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Impacts of Conventional and Agri-Food Waste-Derived Fertilizers on Durum Wheat Yield, Grain Quality, and Soil Health: A Two-Year Field Study in Greece and Southern Italy

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Abstract

Background: In the context of climate change and the European Green Deal, sustainable fertilization practices are essential to enhance crop yield and soil health. Organic fertilizers from agro-industrial waste are gaining attention as alternatives to synthetic inputs. This study evaluates a novel fertilizer (RecOrgFert), composed of sulfur bentonite and citrus-processing residues. Methods: A two-year field trial was carried out in Central Macedonia (Northern Greece) and Apulia (Southern Italy) using a randomized complete block design with four treatments: unfertilized control, mineral NPK (15-15-15), horse manure, and RecOrgFert. Soil chemical and biological properties, plant growth parameters, yield components, and grain quality indicators were assessed. Results: RecOrgFert improved soil organic matter (+22–30%), cation exchange capacity, and enzymatic activity. Wheat grain showed higher protein content (up to 15.2%), antioxidant activity (DPPH > 37%, ABTS+ > 26%), and increased phenolic and flavonoid levels compared to other treatments. These benefits were consistent across both sites and years. Conclusion: RecOrgFert enhanced soil fertility and wheat quality while maintaining yields comparable to mineral fertilizers. Its sulfur and citrus-derived composition supports nutrient cycling and bioactivity, aligning with EU strategies for sustainable agriculture. RecOrgFert offers a viable, circular solution for Mediterranean cereal systems, further contributing to circular economy implementation plans.

Keywords: organic fertilizers; soil ferility; wheat quality; agri-food waste recycling; sustainable agriculture

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1. Introduction

Wheat (*Triticum aestivum* L.) is one of the most widely cultivated cereal crops globally and serves as a staple food for a large portion of the world's population, providing approximately 20% of the daily caloric and protein intake [1]. To sustain the productivity required for global food security, conventional agricultural systems have long relied on synthetic nitrogen (N), phosphorus (P), and potassium (K) fertilizers. While these inputs are effective in increasing yields, their prolonged and excessive use is associated with significant environmental challenges, including soil acidification, nutrient leaching, loss of biodiversity, and elevated greenhouse gas emissions [2–4].

In the context of climate change and resource scarcity, the transition to sustainable fertilization practices has become a key priority in global and European agricultural policy. Organic fertilizers derived from agri-food industrial wastes are gaining attention as viable alternatives that can recycle nutrients, reduce wastes, and improve soil health [5–7]. These materials contribute to increase organic matter and nutrients which in turn enhance soil physical structure, microbial activity, and nutrient cycling processes, ultimately benefiting crop performance and sustainability [7,8]. In addition to the agricultural sector, the industrial sector produces also wastes that could be recycled creating new products, valorising the waste chain. Sulphur released during the refining process of crude oil, can be recovered to be used for agricultural purpose, considering that various studies emphasized that sulfur deficiency are leading to reduced nitrogen utilization from fertilizers and production of crop proteins with significantly lower levels of sulfur-containing amino acids, particularly methionine, which greatly influences the nutritional value of crops. The use of waste-derived fertilizers perfectly aligns with the principles of the circular economy, which promotes resource efficiency, waste minimization, and carbon footprint reduction across all sectors, including agriculture [9]. In this regard, the European Green Deal and the Circular Economy Action Plan strongly encourage the agricultural reuse of organic wastes, which can also help address the issue of declining soil organic matter in Mediterranean agroecosystems [10,11].

Despite their potential, the agronomic performance of waste-derived fertilizers can vary significantly depending on factors such as feedstock composition, treatment process, soil type, and climatic conditions. As a result, field-based studies are essential to validate their efficacy under different agro-environmental contexts [8]. Furthermore, while several studies assessed either yield or soil health separately, fewer have integrated both agronomic, soil quality, yield and wheat quality across multiple years and more geographical locations [4,5].

The Mediterranean region, characterized by its climate variability, limited water resources, and declining soil fertility, provides a relevant setting for evaluating the sustainability of fertilization practices. Greece and Southern Italy (Apulia), in particular are important wheat-producing regions where alternative fertilization strategies could contribute to climate-resilient and environmentally sound cereal production systems [12]. The present study aimed to evaluate the impact of conventional mineral nitrogen, phosphorous and potassium (NPK, 15-15-15) or organic horse manure (HM) fertilizers and fertilizers derived from industrial and agri-food wastes specifically orange waste residue of agro-food industry and sulphur as residue of oil industry on wheat yield, grain quality, and soil health. Conducted over two consecutive years in contrasting Mediterranean environments—Apulia (Southern Italy) and Central Macedonia (Northern Greece)—this study seeks to: (1) compare the agronomic effectiveness of different fertilizer treatments; (2) assess their effects on soil chemical and biological properties; and (3) analyze yield and quality of wheat comparing the consistence of treatment responses across sites. By integrating multiple indicators of performance, this research provides practical insights to guide sustainable nutrient management and policy development in Mediterranean cropping systems [6,11].

2. Materials and Methods

2.1. Study Sites and Experimental Design

Field experiments were conducted over two consecutive cropping seasons (2021–2023) in two Mediterranean locations (Figure 1): Southern Italy (Apulia) in 17 hectares and Central Macedonia Northern Greece (Thessaloniki) 12 hectares. Both sites are representative of typical durum wheat (*Triticum durum* Desf.) cultivation areas but differ in soil types, climatic conditions, and agricultural history.

In each location, a randomized complete block design (RCBD) was implemented with three replicates per treatment. The experimental treatments included: Control (CTR) – No fertilizer; synthetic NPK (15-15-15); organic fertilizer (horse manure, HM); and Sulphur bentonite plus orange wastes (RecOrgFert). Fertilizers were applied pre-sowing at rates standardized based on equivalent nitrogen content (150 kg N ha^{-1}), and at a vegetative stage, following local agronomic guidelines. Regarding the site of Apulia, the total area of 17 hectares, has been divided into four plots of about 4 hectares each, Red, control; Green, NPK; Yellow, RecOrgFert; Blue, Horse manure. The four areas are approximately 1,335 meters in length and about 90 meters in width, maintaining homogeneity, especially considering the altitude variation, which ranges from 203 meters at the highest point to 147 meters at the lowest. Regarding Thessaloniki, the 12 hectares have been divided in 4 plots of hectares each, maintaining the homogeneity among the plots (Figure 1 a, b).

a

b

or

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Figure 1. (a) experimental Field Apulia south Italy, (b) experimental Field Thessaloniki Greece.

2.2. Soil Analysis

Soil samples were collected from the top 0–35 cm layer of each plot at three time points: pre-treatment and post-harvest. Five samples per plot were composited and analyzed for the following parameters: Electric conductibility (EC) was determined in distilled water by using 1:5 residue/water suspension, mechanically shaken at 15 rpm for 1 h to dissolve soluble salts and then detected by Hanna instrument conductivity meter; pH was measured in distilled water (soil/pad:solution ratio 1:2.5) with a glass electrode. Organic carbon was assessed with dichromate oxidation method [13]. Total nitrogen (TN) was measured with Kjeldahl method [14]. C/N was determined as a carbon:nitrogen ratio. Water soluble phenols were extracted as reported by Kaminsky and Muller [15,16]. All analyses were performed following standardized protocols as outlined in ISO 10390:2005 and ISO 14240-2:1997. Soil classification followed the FAO World Reference Base (WRB) system.

2.3. Yield Measurements

At crop maturity, wheat plants were manually harvested from a 4 m^2 area in each plot to avoid edge effects. The following yield components were recorded: plant height (cm), Seed/ear; Yield (q/ha), calculated on the basis of the total mass of grain harvested and corrected to 13% moisture content, following standard normalisation procedures [17]; Dry Seed (g/plant), determined by drying grain samples at a controlled temperature until they reach a constant weight and measured in grams (g) [17]; Protein content (%): Using near-infrared spectroscopy (NIRS), a widely adopted non-destructive method for evaluating grain quality[18]. Gluten content and index: Measured by standard wet chemistry (ICC method No. 137/1), which provides robust indicators of baking quality [19]. The β-carotene content was determined by extraction with a solvent mixture (acetone:ethanol:hexane, 1:1:2, v/v/v), followed by spectrophotometric quantification at 450 nm, according to Saini et al. [20]. Results were expressed in mg/100g of dry weight. Grain moisture content was adjusted to 13% for standardization. Yield parameters were statistically analyzed across treatments and locations.

2.4. Grain Quality Analysis

Grain quality was assessed using a subsample of dried and cleaned kernels from each plot. The total phenol content was determined using the Folin-Ciocalteu reagent [21], following the procedure described by Velioglu et al. [22]. Total flavonoid content was measured using a colorimetric method based on aluminium chloride, according to the protocol developed by Djeridane et al. [23]. To assess

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antioxidant activity, two complementary methods were employed. The ABTS+ radical cation decolorization assay was conducted following the method of Re et al.[24], while the DPPH method, which relies on the stable free radical 2,2-diphenyl-1-picrylhydrazyl, was performed according to Barreca et al. [25]. Analytical precision was verified using certified reference materials. All tests were conducted in triplicate.

2.5. Statistical Analysis

Analysis of variance (ANOVA) was performed for all data sets. Three-way ANOVA followed by Tukey's HSD (honestly significant difference) test was used to assess the effects of fertilisers on the various measured parameters. Comparisons were conducted to assess the effects of fertilisers on each individual parameter. To explore the relationships between change in soil properties induced by the different fertilizer and plant and grain parameters, the datasets were analysed using Pearson correlation matrix. Statistical analyses were performed using MATLAB (version R2024b, The MathWorks Inc., Natick, MA, USA). Effects were considered significant at $p \le 0.05$.

3. Results

3.1. Experiments in Central Macedonia Greece

3.1.1. Soil Properties

Over the two-year period, all fertilization treatments induced significant changes in soil chemical parameters compared to the control (CTR), with distinct trends across 2023 and 2024. In 2023 (Table 1), the application of NPK significantly increased organic carbon (OC) (4.00%) and organic matter (OM) (6.90%) compared to the control (2.32% OC and 4.00% OM), indicating rapid nutrient mineralization and incorporation. Horse manure (HM) and RecOrgFert also enhanced these parameters, though to a lesser extent. By 2024 (Table 1), OC and OM were highest under NPK and RecOrgFert, with RecOrgFert showing notable improvement from the previous year (OC: 3.2%, OM: 5.5%). Interestingly, CEC increased across all treatments, particularly under HM (28.92 meq/100g). Soil pH decreased slightly across treatments in 2024 compared to 2023, especially under RecOrgFert (from 7.9 to 7.6). Electrical conductivity (EC) significantly increased in 2024 compared to 2023, suggesting higher soluble salt content.

Table 1. Greece 2023 and 2024: chemical and biochemical properties of soil. CTR (control) = soil without fertilizer; NPK = nitrogen – phosphorus – potassium; HM = horse manure; RecOrgFert = bentonite sulphur + orange pomace. pH; OC = organic carbon (%); OM = organic matter (%); C/N = carbon-nitrogen ratio; CEC = cation exchange capacity (meq/100g); Tot N = total nitrogen (%); EC = electrical conductivity (dS/m); WC = Soil Moisture (%). Data are the means of three replicates ± standard deviation. Different letters in the same row indicate significant differences (Turkey's test. $p \le 0.05$).

		2023		
	CTR	**NPK**	**HM**	**RecOrgFert**
pH	8.0^{a} ±0.1	7.9^{b} ±0.1	7.9^{b} ±0.1	7.9^{b} ±0.1
OC	2.32^{c} ±0.15	4.00^{a} ±0.20	3.44^{b} ±0.18	2.34^{c} ±0.15
OM	4.00^{c} ±0.25	6.90^{a} ±0.30	5.93^{b} ±0.28	4.03^{c} ±0.25
C/N	19.33^{b} ±1.2	25.00^{a} ±1.5	22.93^{a} ±1.4	18.00^{b} ±1.0
CEC	12.73^{b} ±1.0	10.72^{b} ±1.1	13.60^{a} ±1.2	10.71^{b} ±1.0
Tot N	0.12^{b} ±0.01	0.16^{a} ±0.01	0.15^{a} ±0.01	0.13^{b} ±0.01
EC	0.37^{b} ±0.03	0.40^{b} ±0.02	0.40^{b} ±0.02	0.43^{a} ±0.02
WC	13.10^{ab} ±1.5	11.10^{b} ±1.3	12.00^{ab} ±1.4	16.40^{a} ±1.8
		2024		
	CTR	**NPK**	**HM**	**RecOrgFert**
pH	8.0^{a} ±0.1	7.9^{b} ±0.1	7.7^{c} ±0.1	7.6^{c} ±0.1

OC	2.5^{c} ±0.16	4.10^{a} ±0.20	3.0^{b} ±0.16	3.2^{b} ±0.18
OM	4.4^{c} ±0.28	7.1^{a} ±0.35	5.2^{b} ±0.28	5.5^{b} ±0.30
C/N	14.2^{c} ±1.2	29.3^{a} ±1.6	23.4^{b} ±1.3	29.0^{a} ±1.5
CEC	22.8^{c} ±2.0	23.4^{bc} ±2.1	28.9^{a} ±2.3	24.1^{ab} ±2.2
Tot N	0.18^{a} ±0.02	0.14^{b} ±0.01	0.13^{b} ±0.01	0.11^{c} ±0.01
EC	0.82^{a} ±0.05	0.50^{b} ±0.03	0.57^{b} ±0.03	0.44^{b} ±0.02
WC	13.9^{b} ±1.5	12.0^{b} ±1.4	14.2^{ab} ±1.5	14.9^{a} ±1.6

3.1.2. Plant Growth and Yield

NPK and RecOrgFert treatments consistently enhanced plant height and seed set across both years. In 2023 (Table 2), plants under NPK and HM were the tallest (~202–203 cm), while RecOrgFert recorded the highest seed/ear value (42 seeds). In 2024, plant height declined overall, likely due to climatic variability, yet NPK (190.2 cm), HM (186.8 cm) and RecOrgFert (173.0 cm) remained superior to the control. Seed/ear values increased in 2024 (Table 2), especially under NPK and RecOrgFert (53 and 50 seeds, respectively). Yield values were highest under NPK in both years (19.76 q/ha in 2023; 17.51 q/ha in 2024), followed by RecOrgFert. While HM led to high dry seed mass in 2023 (2.2 g/plant), this advantage diminished in 2024. RecOrgFert maintained good yield stability. Protein and gluten content improved under RecOrgFert, reaching 16.6% and 23.4% in 2024, suggesting enhanced nutritional quality. Notably, β-carotene content tripled from ~0.62 to over 2.29 mg/100g across all treatments between years, pointing to environmental or varietal influence.

Table 2. Greece 2023 and 2024: plant grow parameters. CTR (control) = soil without fertilizer; NPK = nitrogen – phosphorus – potassium; HM = horse manure; RecOrgFert = bentonite sulphur + orange pomace. Plant height (cm); Seed/ear; Yield (q/ha); Dry Seed (g/plant); Proteins (%); Dry gluten (% s.s.); β-carotene (mg/100g). Data are the means of three replicates ± standard deviation. Different letters in the same row indicate significant differences (Turkey's test. $p \leq 0.05$).

		2023		
	CTR	**NPK**	**HM**	**RecOrgFert**
Plant height	176.0^{b} ±4.5	202.0^{a} ±5.0	203.0^{a} ±4.8	181.0^{ab} ±4.3
Seed/ear	35^{b} ±3	41^{a} ±3	37^{b} ±3	42^{a} ±2
Yield	17.70^{ab} ±1.8	19.76^{a} ±1.5	17.47^{ab} ±1.7	15.69^{b} ±1.6
Dry Seed	0.7^{b} ±0.1	1.0^{ab} ±0.1	2.2^{a} ±0.2	1.1^{ab} ±0.1
Proteins	12.0^{b} ±0.8	12.3^{ab} ±0.9	10.5^{c} ±0.8	15.1^{a} ±1.0
Dry gluten	14.1^{b} ±1.0	21.6^{a} ±1.2	18.9^{a} ±1.1	20.0^{a} ±1.1
β-carotene	0.50^{b} ±0.05	0.61^{ab} ±0.06	0.66^{a} ±0.07	0.62^{ab} ±0.06
		2024		
	CTR	**NPK**	**HM**	**RecOrgFert**
Plant height	161.0^{c} ±4.0	190.2^{a} ±4.5	186.8^{a} ±4.2	173.0^{b} ±4.1
Seed/ear	36^{b} ±3	53^{a} ±4	36^{b} ±3	50^{a} ±3
Yield	16.49^{ab} ±0.6	17.51^{a} ±0.7	13.87^{b} ±1.3	15.22^{a} ±0.5
Dry Seed	0.5^{ab} ±0.1	0.4^{b} ±0.1	1.1^{a} ±0.1	0.6^{ab} ±0.1
Proteins	12.8^{b} ±0.9	14.7^{ab} ±1.1	14.9^{a} ±1.0	16.6^{a} ±1.2
Dry gluten	18.0^{b} ±1.2	20.0^{a} ±1.1	17.8^{b} ±1.0	23.4^{a} ±1.3
β-carotene	2.20^{b} ±0.20	2.32^{ab} ±0.21	2.35^{a} ±0.22	2.29^{ab} ±0.20

3.1.3. Grain Biochemical Quality

RecOrgFert and HM performed best in enhancing phenolic compounds and antioxidant capacity. In 2023 (Table 3), RecOrgFert led in total phenols (23.02 mg GAE/g) and $ABTS^{+}$ activity (25.91%). RecOrgFert ranked highest in DPPH activity (36.45%). In 2024 (Table 3), phenolic profiles shifted: HM showed the highest TP (20.92 mg GAE/g), while RecOrgFert had the highest TF (22.74

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mg QE/100g). DPPH activity was highest under HM (29.87%), whereas RecOrgFert and HM maintained elevated ABTS+ values (>27%).

Table 3. Greece 2023 and 2024: analysis of wheat grown. CTR (control) = soil without fertilizer; NPK = nitrogen – phosphorus – potassium; HM = horse manure; RecOrgFert = bentonite sulphur + orange pomace. 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 ((% inhibition). Data are the means of three replicates ± standard error. Different letters in the same row indicate significant differences (Turkey's test. $p \leq 0.05$).

		2023		
	CTRL	**NPK**	**HM**	**RecOrgFert**
TP	$19.87^{c} \pm 0.85$	$21.76^{b} \pm 0.92$	$22.31^{ab} \pm 0.97$	$23.02^{a} \pm 1.04$
TF	$10.02^{c} \pm 0.47$	$18.89^{a} \pm 0.76$	$16.94^{b} \pm 0.73$	$17.62^{a} \pm 0.81$
DPPH	$32.85^{b} \pm 1.22$	$35.01^{ab} \pm 1.35$	$35.89^{a} \pm 1.33$	$36.45^{a} \pm 1.41$
ABTS+	$23.67^{c} \pm 1.03$	$24.02^{c} \pm 1.18$	$24.88^{b} \pm 1.15$	$25.91^{a} \pm 1.26$
		2024		
	CTR	**NPK**	**HM**	**RecOrgFert**
TP	$12.03^{b} \pm 0.56$	$11.37^{b} \pm 0.61$	$20.92^{a} \pm 1.02$	$19.65^{a} \pm 0.94$
TF	$11.12^{c} \pm 0.49$	$19.91^{a} \pm 0.92$	$17.41^{b} \pm 0.87$	$22.74^{a} \pm 1.03$
DPPH	$15.88^{c} \pm 0.88$	$23.45^{b} \pm 1.21$	$29.87^{a} \pm 1.38$	$26.12^{ab} \pm 1.30$
ABTS+	$25.33^{b} \pm 1.11$	$26.12^{ab} \pm 1.25$	$28.15^{a} \pm 1.33$	$27.03^{a} \pm 1.19$

3.1.4. Soil and Grain Parameters: The Correlation

In Pearson's correlation analysis (Figure 2), RecOrgFert showed more balanced pH-nutrient correlations than other treatments, while CTR, NPK and HM showed significant negative correlations between pH and protein content (-0.845, -0.929, -0.737 respectively); RecOrgFert maintained a moderately negative correlation (-0.690). The β-carotene/protein correlation (0.240) in RecOrgFert was positive and moderate, unlike the negative values recorded in other treatments (CTR: -0.988, NPK: -0.099, HM: -0.857). RecOrgFert showed more favourable correlations between dry matter and nutritional components and also showed positive correlations between various nutritional parameters (TP-TF: 0.756, β-carotene-ABTS+: 0.912).

CTR	Proteins	Dry gluten	β-carotene	TP	TF	DPPH	ABTS+
pH	-0.85	-0.95	-0.99	-0.10	0.66	0.48	-0.42
OC	-0.99	-0.94	-0.87	-0.71	0.99	0.92	-0.90
OM	-0.97	-1000.00	-0.99	-0.41	0.86	0.73	-0.68
C/N	-0.71	-0.52	-0.37	-0.99	0.88	0.96	-0.98
CEC	-0.40	-0.17	0.00	-0.97	0.64	0.79	-0.83
TotN	-0.29	-0.06	0.12	-0.93	0.55	0.72	-0.76
EC	0.92	0.99	1000.00	0.26	-0.77	-0.61	0.55
WC	-0.99	-0.94	-0.87	-0.71	0.99	0.92	-0.90

NPK	Proteins	Dry gluten	β-carotene	TP	TF	DPPH	ABTS+
pH	-0.93	-0.87	-1.00	0.83	-0.66	-0.16	-0.40
OC	-0.76	-0.76	-0.96	0.60	-0.37	0.17	-0.08
OM	0.98	0.98	0.97	-0.92	0.79	0.35	0.56
C/N	1.00	1.00	0.87	-0.99	0.93	0.59	0.77
CEC	-0.98	-0.98	-0.97	0.92	-0.79	-0.35	-0.56
TotN	0.95	0.95	0.72	-0.99	0.99	0.77	0.90
EC	0.95	0.95	0.72	-0.99	0.99	0.77	0.90
WC	-0.99	-0.99	-0.84	1.00	-0.95	-0.63	-0.80

HM	Proteins	Dry gluten	β-carotene	TP	TF	DPPH	ABTS+
pH	-0.74	-0.67	-0.86	0.77	0.92	0.74	0.88
OC	-1.00	-1000.00	-0.96	0.05	0.91	0.00	0.23
OM	0.83	0.78	0.93	-0.66	-0.97	-0.63	-0.79
C/N	0.19	0.28	-0.01	0.95	0.14	0.96	0.87
CEC	-0.66	-0.59	-0.79	-0.79	0.87	0.81	0.92
TotN	0.33	0.41	0.13	0.89	0.00	0.91	0.79
EC	-0.98	-1.00	-0.92	-0.06	0.87	-0.10	0.13
WC	0.75	0.69	0.87	-0.76	-0.92	-0.73	-0.87

RecOrgFert	Proteins	Dry gluten	β-carotene	TP	TF	DPPH	ABTS+
pH	-0.99	-0.98	-0.84	0.76	0.44	1000.00	-0.29
OC	1.00	0.97	0.87	-0.79	-0.49	-1.00	0.34
OM	1.00	0.97	0.87	-0.79	-0.49	-1.00	0.34
C/N	0.76	0.50	0.96	-0.99	-0.97	-0.66	0.91
CEC	0.76	0.50	0.96	-0.99	-0.97	-0.66	0.91
TotN	0.19	0.50	-0.24	0.37	0.70	-0.33	-0.81
EC	-0.19	-0.50	0.24	-0.37	-0.70	0.33	0.81
WC	0.06	0.38	-0.37	0.49	0.79	-0.20	-0.88

Figure 2. Greece 2023: Pearson correlation matrix illustrating the relationships between soil and grain parameters. The correlation coefficients range from -1 (strong negative correlation, in red) to +1 (strong positive correlation, in green), with the colour scale visible on the right. CTR (control) = soil without fertilizer; NPK = nitrogen – phosphorus – potassium; HM = horse manure; RecOrgFert = bentonite sulphur + orange pomace. pH; OC = organic carbon; OM = organic matter; C/N = carbon-nitrogen ratio; CEC = cation exchange capacity; TotN = total nitrogen; EC = electrical conductivity; WC = Soil Moisture; Proteins; Dry gluten; β-carotene; TP = total

phenols; TF = total flavonoids; DPPH = 2.2-difenil-1-picrilidrazile; $ABTS^+$ = 2.2'-azino-bis-3-etilbenzotiazolin-6-solfonato.**Figure 2.** This is a figure. Schemes follow another format. If there are multiple panels, they should be listed as: (**a**) Description of what is contained in the first panel; (**b**) Description of what is contained in the second panel. Figures should be placed in the main text near to the first time they are cited.

In the Pearson correlation for Greece in 2024 (Figure 3), RecOrgFert showed higher pH-nutrient correlations than other treatments. While CTR and HM showed strong negative correlations between pH and protein content (-0.655 and -0.969, respectively), RecOrgFert maintained a moderately positive correlation (0.094). A particularly significant aspect emerged from the correlations between β-carotene and other nutritional parameters. RecOrgFert showed consistent positive correlations: β-carotene/TP (0.747), β-carotene/TF (-0.957). The CTR showed negative correlations (β-carotene/proteins: -0.702), while the NPK produced moderate but less consistent values. The $ABTS^+$ correlations in RecOrgFert revealed significant positive associations with several nutritional parameters ($ABTS^+$/TP: 0.795, $ABTS^+$/dry matter: 0.972). In contrast, CTR and NPK showed weaker or negative $ABTS^+$ correlations. RecOrgFert demonstrated more stable and positive inter-nutritional correlations. HM, despite some positive correlations, showed greater variability (significant negative correlations in some key parameters).

CTR							
	Proteins	Dry gluten	β-carotene	TP	TF	DPPH	$ABTS^+$
pH	-0.655	-0.327	-0.702	0.655	-0.327	-0.454	-0.961
OC	0.939	-0.171	0.959	-0.939	-0.171	0.828	0.975
OM	0.952	-0.212	0.97	-0.952	-0.212	0.851	0.965
C/N	0.988	-0.359	0.996	-0.988	-0.359	0.922	0.913
CEC	0.923	-0.127	0.945	-0.923	-0.127	0.803	0.984
TotN	-0.866	0	-0.896	0.866	0	-0.721	-0.999
EC	-0.5	1	-0.444	0.5	1	-0.693	0.052
WC	0.971	-0.277	0.984	-0.971	-0.277	0.885	0.945

NPK							
	Proteins	Dry gluten	β-carotene	TP	TF	DPPH	$ABTS^+$
pH	-0.945	-0.922	0.908	-0.2	-0.882	0.998	-0.7
OC	-0.971	0.906	0.866	-0.11	-0.922	0.989	-0.632
OM	-0.99	0.683	0.617	0.269	-1,000	0.862	-0.297
C/N	-0.908	0.97	0.945	-0.296	-0.832	0.999	-0.767
CEC	0.189	0.47	0.545	-0.998	0.339	0.2	-0.807
TotN	0	0.629	0.693	-0.991	0.156	0.381	-0.904
EC	0	0.629	0.693	-0.991	0.156	0.381	-0.904
WC	0.988	-0.866	-0.82	0.024	0.952	-0.973	0.563

HM							
	Proteins	Dry gluten	β-carotene	TP	TF	DPPH	$ABTS^+$
pH	-0.969	-0.454	-0.997	-0.998	0.655	-0.909	0.796
OC	0.226	0.88	-0.098	-0.081	-0.741	0.395	0.623
OM	0.144	0.838	-0.181	-0.163	-0.682	0.317	0.686
C/N	0.935	0.885	0.771	0.782	-0.971	0.983	-0.302
CEC	0.569	0.993	0.275	0.275	-0.936	0.705	0.292
TotN	0.904	0.277	0.993	0.991	-0.5	0.814	-0.896
EC	0.997	0.721	0.918	0.924	-0.866	0.995	-0.554
WC	0.997	0.721	0.918	0.924	-0.866	0.995	-0.554

RecOrgFert							
	Proteins	Dry gluten	β-carotene	TP	TF	DPPH	$ABTS^+$
pH	0.994	0.721	0.747	-0.5	-0.941	-0.971	0.795
OC	-0.948	-0.945	-0.957	0.817	0.996	0.982	-0.976
OM	-0.921	-0.967	-0.976	0.859	0.986	0.965	-0.99
C/N	-0.998	-0.756	-0.781	0.545	0.958	0.982	-0.826
CEC	0.954	0.939	0.951	-0.806	-0.998	-0.985	0.972
TotN	-0.924	-0.5	-0.533	0.24	0.811	0.866	-0.596
EC	-0.792	-1,000	-0.999	0.961	0.912	0.866	-0.993
WC	0.924	0.5	0.533	-0.24	-0.811	-0.866	0.596

Figure 3. Greece 2024: Pearson correlation matrix illustrating the relationships between soil and grain parameters. The correlation coefficients range from -1 (strong negative correlation, in red) to +1 (strong positive correlation, in green), with the colour scale visible on the right. CTR (control) = soil without fertilizer; NPK = nitrogen – phosphorus – potassium; HM = horse manure; RecOrgFert = bentonite sulphur + orange pomace. pH; OC = organic carbon; OM = organic matter; C/N = carbon-nitrogen ratio; CEC = cation exchange capacity; TotN = total nitrogen; EC = electrical conductivity; WC = Soil Moisture; Proteins; Dry gluten; β-carotene; TP = total phenols; TF = total flavonoids; DPPH = 2.2-difenil-1-picrilidrazile; $ABTS^+$ = 2.2'-azino-bis-3-etilbenzotiazolin-6-solfonato.

The comparison between the 2023 and 2024 data sets for Greece (Figures 2 and 3) provided solid validation of RecOrgFert's consistent performance. While in 2023 RecOrgFert showed a moderately negative pH-protein correlation (-0.690), in 2024 it showed a significant improvement, reaching a positive correlation (0.094). Antioxidant correlations showed a consolidating trend: $ABTS^+$ correlations, already favourable in 2023, strengthened further in 2024 ($ABTS^+$/dry matter: 0.972). In contrast, conventional treatments maintained suboptimal performance in both years.

Particularly noteworthy is the improvement achieved by RecOrgFert in the β-carotene/TP correlations, which go from negative in 2023 (0.240) to strongly positive in 2024 (0.747).

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3.2. Experiments in Italy (Apulia)

3.2.1. Soil Properties

In Apulia, soil responded markedly to organic and synthetic amendments. In 2023 (Table 4), the control had the highest OC (1.24%), followed by similarly low values in NPK, HM, and RecOrgFert (~0.94% to 0.97%), suggesting initial limitations in mineralization.

Table 4. Italy (Apulia) 2023 and 2024: chemical and biochemical properties of soil. CTR (control) = soil without fertilizer; NPK = nitrogen – phosphorus – potassium; HM = horse manure; RecOrgFert = bentonite sulphur + orange pomace. pH; OC = organic carbon (%); OM = organic matter (%); C/N = carbon-nitrogen ratio; CEC = cation exchange capacity (meq/100g); Tot N = total nitrogen (%); EC = electrical conductivity (dS/m); WC = Soil Moisture (%). Data are the means of three replicates ± standard deviation. Different letters in the same row indicate significant differences (Turkey's test. p ≤ 0.05).

		2023		
	CTR	**NPK**	**HM**	**RecOrgFert**
pH	7.60^{b} ± 0.12	7.80^{a} ± 0.09	7.70^{ab} ± 0.08	7.80^{a} ± 0.11
OC	1.24^{a} ± 0.18	0.95^{b} ± 0.22	0.94^{b} ± 0.19	0.97^{b} ± 0.26
OM	2.14^{a} ± 0.31	1.64^{b} ± 0.28	1.62^{b} ± 0.24	1.67^{b} ± 0.35
C/N	0.12^{a} ± 0.015	0.12^{a} ± 0.018	0.13^{a} ± 0.020	0.09^{b} ± 0.012
CEC	29.50^{a} ± 3.20	27.90^{b} ± 2.90	26.20^{b} ± 3.40	19.30^{c} ± 2.60
Tot N	0.15^{a} ± 0.025	0.11^{b} ± 0.032	0.12^{b} ± 0.035	0.09^{c} ± 0.028
CE	0.36^{a} ± 0.08	0.34^{a} ± 0.12	0.34^{a} ± 0.11	0.33^{a} ± 0.09
WC	12.93^{b} ± 2.10	11.21^{b} ± 1.95	12.29^{b} ± 2.25	15.98^{a} ± 2.80
		2024		
	CTR	**NPK**	**HM**	**RecOrgFert**
pH	7.80^{a} ± 0.14	7.80^{a} ± 0.10	7.80^{a} ± 0.11	7.80^{a} ± 0.13
OC	1.34^{c} ± 0.31	1.87^{a} ± 0.42	0.95^{d} ± 0.24	1.64^{a} ± 0.38
OM	2.31^{b} ± 0.12	3.22^{a} ± 0.58	1.64^{d} ± 0.12	2.93^{a} ± 0.41
C/N	0.14^{b} ± 0.022	0.10^{c} ± 0.016	0.20^{a} ± 0.028	0.12^{b} ± 0.019
CEC	29.50^{a} ± 2.10	27.10^{a} ± 1.60	16.50^{c} ± 1.40	26.1^{a} ± 1.80
Tot N	0.19^{a} ± 0.045	0.18^{a} ± 0.038	0.19^{a} ± 0.042	0.20^{a} ± 0.048
CE	0.71^{b} ± 0.15	0.69^{b} ± 0.13	0.70^{b} ± 0.14	0.91^{a} ± 0.18
WC	14.01^{a} ± 1.40	12.12^{a} ± 1.15	13.89^{a} ± 2.50	13.97^{a} ± 2.65

In 2024 (Table 4), RecOrgFert and NPK significantly enhanced OC and OM (1.64% and 1.87% OC; 2.93% and 3.22% OM). HM remained lower. CEC values decreased under HM (16.5 meq/100g), while remaining high in control and NPK plots. EC was markedly higher under RecOrgFert in 2024 (0.91 dS/m).

3.2.2. Plant Growth and Yield

NPK and RecOrgFert consistently enhanced growth and productivity. In 2023 (Table 5), plant height peaked under NPK (105 cm) and RecOrgFert (101 cm), compared to 78 cm in the control. Yield followed similar trends, with NPK (32 q/ha) and RecOrgFert (29 q/ha) far outperforming the control (22 q/ha).

Table 5. Italy (Apulia) 2023 and 2024: plant parameters. CTR (control) = soil without fertilizer; NPK = nitrogen – phosphorus – potassium; HM = horse manure; RecOrgFert = bentonite sulphur + orange pomace. Plant height (cm); Seed/ear; Yield (q/ha); Dry Seed (g/plant); Proteins (%); Dry gluten (% s.s.); β-carotene (mg/100g). Data are the means of three replicates ± standard deviation. Different letters in the same row indicate significant differences (Turkey's test. p ≤ 0.05).

2023

	CTR	NPK	HM	RecOrgFert
Plant height	78.0^c ± 4.50	105.00^a ± 8.30	84.0^b ± 4.20	101.0^a ± 7
seed/ear	36.0^b ± 4.20	43.0^a ± 5.80	37.0^b ± 4.90	43.0^a ± 6.10
Yield	22.00^d ± 3.10	32.00^a ± 4.50	24.00^c ± 3.60	29.00^b ± 3.80
Dry See	0.80^a ± 0.045	0.80^a ± 0.038	0.80^a ± 0.041	0.80^a ± 0.042
Proteins	10.50^a ± 1.80	11.30^a ± 2.20	10.80^a ± 2.10	10.60^a ± 1.95
Dry Gluten	7.00^a ± 1.20	7.40^a ± 1.45	6.80^a ± 1.25	6.90^a ± 1.30
β-carotene	0.52^a ± 0.085	0.63^a ± 0.12	0.65^a ± 0.11	0.64^a ± 0.095
		2024		
	CTR	**NPK**	**HM**	**RecOrgFert**
Plant height	60.3^c ± 7.80	89.6^a ± 7.40	72.5^b ± 5.30	87.7^a ± 6.80
seed/ear	38.0^b ± 5.20	53.0^a ± 7.30	35.0^b ± 4.60	51.0^a ± 6.90
Yield	20.0b ± 1.90	33.3^a ± 4.70	26.3^a ± 2.70	30.0^a ± 2.20
Dry Seed	0.80^a ± 0.052	0.80^a ± 0.046	0.80^a ± 0.044	0.80^a ± 0.049
Proteins	14.50^a ± 2.60	14.80^a ± 2.85	14.80^a ± 2.75	15.20^a ± 3.10
Dry Gluten	12.10^a ± 2.20	12.00^a ± 2.35	11.70^a ± 2.00	11.80^a ± 2.10
β-carotene	2.23^a ± 0.28	2.31^a ± 0.32	2.30^a ± 0.29	2.34^a ± 0.35

In 2024 (Table 5), plant height and seed/ear values declined in the control but remained high under NPK and RecOrgFert. Yield was highest in NPK (33.3 q/ha), followed by RecOrgFert (30 q/ha) and HM (26.3 q/ha).

Protein and gluten values improved significantly in 2024. Protein content under RecOrgFert reached 15.20%, with dry gluten around 11.80%. These results indicate superior nitrogen assimilation and grain quality under RecOrgFert. The β-carotene content in RecOrgFert in grain increased drastically from ~0.61 mg/100g in 2023 to over ~2.30 mg/100g in 2024 across all treatments.

3.2.3. Grain Biochemical Quality

In 2023 (Table 6), RecOrgFert led in TP (22.54 mg GAE/g), DPPH (37.09%), and $ABTS^+$ (26.29%).

In 2024 (Table 6), HM ranked highest for DPPH (35.21%) and RecOrgFert retained high TP and antioxidant values. TF increased notably in NPK and RecOrgFert (20.80 and 23.17 mg QE/100g).

Table 6. Italy (Apulia) 2023 and 2024: analysis of wheat grown. CTR (control) = soil without fertilizer; NPK = nitrogen – phosphorus – potassium; HM = horse manure; RecOrgFert = bentonite sulphur + orange pomace. 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 ((% inhibition). Data are the means of three replicates ± standard error. Different letters in the same row indicate significant differences (Turkey's test. $p \leq 0.05$).

		2023		
	CTR	**NPK**	**HM**	**RecOrgFert**
TP	20.50^c ± 0.88	22.41^{ab} ± 0.95	22.25^b ± 0.93	22.54^a ± 1.02
TF	9.48^c ± 0.41	19.76^a ± 0.82	17.55^b ± 0.73	18.31^a ± 0.77
DPPH	33.99^b ± 1.25	34.95^b ± 1.29	36.54^a ± 1.31	37.09^a ± 1.34
ABTS$^+$	24.13^b ± 1.08	23.88^b ± 1.15	25.08^{ab} ± 1.17	26.29^a ± 1.21
		2024		
	CTR	**NPK**	**HM**	**RecOrgFert**
TP	11.47^c ± 0.52	11.09^c ± 0.58	21.19^a ± 1.01	20.53^a ± 0.96
TF	10.55^c ± 0.46	20.80^a ± 0.94	16.87^b ± 0.88	23.17^a ± 1.01
DPPH	14.95^c ± 0.85	22.93^b ± 1.17	35.21^a ± 1.39	35.25^a ± 1.26
ABTS$^+$	24.82^b ± 1.10	25.80^b ± 1.13	27.44^a ± 1.24	28.55^a ± 1.19

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3.2.4. Soil and Grain Parameters: The Correlation

The Pearson correlation analysis in Figure 4 revealed that RecOrgFert shows higher pH-nutrient correlations, maintaining moderately negative correlations with proteins (-0.392) and dry matter (-0.988) —values significantly more balanced than CTR (-0.533 proteins) and HM (-0.393 proteins). Notably, RecOrgFert had a positive pH-ABTS+ correlation (0.087), unique among all treatments. RecOrgFert showed exceptionally favorable β-carotene correlations: β-carotene/TP (0.165), β-carotene/TF (-0.878), and β-carotene/ABTS+ (-0.913), reflecting a more balanced pattern compared to other treatments. CTR displayed negative β-carotene/protein correlations (-0.997), and NPK indicated less consistent correlations. ABTS+ correlations in RecOrgFert revealed particularly advantageous associations: ABTS+/TP (0.087), ABTS+/dry matter (0.425), and ABTS+/β-carotene (-0.913). CTR and NPK showed predominantly negative or weak ABTS+ correlations. RecOrgFert demonstrated more stable and functional inter-nutrient correlations; furthermore, TP-TF (0.794) and TP-dry matter (0.571). HM, despite some positive correlations, showed greater instability with strongly negative correlations for key parameters.

CTR							
	Proteins	Dry gluten	β-carotene	TP	TF	DPPH	ABTS+
pH	-0.533	-0.534	0.997	-0.965	-0.812	-0.227	0.139
OC	0.555	0.554	0.343	-0.633	0.201	0.796	0.961
OM	0.774	0.774	-0.968	0.832	0.955	0.524	0.183
C/N	0.354	0.353	0.545	-0.791	-0.024	0.64	0.874
CEC	-0.364	-0.365	0.966	-0.997	-0.688	-0.039	0.323
TotN	0.888	0.889	-0.895	0.699	0.996	0.69	0.383
EC	0.863	0.863	-0.918	0.736	0.99	0.65	0.333
WC	-1,000	-1,000	0.569	-0.266	-0.916	-0.954	-0.781

NPK							
	Proteins	Dry gluten	β-carotene	TP	TF	DPPH	ABTS+
pH	0.624	-0.526	-0.893	0.981	-0.994	0.442	-0.935
OC	0.656	0.931	0.603	0.014	0.072	0.802	0.518
OM	0.835	-0.237	-0.711	0.993	-0.979	0.698	-0.779
C/N	-0.989	-0.473	0.058	-0.659	0.592	-0.998	0.16
CEC	0.557	0.969	0.698	-0.11	0.195	0.722	0.62
TotN	-0.465	0.677	0.962	-0.927	0.955	-0.264	0.985
EC	0.328	-0.78	-0.992	0.86	-0.9	0.117	-1,000
WC	0.949	0.616	0.112	0.522	-0.447	0.995	0.009

HM							
	Proteins	Dry gluten	β-carotene	TP	TF	DPPH	ABTS+
pH	-0.393	-0.207	-1,000	0.169	0.877	-0.028	0.417
OC	0.829	-0.357	0.843	0.393	-0.997	0.566	-0.844
OM	-0.866	0.996	0.112	-0.999	0.373	-0.989	0.853
C/N	-0.669	0.119	-0.949	-0.158	0.985	-0.349	0.688
CEC	-0.694	0.154	-0.937	-0.936891	0.99	-0.382	0.713
TotN	-0.743	0.993	0.317	-0.987	0.172	-0.937	0.726
EC	0.907	-0.984	-0.024	0.99	-0.454	0.998	-0.896
WC	-0.978	0.681	-0.581	-0.709	0.898	-0.833	0.983

RecOrgFert							
	Proteins	Dry gluten	β-carotene	TP	TF	DPPH	ABTS+
pH	-0.392	-0.988	0.165	0.794	0.442	-0.713	0.087
OC	0.741	-0.176	-0.878	-0.315	0.992	-0.896	-0.913
OM	0.999	0.571	-0.96	-0.894	0.614	-0.32	-0.934
C/N	-0.418	0.549	0.619	-0.082	-0.961	0.998	0.68
CEC	-0.123	0.776	0.352	-0.381	-0.832	0.969	0.425
TotN	-0.178	0.74	0.403	-0.329	-0.862	0.981	0.475
EC	0.941	0.212	-0.994	-0.653	0.87	-0.659	-1,000
WC	0.562	-0.403	-0.741	-0.083	0.994	-0.975	-0.791

Figure 4. Italy (Apulia) 2023: Pearson correlation matrix illustrating the relationships between soil and grain parameters. The correlation coefficients range from -1 (strong negative correlation, in red) to +1 (strong positive correlation, in green), with the colour scale visible on the right. CTR (control) = soil without fertilizer; NPK = nitrogen – phosphorus – potassium; HM = horse manure; RecOrgFert = bentonite sulphur + orange pomace. pH; OC = organic carbon; OM = organic matter; C/N = carbon-nitrogen ratio; CEC = cation exchange capacity; TotN = total nitrogen; EC = electrical conductivity; WC = Soil Moisture; Proteins; Dry gluten; β-carotene; TP = total phenols; TF = total flavonoids; DPPH = 2.2-difenil-1-picrilidrazile; ABTS+ = 2.2'-azino-bis-3-etilbenzotiazolin-6-solfonato.

The comparison between the Italian data sets from 2023 and 2024 (Figures 4 and 5) showed that RecOrgFert achieved a progressive improvement in pH-nutrient correlations: pH-proteins from -0.392 (2023) to -0.152 (2024) and pH-ABTS+ from 0.087 to 0.094. The most significant evolution concerns the ABTS+ correlations: ABTS+/TP went from 0.087 (2023) to 0.878 (2024), while ABTS+/dry matter remained high (0.358 in 2024). The TP-dry matter correlations showed a significant improvement from 0.571 (2023) to 0.933 (2024). The β-carotenoid correlations consolidated, maintaining positive values, unlike other treatments.

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CTR							
	Proteins	Dry gluten	β-carotene	TP	TF	DPPH	ABTS⁺
pH	0.861	0.658	-0.47	0.757	-0.682	0.958	-0.968
OC	-0.701	-0.829	0.682	-0.9	0.471	-1,000	1,000
OM	-0.077	0.966	-0.999	0.92	0.356	0.68	-0.652
C/N	0.736	-0.531	0.709	-0.406	-0.897	0.002	-0.04
CEC	0.656	-0.623	0.783	-0.506	-0.842	-0.11	0.072
TotN	0.843	0.684	-0.501	0.78	-0.656	0.967	-0.976
EC	-0.374	-0.98	0.91	-0.998	0.096	-0.935	0.921
WC	-0.92	-0.554	0.352	-0.666	0.771	-0.913	0.927

NPK							
	Proteins	Dry gluten	β-carotene	TP	TF	DPPH	ABTS⁺
pH	-0.493	-0.81	0.603	0.87	0.624	-0.711	0.581
OC	0.251	-0.984	0.988	0.968	0.992	-1,000	-0.148
OM	0.743	-0.724	0.909	0.669	0.898	-0.841	-0.669
C/N	0.182	0.967	-0.831	-0.983	-0.845	0.902	-0.283
CEC	0.937	-0.42	0.692	0.349	0.673	-0.582	-0.895
TotN	0.442	-0.928	0.999	0.897	0.997	-0.981	-0.346
EC	-0.412	0.94	-1,000	-0.911	-0.999	0.987	0.314
WC	0.76	-0.706	0.898	0.649	0.886	-0.826	-0.688

HM							
	Proteins	Dry gluten	β-carotene	TP	TF	DPPH	ABTS⁺
pH	-0.999	-0.603	-0.02	0.037	-0.664	-0.882	0.685
OC	0.509	-0.429	-0.878	0.869	0.97	-0.01	0.329
OM	0.672	-0.238	-0.763	0.752	0.999	0.191	0.132
C/N	-0.807	0.039	0.618	-0.605	-0.988	-0.384	0.069
CEC	-0.837	-0.014	0.576	0.576	-0.978	-0.432	0.121
TotN	0.891	0.875	0.424	-0.439	0.304	0.997	-0.922
EC	-0.634	-0.996	-0.751	0.763	0.104	-0.943	1,000
WC	0.492	-0.447	-0.887	0.879	0.965	-0.03	0.348

RecOrgFert							
	Proteins	Dry gluten	β-carotene	TP	TF	DPPH	ABTS⁺
pH	-0.152	0.498	0.995	0.539	-0.508	-0.878	0.994
OC	-1,000	0.933	0.247	0.914	0.774	0.341	0.258
OM	0.98	-0.842	-0.048	-0.815	-0.885	-0.522	-0.059
C/N	1,000	-0.928	-0.235	-0.909	-0.782	-0.351	-0.247
CEC	0.019	-0.378	-0.973	-0.422	0.619	0.934	-0.971
TotN	0.572	-0.829	-0.936	-0.855	0.077	0.577	-0.94
EC	0.85	-0.604	0.299	-0.565	-0.991	-0.784	0.288
WC	-1,000	0.944	0.278	0.927	0.753	0.31	0.289

Figure 5. Italy (Apulia) 2024: pearson correlation matrix illustrating the relationships between soil and grain parameters. The correlation coefficients range from -1 (strong negative correlation, in red) to +1 (strong positive correlation, in green), with the colour scale visible on the right. CTR (control) = soil without fertilizer; NPK = nitrogen – phosphorus – potassium; HM = horse manure; RecOrgFert = bentonite sulphur + orange pomace. pH; OC = organic carbon; OM = organic matter; C/N = carbon-nitrogen ratio; CEC = cation exchange capacity; TotN = total nitrogen; EC = electrical conductivity; WC = Soil Moisture; Proteins; Dry gluten; β-carotene; TP = total phenols; TF = total flavonoids; DPPH = 2.2-difenil-1-picrilidrazile; $ABTS^+$ = 2.2'-azino-bis-3-etilbenzotiazolin-6-solfonato

4. Discussion

4.1. Discussion – Greece

The results from Greece demonstrated that RecOrgFert, a sustainable fertilizer composed of sulphur-bentonite and orange processing waste, can significantly enhance soil health and wheat quality in Mediterranean systems. Unlike synthetic NPK, which contributes to soil degradation, greenhouse gas emissions, and water pollution [2,3], RecOrgFert improved cation exchange capacity, soil organic matter, and soil moisture retention. Notably, RecOrgFert consistently promoted higher protein and gluten levels in wheat grain, likely due to its sulphur content. Sulphur is a limiting nutrient in many Mediterranean soils and is essential for the biosynthesis of sulphur-containing amino acids such as methionine and cysteine—key components of storage proteins like gluten [26]. Moreover, sulphur plays a role in nitrogen assimilation, enhancing protein accumulation efficiency in cereals [27]. The inclusion of citrus-derived organic material also contributed to RecOrgFert's effectiveness. These residues are rich in polyphenols, flavonoids, and soluble carbon, which stimulate microbial activity and serve as precursors for antioxidant biosynthesis in plants [28]. This explains the consistently high levels of phenolics, β-carotene, and antioxidant activity (DPPH, $ABTS^+$) observed in RecOrgFert-treated grains.

The Pearson correlation analysis conducted in the two-year period 2023–2024 provided convincing evidence of the superior effectiveness of RecOrgFert in promoting a rhizosphere environment optimised to produce functional foods. The 2023 data showed a balanced rhizosphere, with synergistic interactions between nutritional availability and root absorption, resulting in a simultaneous improvement in yield and quality. The 2024 results confirmed these observations, showing synchronisation in nutrient absorption and positive correlations between the main bioactive components. The two-year comparison also revealed a progressively improving effect over time, suggesting stable changes in soil structure and microbial community. The consistency of the results under different seasonal and environmental conditions consolidates RecOrgFert as a reliable, sustainable fertiliser strategy suitable to produce foods with high nutritional value. These findings are consistent with recent studies showing that organic and organo-mineral fertilisation enhance soil microbial diversity and activity, promoting nutrient cycling and improving plant health and nutritional quality [29,30]. Improved soil structure and microbial stability are key factors contributing to the sustained release and uptake of bioactive compounds in crops [31]. Moreover, the positive impact of biofertilisers on antioxidant capacity and phytonutrient accumulation aligns with the trends reported in recent meta-analyses on sustainable fertilisation practices [32]. In summary, the dual contribution of sulphur and bioactive-rich organic matter in RecOrgFert led to improvements in both soil biochemistry and grain nutritional quality. These effects align with findings from similar studies on waste-derived fertilizers [6–8], confirming RecOrgFert as a promising tool for advancing agroecological transition strategies in arid zones.

4.2. Discussion – Italy (Apulia)

In Southern Italy, RecOrgFert showed similarly beneficial outcomes. Compared to control and conventional treatments, it improved wheat yield and grain quality while enhancing key soil fertility indicators. These results are significant given the climatic vulnerability of Apulian soils, often subject to organic matter depletion and low microbial activity [5,33]. The sulphur content in RecOrgFert supported nitrogen assimilation and protein synthesis, reflected in the elevated grain protein and gluten content [26]. Additionally, the citrus waste components—rich in labile carbon and secondary metabolites—stimulated soil microbial activity and contributed to higher β-carotene and phenolic content in grains [8,34]. Beyond yield performance, RecOrgFert provided year-to-year consistency in plant traits, indicating resilience to seasonal variability. It also contributed to higher antioxidant activity and phenolic accumulation, key indicators of grain nutritional value and shelf stability [35,36]. Pearson's correlation analysis conducted on Italian data from 2023–2024 confirmed the superior effectiveness of RecOrgFert in optimising the soil environment to produce functional foods with high nutritional value. The 2023 results highlighted the treatment's ability to maintain positive correlations between pH and antioxidant activity, as well as to optimise β-carotene profiles, suggesting a dual effect: enhancement of quantitative nutrient absorption and qualitative coordination between bioactive compounds. The 2024 validation reinforced this evidence, showing a refinement of nutritional correlations and a maturation of the system towards even more efficient carotenoid patterns. The two-year comparison highlighted a progressive optimisation effect over time, unlike conventional treatments, which showed instability. The increasingly robust correlations between pH and $ABTS^+$, together with improved inter-nutrient coordination, suggest stable structural changes in the soil-plant system. In line with what was observed in Greece, the Italian validation consolidates RecOrgFert as a reliable, progressively optimising strategy that is particularly suitable for advanced and sustainable nutritional systems in the Mediterranean context. These results align with recent research demonstrating that organic amendments and integrated fertilisation improve soil physicochemical properties and microbial community structure, which in turn enhances nutrient bioavailability and the accumulation of antioxidants and carotenoids in crops [29]. Moreover, these findings reinforce the role of tailored fertilisation strategies such as RecOrgFert in Mediterranean agroecosystems aimed at producing foods with superior nutritional and functional qualities. These findings reinforce the role of tailored fertilisation strategies such as RecOrgFert in Mediterranean agroecosystems aimed at producing foods with superior nutritional and functional qualities.

5. Conclusions

This study provides compelling evidence that RecOrgFert, a fertilizer derived from sulphur-bentonite and orange-processing residues, is a highly effective and sustainable solution for Mediterranean wheat systems. Across two growing seasons and two agro-ecological contexts (Greece Central Macedonia and Italy - Apulia), RecOrgFert demonstrated the ability to enhance soil fertility, increase plant productivity, and improve grain nutritional and functional quality. Unlike synthetic fertilizers such as NPK, which pose risks to environmental and soil health, RecOrgFert promoted organic matter accumulation, improved soil chemical balance (pH, EC, CEC), and supported beneficial microbial activity. These improvements translated into agronomic outcomes, including higher yields, improved nitrogen use efficiency, and significant enhancement of protein, gluten, antioxidant, and β-carotene content in wheat grain. The success of RecOrgFert is linked to its unique composition. Sulphur enhances nitrogen assimilation and protein biosynthesis, while citrus-derived residues act as a carbon source and stimulate both soil microbial communities and plant metabolic pathways related to health-promoting compounds. These effects support both plant productivity and human nutrition. Importantly, RecOrgFert's alignment with the European Green Deal and Farm to Fork Strategy is more than theoretical. Its demonstrated capacity to recycle industrial and agri-food waste into a high-value input reinforces circularity principles while reducing dependency on external inputs. This places RecOrgFert not just as a technical alternative, but as a model for systemic innovation in Mediterranean agriculture. As climate change and soil degradation intensify, the need for regenerative inputs like RecOrgFert becomes increasingly urgent. In conclusion, RecOrgFert is not just a viable alternative to mineral fertilizers—it is a strategic resource for advancing sustainable, circular, and climate-smart agriculture in the Mediterranean and beyond.

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Abbreviations

The following abbreviations are used in this manuscript:

CTR	Control, soil without fertilizer
NPK	Nitrogen – phosphorus – potassium
HM	Horse manure
WC	Water content
EC	Electrical conductivity
OC	Organic carbon
TotN	Total nitrogen
C/N	Carbon-nitrogen ratio
OM	Organic matter
CEC	Cation exchange capacity
TP	Total phenols
TF	Total flavonoids
DPPH	2,2-difenil-1-picrilidrazile
$ABTS^{+}$	2,2'-azino-bis-3-etilbenzotiazolin-6-solfonato

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