: 210 days), the pH value of the soil treated with compost pellets (OCP) and inorganic fertilizer (MAP+U) had not changed. The SS treatment significantly increased the final pH value. Overall, EC increased in all plots, including the control treatment. There were no statistical differences in OM with fertilization. By the end of the trial, there were no significant differences in available P content. The P extractable content of all treatments decreased by the end of the season, except for the control treatment, which showed a slight increase. In all plots, TN decreased slightly by the end of the experiment (Tf); plots fertilized with SS showed the highest TN value.

The highest soil  $\text{NH}_4^+$  content after harvest was found in the SS plots, followed by the OCP plots, due to the organic N content of this fertilizer. No significant differences were found between the control treatment and the inorganic (IN) fertilizer treatment. The highest soil  $\text{NO}_3^-$  content was found in the SS plots, followed by the control plots and the OCP treatment plots.  $\text{NO}_3^-$  decreased in all plots, probably due to denitrification processes in the soil and  $\text{NO}_3^-$  consumption by the crop.

All treatments showed higher respirometry values ( $p < 0.0001$ ) than the unfertilized control. However, soil biomass C values showed no significant differences between the plots (Table 3). The highest urease value was found in the plots fertilized with sludge (SS) ( $0.88 \mu\text{mol NH}_4^+ \text{ g soil h}^{-1}$ ) followed by plots fertilized with OCP ( $0.83 \mu\text{mol NH}_4^+ \text{ g soil h}^{-1}$ ), with significantly higher values than the control ( $0.68 \mu\text{mol NH}_4^+ \text{ g soil h}^{-1}$ ). Phosphatase activity was significantly higher in plots fertilized with SS, followed by IN.



**Figure 1.** Main soil parameters at sowing (0 days) and at the end of the growing season (210 days). EC: electrical conductivity; OM: organic matter; Pext: extractable phosphorus; Not: total Kjeldahl N;  $\text{NH}_4^+\text{-N}$ : ammonium;  $\text{NO}_3^-\text{-N}$ : nitrate. Different letters within a column indicate significant differences between treatments ( $p < 0.05$ ). Values indicate mean ( $n = 3$ ). For acronyms see Table 1.

**Table 3.** Main enzymatic activities per gram of dry soil ( $\mu\text{mol g}^{-1} \text{h}^{-1}$ ) in wheat growing seasons and respirometry ( $\text{mg CO}_2\text{-C kg}^{-1} \text{h}^{-1}$ ).

| Treatment | Respirometry<br>( $\text{mg CO}_2\text{-C kg soil}^{-1}$ ) | Biomass C<br>( $\text{mg C kg soil}^{-1}$ ) | Urease<br>( $\mu\text{mol NH}_4^+ \text{g soil}^{-1}$ ) | Phosphatase<br>( $\mu\text{moles p-nitrophenol g suelo}^{-1}$ ) |
|-----------|------------------------------------------------------------|---------------------------------------------|---------------------------------------------------------|-----------------------------------------------------------------|
| Control   | 5.88 a                                                     | 962                                         | 0.68 a                                                  | 0.36 a                                                          |
| IN        | 9.21 b                                                     | 997                                         | 0.79 ab                                                 | 2.02 a                                                          |
| SS        | 11.2 b                                                     | 923                                         | 0.88 c                                                  | 6.51 b                                                          |
| OCP       | 9.94 b                                                     | 709                                         | 0.83 b                                                  | 0.78 a                                                          |
| F-ANOVA   | 7.42 *                                                     | 1.21 ns                                     | 6.10 *                                                  | 17.6 ***                                                        |

The data are shown as the means ( $n = 3$ ). \*, \*\*\* significant difference between treatments at  $p < 0.01$  and  $p < 0.0001$ , respectively. Means followed by the same letter indicate no significant difference between treatments by LSD Fisher test ( $p = 0.05$ ). Values indicate mean ( $n = 3$ ). ns = not significant. For acronyms see Table 1.

### 3.2. Yield and Grain Quality

The components of wheat yield are shown in Table 4. The inorganic treatment resulted in the highest aboveground biomass, followed by the compost treatment. Statistically significant differences were observed among the applied treatments. A similar trend was observed for grain and straw yield, with the inorganic treatment produced the highest yields (8169 and 6290  $\text{kg ha}^{-1}$ , respectively). No significant differences were detected between the sewage sludge and control treatments. Additionally, the inorganic treatment showed the highest TGW, although no statistically significant differences were observed compared to the control. Regarding grain quality, plots fertilized with the inorganic

treatment recorded the highest HW, followed by those fertilized with OCP; there were significant differences compared to the control. A similar trend was observed for grain protein content. No significant differences were found between the OCP and SS treatments, in both cases, the results were greater than those of the control treatment ( $p < 0.0001$ ).

**Table 4.** Analysis of variance (ANOVA) of wheat yield components for fertilizer treatments in 2022/23 growing season.

| Treatment | Grain Yield<br>(kg ha <sup>-1</sup> ) | Straw<br>(kg ha <sup>-1</sup> ) | TGW<br>(g) | HW<br>(kg hL <sup>-1</sup> ) | Protein<br>(%) |
|-----------|---------------------------------------|---------------------------------|------------|------------------------------|----------------|
| Control   | 6429 a                                | 4660 a                          | 49.4       | 75.4 a                       | 7.6 a          |
| IN        | 8618 c                                | 6283 b                          | 50.2       | 79.4 c                       | 10.6 c         |
| SS        | 6704 a                                | 5030 a                          | 48.8       | 75.6 ab                      | 7.7 b          |
| OCP       | 7268 b                                | 5310 ab                         | 49.9       | 76.5 b                       | 8.0 b          |
| F-ANOVA   | 78.7 ***                              | 4.3 *                           | 0.3 ns     | 39.7 ***                     | 157 ***        |

TGW: 1000-grain weight, HW: Hectoliter weight. \*, \*\*\* significant difference between treatments at  $p < 0.01$  and  $p < 0.0001$ , respectively. ns = not significant. Different letters within a column indicate significant differences between treatments ( $p < 0.05$ ). Values indicate mean ( $n = 3$ ).

There were no significant differences in the grain content of macronutrients (phosphorus, potassium, calcium and magnesium) between the treatments (Supplementary Materials Table S1). The synthetic fertilization strategy involving chemical fertilizers resulted in higher levels of P and K than the unfertilized control. The inorganic treatment (IN) positively affected the grain Fe content. Additionally, the fertilized treatments exhibited significant variations in Zn grain content in the fertilized treatments compared to the unfertilized control, with the highest value observed in the inorganic strategy.

Significant differences in N uptake and use efficiency were observed between inorganic fertilization (IN) and organic strategies (OCP and SS) (Table 5). Nitrogen uptake was highest in the IN treatment, followed by the OCP treatment. No significant differences were found between the control treatment and the SS treatment. Similarly, nitrogen use efficiency (NUE %) was highest under the inorganic treatment, followed by compost-amended plots (OCP). Phosphorus and potassium uptake were also higher in the inorganic plots, with no significant differences detected between the organic treatments (OCP and SS) and the control. However, phosphorus use efficiency (PUE %) differed significantly between treatments, with the highest values observed in the inorganic plots (IN), followed by the compost-amended plots (OCP). In contrast, there were no statistically significant differences in potassium use efficiency (KUE %) between treatments.

**Table 5.** Nitrogen, phosphorus and potassium use efficiency and agronomic efficiency across fertilizer treatments in 2022/23 growing season.

| Treatment | N Uptake<br>(kg N ha <sup>-1</sup> ) | NUE<br>(%) | P Uptake<br>(kg P ha <sup>-1</sup> ) | PUE<br>(%) | K Uptake<br>(kg K ha <sup>-1</sup> ) | KUE<br>(%) |
|-----------|--------------------------------------|------------|--------------------------------------|------------|--------------------------------------|------------|
| Control   | 84.5 a                               | -          | 21.2 a                               | -          | 29.4 a                               | -          |
| IN        | 158 c                                | 49.2 c     | 30.1 b                               | 18.8 c     | 42.6 b                               | -          |
| SS        | 89.5 a                               | 1.45 a     | 22.4 a                               | 1.4 a      | 31.2 a                               | 5.4        |
| OCP       | 101 b                                | 10.8 b     | 24.1 a                               | 10.3 b     | 32.9 a                               | 2.8        |
| F-ANOVA   | 328 ***                              | 706 ***    | 16.3 *                               | 47 ***     | 22.6 ***                             | 7 ns       |

NUE/PUE/KUE: nitrogen/phosphorus/potassium use efficiency, \*, \*\*\*: significant difference between treatments at  $p < 0.01$  and  $p < 0.0001$ , respectively. ns: not significant. Different letters within a column indicate significant differences between treatments ( $p < 0.05$ ).

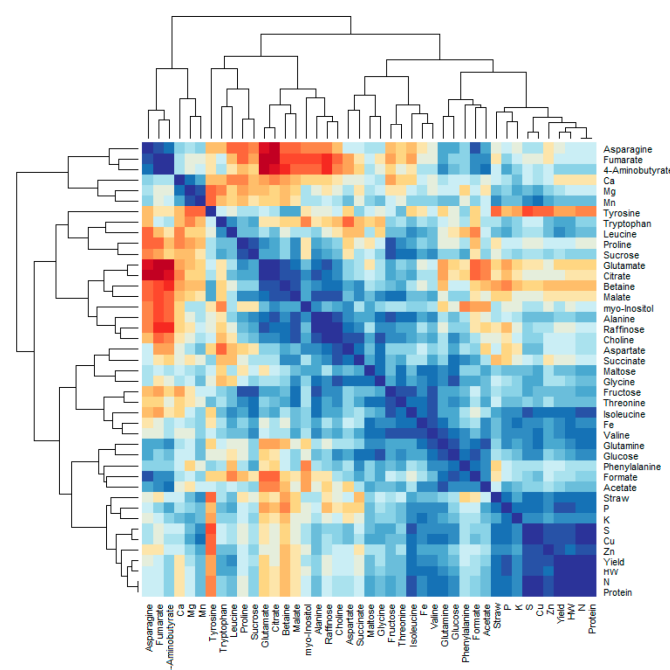
### 3.3. Metabolomics

No statistically significant differences were observed in the concentrations of phenylalanine, proline, threonine, tryptophan and tyrosine across treatments with respect to the amino acid profiles (Supplementary Materials Table S2). However, the inorganic treatment had the highest levels of glutamine, glycine, isoleucine and valine. The highest aspartate levels were observed in the IN and SS treatments. Leucine concentrations peaked in the inorganic and OPC treatments. Notably, the SS treatment exhibited the highest levels of alanine and glutamate, while 4-aminobutyrate and asparagine were most abundant in the control plots.

Significant differences were found in the concentrations of citrate, formate and fumarate (Supplementary Materials Table S2). The SS-treated plants had the highest citrate concentrations, followed by those treated with the inorganic material. Formate and fumarate levels were highest in the control plots, followed by the inorganic plots. No significant differences were observed among the treatments for levels of acetate, malate and succinate.

Regarding sugars (Supplementary Materials Table S2), only significant differences in glucose and raffinose content were observed. The highest glucose concentration was recorded in the inorganic plots, followed by the control plots. Raffinose was most abundant in SS-treated plants, with inorganic treatment ranking second. No statistically significant differences were found in the content of fructose, maltose, myo-inositol or sucrose content. Choline content also varied significantly across the treatments and was highest with the SS treatment (Supplementary Materials Table S2), with the SS plots showing the highest concentration, followed by the IN treatment. Interestingly, the OCP treatment showed lower choline levels than the control treatment.

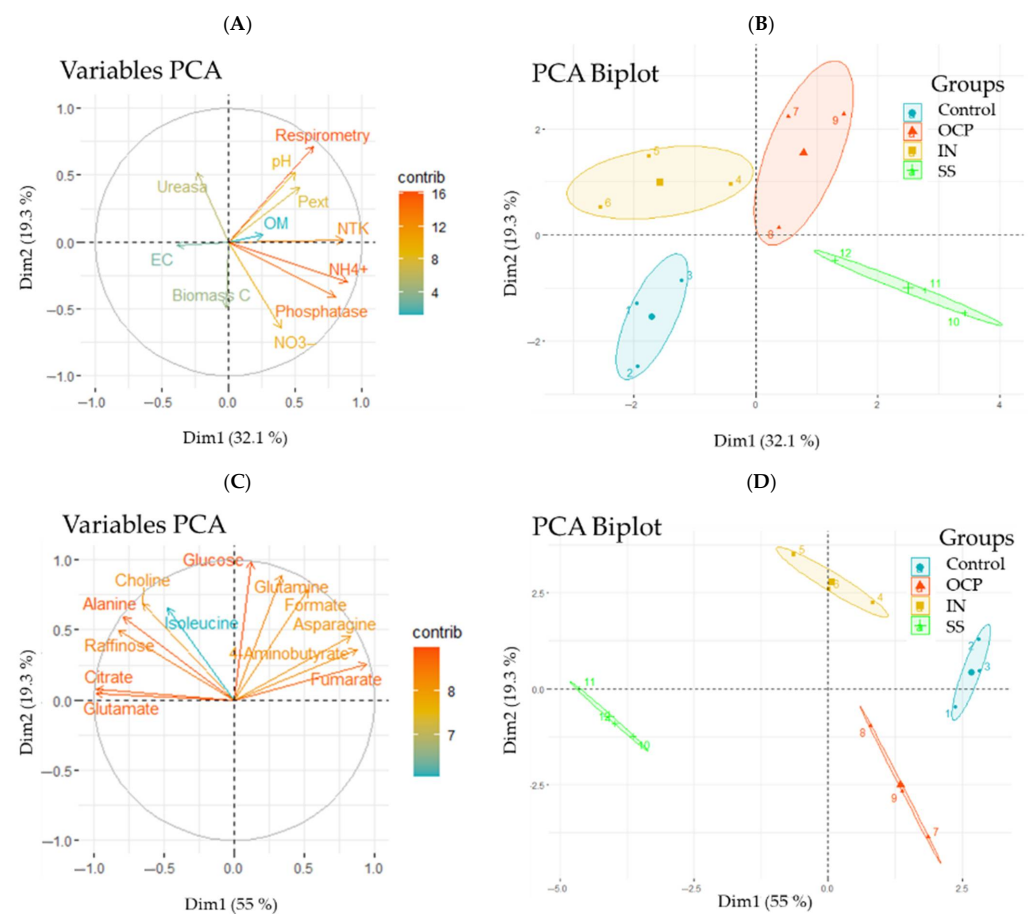
Figure 2 shows a heatmap illustrating the correlation structure between plant metabolic variables. Hierarchical clustering was applied to the variables and the observations independently. The dendrograms along the axes highlight distinct groupings, suggesting patterns of co-regulation and treatment-specific metabolic profiles.



**Figure 2.** Heatmap of the correlation matrix of plant variables. Colors indicate the strength and direction of the correlations, with red tones representing negative correlations, blue tones representing positive correlations, and white indicating no correlation.

Strong positive correlations were observed between asparagine, fumarate and 4-aminobutyrate, respectively, and the elemental concentrations of Ca, Mg and Mn. In contrast, a distinct cluster of metabolites, comprising glutamate, citrate, betaine, malate, myo-inositol, alanine, raffinose, and choline, exhibited strong negative correlations with asparagine, fumarate and 4-aminobutyrate. Additionally, moderate negative correlations were observed between the aforementioned metabolite group and several agronomic and nutrient-related variables. These variables included glutamine, glucose, phenylalanine, formate, acetate, straw biomass, P, K, S, Cu, Zn, grain yield, HW, N, and protein content. Conversely, a cluster in the lower right quadrant of the heatmap showed strong positive correlations among S, Cu, Zn, grain yield, HW, N, and protein content, suggesting close coordination between nutrient status and productivity traits.

A principal component analysis (PCA) of soil variables (Figure 3A,B) revealed a clear separation between the different treatments. Control plots were clustered in the third quadrant and were characterized by lower levels of respirometric activity, pH, and Pext, but relatively higher electrical conductivity (EC) and biomass carbon (Biomass C). The OCP treatment was associated with higher respirometric activity, pH, and Pext, as well as lower EC and biomass C, while the IN treatment was characterized by elevated urease activity and lower concentrations of nitrate ( $\text{NO}_3^-$ ), ammonium ( $\text{NH}_4^+$ ) and phosphatase activity concentrations. In contrast, the SS displayed the inverse pattern, with higher levels of urease,  $\text{NO}_3^-$  and  $\text{NH}_4^+$ , and lower phosphatase and urease activity.



**Figure 3.** (A,C) Observational variables PCA of soil and plants. (B,D) Biplot PCA in four fertilizer treatments. The PCA biplot shows the scores of soils and plants variables of each individual fertilizer: Control (blue circle), IN (yellow circle), SS (green circle); OCP (red circle); the colored ellipses (B,D) (size determined by a probability level of 0.95) emphasize the individuals belonging to each treatment. For acronyms see Table 1.

PCA of plant-related variables (Figure 3C,D) further demonstrated the distinct separation of the IN treatment, reflecting its enhanced plant performance metrics compared to the other treatments. The OCP treatment also showed an improved metabolic and agronomic profile, as indicated by a positive shift along the principal components. In contrast, the SS and control treatments clustered closely together, suggesting limited differentiation in plant responses.

### 3.4. Soil GHG Fluxes and Cumulative Emissions

Nitrous oxide fluxes were higher than the control value during the trial on the fertilized plots (Supplementary Materials Figure S2A). CO<sub>2</sub> fluxes were significantly influenced by the day of sampling, suggesting time-varying emission dynamics, possibly related to the environmental conditions during the experiment (Supplementary Materials Figure S2B). There was a tendency for CO<sub>2</sub> fluxes to increase at the start of irrigation and with rising ambient temperature (Supplementary Materials Figure S1). Specifically, carbon fluxes in the SS treatment were highest on sowing day, decreasing before rising again at the end of the season.

Methane fluxes were initially low, except for emissions from plots with SS (Supplementary Materials Figure S2C), which emitted approximately eight times more than the other treatments. During the trial, methane fluxes varied, emissions from all treatments were low at the beginning, and some even acted as methane sinks. Emissions increased during the last three sampling days, coinciding with an increase in temperature; however the OCP plots acted as a sink.

Significant differences were observed in cumulative N<sub>2</sub>O, CO<sub>2</sub> and CH<sub>4</sub> emissions, (Table 6). Statistical analysis revealed significant differences in cumulative N<sub>2</sub>O emissions, with the highest emissions occurring in plots receiving the SS treatment, followed by those receiving the pelletized compost (OCP). Cumulative CO<sub>2</sub> emissions were significantly higher in the plots fertilized with OCP and SS. The inorganic treatment (IN) and the control produced significantly lower kg CO<sub>2</sub>-C m<sup>-2</sup> than the SS treatment, which presented the highest cumulative value, followed by the OCP treatment. For CH<sub>4</sub> emissions, the OCP treatment acted as a CH<sub>4</sub> sink; however, no significant differences were observed between IN and OCP treatments and the control. Unfertilized plots with the inorganic treatments emitted the least greenhouse gases. Regarding kg CO<sub>2</sub> eq m<sup>-2</sup> unfertilized plots and the inorganic treatment were significantly lower than the other treatments, with the SS treatment having with the highest environmental impact (GWP).

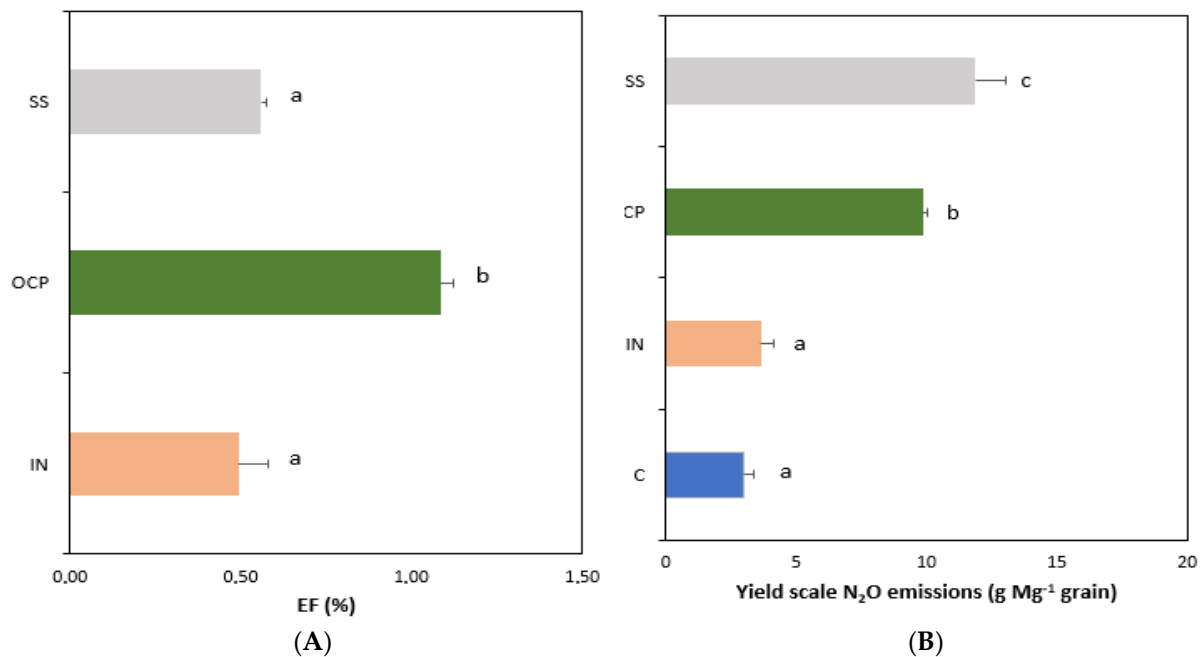
**Table 6.** Cumulative values of soil N<sub>2</sub>O, CO<sub>2</sub>, CH<sub>4</sub> and CO<sub>2</sub> equivalent emissions released from plots soil in the treatments during the entire experimental period (22 November 2022 to 18 April 2023).

| Treatment | g N <sub>2</sub> O-N m <sup>-2</sup> | kg CH <sub>4</sub> -C m <sup>-2</sup> | kg CO <sub>2</sub> -C m <sup>-2</sup> | CO <sub>2</sub> eq (kg m <sup>-2</sup> ) |
|-----------|--------------------------------------|---------------------------------------|---------------------------------------|------------------------------------------|
| Control   | 1.47 a                               | 0.31 a                                | 5869 a                                | 109 a                                    |
| IN        | 3.30 b                               | 0.50 a                                | 6354 a                                | 914 b                                    |
| SS        | 6.25 d                               | 2.83 b                                | 9059 b                                | 1784 d                                   |
| OCP       | 5.48 c                               | -0.60 a                               | 7901 b                                | 1481 c                                   |
| F-ANOVA   | 305 ***                              | 7.46 *                                | 12.1 **                               | 210 ***                                  |

CO<sub>2</sub> equivalent from added N<sub>2</sub>O and CH<sub>4</sub> emissions with the corresponding GWP [46]. \*, \*\*, \*\*\*: significant difference between treatments at  $p < 0.01$ ,  $p < 0.001$  and  $p < 0.0001$ , respectively. Different letters within a column indicate significant differences between treatments LSD Fisher test ( $p < 0.05$ ). For acronyms see Table 1.

The nitrous oxide emission factor (EF) and the yield-scaled N<sub>2</sub>O emissions showed statistical differences between the treatments (Figure 4). The emission factor was significantly higher ( $p < 0.0001$ ) for the OCP treatment, followed by the sewage sludge (SS) treatment. The emissions factor showed values between 0.49 and 1.08%. Yield-scale N<sub>2</sub>O emissions

were significantly higher in the sewage sludge (SS) plots, followed by the OCP, IN and control treatments.



**Figure 4.** (A) Nitrous oxide emission factor; (B) yield scale N<sub>2</sub>O emissions. Different letters within the bars indicate significant differences between treatments LSD Fisher test ( $p < 0.05$ ). For acronyms see Table 1.

## 4. Discussion

### 4.1. Effects of Fertilization on Soil Properties

The pH of the soil was significantly influenced by the fertilization treatments, especially in the SS fertilized soil. These plots had the lowest pH at the beginning of the experiment, due to the initial activity of nitrifying bacteria and the oxidation of  $\text{NH}_4^+$  to  $\text{NO}_3^-$ . This was reflected in a slight decrease in pH compared to the control soil [51]. A buffer effect was observed at the end of the experiment.

Electrical conductivity differed during the sowing phase (Ti), increasing in plots fertilized with sewage sludge (SS) and IN due to the rapid solubilization of inorganic fertilizers and the intrinsic salinity of SS ( $5.68 \text{ dS m}^{-1}$ ). At the end of the experiment (Tf), the EC increased by an average of  $+435 \mu\text{S m}^{-1}$  in the plots, probably due to the higher fertility or salinity. No precipitation or irrigation was recorded in the month before harvest, preventing salt leaching.

The concentration of OM in the soil was not significantly affected by the different fertilization treatments and remained within the same range at the end of the experiment. However, OM in soils with SS decreased compared to the initial values, as SS is a fresh amendment containing more labile organic matter with a low C:N ratio, leading to mineralization [52].

Total nitrogen (TN) in the soil at the time of sowing (Ti) was significantly higher in plots fertilized with SS, as this material has the highest organic nitrogen content; the other treatments had a similar TN content to the control soil. At harvest (Tf), TN decreased slightly, indicating the transformation of organic N into  $\text{NH}_4^+$  and  $\text{NO}_3^-$  following nitrification and mineralization processes [53].

In fact,  $\text{NO}_3^-$  decreased at the end of the experiment, indicating its consumption by the wheat. There were no differences in the ammonium content of the soil between the treatments at the sowing stage. However, at Tf,  $\text{NH}_4^+$  increased, indicating more

intense ammonification. This is reflected in the urease activity of the plots fertilized with SS and OCP, as the organic matter and nutrients provided by these amendments stimulate microbial activity [54].

Significant differences in extractable soil P were observed after fertilizer application (Ti), with the IN treatment showing the highest concentration of available P in the soil, probably due to the type of fertilizer, as compost and sewage sludge can be considered as slow-release fertilizers [55]. By the end of the experiment (Tf), the average decrease in extractable P in the fertilized treatments was 78.05%, indicating that P had been exported to the crop. However, available P increased in the unfertilized plots, probably due to the mineralization of organic P in the soil and the precipitation of  $\text{Ca}_3(\text{PO}_4)_2$ , which can occur in calcareous soils and prevent leaching and loss of P [56,57]. These forms of phosphorus in soil can be detected using the Olsen method, but they must undergo solubilization to be readily available to roots in the form of  $\text{H}_2\text{PO}_4^-$  or  $\text{HPO}_4^{2-}$  [58,59].

In terms of C stock, we can see the control plots have accumulated more organic carbon than the OCP and SS plots. The low C sequestration by OCP and SS is attributed to higher C losses via  $\text{CO}_2$  emission. Although no organic matter was applied to the control plots, soil organic C also comes from exudates in the rhizosphere and microbiota [60].

The urease and acid phosphatase enzymes were more active in the SS than in the other treatments. The introduction of labile carbon by SS improves the carbon balance in agroecosystems by gradually increasing soil OM content, which improves soil microbial properties [61,62]. The higher cellulose content of OCP may slow microbial turnover compared to the labile C of SS, explaining the divergent enzyme activities.

#### 4.2. Effects of Fertilization on Yield

Yield and all other yield parameters (straw, grain protein and HW) increased in the following order  $\text{C} < \text{SS} < \text{OCP} < \text{IN}$ . This behavior has been observed in other studies where the yield of compost was higher than that of SS and other organic amendments [63–65]. As expected, inorganic (IN) fertilizer was the best performer, as found by other authors [66]. However, in other studies [67,68], a combination of sewage sludge and inorganic sources has achieved higher yields than inorganic fertilizers and SS alone at the same N rate. From a practical standpoint, break-even calculations indicate that organic crop production (OCP) could be profitable if priced below inorganic fertilizer, accounting for yield differences and potential soil health benefits. There are policy incentives (e.g., EU CAP subsidies) that could offset yield penalties for sustainable practices.

Regarding nutrient uptake, nitrogen uptake was higher in IN and OCP. Inorganic nitrogen fertilizer urea was applied at tillering. This indicator depends on the availability of N in fertilizers during the cycle [69]. Also, N losses from OCP and SS were higher due to  $\text{N}_2\text{O}$  emissions, and N uptake was higher in plots fertilized with inorganic N. This also contributed to the decrease in the N use efficiency (NUE) of organic treatments [70]. In our case, organic fertilizers were applied during the sowing phase. The inorganic treatment was applied during the first application of MAP during the sowing phase and during the tillering phase of urea. This treatment allowed for a better distribution of N nutrition with respect to the crop cycle. Adjusting N application at the different growth stages also improves NUE and crop yield [71]. Other studies have also reported a relationship between N, P and K availability in soils [72,73].

In general, the use of phosphorus by cereal crops worldwide is low, with an average efficiency of 16% between 1961 and 2013 [74]. PUE is often limited by the immobilization of P when it binds to other ions ( $\text{Al}^{3+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Ca}^{2+}$ ), which inhibits P uptake by plants [75]. The PUE of the inorganic treatment was better than that of the OCP and SS treatments, outperforming them by 8.5% and 17.4%, respectively. However, the P dose was

not normalized in our experimental design. With a dose of 39 kg P ha<sup>-1</sup> and 60 kg P ha<sup>-1</sup>, OCP obtained better PUE than SS, and the IN plots were adjusted to 50 kg P ha<sup>-1</sup>. Therefore, if the application rates had been the same, OCP could probably have improved the PUE, as the P rate applied was lower than that of SS and IN. The type of P source also influences P uptake by plants [76]. Phosphorus in cereals crops is mainly consumed at the early stages of wheat development [77]. Organic fertilizers need to mineralize organic P to the soil to be available to the crop. This influences the P uptake values of crops fertilized with organic fertilizers. In addition, P in the plant must be relocated to the grains, as only the nutrient uptake of the grains was considered when calculating these indicators [78].

There were no significant differences in KUE. The amount of K applied was 160 kg ha<sup>-1</sup> and 19 kg ha<sup>-1</sup> for the OCP and SS, respectively. In this regard, increasing the dose did not affect the K uptake or KUE. The best uptake was obtained in plots with IN, but this uptake is associated with soil K content, as no exogenous K source was applied in this field trial. The homogeneity of KUE likely stems from the uniform availability of soil K across the treatments.

#### 4.3. Effects of Fertilization on Metabolomic, Sugar and Amino Acid Profiles

The amino acid profile has been significantly impacted by fertilizing treatments. Among the non-essential amino acids, the levels of alanine, asparagine, aspartate, glutamate, glutamine and glycine, have changed. These compounds are all related to the plant. Glutamine is synthesized from glutamate through nitrate reduction [79,80], and its production depends on the nitrogen sourced provided to the plant. In our experiment, glutamate levels were higher in plots with SS and IN, while glutamine levels were higher in plots with IN. Amino acids are often higher in plants with easily available nitrogen [6]. In our case, this is reflected in the grain protein content in plots with IN and SS treatments. Some non-essential amino acids, such as asparagine and aminobutyrate, showed the lowest concentrations with organic strategies, as observed in other studies [81]. Conversely, essential amino acids, such as isoleucine and leucine, were found to be higher in the fertilized plots. According to [82,83], the application of nitrogen and microelements has a beneficial effect on the content of these two amino acids in the grains. Valine was higher in the IN fertilized plots, with no differences observed among the other treatments [84].

Regarding the content of organic acid, the treatments affected citrate, formate and fumarate. Citrate increased in the following order: C < OCP < IN < SS. Citrate is synthesized by the oxidation of malate in the mitochondria [85]. This activity is influenced by ATP and participates in the Krebs cycle. Succinate and fumarate are also generated in the Krebs cycle [86]. In trials with *Arabidopsis thaliana*, fumarate accumulation was higher in the control, followed by IN and OCP. Fumarate accumulation is related to soluble sugar content and affects soil–plant interactions [87].

Fertilizing treatments only affected the glucose and raffinose content of the grain. Glucose, sucrose and maltose are often affected by abiotic stresses such as heat and drought [88,89]. Furrow irrigation was performed three times during grain development, but evapotranspiration was high. Slight stress could therefore have occurred. As we can see, the order of increase of sugars (sucrose, maltose, fructose and glucose) is OCP < SS < C < IN. This may be due to the fact that organic amendments generally have a higher water retention capacity and are more resilient to drought [63]. Regarding the oligosaccharide raffinose, it is synthesized from the myo-inositol and sucrose by the enzyme raffinose synthase [90]. This enzyme is also responsible for enhancing stress tolerance, so its content depends on the availability of myo-inositol and sucrose in the grain [91]. Betaine and choline are two methyl donors. Wheat is one of the richest sources of betaine [92]. In

our case, fertilizers did not affect betaine content; however, choline content was higher in plots fertilized with SS, IN and C.

#### 4.4. Effects of Fertilization on Soil GHG Fluxes and Cumulative Emissions

Cumulative CO<sub>2</sub> emissions ranged from 5869 to 9059 kg CO<sub>2</sub>-C ha<sup>-1</sup>. The addition of organic matter to the soil affects CO<sub>2</sub> emissions [93]. Figure 3 shows that the application of SS provokes an immediate CO<sub>2</sub> emission [94], since this amendment contains easily decomposable C, unlike compost (OCP), which is a stabilized material. At the end of the cycle, an increase in emissions was observed, since soil moisture and high temperatures influence CO<sub>2</sub> emissions from the soil [95], and this increase coincided with rising temperatures and irrigation events. CO<sub>2</sub> emissions are also associated with soil microbiota and enzymatic activity in the soil, which promotes the degradation of organic matter (Table 4). The C stock of the plots fertilized with IN was negative, but this did not result in higher CO<sub>2</sub> emissions, probably due to the mineralization of the organic matter already present in these soils.

Methane fluxes from the soil were generally lower and occasionally acted as a sink. Temperature and soil moisture are positively correlated with CH<sub>4</sub> emissions [96]. At the beginning of the season, the application of SS, followed by CH<sub>4</sub> emissions and the addition of fresh, easily decomposable organic matter, as well as the soil conditions at that time (WFPS 50%), limit the supply of O<sub>2</sub> and CH<sub>4</sub> to methanotrophs, thereby inhibiting CH<sub>4</sub> uptake into the soil [97]. The availability of N is also related to CH<sub>4</sub> emissions from the soil, as ammonia-oxidizing bacteria and methane-oxidizing bacteria can compete for O<sub>2</sub> to oxidize NH<sub>4</sub><sup>+</sup> and CH<sub>4</sub>, respectively [98]. Fertilization with SS, as well as introducing mineralizable N and C, may have led to higher cumulative CH<sub>4</sub> emissions.

Nitrous oxide emissions are primarily influenced by soil conditions and denitrification processes. Fluctuations in N<sub>2</sub>O emissions are influenced by environmental factors and soil moisture because the denitrification process is positively correlated with WFPS, soil temperature, and nitrate content [99,100]. The increase in emissions at the beginning of the experiment coincided with the higher WFPS, while the increase at the end coincided with high soil and ambient temperatures, as well as irrigation events. Although the nitrate content of the soil is higher in the inorganic fertilized plots, N<sub>2</sub>O emissions during the experiment and cumulative emissions were lower in these plots, probably due to nitrate leaching or nitrate uptake by the crop [101] (Table 5). Moreover, the inorganic fertilizer was split into two applications: one at the sowing with MAP, and one at the tillering with urea at 150 kg N ha<sup>-1</sup>. Thus, emissions in these plots between sowing and tillering were lower than in the others. Urease activity was also higher in plots fertilized with OCP and SS, which promoted the conversion of organic N to NH<sub>4</sub><sup>+</sup> and the subsequent reduction to N<sub>2</sub>O [102]. Furthermore, SS is an easily decomposable material, enhancing N<sub>2</sub>O emissions [48].

The application of OCP resulted in a greater increase in net GHG emissions than the IN during the crop growing season, likely attributable to the enhanced nutrient availability for microorganisms in the organic fertilizer. Nevertheless, the reuse of olive mill waste and poultry manure has the potential to serve as a viable substitute for chemically synthesized fertilizers, such as urea. This approach has the potential to contribute to a reduction in the environmental impact of agro-industrial production, while simultaneously avoiding the theoretical CO<sub>2</sub>eq emissions associated with the energetically expensive manufacturing of urea [103].

The EF from this trial ranged from 0.78 to 1.08%. A meta-analysis [104] reported an average EF of 0.97 ± 1.17% for solid organic amendments, such as compost and manure, in the Mediterranean region, which is lower than the IPCC Tier 1 EF default value of 1%. Our EF values were within the range proposed by the IPCC Tier 1. Higher EF N<sub>2</sub>O was found in the OCP-fertilized plots, and no differences were observed among the SS- and IN-fertilized

plots. Other authors [105] have suggested that the average EF of N<sub>2</sub>O is not only related to the N rates applied, but also to the N forms applied and crop management, such as the irrigation system or no-till farming. In our case, furrow irrigation may have led to higher N<sub>2</sub>O emission factors. N<sub>2</sub>O emissions on a yield scale followed a similar pattern, with the highest value observed in the SS plots, followed by the OCP. This is associated with higher N uptake by crops fertilized with the IN, and the denitrification in the OCP and SS plots.

## 5. Conclusions

Applying the proposed organic amendments can improve soil properties and enhance carbon storage, thereby contributing to more sustainable agricultural systems. Pelletized compost (OCP) in particular showed strong potential, with only a 15% reduction in yield compared to inorganic treatment, making it a viable option for low-input farming systems that prioritize long-term soil health over maximal short-term yields.

However, the timing of the applications of organic amendments remains critical in order to synchronize the release of nutrients with the demand of the crop, underlining the need for practical, crop-specific guidelines. This is particularly important given that nitrogen from organic sources is slower and more complex to become available, as it relies on microbial mineralization processes. This is in contrast to mineral-N fertilizers, which are immediately available. Our findings suggest that nitrogen mineralization rates from compost may not always align with peak crop nitrogen demand, which could affect early growth and yield from a policy perspective (EU's Farm to Fork strategy). The results of this study support the inclusion of pelletized compost in carbon farming schemes and climate-smart agriculture initiatives, given its potential to promote soil carbon sequestration and reduce greenhouse gas emissions. This study highlights the need for long-term field trials to assess the residual effects of organic amendments on yield stability and carbon sequestration under diverse conditions. Further empirical evidence is required to validate these trends before making recommendations on a large scale.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agriculture15141543/s1>, Figure S1: Edaphoclimatic conditions during the trial; Table S1: Grain nutrient content of wheat at harvest; Table S2: Metabolomic profile of wheat grains at harvest period; Figure S2: Daily emissions of (a) N<sub>2</sub>O, (b) CO<sub>2</sub> and (c) CH<sub>4</sub> in the crop period.

**Author Contributions:** Conceptualization, A.G.-R., L.O. and R.M.; methodology M.Á.B. and R.M.; software, S.S.M. and L.O.; validation, S.S.M. and A.G.-R.; formal analysis, L.O., E.M.-S., M.D.P.-M. and J.A.S.-T.; investigation, A.G.-R., S.S.M., M.Á.M.-U., L.O., M.D.P.-M. and F.J.A.-R.; resources, F.J.A.-R. and R.M.; writing—review & editing, S.S.M., L.O. and A.G.-R.; visualization, J.A.S.-T., E.M.-S., F.J.A.-R., M.D.P.-M. and M.Á.B.; funding acquisition, R.M.; project administration, R.M. All authors have read and agreed to the published version of the manuscript.

**Funding:** This study forms part of the AGROALNEXT/2022/016 program and was supported by MCIN with funding from European Union NextGenerationEU (PRTR-C17.I1) and by Generalitat Valenciana.

**Institutional Review Board Statement:** Not applicable.

**Data Availability Statement:** The data presented in this manuscript are available from the authors upon reasonable request.

**Acknowledgments:** We thank to AGROCOMPOSTAJE Collaboration Agreement between the Generalitat Valenciana, through the Department of Agriculture, Rural Development, Climate Emergency and Ecological Transition and the Miguel Hernández University of Elche, was provided the compost use for the manufacture pelletized fertilizer.

**Conflicts of Interest:** The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

## References

1. Fertilizers Europe. Facts & Figures. June 2023. Available online: <https://www.fertilizerseurope.com/publications/fertilizers-industry-facts-and-figures-2023-edition/> (accessed on 15 July 2025).
2. Grijalva-Contreras, R.L.; Robles-Contreras, F.; Macías-Duarte, R.; Santillano-Cázares, J.; Núñez-Ramírez, F. Nitrogeno en Trigo y su Efecto en el Rendimiento y en la Concentración de Nitratos y Potasio en el Extracto Celular de Tallo (ECT). *Acta Univ.* **2016**, *26*, 48–54.
3. Konopka, I.; Tańska, M.; Faron, A.; Stępień, A.; Wojtkowiak, K. Comparison of the Phenolic Compounds, Carotenoids and Tocochromanols Content in Wheat Grain under Organic and Mineral Fertilization Regimes. *Molecules* **2012**, *17*, 12341–12356. [CrossRef] [PubMed]
4. Saia, S.; Fragasso, M.; De Vita, P.; Beleggia, R. Metabolomics Provides Valuable Insight for the Study of Durum Wheat: A Review. *J. Agric. Food Chem.* **2019**, *67*, 3069–3085. [CrossRef] [PubMed]
5. Zhen, S.; Zhou, J.; Deng, X.; Zhu, G.; Cao, H.; Wang, Z.; Yan, Y. Metabolite Profiling of the Response to High-Nitrogen Fertilizer During Grain Development of Bread Wheat (*Triticum aestivum* L.). *J. Cereal Sci.* **2016**, *69*, 85–94. [CrossRef]
6. Shewry, P.; Rakszegi, M.; Lovegrove, A.; Amos, D.; Corol, D.I.; Tawfike, A.; Mikó, P.; Ward, J.L. Effects of Organic and Conventional Crop Nutrition on Profiles of Polar Metabolites in Grain of Wheat. *J. Agric. Food Chem.* **2018**, *66*, 5346–5351. [CrossRef] [PubMed]
7. IPCC. Climate Change 2013, the Physical Science Basis. 2013. Available online: <http://www.ipcc.ch/report/ar5/wg1/> (accessed on 15 July 2025).
8. Sporchia, F.; Caro, D. Exploring the Potential of Circular Solutions to Replace Inorganic Fertilizers in the European Union. *Sci. Total Environ.* **2023**, *892*, 164636. [CrossRef] [PubMed]
9. European Commission. *The European Green Deal*; COM. 640 final; European Commission: Brussels, Belgium, 2019.
10. European Commission. *A Farm to Fork Strategy for a Fair, Healthy and Environmentally Friendly Food System*; COM/2020/381 final; European Commission: Brussels, Belgium, 2020.
11. International Olive Council. Statistics. 2025. Available online: <https://www.internationaloliveoil.org/what-we-do/statistics/> (accessed on 15 July 2025).
12. Pinho, I.A.; Lopes, D.V.; Martins, R.C.; Quina, M.J. Phytotoxicity Assessment of Olive Mill Solid Wastes and the Influence of Phenolic Compounds. *Chemosphere* **2017**, *185*, 258–267. [CrossRef] [PubMed]
13. Medina, E.; Romero, C.; de los Santos, B.; de Castro, A.; García, A.; Romero, F.; Brenes, M. Antimicrobial Activity of Olive Solutions from Stored *Alperujo* against Plant Pathogenic Microorganisms. *J. Agric. Food Chem.* **2011**, *59*, 6927–6932. [CrossRef] [PubMed]
14. García-Randez, A.; Marks, E.A.N.; Pérez-Murcia, M.D.; Orden, L.; Andreu-Rodríguez, J.; Sabater, E.M.; Cháfer, M.T.; Moral, R. Is the Direct Soil Application of Two-Phase Olive Mill Waste (Alperujo) Compatible with Soil Quality Protection? *Agronomy* **2023**, *13*, 2585. [CrossRef]
15. Sáez, J.A.; Pérez-Murcia, M.D.; Vico, A.; Martínez-Gallardo, M.R.; Andreu-Rodríguez, F.J.; López, M.J.; Bustamante, M.A.; Sanchez-Hernandez, J.C.; Moreno, J.; Moral, R. Olive Mill Wastewater-Evaporation Ponds Long Term Stored: Integrated Assessment of In Situ Bioremediation Strategies Based on Composting and Vermicomposting. *J. Hazard. Mater.* **2021**, *402*, 123481. [CrossRef] [PubMed]
16. Monetta, P.; Sanchez-Montilla, R.; Bosch-Rubia, G.; Rizzo, P.F.; Crespo, D.; Gouiric, S.; Herrera, M.V. Co-composting alperujo with other agroindustrial residues as sustainable practice for its recycling. *Acta Hort.* **2014**, *1057*, 709–716. [CrossRef]
17. García-Rández, A.; Orden, L.; Marks, E.A.N.; Andreu-Rodríguez, J.; Franco-Luesma, S.; Martínez Sabater, E.; Sáez-Tovar, J.A.; Pérez-Murcia, M.D.; Agulló, E.; Bustamante, M.A.; et al. Monitoring of greenhouse gas emissions and compost quality during olive mill waste co-composting at industrial scale: The effect of N and C sources. *J. Waste Manag.* **2025**, *193*, 33–43. [CrossRef] [PubMed]
18. Cucci, G.; Lacolla, G.; Caranfa, G. Use of Composted Olive Waste as Soil Conditioner and Its Effects on the Soil. *Int. J. Agric. Res.* **2013**, *8*, 149–157. [CrossRef]
19. Papandrea, S.F.; Cataldo, M.F.; Palma, A.; Gallucci, F.; Zimbalatti, G.; Proto, A.R. Pelletization of Compost from Different Mixtures with the Addition of Exhausted Extinguishing Powders. *Agronomy* **2021**, *11*, 1357. [CrossRef]
20. Chew, K.W.; Chia, S.R.; Yap, Y.J.; Ling, T.C.; Tao, Y.; Show, P.L. Densification of Food Waste Compost: Effects of Moisture Content and Dairy Powder Waste Additives on Pellet Quality. *Process Saf. Environ. Prot.* **2018**, *116*, 780–786. [CrossRef]
21. Pampuro, N.; Bagagiolo, G.; Priarone, P.C.; Cavallo, E. Effects of Pelletizing Pressure and the Addition of Woody Bulking Agents on the Physical and Mechanical Properties of Pellets Made from Composted Pig Solid Fraction. *Powder Technol.* **2017**, *311*, 112–119. [CrossRef]

22. Usman, K.; Khan, S.; Ghulam, S.; Khan, M.; Khan, N.; Khan, M.; Khalil, S. Sewage Sludge: An Important Biological Resource for Sustainable Agriculture and Its Environmental Implications. *Am. J. Plant Sci.* **2012**, *3*, 1708–1721. [\[CrossRef\]](#)
23. Jamil, M.; Qasim, M.; Umar, M.; Rehman, K. Impact of Organic Wastes (Sewage Sludge) on the Yield of Wheat (*Triticum aestivum* L.) in a Calcareous Soil. *Int. J. Agric. Biol.* **2004**, *6*, 465–467.
24. Brown, S.; Leonard, P. Recycling of Organic Carbon in Organic Wastes Is Also Important for C Sequestration in Soils. *Biocycle* **2004**, *45*, 25–29.
25. Zhou, Z.; Zhang, S.; Jiang, N.; Xiu, W.; Zhao, J.; Yang, D. Effects of Organic Fertilizer Incorporation Practices on Crop Yield, Soil Quality, and Soil Fauna Feeding Activity in the Wheat-Maize Rotation System. *Front. Environ. Sci.* **2022**, *10*, 1058071. [\[CrossRef\]](#)
26. Álvaro-Fuentes, J.; Cantero-Martínez, C.; López, M.V.; Arrúe, J.L. Soil Carbon Dioxide Fluxes Following Tillage in Semiarid Mediterranean Agroecosystems. *Soil Tillage Res.* **2007**, *96*, 331–341. [\[CrossRef\]](#)
27. Soil Survey Staff. *Keys to Soil Taxonomy*, 13th ed.; USDA Natural Resources Conservation Service: Washington, DC, USA, 2022; p. 401.
28. Paredes, C.; Pérez-Murcia, M.D.; Pérez-Espinosa, A.; Bustamante, M.A.; Moreno-Caselles, J. Recycling of Two-Phase Olive-Mill Cake “Alperujo” by Co-Composting with Animal Manures. *Commun. Soil Sci. Plant Anal.* **2015**, *46*, 238–247. [\[CrossRef\]](#)
29. Kabata-Pendias, A. *Trace Elements in Soils and Plants*, 4th ed.; CRC Press: Boca Raton, FL, USA, 2010; p. 548.
30. Bustamante, M.A.; Paredes, C.; Moral, R.; Moreno-Caselles, J.; Perez-Murcia, M.D.; Perez-Espinosa, A.; Bernal, M.P. Co-Composting of Distillery and Winery Wastes with Sewage Sludge. *Water Sci. Technol.* **2007**, *56*, 187–192. [\[CrossRef\]](#) [\[PubMed\]](#)
31. Yeomans, J.; Bremner, J.M. A Rapid and Precise Method for Routine Determination of Organic Carbon in Soil. *Commun. Soil Sci. Plant Anal.* **1989**, *19*, 1467–1476. [\[CrossRef\]](#)
32. Bremner, J.M.; Britebeck, G.A. A Simple Method for Determination of Ammonium in Semimicro-Kjeldahl Analysis of Soils and Plant Materials Using a Block Digester. *Commun. Soil Sci. Plant Anal.* **1983**, *14*, 905–913. [\[CrossRef\]](#)
33. Bremner, J.M.; Keeney, D.R. Steam Distillation Methods for Determination of Ammonium Nitrate and Nitrite. *Anal. Chim. Acta* **1965**, *32*, 485–495. [\[CrossRef\]](#)
34. Olsen, S.; Cole, C.; Watanabe, F.; Dean, L. *Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate*; USDA Circular 939; US Government Printing Office: Washington, DC, USA, 1954.
35. Hao, X.; Ball, B.C.; Culley, J.L.B.; Carter, M.R.; Parkin, G.W. Soil density and porosity. In *Soil Sampling and Methods of Analysis*; Canadian Society of Soil Science: Vancouver, BC, Canada, 2008; pp. 743–759.
36. Nannipieri, P.; Ceccanti, B.; Cervelli, S.; Matarese, E. Extraction of Phosphatase, Urease, Protease, Organic Carbon, and Nitrogen from Soil. *Soil Sci. Soc. Am. J.* **1980**, *44*, 1011–1016. [\[CrossRef\]](#)
37. Anderson, J.P.E.; Domsch, K.H. A Physiological Method for the Quantitative Measurement of Microbial Biomass in Soils. *Soil Biol. Biochem.* **1978**, *10*, 215–221. [\[CrossRef\]](#)
38. Anderson, J.P.E. Soil Respiration. In *Methods of Soil Analysis: Part 2 Chemical and Microbiological Properties*; John Wiley & Sons, Ltd.: Hoboken, NJ, USA, 1983; pp. 831–871. [\[CrossRef\]](#)
39. Wringley, C.; Batey, I. Assessing Grain Quality. In *Food Science, Technology and Nutrition*; Cauvain, S.P., Ed.; Woodhead Publishing: Cambridge, MA, USA, 2003; pp. 71–96. [\[CrossRef\]](#)
40. Marshall, D.R.; Ellison, F.W.; Mares, D.J. Effects of Grain Shape and Size on Milling Yields in Wheat. I. Theoretical Analysis Based on Simple Geometric Models. *Aust. J. Agric. Res.* **1984**, *35*, 619–630. [\[CrossRef\]](#)
41. López-Bellido, L.; López-Bellido, J.; Redondo, R. Nitrogen Efficiency in Wheat under Rainfed Mediterranean Conditions as Affected by Split Nitrogen Application. *Field Crops Res.* **2005**, *94*, 86–97. [\[CrossRef\]](#)
42. Van der Sar, S.; Kim, H.K.; Meissner, A.; Verpoorte, R.; Choi, Y.H. Nuclear Magnetic Resonance Spectroscopy for Plant Metabolite Profiling. In *The Handbook of Plant Metabolomics*; John Wiley & Sons, Ltd.: Hoboken, NJ, USA, 2013; pp. 57–76.
43. Alfósea-Simón, M.; Simón-Grao, S.; Zavala-Gonzalez, E.A.; Navarro-Morillo, I.; Martínez-Nicolás, J.J.; Alfósea-Simón, F.J.; García-Sánchez, F. Ionomics, Metabolic and Hormonal Characterization of the Phenological Phases of Different Tomato Genotypes Using Omics Tools. *Sci. Hortic.* **2022**, *293*, 110697. [\[CrossRef\]](#)
44. Franco-Luesma, S.; Lafuente, V.; Alonso-Ayuso, M.; Bielsa, A.; Kouchami-Sardoo, I.; Arrué, J.L.; Álvaro-Fuentes, J. Maize Diversification and Nitrogen Fertilization Effects on Soil Nitrous Oxide Emissions in Irrigated Mediterranean Conditions. *Front. Environ. Sci.* **2022**, *10*, 914851. [\[CrossRef\]](#)
45. Menéndez, S.; Merino, P.; Pinto, M.; González-Murua, C.; Estavillo, J.M. 3,4-Dimethylpyrazol Phosphate Effect on Nitrous Oxide, Nitric Oxide, Ammonia, and Carbon Dioxide Emissions from Grasslands. *J. Environ. Qual.* **2006**, *35*, 973–981. [\[CrossRef\]](#) [\[PubMed\]](#)
46. IPCC. Chapter 7. The Earth’s Energy Budget, Climate Feedbacks and Climate Sensitivity. In *Climate Change 2021: The Physical Science Basis*; Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change; Cambridge University Press: New York, NY, USA, 2021; p. 132.
47. Yao, Z.; Guo, H.; Wang, Y.; Zhan, Y.; Zhang, T.; Wang, R.; Zheng, X.; Butterbach-Bahl, K. A Global Meta-Analysis of Yield-Scaled N<sub>2</sub>O Emissions and Its Mitigation Efforts for Maize, Wheat, and Rice. *Glob. Change Biol.* **2024**, *30*, e17177. [\[CrossRef\]](#) [\[PubMed\]](#)

48. Aguilera, E.; Lassaleta, L.; Sanz-Cobena, A.; Garnier, J.; Vallejo, A. The Potential of Organic Fertilizers and Water Management to Reduce N<sub>2</sub>O Emissions in Mediterranean Climate Cropping Systems: A Review. *Agric. Ecosyst. Environ.* **2013**, *164*, 32–52. [CrossRef]
49. Di Rienzo, J.A.; Casanoves, F.; Balzarini, M.G.; González, L.; Tablada, M.; Robledo, C.W. *InfoStat, versión 2020*; Centro de Transferencia InfoStat, FCA, Universidad Nacional de Córdoba: Córdoba, Argentina, 2020; Available online: <http://www.infostat.com.ar> (accessed on 15 July 2025).
50. Oksanen, J.; Blanchet, F.G.; Friendly, M.; Kindt, R.; Legendre, P.; McGlinn, D.; Minchin, P.R.; O'Hara, R.B.; Simpson, G.L.; Solymos, P. *Vegan: Community Ecology Package, version 2.6-2*; The R Foundation for Statistical Computing: Vienna, Austria, 2022; Available online: <https://CRAN.R-project.org/package=vegan> (accessed on 15 July 2025).
51. Martínez-Sabater, E.; Pérez-Murcia, M.D.; Andreu-Rodríguez, F.J.; Orden, L.; Agulló, E.; Sáez-Tovar, J.; Martínez-Tomé, J.; Bustamante, M.Á.; Moral, R. Enhancing Sustainability in Intensive Dill Cropping: Comparative Effects of Biobased Fertilizers vs. Inorganic Commodities on Greenhouse Gas Emissions, Crop Yield, and Soil Properties. *Agronomy* **2022**, *12*, 2124. [CrossRef]
52. Sánchez-Monedero, M.A.; Mondini, C.; de Nobili, M.; Leita, L.; Roig, A. Land application of biosolids: Soil response to different stabilization degree or treated organic matter. *Waste Manag.* **2004**, *24*, 325–332. [CrossRef] [PubMed]
53. Orden, L.; Iocoli, G.A.; Bustamante, M.Á.; Moral, R.; Rodríguez, R.A. Nutrient Release Dynamics in Argentinean Pampean Soils Amended with Composts under Laboratory Conditions. *Agronomy* **2022**, *12*, 795. [CrossRef]
54. Antil, R.S.; Bar-Tal, A.; Fine, P.; Hadas, A. Predicting nitrogen and carbon mineralization of composted manure and sewage sludge in soil. *Compost Sci. Util.* **2011**, *19*, 33–43. [CrossRef]
55. Bernal, M.; Alburquerque, J.; Moral, R. Moral Composting of animal manures and chemical criteria for compost maturity assessment. A review. *Bioresour. Technol.* **2009**, *100*, 5444–5453. [CrossRef] [PubMed]
56. Hesterberg, D. Macroscale Chemical Properties and X-Ray Absorption Spectroscopy of Soil Phosphorus. In *Global Change and Forest Soils*; Elsevier: Amsterdam, The Netherlands, 2010; Volume 34, pp. 313–356.
57. Yan, Z.; Chen, S.; Dari, B.; Sihi, D.; Chen, Q. Phosphorus transformation response to soil properties changes induced by manure application in a calcareous soil. *Geoderma* **2018**, *322*, 163–171. [CrossRef]
58. Lizcano-Toledo, R.; Reyes-Martín, M.P.; Celi, L.; Fernández-Ondoño, E. Phosphorus Dynamics in the Soil–Plant–Environment Relationship in Cropping Systems: A Review. *Appl. Sci.* **2021**, *11*, 11133. [CrossRef]
59. Weihrauch, C.; Opp, C. Ecologically relevant phosphorus pools in soils and their dynamics: The story so far. *Geoderma* **2018**, *325*, 183–194. [CrossRef]
60. Lei, X.; Shen, Y.; Zhao, J.; Huang, J.; Wang, H.; Yu, Y.; Xiao, C. Root Exudates Mediate the Processes of Soil Organic Carbon Input and Efflux. *Plants* **2023**, *12*, 630. [CrossRef] [PubMed]
61. Bouhia, Y.; Hafidi, M.; Ouhdouch, Y.; Boukhari, M.E.; Mphatso, C.; Zeroual, Y.; Lyamlouli, K. Conversion of waste into organo-mineral fertilizers: Current technological trends and prospects. *Rev. Environ. Sci. Biotechnol.* **2022**, *21*, 425–446. [CrossRef]
62. Smith, G.H.; Chaney, K.; Murray, C.; Le, S.M. The effect of organo-mineral fertilizer applications on the yield of winter wheat, spring barley, forage maize and grass cut for silage. *J. Environ. Prot.* **2015**, *6*, 103–109. [CrossRef]
63. Li, L.; Li, H.; Tong, L.; Lv, Y. Sustainable Agriculture Practices: Utilizing Composted Sludge Fertilizer for Improved Crop Yield and Soil Health. *Agronomy* **2024**, *14*, 756. [CrossRef]
64. Tejada, M.; Gonzalez, J.L. Application of Different Organic Wastes on Soil Properties and Wheat Yield. *Agron. J.* **2007**, *99*, 1597–1606. [CrossRef]
65. Fernández, J.M.; Plaza, C.; García-Gil, J.C.; Polo, A. Biochemical properties and barley yield in a semiarid Mediterranean soil amended with two kinds of sewage sludge. *Appl. Soil Ecol.* **2009**, *42*, 18–24. [CrossRef]
66. Koutroubas, S.D.; Antoniadis, V.; Fotiadis, S.; Damalas, C.A. Growth, grain yield and nitrogen use efficiency of Mediterranean wheat in soils amended with municipal sewage sludge. *Nutr. Cycl. Agroecosyst.* **2014**, *100*, 227–243. [CrossRef]
67. Antolín, M.C.; Pascual, I.; García, C.; Polo, A.; Sánchez-Díaz, M. Growth, yield and solute content of barley in soils treated with sewage sludge under semiarid Mediterranean conditions. *Field Crops Res.* **2005**, *94*, 224–237. [CrossRef]
68. Motta, S.R.; Maggiore, T. Evaluation of nitrogen management in maize cultivation grows on soil amended with sewage sludge and urea. *Eur. J. Agron.* **2013**, *45*, 59–67. [CrossRef]
69. Deeks, L.K.; Chaney, K.; Murray, C.; Sakrabani, R.; Gedara, S.; Le, M.S.; Tyrrel, S.; Pawlett, M.; Read, R.; Smith, G.H. A new sludge-derived organo-mineral fertilizer gives similar crop yields as conventional fertilizers. *Agron. Sustain. Dev.* **2013**, *33*, 539–549. [CrossRef]
70. Saha, B.K.; Rose, M.T.; Van Zwieten, L.; Wong, V.N.L.L.; Patti, A.F. Slow release brown coal-urea fertilizer potentially influences greenhouse gas emissions, nitrogen use efficiency, and sweet corn yield in Oxisol. *ACS Agric. Sci. Technol.* **2021**, *1*, 469–478. [CrossRef]
71. Sui, B.; Feng, X.; Tian, G.; Hu, X.; Shen, Q.; Guo, S. Optimizing Nitrogen Supply Increases Rice Yield and Nitrogen Use Efficiency by Regulating Yield Formation Factors. *Field Crops Res.* **2013**, *150*, 99–107. [CrossRef]

72. Colomb, B.; Debaeke, P.; Jouany, C.; Nolot, J.M. Phosphorus management in low stockless cropping systems: Crop and soil responses to contrasting P regimes in a 36-year experiment in southern France. *Eur. J. Agron.* **2007**, *26*, 154–165. [\[CrossRef\]](#)
73. Duan, Y.; Xu, M.; Gao, S.; Yang, X.; Huang, S.; Liu, H.; Wang, B. Nitrogen use efficiency in a wheat–corn cropping system from 15 years of manure and fertilizer applications. *Field Crops Res.* **2014**, *157*, 47–56. [\[CrossRef\]](#)
74. Dhillon, J.; Torres, G.; Driver, E.; Figueredo, B.; Raun, W.R. World Phosphorus Use Efficiency in Cereal Crops. *Agron. J.* **2017**, *109*, 1670–1677. [\[CrossRef\]](#)
75. Syers, J.K.; Johnston, A.E.; Curtin, D. Efficiency of soil and fertilizer phosphorus use: Reconciling changing concepts of soil phosphorus behaviour with agronomic information. In *FAO Fertilizer and Plant Nutrition Bulletin*; FAO: Rome, Italy, 2008; Volume 18.
76. Xin, X.; Qin, S.; Zhang, J.; Zhu, A.; Yang, W.; Zhang, X. Yield, phosphorus use efficiency and balance response to substituting long-term chemical fertilizer use with organic manure in a wheat-maize system. *Field Crops Res.* **2017**, *208*, 27–33. [\[CrossRef\]](#)
77. Jones, C.; Olson-rutz, K.; Dinkins, C.P. *Nutrient Uptake Timing by Crops*; Montana State University: Bozeman, MT, USA, 2015.
78. Mnthambala, F.; Tilley, E.; Tyrrel, S.; Sakrabani, R. Effect of Various Organic Fertilisers on Phosphorus Mineralisation, Use Efficiency and Maize Yield. *Resources* **2022**, *11*, 86. [\[CrossRef\]](#)
79. Vita, F.; Giuntoli, B.; Arena, S.; Quaranta, F.; Bertolino, E.; Lucarotti, V.; Guglieminetti, L.; Alessio, M.; Scaloni, A.; Alpi, A. Effects of different nitrogen fertilizers on two wheat cultivars: An integrated approach. *Plant Direct.* **2018**, *2*, e00089. [\[CrossRef\]](#) [\[PubMed\]](#)
80. Qiu, X.M.; Sun, Y.Y.; Ye, X.Y.; Li, Z.G. Signaling Role of Glutamate in Plants. *Front. Plant Sci.* **2020**, *10*, 1743. [\[CrossRef\]](#) [\[PubMed\]](#)
81. Bonte, A.; Neuweget, H.; Goesmann, A.; Thonar, C.; Mader, P.; Langenkamper, G.; Niehaus, K. Metabolite profiling on wheat grain to enable a distinction of samples from organic and conventional farming systems. *J. Sci. Food Agric.* **2014**, *94*, 2605–2612. [\[CrossRef\]](#) [\[PubMed\]](#)
82. Zhang, M.; Ma, D.; Wang, C.; Zhao, H.; Zhu, Y.; Guo, T. Responses of Amino Acid Composition to Nitrogen Application in High-and Low-Protein Wheat Cultivars at Two Planting Environments. *Crop Sci.* **2016**, *56*, 1277–1287. [\[CrossRef\]](#)
83. Isaychev, V.; Andreev, N.; Mudarisov, F. IOP Conf. Ser. Earth Environ. Sci. **2021**, *937*, 022130.
84. Zörb, C.; Langenkämper, G.; Betsche, T.; Niehaus, K.; Barsch, A. Metabolite Profiling of Wheat Grains (*Triticum aestivum* L.) from Organic and Conventional Agriculture. *Agric. Food Chem.* **2006**, *54*, 8301–8306. [\[CrossRef\]](#) [\[PubMed\]](#)
85. Lee, C.P.; Millar, A.H. The plant mitochondrial transportome: Balancing metabolic demands with energetic constraints. *Trends Plant Sci.* **2016**, *21*, 662–676. [\[CrossRef\]](#) [\[PubMed\]](#)
86. Zhang, Y.; Fernie, A.R. The Role of TCA Cycle Enzymes in Plants. *Adv. Biol.* **2023**, *7*, e2200238. [\[CrossRef\]](#) [\[PubMed\]](#)
87. Chia, D.W.; Yoder, T.J.; Reiter, W.D.; Gibson, S.I. Fumaric acid: An overlooked form of fixed carbon in Arabidopsis and other plant species. *Planta* **2000**, *211*, 743–751. [\[CrossRef\]](#) [\[PubMed\]](#)
88. Rizhsky, L.; Liang, H.; Shuman, J.; Shulaev, V.; Davletova, S.; Mittler, R. When defense pathways collide. The response of Arabidopsis to a combination of drought and heat stress. *Plant Physiol.* **2004**, *134*, 1683–1696. [\[CrossRef\]](#) [\[PubMed\]](#)
89. Xie, H.; Shi, J.; Shi, F.; Xu, H.; He, K.; Wang, Z. Aphid fecundity and defenses in wheat exposed to a combination of heat and drought stress. *J. Exp. Bot.* **2020**, *9*, 2713–2722. [\[CrossRef\]](#) [\[PubMed\]](#)
90. Aytenuw, M.; Wolancho, G.B. Effects of Organic Amendments on Soil Fertility and Environmental Quality: A Review. *J. Plant Sci.* **2020**, *8*, 112–119. [\[CrossRef\]](#)
91. Li, S.; Li, T.; Kim, W.D.; Kitaoka, M.; Yoshida, S.; Nakajima, M.; Kobayashi, H. Characterization of raffinose synthase from rice (*Oryza sativa* L. var. Nipponbare). *Biotechnol. Lett.* **2007**, *29*, 635–640. [\[CrossRef\]](#) [\[PubMed\]](#)
92. Shewry, P.R.; Hey, S.J. The contribution of wheat to human diet and health. *Food Energy Secur.* **2015**, *4*, 178–202. [\[CrossRef\]](#) [\[PubMed\]](#)
93. Mejide, A.; Cárdenas, L.M.; Sánchez-Martín, L.; Vallejo, A. Carbon dioxide and methane fluxes from a barley field amended with organic fertilizers under Mediterranean climatic conditions. *Plant Soil* **2010**, *328*, 353–367. [\[CrossRef\]](#)
94. Chadwick, D.R.; Van der Weerden, T.; Martinez, J.; Pain, B.F. Nitrogen transformation and losses from pig slurry applications to a natural soil filter system (solepur process) in Britany, France. *J. Agric. Eng. Res.* **1998**, *68*, 85–95. [\[CrossRef\]](#)
95. Sánchez-Navarro, V.; Shahrokh, V.; Martínez-Martínez, S.; Acosta, J.A.; Almagro, M.; Martínez-Mena, M.; Boix-Fayos, C.; Díaz-Pereira, E.; Zornoza, R. Perennial alley cropping contributes to decrease soil CO<sub>2</sub> and N<sub>2</sub>O emissions and increase soil carbon sequestration in a Mediterranean almond orchard. *Sci. Total Environ.* **2022**, *845*, 157225. [\[CrossRef\]](#) [\[PubMed\]](#)
96. Hansen, J.E.; Kharecha, P.; Sato, M.; Tselioudis, G.; Kelly, J.; Bauer, S.E.; Pokela, A. Global Warming Has Accelerated: Are the United Nations and the Public Well-Informed? *Environ. Sci. Policy Sustain. Dev.* **2025**, *67*, 6–44. [\[CrossRef\]](#)
97. Kravchenko, I.K. Microbial Oxidation of Atmospheric Methane in Natural and Agricultural Upland Soils. In *Agro-Environmental Sustainability*; Singh, J., Seneviratne, G., Eds.; Springer: Cham, Switzerland, 2017. [\[CrossRef\]](#)
98. Sun, B.F.; Zhao, H.; Lü, Y.Z.; Lu, F.; Wang, X.K. The effects of nitrogen fertilizer application on methane and nitrous oxide emission/uptake in Chinese croplands. *J. Integr. Agric.* **2016**, *15*, 440–450. [\[CrossRef\]](#)
99. Butterbach-Bahl, K.; Baggs, E.M.; Dannenmann, M.; Kiese, R.; Zechmeister-Boltenstern, S. Nitrous oxide emissions from soils: How well do we understand the processes and their controls? *Philos. Trans. R. Soc.* **2013**, *368*, 20130122. [\[CrossRef\]](#) [\[PubMed\]](#)

100. Pan, B.; Xia, L.; Lam, S.K.; Wang, E.; Zhang, Y.; Mosier, A.; Chen, D.A. Global Synthesis of Soil Denitrification: Driving Factors and Mitigation Strategies. *Agric. Ecosyst. Environ.* **2022**, *327*, 107850. [[CrossRef](#)]
101. Vico, A.; Sáez, J.A.; Pérez-Murcia, M.D.; Martínez-Tomé, J.; Andreu-Rodríguez, J.; Agulló, E.; Bustamante, M.A.; Sanz-Cobena, A.; Moral, R. Production of spinach in intensive Mediterranean horticultural systems can be sustained by organic-based fertilizers without yield penalties and with low environmental impacts. *Agric. Syst.* **2020**, *178*, 102765. [[CrossRef](#)]
102. Charles, A.; Rochette, P.; Whalen, J.K.; Angers, D.A.; Chantigny, M.H.; Bertrand, N. Global nitrous oxide emission factors from agricultural soils after addition of organic amendments: A meta-analysis. *Agric. Ecosyst. Environ.* **2017**, *236*, 88–98. [[CrossRef](#)]
103. Lombardi, B.; Orden, L.; Varela, P.; Garay, M.; Iocoli, G.A.; Montenegro, A.; Sáez-Tovar, J.; Bustamante, M.Á.; Juliarena, M.P.; Moral, R. Is Dairy Effluent an Alternative for Maize Crop Fertigation in Semiarid Regions? An Approach to Agronomic and Environmental Effects. *Animals* **2022**, *12*, 2025. [[CrossRef](#)] [[PubMed](#)]
104. Cayuela, M.L.; Velthof, G.L.; Mondini, C.; Sinicco, T.; Van Groeningen, J.W. Nitrous oxide and carbon dioxide emissions during initial decomposition of animal by-products applied as fertilisers to soils. *Geoderma* **2010**, *157*, 235–242. [[CrossRef](#)]
105. Cayuela, M.L.; Aguilera, E.; Sanz-Cobena, A.; Adams, D.C.; Abalos, D.; Barton, L.; Ryalsh, R.; Silver, W.L.; Alfaro, M.A.; Pappa, V.A.; et al. Direct nitrous oxide emissions in Mediterranean climate cropping systems: Emission factors based on a meta-analysis of available measurement data. *Agric. Ecosyst. Environ.* **2017**, *238*, 25–35. [[CrossRef](#)]

**Disclaimer/Publisher’s Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.