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Nitrogen effect on zinc biofortification of maize and cowpea in Zimbabwean smallholder farms

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Abstract

Agronomic biofortification of crops with zinc (Zn) can be enhanced under increased nitrogen (N) supply. Here, the effects of N fertilizer on grain Zn concentration of maize (*Zea mays* L.) and cowpea (*Vigna unguiculata* L.) were determined at two contrasting sites in Zimbabwe over two seasons. All treatments received soil and foliar zinc-sulphate fertilizer. Seven N treatments, with three N rates (0, 45, and 90 kg ha^{-1} for maize; 0, 15, and 30 kg ha^{-1} for cowpea), two N forms (mineral and organic), and combinations thereof were used for each crop in a randomized complete block design (n = 4). Maize grain Zn concentrations increased from 27.2 to 39.3 mg kg^{-1} across sites. At 45 kg N ha^{-1}, mineral N fertilizer increased maize grain Zn concentration more than organic N from cattle manure or a combination of mineral and organic N fertilizers. At 90 kg N ha^{-1}, the three N fertilizer application strategies had similar effects on maize grain Zn concentration. Co-application of N and Zn fertilizer was more effective at increasing Zn concentration in maize grain than Zn fertilizer alone. Increases in cowpea grain Zn concentration were less consistent, although grain Zn concentration increased from 39.8 to 52.7 mg kg^{-1} under optimal co-applications of N and Zn. Future cost/benefit analyses of agronomic biofortification need to include information on benefits of agro-fortified grain, complex farmer management decisions (including cost and access to both N and Zn fertilizers), as well as understanding of the spatial and site-specific variation in fertilizer responses.

1 | INTRODUCTION

In Africa, many people with plant-based diets consume foods that often are deficient in Zn. Despite a steady reduction in global dietary Zn deficiency in the last 20 years, more than 25% of the population in sub-Saharan Africa (SSA) is still at risk of inadequate dietary Zn intake (Kumssa et al., 2015). While numerous experimental agronomic biofortification studies using Zn-containing fertilizers have been conducted (e.g., Abdoli, Esfandiari, Mousavi, & Sadeghzadeh, 2014; Cakmak, 2008; Cakmak, Pfeiffer, & McClafferty, 2010a; Manzeke, Mtambanengwe, Nezomba, & Mapfumo, 2014; Manzeke et al., 2017; Zou et al., 2012), fewer studies have been conducted on maize (*Zea mays* L.; e.g., Manzeke et al., 2014; Naveed et al., 2018; Zhang et al., 2013), the staple grain in most Southern African countries. Reducing Zn

Abbreviations: CRM, certified reference material; NR, natural regions; SSA, sub-Saharan Africa.

deficiencies in SSA is confounded by low fertilizer application (Jama & Pizarro, 2008; Kaizzi, Mohammed, & Nouri, 2017) and a scarcity of appropriate fertilizers (Mapfumo & Giller, 2001; Mtambanengwe & Mapfumo, 2005; Nezomba, Mtambanengwe, Rurinda, & Mapfumo, 2018). Most farmers are interested in applying major nutrients (e.g., nitrogen, phosphorus, and potassium; N, P, and K, respectively) rather than minor nutrients such as Zn (MacDonald, Bennett, Potter, & Ramankutty, 2011; Vitousek et al., 2009; Zhang, Wu, & Wang, 2008).

In Southern Africa, agronomic biofortification with soil Zn fertilizer can increase grain Zn concentration in maize (Manzeke, 2013; Manzeke et al., 2014) and cowpea (*Vigna unguiculata* L.; Manzeke et al., 2017). These crops are typically grown in smallholder communities in Zimbabwe on soils of low Zn status, and may contribute to addressing Zn deficiency within the region, if Zn fertilizers are added (Manzeke et al., 2012, 2014, 2019; Moloto, Moremi, Soundy, & Maseko, 2018). While the Zimbabwe Government recently launched the National Food Fortification Program, which regulates mandatory fortification of staple foods with essential micronutrients (WHO, 2015), fortified foods remain unaffordable to the marginalized rural communities.

The application of N fertilizers had been shown to increase grain Zn concentration following foliar Zn fertilizer applications in wheat (*Triticum aestivum* L.; Kutman, Yildiz, & Cakmak, 2011a; 2011b); and rice (*Oryza sativa* L.; Jaksomsak, Rerkasem, & Prom-u-thai, 2017) grown under glasshouse conditions. While these studies revealed that co-application of N and Zn fertilizers may be a promising strategy for the agronomic biofortification of cereal grains, there is limited information from field conditions (Pascoalino et al., 2018) on crops more commonly grown in SSA. Conversely, yield increases resulting from N fertilizer have also been reported to dilute or marginally increase grain micronutrient concentrations (Alloway, 2008; Fan et al., 2008; Garvin, Welch, & Finley, 2006). It is important to find agronomic management techniques that will increase dietary Zn intake in communities reliant on plant-based diets without a concomitant yield penalty. Findings from a large survey of smallholder farmers indicated that the application of N-containing fertilizers increased grain Zn concentration in cereal and legume crops grown even without Zn fertilization (Manzeke et al., 2019). To our knowledge, field research has not confirmed these findings. Therefore, the objective of this study was to determine the influence of different N fertilizer compositions, rates, and application strategies on grain Zn concentration of maize and cowpea that were receiving both soil and foliar Zn fertilizers. Furthermore, while N is important in the remobilization of Zn from the leaves to the grain (Jaksomsak et al., 2017; Kutman, Yildiz, & Cakmak, 2011b), we do not know whether efficacies of N in agronomic biofortification are influenced by N fertilizer composition, that is, mineral, organic, or some combination of both, or the rate of N fertilizer applied.

Core Ideas

- Co-application of N and Zn increased maize grain Zn concentration more than Zn fertilizer alone.
- At smaller N rates, mineral N was more effective at increasing maize grain Zn concentration.
- At larger N rates, all N fertilizer forms and strategies increased maize grain Zn concentration.
- Nitrogen fertilizer did not consistently increase grain Zn concentration of cowpea.

2 | MATERIALS AND METHODS

2.1 | Study sites

Field experiments were conducted in Makwarimba Ward (18°41′ S, 31°42′ E; 1,380 m asl), in Hwedza District, and Honde Valley (18°35′ S, 32°45′ E; 912 m asl) in Mutasa District in Zimbabwe during the 2016–2017 and 2017–2018 cropping seasons (Dec.–May). Hwedza and Mutasa Districts are in the eastern part of Zimbabwe in the Mashonaland East and Manicaland Provinces, respectively (Figure 1). Hwedza and Mutasa Districts were selected based on contrasting Natural Regions (NRs) or agro-ecological zones (Manzeke et al., 2019). Agro-ecological zonation in Zimbabwe is defined in terms of variations in mean annual rainfall, atmospheric temperature and humidity (Vincent & Thomas, 1961; Department of the Surveyor General, 1984). Mean annual rainfall is measured during a unimodal season that occurs between November and April, with NR I receiving the highest annual rainfall of >1,000 mm yr^{-1} and NR V receiving ≤450 mm yr^{-1} (FAO, 2006). Sites selected in each District had the following soil and farming characteristics:

1. Makwarimba Ward in Hwedza District is in NR II. Soils in Hwedza District are broadly classified as Lixisols (FAO, 2006b) with pockets of Luvisol (Anderson, Brinn, Moyo, & Nyamwanza, 1993; FAO, 1988). Maize is the dominant crop under a mixed crop–livestock farming system (Mtambanengwe & Mapfumo, 2009). Legumes such as groundnut (*Arachis hypogaea* L.), cowpea, and common bean (*Phaseolus vulgaris* L.) are typically grown on smaller patches of land compared with the staple maize. Cattle (*Bos taurus*) are the dominant livestock mainly kept for manure (for fuel and/or fertilizer) and draught power provision.

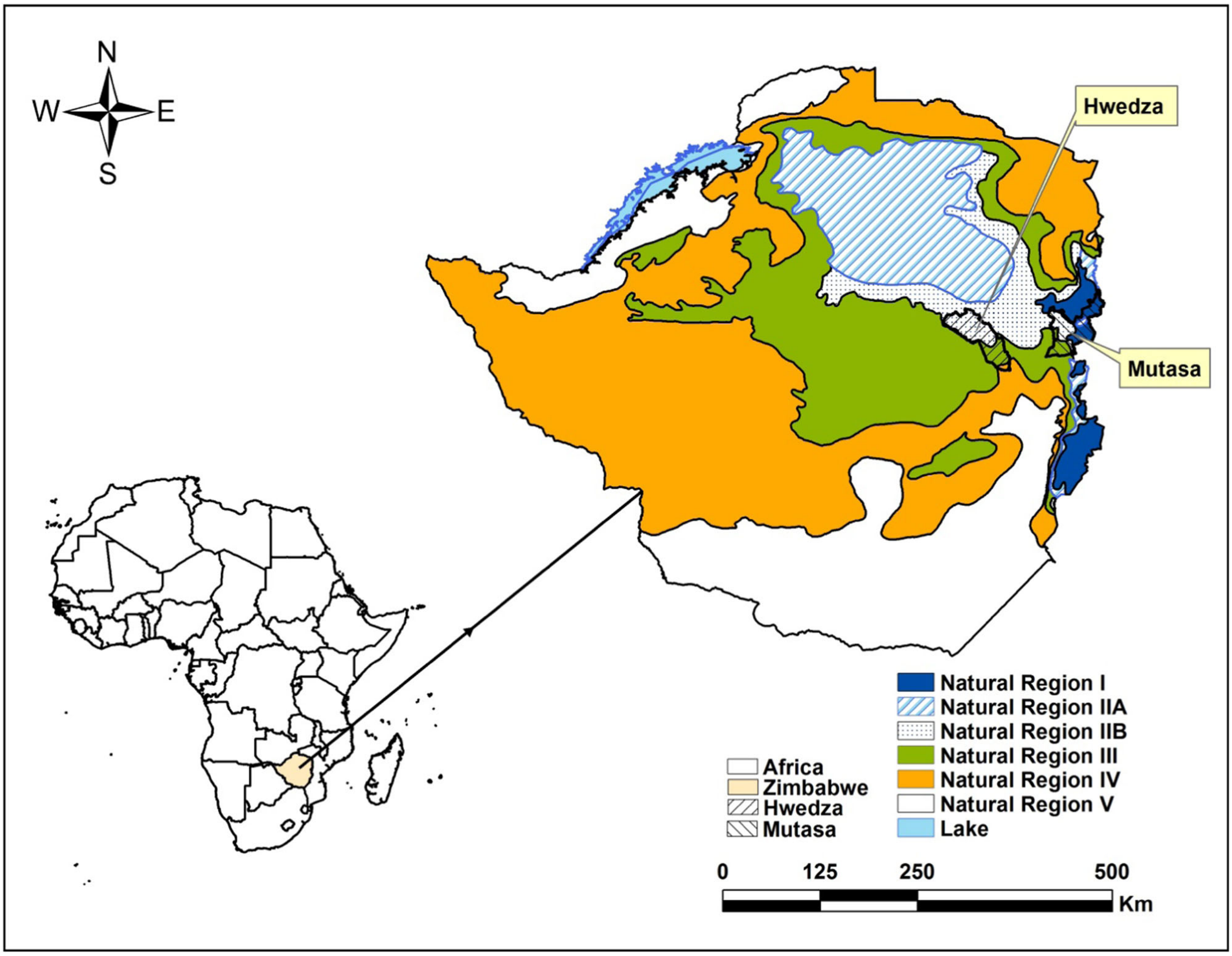

FIGURE 1 Map showing Hwedza and Mutasa Districts, sites where the field experiments were conducted during the 2016–2018 cropping seasons

2. Honde Valley, in Mutasa District, is in NR I and receives mean annual rainfall of >1,000 mm yr^{-1}, mostly between October and May. Honde Valley often receives some precipitation throughout the year, making it the wettest part of the country. Honde Valley extends from the eastern border of Zimbabwe into Mozambique with an average altitude of 900 m asl compared to its immediate surroundings which rise to above 1,800 m asl. Honde Valley has a hot temperate climate (Mugwagwa et al., 2015) and experiences hot and humid weather from late October to the end of April and hot summers averaging 30 °C during the dry months of the year. Soils in this area are broadly classified as Acrisols and Ferralsols with patches of Lixisols and Arenosols (FAO, 1988, 2006b). The main food crops grown are maize and groundnut with banana (*Musa acuminata* Colla) production for income generation. In Honde Valley, few farmers own cattle due to unfavorable terrain and climatic conditions (high temperatures and humidity) within this region (Manzeke et al., 2019).

Using mapping and guidance from agricultural extension workers, potential field sites located over sandy soil types were shortlisted. Sandy soils have inherently low plant-available soil Zn concentration, potentially causing Zn deficiency in plants (Alloway, 2008; Grant, 1981). Therefore, we established the field experiments on sites known to have low concentrations of soil Zn. Using a list of farmers located on sandy soils provided by the agricultural extension workers, 15 field sites were randomly selected in each district. From each of these potential sites, a composited soil sample from the 20 cm top surface was collected from 10 random points in each field measuring ∼0.45 ha. A field site with a diethylenetriamine pentaacetate (DTPA)-extractable soil Zn concentration of <0.8 mg kg^{-1} was then randomly selected from each district. A field with a DTPA-extractable soil Zn concentration of <0.8 mg kg^{-1} is considered to have a low concentration of plant-available soil Zn (Lindsay & Norvell, 1978). Soil pH for the two sites was acidic, where pH of 4.3 and 4.5 were measured in Hwedza and Mutasa, respectively (Table 1). Recent surveys conducted in two major maize-growing

TABLE 1 Physio–chemical properties of selected field site in the Hwedza and Mutasa District at a soil depth of 0–20 cm

Property	Hwedza	Mutasa
Clay content, g kg^{-1}	90 (8)[a]	60 (4)
Sand content, g kg^{-1}	820 (9)	860 (5)
Silt, g kg^{-1}	90 (5)	80 (2)
Available P[b], mg kg^{-1}	7.9 (0.3)	5.6 (0.7)
Available N[c], mg kg^{-1}	24 (2.4)	15 (1.9)
Available Zn[d], mg kg^{-1}	0.7 (0.05)	0.7 (0.03)
Total Zn, mg kg^{-1}	32.8 (4.8)	17.4 (2.9)
SOM[e], g kg^{-1}	28.0 (6)	23.0 (7)
Soil pH (0.01*M* $CaCl_2$)	4.3 (0.2)	4.5 (0.1)

[a]Values in parentheses denote standard error of the mean.
[b]Available P measured using the Olsen method (Olsen, Cole, Watanabe, & Dean, 1954).
[c]Mineralizable N after 2 wk of anaerobic incubation (Anderson and Ingram, 1993).
[d]Measured using the diethylene triamine pent-acetic acid (DTPA) method (Lindsay & Norvell, 1978).
[e]SOM, soil organic matter; measured using the loss on ignition method at a temperature of 105 °C in an oven for 4 h and 450 ± 30 °C in a muffle furnace for a minimum of 4 h (Anderson & Ingram, 1993).

smallholder farms in Zimbabwe showed that most soils had low soil pH between 4.5 to 5.5 (Manzeke et al., 2019). While low soil pH potentially limits crop productivity, it facilitates Zn uptake in soils (Alloway, 2008). Other soil physiochemical properties of selected field sites are presented in Table 1.

2.2 | Establishment of field experiments and experimental design

Mono-cropped maize and cowpea were grown at both field sites. Seven treatments were allocated in a fully randomized complete block design with four replicates. The treatments, which all received soil and foliar Zn fertilizer, were as follows:

Maize

1. T1 = 0 N + Zn
2. T2 = 45 kg mineral N fertilizer ha^{-1} + Zn
3. T3 = 45 kg organic N ha^{-1} + Zn
4. T4 = 22.5 kg mineral N fertilizer + 22.5 kg organic N ha^{-1} + Zn
5. T5 = 90 kg mineral N fertilizer ha^{-1} + Zn
6. T6 = 90 kg organic N ha^{-1} + Zn
7. T7 = 45 kg mineral N fertilizer + 45 kg organic N ha^{-1} + Zn

Cowpea

1. T1 = 0 N + Zn
2. T2 = 15 kg mineral N fertilizer ha^{-1} + Zn
3. T3 = 15 kg organic N ha^{-1} + Zn
4. T4 = 7.5 kg mineral N fertilizer + 7.5 kg organic N ha^{-1} + Zn
5. T5 = 30 kg mineral N fertilizer ha^{-1} + Zn
6. T6 = 30 kg organic N ha^{-1} + Zn
7. T7 = 15 kg mineral N fertilizer + 15 kg organic N ha^{-1} + Zn

Nitrogen was supplied in the readily available form (as mineral N fertilizer) and in the not readily available form (organic N fertilizer). During the first cropping season, Zn fertilizer was applied as $ZnSO_4{\cdot}7H_2O$ (22% Zn, w/w) to all plots, as a basal fertilizer at planting to supply a total of 10 kg of elemental Zn ha^{-1}. Additional Zn was supplied twice as foliar Zn. The amount of Zn applied at each spray was 0.3% w/v $ZnSO_4{\cdot}7H_2O$ in 500 L of water per hectare, which translated to 1.5 kg $ZnSO_4{\cdot}7H_2O$ ha^{-1}. This was equivalent to an additional 3 kg of $ZnSO_4{\cdot}7H_2O$ ha^{-1} (0.66 kg of elemental Zn ha^{-1}) applied as a foliar fertilizer. The Zn foliar spray solution was sprayed twice, at tasseling and silking stage for maize, with a 2-wk interval between sprays. Cowpea was foliar sprayed with Zn at active vegetative growth stage and at flowering stage. Foliar Zn fertilizer was applied twice to vegetative tissues and during grain filling stage to provide a large pool of Zn for increased nutrient uptake and grain Zn concentration (Cakmak et al., 2010b). Assuming residual soil Zn fertility benefits which could last for up to four years (Cakmak, 2008; Martens & Westermann, 1991), basal soil Zn was not reapplied to plots in the second season. Foliar Zn fertilizer application was repeated during the second cropping season as foliar fertilizers do not leave substantial residual effects (Cakmak, 2008). We did not include a non-Zn treatment as we know the differences in productivity and grain Zn concentration between a Zn and non-Zn treatment receiving or not receiving NPK from our previous work and related literature (see Manzeke et al., 2014).

Nitrogen was applied as a Compound D (7% N, 14% P_2O_5, 7% K_2O, w/w) fertilizer and ammonium nitrate (34.5% N w/w) fertilizer in maize and as a Compound D fertilizer only in cowpea. Farmers do not apply ammonium nitrate to cowpea because it fixes its own N. However, legumes require starter N, usually applied as Compound D, on nutrient-depleted sandy soils to boost crop productivity. The organic N source used was cattle manure at rates equivalent to the total N ha^{-1}. The different N fertilizer rates were 45 and 90 kg ha^{-1} in maize and 15 and 30 kg ha^{-1} in cowpea. Details of the treatments are shown in Table 2.

Mineral N and cattle manure treatments and application rates were based on recommendations of the local Zimbabwe Fertilizer Company (see FAO, 2006a) as well as background knowledge on rates applied by different farmer resource groups in maize (Mtambanengwe & Mapfumo, 2005) and cowpea (Kanonge, Mtambanengwe, Nezomba, Manzeke,

TABLE 2 Treatment, fertilizer types and rates applied to determine the influence of optimal N supply on grain Zn concentration in maize and cowpea

Treatment number	Maize		Cowpea	
	Treatment	Form of fertilizer applied	Treatment	Form of fertilizer applied
1	0 N + Zn	No N fertilizer was applied. P and K were supplied as SSP[a] and KCl	0 N + Zn	No N fertilizer was applied. P and K were supplied as SSP and KCl
2	45 kg mineral N fertilizer ha^{-1} + Zn	N was applied as Compound D ($7N:14P_2O_5:7K_2O$)[b] and AN (34.5% N).	15 kg mineral N fertilizer ha^{-1} + Zn	N was applied as Compound D ($7N:14P_2O_5:7K_2O$) only.
3	45 kg organic N ha^{-1} + Zn	Organic N was applied as cattle manure to achieve a N equivalent of 45 kg N ha^{-1}.	15 kg organic N ha^{-1} + Zn	Organic N was applied as cattle manure to achieve a N equivalent of 15 kg N ha^{-1}.
4	22.5 kg mineral N fertilizer + 22.5 kg organic N ha^{-1} + Zn	Organic N was applied as cattle manure depending on the N content of the cattle manure to achieve a N equivalent of 22.5 kg N ha^{-1}. Mineral N fertilizer was applied as Compound D and AN.	7.5 kg mineral N fertilizer + 7.5 kg organic N ha^{-1} + Zn	Organic N was applied as cattle depending on the N content of the cattle manure to achieve a N equivalent of 7.5 kg N ha^{-1}. Mineral N fertilizer was applied as Compound D
5	90 kg mineral N fertilizer ha^{-1} + Zn	N was applied as Compound D ($7N:14P_2O_5:7K_2O$) and AN (34.5% N).	30 kg mineral N fertilizer ha^{-1} + Zn	N was applied as Compound D ($7N:14P_2O_5:7K_2O$) only.
6	90 kg organic N ha^{-1} + Zn	Organic N was applied as cattle manure depending on the N content of the cattle manure (see Table 3) to achieve a N equivalent of 90 kg N ha^{-1}.	30 kg organic N ha^{-1} + Zn	Organic N was applied as cattle manure depending on the N content of the cattle manure (see Table 3) to achieve a N equivalent of 30 kg N ha^{-1}.
7	45 kg mineral N fertilizer + 45 kg organic N ha^{-1} + Zn	Organic N was applied as cattle manure depending on the N content of the cattle manure to achieve a N equivalent of 45 kg N ha^{-1}. Mineral N fertilizer was applied as Compound D and AN.	15 kg mineral N fertilizer + 15 kg organic N ha^{-1} + Zn	Organic N was applied as cattle manure to achieve a N equivalent of 15 kg N ha^{-1}. Mineral N fertilizer was applied as Compound D.

[a]SSP, single superphosphate; AN, ammonium nitrate.
[b]To determine P and K from P_2O_5 and K_2O rates, multiply the composition percentage by 0.44 and 0.83, respectively.

& Mapfumo, 2015; Manzeke et al., 2017). Similar rates of mineral and organic N fertilizer were applied each season. Cattle manure, applied to achieve an equivalent amount of N ha^{-1} (Table 2), was provided by the host farmer in each study site and was applied during both cropping seasons. The manure used in each season was carefully handled by the host farmer in the kraal (cattle pen) and/or through composting to avoid loss in N through leaching and volatilization.

Cattle manure used in Hwedza District was obtained from a pit next to the kraal (composted cattle manure). The farmer in this site would remove the manure from the kraal during the dry season and put it into a pit covered with soil. In contrast, the farmer in Mutasa would clear the manure from the kraal and then heap it nearby. Smallholder farmers in Zimbabwe have different ways of managing cattle manure. Composting of cattle manure is a common practice among smallholder farmers in Zimbabwe. This process involves digging a pit, usually next to the cattle kraal for ease of carrying and handling the manure. Cattle manure is usually put into these pits during the pre-rainy and/or rainy season before application into the fields. The pit, which is usually at the same site each season, is then covered at the end of the season and opened at the onset of the next rainy season. Cattle manure storage influences the nutrient status of manure (Nzuma, Murwira, & Mpepereki, 1998). Heaping manure on open fields, exposure to high ambient temperature, and rainfall affects manure quality through leaching and volatilization (Mugwira & Murwira, 1997). The N content of the cattle manure was used to guide application of an equivalent rate of N ha^{-1} from the manure. While cattle manure has residual fertility benefits which could last for 3–4 years (Mtambanengwe & Mapfumo, 2005), we repeated manure application during the second cropping season as we assumed loss in soil N due to crop uptake, leaching, and volatilization in the first season. Earlier work under similar farming systems showed that soil N content did not significantly increase after 2 yr of cropping when cattle manure was only applied in the first year of cropping (Manzeke et al., 2014).

Plot sizes of 4 by 3.6 m were used for both maize and cowpea. To ensure N was the only limiting factor, all treatments received similar rates of Zn, P (26 kg of P ha^{-1} in both maize and cowpea), and K (30 kg of K ha^{-1} to maize; Kanonge et al., 2015; Kurwakumire et al., 2014) as $ZnSO_4{\cdot}7H_2O$, single super phosphate (SSP) and muriate of potash (KCl; Table 2). In maize, compound D was applied at planting to treatments receiving mineral N fertilizer only to supply 30% of starter N. The remaining mineral N fertilizer for maize was applied as ammonium nitrate.

In cowpea, N was only applied as Compound D to act as starter N. Starter N is N fertilizer applied at the start of the cropping season and is required to kick-start legume productivity under nutrient-degraded sandy soils (Kanonge et al., 2015).

Maize and cowpea were planted after the first effective rains of each cropping season at population densities of ∼37,000 ha^{-1} and 296,000 ha^{-1}, respectively. The crop stands were kept weed-free through hand weeding. Maize stalk borer (*Busseola fusca*) was controlled with thionex (1,9,10,11,12,12-hexachloro-4,6-dioxa-5λ4-thiatricyclo[7.2.1.02,8]dodec-10-ene 5-oxide) granules applied at a rate of 3–4 kg ha^{-1}. To control aphids in cowpea, rogor (2-dimethoxyphosphinothioylsulfanyl-*N*-methylacetamide) was used at a rate of 300 ml ha^{-1}.

2.3 | Cattle manure characterization

Cattle manure was characterized for various physiochemical properties including N and Zn. The nutrient composition of cattle manure (Table 3) was analyzed from a composite sample collected from three different positions within the manure heap in each study site. The two composite samples of cattle manure were air-dried and ground using a stainless-steel grinder (Thomas-Wiley Model 4 Laboratory mill, Thomas Scientific, Swedesboro, NJ). Total N concentration was determined using the micro-Kjeldahl procedure as described previously. Total N, and P, organic C, Zn, and Fe concentrations and exchangeable bases (K, Na, Ca, and Mg) were analyzed using standard operating procedures described by Anderson and Ingram (1993), Murphy and Riley (1962), and Okalebo, Gathua, and Woomer (2002). Readings were read on an atomic absorption spectrophotometer.

TABLE 3 Nutrient composition of cattle manure used for field experimentation

Property	Hwedza	Mutasa
Total Zn[a], mg kg^{-1}	10.0	6.0
Total Fe[a], g kg^{-1}	10.4	5.0
Total N[b], g kg^{-1}	10.1	16.1
Total P[c], g kg^{-1}	2.3	0.8
Total K, g kg^{-1}	4.3	2.6
Total Ca, mg kg^{-1}	454	401
Total Mg, mg kg^{-1}	1,930	1,196
Total Na, mg kg^{-1}	257	101
Organic C[d], g kg^{-1}	243	319
C/N ratio	24.1	19.8

[a]Wet digestion with Aqua Regia.
[b]Kjeldahl procedure.
[c]Wet digestion with Aqua Regia and Murphy Riley.
[d]Walkley–Black method.

2.4 | Grain yield quantification

Cowpea and maize grain were harvested at physiological maturity from net plots of 3 by 1.8 m (5.4 m^2) between February and April. A composite sample of five maize ears was collected from each host farmer's field next to the experimentation field to assess differences in maize grain Zn concentration between grain collected from the experimental site and the farmer's field. We were not able to collect cowpea grain from the host farmers' fields as none of the two host farmers grew cowpea during both cropping seasons. All grain samples were air-dried and adjusted to a moisture content of 9.5% for cowpea and 12% for maize. The dried and shelled grain samples were later ground in a stainless-steel mill to pass through a 0.5 mm-mesh sieve. To mimic smallholder farmers' practice, crop residues were left in the fields after each cropping season and consumed by livestock during the dry season.

2.5 | Plant shoot biomass sampling for N analysis

The biomass of cowpea shoots was quantified at the start of flowering during both cropping seasons using 0.25-m^2 quadrats. During each cropping season, quadrats were placed on three random sampling points per plot. Aboveground cowpea biomass were collected from each of the three quadrats and combined to form a composite sample. The aboveground biomass yield was determined on a dry matter basis after oven-drying at 60 °C to a constant weight and analyzed for N. Maize ear-leaf samples were collected for N analysis before any foliar application of Zn fertilizer.

2.6 | Laboratory analysis

Milled grain sample material (0.2 g dry weight) was digested under a microwave heating system for 90 min (40 min digestion, 20 min ramping, 30 min cooling) at a controlled pressure of 2 MPa in 2.0 ml of 70% trace analysis grade HNO_3, 1.0 ml H_2O_2 and 1.0 ml milli-Q water as described by Manzeke et al. (2017). Digested grain sample solutions were analyzed on an inductively coupled plasma-mass spectrometer (ICP-MS, Agilent 8900 Triple Quad, Santa Clara, CA). Accuracies for Zn measured in the National Institute of Standards and Technology (NIST) 1567b plant certified reference material (CRM) ranged from 89 to 106% with a mean of 97% (standard deviation, SD = 1.4), based on 12 replicates.

Nitrogen analyses in maize and cowpea samples were conducted using the Kjeldahl method (Okalebo et al., 2002) on the VELP micro-Kjeldahl system. Two samples of a CRM (NIST 1573a Tomato leaf), with a certified N concentration of 30.3 g kg^{-1}, and two sample blanks were included in each digestion run of 42 samples for quality control. After digestion, six standards and three technical replicates per sample (including CRMs and blanks) were included in the BioTek EL808 96 well (flat bottom) plate colorimetric reader for absorbance readings which were then converted to g N kg^{-1} concentration. Accuracies for N measured in the NIST 1573a (Tomato leaf) plant CRM ranged from 101 to 112% with a mean of 105% (SD = 4.5) based on 12 replicates in six runs.

2.7 | Data analysis

The experiment entailed repeated measures over two seasons in the same plots. The correlation between the repeated measures on a single plot was dealt with by analysing the data with a linear mixed model in which the random effects included a term for plots and repeated observations within the plots. The fixed effects structure included block, season, and treatment effects. The analysis was done on the open-source R platform (R Core Team, 2014), and using the *lme* procedure from the *nlme* library (Pinheiro, Bates, DebRoy, & Sarkar, 2017). Estimation was done by residual maximum likelihood as each experiment had some missing data. Treatments T2–T7 were regarded as constituting a factorial experiment with N rate (45 or 90 kg ha^{-1} for maize; 15 or 30 kg ha^{-1} for cowpea) and N application strategy (organic, mineral, or combinations) treated as factors in the analysis. The effect of the seven treatments, with six degrees of freedom, were partitioned into up to six orthogonal contrasts with the following specific hypotheses encoded in the contrasts:

1. The application of N influences grain yield and grain Zn and N concentration when Zn fertilizer is also applied. We test this with the following contrast: T1 vs (T2, T3, T4, T5, T6, and T7). This is contrast C1.
2. The effect of applied N on grain yield and grain Zn and N concentration depends on the total amount of N applied. We test this with the following contrast: (T2, T3, and T4) vs (T5, T6, and T7; equivalent to the main effect of N rate in the factorial subset of treatments). This is contrast C2.
3. The effect of applied N on grain yield and grain Zn and N concentration depends on the application strategy (organic, mineral, or mixed). There are two more specific hypotheses under this as follows:
   a. There are differences in grain yield and grain Zn and N concentration between application of N as organic (T3 and T6) and application as mixed organic and mineral N fertilizer (T4 and T7). This is contrast C3.
   b. There are differences in grain yield and grain Zn and N concentration between application of N as mineral (T2 and T5) and application as organic N (including mixed N treatments; T3, T4, T6, and T7). This is contrast C4.
4. The effect of the rate of N application on grain yield and grain Zn and N concentration depends on the strategy. More specifically:
   a. The difference between the effect of applying sole organic N and applying mixed N depends on whether the overall rate of application of N is high (T6 and T7) or low (T3 and T4; this is tested by contrast C5).
   b. The difference between the effect of applying sole mineral N fertilizer and applying sole organic N (including mixed N) fertilizer depends on whether the overall rate of application of N is high (T5, T6, and T7) or low (T2, T3, and T4). This is contrast C6.

A natural $\log_e$ transformation was conducted in R for data transformation. We followed Welham, Gezan, Clark, and Mead (2015) and Webster and Lark (2019) in basing decisions on data transformation on exploratory statistics of residuals. We undertook an exploratory analysis of the data on their original scale of measurement. Examination of summary plots and statistics of the residuals, which showed positive skewness, suggested that a transformation was needed. The data were transformed to natural logarithms, and the repeated exploratory analysis suggested that the assumption of normality for the residuals was plausible on this scale. Confidence intervals for treatment means were computed using the residual mean square from the analysis of variance (ANOVA) output. The ANOVA tables showing grain yield and grain Zn concentration differences among the six tested contrasts are presented throughout the manuscript. Mean values for two cropping seasons for grain yield and grain Zn and grain N concentration are also be presented as graphs and Tables. Relationship between grain yield and grain Zn concentration was explored through simple linear regression using Excel.

TABLE 4 ANOVA contrasts of the effect of N management strategies on maize grain yields in Hwedza and Mutasa during the 2016–2017 and 2017–2018 cropping seasons

		Hwedza			Mutasa		
Contrast	**Comparison**	**Den *df*[a]**	***F* value**	***P*-value**	**Den *df***	***F* value**	***P*-value**
CS	Season effect	19	10.3	.0046	18	30.8	<.0001
C1	0 N vs some N application	18	12.5	.0024	18	6.7	.0185
C2	Low N vs High N	18	2.0	.1733	18	3.0	.1027
C3	Organic N vs mixed N	18	4.9	.0392	18	5.5	.0309
C4	Mineral N vs (mixed N and organic N)	18	8.0	.0113	18	1.0	.3280
C5	(Organic vs mixed N) × (High vs Low N)	18	0.4	.5309	18	0.9	.3603
C6	[Mineral N vs (Mixed and organic N)] × (High vs Low N)	18	0.9	.3460	18	0.2	.6702
CS × C1	Season × (0 N vs some N application)	19	0.006	.9407	18	0.02	.8799
CS × C2	Season × (Low N vs High N)	19	6.0	.0245	18	1.2	.2866
CS × C3	Season × (Organic N vs mixed N)	19	0.7	.4141	18	1.4	.2529
CS × C4	Season × [Mineral N vs (mixed N and organic N)]	19	0.5	.4942	18	4.0	.0594
CS × C5	Season × [(Organic vs mixed N) × (High vs Low N)]	19	2.6	.1236	18	1.3	.2769
CS × C6	Season × {[(Mineral N vs (Mixed and organic N)] × [(High vs Low N)]}	19	3.9	.0632	18	0.8	.3828
Block	Blocking effect	19	1.9	.1617	18	5.4	<.0082

[a]Den *df*, denominator degrees of freedom.

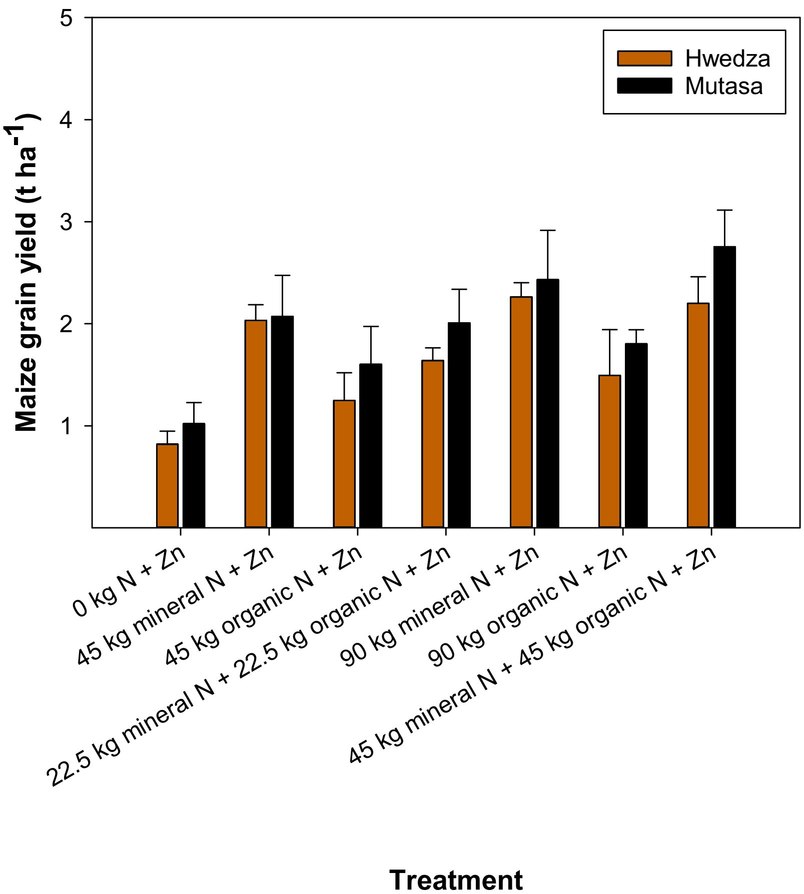

FIGURE 2 Mean maize grain yields over two cropping seasons (2016–2017 and 2017–2018) when receiving zinc (Zn) and different nitrogen (N) management options in Hwedza and Mutasa. Error bars represent standard error of means

3 | RESULTS

3.1 | Maize grain yields

Application of N fertilizer increased maize grain yields in both Hwedza (P = .0024) and Mutasa (P = .0185; Table 4, C1). During both cropping seasons, mean maize grain yields increased from 0.8 to 2.3 t ha^{-1} in Hwedza and from 1.0 to 2.8 t ha^{-1} in Mutasa with N fertilizers (Figure 2). There was no difference in maize yields between the 45 and 90 kg N treatments at Hwedza (P = .1733) and Mutasa (P = .1027; Table 4, C2). Nitrogen fertilizer management strategy influenced maize grain yields differently at both sites (P = .0392 in Hwedza and P = 0.0309 in Mutasa; see Table 4, C3). In Mutasa, the combined organic and inorganic N treatment increased yields to a greater extent than organic N alone; at both 45 and 90 kg ha^{-1} N rates (Figure 2). For example, at 45 kg N ha^{-1}, maize grain yields of 2.0 t ha^{-1} were attained when N was co-applied as mineral N fertilizer and organic N fertilizer compared with yields of 1.6 t ha^{-1} when sole organic N was applied. Similarly, in Mutasa, combinations of organic N and mineral N fertilizer applied at 90 kg N gave higher maize grain yields of 2.8 t ha^{-1} than yields of 1.8 t ha^{-1} when sole organic N was applied (Figure 2). This could be due to relative unavailability of N in cattle manure.

In Hwedza, the sole mineral N-fertilized treatments consistently had larger maize grain yields compared with the sole organic N and combinations of mineral and organic N fertilized treatments (Table 4, C4; P = .0113). For example, when sole mineral N fertilizer was applied at 90 kg N ha^{-1}, maize grain yields were 2.3 t ha^{-1}, compared to yields of 1.5 and 2.2 t ha^{-1} with application of 90 kg organic N, and 45 kg mineral N fertilizer + 45 kg organic N fertilizer ha^{-1}, respectively. Similarly, also in Hwedza, larger maize grain yields of 2.0 t ha^{-1} were attained in treatments receiving 45 kg mineral N fertilizer compared with similar rates of sole organic N or combinations of mineral and organic N fertilizer application which yielded 1.2 and 1.6 t ha^{-1}, respectively. There were no significant interaction effects between N fertilizer application strategy in Hwedza (P = .5309) and in Mutasa (P = .3603; C5), and N fertilizer application rate (P = .3460 in Hwedza; P = .6702 in Mutasa; C6) on maize grain yields (Table 4). There was a significant effect of season (contrast CS) on maize grain yields in Hwedza (P = .0046; Table 4) and Mutasa (P < .0001; Table 4). Maize grain yields were larger in the first cropping season compared with the second cropping season in both study sites. While there was no strong evidence to reject C2 (P > .05) in Hwedza, there was strong evidence to reject the null hypothesis that this contrast was the same in both seasons (P < .05). For example, in the first cropping season, the low N treatments (45 kg ha^{-1}) gave lower grain yields of 1.8 t ha^{-1} compared with mean grain yields of 2.5 t ha^{-1} attained in the high N (90 kg^{-1}) treatments. In contrast, the low N treatments gave larger mean maize grain yields of 1.6 t ha^{-1}, in the second season, compared with maize grain yields of 1.4 t ha^{-1} attained in the high N treatments (data not shown). This might be attributed to differences in rainfall amount between the two cropping seasons which influenced fertilizer availability. For example, Mutasa received almost double the amount of rainfall in Season 1 than Season 2 (Supplemental Figures S1 and S2), potentially resulting in higher rates of leaching in the low N than high N treatments. No significant season × treatment interactions (CS:C; P > .05) effects on maize grain yields were observed in Mutasa indicating a lack of evidence of seasonal differences in treatment contrasts (Table 4).

3.2 | Cowpea grain yields

Nitrogen increased cowpea yields at Hwedza (P = .017; Table 5, C1) but not at Mutasa (P = .557; Table 5, C1). Cowpea grain yields ranged from 0.2 to 0.6 t ha^{-1} in Hwedza (Figure 3). Significant differences between 15 and 30 kg ha^{-1} N rates on cowpea productivity were only evident in Hwedza (Table 5, C2; P = .017) but not in Mutasa (P = .535). For example, the 15 kg ha^{-1} N treatments had average cowpea grain yields of 0.3 t ha^{-1} whereas the 30 kg ha^{-1} N treatments had average cowpea grain yields of 0.5 t ha^{-1}. Nitrogen fertilizer management strategy and/or interaction with N rate had no significant effects on cowpea grain yields (P > .05; Table 5; C3–C6). Although cowpea grain yields were larger in the first cropping season than the second cropping season in Hwedza, no significant season (CS) and season × treatment interaction (CS:C; P > .05) effects on cowpea grain yields were observed (Table 5). In Mutasa, cowpea grain yields ranged from 1.0 to 1.2 t ha^{-1}. There were no significant effects of N fertilizer rate and/or fertilization strategy on cowpea productivity (P > .05; Table 5; C1–C6). Cowpea grain yields were consistently larger in Mutasa than in Hwedza, ranging from 1.1 to 1.4 t ha^{-1} during the first cropping season and from 0.9 to 1.1 t ha^{-1} during the second cropping season (data not shown).

3.3 | Maize grain Zn concentrations

Baseline maize grain Zn concentration in farmers' fields was 23.4 mg kg^{-1} in Hwedza and 21.9 mg kg^{-1} in Mutasa (Figure 4). Maize grain Zn concentration of 27.2 and 26.9 mg kg^{-1} was attained with soil and foliar Zn fertilizer alone in Hwedza and Mutasa, respectively. Grain Zn concentration of up to 38.0 and 31.9 mg kg^{-1} was attained when Zn was co-applied with N fertilizers in Hwedza and Mutasa, respectively. The largest mean maize grain Zn concentration over the two cropping seasons was attained in treatments

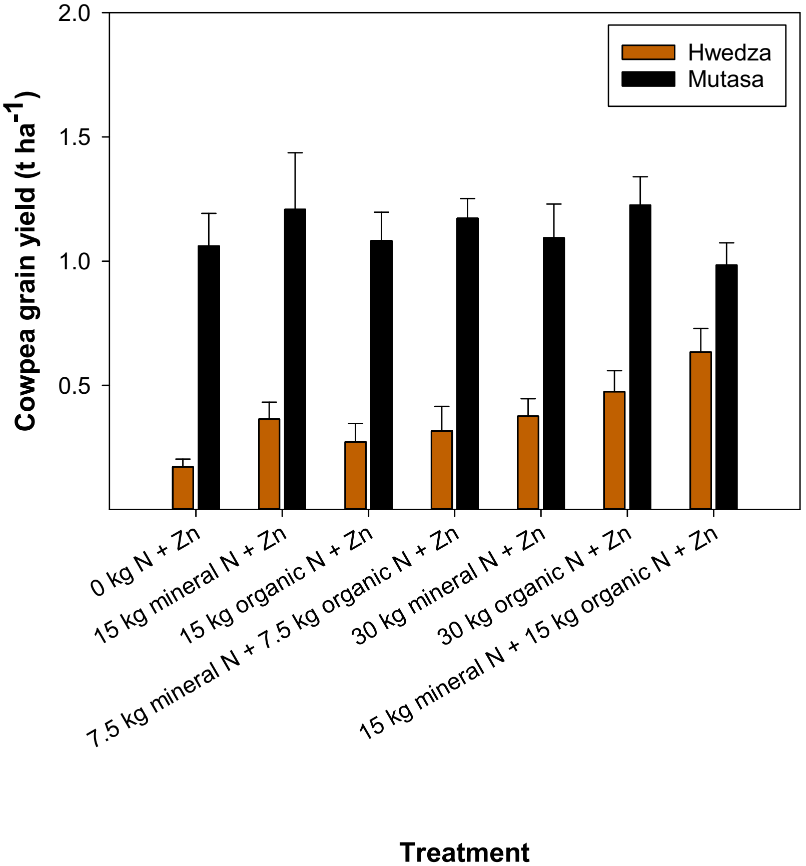

FIGURE 3 Mean cowpea grain yields over two cropping seasons beginning 2016–2018 when receiving zinc (Zn) and different nitrogen (N) management options in Hwedza and Mutasa. Error bars represent standard error of means

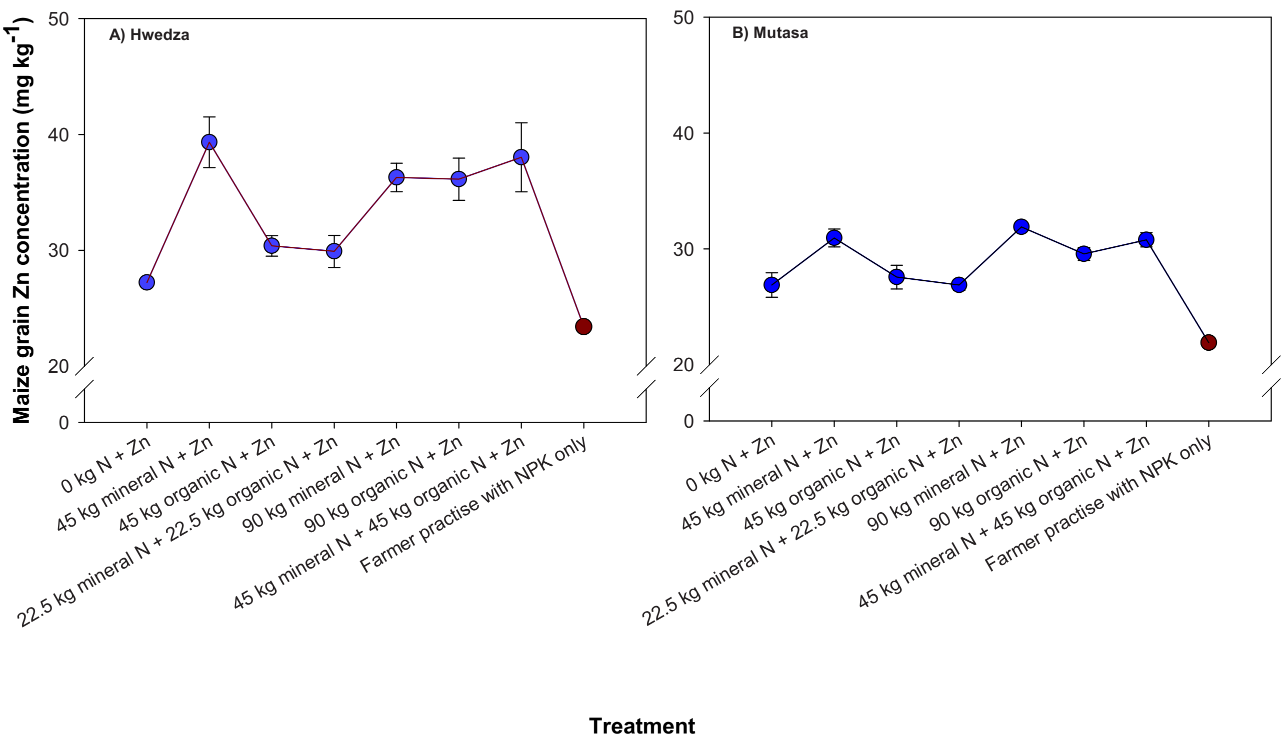

FIGURE 4 Mean maize grain zinc (Zn) concentrations in treatments receiving optimal and suboptimal rates of nitrogen (N) applied as organic, mineral, or in combinations in Hwedza and Mutasa over two cropping seasons beginning 2016–2018. Error bar represent standard error of means. The continuous line joins the treatment means. Farmer practice refers to grain collected from a farmer's field next to the experimental field after being fertilized solely with NPK

TABLE 5 ANOVA contrasts of the effect of N management strategy on cowpea grain yields in Hwedza and Mutasa during the 2016–2017 and 2017–2018 cropping seasons

		Hwedza			Mutasa		
Contrast	**Comparison**	**Den *df*[a]**	***F* value**	***P*-value**	**Den *df***	***F* value**	***P*-value**
CS	Season effect	19	11.4	.079	21	15.1	.0009
C1	0 N vs some N application	18	0.5	.017	18	0.3	.557
C2	Low N vs High N	18	0.2	.017	18	0.4	.535
C3	Organic N vs mixed N	18	0.4	.231	18	0.5	.472
C4	Mineral N vs (mixed N and organic N)	18	0.8	.453	18	0.1	.697
C5	(Organic vs mixed N) × (High vs Low N)	18	1.7	.492	18	2.4	.124
C6	[Mineral N vs (Mixed and organic N)] × (High vs Low N)	18	1.9	.099	18	0.2	.611
CS × C1	Season × (0 N vs some N application)	19	0.3	.9407	21	0.2	.630
CS × C2	Season × (Low N vs High N)	19	0.6	.2450	21	0.2	.599
CS × C3	Season × (Organic N vs mixed N)	19	0.1	.4141	21	0.003	.961
CS × C4	Season × [Mineral N vs (mixed N and organic N)]	19	0.3	.4942	21	0.1	.721
CS × C5	Season × [(Organic vs mixed N) × (High vs Low N)]	19	0.4	.1236	21	0.1	.760
CS × C6	Season × {[(Mineral N vs (Mixed and organic N)] × [(High vs Low N)]}	19	0.05	.0632	21	0.03	.854
Block	Blocking effect	19	4.9	.885	21	5.7	.0063

[a]Den *df*, denominator degrees of freedom.

receiving sole mineral N fertilizer applied at 45 and 90 kg ha^{-1} in Hwedza and Mutasa, respectively (Figure 4).

Nitrogen increased maize grain Zn concentration in Hwedza ($P < .0001$) and Mutasa ($P = .003$; Table 6, C1). In Hwedza, treatments receiving N had a mean concentration of 35 mg kg^{-1}, which was ∼30% larger than a mean of 27.2 mg kg^{-1} attained in the control treatment with Zn fertilizer alone. Similarly, in Mutasa, treatments receiving N had a mean maize grain Zn concentration of 29.6 mg kg^{-1}, which was 10% larger than the control treatments with the least grain Zn concentration of 26.9 mg kg^{-1}. Nitrogen fertilizer application rate had a significant effect on maize grain Zn in Hwedza ($P = .019$) and Mutasa ($P = .001$; Table 6, C2). For example, in Hwedza, the 90 kg ha^{-1} N treatments had a mean grain Zn of 36.8 mg kg^{-1} compared with 33.2 mg kg^{-1} measured in maize grown with 45 kg N ha^{-1}. No effects of N applied as organic or combination of organic and mineral N fertilizer on maize grain Zn were observed in both sites ($P = .685$ in Hwedza and $P = .722$ in Mutasa; see Table 6, C3). In contrast, the sole mineral N-fertilized treatments consistently had larger maize grain Zn compared with the sole organic N and combinations of mineral and organic N fertilized treatments (Table 4, C4). For example, in Mutasa, when sole mineral N fertilizer was applied at 90 kg N ha^{-1}, maize grain Zn was 31.9 mg kg^{-1}, compared to a maize grain Zn of 29.6 mg kg^{-1} and 30.8 mg kg^{-1} when the same rate of N was applied as organic and combinations of organic and mineral N fertilizer, respectively.

The interaction effect between N fertilizer applied as sole organic or mixed N and N fertilizer rate had no significant effects on maize grain Zn concentration at either Hwedza ($P = .497$) or Mutasa ($P = .216$; Table 6, C5). In contrast, the effect of either sole mineral N fertilizer or organic N fertilizer and combinations of mineral and organic N fertilizer was dependent on the rate of N applied in Hwedza alone (Table 6, C6). For example, at 45 kg ha^{-1} N rates, maize receiving sole mineral N fertilizer had larger grain Zn concentration of 39.3 mg kg^{-1}, than the sole organic (30.4 mg kg^{-1}) or the combinations of organic and mineral N-fertilized (29.9 mg kg^{-1}) treatments. At 90 kg N ha^{-1}, the effects of N fertilizer application strategy on maize grain Zn concentration were comparable, with mean maize grain Zn concentrations of 36.3, 36.1, and 38.0 mg kg^{-1} attained in maize receiving sole mineral N fertilizer, sole organic N, and combinations of mineral and organic N, respectively (Figure 4).

There was a significant effect of season (CS) on maize grain Zn concentration in Hwedza ($P < .001$; Table 6) and Mutasa ($P = .0114$; Table 6). There was a larger response to N and Zn fertilizer application in maize during the first cropping season than during the second cropping season. For example, in Hwedza, maize grain Zn concentration ranged from 27.4 to 48.1 mg kg^{-1} during the first cropping season and from 24.0 to 43.1 mg kg^{-1} during the second cropping season (data not shown). In Mutasa, maize grain Zn concentration ranged from 26.4 to 36.7 mg kg^{-1} and from 19.8 to 34.1 mg kg^{-1} during the first and second cropping seasons, respectively (data not shown). In Hwedza, there were no significant season × treatment interactions (CS × C) indicating a lack of evidence of seasonal differences in treatment contrasts (Table 6). In contrast, there was a

TABLE 6 ANOVA contrasts of the effect of N management strategy on grain Zn concentration of maize grown in Hwedza and Mutasa during the 2016–2017 and 2017–2018 cropping seasons

		Hwedza			Mutasa		
Contrast	Comparison	Den *df*[a]	*F* value	*P*-value	Den *df*	*F* value	*P*-value
CS	Season effect	19	242.7	<.0001	15	8.3	.0114
C1	0 N vs some N application	18	23.8	<.0001	18	6.2	.003
C2	Low N vs High N	18	6.7	.019	18	6.6	.001
C3	Organic N vs mixed N	18	0.2	.685	18	0.1	.722
C4	Mineral N vs (mixed N and organic N)	18	8.1	.011	18	8.9	.0004
C5	(Organic vs mixed N) × (High vs Low N)	18	0.5	.497	18	1.4	.216
C6	[Mineral N vs (Mixed and organic N)] × (High vs Low N)	18	11.4	.003	18	2.2	.139
CS × C1	Season × (0 N vs some N application)	19	0.4	.5269	15	1.5	.2368
CS × C2	Season × (Low N vs High N)	19	0.8	.3810	15	6.4	.0230
CS × C3	Season × (Organic N vs mixed N)	19	1.2	.2858	15	0.2	.6874
CS × C4	Season × [Mineral N vs (mixed N and organic N)]	19	0.4	.5336	15	0.3	.6060
CS × C5	Season × [(Organic vs mixed N) × (High vs Low N)]	19	1.2	.2858	15	0.9	.3633
CS × C6	Season × {[(Mineral N vs (Mixed and organic N)] × [(High vs Low N)]}	19	0.4	.5336	15	0.7	.4067
Block	Blocking effect	18	1.3	.317	18	0.07	.794

[a]Den *df*, denominator degrees of freedom.

significant season × treatment interaction effect when low and high N treatments were compared ($P < .05$; CS × C2; Table 6) in Mutasa District. During the first season, the low and high N treatments gave comparable mean maize grain Zn concentrations of 30.0 and 30.4 mg kg^{-1}, respectively. During the second cropping season, the high N treatments gave 16% larger mean maize grain Zn concentration of 31.0 m kg^{-1} than a mean maize grain Zn concentration of 26.7 mg kg^{-1} measured in the low N treatments (data not shown). Maize grain Zn concentration was positively influenced by maize grain yield as shown by a significant positive relationship between grain yield and grain Zn concentration in both Hwedza (Supplemental Figure S3, $r^2 = .49$, $P < .001$) and Mutasa (Supplemental Figure S4; $r^2 = .20$; $P = .015$).

3.4 | Cowpea grain Zn concentrations

Cowpea grain Zn concentrations of 41.6 mg kg^{-1} were attained in Hwedza when soil and foliar Zn fertilizer alone was applied (Figure 5a). In Mutasa, the mean cowpea grain Zn was 50.5 mg kg^{-1} with soil and foliar Zn fertilizer alone (Figure 5b). There were no significant main effects ($P > .05$) of N fertilizer application rate, composition or management strategy on cowpea grain Zn concentration in Hwedza (C1–C5, Table 7). Mean cowpea grain Zn concentrations ranged from 39.8 to 44.5 mg kg^{-1} in Hwedza (Figure 5a) and from 50.5 to 52.7 mg kg^{-1} in Mutasa (Figure 5b). There was a highly significant ($P = .0005$) interaction effect between N fertilizer application rate and management strategy on grain Zn (C6, Table 7) in Hwedza alone with no similar effects in Mutasa ($P = .7782$; Table 7). For example, at 15 kg N ha^{-1}, the sole mineral N-fertilized treatment gave a larger grain Zn concentration of 44.1 mg kg^{-1} compared with grain Zn concentrations of 42.3 and 39.8 mg kg^{-1} when N was applied in sole organic composition or in combination with mineral fertilizer, respectively. In contrast, at 30 N kg ha^{-1}, the sole mineral N fertilizer treatment had 40.9 mg Zn kg^{-1}, which was ∼10% lower than 44.5 mg kg^{-1} measured in both the sole organic N and combinations of organic N and mineral N-fertilized treatments (Figure 5a). In Mutasa, the difference between the effect of applying sole organic N and applying mixed N depended on the overall rate of N applied ($P = .0247$; Table 7, C5). For example, when a low rate of N was applied, the mixed N treatment gave larger mean cowpea grain Zn concentration of 52.7 mg kg^{-1} than a mean cowpea grain Zn concentration of 50.5 mg kg^{-1} attained when N was applied as sole organic. In contrast, at high N rates (30 kg ha^{-1}), the sole organic N gave a larger cowpea grain Zn concentration of 52.4 mg kg^{-1}, outperforming the mixed N treatment by 3% (data not shown).

There was no significant season by treatment interactions (CS × C; $P > .05$; Table 7) in Hwedza. In Mutasa, there was a significant season by treatment interaction effect when organic and mixed N treatments were compared ($P = .0175$; CS × C3; Table 7). During the first season, the organic N treatments had larger cowpea grain Zn concentrations (48.3 mg kg^{-1}) than the mixed N treatments which had a mean cowpea grain Zn concentration of 47.0 mg kg^{-1}. Conversely, the mixed N treatments gave larger cowpea grain Zn

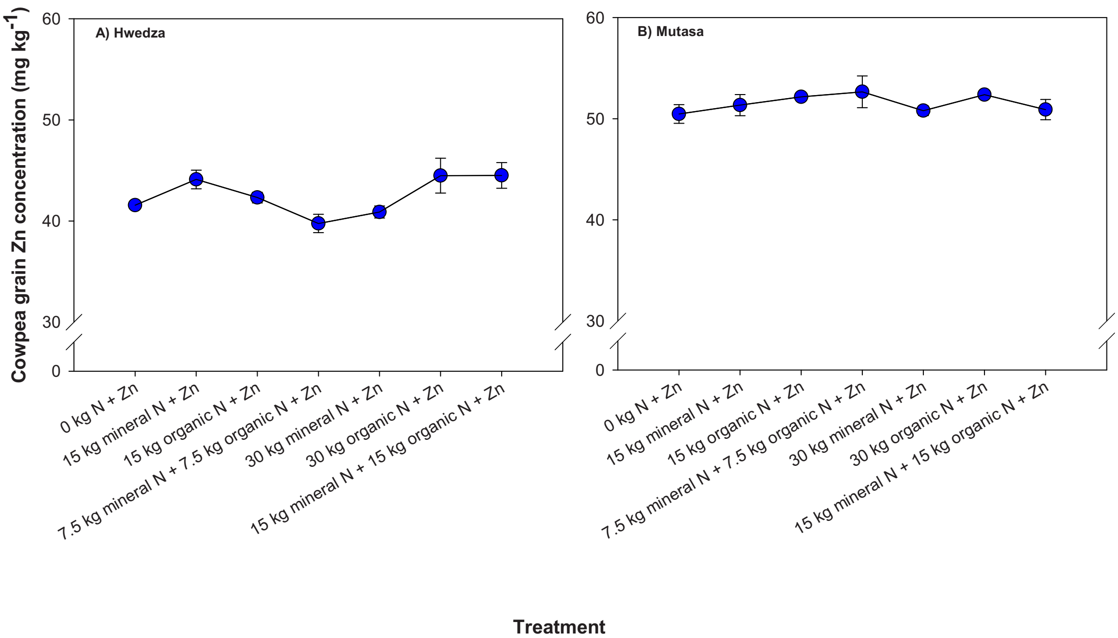

FIGURE 5 Mean cowpea grain zinc (Zn) concentration in treatments receiving optimal and suboptimal rates of nitrogen (N) applied as organic, mineral, or in combinations in Hwedza and Mutasa during two cropping seasons beginning 2016. Error bars represent standard error of means. The continuous line joins the treatment means. No cowpea was available for collection from the farmers' fields

TABLE 7 ANOVA contrasts of the effect of N management strategy on grain Zn concentration of cowpea grown in Hwedza and Mutasa during the 2016–2017 and 2017–2018 cropping seasons

Contrast	Comparison	Hwedza Den *df*[a]	Hwedza *F* value	Hwedza *P*-value	Mutasa Den *df*	Mutasa *F* value	Mutasa *P*-value
CS	Season effect	19	2.6	.1219	20	260.8	<.0001
C1	0 N vs some N application	18	1.2	.304	18	1.2	.2898
C2	Low N vs High N	18	2.5	.120	18	0.07	.7912
C3	Organic N vs mixed N	18	1.3	.164	18	0.2	.6370
C4	Mineral N vs (mixed N and organic N)	18	19.1	.779	18	2.4	.1374
C5	(Organic vs mixed N) × (High vs Low N)	18	0.8	.147	18	6.0	.0247
C6	[Mineral N vs (Mixed and organic N)] × (High vs Low N)	18	0.03	.0005	18	0.1	.7782
CS × C1	Season × (0 N vs some N application)	19	0.7	.4297	20	0.9	.3646
CS × C2	Season × (Low N vs High N)	19	0.6	.4609	20	0.04	.8532
CS × C3	Season × (Organic N vs mixed N)	19	2.4	.1384	20	6.7	.0175
CS × C4	Season × [Mineral N vs (mixed N and organic N)]	19	1.5	.2432	20	0.3	.6101
CS × C5	Season × [(Organic vs mixed N) × (High vs Low N)]	19	0.3	.5872	20	1.4	.2585
CS × C6	Season × {[(Mineral N vs (Mixed and organic N)] × [(High vs Low N)]}	19	0.9	.3550	20	0.1	.7269
Block	Blocking effect	18	2.0	.141	18	1.6	.2215

[a]Den *df*, denominator degrees of freedom.

concentrations of 56.5 mg kg^{-1} than the organic N treatments which had a mean cowpea grain Zn concentration of 54.5 mg kg^{-1}, although not significantly different (data not shown). A simple linear regression analysis showed no relationship between grain Zn concentration and grain yield in cowpea grown in both Hwedza (P = .381; r^2 = .03) and Mutasa (P = .713; r^2 = .005; Supplemental Figures S5 and S6).

3.5 | Nitrogen concentration in biomass and grain

There were no significant effects of N fertilizer application rate, composition, management strategy, and/or their interactions on N concentration in maize ear leaves and cowpea biomass samples. Nitrogen concentration in maize grain

TABLE 8 Mean maize and cowpea grain N concentration (± standard error of the mean) in treatments receiving different N management strategies together with soil and foliar Zn fertilizer in Hwedza and Mutasa over two cropping seasons (2016–2017 and 2017–2018)

Maize			Cowpea		
	Maize grain N concentrationg kg^{-1}		Cowpea grain N concentrationg kg^{-1}		
Treatment	Hwedza	Mutasa	Treatment	Hwedza	Mutasa
0 N + Zn	15.6 ± 0.4	14.6 ± 0.8	0 N + Zn	42.7 ± 0.4	42.6 ± 1.4
45 kg mineral N ha^{-1} + Zn	16.9 ± 0.6	15.6 ± 0.6	15 kg mineral N ha^{-1} + Zn	45.6 ± 1.2	45.2 ± 0.6
45 kg organic N ha^{-1} + Zn	15.9 ± 0.7	15.2 ± 0.2	15 kg organic N ha^{-1} + Zn	40.5 ± 2.6	44.7 ± 0.8
22.5 kg mineral N + 22.5 kg organic N ha^{-1} + Zn	15.7 ± 0.3	15.6 ± 0.8	7.5 kg mineral N + 7.5 kg organic N ha^{-1} + Zn	45.3 ± 1.1	42.6 ± 2.2
90 kg mineral N ha^{-1} + Zn	18.2 ± 0.6	18.9 ± 1.8	30 kg mineral N ha^{-1} + Zn	43.2 ± 1.1	43.4 ± 1.7
90 kg organic N ha^{-1} + Zn	15.7 ± 0.7	14.7 ± 0.5	30 kg organic N ha^{-1} + Zn	42.3 ± 1.4	44.7 ± 1.5
45 kg mineral N + 45 kg organic N ha^{-1} + Zn	16.1 ± 0.5	17.1 ± 0.8	15 kg mineral N + 15 kg organic N ha^{-1} + Zn	43.3 ± 1.8	44.4 ± 1.1
Mean	16.3	15.9	Mean	43.3	44.0
P-value	.03*	.04*	*P*-value	.219ns	.646ns

*Significant at the .05 probability level; ns, no significant treatment differences at P < .05.

ranged from 15.6 to 18.2 g kg^{-1} in Hwedza and 14.6 to 18.9 g kg^{-1} in Mutasa District (Table 8) with the 90 kg mineral N treatment consistently having the largest maize grain N concentration in both Hwedza and Mutasa. Significant differences in grain N concentration across treatments were observed in both Hwedza and Mutasa ($P < .05$; Table 8). When the effect of contrasts on grain N concentration were tested, a significant effect of N fertilizer composition and/or management strategy on maize grain N was evident only in Hwedza but not in Mutasa (ANOVA table of contrasts not shown). The 45 kg mineral N fertilizer rates in Hwedza had a larger grain N concentration of 16.9 g kg^{-1} than application of N as sole organic (15.9 g kg^{-1}) or mixed with mineral N fertilizer (15.7 g kg^{-1}; Table 8), indicating potential N-availability limitation in treatments receiving cattle manure. Nitrogen concentration in cowpea grains ranged from 40.5 to 45.6 g kg^{-1} in Hwedza and 42.6 to 45.2 g kg^{-1} in Mutasa District (Table 8). There were no significant effects of N fertilizer application rate, composition, management strategy, and/or their interactions on grain N concentration of cowpea (ANOVA table of contrasts not shown).

4 | DISCUSSION

4.1 | N management effect on yield

Application of N significantly increased maize grain yield in both sites. Nitrogen management strategy (i.e., sole organic, sole mineral, or combinations of organic and mineral fertilizer) differentially influenced maize grain yield in Hwedza and in Mutasa. Several studies showed the effect of N fertilizer and N management on crop yields. For example, Nyamangara, Mudhara, and Giller (2005) reported an increase in maize grain yield when mineral N was co-applied with cattle manure compared to sole manure treatments. Similarly, findings in Hwedza showed that combinations of organic and mineral N fertilizer resulted in the largest maize grain yields compared with sole organic or mineral N fertilizer. In contrast, the mineral N treatments gave the largest maize grain yields in Mutasa District. Cowpea grain yield was only influenced by N fertilizer rate in Hwedza alone with no significant effects of N on cowpea grown in Mutasa. Our findings show that N fertilizer management is differentially influenced by agro-ecology. This implies that instead of promoting blanket fertilizer use in African smallholder farms (Ichami, Shepherd, Sila, Stoorvogel, & Hoffland, 2019), fertilizer recommendations should consider the climatic and geospatial variations which potentially influence crop response to fertilizer.

4.2 | N management effect on plant Zn

The application of N fertilizer increased grain Zn concentration of maize grown with Zn fertilizer, compared with grains grown with Zn fertilizer alone. Nitrogen fertilizer did not increase grain Zn concentration of cowpea grown with Zn fertilizer as a main treatment effect. However, significant N management × N rate effects on grain Zn concentration were observed in both study sites. In this study, the application of soil and foliar Zn fertilizers alone yielded a maize grain Zn concentration of between 26.9 and 27.2 mg kg^{-1} and a cowpea grain Zn concentration of between 41.6 and 50.5 mg kg^{-1}. When N was co-applied with the Zn fertilizers, maize grain Zn concentration increased between 18.6 and 39.7% and between 4.4 and 7.0% in cowpea. These findings indicate that, under low-Zn soils, N fertilization can be important in improving grain Zn concentration of staple cereal and legume grains grown with Zn fertilizer. Current findings are the first, to our knowledge, to explicitly show the importance of N fertilizer in improving grain Zn concentration of maize and cowpea grown with soil and foliar Zn fertilizer under smallholder cropping systems.

When soil and foliar Zn fertilizers were applied, the largest grain Zn concentration in maize was achieved when N was applied as mineral N fertilizer at a rate of 45 kg N ha^{-1} compared to when N was applied as sole organic or combinations of organic and mineral N fertilizer. In smallholder farming systems, cattle manure, which has additional benefits of supplying micronutrients and increasing soil pH (Manzeke et al., 2012; Mtangadura, Mtambanengwe, Nezomba, Rurinda, & Mapfumo, 2017), is unfortunately only within reach of resource-endowed households (Masvaya et al., 2010; Swift, Frost, Campbell, Hatton, & Wilson, 1989; Zingore, Murwira, Delve, & Giller, 2007). Our findings suggest that intermediate-resourced and resource-constrained farmers who often do not own cattle could still harvest more grain Zn even with smaller additional mineral N fertilizer applications, which would also likely improve cereal and legume grain yields. While cattle manure potentially supplies Zn for improved crop Zn nutrition (Manzeke et al., 2012), the availability of N from manure to augment both N and Zn uptake proved limited. Abbasi, Hina, Khalique, and Khan (2007) reported a net N release capacity of 42% from cattle manure over the control, released within four phases of initial rapid release, slow release, maximum mineralization and decline phase. This N release was also reported by Mubarak, Gali, Mohamed, Steffens, and Awadelkarim (2010) to be strongly influenced by chemical composition of the manure and soil type, with a larger N release in lighter versus heavier textured soils.

While soil Zn fertilizers can augment integrated soil fertility management practices used by smallholder farmers in Zimbabwe through increased maize grain yield and grain Zn concentration (Manzeke et al., 2014), the importance of Zn fertilizers are not yet well known in these communities. It is therefore imperative to disseminate both recent evidence of the importance of traditionally applied N fertilizers in crop Zn nutrition, together with information on the importance of Zn fertilizers in maize–legume cropping systems and their potential contribution to dietary Zn intake of rural households.

Findings from this study revealed that the efficacy of N fertilization in agronomic biofortification of grains with Zn fertilizer is governed by the N fertilizer application rate, composition, and application strategy. Evidence of the importance of N in remobilizing Zn from leaves into wheat grains grown with Zn fertilizer has previously been shown by Kutman et al. (2011a, 2011b) and Pascoalino et al. (2018) under glasshouse conditions. Other authors have shown that N fertilization is important in uptake and accumulation of Zn in grains where it co-localizes as proteins with Zn in the embryo and aleurone layers (Cakmak et al., 2010a; Erenoglu, Kutman, Ceylan, Yildiz, & Cakmak, 2011; Ozturk et al., 2009). This co-localization could potentially increase Zn accumulation in the endosperm to concentrations which exceed current breeding targets (Persson et al., 2016).

Joy et al. (2015) revealed that soil Zn application led to an increase in median Zn concentration in maize, rice, and wheat grains of 23, 7, and 19%, respectively, and foliar application led to increases of 30, 25, and 63%, respectively in the same grains. Soil Zn fertilizers yield lower grain Zn concentrations than foliar sprays possibly due to low uptake efficiencies caused by various soil limiting factors (i.e., high soil pH, $CaCO_3$ content, and water availability; Alloway, 2008; Wang, Mao, Zhao, Huang, & Wang, 2012). Low soil pH promotes Zn bioavailability for plant uptake (Alloway, 2008). In this study, field experiments were established on fields with low soil pH of 4.3 and 4.5 in Hwedza and Mutasa, respectively. Our recent survey on 350 fields showed that most of the fields had a pH range of between 4.2 and 5.5 (Manzeke et al., 2019), with only 4% of farmers applying lime. Such very low soil pH ranges could potentially limit Zn and N uptake and crop productivity. The effect of lime and Zn fertilizer on crop productivity and grain Zn is a potential area warranting further study.

Foliar fertilizers can lead to higher grain Zn concentrations compared to soil-applied Zn (Cakmak, 2008; Joy et al., 2015; Manzeke, 2013; Zou et al., 2012). However, combined application of soil and foliar Zn fertilizers has been reported to be the most effective method in terms of increasing grain Zn concentration (Cakmak, 2008; Cakmak & Kutman, 2018). In this study, a 30% increase in grain Zn concentration was attained when N fertilizers were applied to maize receiving soil and foliar Zn fertilizers compared to a grain Zn concentration of 27.2 mg kg^{-1} when soil and foliar Zn fertilizers alone were applied.

Application of smaller N rates, regardless of composition or management strategy, outperformed the application of sole Zn fertilizer between 12 and 41% in maize grown from both districts. For more resource-endowed farmers who own cattle and often have financial capacity to purchase mineral fertilizer (Mtambanengwe & Mapfumo, 2005), a wider range of N management strategies are possible because at high N fertilizer application rates, increases in maize grain Zn concentration were independent of the N fertilization strategy employed. Increased crop productivity is known to result in a dilution of grain Zn (Alloway, 2008; Cakmak, 2008). However, our findings showed a significant positive relationship between grain Zn concentration and grain yield in maize but not cowpea, with a stronger relationship in Hwedza ($r^2 = .49$) than in Mutasa ($r^2 = .20$). This relationship, which might be influenced by differences in soil type and climatic conditions, might warrant further investigations.

The lack of significant differences in cowpea grain Zn concentration between the N and non-N fertilized treatments is possibly because cowpea inherently fixes N which could minimize the effects of external N fertilization on Zn mobilization (Awonaike, Kumarasinghe, & Danso, 1990). Although N is important in kick-starting crop productivity of grain legumes grown under nutrient-depleted sandy soils in smallholder farming systems, cowpea could still be agronomically biofortified with soil and foliar Zn fertilizers alone, without any external N fertilization. Given the scarcity of nutrient resources within smallholder cropping systems, farmers are better off applying the limited N-supplying fertilizers to staple maize than to N-fixing legumes, in addition to soil and foliar Zn fertilizers.

4.3 | N management on N concentration in plant tissue and grain

Maize grain N concentration, but not maize ear-leaf concentration, was significantly influenced by N fertilizer. Except for the 90 kg mineral N ha^{-1} treatment, the 45 kg mineral N ha^{-1} treatment had largest grain N (as well as grain Zn) concentration in Hwedza, indicating potential co-localization of N and Zn in the grain as evidenced by Kutman et al. (2011a). While developing leaves and seeds are major sinks of N during vegetative growth and reproductive stage, respectively (Tegeder & Masclaux-Daubresse, 2018), there was no evidence of N fertilizer rate or management effect on maize ear leaf, cowpea biomass, as well as cowpea grain N concentration. The absence of N effect on cowpea grain N clearly shows that cowpea's inherent capacity to fix N buffered its response to N fertilizer rate and/or application strategy.

5 | CONCLUSIONS

Nitrogen fertilizer application is important in improving grain Zn concentration of staple maize grown with soil and foliar Zn fertilizer, but not of N-fixing legumes. The increase in grain Zn concentration with low mineral N fertilizer implies that smallholder farmers can still increase the nutritive Zn value of grain produced on-farm, even within current constraints of limited N fertilizer use. Our findings could inform complex farmer decisions on improving crop Zn nutrition as well as on-going biofortification (genetic breeding and agrofortification) efforts through an improved understanding of spatial and site-specific variation in fertilizer response.

DATA AVAILABILITY

Data generated and used in this study are available from the corresponding author upon request.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Muneta G. Manzeke, Paul Mapfumo, Florence Mtambanengwe, Martin R. Broadley, Michael J. Watts were responsible for the conceptualization of the study. Muneta G. Manzeke was responsible for data curation and laboratory analysis and performed data analysis with R. Murray Lark. Paul Mapfumo, Florence Mtambanengwe, Martin R. Broadley, Michael J. Watts were responsible for developing the methodology with Muneta G. Manzeke. Muneta G. Manzeke wrote the original draft, and all authors contributed to the final version of the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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