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Maize response to nitrogen and phosphorus starter fertilisation in mineral-fertilised or manured systems

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ABSTRACT

Phosphorus (P) is an essential nutrient for maize production, but in temperate areas the P uptake during early growing stages can be limited due to low soil temperature, even though the soil was tested high in P. The objective of this study was to assess the effects of nitrogen and phosphorous (NP) starter fertilisation during early growth stages and its carryover until maize harvest, in mineral-fertilised or manured systems. A field experiment was carried out in north-west Italy during the 2019 and 2020 growing seasons. The trial compared sub-surface placement of NP (diammonium phosphate) or N alone (ammonium nitrate) in bands close to the maize seed furrows, in differing long-term (LT) fertilisation managements: two doses of urea (Min-L and Min-H), two doses of bovine slurry (Slu-L and Slu-H) or two doses of farmyard manure (Fym-L and Fym-H). The two rates, low (L) and high (H), corresponded to 170 and 250 kg N ha⁻¹ year⁻¹ respectively. Compared to N fertilisation, NP starter fertilisation improved early maize growth assessed by leaf area index (LAI) and shoot dry weight (SDW) in all systems. The effects differed between the two years (2019: LAI + 63%, SDW + 67%; 2020: LAI + 36%, SDW + 38%), as 2019 was cool during the first growth. Higher LAI and SDW values were confirmed at crop flowering in the mineral-fertilised systems only. As shoot growth was enhanced by NP starter fertilisation, anthesis occurred 1 day earlier in all systems. However, a response to NP starter fertilisation at harvest was recorded in mineral-fertilised systems only (+1.3 and +3.2 t ha⁻¹ in Min-L and Min-H, respectively). The uptake of P, used as a true indicator of soil nutrient availability, increased with increasing soil Olsen P until 39 mg kg⁻¹. These results suggest that soil test thresholds should be revised for points above which P fertilisation should be suspended.

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Abbreviations: Min-L, mineral N at low dose treatment (170 kg N ha⁻¹); Min-H, mineral N at high dose treatment (250 kg N ha⁻¹); Slu-L, bovine slurry at low dose treatment (170 kg N ha⁻¹); Slu-H, bovine slurry at high dose treatment (250 kg N ha⁻¹); Fym-L, farmyard manure at low dose treatment (170 kg N ha⁻¹); Fym-H, farmyard manure at high dose treatment (250 kg N ha⁻¹); NP, banded diammonium phosphate (DAP) treatment; N, banded ammonium nitrate treatment; LT, long-term; GS, growth stage according to BBCH scale; GDD, growing-degree day; DAS, days after sowing; LAI, leaf area index; SDW, shoot dry weight; KSM, number of kernels per square meter; TKW, thousand kernels weight; GPC, grain protein content; FB, fumonisin concentration; AUCDC, Area Under Canopy Development Curve; acP, acid phosphomonoesterase; alkP, alkaline phosphomonoesterase; bisP, phosphodiesterase; piroP, pyrophosphodiesterase; inositolP, inositol-P phosphatase; nona, nonanoate esterase.

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1. Introduction

Phosphorus (P) is an essential nutrient required for crop growth and production; therefore, it plays a fundamental role in soil fertility and world food security [1,2]. Soils contain a large amount of P relative to plant requirements, but most forms of P in the soil have a very low solubility and low availability for plant uptake [3,4]. For these reasons, mineral P fertilisers containing highly-soluble P are traditionally applied to maintain an optimal soil P status in intensively-managed agricultural systems [5]. However, phosphate rocks which are the major current source of P for fertilisers production are non-renewable, which puts the future P fertiliser supply at risk [2].

Globally, maize (*Zea mays* L.) is the most produced cereal as it is used for animal feed, human consumption and industrial or energy uses. It is the main arable crop cultivated in the Po Plain (Northern

Italy), where it is intensively cropped and used for livestock farming [6,7]. Maize is also one of the most sensitive crops to P supply [8]. Indeed, limited P supply usually impairs maize growth, as it delays phyllochron, reduces leaf elongation and final leaf size, and limits aboveground biomass [9,10]. Grant et al. [11] also reported that an adequate P supply is important for early crop establishment and consequently for the final maize yield. In temperate areas, the P uptake of young maize plants can be limited as a consequence of cold stress, even though soils test high in P using standard extraction methods (e.g., Olsen). Reduced plant P utilisation in these circumstances could be due to different factors, such as little P soil mobility, decreased rates of plant P uptake, and limited root system development [12,13]. Low temperatures can also inhibit microbial activity, that plays a key role when P is supplied as organic sources, as they require a biological process to make nutrients available for the plant (e.g., extracellular enzymes release by soil microorganisms and plant roots for P solubilisation) [14].

It is widely acknowledged that P fertilisation is an issue in European agriculture and politics. To date, there is no common regulation on the application of P fertilisers at the European level [15], even though P excess has been recognised in several regions, associated sometimes - but not always - with high livestock density [16,17]. In livestock farming systems, most farmers pay little attention to the P fertiliser value of manures [18]. Indeed, in many European countries, farmers commonly supply P through organic sources in excess of crop requirements, opting instead to manage the nutrient management plan on N fertiliser and/or on the restriction by the Nitrates Directive (91/676/EEC), without considering the disequilibrium between the N/P ratio in organic fertilisers and in crops [19]. This surplus leads to P accumulation in soils and increased risk of P losses to water [20,21]. Aside from the environmental problem, maize growers are keen to avoid early-stage P deficiency to minimize the risk of crop yield penalties, so they routinely apply a dose of mineral P combined with nitrogen (N) near the seeds at sowing even in manured soils, as a starter fertilisation [22]. The combination of N and P was proved as an effective stimulator for both lateral and fine root proliferation in several studies [23,24]. Additionally, plant uptake of ammonium N lowers rhizosphere pH through promoting proton release, and consequently can locally increase P availability of sparingly available P, in particular in calcareous soils [23,25]. In the context of intensive agriculture, it is crucially important to balance the opposing needs of reducing the potential risks of low yields while protecting the environment. Any decision on fertilisation needs to be done while considering soil type, soil P status, and weather conditions.

To this end, this work evaluated the effect of nitrogen and phosphorous starter fertilisation at sowing, in six different fertilisation managements of continuous maize for grain cropping systems. The six systems included long-term (LT) fertilisation with mineral fertilisers or animal manures (i.e., bovine slurry and farmyard manure) that was started in 1992 and resulted in different initial soil P availabilities, as estimated by a standard soil test. The NP starter fertilisation at sowing was compared with a N only addition. Measurements were focused on the early growth phase, but extended until harvest to assess yield quantity and quality. We also tested the extent to which the LT fertilisation of a system influenced the benefits of starter fertilisation on the crop.

2. Materials and methods

2.1. Long-term fertilisation at Tetto Frati

Measurements were carried out in 2019 and 2020 growing seasons on selected plots of the LT experiment of Tetto Frati (44°53'N;

7°41'E; 232 m above sea level) of the University of Turin, north-west Italy.

The LTE, established in 1992, was described by Grignani et al. [26] and Zavattaro et al. [6,27], among others. The LTE, a complete randomised block with three replicates on 75 m² plots, compared four cropping systems based on maize, at five N application levels. Here we analyse only data regarding the continuous maize for grain system at two doses of mineral fertiliser as urea (Min-L and Min-H), two doses of bovine slurry (Slu-L and Slu-H), and two doses of farmyard manure (Fym-L and Fym-H) as sources of N, as LT fertilisation. Each fertiliser was supplied at two rates, low (L) and high (H), corresponding to 170 and 250 kg N ha⁻¹ year⁻¹ respectively, since 2011. The Min and Slu systems also received a supplement of 149 kg K ha⁻¹ as KCl. The plots had also received different amounts of mineral P fertilisers along the years, thus leading to marked differences in the soil P content, as detailed hereafter. The soil background P availability was used to study the interaction of starter P fertilisation with three different LT strategies.

The soil texture is loam and the soil is classified as Typic Udifluent [28]. The ploughed layer (0–30 cm) contains 48.2%, 44.3% and 7.5% of sand, silt and clay, respectively, and has a sub-alkaline soil pH 8.1 (measured in water at 1:2.5 w/v). The Cation Exchange Capacity is also low (10.1 cmol kg⁻¹), and so is the exchangeable K (14.4 mg kg⁻¹).

Soil Olsen P, soil organic carbon (SOC) and total N (N_{tot}) concentrations were measured at each treatment in March 2019 before the start of the experiment here described and are reported in Table S1. The highest soil Olsen P concentration was recorded in Fym-H (91 mg kg⁻¹), followed by Fym-L (52 mg kg⁻¹). On the contrary, Min treatments did not show differences between fertiliser rates and had the lowest values, while both Slu-L and Slu-H were intermediate (about 29 mg kg⁻¹ of Olsen P). As expected, the SOC concentration was highest in the Fym-H treatment (1.13%), and the lowest in either Min-H, Min-L (0.72%) and Slu-L (0.81%). Similarly, the highest N_{tot} concentration was found in the Fym-H treatment (1.33%), and the lowest in either Min-H or Min-L (0.86% and 0.85%). The C/N was about 8.0–8.8 and did not differ between LT fertilisation strategies.

The climate at the site is temperate sub-continental, with by two main rainy periods in spring and autumn. Daily temperature and precipitation were measured at a meteorological station located in the experimental platform. The accumulated growing degree days (GDDs) for maize were calculated considering 10 °C as the minimum base temperature and 30 °C as the maximum temperature threshold.

2.2. Experimental fertilisation and agronomic management

The six LT fertilisation plots (Min-L, Min-H, Slu-L, Slu-H, Fym-L and Fym-H) were split into two hemi-plots (30 m², corresponding to four maize rows) to set two different management options for P fertilisation. At maize sowing, one hemi-plot received the banded application of 27 kg ha⁻¹ of N and 30 kg ha⁻¹ of P as diammonium phosphate (DAP, 18% N and 20% P; hereafter indicated as NP), while the second hemi-plot received 27 kg N ha⁻¹ as ammonium nitrate (34% N; hereafter indicated as N). DAP and ammonium nitrate were deposited 5 cm apart from the seed furrows and at a depth of 10 cm, using a calibrated granular dispenser applied to the planter (Monosem NG, Largeasse, France). No further mineral P fertiliser was distributed in the two experimental years.

The crop was managed equally in all plots. Soil was hoed in autumn and maize residue (stalks, cobs and bracts) were incorporated. All fertilisers, both mineral and organic, were surface supplied in spring and immediately incorporated with disk harrowing. The chemical properties of the bovine slurry and

farmyard manure used for the field experiment are reported in Table S2. Slurry supplied 8 and 12 kg P ha⁻¹ in 2019, or 30 and 44 kg P ha⁻¹ in 2020, in Slu-L and Slu-H treatments, respectively, while farmyard manure supplied 21 and 32 kg P ha⁻¹ in 2019, or 28 and 41 kg P ha⁻¹ in 2020, in Low and High rate treatments, respectively.

The mechanical maize seeding was carried out on 1st April 2019 and 3rd April 2020, using the Corteva Agriscience P1547 hybrid (FAO maturity class 600, 130 days relative to maturity). The distance between the plants and the plant rows were 0.16 and 0.75 m, providing a crop density of 8.3 plant m⁻². Plots were weeded in pre- and post-emergence. Sprinkler irrigation supplied about 40 mm per year.

2.3. Crop development and nutrient uptakes

In order to assess early crop development, different measurements were made on the two central rows of each hemi-plot, over a length of 5 m. Some measurements were planned based on days after sowing (DAS) as the time scale, while others were expressed as related to the plant growth stage (GS), according to the BBCH scale.

A hand-held optical sensing device, GreenSeekerTM (Trimble, Sunnyvale, CA, USA), was used to measure the Normalized Difference Vegetation Index (NDVI) during vegetative stages. The measurement, made holding the instrument about 60 cm above the plant canopy, was performed approximately every 7 days starting from the two-leaf stage (GS12) until tassel emission (GS53). The Area Under Canopy Development Curve (AUCDC) was calculated for each treatment using NDVI measurements at each observation date, following the formula (1) proposed by Capo et al. [29]:

$$\text{AUCDC} = \sum_i^{n-1} \{[(R_i + R_{i+1})/2](t_{i+1} - t_i)\} \quad (1)$$

where R is the NDVI value, t is the time of observation and n is the number of observations.

The maize plant height was monitored during the vegetative stages by measuring five randomly chosen plants from the ground level up to the collar of tallest fully developed leaf (GS13–GS19) or up to the tallest detectable node (GS30–GS53). Plant height was measured five times from 45 to 74 DAS. Plant height was linearly interpolated with time expressed as DAS to derive the growth rate expressed as cm d⁻¹ for each measurement interval, separately at each treatment.

Three plants per plot were sampled at two stages, 50 DAS and flowering, to determine shoot dry weight (SDW), leaf area index (LAI) and tissue P and N concentrations. LAI was measured using a planimeter (Delta-T Devices Ltd., Cambridge, UK). The above-ground plant biomass total P content was quantified after mineralisation in a muffle furnace at 450 °C for 5 h with spectroscopy under continuous-flow conditions (Evolution II, Alliance), while total N was assessed with a CN elemental analyser (Flash EA 1112, Thermoquest, [30]), using atropine (C₁₇H₂₃NO₃, Merk Analytical) as the analytical standard and ERM-BC381 rye flour as the reference material.

The plant flowering date, expressed as DAS, was determined when >50% of the plants had the tips of stigmata visible (GS63).

2.4. Grain yield and sanitary traits

Maize was manually harvested from areas of 7.5 m² from each subplot at maturity, to quantify the grain yield, grain quality and biomass production. Harvest took place on 19th September 2019 and on 16th September 2020. Measurements also included the number of plants and fully developed ears per surface unit. The

number of kernels per square meter (KSM) was calculated multiplying the number of kernels per ear (determined on 7 randomly selected ears) by the number of ears per square meter, as reported by Testa et al. [31]. Sub-samples of 12 ears were shelled using an electric single-ear sheller. Grain moisture was determined using a Dickey-John GAC100 grain analyser (Auburn, IL, USA). Grain, cob and stover were oven dried at 60 °C for 72 h and weighed separately. A sub-sample of 200 kernels was weighed to obtain the thousand kernels weight (TKW).

The grain protein content was obtained by multiplying N grain content (assessed as described above for the tissue N concentration) by a standard 6.25 coefficient. The sanitary traits were evaluated by the fumonisin B₁ and B₂ (FBs) contamination using the ELISA method, by means of direct competitive immunoassays (RIDASCREEN Fumonisin, R-Biopharm, Darmstadt, Germany), according to the manufacturer's instructions.

2.5. Soil N and P measurements

The soil was sampled at 50 DAS, at 75 DAS and at flowering. Three 0–30 cm deep soil cores were collected with an auger along the central rows of each plot and pooled together to obtain a representative sample. The soil mineral N was extracted with 300 g of 1 mol L⁻¹ KCl solution shaken for 1 h with 70 g of wet soil, then filtered and determined by colorimetry with a continuous flow analyser (Evolution II, Alliance Analytical Inc., Menlo Park, CA, USA). Part of the soil sample was air-dried and sieved through a 2-mm mesh screen to analyse the plant-available P using the Olsen method [32].

A linear-plateau model was used to interpolate soil Olsen P vs the true soil available P for the crop, as assessed by the plant uptake (2):

$$\text{Plant P uptake} = \begin{cases} a * (\text{Olsen P}) + b, & \text{Olsen P} < C \\ k, & \text{Olsen P} \geq C \end{cases} \quad (2)$$

where a and b are shape coefficients, k is the predicted total P uptake plateau, and C is the critical value of soil Olsen P (mg P kg⁻¹) after which plant uptake is not influenced by soil availability. The nls function in the R software statistical package was used as fitting procedure.

During 2019, and for the high rate of each LT fertilisation only, six enzymatic activities involved in key steps of P cycle were measured: acid (acP) and alkaline phosphomonoesterase (alkP), phosphodiesterase (bisP), pyrophosphodiesterase (piroP), inositol-P phosphatase (inositP) and nonanoate esterase (nona) involved in the hydrolysis of ester bonds. Enzymatic activities were measured in duplicate at each of the three field replicates, in the 0–30 cm soil layer sampled at 50 DAS. Enzymes were desorbed as described [33] using a heteromolecular exchange method via bead-beating in order to disrupt microbial cells and soil aggregates.

2.6. Statistical analysis

The experiment was analysed through a mixed model as a split-plot design, where the LT fertilisation (i.e., Min-L, Min-H, Slu-L, Slu-H, Fym-L and Fym-H), is the main factor, while the starter fertilisation adopted in each hemi-plot (N or NP) is the sub-factor. A mixed effects model was used, where LT fertilisation, starter fertilisation and year were considered as fixed factors, while block, plot and hemi-plot (as nested effects), as well as the interaction between block and plot with the year, were considered as random factors. A graphical method was used to verify the basic assumptions [34]. When single factors or their interaction determined a significant effect, means were compared using the Bonferroni post hoc

test at the $P \leq 0.05$. The lme function in the nlme statistical package of the R software [35] was used for analyses.

3. Results

3.1. Weather conditions

The two experimental years showed slight differences in the meteorological trends for both temperatures and rainfall during maize growing seasons (Fig. S1). During the first maize growing phase (April and May), the air temperature was lower in 2019 than in 2020, leading to reduced accumulated GDDs ($281\text{ }^\circ\text{C d}^{-1}$ in 2019, and $394\text{ }^\circ\text{C d}^{-1}$ in 2020, as a sum of April and May). During the second maize growth phase, from stem elongation to flowering, corresponding to June and July, the accumulated GDDs were higher in 2019 ($756\text{ }^\circ\text{C d}^{-1}$ in June and July), than in 2020 ($690\text{ }^\circ\text{C d}^{-1}$). Finally, August and September, corresponding to ripening stages, were similar according to the GDD indicator in the two experimental years (about $670\text{ }^\circ\text{C d}^{-1}$ in August and September).

The rainfall amount showed marked differences over the two years in May (+21 mm in 2020), June (+104 mm in 2020), July (−82 mm in 2020) and September (−63 mm in 2020).

3.2. Leaf area index and shoot biomass production

The starter fertilisation influenced early maize development in the three LT fertilisation systems. Generally, leaf area and shoot dry weight (SDW) were significantly higher in the treatment with the sub-surface band application of NP compared to that with N only (Table 1). However, the growing pattern was influenced by external temperature, as indicated by a significant interaction starter fertilization \times year at 50 DAS (data not shown), roughly corresponding to the first growing phase (April and May). Indeed, NP increased the LAI by 63% compared to N in 2019, and only 36% in 2020. Similarly, the SDW was enhanced by NP fertilisation in the year with a cool first phase, 2019, (+67%) compared to 2020 (+38%).

The interaction LT fertilisation \times starter fertilisation, that indicates a different impact of starter P supply over the three LT fertilisation patterns, was significant both at 50 DAS and flowering for SDW, and only at flowering for LAI (Fig. 1a–d). The NP starter fertilisation increased SDW at 50 DAS by 2.1 and 2.3-fold in Min-L and Min-H treatments, and by 1.3 and 1.6-fold in Slu-L and Slu-H treatments, while the increase was 1.1-fold in both Fym treatments. At

flowering, LAI was 31 and 35% greater in NP than in N treatments in Min-L and Min-H systems, respectively, and so was the shoot dry weight (+1.7- and +1.6-fold greater than in the N treatment, respectively). Furthermore, the NP starter fertilisation caused an increase in shoot biomass by 17% in Fym-H, despite no significant differences were found in LAI.

3.3. Crop early vigour, flowering date and grain moisture content

The N and P starter fertilisation enhanced early crop development if compared with N only, when applied to Min systems, but rarely when associated to LT organic fertilisation (Figs. 1e, S2; Table 2). The year also influenced the plant early growth response to starter fertilisation. This was shown both by growing rate and AUCDC indicators. The growing rate increase due to NP compared with N recorded in Min systems was more pronounced in 2019 (+37% and +26% for Min-L and Min-H, respectively) compared to 2020 (+11% and +21%), while within LT organic fertilisations only the Slu-H treatment showed an increased growing rate (+12%) after NP starter fertilisation, during the first year of experiment (data not shown). Similarly, the AUCDC index, that condenses differences in NDVI measurements over time (Fig. S3), showed a significant LT fertilisation \times starter fertilisation interaction (Table 2). The starter fertilisation affected plant development during vegetative stages until flowering in both Min systems and Slu-H, in both growing seasons (Fig. 1f). The AUCDC index pointed out that NP increased by 18%, 23% and 6% the early crop development of Min-L, Min-H and Slu-H treatments, respectively.

The starter fertilisation with NP resulted in a flowering anticipation of 1 day regardless LT fertilisation and year. This reduction in duration from sowing to flowering resulted in a slight reduction in grain moisture content at harvest (−0.7%) in 2019, only. However, neither the flowering date, nor the grain moisture at harvest did show any interaction effect of the starter fertilisation with LT management (Table 2).

3.4. Grain yield, yield components and quality traits

A significant effect of NP starter fertilization on grain yield and on its components was observed in Min-L and Min-H treatments, where grain yield raised of 1.3 and 3.2 t ha^{-1} , while KSM increased by 25% and 30%, respectively (Fig. 1g, h; Table 3). No significant effect was recorded on grain yield in any of the manured

Table 1
Effect of long-term (LT) fertilisation, starter fertilisation and year on leaf area index (LAI) and shoot dry weight production at 50 days after sowing (DAS) and flowering stages.

Treatment	Source of variation	LAI ($\text{m}^2\text{ m}^{-2}$)		Shoot dry weight (g m^{-2})	
		50 DAS	Flowering	50 DAS	Flowering
LT fertilisation	Min-L	0.6 ± 0.1 b	5.2 ± 0.3 a	37.3 ± 10.2 b	1290 ± 114 b
	Min-H	0.5 ± 0.1 b	5.4 ± 0.3 a	30.8 ± 8.5 b	1252 ± 114 b
	Slu-L	0.8 ± 0.2 a	5.3 ± 0.2 a	59.2 ± 13.8 a	1396 ± 64 ab
	Slu-H	0.9 ± 0.2 a	5.7 ± 0.1 a	64.1 ± 16.3 a	1401 ± 47 ab
	Fym-L	0.9 ± 0.2 a	5.4 ± 0.2 a	62.8 ± 14.0 a	1385 ± 39 ab
	Fym-H	1.0 ± 0.2 a	5.7 ± 0.2 a	69.0 ± 16.7 a	1539 ± 57 a
Starter fertilisation	N	0.6 ± 0.1 b	5.1 ± 0.1 b	44.5 ± 7.2 b	1236 ± 47.3 b
	NP	0.9 ± 0.1 a	5.8 ± 0.1 a	63.2 ± 8.5 a	1518 ± 31.6 a
Year	2019	0.4 ± 0.0 b	5.6 ± 0.2 a	14.8 ± 1.1 b	1279 ± 49.0 b
	2020	1.1 ± 0.1 a	5.3 ± 0.1 a	93.0 ± 6.2 a	1475 ± 37.6 a
Analysis of variance					
LT fertilisation		<0.001	>0.05	<0.001	0.021
Starter fertilisation		<0.001	<0.001	<0.001	<0.001
Year		<0.001	>0.05	<0.001	<0.001
LT \times Starter		>0.05	0.003	0.021	<0.001
LT \times Year		<0.001	>0.05	<0.001	>0.05
Starter \times Year		<0.001	>0.05	<0.001	>0.05
LT \times Starter \times Year		>0.05	>0.05	>0.05	>0.05

Different letters separate groups of means according to the Bonferroni post-hoc test, when the linear mixed effects model had highlighted significant differences. The level of significance, $P(F)$, is shown in the table.

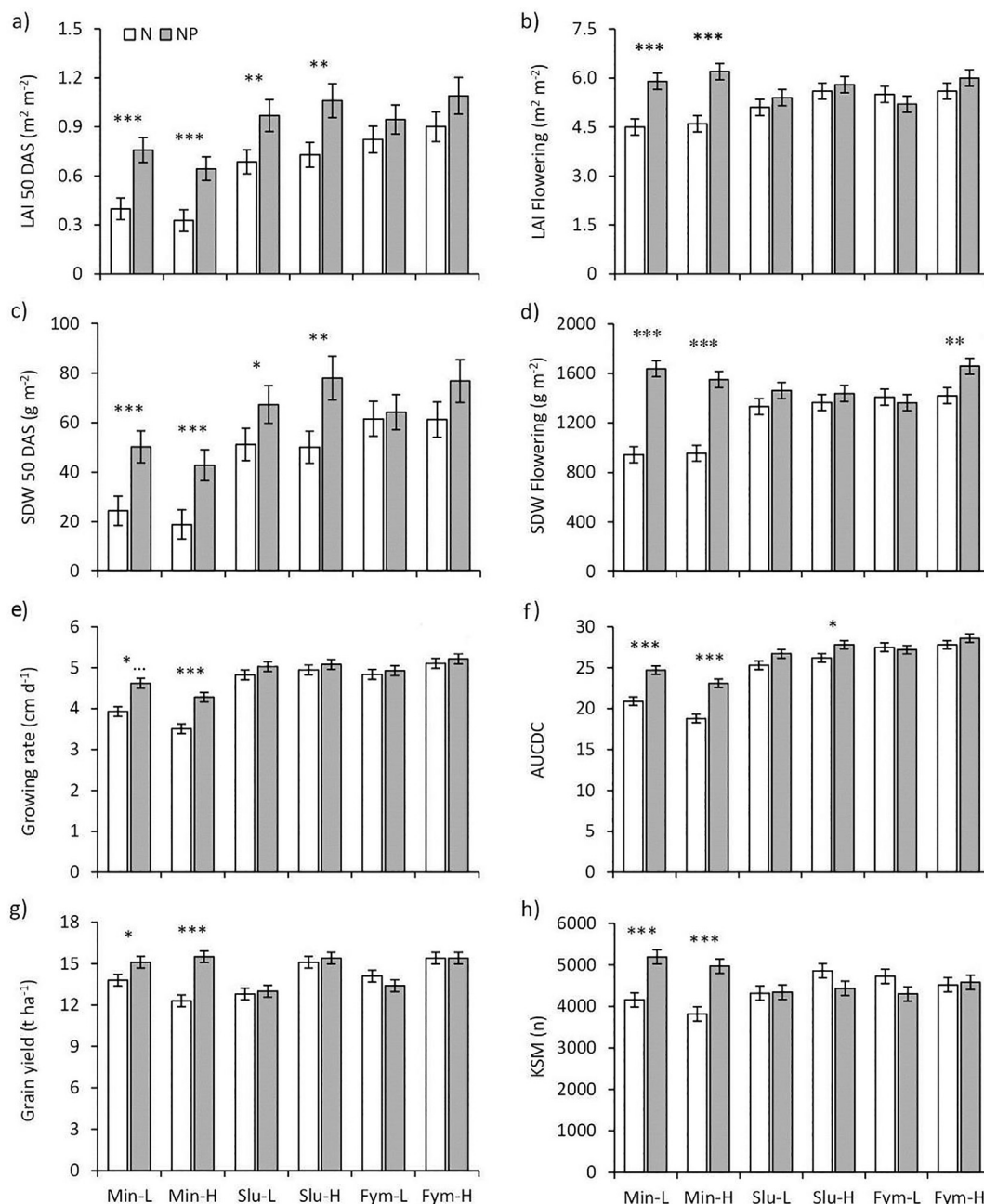


Fig. 1. Effect of starter fertilisation with N (white histograms) and NP (grey histograms) on Leaf area index (LAI) at 50 days after sowing DAS (a) and flowering (b), shoot dry weight (SDW) at 50 DAS (c) and flowering (d), growing rate (e), area under the canopy development curve (AUCDC) (f), grain yield (g) and number of kernels per square meter (KSM) (h) within each long-term (LT) fertilisation. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

treatments (Fym or Slu). Conversely, TKW was not influenced by starter fertilisation (Table 3).

None of the grain quality traits, protein content and contamination by fumonisin, was affected by starter fertilisation, but both were influenced by the year (Table 3). Grain protein content was higher in 2019, when yield was lower, but that year grain was also more contaminated by mycotoxins, due to wet conditions during the ripening phase.

3.5. Nutrient availability in the soil

Fig. 2 reports soil Olsen P (a) and nitrate N (b) concentrations as averaged over the two experimental growing seasons. Soil Olsen P was increased in the NP treatment compared to N in Min-H and

Slu-H treatments, while this trend was observed in Min-L and Slu-L in only one of the experimental years. Conversely, when LT fertilisation included farmyard manure, no significant differences were found in soil Olsen P concentration as a consequence of NP starter fertilisation.

The nitrate-N concentration was highly variable over time and was not influenced by starter fertilisation. The highest values were recorded in Min-H.

The total N and P plant uptake, as indicators of soil nutrient availabilities at 50 DAS, flowering and harvest (Table 4), showed a significant effect of starter fertilisation and also a LT fertilisation × starter fertilisation interaction at all sampling dates (Table S3). When LT fertilisation included mineral fertilisers or bovine slurry, maize P uptake at 50 DAS was higher with NP starter

Table 2

Effect of long-term (LT) fertilisation, starter fertilisation and year on maize growing rate, area under the canopy development curve (AUCDC), date of flowering (expressed as days after sowing, DAS) and grain moisture content.

	Source of variation	Growing rate (cm d ⁻¹)	AUCDC	DAS	Grain moisture content (%)
LT fertilisation	Min-L	4.3 ± 0.7 b	22.8 ± 1.6 c	92.1 ± 1.6 a	23.5 ± 0.6 b
	Min-H	3.9 ± 0.6 b	21.0 ± 1.8 c	93.4 ± 1.5 a	24.6 ± 0.7 a
	Slu-L	4.9 ± 0.7 a	26.0 ± 1.4 b	90.1 ± 1.7 b	22.5 ± 0.5 c
	Slu-H	5.0 ± 0.7 a	27.0 ± 1.2 ab	89.3 ± 1.6 b	23.7 ± 0.4 ab
	Fym-L	4.9 ± 0.7 a	27.4 ± 1.1 ab	89.2 ± 1.6 b	22.9 ± 0.5 bc
	Fym-H	5.2 ± 0.7 a	28.2 ± 0.9 a	88.8 ± 1.6 b	23.6 ± 0.3 b
Starter fertilisation	N	4.5 ± 0.4 b	24.4 ± 1.0 b	91.0 ± 1.0 a	23.7 ± 0.3 a
	NP	4.9 ± 0.4 a	26.4 ± 0.8 a	90.0 ± 0.9 b	23.3 ± 0.3 b
Year	2019	2.6 ± 0.1 b	21.2 ± 0.7 b	96.7 ± 0.3 a	25.0 ± 0.2 a
	2020	6.8 ± 0.1 a	29.6 ± 0.4 a	85.3 ± 0.3 b	21.9 ± 0.2 b
Analysis of variance					
LT fertilisation		<0.001	<0.001	<0.001	<0.001
Starter fertilisation		<0.001	<0.001	<0.001	0.025
Year		<0.001	<0.001	<0.001	<0.001
LT × Starter		0.025	0.004	>0.05	>0.05
LT × Year		>0.05	<0.001	>0.05	0.001
Starter × Year		>0.05	>0.05	>0.05	0.018
LT × Starter × Year		0.029	>0.05	>0.05	>0.05

Different letters separate groups of means according to the Bonferroni post-hoc test, when the linear mixed effects model had highlighted significant differences. The level of significance, $P(F)$, is shown in the table.

Table 3

Effect of long-term (LT) fertilisation, starter fertilisation and year on grain yield, number of kernels per square meter (KSM), thousand kernels weight (TKW), grain protein content (GPC) and fumonisins concentration (FBs).

Treatment	Source of variation	Grain yield (t ha ⁻¹)	KSM (n)	TKW (g)	GPC (%)	FBs (μg kg ⁻¹)
LT fertilisation	Min-L	14.5 ± 0.3 ab	4674 ± 207 a	375 ± 4 cd	8.8 ± 0.1 a	5029 ± 1724 a
	Min-H	13.9 ± 0.7 abc	4392 ± 246 a	389 ± 5 bc	9.4 ± 0.1 a	4207 ± 1321 a
	Slu-L	12.9 ± 0.3 c	4330 ± 148 a	366 ± 4 d	7.7 ± 0.2 b	5500 ± 2075 a
	Slu-H	15.3 ± 0.5 ab	4647 ± 164 a	406 ± 4 ab	9.1 ± 0.1 a	8848 ± 2471 a
	Fym-L	13.8 ± 0.4 bc	4515 ± 126 a	384 ± 2 cd	7.8 ± 0.1 b	4253 ± 1457 a
	Fym-H	15.4 ± 0.4 a	4550 ± 93 a	409 ± 5 a	9.2 ± 0.1 a	6674 ± 2095 a
Starter fertilisation	N	13.9 ± 0.3 b	4399 ± 98 b	388 ± 4 a	8.7 ± 0.1 a	5837 ± 1150 a
	NP	14.7 ± 0.3 a	4637 ± 95 a	389 ± 3 a	8.6 ± 0.1 a	5667 ± 1041 a
Year	2019	13.4 ± 0.3 b	4257 ± 89 b	383 ± 3 b	9.1 ± 0.1 a	10250 ± 1137 a
	2020	15.2 ± 0.2 a	4779 ± 87 a	394 ± 4 a	8.2 ± 0.1 b	1254 ± 151 b
Analysis of variance						
LT fertilisation		0.001	>0.05	<0.001	<0.001	>0.05
Starter fertilisation		0.009	0.031	>0.05	>0.05	>0.05
Year		<0.001	<0.001	0.032	<0.001	<0.001
LT × Starter		0.007	0.001	>0.05	>0.05	>0.05
LT × Year		>0.05	>0.05	0.044	>0.05	>0.05
Starter × Year		>0.05	>0.05	>0.05	>0.05	>0.05
LT × Starter × Year		>0.05	>0.05	>0.05	>0.05	>0.05

Different letters separate groups of means according to the Bonferroni post-hoc test, when the linear mixed effects model had highlighted significant differences. The level of significance, $P(F)$, is shown in the table.

fertilisation than N only. This behaviour was confirmed both at flowering and harvest in Min-L and Min-H, only, while starter fertilisation did not affect the P uptake at flowering and at harvest in all manured systems. In other words, initial positive effects of starter fertilisation were visible in systems with a lower initial P availability, but tended to decline during crop growth. Similarly, even N uptake showed higher values with NP starter fertilisation than N only at both rates of Min and Slu systems at 50 DAS, thus showing a synergic effect of the combined application of the two elements. However, differently to the P uptake, Fym-H recorded an increase of N uptake at 50 DAS when NP was applied at sowing. The N uptake of Min-L and Min-H treatment still differed at flowering, while at harvest only Min-H maintained a difference between the two starter fertilisation treatments.

The LT fertilisation management had significant effects on all enzymatic activities linked to P cycle (Table S4). Specifically, Fym applications led to a significant enhancement of all enzymatic activities compared to Slu and Min, and the latter generally showed the lowest values. Conversely, NP starter fertilisation

was associated with reduced acid (acP) and alkaline phosphomonoesterase (alkP) as well and pyrophosphodiesterase (piroP) activities, if compared with N only. Since the soil pH was sub-alkaline, acid phosphatase was lower than alkaline phosphatase [36].

4. Discussion

The sub-surface application of NP fertilisers at sowing is a common agronomic practice in temperate maize growing areas, but it could be omitted in specific conditions. This study was performed to investigate the advantages of the application of NP starter fertilizer on maize growth, in systems characterised by different fertilisation managements - mineral fertilisers, bovine slurry or farmyard manure at two doses of N supply. At the start of the experiment, the six systems had different soil P contents, since they were the result of LT fertilisation managements, and P availability ranged from medium to very rich (Table S1), according to the Regione Piemonte classification [37]. Likewise, soil organic

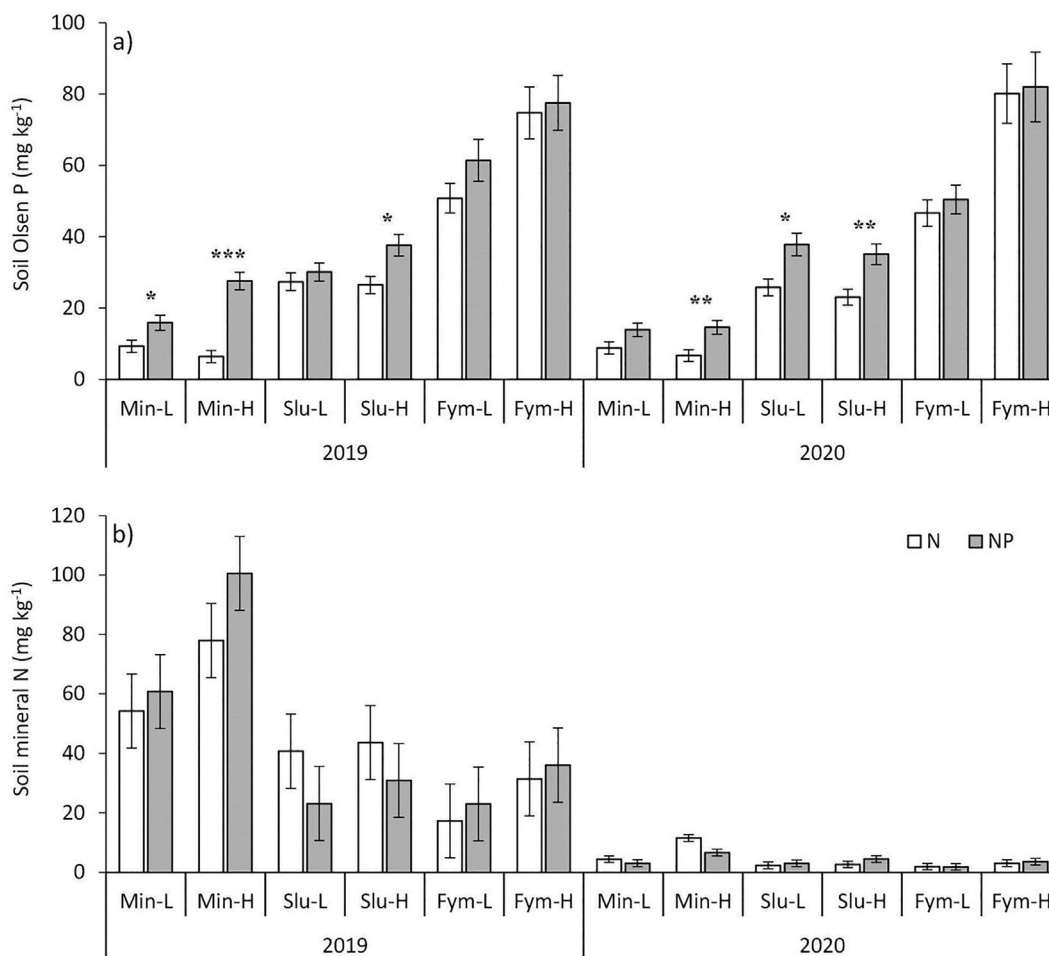


Fig. 2. Effect of starter fertilisation with N (white histograms) and NP (grey histograms) on soil Olsen P (a) and mineral N (b) concentrations (mg kg⁻¹ dry soil) in the 0–30 cm soil layer. Each value is the average of samplings at 50 DAS, 75 DAS and at flowering. *, *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.001. Error bars report ± standard error.

Table 4
Effect of long-term (LT) fertilisation, starter fertilisation and year on phosphorus (P) and nitrogen (N) plant uptake at 50 days after sowing (DAS), flowering stage and harvest.

Treatment	Source of variation	P uptake (kg ha ⁻¹)			N uptake (kg ha ⁻¹)		
		50 DAS	Flowering	Harvest	50 DAS	Flowering	Harvest
LT fertilisation	Min-L	1.4 ± 0.3 b	31.2 ± 3.0 bc	56.9 ± 2.3 b	14.0 ± 3.8 b	211 ± 18.7 a	306 ± 8.9 bc
	Min-H	1.1 ± 0.3 b	29.5 ± 2.5 c	53.0 ± 3.0 b	12.3 ± 3.3 b	231 ± 18.9 a	343 ± 8.9 ab
	Slu-L	2.6 ± 0.6 a	40.4 ± 2.2 ab	72.4 ± 2.6 a	22.6 ± 5.1 a	198 ± 12.7 a	257 ± 8.8 d
	Slu-H	2.6 ± 0.6 a	38.6 ± 1.0 ab	76.9 ± 3.0 a	24.5 ± 5.7 a	226 ± 8.7 a	479 ± 17.6 a
	Fym-L	3.0 ± 0.7 a	42.8 ± 1.8 a	79.1 ± 4.1 a	23.1 ± 4.5 a	203 ± 15.1 a	281 ± 8.9 cd
	Fym-H	3.4 ± 0.8 a	46.8 ± 2.0 a	82.1 ± 3.4 a	27.0 ± 6.0 a	238 ± 13.0 a	489 ± 9.1 a
Starter fertilisation	N	1.9 ± 0.3 b	34.4 ± 1.6 b	67.6 ± 2.8 b	17.1 ± 2.6 b	197 ± 7.4 b	309 ± 8.8 b
	NP	2.8 ± 0.4 a	42.0 ± 1.2 a	72.5 ± 2.2 a	24.1 ± 3.0 a	239 ± 8.6 a	327 ± 9.1 a
Year	2019	0.7 ± 0.1 b	39.3 ± 1.6 a	63.9 ± 1.6 b	6.3 ± 0.5 b	237 ± 8.4 a	322 ± 7.7 a
	2020	4.0 ± 0.3 a	37.1 ± 1.5 a	76.2 ± 2.9 a	34.8 ± 2.0 a	199 ± 7.9 b	314 ± 10.2 a
Analysis of variance							
LT fertilisation		<0.001	<0.001	<0.001	<0.001	>0.05	<0.001
Starter fertilisation		<0.001	<0.001	0.012	<0.001	<0.001	0.022
Year		<0.001	>0.05	<0.001	<0.001	0.010	>0.05
LT × Starter		0.029	0.021	0.017	0.008	0.001	0.021
LT × Year		<0.001	>0.05	<0.001	<0.001	>0.05	>0.05
Starter × Year		<0.001	>0.05	>0.05	<0.001	>0.05	>0.05
LT × Starter × Year		>0.05	>0.05	<0.001	0.024	>0.05	>0.05

Different letters separate groups of means according to the Bonferroni post-hoc test, when the linear mixed effects model had highlighted significant differences. The level of significance, *P* (*F*), is shown in the table.

matter concentration ranged from 1.2% to 1.9%, and a similar trend was observed for total N. Therefore, the six LT treatments allowed to study the effect of NP starter fertilisation in a relatively wide

span of situations differing for the overall fertility, on the same soil. Another important source of variation, that highlighted significant interactions with starter fertilisation, was the weather. The two

observational years were characterised by different trends in temperature, in particular in the first growing phases of the crop. Therefore, interactions between starter fertilisation and LT fertilisation management and with the year are the most relevant results of this work.

The NP starter fertilisation was confirmed to enhance early crop growth, coherently with several studies that document that P is the second key limiting nutrient for maize growth, after N [9,10,11]. Differences were more marked in systems where mineral fertilisers only were used, than in manured ones (Fym or Slu). The cause for this could be simply the fact that soil available P was very different in the observed treatments, and benefits were reduced where a greater soil available P could ensure a good nourishment to the crop. However, a positive effect of starter fertilisation was also observed in systems where the soil P status could indicate that an extra supply was not necessary.

At all systems, differences in crop growth indicators that were detectable in early stages between NP and N treatments, in particular LAI and shoot biomass, progressively reduced until the flowering stage, and became non-significant or negligible in manured systems. However, the enhanced early vigour due to NP significantly reduced time to flowering by 1 day in all LT fertilisation systems. This observation confirmed what was found by other authors, as similar anticipation of anthesis was reported for example by Kaiser et al. [38]. Despite the difference in flowering date was limited in the observed years, the trend is of interest, as early flowering can increase maize yield amount and decrease mycotoxin content [39].

At harvest, NP starter fertilisation induced a higher grain yield than N only, at both rates of Min systems, while no differences were recorded in any manured system. The absence of an effect in maize grain yield due to starter fertilisation in P-rich soils, although the effect was evident in crop growth at early stages, agrees with findings by Bordoli and Mallarino [40], Rehm and Lamb [41], Kaiser et al. [38,42]. In contrast, the increase in grain yield following NP starter fertilisation recorded in Min could be due to boosted LAI at tassel emission, and consequent higher photosynthetic rate, that led to a higher number of KSM and a decrease in barren ear tip lengths. This was coherent with findings of Zhang et al. [43], who indicated a LAI value at flowering of $\sim 4.8 \text{ m}^2 \text{ m}^{-2}$ as critical, and values measured in this study were 4.5 and $4.6 \text{ m}^2 \text{ m}^{-2}$ in Min-L and Min-H N, respectively. Andrade [44] also reported that the reduction of crop growth rate determined by a limited radiation interception during flowering was responsible for small number of grains.

The second important variable that affected plant response to NP fertilisation was air temperature in the first growing stages. Low temperatures recorded in April and May 2019 strongly depressed plant growth, but NP starter fertilisation significantly reduced the negative impact of unfavourable weather conditions. Wortmann et al. [45] and Kaiser et al. [38] reported that the extent of maize response to starter fertilization is significantly influenced by environmental conditions such as meteorological trend, soil texture and their effects on soil moisture and temperature. Past research has shown that plant P requirement is higher with cool weather conditions during seedling [46], as root growth is reduced and this adversely impacts the uptake of nutrients, especially P, which is scarcely mobile in the soil and must be intercepted by roots [47]. P promotes root growth, and Zhang et al. [48] showed that maize root length in a 44 kg P ha^{-1} treatment was significantly greater than that in a no-P treatment in the 50–60 cm soil layer at maize flowering. However, our experimental setup does not allow to test the effect of single P supplies.

In the year characterised by cold temperatures in early growth stages, 2019, maize could compensate a slow establishment with a fast development in the second growth phase, that led to limited

differences among treatments at flowering. The temperature trend was reversed in 2020, when growth conditions were more favourable in the first phase but colder in the second phase. Although less pronounced effects on maize early vigour due to NP starter fertilisation were recorded in the latter case, the maize anthesis occurred earlier in all systems by the same extent recorded in 2019. Similarly, even LAI and shoot dry weight still showed differences at flowering in mineral-fertilised systems.

Another important period whose weather affects yield amount and quality is the ripening phase, that in our study had a further levelling effect on starter fertilisation treatments. The grain moisture content at harvest was significantly decreased by NP fertilisation during the 2019 growing season only. All the other quality and sanitary traits of harvested grain seemed more influenced by N fertilisation level (higher protein content was obtained at higher N rates, owing to a quicker availability of N to plants [49]); or by the meteorological circumstances. In particular, the grain fumonisin contamination was larger in 2019, probably because of wetter conditions in September. It is in fact known that *Fusarium verticillioides*'s growth and biosynthesis of FBs are maximised in warm and wet air conditions [50].

An unexpected finding of this work was that P starter fertilisation was effective in first growth stages also in systems where the soil P test had evidenced a high availability. There are two possible explanations for this. One calls the synergic effect of N and P additions on crop early growth. A growth promotion was in fact observed when both nutrients were supplied, but not when N only was supplied. Duncan and Ohlrogge [51] reported that a rapid development and branching of roots are promoted by combined N and P addition, but not by N only. The synergic addition of N and P has therefore a stimulating effect on maize roots that further helps soil nutrients exploitation. The starter fertilisation also affected the expression of soil enzymes connected with P cycle. In particular, NP addition at sowing caused a general reduction of enzymatic activities at 50 DAS, thus confirming findings by Nannipieri et al. [52], who stated that soil phosphatase activity generally decreases in response to mineral P fertiliser application. However, this is not always true, as, for instance, Margalef et al. [53] found that applying both N and P to soils with low P availability had a positive effect on phosphatase activity, whereas it decreased when N and P were applied to soils with high natural P contents. In contrast, the experiment described here did not show any interaction between starter and LT fertilisation, and the overall reduction of P-related enzymes was observed in all treatments. The LT fertilisation was a great determinant in the P-related enzymes, as the largest enzyme concentrations were found when Fym was used, probably as a consequence of a larger substrate availability. The contrasting behaviour of P enzyme concentrations, that increased across LT systems with increasing soil P test availability, but decreased after P starter fertiliser supply, remains unexplained.

A second hypothesis to interpret the unexpected positive effect of NP fertilisation also in P-rich systems is instead linked to the effectiveness of soil P test in identifying critical levels of P concentrations for crop growth. This held true also when Olsen P was measured close to roots (as in this study) and not on bulk soil (as normally done). Literature reports many studies aimed at identifying critical available P levels for maize, and values ranged from 5 to 40 mg kg^{-1} [54–56], with differences depending on cropping system (continuous or rotational maize) and soil type [57–59]. Our data indicate a critical soil concentration at quite high values of the soil P test. In fact, the total plant uptake at harvest – a true indicator of availability – showed a linear-plateau response to soil Olsen P measured near roots at 50 DAS (Fig. 3). The plant P uptake increased linearly up to the threshold of 39 mg P kg^{-1} of Olsen P, then stabilised, as luxury consumption of P is not typical in maize.

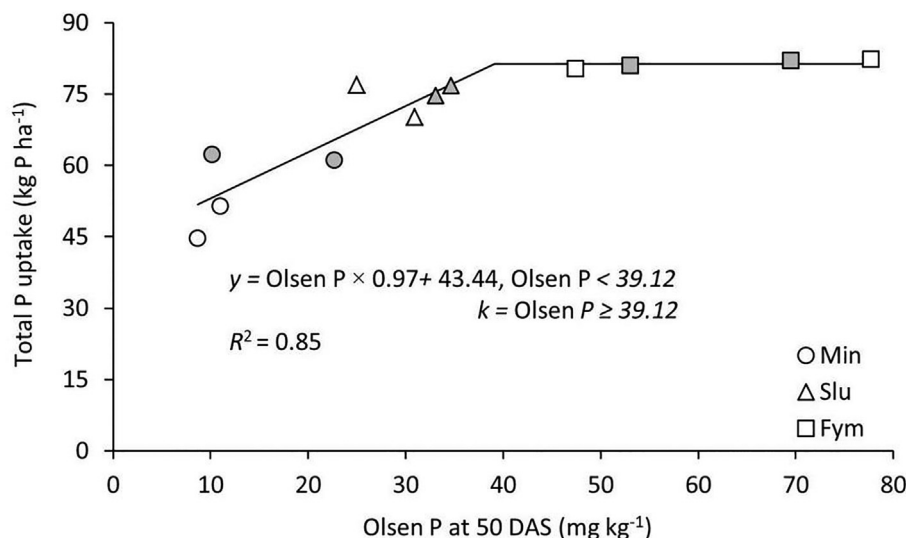


Fig. 3. Total above-ground plant P uptake at harvest in response to soil Olsen P concentration at 50 days after sowing (DAS), averaged over the two years ($n = 6$). Open symbols show N starter fertilisation, while closed symbols show NP starter fertilisation.

Wu et al. [56] also observed a limited plant growth increase due to an additional P application, when soil P availability was higher than a critical value of 40 mg kg^{-1} . Results reported in Fig. 3 indicate that both Min and Slu systems were in the range of soil P test values where a positive effect of fertilisation was expected. The moderate, but positive effect of P starter fertilisation in Fym systems could then be due to a temporary immobilisation of mineralised P in microbial biomass [14], but it did not affect the overall plant uptake. The threshold below which an addition of P was effective in increasing P availability was therefore rather high in our conditions, about 39 mg kg^{-1} of soil Olsen P. Environmental measures set a threshold above which P fertilisation should be suspended in order to better exploit soil P legacy. This value is 25 mg kg^{-1} in the regional legislation and in other European countries [19], or even lower (15 mg kg^{-1} in Colorado and Idaho in USA; [60]), sometimes depending on crop type (for example 25 or 45 mg kg^{-1} in Northern Ireland). Our study provides evidence that the threshold value above which no P should be supplied does not maximise the maize nutrient status.

The optimal dose of mineral fertilisers is a compromise between maximum crop benefit and drawbacks such as economical cost and environmental risk, that is particularly high for P [15,61,62]. Therefore, provided that plant uptake capacity should not be exceeded in order to avoid soil overburden, there is a need to find the right dose that minimises both the risk of dispersing P into the environment and the risk of reducing crop nutrient use efficiency in case of adverse weather conditions. The limited advantages observed in this study in terms of crop growth, anticipation of flowering date, and reduction of grain moisture at harvest could be negligible in years with a normal weather, but could be crucial in ensuring yield amount and quality in adverse weather years. In a context of climate change, this might become relevant soon.

5. Conclusions

This study provides useful guidelines on how to modulate P starter fertilisation in maize, depending on the soil long-term mineral or organic fertilisation history, on the basis of observations all along the maize growth season. The N and P starter fertilisation at maize sowing improved early crop growth and increased grain yield when applied to mineral-fertilised systems where the soil P status was medium. On the other hand, in systems where long-

term farmyard manure applications had increased the soil Olsen P availability to high levels, benefits due to starter fertilisation were relevant in crop early growth, but not visible at harvest. Systems fertilised with bovine slurry were intermediate, with positive effects of NP starter fertilisation in early growth but not in yield.

The crop P uptake increased with increasing soil P status up to the critical value of 39 mg kg^{-1} of soil. Our study provides evidence that the threshold value above which no P should be supplied for environmental reasons does not maximise the maize nutrient status. A limited addition of N and P starter fertilisation at sowing could be useful to boost early maize growth in temperate climates, even in a context of urgent environmental protection concerns. In uncertainties of a rapidly changing climate with increased fluctuations of temperature and rainfall, the adoption of NP starter fertilisation at maize sowing may partially alleviate the negative effects of early cold stress, emphasising the role of this management practice in ensuring stable yields.

CRedit authorship contribution statement

Michela Battisti: writing, conceptualisation, data curation, formal analysis, investigation. **Barbara Moretti:** Conceptualisation, investigation, data curation. **Massimo Blandino:** conceptualisation, methodology. **Carlo Grignani:** supervision. **Laura Zavattaro:** writing - review & editing, conceptualisation, methodology, funding acquisition, project administration.

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.

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Appendix A. Supplementary data

Supplementary data for this article can be found online at <https://doi.org/10.1016/j.cj.2022.09.010>.

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