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The role of zinc fertilization and its interaction with nitrogen and phosphorus starter fertilization on early maize development and grain yield

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ABSTRACT

The early planting of maize in temperate growing areas leads to yield and quality benefits, although a low soil temperature can affect the early nutrient uptake and delay plant development. Not only can the nitrogen (N) and phosphorus (P) uptake be limited, but that of zinc (Zn) can also be affected. Moreover, the application of N and P at early growth stages can result in an enhancement of maize growth. Two field experiment were carried out in North Italy during the 2012–14 period to: i) evaluate the most effective Zn application strategy by comparing seed, soil and foliar applications with an untreated control; ii) investigate the role of a Zn seed treatment on the early development and yield of maize grown in three different types of soils. The effect of Zn application was compared, in both experiments, to starter NP fertilization in bands at planting, according to a full factorial design. Zn fertilization significantly enhanced the early vigor and yield of the maize, although the effects were less pronounced than those of the NP fertilizer. Among the Zn fertilization strategies, maize growth was quicker for the seed and soil applications than for the foliar application or the untreated control. The former application significantly increased the plant height at stem elongation (+32%), shortened the planting-flowering period by 1 day, and increased the grain yield by 4%. Overall, the application of NP starter fertilizer shortened the planting-flowering period by 4.5 days and increased the yield by 10%, compared to the unfertilized control. In the second experiment, higher plant vigor indices were detected in all the soils for the NP starter fertilization (+22–27%) and for the Zn seed treatment (+3–9%) than in the controls. The NP starter fertilization reduced the grain moisture at harvest by 2.3% and increased grain yield by 14%, while the Zn treatment significantly increased the kernel weight but did not affect the grain yield or the moisture content. The interaction between the NP fertilization and the Zn treatment was never significant. Thus, the positive effect of Zn on the early vigor was an additional benefit to that produced by the starter NP fertilization. The study demonstrated that a Zn application at planting, with both a seed dressing and a soil treatment, significantly enhanced the early growth of different maize hybrids, in different growing seasons and soils, although the use of a NP starter fertilization led to more consistent agronomic benefits. Furthermore, the combination of NP and Zn starter fertilization resulted in a further advantage for all the considered production situations.

1. Introduction

Maize (*Zea mays* L.) is the first crop for worldwide production (1210 million tons), and it is increasingly being used in different kinds of food, as well as in the feed, starch, and biofuel sectors. Thus, the expected growth in the demand for this commodity will require a further increase in yield, which may be achieved by focusing on highly efficient crop practices. Breeding programs have increased the tolerance of modern maize hybrids to cold temperatures, thereby allowing a progressive anticipation of the planting date in temperate growing areas. An early planting enhances the interception of solar radiation by the canopy during the crop cycle, reduces the risk of drought and heat stresses at flowering, and leads to an earlier harvest in autumn with a lower drying cost (Battisti et al., 2023; Xu et al., 2022) and a lower risk of mycotoxin contamination (Blandino et al., 2017). Therefore, an early planting date increases the efficiently of the grain yield, as well as the sustainability

Abbreviations: ANOVA, analysis of variance; AUCDC, area under canopy development curve; CEC, cation-exchange capacity; DAS, days after sowing; GDD, growing degree days; DTPA, Di-Ethylene Triamine Penta Acetic acid; GS, growth stage; KSM, kernel square meter; N, nitrogen; NDVI, normalized difference vegetation index; P, phosphorous; REGW-F, Ryan-Einot-Gabriel-Welsh F; TKW, thousand kernels weight; Zn, zinc.

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and economic profitability of the maize cropping system.

However, an early planting time also makes the crop more prone to cold and rainy weather conditions, which can lead to a slowdown in crop growth, thereby reducing the positive effects of this practice. A cold and rainy meteorological trend in the first growth stage can increase the risk of scalar emergencies, dumping-off, root asphyxiation, and root rot, and can make the consequence of insect attacks and weed competition more dangerous, particularly in temperate growing areas. Moreover, low soil temperatures constrain the uptake of phosphorous (P), nitrogen (N), and other macro and micronutrients by the root system, even in soils that have a high nutrient content, thus negatively affecting early crop development (Imran et al., 2013; Battisti et al., 2023). The application of NP fertilizers in bands close to maize seed furrows satisfies the early plant demand by providing nutrients close to the small and developing root system (Zhang et al., 2023). Such agronomic practice provides a significant and positive effect on shortening the planting-flowering period, increasing the grain yield, and enhancing the maize quality, as it minimizes the mycotoxin content (Kaiser et al., 2016; Battisti et al., 2023).

Low temperatures in the soil, apart from effecting the macronutrients, can also have an impact on the uptake of such micronutrients as zinc (Zn), and this can be a further growth-limiting factor (Cakmak and Kutman, 2018). Maize shows a higher impact of Zn deficiency than other crops (Fageria et al., 2002). Since Zn is a constituent of several enzymes, this micronutrient is involved in several plant functions. It affects photosynthesis and the phytohormone activity, pollen formation, seed production, the carbohydrate metabolism, protein synthesis, membrane permeability, and signal transduction, and thus plays an important role in seedling physiology and plant establishment (Alloway, 2008a; Maqbool and Beshir, 2019). Zn also plays an important role in the gibberellic acid metabolism, by inducing the cell division process and in particular by increasing the leaf number and area, but also by improving the photosynthesis process and increasing the total starch and protein contents of the grain and the final ear weight (Suganya et al., 2020).

It has been estimated that half of the world's cereal surface has low levels of available Zn for plants (Cakmak, 2009), mainly in soils where the distribution of animal manure is infrequent (Ogiyama et al., 2005). Zn is taken up from soil primarily as divalent cations (Zn^{2+}) (Fageria et al., 2002), but the soil texture and the soil properties, such as pH, electrical conductivity, organic matter, calcium carbonate ($CaCO_3$) and nutrient interaction (mainly through phosphate fertilizers), can negatively affect the availability of Zn (Hafeez et al., 2013). Among the chemical properties of soil, its pH is the most important factor in controlling Zn mobility. Alkaline calcareous soils, which are characterized by low organic matter, high levels of oxides, and high carbonate contents, favor Zn adsorption and decrease its concentration in the soil (Montalvo et al., 2016). Furthermore, lighter sandy textured soils with a low cation exchange capacity (CEC) have a lower retaining capacity (Alloway, 2008a). The Zn concentration in the soil is also influenced by the P content (native P) and by fertilization: a high rate or prolonged use of P fertilizers can inhibit Zn mobility, thereby reducing its uptake by plants (Akhtar et al., 2019). Karimian (1995) instead reported that N fertilization improved Zn crop nutrition by increasing the Zn concentration and its uptake. However, although a negative interaction between P availability and Zn has been reported, there is still a lack of information on the effect of starter NP fertilization in bands, in soils with a different P content, and on the effectiveness of a Zn supply in promoting the initial development of the crop.

Different Zn fertilization strategies can be applied: directly to soil (broadcast or banded), or as seed or foliar treatments (Cakmak and Kutman, 2018; Martínez-Cuesta et al., 2021). Seed treatment applications can influence plant growth at the earliest growth stages after germination and also during establishment (Maqbool and Beshir, 2019), while the distribution in seed furrows at planting or a foliar application in a mixture with a post-emergence herbicide can result in a delayed effect. Furthermore, these fertilization strategies permit higher nutrient rates than a seed treatment (Montalvo et al., 2016; Cakmak and Kutman, 2018). The role of Zn fertilization has been studied in the literature by focusing on its benefits, in terms of grain yield, in growing areas with a high soil Zn deficiency (Hafeez, et al., 2013. Suganya et al., 2020) or on biofortified cereal grains to establish the content of this micronutrient (Akhtar et al., 2019; Alloway, 2008a; Cakmak and Kutman, 2018).

In the literature, the role of Zn fertilization was studied focusing mainly on their benefit in terms of grain yield in growing areas with high soil Zn deficiency (Hafeez et al., 2013. Suganya et al., 2020) or to biofortify cereal grains for the content of this micronutrient (Akhtar et al., 2019; Alloway, 2008a; Cakmak and Kutman, 2018). Thus, there is a lack of knowledge on the impact of different Zn fertilization strategies (seed, soil or foliar) on the early development of maize under field conditions, as well as a direct comparison with a well-known strategy, such as NP starter fertilization banded at planting.

Our hypothesis was that different Zn applications may have a variable effect in promoting a faster maize development in the early growth stages, and this may have different repercussions on the harvest time and yield capacity. Another hypothesis was that the Zn supply could favor fewer agronomic benefits than those provided by NP starter fertilization, although the potential synergistic role of these practices in enhancing maize growth still needs to be evaluated considering soils with different chemical and physical properties.

Thus, the aims of this study, which was carried out as part of a large set of field experiments, were to: I) study the effects of different Zn application strategies (seed, soil or foliar) on the early vigor and grain yield of maize in a subalkaline calcareous soil; II) compare the effects of a Zn application to the subsurface with an NP starter fertilizer on the early vigor and grain yield of the plant, considering the possible interactions between these practices; III) verify the role of a Zn seed treatment on plant growth in soils with different textures, reactions, and P contents under the same environmental conditions.

2. Materials and methods

The study was carried out at the University of Turin research station in Carmagnola (44° 53′N; 7° 41′E; 245 m a.s.l.) in the North-West of Italy over three growing seasons, from 2012 to 2014. The climate in this area is temperate sub-continental, with a mean annual temperature of 12 °C and an average annual rainfall of 760 mm. The daily temperatures and precipitation were measured over the three growing seasons by a meteorological station located in the experimental center.

Two experiments were set up: the aim of Experiment 1 was to study the most effective Zn application method, while Experiment 2 was conducted to evaluate the effect of a Zn seed treatment on the early growth of maize in three soils with different textures and chemical properties. A banded subsurface NP starter fertilizer was placed at sowing in each experiment, according to a full factorial design, to compare this practice with the Zn fertilization application and evaluate their possible interaction.

2.1. Experiment 1 – Zn fertilizer application methods

The field experiment was carried out in a loam subalkaline soil, with a medium organic matter content (20.2 g kg^{-1}) and low CEC (8.7 cmol (+) kg^{-1}). The soil was sampled to a depth of 0–30 cm, using Eijkelkamp cylindrical augers, just before crop planting and analyzed for the total carbonate (Loeppert and Suarez, 1996), exchangeable K (Summer and Miller 1996), total N (using a CHN elemental analyzer, Flash EA 1112, Thermoquest), available P (Olsen et al., 1954), and Zn-Di-Ethylene Triamine Penta Acetic acid (DTPA, Lindsay and Norvell, 1978). Low total N (1.03 mg kg^{-1}), available P (9 mg kg^{-1}), and Zn (0.77 mg kg^{-1}) contents were observed. The other main soil properties are reported in Table 1.

Different maize hybrids, NP starter fertilization treatments, and Zn application methods were compared each year, according to a factorial

Table 1
Main physical and chemical characteristics of the first 0–30 cm layer of the compared soils for the field experiments carried out in the 2012–2014 period at Carmagnola (North Italy).

Soil Parameter	Measurement unit	Experiment 1	Experiment 2		
		L (loam)	L (loam)	SL (silty loam)	SCL (silty clay loam)
Sand (0.05 – 2 mm)	g kg^{-1}	323	393	203	84
Silt (0.002 – 0.05 mm)	g kg^{-1}	621	542	641	582
Clay (< 0.002 mm)	g kg^{-1}	56	65	156	334
pH (H_20)[a]		8.1	8.2	6.2	8.0
Total carbonate[b]	g kg^{-1}	16	23	-	12
Organic matter[c]	g kg^{-1}	20.2	13.0	12.4	18.2
C/N		11.5	8.6	9.0	7.6
CEC	cmol(+) kg^{-1}	8.7	8.0	11.6	24.1
Exchangeable K[d]	mg kg^{-1}	48	39	41	117
Total N[c]	g kg^{-1}	1.03	0.82	0.70	1.25
Available P[e]	mg kg^{-1}	9	8	37	24
Zn[f]	mg kg^{-1}	0.77	0.70	0.83	0.50

[a] pH 1:2.5 w/v H_2O
[b] Volumetric calcimeter method (Loeppert and Suarez, 1996)
[c] CHN elemental analyzer (Flash EA 1112, Thermoquest)
[d] $BaCl_2$ extraction (Summer and Miller, 1996)
[e] Bicarbonate-extractable-P (Olsen et al., 1954)
[f] Zn-DTPA (Lindsay and Norvell, 1978)

experimental design:

- 2 maize hybrids,

• PR33A46 (FAO maturity class 500; 128 days relative to maturity, Corteva Agriscience, Cremona, Italy),
• P1547 (FAO maturity class 600; 130 days relative to maturity, Corteva Agriscience).

- 2 starter fertilization treatments,

• unfertilized control (unfertilized), without any starter fertilization,
• sub-surface NP starter fertilizer (NP) placed in bands close to the maize seed furrows, with 27 kg N ha^{-1} and 69 kg P_2O_5 ha^{-1} applied as diammonium phosphate (DAP, 18% and 46% for N and P_2O_5, respectively, w/w);

- 4 Zn application treatments,

• untreated check (untreated); without any Zn application,
• Zn seed treatment (seed), (Viener® Zn, 26% Zn w/w; BMS Micro-Nutrients NV, Bornem, Belgium) at a dose of 10 g kg^{-1} of maize seeds (0.07 kg Zn ha^{-1}),
• Zn subsurface fertilization (soil), (Tradecorp® Zn, 14% Zn-chelate with ethylenediaminetetraacetic acid, EDTA, w/w; Trade Corporation International, Madrid, Spain) at a dose of 5 kg ha^{-1} in the seed furrows at maize planting (0.7 kg Zn ha^{-1}),
• Zn foliar treatment (foliar), a single spray of 0.7 kg Zn ha^{-1} (Tradecorp® Zn, 14%, at 5 kg ha^{-1}, Zn-chelate with EDTA) in correspondence to the 4-leaf emission stage (growth stage, GS14, according to the BBCH-scale, Lancashire et al., 1991).

The treatments were assigned to experimental units using a completely randomized block design with four replicates. Each plot consisted of 4 rows 0.75 m apart, separated by two untreated buffer rows, one on either side; the plot length and the alleys between the plots were 10 and 1 m, respectively. The experiment was carried out each year in a different area of the field to avoid any influence of the treatments applied in the previous year.

2.2. Experiment 2 – Zn seed treatment in different soils

The study was carried out on 3 large adjoining plots, each measuring 15 × 150 m, and consisting of different types of soil, as far as the texture, and the physical and chemical properties are concerned:

• a loam (L) soil (Typic Udifluvents, according to the USDA classification, pH 8.2), which is the native soil of the experimental farm, and it has a medium-low CEC (8.0 cmol(+) kg^{-1}), and low N (0.82 mg kg^{-1}) and plant-available P (8 mg kg^{-1}) contents;
• a silty loam (SL) soil (Typic Dystrochrepts, pH 6.2), which was transferred from a site in Riva presso Chieri (44° 59′ N, 7° 52′ E), and it has a medium CEC (11.6 cmol (+) kg^{-1}), a low N content (0.70 mg kg^{-1}) and a high plant-available P (37 mg kg^{-1}) content;
• a silty clay loam (SCL) soil (Typic Hapludalfs, pH 8.0), which was transferred from a site in Sale (44° 58′ N, 8° 48′ E), and it has a high CEC (24.1 cmol (+) kg^{-1}), a medium N content (1.25 mg kg^{-1}) and a medium P (24 mg kg^{-1}) availability.

The SL and SCL soils were transferred in 1992 and were placed to a depth of 60 cm above the native soil (L). The Zn soil content in each soil was low, with the highest value in the SL soil (0.83 mg kg^{-1}) and the lowest value in the SCL soil (0.50 mg kg^{-1}). The other physical and chemical properties are reported in Table 1. Four fertilization strategies were compared for each year and for each soil, according to a factorial experimental design:

- 2 starter fertilization treatments:

• unfertilized control (unfertilized), without any starter fertilization,
• sub-surface NP starter fertilizer (NP) placed in bands close to the maize seed furrows with 27 kg N ha^{-1} and 69 kg P_2O_5 ha^{-1} applied as DAP.

- 2 Zn application treatments:

• untreated check (untreated); without any Zn application,
• Zn seed treatment (seed), (Viener® Zn, 26% Zn w/w) at a dose of 10 g kg^{-1} of maize seeds (0.07 kg Zn ha^{-1});

The treatments were assigned to experimental units in each soil using a split plot design with 3 replications, in which the Zn seed treatment was the main factor, and the NP starter fertilization was the subplot. The subplot consisted of 4 rows 0.75 m apart and 15 m long. The study was performed using the P1543 commercial dent maize hybrid (FAO maturity class 600; 130 days relative to maturity, Corteva Agriscience). The experiment was carried out each year in a different area of the field to avoid any influence of the treatments applied in the previous year.

2.3. Agronomic management of the experiments

The planting was carried out at the end of March – beginning of April (Table S1) in both experiments, according to the prompt planting time of the growing area, after a 0.3 m deep fall ploughing, followed by a suitable disk harrowing. When scheduled, the DAP was placed at a distance of 5 cm from the seed furrows, using a calibrated granular dispenser, at a depth of 10 cm from the soil surface, while the soil Zn application was performed through a calibrated micro-granulator; both dispensers were applied to the planter (Monosem NG, Largeasse, France). The Zn foliar treatment was performed over the entire plot surface using a four-nozzle precision sprayer (Honda Agricultural Sprayer T-Jeet A110/04; Honda Motor Europe, Ltd., London, the UK), held on the shoulders, at the 4 completely unfolded leaf growth stage (GS14).

The conventional crop practices of the growing areas were applied in both experiments. Briefly, the previous crop was maize each year. Before planting, 100 kg ha^{-1} of K_2O was applied (as potassium chloride, 60% K_2O w/w) each year, with no other N or P fertilizers, except for a starter in bands close to the seed furrows. The top-dressing N rates applied as urea (46% N w/w) were obtained as the difference between the crop needs (250 kg N ha^{-1}) and the amount of N supplied with the starter fertilization for the NP treatment.

The planting, harvesting, Zn foliar treatment as well as the N side-dressing fertilization dates are reported in Table S1 for each year and each experiment.

All the seeds were treated with a fludioxonil and metalaxil-m fungicide (Celest XL®, Syngenta Crop Protection S.p.A., Milan, Italy). Systemic granular insecticide clothianidin was applied at planting in the seed furrows (Santana®, Sumitomo Chemical Agro Europe S.A.S, Lion, France) at the dose of 10 kg ha^{--1} to limit and reduce insect damage. The weed control was conducted, at pre-emergence, with ciprosulfamide (66 g AI, Active Ingredient, ha^{--1}), isoxaflutole (100 g AI ha^{--1}), and tiencarbazone metile (40 g AI ha^{--1}) (Adengo®, Bayer Crop Science S.r.l, Milano, Italy) and, at post-emergence, with nicosulfuron (37 g AI ha^{--1}), rimsulfuron (9 g AI ha^{--1}), and dicamba (220 g AI ha^{--1}) (Principal® Mais, Corteva Agriscience). No foliar fungicide or insecticide was applied during the maize growing cycle. Irrigation was performed with a sprinkler, in both experiments, to avoid any drought stress until physiological maturity, according to the farm management system of the research center.

2.4. Crop development

A hand-held optical sensing device, GreenSeekerTM® (Trimble©, Sunnyvale, California, the USA), was used to measure the normalized difference vegetation index (NDVI) during the vegetative stages. The instrument was held approximately 60 cm above each single maize row and its effective spatial resolution was 0.75 m × the full length of the plot (10–15 m). This assessment was performed weekly in the two middle rows of each plot, starting from the three-leaf stage (GS13) until tassel emission (GS55). The NDVI measurement helped to quantify the development of the crop canopy throughout the season, since low values refer to bare soil, while high values are proportional to the maize biomass (Capo et al., 2020). The Area Under Canopy Development Curve (AUCDC) was calculated during the vegetative cycle, for each treatment, starting from the NDVI measurement and using the following formula:

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

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

The plant height was recorded at approximately the 3-leaf stage (GS13) and at stem elongation (GS34, approximately 4 detectable nodes) by measuring 10 consecutive plants randomly selected from the center two rows of each plot. The plant height was measured in centimeters from the ground level up to the collar of the uppermost fully developed leaf (GS13), or from the ground level up to the uppermost detectable node (GS34).

The flowering date of each plot was registered when > 50% of the plants in the two central rows of each plot had reached the beginning of ear flowering (GS61). This parameter was expressed as days after planting (DAS). All the observations described here were carried out in both experiments.

2.5. Grain yield and yield components

Ears were collected by hand at harvest maturity from a 4.5 m^{-2} area in the center of two rows in each plot to quantify the grain yield and to obtain a representative sample. The harvesting was performed on the same day in each experiment for all the compared treatments, when the grain moisture content was between 20% and 30%, according to the conventional harvesting practices in the growing areas (Table S1).

The collected ears from each plot were counted to record the density per square meter of the fully developed ears. The number of kernel rows and the number of kernels per row were also counted on 7 of these randomly selected and de-husked ears, and the theoretical amount of kernels per square meter (KSQ) was then calculated by multiplying the average number of kernels per ear by the number of ears per square meter (Battisti et al., 2023). All the collected ears were shelled using an electric sheller. The kernels from each plot were mixed thoroughly to obtain a random distribution. Grain moisture was analyzed using a Dickey-John GAC2100 grain analyzer (Auburn, IL, the USA). The grain yield results were adjusted to a 14% moisture content. Two hundred dry kernels were randomly collected and weighed to assess the thousand kernel weight (TKW). The same measurements were performed in both experiments.

2.6. Data analysis

The statistical procedure was performed using the lmer function in the LME4 statistical package of the R software package (R Core Team, 2019). The data for Experiment 1 were analyzed using a mixed effects model, where the hybrid, starter fertilization, and Zn treatment were considered as fixed effects, while the year and block were considered as random factors. The data from Experiment 2 were analyzed separately for each of the compared soils, using a mixed effect model in which the starter fertilization and the seed treatment were considered as fixed effects, while the year was considered as a random factor. A graphical method was used to verify the basic assumptions (Onofri et al., 2016). When single factors or their interactions determined a significant effect, the means were compared using the Bonferroni post hoc test at $p \leq 0.05$.

3. Results and discussion

3.1. Weather conditions

The three growing seasons showed slightly different meteorological trends for both rainfall and air temperature (expressed as growing degree days, GDDs) (Table S2). The 2012 and 2013 years showed similar and high amounts of rainfall during the spring months of April and May. Furthermore, the 2012 year had drier and warmer conditions from June to August and, as a result, the harvest was carried out at the beginning of September. Conversely, the 2014 year had less rainfall in the spring months (in particular in March and April), while it had the highest amount of rainfall in June and July, and lower GDDs in August during the ripening stage.

3.2. Experiment 1 – Zn fertilizer application methods

A significant effect of the maize hybrid on plant vigor was only observed at GS13, with a better development of the later genotype (FAO 600) (Table 2) but, as expected, the flowering of the FAO 500 hybrid was 1 day earlier, and this led to a lower grain moisture at harvest. Although the flowering date and, consequently, the yield components of the compared genotypes differed, with a higher KSM for the FAO 500 hybrid, and a higher TKW for FAO 600 (Table 3), no significant effects on grain yield were recorded overall between the maize hybrids.

As far as the fertilization management is concerned, the application of an NP starter fertilizer at planting clearly affected the maize growing cycle each year, from the 3 leaf-stage to tassel emission, as confirmed by

Table 2
Effects of the maize hybrid, the NP starter fertilization and the Zn treatments on the plant height at the leaf emission (GS13) and stem elongation (GS34) stages, the area under canopy development curve (AUCDC), the flowering date, expressed as days after sowing (DAS), and the grain moisture content at harvest for field Experiment 1 carried out in the 2012–2014 period at Carmagnola (North Italy).

Factor	Source of Variation	Plant height GS13 (cm)	Plant height GS34 (cm)	AUCDC (Σ NDVI-day)	Flowering date (DAS)	Grain moisture (%)
Hybrid (H)	FAO 500	7.8 b	43.3	21.7	88.3 a	26.2 b
	FAO 600	8.2 a	44.7	21.8	89.2 b	28.6 a
	p-value	*	n.s.	n.s.	***	***
Starter fertilization (SF)	Unfertilized	7.0 b	22.7 b	19.0 b	91.0 a	28.6 a
	NP	9.1 a	65.3 a	26.4 a	86.5 b	26.5 b
	p-value	***	***	***	***	***
Zn treatment (Zn)	Untreated	7.8 b	37.3 b	21.0 b	89.4 a	27.8
	Seed	8.4 a	49.0 a	22.3 a	88.4 bc	27.4
	Soil	8.1 ab	49.2 a	22.6 a	88.2 c	27.4
	Foliar	7.8 b	40.5 b	21.1 b	89.0 ab	27.7
	p-value	**	***	***	***	n.s.
H × SF	*p*-value	n.s.	n.s.	n.s.	*	n.s.
H × Zn	*p*-value	n.s.	n.s.	n.s.	n.s.	n.s.
SF × Zn	*p*-value	n.s.	n.s.	n.s.	n.s.	n.s.
H × SF × Zn	*p*-value	n.s.	n.s.	n.s.	n.s.	n.s.

Means followed by different letters are significantly different for each factor. The level of significance (*p*-value) is shown in the table: * < 0.01; ** < 0.05; *** < 0.001; n.s, not significant.

Table 3
Effects of the maize hybrid, the NP starter fertilization and the Zn treatments on the maize grain yield and the yield components: number of kernels per square meter (KSM) and thousand kernel weight (TKW) for field Experiment 1, carried out in the 2012–2014 period at Carmagnola (North Italy).

Factor	Source of Variation	Grain yield (t ha^{-1})	KSM (n°)	TKW (g)
Hybrid (H)	FAO 500	15.6	4882 a	387 b
	FAO 600	15.7	4212 b	422 a
	p-value	n.s.	***	***
Starter fertilization (SF)	Unfertilized	14.9 b	4382 b	402 b
	NP	16.4 a	4711 a	406 a
	p-value	***	***	*
Zn treatment (Zn)	Untreated	15.4 b	4462 b	404
	Seed	15.9 a	4564 ab	404
	Soil	16.0 a	4669 a	406
	Foliar	15.3 b	4491 ab	402
	p-value	***	*	n.s.
H × SF	*p*-value	n.s.	n.s.	n.s.
H × Zn	*p*-value	n.s.	n.s.	n.s.
SF × Zn	*p*-value	n.s.	n.s.	n.s.
H × SF × Zn	*p*-value	n.s.	n.s.	n.s.

Means followed by different letters are significantly different for each factor. The level of significance (*p*-value) is shown in the table: * < 0.01; ** < 0.05; *** < 0.001; n.s, not significant.

the canopy development, which was expressed by means of the NDVI measurements (Fig. 1). Overall, the NP starter fertilization significantly influenced ($p < 0.001$) the AUCDC vegetative index (Table 2), which summarized the NDVI development during the vegetative stages, and increased this parameter by 39%, compared to the unfertilized control. The plant height measurements at GS13 and GS34 confirmed the AUCDC differences: the NP fertilized plants were significantly taller ($p < 0.001$, +1.3 – 3 times) than the control with no starter fertilizer.

Furthermore, differences in crop development were also detected at flowering and at grain harvest. On average, when the NP starter fertilization was performed at planting, flowering occurred − 4.5 days before the unfertilized control, although the two maize genotypes led to different results: − 5.1 days for the FAO 600 hybrid and − 4.0 days for the earlier FAO 500. This advantage in the vegetative cycle was maintained and then transformed into a lower grain moisture (−2.1% points) at harvest, and into an increase in the grain yield (+10%), which was influenced by an increase in KSM (+8%) and TKW (+1%) for the starter NP fertilization, compared to the unfertilized control (Table 3).

Among the different Zn fertilization strategies, only the seed Zn treatment was able to affect plant vigor at GS13 (the plants were +8% taller); while both the seed and soil Zn application methods significantly increased plant height at GS34 (+32%), compared to the untreated control. These differences, in terms of early development, were also observed from the increase in the NDVI values during the vegetative stages (Fig. 1) and the AUCDC index (+6% and 8%, respectively), compared to the untreated control (Table 2).

The enhancement in the maize development obtained with the Zn application (seed treatment and soil distribution) in the early growth stages reduced the number of days between planting and silking by 1 day, thus leading to an increase in grain yield (on average of 4%), compared to the untreated control (Table 3), while no differences were reported for grain moisture at harvest. As far as the yield components are concerned, the Zn soil treatment only increased the number of KSM (+5%), in the same way as for the NP starter fertilization. The foliar Zn application did not differ significantly from the untreated control for any of the considered early vigor and grain yield indices. The interactions between the maize hybrid, starter fertilization, and Zn treatment factors were never significant in the early vigor or grain yield assessments, except for the previously mentioned effect on the flowering date.

3.3. Experiment 2 – Zn seed treatment in different soils

In this experiment, the early vigor of maize was mainly affected by the NP starter fertilization banded at planting and secondarily by the Zn seed treatment. The NP fertilization recorded a significantly higher plant height (on average, +52% and +134% at GS13 and GS34, respectively) than the unfertilized control (Table 4) for each soil, whether subacid or subalkaline, or with low and high P contents, and at both the leaf emission and stem elongation growth stages. Apart from the early vigor at GS13 in the SCL soil, the Zn seed treatment always showed a positive and significant effect on the early vigor of the plant, although this effect was less pronounced than that of the NP starter fertilization. The plant height on average increased by 11% and 7% in the L and SL soils, respectively, at GS13, and by 7%, 7%, and 41% in the L, SL, and SCL soils, respectively, at GS34, compared to the untreated control. A significant interaction between the starter fertilization × the Zn seed treatment occurred in the L soil for the plant height at GS13: the Zn seed treatment enhanced the maize growth in a different way when applied in combination with the NP fertilizer (+14%), compared to the Zn treatment without NP (+9%) (data not shown).

Higher AUCDC values were detected in all the soils for the NP starter fertilization (+22–27%) and, albeit to a lesser extent, for the Zn seed

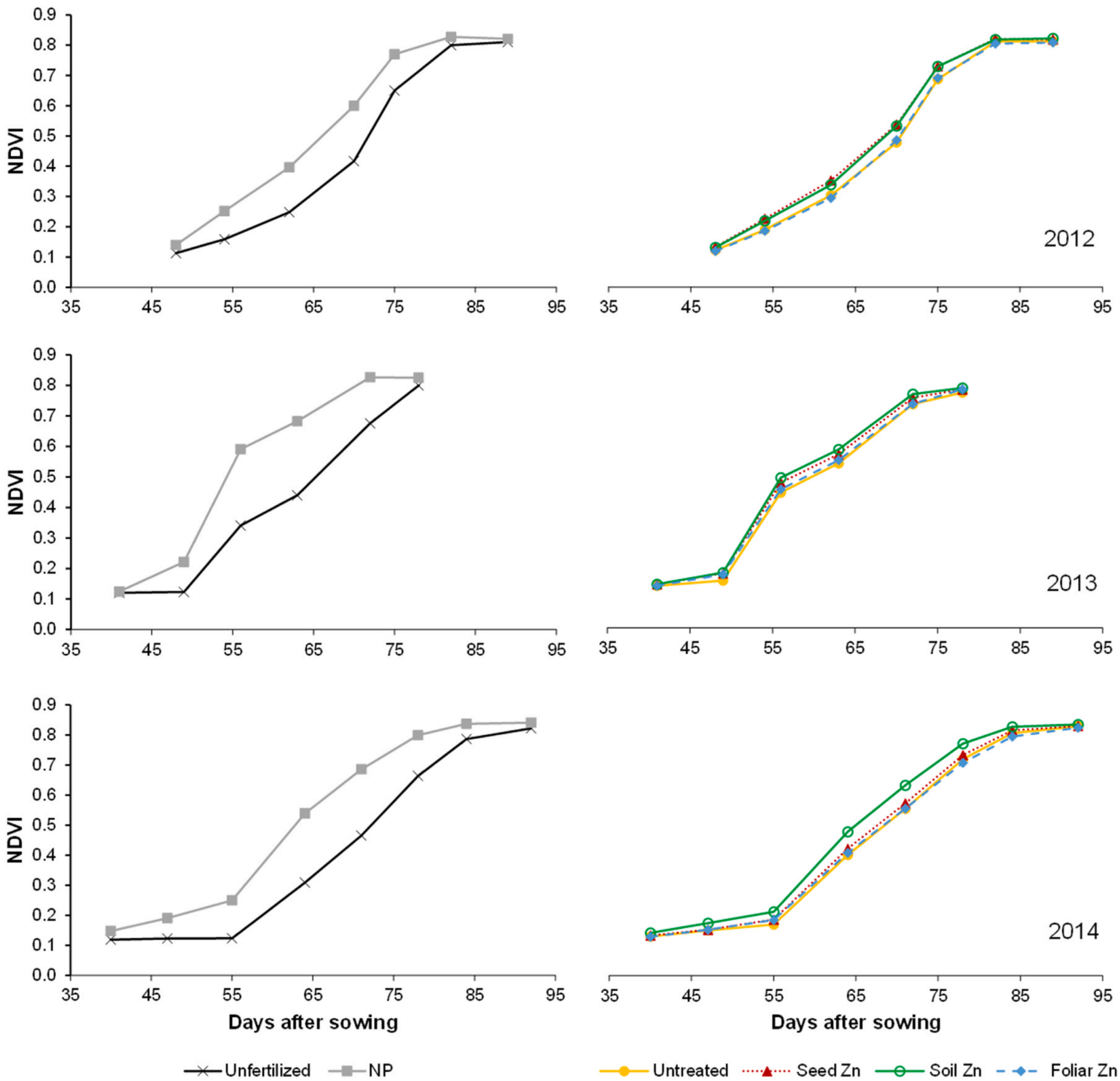

Fig. 1. Effects of the NP starter fertilization (on the left) and the Zn treatments (on the right) on the canopy development of maize, expressed as the Normalized Difference Vegetation Index (NDVI), from the 3-leaf stage to tasseling during the 2012–2014 period for Experiment 1 at Carmagnola (North Italy). Unfertilized: no NP fertilization at planting; NP: 27 kg N ha^{-1} and 69 kg P_2O_5 ha^{-1} applied as diammonium phosphate at planting. Untreated: no Zn application; Seed Zn: Zn applied as a seed coating at a dose of 0.07 kg Zn ha^{-1}; Soil Zn: Zn applied in the maize seed furrows at planting at a dose of 0.7 kg Zn ha^{-1}; Foliar Zn: Zn foliar treatment at the 4-leaf stage at a dose of 0.7 kg Zn ha^{-1}.

Table 4
Effects of the NP starter fertilization and the Zn seed treatment on the plant height at the leaf emission (GS13) and stem elongation (GS34) stages for field Experiment 2 carried out on three natural soils with different textures placed side by side at Carmagnola (North Italy) in the 2012–2014 period.

Factor	Source of Variation	Plant height GS13 (cm)			Plant height GS34 (cm)		
		L	SL	SCL	L	SL	SCL
Starter fertilization (SF)	Unfertilized	8.3 b	9.1 b	7.4 b	23.6 b	34.7 b	13.5 b
	NP	13.0 a	14.1 a	10.6 a	59.9 a	61.1 a	36.8 a
	p-value	***	***	***	***	***	***
Seed treatment (Zn)	Untreated	10.1 b	11.2 b	8.3	40.3 b	46.3 b	20.9 b
	Zn	11.2 a	12.0 a	9.2	43.1 a	49.5 a	29.4 a
	p-value	***	*	n.s.	*	*	***
SF x Zn	*p*-value	**	n.s.	n.s.	n.s.	n.s.	n.s.

Means followed by different letters are significantly different for each factor. The level of significance (*p*-value) is shown in the table: * < 0.01; ** < 0.05; *** < 0.001; n.s, not significant. L: loam, SL: silt loam, SCL: silty clay loam.

treatment (+3–9%), than the controls (Table 5).

In addition to the differences recorded for the plant vigor indices, the banded NP fertilizer and seed Zn applications both significantly affected the flowering date, while only the NP starter fertilization resulted in a significant effect on the grain moisture content. The NP fertilization at planting in the L, SL, and SCL soils reduced the days to flowering by 3.8,

Table 5
Effects of the NP starter fertilization and the Zn seed treatment on the area under the canopy development curve (AUCDC), the date of flowering, expressed as days after sowing (DAS), and the grain moisture content at harvest for field Experiment 2, carried out on three natural soils with different textures placed side by side at Carmagnola (North Italy) in the 2012–2014 period.

Factor	Source of Variation	AUCDC (Σ NDVI-day)			Flowering date (DAS)			Grain moisture (%)		
		L	SL	SCL	L	SL	SCL	L	SL	SCL
Starter fertilization (SF)	Unfertilized	22.3 b	22.3 b	17.9 b	98.7 a	97.0 a	98.8 a	30.1 a	27.9 a	30.3 a
	NP	28.1 a	27.1 a	22.8 a	94.9 b	94.4 b	94.8 b	27.7 b	25.7 b	27.9 b
	p-value	***	***	***	***	***	***	***	***	***
Seed treatment (Zn)	Untreated	24.9 b	24.3 b	19.5 b	97.4 a	96.0 a	97.4 a	28.8	26.6	29.2
	Zn	25.6 a	25.1 a	21.2 a	96.2 b	95.4 b	96.2 b	29.1	27.1	29.0
	p-value	*	***	*	***	***	**	n.s.	n.s.	n.s.
SF x Zn	*p*-value	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Means followed by different letters are significantly different for each factor. The level of significance (*p*-value) is shown in the table: * < 0.01; ** < 0.05; *** < 0.001; n.s, not significant. L: loam, SL: silt loam, SCL: silty clay loam.

2.6, and 4.0 days, respectively, while the Zn seed treatment reduced them by 1.2, 0.6, and 1.2 days, respectively. The shortening of the days to flowering caused by the NP starter fertilization on average reduced the grain moisture content at harvest by 2.3% points, compared to the unfertilized control. A significant effect ($p < 0.001$) of the NP starter fertilization on grain yield and on its components was observed for all the compared soils: the yield rose because of the application of this fertilization practice by 2.1, 1.5, and 1.4 t ha^{-1} in the L, SL, and SCL soils, while KSM increased by 8%, 8.6%, and 9%, respectively, thus suggesting that the earlier flowering occurred under more favorable environmental conditions. In addition, the TKW increased significantly in the L (+2.3%) and SCL (+7.8%) soils (Table 6). Unlike Experiment 1, the grain yield increase and the KSM increase caused by the Zn seed treatment were not significant in any of the soils, while the TKW increased on average by 3% in the L and SL soils, compared to the control. The interactions between starter fertilization and seed treatment were never significant, except for the plant height at GS13 in the L soil.

4. Discussion

This research provides useful information on how different Zn fertilization strategies, i.e. banded in the seed furrow, seed coating, and foliar application affected the early vigor and grain yield of maize crops in a calcareous soil characterized by a low soil Zn-DTPA availability. Furthermore, the effect of a Zn seed treatment was evaluated by considering, at the same time, 3 types of soils, placed side by side, with different pH and Olsen P contents in combination with an NP starter fertilizer banded near the seed furrow at planting.

4.1. Effect of different Zn application strategies

The collected data highlighted that the Zn fertilization significantly enhanced the early vigor and, consequently, the ripening stages and the grain yield in the maize hybrids with different relative maturity, although the observed effect was less marked than the effect of the NP starter fertilization and varied according to the Zn application method. The Zn seed treatment and the Zn banded in the soil seed furrow were the most effective practices in promoting the early vigor of maize in the considered calcareous soil.

Zn covers an essential role for crop development in the early growth stages: in fact, this micronutrient is involved in the activity of such enzymes as alcohol dehydrogenase, which stimulates root development, mainly during seed germination (Cakmak, 2008) and is essential to produce such an auxin hormone as indoleacetic acid, which contributes to cell division (Suganya et al., 2020). Zn is also involved in the carbohydrate metabolism, as it increases the photosynthesis rate (Liu et al., 2016) and the chlorophyll content during the leaf development phase (Zhang et al., 2020). Harris et al. (2007) and Drissi et al. (2015a) reported a positive effect on maize growth in semi-arid areas with a Zn soil deficiency for both seed and soil treatments. Zn deficiency occurs in maize early in the growing season in temperate growing areas due to the low temperatures that limit maize root growth, as well as to prolonged periods of water logging (Alloway, 2008b) and nutrient availability, which lead to interveinal chlorosis and/or white mid-leaf streaking (Cakmak and Kutman, 2018). Furthermore, suboptimal temperatures determine oxidative stress in the exposed seedlings. This leads to an increase in the active oxygen species production involved in cell damage due to membrane lipid peroxidation, protein degradation, enzyme inactivation, and disruption of the DNA strands (Allen and Ort, 2001). Zn, like other micronutrients, represents one of the fundamental co-factors of the enzymes implicated in the detoxification of those compounds that protect plants against the oxidative damage responsible for oxidative cell damage. In addition, Zn is also involved in the carbohydrate metabolism, where it increases the photosynthesis rate, stomatal conductance, the transpiration rate, and the chlorophyll content during the leaf development phase (Liu et al., 2016; Zhang et al., 2020). A low Zn availability affects leaf elongation, and maize growth can be

Table 6
Effects of the NP starter fertilization and the Zn seed treatment on the maize grain yield and the yield components: number of kernels per square meter (KSM), and thousand kernel weight (TKW) for field Experiment 2, carried out on three natural soils with different textures placed side by side at Carmagnola (North Italy) in the 2012–2014 period.

Factor	Source of Variation	Grain yield (t ha^{-1})			KSM (n)			TKW (g)		
		L	SL	SCL	L	SSL	SSL	L	SL	SCL
Starter fertilization (SF)	Unfertilized	12.3 b	11.2 b	12.0 b	4001 b	3633 b	3861 b	349 b	347	344 b
	NP	14.4 a	12.7 a	13.4 a	4319 a	3946 a	4210 a	357 a	353	371 a
	p-value	***	***	***	**	***	*	*	n.s.	***
Seed treatment (Zn)	Untreated	13.1	11.9	12.5	4103	3718	3929	347 b	345 b	357
	Zn	13.6	12.0	12.9	4217	3861	4141	358 a	355 a	359
	p-value	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**	*	n.s.
SF x Zn	*p*-value	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Means followed by different letters are significantly different for each factor. The level of significance (*p*-value) is shown in the table: * < 0.01; ** < 0.05; *** < 0.001; n.s, not significant. L: loam, SL: silt loam, SCL: silty clay loam.

constrained by the reduction of the photosynthetic capacity, due to a net carbon dioxide assimilation rate decrease of as much as 20% as a direct consequence of Zn deficiency (Mattiello et al., 2015). The highest NDVI and AUCDC values recorded in this study were consistent with these effects in the first growth stages. Because of the better early root growth and therefore early seedling establishment, the seed and soil Zn fertilization methods significantly affected the maize flowering date and reduced the number of days between sowing and silking (Drissi et al., 2015a). Zn application could enhance the number and size of ears by increasing the plant photosynthetic rate and providing more photosynthates, due to a higher leaf area index and leaf longevity (Liu et al., 2016). Moreover, Zn deficiency negatively affects the reproductive organs, due to the enhanced formation of abscisic acid in the plant, which causes premature abscission of the leaves and flower buds, as well as disruption of the development and physiology of the anthers and pollen grains. It can also affect the pollination process by impacting pollen tube formation (Alloway, 2008b). Our results showed an increased number of KSM (+5%), albeit only in the Zn soil treatment, probably due to the rise in the pollination rate resulting from an earlier flowering (Potarzycki and Grzebisz, 2009; Liu et al., 2020), while an overall increase in grain yield was reported for both the seed and soil Zn distributions. Stepic et al. (2022) reported a greater yield enhancement from a soil Zn application than from a seed treatment.

The Zn foliar application at the 4-leaf stage did not otherwise affect the plant growth or grain yield of the maize, thus confirming the results reported by Golden et al. (2016). According with the "4Rs" nutrient management stewardship strategy (apply the right source at the right rate, at the right time, and in the right place), a possible explanation for this lack of effect could be related to the application time. An early Zn application (soil and seed) can improve the early growth of seedlings during the most critical stages, under low soil temperature conditions. However, a late application, such as a foliar treatment at the 4-leaf stage, it is not able to improve plant development in Zn sufficient soil. Furthermore, this treatment may not be as effective as a later one (6–8 leaf stage), when the foliar uptake of the plant is expected to be higher. Only a few studies, carried out in Zn deficient soils (Martínez-Cuesta et al., 2021; Drissi et al., 2015b), have shown improvements in the grain yield from a foliar distribution of Zn at the 6–8 leaf stage, when the leaf area index is higher and the Zn amount that reaches the maize plants increases. Later applications, e.g. from tasseling onward, are only able to increase the Zn concentration in the grain (biofortification), without any significant increase in grain yield (Wang et al., 2012). In addition, the effectiveness of an early foliar Zn application could be also related to the different Zn sources (Golden et al., 2016). Potarzycki and Grzebisz (2009) observed a positive grain yield increase after applying Zn through a foliar application at the initial growth stages using Zn as oxysulphate and ammonium acetate, while a Zn-chelate fertilizer was sprayed in the present study.

This study underlines the importance of the Zn application method on plant development in the early stages: the seed treatment, and the banded distribution in the soil seed furrows were the most effective practices in promoting the early vigor of maize and, consequently, the overall agronomic benefits. Although the amount of the micronutrient applied in the seed coating treatment was 10 times lower than in the soil distribution treatment, it was readily available for the seedlings from the very first development stages. Since it is easier for farmers to apply a seed treatment, this practice results to be efficient, feasible, and cost-effective to support the early growth of maize, even under cool conditions (Imran et al., 2013; Gómez-Muñoz et al., 2018).

Moreover, the type of maize hybrid can also affect the effectiveness of a Zn application: modern cultivars respond better to zinc applied before sowing that older one, which instead respond better to a foliar treatment (Potarzycki et al., 2016). Mohsin at al. (2014) observed a significant interaction between the FAO Class of the tested hybrids and the Zn fertilization: a late maturity hybrid (115–125 days) recorded a more marked response to the Zn application, i.e. longer cob length, and higher leaf area index, crop growth rate, and net assimilation rate values than an earlier one (100–105 days). We instead observed no differences in terms of Zn fertilization effectiveness, between the three maize genotypes used in this study, despite the slight differences in their relative maturity.

4.2. Comparison of the effects of Zn and NP starter fertilization on maize growth

The results of our field experiments confirmed the numerous and great benefits of NP starter fertilization as a crop practice to enhance crop development in the early stages (Jing et al., 2010; Zhang et al., 2023). The sub-surface placement of NP fertilizer close to the seed furrows at planting provides immediate nutrient access to emerging maize roots and increases the concentration of such immobile nutrients as P, even in soils with a high P and N availability (e.g., SL soils). In a previous study (Blandino et al., 2022), we observed a synergistic effect on early plant growth for the combined application of N and P. The supply of N and P together in fact enhances the root density and extensions (Gao et al., 2019), increases leaf expansion (Battisti et al., 2023), and enhances the photosynthetic rate (Ma et al., 2014), thereby contributing to boosting the plant biomass in the early vegetative stages, as observed for a more rapid increase in NDVI, expressed as the AUCDC index, and the plant height, even before the stem elongation stages (Roth et al., 2006). Our data are in agreement with those of Kaiser et al. (2016), who found that an NP starter fertilization reduced the number of days between planting and silking, with a consequent reduced grain moisture content at harvest. In addition, the better radiation-use efficiency and the higher percent radiation interception led to a grain yield increase, which was related to the rise in KSM and TKW (Blandino et al., 2022). Even though Bermudez and Mallarino (2002) reported a higher plant growth increase when the starter fertilizer was applied to soil with a low P content, in our study, an improvement in grain yield also occurred in the soils characterized by a high P content (e.g. the SL soil), thereby confirming data reported by Roth et al. (2006).

Carrying out a direct comparison under the same conditions, we observed that the Zn fertilization affected the early development in a similar way, albeit to a lesser extent, to the NP starter fertilization, thereby confirming our hypothesis. Furthermore, the Zn application as a seed or soil treatment resulted in a further quantifiable enhancement of the early vigor of maize in all the considered situations in addition to the NP starter fertilizer effect. Although no specific information was available on the plant development, the combined application of Zn + NP increased the final maize biomass and grain yield more in both the sub-acid and calcareous sub-alkaline soils than their separate application (Drissi et al., 2015c; Imran et al., 2016). Sánchez-Rodríguez et al. (2021) also found an increased final grain yield, due to a greater number of seeds per plant and TKW in a Zn + P treatment than for P and Zn applications on their own.

4.3. Effects of a Zn application on soils with different chemical and physical properties

Our work provides new insights into the interaction between Zn and P, considering soils with different Olsen P contents and other chemical properties. Although some studies (Alloway, 2008a; Hafeez et al., 2013; Akhtar et al., 2019), carried out on calcareous soil with high available P, reported a maize Zn deficiency, the results of Experiment 2 showed that neither the high pH (≥ 8) of the L and SCL soils nor the high soil Olsen P content (37 mg kg^{-1}) of the SL soil negatively influenced the effect of the Zn application as a seed coating. Thus, the effect of Zn in promoting the early development of plants was similar for the three compared soils. Moreover, we observed a further quantifiable enhancement of the early vigor of maize for the combination of the NP starter fertilizer and the Zn application in both experiments (1 and 2), and for all the considered agronomic conditions, thereby confirming a positive and consistent role of this practice in different production situations.

These results could depend on the used fertilization method: the application of Zn as a seed coating shifts the possible involvement of this micronutrient in the soil chemical dynamics. Similarly, the concentrated application of an NP starter fertilizer in bands, close to the plant furrow, guarantees a higher availability for the seedlings, and minimizes the interactions of nutritional elements with the soil properties and the dynamics of other nutrients.

In our experiment, the effect of such fertilization strategies highlighted an additive, but not synergistic, effect on crop development. Recena et al. (2021) suggested a positive effect of soil P on Zn availability to plants, since the Zn uptake by crops resulted to be higher for an increased Olsen P. The use of an N fertilizer that contains ammonium (NH_4^+) could improve the uptake of other macro- and micro-nutrients and their use efficiency in calcareous soils (Jing et al., 2012), as a consequence of rhizosphere acidification due to the release of protons from the roots following N-NH_4^+ acquisition (Jing et al., 2010; Suganya et al., 2020).

5. Conclusions

The study has highlighted that the Zn fertilization of maize seedlings can lead to a significant improvement in the early vigor of maize plants in temperate growing areas. Although the gains in early vigor and, consequently, the reduction in the number of the days between planting and silking and the enhancement in the grain yield were less marked than those observed for the adoption of NP starter fertilization, the application of this micronutrient led to positive and consistent effects for different hybrids, growing seasons, and soils. Furthermore, the study underlined the possibility of adopting the Zn fertilization practice at planting, in combination with the sub-surface banded distribution of NP fertilizer, to achieve a greater enhancement of plant growth and the consequent agronomic benefits.

An early Zn application, involving a seed treatment or soil distribution in seed furrows, resulted to be the most effective practice, while a foliar application at the 4-leaf stage did not result in any significant plant development benefits. Among the possible application methods, a Zn seed treatment is considered a sustainable, low-cost, low-rate strategy, which is easy for farmers to apply in order to support seedling development, especially in those production situations in which the initial development of maize could slow down.

Future studies should focus on evaluating whether the Zn-seed applied technique can positively influence plant development and grain yield, even in soils characterized by high Zn plant availability, and whether a higher dosage could further improve the early vigor and yield of maize or induce Zn phytotoxicity.

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CRediT authorship contribution statement

Capo Luca: Data curation, Investigation, Writing – original draft. **Battisti Michela:** Methodology, Writing – review & editing. **Blandino Massimo:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Visualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Massimo Blandino reports financial support was provided by Piedmont Region.

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.fcr.2023.109245.

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