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Circular Fertilization Strategy Using Sulphur with Orange Waste Enhances Soil Health and Broccoli Nutritional and Nutraceutical Quality in Mediterranean Systems

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Abstract

Fertilization strategies are pivotal in sustainable agriculture, affecting both soil health and crop quality. This study investigated the impact of a circular fertilization approach based on agro-industrial residues—specifically a blend of sulphur bentonite and orange processing waste (RecOrgFert PLUS)—on soil physicochemical and biological properties, as well as the nutritional and nutraceutical quality of broccoli (*Brassica oleracea* var. italica) grown in Mediterranean conditions (Condofuri, Southern Italy). The effects of RecOrgFert PLUS were compared with those of a synthetic NPK fertilizer, an organic fertilizer (horse manure), and an unfertilized control. Results demonstrated that RecOrgFert PLUS significantly improved soil organic carbon (3.37%), microbial biomass carbon (791 μg C g^{-1}), and key enzymatic activities, indicating enhanced soil biological functioning. Broccoli cultivated under RecOrgFert PLUS also exhibited the highest concentrations of health-promoting compounds, including total phenols (48.87 mg GAE g^{-1}), vitamin C (51.93 mg ASA 100 g^{-1}), and total proteins (82.45 mg BSA g^{-1}). This work provides novel evidence that combining elemental sulphur with orange processing waste not only restores soil fertility but also boosts the nutraceutical and nutritional value of food crops. Unlike previous studies focusing on soil or plant yield alone, this study uniquely integrates soil health indicators with bioactive compound accumulation in broccoli, highlighting the potential of circular bio-based fertilization in functional food production and Mediterranean agroecosystem sustainability.

Keywords: Agro-environmental sustainability; sustainable fertilizers; broccoli quality; soil quality

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1. Introduction

Soil fertility is a cornerstone of sustainable agriculture, directly influencing plant productivity, nutrient availability, and the diversity and function of soil microbial communities [1,2]. In recent decades, the global push to increase food production has driven the intensification of agricultural practices, often through a heavy reliance on synthetic fertilizers. While these inputs have contributed to increased yields, their long-term use has led to negative consequences including soil degradation, nutrient imbalances, suppression of beneficial microorganisms, and environmental issues such as nitrate leaching and greenhouse gas emissions [3,4]. To address these challenges, sustainable soil fertility management practices are urgently needed. A promising approach involves the use of organic fertilizers—such as compost, and animal manures—which not only supply nutrients but also improve soil structure, increase organic matter, and stimulate microbial activity [2,5,6]. Organic fertilizers typically release nutrients more slowly than synthetic counterparts, enhancing nutrient use efficiency while reducing the risk of leaching and volatilization losses [7]. Among these organic amendments, those derived from agricultural wastes or biochar—are gaining recognition for their dual role in improving soil fertility and recycling organic wastes [5,8]. These inputs have been shown to enhance soil water retention, aeration, cation exchange capacity, and microbial biomass, which are particularly beneficial in Mediterranean agroecosystems where climatic and edaphic constraints often limit productivity [2,5]. Numerous studies have demonstrated that long-term balanced fertilization—particularly when incorporating sulphur (S) alongside primary macronutrients such as nitrogen (N), phosphorus (P), and potassium (K)—can significantly reduce nitrous oxide (N_2O) emissions without compromising crop productivity, thereby contributing to more sustainable agricultural systems [4,9,10]. These findings highlighted the critical role of elemental Sulphur not only in mitigating greenhouse gas emissions but also in maintaining and enhancing soil fertility. Sulphur is increasingly recognized as an essential nutrient for optimal soil function and plant health. It plays a key role in enzymatic reactions, protein synthesis, and the mobilization of vital nutrients such as N, P, and K, thereby improving nutrient uptake efficiency and overall soil-plant nutrient dynamics [3,7]. Despite its importance, sulphur has often been overlooked in fertilization regimes, largely due to the historic reliance on atmospheric deposition from industrial pollution to meet crop requirements. However, modern agricultural practices—including intensified cultivation, reduced use of organic amendments, widespread adoption of sulphur-free synthetic fertilizers, and significant declines in atmospheric sulphur deposition due to cleaner air policies—have collectively led to widespread sulphur deficiency in many arable soils [8,11]. This deficiency is increasingly associated with reduced crop yields, poor nutrient balance, and diminished soil microbial activity. Consequently, there is a growing consensus that sulphur should be re-evaluated and integrated more systematically into sustainable nutrient management strategies, particularly in regions like the Mediterranean where soil degradation and nutrient imbalances are common. The incorporation of elemental sulphur or sulphur-enriched organic amendments can not only restore sulphur levels but also stimulate microbial communities responsible for sulphur oxidation and nutrient mineralization, enhancing both soil health and plant resilience [4,5]. Thus, sulphur fertilization should be considered a critical component of holistic soil fertility management aimed at improving agroecosystem sustainability, nutrient use efficiency, and environmental stewardship. In addition to improving soil health, fertilization strategies also influence the nutritional and functional quality of crops. Broccoli (*Brassica oleracea* var. *italica*), a widely consumed vegetable in Mediterranean regions, is highly valued for its content of antioxidants, vitamins, and glucosinolates, and is particularly sensitive to soil nutrient dynamics [12,13]. Several studies have shown that organic fertilization can increase the accumulation of health-promoting phytochemicals such as phenolic compounds, and vitamin C in broccoli, thereby improving its nutritional value [13–15]. Moreover, the influence of sulphur (S) fertilization on the accumulation of glucosinolates and other sulphur-containing compounds in *Brassica* species (e.g., broccoli, cabbage, kale, mustard, rapeseed) is well-documented. Sulphur is not just a nutrient—it's a precursor and regulator in the biosynthesis of key secondary metabolites containing sulphur, which are associated with plant defence and human health benefits (e.g., anticancer effects). This is particularly relevant in Mediterranean systems, where climate variability and limited water resources demand resilient cropping systems supported by robust soil microbial networks.

Given the multifaceted benefits that can come by the integration of organic component with sulphur in the optical of sustainable fertilization strategy that is able to increase the health properties of broccoli this study aims to compare the effects of synthetic fertilizers (NPK), horse manure (HM), and a mineral-organic fertilizer (RecOrgFert PLUS) coming from elemental S, residue of hydrocarbon refining processes, and orange waste as residues of food industry on key soil parameters, microbial activity, and nutritional value of broccoli. Previous research by Muscolo et al. [8] using sulphur bentonite plus orange waste primarily emphasized the improvements in soil fertility and nutrient cycling and enhanced plant yield and quality in other crops. This is the first study that tested the efficiency of this new fertilizer in a field scale putting in relation soil biochemical properties related to soil health with the nutraceutical compounds responsible for broccoli quality. By integrating soil health indicators with the nutritional quality parameters of broccoli, this research seeks to provide insights into the potential of waste-based fertilizer as viable alternatives to conventional fertilization practices. The findings contribute to the ongoing debate on sustainable agriculture, highlighting the importance of waste recycle for fertilization strategies to enhance both soil ecosystem function and food quality.

2. Materials and Methods

2.1. Soil Treatment

The experimental trial was carried out in Falcone Farm Condofuri, Reggio Calabria, located at 339 meters above sea level. The soil at the site was sandy loam with a texture composition of 88% sand, 9% silt, and 3% clay, according to the FAO system criteria [16].

The experimental area covered a total of two hectares, subdivided into plots of 20 square meters each. Different fertilization treatments were applied across the plots, with each treatment replicated six times to ensure the reliability and statistical robustness of the results. The fertilization treatments included an unfertilized control soil (CTR), serving as the baseline for comparison; an organic treatment based on horse manure (HM), applied at a rate of 4.300 kilograms per hectare; a synthetic fertilizer with an nitrogen-phosphorous and potassium (NPK) formulation of 20:10:10, distributed at 1.700 kilograms per hectare; and waste based fertilizer (RecOrgFert PLUS) produced recycling elemental sulphur, residue of hydrocarbon refining processes, and orange residues of food industry named RecOrgFert PLUS. This latter formulation was applied at a dose of 476 kilograms per hectare. These treatments were selected to compare traditional, synthetic, and novel sustainable fertilization strategies on Calabrian sprouting broccoli.

2.2. Chemical and Biochemical Soil Analysis

Texture was determined using the hydrometer method of Bouyoucos [17]. Soil moisture content was quantified by oven-drying the samples at 105°C, following the standard method proposed by AOAC [18]. Electrical conductivity (EC) was assessed using a Hanna Instruments conductivity meter on a soil-to-water suspension prepared at a 1:5 ratio and mechanically stirred at 15 rpm for one hour. Soil pH was measured in distilled water using a 1:2.5 soil-to-solution ratio and a glass electrode. Organic carbon and total nitrogen were determined using a LECO CN628 elemental analyser, and the resulting carbon values were converted into organic matter by multiplying for an empiric value of 1.72. The carbon-to-nitrogen (C/N) ratio was then calculated. In addition, cation exchange capacity (CEC) was determined according to the method of Mehlich et al. [19].

Water-soluble total phenols, including both monomeric and polymeric forms, were determined using the Folin-Ciocalteu reagent [20], with gallic acid used as a standard. Results were expressed in terms of gallic acid equivalents (µg GAE per gram of dry weight). Dehydrogenase activity (DHA) was measured using iodonitrotetrazolium chloride, according to the method of von Mersi and Schinner [21]. Catalase activity was assessed volumetrically by quantifying the oxygen released after incubating the soil with hydrogen peroxide for three minutes, as described by Beck [22]. Fluorescein diacetate hydrolase (FDA) activity, which provides a general indication of enzymatic potential in the soil, was analysed following the procedure reported by Dick et al. [23]. Microbial biomass carbon (MBC) was quantified via the chloroform fumigation–extraction method outlined by Vance et al. [24], using field-moist samples equivalent to 20 g dry weight. Both fumigated and unfumigated soil extracts were analysed for soluble organic carbon using the Walkley–Black method [25]. MBC was estimated by calculating the difference between the organic carbon extracted from fumigated and unfumigated soils, applying an extraction efficiency coefficient of 0.38 to convert soluble carbon into biomass carbon [24]. Cations and anions were identified using ion chromatography (DIONEX ICS-1100). For anion analysis, 0.5 g of dried sample was mixed with 50 ml of an anionic extracting solution (3.5 mM $Na_2CO_3/NaHCO_3$) and stirred for 20 minutes. The resulting extract was then filtered before

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undergoing chromatographic analysis. To determine cation content, 1 g of dried sample was incinerated at 550 °C for 5–6 hours in a porcelain crucible. The resulting ash was digested in 1 M HCl at 100 °C for 30 minutes. This solution was filtered and analysed via ion chromatography using a 20 mM methanesulfonic acid eluent.

2.3. Soil Fauna

Soil samples were collected from the designated areas and analysed using the Berlese-Tüllgren extractor, following the method of Bano e Roy [26]. One kilogram of soil was placed in a sieve with a height of sixty millimeters and a diameter of two hundred and twenty millimeters, featuring a two-millimeter mesh, positioned above a funnel that directed the microarthropods into a container filled with an alcohol and glycerin solution in a 2:1 ratio. The extractor was placed under a heat source, a 40- or 60-watt light bulb located twenty centimeters away and kept in operation for ten days to facilitate the migration of microarthropods into the container. The collected specimens were then examined under a stereomicroscope with magnifications ranging from 20X to 40X, identifying the Biological Forms and assigning them a score based on Ecomorphological Indices. In cases where multiple Biological Forms belonged to the same group, only the one with the highest score was recorded. Identification was carried out using a dichotomous key by Angelini et al. [27].

2.4. Broccoli nutritional and nutraceutical analysis

The total phenol content was determined using the Folin-Ciocalteu reagent [20], following the procedure described by Velioglu et al. [28]. Total flavonoid content was measured using a colorimetric method based on aluminium chloride, according to the protocol developed by Djeridane et al. [29]. To assess antioxidant activity, two complementary methods were employed. The ABTS+ radical cation decolorization assay was conducted following the method of Re et al. [30], while the DPPH method, which relies on the stable free radical 2,2-diphenyl-1-picrylhydrazyl, was performed according to Barreca et al. [31]. Total antioxidant capacity (TAC) was quantified based on the method described by Prieto et al. [32]. Vitamin E amount was detected following the Prieto et al. [32] method. The concentration of vitamin C (ascorbic acid) was determined using the colorimetric method as described by Davies and Masten [33]. Total protein has been detected using the Bradford method, following the procedure described by Kruger [34]. Total carbohydrates have been measured using the phenol-sulphuric acid method according to Hedge et al. [35].

2.5. Statistical Analysis

Analysis of variance (ANOVA) was performed for all datasets. One-way ANOVA followed by Tukey's honestly significant difference (HSD) test was used to evaluate the effects of fertilizers on the various measured parameters. Pairwise comparisons were conducted to assess the effects of fertilizers on each individual parameter. To explore the relationships between different fertilizers and broccoli parameters, the datasets were analysed using principal component analysis (PCA). In addition, Pearson's correlation analysis was carried out to assess the strength and direction of linear associations among the considered parameters. Statistical analyses were performed using MATLAB (version R2024b, The MathWorks Inc., Natick, MA, USA). Effects were considered significant at $p \leq 0.05$.

3. Results

3.1. Soil Physicochemical and Biochemical Properties

Table 1 shows the soil characteristics prior to the application of fertilisation, reported in in the first column as time 0 (T0). The low values of water content (8.25%), organic carbon (1.13%), cation exchange capacity (15.0 cmol (+) kg^{-1} ds), and microbial biomass carbon (205 μg C g^{-1}) reflect a condition of limited soil fertility. Fertilisation significantly affected the physico-chemical and

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biochemical properties of the soil, with variable impacts depending on the type of amendment applied.

Table 1. Chemical and biochemical properties of soil located in Condofuri: T0 = time zero; CTR (control) soil without fertilizer; NPK = nitrogen – phosphorus – potassium; HM = horse manure; RecOrgFert PLUS = bentonite sulphur + orange pomace. WC = water content (%); pH; EC = electrical conductivity (dS/m); OC = organic carbon (%); TN = total nitrogen (%); C/N = carbon-nitrogen ratio; OM = organic matter (%); WSP = water-soluble phenols (μg TAE g^{-1} ds); CEC = cation exchange capacity (cmol(+) kg^{-1} ds); DHA = dehydrogenase (μg TTF g^{-1} h^{-1} ds); FDA = fluorescein diacetate hydrolase (μg fluorescein g^{-1} ds); CAT = catalase (O2%/3min/g); MBC = microbial biomass carbon (μg C g^{-1} fs). Data are the means of three replicates ± standard deviation. Different letters in the same row indicate significant differences (Turkey's test, $p \leq 0.05$).

	T0	CTR	NPK	HM	RecOrgFert PLUS
WC	$8.25^c \pm 0.91$	$10.1^b \pm 0.98$	$14.2^a \pm 1.1$	$13.4^a \pm 1.1$	$14.5^a \pm 0.97$
pH	$8.16^a \pm 0.09$	$8.30^a \pm 0.1$	$8.04^b \pm 0.1$	$8.25^a \pm 0.09$	$7.5^c \pm 0.1$
EC	$0.10^c \pm 0.09$	$0.40^b \pm 0.09$	$1.08^a \pm 0.1$	$1.22^a \pm 0.08$	$1.16^a \pm 0.08$
OC	$1.13^d \pm 0.18$	$1.17^d \pm 0.18$	$2.3^c \pm 0.2$	$2.54^b \pm 0.18$	$3.37^a \pm 0.17$
TN	$1.09^a \pm 0.02$	$0.11^d \pm 0.02$	$0.14^c \pm 0.01$	$0.162^b \pm 0.01$	$0.194^b \pm 0.01$
C/N	$1.04^c \pm 1$	$10.6^b \pm 0.9$	$16.5^a \pm 1$	$15.7^a \pm 0.9$	$17.4^a \pm 1$
OM	$1.95^d \pm 0.28$	$2.02^d \pm 0.35$	$3.99^c \pm 0.3$	$4.39^b \pm 0.26$	$5.8^a \pm 0.32$
WSP	$23.3^c \pm 1.7$	$26.7^b \pm 2.0$	$27.5^b \pm 1.6$	$33.4^a \pm 1.7$	$35.9^a \pm 2.0$
CEC	$15.0^b \pm 1$	$16.5^a \pm 1$	$17.1^a \pm 0.98$	$17.3^a \pm 0.97$	$17.1^a \pm 0.85$
DHA	$1.4^b \pm 0.28$	$1.1^b \pm 0.29$	$2.0^a \pm 0.3$	$1.6^b \pm 0.3$	$2.4^a \pm 0.25$
FDA	$13.3^b \pm 0.51$	$14.3^b \pm 0.48$	$15.3^a \pm 0.46$	$15.4^a \pm 0.53$	$16.3^a \pm 0.55$
CAT	$1.3^c \pm 0.33$	$3.3^a \pm 0.27$	$3.2^a \pm 0.29$	$2.1^b \pm 0.31$	$2.2^b \pm 0.33$
MBC	$205^c \pm 17.6$	$218^c \pm 19.1$	$684^b \pm 14.5$	$764^a \pm 14.2$	$791^a \pm 21.1$

Soil water content (WC) increased significantly in the presence of RecOrgFert PLUS (14.5%) and NPK (14.2%) compared to the control (10.1%). Organic carbon (OC) content also increased after fertilization, with RecOrgFert PLUS showing the highest value (3.37%), followed by horse manure (2.54%) and NPK (2.3%). All treatments resulted in significantly higher OC levels than the control (1.17%).

A similar trend was observed for total nitrogen (TN), which was highest in soils treated with RecOrgFert PLUS (0.194%), followed by horse manure (0.162%) and NPK (0.14%). The control soil had the lowest TN value (0.11%). Consequently, the C/N ratio was significantly influenced by treatments. The highest ratio (17.4) was observed in RecOrgFert PLUS-treated soil, suggesting a slower decomposition rate and potential long-term benefits for organic matter stability.

Soil enzymatic activities were also significantly affected by the type of fertilizer applied. DHA, a key indicator of microbial oxidative metabolism, was the highest in RecOrgFert PLUS (2.4 μg TTF g^{-1} h^{-1}), followed by NPK (2.0 μg TTF g^{-1} h^{-1}), HM (1.6 μg TTF g^{-1} h^{-1}), and CTR (1.1 μg TTF g^{-1} h^{-1}). Fluorescein diacetate hydrolase activity showed a similar trend, with RecOrgFert PLUS achieving the highest activity (16.3 μg fluorescein g^{-1}). Catalase activity was the highest in the control and NPK-treated soils. MBC was significantly improved by organic fertilization: RecOrgFert PLUS 791 μg C g^{-1} fs; HM 764 μg C g^{-1} fs; NPK 684 μg C g^{-1} fs; CTR 218 μg C g^{-1} fs.

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PCA analysis of the chemical and physical soil properties (Figure 1) treated with different fertilizers clearly showed that RecOrgFert PLUS generated a distinctive chemical profile that set it apart from all other treatments. In the quadrant 1 RecOrgFert PLUS highlighted a strong correlation with organic matter (OM) and CEC; NPK showed a light correlation with OM, HM correlated lightly with OM and CEC. In the quadrant 2 NPK correlated with pH, in the quadrant 3 a light correlation was observed between CTR and total nitrogen. In the quadrant 4 a light correlation between RecOrgFert PLUS and C/N, EC and water content. Similarly, the PCA of biological properties (Figure 2) revealed that RecOrgFert had a distinctive impact on soil enzyme activity. Its position in the quadrant 4 showed a strongly associated correlation with microbial biomass carbon (MBC) and DHA.

Pearson's correlation analysis (Figure 3) revealed two distinct functional networks within the soil system. The first encompasses parameters associated with organic fertility—such as organic matter content, water retention, carbon storage, and microbial biomass—which showed strong positive correlations, indicating synergistic interactions.

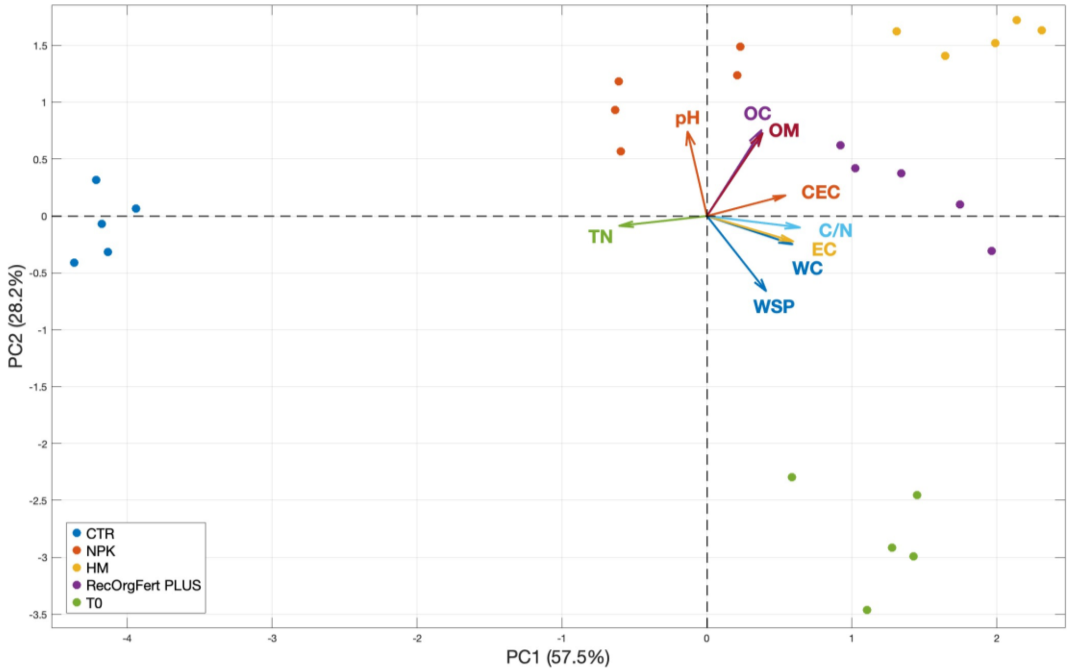

Figure 1. Principal component analysis (PCA) analysis of the chemical and physical properties of the different treatment: CTR (control) = soil without fertilizer; NPK = nitrogen – phosphorus – potassium; HM = horse manure; RecOrgFert PLUS = bentonite sulphur + orange pomace. WC = water content; pH; EC = electrical conductivity; OC = organic carbon; TN = total nitrogen; C/N = carbon-nitrogen ratio; OM = organic matter; WSP = water-soluble phenols; CEC = cation exchange capacity.

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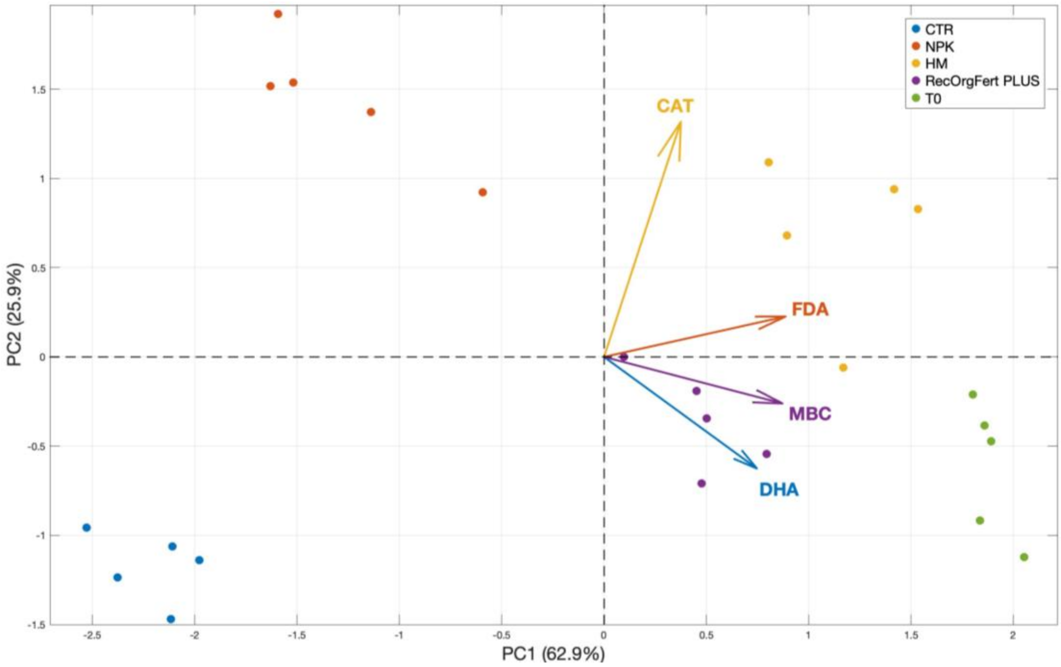

Figure 2. Principal component analysis (PCA) analysis of the biochemical properties of the different treatment: CTR (control) = soil without fertilizer; NPK = nitrogen – phosphorus – potassium; HM = horse manure; RecOrgFert PLUS = bentonite sulphur + orange pomace. DHA = dehydrogenase; FDA = fluorescein diacetate hydrolase; CAT = catalase; MBC = microbial biomass carbon.

Figure 3. Pearson correlation matrix illustrating the relationships between biochemical, chemical and physical soil parameters. The correlation coefficients range from -1 (strong negative correlation, in dark blue) to +1 (strong positive correlation, in yellow), with the colour scale visible on the right. WC = water content; pH; EC = electrical conductivity; OC = organic carbon; TN = total nitrogen; C/N = carbon-nitrogen ratio; OM = organic matter; WSP = water-soluble phenols; CEC = cation exchange capacity; DHA = dehydrogenase; FDA = fluorescein diacetate hydrolase; CAT = catalase; MBC = microbial biomass carbon.

3.2. Soil Cations and Anions

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Cation and anion concentrations varied across the treatments (Table 2). Sodium and potassium were the highest in soils treated with RecOrgFert PLUS, NPK, and HM. Calcium and Magnesium levels remained unchanged compared to the control. Among the anions SO_4^{2-} was the highest in soils treated with RecOrgFert PLUS. NO_3^- was detected only in soil treated with HM, PO_4^{3-}, although present in low amounts, was significantly higher in HM-treated soil compared to the others.

Table 2. T0 = time zero; CTR (control) soil without fertilizer; NPK = nitrogen – phosphorus – potassium; HM = horse manure; RecOrgFert PLUS = bentonite sulphur + orange pomace. Cations and anions concentrations (mg/L): Na^+ (sodium), K^+ (potassium), Ca^{2+} (calcium), Mg^{2+} (magnesium), Cl^-(chloride), NO^{2-} (nitrite), NO^{3-} (nitrate), PO_4^{3-} (phosphate), SO_4^{2-} (sulphate), Data are the means of three replicates ± standard deviation. Different letters in the same row indicate significant differences (Turkey's test$p \leq 0{,}05$).

Cations and anions	T0	CTR	NPK	HM	RecOrgFert PLUS
Na^+	0.012^b± 0.0003	0.016^b± 0.0003	0.025^a± 0.0003	0.024^a± 0.0002	0.026^a± 0.0003
K^+	0.013^b± 0.0003	0.012^b± 0.0003	0.027^a± 0.0003	0.025^a± 0.0002	0.030^a± 0.0003
Ca^{2+}	0.02^a± 0.005	0.03^a± 0.005	0.03^a± 0.005	0.03^a± 0.004	0.03^a± 0.005
Mg^{2+}	7.6^b ± 0.82	9.3^a ± 0.79	9.3^a ± 0.85	9.3^a ± 0.83	9.7^a ± 0.78
Cl^-	0.55^b ± 0.14	0.68^a ± 0.16	0.66^a ± 0.15	0.67^a ± 0.13	0.66^a ± 0.14
NO^{2-}	nd	0.001^b± 0.0001	0.007^a± 0.0001	0.001^b± 0.0001	0.007^a± 0.0001
NO^{3-}	nd	nd	nd	0.026^a± 0.0105	nd
PO_4^{3-}	nd	nd	0.002^b± 0.0001	0.006^a± 0.0001	0.002^b± 0.0001
SO_4^{2-}	0.001^c±0.0003	0.001^c± 0.0003	0.002^b± 0.0003	0.002^b± 0.0002	0.003^a± 0.0001

3.3. QBS-ar index

Soil Biodiversity, assessed through QBS-ar values (Figure 4), was significantly influenced by the type of fertilizer. Soils treated with RecOrgFert PLUS showed the highest QBS-ar scores, indicating a biologically more active and structured soil community compared to NPK and HM. The soil treated with NPK showed the lowest biodiversity index, inducing among the fertilizers used a less favourable environment for soil fauna.

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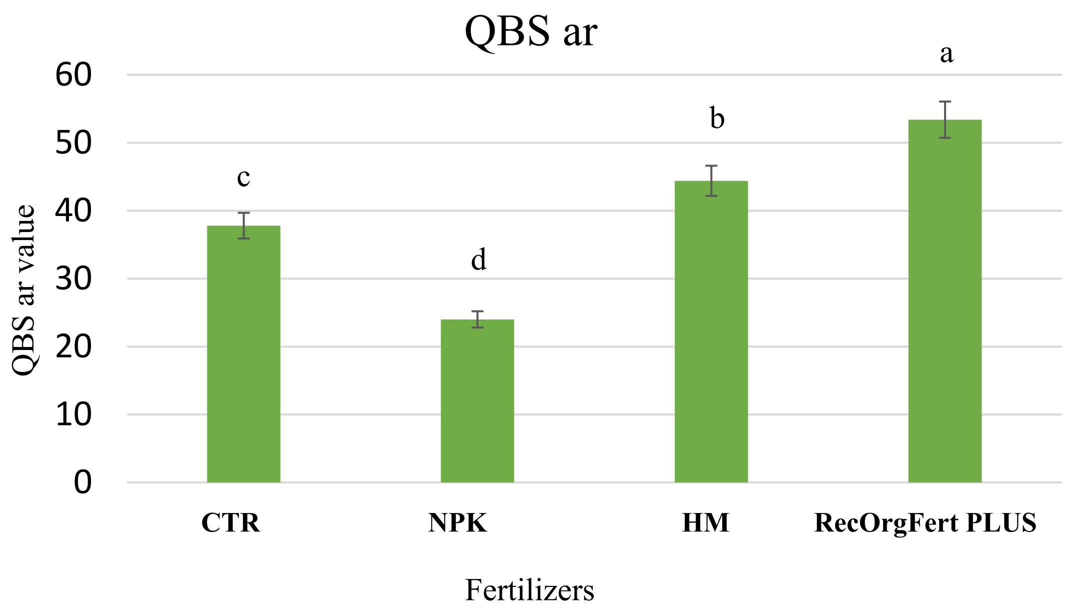

Figure 4. Figure 4 Shows the different QBS-ar values. CTR (control) = soil without fertilizer; NPK = nitrogen – phosphorous - potassium; HM = horse manure; RecOrgFert PLUS = bentonite sulphur + orange pomace. Data are the means of three replicates ± standard deviation. Different letters in the same row indicate significant differences (Turkey's test p ≤0,05).

3.4. Fertilization Effects on Broccoli Quality

Fertilization strategies significantly influenced the biochemical composition of broccoli (Table 3). Water content was not significantly affected by the treatments. Total phenolic content was the highest in broccoli treated with RecOrgFert PLUS (48.87 mg GAE g^{-1}), followed by HM (43.86 mg GAE g^{-1}), NPK (43.35 mg GAE g^{-1}), and CTR (42.09 mg GAE g^{-1}). Total flavonoid content was significantly higher in broccoli treated with RecOrgFert PLUS and HM, compared to CTR and NPK. DPPH antioxidant activity did not vary significantly among the treatments. However, $ABTS^+$ and TAC antioxidant capacities were significantly higher in broccoli treated with RecOrgFert PLUS and HM respect to CTR and NPK**.** Vitamin C content increased significantly compared to the control, reaching the highest levels in RecOrgFert PLUS (51.93 mg ASA 100 g^{-1}) and HM (50.97 mg ASA 100 g^{-1}) treatments. Vitamin E was higher in all fertilized treatments than in CTR. Total proteins did not change among the treatments. Total carbohydrate content was also the highest in broccoli treated with RecOrgFert PLUS and HM.

Table 3. Analysis of broccoli grown in the soils of Condofuri: CTR (control) soil without fertilizer; NPK = nitrogen – phosphorus – potassium; HM = horse manure; RecOrgFert PLUS = bentonite sulphur + orange pomace. WC = water content (%); DW = dry weight (%); TP = total phenols (mg GAE g^{-1}); TF = total flavonoids (mg QE g^{-1}); DPPH = 2,2-difenil-1-picrilidrazile (% inhibition); $ABTS^+$ = 2,2'-azino-bis-3-etilbenzotiazolin-6-solfonato (μM Trolox g^{-1}); TAC = total antioxidant capacity (mg α-tocopherol 100 g^{-1}); Vit C = vitamin C (mg ascorbic acid 100 g^{-1}); Vit E = vitamin E (mg α-tocopherol 100 g^{-1}), TPRO = total protein (mg Bovine Serum Albumin (BSA) g^{-1}), TCARB = total carbohydrates (mg glucose g^{-1}). Data are the means of three replicates ± standard error. Different letters in the same row indicate significant differences (Turkey's test, $p \leq 0.05$).

	CTR	NPK	HM	RecOrgFert PLUS
WC	90.65^a±2.1	89.14^a±2.5	87.24^a±2.4	86.32^a±2.6
DW	9.35^a±0.9	10.86^b±0.5	12.76^b±0.6	13.68^c±0.4

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TP	$42.09^{b}\pm1.8$	$43.35^{b}\pm1.9$	$43.86^{b}\pm1.9$	$48.87^{a}\pm2.1$
TF	$5.29^{b}\pm0.3$	$5.76^{b}\pm0.3$	$6.28^{a}\pm0.4$	$6.29^{a}\pm0.4$
DPPH	$23.25^{a}\pm1.2$	$23.54^{a}\pm1.2$	$23.37^{a}\pm1.2$	$24.85^{a}\pm1.3$
ABTS⁺	$3.39^{c}\pm0.2$	$4.37^{b}\pm0.2$	$5.26^{a}\pm0.3$	$5.29^{a}\pm0.3$
TAC	$4.43^{c}\pm0.3$	$5.62^{b}\pm0.3$	$6.98^{a}\pm0.4$	$7.13^{a}\pm0.4$
Vit C	$18.98^{c}\pm1.1$	$42.17^{b}\pm2.3$	$50.97^{a}\pm2.8$	$51.93^{a}\pm2.9$
Vit E	$1.52^{b}\pm0.1$	$1.87^{a}\pm0.1$	$1.89^{a}\pm0.1$	$1.93^{a}\pm0.1$
TPRO	$80.21^{a}\pm3.2$	$83.05^{a}\pm3.3$	$82.42^{a}\pm3.3$	$82.45^{a}\pm3.3$
TCARB	$124.07^{c}\pm12.4$	$220.65^{b}\pm12.1$	$237.93^{a}\pm12.8$	$239.93^{a}\pm13.0$

PCA analysis of the nutraceutical properties of broccoli grown on soil treated with different fertilizers (Figure 5) clearly showed that RecOrgFert PLUS generated a distinctive chemical profile that differentiated it from all other treatments. In quadrant 1, RecOrgFert PLUS showed a strong correlation with DPPH and total phenolics; NPK showed a strong correlation with total phenolics. In quadrant 4, a slight correlation was observed between RecOrgFert PLUS and Vitamin E, TAC, ABTS⁺, Vitamin C; in the same quadrant a weak correlation was shown between Horse Manure with Vitamin C, total flavonoids, total carbohydrates; NPK present in quadrant 4 had a strong correlation with total proteins.

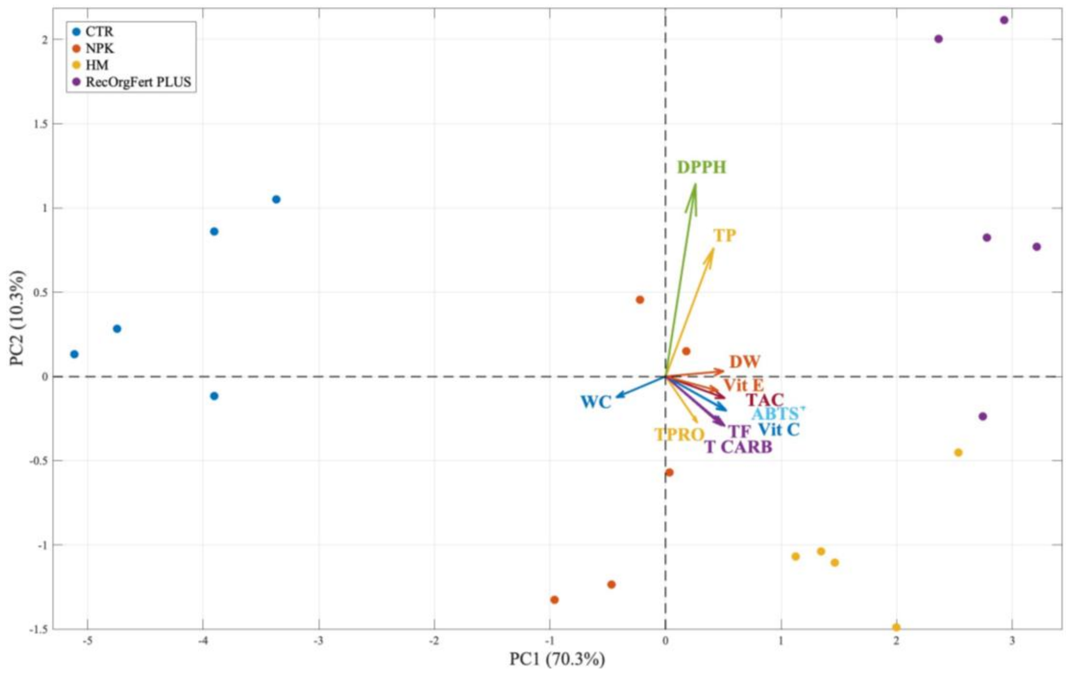

Figure 5. Principal Component Analysis (PCA) of nutraceutical properties of broccoli grown on soils treated with different fertilizers. Treatments include CTR (control) = soil without fertilizer; NPK = nitrogen – phosphorus – potassium; HM = horse manure; RecOrgFert PLUS = bentonite sulphur + orange pomace. WC = water content; DW = dry weight; TP = total phenols; TF = total flavonoids; DPPH = 2,2-difenil-1-picrilidrazile; ABTS⁺ = 2,2'-azino-bis-3-etilbenzotiazolin-6-solfonato; TAC = total antioxidant capacity; Vit C = vitamin C; Vit E = vitamin E; TPRO = total protein; TCARB = total carbohydrates.

Pearson's correlation analysis (Figure 6) revealed a well-defined network of interactions among variables related to antioxidant capacity. Strong positive correlations were observed between ABTS⁺,

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total antioxidant capacity (TAC), vitamin C, and total flavonoids (TF), suggesting that these parameters were functionally interconnected and likely reflected a shared underlying antioxidant mechanism. Similarly, total carbohydrates (TCARB) showed strong associations with ABTS⁺, vitamin C, TAC, and TF, indicating a possible supportive role of carbohydrates in the stabilization, transport, or accumulation of antioxidant compounds.

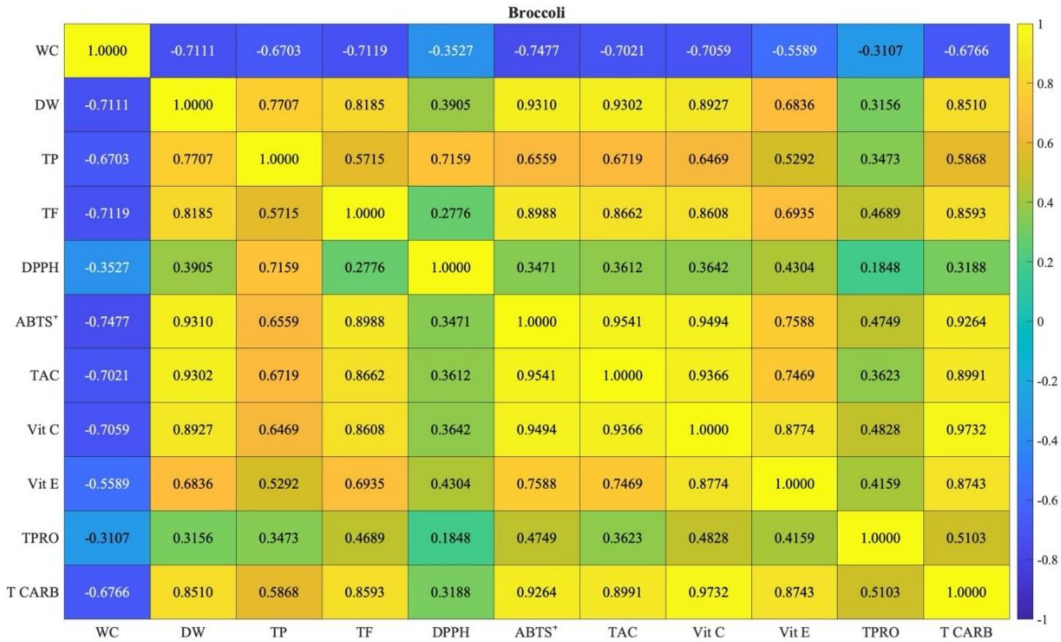

Figure 6. Pearson correlation matrix of broccoli quality parameters. The color gradient indicates the strength and direction of the correlations, with positive correlations shown in blue and negative correlations in red. Strong correlations highlight potential interdependencies among quality traits influenced by different soil fertilization treatments. WC = water content; DW = dry weight; TP = total phenols; TF = total flavonoids; DPPH = 2,2-difenil-1-picrilidrazile; ABTS⁺ = 2,2'-azino-bis-3-etilbenzotiazolin-6-solfonato; TAC = total antioxidant capacity; Vit C = vitamin C; Vit E = vitamin E; TPRO = total protein; TCARB = total carbohydrates.

3.5. Soil and Broccoli Correlation

Pearson correlation matrix between soil parameters and broccoli quality traits (Figure 7) revealed significant relationships. Fluorescein diacetate hydrolysis (FDA) exhibited consistently strong positive correlations with all broccoli quality parameters (all values > 0.8812), particularly with total phenols (TP, 0.9180), ABTS⁺ radical scavenging activity (0.8918), and vitamin E content (0.8982). Soil organic matter (OM) also showed high correlations across most broccoli traits (≥ 0.8886), with the strongest associations observed for total antioxidant capacity (TAC, 0.9286) and total flavonoids (TF, 0.9183). Microbial biomass carbon (MBC) exhibited very high correlations, especially with vitamin E (0.9969) and total carbohydrate content (TCARB, 0.9996). Correlation with TP was slightly lower (0.6475), though still moderate. MBC emerged as particularly relevant for vitamin E and carbon accumulation. Water-soluble fraction (WSP) was strongly correlated with TAC (0.9348) and ABTS⁺ (0.9051). Cation exchange capacity (CEC) displayed moderate to strong correlations with most quality parameters, with the exception of TP (0.4312—weak), while showing strong correlations with vitamin E (0.9472) and TCARB (0.9681). Dehydrogenase activity (DHA) showed variable performance, with weaker correlations with TF (0.6979) and TAC (0.7122), but better correlation with TP and vitamin E.

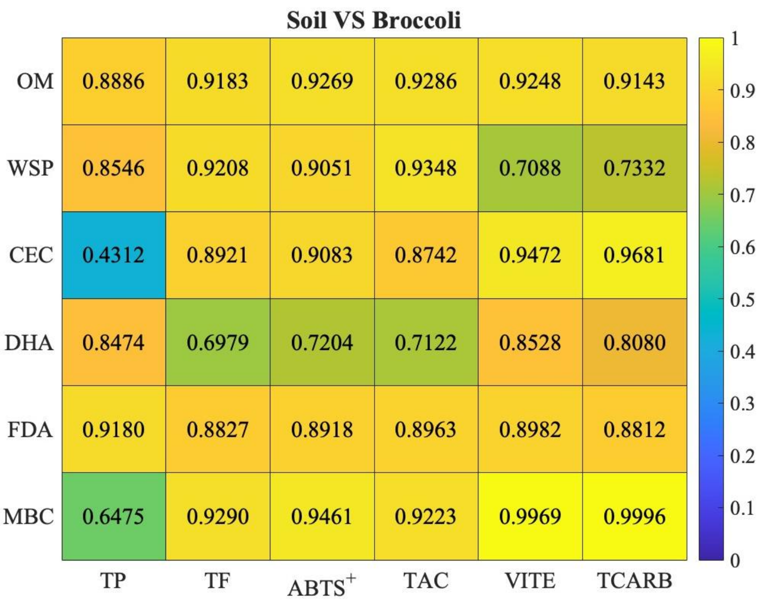

Figure 7. Pearson correlations matrix between soil quality and broccoli quality. The color gradient indicates the strength and direction of the correlations, with positive correlations shown in blue and negative correlations in red. OM = organic matter; WSP = water-soluble phenols; CEC = cation exchange capacity; DHA = dehydrogenase; FDA = fluorescein diacetate hydrolase; MBC = microbial biomass carbon; TP = total phenols; TF = total flavonoids; ABTS+ = 2,2'-azino-bis-3-etilbenzotiazolin-6-solfonato; TAC = total antioxidant capacity; Vit E = vitamin E; TCARB = total carbohydrates.

When analyzing correlations between soil quality and broccoli quality by fertilization treatment (Figure 8), we observed:

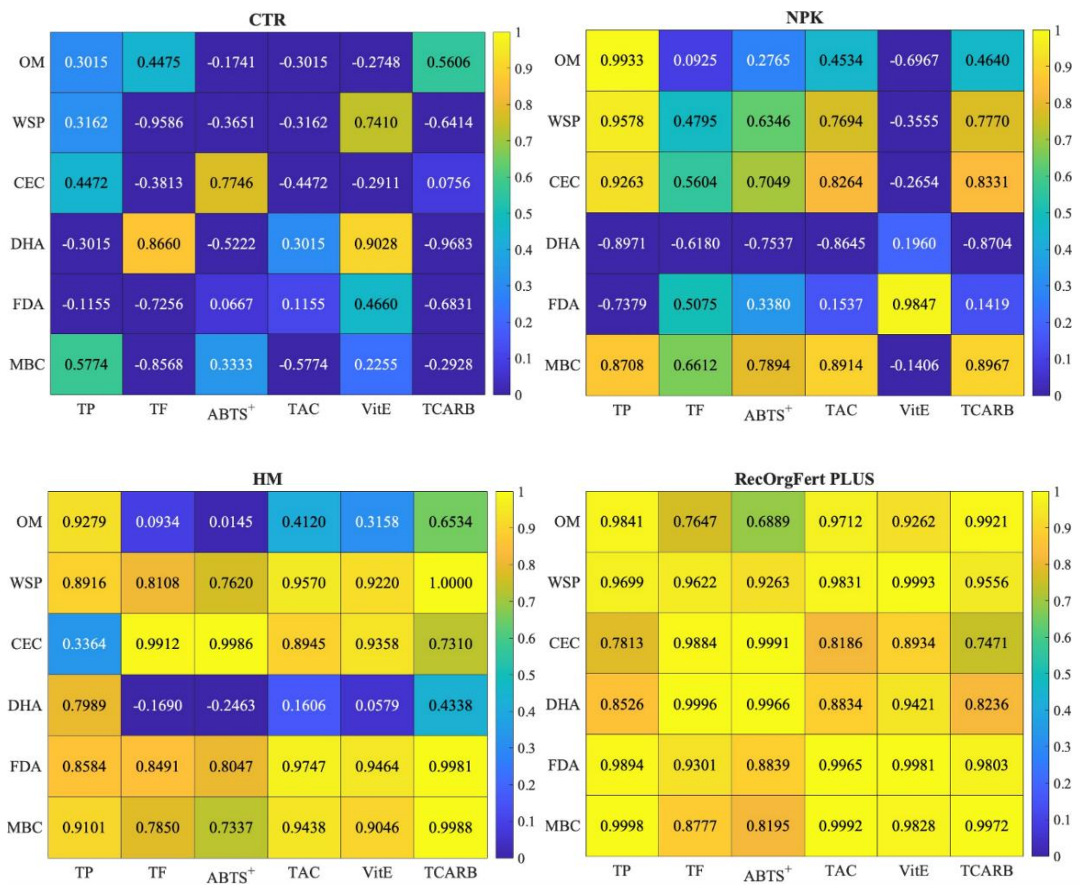

Figure 8. Pearson correlations matrix between soil quality and broccoli quality treated with different fertilizers: CTR (control) = soil without fertilizer; NPK = nitrogen – phosphorus – potassium; HM = horse manure; RecOrgFert PLUS = bentonite sulphur + orange pomace. The color gradient indicates the strength and direction of the correlations, with positive correlations shown in blue and negative correlations in red. OM = organic matter; WSP = water-soluble phenols; CEC = cation exchange capacity; DHA = dehydrogenase; FDA = fluorescein diacetate hydrolase; MBC = microbial biomass carbon; TP = total phenols; TF = total flavonoids; $ABTS^+$ = 2,2'-azino-bis-3-etilbenzotiazolin-6-solfonato; TAC = total antioxidant capacity; Vit E = vitamin E; TCARB = total carbohydrates.

In untreated soil (CTR), Pearson correlations were generally weaker and more variable compared to treated soils. OM showed modest correlations with TP (0.3015) and TCARB (0.5606). WSP showed a strong negative correlation with TF (–0.9586) and a positive correlation with vitamin E (0.7410). CEC was moderately correlated with $ABTS^+$ (0.7746). DHA was strongly correlated with TF (0.8660) and vitamin E (0.9028), but showed a marked negative correlation with TCARB (–0.9683). FDA exhibited weak or negative correlations with most parameters (TF: –0.7256; TCARB: –0.6831). MBC had only moderate positive correlations with TP (0.5774) and $ABTS^+$ (0.3333), while showing negative correlations with TF (–0.8568) and TAC (–0.5774). Overall, in the absence of fertilization, correlations between soil quality indices and broccoli nutritional quality were weaker and less consistent.

The application of horse manure substantially enhanced correlations between biological soil parameters and antioxidant components of broccoli. SOM showed strong correlations with TP (0.9279) and TCARB (0.6534). WSP displayed high correlations with all quality traits, particularly TAC (0.9570) and TCARB (1.0000). CEC showed very high correlations with TF (0.9912), $ABTS^+$ (0.9986), and Vitamin E (0.9358). DHA generally showed low or negative correlations, with TF (–0.1690) and $ABTS^+$ (–0.2463). FDA was strongly correlated with TAC (0.9747), vitamin E (0.9464), and TCARB (0.9981). MBC was highly correlated with TP (0.9101), TAC (0.9438), and TCARB (0.9988). Overall, WSP, CEC, FDA, and MBC were the best predictors of broccoli nutritional quality, especially total carbohydrate content, TAC, and antioxidant compounds.

Under NPK treatment, strong positive correlations were observed between OM and TP (0.9933), WSP and TP (0.9578), CEC and TP (0.9263), MBC and TAC (0.8914), and MBC and TCARB (0.8967), indicating a direct influence of soil quality on total antioxidants and carbohydrate content. DHA showed strong negative correlations with TP (−0.8971), TAC (−0.8645), and TCARB (−0.8704), suggesting a potentially adverse effect of dehydrogenase activity on nutritional quality. A high correlation between FDA and vitamin E (0.9847) indicates a strong association between enzymatic activity and vitamin E content. These results suggest that chemical and biochemical soil properties significantly influence the nutritional quality of broccoli under NPK fertilization.

Pearson correlations between soil properties and broccoli quality under RecOrgFert PLUS treatment were very high across nearly all combinations, indicating a strong association between soil quality and nutritional quality. Specifically, MBC was highly correlated with TP (0.9998), TAC (0.9992), vitamin E (0.9828), and TCARB (0.9972). FDA showed strong correlations with vitamin E (0.9981), TAC (0.9965), and TCARB (0.9803). DHA exhibited extremely high correlations with TF (0.9996) and $ABTS^+$ (0.9966), in contrast to the negative correlations observed under NPK. WSP and CEC also showed high values, with WSP–vitamin E at 0.9993 and CEC– $ABTS^+$ at 0.9991. These findings suggest that RecOrgFert PLUS createed a pronounced synergistic effect between soil quality and the nutritional profile of broccoli, outperforming other treatments.

4. Discussion

Global conventional agriculture practices optimize agricultural production via the use of synthetic inputs, energy resources, and diverse industrial materials, frequently at the expense of biodiversity, soil fertility, and overall ecosystem health. With projections indicating that the global population may reach 9 billion by 2050, food security concerns are intensifying, necessitating soil health preservation and degradation mitigation. Numerous anthropogenic activities have created challenges to soil ecosystem health, leading to biodiversity loss, decline in organic matter, increased diffuse pollution, and irreversible salinization [36]. Therefore, the global community is actively seeking viable strategies to enhance soil health for sustained agricultural productivity, designed to respect the environment, improve resource efficiency, and promote human well-being. The circular economy offers nowadays a transformative pathway for the fertilizer industry to achieve sustainability, reduce wastes, and enhance agricultural productivity. By adopting innovative recycling practices, it is possible to transition to a model that benefits both environment, health and economy. Embracing a circular economy isn't just an option—it's a necessity for the future of agriculture. The results of this study evidenced that RecOrgFert PLUS the fertilizer produced using wastes led to the highest increases in soil organic carbon (OC), total nitrogen (TN), and microbial biomass carbon (MBC), compared to NPK and HM demonstrating that its formulation containing elemental sulphur plus organic components is able to contribute to long-term carbon sequestration in the soil and to improve nutrient availability as already demonstrated by other authors [5] for different matrices containing organic components. Compared to other sustainable fertilizers such as compost and vermicompost, which generally increase soil microbial biomass and enzymatic activity [37,38], RecOrgFert PLUS has demonstrated equal or even greater improvements in soil microbial health. Unlike compost, which requires long maturation periods to prevent phytotoxicity and ensure microbial stability [39], RecOrgFert PLUS provides readily available nutrients, promoting a rapid and efficient microbial response. Furthermore, due to its controlled formulation, it avoids the uncontrolled ammonia emissions and nutrient dilution often observed in low-quality composts [11,40,41]. Any changes in soil MBC have a significant impact on soil carbon, nitrogen, phosphorus, plant species and the dynamics of the terrestrial ecosystems. MBC can be used as the early prediction index of soil quality and the change of soil total organic matter. Soil enzyme activities reflect the rate of soil nutrient cycling and utilization and may be an index of soil biodiversity, productivity and potential microbial activity. In the present study, soil enzyme activities, particularly dehydrogenase and fluorescein diacetate hydrolase, significantly increased in soils treated with RecOrgFert PLUS. These enzymes are widely recognized as indicators of soil microbial activity and biochemical fertility [42,43]. The observed increase in enzymatic activity suggested that RecOrgFert PLUS is able to provide a favourable environment for microbial communities, likely due to its organic composition and slow nutrient release [41]. The lower level of Catalase activity supports this hypothesis suggesting a reduction of soil stress levels, that is in line with previous results of Song et al. [44] reporting that catalase can serve as biochemical markers for providing insights into the community responses to exogenous stress. Furthermore, it is interesting to note that catalase activity was significantly higher in the NPK treatment than in the others, particularly compared to RecOrgFert PLUS, indicating a higher presence of oxidative stress in soils treated with synthetic fertilisers, as suggested by studies that associate increased catalase activity with the need to detoxify free radicals, generated by oxidative reactions induced by intensive chemical inputs [44]. Conversely, the lower CAT values found in the RecOrgFert PLUS treatment may reflect a more stable microbial environment that is less subject to environmental stress, confirming the hypothesis of a better ecological balance in the soil. Like biochar and vermicompost, which improve soil structure and faunal diversity [45], RecOrgFert PLUS supported a more structured soil fauna community. The higher QBS-ar values observed with RecOrgFert suggest it is at least as effective as biochar–vermicompost blends—and avoids potential antagonistic interactions noted in combined amendments [46].

PCA analysis of the chemical and physical soil properties treated with different fertilizers clearly showed that RecOrgFert PLUS generated a distinctive chemical profile that set it apart from all other treatments. The distribution of RecOrgFert PLUS variables in the quadrants evidence a greater dispersion suggesting more heterogeneous effects of this treatment, with unique chemical changes in soil. These effects are likely due to its specific formulation, which synergistically combines organic and mineral components. The distance from control samples further confirms the effectiveness of the

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treatment in modifying soil chemistry, while the tight clustering of the treated samples demonstrates the consistency and reproducibility of its effects.

Similarly, the analysis of biological properties indicates that RecOrgFert has a distinct influence on soil enzyme activity. It is closely associated with increased levels of microbial biomass carbon (MBC) and dehydrogenase activity (DHA), suggesting that this fertilizer effectively promotes the growth and functional activity of the soil microbial community. The clear separation from NPK and HM treatments further indicates that RecOrgFert PLUS creates a unique enzymatic profile, likely due to its ability to provide specific substrates that enhance microbial activity. Microbial biomass is a key indicator of soil vitality, as it represents the active fraction of organic matter responsible for the decomposition of organic matter and the release of essential nutrients for plants, thus contributing to soil fertility and health [47].

Pearson's correlation analysis revealed a tightly interconnected group of soil properties associated with organic fertility. The strong positive relationships among these variables suggest that soil amendments can trigger coordinated improvements across key biological and physicochemical functions, reflecting the activation of integrated soil processes. These relationships suggest that fertilizer treatments have activated integrated biogeochemical processes, whereby the improvement of one parameter promotes enhancements in the others. This is supported by recent studies demonstrating that the application of organic fertilizers, such as cow manure and straw residues, significantly enhances soil organic carbon pools and microbial activity, leading to improved soil fertility and sustainability [48]. On the other hand, the consistent negative correlation of pH with organic and enzymatic parameters suggests that the fertilizers caused a slight acidification of the soil. Rather than being detrimental, this acidification reflects the intensification of organic matter decomposition and nutrient mineralization, which naturally release organic acids. The resulting mildly acidic environment appears to stimulate microbial activity—particularly of dehydrogenase, a key enzyme in microbial metabolism—as confirmed by its strong negative correlation with pH [4].

In line with our results, Liu et al., [40] demonstrated that organic fertilizers improved microbial diversity and functionality, enhancing soil health compared to conventional fertilization. The increased concentrations of Na^+, K^+, and SO_4^{2-} in soils treated with RecOrgFert PLUS indicated a better nutrient supply compared to the control. These results are consistent with studies highlighting that fertilizers containing organic components contribute to an increased cation exchange capacity (CEC) of the soil, thus improving nutrient retention and reducing losses due to leaching [5]. Interestingly, NO_3^- was detected only in the horse manure treatment, suggesting a different nitrogen mineralization pattern. Synthetic fertilizers, such as NPK, tend to release nitrogen rapidly, leading to potential leaching [11]. In contrast, RecOrgFert PLUS releasing nutrients more gradually, could improve nitrogen use efficiency reducing nitrogen environmental impact [49].

The increase in biodiversity observed in soils treated with RecOrgFert PLUS, as indicated by QBS-ar values, supports the finding that RecOrgFert Plus created a more favorable habitat for soil fauna. Several studies reported similar findings, demonstrating that organic fertilization or fertilizers with organic components improved soil biological quality by increasing microbial biomass, enzymatic activity, and macrofauna diversity [5,41]. Soil biodiversity is a crucial indicator of soil resilience and functionality, as it contributes to organic matter decomposition, nutrient cycling, and the maintenance of soil structure [50]. The improvement in the QBS-ar index in soils treated with RecOrgFert PLUS suggests that waste-based fertilizers can play a key role in promoting more stable and self-sufficient soil ecosystems, a feature that is increasingly relevant in sustainable agriculture.

The different types of fertilizers used significantly influenced the biochemical composition of broccoli, plants treated with RecOrgFert PLUS showed the highest levels of total phenols, flavonoids, and vitamin C. In particular, the increase in total phenolic content observed in broccoli treated with RecOrgFert PLUS aligns with studies demonstrating that organic amendments can improve the antioxidant profile of crops [51]. This effect can be attributed to the improved soil organic matter content and microbial activity, which facilitate the uptake of micronutrients essential for phenolic biosynthesis [41]. Notably, the significantly higher vitamin C content in RecOrgFert PLUS and horse manure treated broccoli (compared to synthetic fertilization, NPK) is also worth mentioning. Organic fertilization has been shown to enhance ascorbic acid accumulation in vegetables due to lower nitrogen availability, which promotes secondary metabolite synthesis rather than excessive vegetative growth [52]. This is in line with our results, suggesting that waste-based fertilizers may offer advantages in improving both yield and nutritional quality. Similarly, the increase in total protein and carbohydrate content in broccoli treated with RecOrgFert PLUS supports previous evidence that organic fertilization enhances the accumulation of macronutrients in crops [49]. The increase in protein content could be related to better nitrogen absorption efficiency, while the increase in carbohydrate levels could be linked to greater microbial interaction in the soil, which has been shown to promote photosynthetic efficiency and carbon fixation [40].

The results of the PCA analysis highlighted how the choice of fertilizer profoundly affected not only plant growth but also the nutritional quality of broccoli. The distinct placement of RecOrgFert PLUS in the upper right quadrant, correlated with higher contents of bioactive compounds, suggesting that this fertilizer could specifically stimulate the metabolic pathways responsible for the synthesis of flavonoids and other phenolic compounds in broccoli. These compounds are known for their antioxidant properties and potential human health benefits. The clear separation between RecOrgFert PLUS treated and control samples indicates that intervention with this biofertilizer produces a substantial improvement in nutritional quality compared with standard growing conditions. This suggests that RecOrgFert PLUS could be a significant innovation to produce vegetables with enhanced nutritional profiles. The intermediate placement of samples treated with horse manure indicates that this conventional organic fertilizer offers some nutritional benefits over the control but does not achieve the quality levels obtained with RecOrgFert PLUS. This observation is particularly relevant to organic farming systems seeking sustainable alternatives to synthetic fertilizers. The variability observed in NPK samples might reflect a less consistent plant response to this type of chemical fertilizer, suggesting that immediate availability of inorganic nutrients may not necessarily translate into uniform improvement in nutritional quality. The positive correlation between several nutritional parameters (total phenols, DPPH, VITE, TAC and $ABTS^+$) in RecOrgFert PLUS-treated samples suggests that this fertilizer may activate complex physiological mechanisms that simultaneously improve several aspects of broccoli nutritional quality, rather than affecting individual isolated parameters. These results have important implications for agricultural practices oriented not only to productivity but also to nutritional quality of food, highlighting how RecOrgFert PLUS may represent an innovative solution for growing brassicas with enhanced nutraceutical properties.

Correlation matrix analysis reveals significant relationships between the different biochemical parameters examined. The high concordance between the different antioxidant assays ($ABTS^+$, TAC, DPPH) confirms the validity of the measurements, while the slight variations in their correlations highlight the specificity of each method towards antioxidants. The extraordinary correlation between vitamin C and total carbohydrates (0.9732) suggests potential metabolic or structural relationships between these components. Carbohydrates in fact act as precursors for vitamin C synthesis, primarily through the Smirnoff-Wheeler pathway. Specifically, D-glucose and D-mannose, both simple sugars, are converted into compounds like GDP-D-mannose, L-galactose, and L-galactono-1,4-lactone, which are then used to produce vitamin C [53]. The differential correlations of phenolic compounds with the different antioxidant assays (are strongly correlated with DPPH activity and moderately correlated with TAC, $ABTS^+$ and Vitamin C) indicate an integrated and synergistic antioxidant system in the sample analysed, where vitamin C, phenolic compounds and potentially some carbohydrate fractions collectively contribute to the total antioxidant activity through complementary mechanisms. Flavonoids correlated strongly with $ABTS^+$, TAC and Vitamin C indicating that crops with higher flavonoid levels may also exhibit greater antioxidant activity.

Pearson correlation analysis revealed that FDA, OM, and MBC were the most influential soil properties associated with broccoli quality. These parameters showed strong and consistent correlations with key broccoli traits, including total phenols (TP), total flavonoids (TF), antioxidant

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activity (ABTS$^+$, TAC), vitamin E, and total carbohydrate content. Among these, FDA emerged as the primary indicator, reflecting high microbial activity and robust associations with all broccoli quality parameters. Similarly, OM contributed significantly to nutrient availability and soil structure, enhancing antioxidant and nutrient levels in broccoli. MBC particularly influenced vitamin E content and carbohydrate accumulation.

Conversely, CEC and DHA showed more variable correlations, with CEC performing poorly for TP and DHA being the most inconsistent overall. WSP played a more targeted role, primarily benefiting antioxidant properties. These results suggest that soil biological activity and organic matter content are key determinants of broccoli's nutritional quality and may represent strategic targets for improving crop value.

Correlational differences among fertilization treatments highlighted the pivotal role of soil management in shaping the nutritional and functional attributes of horticultural crops. In the control treatment (CTR), devoid of fertilization, correlations between soil biological parameters and broccoli quality were weak and inconsistent, reflecting limited microbial activity and nutrient availability—typical of unmanaged, low-fertility soils. This is consistent with recent studies showing that the absence of organic or mineral inputs reduces microbial biomass and nutrient-use efficiency in crops [54].

Horse manure application increased the correlations between soil biological parameters and broccoli antioxidant components, indicating its capacity to stimulate microbial processes and provide substrates for secondary metabolite biosynthesis in plants [6,52]. However, performance was not uniformly high, suggesting possible variability in organic matter mineralization.

Mineral fertilization (NPK) enhanced correlations between soil chemical parameters and broccoli nutrients such as carbohydrates and vitamin E, but correlations with antioxidant parameters were weaker. This suggests that mineral fertilization supports vegetative growth and macronutrient content, but does not effectively stimulate the synthesis of bioactive metabolites such as phenols and flavonoids. These findings align with those of Liu at al. [52] and meta-analyses indicating that mineral nutrition may reduce antioxidant content compared to organic practices [52].

The RecOrgFert PLUS treatment produced the highest, most consistent, and most significant correlations between soil quality and broccoli quality. The coherence and strength of these associations indicate a strong synergistic effect between soil microbiological vitality and the expression of nutraceutical compounds in broccoli.

These findings are supported by recent evidence showing that regenerative organic fertilizers—rich in stable carbon, microbial substrates, and slowly available nutrients—enhance soil microbial biomass, enzymatic activity (e.g., FDA), and crop antioxidant capacity [55]. Furthermore, the high correlations with MBC and OM underscore that increasing soil organic matter and microbial biomass is crucial for improving broccoli's nutritional quality. This confirms that regenerative approaches are not only sustainable but also beneficial from a nutraceutical standpoint [54].

Therefore, RecOrgFert PLUS emergeed as a high-potential strategic solution for improving the nutritional quality of horticultural products through the restoration of biological and biochemical soil fertility, outperforming both conventional (NPK) and traditional organic (HM) fertilization approaches.

Our results strengthen the growing body of evidence supporting the use of waste-based fertilizers as sustainable alternatives to synthetic fertilization (NPK). The significant improvements in soil health indicators (organic carbon, microbial activity, and enzymatic functions) and crop quality parameters (antioxidants, vitamins, and proteins) suggest that RecOrgFert PLUS has strong potential for application in sustainable agriculture. Given the environmental concerns associated with synthetic fertilizers, such as nitrate leaching and soil degradation [56], waste-based fertilizers represent a promising strategy for improving soil fertility while minimizing ecological impacts.

5. Conclusions

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This study highlights the strategic role of waste-based fertilizers in enhancing soil fertility and crop quality within a sustainable agriculture framework and a circular economy approach. The application of RecOrgFert PLUS favours a more biologically active and structured soil environment, reflecting improved ecological quality. These effects suggest greater organic matter stability and optimized nutrient availability, which are essential for long-term agricultural productivity.

Simultaneously, broccoli cultivated with RecOrgFert PLUS exhibited a superior nutritional profile, with marked increases in total phenols, vitamin C, and protein content compared to other treatments. The enhanced soil biodiversity likely promoted beneficial microbe-plant interactions, aiding nutrient uptake and the synthesis of bioactive compounds with nutraceutical benefits.

These findings underscore the potential of this waste-based fertilizers to improve nutritional quality, supporting healthier and more sustainable food production. The use of the waste-based fertilizers embodies a circular economy strategy that reduces environmental impact by recycling waste into valuable resources, improves soil health, and increases the resilience of agricultural ecosystems.

Further long-term research is needed to assess the effects of RecOrgFert PLUS across various soil types and crops, aiming to optimize its large-scale application and integration into sustainable and circular agricultural practices.

Author Contributions: "Conceptualization, Adele Muscolo (A.M.); methodology, F.M..; software, L.S.; validation. L.S.; S.B., F.M., Mt., O., C.M..; investigation, S.B..; resources, Adele Muscolo (A.M.); data curation, Mt.O.; writing—original draft preparation, Adele Muscolo (A.M.); writing—review and editing, Mt.O. L.S. and Adele Muscolo (A.M.); visualization, S.B.; supervision, All authors have read and agreed to the published version of the manuscript.".

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Data Availability Statement: data is unavailable due to privacy

Conflicts of Interest: "The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

T0	Time zero
CTR	Control, soil without fertilizer
NPK	Nitrogen – phosphorus – potassium
HM	Horse manure
WC	Water content
EC	Electrical conductivity
OC	Organic carbon
TN	Total nitrogen
C/N	Carbon-nitrogen ratio
OM	Organic matter
WSP	Water-soluble phenols
CEC	Cation exchange capacity
DHA	Dehydrogenase
FDA	Fluorescein diacetate hydrolase
CAT	Catalase
MBC	Microbial biomass carbon
DW	Dry weight
TP	Total phenols
TF	Total flavonoids
DPPH	2,2-difenil-1-picrilidrazile
$ABTS^+$	2,2'-azino-bis-3-etilbenzotiazolin-6-solfonato
TAC	Total antioxidant capacity

Vit C	Vitamin C
Vit E	Vitamin E
TPRO	Total proteins
TCARB	Total carbohydrates
PCA	Principal component analysis

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