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Mehlich 3 as an indicator of grain nutrient concentration for five cereals in sub-Saharan Africa

S.M. Haefele [a,*], A.W. Mossa [b], D. Gashu [c], P.C. Nalivata [d], M.R. Broadley [a,e], S.P. McGrath [a], C.L. Thomas [a]

[a] Sustainable Soils and Crops, Rothamsted Research, West Common, Harpenden AL5 2JQ, UK
[b] ETH Zurich, Swiss Federal Institute of Technology, Department of Environment Systems Sciences, Inorganic Environmental Geochemistry, Universitätstrasse 16, Zurich 8092, Switzerland
[c] Centre for Food Science and Nutrition, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia
[d] Lilongwe University of Agriculture and Natural Resources (LUANAR), Lilongwe, Malawi
[e] School of Biosciences, University of Nottingham, Sutton Bonington, Leicestershire LE12 5RD, UK

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ABSTRACT

Context or Problem: Soil testing for available nutrients is an important tool to determine fertilizer rates, however many standard methods test the availability of a single nutrient only. In contrast, Mehlich 3 (M3) is a multi-element test for predicting crop yield responses to the addition of macro and micronutrients. However, the M3 test has rarely been validated against crop nutrient concentrations, which limits its application for dietary improvement studies in sub-Saharan Africa.
Objective or Research Question: The primary objective was to test how well the M3 nutrient concentrations corresponds to grain nutrient concentrations as an indicator of plant nutrient status and grain quality. A secondary objective was to compare the performance of the M3 test with other extraction tests.
Methods: This study used 1096 paired soil and crop samples of five cereals: maize, rice, sorghum, teff and wheat, covering a broad range of soil types and soil properties in Ethiopia and Malawi (e.g., pH 4.5 - 8.8; Olsen P < 1 - 280 ppm). The samples were selected from a larger collection based on "high" or "low" grain nutrient concentrations in the crop, and the respective soil available nutrients were measured with M3 and other extraction tests: $CaCl_2$ (P, K, Mg, Mn), $Ca(NO_3)_2$ (K and Mg), Olsen P, sequential extraction (S), and DTPA (Mn, Fe and Zn).
Results: The M3 concentrations followed the trend of the "high" and "low" grain concentrations in nearly all nutrients and crops, and this was statistically significant in teff and wheat for all nutrients. The results were best for macronutrients, and slightly less good for micronutrients, probably because the concentration of micronutrients in the selected soil samples was generally quite low. Compared to the other multi-element extractant ($CaCl_2$), the M3 test corresponded better to grain concentrations of K and Mg, and equally well to Olsen P, sequential extraction (S), and DTPA predictions of P, S, Zn and Fe, respectively. M3 extracted much greater concentrations than the other tests, and this was more pronounced in alkaline soils.
Conclusions: Given that the M3 test corresponded well to grain nutrient concentrations across a range of soils and crops in sub-Saharan Africa (SSA), we conclude that it can be considered a universal test for plant nutrients. We also proposed thresholds for M3 values, defining below optimum, optimum and above optimum soil fertility status.
Implications or Significance: These results validate the use of the M3 test to assess soil fertility and develop fertilizer recommendations for improved produce quality to enhance diets in SSA.

1. Introduction

Soil testing is an essential tool in agronomy (Ros et al., 2021), enabling the evaluation of soil fertility and the subsequent adjustment of fertilizer applications to promote optimal crop growth and to avoid pollution of the soil as well as surface and ground water bodies (Sharpley

* Corresponding author.
E-mail address: stephan.haefele@rothamsted.ac.uk (S.M. Haefele).

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et al., 2006; Sapkota et al., 2021). In general, soil tests do not extract specific fractions of nutrients in the soil but vary in their extraction efficiency (Wuenscher et al., 2015). To be useful for agronomy, the soil nutrient test results must relate to the plant available pool, i.e. the amount of a nutrient in the soil that is available for plant uptake during the growing season. Therefore, the relationship between the soil test result and the actual crop uptake or performance must be established through field experiments, plant analysis or based on the observation of deficiency symptoms in plants. However, given the complexity of soils—in particular, the dynamic equilibrium if nutrients between the solid phase and soil pore water— and the interaction of crops with a range of environmental factors (including other nutrients and rhizobacteria), the relations between soil tests and crop performance are often weak and prone to considerable error (Dobermann et al., 1996, 2003; Mason et al., 2010; Schut and Giller, 2020). Nevertheless, soil tests are indispensable because they are the only tool allowing a fast evaluation of nutrient availability before the cropping season and lead to the implementation of comprehensive nutrient management strategies.

Another issue with soil nutrient testing is that there are a wide variety of methods, many whose use is limited to specific countries or crops, and few are universal. This makes the comparison of soil nutrient test results across countries or crops difficult, often requiring transfer functions from one test to another. Traditionally, tests for individual nutrients were common, but due to modern analytical methods like ICP (inductively coupled plasma) spectroscopy, multi-element extractions have gained popularity in recent years. Their convenience and lower costs make them more attractive than the use of separate single element extractions (Iatrou et al., 2014). Consequently, there is growing international interest in multi-element extraction methods like the Mehlich 3 (M3) test. The M3 test was developed in 1984 (Mehlich, 1984) containing a combination of chemicals (CH_3COOH, NH_4NO_3, NH_4F, HNO_3 and EDTA) designed to extract both macro- and micronutrients from soils with a wide range of pH. It is similar to the Bray P-1 test (a dilute acid and fluoride extraction for assessing phosphate; Bray and Kurtz, 1945), but includes a chelating agent (EDTA) to enhance the extraction of trace metals (Mylavarapu et al., 2002). The test is popular in the United States and several other countries (Wuenscher et al., 2015), and is used to assess the availability of a range of nutrient elements (e.g., phosphorus, potassium, calcium, magnesium, sodium, manganese, zinc, copper, aluminium and boron) (Sims, 1989).

The most common limiting nutrients in crop production worldwide are N, P and K, consequently most countries have well established soil tests and threshold values for these nutrients. In contrast, micronutrient deficiencies are rarely identified and addressed, partly because they are less common in soils of temperate regions. However, at global scale, about one-third of arable soils are deficient in micronutrients, particularly in zinc (Zn) (Alloway, 2008; Cakmak et al., 2017). In sub-Saharan Africa (SSA), widespread soil micronutrient deficiencies and multiple element deficiencies have been reported (Kihara et al., 2020; Hengl et al., 2017). The same authors found that in SSA zinc (Zn) was the most common soil micronutrient deficiency, followed by boron (B), iron (Fe), molybdenum (Mo), manganese (Mn) and copper (Cu). Hengl et al. (2017) estimated macro- and micro-nutrient availability in soils of SSA based on M3 data from the Africa Soil Information Service (AfSIS) project but the availability classes were based on other testing methods (Roy et al., 2006), which nevertheless predicted yields well in a validation test. High-resolution data (30 m) for general soil characteristics and nutrient availability in Africa based on M3 was established by Hengl et al. (2021), and the continuous updating of these maps is ongoing as new data become available (iSDA, personal communication).

The combination of this soil information on macro- and micronutrient availability together with an evaluation of the predictive value of the M3 test in SSA is therefore of considerable practical value. It could help agronomists to predict nutrient deficiencies at farm and regional level, and contribute to optimize fertilizer recommendations by adding micronutrients where needed. It also could help fertilizer producers and blenders to decide where the use of fertilizers including micronutrients could improve crop performance and farmers income, whilst contributing to improved produce quality and human health based on agronomic biofortification (Kiran et al., 2022).

Our objective was therefore to use an existing collection of paired soil – crop samples to evaluate the capacity of the M3 test to predict crop nutrient concentrations. The sample collection covered a variety of cereal crops (maize, wheat, teff, sorghum, rice) and represented the major agricultural areas of Ethiopia and Malawi, collected as part of the "GeoNutrition" project (Gashu et al., 2021; Kumssa et al., 2022). We re-analysed a subset of the soil samples (n = 1096) with the M3 method and compared the results for a range of nutrients with their concentration in the grain samples. In addition, we evaluated the results of the M3 method with other nutrient specific and multi-element soil extraction tests to further validate the M3 test.

2. Materials and methods

2.1. Site and sample selection

This study used a sub-sample of the soil and grain samples collected by the GeoNutrition project funded by the Bill and Melinda Gates foundation and described by Gashu et al., (2020, 2021). These authors collected soil (0–15 cm) and grain samples from 1389 locations in Ethiopia during late-2017 and late 2018 harvest seasons and from 1812 locations in Malawi, which were sampled during the April – June 2018 harvest season. At each location, a grain sample and a co-located composite soil sample were taken with the informed consent of the farmer. Sampling designs and geostatistical methods are described in Gashu et al. (2020), (2021). Soils were analysed for a wide range of chemical parameters (up to 84 parameters) and grains were analysed for total elemental concentration (29 elements) using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). All analytical data and meta-data are available (Kumssa et al., 2022).

Of these samples, we selected a subsample based on known concentrations of P, K, S, Mg, Ca, Mn, Fe, and Zn in paired soil/grain samples. Our hypothesis was that soil extractable nutrient concentrations as determined with the Mehlich 3 (M3) method (Mehlich, 1984) would be a useful proxy of actual grain concentrations in mature crops. As described above, the M3 method has the theoretical advantage of determining the availability of many nutrients in the soil in one extraction, but this has not been validated at a relevant scale in sub-Saharan Africa. Due to limited sample material in some locations and limited funds for the M3 extraction, we aimed to re-analyse about 1000 soil samples with M3, to cover areas that are representative of where the major crops grown in the two target countries. The original survey included grain samples from the following crops in Ethiopia: teff (*Eragrostis* tef (Zucc.) Trotter; n = 373), wheat (*Triticum aestivum* L.; n = 328), maize (*Zea mays* L.; n = 302), sorghum (*Sorghum bicolor* (L.) Moench; n = 138), barley (*Hordeum vulgare* L.; n = 181) and finger millet (*Eleusine coracana* (L.) Gaertn.; n = 39), with a smaller number of triticale (× *Triticosecale* Wittm. ex A. Camus; n = 20) and rice (*Oryza sativa* L.; n = 8); and in Malawi: maize (n = 1608), sorghum (n = 117), rice (n = 54), and pearl millet (*Pennisetum glaucum* (L.) R. Br.; n = 32). Given these sample numbers and distribution, we decided to focus on teff, maize, wheat, sorghum and rice. There were only few samples of sorghum and rice grains available, so all paired soil and grain samples from these crops were included in the final selection.

Following this identification of crops to be included, corresponding soil samples were identified for M3 analysis. To reduce the number of paired soil/grain samples for maize, wheat and teff, a selection was made, aiming for ~250 paired samples from each crop. The grain sample data from Ethiopia and Malawi were pooled, the combined sample data were then ranked by the concentration of each nutrient independently, and the top ("high") and bottom ("low") ranking 25 samples for the concentration of each nutrient were selected from each crop. Often the same sample was in the ranked selection for multiple nutrients, hence there being often more or less than 250 samples included per crop. The final total number of crop/soil sample pairs selected was 1036 (n = 641 from Ethiopia and n = 395 from Malawi, Fig. 1). There were five crop types included from Ethiopia: maize (n = 59), rice (n = 8), sorghum (n = 138), teff (n = 219) and wheat (n = 217); and three crop types from Malawi: maize (n = 224), rice (n = 54), and sorghum (n = 171). The breakdown of sample number per nutrient and crop can be seen in Appendix 1 and distribution plots of grain nutrient concentrations in the whole sample set and the selected sub-set are shown in Appendix 2.

2.2. Soil analysis methods

For the M3 extraction, 4 g of air-dried, 2 mm sieved soil were mixed with 40 ml of the Mehlich 3 extracting solution (Mehlich, 1984) and shaken for 5 min on a reciprocating shaker. The filtrate was then analysed for P, K, Ca, Mg, Na, Mn, Fe, Cu, Zn, B, Mo, S, Al using ICP Optical Emission Spectrometry (ICP-OES) and ICP-MS. Available data on standard soil characteristics were reported in Kumssa et al. (2022) and included: Soil reaction (pH) determined in 1:2.5 soil: water suspension (ISO, 2005). Total carbon and nitrogen content were analysed with a LECO TruMac Combustion Analyser (LECO, Michigan, USA). Inorganic carbon was analysed using combustion with a Skalar Primacs (Skalar Analytical BV, Breda, Netherlands). Organic carbon was then calculated by subtracting inorganic carbon from total carbon. Effective cation exchange capacity (eCEC) was determined using the cobalthexamine method of extraction (ISO, 2018) followed by ICP-OES analysis of Co. The exchangeable cations Ca, K, Mg and Na in the extract were also analysed using ICP-OES.

In addition to the comparison of M3 extracted nutrients with grain concentrations, we also compared the M3 extracted nutrients with selected other extraction methods regularly used for the evaluation of nutrient availability, which again were previously reported in Kumssa et al. (2022) (additional information; Tables: 'ETH CropSoilData' and 'MWI CropSoilData'). The methods considered were the Olsen P extraction for available P (Olsen, 1954), the 0.01 M $Ca(NO_3)_2$ (1:10 soil: solution ratio) extraction for determination of available K and Mg (Mossa et al., 2021), the 0.01 M $CaCl_2$ (1:10 soil:solution ratio) extraction for available P, K, Mg and Mn (based on Houba et al., 1996; only available for a subset of Ethiopia samples), a three-step sequential extraction scheme for available S (adapted from Mathers et al., 2017 and Shetaya et al., 2012), and the DTPA extraction for available Mn, Fe and Zn (Lindsay and Norvell, 1978).

2.3. Statistical analysis

To assess whether the M3 nutrient concentrations of the soils differed between those selected based on "high" and "low" grain concentrations a t-test ($p \leq 0.1$) was performed. Secondly, the M3 extraction tests were related to the extraction test understood to be optimal for that particular nutrient: K, Mg and Mn with $CaCl_2$ and K and Mg also with $Ca(NO_3)_2$ extraction; P with Olsen P; S with sequential extraction (adapted from Mathers et al., 2017); and Zn and Fe with DTPA. The analyses were made separately for acid/neutral soils (pH ≤ 7.3, n = 788) and alkaline soils (pH > 7.3, n = 236), because M3 is considered most suitable for acid-neutral soils (Zhang et al., 2014, 2019; Mallarino, 2003a, 2003b; Watson and Mullen, 2007) and has been found to be more reliable on soils with pH ≤ 7.3. T-tests were performed using Genstat (18th edition, VSN International Ltd., UK). Correlation analyses were performed in the R environment (R Core Team, 2019).

3. Results

3.1. General observations

Although the sample sites were selected primarily based on the grain nutrient concentrations, they were well distributed across both countries (Fig. 1). However, some crops were grown more widely than others; particularly well distributed were wheat and teff in Ethiopia and maize in Malawi. More selective use of some crops caused clusters of sample sites for sorghum in the NW and SE of Ethiopia and in South Malawi, and for rice preferentially along the coast of Lake Malawi. It should also be noted that teff and wheat were not grown at any selected site in Malawi and only very few rice sites were located in Ethiopia (Fig. 1; Appendix 1).

Basic soil characteristics are shown in Table 1. In both countries these covered a wide range of soil reaction (pH) from very acidic to

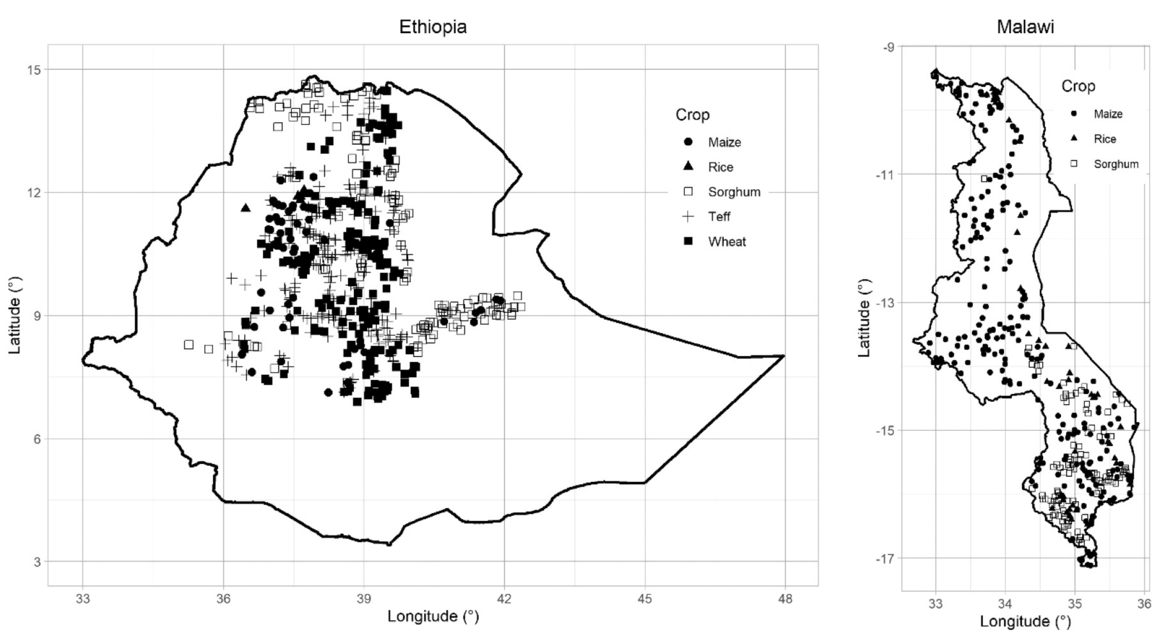

Fig. 1. The geographical distribution of the soil samples selected for Mehlich 3 extraction across Ethiopia (**a**) and Malawi (**b**). Soil sample numbers selected were n = 641 from Ethiopia and n = 395 from Malawi. The minimum and maximum latitude and longitude values on the plot/axes are the minimum and maximum of the whole sample set. Note that the two countries are not in the same scale.

Table 1
Descriptive statistics of general soil characteristics for the selected sites in Ethiopia and Malawi. Composite topsoil samples (0–15 cm) were sampled from a 100 m^2 area as described by Gashu et al. (2020), (2021).

	pH $_{H2O}$	C inorg	C org	N tot	Olsen P	eCEC	exch. Ca	exch. Mg	exch. K	exch. Na
	(-)	(%)	(%)	(%)	(mg kg^{-1})	(cMolc kg^{-1})				
Ethiopia (n = 640)										
Mean	6.60	0.16	1.92	0.17	17.0	29.7	20.1	6.4	0.72	0.11
Min	4.57	0.01	0.11	0.01	0.9	1.6	1.6	0.2	0.05	0.02
Max	8.75	6.73	7.24	0.63	280.5	55.7	41.9	23.2	7.91	0.92
Malawi (n = 396)										
Mean	6.48	0.02	1.25	0.09	22.3	10.9	7.6	2.5	0.48	0.19
Min	4.67	0.01	0.21	0.01	0.7	0.2	0.2	0.0	0.01	0.00
Max	8.76	0.86	8.59	0.71	123.7	49.9	33.8	19.5	2.75	8.09

Table 2
Descriptive statistics of grain nutrient concentrations in the selected crops. Data for maize, rice and sorghum is across Ethiopia and Malawi whereas data for teff and wheat is from Ethiopia only.

Crop	Value	P	K	Ca	Mg	S	Mn	Fe	Zn
				mg kg^{-1}					
Maize	Median	2798	3850	56	1164	1713	6	20	21
n = 283	Min	1077	2049	24	480	738	2	2	10
	Max	5368	7463	1621	2172	4257	384	657	50
Rice	Median	2938	2865	94	1321	1816	20	46	23
n = 62	Min	1504	1575	47	762	682	6	7	12
	Max	4680	5124	336	2402	2493	127	376	41
Sorghum	Median	3353	4490	163	1664	1614	14	40	21
n = 309	Min	1634	2587	52	908	565	6	18	11
	Max	5140	7793	1587	2954	3603	40	718	38
Teff	Median	3947	4426	1470	1913	2051	68	96	28
n = 219	Min	2087	2897	47	912	766	5	16	17
	Max	5501	6352	7925	3043	3121	431	2574	50
Wheat	Median	3164	4439	414	1164	1653	36	39	25
n = 217	Min	1434	2931	249	806	826	13	22	11
	Max	4768	7834	1203	1796	2739	80	273	66

alkaline. Only few soils had significant amounts of inorganic carbon but there were some calcareous soils in Ethiopia. The average soil organic carbon (SOC) concentration in Ethiopia was very good for arable soils, and considerably lower in Malawi. Total soil N mirrored the SOC values resulting in mean C/N ratios of 11.5 in Ethiopia and 14.3 in Malawi. Mean available P (Olsen P) was considerably higher in Malawi and the values indicated generally good P supplies even for European standards (Steinfurth et al., 2022). Effective cation exchange capacity (eCEC), measured at the actual soil pH, was on average three times higher in Ethiopia, mostly due to higher SOC, finer textured soils with higher clay content and more three-layer clay minerals (less weathered soils). As a consequence, soils in Ethiopia had a much better supply of exchangeable Ca, Mg and K. However, mean base saturation was above 90% in both countries (data not shown).

Basic statistics of grain nutrient concentrations for selected macro and micronutrients are shown in Table 2. Data for maize, sorghum and rice are presented across both countries, whereas data for teff and wheat are only for Ethiopia. Generally, high or highest nutrient concentrations were observed in teff, which is a low yielding crop with grain yields of usually below 2 t ha^{-1} (Desta et al., 2021). Low or lowest concentrations are often found in maize which, particularly in Ethiopia, is often a medium yielding crop (about 4 t ha^{-1}; FAO, 2023), whereas low maize yields around 2 t ha^{-1} (FAO, 2023) are common in Malawi. Rice, sorghum and wheat are intermediate for most nutrients shown. Maximum Fe values were very high in all crops, but particularly in teff, and are most likely caused by contamination of the grain samples with soil dust.

Literature-based thresholds for M3 values are shown in Table 3. They were established based on soil fertility research conducted on soils of the

Table 3
Availability of nutrients according to soil extraction concentrations of the Mehlich 3 method, based on Rutgers (2015) and Heckman (2004), modified by Ajumako/Kiberashi (AfSIS1, unpublished) and Ethiosis (AfSIS2 unpublished). The "high" category is considered optimum fertility. Categories "very low", "low", and "medium" are below optimum; "very high" is above optimum (excessive). The thresholds were developed for cereals.

Nutrient	Source	Very Low	Low	Medium	High	Very High
		mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
Phosphorus	AfSIS1	< 5	5-15	16-30	30-50	> 50
Potassium	AfSIS2	< 90	90-190	191-600	600-900	> 900
Sulfur	Rutgers*	< 5	5-10	11-40	41-50	> 50
Magnesium	Rutgers*	< 22	22-41	42-71	72-148	> 148
Calcium	Rutgers*	< 307	307-503	504-699	700-895	> 895
Zinc	AfSIS	< 1.0	1.0 – 10	10 - 50	> 50	
Copper	AfSIS	< 0.5	0.5 – 1.0	1.0 - 20	> 20	
Boron	AfSIS	< 0.5	1.0 – 1.5	1.5 - 20	> 20	
Iron	AfSIS	< 60	60-80	80 - 300	300 - 400	> 400
Manganese	AfSIS	< 60	60-100	100 - 300	300 – 500	> 500

* Rutgers values are sometimes reported in lbs $acre^{-1}$ and were transformed to mg kg^{-1} by dividing by two according to Hannan (2023).

MidAtlantic Region of the USA, which lays on the east coast between the northeast and the southeast region (Heckman, 2004; Rutgers, 2015). The categories were developed from crop (cereal) yields that were observed during nutrient response studies conducted over a range of soil test levels (unpublished). In this system, observed M3 test values are classified into five levels (very low, low, medium, high, very high), corresponding to three fertility categories, i.e. below optimum (very low, low, medium), optimum (high) and excessive (very high). These values were adjusted for cereals in Africa by the AfSIS project for SSA (Vågen et al., 2010; Ethiosis, unpublished). There are variations of these thresholds for individual nutrients, different crops, soils and regions particularly in the USA, but we are not aware of a published general list as presented in Table 3 for any region outside of the USA, particularly for more tropical soils and crops.

3.2. Relationship between Mehlich 3 (M3) extractable nutrients and grain nutrient concentrations

The main objective of the study was to investigate if the M3 soil test could reliably predict availability of nutrients to the crop as measured in the grain nutrient concentration. Fig. 2 shows the mean M3 extraction nutrient concentrations and the corresponding grain nutrient

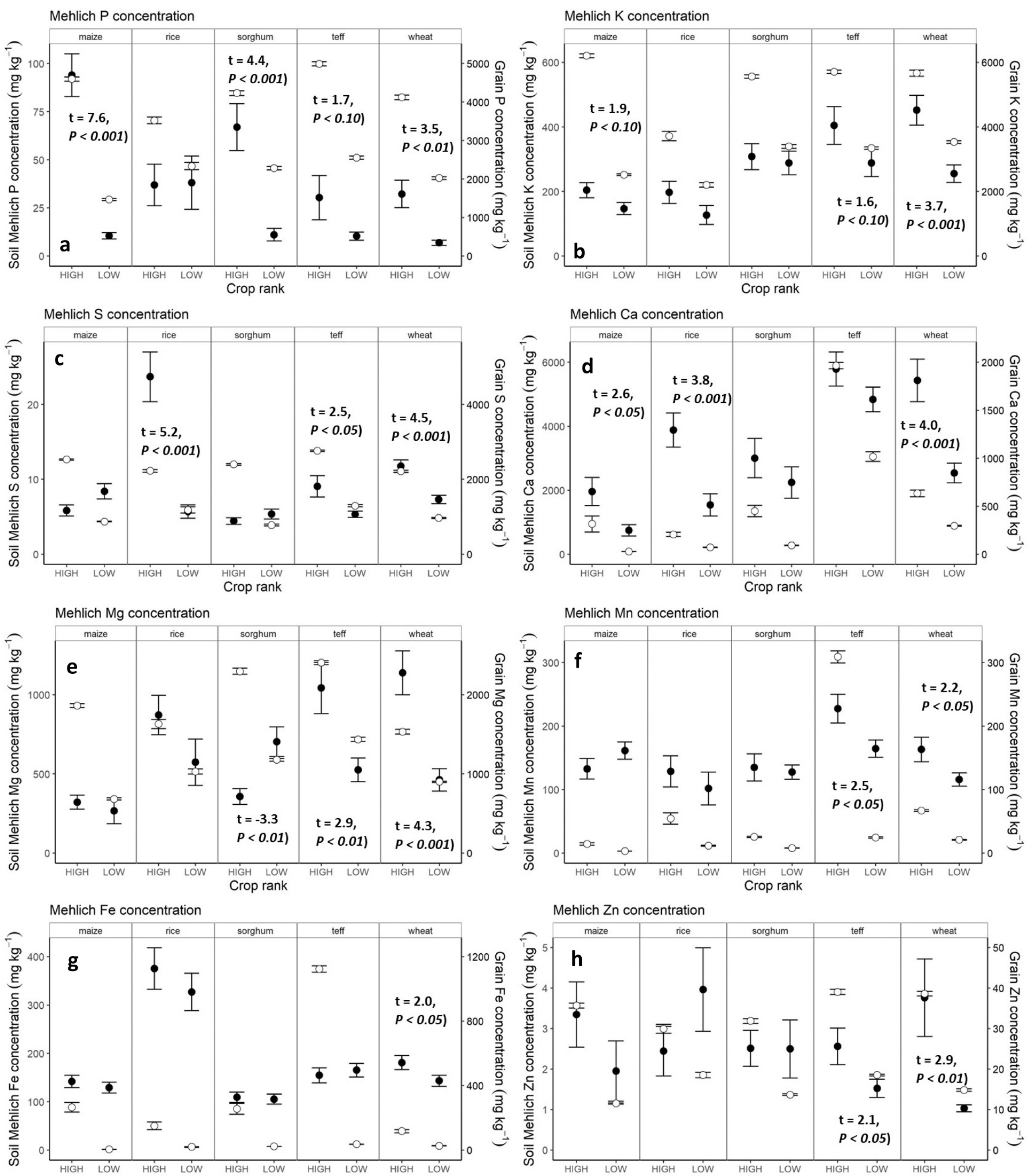

Fig. 2. Mean (± SEM error bars) of grain (open circles) and corresponding soil Mehlich 3 extraction (filled circles) concentration of **a**. P, **b**. K, **c**. S, **d**. Ca, **e**. Mg, **f**. Mn, **g**. Fe and **h**. Zn. The sites were selected across Ethiopia and Malawi to have either "high" or "low" grain nutrient concentrations. The selection was made independently for maize, rice, sorghum, teff and wheat (see sample size per nutrient per crop in Table 1). Also showing the standard error of the mean (± SEM error bars) and t-test results of the difference in the Mehlich 3 extractable nutrient concentration between the "high" and "low" selected sites. NB. There were no teff and wheat samples from Malawi. Note that the scaling on the left and right y-axis are often different.

concentrations in the selected “high” and “low” samples. Given that the plant samples were selected for “high” and “low” concentrations, they are always significantly different sample groups. The M3 concentrations of the macronutrients P and K show generally good agreement with the grain concentrations i.e. following the “high” and “low” trend, with the exception of P in rice and K in sorghum, and this was significant in 7 out of 10 cases (Fig. 2a and b). The results are similar for the secondary nutrients S, Ca and Mg (Fig. 2c, d and e); again, the M3 soil extracts follow the “high” and “low” trend in 12 out of 15 cases. Exceptions were S in maize, and S and Mg in sorghum, and the difference was significant in 8 out of 15 cases. For micronutrients, the M3 test corresponds well to grain Zn concentrations in teff and wheat (Fig. 2h). However, only small differences in M3 concentrations of Mn and Fe between the “high” and the “low” samples (Fig. 2f and g) were found (and there was an inverse trend of Mn in maize and of Fe in teff). Among crops, the positive correlation between “high” and “low” grain nutrient concentration and the M3 concentrations was lowest for sorghum, intermediate for maize and rice, and highest for wheat and teff. Note that in the case of teff and wheat, all points were in Ethiopia only, possibly representing a more homogeneous environment and group of varieties. It should also be noted that for rice and sorghum, there was no clear separation of “high” and “low” samples because all samples were used, which may explain why for these crops the distinction in the M3 values between the “high” and “low” samples is less clear.

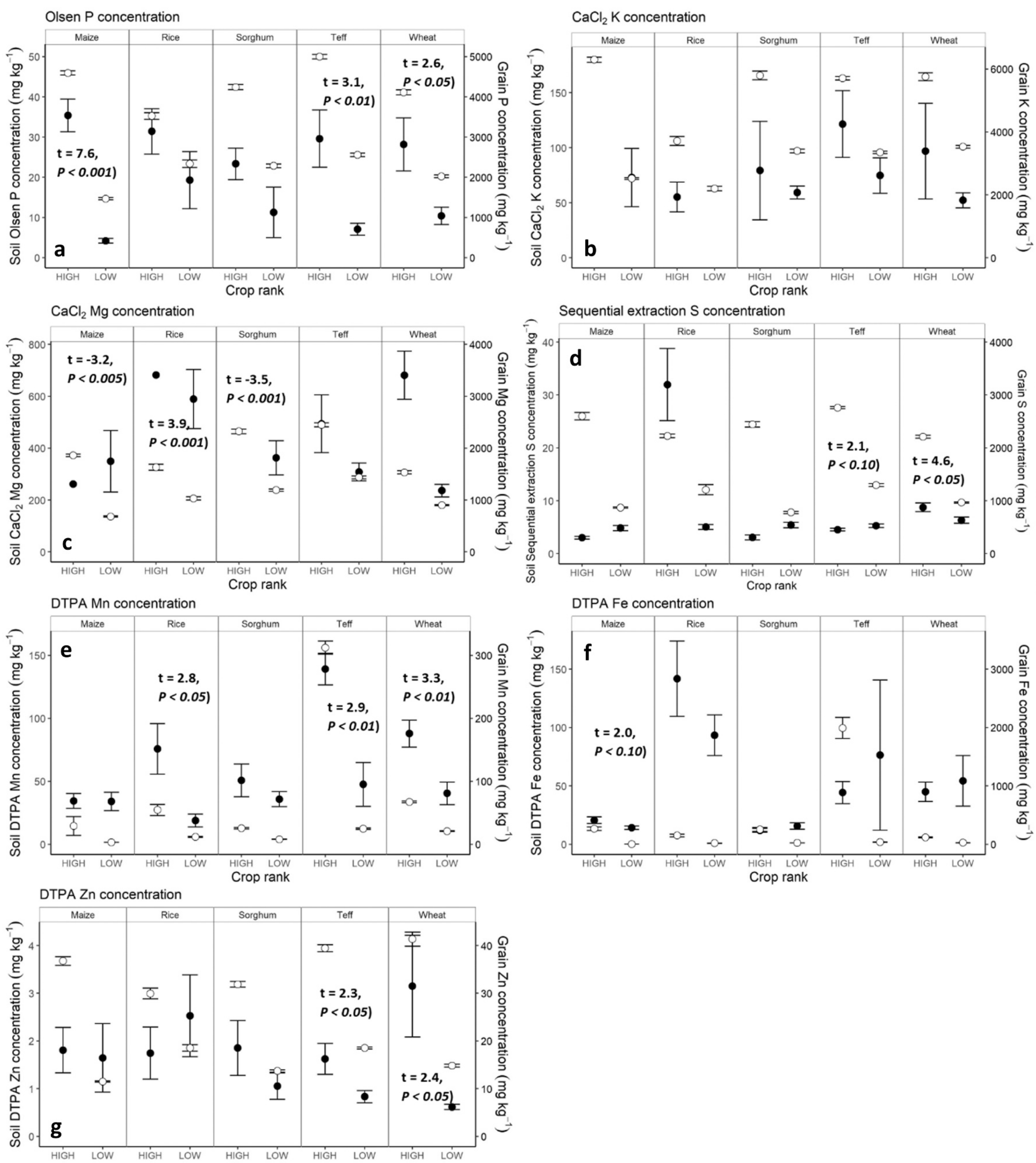

Fig. 3. Mean (± SEM error bars) of grain concentration (open circles) and corresponding soil extraction test concentration (filled circles) of **a**. P with Olsen, **b**. K with $CaCl_2$, **c**. S with sequential extraction, **d**. Mg with $CaCl_2$, **e**. Mn with DTPA, **f**. Fe with DTPA and **g**. Zn with DTPA. The sites were selected across Ethiopia and Malawi to have either high or low grain nutrient concentrations. The selection was made independently for maize, rice, sorghum, teff and wheat (see sample size per nutrient per crop in Table 1). Also showing t-test results of the difference in the soil extractable nutrient concentration between the high and low selected sites. NB. There were no teff and wheat samples from Malawi. Note that the scaling on the left and right y-axis is often different.

The dataset published in Kumssa et al. (2022) included a range of other measures of nutrient availability determined for the soil samples used in the present study, allowing a comparison of these with the M3 test used in the present study (Figs. 2 and 3). In terms of significance of the difference between the "high" and "low" samples, the M3 test discriminated more effectively between "high" and "low" samples than the Olsen extraction of P (significant in four and three cases, respectively), the M3 test performed better than $CaCl_2$ extraction of K (significant in three and no cases, respectively), M3 performed better than the sequential extraction of S (significant in three and two cases, respectively), M3 performed comparably to $CaCl_2$ extraction of Mg (both significant in two cases), but M3 performed worse than DTPA extraction of Mn (significant in two and three cases, respectively), and performed comparably to DTPA extraction of Fe and Zn, (both significant in one and two cases, respectively). These results indicate that the M3 test was better or equally good as any of the other tests. However, in some cases the M3 method was not very precise with small crop differences between "high" and "low" soil samples.

3.3. Relationship between M3 extractable nutrients and other soil tests

In a second step, we analysed direct relationships between the M3 and other soil extraction tests. To rate this regression analyses we used qualitative terms: poor ($R^2 < 0.50$), moderate ($R^2 = 0.51$ to 0.65), good ($R^2 = 0.66$ to 0.80), and very good ($R^2 > 0.81$). The regression analyses was performed separately for acid to neutral soils (pH ≤ 7.3, n = 788) and alkaline soils (pH > 7.3, n = 236), because M3 is considered most suitable for acid to slightly above neutral soils (Zhang et al., 2014; Rutter et al., 2021).

The first element evaluated was available P, measured with M3, Olsen P and the $CaCl_2$ extraction (Fig. 4). The relationship between M3 P and Olsen P was moderate to good ($R^2 = 0.57$ and 0.70 in acid/neutral and alkaline soils, respectively) across Malawi and Ethiopia. In general, the M3 values were around twice as high as the Olsen P values, but closer to the Olsen values in the acid soils.

On the other hand, the relationship between M3 P and $CaCl_2$ P was very good ($R^2 = 0.84$ and 0.88 in acid/neutral and alkaline soils, respectively; only Ethiopian sites), but the absolute M3 values were higher by a factor of about 40. Available K was compared between M3, the $Ca(NO_3)_2$ extraction and the $CaCl_2$ extraction methods (Fig. 3c,d). The relationship between M3 K and $CaCl_2$ K was again good to very good ($R^2 = 0.86$ and 0.77 in acid/neutral and alkaline soils, respectively; only Ethiopian sites), and better than the relationship with the $Ca(NO_3)_2$ extraction ($R^2 = 0.65$ and 0.54 in acid/neutral and alkaline soils, respectively; across Ethiopia and Malawi).

Again, the M3 values were much higher (factor 1.7 for the $Ca(NO_3)_2$ extraction; factor 2.5 for the $CaCl_2$ extraction) and the M3 values were closer to the $CaCl_2$ extraction in the acid soils. The relationships between M3 Mg and both $Ca(NO_3)_2$ Mg ($R^2 = 0.87$ and 0.86 in acid/neutral and alkaline soils, respectively; across Ethiopia and Malawi) and $CaCl_2$ Mg ($R^2 = 0.87$ and 0.88 in acid/neutral and alkaline soils, respectively; in Ethiopia) were very good, about 3 times higher than with the two other methods, and again closer to other extractions in acid/neutral soils (Fig. 4e,f). A not commonly used method for available S is the three-step sequential extraction scheme (adapted from Mathers et al., 2017 and Shetaya et al., 2012). The comparison of that method with M3 is shown in Fig. 4g, indicating a poor relationship in acid/neutral soils ($R^2 = 0.40$, across Ethiopia and Malawi) and only slight better, moderate results in neutral/alkaline soils ($R^2 = 0.53$, across Ethiopia and Malawi). Absolute values of both methods were very similar. However, in contrast to the other nutrients, the M3 values of the alkaline soils were closer to the sequential extraction values and much higher concentrations were extracted in acid soils.

Fig. 5 shows comparisons of methods used for the evaluation of micronutrients, including the M3 method, the DTPA method and the $CaCl_2$ extraction. All three methods were used to evaluate available Mn (Fig. 4a,b). In acid/neutral soils, the relationship between M3 Mn and the DTPA Mn extraction was poor ($R^2 = 0.40$ in Ethiopia and Malawi), as for the $CaCl_2$ Mn extraction ($R^2 = 0.49$, in Ethiopia only); however, relationships were very poor in the alkaline soils ($CaCl_2$ extraction $R^2 = 0.01$; DTPA extraction $R^2 = 0.06$). Better results were observed for the evaluation of micronutrient availability with the M3 and the DTPA method for Zn and Fe. The relationship between M3 Zn and DTPA Zn was moderate ($R^2 = 0.64$ and 0.79 in acid/neutral and alkaline soils, respectively; Ethiopia and Malawi), and absolute values of the M3 method were close to the DTPA values in the acid soils (Fig. 5c). The relationship between M3 and DTPA Fe values was also moderate ($R^2 = 0.62$ and 0.60 in acid/neutral and alkaline soils, respectively; Ethiopia and Malawi). The M3 Fe values were about twice as high as the DTPA values, and much higher in alkaline soils (Fig. 5d).

4. Discussion

4.1. General observations

The data on the sampling sites did not include a soil type description but the wide spread of the sampling sites included in the original design, across the arable soils in both countries (Fig. 1), implies a wide coverage of soil types. The representation of a diverse range of soils is also confirmed by the large ranges of analytical results for all soil characteristics analysed (Table 1). The major soil types in Malawi are Luvisols, Lixisols, and Cambisols (Vargas and Omuto, 2016), with Lixisols dominating in the northern region, Luvisols in the central, and Cambisols along the Rift Valley and largely in the southern regions. In Ethiopia, dominant soil types are Lithosols, Cambisols, Nitosols, Vertisols, Xerosols, Solonchaks, Fluvisols and Luvisols, covering about 80% of the country (IUSS Working Group WRB, 2007), but especially Vertisols are very important soils in Ethiopian arable agriculture. This soil type distribution indicates generally less weathered soils in Ethiopia which is also confirmed by the slightly better soil characteristics (Table 1). But across all sampling sites, the difference between the two countries is small for all characteristics.

The grain nutrient concentrations observed are of course within the range of the full sample set from both countries described by Gashu et al. (2021). Generally high nutrient concentrations in teff as well as typically low concentrations in maize were already reported by the same authors. These differences are most likely caused by a combination of genetic effects as well as a dilution effect from much higher yields in maize (Jarrell and Beverly, 1981). Some high grain concentration values, especially for Fe and Mn, are most likely due to a contamination with soil dust, as the Fe and Mn concentrations are often magnitudes higher in soil than in grains (Gashu et al., 2021). However, this is not the case for the other elements investigated (P, K, Ca, Mg, S, Zn and Se) because their grain concentrations are usually equal or higher in crops than in soils.

4.2. Mehlich 3 soil deficiency thresholds

The M3 test has been used in the USA for several decades but most related studies focused on the availability of P and few other macronutrients for several crops (e.g., Watson and Mullen, 2007; Mallarino, 2003a; Grewal et al., 2017; Sawyer and Mallarino, 1999). Studies on the determination of threshold values for macro and micronutrients in tropical environments are rare and we compiled a list (Table 3) of threshold values and/or availability ranges based on Rutgers (2015) and Heckman (2004), adjusted by internal "grey" literature from the AfSIS project (AfSIS 1 and 2). These values were compared to thresholds in available reports. Critical limits of Mehlich 3 for the nutrition of rice as determined by Seth et al. (2018) and applied by Haefele et al. (2021) varied between the two soil orders studied and were for P, K and S 14.7, 51.2 and 22.9 mg kg^1 in Inceptisols, but 8.2, 117.3 and 21.9 mg kg^{-1} in Alfisols, respectively. All these thresholds are within the very low to

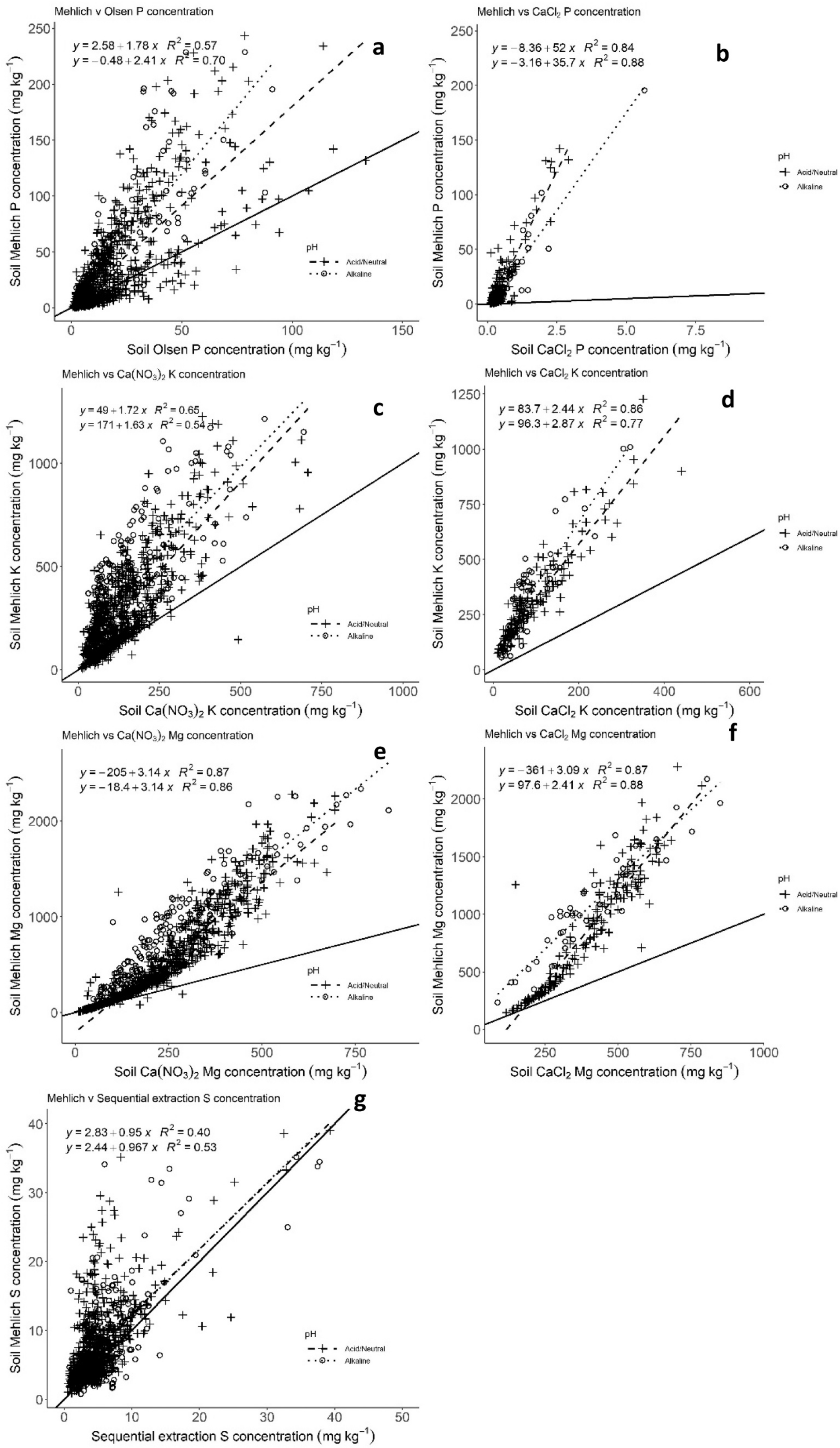

Fig. 4. Relationships between macronutrient availability as determined by Mehlich 3 extractions and other extraction methods: **ab**. Olsen P and $CaCl_2$ extraction of P; **cd**. $Ca(NO_3)_2$ and $CaCl_2$ extraction of K; **ef.** $Ca(NO_3)_2$ and $CaCl_2$ extraction of Mg, and **g.** sequential extraction of S. The relationships were across all sites in Ethiopia and Malawi (**aceg**) or for Ethiopia only (**bdf**). Solid line = 1:1 line. NB. $CaCl_2$ extraction data was only available for Ethiopia. Separate regression functions and R^2 values for acid/neutral (top equation) and alkaline soils (bottom equation) are shown.

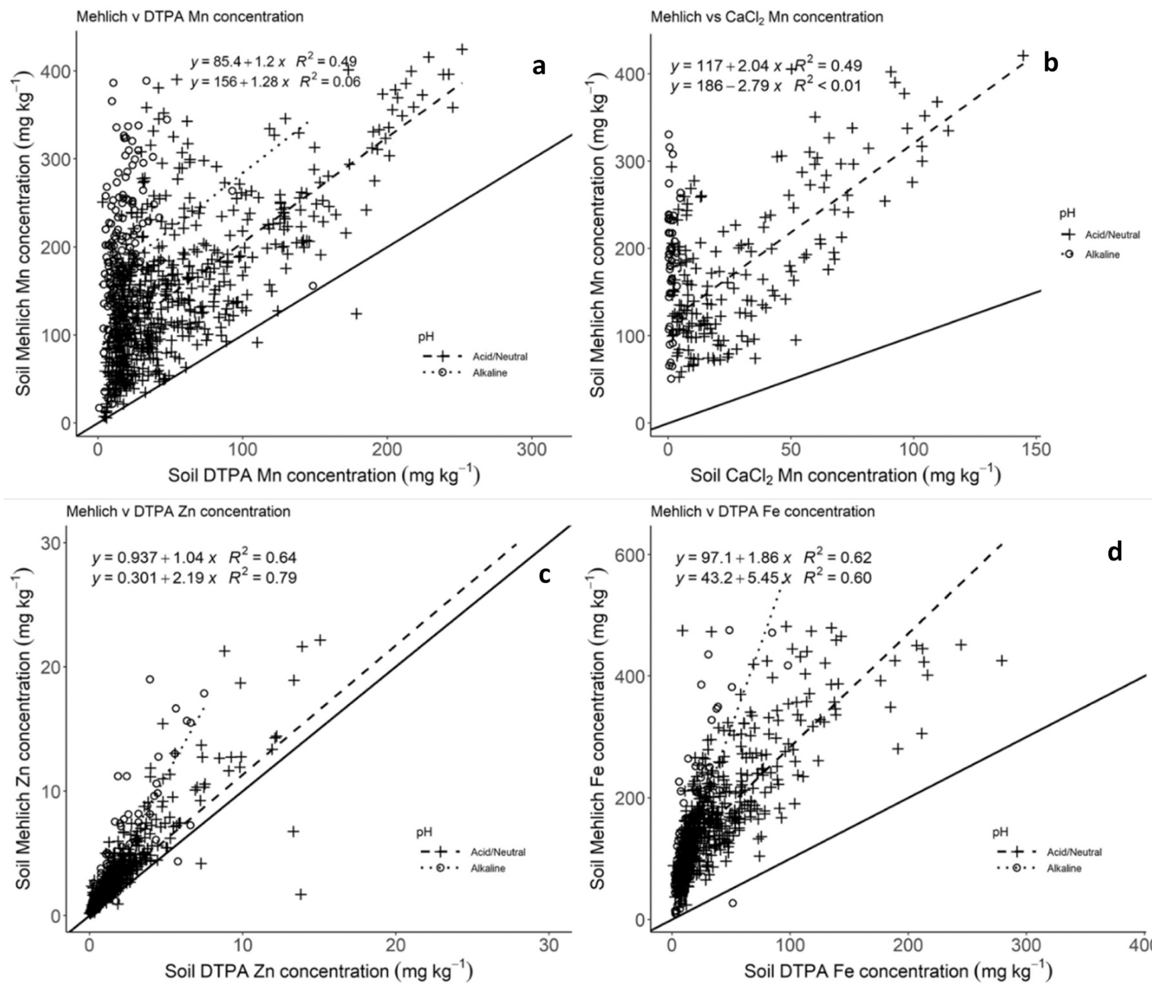

Fig. 5. Relationships between micronutrient availability as determined by Mehlich 3 extractions and other extraction methods: **ab**. DTPA and $CaCl_2$ extraction of Mn; **c**. DTPA extraction of Fe, and **d.** DTPA extraction of Zn. The relationships were across all sites in Ethiopia and Malawi (**acd**) or for Ethiopia only (**b**). Solid line = 1:1 line. NB. $CaCl_2$ extraction data was only available for Ethiopia. Separate regression functions and R^2 values for acid/neutral (top equation) and alkaline soils (bottom equation) are shown.

medium range in Table 3, therefore infer requirement for fertiliser inputs. The critical limits computed for B and Zn by the same authors were 0.65 and 0.40 mg kg^{-1} and 1.27 and 2.15 mg kg^{-1} in Inceptisols and Alfisols, respectively. Again, these values are close to the critical thresholds in Table 3. Compared to Table 3, similar but lower M3 thresholds values (about half) for P and K, and the same threshold for Zn were recommended by Mallarino (2003a) for corn in the US. Alvey (2013) reported 18 mg kg^{-1} of M3 sulfur as sufficient for North American crops, which is considerably lower than the value proposed here of 40 mg kg^{-1}. Similar M3 threshold values for B, Cu, Zn, Mn and Fe as in Table 3 were also proposed by Zbiral (2016) for cereals in Europe. We therefore concluded that although there is a range of threshold M3 values for deficiency (very low category) and the upper boundary where some crop yield losses are expected (medium category) for most nutrients, the values shown in Table 3 can serve as guidelines for the prediction of likely deficiencies and identification of soils were macro or micronutrient application will be beneficial for intensive farming. Comparing observed M3 values in both countries (Fig. 2) with the proposed thresholds (Table 3) indicates common soil fertility limitations for S (< 40 mg kg^{-1}), Mn (< 300 mg kg$^{-1)}$, Fe (< 300 mg kg^{-1}), and Zn (< 50 mg kg^{-1}). Surprisingly, few limitations were indicated for P (< 30 mg kg^{-1}) and Mg (< 71 mg kg^{-1}); Ca (< 699 mg kg^{-1}) and K (< 600 mg kg^{-1}) had an intermediary fertility status. The cited reports also confirm that local conditions and crop type will modify the thresholds so that if M3 values are close to the upper thresholds in the medium category, field observations and/or experimentation should be used to decide on rate and frequency of nutrient applications. The proposed threshold values for M3 can of course be updated when more data from field observations become available.

4.3. Mehlich 3 and grain nutrient concentrations

One basic hypothesis underlying this study was that grain nutrient concentrations are representative of nutrient availability in the soil. This is different from the normal validation of soil tests against yield response (grain or biomass) or nutrient concentrations in leaves, and assumes equally free nutrient flow from the vegetative to the reproductive organs. Preferential transport of nutrients into the grain as well as relative immobility are known (Marschner, 2011) but all essential plant nutrients are found in the grain of cereals. Thus, even if nutrients might be accumulated or diluted in the grain, their relative concentration differences between different sites and, for the same crop, can still be representative of the availability of each individual nutrient. Another effect changing the nutrient concentrations in grains which we could not take into consideration is the dilution effect of high yields (Jarrell and Beverly, 1981). With increasingly optimum conditions for growth, the plant accumulates a relatively greater proportion of assimilated carbohydrates in the grains, causing a dilution of other nutrients. However, the sampling conducted for the “GeoNutrition” project did not collect any yield data and the samples represent a wide range of crop yields.

This effect will have increased the error in the M3 value/grain relationship. Another potential error is that different varieties of the same crop species can also differ in their grain nutrient concentrations which could also not be considered here, as was drought, which can also affect nutrient uptake and transport in the plant. But despite all these caveats, we found a good correspondence between the M3 soil extraction concentrations and grain nutrient concentrations in the samples selected as "high" and "low" (Fig. 2) although the main target of the M3 test was for yield response, not nutrient concentration in the grain. For macronutrients (P, K, S, Mg and Ca) the grain concentration trend was always correct and significant in maize, teff and wheat (the only exception being S in maize). Furthermore, the difference between the "high" and "low" samples was often more significant with M3 than with other traditional extraction tests; being equal or superior to the other tests for P, K, Mg and S (Figs. 2, 3). Therefore, M3 could be regarded as a suitable universal test for macronutrients, able to replace common single nutrient tests and performing equal or better than other multi-element tests. This has already been shown for a few elements, crops and environments (e.g., Rutgers, 2015; Zbiral, 2016; Seth et al., 2018) but is new for crops and environments across SSA. It should be noted that this has been tested here for selected "high" or "low" availability soils only, and might not be precise enough for fine-resolution nutrient requirement estimations, but predictions of the basic three soil supply levels (below optimum, medium and excessive fertility) are typical for most fertiliser recommendations.

For micronutrients (Mn, Zn and Fe in this study), the M3 extractions were less indicative/predictive of grain concentrations (Fig. 2). As with all soil trace element tests, the relationship between soil extractable levels and plant uptake is not very strong due to the low concentration and complex interactions with soil properties, crop uptake mechanisms and general environment factors. For example, when pH and CEC were included as terms along with M3 Zn in the prediction of crop Zn, the relationship between the soil and crop Zn were significantly improved compared to using the soil M3 test alone (Junus and Cox, 1987). Consequently, interpretation of test results for micronutrients should be made with caution, including other sources of information if possible (e.g., visual plant symptoms). But the M3 test usually mirrored the trend in grain concentrations, even if rarely significant, and it should be noted that the M3 soil values of the "high" and the "low" grain concentration group were often both in the below-optimum range, indicating deficiencies in both groups. Extractable Mn is generally considered an unreliable measure of crop available Mn because availability is very dependent on recent soil moisture conditions (McGrath et al., 2013). But it is encouraging that the M3 and DTPA tests corresponded equally well to grain concentrations of Fe and Zn in teff and wheat, and that the differences between the "high" and "low" samples were significant, possibly because all teff and wheat samples were from Ethiopia, thus representing less heterogeneity in environmental conditions affecting crop nutrient uptake (Marschner and Rengel, 2012; Chen and Barak, 1982; Wang et al., 2019). These results support the decision of the Ethiopian Soil Information System (EthioSIS) that the M3 soil test can be used as a measure to evaluate the need for micronutrient fertilizer recommendations. It is also supported by studies of the Hill laboratories in New Zealand which have shown good relationships between the M3 test with the standard EDTA test for Mn, Zn, Cu and Co (Hill Laboratories, 2023). However, Vidal-Vázquez et al. (2005) did not find good relationships between the DTPA test and M3 for micronutrients, whereas Pradhan et al. (2015) found good relationships between both tests for Cu but not for Zn.

4.4. M3 relationship with other extraction tests

Comparing the results of different soil testing methods is often not straightforward and requires transfer functions that can include specific soil properties to translate the outcome of one soil test into another. Nevertheless, transfer functions can help to harmonize data collections that used different methods and can therefore be very useful. We did not intend to develop transfer functions but wanted to confirm the M3 performance in comparison with other established soil tests.

In general, the M3 test extracted consistently greater concentrations of P, K, Mg, Mn, Zn and Fe than the other extraction tests (Figs. 4 and 5). This effect was greater in alkaline than acid/neutral soils and was particularly strong for micronutrients. Similar results were reported for the M3 compared to Olsen extraction (Zbiral and Nemec, 2002) and for the Bray 1 test (Mallarino, 2003b). This pH effect might have affected our results, because the soils included in our analysis did span a wide range of soil reaction values. However, most of the soils tested were in the acid/neutral range (n = 788) as compared with the alkaline range (n = 236).

In general, the M3 concentrations related best (good to very good) with the $CaCl_2$ extraction test of macronutrients ($R^2 > 0.86$ for P, K and Mg in acid/neutral soils, and $R^2 > 0.77$ in K and Mg in alkaline soils (Fig. 3). However, M3 did relate moderately well with the single nutrient tests Olsen P and sequential S extraction: the R^2 was about 0.60 in both cases. In comparison, Breure et al. (2022) found very good relationships between the M3 test and 1 M ammonium acetate extraction for K and between M3 and Olsen P for P. Likewise, Kleinman and Sharpley (2002) observed strong relationships between M3 P and the bicarbonate P test in both acid and alkaline soils. In addition, we found that the M3 test was moderately related to the DTPA test results for micronutrients ($R^2 \sim 0.60$; Fig. 5), which is contrast to Vocasek and Friedericks (1994) who found strong relationships between M3 and DTPA extractions of Fe and Zn ($R^2 > 0.85$). But in their study, all soils were from the Great Plains in the US, perhaps representing less physical and chemical heterogeneity than in the soils tested here.

Another study showed that M3 was not better at predicting Cu, Mn and Zn than Mehlich1 (Mehlich, 1953), despite the addition of EDTA to M3 (Sims, 1989). However, when pH and CEC were included in the prediction along with M3, good relationships between the soil test and crop uptake were observed (Junus and Cox, 1987; Moraghan and Mascagni, 1991). This confirms that soil pH is an important covariate for making accurate micronutrient availability predictions with the M3 test because pH is an important determinant of micronutrient availability (Rengel, 2015; Botoman et al., 2022).

5. Conclusions

Soil nutrient tests are rapid assessment tools for the nutrient status of soils, and as such indispensable for soil fertility management. But the hypothesis for this study was that they can also be used to indicate/predict the nutrient concentrations in grain which is an important measure of grain quality and the contribution of crops to a healthy diet containing sufficient (micro)nutrients. And in the specific case of the M3 soil test, this would allow the evaluation of all macro and micronutrients (except N) with one measurement.

In general, we believe that this hypothesis was confirmed even though the M3 test (and soil tests in general) is not a precise measure of available nutrient supply. Soils are very heterogenous and many soil characteristics like soil organic carbon content, soil pH, and clay concentration, as well as soil rhizosphere processes affect nutrient availability to crops. Further variability in nutrient uptake is added by different crop species, crop varieties and crop yield, which can cause an accumulation/dilution effect. Therefore, we do think it is very promising that the M3 test indicated/predicted high and low nutrient concentrations well in the grains of the five crops tested and across a wide variety of soils. The predictions were reliable for macronutrients, but less so for micronutrients. However, the latter limitation might have been caused by generally low micronutrient concentrations in most soils analysed. Inclusion of soil pH in the interpretation of the M3 test results could further improve the predictive power for grain micronutrient concentration.

The M3 results were also confirmed by comparison with several other standard tests. Based on all these results, we believe that the M3 test can be regarded as a suitable universal test of nutrients available to different cereals across a range of soils in sub-Saharan Africa. Towards this goal, we also compiled M3 threshold values for the most important macro and micronutrients in sub-Saharan Africa, which seems currently only available in grey literature.

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CRediT authorship contribution statement

Haefele Stephan M.: Conceptualization, Methodology, Writing – original draft. **Mossa AW:** Data curation, Writing – review & editing. **Gashu D:** Writing – review & editing. **Nalivata PC:** Writing – review & editing. **Broadley MR:** Project administration, Writing – review & editing. **McGrath SP:** Methodology, Writing – review & editing. **Thomas CL:** Methodology, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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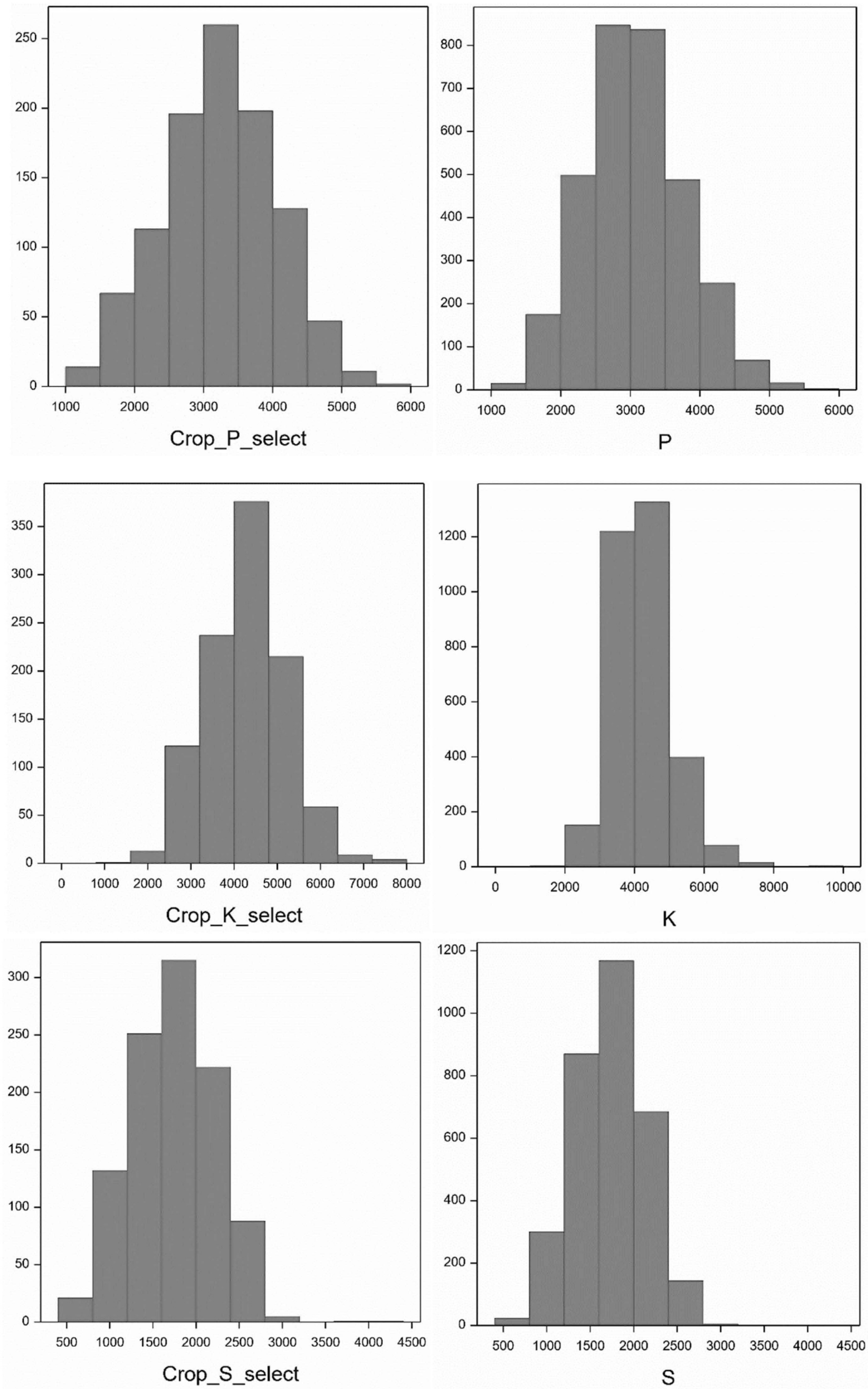
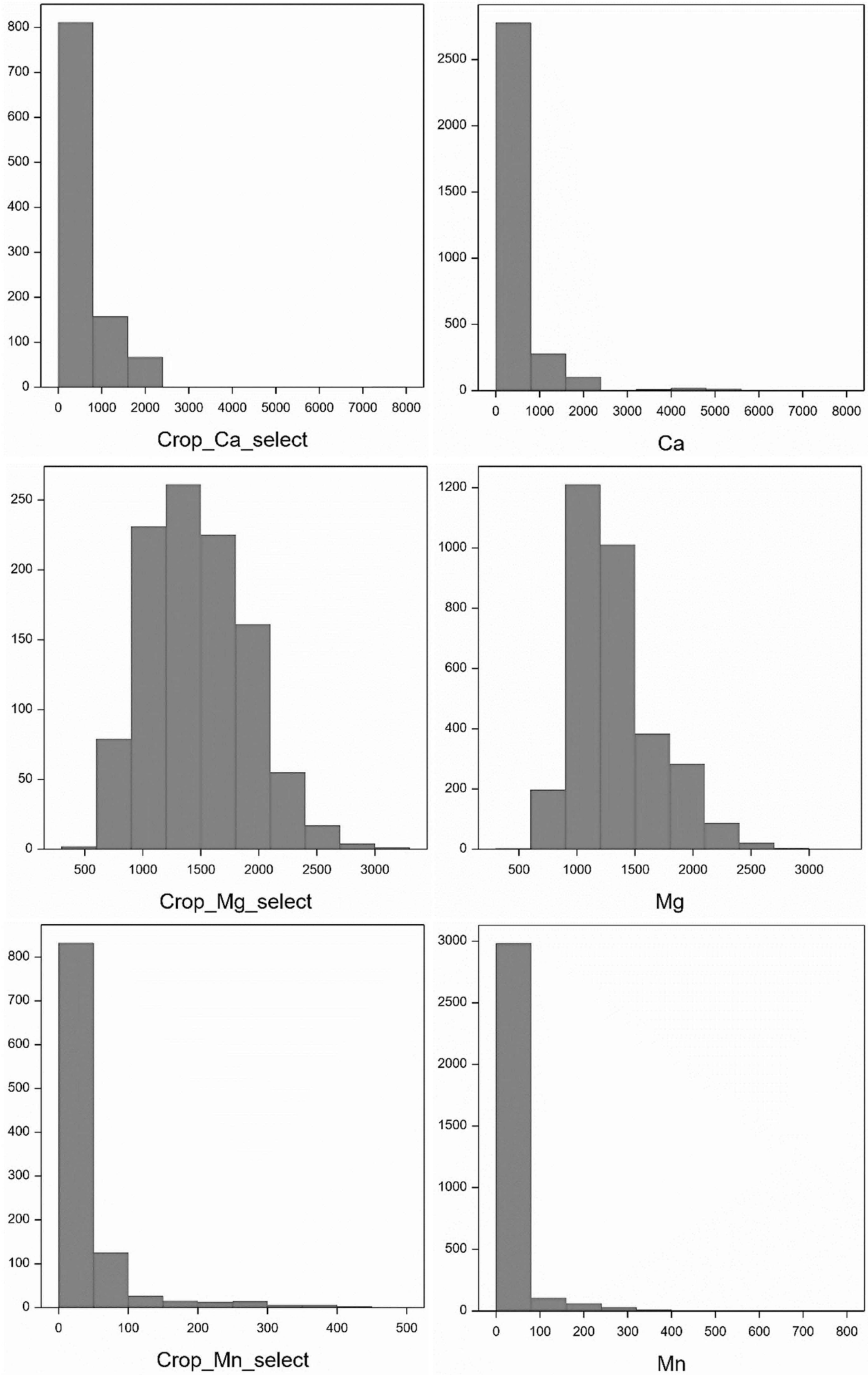
Appendix 1. The number of soil samples selected for Mehlich 3 extraction from Ethiopia (ETH) and Malawi (MW) for each nutrient. The selection was made from the whole set based on "high" and "low" grain nutrient concentrations, and was made per nutrient and per crop

CROP	"HIGH"		"LOW"		Total
	ETH	MW	ETH	MW	
	P				
maize	4	21	5	20	50
rice	1	12	1	14	28
sorghum	4	19	20	4	47
teff	22	\	24	\	46
wheat	24	\	25	\	49
	K				
maize	0	25	5	19	49
rice	3	9	1	14	27
sorghum	7	18	10	14	49
teff	23	\	24	\	47
wheat	24	\	25	\	49
	S				
maize	1	24	20	5	50
rice	0	12	4	8	24
sorghum	0	25	21	2	48
teff	24	\	24	\	48
wheat	25	\	23	\	48
	Mg				
maize	2	23	7	16	48
rice	0	14	2	13	29
sorghum	0	25	21	2	48
teff	20	\	24	\	44
wheat	23	\	25	\	48
	Mn				
maize	3	22	10	14	49
rice	3	10	1	13	27
sorghum	6	17	19	3	45
teff	19	\	25	\	44
wheat	23	\	25	\	48
	Zn				
maize	3	21	10	14	48
rice	0	15	5	9	29
sorghum	10	13	18	5	46
teff	26	\	24	\	50
wheat	23	\	23	\	46
	Fe				
maize	3	22	0	25	50
rice	2	4	0	11	17
sorghum	18	7	13	10	48
teff	24	\	23	\	47
wheat	25	\	23	\	48

Appendix 2. Nutrient concentrations in the grain of the selected set (left column) and whole set (right column)

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0 1000 2000 3000 4000 5000 6000 7000 8000
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0 1000 2000 3000 4000 5000 6000 7000 8000
Ca
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500 1000 1500 2000 2500 3000
Crop_Mg_select
0 200 400 600 800 1000 1200
500 1000 1500 2000 2500 3000
Mg
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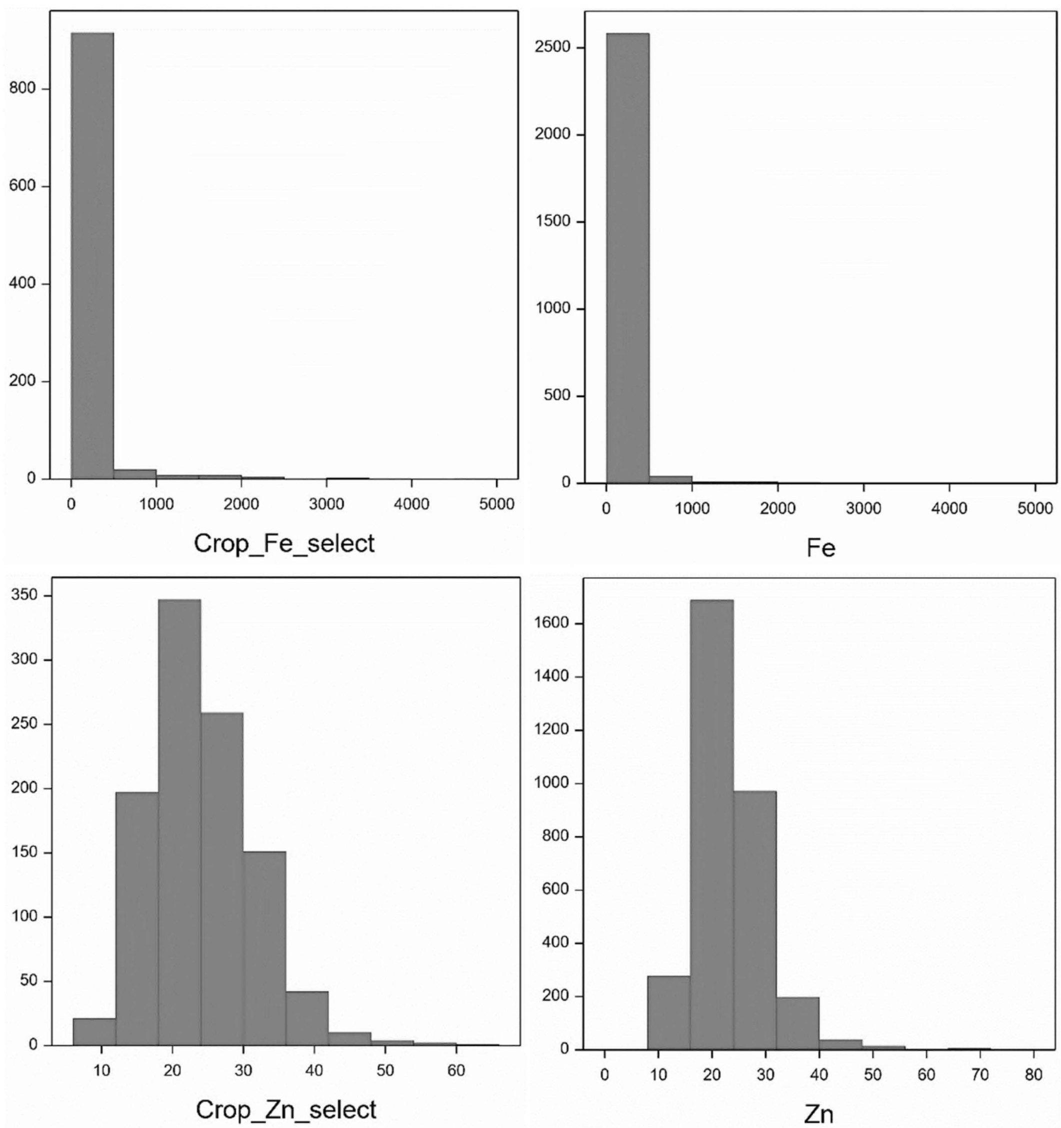
0 100 200 300 400 500
Crop_Mn_select
0 500 1000 1500 2000 2500 3000
0 100 200 300 400 500 600 700 800
Mn



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