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Residual soil-applied zinc improves grain zinc nutritional quality of maize grown under contrasting soil types in Malawi

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Introduction: A proper understanding of the residual value of zinc (Zn) is essential for sustainable biofortification of food crops. This study hypothesized that Zn applied at rates higher than the current national recommendation would have significant residual benefits on maize productivity and Zn uptake.

Methods: The residual effects of soil-applied Zn on maize grain Zn concentration and uptake were evaluated at two Malawian agricultural research stations, Chitedze and Chitala, each with Lixisols and Vertisols soil types. The experiment used three Zn fertilizer rates (1, 30, and 90 kg Zn ha^{-1}) applied as $ZnSO_4.7H_2O$ in the previous season, arranged in a randomized complete block design (RCBD) with 10 replications per site. Maize grain yield, and Zn concentrations in grain and stover were measured at harvest. Data were analyzed to assess crop yield and Zn uptake relative to Zn application rates and soil types.

Results: Maize grain yield in the second season was 24.6% higher on plots receiving 30 kg Zn ha^{-1} compared to those with 1 kg Zn ha^{-1}. Grain Zn concentration and Zn uptake increased by 12.5% and 29.6%, respectively, on plots with 30 kg Zn ha^{-1} versus the lowest rate. Application of 90 kg Zn ha^{-1} did not provide additional benefits over 30 kg ha^{-1} for yield, Zn concentration, or Zn uptake. Residual Zn benefits did not vary between soil types.

Discussion: These results demonstrate that residual application of 30 kg Zn ha^{-1} significantly enhances maize productivity and Zn biofortification compared to the current national recommendation of 1 kg Zn ha^{-1}. Given no added advantage with 90 kg Zn ha^{-1}, revising Zn fertilizer guidelines to higher but optimized rates could improve the effectiveness of biofortification programs without environmental or economic drawbacks.

KEYWORDS

grain yield, grain zinc concentration, residual zinc, soil type, zinc uptake

1 Introduction

Maize (*Zea mays* L.) is the significant food security crop in the world (1, 2). It is a staple cereal crop for many people in sub-Saharan Africa (SSA), South Asia and Latin America (3). Published reports indicate that maize is an important food crop for approximately 1.2 billion people in SSA and Latin America (4). The statistics show that about 300 million people in Africa depend on maize and more than 30% of the total calorie dietary intake of people in SSA comes from maize (5). Because maize is a highly consumed crop in SSA, more than 40% of the cereal production in the region is accounted by maize (5).

In Malawi, maize contributes significantly to diets of more than 80% of the population, with highest per capita consumption in Africa of 129 kg per year (6, 7). It is a crop with significant impact on national food and nutritional security. The adequate availability of maize in Malawi equates to food security (8). It is estimated that more than 75% of agricultural land in Malawi is allocated to maize production (9). The crop has wide range of adaptability and is grown by 97% of farming households, and accounts for more than 60% of total food consumption in Malawi (10).

Cereal based diets are the dominant source of nutrients for the majority of the world's population (11). However, cereals such as maize grown on Zn-deficient soils often have low grain Zn concentrations, leading to widespread Zn deficiency and associated health problems in humans. Zinc concentrations in maize have been reported to be as low as 20 mg kg^{-1} (12, 13) against the human requirement of 40–50 mg kg^{-1} (14–16). Recent reports suggest that Zn deficiency is widespread, with high prevalence rate among women of reproductive age and children of under 5 years, especially in the developing countries (17). It is estimated that nearly 1 billion people worldwide suffer from Zn malnutrition (18). The deficiency of Zn in humans is associated with multiple health problems that include immune system impairments, retarded physical growth and brain development among children under 5 years of age, and poor birth outcomes in women (19–21). In Malawi, recent studies indicate that over 60% of the population is Zn deficient, largely due to low Zn levels in staple cereals grown on Zn-deficient soils (22, 23). This nutritional concern underscores the importance of agronomic strategies to enhance zinc availability in soils for improving human nutrition. Various interventions such as application of Zn fertilizers are suggested to be possible means of alleviating Zn deficiency in humans through increasing the concentration of Zn in the edible parts of the crops, a process termed agronomic biofortification or agro-fortification (24–27). This is achieved either through sole or co-application of foliar and soil Zn fertilizers (28–31).

In Malawi, Zn-enriched fertilizers are recommended for basal application in maize cropping system at the rate of 92 kg N ha^{-1}, 10 kg P_2O_5 ha^{-1}, 5 kg K_2O ha^{-1} and 6 kg S ha^{-1} in NPKS fertilizers applied immediately after seedling emergence (32). It is reported that the effectiveness and efficiency of soil-applied Zn in improving grain Zn nutritional quality of staple crops is influenced by fertilizer form, soil and environmental factors such as pH, moisture, temperature, organic matter and clay content (33–35). These factors also determine whether the nutrient will be available to the succeeding crop (36). Previous studies have reported that only a small fraction of Zn applied to the soil under field conditions is taken up by crops with a recovery rate ranging from 0.5 to 5% of the annually applied Zn depending on soil type, fertilizer types and application rates (37, 38). This means that a considerable amount of applied Zn remains in the soil, some of which may be available to crops in subsequent seasons (39–41). However, the extent to which residual soil-applied Zn benefits subsequent maize crops, particularly across different soil types like Lixisols and Vertisols in Malawi, remains unclear.

A pragmatic way to assess the residual benefit of nutrients is by growing a second crop in the subsequent year and determining their nutrient uptake (42). This approach provides a direct measure between the original amount of fertilizer nutrient applied and the crop uptake. Measuring the amount of residual nutrient in the soil through chemical extraction is another option for predicting the benefit to a subsequent crop (43), however, this approach can be ambiguous as it may over or under estimate plant available nutrients (42). This is partly due to chemical transformations of the nutrients in the soil. Trace metals such as Zn exist in soil adsorbed within different chemical pools (fractions) which affects their bioavailability for crop uptake (44). These operationally defined fractions include water soluble and exchangeable Zn, organic matter-bound Zn, carbonate-bound Zn, iron and manganese oxide-bound Zn and residual Zn (45). Other studies further indicate that the availability of Zn for crop uptake varies between soil types due to various underlying soil physico-chemical properties (35, 46, 47).

In the current study, the focus was to assess the residual benefit of soil-applied Zn under contrasting soil types by growing a second maize crop in the subsequent cropping season following application of Zn fertilizer. The two soil types compared are Lixisols and Vertisols, classified according to the World Reference Base by the (48) and applied in Malawi by (49). Vertisols are primarily composed of 2:1 alumino-silicate clay minerals, have a pH greater than 7, and exhibit variable organic matter content (50). Due to the nature of these 2:1 clay mineral, Vertisols experience (51, 52) significant shrinkage and swelling during drying and rewetting cycles, which leads to the formation of large, deep cracks when dry; these cracks only close after extended periods of wetting. In contrast, Lixisols have a pH ranging from 5.6 to 6.7, contain a moderately high level of basic cations such as calcium, magnesium, potassium, and sodium, have a low available water holding capacity, and possess low-activity clays down to a depth of 100 cm (48).

Our previous experiments in Malawi have shown that agronomic biofortification is a viable way of improving the Zn nutritional quality of maize in the first season of application (51, 52). These results showed that Zn fertilizer application rates of 1, 30 and 90 kg Zn ha^{-1} yielded average maize grain Zn concentrations of 26.5, 30.3 and 31.2 mg kg^{-1}, respectively (51). The choice of the 30 and 90 kg Zn ha^{-1} treatment in the original experiment was based primarily on observed low Zn grain concentration in the pilot study, where a lower maximum rate of Zn application of 20 kg ha^{-1} was used. Following large Zn application rates in the previous study (52), we could examine residual benefit to a subsequent crop. In the present study, field experiments were conducted in the 2020–21 growing season to assess the residual benefit of soil Zn fertilization on maize grain Zn quality. The aim of study was to evaluate the residual benefit of soil-applied Zn fertilizer on the Zn concentration and nutritional quality of maize grain grown on contrasting soil types in Malawi. Specific objectives were to quantify the residual effect of Zn fertilizer applied in the previous season on maize grain Zn concentration in the subsequent cropping season, and compare the residual availability and uptake of Zn by maize grown on two contrasting soil types, Lixisols and Vertisols The current study, therefore, sought to address the following hypothesis: soil residual Zn fertilization can increase Zn concentration in maize grain thereby improving the Zn nutritional quality of maize. The study was important to assess the residual value of Zn fertilizer given that there may be widespread future use of Zn biofortification.

2 Materials and methods

2.1 The description of the location where the original experiment was conducted

The original experiment took place during the 2019–20 cropping season at the Chitala, Chitedze, and Ngabu Agricultural Research Stations, located in the Lilongwe, Salima, and Chikwawa districts, respectively (51). Table 1 provides details about the locations of these experimental sites. All three sites experience a unimodal rainfall pattern, with rain typically occurring from November to April. In Malawi, maize is usually planted in November at the start of the rainy season and harvested in May or June, with planting beginning earlier in the southern and low-lying regions. The sites generally undergo a cool, dry period from May to mid-August, with temperatures ranging between 16°C and 27°C, followed by a warmer phase from mid-August to October, when temperatures range from 20°C to 40°C. From November to April, the climate is warm and wet, with temperatures fluctuating between 25°C and 36°C. A detailed study area map that illustrates the research's geographical location, sampling sites, and spatial context is presented in Figure 1.

Before setting up the original experiment, soil samples were collected across the entire experimental area for each soil type at each site (51). The soil sampling procedure was carefully designed to capture the variability across each experimental area by selecting five evenly spaced, random points per site. Soil was collected uniformly from the top 0–20 cm layer using clean tools to prevent contamination. Samples from these points were combined into a 500-gram composite sample representing the average soil conditions for each soil type and site. These composites were then analyzed in the laboratory for key soil properties—such as pH, organic carbon, total nitrogen, DTPA Zn, cation exchange capacity, and exchangeable bases (calcium, magnesium and potassium) to establish baseline fertility and nutrient status before planting. The analysis results presented in Table 2 revealed considerable variation in soil characteristics between the different soil types. Notably, Vertisols consistently showed higher average values for pH, organic carbon, total nitrogen, and exchangeable bases compared to Lixisols across all sites. This trend suggested that Vertisols generally possess greater inherent fertility, making them more favorable for crop growth under the conditions studied. Understanding these baseline differences was critical for interpreting experiment outcomes.

2.2 The design of the original experiment

As previously stated, the original study was conducted at Chitedze, Chitala and Ngabu Agricultural Research Stations. Since larger Zn application rates were considered in the original study, this experiment was conducted to examine residual effects in a second cropping season at the same locations. The experiment involved three Zn fertilizer rates;1, 30, and 90 kg Zn ha^{-1} applied as $ZnSO_4.7H_2O$ in the previous season, arranged in a randomized complete block design (RCBD) with 10 replications per site. Subsequent trials were, however, not successful at Ngabu Agricultural Research Station due to drought and failure of trial establishment.

2.3 Measurements of residual availability of zinc in soil

The residual benefit of soil-applied Zn to subsequent crops has previously been noted (39, 43, 53, 54). Measurement of residual Zn in the soil prior to another crop being planted can determine the extent of

TABLE 1 Description of the experimental sites.

Location	+Selected soil type	Geo-reference	Elevation (*masl)	Agro-ecology
Chitedze	Lixisol	13.99 S, 33.64 E	1150 m	Mid-altitude plateau
	Vertisol	13.98 S, 33.65 E		
Chitala	Lixisol	13.69 S, 34.25 E	600 m	Lakeshore plain
	Vertisol	13.68 S, 34.26 E		
Ngabu	Lixisol	16.50 S, 34.86 E	100 m	Lower Shire valley
	Vertisol	16.45 S, 34.89 E		

+soil type based on World Reference Base (48) soil classification, *masl, meters above sea level.

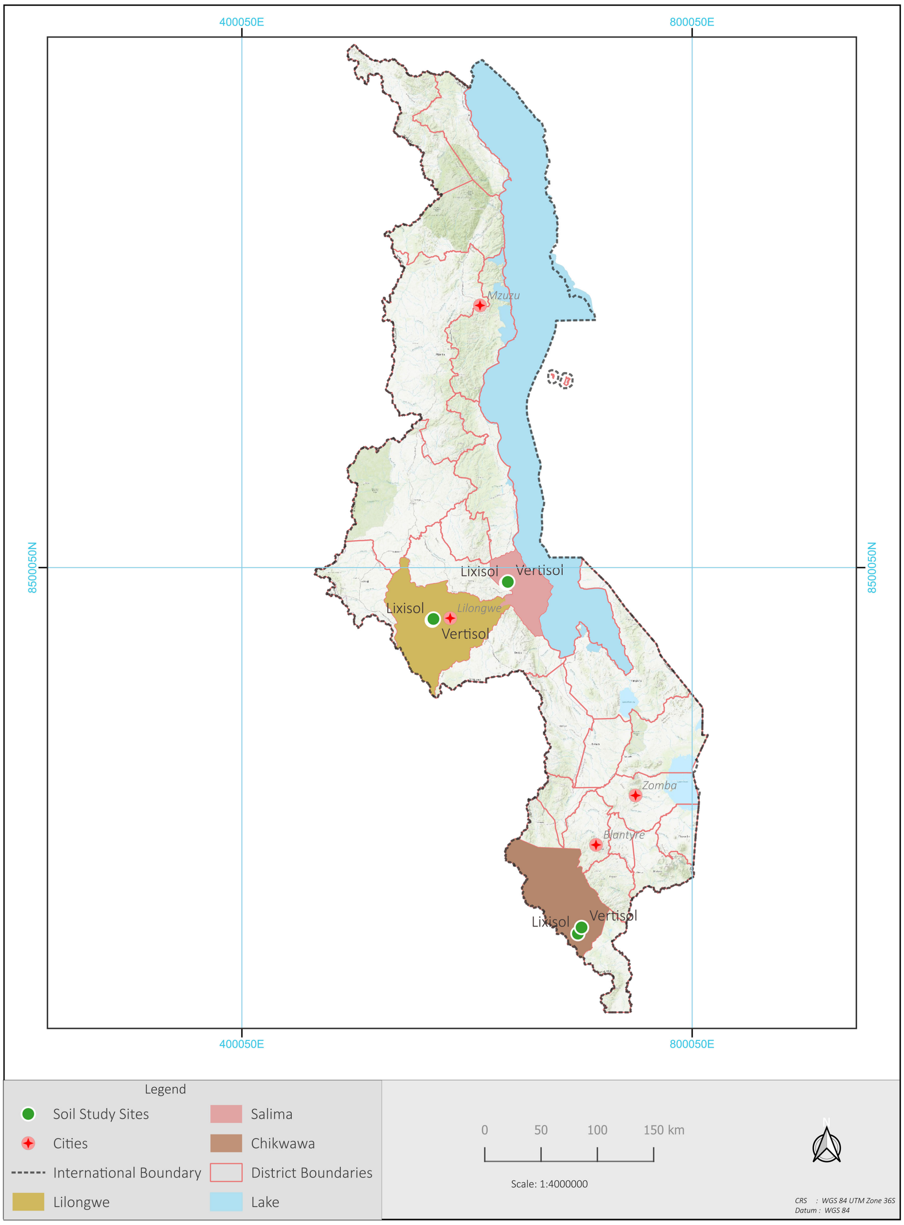

FIGURE 1
Map of Malawi showing the geographical locations of the study sites.

its availability for the next crop. Samples were analyzed as described by (52). Soil samples from the depth of 0–20 cm were collected at the final harvest in 2020 from all the plots at Chitedze Research Station. The samples were collected at ten points along the summit of one of the peripheral ridges, which were selected at random from each net plot, using a Dutch soil auger with a flight length of 15 cm and a diameter of 3.5 cm, and the 10 samples from each plot were bulked. The samples were air-dried, sieved (<2 mm) and homogenized before determination of extractable Zn as a measure of plant-available Zn using the diethylenetriaminepentaacetic acid (DTPA) method (55). The extraction procedure was undertaken on duplicate subsamples from each plot, using 5 g of soil extracted with 10 mL of 0.005 M DTPA, 0.1 M triethanolamine and 0.01 M $CaCl_2$ at pH = 7.3 shaken for 2 h on an end-over-end shaker. Thereafter, the samples were centrifuged at 3000 rpm for 15 minutes and the supernatant filtered through <0.22 µm syringe filters prior to analysis using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS).

2.4 Trial establishment and management

The field trials were conducted at Chitedze and Chitala Agricultural Research Stations on two contrasting soil types: Lixisols and Vertisols. The original experiment was laid out in a randomized complete block design (RCBD) with each Zn fertilizer treatment replicated 10 times for each soil type at the experimental

TABLE 2 Initial soil characteristics of the experimental sites.

Soil property	Chitala		Chitedze		Ngabu	
	Lixisol	Vertisol	Lixisol	Vertisol	Lixisol	Vertisol
Organic carbon (%)	0.98	1.46	1.33	1.64	0.99	2.05
$pH_{(water)}$	5.44	6.87	5.21	6.23	5.33	7.52
Total N (%)	0.05	0.16	0.07	0.23	0.09	0.39
Total Zn (mg kg^{-1})	88.5	89.0	77.5	97.0	155.0	196.5
⁺DTPA Zn (mg kg^{-1})	1.60	3.34	0.76	1.17	2.61	4.82
Available P (mg kg^{-1})	13.6	15.1	12.4	14.7	16.9	19.4
*CEC ($cmol_c$ kg^{-1})	18.1	26.8	11.5	24.5	17.5	29.5
Exchangeable Ca ($cmol_c$ kg^{-1})	2.7	4.9	1.8	3.1	5.6	9.1
Exchangeable Mg ($cmol_c$ kg^{-1})	0.8	1.6	0.6	1.5	1.3	1.9
Exchangeable K ($cmol_c$ kg^{-1})	0.5	0.7	0.1	0.4	0.9	1.2

⁺Diethylenetriamine pentaacetic acid.
*Cation exchange capacity.

sites (51, 52). The recommended planting pattern was followed as described in the Guide to Agricultural Production and Natural Resource Management of the Ministry of Agriculture (32). The detailed procedures of Good Agronomic Practices (GAP) followed in the current study generally included the following key steps: site selection and preparation, seed selection and sowing, crop management, pest and disease control, and harvesting and post-harvest handling of the grain. The residual benefit of Zn to the maize crop was assessed by growing the crop in the subsequent cropping season (2020–2021) on the same plots and ridges without ploughing or any added Zn. The gross plot size was 5 ridges, each 5 m long, with the net plot being the 3 middle ridges, each 3 m long. The ridges were spaced at 75 cm apart. Maize seeds were sown at 25 cm spacing along the ridge, giving an expected plant density of 53,333 plants ha^{-1}. Good agronomic practices were followed except for avoiding creating new ridges. The SC 403 maize variety, locally known as Kanyani, was used. General information about the maize variety and climatic conditions of the sites is provided in Botoman et al. (2020). Kanyani is a F_1 hybrid variety widely grown in Malawi, can mature in ~90 days and adapts to a wide range of environmental conditions. Critical nutrients including nitrogen (N), phosphorus (P), potassium (K) and sulphur (S) were adequately applied as straights to avoid extra Zn coming in following the guidelines outlined in the Guide to Agricultural Production and Natural Resource Management of the Ministry of Agriculture (32).

2.5 Data collection and sample laboratory analyses

Maize was sown in December 2020 when effective rain started and harvested in April 2021 at Chitala, and in May 2021 at Chitedze. At harvest, grain and stover samples were collected. Grain yield (kg) and dry weight of stover (kg) were recorded from the net plots and used to calculate Zn uptake and harvest index of the crop. The Zn harvest index is a ratio between Zn accumulated in the grain to the sum of the Zn accumulated in the grain and stover (56), expressed as a percentage. Daily rainfall (mm) was also recorded using rain gauges stationed in each of the research stations where the experiment was conducted (Supplementary Figure S1). Generally, rainfall was adequate at both Research Stations and additional irrigation was not used given that rain-fed agriculture is the common practice in Malawi.

Grain and stover samples were prepared and Zn concentrations determined as described by (52, 57). A portion of finely ground plant material (c. 0.2 g of grain or stover) was digested with 6 mL of 70% HNO_3 (trace analytical grade) using a microwave system comprising a Multiwave Prom 41HVT56 Rotor and pressure-activated-venting vessels made of modified polytetrafluoroethylene (PTFE-TFM, 56 mL 'SMART VENT', Anton Paar GmbH, Graz, Austria). A Certified Reference Material, (CRM; Wheat flour SRM 1567b, National Institute of Standards and Technology, Gaithersburg, MD, US; 11.61mg kg^{-1}) and 12 operational blank digestions were used to determine the accuracy of the analyses and the limit of detection (LOD) for quality control. The Zn elemental recovery for SRM 1567b was 105%. After the extraction procedure was complete, grain and stover samples were analyzed for Zn by inductively coupled plasma mass spectrometry (ICP-MS).

2.6 Statistical data analysis

Data analyses were conducted using the linear and non-linear mixed effects (nlme) package for the R platform (58). The analysis of data was done after validating the assumptions of normal distribution of residuals and homogeneity of variances by checking the model plots. After estimation of the model parameters, histograms were plotted of the random effects estimates at each level, the marginal residuals were plotted against the fitted values (Supplementary Figures S2–S7) and summary statistics (Supplementary Tables S1–S6) were computed. The outputs for maize grain yields, grain and stover Zn concentrations and uptake met these assumptions. For harvest index, these assumptions were not valid, and data were transformed using a natural log. A linear mixed model (LMM) was used with a random effects structure to reflect how the fertilizer rate was randomized among plots within sets of blocks all within one sub-site of a single soil type. A fixed effects model was used comprising main effects of fertilizer rate, soil type and their interaction. Further, the main effect of fertilizer rate was partitioned into linear and non-linear components with an appropriate choice of orthogonal polynomials, and the soil type by fertilizer rate interaction was similarly partitioned into contrasts between the linear and non-linear responses to Zn applicate rate on the different soils. The output of the analysis tested the hypothesis concerning the differences between soil types and Zn fertilizer rates with respect to the response variable.

3 Results

3.1 Residual availability of zinc in soil after harvest in the first growing season

The residual Zn availability, at the end of the growing season in the year of application, typically increased with an increase in applied Zn fertilizer rate (Figure 2). There were no significant differences in the concentration of DTPA-extractable Zn between the application rates of 1 and 30 kg ha^{-1}. However, the differences were significant when the rate increased to 90 kg ha^{-1}. There was an apparent drastic increase in soil zinc concentration from 0.76 mg kg^{-1} to over 300 mg kg^{-1} following the application of 90 kg ha^{-1}. It is important to note that the values represent DTPA-extractable zinc, which reflects the readily available zinc fraction rather than total soil zinc content. The high zinc application rate likely created localized zones of elevated zinc availability, which the DTPA extraction sensitively detected. Additionally, it is possible that zinc fertilizer residues on the soil surface contributed to the inflated extractable zinc values immediately post-application. No observable toxic effects of Zn on the maize crops were noticed.

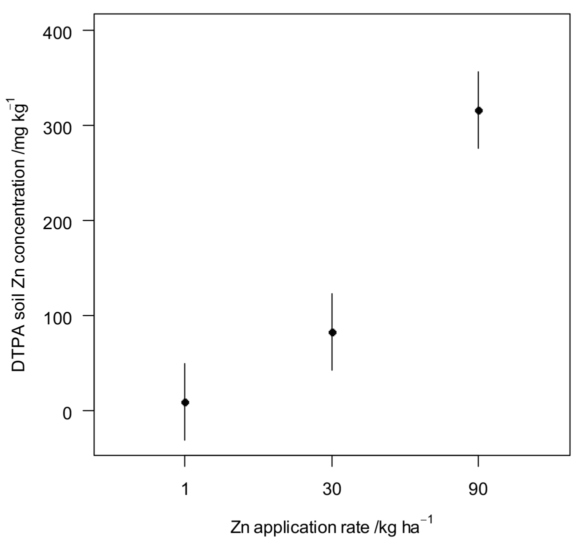

FIGURE 2
Residual DTPA-extractable Zn concentration measured at the end of the growing season in which the fertilizer was applied (1, 30 and 90 kg ha^{-1}) for the experimental sub-sites at Chitedze Agricultural Research Station. The error bars show the standard error of the mean (±SEM).

3.2 Effect of soil type and residual Zn fertilizer on maize grain yields

The maize grain yields obtained over all experimental sites are presented in Figure 3a, error bars show the standard error. Some of the soil-applied Zn appeared to remain in an available form to the succeeding maize crop resulting in a positive grain yield response. Soil type is not replicated within sites, and so we can make inferences only about an additive soil effect over all the sites. A LMM framework was used to fit the data as proposed by (52). The main effect of Zn fertilizer rate was partitioned into linear and non-linear components. A positive response of maize grain yield to residual Zn for each Zn fertilizer rate was observed at each site except at Chitala. The observed decrease in yield at Chitala with 90 kg Zn application compared to 30 kg is likely due to antagonistic nutrient interactions and nutrient imbalance rather than simply excessive Zn. The interaction effects vary strongly with soil type, nutrient status, and local environmental factors. For example, calcareous soils with high pH commonly limit Zn solubility, and excessive Zn application may not translate linearly into higher plant availability or yield. Because of these factors, the large yield difference between 30 kg and 90 kg Zn application suggests that the optimal Zn rate lies somewhere between these values, where the beneficial residual Zn effect enhances yield without provoking antagonistic deficiencies of P, N, or K. Overall, the mean grain yield increased by 1490 kg ha^{-1} in response to residual Zn from the 30 kg ha^{-1} Zn fertilizer rate relative to the 1 kg Zn ha^{-1} rate (24.6% higher). However, no further increases in yield was observed at Chitedze when the Zn application rate was increased to 90 kg ha^{-1}.

The analysis of variance (ANOVA) for the maize grain yield is shown in Table 3. There is strong evidence for an effect of residual Zn in soil for both linear and non-linear components. The linear component ($p < 0.05$) represents the positive effect of residual Zn on grain yield, while the non-linear component ($p < 0.05$) shows the diminishing marginal returns of 90 kg ha^{-1} rate, relative to the response at 30 kg ha^{-1}. However, there was no significant differences among the soil types ($p > 0.05$). Furthermore, an interaction of linear response of Zn fertilizer rate with soil type ($p > 0.05$) and the non-linear response with soil type ($p > 0.05$) was not significant. This, therefore, suggests that maize grain yield response to Zn fertilizer rate did not depend on soil type.

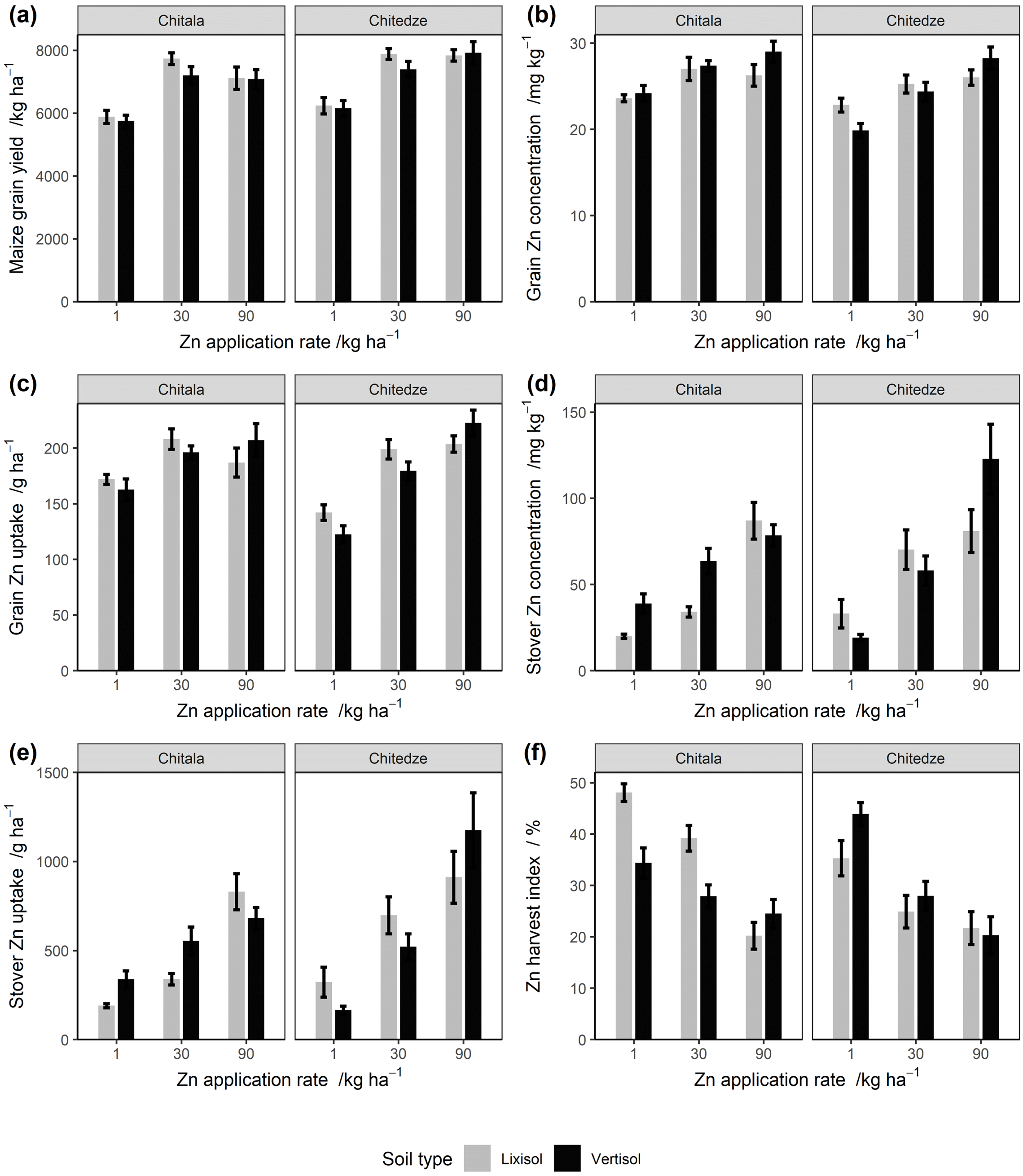

FIGURE 3
Effects of residual Zn fertilizer rate and soil type on **(a)** maize grain yield, **(b)** grain Zn concentration, **(c)** grain Zn uptake, **(d)** stover Zn concentration, **(e)** stover Zn uptake, and **(f)** Zn harvest index at the experimental sites during the 2020–21 cropping season. The error bars show the standard error of the mean (±SEM).

3.3 Effect of residual soil Zn on maize grain Zn concentration and uptake

The grain Zn concentrations and uptake for each fertilizer rate at all experimental sites are presented in Figures 3b, c, along with the standard errors calculated for each treatment level. The uptake of residual Zn from soil was clearly observed in the subsequent maize crop. As observed for grain yield, positive responses of grain Zn concentration and uptake to residual Zn fertilizer were apparent. The overall mean grain Zn concentrations at 1, 30 and 90 kg ha^{-1} Zn fertilizer rate were 22.6, 26.1 and 27.4 mg kg^{-1} respectively, with their standard errors, for the three Zn fertilizer rates as estimated in the LMM. Similarly, maize grain Zn uptake at 1, 30 and 90 kg ha^{-1} Zn fertilizer rate were 149, 195 and 205 g ha^{-1} respectively. The estimated additional grain Zn concentration and uptake arising from residual soil Zn following the 30 kg ha^{-1} were 3.4 mg kg^{-1}

TABLE 3 ANOVA output table for maize grain yield, grain Zn concentrations, grain Zn uptake, stover Zn concentrations, stover Zn uptake and natural log of Zn harvest index at Chitala and Chitedze agricultural research stations.

Factor	Num DF	Den DF	Grain yield (kg ha^{-1})		Grain Zn conc. (mg kg^{-1})		Grain Zn uptake (g ha^{-1})		Stover Zn conc. (mg kg^{-1})		Stover Zn uptake (g ha^{-1})		Zn HI	
			F-value	*P*-value	F-value	*P*-value	F-value	*P*-value	F-value	*P*-value	F-value	*P*-value	F-value	*P*-value
Soil type	1	1	1.3271	0.4551	0.1587	0.7586	0.3681	0.6528	2.56236	0.3555	0.16819	0.7522	0.085	0.8194
Zn lin	1	76	70.7532	<.0001	38.5574	<.0001	53.4562	<.0001	81.4031	<.0001	80.6168	<.0001	71.68	<.0001
Zn rem	1	76	47.6878	<.0001	8.4122	0.0049	21.2399	<.0001	1.497	0.2249	1.01045	0.318	1.922	0.1697
Soil type ● Zn lin	1	76	0.0474	0.8283	7.1444	0.0092	7.5350	0.0075	0.9747	0.3266	0.1837	0.6694	0.526	0.4705
Soil type ● Zn rem	1	76	0.9745	0.3267	0.0473	0.8283	1.0339	0.3125	0.01479	0.9035	0.00125	0.9719	0.465	0.4972

A dot,●, denotes interaction; Zn lin, linear effect of Zn application rate and Zn rem, non-linear effect of Zn application rate; Num DF, Numerator degrees of freedom; Den DF, Denominator degrees of freedom.

(12.5% higher than for 1 kg ha^{-1}) and 44 g ha^{-1} (29.6% higher than for 1 kg ha^{-1}), respectively; no further increases were observed when the Zn fertilizer rate was increased to 90 kg ha^{-1}.

The ANOVA for maize grain Zn concentration and Zn grain uptake are presented in Table 1. There was a significant response of maize grain Zn concentration and uptake to Zn fertilizer rate for the linear ($p < 0.05$) and non-linear ($p < 0.05$) components of the response. Over all sites, there was no evidence for differences in grain Zn concentration ($p > 0.05$) and grain Zn uptake ($p > 0.05$) between soil types. The linear response was noticed when the Zn fertilizer rate increased from 1 to 30 kg ha^{-1} whereas increasing Zn application from 30 to 90 kg ha^{-1} resulted in a non-linear response. Thus, increasing the Zn fertilizer rate from 1 to 30 kg ha^{-1} results in a proportional increase in maize grain Zn concentration and uptake from residual Zn in the subsequent growing season, while an increase from 30 to 90 kg ha^{-1} results in a proportionally smaller increase in grain Zn concentration and uptake. Furthermore, the interaction of soil type and linear response for grain Zn concentration ($p < 0.05$) and grain Zn uptake ($p < 0.05$) was significant. For both response variables, no significant differences ($p > 0.05$) in grain Zn concentration and ($p > 0.05$) in grain Zn uptake were observed with the interaction of non-linear response and soil types. This suggests that maize grain Zn concentration and uptake depended on soil type when the rate was increased from 1 to 30 kg ha^{-1} while from 30 to 90 kg ha^{-1}, soil type did not have any effect over all sites.

3.4 Effect of residual soil Zn on maize stover Zn concentration and uptake

The results on the effect of residual available Zn for each Zn fertilizer rate on stover Zn concentration, uptake, and harvest index at all experimental sites are shown in Figures 3d–f. When the main effect of Zn fertilizer rate was partitioned into linear and non-linear components, a positive response of stover Zn concentration and uptake to Zn fertilizer rate was observed at all sites. The stover Zn concentrations at applications of 1, 30 and 90 kg ha^{-1} were 27.8, 56.5 and 92.3 mg kg^{-1}, respectively while the stover Zn uptake at these rates were 254, 528 and 900 g ha^{-1}, respectively. Thus, over all sites, increasing the Zn fertilizer rate from 1 to 90 kg ha^{-1} resulted in a linear correlation between the fertilizer rate and stover Zn concentration and stover Zn uptake.

The ANOVA output for stover Zn concentration and uptake are presented in Table 1. There was a significant response of stover Zn concentration and uptake to Zn fertilizer rate for the linear ($p < 0.05$) response. However, the non-linear response was not statistically significant for both stover Zn concentration ($p > 0.05$) and stover Zn uptake ($p > 0.05$). Similarly, over all sites, there was no evidence for differences in stover Zn concentration ($p > 0.05$) and stover Zn uptake ($p > 0.05$) between soil types. There was no significant interaction of the linear response ($p > 0.05$) and non-linear component of the response ($p > 0.05$) with soil type in stover Zn concentration over all sites. Similarly, there was no evidence for an effect of the interaction of linear ($p > 0.05$) and non-linear responses ($p > 0.05$) with soil type in stover Zn uptake. This suggests that stover Zn concentration and uptake did not depend on soil type when the rate was increased from 1 to 90 kg ha^{-1}.

3.5 Effect of soil type and residual Zn fertilizer on Zn harvest index

The mean Zn harvest indices (Zn_{HI}) for each fertilizer rate at all sites are presented in Figure 4, accompanied by their standard errors estimated for each treatment level. The effects of soil type, Zn fertilizer rate and their interaction on Zn_{HI} were analyzed using the LMM. Prior to analysis, Zn_{HI} was tested for normality of the residuals and the outputs showed a skewed distribution and, therefore the response variable was transformed into natural logarithm values. After the transformation, the assumption of a normal distribution and homogeneity of variances of the residuals were valid. Generally, mean Zn_{HI} decreased for all the soil types in response to the increase of Zn fertilizer rate (Figure 4). Note that no statistical inference about soil type at each site could be made since soil type was not replicated within each experimental site. There was no observed effect in Zn_{HI} between soil types when the rate was increased from 1 to 30 kg ha^{-1} while at 90 kg ha^{-1} the decrease was statistically different. The observed variations in Zn_{HI} response to Zn fertilizer rate over all sites might be due to differences in soil physical and chemical behavior.

Table 1 shows the ANOVA output for the natural log of Zn harvest index. There was strong evidence for an effect on Zn_{HI} of Zn fertilizer rate for the linear ($p < 0.05$) component of the response. However, there was no evidence for an effect on Zn_{HI} of Zn fertilizer rate for the non-linear ($p > 0.05$) component of the response. Normally, when the rate was increased from 1 to 90 kg ha^{-1}, there was a negative response for both linear and non-linear components. The observed reduction in Zn_{HI} with Zn fertilizer rate shows that Zn partitioning efficiency to the grain was negatively affected by the physiological response of the crop to Zn availability in the soil through reduction of Zn uptake by the crop roots. Over all sites, there were no differences in Zn_{HI} reduction as the Zn fertilizer rate was increased from 1 to 30 kg ha^{-1} while noticeable differences were observed as the rate was increased from 30 to 90 kg ha^{-1}.

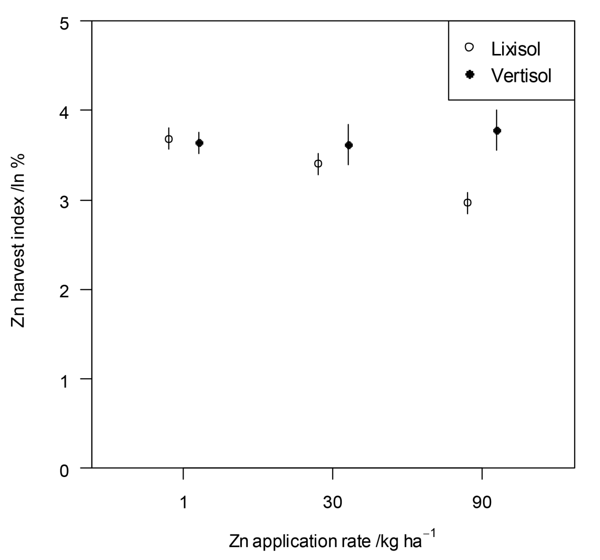

FIGURE 4
Mean Zn harvest index of maize in response to residual Zn fertilizer during the 2020–21 cropping season. The error bars show the standard error of the mean (± SEM).

4 Discussion

4.1 Residual Zn fertilizer improved maize grain yields

Increased maize grain yield resulting from residual available Zn in the soil has been documented in previous studies (59). For instance, research conducted in Brazil (39) demonstrated that maize grain yield increases were consistently observed during the second year of maize cropping when Zn fertilizer was applied at rates of 2, 4, and 8 kg Zn ha^{-1}. These results highlight the persistence of Zn in the soil and its continued availability to crops beyond the initial application year. Further supporting evidence comes from another study (43), which reported pronounced residual effects in terms of both plant Zn uptake and increased plant extractable Zn, with benefits lasting up to six years after Zn application at rates of 5.6, 11.2, 16.8, and 22.4 kg ha^{-1}. These findings underscore the long-term value of Zn fertilization in enhancing soil fertility and crop productivity.

Similarly, the findings of the present study confirm the positive impact of residual Zn fertilizer on maize grain yields. When Zn fertilizer was applied at a rate of 30 kg ha^{-1}, maize grain yields increased by 24.6% compared to the national recommendation rate of 1 kg ha^{-1}. This improvement translates to an additional 1,500 kg ha^{-1} of maize grain produced because of increasing the Zn fertilizer rate from 1 to 30 kg ha^{-1}. Such results emphasize the importance of adequate Zn management in maize production systems, particularly in regions where Zn deficiency is a limiting factor for crop yields. The sustained benefits of Zn fertilization not only boost immediate yields but also contribute to improved soil health and long-term agricultural productivity.

The use of residual Zn fertilizer offers potential benefits for enhancing the food security of farmers by improving crop yields over time. However, research findings indicate that the maize grain yields obtained at a low Zn application rate of 1 kg ha^{-1} were noticeably lower than yields achieved with a higher Zn application rate of 30 kg ha^{-1} during the subsequent cropping season. This difference suggests that the amount of plant-available residual Zn remaining in the soil after applying 1 kg ha^{-1} in the previous season is very limited and insufficient to sustain optimum crop growth.

One possible explanation for the poor residual effect at the lower Zn rate is the chemical interactions in the soil. Zinc tends to form stable complexes with soil organic matter, which can reduce its availability to plants (60). Additionally, Zn ions are prone to adsorption onto soil minerals such as iron (Fe) and manganese (Mn) oxides as well as aluminosilicate clays (61). These adsorption processes effectively immobilize Zn, preventing it from being easily taken up by plant roots (61). Such mechanisms largely explain why the application of 1 kg ha^{-1} Zn fertilizer does not result in a meaningful residual yield benefit because much of the Zn applied becomes fixed in unavailable forms shortly after application. In contrast, the impact of Zn adsorption on residual availability appears to be less pronounced at higher Zn fertilizer rates such as 30 kg ha^{-1}. At this elevated level, the quantity of Zn applied likely exceeds the soil's capacity to fix or adsorb it entirely, leaving more plant-available Zn in the soil for the following cropping season. This increased residual availability contributes to the improved maize grain yields observed at the higher fertilizer rate.

4.2 Residual effect of Zn fertilizer on maize crop Zn uptake

When the initial Zn fertilizer rate was set at 30 kg ha-1, the grain Zn concentration measured in the residual year showed a notable increase of 12.5% compared to the grain Zn concentration under the lower Zn fertilizer rate of 1 kg ha^{-1}. This sustained enhancement in Zn concentration is particularly significant because it demonstrates that the effects of higher Zn application persist beyond the initial cropping season. Interestingly, this difference is quite consistent with the 15% increase in grain Zn concentration observed between these two treatments during the very first year of Zn application, as documented in our previous study (51). This consistency suggests that initial Zn application rates can have lasting impacts on Zn bioavailability in the soil, thereby maintaining grain Zn enrichment over multiple seasons.

In parallel, grain Zn uptake by the plants was also substantially influenced by the initial Zn fertilizer rates. Specifically, grain Zn uptake was 29.6% greater following application of 30 kg ha^{-1} Zn fertilizer compared to the minimal application rate of 1 kg ha^{-1}. This increase in Zn uptake exceeds the previously reported difference of 23% observed during the initial application period, implying that plants not only concentrate more Zn in their grains but also assimilate higher total Zn amounts when higher initial fertilization rates are employed. This enhancement in Zn uptake could be indicative of improved Zn availability and root uptake mechanisms persisting into subsequent crop cycles.

Overall, these findings reinforce the agronomic value of applying Zn fertilizer at adequate rates, highlighting that an initial Zn input of 30 kg ha-1 not only maximizes Zn enrichment in harvested grains during the first year but also sustains this nutritional benefit in subsequent seasons. This residual effect is crucial for strategies aiming to improve Zn biofortification in staple crops, especially in Zn-deficient soils prevalent in many regions. Furthermore, the pronounced increase in Zn uptake emphasizes the soil-plant Zn dynamics' responsiveness to fertilization, which could inform more efficient and cost-effective Zn management practices in cereal production systems.

The data presented in this study clearly demonstrate that the application of residual Zn fertilizer not only enhances grain Zn levels but also significantly increases the Zn concentration and uptake in crop stover. Specifically, raising the Zn fertilizer rate from 1 to 90 kg ha^{-1} resulted in a proportional increase in Zn content within the stover biomass. This finding holds considerable practical implications for livestock farmers, as the stover commonly used as fodder for ruminants can serve as an enriched source of dietary Zn. Improved Zn intake in animals is essential for maintaining optimal immune function, growth, and reproduction, ultimately enhancing overall livestock health and productivity.

Moreover, the elevated Zn levels in crop residues carry further importance for farmers practicing conservation agriculture (CA) systems. CA emphasizes maintaining and incorporating organic residues into the soil to improve soil health and fertility. The higher Zn concentration in the stover means that when these residues are returned to the soil, they contribute to replenishing soil Zn levels, which is critical for sustaining soil nutrient balance and long-term fertility. This cycle of residue incorporation can gradually enhance soil Zn availability without the immediate need for additional Zn fertilizer inputs each season.

Consequently, these improvements in soil Zn status through residue incorporation may lead to a reduction in Zn fertilizer requirements over the medium to long term. Less frequent or lower-dose fertilizer applications translate into lower production costs for farmers, which can directly boost their net economic returns. Thus, residual Zn fertilization coupled with conservation agriculture practices offers a synergistic approach that benefits both crop and livestock production systems while also promoting sustainability and cost-efficiency in smallholder farming contexts. This integrated strategy holds promise for enhancing nutrient cycling, farm resilience, and economic viability simultaneously.

Our findings on the residual effects of soil-applied Zn are consistent with previous studies from sub-Saharan Africa and globally, which indicate that applying zinc at higher rates has lasting benefits for following crops by enhancing grain yield, Zn concentration in grains, and Zn uptake (39, 40, 62). Comparable research confirms that these residual effects endure for at least two cropping seasons and occur across different soil types. These findings underscore the value of Zn fertilization for both improving crop productivity and achieving Zn nutritional biofortification in the region. Improving maize grain Zn concentration through agronomic biofortification in Malawi has direct potential to enhance dietary Zn intake among rural populations, who rely heavily on maize as a staple food and commonly experience Zn deficiency. Our previous studies show that Zn-enriched fertilizer application can increase grain Zn concentrations by 15% or more (51), which can meaningfully contribute to alleviating dietary Zn deficiency, supporting better immune function and growth. These improvements provide a cost-effective, food-based strategy to reduce Zn deficiency-related health risks in vulnerable communities without requiring dietary changes.

4.3 Residual Zn fertilizer affected maize grain Zn partitioning efficiency

The Zn harvest index (ZnHI), which reflects the efficiency with which Zn is partitioned and accumulated in the grain relative to the total zinc taken up by the crop, was estimated in this study to understand how different Zn fertilizer application rates affect grain Zn loading. Notably, increasing Zn fertilizer application rates resulted in decreases in ZnHI, indicating that as more Zn was applied, the proportion of Zn ultimately allocated to the grain declined. Specifically, the grain Zn loading efficiencies at application rates of 1, 30, and 90 kg ha^{-1} were found to be 40%, 30%, and 22%, respectively. This gradual decline in Zn loading efficiency suggests diminishing returns in grain Zn accumulation as fertilization intensifies. A closer examination reveals that when the Zn fertilizer rate increased from 1 to 30 kg ha^{-1}, there was a 10% reduction in grain Zn loading efficiency. This trend aligns well with observations made during the first season of the study, indicating a consistent response across different growing periods (51). However, with a further increase in fertilizer rate from 30 to 90 kg ha^{-1}, the loading efficiency decreased more sharply to 22%, a larger decline compared to the 30% reported in the previous study for the same increase. This sharper decline might be explained by changes in soil chemistry and zinc dynamics at higher application rates.

One plausible explanation for the decrease in ZnHI at higher fertilizer rates is related to the residual available Zn in soil. At lower Zn application rates, residual Zn that remains available in the soil possibly interacts with soil geo-colloids, components such as clay and organic matter that have high cation exchange capacity thereby affecting Zn retention and availability. However, as Zn inputs increase, the residual Zn may become susceptible to losses through leaching, especially in soils prone to water movement, and fixation where Zn becomes tightly bound to soil particles or converted into forms unavailable to plants. These processes reduce the pool of Zn accessible for root uptake and subsequent translocation to grains.

This study's findings resonate with similar research conducted in China on maize, where ZnHI decreased from 74% to 52% when zinc fertilizer rates increased from 2.3 to 34.1 kg ha^{-1} under field conditions (63). Similar results across different agroecological zones underscore a common physiological and soil chemical limitation in Zn uptake and partitioning under high fertilization regimes. Furthermore, an additional physiological mechanism may contribute to this observation. The delivery of Zn to the root xylem, which translocates nutrients from roots to shoots, could be adversely affected by excessive available Zn in the soil. High Zn concentrations have been reported to suppress both the loading of Zn into the xylem and its unloading within the plant tissues, possibly as a regulatory mechanism to prevent Zn toxicity (64, 65). This suppression effectively lowers the transport of Zn to the aerial parts and grains, further reducing ZnHI.

5 Conclusion

This study evaluated the effect of residual Zn fertilizer on the nutritional quality of maize grain cultivated on two different soil types in Malawi. The findings demonstrated a significant increase in both maize grain yield and Zn uptake with higher initial Zn fertilizer application rates. Residual Zn effects were observed in the subsequent cropping season, resulting in yield and grain Zn concentrations that were 24.6% and 12.5% higher, respectively, than the national recommended rate. These responses were consistent across both soil types studied. The 12.5% increase in grain Zn concentration underscores the potential to mitigate Zn deficiency among rural populations, enabling less frequent Zn fertilizer applications, thereby reducing input costs and improving farmer profitability. Furthermore, the benefits of residual Zn fertilizer persisted in the following season, indicating continued maize responsiveness to residual Zn in the soil. Notably, the similar maize response across different soil types suggests that soil characteristics did not significantly affect the residual Zn effect. Overall, the enhanced grain Zn concentration due to residual Zn fertilization could contribute to alleviating Zn deficiency in rural communities of developing countries like Malawi, allowing farmers to lower fertilizer application frequency and achieve improved economic returns over the medium to long term.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Author contributions

LB: Data curation, Formal Analysis, Writing – original draft, Conceptualization, Investigation. PN: Writing – review & editing, Supervision, Project administration. JC: Writing – review & editing, Supervision. EB: Writing – review & editing, Supervision. MM: Supervision, Writing – review & editing. EA: Supervision, Writing – review & editing. A-WM: Supervision, Writing – review & editing. RL: Conceptualization, Data curation, Writing – review & editing, Supervision. MB: Funding acquisition, Supervision, Writing – review & editing, Resources, Conceptualization.

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Conflict of interest

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

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