Article

Landscape and Micronutrient Fertilizer Effect on Agro-Fortified Wheat and Teff Grain Nutrient Concentration in Western Amhara

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Abstract: Agronomic biofortification, encompassing the use of mineral and organic nutrient resources which improve micronutrient concentrations in staple crops is a potential strategy to promote the production of and access to micronutrient-dense foods at the farm level. However, the heterogeneity of smallholder farming landscapes presents challenges on implementing agronomic biofortification. Here, we test the effects of zinc (Zn)- and selenium (Se)-containing fertilizer on micronutrient concentrations of wheat (*Triticum aestivum* L.) and teff (*Eragrostis tef* (Zucc.) Trotter) grown under different landscape positions and with different micronutrient fertilizer application methods in the western Amhara region of Ethiopia. Field experiments were established in three landscape positions at three sites, with five treatments falling into three broad categories: (1) nitrogen (N) fertilizer rate; (2) micronutrient fertilizer application method; (3) sole or co-application of Zn and Se fertilizer. Treatments were replicated across five farms per landscape position and over two cropping seasons (2018 and 2019). Grain Zn concentration ranged from 26.6 to 36.4 mg kg^{-1} in wheat and 28.5–31.2 mg kg^{-1} in teff. Grain Se concentration ranged from 0.02 to 0.59 mg kg^{-1} in wheat while larger concentrations of between 1.01 and 1.55 mg kg^{-1} were attained in teff. Larger concentrations of Zn and Se were consistently attained when a foliar fertilizer was applied. Application of $^1/_3$ nitrogen (N) yielded significantly larger grain Se concentration in wheat compared to a recommended N application rate. A moderate landscape effect on grain Zn concentration was observed in wheat but not in teff. In contrast, strong evidence of a landscape effect was observed for wheat and teff grain Se concentration. There was no evidence for any interaction of the treatment contrasts with landscape position except in teff, where an interaction effect between landscape position and Se application was observed. Our findings indicate an effect of Zn, Se, N, landscape position, and its interaction effect with Se on grain micronutrient concentrations. Agronomic biofortification of wheat and teff with micronutrient fertilizers is influenced by landscape position, the micronutrient fertilizer application method and N fertilizer management. The complexity of smallholder environmental settings and different farmer socio-economic opportunities calls for the optimization of nutritional agronomy landscape trials. Targeted application of micronutrient fertilizers across a landscape

gradient is therefore required in ongoing agronomic biofortification interventions, in addition to the micronutrient fertilizer application method and the N fertilizer management strategy.

1. Introduction

Dietary micronutrient deficiency remains a worldwide challenge. Recent evidence from population-based individual-level datasets reported micronutrient deficiencies in >50% preschool-aged children and two-thirds of non-pregnant women of reproductive age globally, with the most deficient micronutrients being zinc (Zn), iron (Fe) and essential vitamins [1]. This calls for more cost-effective and site-specific agronomic technologies which promote access of micronutrient-dense foods at the farm level. Advances in agronomic biofortification research encompassing use of micronutrient-based fertilizers for improved crop nutrition have been ongoing for over a decade, with a focus on Zn among other micronutrients [2–5]. Selenium (Se) is among the essential micronutrients required for human health but has no proven function in plants [6,7]. Projections of dietary Se deficiency predicted that more than one billion people are at risk of Se deficiency, with changes in climate and decreases in soil organic carbon (SOC) estimated to intensify this challenge by 2099 [7]. Over 90% of children under five years of age and ~70% of women of reproductive age were reported to be at risk of Se deficiency [8]. This is evidence from a recent household survey conducted in three contrasting agro-ecological regions in Zimbabwe, where blood samples were collected from consenting participants.

Crop production systems have a role to play in alleviating dietary micronutrient deficiencies [9]. However, the heterogeneity of farming systems in Sub-Saharan Africa (SSA) still presents challenges on how best to target micronutrient fertilizers. Farmer soil fertility management practices emanating from differences in access to nutrient resources have been reported to influence the availability of micronutrients in soils and grains. Manzeke et al. [10] reported larger Zn concentrations in soils and grains collected from the most productive fields which often preferentially receive organic nutrient resources compared to the least productive fields which are often unfertilized by farmers due to farmers' socio-economic constrains and a lack of response to fertilization from these fields. Similarly, Wood et al. [11] reported a relationship between soil organic matter (SOM) content largely from organic nutrient resources, and grain Zn concentration in wheat (*Triticum aestivum* L.). Recent evidence from a wheat biofortification glasshouse study showed increases in grain Se concentration due to improved assimilation of inorganic Se into selenomethionine from nitrogen (N) application [12].

Apart from farmer management effects on micronutrient supply, micronutrient availability to crops is also affected differently by soil pH, SOM content, and various climatic and environmental factors such as temperature, rainfall, and topography [13]. This is based on recent evidence from surveillance work on geospatial variation in micronutrients conducted at sub-national scale in Ethiopia and Malawi. Crop genotype and variety also differentially influence grain micronutrient concentrations. A conventionally bred Zincol-2016 wheat variety yielded larger concentrations of Zn (~36 mg kg^{-1}) compared to a local Faisalabad-2008 variety (~25 mg kg^{-1}), when both varieties were grown on Zn-sufficient soils [14]. Zincol-2016 is a Zn-efficient wheat variety released by HarvestPlus. A recent study by Hafeez et al. [15] showed Zincol-2016′s superiority in yielding larger concentrations of Zn, Fe, starch, and wet gluten content when fertilized with these two micronutrients compared to a Zn-inefficient variety.

Whilst agronomic biofortification has potential to ameliorate micronutrient deficiencies in a cost-effective way [5,16], it is important that fertilization is employed with an understanding behind responsiveness of the soil and crop to micronutrients. In SSA, most

on-farm agronomic biofortification experiments with iodine (I), Fe, Se and Zn fertilizers have been conducted under uniform field landscape positions, e.g., [17–20] with fewer studies employed on contrasting field landscape positions and in different agro-ecological regions. Landscape positions have shown significant effects on soil physico-chemical properties [21], thus calling for site-specific fertilizer recommendations for improved fertilizer cost efficiency [22].

In Malawi, maize grain Zn concentration showed evidence of spatially correlated variation [23]. Some of this variation was associated with soil and environmental covariates implying that beyond localized sources of variation (e.g., crop variety and agronomic practices), the geographical location of a household can sometimes be the largest factor influencing grain micronutrient concentrations in cereals. It is within this background context that cropping systems, including agronomic biofortification interventions ought to be redesigned to consider targeted application of essential micronutrients, considering the heterogeneity of farming systems and different farmer socio-economic opportunities. We aimed to explore the effects of Zn- and Se-containing fertilizer, field landscape positions and their interactions on grain Zn and Se concentration of wheat and teff (*Eragrostis tef* (Zucc.) Trotter) grown under different topographic positions and with different micronutrient fertilizer application methods and mineral N fertilizer rates in the western Amhara region of Ethiopia. Farming systems and landscape positions are highly variable in Ethiopian highlands, resulting in considerable variations in soil and plant nutrients.

2. Materials and Methods

2.1. Study Sites

This study was conducted in Ethiopia's western Amhara region. Trials were implemented in three districts, Bahir Dar Zuria, Yilmana Densa, and Womberma, with one site in each district, Aba Gerima, Debre Mewi and Markuma, respectively (Figure 1). The western Amhara region has a unimodal rainfall system comprising a main growing season (*Kiremt*) which spans from June to September [24]. Mixed crop-livestock systems are typically practiced, with sparsely distributed tree cover. Crop production in this region is diverse, with a wide range of cereals namely teff, wheat and maize (*Zea mays* L.), while Triticale (*Triticosecale* Wittm. ex A. Camus) and rice (*Oryza sativa* L.) are produced at a much smaller scale. Pulses, including chickpea (*Cicer arietinum* L.), faba bean (*Vicia faba* L.), common bean (*Phaseolus vulgaris*) and lentil (*Lens culinaris* Medik.) are also major components of the cropping system. Western Amhara has a subtropical climate, receiving average annual rainfall of 1022–1450 mm and is situated 1800–2200 m above sea level (masl). The total amount of rainfall received during the 2018 and 2019 cropping seasons was 1431.8 and 1591.8 mm, respectively (Figure 2). The most dominant soils are Nitisols, Andosols, Cambisols, Vertisols and Luvisols. High soil degradation, aggravated by soil acidity and nutrient mining, is apparent in this region.

2.2. Test Sites Agroecological Characteristics

Abagerima and Debre-Mewi have a tepid moist mid-highlands agroecology character and Markuma test sites have a tepid sub-humid-mid-highlands agroecology [25]; (Table 1). The longest landscape represented in this study was Abagerima followed by Debre-Mewi and Markuma sites.

2.3. Experimental Procedure

The experiments were undertaken in the main cropping seasons of both 2018 and 2019. There were two sets of experiments in each season, one with wheat as the experimental crop, and the second with teff. The same treatments established on farmers' fields, were applied to both crops. The fields were on farms in specified communities ("sites"). Wheat experiments were performed in Debre Mewi and Markuma, and experiments with teff were performed in Debre Mewi and Aba Gerima.

Each farmer provided a single field for the experiment, and one replicate of each treatment was established in that field. Treatments were thus laid out in randomized complete block design, replicated across farms. Farm fields were selected at random from among those in each of three landscape positions: foot slope, mid-slope, and hillslope [22,26]. There were five farms per landscape position in each of the three districts, resulting in a total of 45 farms. Each treatment plot had 5 × 5 m dimensions.
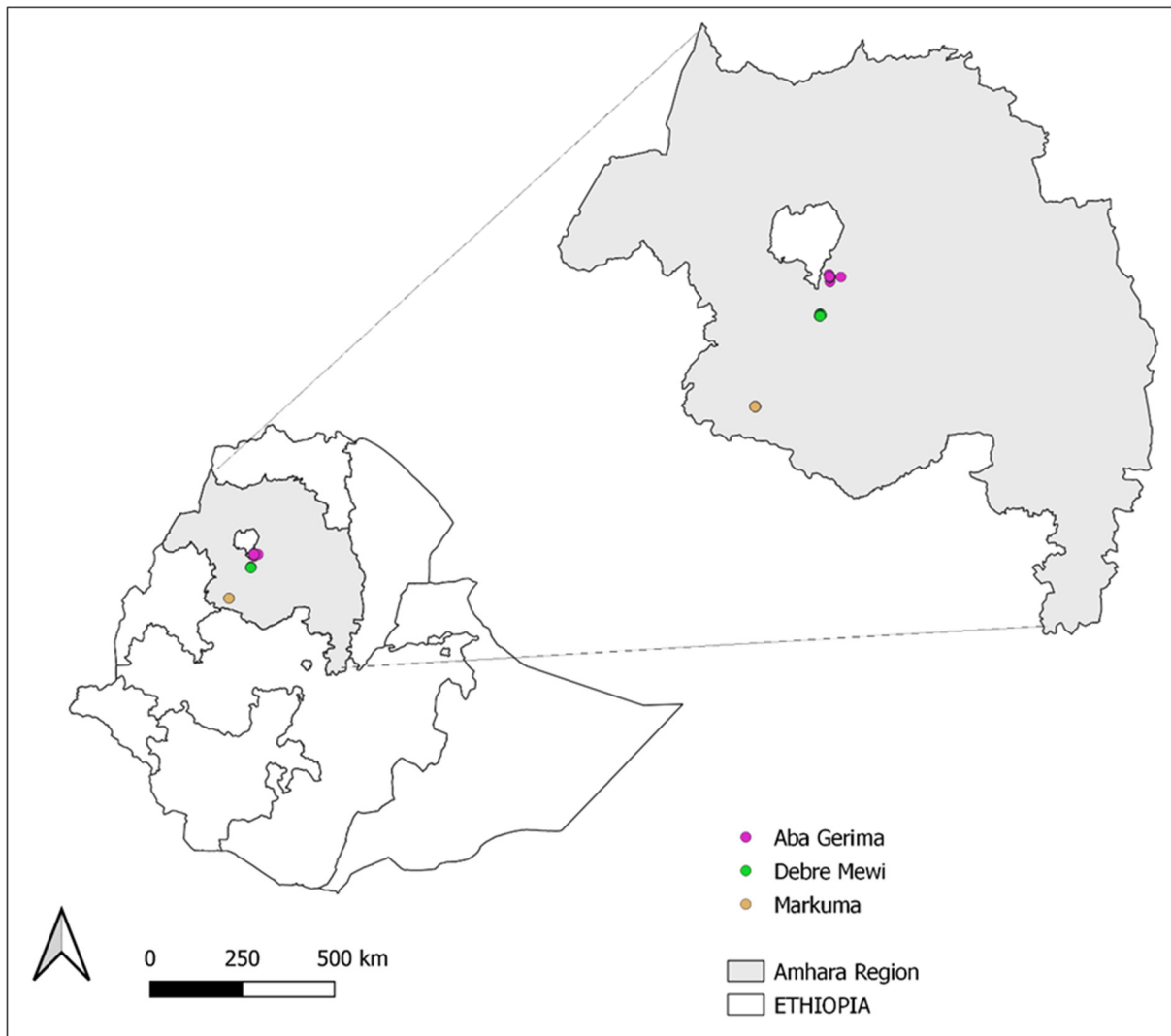
Figure 1. Map showing study sites locations in western Amhara.

In 2019, the site locations were revisited, but the treatments were established on different farms from those used in 2018 following similar patterns of landscape positions. In some cases, the same farmer was involved in both seasons, but with a different parcel of land. This was performed to avoid adding residual effects of fertilizer treatment into the factors to be considered when interpreting the results for the second season. There were 300 observations for teff (2 sites × 2 years × 3 landscapes × 5 farmers' fields × 5 treatments) and for wheat (2 sites × 2 years × 3 landscapes × 5 farmers' fields × 5 treatments), respectively. We also established farm history before selecting target plots and excluded farms where high fertilizer rates (>50 kg ha^{-1}) were applied in the previous year. The replication over seasons therefore provides information simply about the effect of adding fertilizer to soil which mainly contains the background concentration of those nutrients attributable to organic sources and minerals in its parent material. Similarly, a single replication of each treatment was established in each farm during the second season.

The treatment was allocated to a plot independently and at random. The randomization was performed on the R platform [27], and the R code produced a plot showing the randomization which could be used in the field to record landmarks and other features to facilitate orientation on visits to the plots. Table 2 shows the soil physico-chemical properties for the three sites across farms on different landscape positions. These soil properties, and a detailed presentation on soil analytical methods used, have been presented earlier by Desta et al. [28].
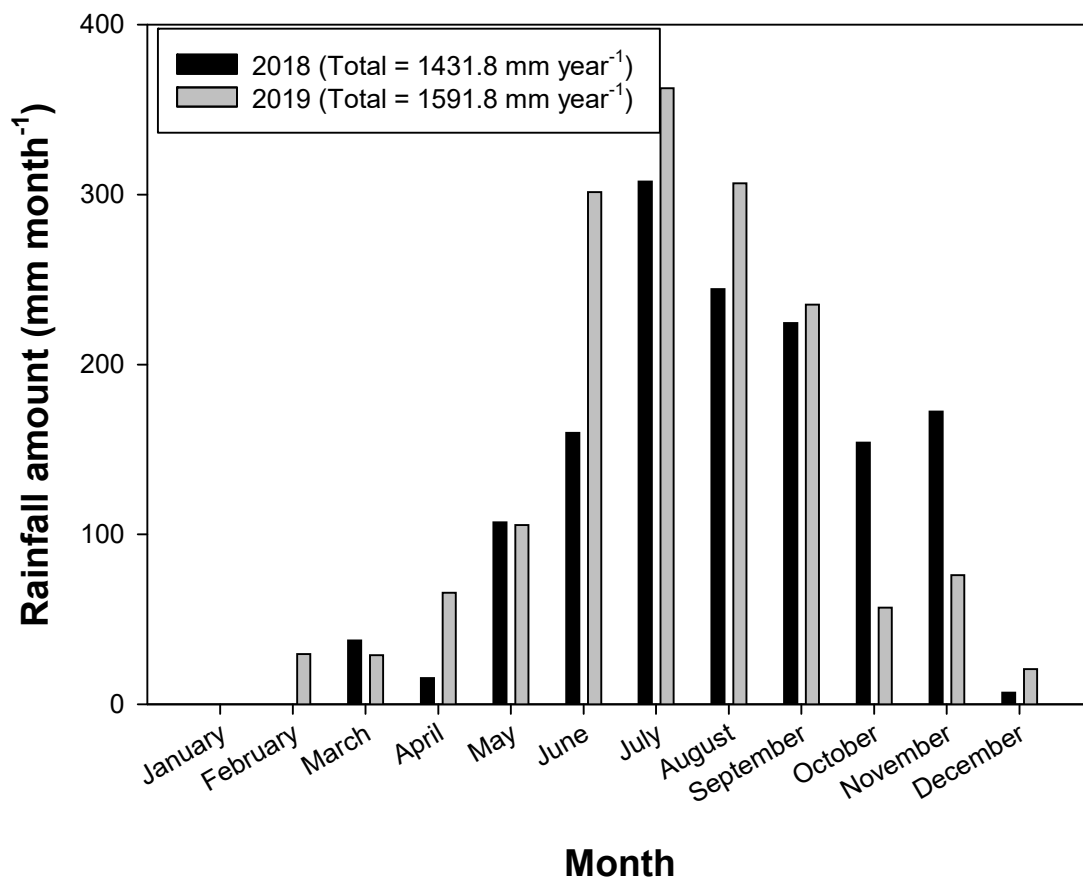
Figure 2. Monthly rainfall received during the 2018 and 2019 cropping seasons in western Amhara, Ethiopia. Graph plotted using SigmaPlot 15.

Table 1. Test crops and agro-ecologies of the test sites.

Site	Crops	Elevation Range in the Landscape (masl)	Landscape Length (m)	Agro-Ecological Zones
Debre-mewi (Yilmana Densa)	Teff and Wheat	2197–2287	2380	Tepid moist mid-highlands
Markuma (Wonberima)	Wheat	2052–2081	720	Tepid sub-humid-mid-highlands
Abagerima (Bahir Dar Zuria)	Teff	1899–1994	3405	Tepid moist mid-highlands

Table 2. Average soil physico-chemical properties from field sites in the same landscape position.

Location	Crop	Landscape Position	pH [1]	Total N [2]	SOC [2]	Clay [3]	Olsen P	Total Zn [4]	Available Zn [5]	eCEC [6]
				(%)				(mg kg^{-1})		(cmol$_c$ kg^{-1})
Aba Gerima		Hillslope	6.0	0.11	1.34	38	4.2	94	1.15	29.8
	Teff	Mid-slope	5.8	0.08	0.94	36	3.2	114	0.98	27.8
		Footslope	4.9	0.13	1.41	50	5.4	96	1.13	13.0
Debre Mewi		Hillslope	5.1	0.17	1.90	50	5.0	101	2.24	17.0
	Teff and Wheat	Mid-slope	5.6	0.12	1.37	57	3.2	91	0.96	25.8
		Footslope	6.2	0.12	1.51	70	3.3	99	1.64	37.3
Markuma		Hillslope	4.8	0.18	2.44	39	3.9	55	0.66	12.7
	Wheat	Mid-slope	4.9	0.17	2.27	42	2.5	54	0.56	13.0
		Footslope	4.9	0.15	2.09	44	2.0	59	0.30	12.5

Adapted from Desta et al. [28]. [1] = deionized water, [2] = dry combustion, [3] = laser scattering particle size distribution analyzer, [4] = aqua regia, [5] = Mehlich 3, and [6] = 0.0166 M cobalt (III) hexamine chloride solution (Cohex) $[Co[NH_3]_6]Cl_3$.

The treatments and their coding are set out in Table 3. The control (Treatment 1) is a recommended application rate of N, phosphorus (P), sulfur (S), potassium (K), and boron (B) fertilizer. Recognizing that this recommended application rate might often not be practiced, an additional treatment (Treatment 5, reduced control) was implemented with N and P applied at one-third ($^1/_3$) of the control rate. The remaining treatments, 2 to 4, entailed application of Zn, or Zn and Se. In Treatment 2 there was a basal Zn application at a rate of 8.25 kg Zn ha^{-1} (25 kg zinc sulfate monohydrate per hectare), this was also applied in Treatments 3 and 4. Treatment 3 also entailed, in addition to the basal Zn application, a foliar application of Zn (at rates of 4.13 kg Zn ha^{-1} or 12.5 kg zinc sulfate monohydrate per hectare) and Se. Foliar Se was applied as 20 g sodium selenate (Na$_2$SeO$_4$; 40.8% Se) ha^{-1} at the knee height stage. Treatment 4 entailed a side dressing of Zn at a rate of 4.13 kg Zn ha^{-1} (12.5 kg Zn sulfate monohydrate per hectare) in addition to the basal application.

In the 2018 harvest season, a foliar Zn application was included in Treatment 3. In 2019, this was changed to Treatment 4 because of challenges with leaf scorching experienced with co-application of foliar Zn and Se.

Fertilizer application rates were applied based on soil and crop type. Similar N, P, S, K and B rates of 138 kg N ha^{-1}, 92 kg P$_2$O$_5$ ha^{-1}, 16.96 kg SO$_3$ ha^{-1}, 40 K$_2$O ha^{-1} and 0.24 kg B ha^{-1} were applied in wheat grown on Nitisols and Vertisols; two soil types on which selected farms were situated (Table 3). Fertilizer elements were supplied from an NPSB blend with 18.9 N: 37.2 P$_2$O$_5$: 6.95 S: 0.1 B at planting. Half of the N was supplied from the blend and from urea (46% N) as a basal dressing. The remaining N was supplied as a top-dressing fertilizer. Potassium was supplied from muriate of potash (60% K$_2$O). In contrast, different fertilizer rates were applied in teff grown on the two different soil types. Fertilizer rates of 40 kg N ha^{-1}, 60 kg P$_2$O$_5$ ha^{-1}, 11.06 kg SO$_3$ ha^{-1}, 40 K$_2$O ha^{-1} and 0.16 kg B ha^{-1} were applied in teff grown on Nitisols (Table 4). Fertilizer rates of 80 kg N ha^{-1}, 46 kg P$_2$O$_5$ ha^{-1}, 8.48 kg SO$_3$ ha^{-1}, 40 K$_2$O ha^{-1} and 0.12 kg B ha^{-1} were applied in teff grown on Vertisols (Table 4). Larger rates of N were applied to teff grown on Vertisols due to increased leaching and volatilization rates in these soils. Due to the change in treatment from 2018 to 2019 and dropping of the foliar Zn application, treatments were relabeled to consider Se response as detailed in Table 5. Agronomic measurements (i.e., above ground total biomass, grain and stover yields) were collected. Harvesting was performed at physiological maturity. All fields were geolocated.

A complete set of prior orthogonal contrasts among these treatments were identified as encoding specific hypotheses of interest. For the analysis of the data on Zn concentration, the following contrasts were considered:

Contrast C1: No Zn input vs. Zn (i.e., {Treatment 1 and Treatment 5} vs. {Treatment 2, Treatment 3, and Treatment 4}). Here, it was hypothesized that there would be a difference in the Zn concentration of grain between those treatments which receive no Zn fertilizer, and those to which Zn is applied.

Contrast C2: Soil applied Zn vs. soil + foliar applied Zn (i.e., {Treatment 2, Treatment 4} vs. Treatment 3). Here, it was hypothesized that there would be a difference in Zn grain concentration between those crops receiving just soil applications of Zn, and those which also receive a foliar application.

Contrast C3: Basal vs. basal + side (i.e., Treatment 2 vs. Treatment 4). Here, it was hypothesized that there would be a difference in grain Zn concentration between those crops receiving just basal Zn, and those receiving both basal and side-dressing (i.e., no foliar) Zn.

Contrast C4: Control vs. 0.33 control (i.e., Treatment 1 vs. Treatment 5). Here, it was hypothesized that, in the absence of any Zn application, the rate of fertilizer application of macronutrients would affect the Zn concentration in grain. This could happen because of increased dilution of the Zn available from the native soil supply when the crop has a better macronutrient supply increasing the grain dry-matter content.

Table 3. Treatments and nutrients applied to wheat grown on both Nitisols and Vertisols during the 2018 and 2019 cropping seasons.

Treatment Label	Harvest Season			
	2018	Nutrient Amount (kg ha^{-1})	2019	Nutrient Amount (kg ha^{-1})
Treatment 1	NPSKB + urea "control"	138 kg N + 92 kg P_2O_5 + 16.96 kg SO_3 + 40 K_2O + 0.24 kg B	NPSBK + urea "control"	138 kg N + 92 kg P_2O_5 + 16.96 kg SO_3 + 40 K_2O + 0.24 kg B
Treatment 2	Control + basal Zn	Treatment 1 + 8.25 kg basal Zn	Control + basal Zn	Treatment 1 + 8.25 kg basal Zn
Treatment 3	Control + basal Zn+foliar Zn and Se	Treatment 1 + 8.25 kg basal Zn + 4.13 kg foliar Zn + 20 g sodium selenate (Na_2SeO_4)	Control + basal Zn+foliar Zn	Treatment 1 + 8.25 kg basal Zn + 4.13 kg foliar Zn
Treatment 4	Control + basal Zn+side Zn	Treatment 1 + 8.25 kg basal Zn + 4.13 kg side Zn	Control + basal Zn + side Zn + foliar Se	Treatment 1 + 8.25 kg basal Zn + 4.13 kg side Zn + 20 g sodium selenate (Na_2SeO_4)
Treatment 5	$^1/_3$ control rate	0.33 × (138 kg N + 92 kg P_2O_5 + 16.96 kg SO_3 + 40 K_2O + 0.24 kg B)	$^1/_3$ control rate	0.33 × (138 kg N + 92 kg P_2O_5 + 16.96 kg SO_3 + 40 K_2O + 0.24 kg B)

These treatment labels were used throughout field work.

Table 4. Treatments and nutrients applied to teff grown on Nitisols and Vertisols during the 2018 and 2019 cropping seasons.

Treatment Label	Nitisols		Vertisols *
	2018	Nutrient Amount (kg ha^{-1})	Nutrient Amount (kg ha^{-1})
Treatment 1	NPSKB + urea "control"	40 kg N + 60 kg P_2O_5 + 11.06 kg SO_3 + 40 K_2O + 0.16 kg B	80 kg N + 46 kg P_2O_5 + 8.48 kg SO_3 + 40 K_2O + 0.12 kg B
Treatment 2	Control + basal Zn	Treatment 1 + 8.25 kg basal Zn	Treatment 1 + 8.25 kg basal Zn
Treatment 3	Control + basal Zn + foliar Zn and Se	Treatment 1 + 8.25 kg basal Zn + 4.13 kg foliar Zn + 20 g sodium selenate (Na_2SeO_4)	Treatment 1 + 8.25 kg basal Zn + 4.13 kg foliar Zn + 20 g sodium selenate (Na_2SeO_4)
Treatment 4	Control + basal Zn + side Zn	Treatment 1 + 8.25 kg basal Zn + 4.13 kg side Zn	Treatment 1 + 8.25 kg basal Zn + 4.13 kg side Zn
Treatment 5	$^1/_3$ control rate	0.33 × (40 kg N + 60 kg P_2O_5 + 11.06 kg SO_3 + 40 K_2O + 0.16 kg B)	0.33 × (80 kg N + 46 kg P_2O_5 + 8.48 kg SO_3 + 40 K_2O + 0.12 kg B)
Treatment Label	**Nitisols**		**Vertisols ***
	2019	Nutrient Amount (kg ha^{-1})	Nutrient Amount (kg ha^{-1})
Treatment 1	NPSBK + urea "control"	40 kg N + 60 kg P_2O_5 + 11.06 kg SO_3 + 40 K_2O + 0.16 kg B	80 kg N + 46 kg P_2O_5 + 8.48 kg SO_3 + 40 K_2O + 0.12 kg B
Treatment 2	Control + basal Zn	Treatment 1 + 8.25 kg basal Zn	Treatment 1 + 8.25 kg basal Zn
Treatment 3	Control + basal Zn + foliar Zn	Treatment 1 + 8.25 kg basal Zn + 4.13 kg foliar Zn	Treatment 1 + 8.25 kg basal Zn + 4.13 kg foliar Zn
Treatment 4	Control + basal Zn + side Zn + foliar Se	Treatment 1 + 8.25 kg basal Zn + 4.13 kg side Zn + 20 g sodium selenate (Na_2SeO_4)	Treatment 1 + 8.25 kg basal Zn + 4.13 kg side Zn + 20 g sodium selenate (Na_2SeO_4)
Treatment 5	$^1/_3$ control rate	0.33 × (40 kg N + 60 kg P_2O_5 + 11.06 kg SO_3 + 40 K_2O + 0.16 kg B)	0.33 × (80 kg N + 46 kg P_2O_5 + 8.48 kg SO_3 + 40 K_2O + 0.12 kg B)

* Larger rates of N were applied to teff grown on Vertisols due to increased leaching and volatilization rates in these soils.

Table 5. Relabeling of treatments for analysis of the data on Se concentrations.

	Harvest Season	
	2018	2019
TR1	* NPSKB + urea "control" (Treatment 1)	NPSBK + urea "control" (Treatment 1)
TR2	$^1/_3$ control rate (Treatment 5)	$^1/_3$ control rate (Treatment 5)
TR3	Control + basal Zn + side Zn (Treatment 4)	Control + basal Zn + foliar Zn (Treatment 3)
TR4	Control + basal Zn + foliar Zn and Se (Treatment 3)	Control + basal Zn + side Zn + foliar Se (Treatment 4)

* Recommended rates of NPSKB for wheat and teff. The original treatment label is included for each season.

At the analysis stage the treatment labels were changed for examination of the response to Se, as shown in Table 5.

Treatment 2 was dropped for the analysis of Se concentration data and Treatment 4 (2018) and Treatment 3 (2019) constitute TR3 (double dose of Zn with no Se). Treatment 3 (2018) and Treatment 4 (2019) constitute TR5 (double dose of Zn with foliar Se).

As for the Zn concentration, specific hypotheses about the treatment effects on grain Se concentration were encoded in a complete set of orthogonal contrasts. These were as follows:

Contrast C1: No Se input vs. Se input (i.e., {TR4 } vs. {TR1,TR2, TR3}). Here, it was hypothesized that the Se concentration in grain of the experimental crop would be increased by a foliar application of Se.

Contrast C2: Zn applied vs. no Zn applied, no Se (i.e., TR3 vs. {TR1, TR2}). In this contrast none of the treatments entail a Se treatment. However, it was hypothesized that the application of Zn, a plant nutrient, would increase dry matter production in the grain and so reduce the concentration of Se available from the soil through a dilution effect.

Contrast C3: Control vs. reduced control, no Se (i.e., TR1 vs. TR2). As in C2, it was hypothesized that a dilution of available Se, where none is applied experimentally, is seen in those crops receiving the larger control application of macronutrient fertilizer.

2.4. Implementation in the Field

Sowing was performed for each test crop by location following the onset of rainfall, moisture contents for each specific soil type and local rainfed cropping calendar. Farmers, who rented out their farmlands, were responsible for the seedbed preparation using oxen plough which was performed two to three times based on soil type and soil moisture content. Weed control was carried out two to four times by hand as necessary per farmland. Teff 'Kuncho' and wheat 'TAY' varieties were used for all sites. Row planting was preferred for Nitisols whereas ridging and broadcasting was preferred for Vertisols.

2.5. Grain Quality Analysis

Grain samples were collected from representative heads of each treatment at physiological maturity, and a composite sample was considered for nutrient analysis. Grain samples were oven dried, cleaned and milled. Approximately 0.2 g (dry weight, dw) of each finely ground plant sample was weighed and microwave digested in 6 mL trace analysis grade HNO_3 in perfluoroalkoxy (PFA) vessels (Multiwave; Anton Paar GmbH, St. Albans, UK). The digested samples were diluted 1-in-10 with Milli-Q water immediately prior to multi-element analysis by inductively coupled plasma mass spectrometry (ICP-MS). Each digestion batch included a minimum of 6 blanks and a certified wheat flour standard (NIST 1567a) for QA purposes; recoveries were 94% for Zn and 95% for Se.

Zinc and Se elemental analysis of diluted solutions was undertaken by ICP-MS (Thermo-Fisher Scientific iCAP-; Thermo Fisher Scientific, Bremen, Germany). Samples were introduced (flow rate 1.2 mL min^{-1}) from an autosampler (Cetac ASX-520 Teledyne

CETAC Technologies, Omaha, NE, USA) incorporating an ASXpress™ rapid uptake module through a perfluoroalkoxy (PFA) Microflow PFA-ST nebulizer (Thermo Fisher Scientific, Bremen, Germany). Sample processing was undertaken using Qtegra™ software (Thermo-Fisher Scientific) utilizing external cross-calibration between pulse-counting and analogue detector modes when required. The iCAP-Q employed in-sample switching between two modes using a collision cell (i) charged with He gas with kinetic energy discrimination (KED) to remove polyatomic interferences and (ii) using H_2 gas as the cell gas. The latter was used only for Se determination. Internal standards Sc, Ge, Rh, and Ir, to correct for instrumental drift, were introduced to the sample stream on a separate line. Calibration standards included a multi-element solution including Zn and Se, in the range 0–100 μg L^{-1} (Claritas-PPT grade CLMS-2 from SPEX Certiprep Inc., Metuchen, NJ, USA), a bespoke external multi-element calibration solution (PlasmaCAL, SCP Science, Courtaboeuf, France) with Ca, Mg, Na and K in the range 0–30 mg L^{-1} and, a mixed phosphorus, boron and sulfur standard made in-house from salt solutions (KH_2PO_4, K_2SO_4 and H_3BO_3). The matrices used for internal standards, calibration standards and sample diluents were 2% Primar grade HNO_3 (Fisher Scientific, Loughborough, UK) with 4% methanol (to enhance ionization of Se).

2.6. Analysis of Data

The data were analyzed with a linear mixed model to reflect the structure of the experimental design. In this experiment, as described above, the treatments were randomized at the level of plots within farms. Farms were selected at random from within each of three landscape units within each site, but in specified numbers and so landscape units are treated as fixed effects. We therefore have a nested design with plots within farms (the residual level), and farms within sites. The same design was repeated in the second season, but on new farms, and so we treated the season as a random effect crossed with the nested random effects.

This model was fitted on the R platform [27] using the mixed function from the afex library for R [29]. This uses the lme4 library [30]. After model fitting the residuals were extracted and examined to check the plausibility of the assumption that they were normally distributed. This was performed with summary statistics, histograms and QQ plots (Figures S1–S9). Null hypotheses related to the effects of landscape position, the treatments (expressed as orthogonal contrast sets) and the interaction of treatment with landscape position were tested using variance ratios with degrees of freedom computed by the method of Kenward and Roger [31]. This adjusts the denominator degrees of freedom for dependence. Note that the denominator degrees of freedom may therefore be fractional.

3. Results

3.1. Wheat Grain Zn Concentration

Grain Zn concentration in wheat ranged from 26.6 to 36.4 mg kg^{-1} (Figure 3) with largest concentrations attained with co-application of basal and foliar Zn fertilizer. Strong evidence of fertilizer application effects on grain Zn concentration in wheat was evident in both C1 (no Zn input vs. Zn) and C2 (soil vs. soil + foliar application); ($p < 0.001$) (ANOVA Table S1 Supplementary File). There was no evidence for any interaction of the treatment contrasts with landscape unit ($p > 0.05$; ANOVA Table S1 Supplementary File). For this reason, mean Zn concentrations can be plotted for each treatment with foot slope as the reference position in Figure 3 (i.e., the expected concentration under each treatment at a foot slope position), and the additive effects of landscape position with the control fertilizer treatment as reference are shown in Figure 4 (i.e., the expected concentration at each landscape position if the control fertilizer treatment is applied).
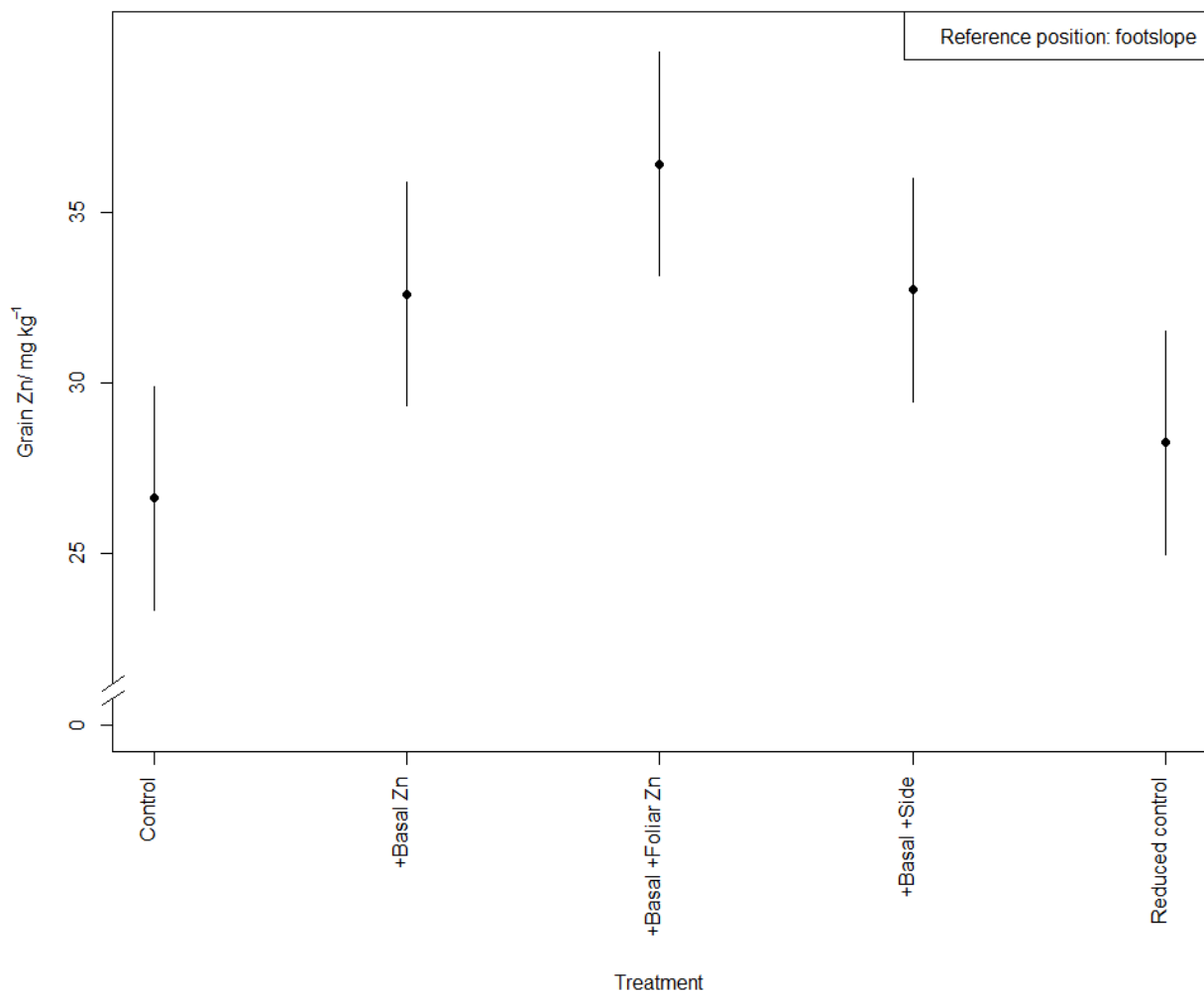
Figure 3. Mean wheat grain zinc concentration attained under different fertilizer treatments with foot slope as the reference landscape position. The bars show ±1 standard error.

Figure 3 supports the interpretation of the significant contrasts. The concentration of grain Zn over all treatments where Zn is applied in the fertilizer exceeds that in treatments where no fertilizer Zn is applied (C1). The application of Zn in foliar form in addition to soil Zn application resulted in larger Zn concentration in wheat compared to the application of soil Zn fertilizer alone (C2; $p < 0.001$). For example, the application of basal and foliar Zn fertilizer yielded grain Zn concentration of 36.4 mg kg^{-1} compared to grain Zn concentrations of 32.6 and 32.7 mg kg^{-1} attained in the basal only and basal + side dressing treatments, respectively (Figure 3). No strong evidence of an effect of the soil Zn application method (basal vs. basal + side; C3; $p = 0.93$) or macronutrient fertilizer application rate (control vs. $^1/_3$ control; C4) was observed ($p = 0.073$). The basal and basal + side dressing treatments yielded comparable grain Zn concentrations of 32.6 and 32.7 mg kg^{-1}, respectively, while the control and 0.33 control yielded 26.6 and 28.3 mg kg^{-1}, respectively (Figure 3).
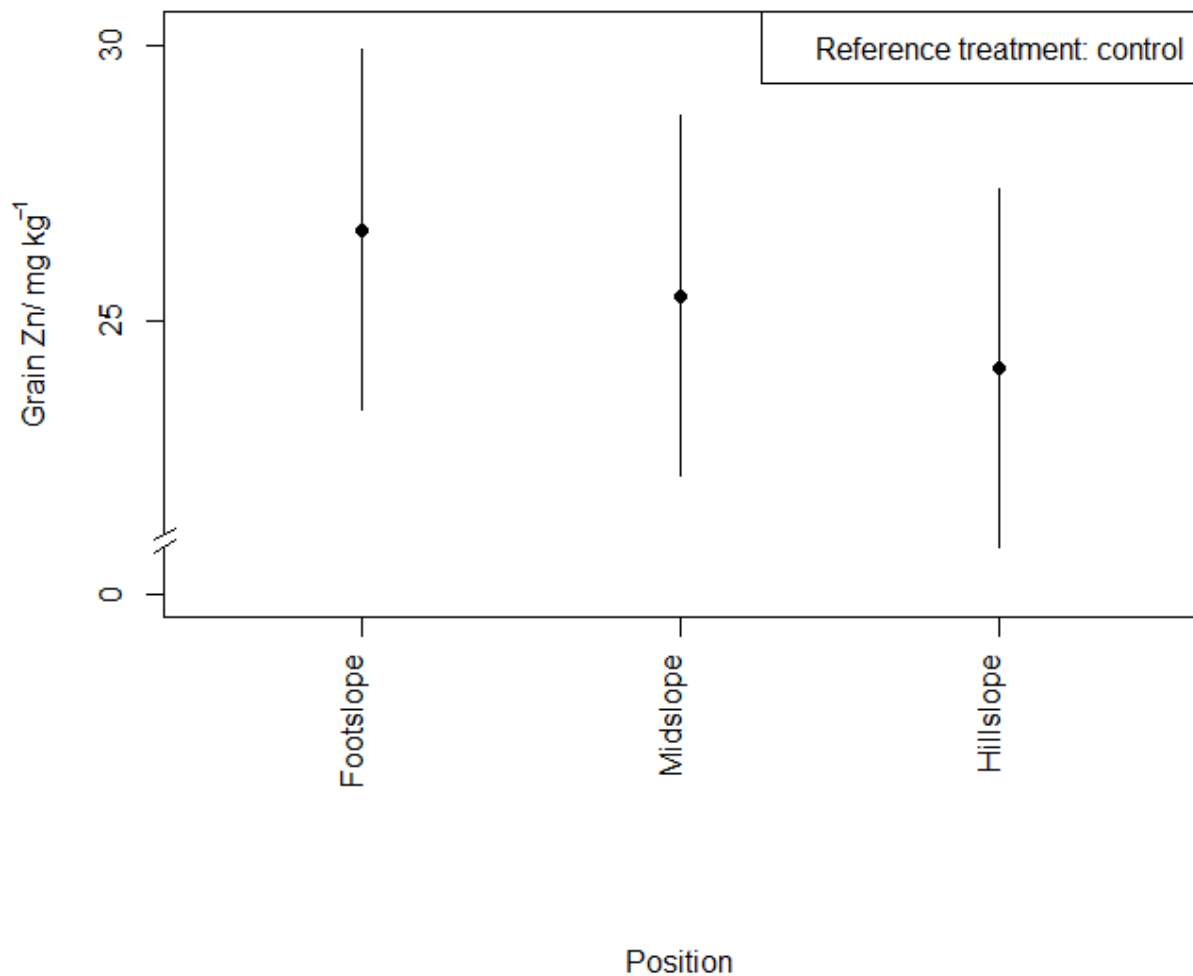
Figure 4. Mean grain zinc concentration of wheat grown on different landscape positions, with control as the reference fertilizer treatment. The bars show ± 1 standard error.

Moderate evidence for an effect of landscape unit on grain Zn concentration in wheat was observed ($p = 0.029$; ANOVA Table S1 Supplementary File). Wheat grown on foot slope positions had a larger grain Zn concentration of 26.6 ± 3.3 mg kg^{-1} compared to 25.5 and 24.1 ± 3.3 mg kg^{-1} attained on mid-slope and hillslope, respectively, when the control was used as the reference fertilizer treatment (Figure 4).

3.2. Teff Grain Zn Concentration

As with wheat grain Zn concentration, there was no evidence for an interaction of fertilizer treatment and landscape position, and so mean values for each level of these factors can be plotted for a reference level of the other. Mean teff grain Zn concentration (foot slope as reference position) ranged from 28.5 to 31.2 mg kg^{-1} (Figure 5). The application of Zn fertilizer irrespective of form, resulted in increased teff grain Zn concentration compared to no Zn input (C1). A strong effect of applying Zn in foliar form in addition to soil application yielded the largest grain Zn concentration in teff compared to application of soil Zn fertilizer alone (C2; $p < 0.031$), a similar trend was observed for wheat. For example, the application of basal and foliar Zn fertilizer yielded grain Zn concentration of 31.2 mg kg^{-1} compared to grain Zn concentrations of 30.0 and 30.9 mg kg^{-1} attained in the basal only and basal + side dressing treatments, respectively (Figure 5). While the margins between the basal + side dressing treatment and the basal Zn only treatment were

small, the earlier treatment yielded significantly larger grain Zn concentrations (C3). No evidence of an effect of macronutrient fertilizer application rate (control vs. $^1/_3$ control; C4) on teff grain Zn concentration was observed ($p > 0.05$), hence the control and $^1/_3$ control yielded comparable concentrations of 28.5 and 29.0 mg kg^{-1}, respectively (Figure 5). The $^1/_3$ control macronutrient fertilizer application consistently yielded a few milligrams of Zn more than the full control in both wheat and teff. No evidence for an effect of landscape unit on teff grain Zn concentration was reported ($p > 0.05$; ANOVA Table S2 Supplementary File). The main effect of treatment (within the foot slope position), with its standard error, is shown in Figure 5 for a model with no interaction. It is worth noting that although the teff control treatment yielded a few milligrams more than wheat, the absolute treatment effect on grain Zn concentration is much smaller for teff than wheat.
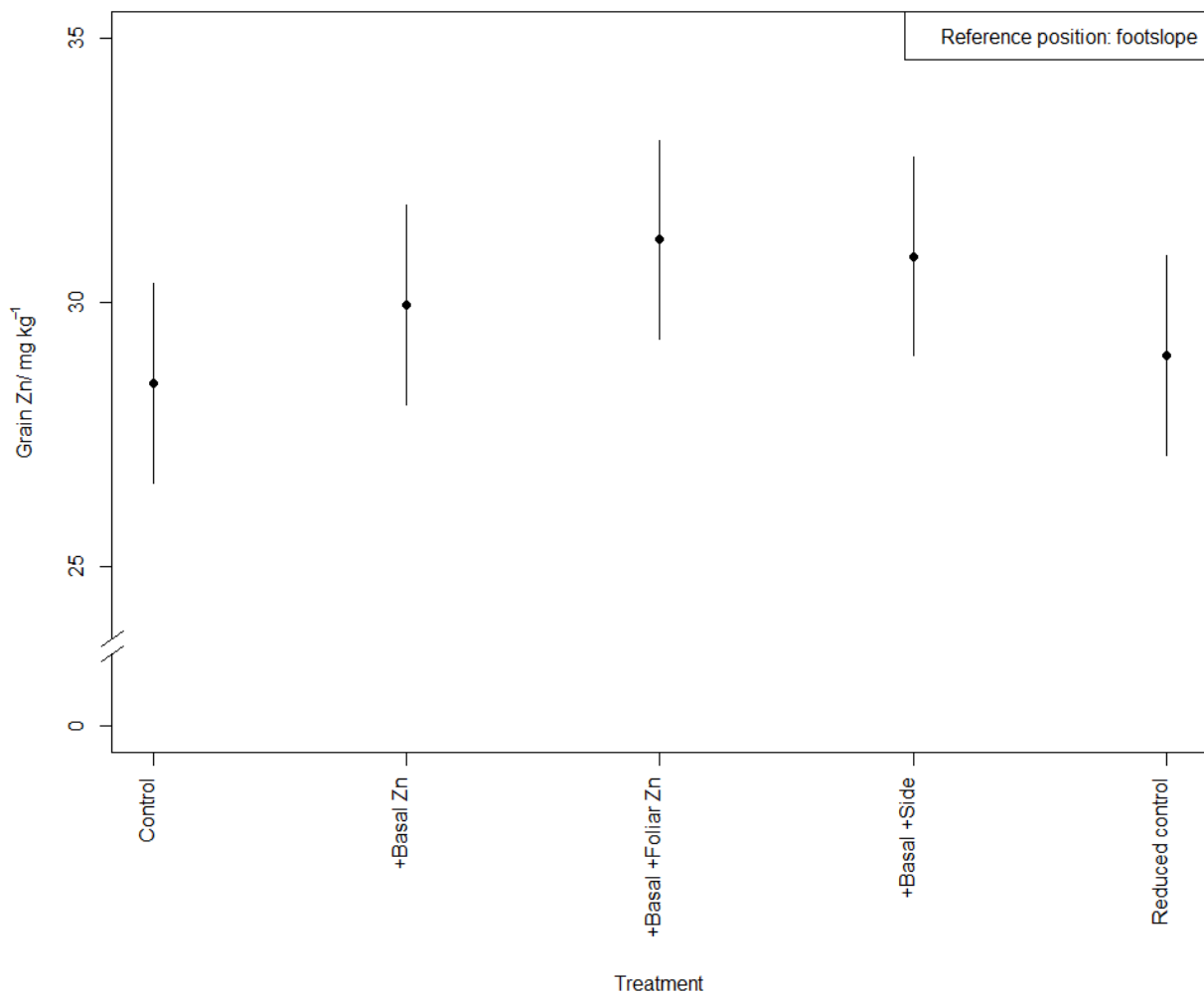
Figure 5. Teff grain zinc concentration attained under different treatments in western Amhara. The bars show ±1 standard error.

3.3. Wheat Grain Se Concentration

There was strong evidence to reject the null hypothesis of no difference among landscape positions ($p < 0.001$), but no evidence for any interaction of fertilizer treatment effects with landscape position. The fertilizer treatment means, with foot slope as reference position, are therefore shown in Figure 6. There was strong evidence to reject the null hypothesis for C1 (Se application effect; $p < 0.001$) and C3 (control vs. $^1/_3$ control; $p < 0.001$; ANOVA Table S3 Supplementary File). Application of Se significantly increased grain Se

concentration in wheat to 0.59 mg kg^{-1} compared to non-Se treatments (0.02–0.03 mg kg^{-1}; C1). Without Se fertilization, the $^1/_3$ control macronutrient fertilizer application rate significantly increased grain Se concentration by 50% from 0.02 (full control) to 0.03 mg kg^{-1} (0.33 control; C3). No evidence of Zn fertilizer effect on wheat grain Se concentration was reported when Se was not applied. Treatment means in Figure 6 are back transformed to the original units, so the mean values are median unbiased. The largest grain Se concentrations were found on foot slope positions, followed by hillslopes and lastly mid-slopes (Figure 7).
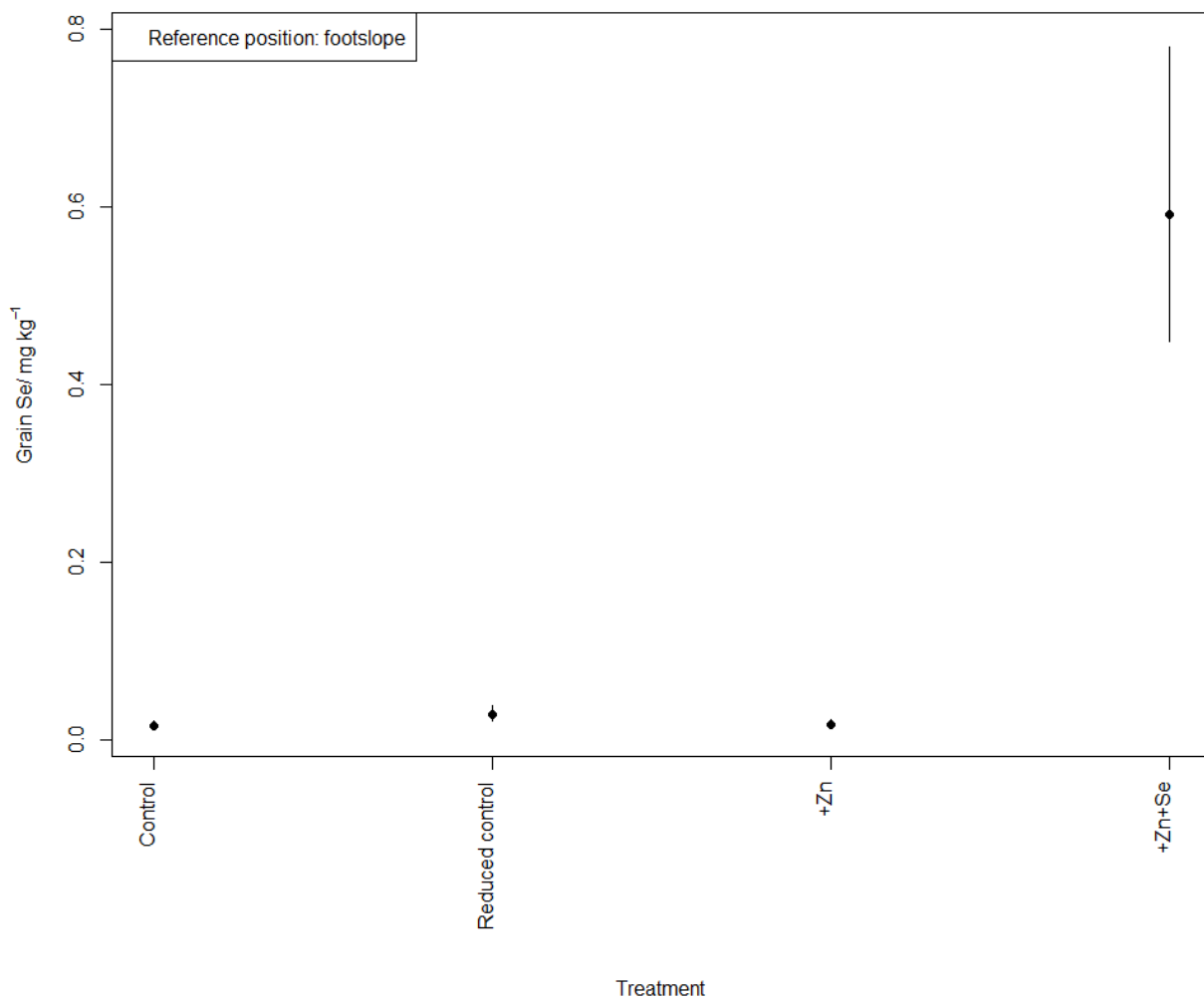
Figure 6. Wheat grain Se concentration in different fertility treatments in foot slope positions of western Amhara. The bars show ±1 standard error back-transformed from the log scale.
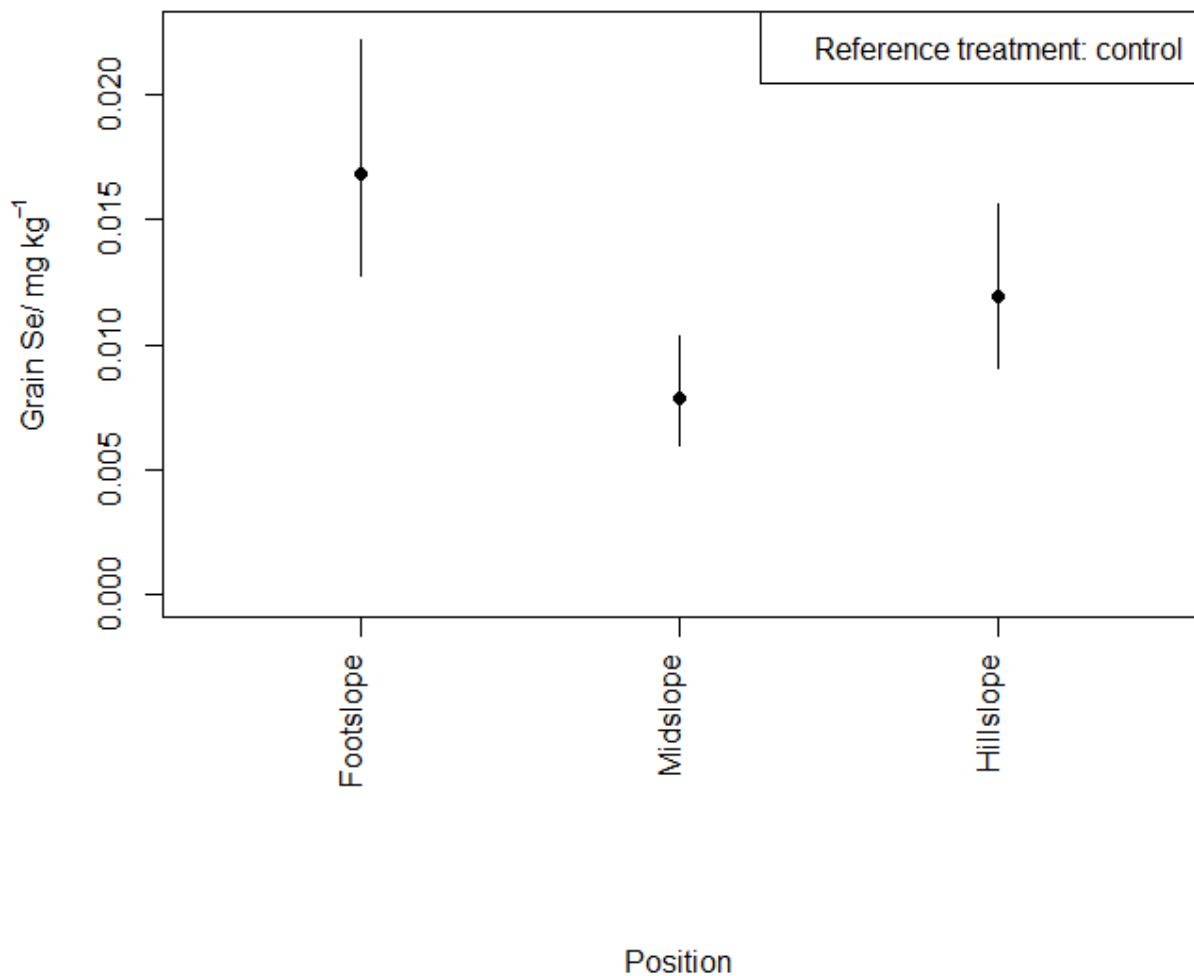
Figure 7. Mean wheat grain Se concentrations on different landscape positions with control as the reference fertilizer treatment. The bars show ±1 standard error back-transformed from the log scale.

3.4. Teff Grain Se Concentration

Teff grain Se concentration ranged from 1.01 to 1.55 mg kg^{-1} in different treatments and on different landscape positions (Figure 8). The application of Se fertilizer significantly resulted in the largest grain Se concentration compared to treatments which did not receive Se fertilizer (C1; $p < 0.001$) indicating strong evidence to reject the null hypothesis for C1. No evidence of Zn fertilizer effect (C2) or an effect of $^1/_3$ control rate application of macronutrient fertilizers (C3) on grain Se concentration in teff was observed ($p > 0.05$; ANOVA Table S4 Supplementary File). Strong evidence for a landscape unit effect on grain Se concentration in teff was reported ($p < 0.001$). Similarly, strong evidence for an interaction effect of C1 (Se fertilizer application) with landscape unit on grain Se concentration in teff was observed ($p < 0.001$). Because of the interaction term, Figure 8 shows the means and SE for all factorial combinations, back transformed to the original units, so the mean values are median unbiased. In the absence of applied Se, the concentrations are slightly larger on foot slopes than other slope positions. However, the response to applied Se is rather larger on mid-slopes than other landscape positions. In contrast, teff grain Se concentrations at foot slope position were similar to hillslope (Figure 8).
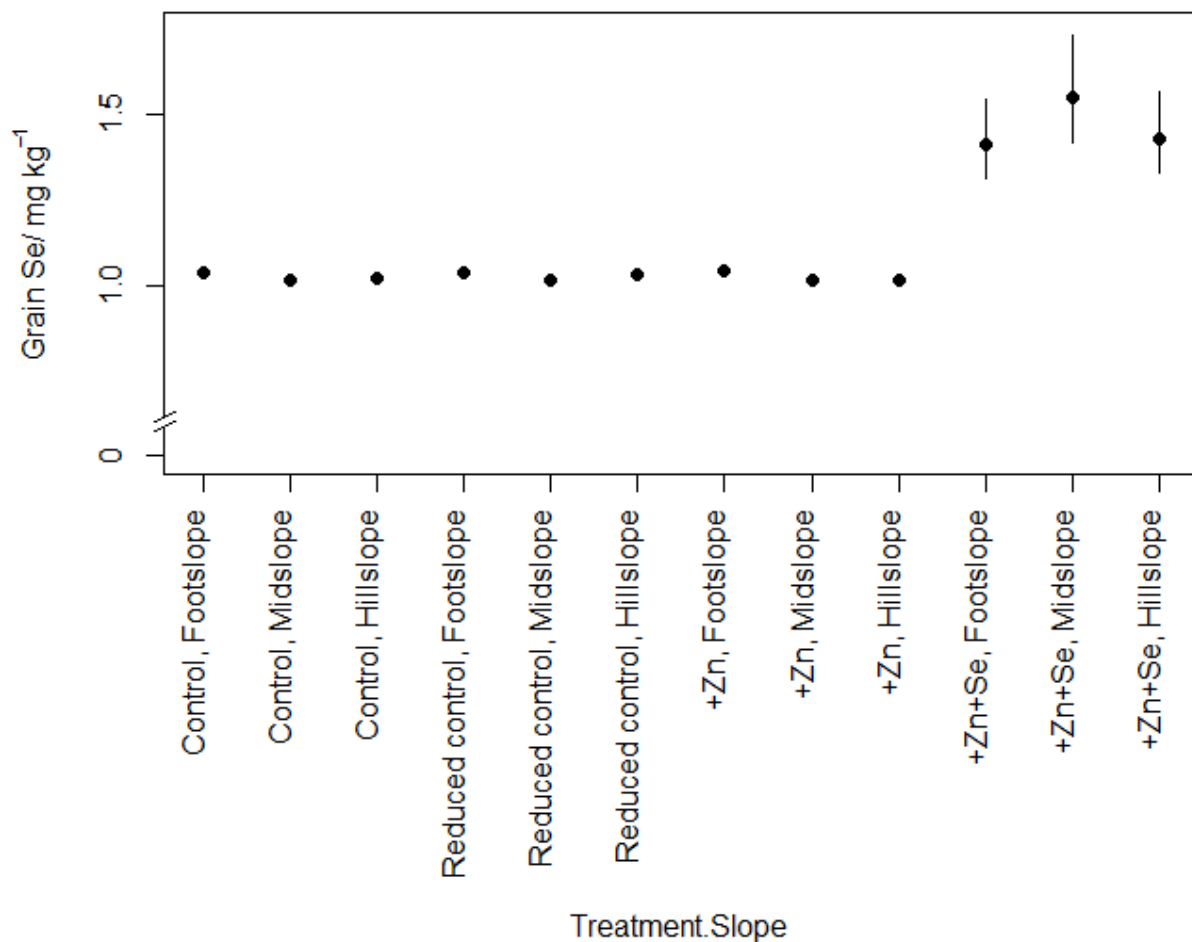
Figure 8. Mean grain Se concentrations in Teff for combinations of fertilizer treatment and landscape position. The bars show ±1 standard error back-transformed from the log scale.

4. Discussion

4.1. Micro- and Macronutrient Fertilizer Effect on Grain Nutritional Composition

Findings from this study confirms that Zn and Se fertilizer applications can increase grain Zn and Se concentrations, respectively. Zinc fertilizer improved grain Zn concentration the most when co-applied as a soil and foliar fertilizer. Similar findings have been reported earlier in maize [32] and wheat [2]. Co-application of soil and foliar Zn fertilizers has repeatedly shown superior grain micronutrient concentrations compared to application of soil and/or soil + side dressing. However, the practicality of using foliar fertilizers in smallholder farming systems dominated by cereals, (i.e., maize production) is likely to constrain their use in agronomic biofortification strategies. Concentrated forms of Se would carry significant risks of toxicity if used incorrectly, therefore, products blended/granulated at source would be the appropriate method to adopt as used in other countries [6].

Recent evidence from a powered on-station study in Malawi reported significant increases in maize grain Zn concentration when soil Zn fertilizer was applied at 30 kg Zn ha^{-1} compared to a 0 kg ha^{-1} control treatment. No significant differences were reported between 30 kg soil Zn and 90 kg soil Zn ha^{-1} treatments [33]. Foliar Zn application is potentially more efficient than soil application in terms of Zn quantities, as shown in this study and in other studies [34–36]. However, the stature of some crops can impede use of foliar Zn fertilizers. Additionally, there is risk of leaf scorch. Thus, soil applications using standard farmer practice would be a preferred practice. Notably, residual Zn fertility benefits can accrue from soil applications in subsequent cropping season [33] and for up to four years [2,37].

While research on Se biofortification is recent, several studies have been conducted to date. Our current work confirmed the contribution of Se fertilization to grain micronutrient concentrations. Ligowe et al. [38] similarly reported increases in grain Se concentration in maize and staple grain legumes in Malawian Alfisols under conservation agriculture. The effect of N management on grain micronutrient concentrations was also evident in this study with larger concentrations reported under reduced application rates of macronutrient fertilizer in both wheat and teff. Manzeke et al. [32] similarly reported larger concentration of grain Zn in maize grown with 45 kg N ha^{-1} compared to maize receiving 90 kg N ha^{-1} with no such effects in grain legumes. Improved grain micronutrient concentrations observed even with reduced amounts of macronutrient fertilizer is a good incentive to encourage farmers to adopt Zn fertilizers.

4.2. Landscape and G × E effect in Agronomic Biofortification

Evidence of spatial factors affecting Zn bioavailability in soils and grains is widely known. For example, Desta et al. [28] reported effects of landscape position on plant-available Zn through a series of adsorption and desorption studies. They reported that changes in soil pH across a landscape gradient influences the solubility of native soil Zn, with increases in adsorption evident under high soil pH and vice versa. Additionally, soil pH, plant-available Zn and temperature were also reported as factors influencing grain Zn concentration from a linear mixed model on >1500 soil and grain samples collected across Malawi [23]. The current study tested effects of micronutrient fertilizer on wheat and teff grain micronutrient concentrations over variable landscape positions. Evidence of a landscape effect on grain Zn concentration in wheat but not in teff, and grain Se concentration in both wheat and teff with an interaction effect with Se fertilization was reported. Apart from crop physiological differences which could have influenced the two crops' response to micronutrient fertilization, landscape showed effects on crop response to agronomic biofortification.

Topography influences soil erosion and movement of nutrients from the hillslope to the foot slope [21]. Xu et al. [39] reported decreases in grain Zn and Se concentration in rice with increase in elevation. For example, a decline in Zn and Se concentration of 0.912 mg kg^{-1} and 0.022 mg kg^{-1} was reported for every 100 m increase in elevation. In our study, larger concentrations of Zn and Se were reported in grains grown on foot slopes showing a potential fertility gradient from the hillslope to foot slope which might have influenced uptake of applied fertilizers. For example, larger concentrations of SOC were reported on footslopes in Aba Gerima and Debre Mewi compared to hillslopes and mid-slopes in Aba Gerima and mid-slopes in Debre Mewi (Table 1); [28]. Approximately 60% of SOM is available as SOC on a mass basis. Soil organic matter increases available forms of micronutrients in the soil (i.e., water soluble and exchangeable fractions), thus increasing micronutrient uptake by plants [40]. Variations in soil type among slope positions could have also resulted in differences in grain micronutrient concentrations. The hillslopes were dominated by Nitisols while the mid-slopes were in a transition between the Nitisols and Vertisols. Foot slopes were dominated by Vertisols. Ligowe et al. [41] reported larger concentrations of bioavailable Se on calcareous Vertisols compared to moderately acidic Alfisols and acidic Oxisols. In addition to soil pH, Zn availability in soils is influenced by organic matter content [42]. It is possible that larger concentrations of Zn obtained on foot slopes could be due to larger amounts of SOM deposited in the lower slope positions which improved bioavailability and uptake of Zn. While SOM build-up increases conversion of adsorbed fractions of micronutrients to plant-available forms, Se bioavailability becomes limited under high-organic-matter soils as it is organically bound in humus [43].

Grain micronutrient concentrations are also affected by shorter distance factors including farmer management of organic nutrient resources and crop type. Across treatments, teff had a smaller range of grain Zn concentration (28.5–31.2 mg kg^{-1}) compared to wheat (26.6 to 36.4 mg kg^{-1}). The teff control treatment yielded 2 mg kg^{-1} more Zn than wheat possibly implying teff could take up Zn in low nutrient conditions (i.e., Zn efficient) al-

beit with lower grain yield potential. Similarly, teff had larger concentrations of grain Se (1.01–1.55 mg kg^{-1}) compared to wheat (0.02–0.59 mg kg^{-1}). These differences between teff and wheat could be attributed to crop physiology which could potentially play a bigger role in crop micronutrient variations. Cakmak and Kutman [35] reported superiority of wheat in responding to foliar Zn application and yielding larger grain Zn compared to moderate responses in rice and less response in maize. Similarly, cowpea grown without external Zn fertilization under low-nutrient soils yielded larger grain Zn concentrations compared to maize [44]. There is therefore a need to exploit site-specific crops with the capacity to extract micronutrients in poorer soils as a complimentary approach to agronomic biofortification.

The complexity of environmental settings and different farmer socio-economic opportunities calls for the optimization of nutritional agronomy landscape trials. The design of the study, the number of sampling locations and the sample sizes allow for the representativeness of farming systems and the detection of small increases in grain nutritional quality. Findings from this work, generated from farms across western Amhara, were able to show variations in micronutrient concentrations across treatments and landscapes. It is therefore imperative that landscape trials are adequately powered to detect relatively small effect sizes, which can be impactful in terms of micronutrient provisioning in food systems.

5. Conclusions

Targeting of micronutrient fertilizer application in ongoing agronomic biofortification interventions is likely to be influenced by field landscape position but more so by micronutrient and N fertilizer application, as well as the method of micronutrient fertilizer application. Findings from this study will guide ongoing agronomic biofortification interventions in Sub-Saharan Africa. It is therefore imperative that nutritional agronomy landscape trials are optimized to represent heterogeneity in farming systems and detect small but impactful changes in micronutrient supply.

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