

Article

Looking for New P Fertilizers: Comparative Study of Mineral-, Organomineral- and Organic-Based Fertilizers for Lettuce (*Lactuca sativa* L.)

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Abstract

In this study several phosphorus fertilizers were evaluated under controlled production conditions using *Lactuca sativa* var. baby leaf and a clay-loam soil of pH 6.5 as a plant–soil model system. Various inorganic (phosphate rock, monoammonium phosphate, struvite), organic (bone meal and bone meal pelletized with compost) and organomineral fertilizers (phosphate rock, monoammonium phosphate, struvite pelletized with compost) were compared. The soil properties, crop yield, morphological aspects and metabolomics of the plants were analyzed. After 45 days of the growing cycle, the organomineral fertilizers (OMFs) composed of compost and monoammonium phosphate (OMF₂(MAP+C)) or struvite (OMF₃(STR+C)) exhibited the best yield results: 101.37 g and 83.21 g, respectively. These treatments also exhibited the best phosphorus use efficiency (PUE) results: 7.40% and 8.33%, respectively. The yield of plants treated with MAP was 56.01 g, and its PUE was 5.33%. The yield of plants treated with STR was 62.10 g and the PUE was 4.67%. Accordingly, the development of OMFs with compost had a positive effect regarding MAP and STR fertilization. Lettuce fertilized with organic bone meal fertilizers had the lowest yield and nutrient use efficiency. The non-targeted metabolic study of green tissue revealed an overactivation of the TriCarboxylic Acids-TCA cycle and amino acid biosynthesis in plants fertilized with bone meal and phosphate rock treatments, likely as a plant stress response. The overall conclusion of this work is that the development of OMFs with compost is a good strategy to increase soil P availability and, accordingly, plant P uptake and %PUE.

Keywords: phosphorus; lettuce; struvite; organomineral fertilizers; yield; NUE; PUE; metabolomics



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1. Introduction

Phosphorus is essential in crop nutrition and to ensure crop productivity. However, P for fertilizer production is mostly obtained from the mining of phosphate rock (PR) [1], which is a non-renewable resource and is highly concentrated in few countries, generating dependency on imports. The major concentration of these reserves is found in Morocco,

accounting for more than 70% of the world's phosphate reserves (50,000 Mt) [2]. Morocco dominates PR mining together with other countries such as China and Russia [3]. It is mandatory to find alternative P supplies to eliminate the uncertainty on P fertilizer availability in the future.

After years of conventional inorganic fertilizer use in an intensive agriculture context, P is considered an ecological contaminant. NPK fertilizers contain usually 60–70% P in its water-soluble form. P not immediately absorbed by plant roots is immobilized by the soil [4]. In acidic soils, most P is readily adsorbed on the surfaces of iron (Fe) and aluminum (Al) (hydr)oxides. In calcareous soils P is predominantly adsorbed on calcite surfaces or precipitated as calcium phosphates, predominantly as dicalciumphosphate. In soils oversaturated with P due to overfertilization practices over prolonged times, it partially leaches towards water bodies. This results in eutrophication, a huge environmental concern [5].

It is urgent to close the agricultural P cycle in order to avoid side-effects caused by excess P accumulating in soil. The strategy to be addressed is to develop sustainable fertilizers which ensure complete P utilization. Slow-release fertilizers reduce leaching losses [6,7]. An interesting slow-release fertilization strategy is the use of organomineral fertilizers (OMFs) [8], which combine a high nutrient dosage in an organic matrix. OMFs provide the soil with organic matter which improves the soil's water retention capacity and decreases erosion [9]. OMFs with compost help to restore soil fertility [10]. The technology employed to develop OMFs is pelletization, which is a widespread strategy in animal feed production [11]. The pelletization of compost enables easier handling, transport and storage. Through pelletization, compost can be combined with other fertilizing materials to achieve nutrient concentration and uniform amending properties [12].

Currently, <50% of phosphorus wastes/residues are recycled back into the global food system [13]. Biowaste, due to its high generation rate, is a critical issue at a global level. P recovery from residues such as sludge or manure is a desirable strategy for recycling nutrients, avoiding environmental pollution and contributing to a circular bioeconomy model. Some available P sources include municipal and industrial wastewater, meat and bone meal (MBM) and other organic wastes [14]. One widespread approach being implemented at lab and industrial scales is struvite crystallization [15].

The aim of this work was to study the potential of compost-based OMFs as slow-release fertilizers in comparison to mineral and organic fertilizers in a pot study, together with the evaluation of the fertilizing capacity of recovered P sources. We comparatively studied the effect of the treatments on (1) soil properties, (2) crop yield, (3) nutrient use efficiency and (4) the plant metabolic state at harvest. For the present research we developed various OMFs with organic matrix compost, combined with several P sources: conventional mineral fertilizers like monoammonium phosphate (MAP) and phosphate rock (PR), organic recovered fertilizers like bone meal (BM), and struvite (STR) recovered from a wastewater treatment plant (WWTP). The plant model employed in this work was *Lactuca sativa* var. baby leaf. According to the most recent data from FAOSTAT [16] corresponding to the year 2023, in Europe, lettuce crop represented an extension of 127,311 ha, accounting for a total production of 3,332,479.7 tons. Specifically in Spain, lettuce crop production was 864,570 tons, which represents 26% of total European lettuce production. *Lactuca sativa* is a desirable plant model, since it has a short vegetative cycle; the duration of the crop is usually between 45 and 60 days.

2. Materials and Methods

2.1. Experimental Design

The P treatments studied are classified into 2 groups: simple and complex OMF treatments. The simple treatments include inorganic materials, such as phosphate rock (PR, 0-33-0 %N-P₂O₅-K₂O), monoammonium phosphate (MAP, 11-61-0) and struvite recovered from a wastewater treatment plant (STR, 5-33-0), and organic materials, including meat industry by-product bone meal (BM, 3-30-0). The complex OMF treatments involve organomineral fertilizers with the addition of compost at a 50:50 ratio. We used the conventional inorganic fertilizer ComplexIN (15-15-15) as a reference for 3 doses: 100 (IN100), 200 (IN200) and 300 (IN300) kg NPK ha⁻¹. In all cases an isodose of potassium nitrate (KNO₃, 13-0-46) was supplied to satisfy the N demand of the crop. The N and P sources for all the treatments included in the experiment are shown in Table 1. The fertilizing treatments were applied according to a normalized N application rate of 200 kg N ha⁻¹ and 120 kg P ha⁻¹.

Table 1. Treatment summaries and NPK sources.

Treatment Group	Treatment	N Source	P Source	K Source
Control	Control	--	--	--
Conventional inorganic treatments	IN100	IN100	IN100	IN100
	IN200	IN200	IN200	IN200
	IN300	IN300	IN300	IN300
Simple treatments	PR	KNO ₃	Phosphate rock	KNO ₃
	MAP	KNO ₃	Monoammonium phosphate	KNO ₃
	STR	KNO ₃	Struvite	KNO ₃
	BM	KNO ₃	Bone meal	KNO ₃
Complex OMF treatments	OMF ₁ (PR+C)	KNO ₃ + compost	Phosphate rock + Compost	KNO ₃ + compost
	OMF ₂ (MAP+C)	KNO ₃ + compost	Monoammonium phosphate + Compost	KNO ₃ + compost
	OMF ₃ (STR+C)	KNO ₃ + compost	Struvite + Compost	KNO ₃ + compost
	OMF ₄ (BM+C)	KNO ₃ + compost	Bone meal + Compost	KNO ₃ + compost

A pot trial was performed using lettuce (*Lactuca sativa* var. baby leaf) as a plant model. The soil used was a clay-loam soil collected from the EPS-Orihuela experimental farm (OECD 208:2006). The soil was mixed with 4% FeSO₄ to acidify soil pH from a value of 8.5 to 6.5 to improve nutrient availability. The soil had an initial electrical conductivity of 3.75 dS m⁻¹, a total N content of 0.86 g kg⁻¹, 1.62 mg kg⁻¹ N-NH₄⁺, 26.2 mg kg⁻¹ N-NO₃⁻, 15.6 mg kg⁻¹ available P and 0.59% organic matter. The soil was sieved through 5 mm mesh to remove large particles and roots. The pots used were filled with 1500 g of the soil mentioned. The pots were placed in an environmentally controlled room with an average temperature of 21 °C, 60% relative humidity and a 12 h photoperiod with artificial lightning. The experimental design was randomized (*n* = 36) for 11 treatments in triplicate, in addition to a non-fertilized control.

2.2. Treatments Characterization

Previously to the experimental setup, a thorough characterization of the fertilizers to be assayed was performed according to Paredes et al.'s [17] methodology. The results are presented in Table 2.

Table 2. Treatment characterization.

Nutrient	PR	MAP	STR	BM	OMF ₁ (PR+C)	OMF ₂ (MAP+C)	OMF ₃ (STR+C)	OMF ₄ (BM+C)
pH	6.5	4.2	7.3	6.9	9.4	5.6	6.7	8.6
EC (dS m ^{−1})	0.97	22.8	1.3	0.7	2.05	3.02	3.9	1.9
Total C (%)	0.48	0.031	0.14	15.1	17.5	21.7	17.9	29.4
Total N (%)	0.044	12.1	5.7	3.00	1.16	7.06	3.73	2.76
K (%)	0.11	0.001	0.06	0.05	1.25	1.44	1.20	1.57
P (%)	2.0	2.3	14.2	2.1	6.5	10.3	7.3	4.6
Ca (%)	25.1	0.11	0.09	23.2	16.3	2.0	5.3	11.6
Mg (%)	0.20	0.003	11.7	0.49	0.24	0.22	2.11	0.41
Na (%)	0.07	0.002	0.004	0.44	0.09	0.06	0.22	0.28
Fe (mg kg ^{−1})	10,930	16.5	156	47.9	4808	1177	4173	1216
Cu (mg kg ^{−1})	78	0.16	0.5	0.24	84.4	51.1	51.3	51.5
Mn (mg kg ^{−1})	470	<0.01	642	42.6	388	159	272	165
Zn (mg kg ^{−1})	413	1.33	1.2	136	244	120	184	168

EC: Electrical conductivity. Simple treatments: PR: Phosphate rock; MAP: Monoammonium phosphate; STR: struvite; BM: Bone meal. Complex OMF treatments: OMF1(PR+C): Phosphate rock + Compost; OMF2(MAP+C): Monoammonium phosphate + Compost; OMF3(STR+C): Struvite + Compost; OMF4(BM+C): Bone meal + Compost.

To obtain the OMFs, organic compost was used (prepared from a mixture of olive mill waste–poultry manure–olive leaf waste in a 60:20:20 ratio). The OMFs were manufactured at Compolab-EPSO UMH (Orihuela, Alicante, Spain) through the extrusion of the mixtures using a low-power (4 kW) three-phase small scale pelletizer machine (50 kg h^{−1} capacity) with two rotating rollers (78 mm) operating on a fixed flat die of 119 mm diameter H-24, with holes of 6 mm diameter. Mixtures were prepared combining compost with various sources of high P content.

2.3. Crop Management

The first lettuce cycle began on 4 April 2023. Previously at day 0 the treatments were applied and distributed along the surface of the pot soil followed by irrigation with 200 g of deionized water. Then, lettuce seedlings at 2–3-true-leaves phenological stage were planted. Following the infiltration of the first watering, 75 g was added to each pot. Periodical watering with deionized water was performed in order to keep soil moisture at 60% field capacity.

At days 15, 25, and 45, the green cover factor (fCOVER) was determined using the *Canopeo* app developed for Matlab by Patrignani and Ochsner [18], and the chlorophyll content (CCC) of the plants was determined using a hand-held chlorophyllmeter (SPAD Minolta 502, Konica Minolta Sensing, Inc., Sakai, Osaka Prefecture, Japan) [19].

Lettuce plants were harvested on 19 May 2023 at phenological stage 45.

2.4. Plant Analysis

At the time of harvest, the aerial part of the plant was cut. In order to establish the crop yield, fresh biomass was weighed, then it was dried at T = 60 °C for 48 h, and its weight was recorded again for dry biomass determination [20]. The dry lettuces were milled in a RETSCH mill to prepare them for physicochemical determinations.

Physicochemical determinations were conducted as in the work by Martínez-Sabater et al. [21]. The C and N of the green tissue were measured in an automatic elemental micro analyzer (EuroVector Elemental Analyzer, EuroVector S.p.A., Pavia, Italy). For other nutrients, samples were digested with nitric acid 67% (0.2 g in 10 mL) in a Mars 6 digester [22]. Subsequently, total P (Pt) was determined by colorimetry in an UV-V

spectrophotometer, K and Na were determined in a flame photometer and the rest of the macronutrients (Ca, S, Mg) and micronutrients (Na, Cu, Fe, Mn, Zn) were analyzed via induced coupled plasma mass spectroscopy (ICP-MS).

For chlorophyll quantification we performed an extraction in 80% acetone followed by its spectrophotometric determination on a Thermoscientific Multiskan plate reader [23].

Nutrient use efficiency was also calculated for the main nutrients involved, N (NUE), P (PUE) and K (KUE), as the ratio of the nutrient application rate of the fertilizers and the nutrient uptake by the plant tissue, expressed as a percentage [24].

Through a non-targeted metabolomic study, a set of 29 metabolites were studied, belonging to 4 groups: amino acids (glutamate, glutamine, alanine, arginine, GABA, leucine, valine, isoleucine, asparagine, aspartate, phenylalanine, proline and threonine), organic acids (formate, malate, succinate, citrate, fumarate, acetate, ascorbate, 2-oxoglutarate, tartrate) and sugars (glucose, fructose, sucrose, myo-inositol). The samples were extracted following the protocol by Van der Sar et al. [25]. They were analyzed with an RMN Ascend 500 MHz AVANCE III HD H-NMR (Weinheim, Germany). The resulting spectra were evaluated with the 'Chenomx NMR Suite' version 8.3 program [26].

2.5. Soil Analysis

After harvest, soil from the pots was processed in order to remove the roots and was spread out to dry. Once dried, it was sieved through a 2 mm mesh. The soil parameters analyzed were pH, electrical conductivity (EC), total N (TN), available P (Pext) and organic matter content (OM). The pH and EC of the soil samples were measured in extracts with a 1:2.5 and 1:5 soil–water (*w/v*) ratio, respectively. For TN, we used the Kjeldahl method [17] of prior digestion with sulfuric acid. Pext was determined colorimetrically through the Olsen–Watanabe method [27]. For OM determination, organic carbon (COT) oxidization was measured according to Yeomans and Bremner [28].

2.6. Statistical Analysis

Statistical analyses were performed using the Infostat v.2020 statistical software package linked to the R program [29]. Differences in soil and lettuce characteristics between the different fertilization treatment groups were evaluated using Analysis of Variance (ANOVA) models with a significance level of $p < 0.05$. The Shapiro-Wilks test and Levene's test were previously applied to confirm the normality and homoscedasticity of the data across groups. Multiple comparisons between means were also performed using Fisher's LSD test ($\alpha = 0.05$). Variables were grouped in chemical parameters of the soil and biochemical parameters of the lettuce. R software [version 4.3.1 (16 June 2023 ucrt)] was employed to generate a heat map of correlations between lettuce biochemical variables. Principal components analysis (PCA) was performed to identify patterns and highlight similarities and differences between the different treatments and their outcomes on soil parameters.

3. Results

3.1. Yield

All conventional inorganic treatments, MAP, STR, OMF₂(MAP+C) and OMF₃(STR+C), performed significantly better than the control, whereas PR and BM as well as the corresponding complex OMF treatments showed yield values comparable to the control. The best yield results corresponded to OMF₂(MAP+C) (101.37 g), followed by OMF₃(STR+C) (83.21 g), with significant differences with respect to the best-performing conventional inorganic treatment: IN300 (60.42 g) (Figure 1).

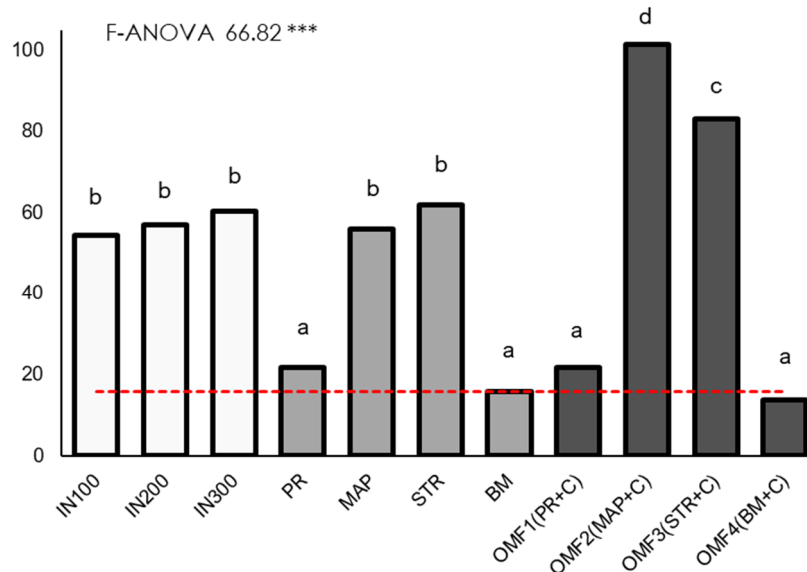


Figure 1. Fresh mass yield (FMY) (g) (t = 45 days). The red dotted line corresponds to the reference value of control plants. ***: significant difference between treatments at $p < 0.0001$. Different letters within a column indicate significant differences between treatments ($p < 0.05$). Values indicate mean ($n = 3$). Conventional inorganic treatments: IN100: 100 kg NPK ha⁻¹; IN200: 200 kg NPK ha⁻¹; IN300: 300 kg NPK ha⁻¹; Simple treatments: PR: Phosphate rock; MAP: Monoammonium phosphate; STR: struvite; BM: Bone meal. Complex OMF treatments: OMF1(PR+C): Phosphate rock + Compost; OMF2(MAP+C): Monoammonium phosphate + Compost; OMF3(STR+C): Struvite + Compost; OMF4(BM+C): Bone meal + Compost.

3.2. Morphological Aspects

The crop's morphological parameters studied during the plant's growing cycle were green cover (fCOVER) and chlorophyll content (CCC). By the end of the study, the chlorophyll concentration in the plant's leaves was determined by spectrophotometry. The results of these parameters at harvest date are shown in Table 3.

Dynamics of the green cover factor (fCOVER) for the whole duration of this study were established from the measurements performed at days 10, 25 and 45. By day 10, all treatments presented similar fCOVER values, but by day 25, it already demonstrated a divergence of these values, with this tendency being clearly established by the end of this study (Figure 2). Control (C) PR-based simple and complex treatments, and BM-based simple and complex treatments, presented the lowest values of fCOVER during the whole period of study, with a remarkable decrease by the end of the growing cycle. OMF₂(MAP+C) presented the highest fCOVER value by day 45, with significant differences with respect to the rest of treatments. Conventional inorganic treatments, specially IN200, MAP, STR and OMF₃(STR+C), presented more similar fCOVER values by day 45. These results correlate with the yield results.

The chlorophyll concentration in the plants' leaves measured by spectrophotometry allowed us to study the concentration of chlorophyll a and chlorophyll b separately together with total chlorophyll levels. In general, the levels of chlorophyll a were greater than those of chlorophyll b. The results show that MAP and STR were the best performing simple treatments, with significant differences with respect to the other simple treatments (PR, BM). Among the complex treatments, the chlorophyll concentration in plants treated with OMF₂(MAP+C) and OMF₃(STR+C) was significantly higher than in plants treated with OMF₁(PR+C) and OMF₄(BM+C). There were no significant differences in chlorophyll concentration between simple and complex treatments. Plants treated with the conventional inorganic treatments IN200 and IN300 had the highest chlorophyll concentration (Figure 3).

Table 3. Morphological parameters at harvest.

Treatment Group	Treatment	SPAD t_f	Canopy t_f (%)	Chl a (mg g^{-1})	Chl b (mg g^{-1})	Chl (mg g^{-1})
Control	C	5.61 b	2.68 a	0.32 b	0.13 a	0.43 ab
Conventional inorganic treatments	IN100	10.29 c	10.01 b	0.44 c	0.20 b	0.64 c
	IN200	16.03 f	11.67 cd	0.55 e	0.24 cd	0.80 f
	IN300	12.99 d	10.75 bc	0.53 de	0.25 cd	0.78 ef
Simple treatments	PR	3.82 a	2.56 a	0.24 a	0.10 a	0.37 a
	MAP	12.26 d	10.27 b	0.52 de	0.27 d	0.70 cde
	STR	12.87 d	10.73 bc	0.43 c	0.23 bc	0.68 cd
	BM	3.82 a	2.23 a	0.33 b	0.13 a	0.46 ab
Complex OMF treatments	OMF ₁ (PR+C)	3.61 a	2.36 a	0.30 ab	0.11 a	0.41 ab
	OMF ₂ (MAP+C)	14.33 e	13.88 e	0.47 cd	0.26 d	0.73 def
	OMF ₃ (STR+C)	20.14 g	10.73 bc	0.44 c	0.24 cd	0.68 cd
	OMF ₄ (BM+C)	4.29 a	2.69 a	0.35 b	0.13 a	0.48 b
F-ANOVA		177 ***	116 ***	17 ***	36 ***	24 ***

SPAD: Non-destructive chlorophyll content measurement, t_f : final time, 45 days, Chl: Chlorophyll. Different letters within a column indicate significant differences between treatments ($p < 0.05$). ***: significant difference between treatments at $p < 0.0001$. Values indicate mean ($n = 3$). C: Control; Conventional inorganic treatments: IN100: 100 kg NPK ha^{-1} ; IN200: 200 kg NPK ha^{-1} ; IN300: 300 kg NPK ha^{-1} ; Simple treatments: PR: Phosphate rock; MAP: Monoammonium phosphate; STR: struvite; BM: Bone meal. Complex OMF treatments: OMF₁(PR+C): Phosphate rock + Compost; OMF₂(MAP+C): Monoammonium phosphate + Compost; OMF₃(STR+C): Struvite + Compost; OMF₄(BM+C): Bone meal + Compost.

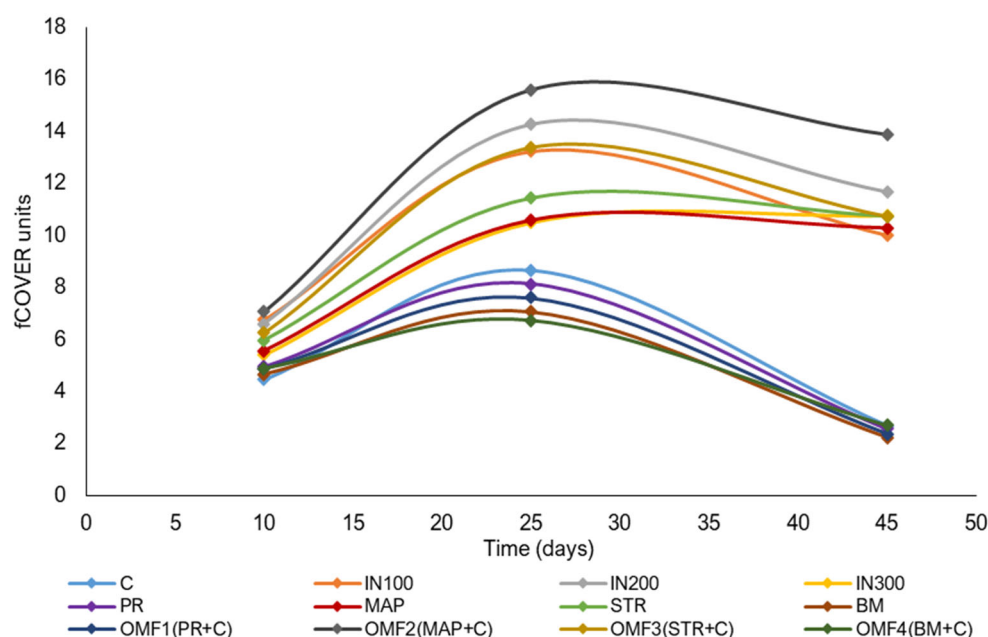


Figure 2. Green cover (fCOVER) dynamics ($t_1 = 10$, $t_2 = 25$, $t_f = 45$ days). C: Control; Conventional inorganic treatments: IN100: 100 kg NPK ha^{-1} ; IN200: 200 kg NPK ha^{-1} ; IN300: 300 kg NPK ha^{-1} ; Simple treatments: PR: Phosphate rock; MAP: Monoammonium phosphate; STR: struvite; BM: Bone meal. Complex OMF treatments: OMF₁(PR+C): Phosphate rock + Compost; OMF₂(MAP+C): Monoammonium phosphate + Compost; OMF₃(STR+C): Struvite + Compost; OMF₄(BM+C): Bone meal + Compost.

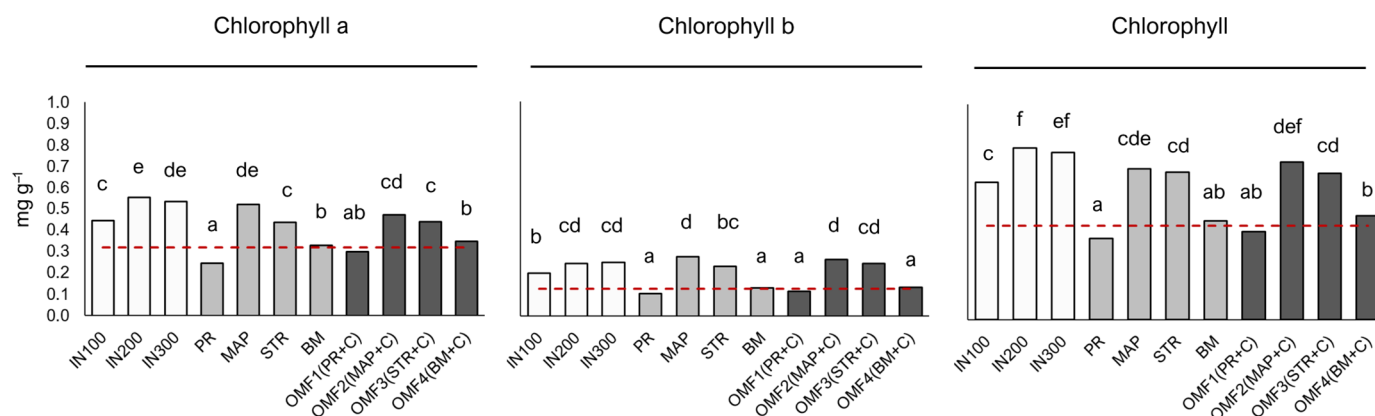


Figure 3. Chlorophyll content in plants at harvest (mg g^{-1}). Chl: chlorophyll. Different letters within a column indicate significant differences between treatments ($p < 0.05$). Values indicate mean ($n = 3$). The red dotted line corresponds to the reference value of control plants. Conventional inorganic treatments: IN100: 100 kg NPK ha^{-1} ; IN200: 200 kg NPK ha^{-1} ; IN300: 300 kg NPK ha^{-1} ; Simple treatments: PR: Phosphate rock; MAP: Monoammonium phosphate; STR: struvite; BM: Bone meal. Complex OMF treatments: OMF1(PR+C): Phosphate rock + Compost; OMF2(MAP+C): Monoammonium phosphate + Compost; OMF3(STR+C): Struvite + Compost; OMF4(BM+C): Bone meal + Compost.

3.3. Soil Parameters

Soil pH at day 45 had values of 7.6–7.8 (Table 4) for all treated soils, with no significant differences between treatments. There were significant differences with the control, with a pH of 7.37. The soil EC was significantly lower in soils treated with OMF₂(MAP+C) and OMF₃(STR+C) with respect to the rest of treatments and the control.

Table 4. Effect of fertilizer treatments on soil properties at harvest.

Treatment Group	Treatment	pH	EC (dS m^{-1})	OM (%)	Pext (mg kg^{-1})	TN (g kg^{-1})	$\text{NH}_4^+\text{-N}$ (mg kg^{-1})	$\text{NO}_3^-\text{-N}$ (mg kg^{-1})
Control	C	7.37 a	3.25 cd	0.51 a	138 d	0.34 a	2.2 ab	1 a
Conventional inorganic treatments	IN100	7.62 bc	3.17 bc	0.66 d	190 e	0.41 b	5.6 cd	7 ab
	IN200	7.66 bc	3.19 c	0.39 b	218 ef	0.41 b	7.0 de	18 bc
	IN300	7.69 bc	3.14 abc	0.42 bc	260 g	0.49 c	8.5 e	44 d
Simple treatments	PR	7.81 c	3.20 cd	1.12 f	85 ab	0.52 c	7.0 d	85 e
	MAP	7.55 ab	3.18 bc	0.10 a	263 g	0.49 c	1.2 a	55 d
	STR	7.75 bc	3.20 cd	0.90 e	100 abc	0.54 c	11.2 f	43 d
	BM	7.75 bc	3.22 cd	0.98 e	78 a	0.49 c	5.4 c	111 f
Complex OMF treatments	OMF ₁ (PR+C)	7.77 bc	3.20 cd	1.14 f	128 cd	0.51 c	3.5 b	94 e
	OMF ₂ (MAP+C)	7.77 bc	3.02 a	1.17 f	231 fg	0.42 b	3.0 b	26 c
	OMF ₃ (STR+C)	7.73 bc	3.04 ab	1.11 f	114 bcd	0.41 b	11.1 f	22 c
	OMF ₄ (BM+C)	7.77 bc	3.34 d	1.35 g	112 bcd	0.67 d	5.3 c	111 f
F-ANOVA		3 *	3 *	120 ***	36 ***	17 ***	37 ***	89 ***

EC: Electrical conductivity; OM: Organic matter; Pext: available phosphorus; TN: total Kjeldahl N; $\text{NH}_4^+\text{-N}$: ammonium; $\text{NO}_3^-\text{-N}$: nitrate. *, ***, significant difference between treatments at $p < 0.01$ and $p < 0.0001$, respectively. Different letters within a column indicate significant differences between treatments ($p < 0.05$). Values indicate mean ($n = 3$). C: Control; Conventional inorganic treatments: IN100: 100 kg NPK ha^{-1} ; IN200: 200 kg NPK ha^{-1} ; IN300: 300 kg NPK ha^{-1} ; Simple treatments: PR: Phosphate rock; MAP: Monoammonium phosphate; STR: struvite; BM: Bone meal. Complex OMF treatments: OMF1(PR+C): Phosphate rock + Compost; OMF2(MAP+C): Monoammonium phosphate + Compost; OMF3(STR+C): Struvite + Compost; OMF4(BM+C): Bone meal + Compost.

The percentage of soil organic matter at harvest was significantly higher in soils treated with OMF₄(BM+C) than with the rest of the treatments. Soils treated with MAP had the lowest %OM, even lower than the control.

In the case of extractable phosphorus, of soils treated with conventional inorganic treatments, IN300 had the highest P concentration. In the group of simple treatments, the soil of plants treated with MAP had the highest P extractable value, comparable to IN300. In the group of complex treatments, OMF₂(MAP+C) had the highest P extractable value. Organomineral fertilizers OMF₁(PR+C) and OMF₄(BM+C) exhibited significantly higher available *p* values than the corresponding simple treatments. In general, there seems to be an effect of compost addition on P availability increase in the soil.

Soils treated with OMF₄(BM+C) had the highest total N value. There were no significant differences between the simple treatments, with values comparable to IN300, which had a significantly higher TN value than the conventional inorganic fertilizers IN100 and IN200. Ammonia levels were significantly higher in soils treated with both STR (11.2 mg kg⁻¹) and OMF₃(STR+C) (11.1 mg kg⁻¹), followed only by IN300 (8.5 mg kg⁻¹). The lowest ammonia concentration was detected in soils treated with MAP (1.2 mg kg⁻¹). The soils with the highest content of nitrates were soils treated with BM and OMF₄(BM+C). Soils treated with the complex treatments OMF₂(MAP+C) and OMF₃(STR+C) had a significantly lower NO₃⁻ concentration than soils treated with the simple treatments MAP and STR (Table 4).

When comparing the mean values of each group, differences in soil parameters were observed between groups of treatments (Table 5). The percentage of organic matter in the soil at the end of this study was significantly higher in soils treated with OMFs thanks to the effect of compost, thus helping to improve soil properties. In any case, soils treated with both simple and complex OMF treatments exhibited greater OM content than those treated with conventional inorganic fertilizers, except the inorganic fertilizer MAP.

Table 5. Comparison between treatment groups on soil properties at harvest.

Treatment Group	pH	EC (dS cm ⁻¹)	OM (%)	Pext (mg kg ⁻¹)	TN (g kg ⁻¹)	NH ₄ ⁺ -N (mg kg ⁻¹)	NO ₃ ⁻ -N (mg kg ⁻¹)
Control	7.37 a	3.25	0.51 ab	138 a	0.41 a	2.16	1.25 a
Conventional inorganic treatments	7.66 b	3.17	0.49 a	222 b	0.41 a	7.02	22.81 a
Simple treatments	7.72 b	3.20	0.78 b	131 a	0.51 b	6.18	73.65 b
Complex OMF treatments	7.76 b	3.15	1.19 c	146 a	0.50 ab	5.73	63.27 b
F-ANOVA	7.8 ***	0.93 ns	14.07 ***	4.48 **	3.93 *	1.91 ns	7.78 ***

EC: Electrical conductivity; OM: Organic matter; Pext: available phosphorus; TN: total Kjeldahl N; NH₄⁺-N: ammonium; NO₃⁻-N: nitrate. *, **, ***: significant difference between treatments at $p < 0.01$, $p < 0.001$ and $p < 0.0001$, respectively; ns: not significant. Different letters within a column indicate significant differences between treatments ($p < 0.05$). Values indicate mean ($n = 3$).

The concentration of available P was significantly higher in the conventional inorganic fertilizer group. Though not significantly, available P (Pext) concentration had a greater value in the complex OMF treatment group (146 mg kg⁻¹) than in the simple treatments group (131 mg kg⁻¹). Soils treated with simple treatments had a significantly higher total N concentration. Nitrate concentration in the soil was significantly higher in both the simple and complex treatment groups when compared to the conventional inorganic fertilizers and the control. Though not significantly, nitrate concentration in soils treated with complex OMF treatments was lower than in those treated with simple treatments.

3.4. Nutrient Use Efficiency

OMF₂(MAP+C)- and OMF₃(STR+C)-treated plants presented significantly higher P uptake values compared to the rest of the treatments and the control, followed by inorganic treatments IN200 and IN300 (Table 6). PR and BM in simple and complex form exhibited P uptake values comparable to those of control plants.

Table 6. Lettuce N, P and K nutrient uptake and nutrient use efficiency at harvest.

Treatment Group	Treatment	P Uptake (g P pot ^{−1})	PUE (%)	N Uptake (g N pot ^{−1})	NUE (%)	K Uptake (g K pot ^{−1})	KUE (%)
Control	C	0.001 a	-	0.05 a	-	0.012 a	-
Conventional inorganic treatments	IN100	0.006 b	11.60 e	0.11 c	71.33 gh	0.032 d	24.80 d
	IN200	0.009 d	9.33 d	0.18 de	69.67 gh	0.053 g	25.80 d
	IN300	0.011 e	7.97 c	0.20 ef	53.67 de	0.039 e	11.17 c
Simple treatments	PR	0.002 a	0.97 a	0.10 c	30.33 c	0.020 c	1.37 a
	MAP	0.007 c	5.33 b	0.20 f	64.33 fg	0.045 f	5.97 b
	STR	0.006 bc	4.67 b	0.16 d	47.00 d	0.040 ef	5.13 b
	BM	0.001 a	0.23 a	0.07 b	13.00 ab	0.014 ab	0.30 a
Complex OMF treatments	OMF ₁ (PR+C)	0.001 a	0.30 a	0.10 c	18.33 b	0.018 bc	0.77 a
	OMF ₂ (MAP+C)	0.016 g	7.40 c	0.25 g	75.33 h	0.071 h	10.43 c
	OMF ₃ (STR+C)	0.013 f	8.33 cd	0.20 f	59.33 ef	0.054 g	7.37 b
	OMF ₄ (BM+C)	0.001 a	0.13 a	0.07 ab	8.67 a	0.013 ab	0.13 a
F-ANOVA		241.51 ***	120.93 ***	112.31 ***	57.61 ***	99.60 ***	89.64 ***

NUE: Nitrogen use efficiency, PUE: phosphorus use efficiency, KUE: potassium use efficiency. ***: significant difference between treatments at $p < 0.0001$. Different letters within a column indicate significant differences between treatments ($p < 0.05$). Values indicate mean ($n = 3$). C: Control; Conventional inorganic treatments: IN100: 100 kg NPK ha^{−1}; IN200: 200 kg NPK ha^{−1}; IN300: 300 kg NPK ha^{−1}; Simple treatments: PR: Phosphate rock; MAP: Monoammonium phosphate; STR: struvite; BM: Bone meal. Complex OMF treatments: OMF₁(PR+C): Phosphate rock + Compost; OMF₂(MAP+C): Monoammonium phosphate + Compost; OMF₃(STR+C): Struvite + Compost; OMF₄(BM+C): Bone meal + Compost.

Regarding nutrient use efficiency indexes, the best phosphorus use efficiency (PUE) results correspond to the complex treatment of OMF₃(STR+C) followed by OMF₂(MAP+C), with values of 8.33% and 7.40%, respectively. These results are significantly better than those obtained with the simple treatments MAP and STR, with values of 5.33% and 4.67% PUE, respectively. The complex treatments OMF₃(STR+C) and OMF₂(MAP+C) exhibited %PUE values close to those of the inorganic treatments IN100, IN200 and IN300 (11.60%, 9.33%, 7.97% PUE, respectively). PR and BM did not present significant differences between their simple and complex forms. BM-based treatments presented the lowest PUE (Table 6).

Nitrogen use efficiency (NUE) results are comparable to PUE. The complex treatment OMF₂(MAP+C) obtained the best NUE (75.33%), followed by the simple treatment MAP (64.33%) and OMF₃(STR+C) (59.33%). As in the case of P, the addition of compost had a significant effect improving N uptake in OMFs with respect to the simple treatments MAP and STR. Pelletization with compost had no effect in the case of the PR and BM fertilizers. The organic fertilizers BM and OMF₄(BM+C) had the lowest NUE (13.00% and 8.67%, respectively). In general, NUE had greater values than PUE.

The highest values of potassium use efficiency (KUE) were observed for the inorganic treatments IN 100 and IN 200 (24.80% and 25.80%, respectively). As in the cases of NUE and PUE, the complex treatments OMF₃(STR+C) and OMF₂(MAP+C) showed better KUE values compared to the simple treatments.

Comparing the different treatment groups, the inorganic treatments show the highest NUE, PUE and KUE values. Among the phosphorus treatments, though not significantly, the complex treatments group shows the greatest values, indicating a trend in higher nutrient use efficiency as a consequence of compost addition in OMF treatments (Table 7).

Table 7. Comparison between treatment groups on lettuce N and P nutrient uptake and nutrient use efficiency at harvest.

Treatment	N Uptake (g N pot ^{−1})	NUE (%)	P Uptake (g P pot ^{−1})	PUE (%)	K Uptake (g N pot ^{−1})	KUE (%)
Control	0.05 a		0.001 a		0.01	
Conventional inorganic treatments	0.16 b	7.42 b	0.009 c	9.63 b	0.03	20.59 b
Simple treatments	0.14 b	6.42 a	0.004 ab	2.80 a	0.04	3.19 a
Complex OMF treatments	0.15 b	6.42 a	0.008 bc	4.04 a	0.04	4.68 a
F-ANOVA	3.04 *	4.26 *	3.10 *	14.76 ***	2.49 ns	36.03 ***

NUE/PUE/KUE: nitrogen/phosphorus/potassium use efficiency, respectively. *, ***: significant difference between treatments at $p < 0.01$ and $p < 0.0001$, respectively. ns: not significant. Different letters within a column indicate significant differences between treatments ($p < 0.05$). Values indicate mean ($n = 3$).

3.5. Metabolomic Study

The metabolomics data obtained provides a picture of the metabolic state of the plants at the moment of harvest.

We studied the levels of fundamental metabolites involved in energetic metabolism (glycolysis and TCA cycle). Sugars like glucose, which is the primary substrate for glycolysis, are significantly augmented in plants treated with OMF₃(STR+C) followed by OMF₂(MAP+C), STR and MAP (Table S3). This means that in these plants glucose is not being degraded through glycolysis, so it is being accumulated. On the contrary, significantly lower glucose levels are detected in plants treated with PR, BM, OMF₁(PR+C) and OMF₄(BM+C), with no significant differences among them, nor with control plants. This result suggests a higher energy demand in these plants. The TCA cycle's initial intermediate citrate levels are significantly higher in plants treated with BM, OMF₁(PR+C), OMF₄(BM+C) and PR, which reinforces the idea of a more active energetic metabolism in these plants (Table S2). Other TCA cycle intermediates such as succinate also accumulated in these plants.

Among organic acids, tartrate concentration was significantly higher in the simple treatment group than in the complex OMF treatment group.

In general, amino acids presented significantly higher levels at harvest in plants treated with PR and BM simple and complex OMF treatments (Table S1).

GABA and myoinositol presented significantly higher concentrations in plants treated with OMF₂(MAP+C) and OMF₃(STR+C).

4. Discussion

4.1. Effects of Fertilization on Quality and Yield

The organomineral MAP and STR with compost fertilizers showed significantly higher yield with respect to fertilizers in their inorganic form. These results are in concordance with those obtained by Carciochi et al., in which OMFs exhibited a 13% increase in wheat yield with respect to inorganic MAP plus urea in a 3 year field experiment on a wheat crop [30]. In a pot trial using lettuce and cabbage, a 50% dose of OMF-P produced at least 11% higher fresh matter and dry matter than 100% mineral fertilization [31].

De Moraes et al. found that OMFs formulated with MAP^{25%}ChickenManure^{37.5%}CoffeeHusk^{37.5%} increased maize shoot biomass in ~26%, and shoot P in ~22% over MAP-fertilized plants [32]. Araújo et al. evaluated the growth of millet cultivated in sandy soil in

a greenhouse experiment under fertilization with MAP, and OMF prepared with MAP and organic compost of waste from small ruminant production. They found that, considering the total accumulated in the two cuts of millet, the OMF promoted a higher nutrient content compared to MAP [33]. In the study by Erenoğlu et al., the OMF-P-fertilized plants showed higher plant P concentrations and P uptake than the mineral fertilizers, with a more noticeable effect at the P rates of 25 and 50 mg kg⁻¹ soil [34]. De Sousa and Alleoni, in a greenhouse experiment of two successive maize crops, concluded that STR and an OMF composed of chicken manure and triple superphosphate (TSP) had higher agronomic efficiencies than TSP in sandy soils. STR application increased plant tissue P concentrations. The OMF also benefited P uptake, mainly in the second crop cycle. The authors discuss that this effect may be explained by their slow nutrient release properties that would enable sustained nutrient availability over multiple crop cycles [35].

In general, struvite performed similarly to MAP in terms of yield and P uptake. In various assays using acidic soils, struvite performed similarly to inorganic soluble P fertilizers in terms of P uptake [36,37]. Erdal et al. also obtained comparable yield and P uptake results in lettuce plants fertilized with struvite and MAP in a 60 day experiment [38].

N-use efficiency (NUE) was higher in the inorganic treatments (IN100, IN200) and in complex fertilizer OMF₂(MAP+C), outperforming its correspondent simple fertilizer MAP in 11%; also, OMF₃(STR+C) was higher than STR alone. This indicates that simple inorganic fertilizer improves its productivity when combined with compost. This effect has also been observed in other studies comparing urea and blended fertilizers of urea and biochar as organic matter [39,40].

The addition of compost seems to have had a positive effect on P use efficiency in the case of MAP and STR, but not in the case of PR and BM. The effects of the different OMFs are dependent on the P sources used [41]. The P uptake of OMF₁(PR+C) and OMF₄(BM+C) was lower than that of OMF₂(MAP+C) and OMF₃(STR+C), probably due to the high Ca content of these OMFs, which can form bonds with P in the organic matrix, thereby decreasing its availability [42].

KUE was higher in the case of conventional inorganic treatments, probably due to overfertilization. As K fertilization was imbalanced, this could lead to the leaching of soluble potassium, which would prevent its absorption by the plant [43]. As reported by Yin et al. [44], higher doses of K present lower KUE.

According to Uddin et al. [10], organomineral fertilizers release nutrients slowly according to plant needs throughout the growing season, which enables the plants to uptake nutrients more efficiently, resulting in better plant growth and development.

4.2. Effects of Fertilization on Soil Properties

The pH was slightly alkaline in all the soils, and no significant differences were observed between treatments for pH values. Similar results were observed in a study about the effect of soil quality on lettuce production using alternative sources of P (struvite) compared with the use of other conventional P fertilizers, including monoammonium phosphate (MAP) [45]. The conductivity values were slightly lower in the treatment groups compared to the control group, possibly due to the leaching of salts due to irrigation.

Soils treated with OMFs had the highest OM percentages, higher than those of conventional inorganic and simple treatments, probably due to the effect of compost. Phosphorus OMF treatments also had higher OM values than the control in a pot experiment with perennial ryegrass [46]. According to Toprak and Seferoğlu's research, p-OMFs increased the soil organic matter (SOM) by 60.1% [47].

Soil nitrate content was significantly lower in soils treated with OMF₃(STR+C) and OMF₂(MAP+C), matching high NUE values, indicating high nitrate consumption by the

plant for these treatments. Nitrate content was higher in organic simple and complex OMF fertilizers, suggesting an immobilization of mineral N in the soil.

The concentration of available phosphorus in the soil after the assay was higher for all treatments and the control compared to the initial soil. The soils fertilized with MAP and OMF-MAP treatments had the highest concentration of available phosphorus at the end of the experiment. The available phosphorus value of the MAP treatment was similar to that of the conventional inorganic treatment IN300. Although not significantly, Mancho et al. observed that the highest concentration of available P in the soil was found for the MAP treatment [45].

In our study, available P significantly increased in the soils treated with the organomineral fertilizers OMF₁(PR+C) and OMF₄(BM+C) when compared to simple treatments. In the case of OMF₃(STR+C), there was a slight increase in available P with respect to STR, though it was not significant. These results are in concordance with those obtained by Erenoğlu et al. in a pot experiment in wheat [34], in which, after 90 days, available P in soils treated with the OMF treatment at all studied rates was significantly higher than in plants treated with the mineral fertilizer. This effect was also described by Adebayo et al. in a field experiment with *Moringa oleifera*. They described higher P soil concentrations for the OMF applied at 30 tons ha⁻¹, than the rest of the fertilizers evaluated: NPK (15:15:15), cow dung and poultry manure [48]. Frazão et al. observed that significantly higher amounts of available P to maize plants were obtained using organomineral phosphate fertilizers in a study comparing these OMFs with triple superphosphate [49]. Similarly, biochar-based OMFs showed greater agronomic efficiency and greater P availability after harvest in maize [50]. P availability increase by OMF fertilizers was also described by Borges et al. in sugarcane [51]. This effect may be caused by the extra supply of OM with complex OMF treatments (see Table 4).

As seen in the individual PCA of soil parameters (Figure 4), clearly significant differences are observed between treatments. Treatments IN100, IN200, IN300 and OMF₂(MAP+C) are located in the left region, close to the horizontal axis, indicating high amounts of assimilable P. MAP and C treatments are located in the lower left region, which means that they present low amounts of OM, pH and NH₄⁺ and high amounts of assimilable P. The treatments OMF₃(STR+C), STR and PR are located in the upper right region, indicating that these present high amounts of OM, pH and NH₄⁺. In addition, a downward diagonal trend is observed between these treatments, suggesting that the OMF₃(STR+C) treatment presents medium levels of assimilable P, and higher amounts of NH₄⁺, while the PR treatment presents lower amounts of assimilable P and higher amounts of OM. Finally, BM, OMF₁(PR+C) and OMF₄(BM+C) treatments are located in the lower right region, indicating higher amounts of NO₃⁻, TN and EC, as well as lower amounts of extractable P.

4.3. Effects of Fertilization on Metabolomics

Plants fertilized with PR and, especially, BM treatments had a significantly lower yield than plants fertilized with MAP and STR treatments at the end of the assay, which is consistent with a greater activation of anabolic metabolism for molecule synthesis and growth promotion in the first plants. This is in concordance with the results by Matamoros et al., who concluded that under low nutrient availability, the activity of the TCA cycle was up-regulated [52], since it is necessary for ATP production and for providing the precursors used in many biosynthetic pathways [53,54]. Gao et al. proposed increased citrate and succinate levels as low-phosphorus stress biomarkers in lettuce plants [55]. On the contrary, sugars including glucose, fructose and sucrose were found to be significantly augmented in plants treated with MAP and STR simple treatments, and to a greater extent, in the case of complex-OMF treatments. This is consistent with the results of Hurtado

et al., who suggested that the use of high-nutrient-content fertilizers promotes carbon accumulation [56].



Figure 4. Individual PCA of soil parameters. C: Control; Conventional inorganic treatments: IN100: 100 kg NPK ha⁻¹; IN200: 200 kg NPK ha⁻¹; IN300: 300 kg NPK ha⁻¹; Simple treatments: PR: Phosphate rock; MAP: Monoammonium phosphate; STR: struvite; BM: Bone meal. Complex OMF treatments: OMF1(PR+C): Phosphate rock + Compost; OMF2(MAP+C): Monoammonium phosphate + Compost; OMF3(STR+C): Struvite + Compost; OMF4(BM+C): Bone meal + Compost.

As is the case in the present study, Chandrou et al. also found augmented amino acid concentrations in plants treated with phosphate rock and bone meal [57], indicating an overactivation of amino acid biosynthesis in these plants. Among the 21 proteinogenic amino acids, arginine has the highest nitrogen-to-carbon ratio, making it especially suitable as a storage form of organic nitrogen [58]. The increase in alanine concentration, as a precursor of acetyl-CoA, is another indicator of metabolism activation [59]. Both amino acids presented significantly higher levels in plants treated with bone meal and phosphate-rock-based treatments.

Myoinositol levels are implicated in growth and development regulation through their participation in key cellular processes, including the biogenesis of the cell wall and membrane structures, phosphate storage, cell signaling, and cell resistance to external stress factors [60,61]. Conventional inorganic treatments, OMF₂(MAP+C) and OMF₃(STR+C), presented the highest levels of this metabolite.

Another crucial molecule in plant growth regulation is the non-protein amino acid gamma-aminobutyric acid (GABA) [62]. Similarly, to myoinositol concentration, a higher GABA concentration was detected in plants treated with OMF₂(MAP+C) and OMF₃(STR+C).

Tartrate is a product of ascorbic acid oxidation. Its increased levels could be an indicator of antioxidant mechanisms. Tartaric acid derivatives have been described as the main up-regulated phenolic acid components in stressed plants [42,63]. Phosphate rock-based treatments presented high tartrate levels. Tartrate concentration was, in general,

significantly augmented in the simple treatment group with respect to the complex OMF treatment group. Interestingly, the levels of this metabolite presented a lower concentration in plants treated with MAP and STR complex OMF treatments than in plants treated with MAP and STR simple treatments.

When correlating plant metabolites and macro and microelements at harvest (Figure 5), it was observed that P (%) positively correlated with sucrose, glucose, fructose, myo-inositol, chlorophyll, GABA and K (%), while it correlated negatively with the vast majority of amino acids and organic acids, including TCA cycle intermediates and tartrate.

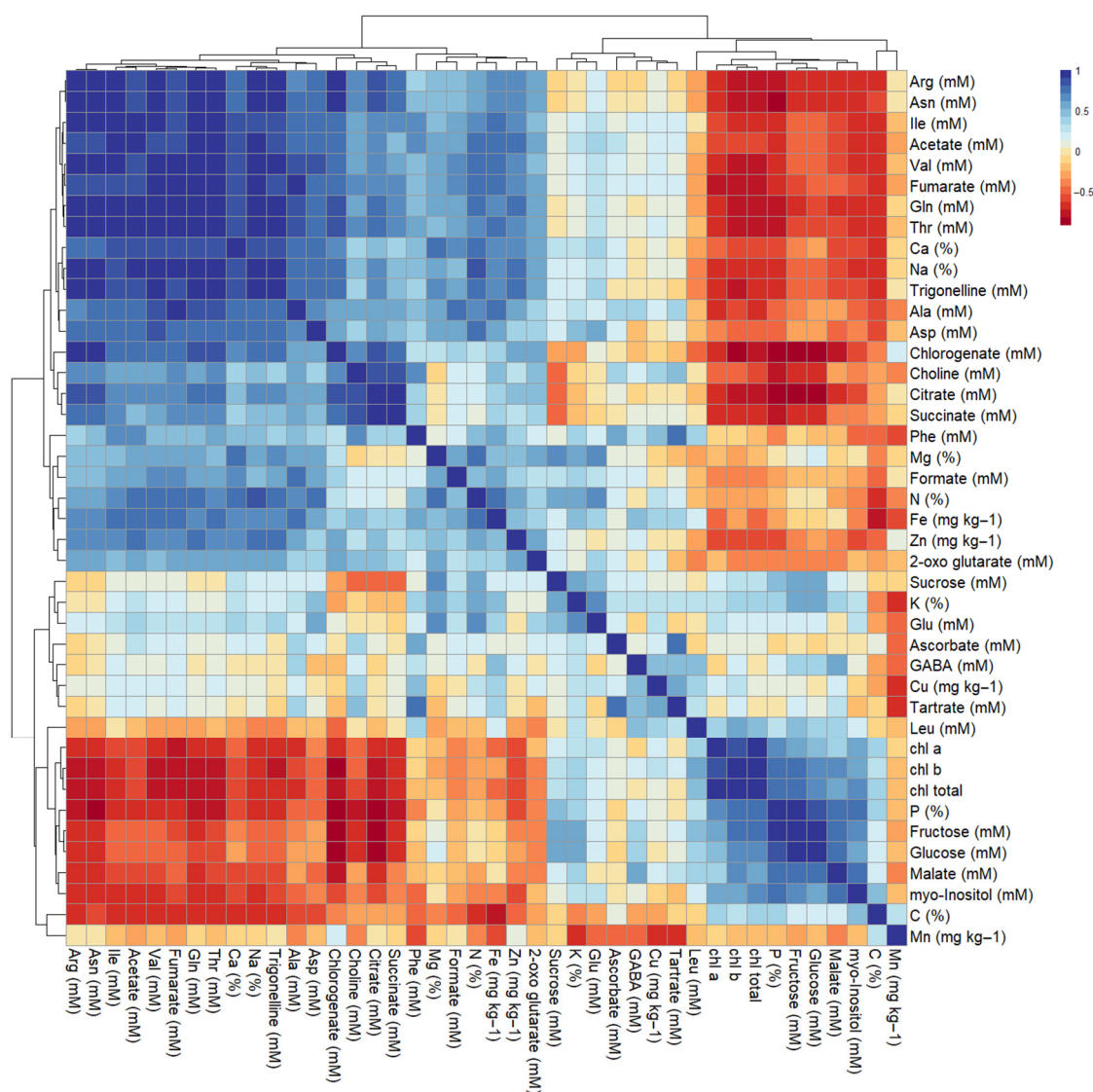


Figure 5. Heatmap correlating plant metabolites and macro and microelements.

5. Conclusions

The complex OMF STR and MAP treatments produced the best results in terms of yield, plant phosphorus (P) uptake and percentage phosphorus use efficiency (PUE), with significant differences with respect to simple MAP and STR treatments, and PR- and BM-based treatments.

The application of complex treatments incorporating compost improved levels of OM and Pext in soils when compared to simple treatments.

Significantly higher levels of GABA and myo-inositol were found in plants treated with OMF₂(MAP+C) and OMF₃(STR+C) than in plants treated with the remaining simple and

complex phosphorus treatments. High levels of these metabolites are important biomarkers of plant homeostasis.

High levels of TCA cycle intermediates, amino acids, and tartrate may be indicators of plant low-phosphorus stress at harvest. These metabolites were augmented in plants treated with PR and BM simple and complex-OMF treatments.

According to their metabolic state, plants treated with OMF₂(MAP+C) and OMF₃(STR+C) better activated protective mechanisms towards oxidative stress, while the contrary happened in plants treated with PR- and BM-based treatments.

The results of this work demonstrate the efficiency of compost addition through pelletization as a novel fertilizing strategy for improved plant and soil quality post-harvest, specifically in combination with mineral P sources like monoammonium phosphate (MAP) and struvite (STR). In addition, it proves the fertilizing potential of struvite, with results equivalent to those obtained with the widespread mineral fertilizer MAP.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/agronomy15071661/s1>: Figure S1: Experimental setup at EPSO-UMH greenhouse; Figure S2: Chlorophyll content dynamics measured non-destructively during this study ($t_1 = 10$ days, $t_2 = 25$ days, $t_f = 45$ days). Table S1: Amino acid concentration (mM); Table S2: Organic acid concentration (mM); Table S3: Water-soluble sugar and alcohol concentration (mM).

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