



# Differential Effects of Organic Amendments on Soil P Availability, Plant P Uptake Efficiency and Root Traits of Durum Wheat at its Earliest Stage in Calcareous and Non-Calcareous Soils: A Microcosm Approach

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## Abstract

This study assesses the effect of commonly used organic amendments on soil phosphorus (P) availability, P uptake, growth and root traits of durum wheat seedlings grown in two soils with contrasting properties. After a phytotoxicity assay developed to evaluate potential negative effects on plant growth, durum wheat was grown in soil microcosms amended with composted olive mill pomace (COP), vermicompost (VEC), compost from solid anaerobic digestate (DIG) or municipal solid waste compost (CSW) at an equivalent dose of 30 mg P kg<sup>-1</sup>. Diammonium phosphate and no P application were included as positive and negative control. A calcareous Vertisol and a non-calcareous Luvisol were used to evaluate alterations in soil (pH, electrical conductivity, available P) and plant responses (leaf chlorophyll content, biomass, P uptake, root traits, nutrient contents) after a 21-d growing period. Tested organic amendments were non-phytotoxic. In the microcosm experiment, DIG and CSW produced the largest soil P availability (22.7–28.6% higher than control treatment-CON) and P uptake efficiency (3.2–5.1%) in both soils. In comparison with CON, DIG increased the total (+ 82.9%) and specific root length (+ 104.0%) in both soils, while VEC stimulated the specific root length (+ 39.4%) in the calcareous soil only. COP, VEC and CSW negatively affected root traits in wheat seedlings growing in the non-calcareous soil, as well as leaf chlorophyll content and / or plant height in both soils. DIG and CSW were a reasonable alternative to inorganic fertilizers in the soils tested in terms of P provision and uptake efficiency, and in soil exploration.

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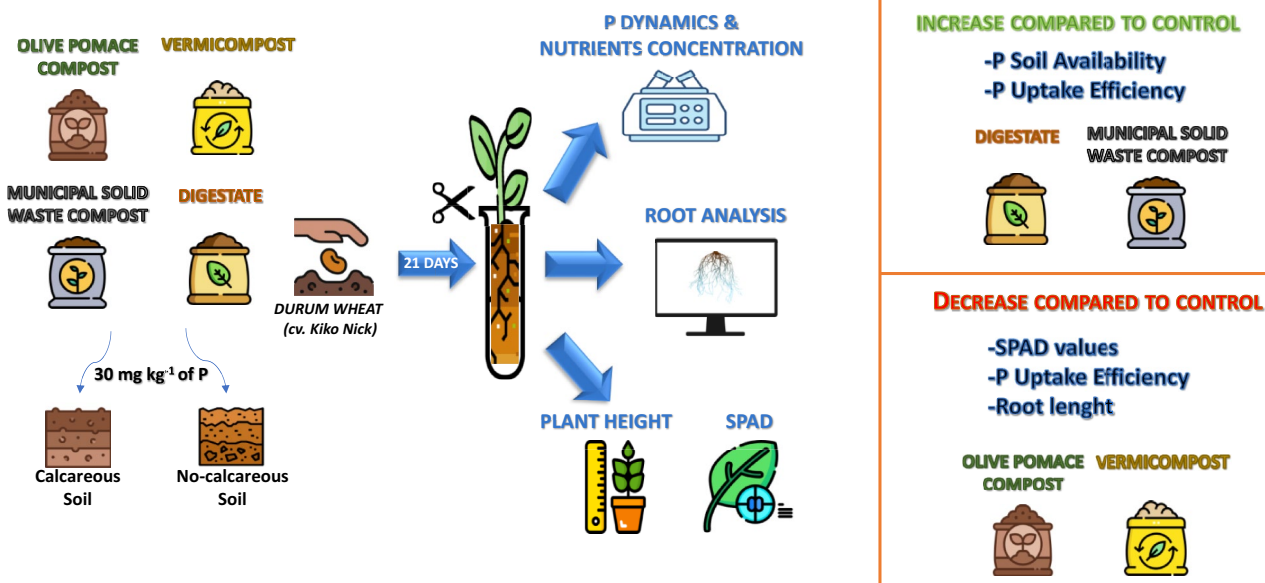
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## Graphical Abstract

## Differential effects of organic amendments on soil P availability, plant P uptake efficiency and root traits of durum wheat at its earliest stage in calcareous and non-calcareous soils: a microcosm approach



**Keywords** Phosphorus uptake · Nutrients interaction · Root traits · Compost · Nutrient balance · Digestate

### 1 Introduction

Wheat is one of the world's most widespread crops, which provides a global yield of 2.2 10<sup>6</sup> tons and extends over a cultivated area of 673,655 ha (FAO 2024). To be productive, durum wheat cultivation globally requires the application of chemical fertilizers including phosphorus (P), which is often provided at a less-than-sufficient level (Gadaleta et al. 2022) or whose availability to plants is reduced due to complex edaphic processes. The functional role of P in plant growth is significant and multifaceted. P is an essential plant nutrient element, a macronutrient, involved in several physiological and biochemical processes, particularly in the context of energy transfer, photosynthesis, flowering, fruiting, nucleic acid synthesis, and cell division (López-Arredondo et al. 2014). In cereal plants at early phenological stage, it is necessary for the available P concentration in soil to be large enough to ensure the development of the root system and the primordia of the reproductive parts (Balo and Maha 2022). In soil, the total amount of P is relatively low and widely variable with values ranging from 500 to 10,000 kg ha<sup>-1</sup> in the upper 50 cm (Weil and Brady 2017). Soil P is represented by both organic (broadly spanning from 20 to 80% of total content, mainly inositol phosphate and phospholipidic,

which require enzymatic hydrolysis to become available to plants) and inorganic forms. Inorganic P forms can generally be divided into mineral (non-available, fixed), adsorbed onto particle surfaces (slowly available), and water soluble (readily available). Since most of the P pool in soil occurs in high insoluble (both organic and inorganic) forms, it presents problems in the aspects related to soil fertility. Moreover, following P application with mineral fertilizers or organic amendments, it can become fixed or precipitated, and then not be readily absorbable by the root system (Weil and Brady 2017).

Soil availability of P strongly depends on pedochemical conditions, such as the type and quantity of clay minerals, calcium carbonate or organic matter content. Particularly, in acid soils (pH < 6.0), soluble P reacts with free aluminium (Al) and iron (Fe) ions, thus forming insoluble Al (AlPO<sub>4</sub>) and Fe phosphates (FePO<sub>4</sub>), respectively. Instead, under alkaline conditions (pH ~ 8.0), and in calcareous soils characterised by the presence of significant amounts of excess free calcium lime (calcium carbonate or magnesium), soluble H<sub>2</sub>PO<sub>4</sub><sup>-</sup> ions supplied with phosphate fertilizers readily react with Ca<sup>2+</sup> ions to generate a sequence of products of decreasing solubility (Hopkins and Ellsworth 2005). The largest availability of soluble P forms for plant uptake is

found at a soil pH close to 6.5, a condition which is rarely found in arable soils (Weil and Brady 2017).

As agricultural production demands rise and concerns over a potential global production peak in the coming decades persist, albeit debated, agricultural P is gaining increasing recognition as a fast-depleting nutrient (Shen et al. 2019). In fact, there are growing concerns about current rates of phosphate mining and utilisation practices, which pose at risk the global food security (Chowdhury et al. 2016). Studies on the availability of phosphate rocks estimate that they will become exhausted within the next few decades (Koppehaar and Weikard 2013), or less pessimistically by the end of the twenty-first century (Sverdrup and Ragnarsdottir 2011). Other research has offered a contrasting view on the issue, supporting the view that there will be enough mine-derived P to sustain agricultural production beyond the twenty-first century (Cooper et al. 2011; van Vuuren et al. 2010). In both cases, this indicates that the potential exhaustion of phosphate rock reserves is a topic of significant present-day concern.

Considering this scenario and encouraged by national and European policies and strategies (the Green Deal, from farm to fork, the EU Mission: A soil deal for Europe), the use of organic by-products such as compost, vermicompost and digestates can be considered as a feasible alternative and sustainable strategy, in view of a circular economy process. Some of these organic by-products with an appreciable content in organic matter and nutrients could provide an important portion of available P needed for plant growth (Verma 2013), thus maintaining adequate soil fertility levels and ensuring food security. Organic amendments can increase the amount of available soil P through various ways: direct release of soluble P forms, induced changes in soil pH during the mineralization process, stimulation of microbial and enzyme activities, complexation of Al and Fe ions with the consequent release of soluble phosphates (Khan and Joergensen 2009). Moreover, negatively charged humic-like compounds entering the soil with the addition of organic matter can reduce the phosphate adsorption on positively charged particle surfaces rich in Fe (or Al) hydroxides, thus mitigating the bounding effect due to the recipient soil mineralogy (Weil and Brady 2017). Nowadays, a wide range of organic by-products have become available as soil conditioners, and, besides their differences in origin and major chemical properties, they can provide a significant supply of soluble nutrients and contribute to manage the soil fertility status.

The impact on soil fertility and plant growth resulting from the addition of organic amendments is also linked to their ability to increase the volume of soil explored by the root system, as observed in wheat plants by Zhao et al. (2019). This response was assumed as related to a direct compost-induced effect on physical (i.e. increased

aggregates formation, and porosity; Zhao et al. 2019), chemical and biological soil properties (i.e. stimulation of microbial and enzyme activities; Martínez et al. 2018). The developmental plasticity of the root system, however, is not a common property for all plant species, and depends on genotypic adaptability (Yuan et al. 2016). In this regard, Lyu et al. (2016) classified cereals as crop species capable of exhibiting a considerable and significant root morphological response to P deficiencies. Moreover, Toderi and D'Antuono (2000) went on to show that in cereals i) P uptake is larger in young seedlings, and ii) root responses during the early stage are critical for both plant development and crop productivity. This becomes particularly true in durum wheat, which is generally cultivated under rainfed conditions in soils with a low fertility level and combined with a substantial use of chemical fertilizer that are often characterized by a low efficiency. Moreover, there is still lack of information regarding how the available wide range of organic amendments could affect root architecture and morphology, and, as a result, plant nutrient (P) uptake under different pedological conditions (calcareous and non-calcareous soils). According to Bindraban et al. (2020), sustainable fertilization strategies should include technologies and fertilizers that reduce the negative environmental impacts of potential excess of P in soil while enhancing plant P uptake. It has been seen that certain organic amendments can modify root traits and stimulate the proliferation of fine roots, which in turn enhance water and nutrient absorption (Zhao et al. 2019). However, it is also true that P uptake is a function of complex dynamics occurring at soil–plant interface.

The main purpose of this research was to investigate the combined effect of pedogenic calcium carbonate ( $\text{CaCO}_3$ ) content and the incorporation of four different commonly available organic amendments (i.e. three compost types and a vermicompost) on soil P availability and P uptake, biomass production and root traits of durum wheat seedlings at the early stage of their growth by using a lab-scale microcosm approach. Our working hypotheses were: i) soil P availability and plant P uptake are primarily affected by the presence of  $\text{CaCO}_3$ , more than by the nature of the organic amendment, and ii) according to their nature, organic amendments selectively stimulate root traits development in view of an adequate uptake of P (and other nutrients). For that, an initial phytotoxicity assay was carried out to assess whether the organic amendments could have negatively affected plant growth. Then, a microcosm-scale experiment using soils with different properties and availability of P (a calcareous Vertisol and a non-calcareous Luvisol with similar soil pH but contrasting calcium carbonate content) was set-up to grow durum wheat under controlled conditions for a 21-d period. A positive (inorganic P fertilizer) and a negative (no P application) control references were also included to investigate opposite conditions of inorganic P availability in soil.

## 2 Materials and Methods

### 2.1 Soils and Organic Amendments

Two agricultural soil types with contrasting  $\text{CaCO}_3$  content were selected for the experiments. The first soil was a calcareous Vertisol from Adamuz, Córdoba ( $38^\circ 04' 57''$  N,  $4^\circ 31' 33''$  W), and the second, a non-calcareous Luvisol from Puertollano, Ciudad Real ( $38^\circ 42' 07''$  N,  $4^\circ 08' 50''$  W) (Soil Survey Staff 2022). After collection (0–25 cm), soils were air-dried at room temperature for one week, and then each split into two aliquots. A subsample of each soil was sieved to a 1 cm particle size to remove small roots and rock fragments, and then used for filling the microcosms used in the pot experiment. A second aliquot was sieved to a 2 mm particle size, and used for physical and chemical characterization according to international standard methods (Klute et al. 1986; Sparks et al. 1996). Briefly, soil texture was determined by the pipette method (Gee and Bauder 1986); organic carbon (OC) was determined by rapid oxidation with an excess of 1 M potassium dichromate according to the Walkley and Black (1934) method; pH was potentiometrically measured in a 1:2.5 (w/v) soil-to-water slurry; electrical conductivity ( $\text{EC}_{1:5}$ ) was measured in a 1:5 (w/v) soil-to-water slurry; total calcium carbonate ( $\text{CaCO}_3$ ) was determined according to van Wesemael (1955); cation exchange capacity (CEC) was determined by using 1 M ammonium acetate buffered to pH 7; soil available P was extracted with 0.5 M sodium bicarbonate (pH 8.5; Olsen et al. 1954) and measured following the ascorbic acid method (Murphy and Riley 1962) ( $P_{\text{Olsen}}$ ). Major soil characteristics are shown in Table 1.

Mature compost from a 5-month bioconversion of olive mill pomace (COP) was obtained from Vadolivo, an olive oil processing plant located in Cazorla (Jaén, Spain). Vermicompost (VEC) was gently provided by the University of Seville after 4-month vermicomposting of horse manure with *Eisenia fetida*. The solid fraction resulting from the anaerobic digestion of a mixture of manure/wastewater (DIG) was obtained by BIOVEC (Valencia, Spain). Compost from the organic fraction of municipal solid waste (CSW) was obtained from the local municipal solid waste processing plant, Sadeco (Córdoba, Spain).

Before use, the organic materials were air-dried, finely ground at <0.5-mm particle size, and fully characterized according to Thompson et al. (2002). Briefly, pH and electrical conductivity (EC) were measured in the 1:25 ratio (soil:water). The supernatant obtained in these determinations was subjected to microfiltration and the remaining product was used to determine total soluble nitrogen (TSN) in a high-temperature combustion equipment (TOC Analyzer: Shimadzu TOC L). Organic C was measured as detailed for soil. Total nitrogen was determined according to the Kjeldahl method (N-NTK). Moreover, volatile solids content (VS) was calculated by Weight difference, taking 1 g of homogenised sample of each amendment that was first subjected to 105 °C for 24 h in an oven and then at 550 °C in a muffle for additional 3 h. The total P content was determined by oxidation ( $\text{P-P}_2\text{O}_5$ ) according to Thompson et al. (2002). Total Cu and Zn were measured by atomic absorption spectroscopy after digestion with concentrated HCl. Major characteristics of COP, VEC, DIG and CSW are reported in Table 2 along with the analysis of the positive control, diammonium phosphate (DAP), a commonly used NP-containing fertilizer.

### 2.2 Phytotoxicity Assay

Seeds of cress (*Lepidium sativum* L.) and durum wheat (*Triticum durum* L. cv Kiko Nick) were used for the phytotoxicity assay and the microcosm experiment, respectively. They were provided by the local company Sehicor (Córdoba, Spain). Firstly, seeds were surface sterilized by soaking them in 15% (v/v) NaClO solution for 15 min, and then thoroughly rinsed with deionized water to remove excess NaClO. Before their use and in compliance with Italian (Legislative decree n. 75/2010) and European (Reg. EU n. 2019)/1009) regulations, the phytotoxicity of the four organic amendments (COP, VEC, DIG and CSW) was assessed according to the bioassay method based on the germination and root growth of cress seeds exposed to the aqueous extracts of the organic matrices (Di Maria et al. 2014; Zucconi et al. 1985). Briefly, each organic matrix was adjusted to a moisture content of 85% (wet weight) by addition of deionized water. After a 2-h incubation in darkness at room temperature (25 °C), the suspension was centrifuged (8000 g at 4 °C, 20 min) and filtrated (Whatman® n. 42 filter paper). Clean extracts

**Table 1** Major physical and chemical properties (mean of two replicates) of the two tested soils: a calcareous Vertisol and a non-calcareous Luvisol

Soil type	Clay $\text{g kg}^{-1}$	OC $\text{g kg}^{-1}$	$\text{CaCO}_3$ $\text{g kg}^{-1}$	$\text{pH}_w$	$\text{EC}_{1:5}$ $\text{dS m}^{-1}$	CEC $\text{cmol}_+ \text{kg}^{-1}$	$P_{\text{Olsen}}$ $\text{mg kg}^{-1}$
Calcareous soil	240	11	520	8.3	0.354	23.5	14
Non-calcareous soil	540	14	-	7.8	0.173	42.2	20

Clay clay content, OC organic carbon content,  $\text{CaCO}_3$  total calcium carbonate,  $\text{pH}_w$  soil pH measured in 1:2.5 (v/w) soil:water,  $\text{EC}_{1:5}$  electrical conductivity measured in 1:5 (v/w) soil:water, CEC cation exchange capacity,  $P_{\text{Olsen}}$   $\text{NaHCO}_3$  extractable P

**Table 2** Application rate (grams of product per kg of soil) and major chemical (mean,  $n=2$ ) properties of diammonium phosphate (DAP) and the four organic amendments (COP: composted olive pomace; VEC: vermicompost from horse manure treated with *Eisenia fetida*; DIG: compost from solid anaerobic digestate; CSW: municipal solid waste compost) used in the microcosm experiment

		DAP	COP	VEC	DIG	CSW
Application rate	g kg <sup>-1</sup>	0.150	25.9	3.2	1.3	7.8
pH <sub>w</sub>		7.44	8.81	8.42	8.33	8.09
EC <sub>1:25</sub>	dS m <sup>-1</sup>	29.7	0.996	3.83	3.03	2.38
OC	g kg <sup>-1</sup>	0.80	440	240	422	290
NTK	g kg <sup>-1</sup>	180	16	14	34	23
C/N		0.004	27.5	17.1	12.4	12.6
VS	g kg <sup>-1</sup>	-	750	408	726	500
TSN	g kg <sup>-1</sup>	180	0.89	7.57	16.7	7.28
P	g kg <sup>-1</sup>	200	1.16	9.29	22.69	3.85
Cu	mg kg <sup>-1</sup>	44	32	125	8	13
Zn	mg kg <sup>-1</sup>	59	85	90	58	124

pH<sub>w</sub> pH measured in 1:25 (w/v) biomass:water, EC<sub>1:25</sub> electrical conductivity measured in 1:25 (w/v) biomass:water, OC total organic carbon, NTK total nitrogen by Kjeldahl method, C/N carbon/nitrogen ratio, VS volatile solids, TSN total soluble nitrogen, P total phosphorus, Cu and Zn total copper and zinc

(representing 100%) were diluted with deionized water to obtain a final 30% concentration. Ten surface sterilized seeds of cress were evenly placed into a Petri dish (9 cm diameter) over a double layer of filter paper previously moistened with 2 mL of the 30%-diluted aqueous extract. Deionized water served as a control (0%). Petri dishes were placed in the dark in a growth chamber set at  $27 \pm 1$  °C. Treatments (0, 30%) from each organic matrix were replicated three times and arranged in a completely randomized design. After 48 h, germinated seeds were counted, and their root length measured. The Germination Index [GI (%)] was calculated by multiplying the germination percentage by the root length percentage, and then, dividing by 100 as reported by Murillo et al. (1995).

### 2.3 Experimental Set-up

Soil microcosms consisted of Falcon™ tubes (30-mm diameter and 115-mm height) with a draining hole (5-mm diameter) closed at the bottom by a cellulose acetate filter to ensure an adequate drainage. The use of small-sized containers as soil microcosms was chosen not to replicate (or to be immediately extrapolated to) field conditions, but to create a simplified and standardised experimental model system suitable to impose a close contact in the soil–plant system, and promote a complete exploitation of soil nutrient resources, thus allowing for controlled and highly replicable assessments of root responses and P uptake in following immediate (up to 21 days) soil addition of different types of organic amendments. Each microcosm was filled with a freshly prepared potting mixture made up of an amount of soil (equivalent to 50 g dry weight) amended with COP, VEC, DIG or CSW in varying absolute quantities, depending on their P content (Table 2), to provide an amount of 30 mg P kg<sup>-1</sup> soil. The experimental set-up included also

a treatment with diammonium phosphate (DAP, 18–46, N-P<sub>2</sub>O<sub>5</sub>), a commonly applied inorganic NP-containing fertilizer here used as a reference for conventional management of P fertilization of wheat crop. At the rate applied to supply 30 mg P kg<sup>-1</sup> soil, DAP also provided the soil with 26.9 mg N kg<sup>-1</sup>. A control soil with no additional P application was included a control treatment (CON). Soon after, microcosms were sown with 3 surface sterilized seeds of durum wheat each and placed in a growth chamber under controlled conditions (photosynthetically active radiation of 350 μmol m<sup>-2</sup> s<sup>-1</sup>, 16/8 h light/darkness photoperiod, 24/20 °C day/night temperature, 60% relative humidity) in a completely randomized block design with 5 replications. To sum up, the experimental set-up consisted of 60 microcosms (2 soils × 6 treatments × 5 replicates).

Six days after sowing (DAS), two out of three durum wheat seedlings were manually removed, and the one left was harvested 21 DAS. Moisture was maintained at ~70% total soil volume by periodical addition of de-ionized water. Moreover, 1 ml of 0.5 M Ca(NO<sub>3</sub>)<sub>2</sub> per microcosm was applied daily since 10 DAS to the end of the trial (corresponding to a total provision of 40.1 mg Ca<sup>2+</sup> and 28 mg N-NO<sub>3</sub><sup>-</sup>) to ensure optimal N supply and vegetative development in all treatments for the length of the experiments.

#### 2.3.1 Plant Analysis

The seedling emergence percentage was determined by the number of durum wheat seedlings emerging 6 DAS. This evaluation was conducted through a meticulous visual analysis of individual replicates across the different experimental treatments, and aimed at assessing any inhibitory effects of the organic amendments applied.

At the end of the 21-day experimental period, the height of each wheat seedling was accurately measured and the leaf

chlorophyll index of the two youngest fully expanded leaves (3 measurements per leaf) was estimated using a SPAD 502 Portable Chlorophyll Meter (Minolta Camera Co., Osaka, Japan). Then, microcosms were carefully opened, durum wheat seedlings gently removed from the potting substrate, separated into root and shoots portions, gently rinsed with deionised water, and their fresh Weight measured. The root system was stained with 0.1% (w/v) *o*-toluidine blue staining solution for 5 min, thoroughly washed with deionised water and then scanned (Epson Perfection V850 Pro) at a resolution of 600 dpi for image analysis of root morphology. Scanned images were processed using the WinRhizo® root analysis software (WinRhizo® STD 1600, Instruments Régents Inc., Canada) to estimate the total surface area, the average diameter, the total root length, and the total root length within diameter classes. Then, root and shoot dry weight was determined by oven-drying (65 °C, 72 h). Based on the measurements above, the following derived parameters were calculated: the specific root length (SRL), defined as the root length per root dry weight, and the shoot/root ratio, defined as the dry weight of the aerial part divided by the dry weight of the root system.

The aerial portion of the plant was finely ground in a mill and the dry powder (0.2 g aliquot) digested with 3 mL of 65% HNO<sub>3</sub> and 1 mL of 60% HClO<sub>4</sub> (Zasoski and Burau 1977). Then, P content in aerial plant tissues was measured by the molybdate blue method (Murphy and Riley 1962); while Ca (422.67 nm), Mg (285.21 nm), Fe (248.33 nm), Cu (324.75 nm), Zn (213.86 nm) and Mn (279.48 nm) were determined by flame atomic absorption spectrophotometry, and K by flame photometry. Analytical readings allowed estimate P uptake efficiency (PUpE) of wheat seedlings according to the following equation:

$$PUpE(\%) = \frac{P_{\text{uptake}}(\text{treatment}) - P_{\text{uptake}}(\text{control})}{P_{\text{applied}}} \times 100$$

where  $P_{\text{uptake}}(\text{treatment})$  is the amount of P content in wheat seedlings exposed to a different organic amendment (aerial biomass multiplied by P concentration in shoot);  $P_{\text{uptake}}(\text{control})$  is the P content in wheat seedlings grown in the control (no P applied) soil (aerial biomass multiplied by P concentration in shoot); and  $P_{\text{applied}}$  is the amount of P supplied to the soil with each treatment, which equals, in this experiment, 1.5 mg of P per pot (30 mg P kg<sup>-1</sup> soil multiplied by 0.05 kg of soil), whatever the P form.

### 2.3.2 Soil Analysis

Following wheat plant harvest, soil aliquots from all microcosms were collected, air dried, and used to determine pH<sub>w</sub>, EC<sub>1:5</sub> and P<sub>Olsen</sub> according to the above-mentioned methods (in § 2.1).

## 2.4 Statistics

All data were initially checked for normality and homogeneity of variance. Readings from the phytotoxicity assay were subjected to a one-way analysis of variance (ANOVA, amendment type). Multiple pairwise comparison of means was done by least significance difference (LSD) test at  $P < 0.05$ . Plant and soil data collected at the end of the 21-day observation period were analysed by two-way ANOVA (soil type × treatment). As multiple interactions occurred, a one-way ANOVA was done for each soil, independently. Multiple pairwise comparison of means was done by least significance difference (LSD) test at  $P < 0.05$ . Statistical analyses were run by using the software Statistix v 10.0 (Analytical Software, Tallahassee, Florida, USA). Graphs were drawn by using the SigmaPlot 10.0 software (SYSTAT Software Inc.).

## 3 Results

### 3.1 Soils

The two agricultural soils showed contrasting properties. Their pH<sub>w</sub> and CaCO<sub>3</sub> content was different (520 vs 0 g kg<sup>-1</sup>), as was their clay content, CEC, OC, EC<sub>1:5</sub>, and P<sub>Olsen</sub> (Table 1).

### 3.2 Organic Amendments and Phytotoxicity Assay

All tested amendments were markedly different in their chemical properties, but not in the pH<sub>w</sub> that resulted alkaline in all cases (Table 2). The total Cu and Zn contents were in all cases below the mandatory limits reported by national (Legislative decree n. 75/2010) and European (Reg. EU n. 2019/1009) regulations. Further inside, COP showed the highest OC content, followed by DIG. Additionally, COP had a low content of total P, and the lowest electrical conductivity. On the contrary, VEC has the highest salinity (3.83 dS m<sup>-1</sup>) and the lowest NTK. DAP was the richest in NTK. Finally, DIG revealed an adequate NTK content accompanied by a significant P, Cu and Zn load (Table 2).

A stimulatory effect was found when the aqueous extracts from the organic amendments were tested in the phytotoxic assay, with no significant differences (Table 3;  $P = 0.3415$ ).

### 3.3 Soil Responses: Soil pH, Electrical Conductivity and Soil Available Phosphorus

Variations in pH<sub>w</sub> were found to be statistically affected by the soil type, the treatment and their interaction (Table S1). No significant differences among treatments were observed in the calcareous soil, and the resulting

**Table 3** Germination Index (GI, %) (mean  $\pm$  SEM,  $n = 3$ ) of seeds of *Lepidium sativum* L. exposed to 30% diluted organic amendment (COP: composted olive pomace; VEC: vermicompost from horse manure treated with *Eisenia fetida*; DIG: compost from solid anaerobic digestate; CSW: municipal solid waste compost) water extracts. When present, different letters indicate significant differences according to the LSD post hoc test (significance at  $P < 0.05$ ). Factorial one-way ANOVA is indicated as  $P$ -value<sup>a</sup>

Organic amendment	GI (%)
COP	119.0 $\pm$ 7.4
VEC	105.5 $\pm$ 3.9
DIG	103.5 $\pm$ 3.2
CSW	109.0 $\pm$ 8.1
$P$ value	<b>0.3415</b>

<sup>a</sup> Significant values are in bold

value found in the microcosms was slightly below the initial  $pH_w$  (Table 4). On the contrary,  $pH_w$  was significantly reduced with DAP and DIG related to CON in the non-calcareous soil (Table 4). In the calcareous soil,  $EC_{1:5}$  was significantly higher with COP and CSW; whereas in the non-calcareous soil, it was significantly lower with DAP and VEC, in comparison with CON (Table 4).

$P_{Olsen}$  after harvest was significantly affected by the soil type, the treatment, but not by their interaction (Table S1). DAP addition produced a strong increase of  $P_{Olsen}$  in both soils (+ 53% in the calcareous soil and + 59% in the non-calcareous soil in comparison with CON; Fig. 1a-b). With a similar trend in both soils, DIG and CSW addition caused a significant increase in  $P_{Olsen}$  (+ 27 and + 19% in calcareous, and + 31% and + 26% in non-calcareous soil compared to CON). The addition of compost (COP) or vermicompost (VEC) did not significantly vary  $P_{Olsen}$  irrespective of the soil type (Fig. 1a-b).

**Table 4** Soil  $pH_w$  [soil pH measured in 1:2.5 (v/w)] and  $EC_{1:5}$  [electrical conductivity measured in 1:5 (v/w) soil:water] (mean  $\pm$  SEM,  $n = 5$ ) in soil microcosms filled with two soil types (calcareous or non-calcareous) and exposed to different treatments (CON: control treatment without P addition; DAP: diammonium phosphate; COP: composted olive pomace; VEC: vermicompost from horse manure

	$pH_w$	$EC_{1:5}$ (dS $m^{-1}$ )	$pH_w$	$EC_{1:5}$ (dS $m^{-1}$ )
<i>Treatment</i>	Calcareous soil		Non-calcareous soil	
CON	8.19 $\pm$ 0.03 a	0.30 $\pm$ 0.02 bc	7.84 $\pm$ 0.01 ab	0.23 $\pm$ 0.02 a
DAP	8.14 $\pm$ 0.02 a	0.29 $\pm$ 0.01 c	7.74 $\pm$ 0.02 c	0.20 $\pm$ 0.01 b
COP	8.15 $\pm$ 0.01 a	0.35 $\pm$ 0.01 a	7.92 $\pm$ 0.03 a	0.22 $\pm$ 0.01 ab
VEC	8.13 $\pm$ 0.02 a	0.29 $\pm$ 0.01 bc	7.87 $\pm$ 0.02 ab	0.20 $\pm$ 0.01 b
DIG	8.15 $\pm$ 0.02 a	0.33 $\pm$ 0.01 ab	7.74 $\pm$ 0.03 c	0.23 $\pm$ 0.01 ab
CSW	8.17 $\pm$ 0.01 a	0.36 $\pm$ 0.01 a	7.82 $\pm$ 0.04 bc	0.24 $\pm$ 0.01 a
$P$ value	<b>0.5042</b>	<b>0.0017</b>	<b>0.0022</b>	<b>0.0210</b>

<sup>a</sup> Significant values are in bold

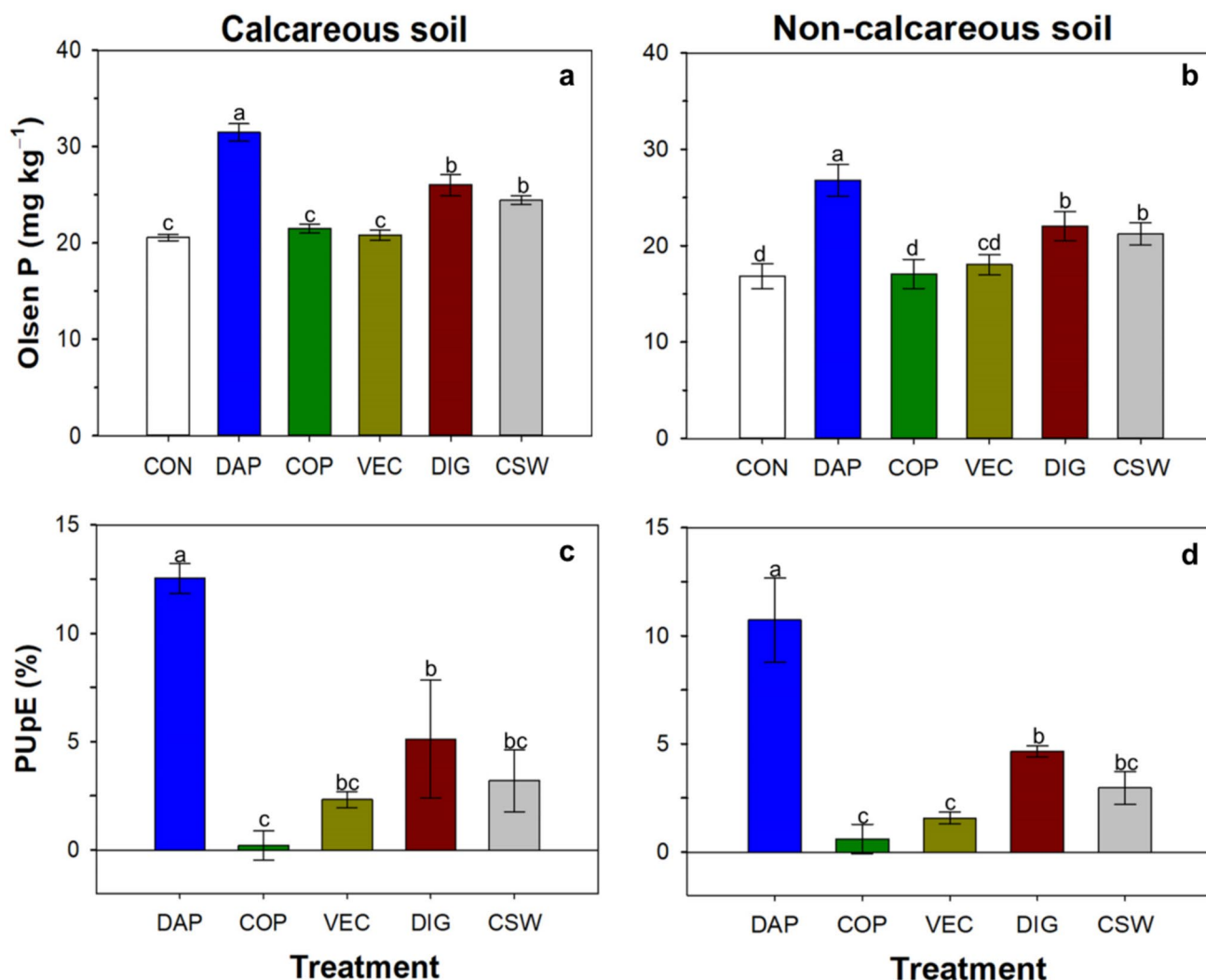
### 3.4 Plant Responses: Seedlings Emergence Percentage, SPAD, Seedling Height, P Use Efficiency

Seedling emergence percentage (6 DAS), leaf chlorophyll index and seedling height (both measured 21 DAS) were all significantly influenced by the soil type, the nature of the organic conditioner, and their interaction (Table S1). It was observed that seedling emergence was higher in the calcareous soil (86.6% on average) than in the non-calcareous one (70.0% on average) (Fig. 2a). Further inside, the lowest emergence rate was found in seedlings exposed to DAP in the calcareous soil; whereas in the non-calcareous soil seedling emergence provided more variable (and lower) values being significantly decreased only by the VEC addition (Fig. 2b).

Conversely, wheat seedlings grown in the non-calcareous soil provided higher values of both leaf chlorophyll index (SPAD) and plant height (cm) which were higher (43.5 and 23.3, respectively on average) than those grown in the calcareous soil (31.1 and 20.4, respectively, on average), even though it was in that soil that they appeared more selectively affected by the type of the organic amendment (Fig. 2c-f). In other words, COP and CSW treatments determined a significant reduction of either SPAD (Fig. 2d) and plant height values (Fig. 2f) respect to CON in the non-calcareous soil. Moreover, SPAD values were decreased only with COP- and VEC related to CON in the calcareous soil (Fig. 2c).

Only the treatment exerted a significant effect on PUpE ( $P < 0.0001$ ) (Table S1). Same as seen for  $P_{Olsen}$ , DAP addition induced the highest PUpE compared to CON in both soils (+ 12.5% in the calcareous soil and + 10.7% in the non-calcareous soil; Fig. 1c-d). Focusing on the organic treatments, DIG produced the largest increase in PUpE in both soils (+ 5.1% and + 4.6% in the calcareous

treated with *Eisenia fetida*; DIG: compost from solid anaerobic digestate; CSW: municipal solid waste compost) at the end of the 21-day observation period. When present, within each soil type different letters indicate significant differences among treatments according to the LSD post hoc test (significant at  $P < 0.05$ ). Factorial one-way ANOVA (treatment) is indicated as  $P$ -value<sup>a</sup>



**Fig. 1** Soil available P ( $P_{\text{Olsen}}$ , **a** and **b**) and P uptake efficiency (PUPE, **c** and **d**) (mean  $\pm$  SEM,  $n=5$ ) of wheat seedlings grown in two soil types (calcareous or non-calcareous) and exposed to differing treatments (CON: control treatment without P addition; DAP: diammonium phosphate; COP: composted olive pomace; VEC: vermicom-

post from horse manure treated with *Eisenia fetida*; DIG: compost from solid anaerobic digestate; CSW: municipal solid waste compost) at the end of the 21-day microcosm experiment. When present, different letters indicate significant differences among treatments according to the LSD post hoc test (significant at  $P < 0.05$ )

and non-calcareous soil, respectively; Fig. 1c-d). On the contrary, VEC (1.5–2.3%), CSW (3.0–3.2%) and COP (0.2–0.6%) treatments exerted a reduced increase of PUPE, although with no statistically significant differences among them and CON (Fig. 1c-d).

### 3.5 Plant Responses: Root-shoot Ratio, Root Traits

Only the soil type and the treatment affected the dry biomass shoot–root ratio (Table S2). It must be first noticed that a larger biomass allocation towards the root system was found in the non-calcareous soil (shoot/root < 1), whereas an opposite trend was clear in the calcareous one (shoot/root > 1)

(Fig. 3a-b). Moreover, only the addition of DAP produced an increase of this variable (shoot–root ratio) in both soils.

The total root length and SRL were significantly affected by the treatment and by the soil type  $\times$  treatment interaction (Table S2). In the calcareous soil, the total root length markedly raised in DIG (+83%), DAP (+70%) and VEC (+40%) treatments compared to CON; whereas COP- and CSW-treated soils provided readings like those of the CON (Fig. 3c). A different wheat plant response was observed in the non-calcareous soil, where root length generally decreased, especially with COP (–60% compared to CON), while this variable remained unaffected in DIG and DAP (Fig. 3d). Moreover, only DIG significantly increased SRL (+104% from the control) in the calcareous soil (Fig. 3e). On the contrary, COP, VEC and CSW

treatments led to a statistically significant reduction of SRL in the non-calcareous soil (−81%, −62% and −65%, respectively, compared to the control) (Fig. 3f).

Percent distribution of root length of durum wheat seedlings according to diameter classes in the two soil types is shown in Fig. 4. The two assessed factors and their interactions influenced these variables (Table S2). It is worth noting that the highest percentage of thinner roots (0.03–0.06 mm) was observed in the non-calcareous soil (51.1% vs 35.9% found in the calcareous soil) (Fig. 4a–b). Whereas an opposite trend was noticed when considering the percentage of thicker roots (0.12–0.24 mm, 24.9% vs 37.4%; Figs. 4e–f). To note also that in most cases (especially in the calcareous soil), DAP, VEC and DIG treatments differed from CON and were responsible for these contrasting responses between the two soils. On the other side, addition of organic amendments similarly acted on the morphology of mid (0.06–0.12 mm) and thick (>0.24 mm) roots (Fig. 4c–d, and g–h). Finally, durum wheat plants grown in the microcosms fertilized with CSW were scarcely affected by the amendment: higher values than the control were observed only for the 0.06–0.12 mm class in the non-calcareous soil and for the coarse root (>0.24 mm) class in the calcareous one (Fig. 4d and g).

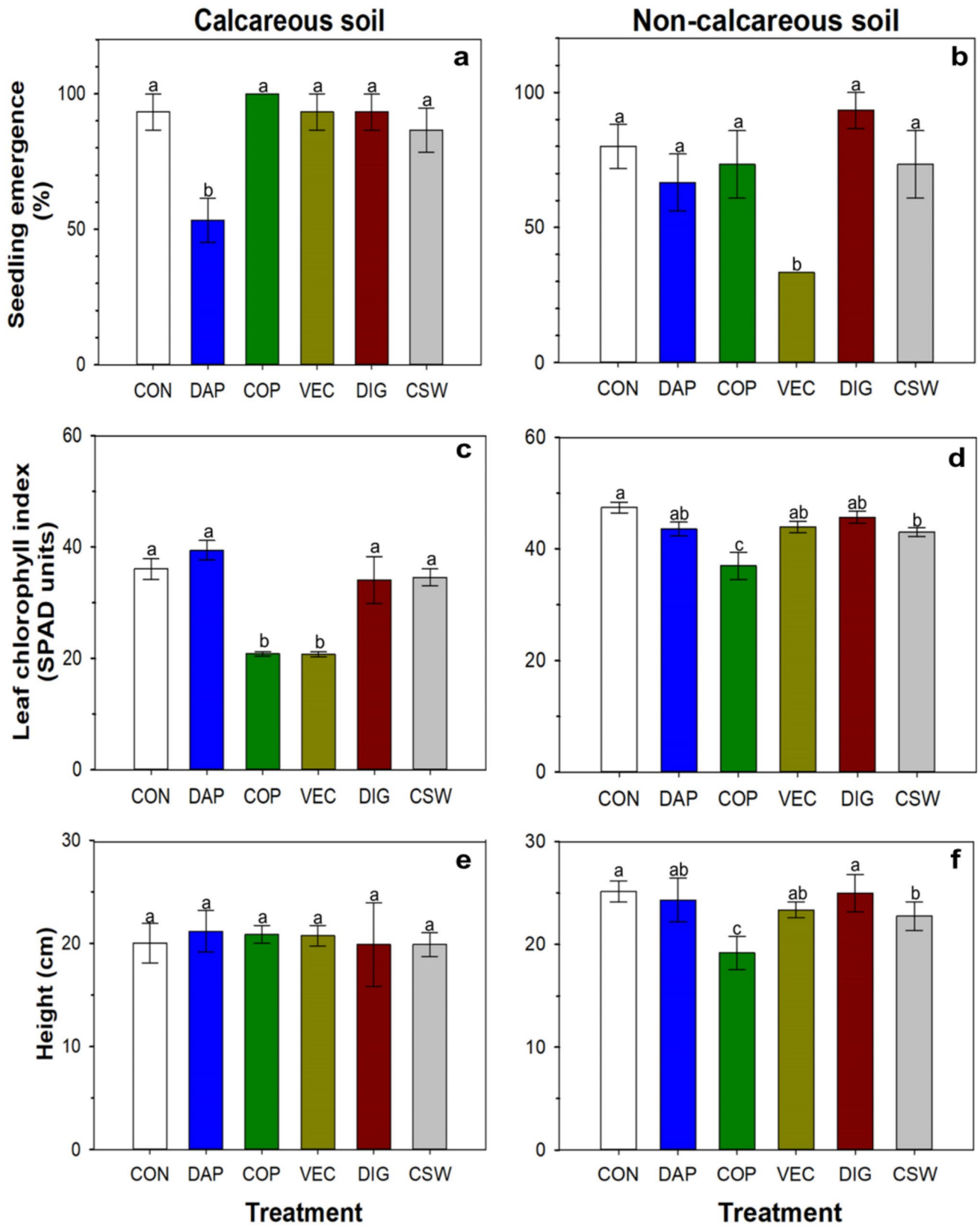
### 3.6 Plant Responses: Nutrient Elements in the Aerial Biomass

The nutrient element concentration in aerial tissues of durum wheat seedlings was statistically influenced by the soil type (K, Mg, Fe, Zn, Mn, Cu), the treatment (Ca, Mn and Cu) and by the soil × treatment interaction (only K and Zn) (Table S3). Plants grown in the calcareous soil showed a declining trend of aerial Cu concentration that became significant in DIG and CSW-treated plants related to CON (Table 5). Moreover, Mn concentration in aerial tissues markedly increased with all treatments, but especially with CSW and DIG, reaching values 2- or 3-times higher than that of the CON (Table 5). Conversely, most significant changes in K concentration in wheat occurred when DAP and DIG were applied, producing a significant increase and decrease, respectively, related to CON, in the non-calcareous soil. Moreover, Ca concentration in wheat was significantly reduced with the four organic amendments in comparison with CON, while Zn concentration was significantly increased with all treatments except for VEC, in the non-calcareous soil (Table 5).

## 4 Discussion

Based on the phytotoxicity assay, the four organic amendments were found to be non-phytotoxic and hence their use could be considered suitable as organic conditioners

in agricultural soils. It is also worth noting that, despite being potentially limiting due to their large salinity content (Raviv 2015), all amendments here tested showed a higher GI (%) than that of the control treatment. This finding is not surprising since it has been already reported that organic matrices can have a stimulatory effect on germination of wheat (Sheikh and Dwivedi 2017). However, differently from what seen in the *in vitro* test, the addition of the horse manure-derived vermicompost (VEC) negatively affected the seedling emergence in soil microcosms, particularly in the non-calcareous soil, suggesting that the material's properties acted as primary determinant of the plant response. Since the  $EC_{1:25}$  of the VEC was found to be as high as  $3.83 \text{ dS m}^{-1}$  it could be argued that the high soluble salts content was responsible for the low emergence of wheat seedling, in line with Al-Tabbal et al. (2024). However, by considering that: i) a similar finding was not observed in the calcareous soil, ii) the  $EC_{1:5}$  observed in the VEC-treated soil, irrespective of the carbonate content, never was higher than that of the CON, iii) addition of the mineral fertilizer did not affect the soil  $EC_{1:5}$ , but produced an opposite effect on seedling emergence in the two tested soils, then it can be concluded that factors other than a high salt content influenced the seed responses such as those linked to the nature of the organic matrix. Consistent with that, findings of SPAD and plant height confirm that soil incorporation of organic by-products produced a different response in wheat seedlings as a function of both soil and amendment properties, as seen for COP and VEC. Since plants are known to be strongly affected by the availability of N (Vishwakarma et al. 2023), we hypothesize that, beside other factors, the low total N content of these two matrices could have negatively affected these plant parameters. Moreover, certain components of the organic amendments could negatively impact on plant development such as the polyphenols content in olive oil wastes (Rodis et al. 2002), which could be high if the composting process of the olive mill pomace is not adequate. As for the  $\text{CaCO}_3$  content, the different soil type clearly affected the biomass allocation between the shoot and the root systems, notwithstanding the organic addition. The shoot-to-root ratio was lower in plants grown in our calcareous soil, consistent with findings from a previous study by Bavaresco and Poni (2003) on grapevines. However, a lower P availability for plants is associated with a higher shoot-to-root ratio in other studies (Hadir et al. 2021; Hayes et al. 2019). This effect is particularly pronounced in seedlings, as P is a key element for root development during the early stages of growth (Toderi and D'Antuono 2000). This is further confirmed by the shoot-to-root ratio results in seedlings treated with DAP, which, due to the higher amount of readily assimilable P, exhibited the highest values for this parameter. It is therefore fundamental not only to develop phytotoxicity tests with specific plant species as proposed in the legislation, but also to assess the



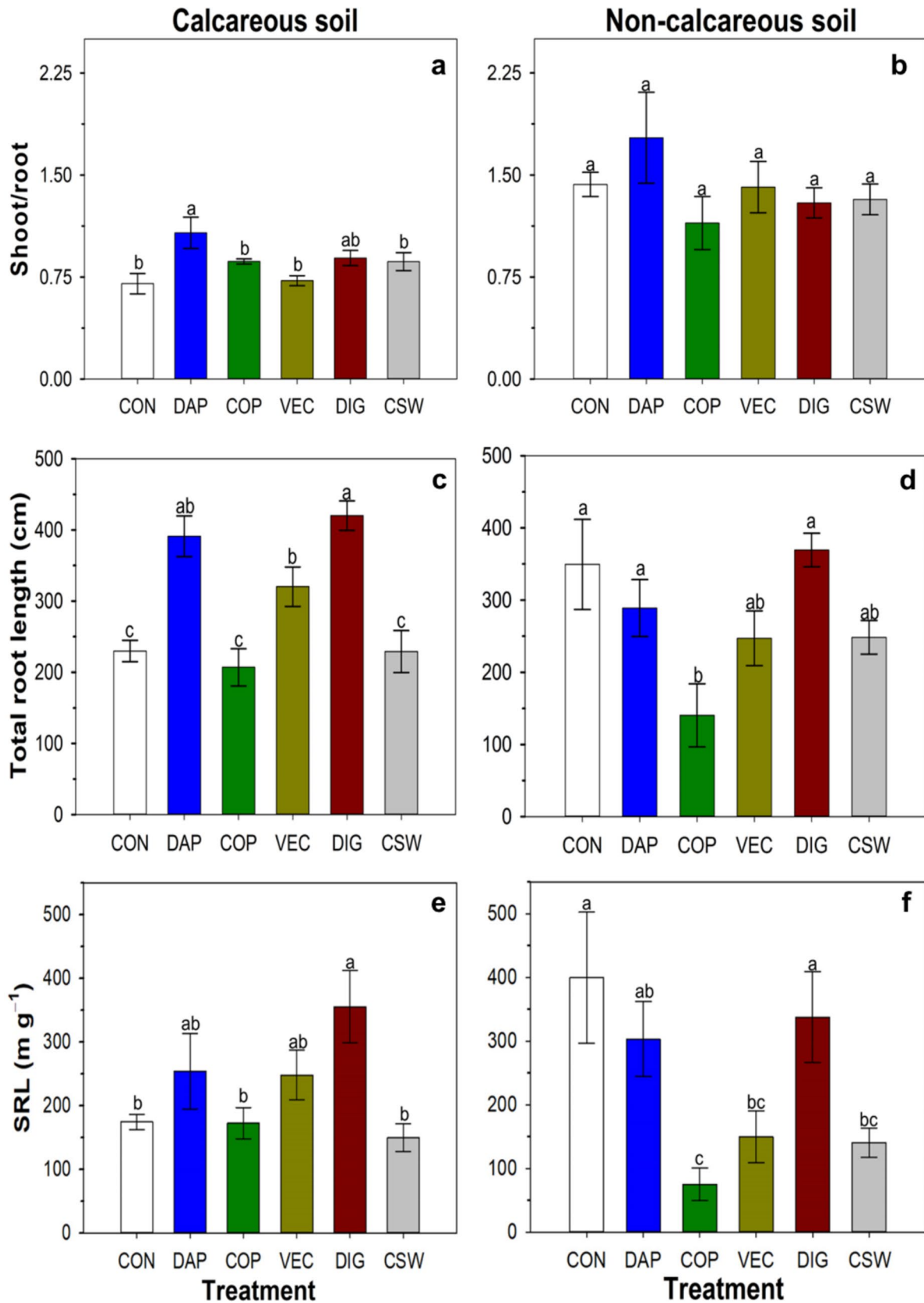
**Fig. 2** Seedling emergence 6 days after sowing (**a** and **b**), leaf chlorophyll index (SPAD, **c** and **d**) and plant height (**e** and **f**) (mean  $\pm$  SEM,  $n=5$ ) of wheat seedlings grown in two soil types (calcareous or non-calcareous) and exposed to differing treatments (CON: control treatment without P addition; DAP: diammonium phosphate; COP: composted olive pomace; VEC: vermicompost from horse manure treated with *Eisenia fetida*; DIG: compost from solid anaerobic digestate; CSW: municipal solid waste compost) at the end of the 21-day microcosm experiment. When present, different letters indicate significant differences among treatments according to the LSD post hoc test (significant at  $P < 0.05$ )

effects of organic amendments on the plant of interest at germination and at advanced phenological stages.

Moreover, the addition of DIG to both soils produced an immediate increase of  $P_{Olsen}$ , which met wheat requirement during a critical stage for further development. In fact, wheat typically takes up most (about 50%) of required P during the initial growth stages (from germination until the end of shooting), with no luxury consumption (Römer and Schilling 1986). A similar immediate increase of  $P_{Olsen}$  was not observed in COP and VEC treatments, indicating that a larger amount of chemically complex humic-like compounds occurring in these matrices require a longer than 21-day mineralization process before soluble mineral forms are released and become available to plants. However, plants can benefit from the slow releasing process since loss of soluble P forms via precipitation or occlusion by soil minerals is reduced, as demonstrated by similar trends observed in both the calcareous and the non-calcareous soil. It is important to point out that DAP was included in the experiments as a reference of an immediate release of readily soluble P. This obviously makes plant P-induced responses not fully comparable to organic amendments, since microbial mineralization is required before organic-derived soluble P becomes available to plants. Having said that, DAP-derived  $P_{Olsen}$  shows several environmental drawbacks because it is potentially leachable under heavy rainfall in coarse-textured soils, and prone to precipitation/immobilization of phosphates. Within this perspective, organic-derived P shows a longer lasting release that preserves this essential plant nutrient from environmental losses.

Plant responses to varying treatment-released  $P_{Olsen}$  were captured by the PUpE. At this regard, it is worth noting that inclusion of a negative control treatment (CON) where no P was supplied regardless of its chemical forms (either inorganic or organic) allowed to establish a baseline level of P uptake that reflects endogenous sources only. Since the PUpE was estimated as the percentage increase in the amount of P in the aerial biomass of plants treated with DAP or one of the four organic amendments, compared to their respective untreated soils (CON), calculated PUpE values represent the net contribution of externally applied P, excluding the P fraction derived from both soil and wheat seed. Thus, using

CON-treated plants as a quantitative reference allowed us to control for the contribution of soil and seed P, thereby isolating the specific effect of each treatment (organic or inorganic) on plant P uptake. The values observed in wheat PUpE when DAP was applied are in line with the values obtained in the year of application (10–25%), as most of the P introduced to the soil becomes immobilized (Mihoub et al. 2019). Similarly, the low PUpE from organic amendments has been confirmed, primarily due to the gradual release of soluble P forms through mineralization (Muktamar et al. 2020). However, the significant differences observed among the treatments in the two tested soils could be attributable to variations in the composition of the raw materials and the processing methods employed, as highlighted by Regelink et al. (2021). In particular, DIG provided a larger amount of P, especially when compared to N (Tambone et al. 2017). Conversely, COP, VEC and CSW exerted a minor positive effect on PUpE and provided a less evident contribution to  $P_{Olsen}$ . This effect has a dual cause. Firstly, the fraction of labile P, and therefore P available for plant uptake, is reduced in composts derived from matrices such as manure in accordance with Wei et al. (2015). Secondly, P availability can significantly decline during the composting process due to microbial immobilization, which depletes available P and promotes the conversion of labile P into more recalcitrant forms (Aboutayeb et al. 2023). Certainly, the availability of plant-assimilable P, and consequently PUpE, would increase over a longer period due to the mineralization of organic matter (Hayatu et al. 2023). The significant positive correlation between  $P_{Olsen}$  levels and PUpE here found (Table S4) contrasts with what was shown by Wu et al. (2018), who stated that when soil  $P_{Olsen}$  content is near agronomic critical values, PUpE may either peak or exhibit minimal variation. However, when  $P_{Olsen}$  levels exceed the critical values, PUpE tends to decline noticeably with increasing  $P_{Olsen}$  content. Moreover, the observed changes in soil  $pH_w$  in the non-calcareous soil but not in the calcareous soil (in which the  $pH_w$  is buffered) in response to the addition of the organic amendments are expected (Angelova et al. 2013; Mitran et al. 2018), and depend on the composition of the organic amendments, even though they were found to be not persistent and enough to significantly affect plant responses for long time (Zhang et al. 2023). These alterations in soil  $pH_w$  should have influenced the availability of P for plants, such as in the case of DIG in the non-calcareous soil where  $P_{Olsen}$  could have increased due to the observed soil  $pH_w$  decrease. We should highlight that the content of  $CaCO_3$  did not affect the availability of P in soil nor the PUpE of wheat plants in this short-term experiment. For that, our results do not support our first hypothesis, as the nature of the organic amendments more than the pedological content of  $CaCO_3$  primarily affected both soil P availability and plant P uptake in the short-term. Furthermore, previous research as that



**Fig. 3** Dry biomass shoot/root ratio (**a** and **b**), total (**c** and **d**) and specific root length (SRL, **e** and **f**) (mean  $\pm$  SEM,  $n=5$ ) of wheat seedlings grown in two soil types (calcareous or non-calcareous) and exposed to differing treatments (CON: control treatment without P addition; DAP: diammonium phosphate; COP: composted olive pomace; VEC: vermicompost from horse manure treated with *Eisenia fetida*; DIG: compost from solid anaerobic digestate; CSW: municipal solid waste compost) at the end of the 21-day microcosm experiment. When present, different letters indicate significant differences among treatments according to the LSD post hoc test (significant at  $P < 0.05$ )

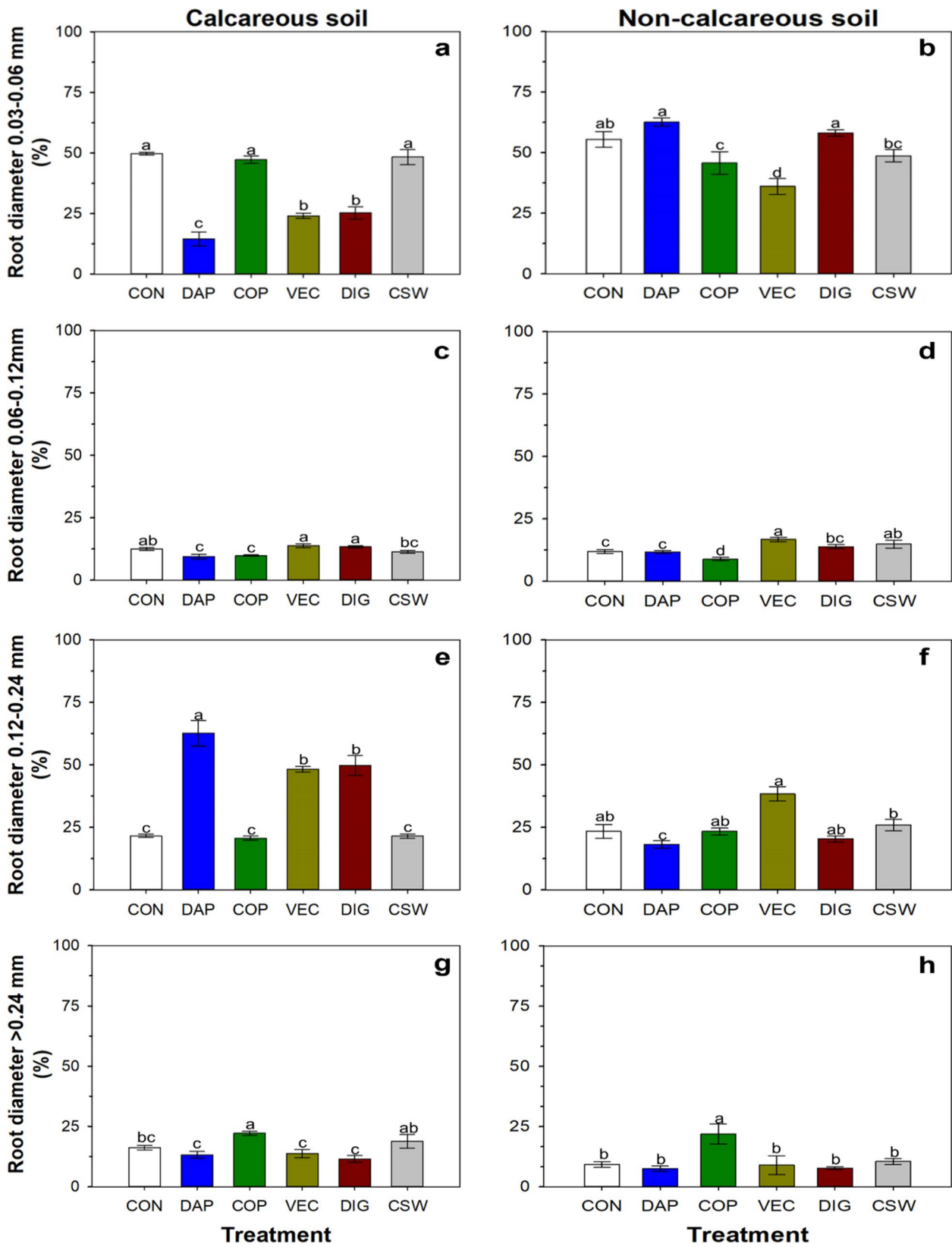
reported by Wu et al. (2018) is commonly focused on long-term fertilization experiments, whereas immediate responses after addition of P from sources of different origin (inorganic and organic) were here investigated.

Root development and traits are essential factors for plant P uptake (Lynch 1995; Niu et al. 2012). When grown under limited P availability, plant root adaptations may involve increased development of adventitious roots and lateral root branching, as well as greater formation of root hairs and cluster roots (Teng et al. 2013). In line with the second hypothesis, the traits and development of the root system of durum wheat seedlings varied according to the nature of the amendments applied and their different turnover in relation to the recipient soil types, as also reported by Yuan et al. (2016). A well-developed root system provides a larger surface area for the adsorption of water and nutrients from the soil, which is particularly crucial for P, as phosphate ions are among the least mobile in soil (Singh Gahoonia and Nielsen 2004). The total root length, in this study, provided similar readings between the calcareous and the non-calcareous soil irrespective of the organic addition, except in the case of CON, which was characterized by the lowest soluble nutrient content. We hypothesize that in the control soil, which received neither organic nor inorganic additions, wheat plants had to increase their root length to explore a larger soil volume in search of nutrients. Similar results were obtained by Sánchez-Rodríguez et al. (2014) in non-P-fertilized plants grown in calcareous soils. Moreover, the root systems of wheat seedlings selectively responded to the organic addition, being increased (DIG) or unaffected/decreased (COP, VEC, CSW) according to the characteristic of the added biomass. The greatest increase in total root length and SRL in DIG-treated calcareous soil can be attributed to an enhanced release of soluble nutrients and, in addition, to a bio-stimulating effect on plant growth due to hormones and hormone-like molecules released from the solid digestate once introduced into the soil (Scaglia et al. 2015, 2017). Again, these results support our second hypothesis. The positive impact of DIG is further corroborated by findings reported in a study by Ross et al. (2018). Conversely, the commented inhibitory effect observed on the root system after compost addition (COP, VEC and/or CSW) could be attributed to their chemical properties. Olive mill

pomace, being naturally rich in polyphenols, can adversely affect the root system development, particularly during the early growth stages of the plant, as also noted in a previous study by Omer and El-Sayed (2012). SRL mirrored what was seen for total root length. This finding aligns with several studies (Loudari et al. 2022; Nguyen and Stangoulis 2019; Shen et al. 2018; Wang et al. 2016), who observed that varying the P supply, and consequently, its availability to plants, as seen in this study depending on the type of amendment used, leads to an increase in total root length and in SRL.

Root diameter indicates the soil volume roots can access to take up nutrients and water. Indeed, low soil P availability typically stimulates the development of finer roots (Christie and Moorby 1975; Schroeder and Janos 2005; Shen et al. 2018), which allows the plants acquire more nutrients (and water). However, our findings contrast with those reported in previous studies; specifically, the finest roots in the first diameter class ( $\varnothing = 0.03\text{--}0.06$  mm) exhibited differences between the two soil types, with higher root fineness observed in the non-calcareous soil. A higher availability of soluble P in non-calcareous soils would correlate with a less fine root system, as finer roots are typically stimulated under low P availability (Christie and Moorby 1975; Shen et al. 2018; Yuan et al. 2016). In calcareous soils, P forms insoluble complexes with Ca, thus limiting available P uptake. This leads to root responses such as the development of roots smaller in diameter, as seen in DIG- and CSW-treatments, which also showed high  $P_{\text{Olsen}}$  and  $P_{\text{UpE}}$  values. This suggests that reducing root diameter may be a strategy to enhance P acquisition under limited nutrient availability. Beyond a threshold of P deficiency, root growth may be suppressed due to metabolic constraints or resource allocation shifts (Richardson et al. 2009; Wissuwa et al. 2015). Fine roots, efficient in nutrient and water uptake due to their high surface area-to-volume ratio, require more carbon due to faster turnover rates (Marschner 2012; Persson 1983). In addition, although finer roots are common under nutrient scarcity, their maintenance costs can outweigh their benefits, thus inhibiting their growth (Li et al. 2012; Yuan et al. 2016). This trade-off aligns with our findings in the calcareous soil, where P limitations did not stimulate finer root production, likely due to their high maintenance costs. Hence, this study confirms that replacing inorganic P fertilizer with an equivalent organic-derived P affects root morphology even in the short-term (21 days), when plants exhibit high root plasticity.

The different nutrients concentration measured in the aerial part of wheat plants is likely due to both soil properties and the nature of the organic amendments. For instance, unexpectedly K accumulation was larger in plants grown in the calcareous soil, irrespective of the negative interaction between K and Ca during the ion uptake (Rhodes et al. 2018). However, the organic treatments produced a lower



**Fig. 4** Length percentage (mean  $\pm$  SEM,  $n=5$ ) within diameter classes of roots of wheat seedlings grown in two soil types (calcareous or non-calcareous) and exposed to differing treatments (CON: control treatment without P addition; DAP: diammonium phosphate; COP: composted olive pomace; VEC: vermicompost from horse manure treated with *Eisenia fetida*; DIG: compost from solid anaerobic digestate; CSW: municipal solid waste compost) at the end of the 21-day microcosm experiment. When present, different letters indicate significant differences among treatments according to the LSD post hoc test (significant at  $P < 0.05$ )

absorption of the nutrient especially under non-calcareous conditions. Having said this, despite the wide range of variability existing in the accumulation of nutrients, recorded levels are consistent with those reported in the literature by Khan and Shewry (2009) (K  $\sim 20$ – $40$  g kg<sup>-1</sup>; Ca  $\sim 2$ – $5$  g kg<sup>-1</sup>; Mg  $\sim 1$ – $3$  g kg<sup>-1</sup>). Fe, Zn, Mn and Cu detected in the aerial part of durum wheat seedlings are in accordance with literature (Khan and Shewry 2009), since they are ranging within expected values: Fe  $\sim 20$ – $50$  mg kg<sup>-1</sup>; Zn  $\sim 30$ – $80$  mg kg<sup>-1</sup>; Mn  $\sim 50$ – $150$  mg kg<sup>-1</sup>, Cu  $\sim 5$ – $15$  mg kg<sup>-1</sup> and always above the deficiency limits set by Korzeniowska et al. (2020). In general, lower Fe, Zn and Mn concentrations were found in wheat seedlings grown in the non-calcareous soil, probably due to the dilution effect previously reported by González-Caballo et al. (2022) under similar conditions. To note that the larger Zn content observed in the aerial part of plants grown under calcareous conditions corroborates the antagonistic inhibition between P and Zn uptake in plants, as also highlighted by Sánchez-Rodríguez et al. (2021). Typically, calcareous soils show a lower soluble P content than non-calcareous soils due to P fixation dynamics, which reduces the inhibitory interaction between P and Zn uptake. Relatively to organic amendments effect in non-calcareous soil, even if a large amount of Zn was found in COP and VEC, the higher value for Zn present in aerial part was detected in DIG and CSW treatment, and this can be caused by a faster mineralization, as also noted above in the case of P. Additionally, all the organic amendments produced plant biofortification except VEC in the non-calcareous soil, while DIG produced the highest Zn concentration in the calcareous soil, which is an extra benefit of these treatments. In our results, the Cu concentration in the aerial part was lower when the concentrations of Fe, Zn, and Mn were higher, particularly in calcareous soil. In contrast, the analysis revealed higher Cu concentrations in non-calcareous soil, where the concentrations of the other tested micronutrients were lower. This could be explained by the known antagonism between Cu, Fe, Mn and Zn, because are all transition metal ions compete for the same transporters in plant cells (Mengel et al. 2001; Hänsch and Mendel 2009). Moreover, the organic amendments treatment influenced Cu concentration in the aerial part only in the calcareous soil, with lower values observed in organic DIG and CSW treatments, displaying an opposite

trend compared to the general pattern of other nutrients. In contrast, CON and DAP treatments resulted in the highest Cu concentrations in aerial part. This could be attributed to the greater capacity of organic chemical compounds to adsorb Cu (and other metals), thereby inhibiting its entering plant tissues. Moreover, the alterations in nutrient concentrations caused by the organic amendments should be considered when designing strategies in which these potential fertilizers are included.

The contrasting responses of the organic amendments observed in our study can be attributed to their distinctive features such as, along with biochemical features, chemical properties, nutrient content and balance, type and stability of carbon substrates, and occurring bioactive compounds. Summarizing, DIG consistently improved P availability, root traits, and micronutrient uptake, likely due to its elevated soluble P levels, a balanced N/P ratio, and the presence of hormone-like substances such as auxin analogues, which are known to enhance root proliferation and nutrient uptake. Additionally, the occurrence of partially degraded organic compounds with a relatively low C/N ratio promote C substrates mineralization and consequent soluble nutrient release, particularly under short-term conditions. VEC enhanced SRL in the calcareous soil where the buffering capacity might have mitigated its high salinity ( $EC > 3.8$  dS m<sup>-1</sup>). On the contrary, VEC showed adverse effects in the non-calcareous soil, likely due to osmotic stress and insufficient N and P supply during early seedling growth. Despite being rich in hydrolytic enzymes and microbial communities, the mineralization process in VEC-amended soils may proceed slower and less synchronized with plant demand, reducing its immediate effectiveness. CSW provided moderate improvements in available P in soil and Zn uptake, especially in the non-calcareous soil. These effects might stem from the occurrence of either inorganic contaminants (albeit below the mandatory limits) or highly stabilized organic matter. The limited influence on root traits and SPAD index suggests that the microbial dynamics of nutrient release proceeds slower in CSW-treated soils, and the biological stimulation is less pronounced when compared to digestate-treated soils. Among tested amendments COP provided lower and lessen responses, particularly in the non-calcareous soil, where negative effects on SRL, plant height and leaf chlorophyll content were observed. This finding is most likely due to its reduced P availability and the presence of residual phenolic compounds, which can exert phytotoxic or inhibitory effects on both plant roots and soil microbiota. Moreover, its high C/N ratio further limits the mineralization process, especially in the short-term following addition. Altogether, findings collected from model soil-plants systems soon after addition of different types of organic amendments emphasize the need

**Table 5** Concentration of nutrient elements in the aerial part (mean  $\pm$  SEM,  $n=5$ ) of wheat seedlings grown in microcosms filled with two soil types (calcareous or non-calcareous) and exposed to different treatments (CON: control treatment without P addition; DAP: diammonium phosphate; COP: composted olive pomace; VEC: vermicompost from horse manure treated with *Eisenia fetida*; DIG:

compost from solid anaerobic digestate; CSW: municipal solid waste compost) at the end of the 21-day observation period. When present, within each soil type different letters indicate significant differences among treatments according to the LSD post hoc test (significant at  $P < 0.05$ ). Factorial one-way ANOVA (treatment) is indicated as  $P$ -value<sup>a</sup>

Treatment	K (g kg <sup>-1</sup> )	Ca (g kg <sup>-1</sup> )	Mg (g kg <sup>-1</sup> )	Fe (mg kg <sup>-1</sup> )	Zn (mg kg <sup>-1</sup> )	Mn (mg kg <sup>-1</sup> )	Cu (mg kg <sup>-1</sup> )
Calcareous soil							
CON	41.2 $\pm$ 4.6 a	2.2 $\pm$ 0.4 a	1.6 $\pm$ 0.2 a	159.4 $\pm$ 42 a	76.8 $\pm$ 9.7 a	56.7 $\pm$ 6.4 a	12.6 $\pm$ 1.9 a
DAP	42.8 $\pm$ 4.2 a	3.3 $\pm$ 0.1 a	1.6 $\pm$ 0.1 a	128.7 $\pm$ 4.5 a	56.1 $\pm$ 6.3 a	59.3 $\pm$ 4.3 a	11.7 $\pm$ 1.4 a
COP	53.3 $\pm$ 7.7 a	2.4 $\pm$ 0.7 a	1.7 $\pm$ 0.3 a	100.5 $\pm$ 9.8 a	48.7 $\pm$ 3.0 a	66.1 $\pm$ 3.4 a	9.4 $\pm$ 1.2 ab
VEC	45.1 $\pm$ 4.7 a	2.3 $\pm$ 0.1 a	1.5 $\pm$ 0.1 a	128.6 $\pm$ 5.8 a	55.6 $\pm$ 7.6 a	81.6 $\pm$ 5.3 a	9.8 $\pm$ 1.5 ab
DIG	48.5 $\pm$ 5.4 a	2.0 $\pm$ 0.5 a	1.6 $\pm$ 0.3 a	134.0 $\pm$ 32.9 a	80.4 $\pm$ 15.1 a	153.1 $\pm$ 54.9 a	6.5 $\pm$ 1.7 b
CSW	52.1 $\pm$ 1.9 a	2.2 $\pm$ 0.1 a	1.3 $\pm$ 0.1 a	153.1 $\pm$ 14.9 a	53.8 $\pm$ 6.8 a	108.1 $\pm$ 8.7 a	5.6 $\pm$ 0.8 b
$P$ value	<i>0.4585</i>	<i>0.2247</i>	<i>0.8060</i>	<i>0.5545</i>	<i>0.0851</i>	<i>0.0502</i>	<b>0.0145</b>
Non-calcareous soil							
CON	34.7 $\pm$ 4.6 bc	3.7 $\pm$ 1.4 a	1.4 $\pm$ 0.2 a	132.2 $\pm$ 8.8 a	29.2 $\pm$ 2.0 c	38.7 $\pm$ 1.7 a	15.6 $\pm$ 0.4 a
DAP	47.6 $\pm$ 3.5 a	2.9 $\pm$ 0.2 ab	1.4 $\pm$ 0.2 a	94.1 $\pm$ 9.8 a	37.0 $\pm$ 3.2 ab	45.6 $\pm$ 3.3 a	14.1 $\pm$ 1.1 a
COP	35.4 $\pm$ 0.9 bc	0.9 $\pm$ 0.2 c	1.0 $\pm$ 0.1 a	115.8 $\pm$ 8.7 a	36.8 $\pm$ 3.2 ab	49.7 $\pm$ 6.2 a	13.3 $\pm$ 0.8 a
VEC	38.8 $\pm$ 2.4 b	1.7 $\pm$ 0.2 bc	1.0 $\pm$ 0.1 a	121.4 $\pm$ 12.6 a	32.8 $\pm$ 1.3 bc	42.1 $\pm$ 2.6 a	14.0 $\pm$ 0.6 a
DIG	27.7 $\pm$ 1.3 c	2.0 $\pm$ 0.1 bc	1.4 $\pm$ 0.1 a	96.9 $\pm$ 10.7 a	41.3 $\pm$ 2.3 a	45.7 $\pm$ 3.1 a	14.2 $\pm$ 0.6 a
CSW	38.1 $\pm$ 0.8 b	1.7 $\pm$ 0.1 bc	1.3 $\pm$ 0.1 a	117.9 $\pm$ 6.7 a	43.1 $\pm$ 2.3 a	42.7 $\pm$ 2.4 a	14.0 $\pm$ 0.5 a
$P$ value	<b>0.0010</b>	<b>0.0322</b>	<i>0.1351</i>	<i>0.0773</i>	<b>0.0064</b>	<i>0.3587</i>	<i>0.3308</i>

<sup>a</sup> Significant values are in bold

to evaluate organic amendments not only for their well-known long-term beneficial actions on physical, chemical and biological properties (benefits should be accounted for but not in the present research), but also considering their biochemical stability, salinity, potential stimulatory or inhibitory compounds, and interactions with specific soil properties when designing integrated plant nutrition strategies. This is particularly relevant at early stages of growth when plant enters a fully vegetative autotrophic stage, after seed resources have become exhausted.

Our findings can serve as a basis for designing fertilization strategies targeting soils in semi-arid regions, with low organic matter content and limited nutrient availability (in this case, P). Moreover, they highlighted the potential of some organic amendments (DIG and, to a lower extent, CSW) to supply P during the early growth stages of wheat (cereal) and their capacity to modify root traits and enhance P acquisition. They suggest that organic amendments could be integrated into sustainable P management practices, particularly in soils with low availability in this nutrient. While this study focused on early seedling responses to organic amendments, we acknowledge that our experiments (21 days length) does not fully capture long-term P availability or its effects on wheat development. However, this approach is valuable for identifying whether the tested organic amendments

can promptly release P and induce modifications in root traits, which are indicators of their potential as sustainable fertilizers. Future research should evaluate the impacts of these amendments on plant growth and soil nutrient dynamics in a longer-period.

## 5 Conclusions

Although similar patterns were observed in terms of soil P availability and P uptake efficiency (PUpE) at the end of the microcosms experiments in both soils (short-term), only compost from solid anaerobic digestate (DIG) followed by municipal solid waste compost (CSW) could be proposed as a feasible alternative to diammonium phosphate (DAP) in the tested soils. We also demonstrated that the magnitude of the morphological responses at the root system level were dependent on soil type. This means that agricultural use of organic amendments might results in different, sometimes contrasting, plant responses in terms of nutrient use efficiency, depending on soil properties. In other words, main characteristics of the soil should be duly considered when adopting organic farming practices. Even though the observation period was as short as 21 days, similar studies focusing on PUpE and root traits responses under contrasting soils during the first phenological stages, which are

critical for cereals P acquisition, are not common. Additionally, findings of this study confirm that the effect of organic amendments on nutrient uptake varies depending on soil type, with significant differences observed between calcareous and non-calcareous soils. The treatments with organic amendments, such as DIG and CSW, showed distinct patterns of nutrient concentration, highlighting the key role of the nature of organic amendment in influencing nutrient availability and plant nutrition. Further research is needed to unravel how these dynamics would affect soil and plant relationships in a longer term.

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**Data Availability** The data will be available on reasonable request.

**Code Availability** Not applicable.

## Declarations

**Ethics Approval** Not applicable.

**Consent to Participate** Informed consent was obtained from all individual participants included in the study.

**Consent for Publication** It is stated that we wanted to publish this manuscript in “Journal of Soil Science and Plant Nutrition”. I give my consent for the publication of identifiable details, which can include a photograph(s) or details within the text to be published in the proposed journal. I confirm that I have seen and been allowed to read both the Material and the Article to be published by the “Journal of Soil Science and Plant Nutrition”. I have discussed this consent form with all my co-authors.

**Competing interests** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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