

Article

From Water Buffalo (*Bubalus bubalis*) Manure to Vermicompost: Testing a Sustainable Approach for Agriculture

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Abstract: The application of organic amendments in agriculture has gained increasing attention as a sustainable approach to improving soil fertility and crop productivity. This study assessed the effects of vermicompost derived from water buffalo (*Bubalus bubalis*) manure on the yield and biochemical quality of cauliflower cultivated in soil types typical of the Campania region: loam and clay. Three fertilization treatments were tested, an unfertilized control, vermicompost (140 kg N ha^{−1}), and mineral fertilizer (MIN), at the same nitrogen rate. The results showed that vermicompost more significantly improved plant growth compared to the unfertilized control, particularly in loam soil, where the biomass and the leaf number increased by 160% and 335%, respectively. In clay soil, vermicompost enhanced nutrient availability, leading to a 159% biomass increase relative to the control. While mineral fertilization resulted in the highest yields, vermicompost improved the antioxidant capacity and influenced the amino acid composition, particularly in clay soil, where it enhanced the total amino acid content by 35% over that of the control. Additionally, vermicompost increased the quantity of soil organic matter and moderated the oxidative stress responses, suggesting long-term benefits for soil health. These findings highlight the potential of vermicompost as an effective and sustainable soil amendment, particularly in regions with intensive livestock farming and nitrate-sensitive environments. Further research is needed to optimize its integration with conventional fertilization strategies to maximize the agronomic and environmental benefits.

Keywords: organic waste; vermicomposting; sustainable agricultural practices; soil improvement; nutrient optimization



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1. Introduction

Sustainability has become a key global focus, forcing production sectors to adopt environmentally, socially, and economically viable practices. However, making agriculture truly sustainable is a highly complex challenge. Agri-food systems are responsible for roughly one-third of global greenhouse gas emissions, amounting to an estimated 16.2 billion tonnes of CO₂ equivalent per year between 2020 and 2022 [1,2]. Around 7 billion tonnes

of these emissions come directly from agricultural activities, while the rest are linked to deforestation, land use changes, processing, transportation, and waste management [3]. This underscores the dual challenge agriculture faces, ensuring food security, while also representing a significant source of emissions, highlighting the urgency of adopting more sustainable practices. One possible way to tackle these challenges is by applying the principles of a circular economy, especially regenerative agriculture, to make food production more sustainable. A circular economy focuses on reducing waste, reusing resources, and using them more efficiently. This approach aligns with the European Union's (EU) Green Deal [4] and the Circular Economy Action Plan (CEAP) [5], which aim to achieve climate neutrality by 2050. Agricultural waste, particularly the residual biomass from livestock farming, holds immense potential for innovative applications. Buffalo manure is a prime example, especially in southern Italian regions like Campania, where Mediterranean water buffalo farming (*Bubalus bubalis*) has long been a cornerstone of the local economy. Driven by the global demand for buffalo milk and mozzarella cheese, the Campania region is home to one of Italy's largest concentrations of water buffaloes. As of December 2024, the area hosts approximately 307,000 buffaloes, distributed across 1182 farms, with the largest concentrations in the provinces of Caserta (60.5%) and Salerno (33.9%) [6]. Each adult buffalo produces between 4 and 6 tonnes of wet manure annually, which represents a severe challenge due to the environmental restrictions imposed by the EU Nitrates Directive [7]. This directive, designed to protect water resources from agricultural pollution, limits the application of nitrogen-rich residual biomass to soil, capping nitrogen inputs at 170 kg per hectare annually in designated vulnerable zones. These constraints aim to reduce the leaching of nitrates into groundwater and surface water. In Campania region, the management of buffalo manure is governed by an updated regional regulation for the agronomic use of livestock effluents, wastewater, and digestates [8]. Based on Regional Council Resolution No. 585/2020, this updated framework strengthens compliance with the EU Nitrates Directive by introducing stricter guidelines to minimize the environmental risks from manure application. The key measures include restrictions on spreading manure near ecologically sensitive water bodies, the mandatory nitrate analysis of irrigation water, and the use of cover crops during autumn and winter to reduce nitrate leaching. While these provisions address environmental concerns, they also highlight the challenges of managing the significant quantities of manure produced in intensive buffalo farming systems, underscoring the need for sustainable and innovative solutions.

In this context, vermicomposting can represent an efficient method for converting biodegradable solid waste into a nutrient-rich and stable material known as vermicompost (VC). Compared to traditional composting or anaerobic digestion, it offers several distinct advantages. Vermicomposting operates at ambient temperatures, reducing energy inputs, and produces a highly stable product with elevated levels of humified organic matter and plant-available nutrients [9,10]. The resulting VC is a dark-colored, humus-like substance, free from unpleasant odors, typically characterized by low levels of pathogens and heavy metals, a balanced carbon-to-nitrogen ratio, and enhanced microbial activity, contributing to better soil structure and fertility [11,12]. Unlike anaerobic digestion, which is mainly suited to liquid substrates and requires complex equipment, vermicomposting is relatively simple, scalable, and adaptable to various types of solid organic waste, including manure, crop residues, and food waste [13]. These features make it an attractive strategy for low-input, sustainable agriculture both in industrial and smallholder contexts [10]. Thanks to the digestive activity of earthworms, particularly *Eisenia fetida*, VC is naturally enriched with plant-available nutrients, beneficial enzymes, and growth-stimulating hormones, making it suitable for use in agriculture as both a soil enhancer and an organic fertilizer [9]. VC, used as an amendment, improves aggregate stability, porosity, and mois-

ture retention, creating more resilient growing conditions [14]. These properties reduce reliance on synthetic fertilizers by ensuring slow nutrient release and minimizing leaching losses. Moreover, vermicomposting contributes to mitigating the environmental impact of agricultural practices by recycling organic waste, reducing greenhouse gas emissions, and supporting the development of disease-resistant cropping systems. This holistic approach is fully aligned with the principles of a circular economy and sustainable agriculture [11,15]. Vermicomposting has been successfully applied to various livestock manures, including cattle, pig, poultry, and goat waste. Some studies have shown that cow manure vermicompost improves nitrogen availability and soil microbial activity [12], pig and poultry manure treatments reduce pathogen loads and improve compost maturity [11], and goat manure supports high-quality VC production with improved carbon stabilization and plant growth promotion [10]. These results demonstrate the broad applicability of the process and reinforce its potential as a sustainable waste management strategy for different livestock systems. Beyond its benefits as a soil conditioner, VC from animal manure is rich in carbon, nitrogen, and essential nutrients, making it a valuable organic fertilizer. When properly integrated into soil at appropriate proportions, it functions as a slow-release fertilizer, providing a steady supply of nutrients to crops over time. This slow-release property reduces nutrient leaching and improves the long-term nutrient availability in the soil, fostering healthier and more sustainable crop growth [10,13].

This study evaluated the feasibility of transforming untreated buffalo manure into high-quality VC through the vermicomposting process using two species of earthworms, *Eisenia fetida* and *Eisenia andrei*. Once the VC was produced, its efficacy was assessed in cultivating cauliflower, a nutrient-demanding vegetable widely grown in the Campania region. With Campania contributing 18% of Italy's national brassica production and the sector generating EUR 805 million annually, cauliflower holds both economic and agricultural significance [16]. Due to its substantial requirements for nitrogen, phosphorus, and potassium [17], cauliflower was selected as a model crop to evaluate the performance of the organic amendment VC compared to that of a conventional mineral fertilizer. Its importance is further highlighted by its adaptability to environmental stresses and growing recognition as a high-value crop in domestic and export markets. This research focused on two prevalent soil types in Campania, loam and clay, each presenting a distinct challenge. Loam soils, with moderate fertility and drainage issues, and clay soils, characterized by compaction and low permeability, provided a comprehensive basis for assessing the adaptability and benefits of VC application. The primary objective was to determine whether the VC derived from water buffalo manure could enhance soil fertility and sustainably improve cauliflower yields without increasing nitrate leaching into aquifers.

2. Materials and Methods

2.1. Pre-Treatment of Buffalo Manure

Buffalo manure, the primary substrate for the vermicomposting process, was collected directly from the farm's manure storage system "Caseificio Polito" located in Agropoli, Salerno, Italy (40°23' N, 15°02' E; 24 m above sea level) (Figure 1A,B). With a high organic matter content (76.1%) and a C-to-N ratio of 20.4, the substrate provided optimal conditions for bacterial growth and microbial activity, supporting the initial phase of aerobic respiration. It was also rich in essential nutrients, containing 2.2% total nitrogen, 7920 mg kg⁻¹ (0.79%) phosphorus, and 33,744 mg kg⁻¹ (3.37%) potassium, making it well suited for the vermicomposting process (Supplemental File S1). The pre-treatment of buffalo manure and the subsequent vermicomposting process were carried out in separate, but controlled setups within the same farm. The stalls (Figure 1C) are frequently washed in an intensive buffalo farming system, and as a result, slurry, a highly diluted mixture of feces, urine,

and water, is produced. To obtain a substrate suitable for aerobic stabilization and vermicomposting, mechanical separation was performed to remove the excess liquid fraction, including urine. This separation was carried out using a screw press separator (Doda, Mantova, Italy, Figure 1D), applying mechanical pressure to the raw material and forcing the liquid portion to pass through a filtering screen, while the solid fraction was gradually extruded (Figure 1E). The resulting manure solid fraction, used for pre-composting and subsequent vermicomposting, had an average moisture content of approximately 70%, suitable for aerobic microbial activity (Figure 1F). This material then underwent a stabilization phase through aerobic respiration for approximately 40 days. This step facilitated pathogen reduction (*Escherichia coli* and *Salmonella* spp.), the degradation of phytotoxic compounds (e.g., phenols and organic acids), and the stabilization of organic matter while minimizing nitrogen volatilization. During this aerobic pre-stabilization phase, temperature was continuously monitored using digital thermocouple sensors placed in the core of the manure piles. The material naturally entered the thermophilic range (55–65 °C) within the first days, without any external heat source. Daily temperature recordings confirmed sustained microbial activity and were used to define the end of the thermophilic phase before vermicomposting. Microbial activity accelerated the decomposition of easily degradable organic matter, improving substrate quality. The manure was periodically mixed to ensure homogeneous nutrient distribution and aeration (Figure 1G), promote aerobic microbial activity, and reduce ammonia accumulation. This process prevented anaerobic conditions, mitigating the risk of methane and hydrogen sulfide formation. At the end of this phase, the manure naturally cooled down until it reached 25 °C, making it suitable for earthworm survival (Figure 1H). During this period, the ammonia and urea concentrations naturally decreased to non-toxic levels, ensuring a stable substrate for vermicomposting.

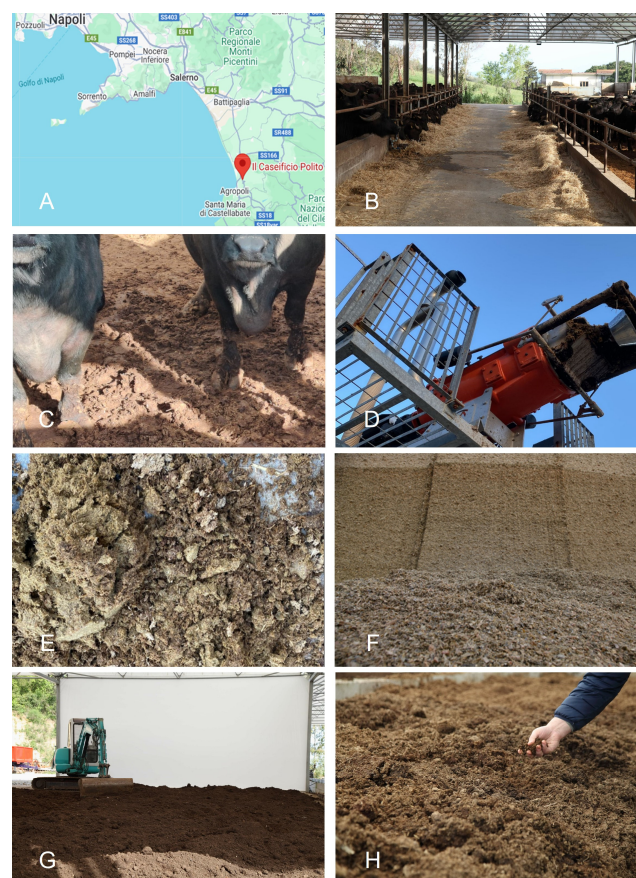


Figure 1. Overview of buffalo farm and aerobic pre-treatment. (A) Geographical location of Caseificio Polito (Agropoli, Salerno, Italy); (B) covered stalls of water buffalo farm where animals are housed

and manure accumulates; (C) water buffalo livestock, primary source of manure used for vermicomposting; (C) buffalo slurry; (D) mechanical screw-press separator used to separate liquid fraction (urine + water) from solid manure; (E,F) extruded solid fraction of buffalo manure used as starting substrate with suitable moisture content (~70%); (G) manure in aerobic stabilization area, where mechanical shovel is used to mix material, ensuring aeration and uniform decomposition; (H) manure naturally cooled down suitable for vermicomposting Photos kindly provided by Caseificio Polito (www.caseificiopolito.it, accessed on 6 May 2025).

2.2. Vermicomposting Process

Once stabilized, the manure was transferred to dedicated vermicomposting beds (Figure 2A) specifically designed to optimize the process. These beds were equipped with perforations to facilitate aeration and microbial activity. The environmental conditions were continuously monitored using a datalogger system (Delta OHM HD35AP4G Base Unit with 4G connection and several HD35EDW dataloggers) to check the climate conditions for earthworm activity. This system provided real-time data acquisition and storage, enabling the monitoring of key environmental variables, including soil and air temperature, soil and air humidity, pH, CO₂ concentration, and solar radiation, within the vermicomposting beds. The earthworm species *Eisenia fetida* and *Eisenia andrei* were introduced into the substrate after an acclimatization period of two weeks, during which the worms adapted to the conditions of the pre-conditioned buffalo manure (Figure 2B,C). These two epigenic species are widely used in vermicomposting due to their high efficiency in decomposing organic matter. Although *E. andrei* is generally more prolific and shows faster growth and cocoon production under optimal conditions, it tends to be more sensitive to environmental fluctuations. In contrast, *E. fetida* is known for its higher tolerance to variable temperature, moisture, and substrate conditions. It is particularly suitable for large-scale or field-based systems where the environmental parameters are not tightly controlled. According to the previous studies [18,19], by using both the species together, the robustness and stress tolerance of *E. fetida* were combined with the strong reproductive performance of *E. andrei*, ensuring adaptability and efficiency throughout the vermicomposting cycle. The earthworm-to-substrate ratio was approximately 1:5 (*w/w*, fresh worm biomass to fresh manure), corresponding to an estimated density of 250–330 individuals per kilogram of substrate, assuming an average individual weight of 0.6–0.8 g, according to the optimal range recommended in the literature for efficient vermicomposting [18]. It ensures a good balance between the decomposition rate, worm health, and process scalability under semi-controlled farm conditions. It is important to specify that the population included adult worms, juveniles, and cocoons, reflecting the natural developmental variability of *Eisenia* species, which typically live up to 18 months. At the end of the vermicomposting cycle, plastic trays with small perforations at the bottom were filled with fresh manure and placed on top of the processed material. These perforated trays allowed for some of the worms to migrate upward in search of fresh substrate, taking advantage of their natural behavior. This method enabled the passive and efficient recovery of the needed earthworm biomass, minimizing disturbance and preserving the population for reuse in subsequent cycles. The vermicomposting process itself lasted 6–12 weeks and adhered to the standards outlined in Italian Legislative Decree 75/2010. This controlled setup provided the conditions for effective vermicomposting, creating a final stable and odorless bioproduct (Figure 2D). The obtained VC exhibited a pH of around 7.9, but always less than 8.0, a C-to-N ratio of 16, and an organic nitrogen content of 2.1%. It contained 33% total organic carbon (TOC), with 39% of it being extractable organic matter (TEC/TOC) and 71% classified as humified organic matter, indicating a high degree of humification (Supplemental File S2).

The VC was subsequently tested in cauliflower cultivation experiments, as detailed in the following sections.



Figure 2. Overview of vermicomposting process for buffalo manure. (A) Vermicomposting beds prepared for earthworm introduction; (B,C) earthworms (*Eisenia fetida* and *Eisenia andrei*) actively processing manure; (D) final vermicompost product, characterized by earthy texture and lack of odor, indicating proper stabilization and maturation. Photos kindly provided by Caseificio Polito (www.caseificiopolito.it, accessed on 6 May 2025).

2.3. Plant Cultivation

The experiments took place from 14 December 2023 (amendments application) to 26 April 2024 (final harvest) at the Department of Agricultural Sciences, University of Naples Federico II, Portici, Italy (40°49' N, 14°15' E; 70 m a.s.l.). This study aimed to assess the effectiveness of VC as both an organic amendment and a biofertilizer in cauliflower (*Brassica oleracea* var. Freedom “Seminis”) cultivation. Field plots were established outdoors, using two representative soil types from the Campania Plain (loam and clay) selected for their contrasting physicochemical properties, affecting nutrient dynamics and plant growth. The amendments were uniformly incorporated into the soil to ensure consistent treatment conditions. Cauliflower seedlings were transplanted on 18 December 2023, with a density of 2.5 plants per square meter.

The plants were subjected to three fertilization regimes, an unfertilized control (NF) to establish baseline growth, a VC treatment, and a mineral fertilizer (MIN) treatment as ammonium nitrate (34% N), applied in three doses.

The nitrogen dose was calculated with the plan of fertilization of Campania Region, and it was 140 kg N ha^{−1}; then, the organic and mineral fertilizers were applied at rates of 7.0 and 0.4 t ha^{−1}, respectively. Phosphorus and potassium were not added due to their high initial soil levels. Evapotranspiration was estimated using the Hargreaves method, and irrigation fully compensated for water loss through a drip system. No pesticides were used. The soil parameters, including organic matter (O.M. %) [20], total nitrogen (N-Kjeldah %) [21], nitrate nitrogen (NO₃-N), and ammonium nitrogen (NH₄-N) [22], were monitored at the beginning and end of the experiment to assess nutrient transformations under the different fertilization treatments (Table 1). These measurements provided insights into how

VC and mineral fertilizers influenced soil fertility over the growing season. The cauliflower plants were harvested at marketable maturity (16–26 April 2024), determined by measuring the fresh weight of the corymbs from each experimental plot.

Table 1. Soil parameters, including organic matter (O.M. %), total nitrogen (N-Kjeldahl %), nitrate nitrogen (NO₃-N, ppm), and ammonium nitrogen (NH₄-N, ppm), were measured before transplanting (start) and after the final harvest (end) for unfertilized control (NF), mineral fertilizer (MIN), and vermicompost (VC) in loam and clay soils.

Soil Parameters	O.M. %		N-Kjeldahl %		NO ₃ -N ppm		NH ₄ -N ppm	
	Start	End	Start	End	Start	End	Start	End
Loam NF	2.2	2.3	0.118	0.145	28.9	40.7	10.0	12.4
Loam MIN	2.2	2.6	0.118	0.150	28.9	85.8	10.0	12.0
Loam VC	2.2	2.7	0.118	0.150	28.9	84.1	10.0	9.1
Clay NF	1.9	1.7	0.122	0.106	17.0	17.4	7.7	5.6
Clay MIN	1.9	2.4	0.122	0.138	17.0	96.5	7.7	12.1
Clay VC	1.9	2.6	0.122	0.150	17.0	30.4	7.7	6.1

2.4. Morphological, Color, and Leaf Chlorophyll Measurements

In 2011, Corymb diameter and height were measured for 5 plants per treatment in each experimental condition. These measurements were performed using a digital caliper to ensure precision and consistency. Corymb color was measured on the upper surface using a Minolta CR-300 Chroma Meter (Minolta Camera Co., Ltd., Osaka, Japan) to obtain color space parameters, specifically L* (brightness), a* (from green to red chroma component), and b* (from blue to yellow chroma component). The SPAD index, a non-destructive technique for estimating in vivo chlorophyll content, was used to evaluate plant health and nitrogen status during the growth cycle. Measurements were taken using a portable SPAD-502 chlorophyll meter (Konica Minolta, Tokyo, Japan), allowing for real-time monitoring without causing damage to the plants. Each biological replicate, consisting of three replicates per treatment, was calculated as the average of measurements taken from six fully expanded leaves per plant, and the mean SPAD value was determined for each treatment.

2.5. Chemicals and Reagents

All biochemical analyses were performed using authenticated standards and certified analytical-grade reagents (ACS or CertiPUR grade). Amino acid standards (HPLC-grade), 2-mercaptoethanol (HPLC-grade), tetrahydrofuran (HPLC-grade), sodium acetate (HPLC-grade), sodium borate (HPLC-grade), ninhydrin, Trizma[®] base, multielement anion and cation standards (HPLC-grade), bovine serum albumin (BSA), HEPES, adenosine 5'-triphosphate (ATP) disodium salt hydrate, dithiothreitol, potassium hydroxide hydrate, glucose, α -amylase, amyloglucosidase, hexokinase, phosphoglucose isomerase, invertase, glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroides*, β -nicotinamide adenine dinucleotide hydrate, glacial acetic acid, gallic acid, and Folin–Ciocalteu's phenol reagent were purchased from Merck KGaA (Darmstadt, Germany). The protein assay reagent was obtained from Bio-Rad Laboratories (Hercules, CA, USA). Methanol and ethanol (HPLC-grade) were purchased from VWR International (Radnor, PA, USA). Ultra-pure water was obtained from a Milli-Q Gradient A10 water purification system (Millipore, Billerica, MA, USA).

2.6. Starch and Soluble Carbohydrates Content

Soluble sugars were quantified from ethanolic extracts of fresh cauliflower corymbs using the method of Fusco, et al. [23]. Samples (20 mg) were extracted with 250 μL of 80% ethanol (*v/v*) and incubated at 80 °C for 20 min, and then centrifuged at 13,000 rpm for 10 min at 4 °C. Two additional extractions were performed using 150 μL of 80% and 50% ethanol (*v/v*), respectively, and the supernatants were pooled for soluble sugar analysis. The starch in the remaining pellet was hydrolyzed by adding 100 μL of 0.1 M KOH and heating at 90 °C for 2 h. The pH was adjusted to 4.5 with acetic acid, followed by mixing at 1:1 with a hydrolysis buffer containing 50 mM sodium acetate (pH 4.5), α -amylase (2 U mL^{-1}), and amyloglucosidase (20 U mL^{-1}). This mixture was incubated at 37 °C for 20 h. After centrifugation at 13,000 rpm for 10 min at 4 °C, the supernatant containing glucose from hydrolyzed starch was used for analysis. An enzymatic assay coupled with pyridine nucleotide reduction was applied to measure glucose, fructose, and sucrose concentrations, with absorbance at 340 nm recorded using a Synergy HT spectrophotometer (BioTEK Instruments, Bad Friedrichshall, Germany). Sugar content was expressed as $\mu\text{mol g}^{-1}$ DW.

2.7. Protein and Free Amino Acid Contents

Proteins were extracted from 20 mg of lyophilized tissue using TRIS-HCl buffer (200 mM, pH 7.5) with 500 mM MgCl_2 , stored at 4 °C for 24 h, and centrifuged (13,000 rpm, 5 min). The protein content was determined using the Bio-Rad assay and BSA standard curve (595 nm) and is expressed as mg g^{-1} FW [24]. Free amino acids were quantified by HPLC after OPA derivatization. Proline was measured calorimetrically using a ninhydrin-based assay, and the results are expressed as $\mu\text{mol g}^{-1}$ DW [23].

2.8. Polyphenols, Hydrogen Peroxide and ABTS Activity

Polyphenols were quantified using the Folin–Ciocalteu method [25], with the following modifications. Lyophilized samples (30 mg) were extracted with 60% methanol (700 μL), centrifuged (800 rpm, 5 min), and analyzed by mixing with Folin–Ciocalteu reagent and sodium carbonate. Absorbance at 760 nm was measured, and the results are expressed as gallic acid equivalents (GAEs) in $\mu\text{g g}^{-1}$ FW. The flavonoids content was measured using a Dualex Scientific+ (Force A, Orsay, France) portable device according to [26]. The H_2O_2 levels were measured following Jana, et al. [27] with some modifications. Lyophilized tissues (20 mg) were homogenized in 230 μL of 0.1% (*w/v*) trichloroacetic acid and centrifuged at 12,000 rpm at 4 °C for 10 min. Clear supernatants (50 μL) or H_2O_2 standards (0.5–4 mM) were mixed with 50 μL of 10 mM phosphate buffer (pH 7.0) and 100 μL of 1 M KI in a polypropylene microplate. Absorbance at 390 nm was measured using a Synergy HT spectrophotometer. The H_2O_2 levels were calculated using a standard curve and expressed as $\mu\text{mol g}^{-1}$ DW. ABTS radical scavenging was assessed as described by Re, et al. [28]. Lyophilized tissues (50 mg) were extracted with ethanol, cooled for 2 h, centrifuged (12,000 rpm, 10 min, 4 °C), and mixed with ABTS reagent. Absorbance at 745 nm was measured, and the results are expressed as Trolox equivalents ($\mu\text{mol Trolox g}^{-1}$ DW).

2.9. Statistical Analysis

The effects of soil type (S), loam and clayey, were compared using Student's t-test. One-way ANOVA was applied to evaluate the mean effects of the fertilization treatments (F), including no fertilization (NF), vermicompost (VC), and mineral fertilization (MIN). To determine the significance of main effects and interactions between the factors, a two-way ANOVA was conducted for the combinations of soil (loam or clayey) $\times$ fertilization (NF, VC, and MIN). Statistical significance was assessed at $p < 0.05$, and post-hoc comparisons for the interactions (e.g., loam $\times$ NF, loam $\times$ VC, loam $\times$ MIN, clayey $\times$ NF, clayey $\times$ VC,

and clayey $\times$ MIN) were performed using the Tukey–Kramer HSD test. All the results are presented as mean $\pm$ standard deviation. Statistical analyses were carried out using the IBM SPSS Statistics 20 software package (Armonk, NY, USA) on Microsoft Windows 11.

3. Results

3.1. Fertilization Predominantly Shapes Yield and Morphometry

The results for the marketable yield and morphometric parameters of cauliflower are presented in Figure 3 and Table 2. Marketable yield, expressed as corymb fresh weight, along with the other morphometric traits, such as total fresh weight, corymb diameter, and the harvest index (HI), was significantly influenced by the interaction between the fertilization treatments and the soil type. Conversely, corymb height was affected only by fertilization ($p < 0.001$) (Figure 3; Table 2). Mineral fertilization (MIN) consistently resulted in the highest values across all the parameters, with corymb fresh weight exceeding those of the vermicompost (VC)-treated plants by 68% and surpassing that of the unfertilized control (NF) by more than 520% (Figure 3A). In the loam soil, MIN fertilization further enhanced the performance, yielding a corymb fresh weight 56% higher than that of the corresponding VC treatment (Figure 3A). The increase in corymb fresh weight under the MIN treatment was primarily due to the greater corymb diameter, irrespective of the soil texture. The plants treated with VC showed intermediate values, while those left unfertilized had corymb diameters nearly 50% smaller than those under the MIN treatment (Table 2). Similarly, total fresh weight was significantly higher under MIN fertilization, averaging 53.7% more than that of VC and over threefold compared to those of the NF plants (Table 2). The highest HI values were recorded in the loamy soil, with no significant differences between mineral and organic fertilization, followed by the MIN treatment in the clay soil. The NF plants exhibited the lowest HI values (Table 2). Finally, MIN fertilization significantly increased the corymb height by 8.4% compared to that of VC and by 45.9% relative to that of NF (Table 2).

Table 2. Effects of interaction between fertilization treatments (NF: no fertilization; VC: vermicompost; MIN: mineral fertilizer) and soil type (loam and clayey) on total fresh weight (total FW), individual relative harvest index (IRHI), corymb diameter, and corymb height of cauliflower (*Brassica oleracea* var. Freedom “Seminis”). Different letters indicate significant differences according to Tukey’s HSD test ($p < 0.05$). ns, *, **, *** Nonsignificant or significant at $p \leq 0.05$, 0.01 and 0.001, respectively. All data are expressed as mean $\pm$ SD, $n = 5$.

Source of Variance	Total FW (g)	IRHI	Corymb Diameter (cm)	Corymb Height (cm)
Soil (S)				
Loam	1095 $\pm$ 495	35.8 $\pm$ 8.8	13.23 $\pm$ 3.74	9.84 $\pm$ 1.80
Clayey	1080 $\pm$ 529	34.6 $\pm$ 4.8	12.73 $\pm$ 3.21	9.80 $\pm$ 1.60
	ns	ns	ns	ns
Fertilization (F)				
Control (NF)	498 $\pm$ 34 c	26.8 $\pm$ 3.4 c	8.68 $\pm$ 0.69 c	7.73 $\pm$ 0.91 c
Vermicompost (VC)	1089 $\pm$ 16 b	38.3 $\pm$ 4.1 b	13.75 $\pm$ 0.77 b	10.45 $\pm$ 0.37 b
Mineral (MIN)	1674 $\pm$ 320 a	40.5 $\pm$ 2.2 a	16.52 $\pm$ 1.70 a	11.28 $\pm$ 0.63 a
	***	**	***	***
S $\times$ F				
Loam $\times$ NF	532 $\pm$ 34 c	24.5 $\pm$ 3.2 d	8.50 $\pm$ 0.95 e	7.67 $\pm$ 1.19
Loam $\times$ VC	1080 $\pm$ 12 b	41.3 $\pm$ 3.5 a	14.40 $\pm$ 0.40 c	10.47 $\pm$ 0.40

Table 2. Cont.

Source of Variance	Total FW (g)	IRHI	Corymb Diameter (cm)	Corymb Height (cm)
Loam × MIN	1672 ± 126 a	41.5 ± 2.1 a	16.80 ± 0.30 a	11.40 ± 0.10
Clayey × NF	463 ± 28 d	29.1 ± 1.5 c	8.87 ± 0.40 e	7.80 ± 0.80
Clayey × VC	1099 ± 95 b	35.3 ± 1.6 b	13.10 ± 0.26 d	10.43 ± 0.42
Clayey × MIN	1677 ± 120 a	39.4 ± 2.1 a	16.23 ± 0.06 b	11.17 ± 0.21
	*	**	*	ns

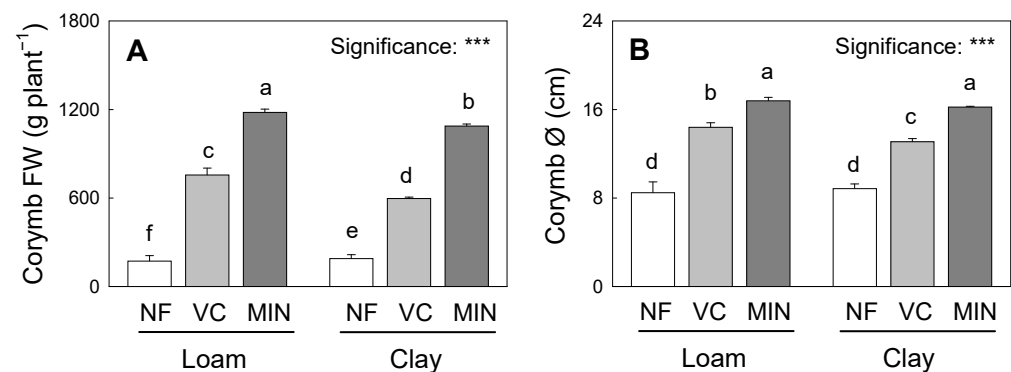


Figure 3. Effects of interaction between fertilization treatments (NF: no fertilization; VC: vermicompost; MIN: mineral fertilizer) and soil type (loam and clayey) on corymb fresh weight (A) and corymb diameter (B) of cauliflower (*Brassica oleracea* var. *botrytis*). Data are presented as mean ± SD (n = 5). Different lowercase letters above bars indicate significant differences between treatments according to Tukey's HSD test ($p < 0.05$). *** Significant at $p \leq 0.001$.

3.2. Fertilization Enhances SPAD Index, Chlorophyll, and Colorimetry

The interaction between the soil type and fertilization ($S \times F$) was significant for the colorimetric parameters (L^* , a^* , and b^*), but not for the SPAD index or the total chlorophyll content (Table 3). No significant differences were observed between the loam and clayey soils for the SPAD index, the total chlorophyll content, or the colorimetric parameters when considering the soil type alone. However, fertilization significantly influenced all the measured variables. MIN led to the highest SPAD index and total chlorophyll content, with increases of over 70% and 30%, respectively, compared to those of the NF. The VC treatments showed intermediate values, with increases of approximately 40% for the SPAD index and 13% for the chlorophyll content compared to those of NF (Table 3). Regarding the colorimetric parameters, MIN significantly increased L^* , with corymbs appearing 13% brighter than those in NF. The VC treatments also improved brightness, but to a lesser extent. The interaction $S \times F$ was particularly evident for L^* , where MIN had a similar effect in both the soils, whereas the NF plants exhibited lower L^* values in loam compared to the clayey soil. For a^* , MIN resulted in the highest values, indicating a shift towards a less green and more reddish hue, while the VC-treated plants showed intermediate values. NF had the lowest a^* values, especially in the loam soil, significantly lower than that in the clayey soil. Similarly, b^* was highest in the NF plants, particularly in the loam soil, suggesting a stronger yellow hue. MIN fertilization led to the lowest b^* values, indicating a reduction in yellow intensity.

Table 3. Leaf SPAD index, total chlorophyll content, and corymb colorimetric parameters (L^* = brightness (black/white), a^* (green/red), b^* (blue/yellow)) of cauliflower (*Brassica oleracea* var. Freedom “Seminis”) in relation to fertilization treatments (NF: no fertilization; VC: vermicompost; MIN: mineral fertilizer) and soil type (loam and clayey). Different letters indicate significant differences according to Tukey’s HSD test ($p < 0.05$). ns, *, **, *** Nonsignificant or significant at $p \leq 0.05$, 0.01 and 0.001, respectively. All data are expressed as mean $\pm$ SD, $n = 3$.

Source of Variance	L^*	a^*	b^*	SPAD Index	Total Chlorophylls (mg g^{-1} FW)
Soil (S)					
Loam	83.6 ± 5.62	-0.91 ± 1.96	18.11 ± 3.13	36.52 ± 7.38	0.80 ± 0.12
Clayey	83.7 ± 4.76	-0.92 ± 1.26	17.10 ± 2.11	33.39 ± 8.57	0.73 ± 0.09
	ns	ns	ns	ns	ns
Fertilization (F)					
Control (NF)	79.4 ± 2.51 b	-2.47 ± 0.50 c	20.93 ± 1.42 a	25.52 ± 2.58 c	0.67 ± 0.07 c
Vermicompost (VC)	81.5 ± 2.13 c	-1.36 ± 0.29 b	16.57 ± 0.57 b	35.65 ± 2.49 b	0.76 ± 0.07 b
Mineral (MIN)	89.9 ± 5.23 a	1.08 ± 1.38 a	15.32 ± 0.92 c	43.70 ± 4.74 a	0.87 ± 0.04 a
	***	***	***	***	**
S $\times$ F					
Loam $\times$ NF	77.7 ± 2.10 e	-2.89 ± 0.29 e	22.10 ± 0.40 a	27.47 ± 1.06	0.71 ± 0.04
Loam $\times$ VC	82.8 ± 1.67 b	-1.34 ± 0.12 b	16.93 ± 0.47 b	37.87 ± 0.75	0.77 ± 0.10
Loam $\times$ MIN	90.1 ± 1.63 a	1.50 ± 0.54 a	15.30 ± 0.96 e	44.23 ± 1.19	0.93 ± 0.07
Clayey $\times$ NF	81.1 ± 1.67 c	-2.04 ± 0.05 d	19.77 ± 0.90 a	23.57 ± 2.01	0.62 ± 0.06
Clayey $\times$ VC	80.2 ± 1.89 d	-1.37 ± 0.44 c	16.20 ± 0.44 c	33.43 ± 0.47	0.76 ± 0.05
Clayey $\times$ MIN	89.7 ± 1.29 a	0.66 ± 0.45 a	15.33 ± 0.40 d	43.17 ± 1.19	0.80 ± 0.05
	*	**	*	ns	ns

3.3. Fertilization Influences Antioxidant Compounds and Oxidative Stress Markers

The interaction $S \times F$ significantly affected ABTS antioxidant activity, but did not significantly influence the flavonoid or polyphenol content, nor hydrogen peroxide (H_2O_2) accumulation (Table S1). When considering the soil type alone, the loam soil exhibited slightly higher flavonoid concentrations than the clayey soil, while the polyphenol content remained statistically similar between the soil types. However, fertilization significantly impacted all the measured biochemical parameters. MIN application resulted in the highest flavonoid accumulation, showing a 27% increase compared to NF and a 15% increase compared to VC. Conversely, the VC treatments led to intermediate flavonoid levels, while NF had the lowest content. The polyphenol content, in contrast, was highest in the NF-treated plants (Table S1). H_2O_2 accumulation was markedly influenced by fertilization, with the VC-treated plants exhibiting the highest H_2O_2 levels, nearly doubling those observed in the MIN and NF treatments (Table S1). Regarding ABTS antioxidant activity, the interaction between soil and fertilization played a significant role (Table S1). The NF-treated plants in the clayey soil and the VC-treated plants in the loam soil exhibited the highest antioxidant capacity, with the ABTS values significantly higher than those recorded under the MIN treatments.

3.4. Mineral Fertilization and Loam Soils Boost Nitrogen Assimilation and Amino Acid Profiles

The interaction $S \times F$ was significant for the nitrate concentration, N-Kjeldahl, and the protein content, but not for starch and the total non-structural carbohydrates (TNSCs)

(Table 4). Nitrate accumulation was substantially higher in the loam soils, with the concentrations more than three times greater than in the clayey soils. Among the fertilization treatments, MIN resulted in a 210% increase compared to NF and 45% more than VC. However, in loam, the MIN-treated plants showed the highest nitrate levels, while the lowest values were recorded in the clayey soil under the NF treatment. Despite higher nitrate accumulation under MIN, the N-Kjeldahl content was highest in the clayey soil treated with MIN, surpassing loam by nearly 70%. In contrast, the loam soil under the MIN treatment recorded the lowest values, suggesting less efficiency in nitrogen incorporation. The protein content followed a similar trend, with the loam soils showing a 29% increase over that of the clayey soils. Among the fertilization treatments, MIN led to the highest protein accumulation. Still, when considering interactions, the increase was most pronounced in the loam soil under the MIN treatment, where the protein content was nearly 70% higher than that in the clayey NF-treated plants, which had the lowest values. The starch content was about 20% higher in the clayey soils, but the fertilization treatments had no significant effect on either starch or the TNSCs (Table 4).

Table 4. Nitrate (NO_3^-) concentration, total nitrogen (N-Kjeldahl), protein, starch, and total non-structural carbohydrate (TNSC) contents of cauliflower (*Brassica oleracea* var. Freedom “Seminis”) corymbs in relation to fertilization treatments (NF: no fertilization; VC: vermicompost; MIN: mineral fertilizer) and soil type (loam and clayey). Different letters indicate significant differences according to Tukey’s HSD test ($p < 0.05$). ns, *, **, *** Nonsignificant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively. All data are expressed as mean $\pm$ SD, $n = 3$.

Source of Variance	NO_3^- (mg Kg^{-1} FW)	N-Kjeldahl (%)	Proteins (mg g^{-1} DW)	Starch (mg g^{-1} DW)	TNSCs (mg g^{-1} DW)
Soil (S)					
Loam	585.8 $\pm$ 258.5	2.32 $\pm$ 0.45	63.53 $\pm$ 9.70	1.49 $\pm$ 0.34	23.73 $\pm$ 2.40
Clayey	176.8 $\pm$ 104.6	2.49 $\pm$ 0.59	49.34 $\pm$ 10.10	1.79 $\pm$ 0.59	22.00 $\pm$ 3.11
	***	ns	**	***	ns
Fertilization (F)					
Control (NF)	183.5 $\pm$ 124.6 c	2.44 $\pm$ 0.38	55.07 $\pm$ 16.49 b	1.69 $\pm$ 0.46	23.26 $\pm$ 2.05
Vermicompost (VC)	392.0 $\pm$ 284.9 b	2.26 $\pm$ 0.41	51.72 $\pm$ 4.82 c	1.70 $\pm$ 0.54	23.42 $\pm$ 2.99
Mineral (MIN)	568.3 $\pm$ 331.5 a	2.52 $\pm$ 0.47	62.53 $\pm$ 10.14 a	1.55 $\pm$ 0.38	21.91 $\pm$ 1.90
	*	ns	***	ns	ns
S $\times$ F					
Loam $\times$ NF	295.0 $\pm$ 37.2 d	2.51 $\pm$ 0.49 c	68.24 $\pm$ 6.30 b	1.30 $\pm$ 0.17	21.98 $\pm$ 1.49
Loam $\times$ VC	622.7 $\pm$ 200.1 b	2.60 $\pm$ 0.26 b	51.93 $\pm$ 5.35 d	1.44 $\pm$ 0.29	25.30 $\pm$ 2.58
Loam $\times$ MIN	839.7 $\pm$ 15.0 a	1.86 $\pm$ 0.05 f	70.43 $\pm$ 1.21 a	1.74 $\pm$ 0.45	23.91 $\pm$ 2.39
Clayey $\times$ NF	72.0 $\pm$ 11.14 f	2.37 $\pm$ 0.32 d	41.90 $\pm$ 10.94 f	2.07 $\pm$ 0.21	24.54 $\pm$ 1.84
Clayey $\times$ VC	161.3 $\pm$ 57.5 e	1.92 $\pm$ 0.06 e	51.51 $\pm$ 5.42 e	1.95 $\pm$ 0.67	21.55 $\pm$ 2.25
Clayey $\times$ MIN	297.0 $\pm$ 42.6 c	3.18 $\pm$ 0.20 a	54.62 $\pm$ 11.27 c	1.36 $\pm$ 0.68	19.90 $\pm$ 3.70
	*	***	*	ns	ns ($p < 0.1$)

The interaction $S \times F$ significantly influenced the total amino acids (total AAs), essential amino acids (EAAs), alanine (ALA), glutamate (GLU), glutamine (GLN), proline (PRO), and GABA (Figure 4), as well as the other free amino acids reported in Table S2. In general, MIN fertilization led to the highest amino acid accumulation, with consistent effects in both the loam and clayey soils. The total AAs and EAAs were the most responsive, increasing by 102% and 133%, respectively, under MIN. Loam favored more accumulation, with the quantities of ALA and GLU increasing by 65% and 103%, respectively, compared to those of the clayey soil. Among the individual amino acids, the PRO levels increased by 90% under MIN, particularly in the loam, while ALA and GABA also showed significant increases (Figure 4). GLN and GLU reached their highest levels in loam $\times$ MIN, confirming a strong

interaction between fertilization and the soil type. VC resulted in intermediate values, though its effect varied across the amino acids. Notably, the total AA levels in loam $\times$ VC were lower than those of NF, while in the clayey soil, the VC-treated plants showed 35% higher total AA concentrations than those of NF (Figure 4). The other amino acids showed distinct responses (Table S2). Asparagine and aspartate, involved in nitrogen metabolism and transport, reached their highest levels in loam $\times$ MIN (+240% and +280% vs. NF), while VC significantly increased their accumulation in the clayey soil (+75% and +50% vs. NF). The amino acids linked to stress responses, such as serine and glycine, followed a similar trend, increasing by 85% and 110% under MIN, with VC also increasing the level of glycine in the clayey soil (+58% vs. NF). The level of arginine and ornithine, key in nitrogen recycling, increased under MIN (+130% and +95% vs. NF), especially in the loam, while VC had a moderate effect in the clayey soil (+55% and +40% vs. NF). Among the essential amino acids, including the BCAAs and tryptophan, MIN induced the highest accumulation (BCAAs +130%, tryptophan +140% vs. NF), with VC leading to a moderate BCAA increase (+60% vs. NF) (Figure 4; Table S2).

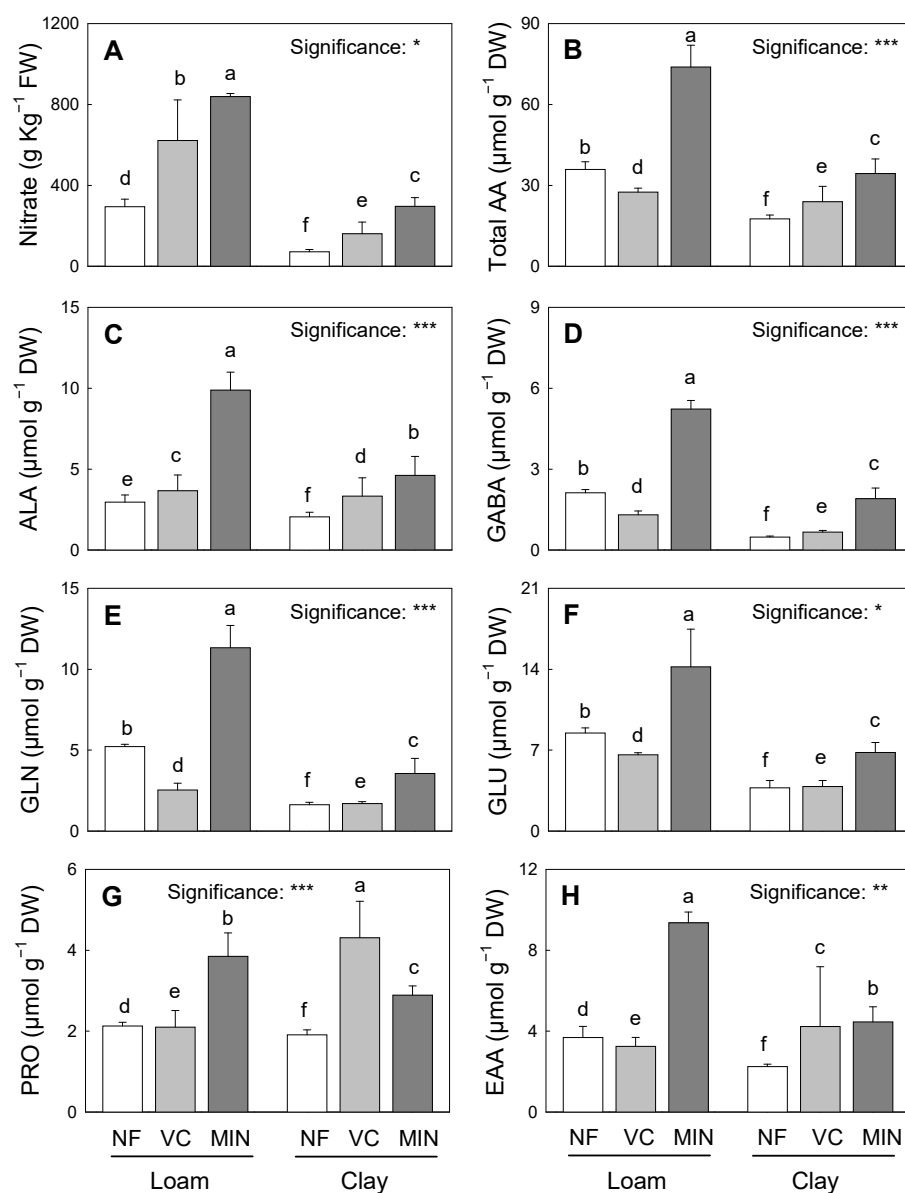


Figure 4. Effects of interaction between fertilization treatments (NF: no fertilization; VC: vermicompost; MIN: mineral fertilizer) and soil type (loam and clayey) on nitrate concentration (A), total amino

acids (total AAs) (B), alanine (ALA) (C), γ -aminobutyric acid (GABA) (D), glutamine (GLN) (E), glutamate (GLU) (F), proline (PRO) (G), and essential amino acids (EAAs) (H) in cauliflower (*Brassica oleracea* var. Freedom “Seminis”). Data are presented as mean $\pm$ SD ($n = 3$). Different lowercase letters above bars indicate significant differences between treatments according to Tukey’s HSD test ($p < 0.05$). Significance levels: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

3.5. Soil Type and Fertilization Were the Main Drivers of PCA Clustering

Principal component analysis (PCA) identified the primary sources of variability in the dataset, highlighting the effects of the soil type and the fertilization treatments (Figure 5). The first four principal components (PCs) had eigenvalues greater than one, accounting for 94.9% of the total variance. Specifically, PC1, PC2, PC3, and PC4 explained 61.9%, 16.2%, 8.8%, and 8.0% of the variance, respectively. PC1 was positively correlated with the total amino acids, including alanine, arginine, asparagine, glutamate, and proline, along with the essential amino acids and the branched-chain amino acids (BCAAs). These variables were also associated with the total chlorophylls, the nitrate content, and the SPAD index. Notably, the MIN treatments clustered along the positive side of PC1, indicating their strong impact on nitrogen assimilation and amino acid metabolism. PC2 primarily reflected the soil effects, separating the clayey and loam soils. Variables such as starch and N-Kjeldahl nitrogen were positively associated with PC2 under the VC treatments, whereas the proline and corymb morphometric parameters (fresh weight, diameter, and height) were prominent under the MIN treatments. The negative associations with PC2 included antioxidant-related variables, such as ABTS and polyphenols. The PCA biplot reveals the clear clustering of fertilization treatments within the specific soil types. The MIN treatments are associated with enhanced corymb metrics and amino acid synthesis, while VC is linked to balanced nitrogen levels and the antioxidant properties. The non-fertilized control samples show weaker associations with the productivity and metabolic markers.

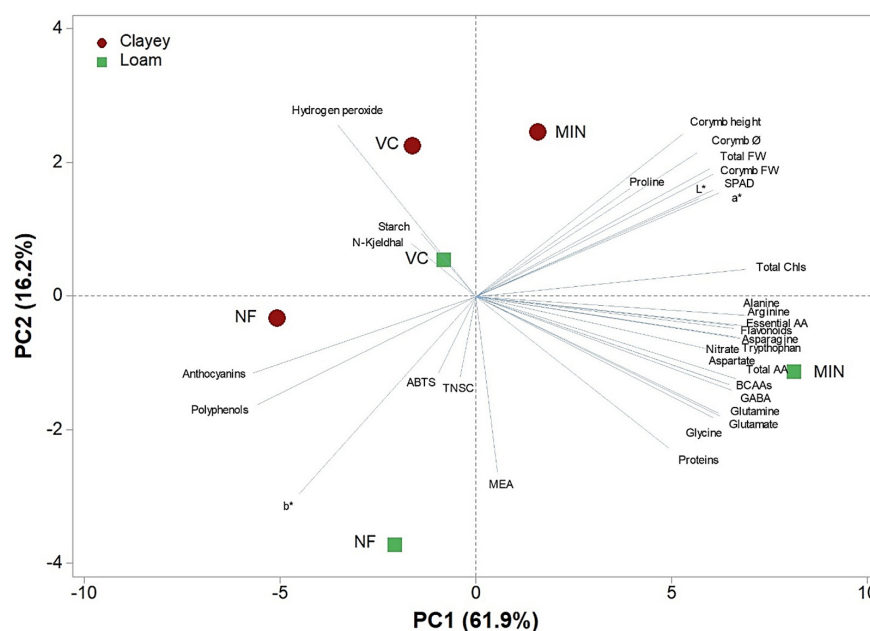


Figure 5. Principal component loading plot and scores of principal component analysis (PCA) of analyzed parameters in cauliflower (*Brassica oleracea* var. Freedom “Seminis”), as influenced by fertilization treatments (NF: no fertilization; VC: vermicompost; MIN: mineral fertilizer) and soil type (loam and clayey). PC1 and PC2 represent first and second principal components, linear combinations of original variables and account for 61.9% and 16.2% of total variance, respectively. These components summarize main patterns of variation in dataset, allowing for dimensionality reduction and visualization of treatment effects. All variables included in PCA are significant for at least one factor (soil type, fertilization, or their interaction).

4. Discussion

The Campania region in Southern Italy faces considerable challenges related to nitrate pollution, particularly in its coastal plains, where the nitrate concentrations in groundwater have occasionally exceeded 200 mg L^{-1} . This issue is predominantly linked to intensive agricultural practices, urban expansion, and the extensive application of chemical fertilizers [29]. To address this problem, the region has identified specific nitrate-vulnerable zones (NVZs) and adopted mitigation measures in accordance with the European Union directives [30,31]. To strengthen its approach and address nitrate pollution in vulnerable zones, the Campania Regional Government implemented a new policy in 2023 for the agronomic use of livestock effluents, wastewater, and digestates [8]. This policy replaced the 2020 one and introduced several updates, including the expansion of no-spread zones near water bodies with poor ecological status, the adoption of more efficient fertilization techniques adapted to land slopes, the mandatory analysis of nitrate levels in irrigation water for fertilization planning, and the requirement for farmers to grow cover crops during the autumn–winter seasons on specific agricultural land.

In the context of Campania's NVZs, where groundwater contamination poses a persistent threat, water buffalo manure, a by-product of the region's robust dairy industry, represents both a challenge and an opportunity. As a potential source of pollution, improperly managed buffalo manure could exacerbate nitrate leaching; however, its transformation through vermicomposting may offer benefits. In fact, this study demonstrated that vermicomposting effectively transformed raw water buffalo manure into a high-quality organic amendment and biofertilizer, meeting the regulatory standards. Using *Eisenia fetida* and *Eisenia andrei*, the manure stabilized through aerobic oxidation and was converted into nutrient-rich vermicompost in just 6–12 weeks. The process was fine-tuned with strategic manure distribution to prevent nitrogen loss, the regular monitoring of pH and temperature, and systematic harvesting to ensure consistency and quality. This vermicompost, naturally enriched with macro- and micronutrients, beneficial microbes, and plant growth regulators, was evaluated in experimental trials on cauliflower, highlighting its potential as a dual-purpose amendment and biofertilizer for sustainable agriculture in nitrate-sensitive regions. The consequent field study underscored the comparative benefits of vermicomposting and mineral fertilization in improving yield, nitrogen assimilation, and crop quality in cauliflower, while also stressing their differential performances across the soil types.

Mineral fertilization (MIN) resulted in the highest yields due to the immediate availability of nutrients, particularly nitrogen. However, this increase in productivity came with higher nitrate accumulation, raising concerns about environmental sustainability and food safety. In contrast, vermicompost (VC) provided a more gradual nutrient release, leading to intermediate yields, but offering significant benefits in terms of soil quality and reduced nitrate leaching. These findings align with the previous studies on organic amendments, which have consistently shown that VC enhances soil fertility and structure over time [32,33]. Fertilization also significantly influences soil characteristics, which are crucial for long-term agricultural sustainability. Our analysis revealed that VC application improved the soil organic matter content, particularly in the clay soils, where it increased by 59% compared to that of NF and around 8% compared to that of MIN. In the loam soils, the increment is smaller, with values equal to around 17% and 3.8%, respectively. Organic matter enrichment enhances soil structure, moisture retention, and microbial activity, fostering a healthier rhizosphere. This result is consistent with previous research indicating that vermicompost promotes microbial diversity and enzymatic activity, leading to improved nutrient cycling [34]. Additionally, the N-Kjeldahl levels rose more consistently under VC, indicating improved nitrogen stabilization and a reduced risk of leaching

losses. These effects were more pronounced in the clay soils, as it is well known that VC enhances soil aeration and microbial diversity [14,34], counteracting the typical compaction associated with a high clay content. In contrast, in the loam soils, VC helped maintain a balanced soil structure, while enhancing nutrient retention, demonstrating its adaptability across different soil textures. These findings are aligned with the previous studies indicating that VC is particularly beneficial in improving soil tilth and structure in fine-textured soils [35]. The impact of fertilization on nitrogen dynamics was particularly evident in the accumulation of nitrate nitrogen ($\text{NO}_3\text{-N}$). While MIN application caused a sharp rise in $\text{NO}_3\text{-N}$ concentrations, especially in the clay soils, the VC treatment resulted in more moderate nitrate levels. This suggests that vermicompost enhances nitrogen retention in organic forms, mitigating the risk of nitrate leaching, one of the primary concerns in NVZs. The previous studies have also shown that organic amendments, including VC, reduce nitrate leaching by improving nitrogen use efficiency. Additionally, the level of ammonium nitrogen ($\text{NH}_4\text{-N}$) decreased under the VC treatment in both the soil types, likely due to enhanced microbial nitrification and the slower mineralization of organic nitrogen sources. While MIN ensured higher productivity, it is crucial to consider its impact on both soil and plant nitrate levels. Excessive nitrogen application can lead to elevated nitrate content in vegetables, posing potential health risks such as the formation of carcinogenic nitrosamines [36]. Our data confirm that the nitrate level in the MIN-treated corymbs was significantly higher than in those fertilized with VC, reinforcing the previous findings [37]. Some studies on nitrate accumulation in leafy vegetables suggest that controlled nitrogen supply through organic fertilization can significantly reduce the nitrate levels, while maintaining productivity [15]. Cauliflower is generally considered a low-nitrate vegetable, with typical concentrations below $500 \text{ mg NO}_3\text{- kg}^{-1}$. However, other studies have reported a wide variability in nitrate content, ranging from 145.9 to $1124 \text{ mg NO}_3\text{- kg}^{-1}$, depending on the cultivar, the soil characteristics, the fertilization methods, and environmental factors [38,39]. The current European Union regulations (e.g., Regulation (EC) No 1881/2006) set nitrate limits for specific leafy vegetables, such as spinach, lettuce, and rocket, but no explicit threshold has been established for cauliflower. Instead, nitrate levels are assessed through broader food safety classifications and regional evaluations. Given this regulatory gap, adopting sustainable fertilization strategies, such as integrating VC with reduced mineral fertilization is essential to control nitrate levels, ensuring both food safety and environmental sustainability, particularly in NVZs [15,40,41].

The physiological response of plants to fertilization was also evident in the chlorophyll content and nitrogen assimilation. MIN significantly improved the SPAD index, reflecting enhanced photosynthetic efficiency. However, this rapid nitrogen uptake resulted in the accumulation of free amino acids, such as alanine and gamma-aminobutyric acid (GABA), suggesting that the plants redirected excess ammonium to maintain cellular pH and mitigate oxidative stress [42]. While this metabolic adjustment helps avoid ammonium toxicity, excessive nitrogen availability can still lead to chlorosis, reduced growth, and impaired root development [43]. In contrast, VC facilitated a more gradual nitrogen uptake, reducing metabolic stress and promoting steady growth patterns. Moreover, we observed a significant increase in free amino acids, particularly proline and glutamate, under the VC treatment. These amino acids play essential roles in osmoprotection, stress resilience, and flavor enhancement. Additionally, GABA, which acts as a key regulator in nitrogen metabolism and stress responses [44], showed increased accumulation under VC, suggesting improved nitrogen efficiency and adaptive responses in the plants. Similar physiological and metabolic benefits have also been reported in strawberry plants grown with vermicompost, where enhanced photosynthetic activity, chlorophyll contents, and antioxidant enzyme function were accompanied by increased fruit quality parameters such

as vitamin C and soluble sugars. These improvements were closely linked to elevated root activity and microbial-driven changes in the soil environment, supporting the idea that VC contributes to both the stress regulation and nutritional enhancement of crops [45]. The metabolic changes associated with fertilization were also reflected in polyphenol accumulation and antioxidant activity. The NF-treated plants exhibited the highest polyphenol content, while MIN reduced the accumulation of these secondary metabolites. On the other hand, H_2O_2 accumulation was markedly influenced by fertilization, with the VC-treated plants exhibiting the highest H_2O_2 levels, nearly doubling those observed in the MIN and NF treatments. This trend suggests that organic amendments may stimulate a controlled oxidative response, potentially linked to enhanced stress resilience and metabolic activity. For instance, the application of organic amendments has been shown to improve plant stress tolerance by enhancing antioxidant systems and reducing oxidative damage [46]. Similarly, the co-application of organic amendments and natural biostimulants has been reported to alleviate stress and improve soil properties and microbial structures, thereby enhancing plant resilience [47]. Similar findings were reported for *Vicia faba*, where vermicompost application, particularly when combined with *Rhizobium* inoculation, significantly enhanced plant height, leaf number, and total biomass under greenhouse conditions in Ethiopia [48]. Interestingly, the VC-treated plants in the loam soil exhibited the highest antioxidant capacity, as indicated by ABTS values significantly higher than those recorded under the other two treatments. This suggests that organic amendments in loam soil may enhance antioxidant potential and defense capacity, as also suggested by the lower proline levels, a symptom of reduced physiological stress, aligning with the higher ABTS antioxidant capacity observed. Since proline often accumulates under stress conditions, lower levels indicate that the plants experienced a more favorable environment, likely due to a more balanced nutrient supply and microbial activity from vermicompost. Similar effects have been reported in *Vicia faba* plants under salt stress, where vermicompost application significantly reduced oxidative stress markers and lowered proline accumulation compared to those of untreated controls. These findings support the hypothesis that VC mitigates stress-induced metabolic imbalances, enhancing plant resilience even under suboptimal conditions [49].

From a regulatory perspective, intensifying the restrictions in NVZs necessitates the integration of sustainable fertilization practices. With the European policies progressively lowering the nitrogen application limits (EC Directive 91/676/EEC), a shift toward organic amendments such as VC may become essential for compliance while maintaining soil productivity. Some studies on NVZ management strategies indicate that a combination of organic amendments and cover crops is the most effective way to reduce nitrogen leaching, while preserving soil fertility [39]. Adopting VC as a standalone fertilizer or combined with reduced mineral inputs could offer a viable strategy to balance productivity with environmental responsibility. In addition to environmental and agronomic considerations, consumer preferences are an essential aspect of fertilization strategies. While high productivity is the primary goal, marketability increasingly depends on attributes such as texture, flavor, and post-harvest stability. Organic amendments like VC have been shown to enhance sensory properties, antioxidant potential, and health benefits. For instance, a study on guava (*Psidium guajava* L.) reported that the combined application of VC, biochar, and jaggery significantly increased the content of antioxidants, phenols, and flavonoids in fruit, which are associated with improved taste and nutritional value [50]. Similarly, the use of sheep manure vermicompost in tomato cultivation has been shown to enhance plant height and improve fruit quality traits, such as soluble and insoluble solid and carbohydrate concentrations, while also reducing fruit acidity and soil PH. These findings support the

role of VC in modulating not only yield components, but also key nutritional and sensory attributes of edible plant products [51].

Visual quality is a key determinant of consumer preference for cauliflower, where attributes such as brightness, uniformity, and the absence of yellowing play a crucial role in marketability. Our findings suggest that fertilization strategies significantly influence these traits, with the MIN-treated plants exhibiting the brightest and most uniform curds, while the VC-treated plants displayed intermediate visual quality, and the NF curds were more prone to dullness or slight discoloration. This aligns with research indicating that nitrogen availability can affect structural integrity and oxidative processes, influencing post-harvest appearance [52]. However, while bright, uniform curds are traditionally favored in commercial markets, consumer preferences are shifting. A growing segment of buyers prioritizes organic and low-impact farming methods, often accepting minor aesthetic imperfections and higher prices in exchange for perceived health and environmental benefits [53].

5. Conclusions

The successful transformation of buffalo manure into high-quality vermicompost (VC) emphasizes its potential as a sustainable soil amendment, particularly in nitrate-vulnerable regions such as Campania, Southern Italy, where intensive buffalo farming generates significant organic waste. Compared to those of the unfertilized control, VC significantly increased the total fresh biomass by 119%, the corymb diameter by 58%, the SPAD index by 40%, and the chlorophyll content by 13%, confirming its beneficial effects on crop growth and physiology. VC enhanced amino acid accumulation in the clayey soil, leading to a 35% increase in total amino acids and a 60% increase in BCAAs over those of the control.

While mineral fertilization (MIN) produced the highest yields, it also caused a marked accumulation of nitrates in edible tissues, with the concentrations 45% higher than under VC and over 210% higher than those of the control. In contrast, VC maintained lower nitrate levels, indicating more balanced nitrogen assimilation. At the soil level, VC improved the organic matter content by +23% in the loam and +37% in the clayey soil and increased the total Kjeldahl nitrogen by +27%, confirming its contribution to long-term soil fertility. These results support the integration of vermicompost into fertilization strategies as an environmentally sound alternative to mineral inputs, particularly in livestock-intensive and nitrate-regulated agroecosystems. Overall, vermicompost emerged as a valuable solution for manure valorization, improving yield and crop quality, while reducing the environmental risks. Its use aligns with the principles of the circular economy and offers a viable alternative to mineral fertilizers, particularly in nitrate-sensitive agroecosystems.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/su17104253/s1>, Supplemental File S1: Certified analysis of buffalo manure; Supplemental File S2: Certified analysis of vermicompost; Table S1: Flavonoids, polyphenols, H₂O₂, and antioxidant activity in cauliflower under Fertilization × Soil interaction; Table S2: Amino acid profile in cauliflower under Fertilization × Soil interaction.

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Conflicts of Interest: Author Fulvio Trasacco was employed by the company BIOS MIMESIS Società Agricola Innovativa S.r.l. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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