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Scarcity of P-fertilisers: Humic-complexed phosphate as an adaptive solution for wheat and maize under rainfed conditions

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ABSTRACT

The current context of food security and global geopolitical crises calls for proactive efforts to seek adaptive strategies for limited resources in agriculture. The production of phosphatic-based fertilisers has caused a substantial depletion of natural phosphorus (P) reserves, raising concerns about price increases due to the growing demand for P. The aim of the study is to assess whether the use of liquid organo-mineral P-complexes can be an adaptive strategy to address the issue of the limited P-fertiliser resource. A complete randomised block design was implemented with three replicates, spanning two contrasting cropping seasons under a Mediterranean climate with reduced tillage and rainfed conditions. The study aimed to assess the response of a durum wheat–maize rotation to various rates of liquid organo-mineral P-complexes (humic-complexed phosphate; HCP) in comparison to granular triple superphosphate (TSP). The evaluation focused on several parameters, including P availability, plant architecture, dry matter accumulation, grain yield and yield components and P use efficiency (PUE) for both durum wheat and maize. The results revealed that applying HCP at a rate of 4 or 8 kg ha^{-1} increased soil P availability on average by 66% and 144%, respectively, compared to the control (no P-fertilisation), although a significant interaction with the monitoring year emerged. For wheat, applying HCP at a rate of 8 kg ha^{-1} of P resulted in higher grain yield (+15%), protein content (+0.9%) and P uptake (+48%) than the control on average considering both monitoring years. In the season characterised by favourable rainfall patterns, the application of HCP at 5 or 10 kg ha^{-1} of P for maize showed similar effects in terms of soil P availability, dry matter accumulation, grain yields and PUE. Conversely, in the season marked by a deficiency in summer precipitation, low PUE for maize was observed for HCP fertilisers, although they remained positive, unlike the case of TSP. Using HCP in conservation agriculture appears promising as an adaptive solution to address P-fertiliser scarcity, especially amid food security challenges and global crises. However, further studies are required to validate these findings in diverse pedo-climatic contexts and cropping systems.

1. Introduction

Currently, despite recent studies suggesting that improvements in grain yield do not require proportional increases in the use of chemical phosphorus (P) fertilisers (Gao et al., 2023; Wu et al., 2015), incorrect recommendations on P-fertilisation have misled farmers into believing that increasing mineral inputs increases crop production. This caused a general overuse of P-fertilisers in crop systems, especially in developed regions where the accumulation of P in soils and eutrophication of natural aquifers have been shown to be a threat to the environment in many studies (Alewell et al., 2020; Piegholdt et al., 2013; Torrent et al., 2007; Wang et al., 2013). The current context of food security and global geopolitical crises calls for proactive efforts to seek adaptive strategies for limited resources in agriculture. This aspect becomes even more significant when considering that Russia is the world's third-largest exporter of P-fertilisers (Gross, 2022) and that the current geopolitical

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tensions will likely exacerbate the P-issue. Thus, there is a need to seek solutions that act preventively and in relatively short time frames. In the context of P-fertilisers, a more integrated and sustainable approach to P-management in agricultural systems is highly required (Alewell et al., 2020; Bindraban et al., 2020; Reijnders, 2014; Sharpley et al., 2018).

Globally, maize and wheat are the major contributors to food security, and frequently, maize is grown in rotation with wheat in many countries, such as China, India, Mexico and Pakistan (Prasanna et al., 2020). In addition, in Italy, maize is usually grown in rotation with wheat under rainfed and conservation agriculture practices (Piccoli et al., 2021). This helps to reduce inputs and gain environmental sustainability based on conservation agriculture, although reduced crop yields are also expected (Di Paolo and Rinaldi, 2008). It is well known that in addition to nitrogen and potassium, adequate P concentration in plant tissues is crucial to maintain a high rate of photosynthesis in cereal crops, and P deficiency may reduce total leaf area, plant dry matter, root hair and grain yield (Marschner and Rengel, 2011; Mazlouzi et al., 2020; Rychter and Rao, 2016; Zhu et al., 2012).

Under the regime of conservational agriculture, reduced tillage, among other crop management practices, aims to significantly reduce the mineralisation of soil organic matter and mitigate soil greenhouse gas emissions (Francioni et al., 2020; Rutkowska et al., 2018; Toderi et al., 2021). In climate change ‘hotspot’ areas such as the Mediterranean Basin, it is expected that the increase in temperature, decrease in precipitation and intensification of extreme events will significantly affect soil carbon and water reserves (Almagro et al., 2009; González-Ubierna and Lai, 2019; Zhao et al., 2021). Climate change is anticipated to impact crop production, especially during the dry season (Abd-Elmabod et al., 2020; Mereu et al., 2021; Zittis et al., 2019). In this climate change perspective, it becomes important to move towards the application of more sustainable practices, including reduced tillage and the sparing use of water resources (Di Paolo and Rinaldi, 2008; Francaviglia et al., 2018; Huang et al., 2020). However, achieving a homogenous distribution of immobile elements, such as P, for plant nutrition can become critical, especially in highly calcareous soils in the Mediterranean area (Alewell et al., 2020; Reijnders, 2014; Sharpley et al., 2018; Torrent et al., 2007).

Given the current geopolitical crisis and concerns regarding food security, a significant contribution to maintaining satisfactory crop yields could come from the introduction of innovative P-fertilisers aimed at improving P availability in the soil. Liquid organo-mineral P-complexes, designed for this purpose, are formulated to enhance the available fraction of P in the soil, promoting increased efficiency and uptake by crops. This complexation process involves a reaction between sulphuric acid and phosphate rock in the presence of specific quantities of humic extracts, resulting in the formation of soluble monocalcium phosphate. Subsequently, this compound is intricately bound to humic substances through metal–oxygen binding sites (Erro et al., 2012; Herrera et al., 2016). Recent studies proved that the complexation of humic substances with the phosphate fraction can decrease phosphate fixation in the soil and increase phosphate uptake, ultimately benefitting plant growth. For instance, Herrera et al. (2016) conducted a study comparing the cumulative application of a humic-complexed superphosphate with a commercial simple superphosphate in a temperate climate characterised by the absence of a rainy season. The research was conducted in a sandy clay loam typic hapludox soil over a 5-year crop rotation involving maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), soybean (*Glycine max* (L.) Merr.), white oat (*Avena sativa* L.) and soybean. The findings revealed that humic-complexed superphosphate led to an increase in the labile P pool within the soil compared to simple superphosphate. This enhancement contributed to improved soil P availability, resulting in enhanced crop grain yield and increased agronomic efficiency (AE). In a loam soil with alkaline calcareous characteristics and low organic matter content in Pakistan, Izhar Shafi et al. (2020) conducted a study to assess the impact of single superphosphate both with and without the addition of humic acids. The results revealed a significant interaction effect between P and humic acids on wheat plant height, grain yield, biological yield and soil extractable P and potassium levels. However, some other studies have presented conflicting results regarding the humic complexation of P-fertilisers. For instance, in an experimental trial with sugarcane (*Saccharum* spp.) conducted in clayey soil under tropical climate conditions, Zavaschi et al. (2020) discovered that applications of simple superphosphate increase both the dry matter and P content of the sugarcane plant. This effect had a more substantial residual impact than applications of superphosphate complexed with humic acid. Conversely, superphosphate complexed with humic acid exhibited greater efficiency in P assimilation. Among the limited studies currently available on P-fertilisers complexed with humic acids, there is a lack of research examining the use of these new P-fertilisers in clayey calcareous soils, which are increasingly managed through conservation agriculture in the Mediterranean Basin.

This study aims to address these knowledge gaps by investigating how a rainfed durum wheat–maize rotation responds to different rates of humic-complexed phosphate (HCP) fertilisers over two cropping seasons. The hypothesis was that reduced rates of P applied in the form of HCP can ensure comparable soil P availability and crop yields when juxtaposed with the effects of triple superphosphate (TSP). To test this hypothesis, rates of 4 and 8 ha^{-1} of P in the form of liquid HCP fertiliser were compared to equivalent rates of P in the form of granular TSP for wheat. Similarly, for maize, the same comparison was conducted with rates of 5, 10 and 20 kg ha^{-1} of P. For both crops and all P-fertilisation treatments, the effects were assessed in terms of i) soil P availability; ii) plant architecture; iii) dry matter accumulation; iv) grain yield and yield components and v) P use efficiency (PUE).

2. Materials and methods

2.1. Study area

The study was conducted at the experimental farm ‘Pasquale Rosati’ of the Università Politecnica delle Marche (43° 32′ 24.5″N, 13° 22′ 30.2″E, Agugliano, Ancona province, Italy). The climate is a temperate oceanic sub-Mediterranean variant with an average annual rainfall of approximately 800 mm and a mean temperature of 15.4 °C (reference period: 1998–2012). The meteorological data of the experimental site recorded during the study period were recorded by a weather station located at approximately 2 km and combined with pluriannual data obtained from the Agency for Innovation in the Agri-Food Sector (in Italian, Marche Agricoltura Pesca, Agenzia per l’innovazione nel settore agroalimentare e della pesca, AMAP) (Fig. 1). The soil was classified as Vertic Cambisols and had a silty-clay loam texture (Food and Agriculture Organization of the United Nations, 2014). At the beginning of the study, the soil at 0–20 cm depth was characterised by a pH of 8.2, a soil organic matter of 1.8%, a total N of 1.2 g kg^{-1}, an available P of 8.6 mg kg^{-1} and a cation exchange capacity of 19.45 meq 100 g^{-1}. These measurements adhere to the guidelines of the Italian Ministry of Agriculture and Forestry (DM 13/09/99 GU 248), which serves as the official reference for soil chemical analyses in Italy. In the study area, winter cereals (mainly durum wheat) are normally placed in rotation with spring crops such as sunflower and maize, sometimes alternated with forage crops such as alfalfa (*Medicago sativa* L.) (Francioni et al., 2020).

2.2. Field management

The experimental field was obtained from a field subjected to sunflower–wheat–maize–alfalfa rotation for at least 7 years, therefore representative of the local cropping systems. In 2015, the whole field was cultivated with sunflowers, and after the sunflower harvesting (first half of September 2015), it was divided into two adjacent subfields (‘Field A’ and ‘B’). The detailed soil chemical properties of Fields A and B are reported in the supplementary file (Table S1).

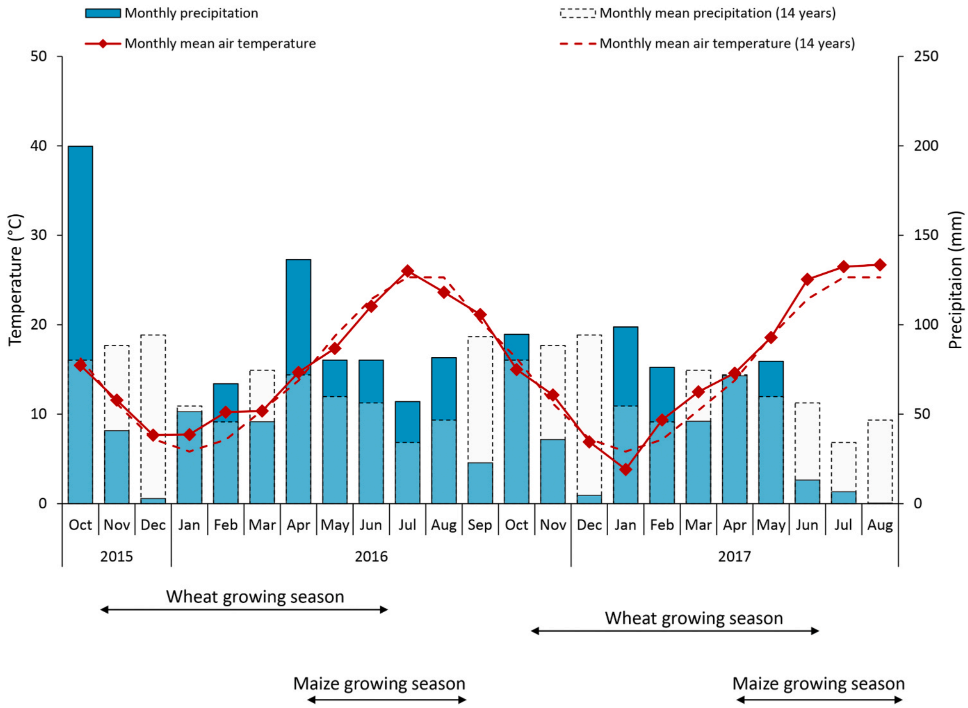

Fig. 1. Monthly precipitation and air mean temperature during the study period (November 2015–August 2017) and over the long-term (1998–2012) period for Agugliano (Ancona province, Italy). Source: Agency for Innovation in the Agri-Food Sector.

During the 2015 growing season, Field A was planted with durum wheat (*Achille* cultivar, Isea-Agroservice Co. spa, Macerata, Italy) in the first week of November, using 17 cm of row spacing with approximately 425 seeds per square metre. The harvest occurred in June. Field B was planted with maize (DKC4316 hybrid cultivar, Monsanto Agriculture Italia spa, Milano, Italy) in the third week of April on ridges, with a row-to-row spacing of 60 cm and a plant-to-plant spacing of 20 cm, with eight seeds per square metre. Harvesting occurred in October.

In the following year, the same crops were sown with the same seeding rate in the same periods but in inverted fields (i.e. maize in 'Field A' and durum wheat in 'Field B'). The monitoring period lasted for two cropping seasons (Fig. 2). The study was conducted under a reduced tillage regime, which involved chisel ploughing to a depth of 0.25 m and double harrowing for seedbed preparation before the sowing date. Nitrogen was applied at rates of 160 kg ha^{-1} for wheat and 184 kg ha^{-1} for maize using urea. Nitrogen was equally distributed in two splits: at

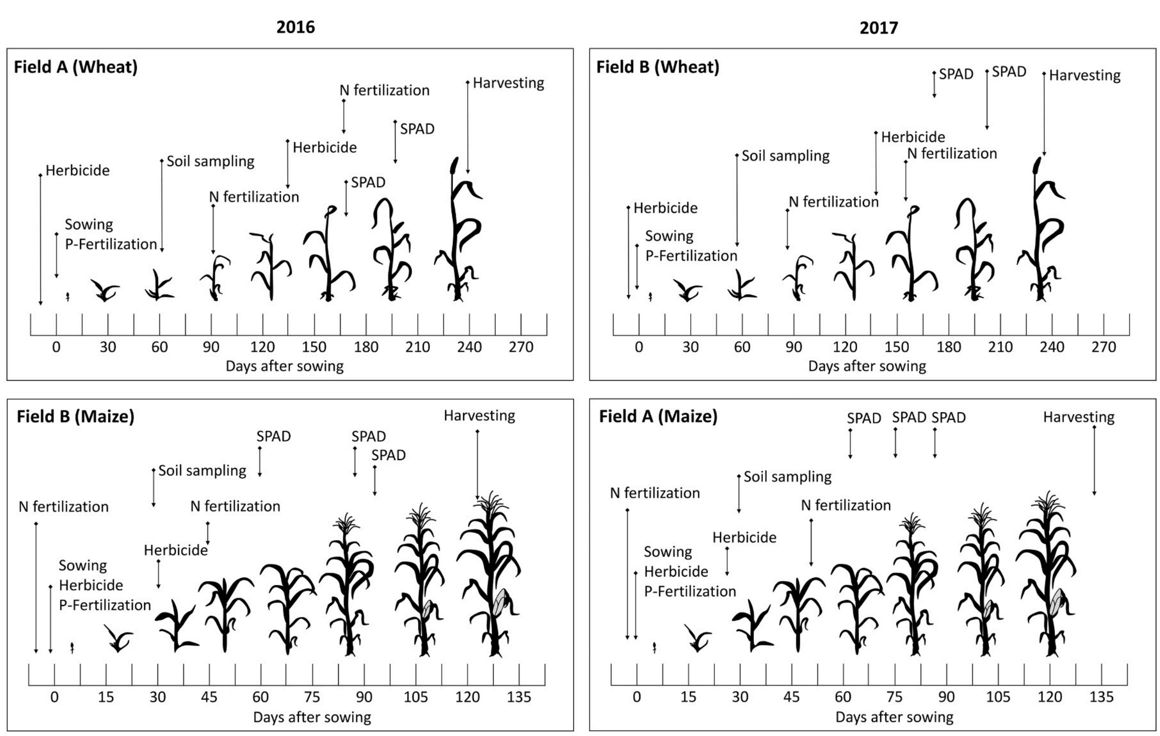

Fig. 2. Layout of the experimental field and the sequence of surveys and management operations during the 2-year study.

tillering and before booting in wheat and 1 week before sowing and at the pre-tasselling stage (47–51 days after sowing at the eight-leaf stage, i.e. the third week of May for both years) in maize. All TSP fertiliser rates were applied in marked-out bands during the seeding operation using a precision seeder (Direttissima 250, Gaspardo, Italy for wheat and Sigma 5, Sfoggia, Italy for maize) in both years. For HCP fertilisers, a fertiliser injector (Ecofert, Startec, Italy) was used. Rates and the source of P varied based on the applied treatments, as detailed in the Experimental design section, and were buried at a depth of 3 cm for wheat and 5 cm for maize. As HCP fertilisers contain a variable nitrogen component, the effects of the tested P-fertilisers were studied under conditions where the nutritional requirements of plants for nitrogen were fully met. Weed growth was controlled by a total herbicide spraying application of 5 L ha^{-1} of glyphosate before sowing. Additionally, 500 g ha^{-1} of mesosulfuron-metile + iodosulfuron-metile-sodium and 1.5 l ha^{-1} of florasulame + fluroxypyr were applied during stem elongation in wheat, and 1.5 l ha^{-1} of thiencarbazone-methyl and isoxaflutole were applied in the second week of May each year for maize. The study was conducted under rainfed conditions.

2.3. Experimental design

In each of the fields (Field A and Field B), a complete randomised block design with three replicates and individual plots measuring 20 m^2 (5 m × 4 m) was utilised to assess soil P availability and the agronomic responses of maize and wheat (i.e. plant architecture, dry matter accumulation, grain yield and yield components and PUE) when fertilised with two different P sources. The first P source was a fertiliser based on orthophosphate, organically complexed with leonhardite (total N 7%, ammoniacal N 6%, urea N 1%, water-soluble P_2O_5 21%, water-soluble Zn 0.2%, activator: humic extracts 7%), in liquid form (Vital Power $Phos^{7-21}$, Siriac, Italy). The second was a commercial TSP in granular form (water-soluble P_2O_5, 43%).

For wheat, fertilisation rates were i) control (CT, no P-fertilisation), ii) 4 kg ha^{-1} P applied as HCP (HCP-4), iii) 8 kg ha^{-1} P applied as HCP (HCP-8), iv) 4 kg ha^{-1} P applied as TSP (TSP-4) and v) 8 kg ha^{-1} P applied as TSP (TSP-8). For maize, the design included the following treatments: i) control (CT, no P-fertilisation); ii) 5 kg ha^{-1} of P applied as HCP (HCP-5); iii) 10 kg ha^{-1} of P applied as HCP (HCP-10); iv) 5 kg ha^{-1} of P applied as TSP (TSP-5); v) 10 kg ha^{-1} of P applied as TSP (TSP-10) and vi) 20 kg ha^{-1} of P applied as TSP (TSP-20).

To avoid cumulative effects of P-fertilisers, the plots were relocated within Fields A and B but in a different position, where the previous year maize or wheat had been cultivated as a control (i.e. without P-fertilisation).

2.4. Sampling and analysis

2.4.1. Soil P availability

From each plot, five soil cores were collected at 0–10 and 10–20 cm depth using a 5-cm diameter auger following a non-systematic 'W' pattern. The sampling was performed 1 or 2 months after the sowing of maize and wheat, respectively. Subsamples were air-dried, mixed as one composite sample for each plot and then gently sieved through a 2-mm sieve. The analysis of the available soil P was conducted using the method described by Olsen (1954). Briefly, 2.5 g of soil (2 mm sieved) was added with 50 mL of 0.5 M of $NaHCO_3$ (pH = 8.5) extracting solution, shaken for 30 min, and filtered through 0.5 g of charcoal. The filtrate (10 mL) was added with 1 mL of H_2SO_4 and 30 mL of the ammonium molybdate-antimony potassium tartrate-ascorbic acid (Murphy-Riley solution). It consists of an acidified solution of ammonium molybdate containing ascorbic acid and a small amount of antimony that reacts rapidly with phosphate ion yielding a blue-purple compound. Concentration of P was colorimetrically determined by measuring the absorbance of solution at 700 nm in a Cary 50 Scan (Varian) UV–vis spectrophotometer. Sample blanks were prepared to estimate absorbance interferences.

2.4.2. Leaf chlorophyll content

Chlorophyll content was determined in the field using a chlorophyll metre (SPAD-502, Minolta, Tokyo, Japan) on the leaves of 30 plants from each plot. For wheat, the SPAD measurements were conducted 169 and 196 days after sowing in 2016 and 174 and 195 days after sowing in 2017. For maize, the SPAD measurements were conducted 58, 89 and 98 days after sowing in 2016 and 67, 79 and 88 days after sowing in 2017 (Fig. 2).

2.4.3. Plant dry matter accumulation

Above-ground dry matter accumulation was assessed as follows: for wheat by cutting the plants at ground level in a sample area of 1 m^2 within each plot. This process was repeated twice, at the flowering stage and at the stage of physiological maturity. For maize, 6 consecutive plants were selected and cut at ground level twice, at the silking stage and at physiological maturity (Fig. 2). To determine dry matter, all plant parts were dried in an oven at 70 °C until a constant weight was achieved.

2.4.4. Grain yield and yield components

For wheat, an area of 1 m^2 was manually harvested to determine grain yield, yield components and grain protein content. Yield components (i.e. number of spikelets per spike, 1000-grain weight, number of grains per spike, test weight and Harvest Index) were also determined after threshing with a Wintersteiger LD 180 thresher. For dry weight determination, all plant parts were oven dried at 70 °C to a constant weight. Grain protein content was determined through an Infratec 1241 analysis at the Research and Experimental Centre for Crop Improvement (in Italian, Centro Ricerche e Sperimentazione per il Miglioramento Vegetale 'N. Strampelli', CERMIS, Tolentino, Italy). The total P content of grain was measured at the AMAP research laboratory (Jesi, Italy).

Maize grain yield (15% moisture) and components (i.e. ear length, number of rows and kernels per ear, 100-kernel weight, test weight and Harvest index) were determined by manually harvesting six consecutive plants in each plot at physiological maturity (123 and 135 days after sowing in 2016 and 2017, respectively). The 100-kernel weight was determined over 300 random kernels obtained from the same sampled plants.

The Harvest index was calculated as the grain-to-above-ground dry biomass ratio for both wheat and maize.

2.4.5. P uptake and use efficiency

Grain P content for both wheat and maize was determined with an inductively coupled plasma-optical emission spectroscope (Horiba Ultima) on ground grains. Grain P uptake was determined by multiplying the grain P content by the grain yield for various P treatments. PUE (%) was calculated using the following equation:

$$PUE = \frac{PF - P0}{PA} \times 100 \quad (1)$$

where PF is the P content in the grains of the fertilised treatments, $P0$ is the P content in the grains of the control treatment and PA is the annual P input as fertiliser. AE (kg grain yield increase per kg P applied) was calculated using the following equation:

$$AE = \frac{YF - Y0}{PA} \quad (2)$$

where YF is the grain yield of the fertilised treatments, $Y0$ is the grain yield of the control treatment and PA is the annual P input as fertiliser.

2.5. Statistical analysis

Statistical analysis was performed using SPSS Statistics, version 25.0

(SPSS Inc., IBM, Chicago, IL, USA). A paired T-test (matching criterion: block) was used to determine differences in soil P availability between soil depths within each P-treatment. A two-way repeated-measures analysis of variance (RMANOVA, GLM procedure) was used to determine the effects of year (within-factor), P-fertilisers (between-factor) and interactions between factors on soil P availability at 0–10 and 10–20 cm depth, chlorophyll content, dry matter accumulation, grain yield and yield components, P uptake, PUE, and AE. When the data did not meet normality and/or homoscedasticity assumptions, the box-cox transformation was applied. The data in the figures within the main text show the non-transformed means for each treatment differentiated by the Tukey HSD test. The complete RMANOVA tables are available in the supplementary file.

3. Results

3.1. Soil P availability

A significant interaction between the monitoring year and P-fertilisation emerged for both wheat and maize at depths of 0–10 cm and 10–20 cm (Table S2 and S3). In general, for both wheat and maize, higher values of soil available P were observed at a depth of 0–10 cm than those observed at 10–20 cm, although these differences did not always reach statistical significance (Fig. 3). The only exception was TSP-8, which in 2016 showed a higher content of available P at 10–20 cm than at 0–10 cm for wheat (Fig. 3A).

For wheat in 2016, the highest values of soil available P at 0–10 cm depth were observed for HCP-8, which, however, did not show significant differences compared to TSP-4 in 2017. Despite the fact that in 2016, the values of soil available P at 10–20 cm were in the same range as those observed at 0–10 cm depth, in 2017, HCP-8 at 0–10 cm was approximately four times higher than that at 10–20 cm depth. Furthermore, in 2017, the soil available P at 10–20 cm showed significantly higher values only for HCP-4 than for the control (Fig. 3A, B).

For maize, higher values of soil available P at 0–10 cm depth were observed in 2016 than those observed in 2017, regardless of dose and type of P-fertiliser. Notably, TSP-5 and TSP-10 showed similar values at 0–10 and 0–20 cm (not significantly different from the control) and, in any case, were always lower than HCP-5 and HCP-10; this generally emerges for both depths and for both years. In 2016, the soil available P at 0–10 cm for HCP-10 and TSP-20 showed the highest values, being almost 6.5 and 6.2 times higher than the control. The trend was also confirmed for 10–20 cm, where a higher soil available P was again observed for HCP-10 and TSP-20, which showed values almost double those of the control (Fig. 3C). In 2017, the soil available P at 0–10 cm was found to be highest for TSP-20, although this did not show significant differences with neither HCP-5 nor HCP-10. The trend was confirmed again for the 10–20 cm depth, where TSP-20 and HCP-10 showed higher values than TSP-5, TSP-10 and control (Fig. 3D).

Notably, in some cases (e.g. HCP-5, HCP-10 and TSP-20 for maize in 2016, Fig. 3C), the considerably higher values exhibited by certain treatments at 0–10 cm depth than at 10–20 cm depth do not reach significance due to the strong gradient controlled by the block in the experimental design (paired T-test).

3.2. Chlorophyll content

A significant interaction between the monitoring year and P-fertilization on the chlorophyll content of plants emerged for wheat at the flowering stage (Table S4) and for maize at the stem elongation and silking stages (Table S5).

In 2016, the chlorophyll content of wheat leaves was highest for both the HCP treatments compared to the TSP treatments and the control 169 days after sowing. No differences emerged 196 days after sowing. In 2017, the chlorophyll content of HCP-8 was found to be higher than that of the control only 195 days after wheat sowing (Table S6).

In 2016, the chlorophyll content of maize leaves was found to be significantly higher for HCP-10 than for the control 89 days after sowing, with differences maintained even 98 days after sowing. Conversely, in 2017, no significant differences emerged on any of the sampling days for any treatment (Table S6).

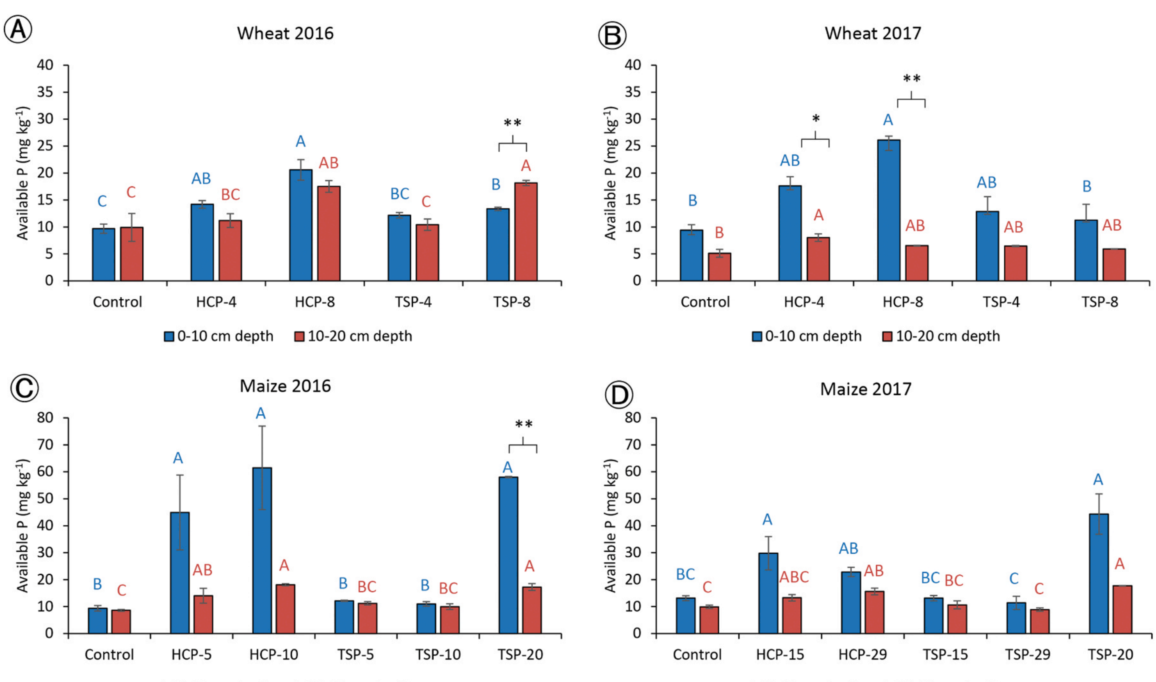

Fig. 3. Available soil phosphorus (P) at 0–10 and 10–20 cm soil depth for wheat and maize in the 2 years of monitoring. Different letters indicate significant differences at $p \leq 0.05$ (Tukey's test, within soil depth). Asterisks indicate significant differences at $p \leq 0.05$ (*) or ≤ 0.01 (**) based on paired T-tests between soil depths within P-treatment. Bars represent the standard error of the mean.

3.3. Plant dry matter accumulation

A significant interaction between the monitoring year and the P-fertilisation on plant dry matter accumulation emerged for wheat and for maize at the anthesis stage (Tables S7 and S8).

In 2016, the dry matter accumulation for wheat at the anthesis phase was highest for the two HCP treatments and for TSP-8 compared to TSP-4 and the control. However, at the maturation phase, only the two HCP treatments maintained a higher dry matter accumulation. In 2017, only HCP-8 showed higher dry matter accumulation than the control (+45%) but that emerged only during the anthesis phase (Table S9).

In 2016, for maize plants, TSP-20, HCP-10 and HCP-5 showed a higher dry matter accumulation than the control (+50%, +44% and +42%, respectively) at the silking stage. At physiological maturity, all fertilised treatments showed higher values than the control for all the parameters analysed, but these differences never reached significance ($p = 0.08$, Table S9). In 2017, at the plant silking stage, significant differences emerged for the two HCP treatments compared to the control (Table S9). At physiological maturity, HCP-5 showed a significant increase in dry matter accumulation compared to TSP-5, TSP-10 and the control (+23%, +25% and +40%, respectively) (Table S9).

3.4. Grain yield and yield components

A significant interaction between the monitoring year and P-fertilization on the number of grains per spike, spike density and number of spikelets per spike emerged for wheat (Table S10). For maize, significant interactions emerged for grain yield, number of kernels per spike and 100-grains weight (Table S11).

In 2016, only HCP-8 resulted in a higher wheat grain yield than the control (+14%), whereas protein content was highest for HCP-8 and TSP-8 (Table S12). The number of grains per spike was higher for HCP-4 than for TSP-4, but the number of spikes per square metre was higher for HCP-4, HCP-8 and TSP-8 than for TSP-4 and the control. Furthermore, the two HCP treatments had a higher number of spikelets per spike than the control. No differences emerged among the different treatments in terms of wheat Harvest Index in 2016. In 2017, both HCP treatments resulted in significantly higher wheat grain yields than the control (+16% and +14%, respectively), but only HCP-8 resulted in the highest protein content (i.e. +6% compared to the control). Both HCP-4 and HCP-8 resulted in a higher number of grains per spike than the control, but no difference emerged in terms of the number of spikes per square metre. A similar number of spikelets per spike emerged for all P-fertilised treatments. Both HCP treatments and TSP-8 resulted in a higher Harvest Index than the control in 2017 (Table S12).

In general, higher maize grain yields and related components were observed in 2016 than those observed in 2017 (Table S13). In 2016, the highest gain yield was observed for HCP-10 (+19% compared to the control), TSP-20 (+18% compared to the control) and HCP-5 (+15% compared to the control). The number of kernels per ear was found to be higher only for HCP-10 and TSP-20 than for the control. No significant differences emerged between treatments in terms of the number of rows per ear, test weight or Harvest Index. In 2017, higher maize grain yields were observed for HCP-5 and HCP-10 than for TSP-20, which recorded comparable values to the control. Both the ear length and the number of kernels per ear were found to be higher for HCP-5 than for the control. The highest Harvest Index values were observed for HCP-5 and for the control, whereas HCP-10 showed the lowest values (Table S13).

3.5. P uptake and use efficiency

No significant interaction between the monitoring year and P-fertilization emerged for wheat in terms of grain P content, P uptake, PUE, and AE (Table S14). For maize, a significant interaction emerged for P uptake (Table S15).

In 2016, no differences emerged among the different treatments in terms of P content in the wheat grains (Fig. 4A). However, both HCP treatments showed the highest P uptake (Fig. 5A), with HCP-4 resulting in the highest PUE and AE (Figs. 6A and 7A). Similar to 2016, in 2017, no differences emerged among the different treatments in terms of wheat grain P content (Fig. 4B). A higher P uptake and PUE were observed for HCP-8 than those observed for the two TSP treatments and the control in 2017 (Figs. 5B and 6B). Additionally, in the same year, HCP-4 exhibited greater AE than TSP-8 (Fig. 7B).

In 2016, no differences emerged among the different treatments in terms of P content in the maize grains (Fig. 4C). Nevertheless, significant differences were evident in terms of P uptake, with HCP-5 and HCP-10 showing a +17% and +19% increase in P uptake, respectively, compared to the control (Fig. 5C). A higher PUE was observed for HCP-5 than for TSP-10, whereas the other treatments exhibited intermediate values (Fig. 6C). The highest AE was again observed for HCP-5, whereas the lowest was observed for all the TSP treatments (Fig. 7C). Similar to 2016, in 2017, no differences emerged among treatments for the maize grain P content (Fig. 4D). The P uptake in 2017 was higher for HCP-10 plots than for the control, with the other treatments showing intermediate values (Fig. 5D). However, both the PUE and AE were found to be not different among the treatments (Figs. 6D and 7D).

4. Discussion

4.1. Soil P availability

The general lower values of P availability, particularly those observed at a depth of 0–10 cm during the second year of the experiment, may be attributed to a single heavy rainfall event that occurred after the fertiliser distribution in the third week of April 2017 (Figure S1). This likely prevented the gradual and proper dissolution of TSP granules, resulting in the loss of fertiliser due to water runoff (Torrent et al., 2007; Wang et al., 2013). Similarly, the HCP, which is already available in liquid form, might have been subjected to water runoff before it penetrated the first 0–10 cm of soil.

Interestingly, the available P at 10–20 cm observed for wheat in 2016 was generally higher than the one observed for wheat in 2017 (Fig. 3A, B). One reason that might explain this trend is the high precipitation registered in October 2015 (i.e. before the seeding), which affected the soil capability and improved the mobility of P along the 10–20 cm depth layer. Another possible reason is the fact that the soil typology of the area under study is characterised by the presence of a concentration of gypsum in layers of depth close to 20 cm, which may, according to Pauletti et al. (2014), have promoted the increased availability of P in these layers. At the same time, Vertic Cambisols (Inceptisols), such as those in the study area, traditionally contain iron oxyhydroxide nodules, which exhibit a strong affinity for P, reducing its capacity to be displaced within the soil solution and thus limiting its availability to plants, owing to the variability in their binding energy (Fink et al., 2016). A third possibility could be a soil characteristic heterogeneity between Fields A and B, but given their contiguity and the characteristics observed before the trial installation (Table S1), this hypothesis is considered the least likely.

Overall, the general scarce availability of P observed at 10–20 cm depth could be attributed to the scarce mobility of P in alkaline calcareous soils (Hopkins and Ellsworth, 2005). Indeed, in soils rich in carbonates ($CaCO_3$), P solubility may be controlled by the formation of the solid-phase dicalcium phosphate or by the chemisorption of P on calcite, with the formation of a surface complex of calcium carbonate-P with a well-defined chemical composition (Samadi and Gilkes, 1999).

A further explanation for the low presence of available P at 10–20 cm depth could be that P sorption is highly correlated with the clay content and with amorphous Fe and Al oxides content (Börling et al., 2001). However, comparing by P dose, HCP formulation has allowed a higher

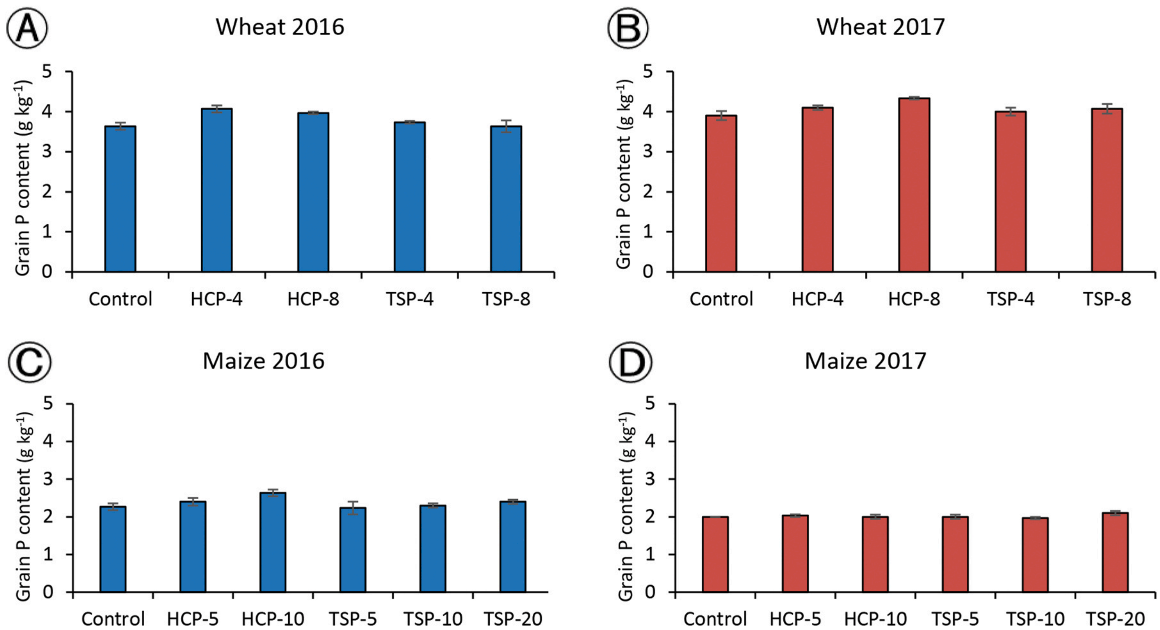

Fig. 4. Wheat and maize grain phosphorous (P) content in the 2 years of monitoring. Different letters indicate differences at $p \leq 0.05$ (Tukey's test, within crop). Bars represent the standard error of the mean.

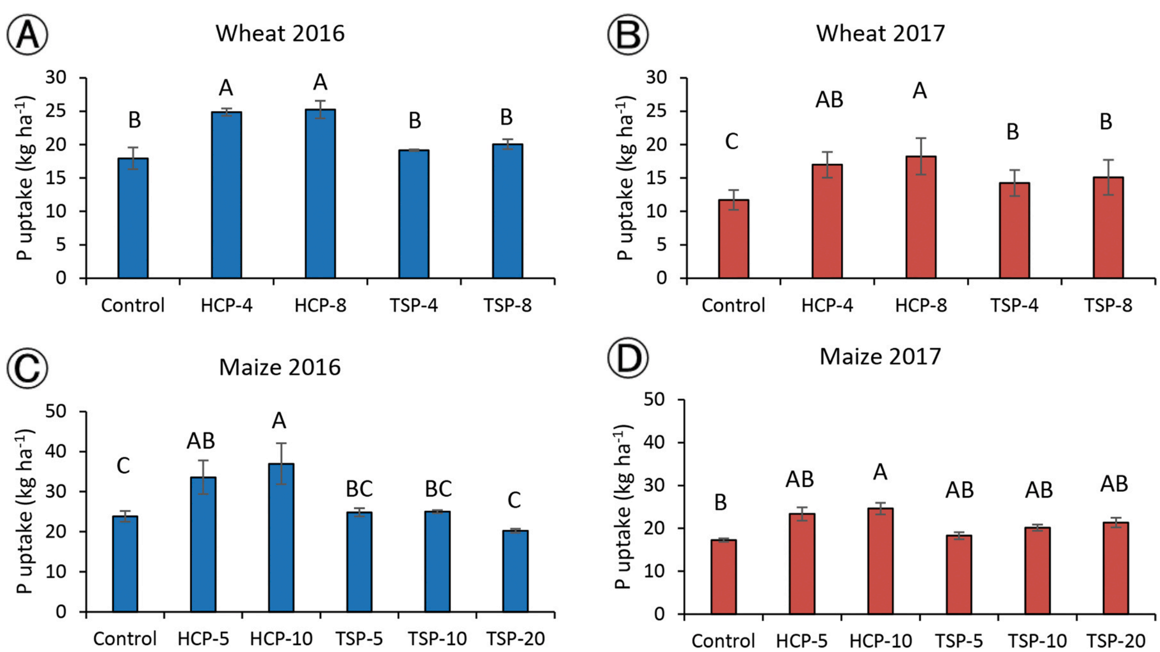

Fig. 5. Wheat and maize grain phosphorous (P) uptake in the 2 years of monitoring. Different letters indicate differences at $p \leq 0.05$ (Tukey's test, within crop). Bars represent the standard error of the mean.

mobility of P at 10–20 cm than TSP formulation.

The different availability of P observed for the two P formulations, especially at 10–20 cm, can be likely attributed to the influence of the organic binding agents that allow for a higher bioavailability of P in poor organic matter and calcareous agricultural soils when fertiliser is applied in the markout band as a pre-plant starter (Hopkins and Ellsworth, 2005; Zhen-Yu et al., 2013).

Other studies confirmed the highest efficiency of HCP fertiliser applied under different pedo-climatic conditions. For example, higher soil available P was reported by Herrera et al. (2016) for sandy clay loam Typic Hapludox under temperate and no-dry season conditions. Higher available soil P was also observed by Erro et al. (2012) for both acidic and alkaline soils under the recommendation to select appropriate humic substances to be used in HCP production based on soil characteristics. A higher soil P availability of HCP fertilisers than of traditional P-fertilisers was also reported in a recent study conducted on wheat-cultivated calcareous fluvo-aquic soils (Gao et al., 2023).

In the present study, the greater soil P availability observed for HCP might be attributed to the ability of these complexed fertilisers to inhibit phosphate fixation and retrogradation in soil (Erro et al., 2012; Urrutia et al., 2013). Indeed, due to the high number of functional groups on their surface (i.e. carboxyl, phenolic hydroxyl, alcoholic hydroxyl, ketone and quinoid), humic acids can enhance the availability of P by complexing ions into stable compounds. The presence of humic acids

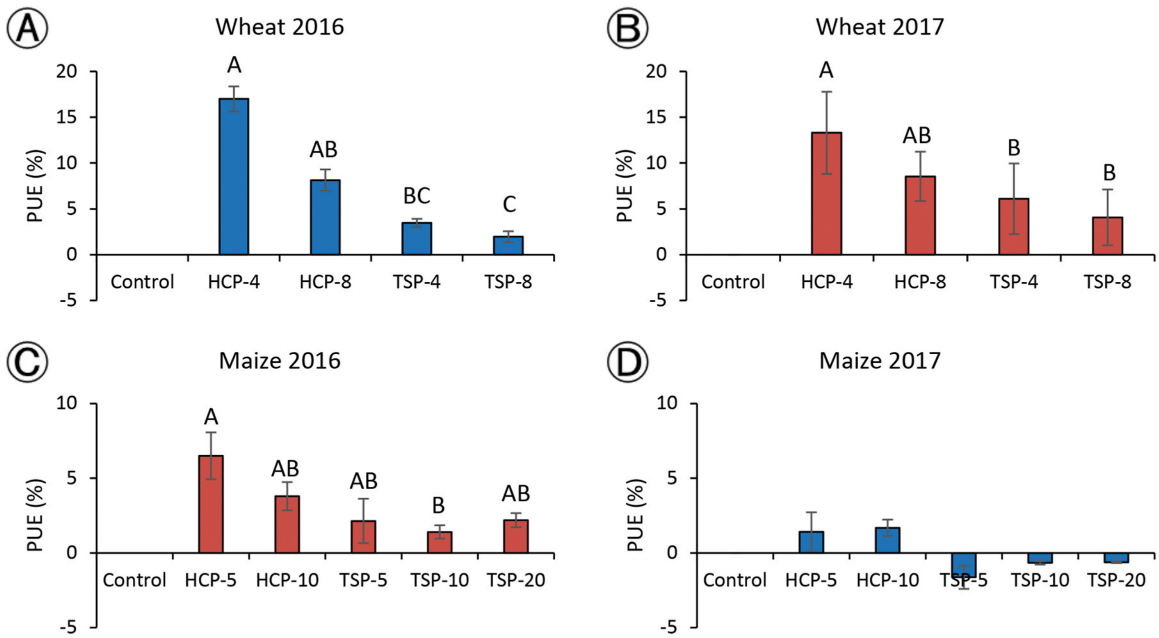

Fig. 6. Wheat and maize phosphorous use efficiency (PUE) in the 2 years of monitoring. Different letters indicate differences at $p \leq 0.05$ (Tukey's test, within crop). Bars represent the standard error of the mean.

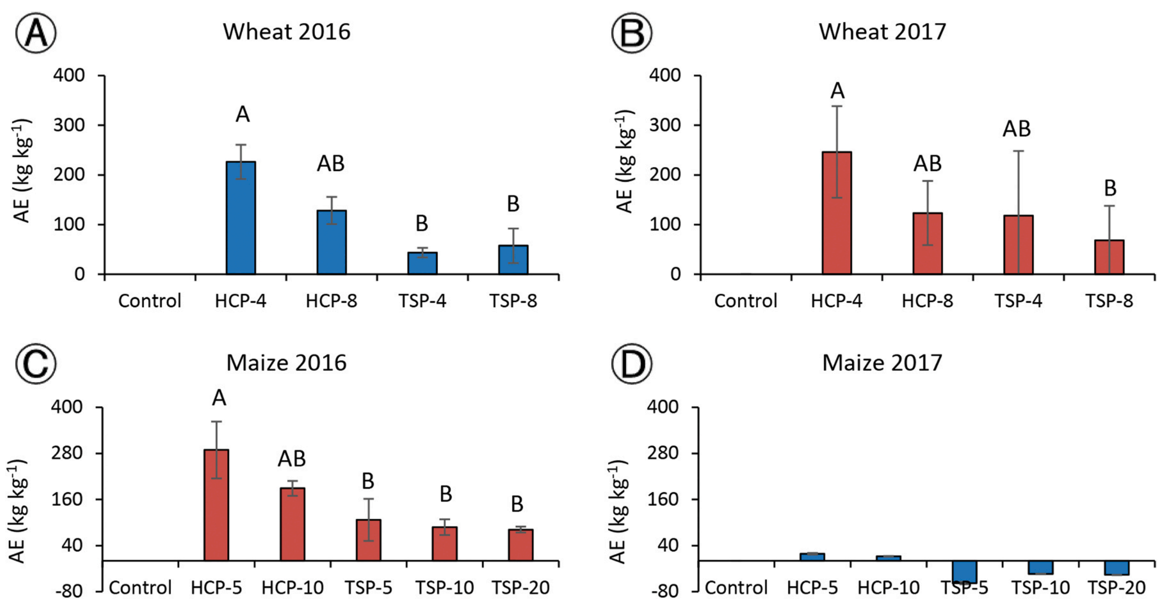

Fig. 7. Wheat and maize agronomic efficiency (AE) in the 2 years of monitoring. Different letters indicate differences at $p \leq 0.05$ (Tukey's test, within crop). Bars represent the standard error of the mean.

can keep P-ions in an exchangeable state, preventing the combination of P with soil metal elements (Yang et al., 2021), a desirable condition in alkaline and calcareous soils characterised by the scarce mobility of P.

4.2. Leaf chlorophyll content, dry matter accumulation and grain yields

It is well known that P-fertilisation is crucial for crop growth because the presence of both N and P in the leaf is essential for photosynthesis and plant development (Bindraban et al., 2020). Despite the limited number of field studies on HCP fertilisers (Herrera et al., 2016), recent studies conducted in calcareous soils have reported a significant interactive effect between P and humic acid, leading to an overall improvement of the soil biochemical environment. Such improvement was attributed to improved soil enzymatic activities, microbial activities, population, cation exchange capacity and water retention of soil that ultimately enhance plant growth and nutrient uptake (Baigorri et al., 2013; Izhar Shafi et al., 2020; Urrutia et al., 2013). The same hypothesis could also be applied to the present study, although the effects were less pronounced in the second year of monitoring. During this period, an unfavourable precipitation pattern (as shown in Fig. 1 and S1) and a generally reduced soil P availability (as shown in Fig. 3) might have contributed to the lower chlorophyll content observed in all treatments (Plénet et al., 2000; Zhu et al., 2012). Recent studies conducted in alkaline soils have indicated that the combination of P deficiency and drought stress can lead to a significant reduction in maize chlorophyll content. However, these studies have also shown that the combination of humic acids with S-enriched leonhardite may alleviate this negative effect (Kaya et al., 2020; Plénet et al., 2000; Zhu et al., 2012).

Studies have shown that P-fertilisation and the subsequent improvement in soil P availability have a positive impact on dry matter accumulation in various crops, including sugarcane (Zavaschi et al., 2020), spring wheat (Zhu et al., 2012) and maize (Corrêa et al., 2005). Andrade et al. (2007) reported the positive effect of applying humic acids before P-fertilisation on dry matter accumulation in both shoots and roots of maize. Consistent with the findings of the present study, the highest dry matter accumulation was associated with increases in soil available P, primarily observed in maize at a depth of 0–10 cm for HCP-5, HCP-10 and TSP-20 (Fig. 3). However, no clear relationship emerged between the various P-fertilisation treatments and the dry matter accumulations of wheat or maize. These accumulations also exhibited variations within each crop during different growth phases (e.g. anthesis *versus* maturation) and between the monitoring years. Indeed, in 2016, the effect of HCP fertilisers was evident only at the anthesis and silking stages for wheat and maize, respectively (Table S9). Conversely, the unfavourable rainfall pattern that occurred in 2017 (Fig. 1) resulted in the highest maize plant dry matter accumulation for HCP-5 (Table S9). This finding partially supports what was reported by Kaya et al. (2020), suggesting that a combination of water stress and soil P deficiency may enhance HCP efficiency, leading to higher maize yields and related traits.

It has been estimated that, with adequate N and K fertilisation, P can moderately increase crop yield by 10%–20% across different crops and soil types (Bindraban et al., 2020). Since in the present study N fertilisation was provided in sufficient quantity, the low grain yields observed, especially for maize in the second year, can be mainly attributed to the unfavourable rainfall pattern during crucial plant stages (Tables S13, Fig. 1). It is known that nitrogen taken up before flowering and nitrogen supplied during grain filling are fundamental to increase the protein content of wheat (Ravier et al., 2017).

The results obtained in this study indicate that wheat protein content can be significantly enhanced by HCP (Table S12). This could be explained by the fact that HCP may increase nitrogen uptake (Takahashi and Anwar, 2007), as it enables plants to develop an extensive root system, thereby enhancing their capacity to absorb more nitrogen (Dordas and Sioulas, 2008). However, it has also been suggested that HCP fertilisation has positive effects on wheat only when the humic acid component falls within a specific range, such as the 1%–2% range recommended by Gao et al. (2023). This highlights the need for further research to determine the optimal proportion of humic acids in the production of HCP fertilisers to optimise crop yields.

The maize grain yields and components in 2016 and 2017 showed contrasting results, irrespective of the source of the applied P-fertilisers (Table S13). Similar to wheat, these variations can be attributed to water scarcity coinciding with the most vulnerable stages of maize growth in 2017, particularly during flowering and silking, which occurred in the first decade of July. While climate change features such as water shortages and increased soil temperatures are expected to lower maize yields in the Mediterranean Basin (Mereu et al., 2021), some long-term studies have shown that in areas where rainfall is expected to slightly increase, shifts in the rainfall pattern can lead to water deficits that may affect yield components (Mastachi-Loza et al., 2016). It is well known that water stress can impact the growth, development and physiological processes of maize, resulting in a significant reduction in dry matter production and yield components (Di Paolo and Rinaldi, 2008; García-Lara and Serna-Saldivar, 2019; Payero et al., 2008).

In the present study, despite the unfavourable weather conditions observed in 2017, the application of HCP-5 yielded results substantially comparable to those of HCP-10. Surprisingly, the highest rate of P provided in TSP-20 plots resulted in some of the lowest grain yields (Table S13). This discrepancy could be primarily attributed to P loss due to water runoff, which may have been higher for TSP fertilisation provided in granule form. This phenomenon likely occurred due to a high precipitation event registered in 2017 following a prolonged drought period during the summer (Francaviglia et al., 2018; Wang et al., 2013). These findings suggest that in cases of asynchrony between P-fertilisation and precipitation, conditions for significant P surface erosion may arise, especially in hilly areas such as those of Central Italy (Francioni et al., 2020; Toderi et al., 2021). This underscores the importance of developing climate change adaptative tools for management options for farmers (Mereu et al., 2021).

4.3. P uptake and use efficiency

Despite none of the P-fertilisation resulting in significant wheat grain P content in any of the monitoring years (Fig. 4), HCP-8 resulted in the highest P uptake for wheat in both years of monitoring, with comparable results for HCP-10 for maize in 2016 (Fig. 5A, C). Similar results were observed in 2017, but significant differences emerged only for wheat (Fig. 5B, D). This result is in line with previous studies that reported higher P uptake by wheat with HCP fertilisers than with conventional P-fertilisers (e.g. Gao et al., 2023).

It has been suggested that P remobilisation in post-anthesis wheat is enhanced in plants with a low P status, whereas it is significantly reduced in plants with a high P status (Mazlouzi et al., 2020). The similar grain P content observed for all the P-fertilisers for both wheat and maize (Fig. 4) suggests that plants did not remobilise the P from other organs to the grains. However, the highest wheat and maize yields (observed for HCP-8 and for HCP-10, respectively, Tables S12 and S13) could be partially attributed to the higher efficiency of plant photosynthetic tissue enhanced by the presence of P (Rychter and Rao, 2016; Zhang et al., 2018).

Phosphorous uptake in maize was generally far higher in 2017 than in 2016 (Fig. 5C, D), despite the highest P availability at 0–10 cm depth observed in 2016 for HCP fertilisers (Fig. 3C, D). This could be attributed to the weather conditions of 2017, particularly the unfavourable rainfall during critical phenological stages of maize, which led to increased P uptake to compensate for or enhance photosynthetic activity. Additionally, it is likely that the maize roots developed more extensively in 2017 (a significantly drier year than in 2016), resulting in a well-developed root system capable of exploring considerably larger soil volumes (Bindraban et al., 2020).

Interestingly, the highest PUE and AE for both wheat and maize were observed for the lowest dose of HCP fertilisers (Figs. 6 and 7). Other studies have observed similar results, showing higher AE of humic complex fertilisers than of single superphosphates for different crops in different acid or alkaline soils (Erro et al., 2012; Herrera et al., 2016; Zavaschi et al., 2020).

In addition to HCP fertilisation, new combined strategies to enhance crop P uptake and efficiency might be represented by favouring crop mycorrhisation. Indeed, recent studies have reported several beneficial effects of mycorrhisation, including root volume extension, ease of P translocations and promotion of P solubilisation by root excretion (Bindraban et al., 2020; Klamer et al., 2019; Schachtman et al., 1998). Some other studies have suggested that P-metal-humic acid complexes can also stimulate the plant root system, allowing the plant itself to adjust P utilisation efficiency (Yang et al., 2021).

5. Conclusion

This study analysed the responses of wheat and maize to different P-fertilisers, specifically liquid humic-complexed phosphate and granular triple superphosphate, under reduced tillage and Mediterranean conditions in two contrasting years with varying rainfall patterns.

Humic-complexed P-fertilisation led to higher soil P availability, particularly at a depth of 0–10 cm, with maize showing significantly higher levels than wheat. In the case of wheat, humic-complexed P-fertilisation applied at a rate of 8 kg ha^{-1} of P resulted in a higher grain yield and protein content, increased P uptake and improved use and AE compared to the control (no P-fertilisation) in both years of monitoring. The highest PUE was recorded for wheat fertilised with humic-complexed P-fertilisation applied at a rate of 4 kg ha^{-1} in both years.

For maize, the application of humic-complexed P-fertilisation at rates of 5 or 10 kg ha^{-1} of P had a similar effect in terms of soil P availability, dry matter accumulation, grain yields, PUE and AE in 2016, a year characterised by favourable rainfall patterns. In contrast, in 2017, a year marked by a deficiency in summer precipitation, applying humic-complexed P-fertilisation at a rate of 10 kg ha^{-1} of P resulted in higher P uptake than the control; however, it did not lead to a significant increase in yield compared to the control. In the same year, low PUE and AE were observed, although they remained positive, unlike the case of granular triple superphosphate fertilisations.

The use of a liquid humic-complexed phosphate as a source of P in the context of conservation agriculture shows promise as an adaptive solution to address the scarcity of P-fertiliser, particularly in the face of food security challenges and global geopolitical crises. Further studies are required to confirm the results in different pedo-climatic contexts and cropping systems.

CRediT authorship contribution statement

Matteo Francioni: Formal analysis, Writing – original draft, Visualisation. **Matteo Palmieri**: Investigation, Writing – original draft. **Marco Fiorentini**: Writing – review & editing. **Paola Antonia Deligios**: Writing – review & editing. **Elga Monaci**: Methodology, resources, Writing – review & editing, Supervision. **Costantino Vischetti**: Resources, Writing – review & editing, Supervision. **Überson Boaretto Rossa**: Writing – review & editing. **Laura Trozzo**: Writing – review & editing. **Marco Bianchini**: Writing – review & editing. **Chiara Rivosecchi**: Writing – review & editing. **Luigi Ledda**: Writing – review & editing. **Roberto Orsini**: Writing – review & editing. **Rodolfo Santilocchi**: Conceptualisation, Resources, Project administration. **Paride D'Ottavio**: Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.eja.2024.127143.

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