



RESEARCH ARTICLE

Boron availability and fertilizer response of maize in soils from sub-Saharan Africa

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Abstract

Background and aims: Low boron (B) availability is associated with strongly weathered, coarse-textured, and low organic matter soils, widespread in sub-Saharan Africa (SSA). It is unknown to what extent B fertilization can increase maize yields in SSA. This study aims to understand the soil properties controlling B availability to field-grown maize.

Methods: Boron fertilizer omission trials with maize were executed at 15 sites in Kenya, Zambia, and Zimbabwe. Yield, B uptake, and soil parameters potentially relevant for B availability, including extractable soil B (hot water, 0.01 M CaCl₂, and 0.43 M HNO₃), were determined.

Results: Soil B pools were strongly intercorrelated and were positively correlated with organic carbon, suggesting the relevance of organic matter for soil B availability. Soil parameters described limited variation in B uptake and the yield response to B fertilization. Boron fertilization did not increase yields in any of the 15 sites but increased uptake in 11 sites. Yields were reduced through B fertilization in five sites, likely because B application induced toxicity. No clear critical soil or plant B concentrations indicating deficiency could be derived, but positive yield responses to B fertilization were absent with hot water B levels above 0.69 mg kg⁻¹.

Conclusion: Assessing B fertilizer needs in maize grown in tropical soils based on soil or plant tissue concentrations remains challenging. Improving soil organic matter status could potentially alleviate B deficiency in crops when present. Recommendations are given to overcome the identified challenges associated with studying B availability in tropical soils.

KEYWORDS

availability, boron, fertilizer response, maize, sub-Saharan Africa

1 | INTRODUCTION

Boron is an essential micronutrient for plants. It is required for various processes in plant metabolism, such as root elongation, flower and seed formation, and membrane functioning (Gupta, 2007). The occurrence

of boron (B) deficiency is believed to be widespread globally as positive yield responses to B fertilization have been reported for a variety of crops in at least 80 countries (Shorrocks, 1997). Low soil B availability has been associated with strongly weathered and coarse-textured soils, as well as soils with low quantities of organic matter (Shorrocks,

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1997). In sub-Saharan Africa (SSA), such soils are widespread (Hengl et al., 2015). The numbers of studies addressing B deficiency in maize grown in SSA are limited (Shorrocks, 1997; Sillanpää, 1990; Tamene et al., 2016; Vanlauwe et al., 2015; Wendt & Rijpma, 1997). Generally, gramineous species, including maize, are known for their low B demand (Lordkaew et al., 2011). However, maize is an important staple crop (Goredema-Matongera et al., 2021), and several fertilizer response trials across several countries in SSA indicate that fertilization with secondary and micronutrients, including B, can lead to higher maize yields in some cases (Kihara et al., 2017; Rurinda et al., 2020; Wortmann et al., 2019). However, the incidence of B deficiency could not be separated from the other nutrients, as they were applied as a mixture. It is therefore currently unknown where B fertilization can improve maize yields in SSA.

Fertilizer response trials are the benchmark for calibration and evaluation of critical plant and soil concentrations that indicate B deficiency (Bell, 1997). Critical plant B concentrations are difficult to establish as they strongly depend on the plant part and maturity of the tissue sampled (Bell, 1997). The critical B concentrations in maize plants show a wide range between 2 and 12 mg kg⁻¹ for various plant parts (de Souza Lima et al., 2007; Gupta, 2007; Joshi et al., 2014; Kumar et al., 2018; Reuter & Robinson, 1997). In soils, hot water extractable B (B-HW) is seen as a good measure for B availability, and good relations with yield and plant B concentrations have been reported for maize and other crops (Aitken et al., 1987; Chaudhary & Shukla, 2004; de Souza Lima et al., 2007; Jin et al., 1988; Kumar et al., 2018). Based on field trials in India, critical B-HW concentrations were estimated to be 0.50 mg kg⁻¹ soil for maize (Kumar et al., 2018). In the USA, B application to maize is recommended only when B-HW levels are below 0.25 mg kg⁻¹ (WARD Laboratories, 2020). However, soil concentrations below thresholds established for maize do not guarantee a positive yield response to B fertilization (Wendt & Rijpma, 1997). Furthermore, critical soil B limits could depend on soil properties, such as soil texture, organic matter content, and pH (Bell, 1997).

Understanding the soil, environmental, and biotic factors that determine availability of B, as well as the yield responses to B fertilization in field trials, is key to deciding whether or not to apply B fertilizers. Boron availability in soils is, however, still poorly understood. Generally, B adsorption to the soil reactive surfaces such as organic matter, iron and aluminum oxides, and clay minerals increases with pH (Goldberg, 1997). However, Van Eynde et al. (2020) found that most of the reactive B was found in solution and that adsorption thus plays only a minor role in controlling B availability in soils from SSA. Weathering of soil minerals and mineralization of organic matter are believed to be the primary sources of B found in the soil solution besides adsorption/desorption processes and atmospheric deposition (Kot et al., 2016; Park & Schlesinger, 2002). But the relevance of each of these processes may be different, depending on the soil system (Van Eynde et al., 2020).

This study aims to increase the understanding on the soil properties that affect the availability of B to field-grown maize in SSA. Using B fertilizer omission trials at several sites within three African coun-

tries, selected based on low soil B availability, we address the following research questions: (1) Which soil parameters determine availability of B? (2) Which soil parameters determine the yield response to B fertilization? (3) Which critical soil and plant B concentrations indicate B deficiency in maize?

2 | MATERIALS AND METHODS

Situations where B availability is the growth-limiting factor are required to address the research questions. Therefore, B fertilizer omission trials were set up in locations with low soil B concentrations. These trials were executed as part of a larger experiment, with several nutrient omission treatments. Layout of the experimental plots was based on a randomized block design. Below we present the relevant methodology for the B fertilizer omission trials and data analysis described in this article. For a full overview of the materials and methods of the larger experiment, we refer to Van Eynde et al. (2023).

2.1 | Field trials

Field trials were conducted at 15 on-farm sites in Kenya (5 farms, 5 replications each), Zambia (4 farms, 4 replications each), and Zimbabwe (6 farms, 6 replications each). In Zimbabwe, the B fertilizer omission treatment was included in six out of ten farms of the larger experiment; farm names correspond to those used in Van Eynde et al. (2023). Most farms have a history of low input use, except Kenya, where N, P, and K were applied as mineral fertilizers in previous seasons. Within countries, most farms were located in relatively close proximity of each other (<5 km). Of several locations sampled, farms with low B availability were selected for field trials. In 11 out of 15 farms, B-HW values (Table 1) were below the critical threshold of 0.50 mg kg⁻¹ reported by Kumar et al. (2018) for maize. The trials included a full fertilizer and a B omission treatment. For the full treatment, nutrients potentially limiting yields were applied at high rates (in kg ha⁻¹: 180–350 N, 35–180 P, 100–120 K, 26–61 Ca, 2.3–20 Mg, 6–31 S, 3–5 Zn, 5 Cu, and 3–5 B), with rates varying among countries (see Van Eynde et al., 2023 for details). The B omission treatment was similar to the full treatment, except that B was omitted. Maize variety, planting densities, and plot sizes varied among countries based on the availability of resources and local practices.

In Kenya, B was applied as Solubor at a rate of 5 kg B ha⁻¹ in the planting hole together with the other fertilizers, right before sowing. Boron application rates of 5 kg ha⁻¹ are not uncommon for maize (e.g., Rurinda et al., 2020; Gotz et al., 2021). Negative yield effects to application of 5 kg B ha⁻¹ were, however, observed in the Kenyan trials. It was therefore decided to apply B at a rate of 3 kg ha⁻¹ in Zambia and Zimbabwe. In Zambia, 1.5 kg B ha⁻¹ in the form of Borax was co-applied with urea during two topdressings, at respectively, 3 and 5–6 weeks after planting. In Zimbabwe, 3 kg ha⁻¹ B was applied as Solubor in the planting hole together with the other fertilizers, right before sowing.

TABLE 1 Soil characteristics of the farms.

	Site	pH H ₂ O	SOC (g kg ⁻¹)	B-HNO ₃ (mg kg ⁻¹)	B-CaCl ₂ (μg kg ⁻¹)	B-HW (mg kg ⁻¹)	Ca-M3 (mg kg ⁻¹)	AlFe (mmol kg ⁻¹)	Clay (%)
Kenya	Farm 1	5.9	15.6	0.34	74	0.67	1266	91	29
	Farm 2	5.7	15.8	0.18	34	0.34	1139	103	30
	Farm 3	5.6	11.3	0.21	50	0.29	537	51	29
	Farm 4	5.6	9.9	0.14	40	0.21	369	60	35
	Farm 5	5.3	16.2	0.26	75	0.65	622	97	40
Zambia	Farm 1	6.2	10.3	0.11	16	0.21	591	52	-
	Farm 2	6.1	8.3	0.08	17	0.05	307	34	-
	Farm 3	5.9	6.3	0.07	14	0.10	188	37	-
	Farm 4	6.3	6.5	0.09	16	0.11	243	25	-
Zimbabwe	Farm 4	5.6	13.0	0.32	132	0.91	1112	69	35
	Farm 6	5.6	5.2	0.10	48	0.07	123	11	7
	Farm 7	5.5	5.8	0.10	50	0.17	94	17	6
	Farm 8	5.3	4.8	0.11	50	0.12	83	22	2
	Farm 9	5.4	6.2	0.09	41	0.09	155	14	8
	Farm 10	6.2	4.7	0.36	93	0.50	444	21	2

Note: Values represent averages per farm, except for clay content, which was analyzed on farm level.

Abbreviations: B-HW, hot water extractable B; Ca-M3, Calcium concentrations in M3; SOC, Soil organic carbon.

2.2 | Data collection

Field-dry stover and grain biomass were measured for each plot during harvest. A few plants were randomly selected from each plot, omitting plants from the border rows. The plants were chopped into small pieces and mixed, and a subsample was taken. Dry matter contents of these subsamples were used to convert biomass weights to dry weights. Throughout this study, grain yield is expressed at a standardized moisture content of 13%. A range of plant essential elements was analyzed in both stover and grain biomass samples using a digestion with 0.8 M H₂SO₄/Se/H₂O₂ for N and a microwave digestion with concentrated HNO₃ for the other elements (Novozamsky et al., 1983). Boron uptake was derived from dry matter production of stover and grains, multiplied by their respective B concentrations. Pooled topsoil samples were collected per block at a depth of 0–20 cm during harvest. Soil samples were taken between the rows, as fertilizers were applied in the planting hole.

Daily precipitation was derived from satellite data (WAPOR, 2020) as rainfall can affect B leaching and plant availability (Degryse, 2017). To estimate whether rainfall may have affected plant B uptake, the sum of precipitation in the 100 days after the first B fertilizer application was used.

2.3 | Soil analysis

In addition to hot water (B-HW), soil B availability was assessed using 0.01 M CaCl₂ (B-CaCl₂) and 0.43 M HNO₃ (B-HNO₃) soil extrac-

tions. The B-CaCl₂ can be viewed as a measure of B present in the soil solution, whereas B-HNO₃ may represent both the concentration in solution as well as B not directly available for plant uptake but reversibly bound to the soil solid phase (Groenenberg et al., 2017; Van Eynde et al., 2020), representative of the B pool that becomes available for plant uptake during a growing season (i.e., the reactive pool). Like HNO₃, the HW method extracts B in solution as well as adsorbed B (Marupaka et al., 2022).

Soils were air-dried and passed through a 2-mm sieve prior to analysis. Solutions were freshly prepared for each extraction. For the HW method, soils were extracted with 0.01 M CaCl₂ with a solution-to-solid ratio of 2 L kg⁻¹ and a boiling time of 10 min (Aitken et al., 1987). Suspensions were heated in Teflon destruction tubes in a Mars 6 Microwave Digestion System (CEM corporation). The ramping time was set to 5 min before holding the suspensions at a temperature of 105 ± 5°C for 10 min. Tubes were removed immediately from the microwave when the program was finished and suspensions were decanted in 50-mL Greiner tubes for centrifugation. For the CaCl₂ method, soils were extracted with 0.01 M CaCl₂ at a solution-to-solid ratio of 10 L kg⁻¹ and an equilibration time of 2 h (Houba et al., 2000). After centrifugation and filtration, extracts were acidified with concentrated HNO₃ before analysis. A HNO₃ soil extraction was done using a solution-to-solid ratio of 10 L kg⁻¹ and an equilibration time of 4 h, according to the International Organization for Standardization (ISO) standard (ISO, 2016). Boron concentrations were measured using high resolution Inductively Coupled Plasma-Mass Spectrometry (ICP-MS, Element 2, Thermo Scientific) in all four soil suspensions, after centrifugation and filtration of the suspensions over a 0.45-μm

membrane filter. Soil B concentrations are expressed per kg of dry soil.

Several other soil properties potentially affecting B adsorption were determined. Soil pH was measured with a glass electrode in a distilled water extract, with a solution-to-solid ratio of 5 L kg⁻¹, after shaking for 1 h on a linear shaker at 180 strokes min⁻¹, and letting suspensions settle for 1 h (ISO, 2005). Soil organic carbon (SOC) content was spectrophotometrically determined after the Kurmis wet oxidation method (Walinga et al., 1992). Soil contents of Al and Fe hydroxides were determined with an ammonium oxalate soil extraction (ISO, 2012). For further data analysis, the sum of Al and Fe hydroxides in ammonium oxalate (AlFe-AO; in mmol kg⁻¹) was used. Clay content was determined with various methods (see Van Eynde et al., 2023). Soil calcium, in the form of carbonates, calcium clays, and free ions, is also known to affect B adsorption (Goldberg, 1997). Calcium concentrations in M3 (Ca-M3) were analyzed as a proxy for soil calcium levels (Mehlich, 1984). Concentrations in the extraction solutions were measured using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES, Thermo Scientific iCAP6500).

2.4 | Analytical limits

The determination limits for the different methods were calculated as the average value of the blanks + three times its standard deviation, across different analytical series (Keskinen et al., 2019). Determination limits for B in soils were found to be 0.78 µg kg⁻¹ for hot water ($n = 10$ blanks), 4 µg kg⁻¹ for 0.01 M CaCl₂ ($n = 28$), and 0.03 mg kg⁻¹ for 0.43 M HNO₃ ($n = 14$). None of the samples had B concentrations below the determination limits for HW, CaCl₂, or HNO₃.

2.5 | Data analysis

Data analysis was done using R software, version 4.0.2 (R Core Team, 2020). Results were visualized with the *ggplot2* package (Wickham, 2016).

2.6 | Treatment effects

The effect of fertilizer treatment (i.e., full or B omission) on maize grain yields (Mg ha⁻¹) and B uptake (g ha⁻¹) was assessed with linear mixed effect models (LME) using the *lme* function from the *nlme* package (Pinheiro, 2013) at farm and country level. These analyses were first done per farm, with treatment as fixed factor, and block as random factor (random = ~1|Block). At country level, differences between farms were also assessed using an LME model with farm as additional fixed factor. Significance of factors, as well as their interaction, was tested with the ANOVA function from the *car* package (Fox & Weisberg, 2019). Individual differences were analyzed with Tukey's post hoc test, using the *glht* function from the *multcomp* package, version 1.4.17 (Hothorn et al., 2008). Normality of model

residuals was checked with the Shapiro–Wilk test using the Shapiro-test function from the *stats* package, version 4.1.0 (R Core Team, 2020). Homogeneity of variance across groups was tested with Levene's test, using the *leveneTest* function from the *car* package (Fox & Weisberg, 2019). In case assumptions of normality of residuals or homogeneity were violated, data were transformed, or the non-parametric Kruskal–Wallis test was used (*kruskal.test* from the *stats* package).

The effect of fertilizer treatment was also assessed by calculating the yield response as the yield of the full treatment divided by the yield in the B omission treatment.

2.7 | Soil–plant relations

Boron uptake in the B omission plots was used as a proxy for B availability. Relations between soil properties and B uptake in the B omission treatment, as well as the yield response, were assessed using LME modeling with method specified as maximum likelihood. In these models, soil properties were used as fixed effects, and the effect of agroecological zone and maize variety was represented by including country as a random factor (random = ~1|Country). As farm was not included as a fixed or random factor, these models treat all blocks within a country as pseudo-replicates. This was considered suitable given the large variability in soil and plant parameters within farms. The analyses were done on two datasets. The first dataset comprises all available data, with a total of 72 blocks. The second dataset comprises only the blocks with a positive yield response to B fertilization ($n = 24$). In the later dataset, B uptake is assumed to equal soil supply, as B was likely growth-limiting (Janssen et al., 1990).

Based on Van Eynde et al. (2020) and Goldberg (1997), the soil properties considered relevant for B availability were pH, B-CaCl₂, B-HNO₃, B-HW, SOC, Ca-M3, and AlFe-AO. Although multicollinearity was observed among the explanatory soil parameters, no variable selection was made, as all of the variables are potentially relevant for controlling B availability. Model selection was done based on the Akaike's Information Criterion value (Webster & McBratney, 1989) using the *dredge* function from the *MuMIn* package (Barton, 2020). The yield response was modeled on a log₁₀-scale (Marcillo & Miguez, 2017), where each of the independent parameters was also log-transformed, except pH. Boron uptake was modeled on a normal scale for the subset ($n = 24$) and on a log scale for the full set ($n = 72$), to normalize residuals.

Normality of residuals was checked with the Shapiro–Wilk test. The variance explained by the regression models was calculated using the *R*² function from the *performance* package in R (Lüdtke et al., 2021), which reports the variance explained by the fixed factors only (marginal *R*²) and the variance explained by both the fixed and random effects (conditional *R*²). Throughout this manuscript, these will be referred to as fixed and total *R*², respectively. The relative contribution of each variable in the final LME model was tested using the *r2beta* function from the *r2glmm* package (Jaeger, 2017).

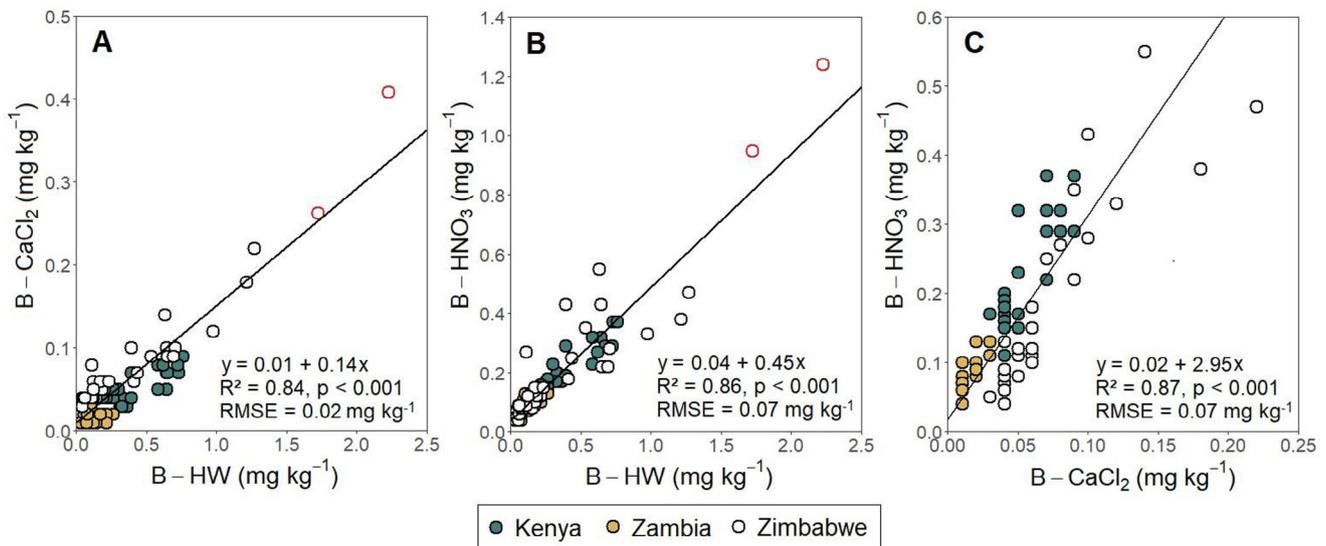


FIGURE 1 Relations among soil B concentrations in hot water (HW) and CaCl₂ (A), HW and HNO₃ (B) and CaCl₂ and HNO₃ (C) extracts. Points represent individual blocks, with colors indicating country. The red circles belong to the Zn omission trial of Farm 5 in Zimbabwe (Van Eynde et al., 2023) and were included for discussion purposes.

3 | RESULTS

3.1 | Soil parameters

The soils of the field trial sites generally had low SOC contents with no values exceeding 20 g kg⁻¹ (Table 1). The Zambian and most of the Zimbabwean farms had the lowest SOC contents, which ranged from 4 to 11 g kg⁻¹, whereas the Kenyan farms had higher SOC contents, between 9 and 20 g kg⁻¹. The field trials covered a limited range in soil pH, with most of the values between 5.0 and 6.5, with the exception of two blocks in Zimbabwe (both on Farm 10). Within countries, the range in pH values was even more limited, with the Kenyan farms covering pH 5.1–6.3 and the Zambian farms covering pH 5.7–6.4. The pH values of the farms in Zimbabwe ranged between 5.0 and 7.0, covering the entire range in pH values reported in this study.

The soils had B concentrations of 0.03–1.27 mg kg⁻¹ for B-HW, 0.05–0.22 mg kg⁻¹ for B-CaCl₂, and 0.04–0.55 mg kg⁻¹ for B-HNO₃ (Figure 1). Boron concentrations were lowest for the Zambian and some of the Zimbabwean soils; the Zimbabwean soils generally covered the widest range. Concentrations of B-HW were highest, followed by B-HNO₃ (approx. 45% of B-HW), and B-CaCl₂ (approx. 14% of B-HW). Boron concentrations in the different extracts were strongly correlated, but correlations were relatively poor at lower concentrations (i.e., below approx. 0.1 mg kg⁻¹ B-CaCl₂; Figure 1). Despite these strong correlations, the fractions of soil B exhibited different relations with different other soil properties. Of the three extraction methods, B-HW correlated best with SOC ($r = 0.63$, $p < 0.001$), AlFe-AO ($r = 0.61$, $p < 0.001$), and Ca-M3 ($r = 0.76$, $p < 0.001$). In contrast to B-HW, B-HNO₃ was also correlated with pH ($r = 0.29$, $p = 0.01$), in addition to SOC ($r = 0.44$, $p < 0.001$), AlFe-AO ($r = 0.43$, $p < 0.001$), and Ca-M3 ($r = 0.66$, $p < 0.001$). B-CaCl₂ was only significantly correlated with SOC ($r = 0.26$, $p = 0.022$) and Ca-M3 ($r = 0.44$, $p < 0.001$). The ratio

between B-HW/B-HNO₃ increased with SOC ($r = 0.63$, $p < 0.001$), AlFe ($r = 0.66$, $p < 0.001$), and Ca-M3 ($r = 0.60$, $p < 0.001$) concentrations. Similarly, the ratio between B-HW/B-CaCl₂ increased with SOC ($r = 0.56$, $p < 0.001$), AlFe ($r = 0.62$, $p < 0.001$), and Ca-M3 ($r = 0.54$, $p < 0.001$) concentrations, as well as pH ($r = 0.29$, $p = 0.011$).

3.2 | Yield response

The majority of maize yields ranged between 4 and 8 Mg ha⁻¹. Within each of the countries, significant differences in maize yields among sites were found (Figure 2). Fertilization with B led to lower yields ($p < 0.05$) on 5 out of 15 sites (Farms 1 and 3 in Kenya, Farm 3 in Zambia, and Farms 6 and 8 in Zimbabwe), indicating potential B toxicity. However, no visual symptoms of toxicity (or deficiency) were observed in the field. A negative yield response (i.e., a ratio below 1) to B fertilization was found for 48 out of 72 blocks (67%) across all sites, although the majority of blocks ($n = 52$, 72%) had a yield response between 0.8 and 1.2 (Figure 3). We considered this as natural variation, as a variation of 20% from the mean yield response roughly corresponds to the variation in yield response within a farm and treatment (Figure 2).

The yield response could not be explained based on any soil parameter or rainfall data. Further inspection of the data showed three observations with an outlier in yield response (<0.6), at Farms 2 and 3 in Zambia and Farm 8 in Zimbabwe. At Farm 3 in Zambia and Farm 8 in Zimbabwe, the negative effect of B fertilization on yield was significant (Figure 2), although not at Farm 2 in Zambia. These outliers could not be explained by any soil parameter or observations made in the field. Regressions were rerun after removing these three outliers, however, again no soil parameter or rainfall was retained in the final model to explain the variation in the yield response to B. When fitting LME models to the subset of data with a yield response above 1 ($n = 24$),

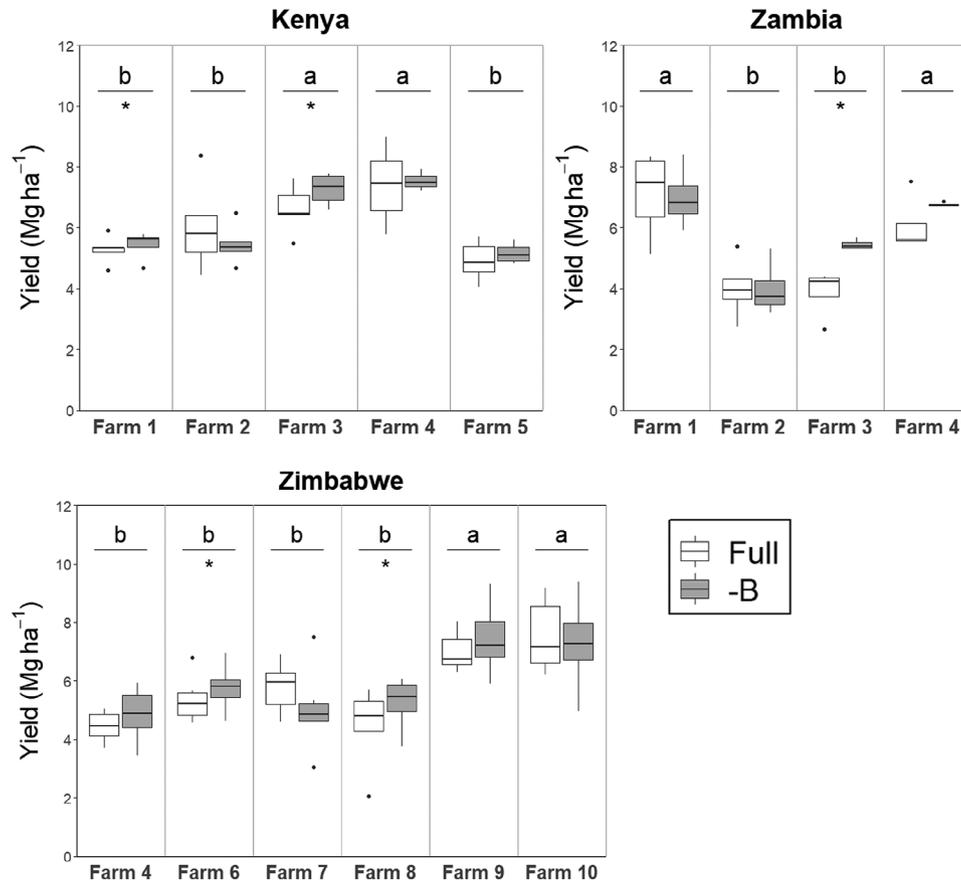


FIGURE 2 Boxplots of maize yield for individual farms, grouped per country. The boxplots show the median (line), first and third quartiles (hinges), the minimum and maximum based on the interquartile range (whiskers), and the outliers (points). Letters indicate significant differences among sites within a country, asterisks indicate a significant difference between treatments.

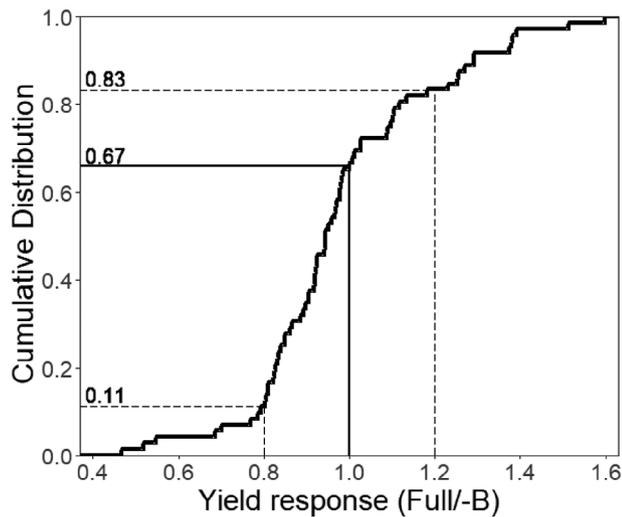


FIGURE 3 Cumulative distribution of the yield response per block ($n = 72$). A yield response value of 1 indicates no yield difference between both treatments, a value below 1 a negative yield response (higher yield for the B omission treatment) and a value above 1 a positive yield response (higher yield for the full treatment). Dashed lines indicate yield responses of 0.8 and 1.2.

similar results were obtained. For both datasets, model residuals were strongly related to yields in both treatments.

Although auto-correlated with the yield response, to normalize model residuals and gain insights in additional explanatory variables, regressions were rerun using yields of the B omission treatment as an explanatory variable for the yield response. This resulted in models with yields in the B omission plots as primary (explaining 68% of R^2 ; full dataset) or single (subset of data with yield response > 1) significant parameter in describing the yield response. In addition, limited variation in the yield response was described (R^2 of 0.32 for the full dataset and 0.23 for the subset). For the full dataset, when rainfall was added as an explanatory variable, it was the most important parameter in explaining the yield response (positive coefficient), followed by yield, B-HW, and B-CaCl₂. Fixed and total R^2 were 0.538 and 0.944, indicating that country as a random effect was significant when rainfall was included in the model. Despite more variation in the yield response explained by the model including rainfall, it was not significantly different from the model without ($p = 0.064$). In the subset of data with a yield response > 1 , rainfall was not a significant factor describing the yield response.

No clear linear trends between the yield response and soil B concentrations analyzed with HW, CaCl₂, and/or B-HNO₃ were found as variation in yield responses was large (Figure 4A–C). Exclusion of

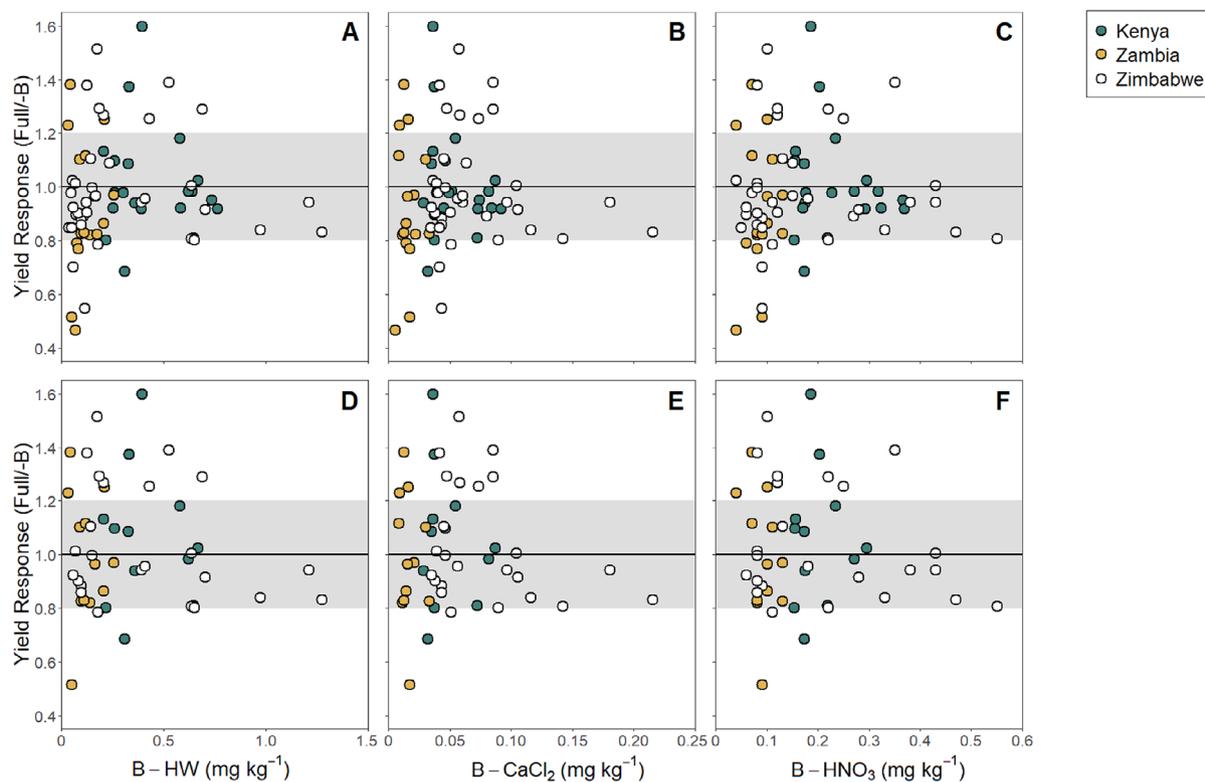


FIGURE 4 The yield response per block ($n = 72$) plotted against soil B concentrations in (A) hot water, (B) CaCl_2 and (C) HNO_3 . Similar plots are presented in (D), (E) and (F) without data from the farms where B application led to a negative yield response ($n = 48$). Gray marked areas represent natural variation in the yield response.

TABLE 2 Plant B concentrations and concentration response to fertilization (full/B omission).

Country	Grain [B] mg kg^{-1}		Stover [B] mg kg^{-1}		Response	
	Full	B omission	Full	B omission	Grain	Stover
Kenya	1.77 (1.52–2.16)	1.27 (0.99–1.63)	6.40 (3.48–10.50)	3.68 (2.44–4.64)	1.40 (0.99–2.03)	1.74 (1.12–2.58)
Zambia	2.08 (1.65–2.43)	1.06 (0.69–1.66)	6.65 (4.70–10.60)	4.50 (3.70–6.50)	1.86 (1.24–3.10)	1.45 (0.91–1.94)
Zimbabwe	2.07 (1.37–4.89)	1.29 (0.90–1.88)	7.00 (3.36–20.0)	3.59 (2.71–5.00)	1.55 (0.94–4.07)	1.85 (0.95–5.66)
All	1.92 (1.37–4.89)	1.25 (0.69–1.88)	6.60 (3.36–20.0)	3.71 (2.44–6.50)	1.54 (0.94–4.07)	1.75 (0.91–5.66)

Note: Values represent medians, range between brackets. Within countries, average concentrations were all significantly different between the full and B omission treatments, with p -values below 0.001.

the five farms where B application led to a significant yield reduction strongly reduced the variation in yield response at lower soil B concentrations (Figure 4D–F). For individual blocks, positive yield responses (>1.2) to B fertilization were found when B-HW was below 0.69 mg kg^{-1} , B- CaCl_2 below 0.085 mg kg^{-1} , and B- HNO_3 below 0.35 mg kg^{-1} (Figure 4). However, below these concentrations, negative yield responses (<0.8) were still found.

3.3 | Plant B concentrations

Both grain and stover B concentrations increased significantly as a result of B fertilization (Table 2). Across countries, median grain B con-

centrations increased from 1.25 to 1.92 mg kg^{-1} as an effect of B fertilization, whereas median stover B concentrations increased from 3.71 to 6.60 mg kg^{-1} . Grain B concentrations were less affected by B fertilization than stover B concentrations (1.54-fold increase for grain vs. 1.75-fold increase for stover). Differences among countries were observed; however, in Kenya and Zimbabwe, stover B concentrations increased relatively more than grain B concentrations, whereas the opposite was observed in Zambia (Table 2).

Across countries, grain B concentrations ranged between 0.69 and 1.88 (B omission) and 1.37 and 4.89 mg kg^{-1} (full treatment; Figure 5A, Table 2). Boron concentrations in stover were generally 2–6 times larger than in grains and ranged between 2.44 and 6.50 (B omission) and 3.36 and 20.00 mg kg^{-1} (full treatment; Figure 5B, Table 2). The

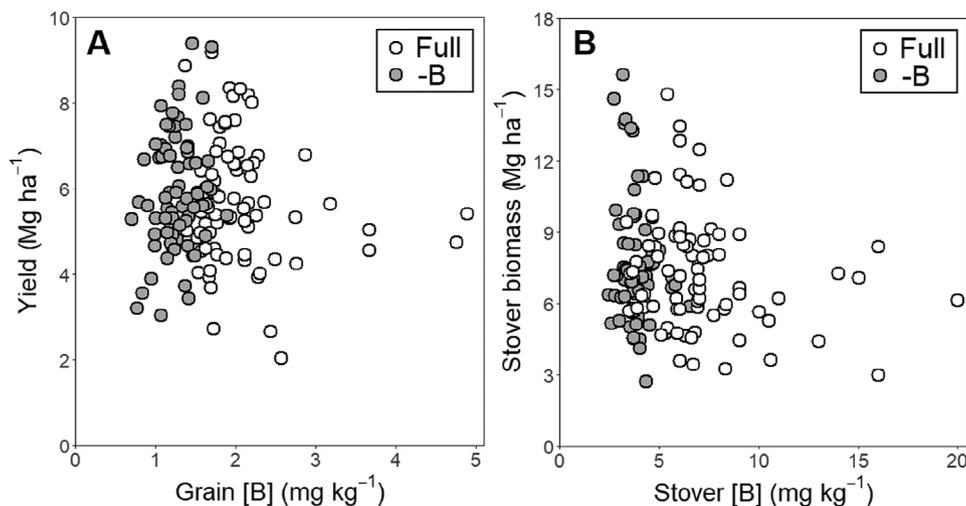


FIGURE 5 B concentrations in (A) grain and (B) stover, plotted against the biomass production of each fraction. Points represent individual blocks.

highest stover B concentrations were associated with relatively low stover biomass production, potentially indicating B accumulation in the crop due to reduced biomass productivity (Figure 5B). For grain B concentrations, a similar trend was visible, but less clear than for stover B concentrations (Figure 5A). The 10 highest grain B concentrations ($>2.49 \text{ mg kg}^{-1}$) and the 7 highest stover B concentrations ($>11 \text{ mg kg}^{-1}$) were found in the full treatment for Farms 6 and 8 in Zimbabwe, where a significant negative yield response to B fertilization was found (Figure 2). In Kenya and Zambia, however, no clear differences in plant B accumulation were found between farms where B fertilization led to a significant yield reduction and farms where it did not.

No clear relations between grain or stover B concentrations of the B omission plots and the yield response were found (results not presented). It was therefore not possible to derive critical plant concentrations that could indicate potential B deficiency.

3.4 | B uptake

Across countries, B fertilization led to a significant increase in B uptake for most farms (Figure 6). No increase in B uptake was observed in Farm 1 in Kenya, Farms 3 and 4 in Zambia, and Farm 4 in Zimbabwe. For Farm 1 in Kenya and Farm 3 in Zambia, the absent uptake response can be explained by the lower yields (and stover biomass production) in the full treatment (Figure 2), which translates into lower B uptake. Boron fertilization led to an average increase in B uptake of 21 g ha^{-1} for Kenya, 9 g ha^{-1} for Zambia, and 35 g ha^{-1} for Zimbabwe. Given the fertilization rates of 5 kg ha^{-1} for Kenya and 3 kg ha^{-1} for Zambia and Zimbabwe, the fertilizer use efficiency was low (0.3%–1.2%).

Boron uptake (g ha^{-1}) in the B omission plots was used as a proxy for B availability. LME models were fitted to find the soil parameters most relevant in describing variation in B uptake. This was done again for the full dataset ($n = 72$) and the subset of data with yield response > 1

($n = 24$). For the full dataset, B uptake data needed to be transformed to normalize residuals.

$$\log_{10}(\text{Buptake}) = 1.537 + 0.998 \times \text{B} - \text{HNO}_3 - 1.804 \times \text{B} - \text{CaCl}_2 - 0.009 \times \text{SOC}. \quad (1)$$

The final model for the full dataset included a positive effect of B-HNO₃, and negative effects of B-CaCl₂ and SOC to describe the variation in B uptake (Equation 1; Figure 7). Fixed and total R² of this model were both 0.18, and residuals were normally distributed ($p = 0.954$). The B-HNO₃ was the most important parameter in describing B uptake (67% of R²), followed by B-CaCl₂ (21%), and SOC (13%). For the subset with yield response > 1 , B-HNO₃ was the only significant parameter in describing B uptake. Fixed and total R² of this model were both 0.21, leaving much of the variation in B uptake unexplained. For both the full dataset and the subset with yield response > 1 , models including rainfall as an explanatory variable had slightly higher R² and RMSE values compared to models without; rainfall had a positive coefficient in both cases. The models including rainfall were, however, not significantly better in explaining variation in B uptake compared to models without rainfall.

4 | DISCUSSION

4.1 | Response to fertilization

Soil B-HW concentrations in 11 out of 15 farms were below the formerly derived critical concentrations of 0.50 mg kg^{-1} for maize (Kumar et al., 2018). Nonetheless, B fertilization did not significantly increase maize yields at any farm. In contrast, B uptake increased in most farms as an effect of fertilization, indicating luxury consumption. These findings are in line with several other studies reporting that B fertilization

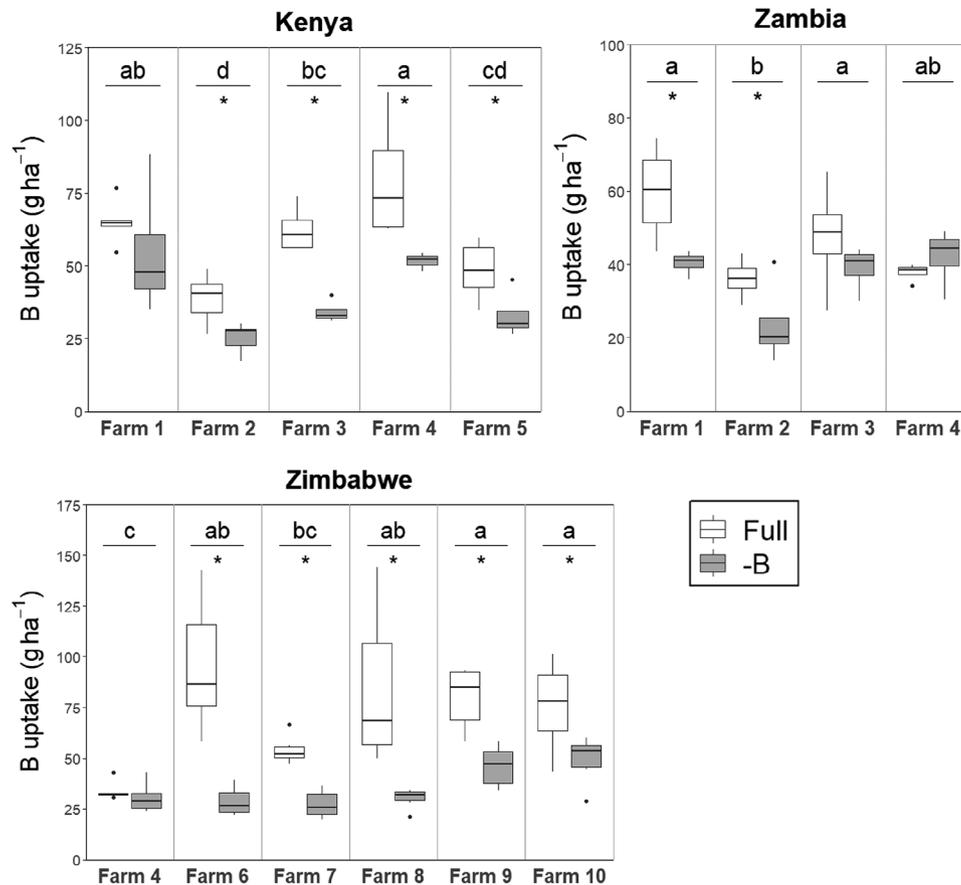


FIGURE 6 Boxplots of B uptake for individual farms, grouped per country. The boxplots show the median (line), first and third quartiles (hinges), the minimum and maximum based on the interquartile range (whiskers), and the outliers (points). Letters indicate significant differences among sites within a country, asterisks indicate a significant difference between treatments within a site.

mainly increases maize B concentrations, but not biomass production (Andrić et al., 2016; Jin et al., 1988; Kaur & Nelson, 2015; Lordkaew et al., 2011; Mozafar, 1987).

The B fertilizer application rates used in this study likely were too high. In the majority of plots (48 out of 72), a negative yield response to B fertilization was observed, with significant yield reductions at 5 out of 15 farms. Fertilizer B will largely remain in solution as adsorption is limited, especially in acidic soils (Degryse, 2017; Van Eynde et al., 2020). As the range between deficient and toxic levels of soil available B is very narrow, this can easily lead to toxicity, especially when large quantities of B are applied (Gupta et al., 1985). In this study, B was fertilized as 5 kg ha⁻¹ basal application in Kenya, 3 kg ha⁻¹ split application in Zambia, and 3 kg ha⁻¹ basal application in Zimbabwe, all applied in or close to the planting hole. Previously, application of 5 kg B ha⁻¹ did not result in notable maize yield reductions in SSA (Rurinda et al., 2020) and was found to be the optimal B application rate for field-grown maize in Brasil (Gotz et al., 2021), although B was broadcasted in the latter study, rather than applied in the planting hole. On the other hand, B application rates were considerably higher than the 0.5–1 kg ha⁻¹ used in other field trials with maize in SSA (Kihara et al., 2017; Lisuma et al., 2006; Vanlauwe et al., 2015; Wendt & Rijpma, 1997; Wortmann et al., 2019). Wendt and Rijpma (1997), furthermore, also noted potential B

toxicity in maize grown in Malawi at a B application rate of 3 kg ha⁻¹, despite soil testing values indicating B deficiency. We therefore recommend to study that rates of B fertilization can improve maize yields in cases of low soil B availability while minimizing the risk of toxicity. The findings of this study indicate that maximum rates should be below 3 kg ha⁻¹ for maize when using soluble B fertilizer sources and split application. Based on Degryse (2017) and Abat et al. (2015), we furthermore recommend to apply B either through broadcasting or in the planting hole as a slow-release fertilizer, which prevents B toxicity as well as losses through leaching.

4.2 | Soil properties

The sources of soil available B for crop uptake are believed to be mineralization of soil organic matter, weathering of soil minerals, and atmospheric deposition with precipitation, in particular close to coastal areas (Park & Schlesinger, 2002; Shorrocks, 1997). All trials were located far away from any coast, and as weathering is regarded as a slow process in comparison with mineralization of soil organic matter (Kot et al., 2016), the latter is expected to be the primary source of available B in the soils from this study. Not surprisingly, all B pools (HW,

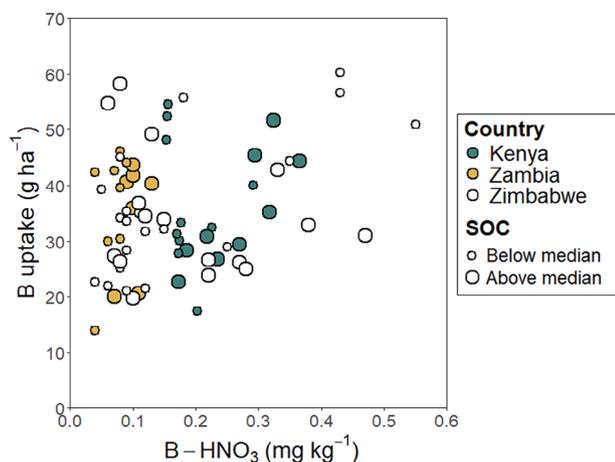


FIGURE 7 B uptake of the B omission plots plotted against B-HNO₃, with size of the data points indicating whether soil organic carbon (SOC) concentrations were below or above the median, within each respective country. Data points represent individual blocks ($n = 72$).

HNO₃, and CaCl₂) were positively related to SOC. In contrast to what was expected, however, higher levels of SOC were associated with lower levels of B uptake, which points to adsorption rather than mineralization processes that control B availability (Van Eynde et al., 2020). However, the model only explained 18% of variation in B uptake and SOC contributed relatively little compared to B-HNO₃. Furthermore, the two were positively correlated ($r = 0.44$), whereas higher levels of B-HNO₃ were associated with higher levels of B uptake. These findings confirm that availability of B is still poorly understood, and no straightforward identification of soil processes that control B availability could be obtained from our results.

Generally, soil parameters had limited explanatory power in describing yield response and B uptake. We have several hypotheses for this result. First, natural variation in the data may have hampered the ability to draw meaningful relations between soil parameters and yield response or B uptake. The majority of data points (72%) had a yield response that could be considered natural variation (i.e., deviating less than 20% from no effect). For these plots, no consistent relation between soil parameters and the yield response is expected. Second, the sites described in this study covered limited ranges in soil parameters that were previously found to affect B availability, such as SOC and pH. Inclusion of soils with a wider range in SOC might have revealed stronger (and possibly positive) trends between SOC and B uptake. In addition, most soils had pH values between 5.0 and 6.5, a range in which pH-dependent adsorption of B plays a limited role (Goldberg, 1997). Third, factors other than soil properties could play an important role in explaining the yield response to B fertilization, such as water availability (Degryse, 2017). Boron is relatively immobile within plants and therefore requires a constant supply of soil B (Kaur & Nelson, 2015; Mozafar, 1987). Drought can cause B deficiency as uptake of water and thus uptake of B by the roots is limited (Bell, 1997; Gupta et al., 1985). Heavy rainfall is also associated with leaching of B, especially in sandy soils (Degryse, 2017). Heavy rainfall after B fertilization could there-

fore potentially ameliorate the effect of B toxicity. Our data neither disprove nor confirm the effect of rainfall on B uptake or yield response. Rainfall data were available per farm, leading to only 5 (Kenya), 4 (Zambia), and 6 (Zimbabwe) observations per country. Rainfall furthermore did not differ strongly within a country, as most farms were located in relatively close proximity of each other. For future research, we recommend to study the effect of water availability in combination with B fertilization to gain insight on the best timing for B application with regard to expected rainfall. Finally, B does not appear to have been growth-limiting in these field trials, as positive yield responses to B fertilization were absent on a farm level. A lacking response may have been caused by the low B demand of maize (Lordkaew et al., 2011). When B is not the yield-limiting nutrient, B uptake does not equal B availability, as other nutrients or biophysical factors constrain biomass production and therefore B uptake (Janssen et al., 1990), and soil parameters are expected to describe only limited variation in nutrient uptake.

4.3 | Critical concentrations

In this study, positive maize yield responses to B fertilization on plot level were associated with B-HW concentrations below 0.69 mg kg⁻¹, B-CaCl₂ below 0.085 mg kg⁻¹, and B-HNO₃ below 0.35 mg kg⁻¹. This could imply that B fertilization should not be recommended with soil B levels above these thresholds. The value of 0.69 mg kg⁻¹ for B-HW is in the same order of magnitude compared to the 0.50 mg kg⁻¹ reported by Kumar et al. (2018). However, B fertilization did not lead to significant yield increases at farm level, despite many of the plots having B concentrations below this threshold. In line with our results, Wendt and Rijpma (1997) reported that B fertilization significantly increased maize yields at only two out of eight locations in Malawi, whereas each of the locations had low (<0.32 mg kg⁻¹) B-HW concentrations. These findings could indicate that a single soil extraction method is not sufficient to identify whether B fertilization is required. Furthermore, as B is prone to leaching, B concentrations in the subsoil often exceed those in the topsoil (Gupta et al., 1985). Maize roots can reach a depth of 50 cm approximately 5 weeks after sowing (Hund et al., 2009). In practice and as well as in this study, often only the topsoil is sampled for analysis. For future studies, we recommend to also analyze subsoil samples (25–50 cm), as B concentrations in this soil layer may be relevant for diagnosis of B deficiency.

No critical plant B concentration indicating potential B deficiency in maize could be derived in this study either. Establishing a universal critical plant B concentration may be difficult, given the variation among maize varieties and plant parts (Andrić et al., 2016; Gotz et al., 2021; Mozafar, 1987). Furthermore, although deficiency of several plant-essential nutrients can be derived from maize ear leaf concentrations to some extent, this does not apply to B (Kovács & Vyn, 2017). Establishment of critical plant concentrations is also complicated by the fact that B is highly immobile within plants and that soil supply of B is not constant (Bell, 1997); plant concentrations therefore do not give a clear representation of the (actual) nutritional status of the crop.

Timing of fertilization may therefore affect plant B concentrations. Boron fertilization increased stover B concentrations relatively more than grain B concentrations in Kenya and Zimbabwe, but not in Zambia. This could be due to the moment of B application, as B was fertilized right before sowing in Kenya and Zimbabwe, but later in the growing season in Zambia. Gotz et al. (2021) found significantly higher B concentrations in maize cob leaves when B was applied at growth-stage V6 compared to application at sowing (at rates of 4 and 12 kg ha⁻¹). We therefore hypothesize that, as B leaches easily and is not mobile within plants, B concentrations of plant tissues that are formed within a few weeks after fertilization are most strongly affected by B application. For future research, we recommend to study the effect of B fertilization at different times in the growing season on maize yields.

4.4 | Soil B pools

Boron concentrations in HW, HNO₃, and CaCl₂ methods were strongly correlated (Figure 1). Novozamsky et al. (1990) also found a good correlation between B-HW and B-CaCl₂ in 100 Dutch soils ($R^2 = 0.74$). They, however, found that CaCl₂ extracted around 27% of B compared to HW, in comparison to the 14% found in this study. These differences may be due to differences in the HW protocol (not clearly specified in Novozamsky et al., 1990) or soil properties such as organic matter content, pH, or phosphate loading (Van Eynde et al., 2020).

The HW extraction method overestimates the reactive B pool, which is a representative of the B that becomes available for plant uptake during a growing season. Previously, B-HNO₃ was shown to overestimate the reactive B pool, which could be due to dissolution of silicate minerals (Van Eynde et al., 2020). In this study, B concentrations in HW were roughly twice as high as in HNO₃. The HW method was relatively more efficient in extracting B compared to HNO₃ with increasing SOC. We hypothesize that as suspensions are heated to boiling in the HW method, B from organic matter is disclosed, which is not extracted by the acidic (pH ≈ 0.5–1.0) HNO₃ extract. Although the difference between the HW- and HNO₃-extractable B seems to point at B not bound to external functional groups in soil organic matter, and possibly to B in undecomposed biomass, the exact nature of this source of B, and whether it is mineralized during the growing season, remains unclear. For future studies, we recommend to explore which soil B pool is extracted with the HW method, which may help to better understand the relevance of this pool for plant B uptake.

To use the HW method as a proxy for B uptake or yield response in future studies and applications, we urge the need for standardization of the method. Many variations of the HW method are in use, and protocol details are often not explicitly specified in studies, which complicates interpretation of results. For example, soils can be extracted with distilled water, CaCl₂ (Bingham, 1982), or BaCl₂ (Wear, 1965) and sometimes with the addition of activated charcoal to obtain a clear extract for colorimetric determination; all variations affect extractable B (Chaudhary & Shukla, 2004; Jeffrey & McCallum, 1988; Joshi et al., 2014; McGeehan et al., 1989; Sahrawat et al., 2012). Many studies do not report cooling time, although B re-adsorbs to the soil dur-

ing cooling over time (Jeffrey & McCallum, 1988; McGeehan et al., 1989). In addition, colorimetric analysis likely overestimates B concentrations due to interferences, leading to different results compared to using ICP (Gestring & Soltanpour, 1981; Jeffrey & McCallum, 1988; Sahrawat et al., 2012). As ICP devices do not require colorless extracts, charcoal additions are not needed (McGeehan et al., 1989). We therefore recommend ICP-AES or ICP-MS for the analysis of B-HW, in addition to detailed descriptions of protocol details when using this method.

5 | CONCLUSIONS

Despite low soil and plant B levels, B fertilization did not increase maize yields in any of the 15 trial sites in SSA, although B uptake increased in 11 sites. Our findings show that assessing the need for B fertilization in maize grown in tropical soils based on soil or plant tissue concentrations is challenging. Critical thresholds in hot water extractable B previously derived for maize were not sufficient to predict a positive yield response to B fertilization. As a result, it remains unclear to what extent maize yields in SSA can be improved through application of B fertilizers. However, all soil B pools were positively correlated with SOC. Maintaining or improving soil organic matter status thus potentially can prevent or alleviate B deficiency in crops grown in tropical soils.

In this study, soil parameters described only limited variation in B availability/uptake and the yield response to fertilization. The main reasons identified were high B application rates (5 and 3 kg ha⁻¹) causing toxicity and large natural variation in the yield response to B fertilization. We presented a number of recommendations for future studies that aim to understand availability of B to field-grown maize in tropical soils, including (split) B fertilizer application at rates below 3 kg ha⁻¹ to prevent toxicity, as well as collection of data on factors such as rainfall (distribution) and B availability in deeper soil layers, which potentially explain variation in B uptake and yield response.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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