**RESEARCH ARTICLE** 



# **Exploring options for increasing maize yields and grain Zn concentrations in sub-Saharan Africa**

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# Abstract

*Background* Increasing zinc (Zn) concentrations in maize grains could contribute to alleviating widespread human Zn deficiency in sub-Saharan Africa (SSA). However, trade-offs between grain Zn concentrations and maize yields have been observed.

*Scope* Using data from researcher-managed, onfarm and on-station field trials in Kenya, Zambia and Zimbabwe, we aimed (i) to confirm whether this trade-off is found in current farming systems in SSA and (ii) to explore whether genotypic and management options, relevant for the African context, can increase both yields and grain Zn concentrations across several environments.

*Results* An overall negative, but weak relation between maize yields and grain Zn concentrations was found. High yields and high grain Zn concentrations did not co-occur. The negative relation between grain Zn concentrations and yields cannot be

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H. R. Pasley CSIRO, Toowoomba, Australia bypassed by selecting one of the commercially available varieties used in this study. Nitrogen application increased yields, but had contrasting effects on grain Zn concentrations depending on variety and site. Grain Zn concentrations were positively related with soil organic carbon and P and K availability.

*Conclusions* Attaining grain Zn concentrations above the HarvestPlus target of 38 mg kg<sup>-1</sup>, considered adequate for reducing human Zn deficiency, with current commercially available maize varieties and presented management options, is not possible without compromising yield levels. Increasing soil organic matter content and balanced application of N, P and K fertilisers could increase grain Zn concentrations. These practices likely will also increase yields and could be a viable option to bypass the trade-off between maize yields and grain Zn concentrations.

# Introduction

Human zinc intake needs to increase in sub-Saharan Africa (SSA) in order to alleviate widespread and severe health problems related to zinc deficiency. An estimated 40% of the African population does not have sufficient intake of zinc (Zn). Zinc deficiency can lead to several diseases, growth and

developmental issues and cognitive problems (Das and Green 2016; Joy et al. 2014). The prevalence of Zn deficiency can be attributed to unbalanced diets, rich in staple cereal crops and low in animal products, as well as low Zn availability in soils leading to low Zn concentrations in the crops that grow on them (Cakmak and Kutman 2018; de Valença et al. 2017; Joy et al. 2014). Increasing Zn concentrations in the edible parts of staple crops such as maize can contribute to reaching a sufficient dietary intake of Zn in African countries (Joy et al. 2015). However, Zn concentrations in cereals are inherently low and have declined even further as a result of long-term breeding efforts to increase yields (Cakmak and Kutman 2018). In the past two decades, Zn concentrations of staple crops have increasingly received agronomists' attention. Agronomically, Zn concentrations above 20 mg kg<sup>-1</sup> are considered adequate for optimal cereal production (Alloway 2008; Cakmak and Kutman 2018). The HarvestPlus biofortification programme, however, has set a target of 38 mg Zn kg<sup>-1</sup> maize grains in order to alleviate Zn malnutrition (Bouis and Welch 2010).

While low grain Zn concentrations are a concern, closing the large yield gaps faced by African smallholder farmers, a key contributor to food insufficiency (Van Ittersum et al. 2016), is prioritised. Although micronutrient bioavailability-induced yield limitations occur (Kihara et al. 2017; Wortmann et al. 2019), main solutions for closing yield gaps are considered to be fertilisation with macro- (and secondary) nutrients, improved varieties, as well as good agronomic practices (Ichami et al. 2019; Tittonell and Giller 2013). Farmers are unlikely to engage in management practises that increase grain Zn concentrations if they do not also increase yields. Approaches that improve grain yields and increase grain Zn concentrations simultaneously may therefore be best suited in helping African farmers address these two key concerns.

Efforts for increasing grain Zn concentrations have mainly focussed on biofortification through breeding for higher grain Zn concentrations, fertilisation with Zn or a combination of both strategies (de Valença et al. 2017). Breeding for cultivars with higher Zn concentrations is thought to be more cost-effective than a fertiliser based approach (Joy et al. 2015). Sufficient genetic variation has been demonstrated to increase maize grain Zn concentrations through breeding, but genetic trade-offs between yield and grain Zn concentrations have been demonstrated (Bänziger and Long 2000; Garcia-Oliveira et al. 2018).

Maize is considered very sensitive to low soil Zn bioavailability (Alloway 2008). Several studies have shown that the use of Zn fertilisers can increase yields (Chilimba et al. 1999; Eteng et al. 2014; Kihara et al. 2017; Vanlauwe et al. 2015; Wendt and Rijpma 1997), grain Zn concentrations (Joy et al. 2015; Kihara et al. 2020; Van Eynde et al. 2023) or both (Liu et al. 2017; Manzeke et al. 2014, 2020). However, fertilisation with Zn does not always result in increased yields (Chilimba et al. 1999; Kihara et al. 2016; Lisuma et al. 2006; Rurinda et al. 2020; Uyovbisere and Lombin 1990; Van Eynde et al., 2023; Wendt and Rijpma 1997), likely depending on the soil Zn availability status. Based on soil testing, it is currently difficult to assess whether Zn availability is yield-limiting (Van Eynde et al., 2023). Economically, Zn fertilisation is an unattractive option for farmers if yields do not increase.

Nitrogen (N) fertilisation, either with or without Zn, has been found to increase yields and grain Zn concentrations in cereals (Kutman et al. 2011; Losak et al. 2011; Xue et al. 2014, 2019). In cereals, Zn in the grain is associated with proteins (Cakmak and Kutman 2018). In maize, N fertilisation leads to a small increase in grain protein content and large increase in biomass i.e. carbohydrates, which could potentially lead to dilution of grain Zn concentrations (Bänziger and Long 2000). A recent global metaanalysis by Zhao et al. (2022) however showed that maize grain Zn concentrations are maintained when yields increase as an effect of N fertilisation, as the dilution effect is counteracted by increased remobilisation of Zn from the stover during grain filling. This meta-analysis also revealed that limited data from SSA are available on grain Zn concentrations in relation to N fertilisation. Given the low soil Zn availability in large parts of SSA (Alloway 2008), application of N without addition of Zn could potentially also lead to excessive growth and dilution of grain Zn concentrations (Cakmak and Kutman 2018; de Valença et al. 2017; Zhang et al. 2021).

A trade-off between maize yields and grain Zn concentrations has been reported. The first aim of this study is to confirm whether this trade-off is found in current farming systems in SSA. The second aim of

this work is to explore whether genotypic and management options, relevant for the African context, can increase both yields and grain Zn concentrations across several environments. Data from several field experiments focussing on the effects of variety, N fertilisation, Zn fertilisation and NPK fertilisation on maize yield across different sites in Kenya, Zambia and Zimbabwe were analysed. The outcomes of this study will help to develop an integrated approach for increasing yield levels while at the same time increasing grain Zn concentrations in maize.

# Materials and methods

Field trials

# Embu, Kiboko, Harare

The first set of researcher-managed on-station field trials was executed in Embu (-0.52, 37.48) and Kiboko (-2.22, 37.70) in Kenya, and in Harare (-17.72, 31.08), Zimbabwe. These trials focused on the effect of N fertilisation and maize variety on plant uptake and soil depletion of N and other nutrients. Complete details of these trials are described in Pasley et al. (2019). Briefly, experiments consisted of a split-plot design, replicated four times, with N rate as the main plot and variety as the sub-plot. In each site, six varieties were used, with one duplicated variety in Embu and Kiboko as well as in Embu and Harare. Of the six varieties, three were improved in regard to high tolerance to stress (drought, heat and/ or nutrient), whereas the other three were not. All varieties were commercially available and commonly used by farmers in Kenya and Zimbabwe. Fertiliser application rates were 0, 30, 60, and 90 kg N  $ha^{-1}$  in Embu and 0, 40, 80, and 160 kg N ha<sup>-1</sup> in Kiboko and Harare. A basal application of P at a rate of 20 kg ha<sup>-1</sup> was also applied. Plant and soil samples were taken in the 2013 short rainy season in Kiboko, the 2014/2015 season in Harare and the 2015 short rainy season in Embu. Soil samples were collected per sub-plot in most cases.

# Sidindi

The second set of researcher-managed on-farm field trials was executed in Sidindi (0.15, 34.40), Kenya.

These trials (executed from 2013 to 2018) focused on the spatial and temporal patterns of maize yield responses to N, P and K omission and the recovery of past N, P and K applications. Complete details of the trials are described in Njoroge Kinyanjui (2019). Data on yields and grain Zn concentrations were available for 10 farms, collected in the 2016 and 2018 long rainy seasons. In 2016, nutrient omission experiments included five treatments namely: a control (no fertiliser), PK, NK, NP and NPK, with nutrients applied at the rates of 150 kg N ha<sup>-1</sup>, 40 kg P ha<sup>-1</sup> and 60 kg K ha<sup>-1</sup>. Treatments were not replicated on-farm. In 2018, all five plots at each farm received NPK applied at the rates of 150 kg N ha<sup>-1</sup>, 40 kg P ha<sup>-1</sup> and 60 kg K ha<sup>-1</sup>. Short season maize variety DK8031 was planted at all farms and in both years.

# Kenya, Zambia, Zimbabwe

The third set of researcher-managed on-farm field trials was executed on 19 locations in Kenya (5 farms, 5 replications each, coordinates: 0.15, 34.40), Zambia (4 farms, 4 replications each, coordinates: -13.36, 28.46) and Zimbabwe (10 farms, 6 replications each, various locations). Complete details of these trials, which focussed on availability of several micronutrients, are described in Van Eynde et al. (2023). The trials were laid out as a randomized block design. For this study, only the Zn omission treatment was included. Briefly, nutrients N, P, K, S, Ca, Mg, Cu and B were applied at relatively high rates to prevent yield-limitations. Fertiliser application rates differed among countries. The maize varieties also differed among countries: DK8031 was planted in Kenya, Afric1 in Zambia and SC637 in Zimbabwe.

#### Plant and soil analysis

Plant samples were analysed for the following nutrient contents: N, P, K, S, Zn, Cu and Mn. Soil samples were analysed for the following parameters:  $pH-H_2O$ , soil organic carbon (SOC), nutrient availability as determined by a Mehlich 3 (M3) extraction and clay content. For details on analytical methods, see Pasley et al. (2019), Njoroge Kinyanjui (2019) and Van Eynde et al. (2023). Median values for each of the soil properties of the N application trials are presented per site in Table 1. Table 1Soil characteristicsfor Embu, Kiboko andHarare (0-30 cm), valuesrepresent medians

		_	${\rm g \ kg^{-1}}$	%	Mehlich 3 (mg kg <sup>-1</sup> )				
Site	Soil type	pН	SOC	Clay	Р	K	Zn	Cu	Mn
Embu	Nitisol	5.1	28	39	16	343	14.8	1.1	270
Kiboko	Ferralsol	7.8	12	24	77	387	2.1	3.9	70
Harare	Luvisol	5.7	13	42	15	101	11.9	10.0	135

# Data analysis

#### Data cleaning

Data with Harvest Indices (HI) below 0.25 were removed from the dataset, as these crops were considered to have failed and therefore unsuitable for addressing the objectives of this study. Yield was expressed at a standardised moisture content of 13%. Grain concentrations, as well as nutrient uptake are expressed based on dry weight. Nutrient uptake is calculated as the sum of stover and grain biomass, multiplied with their respective nutrient concentrations.

#### Analyses

To test for differences in yield and grain Zn concentration among varieties within each site, linear mixed effect models (LME) were fitted to the first set of field trials. N application rate, maize variety and their interaction were set as fixed factors and N application rate nested in block as a random factor (ran $dom = \sim 1|Block/N rate)$  to account for the split-plot design. In case the interaction among the fixed factors was not significant, the model was rerun without the interaction factor. This analysis was done separately for Embu, Kiboko and Harare. In addition, correlations among soil parameters, yield and grain Zn concentrations were explored. The soil factors earlier associated with Zn availability, i.e. Zn-M3, pH and SOC (Alloway 2008; Chilimba et al. 1999; Van Eynde et al., 2023), were used for this analysis.

#### Software

Statistical software R, version 3.6.3 (R Core Team 2020) was used for all analyses. Plots were made with the ggplot function from the ggplot2 package, version 3.3.2 (Wickham 2016). LMEs were fitted using the lme function from the nlme package (Pinheiro et al. 2013). Significance of factors was tested with the

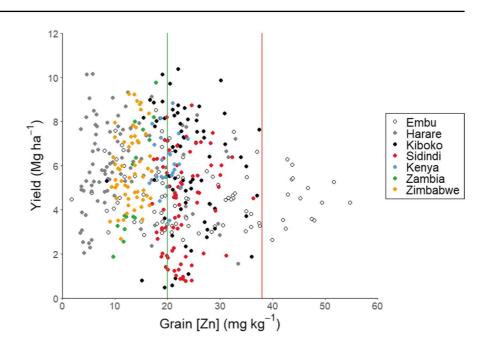
Anova function from the car package (Fox and Weisberg 2019). Individual differences were analysed with Tukey's post hoc test, using the glht function from the multcomp package, version 1.4-17 (Hothorn et al. 2008). Normality of model residuals was checked with the Shapiro-Wilk test using the shapiro.test function from the stats package, version 4.1.0 (R Core Team 2020). Homogeneity of variances was tested with Levene's test, using the leveneTest function from the car package. In case assumptions of normality of residuals or homogeneity were violated, the data were transformed using log10-transformation. Correlations were explored using the rcorr function from the Hmisc package, version 4.5-0 (Harrell et al. 2021).

#### Results

# Overview

Maize yields ranged between 0.5 and 10.4 Mg ha<sup>-1</sup> across all field trials, while grain Zn concentrations ranged between 1.7 and 55 mg kg<sup>-1</sup> (Fig. 1). Across all data, yields and grain Zn concentrations were negatively correlated (r=-0.16, p=0.001). These results could point towards a trade-off between yields and grain Zn concentrations, caused by differences in genotype, environments and/or management. Some clustering per site was visible and the overall negative correlation between yields and grain Zn concentrations was likely caused by differences among sites. Indeed, negative correlations were not present within individual sites (all p-values above 0.17) and was positive for the trials in Zambia (r=0.89, p<0.001). Generally, combinations of high yields (> 6 Mg  $ha^{-1}$ ) and high grain Zn concentrations (> 38 mg kg<sup>-1</sup>) were absent. Only 15 out of 301 datapoints for which Zn was not applied, met the HarvestPlus grain Zn target concentration of 38 mg kg<sup>-1</sup>. These 15 datapoints all corresponded to maize crops grown in Embu, and consisted of four different varieties and three N

**Fig. 1** Maize yields plotted against grain Zn concentrations per site or country. The green line represents the adequate Zn concentration for optimal growth of maize, the red line the HarvestPlus grain Zn target concentration for maize



application rates (30, 60 and 90 kg  $ha^{-1}$ ). In all 15 cases, yields did not surpass 6.3 Mg  $ha^{-1}$  and were relatively low compared to the maximally obtained yield in these trials.

# Genetic factors

To compare relations between yields and grain Zn concentrations among different maize varieties, data from the N application trials in Embu, Kiboko and Harare were used. Analysis was done per site, as there was limited overlap in varieties. Differences in grain Zn concentrations and yields were found among varieties in Embu, but not in Harare and Kiboko (Table 2; Fig. 2). Although the ANOVA output (Table 2) suggested that maize variety was a significant factor affecting yields in Kiboko, post hoc analysis did not identify significant differences among maize varieties.

In Embu, four varieties had significantly higher grain Zn concentrations compared to the other two (Fig. 2). Two of those four varieties were improved, the two others were not. However, these four varieties had significantly lower yield levels compared to the variety with the lowest grain Zn concentrations (DUMA43). Furthermore, the variety with the highest grain Zn concentrations (WH403) had the lowest yields. These results could point towards a trade-off between yields and grain Zn concentrations.

**Table 2** Results of the ANOVA analysis, with *p*-values for main and interaction effects. In case interactions were not significant, only significance of main factors was tested

Site	Main factors	Yield	Grain [Zn]	
Embu	N rate	0.08	< 0.001	
	Variety	< 0.001	< 0.001	
	N rate * variety	n.s.	n.s.	
Harare	N rate	< 0.001	0.23	
	Variety	0.90	0.34	
	N rate * variety	n.s.	0.02	
Kiboko	N rate	< 0.001	0.01	
	Variety	0.01	0.98	
	N rate * variety	n.s. <sup>a</sup>	n.s. <sup>a</sup>	

<sup>a</sup>Due to HI restrictions, limited data were available for the 0 N treatment (n=3, only one variety). To test the interaction between N rate and variety, data from the 0 N treatment were removed

# Environmental factors

To explore whether soil characteristics explain grain Zn concentrations and yields, correlations among these factors were explored using data from the Embu, Kiboko and Harare trials. For the Sidindi trials, no soil data were available. Data from the Kenya, Zambia and Zimbabwe trials have been presented in Van Eynde et al. (2023).

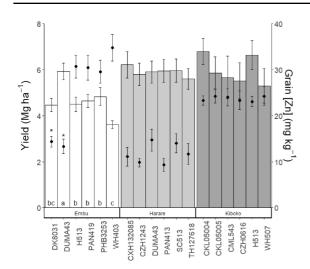


Fig. 2 Yields (bars) and grain Zn concentrations (points) per variety for Embu, Harare and Kiboko (all N rates). Letters indicate significant yield differences within sites, asterisks indicate significant differences in grain Zn concentrations within sites. Error bars represent standard errors

Yields were positively correlated with pH and negatively correlated with Zn-M3 and SOC (Fig. 3A-C). These correlations most likely do not indicate causality, as yields were strongly determined by N application rate (section 3.4). In addition, the soil parameters, mainly pH and SOC, showed strong clustering per site. Correlations could consequently be affected by other site-related factors, such as variety or climate. Grain Zn concentrations were only (positively) correlated with SOC (Fig. 3F). When correcting grain Zn concentrations through dilution, SOC levels were also positively related with grain Zn concentrations.

To exclude the effect of maize variety on relations between soil parameters, yields and grain Zn concentrations, data from two duplicated varieties (H513 and DUMA43) were investigated, using the same N application treatment (0 kg ha<sup>-1</sup>). For variety H513, grown in Embu and Kiboko, data from the 0 N treatment in Kiboko had been removed based on HI restrictions, so no comparison was possible. For variety DUMA43, grown in Embu and Harare, no differences in yield (p=0.798) and grain Zn concentrations (p=0.832) were found. Differences in Zn-M3 and pH between sites were limited (Table 1). Although SOC levels differed between sites, data were insufficient to draw conclusions on the effect of soil parameters on grain Zn concentrations.

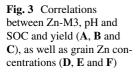
Identical N treatments were used in Kiboko and Harare and differences among varieties were absent (Fig. 2). This allows for a comparison between these sites, with regard to a potential effect of soil characteristics on yield and grain Zn concentrations. The soil in Kiboko was alkaline (pH 7.8) and Zn-M3 levels were low (Table 1; Fig. 3). The soil in Harare was acidic (pH 5.7) and was characterised by relatively high Zn-M3 levels; SOC contents were similar in both sites. This suggests that soil Zn availability was higher in Harare and higher grain Zn concentrations would therefore be expected compared to Kiboko. However, average grain Zn concentrations in Kiboko  $(24 \text{ mg kg}^{-1})$  were higher than Harare  $(12 \text{ mg kg}^{-1})$ , whereas yield levels were similar (Fig. 2). In addition, Zn uptake was higher (p < 0.001) for the maize crops grown in Kiboko (between 171 and 247 g  $ha^{-1}$ , depending on the N application rate) compared to Harare (between 91 and 215 g  $ha^{-1}$ ). These findings do not point towards the relevance of the tested soil characteristics, but rather to other factors affecting grain Zn concentrations.

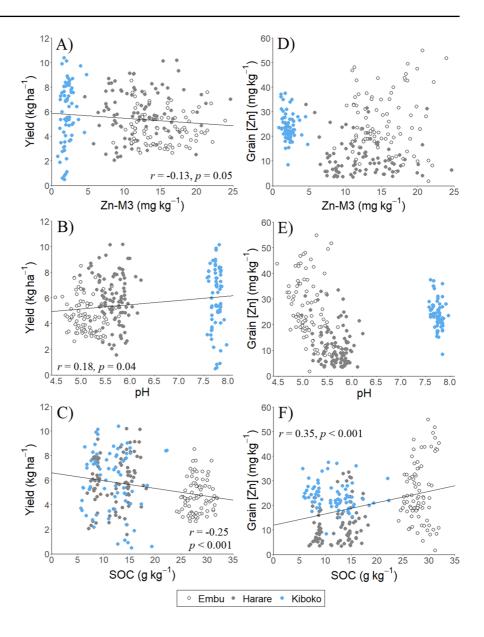
#### Management factors

To analyse the effect of N fertilisation on yields and grain Zn concentrations, data were analysed per site, as different N application levels were applied and most varieties were not duplicated. Main effects of N application rate on yields were positive in Kiboko (p < 0.001), Harare (p < 0.001) and Embu (p=0.08; Table 1, Fig. 4).

The effect of N application rate on grain Zn concentration was less straightforward and had contrasting effects. Main effects of N application treatment on grain Zn concentrations were significant for Embu and Kiboko, but not Harare (Table 1). In Embu, contrasting effects of N treatment on grain Zn concentrations were found. For four varieties, an increasing trend of grain Zn concentrations with N application rate was found, whereas for the other two varieties, grain Zn concentrations remained constant (a representative variety of both groups presented in Fig. 4). In Kiboko, generally a negative trend of grain Zn concentrations with N application rate was found.

The response to N application of varieties DUMA43 and H513 was tested in two

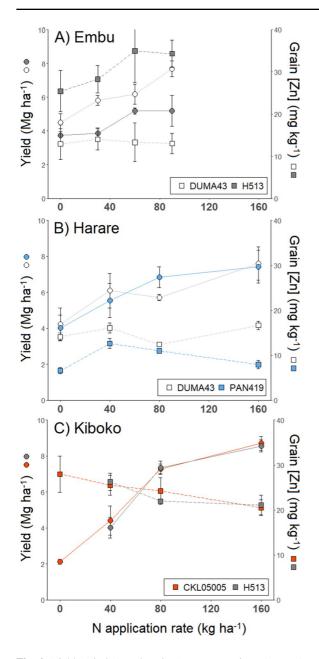




locations. For variety H513, contrasting trends were observed: an increase in both yields and grain Zn concentrations with N application rate was visible in Embu (Fig. 4A), whereas a trade-off between both parameters was visible in Kiboko (Fig. 4C). The response to N application of variety DUMA43 seems consistent, as yields increase and grain Zn concentrations are maintained at a similar level in both Embu (Fig. 4A) and Harare (Fig. 4B).

# Discussion

In this study, combinations of high yields and grain Zn concentrations above target grain Zn concentrations were not observed. This could indicate a potential trade-off between maize yields and grain Zn concentrations as also reported by Bänziger and Long (2000) and White and Broadley (2011) in commercially available varieties grown under different



**Fig. 4** Yields (circles) and grain Zn concentrations (squares) plotted against N application rate. For each site, results from two representative varieties are presented (different colours). Error bars represent standard error

environmental and management conditions in SSA. Grain Zn concentrations above target grain Zn concentrations (38 mg kg<sup>-1</sup>) were only observed when yields were below 6.3 Mg ha<sup>-1</sup>. These findings are in line with Manzeke et al. (2014, 2020), who reported maize grain Zn concentrations up to 35-40 mg kg<sup>-1</sup>,

corresponding with relatively low yield levels (below  $4 \text{ Mg ha}^{-1}$ ).

As our dataset comprised several varieties, grown on several soils and under different fertiliser treatments, there can be multiple causes for this observed trade-off: genotype, environment and/or management. Due to the nature of the dataset, it is impossible to separate the roles of these three mechanisms. However, these data provide an overview of options within common practices for increasing yields and grain Zn concentrations.

#### Genetic factors

The set of commercially available varieties used in this study seem to provide no option for increasing both yields and grain Zn concentrations by selecting a particular variety. The similarity in yields and grain Zn concentrations among varieties in Harare and Kiboko indicate that the genetic factors play a relatively minor role at these sites. Findings from the Embu trials show that selection of variety can matter when it comes to increasing grain Zn concentrations: four varieties had higher grain Zn concentrations (above 30 mg  $kg^{-1}$ ) compared to the other two varieties (~13-14 mg kg<sup>-1</sup>). However, the varieties with high grain Zn concentrations had relatively low yields. The relatively low yields in Embu likely are caused by the N application rates, which did not exceed 90 kg ha<sup>-1</sup>. However, increasing N application rates could lead to a reduction in grain Zn concentrations due to dilution, as shown for variety H513 grown in Kiboko.

#### Environmental factors

The negative relation between yields and grain Zn concentrations was absent within individual sites. The clustering of data per site in Fig. 1 indicates that environmental factors, such as soil and/or climate, affect the trade-off between yield and grain Zn concentrations, as also shown by Bänziger and Long (2000). None of the soil factors earlier associated with Zn availability (i.e. Zn-M3, pH and SOC), were related with both high yields as well as high grain Zn concentrations (Fig. 3). However, the clustering in soil parameters hampers drawing meaningful conclusions from statistical relations. For instance, the negative correlation between yield and SOC can most likely be explained by the low

N application rates in Embu, where the highest SOC levels were found (Fig. 3C, Table 1). However, our results indicate that grain Zn concentrations increased with SOC levels, in agreement with Gashu et al. (2021), Zhao et al. (2022). and Van Eynde et al. (2023). In contrast to the findings of Van Eynde et al. (2023), Manzeke et al. (2012) and Kihara et al. (2020), grain Zn concentrations were not related with extractable soil Zn. This may have been because soil Zn availability was not a limiting factor, as most soil Zn-M3 concentrations were above critical levels (Chilimba et al. 1999; Cuesta et al. 2021), except for Kiboko.

Results of Kiboko and Harare may indicate that other soil factors are relevant in the trade-off between yield and grain Zn concentrations. In Kiboko, consistently higher grain Zn concentrations and plant uptake were observed compared to Harare, despite similar yield levels (Fig. 2). We cannot exclude the role of genetic factors in these findings, as no identical variety was grown in both locations, but the role of environmental factors in these results seem likely. The higher grain Zn concentrations and plant uptake in Kiboko cannot be explained by soil Zn availability, which was much lower in Kiboko than Harare (Table 1).

We hypothesise that the higher Zn uptake and grain concentrations in Kiboko can be explained by two mechanisms: facilitation by P and/or K and competition with Cu and/or Mn. We hypothesise that maize Zn uptake was higher in Kiboko than Harare due to higher levels of P-M3 and K-M3 (Table 1). The higher availability of soil K and P in Kiboko may have enabled a larger root system (de Valença et al. 2017), which can explore a larger volume of soil and therefore access more Zn, as well as other nutrients. This hypothesis is supported by a study with rice, where soil K concentrations were positively correlated with root length and diameter (de Almeida Carmeis Filho et al. 2017). Alternatively, the higher levels of Cu-M3 and Mn-M3 in Harare compared to Kiboko (Table 1) may have reduced Zn uptake through competition (Adiloglu 2007; Alloway 2008; Singh and Steenberg 1974).

#### Management factors

#### N application

Application of N increased yields, but had contrasting effects on grain Zn concentrations (Fig. 4). Grain Zn concentrations can increase (Embu), remain constant (Embu and Harare) or decrease (Kiboko) with increasing N application rates. Our results are in line with a recent meta-analysis, showing that maize grain Zn concentrations can be both positively and negatively affected by N fertilisation (Zhao et al. 2022). Several other studies reported that N fertilisation increased maize grain Zn concentrations (Losak et al. 2011; Manzeke et al. 2020; Xue et al. 2014, 2019), whereas others report the opposite (Feil et al. 2005; Miner et al. 2018). Our results provide several insights in the mechanisms that could explain the contrasting effects of N fertilisation on grain Zn concentrations.

First of all, N application can reduce grain Zn concentrations through increased biomass production, i.e. a dilution effect. This dilution effect has been shown for many nutrients, including Zn (e.g. Raymond et al. 2009; Hertzberger et al. 2021; Zhang et al. 2021). The results of Kiboko (Fig. 4C) provide support for a dilution effect of grain Zn concentrations. However, the dilution effect was not observed in Embu and Harare (Fig. 4A and B). For Embu, these results may be explained by the low N application rates, which did not exceed 90 kg ha<sup>-1</sup>. However, the absence of a dilution effect in Harare, which had similar N application rates as Kiboko, points towards the relevance of additional factors.

Second, genetic factors may influence the effect of N application on grain Zn concentrations. This was shown for Embu. For four varieties, an increasing trend between N application and grain Zn concentrations was found, whereas two other varieties maintained grain Zn concentrations. For variety DUMA43, the same trends in yields and grain Zn concentrations across N application rates were observed when grown in Harare, indicating this may be a variety characteristic (Fig. 4).

Third, environmental factors, in particular soil Zn availability, could explain relations between N application rates and grain Zn concentrations. Dilution of grain Zn concentrations can be observed when biomass production increases strongly, which is not compensated for by a higher Zn uptake (Zhao et al. 2022). In Kiboko, the trade-off between yield and grain Zn concentrations as an effect of N application was clearly present, in contrast to Embu and Harare (Fig. 4). Soil Zn availability in Kiboko was lower compared to Embu and Harare (Table 1) and Zn-M3 levels were close to critical values reported in literature. Soil Zn availability may thus have constrained Zn uptake in Kiboko, leading to a dilution effect of grain Zn concentrations at higher N levels. These findings could imply that N fertilisation should be complemented with Zn fertilisers in soils with low Zn availability, to avoid this trade-off.

# Zn fertilisation

Although not covered in this study, application of Zn fertilisers is another management option that has the potential to increase both maize yields and grain Zn concentrations. Zinc fertilisation has been shown to increase maize grain concentrations to a maximum of 23-27 mg kg<sup>-1</sup> (Joy et al. 2015; Kihara et al. 2020; Liu et al. 2017; Manzeke et al. 2020; Van Eynde et al., 2023). This is well below the HarvestPlus target concentration of 38 mg kg<sup>-1</sup>. Zinc fertilisation alone therefore cannot help to increase both yields and grain Zn concentrations, in particular when soil Zn availability is limiting.

# Conclusions

In this study, we demonstrated that attaining grain Zn concentrations above the HarvestPlus target of 38 mg kg<sup>-1</sup>, with the current commercially available maize varieties and presented management options, is not possible without compromising yield levels. This implies that improved maize varieties could play a key role in increasing grain Zn concentrations without compromising yields. Recently, improved maize varieties with grain Zn concentrations close to the target value have been released in Latin America (Virk et al. 2021).

However, increasing grain Zn concentrations, even if below the HarvestPlus target, can contribute to reducing human Zn deficiency in SSA (Manzeke-Kangara et al. 2021). This can potentially be achieved without the use of improved varieties. Our results suggest that grain Zn concentrations increase with soil organic matter contents and could be affected by soil P and K availability. Our results furthermore indicate that maize grown on soils with low Zn availability, may require Zn fertilisation to prevent dilution of grain Zn concentrations at high N application rates. As farmers are incentivised by economic returns, options for increasing grain Zn concentrations are more likely to be implemented when they also lead to higher yields. Our results imply that management could play a role in increasing both maize yields and grain Zn concentrations. Options include increasing soil organic matter contents and/or optimising N, P and K fertilisation. In contrast to using Zn fertilisers, these management practices likely will also improve yields, especially in nutrient-depleted soil with low SOC contents (Manzeke et al. 2012; Njoroge et al. 2017; Tittonell and Giller 2013). Future studies should confirm if these management options indeed have the potential to increase both maize yields and grain Zn concentrations, in several maize varieties and under various field conditions relevant for the SSA context.

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#### Declarations

**Competing interests** The authors declare that they have no conflict of interest.

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