

Combining genetic and agronomic fortification is essential to meet human health targets for zinc, iron, and protein concentrations in rice grains: A meta-analysis

Kalimuthu Senthilkumar

k.Senthilkumar@cgiar.org

Africa Rice Center (AfricaRice)

Dominic Mutambu

International Center for Tropical Agriculture (CIAT), ICIPE Duduville Complex, Nairobi, Kenya

Gudeta Weldesemayat Sileshi

Addis Ababa University

Panneerselvam Peramaiyan

International Rice Research Institute, South Asia Regional Center, Varanasi, 221106, India

Kazuki Saito

International Rice Research Institute

Mina Devkota

International Center for Agriculture Research in Dry Areas (ICARDA)

Ali Ibrahim

AfricaRice

Justin Fagnombo Djagba

AfricaRice

Sali Atanga Ndindeng

AfricaRice

Prem S Bindraban

AfricaRice

Job Kihara

International Center for Tropical Agriculture (CIAT), ICIPE Duduville Complex, Nairobi, Kenya

Systematic Review

Keywords: Agronomic fortification, bioavailability, genetic biofortification, nutrient uptake

Posted Date: April 13th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9267207/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Rice, consumed by half of the world's population, is inherently low in zinc (Zn), iron (Fe), and protein. We synthesized data from 245 studies across 34 countries to evaluate the impact of genetic, agronomic, and processing interventions on rice grain Zn, Fe, and protein concentrations. Zn-biofortified cultivars had 9.8% higher grain Zn concentrations than non-biofortified cultivars, while Fe-biofortified cultivars did not exhibit a significant improvement in Fe content. The probabilities of achieving the breeding target concentrations of Zn (28 mg kg^{-1}) and Fe (15 mg kg^{-1}) in polished rice were only 4.0% and 10.5%, while Zn and Fe fertilization increased the probability to 41.3% and 67.7% for Zn and Fe, respectively. Milling and polishing of brown rice grains reduced Zn, Fe, and protein concentrations by 21%, 70%, and 6.5%, respectively. Our findings emphasize the need for combined use of genetic and agronomic fortification, and consumption of parboiled rice to attain desired health impacts.

Introduction

Rice is a staple food for more than half of the world's population, and the single largest food source for the poor^{1–4}. It provides 19–21% of the global per capita energy and about 15% of the protein requirement for humans^{3–6}. Rice is consumed primarily in a polished form which is loaded with easily digestible starch ($\sim 90\%$ of dry weight)¹, but low in zinc (Zn), iron (Fe), and protein concentrations compared with other staple cereals such as wheat and maize^{7,8}. In the past, research and development efforts have focused most on yield improvement, and less on improvement of the nutritional quality. Decades of breeding for higher yields have led to a decline in grain concentrations of Zn and Fe in staple cereals such as rice and wheat^{9,10}. The grain Zn and Fe concentrations reported in the literature are often lower than the breeding targets proposed for human health¹¹. Initially, the CGIAR biofortification challenge program (HarvestPlus) set the breeding target at 15 mg Fe kg^{-1} in polished (white) rice grains¹¹. However, this breeding target appears to have been abandoned and no longer appears in the recent literature¹². The breeding target for Zn is 28 mg kg^{-1} in polished rice^{11,12}. These targets are aspirational goals set to achieve a realistic level of the daily requirement of Zn and Fe for human health. However, these targets do not consider the bioavailability of Zn and Fe in the human gut. The bioavailability and uptake of Zn, Fe, calcium (Ca), magnesium (Mg), and manganese (Mn) in the human gut depend on the phytic acid concentrations in the processed grain^{13–15}. Plants often store excess phosphorus (P) as phytate (the salt form of phytic acid), which accounts for 60–80% of total P in cereal grains¹⁶. While it has beneficial health effects as an anticarcinogen, antioxidant, and inhibitor of kidney stone formation, phytate is the most abundant anti-nutritional factor in cereal grains with regard to the uptake of bivalent cations^{13–15}. Earlier work has emphasized the need for improvement of the nutritional value of cereals through reduction of phytate and increased Zn and Fe concentrations in the grain¹⁶. Generally, whole-grain rice also has one of the lowest protein concentrations among cereals¹³. The protein, nitrogen (N), and amylose concentrations in rice grains also play a key role in the nutritional, cooking, and eating qualities of rice^{17–19}. Nevertheless, there are no breeding targets for grain protein or phytate

concentrations in rice. Recent findings in breeding rice varieties with higher protein content and low glycemic index are opening new opportunities for breeding more nutritious rice¹. These discoveries highlight the potential to develop rice varieties with improved protein content and better health features, especially for people with diabetes.

Our ability to improve grain yield, grain Zn, Fe, and protein concentrations will depend on understanding the complex interactions and correlations between traits in soils and plants. The interaction between two elements in soils may be antagonistic or synergistic, thereby influencing nutrient uptake and use efficiency²⁰. For example, P fertilization is expected to increase crop yields due to increased P uptake (Fig. 1), which may increase phytate concentrations in grains, but depresses Zn uptake¹⁶. The plant physiological process of absorption by phytate, decreases the availability of Zn even further. However, information on the entire spectrum of how nutrient uptake and physiological processes would interact with regards to P, Zn, Fe, sulfur (S) (relevant for amino acids in proteins), and proteins, and hence their concentrations in rice grains is lacking. Figure 1 presents a schematic representation of our hypothesized relationships between nutrient uptake, grain yield, and the concentrations of nutrients, protein, and phytate in rice grains. Quantifying the correlations between these variables could inform efforts to simultaneously improve yield and minerals such as Zn and Fe^{12,21}. According to Senguttuvel and co-workers²¹, the correlation between grain yield and grain Zn concentrations is a key factor in identifying and releasing Zn-biofortified rice varieties. Genotype-by-environment (G×E) interactions also play a crucial role in identifying stable lines for Zn and Fe concentrations²¹. However, the strength of correlations and the effect of G×E interactions on the traits targeted for rice biofortification are not fully understood. It is also unclear to what degree agronomic and breeding efforts have achieved the initial breeding targets for Zn and Fe in rice. The impact of the production environments (e.g. irrigation vs rainfed) and agronomic interventions (e.g. fertilization) on grain Zn, Fe, and protein concentrations has not been fully quantified.

Although several primary studies have measured concentrations of Zn, Fe, and protein in rice grain at the local level, a global synthesis of this information is lacking. Such syntheses are urgently needed for setting target concentrations and identifying management practices to increase the concentration of essential micronutrients and reduce the concentrations of antinutritional factors such as phytic acid in rice grain. Therefore, the overarching aim of this synthesis is to inform global efforts in genetic and agronomic fortification toward achieving the target concentrations¹¹ of Zn (28 mg kg⁻¹) and Fe (15 mg kg⁻¹) and increasing protein concentration in rice grains. The specific objectives of this synthesis are to: (1) determine the probability of achieving the target concentrations of Zn and Fe in white rice grains; (2) determine trait correlations, the influence of G×E interactions and heritability toward the simultaneous improvement of grain yield, grain Zn, Fe, and protein concentrations; (3) quantify variations in response ratios of grain yield, grain Zn, Fe, and protein concentrations to genotypes, production environments, agronomic practices, and soil and climate variables. The main hypotheses being tested in this analysis are: (1) grain Zn and Fe concentrations in rice cultivars are below the target concentrations for human nutrition; (2) grain Zn and Fe concentrations can be increased with genetic biofortification and Zn and Fe

fertilization; (3) the correlations between grain yield, grain Zn, Fe, and protein concentrations are positive across rice genotypes and fertilization treatments; and (4) Zn and Fe fertilization significantly increase grain Zn, Fe, and protein concentrations across climates, soils, and rice growing environments.

Results and discussion

Distributions of grain Zn, Fe, protein, and phytate concentrations

By consolidating data from 245 publications in studies conducted across 34 countries, and using over 3,600 rice genotypes, this analysis has established benchmark concentrations of Zn, Fe, and protein in rice grain on dry matter basis (Table 1). So far, such baselines have not been available, except for Zn and Fe, which were based on limited data generated in the early 2010s. Grain Zn, Fe, and protein concentrations were available in 191, 94, and 89 studies, respectively (Table 1), and these were used to establish their empirical distributions (Figure S1).

Table 1

The mean, median, 25th percentile (Q1), and 75th percentile (Q3) concentrations and the coefficients of variation (CV) of grain zinc, iron, nitrogen, phosphorus, sulfur, protein, and phytate concentrations in rice across both brown and white rice samples

Elements (unit)	No. studies and observation†	Concentration on dry matter basis				
		Mean	Median	Q1	Q3	CV (%)
Zinc (mg kg ⁻¹)‡	[191; 9801]	25.1	24.8	20.0	29.4	36.9
Iron (mg kg ⁻¹)	[94; 7104]	14.9	11.6	9.7	13.9	103.6
Nitrogen (%)	[89; 1652]	1.4	1.4	1.2	1.6	29.6
Phosphorus (%)	[48; 5282]	0.34	0.35	0.31	0.38	25.8
Sulfur (%)	[21; 3888]	0.128	0.127	0.112	0.143	20.4
Protein (%)	[89; 1652]	8.5	8.1	7.2	9.4	29.6
Phytate (%)	[27; 813]	0.94	0.72	0.41	1.20	73.7
CV, coefficient of variation; Q1, lower quantile; Q3, upper quantile.						
† Figures in brackets represent the number of studies (n) and total sample size (s) available for analysis						
‡ 1 mg kg ⁻¹ = 1 µg g ⁻¹ .						

Using data from a total of 38 and 21 studies reporting measurements in polished rice on Zn and Fe concentrations, we estimated the median grain concentrations in white rice at 17.5 mg Zn kg⁻¹

(confidence interval [CI]: 17.0–18.0) and 3.6 mg Fe kg⁻¹ (CI: 3.4–3.8). These values are much lower than the breeding targets (28 mg Zn kg⁻¹ and 15 mg Fe kg⁻¹)¹¹. We estimated the probability of exceeding the targets of Zn and Fe in white rice using their cumulative frequency distributions. This was 4.0% in white rice for grain Zn concentration, and 10.5% for grain Fe in the absence of Zn and/or Fe fertilization. These low figures indicate that the progress toward the targets through genetic biofortification has been slow. Where Zn and/or Fe fertilizers were applied, the probabilities of achieving the targets were 41.3% for Zn and 67.7% for Fe. On the basis of these findings, we argue the necessity of complementary efforts in breeding and agronomic interventions to create synergistic effects.

The median phytate concentration in rice grain was 0.72% across the 27 studies. The median phytate concentration in white rice (0.38%) was significantly lower than concentrations in brown rice (0.92%) (Table S2). According to Kumar and colleagues²², high Zn bioavailability is associated with rice cultivars with low grain phytate concentrations (< 0.82%), while those with 2.62% or more phytate concentrations have low Zn and Fe bioavailability. Our analysis reveals that the processing of rice also impacts phytate concentrations, and consequently the bioavailability of Zn and Fe.

Trait correlations, G×E interactions, and heritability

Figure 1, Table 2, and Supplementary Table S3 provide an overview of the relationships and correlations between grain yield and nutritional qualities. The analysis of data aggregated across studies on germplasm and fertilization revealed significantly positive correlations between grain yield and grain Zn concentrations ($r = 0.091$; $P < 0.001$), grain yield and Fe concentrations ($r = 0.198$; $P < 0.001$), and grain Zn and Fe concentrations ($r = 0.418$; $P < 0.001$), but not between grain yield and protein or phytate concentrations (Table 2). Significant positive correlations were also observed between grain yield and grain Zn uptake ($r = 0.741$; $P < 0.001$), grain yield and grain Fe uptake ($r = 0.494$; $P < 0.001$), grain yield and grain P uptake ($r = 0.941$; $P < 0.001$), grain Zn uptake and Fe uptake ($r = 0.688$; $P < 0.001$), grain Zn uptake and N uptake ($r = 0.714$; $P < 0.001$), grain Zn uptake and P uptake ($r = 0.643$; $P < 0.001$), grain yield and grain S uptake ($r = 0.671$; $P < 0.001$), and grain Fe uptake and Zn uptake ($r = 0.688$; $P < 0.001$) (Table 2). Table S3a provides the 95% confidence intervals and information on the robustness of the trait correlations.

Table 2

Pearson correlation coefficients of the associations between grain yield, grain Zn, Fe, protein, and phytate concentrations (conc), grain Zn uptake, Fe uptake, N uptake, P uptake, and S uptake in rice grains (for details of tests of statistical significance please refer to Table S3a)

Variable	Grain yield	Zn conc	Fe conc	Protein conc	Zn uptake	Fe uptake	P uptake
Zn conc	0.091***						
Fe conc	0.198***	0.418***					
Protein conc	0.002ns	0.179***	0.097*				
Phytate conc	-0.067ns	-0.069*	0.371***	0.268**	-0.555***	0.026ns	
P conc	0.346***	-0.058ns	-0.119ns	-0.125ns	0.192**	0.009ns	0.642***
S conc	0.142*	-0.213**	0.423***	-0.164*	-0.051ns	0.751***	0.434***
Zn uptake	0.741***	0.729***	0.468***	0.207***		0.688***	
Fe uptake	0.494***	0.555***	0.944***	-0.266***	0.688**		
N uptake	0.797***	0.364***	-0.192**	0.603***	0.714***	0.301**	0.580***
P uptake	0.941***	0.140ns	0.077ns	-0.073ns	0.643***	0.396***	
S uptake	0.671***	-0.149*	-0.030ns	-0.035ns	0.340***	0.631***	0.745***
Statistical significance: * $\alpha = 0.05$; ** $\alpha = 0.001$; *** $\alpha = 0.0001$; ns = not significant							

The findings above generally confirm the relationships depicted in Fig. 1. We were unable to establish the correlations between concentrations of grain phytate and P uptake or S uptake due to lack of matching data on phytate concentrations. Given the correlations between traits, we posit that improvement of nutritional quality is possible through complementary use of agronomic and genetic fortification of rice. Genetic fortification relies on the inherent genetic potential of crop varieties to accumulate nutrients in the grains. However, the effectiveness of this process depends on nutrient availability, which must be ensured through agronomic fortification via soil and/or foliar application, as their uptake and subsequent accumulation in grains are influenced by soil properties and prevailing weather conditions. In the individual studies that evaluated rice germplasm, the correlations between grain yield, grain Zn, Fe, and protein concentrations were significantly positive in 25–33% of the studies and non-significant in 35–75% of the studies. On the other hand, the correlation between grain Zn and Fe was significantly positive in 59% of the studies. In the meta-analysis, the majority (88%) of Fisher r-to-z transformed correlation coefficients between grain Zn and Fe were positive and the random-effects model estimate (0.368) was significantly different from zero. However, between-study heterogeneity was significant (Table S3b).

From the individual studies on fertilization effects, the correlations between grain yield, grain Zn concentrations, Fe concentrations, and protein concentrations were all significant. The fail-safe number, a tests of publication bias, indicate that the pooled effect size estimates are robust. However, significant between-study heterogeneity was evident in all estimates (Table S3b). Taken together these positive correlations suggest that grain yield, grain Zn, Fe, and protein concentrations can be simultaneously improved through Zn and Fe fertilization, and (but to a lesser extent) by breeding.

We found 7, 5, and 4 studies on G×E interactions for grain yield, grain Zn, and grain Fe concentrations, respectively. The review of these studies suggests that the environment and the G×E interactions account for a significant percentage of the variance in grain yield, grain Zn, and grain Fe concentrations than the genotype effect (Table S4a). In almost all cases, the environment and G×E interaction effects on Zn and Fe concentrations were statistically significant (Table S4a). This indicates a strong influence of the environment on the expression of grain Zn and Fe concentrations.

Our review of 20 studies on heritability indicated low to high broad-sense heritability (H^2) for grain yield (median 80%; range: 12–95%), grain Zn concentrations (median 86.5%; range: 7–99.5%), and grain Fe concentrations (median 72.8%; range: 7–99.5%) (Table S4b). Low heritability ($H^2 < 40\%$) was found in less than 20% of the observations (Table S4b). In the majority of cases, heritability appears to be high enough for genetic biofortification of Zn and Fe through conventional breeding techniques.

Variations in grain yield, grain Zn, Fe, and protein concentrations with varietal release year

Grain yields of newly released rice varieties significantly increased between 1960 and 2024 (Figure S2a), while grain Zn concentrations significantly declined over the same period (Figure S2b). Grain Fe concentrations were significantly higher in varieties released after 2000 than earlier years (Figure S2c) but with a very wide confidence interval reflecting greater uncertainty in varieties released during the 2001–2024 period. Grain protein concentrations did not vary significantly with the year of release of varieties (Figure S2d). Fan and colleagues²³ report a similar decline in micronutrient concentrations of Zn, Fe, copper (Cu), and Mg in wheat over the years.

Ecotypic differences

Only one study reported measurements of nutrient concentrations in African rice (*Oryza glaberrima*). Therefore, the rest of this analysis focuses on comparing ecotypes and admixtures of Asian rice (*O. sativa*) in terms of grain yield and nutrient concentrations in brown rice. Data on white rice were excluded because of the small sample size available for the different ecotypes. Violin plots and Kruskal–Wallis test revealed significant differences between sample medians for all variables used in the comparison of rice ecotypes (Table S5). The highest median grain yield was recorded in Japonica cultivars (8.3 t ha⁻¹) followed by admixtures (6.1 t ha⁻¹), which were significantly higher than yield recorded in AUS (3.2 t ha⁻¹)

¹) and Indica cultivars (3.3 t ha⁻¹) (Table S5). This may be attributed to the fact that significant increases in harvest index (HI) have been achieved in Japonica cultivars (Table S5) contributing to the improvements in grain yield²⁴. Grain Zn concentrations were significantly higher in AUS (27.7 mg kg⁻¹) and Japonica cultivars (27.5 mg kg⁻¹) than in Indica cultivars (23.9 mg kg⁻¹) (Table S5) across all treatments. Indica cultivars had significantly lower Fe concentrations than the other ecotypes (Table S5). Grain protein concentrations did not differ significantly between the different ecotypes of rice (Table S5).

Differences between genetically biofortified and regular cultivars

A total of 43 cultivars have been reported to be genetically biofortified with Zn and one cultivar (NSIC Rc172 [MS 13]) biofortified with Fe. Data on Zn and Fe concentrations in brown rice were available for only 11 cultivars from 13 field studies comparing biofortified with regular cultivars side by side. Comparisons of grain yields were reported in 7 studies, grain Zn concentrations in 13 studies, and grain Fe concentrations in 4 studies that trialed biofortified and regular cultivars side by side. All of the studies report grain yields and grain Zn and Fe concentrations in brown rice but not in white rice. Our analysis did not reveal significant differences in grain yield (Table 3; Figure S3g), and a marginal difference in Fe concentrations between the genetically biofortified and regular cultivars (Table 3). On the other hand, the median grain Zn concentration of genetically biofortified cultivars (24.8 mg kg⁻¹; CI: 24.3–25.7 mg kg⁻¹) was significantly higher than that in regular cultivars (22.6 mg kg⁻¹; CI: 22.2–23.0 mg kg⁻¹), but the difference was only 9.7 (Table 3; Figure S3h).

Table 3

Differences between genetically biofortified and regular cultivars in median grain yield, grain Zn and Fe concentrations of brown rice for studies that tested biofortified and regular cultivars side by side in the same trial.

Variable	Biofortified cultivars (95% CI) [†]	Regular cultivars (95% CI) [†]	% change	<i>P</i> value (M-W test) [‡]	<i>P</i> value (K-S test) ‡
Grain yield (t ha ⁻¹)	3.5 (3.2; 3.7)	3.7 (3.4; 4.1)	-5.4	0.071	0.016
Grain Zn (mg kg ⁻¹)	24.8 (24.3; 25.7)	22.6 (22.2; 23.0)	9.7	< 0.001	< 0.001
Grain Fe (mg kg ⁻¹)	9.4 (7.8; 11.6)	11.3 (11.1; 11.6)	-16.8	0.011	0.007
[†] Figures in parentheses represent the 95% confidence intervals (CI) of median values estimated using bias-corrected and accelerated boot strapping. Two or more medians are deemed significantly different if their 95% CIs do not overlap and the Mann-Whitney test and the Kolmogorov-Smirnov tests produce <i>P</i> < 0.05.					
[‡] The <i>P</i> values are for the Mann-Whitney (M-W) test of equality medians and the Kolmogorov-Smirnov (K-S) test of equality of distributions					

Loss of nutrients with grain processing

Using two independent estimation methods, we found that milling and polishing can reduce grain Zn concentrations by 21.2–30.9%, Fe concentrations by 69.3–70.3%, protein concentrations by 6.5–7.1%, and phytate concentrations by 47.4–59.3% in rice grain (Table 4).

Table 4

Percentage loss of grain concentrations of zinc, iron, phosphorus, protein, and phytate with processing of brown rice to white rice estimated using two different methods.

Nutrient [n, s] [†]	Method 1*	Method 2*
	% loss (95% CI) [‡]	% loss
Zinc [25; 1366]	-21.2 (-21.9; -20.4)	-30.9
Iron [9; 640]	-70.3 (-71.2; -68.3)	-69.3
Phosphorus [1; 6]	-52.3 (-54.0; -49.0)	-63.5
Protein [3; 36]	-6.5 (-8.1; -4.2)	7.1
Phytate [8; 146]	-47.4 (-51.0; -46.0)	-59.3
* Method 1 is based on percentage changes between mean values from brown and white rice reported in the same study, while Method 2 is based on the percentage change between the median values in brown rice and white rice from all studies.		
[†] Figures in brackets represent the total number of studies (n) and the total sample size (s) available for the analysis in Method 1. See the sample size for Method 2 in Supplementary Table S2.		
[‡] Figures in parentheses represent the 95% confidence intervals (CI) of median values estimated using bias-corrected and accelerated boot strapping.		

Some studies show that limiting the degree of milling to 5% can reduce losses of Zn and Fe substantially. For example, in an analysis of 30 landraces from Manipur in India, Longvah and co-workers²⁵ found 12.5% reduction in Zn concentrations with 5% milling but 19.8% reduction with 10% milling. In the case of Fe concentrations, they found 41.8% reduction with 5% milling but 61.3% reduction with 10% milling²⁵. There is growing interest in promoting the consumption of whole-grain brown rice^{26,27}. However, brown rice is often considered as “peasant food,” and only consumed by the elderly among Asians²⁸. Meanwhile, parboiled rice is known to have higher nutrient and vitamin B6 concentrations but lower phytic acid concentrations than non-parboiled rice²⁹. Parboiling involves soaking of brown rice in hot water, followed by steaming and drying to a moisture content of 12–14%. During the parboiling process, vitamins and micronutrients are infused into the starch endosperm, thereby reducing nutrient losses during milling and making parboiled rice nutritionally superior to non-parboiled white rice³⁰.

Bioavailability of Zn and Fe

The bioavailability of Fe and Zn in rice grain is estimated at 10–25%¹¹, which is largely determined by the concentration of chelating molecules such as phytate (phytic acid) in the grain. The phytate-to-Zn and phytate-to-Fe molar ratio is considered the first proxy for bioavailability of Zn, Fe, and P to humans¹⁶.

Phytate-to-Zn molar ratios greater than 15 are associated with low bioavailability, while ratios of 5–15 and below 5 are associated with moderate and high Zn bioavailability³¹. Zn fertilization reduced the phytate-to-Zn molar ratio by 29.4% and phytate-to-Fe molar ratio by 55% in rice grain relative to treatments without Zn fertilization (Table 5). This is because Zn fertilization significantly increases grain Zn uptake without significantly affecting grain phytate concentrations (Fig. 3a). The negative correlation between grain Zn uptake and phytate concentrations (Table 2) is also supportive of this finding. Similarly, Fe fertilization reduced the phytate-to-Zn and phytate-to-Fe molar ratios by 60 and 51%, respectively (Table 5). The results in Table 5 further indicate significant improvements in the bioavailability of Zn and Fe with Fe fertilization.

Table 5

Comparison of phytate-to-zinc and phytate-to-iron molar ratios in rice grain without Zn fertilization (Without Zn) and with Zn fertilization (With Zn) or without Fe fertilization (Without Fe) and with Fe fertilization (With Fe).†

Molar ratio	Treatments [n; s]‡	Median (95% CI)	Equality of medians§	Equality of distributions§
Phytate : Zn	Without Zn [25; 427]	31.3 (28.6; 33.3)	< 0.001	< 0.001
	With Zn [13; 322]	22.1 (21.0; 23.8)		
	Without Fe [25; 717]	27.1 (26.2; 28.5)	< 0.001	< 0.001
	With Fe [5; 32]	10.8 (9.7; 17.4)		
Phytate : Fe	Without Zn [18; 321]	36.2 (31.7; 42.1)	< 0.001	< 0.001
	With Zn [6; 103]	12.2 (11.6; 14.8)		
	Without Fe [18; 380]	34.7 (30.5; 39.8)	< 0.001	< 0.001
	With Fe [6; 44]	16.9 (14.9; 19.3)		
† The median value of 31.3 indicates that for each mole of Zn there were 31.3 mols of phytate. The Mann-Whitney test of equality of medians and Kolmogorov–Smirnov test of equality of distributions are shown on the right.				
‡ Figures in brackets represent the number of studies (n) and the total sample size (s) available for analysis.				
§ P values estimated with 999 Monte Carlo permutations.				

Variations in response ratios

Variation with nutrient inputs

Among the various nutrient inputs tested (Table S6), significantly greater increments in grain yield were achieved with Zn seed treatment (response ratio [RR] = 1.29), followed by a combination of soil and foliar application of Zn (RR = 1.25), and soil application of Fe (RR = 1.24); these gave 29, 25, and 24% increase in grain yield over the recommended NPK fertilizer (Fig. 2a). The highest increment in grain Zn concentrations (RR = 1.88) was achieved with the combination of soil and foliar application of Zn (Fig. 2b). This was followed by foliar application of Zn (RR = 1.36), seed treatment with Zn (RR = 1.34), and soil application of Zn (RR = 1.23). The highest increment in grain Fe concentrations (RR = 1.40) was achieved with growth-promoting rhizobacteria/zinc solubilizing bacteria (GPR + ZSB), followed by soil application of Fe (RR = 1.27), and foliar application of Fe (RR = 1.24) (Fig. 2c). Greater increments in grain protein concentrations were achieved with soil application of Fe (RR = 1.17), foliar application of Zn (RR = 1.13), and foliar application of Fe (RR = 1.12) than the other treatments (Fig. 2d). Treatments involving other inputs ("Other") did not significantly increase grain yields over the recommended fertilizer, but slightly increased grain Zn, Fe, and protein concentrations. Significant reduction in grain yield and grain Zn, Fe, and protein concentrations was noted in the absence of external inputs ("Noinput") (Fig. 2). These findings highlight the value of applying Zn, Fe, and other micronutrients in addition to the recommended NPK fertilizer.

Application of Zn fertilizer ("WithZn") together with the recommended NPK fertilizer achieved 17% increment in grain yields (RR = 1.17) relative to the recommended NPK fertilizer alone. But the 2% yield increment (RR = 1.02) achieved with all other treatments without Zn ("WithoutZn") relative to the recommended NPK fertilizer was negligible (Fig. 3a). Zn fertilization also increased grain Zn concentrations by 44%, Fe concentrations by 5%, and protein concentrations by 7%, and reduced phytate concentrations by 8% compared with the recommended fertilizer (Fig. 3a). Between-study heterogeneity was high for all effect sizes with both with and without Zn (Figure S6). The fail-safe numbers from the random effects models indicated that the effect size estimates are all robust (Figure S6). It is likely that Zn fertilization increases grain yields and grain protein concentrations via increased grain Zn uptake, N uptake (see positive correlations in Table 2), and better agronomic use efficiency of the applied N fertilizers. For example, grain Zn uptake increased by 68% and N uptake by 29% with Zn fertilization (Fig. 3a). Zn fertilization also increased the agronomic efficiency of N fertilizers by 4.8 kg per kg of applied N fertilizer (CI: 4.4–5.2) relative to the recommended fertilizer, while treatments without Zn inputs did not significantly increase the agronomic efficiency of N use (median: 1.4; CI: 1–2 kg per kg). In addition, Zn fertilization significantly increased the harvest index, which is a predictor of water productivity and nutrient use efficiency in crops²⁴.

Like Zn fertilization, Fe fertilization achieved significant increments in almost all variables except grain phytate concentrations relative to the recommended NPK fertilizer (Fig. 3b). For example, Fe fertilization increased grain yields by 20%, grain Zn by 22%, grain Fe by 25%, Zn uptake by 39%, and N uptake by 29% (Fig. 3b). It also increased protein concentrations by 31% and reduced phytate concentrations by 10% over the recommended fertilizer (Fig. 3b). Fe fertilization also significantly increased the agronomic

efficiency of N by 3.7 kg per kg of applied N (95% CI: 3.3–4.9) relative to the recommended fertilizer. The agronomic efficiency of soil-applied Zn fertilizer also increased by 50 kg per kg of applied Zn.

The results above confirm our hypothesis that grain Zn and Fe concentrations can be increased with Zn and Fe fertilization.

Variation with Zn, N, and P fertilizer rates

The trends in response ratios indicated significant improvements in grain yield, grain Zn concentrations, Zn uptake, and N uptake with increasing Zn application to soil at rates up to 30 kg ha⁻¹. Further increases in Zn application rates above 30 kg/ha appeared to reduce grain Zn uptake and N uptake (Figure S7). Since Zn is not easily available to smallholder farmers and is also costly, soil application of modest amounts (e.g. 5–10 kg ha⁻¹ Zn) may be recommended, as can be inferred from Figure S7. Zn oxide nanoparticles (ZnO NPs), a new class of Zn fertilizers, have been shown to be more effective than common Zn fertilizers due to their high specific surface area, and are easily absorbed and utilized by rice roots³². ZnO NPs have also been shown to increase rice yields, grain Zn content, and many quality traits compared to ionic Zn at the same application rate^{32,33}.

The LOESS (locally estimated scatterplot smoothing) regression also indicated that N application rates up to 150 kg ha⁻¹ can significantly improve grain yields, grain Zn and Fe concentrations, Zn, Fe, and N uptake beyond which response was flat (Figure S8a–f). On the other hand, grain protein concentrations did not significantly improve with increasing N application rates (Figure S8h). The response ratios of grain yield, and Zn uptake appear to diminish with increasing P application rates higher than 80 kg P ha⁻¹ (Figure S9a,c). Although P fertilization increases yield, it also reduces the bioavailability of Zn, Fe, and other nutrients to humans due to increased phytate content (it also has negative environmental impacts)¹⁶. This highlights the need for reconsideration of P fertilizer strategies. The trends in response ratios of Fe concentrations and Fe uptake with P application rates were somewhat idiosyncratic due to the small sample sizes (Figure S9d,e).

Variation with production systems

Global syntheses of the effects of rainfed and irrigated rice, which are two contrasting production environments, on grain nutrient concentrations are lacking. The magnitude of Zn and Fe fertilization on grain Zn and Fe concentrations in these systems are also not fully understood. In this meta-analysis, soil application of Zn significantly increased grain yields, grain Zn, and protein concentrations in both production systems (Fig. 4a,b,d). Grain yield and grain protein concentrations did not significantly differ between rainfed and irrigated rice with soil-application of Zn (Fig. 4a,d). On the other hand, grain Zn concentrations were significantly higher in irrigated rice (RR = 1.5) than in rainfed rice (RR = 1.36) (Fig. 4b), while the reverse was true for grain Fe concentrations (Fig. 4c). The significant increase in grain Zn concentrations with Zn fertilization in irrigated rice may be attributed to its widespread deficiency and lower bioavailability due to precipitation of Zn in flooded soils³⁴. Thus, Zn fertilization is a critical agronomic practice in irrigated systems. On the other hand, flooding of the soil is known to increase the

availability and total uptake of other nutrients such as N, P, K, S, Fe, and Mn³⁵. The availability of Fe is high in flooded conditions because of the lower redox potential³⁶, while the availability of Fe is limited in rainfed conditions, particularly when soil pH is high³⁷.

Variation with climatic factors

Compared with the recommended NPK fertilizers, soil application of Zn significantly increased grain yield, grain Zn, and protein concentrations across all categories of aridity and climate zones (Fig. 4a,b,d). This means that soil application of Zn can improve yield and grain Zn and protein concentrations over the recommended NPK fertilizer regardless of the climate and aridity zones. Greater increments in grain yields were achieved in subhumid zones and subtropical climates than in other zones (Fig. 4a). On the other hand, greater increments in grain Zn concentrations were achieved in humid zones and tropical climates (Fig. 4b). No clear patterns could be claimed in the case of grain Fe concentrations partly due to the small number of studies available for meta-analysis (Fig. 4c).

Variation with soil factors

Soil application of Zn significantly increased grain yield, grain Zn, and protein concentrations across all categories of soil parent material, texture, soil pH, soil organic carbon (SOC), Olsen P, and soil Zn concentrations (Fig. 5a,b,d). This means that soil application of Zn can improve yields and grain Zn and protein concentrations over NPK fertilizer regardless of the soil conditions. The exception was grain Fe concentrations, for which there were too few studies and observations to make firm conclusions. Significantly greater increments in grain yield were achieved on soils that are deficient in Zn than on those considered as having sufficient Zn (Fig. 5a). This is probably because Zn is a yield-limiting soil nutrient whose deficiency is widespread especially in wetland rice^{38,39}. On the other hand, grain Zn and protein concentrations were equally improved on both Zn-deficient and Zn-sufficient soils (Fig. 5b,d). This implies that soil application of Zn can significantly improve grain Zn and protein concentrations regardless of the Zn status of the soil. Taken together, the results above confirm our hypothesis that “Zn and Fe fertilization significantly increases grain Zn, Fe, and protein concentrations over control (NPK fertilizer only) across climates, soils, and rice growing environments.” Greater improvements in grain yield, grain Zn, and protein concentrations, and N and P uptake due to soil application of Zn occurred where soil available Zn concentrations fell below 1.0 mg kg⁻¹ (diethylenetriaminepentaacetic acid [DTPA] method). This is consistent with the argument that the optimum range of soil available Zn for rice is 0.38–0.90 mg kg⁻¹ (DTPA-extractable Zn)⁴⁰.

Challenges faced and limitations of the meta-analysis

The major challenges faced in this analysis were (1) the small number of studies reporting nutrient concentrations in white rice; (2) incomplete description of the study sites; (3) scarcity of information on the rice cultivar used, fertilizer rates, and irrigation regimes; (4) lack of directly measured soil properties and climate variables; and (5) selective reporting of results of statistical analyses. In many publications, main effects were reported and interaction effects were missing. These challenges have limited in-depth

analysis of the association between grain nutrient concentrations and climate and soil variables. These limitations are presented in detail in the Supplementary Methods.

Conclusion

Based on the various analyses, it is concluded that (1) modern rice cultivars have inadequate concentrations of Zn, Fe, and protein for healthy human nutrition; (2) processing of brown rice to white rice significantly reduces Zn, Fe, and protein concentrations; (3) current rates of increments in grain Zn and Fe concentrations with genetic biofortification are not likely to achieve the target Zn and Fe concentrations without complementary use of agronomic interventions; (4) application of Zn and Fe fertilizers can significantly increase the nutritional value of rice grains while also increasing grain yields; and (5) soil application of Zn can improve yields and grain Zn and protein concentrations over the recommended NPK fertilizer regardless of production systems, climate zones, soil conditions, and soil Zn status. We recommend, (1) complementary efforts in genetic and agronomic fortification of rice to increase grain Zn and Fe concentrations; (2) emphasis on consumption of parboiled rice among populations whose staple diet is rice; and (3) reducing milling and polishing of rice grains to reduce loss of micronutrients in rice grain.

Materials and Methods

Scope and context of this review and meta-analysis

The scope of this meta-analysis is global, covering all rice growing regions, cultivated rice species and their crosses, subspecies, and cultivars. Rice was chosen for this analysis because of its strategic importance for food security especially in Asia and Africa^{41,42}. For example, rice ranks first in Asia, and second in Africa in terms of area and production in 2022.⁴³ Historically cultivated rice is diverse, and consists of two species, namely, *Oryza sativa* (Asian rice) and *O. glaberrima* (African rice)⁴⁴ and their crosses. Asian rice is dominant in terms of area under cultivation, whereas African rice remains important in some parts of Africa. The latter is also an essential source of genes for resistance to biotic and abiotic stresses⁴⁴. Asian rice consists of two sub-species, namely *O. sativa indica* (Indica hereafter) and *O. sativa japonica* (Japonica), each with different ecotypes^{45,46}. Indica rice is mainly found in tropical and subtropical rice growing regions, at low latitudes or low altitudes, whereas Japonica rice is mostly cultivated in high-latitude temperate regions^{45,46}.

Literature search, retrieval, and data extraction

We followed the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA-S)⁴⁷ guidelines for literature searches in systematic reviews and meta-analysis. We conducted searches in Google Scholar and Web of Science for peer-reviewed publications. In Google Scholar, we used the keywords: (Rice and mineral or ferti* and grain yield or produc*) and (grain quality or zinc or iron or protein or phosphorous or selenium), retrieving 998 titles. In Web of Science, the search string was: "Rice

or (Oryza) (All Fields) AND Zinc or iron ferti* (All Fields) AND grain yield or grain produce or bio-forti* or bioforti or grain zinc or grain iron or grain phosphorous” resulting in 9,379 titles. After refining by document type (Article) and language (English), 8,998 titles were retained. Initial title screening excluded 8,184 publications (Figure S4). After removal of duplicate studies, 670 publications were subjected to abstract and full-text screening. At this stage, 388 studies were excluded based on the exclusion criteria. A total of 38 studies were found by reading the reference lists of the selected studies. In total, 245 publications were retained for data extraction (Figure S4). A map of the study sites included in this meta-analysis is presented in Figure S5.

Two reviewers screened each record independently. No automation tools were used in the process. All retrieved publications were compiled into an MS Excel spreadsheet with metadata including author, title, journal name, publication year, DOI, geographic coordinates of the site, site name, and country. All relevant data were extracted from the 245 publications and organized in Excel after extracting information from tables directly and digitizing figures using Web PlotDigitizer. Studies reporting grain yield and mineral contents with clearly defined fertilizer management strategies were categorized as “Treatment”; otherwise, they were identified as “Germplasm” studies. In cases where the same data were reported in multiple publications by the same authors, we selected the most comprehensive source for data extraction. Where geographical coordinates of the experimental site were not provided, we used Google Maps (<https://www.google.com/maps/>) to determine the latitude and longitude based on the location of the nearest city or the experimental station where the study was conducted. Where initial soil properties of the test site were not provided, we extracted some of them for the approximate study locations from the ISRIC Soilgrids.

Inclusion and exclusion criteria

The following criteria were used for inclusion of a study in the meta-analysis: (1) the study was published in a peer-reviewed journal; (2) the study compared application of either Zn or Fe alone or in combination with other micronutrients as treatments and/or rice genotypes; (3) the study reported rice grain yield and grain nutrient concentrations on a dry matter basis; (4) all recommended agronomic management practices were performed uniformly; (5) the study was performed in randomized and controlled field trials; (6) the treatments were replicated; (7) the study reported survey results that sampled crops directly from farmers’ fields on a dry mass basis. A study was excluded if it: (1) was conducted under greenhouse conditions, as a pot experiment, or in a hydroponic medium; (2) reported nutrient concentrations of rice grains sampled in the market; (3) reported nutrient concentrations of rice grains sampled after long storage period; (4) did not report treatment means, but reported other measures such as median and the corresponding author was not willing to share raw data; (5) is a review paper; (6) is not published in a peer-reviewed journal; (7) presented results in a manner that treatment means cannot be extracted, for example boxplots without mean values or in 3D graphs or in broken bar charts; (8) is based on genetically modified (transgenic) rice varieties (but genetically biofortified varieties were included); and (9) was retracted by the journal after publication.

Quality control of the data used in this meta-analysis was assured by: (1) including only publications in peer-refereed journals; (2) ensuring that all selected publications are independently reviewed by the co-authors; (3) full article reading of the publications to substantiate each finding; (4) thorough training of the data extraction team; and (5) counterchecking the extracted data by co-authors before pre-processing for statistical analysis.

Choice of response variables and calculation of effect sizes

For meta-analysis, we used the response ratios (RR) of grain yield, grain Zn, Fe, protein, and phytate concentrations as the response variables. Particular attention was given to grain Zn, Fe, and protein concentrations in the meta-analysis because rice is deficient in these nutrients among the staple cereal crops^{8,15}. We also chose harvest index and thousand-grain weight (TGW) as response variables. We chose harvest index specifically because it is considered as an informative indicator of the sink–source balance, and a measure of biological success in partitioning photosynthates to the harvestable product⁴⁸. Over the years, breeding efforts have achieved great increases in yield by improving the harvest index. Therefore, the harvest index is considered as a useful indicator to consider in the breeding of new high-yielding varieties and improving resource use efficiency⁴⁸, and the amount of nutrients recovered in harvested products is also a function of the harvest index.

Data processing

In preparation for statistical analysis, some variables were calculated from the available data and gaps were filled: harvest index, Zn, Fe, and protein concentrations, Zn and Fe bioavailability. Nutrient uptake and agronomic use efficiency of N fertilizer were not reported in some studies. These were calculated from studies that have reported the requisite variables. For example, where the harvest index (HI) was not provided, it was calculated from grain yield, straw yield, or total aboveground biomass as follows: $HI = 100 * (\text{grain yield} / \text{total aboveground biomass})$ or $HI = 100 * (\text{grain yield} / (\text{grain yield} + \text{straw yield}))$. In studies where only grain nutrient uptake was reported, grain N, Zn, and Fe concentrations were calculated from uptake as follows:

$N \text{ concentration (in \% or g kg}^{-1}\text{)} = (\text{Uptake} * 100) / \text{grain yield (in kg ha}^{-1}\text{)}$

$Zn \text{ or Fe concentration (in mg kg}^{-1}\text{)} = (\text{Uptake} * 1000) / \text{yield (in kg ha}^{-1}\text{)}$

In studies where protein concentration was not reported but grain N concentration was reported, grain protein concentration was calculated by multiplying N concentration (in %) by 5.95 as per Food and Agriculture Organization of the United Nations (FAO) guidelines⁴⁹. Conversely, N concentration was calculated by dividing the protein concentration (%) by 5.9.

In studies where nutrient yield alone was reported, grain Zn and Fe concentrations were calculated from nutrient yield as follows:

$\text{Zn or Fe concentration (in mg kg}^{-1}\text{)} = ((\text{Nutrient yield (kg ha}^{-1}\text{)}/\text{grain yield (in kg ha}^{-1}\text{))}/10.$

Grain N, Zn, Fe, P, and S uptake were calculated from their respective concentrations as follows:

$\text{N, P, or S uptake (in kg ha}^{-1}\text{)} = \text{N, P, or S concentration (in \%)} * \text{grain yield (in kg ha}^{-1}\text{)}/100$

$\text{Zn or Fe uptake (in g ha}^{-1}\text{)} = \text{concentration (in mg kg}^{-1}\text{)} * \text{grain yield (in kg ha}^{-1}\text{)}/1000.$

The agronomic efficiency of N fertilizer was calculated as follows:

$(\text{treatment yield} - \text{control yield})/\text{N rate},$

where the control yield is the yield recorded in the recommended NPK treatment.

To facilitate the meta-analyses, continuous explanatory variables were also converted into categories and dummy variables were created. Using the soil texture provided and/or the soil clay and silt concentrations reported, three soil texture classes (sandy, loam, and clayey) were created following Chivenge and co-workers⁵⁰. Soils were classified as calcareous, silicic, or mafic based on their parent materials⁵¹. Calcareous parent materials include limestone, dolomite, calcareous shale, and sands with > 50% CaCO_3 or MgCO_3 , while silicic (also called acidic) refers to rocks that contain significant amounts of silica (> 68% Si), and intermediate parent materials contain 52–68% Si⁵¹. The term mafic (basic) is applied to parent material with relatively low amounts of silica (45–52% Si)⁵¹.

Soil organic matter (SOM) data were converted to SOC by dividing SOM by 2 following Pribyl⁵². Then, the SOC values were grouped into two categories: low ($\text{SOC} \leq 1\%$) and medium to high ($\text{SOC} > 1\%$).

Wherever primary studies used different soil extraction methods of soil pH, available P, and cations, and reported in different units, these were converted into a single unit before combining the data for meta-analysis. All soil pH reported in different extraction methods were converted into pH in H_2O . To facilitate meta-analysis, soil pH data were grouped into three categories as acidic ($\text{pH} < 6.5$), neutral ($\text{pH} 6.5\text{--}7.5$), and alkaline ($\text{pH} > 7.5$) in H_2O following Xu and co-workers⁵³. Similarly, P extracted using different methods were converted into Olsen P equivalents using regression equations. The Olsen P (bicarbonate extraction) was used in preference to the other methods as it is the most used indicator for soil P status⁵⁴. Olsen P values were grouped into three categories: low ($< 15 \text{ mg kg}^{-1}$), medium ($15\text{--}22 \text{ mg kg}^{-1}$), and high ($> 22 \text{ mg kg}^{-1}$) based on application in earlier studies. Following Wissuwa and co-workers⁵⁵, we classified study sites as Zn-deficient ($\leq 0.8 \text{ mg kg}^{-1} \text{ Zn}$) and Zn-sufficient ($> 0.8 \text{ mg kg}^{-1} \text{ Zn}$) using the DTPA soil available Zn reported.

Based on existing convention, climate zones were classified into tropical (between latitudes 23.5° north and 23.5° south), sub-tropical ($23.5\text{--}35^\circ$ north and south), temperate ($35\text{--}50^\circ$ north and south), and boreal ($> 50^\circ$ north) using the global positioning system (GPS) coordinates of the study sites. Study sites

were also classified based on the aridity index (AI) as arid ($AI < 0.2$), semiarid ($AI = 0.2-0.5$), subhumid ($AI = 0.51-0.65$), and humid ($AI > 0.65$)⁵⁶.

Rice is produced in two contrasting production systems: rainfed (aerobic conditions) and irrigated (mostly anaerobic). Rainfed rice is grown in unsaturated soil under rainfed conditions but sometimes with supplemental irrigation, while irrigated rice is conventionally produced in flooded and puddled soil, and seedlings are normally transplanted^{2,41}. Accordingly, the rice production systems were grouped into rainfed and irrigated to facilitate the meta-analysis.

Statistical analysis

Quantifying baseline grain nutrient concentrations and antinutritional factors

To establish the baseline grain Zn, Fe, and protein concentrations, the median values, their 95% confidence intervals (CIs), and the lower and upper quantiles (25th and 75th percentiles; Q1 and Q3) were estimated. The uncertainty around median values was represented by 95% CIs estimated using bias-corrected and accelerated (BCa) bootstrapping with 9,999 replicates. BCa is a non-parametric method that accounts for skewness and allows one to set CIs through resampling with replacement⁵⁷. Violin and box plots were also generated to aid visualization and comparison of brown rice with white rice in terms of distributions. To ensure rigorous statistical inferences, tests of equality were performed for both medians and the data distributions. Accordingly, two or more medians or distributions were deemed significantly different if the 95% confidence intervals of medians did and/or overlap and the tests of equality of medians and distributions yielded $P < 0.05$. For the equality of medians, the Mann-Whitney test was used for two samples and the Kruskal–Wallis test for several samples. The Kolmogorov–Smirnov test was used for equality of distributions. In all cases, the P values were generated via Monte Carlo permutation.

The overall distributions of grain Zn, Fe, and protein concentrations were derived using all datasets covering field experiments and farm surveys. Farm survey data were not treated differently from experimental data since means of a given number of samples were reported on a dry mass basis. Before formal analysis of the distributions, outliers were identified using the Extreme Studentized Deviate test. Then, histograms of rice grain Zn, Fe, and protein concentrations were generated for brown rice and white rice separately. Histograms were generated by setting the number of bins to optimal following the zero-stage rule. The Gaussian kernel density and the normal distribution curves were added on the histograms. To test the first hypothesis that “grain Zn and Fe concentrations in rice cultivars are below the target concentrations for human nutrition,” the probability (ϕ) of exceeding the target values of Zn (28 mg kg^{-1}) and Fe (15 mg kg^{-1}) in brown and white rice were estimated using their cumulative frequency distributions.

Correlations, G×E interactions, and heritability traits

Although the effects of genotype-by-environment (G×E) interactions on grain yield, grain Zn, Fe, and protein concentrations and the heritability of these traits in rice have been widely studied, syntheses were lacking. To gain insights into the genetic and environmental control of grain yield, grain Zn, Fe, and protein concentrations, we reviewed studies that have quantified G×E interactions and heritability, and we compiled the variance explained by the genotype, environment, and the G×E interactions and the broad-sense (H^2) and narrow-sense (h^2) heritability estimates. Details of the steps taken are described in detail in the Supplementary Methods. In addition, we performed correlation analyses between grain yield, grain Zn, Fe, and protein concentrations, and grain N uptake, Zn uptake, Fe uptake, P uptake, and S uptake using the data compiled for the meta-analysis. We limited the correlation analysis to studies that have reported two target variables measured concurrently and where the number of means was more than 10 to correctly estimate the Pearson correlation coefficient. We then performed a random effects meta-analysis of the correlation coefficients between grain yield, and concentrations of Zn, Fe, protein, and phytate using the Fisher r-to-z transformed values as the outcome measure. We performed the random effects modeling using restricted maximum likelihood method (REML) implemented in the MAJOR module of the JAMOVI software. This is an open-source software that performs random effects meta-analysis using an extension of the *metafor* package of R⁵⁸. The random effects meta-analysis provides an estimate of the pooled correlation coefficient, the amount of heterogeneity (i.e. T^2 , I^2 , H^2 , and Cochran's Q statistics), and tests of publication bias (Rosenthal's fail-safe N, Begg and Mazumdar Rank Correlation, Egger's Regression and Trim and Fill Number of Studies). The procedure uses Studentized residuals and Cook's distances to identify outliers and/or influential. To check for funnel plot asymmetry, the procedure uses rank correlation test and regression test using the standard error of the observed outcomes as the predictor.

Variations in grain yield, grain Zn, Fe, and protein concentrations with variety age

For this analysis, released rice varieties were classified by the year of release, and the median concentrations and their 95% CIs were determined. Due to uncertainty about some exact dates, release years were grouped into four 20-year periods: pre-1960, 1960–1980, 1981–2000, and 2001–2022 for varieties for which the years of release could be verified. Statistical inferences were based on medians and their 95% CIs, estimated using BCa bootstrapping with 9,999 replicates.

Analysis of genotypic differences in grain Zn, Fe, and protein concentrations

Of the 245 studies, 20 (8%) did not report the cultivar used in the study. In the remaining 225 studies, over 3,600 cultivars (including landraces, advanced lines, recombinant inbred lines, and high-yielding improved varieties) were used. It was not possible to make valid statistical comparisons between individual cultivars due to the large number of cultivars with small sample sizes. Therefore, cultivars were grouped into smaller and manageable categories to facilitate the comparison and establish genotypic differences in terms of grain Zn, Fe, and protein concentrations. Accordingly, rice cultivars

were grouped based on known ecotypes of rice (i.e. aromatic, AUS, indica, japonica, admixtures vs. unknown), improvement status (landraces vs. improved cultivars), and biofortification status of improved cultivars (regular vs. genetically biofortified). Admixtures comprised crosses between Asian and African rice species (e.g. New Rice for Africa [NERICA]) and crosses between indica and japonica, indica and AUS, etc. Cultivars that could not be reliably placed in any one of these categories were categorized as “unknown.” To facilitate statistical comparisons, the median values, their 95% CIs, and the lower and upper quantiles (Q1 and Q3) were presented. Violin plots were also generated to aid visualization of the distributions.

Estimating loss of nutrients through milling and polishing

The magnitude of loss in grain nutrients was estimated using two methods. Method 1 involved estimations using data from 26 studies that have reported nutrient concentrations of brown rice and the corresponding values for white rice from the same sample. Method 2 involved calculations of the loss from the median concentration in brown rice and white rice. In both cases, losses were estimated as:

$$100 * (\text{concentration in white rice} - \text{concentration in brown rice}) / (\text{concentration in brown rice}).$$

Although Method 1 is expected to be more reliable than Method 2, estimates from Method 2 are expected to give an approximate idea of the potential loss in the absence of directly measured data.

Estimating bioavailability of Zn and Fe

The bioavailability of Zn and Fe in grains was estimated as the phytate (PA)-to-Zn (PA: Zn) and phytate-to-Fe (PA: Fe) molar ratios calculated as follows:

$$\text{PA: Zn} = ((\text{phytic acid content in mg kg}^{-1}) / 660) / ((\text{Zn content in mg kg}^{-1}) / 65.38)$$

$$\text{PA: Fe} = ((\text{Phytic acid content in mg kg}^{-1}) / 660) / ((\text{Fe content in mg kg}^{-1}) / 55.845)$$

Meta-analysis of response to fertilization

The meta-analysis focused on quantifying the magnitude of effect on the target variables following a given treatment. The bulk of the meta-analysis focused on grain yield, grain Zn, Fe, and protein concentrations because the majority of the studies involved treatments with either Zn and Fe fertilizers or their combination. Additional meta-analyses were performed on response ratios of grain Zn uptake, Fe uptake, and N uptake. The number of studies and total number of observations used in the meta-analysis for each treatment are summarized in Table S6. Meta-analyses were performed on a subset of the data where the desired “control” defined as the treatment receiving the recommended inorganic nitrogen, phosphorus, and potassium (NPK) fertilizer could be reliably identified. The recommended NPK fertilizer was specifically chosen as the control because Zn, Fe, and other micronutrients are almost always combined with NPK. As such, the recommended NPK fertilizer is expected to provide a reasonable

baseline against which gains in crop yields and nutrient concentrations in response to the micronutrient inputs can be compared.

Grain yield, grain Zn, Fe, and protein concentrations were chosen as the target variables for most of the meta-analysis because sufficient data were available. Other variables such as the harvest index, thousand-grain weight, and the phytate and amylase concentrations were used in the meta-analysis only where sufficient data existed. Meta-analysis of phytate concentrations was not performed due to lack of sufficient data.

All meta-analyses were performed using the response ratio (RR) as the effect size metric. The RR was calculated as the ratio of the value of the target variable in the treatment and the corresponding control. To normalize the RR, its natural logarithm (lnRR) was calculated as follows:

$$\ln RR = \ln \left(\frac{T}{C} \right)$$

where T and C are the values of the target variable from the treatment and control, respectively. Once analyses were completed, lnRR and its 95% CIs were back-transformed into the arithmetic domain. The effect sizes estimated in percentage terms were used for inference because percentage change can be readily understood by non-technical readers. For this purpose, lnRR values were re-expressed in percentage change in the target variables as follows:

$$\% \text{ change} = 100 \times (e^{\ln RR} - 1) \text{ or } 100 \times (RR - 1)$$

Accordingly, an RR value of 1.0 represents no change, while 1.1 represents a 10% increment over the recommended NPK fertilizer.

Prior to meta-analysis, we evaluated publication bias using funnel plots of the response ratios (Figure S6) generated using the random effects models implemented in the MAJOR module of the JAMOVI software. Figure S6 also presents tests of heterogeneity (i.e. T^2 , I^2 , H^2 , and Cochran's Q statistics) and publication bias (Rosenthal's fail-safe N, Kendal's tau, and Egger's Regression) for the variables presented in the funnel plots. P values of these tests greater than 0.05 indicate no publication bias in the effect sizes, and these are indicated by "ns" (Figure S6).

We also performed sub-group analyses within a linear mixed-effects modeling framework to estimate the variation in effect sizes with genotypic, agronomic practice, soil, and climate variables. The genotypic variables were subspecies (e.g. AUS, indica, japonica) and genetic biofortification status (biofortified vs. unfortified). Comparison of rice species (Asian vs. African) was not possible because African rice was reported in only one study. To ensure the comparability of results, a subset of studies on regular and genetically fortified cultivars under the same growth conditions under the same agronomic management were analyzed. The agronomic practices included methods of Zn and Fe application (e.g. seed coating, soil application, foliar application, and a combination), application of manures, Zn solubilizing bacteria

(ZnSolB) and/or growth promoting rhizobacteria (GPR), and growth condition (aerobic vs. anaerobic) (Table S6). To test the hypothesis that Zn and Fe fertilization significantly increase grain Zn, Fe, and protein concentrations over the recommended fertilizer, all treatments involving Zn and Fe were grouped into WithZn and WithFe, respectively, and compared with those that did not contain any Zn or Fe input (WithoutZn and WithoutFe). This grouping was also aimed at overcoming statistical artefacts that arise due to small sample sizes when modes of application (foliar, seed, soil, etc.) were analyzed individually. Other treatments for which sample sizes were too small were bulked together as “Other” to reduce data fragmentation and small sample size artefacts.

Meta-analysis of the effect of production-system, climatic, and soil factors was limited to soil application of Zn because there were adequate numbers of studies ($n = 93$) and observations ($S = 1013$). A comparable meta-analysis could not be performed on the effect of the other treatments due to the small number of studies (see Table S6). The focus on soil application of Zn was further motivated by the fact that Zn deficiency is a significant factor limiting grain yield of wetland rice⁵⁹. Zn deficiency has also increased due to the substitution of traditional rice varieties by modern varieties that are less tolerant to Zn deficiency, removal of large amounts of Zn by modern high-yielding cultivars, high phosphorus fertilization, and other factors such as pH⁵⁹. Meta-analysis of effects of climate variables was limited to climate zone and aridity of the study sites (arid, semiarid, subhumid, humid). Meta-analysis of the effects of soil variables was performed primarily on soil texture (sandy, loamy, clay), parent material (calcareous, mafic, intermediate, silicic), soil pH (acidic, alkali, neutral), Olsen P (high, low, medium), and soil organic carbon levels (high, low, medium). The climatic and soil variables mentioned above were entered into the model as fixed effects. The studies (each publication) were entered as the random effect because they represent the clustering structure in the population. In all presentations, the marginal least square means and the uncertainty around means were represented by the 95% CI estimated using linear mixed effects models.

Uptake of soil-applied N, Zn, and Fe by crops and their subsequent translocation to the grain may depend on rate of application and the interactions between N, P, and Zn (synergistic or antagonistic). To reveal the trends in response ratios with Zn, N, and P application rates, we isolated treatments involving soil-applied Zn and we performed locally estimated scatterplot smoothing (LOESS), a non-parametric regression analysis. LOESS regression was performed in preference to linear or non-linear regression because a parametric form of the relationships could not be established in the exploratory analyses.

Declarations

Acknowledgements

This work is part of the CGIAR Research Initiative on Excellence in Agronomy and Sustainable Farming Science Program. We would like to acknowledge all funders who supported this research through their contributions to the CGIAR Trust Fund.

Funding information

This study was funded through the Excellence in Agronomy initiative and the Sustainable Farming Science Program of One CGIAR.

Author contributions

Conceptualization and design: SK, GWS, DM, JK, PSB

Data collection, curation and analysis: DM, GWS, PP, JFD

Technical review and writing: SK, GWS, KS, MD, PP, DM, AI, SAN, PSB, JK

All authors read and approved submission of the manuscript to *npj Sustainable Agriculture*.

Competing interests

The authors declare no competing interests.

Additional information

All additional information has been supplied in the Supplementary Materials

Data availability

The raw data used for this analysis, template used for data collection forms, and any other materials used in the review will be made available upon reasonable request.

References

1. Badoni, S. et al. Multiomics of a rice population identifies genes and genomic regions that bestow low glycemic index and high protein content. *Proc. Nat. Acad. Sci. USA* 121, e2410598121 <https://doi.org/10.1073/pnas.2410598121> (2024).
2. Devkota, K. P. et al. Economic and environmental indicators of sustainable rice cultivation: a comparison across intensive irrigated rice cropping systems in six Asian countries. *Ecol. Indic.* 105, 199–214 <https://doi.org/10.1016/j.ecolind.2019.05.029> (2019).
3. Elert, E. Rice by the numbers: a good grain. *Nature* 514, S50–S51 <https://doi.org/10.1038/514S50a> (2014).
4. Wairich, A., Ricachenevsky, F. K. & Lee, S. A. tale of two metals: biofortification of rice grains with iron and zinc. *Front. Plant Sci.* 13, 944624 <https://doi.org/10.3389/fpls.2022.944624> (2022).
5. Carrijo, D. R., Lundy, M. E. & Linquist, B. A. Rice yields and water use under alternate wetting and drying irrigation: a meta-analysis. *Field Crop. Res.* 203, 173–180 <https://doi.org/10.1016/j.fcr.2016.12.002> (2017).

6. IRRI. The importance of rice: 1. Rice as a global staple food.
http://www.knowledgebank.irri.org/ericeproduction/Importance_of_Rice.htm (2024).
7. Kihara, J. et al. Maize grain zinc and iron concentrations as influenced by agronomic management and biophysical factors: a meta-analysis. *Food Secur.* 16, 1147–1173
<https://doi.org/10.1007/s12571-024-01478-5> (2024).
8. Mahender, A. et al. Rice grain nutritional traits and their enhancement using relevant genes and QTLs through advanced approaches. *Springerplus* 5(1), 2086 <https://doi.org/10.1186/s40064-016-3744-6> (2016).
9. Debnath, S. et al. Historical shifting in grain mineral density of landmark rice and wheat cultivars released over the past 50 years in India. *Sci. Rep.* 13, 21164 <https://doi.org/10.1038/s41598-023-48488-5> (2023).
10. Devkota, M. et al. Grain zinc, iron and protein concentrations of contemporary wheat cultivars fall short of targets for human health: global meta-analysis. *Nat. Food.* 7, 283–295.
<https://doi.org/10.1038/s43016-026-01314-3> (2026).
11. Bouis, H. E. et al. Biofortification: a new tool to reduce micronutrient malnutrition. *Food Nutr. Bull.* 32, S31–S40 <https://doi.org/10.1177/15648265110321S105> (2011).
12. Andersson, M. S. et al. Progress update: crop development of biofortified staple food crops under HarvestPlus. *Afr. J. Food Agric. Nutr. Dev.* 17, 11905–11935
<https://doi.org/10.18697/ajfand.78.HarvestPlus05> (2017).
13. Juliano, B. O. Rice: role in diet. In *Encyclopedia of Food and Health*, Caballero, B., Finglas, P. M. & Toldrá, F. (eds.), 641–645 <https://doi.org/10.1016/B978-0-12-384947-2.00595-X> (Academic Press, 2016).
14. Nakandalage, N. et al. Improving rice zinc biofortification success rates through genetic and crop management approaches in a changing environment. *Front. Plant Sci.* 7, 764
<https://doi.org/10.3389/fpls.2016.00764> (2016).
15. Wairich, A., Ricachenevsky, F. K. & Lee, S. A tale of two metals: biofortification of rice grains with iron and zinc. *Front. Plant Sci.* 13, 944624 <https://doi.org/10.3389/fpls.2022.944624> (2022).
16. Bindraban, P. S., Dimkpa, C. O. & Pandey, R. Exploring phosphorus fertilizers and fertilization strategies for improved human and environmental health. *Biol. Fertil. Soils* 56, 299–317
<https://doi.org/10.1007/s00374-019-01430-2> (2020).
17. Chen, P. et al. The genetic basis of grain protein content in rice by genome-wide association analysis. *Mol. Breed.* 43, 1 <https://doi.org/10.1007/s11032-022-01347-z> (2023).
18. Shi, S. et al. Comprehensive evaluation of 17 qualities of 84 types of rice based on principal component analysis. *Foods* 10, 2883 <https://doi.org/10.3390/foods10112883> (2021).
19. Yang, Y. et al. Natural variation of *OsGluA2* is involved in grain protein content regulation in rice. *Nat. Commun.* 10, 1949 <https://doi.org/10.1038/s41467-019-09919-y> (2019).
20. Rietra, R. P. J. J. et al. Effects of nutrient antagonism and synergism on yield and fertilizer use efficiency. *Commun. Soil Sci. Plant Anal.* 48, 16 <https://doi.org/10.1080/00103624.2017.1407429>

(2017).

21. Senguttuvel, P. et al. Rice biofortification: breeding and genomic approaches for genetic enhancement of grain zinc and iron contents. *Front. Plant Sci.* 14, 1138408 <https://doi.org/10.3389/fpls.2023.1138408> (2023).
22. Kumar, A. et al. Bioavailability of iron and zinc as affected by phytic acid content in rice grain. *J. Food Biochem.* 41, e12413 <https://doi.org/10.1111/jfbc.12413> (2017).
23. Fan, M.-S. et al. Evidence of decreasing mineral density in wheat grain over the last 160 years. *J. Trace Elem. Med. Biol.* 22, 315–324 <https://doi.org/10.1016/j.jtemb.2008.07.002> (2008).
24. Yang, J. & Zhang, J. Simultaneously improving grain yield and water and nutrient use efficiencies by enhancing the harvest index in rice. *Crop Environ.* 2, 157–164 <https://doi.org/10.1016/j.crope.2023.07.001> (2023).
25. Longvah, T. et al. Nutrient variability of rice landraces (*Oryza sativa* L.) from Manipur, northeast India and its nutrients supply potential in rice-based diets. *Nutr. Food Sci.* 52, 1100–1115 <https://doi.org/10.1108/NFS-10-2021-0296> (2021).
26. Carcea, M. Value of wholegrain rice in a healthy human nutrition. *Agriculture* 11, 720 <https://doi.org/10.3390/agriculture11080720> (2021).
27. Xia, Q. et al. Innovative processing techniques for altering the physicochemical properties of wholegrain brown rice (*Oryza sativa* L.) – opportunities for enhancing food quality and health attributes. *Crit. Rev. Food Sci. Nutr.* 59, 3349–3370 <https://doi.org/10.1080/10408398.2018.1491829> (2018).
28. Lee, H. H. et al. Impact of phytic acid on nutrient bioaccessibility and antioxidant properties of dehusked rice. *J. Food Sci. Technol.* 52, 7806–7816 <https://doi.org/10.1007/s13197-015-1918-9> (2015).
29. Bhar, S. et al. A perspective on the benefits of consumption of parboiled rice over brown rice for glycaemic control. *Eur. J. Nutr.* 61, 615–624 <https://doi.org/10.1007/s00394-021-02694-x> (2022).
30. Ndindeng, S. A. et al. Rice milling and parboiling trade-offs for economic and nutritional gains with special attention to sub-Saharan Africa: a comprehensive review. *Appl. Food Res.* 5, 101274 <https://doi.org/10.1016/j.afres.2025.101274> (2025).
31. Castro-Alba, V. et al. Phytate, iron, zinc, and calcium content of common Bolivian foods and their estimated mineral bioavailability. *Food Sci. Nutr.* 7, 2854–2865 <https://doi.org/10.1002/fsn3.1127> (2019).
32. Mi, K. et al. Zinc oxide nanoparticles enhanced rice yield, quality, and zinc content of edible grain fraction synergistically. *Front. Plant Sci.* 14, 1196201 <https://doi.org/10.3389/fpls.2023.1196201> (2023).
33. Yang, G. et al. Effect of ZnO nanoparticles on the productivity, Zn biofortification, and nutritional quality of rice in a life cycle study. *Plant Physiol. Bioch.* 163, 87–94 <https://doi.org/10.1016/j.plaphy.2021.03.053> (2021).

34. Johnson-Beebout, S. E., Lauren, J. G. & Duxbury, J. M. Immobilization of zinc fertilizer in flooded soils monitored by adapted DTPA soil test. *Commun. Soil Sci. Plant Anal.* 40, 1842–1861 <https://doi.org/10.1080/00103620902896738> (2009).
35. Dass, A. et al. Agronomic fortification of rice grains with secondary and micronutrients under differing crop management and soil moisture regimes in the north Indian Plains. *Paddy Water Environ.* 15, 745–760 <https://doi.org/10.1007/s10333-017-0588-9> (2017).
36. Fageria, N. K. et al. Chemistry of lowland rice soils and nutrient availability. *Commun. Soil Sci. Plant Anal.* 42, 1913–1933 <https://doi.org/10.1080/00103624.2011.591467> (2011).
37. Kreye, C. et al. Biotic and abiotic causes of yield failure in tropical aerobic rice. *Field Crops Res.* 112, 97–106 <https://doi.org/10.1016/j.fcr.2009.02.005> (2009).
38. Awio, T. et al. Micronutrients in East African lowlands: are they needed to intensify rice production? *Field Crop. Res.* 270, 108219 <https://doi.org/10.1016/j.fcr.2021.108219> (2021).
39. Senthilkumar, K. et al. Rice yield and economic response to micronutrient application in Tanzania. *Field Crop. Res.* 270, 108201 <https://doi.org/10.1016/j.fcr.2021.108201> (2021).
40. Takkar, P. N., Singh, M. V. & Ganeshmurthy, A. N. A critical review of plant nutrient supply needs, efficiency and policy issues for Indian agriculture for the year 2000: micronutrient and trace elements. In *Plant Nutrient, Supply and Policy Issues: 2000–2025*, Kanwar, J. S. & Katyal, J. C. (eds.), 238–264 (NAAS, 1997).
41. Ibrahim, A. et al. Thirty years of agronomy research and development in irrigated rice-based cropping systems in the West African Sahel: achievements and perspectives. *Field Crop. Res.* 266, 108149 <https://doi.org/10.1016/j.fcr.2021.108149> (2021).
42. Trijatmiko, K. R. et al. Biofortified *indica* rice attains iron and zinc nutrition dietary targets in the field. *Sci. Rep.* 6, 1–13 <https://doi.org/10.1038/srep19792> (2016).
43. FAOSTAT. Crops and livestock products. <https://www.fao.org/faostat/en/#data/QCL> (2024).
44. Manful, J. T. & Graham-Acquaah, S. African rice (*Oryza glaberrima*): a brief history and its growing importance in current rice breeding efforts. In *Encyclopedia of Food Grains*, Second Edition, Wrigley, C. et al. (eds.), 140–146 <https://doi.org/10.1016/B978-0-12-394437-5.00016-4> (Academic Press, 2016).
45. Lu, B. R., Cai, X. & Xin, J. Efficient *indica* and *japonica* rice identification based on the InDel molecular method: its implication in rice breeding and evolutionary research. *Prog. Natur. Sci.* 19, 1241–1252 <https://doi.org/10.1016/j.pnsc.2009.01.011> (2009).
46. Shi, S. et al. Relationship between rice grain protein content and key phenotypes in rice. *Agron. J.* 115, 1257–1264 <https://doi.org/10.1002/agj2.21250> (2023).
47. Rethlefsen, M. L. et al. PRISMA-S: an extension to the PRISMA Statement for Reporting Literature Searches in Systematic Reviews. *Syst. Rev.* 10, 39 <https://doi.org/10.1186/s13643-020-01542-z> (2021).
48. Saito, H. et al. Two novel QTLs for the harvest index that contribute to high-yield production in rice (*Oryza sativa* L.). *Rice* 14, 18 <https://doi.org/10.1186/s12284-021-00456-1> (2021).

49. FAO. *Food Energy – Methods of Analysis and Conversion Factors*. FAO Food and Nutrition Paper 77. <https://www.fao.org/4/y5022e/y5022e03.htm> (Food and Agriculture Organization of the United Nations, 2003).
50. Chivenge, P., Vanlauwe, B. & Six, J. Does the combined application of organic and mineral nutrient sources influence maize productivity? A meta-analysis. *Plant Soil* 342, 1–30 <https://doi.org/10.1007/s11104-010-0626-5> (2011).
51. Gray, J. M., Humphreys, G. S. & Deckers, J. A. Distribution patterns of World Reference Base soil groups relative to soil forming factors. *Geoderma* 160, 373–383 <https://doi.org/10.1016/j.geoderma.2010.10.006> (2011).
52. Pribyl, D. W. A critical review of the conventional SOC to SOM conversion factor. *Geoderma* 156, 75–83 <https://doi.org/10.1016/j.geoderma.2010.02.003> (2010).
53. Xu, H. et al. Effects of biochar application on crop productivity, soil carbon sequestration, and global warming potential controlled by biochar C:N ratio and soil pH: a global meta-analysis. *Soil Tillage Res.* 213, 105125 <https://doi.org/10.1016/j.still.2021.105125> (2021).
54. Chen, X.-P. et al. Harvesting more grain zinc of wheat for human health. *Sci. Rep.* 7, 7016 <https://doi.org/10.1038/s41598-017-07484-2> (2017).
55. Wissuwa, M., Ismail, A. M. & Graham, R. D. Rice grain zinc concentrations as affected by genotype, native soil-zinc availability, and zinc fertilization. *Plant Soil* 306, 37–48 <https://doi.org/10.1007/s11104-007-9368-4> (2008).
56. Zomer, R. J., Xu, J. & Trabucco, A. Version 3 of the Global Aridity Index and Potential Evapotranspiration Database. *Sci. Data* 9, 409 <https://doi.org/10.1038/s41597-022-01493-1> (2022).
57. Efron, B. & Narasimhan, B. The automatic construction of bootstrap confidence intervals. *J. Comput. Graph. Stat.* 29, 608–619 <https://doi.org/10.1080/10618600.2020.1714633> (2020).
58. JAMOVİ. The jamovi project. *jamovi*. (Version 2.6) [computer software]. Retrieved from <https://www.jamovi.org> (2024).
59. Fahad, S. et al. Effects of tire rubber ash and zinc sulfate on crop productivity and cadmium accumulation in five rice cultivars under field conditions. *Environ. Sci. Pollut. Res.* 22, 12424–12434 <https://doi.org/10.1007/s11356-015-4518-3> (2015).

Figures

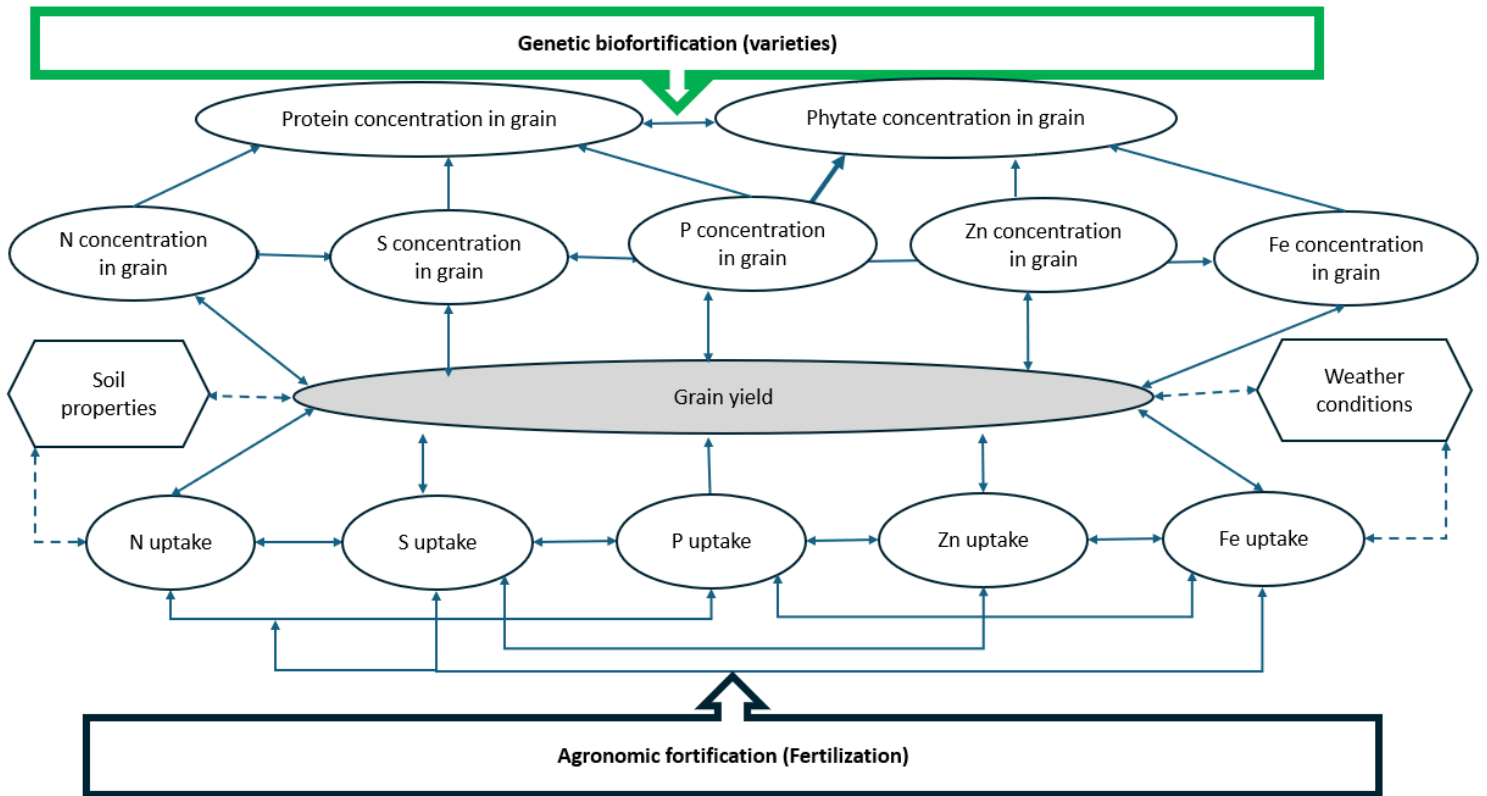


Figure 1

Schematic representation of hypothesized relationships between uptake of various nutrients, grain yield, and concentrations of the nutrients in the grain. Solid and dashed lines indicate direct and indirect relationships.

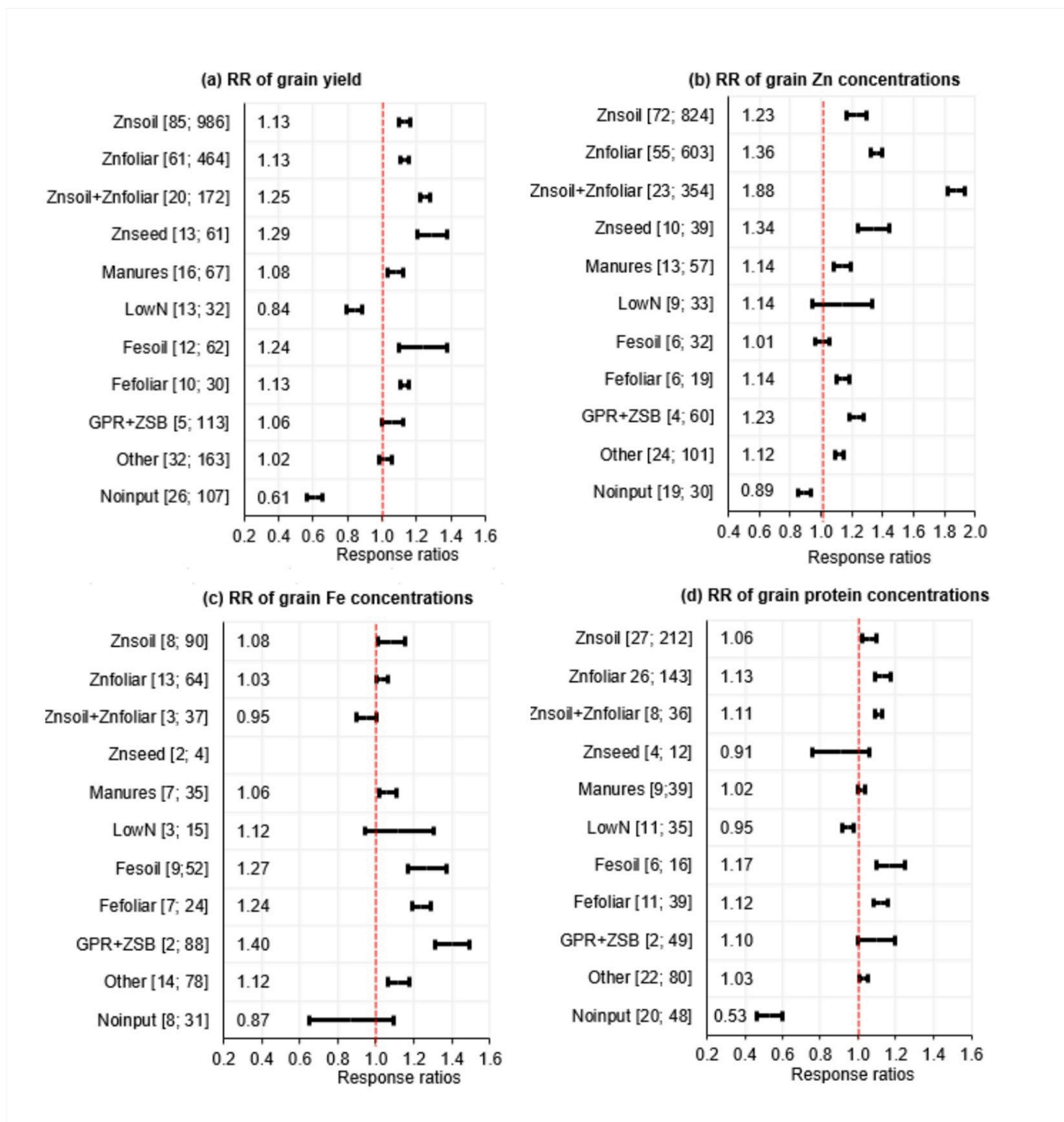


Figure 2

Variations in the response ratios of grain yield, grain Zn, Fe, and protein concentrations with soil treatments. Horizontal bars represent the 95% confidence intervals (CIs). CIs falling above the red line (RR = 1) indicate significant improvement in response to the treatment over the control (e.g. soil application of Zn over the recommended fertilizer). Figures in brackets in front of each category represent the number of studies (n) and total sample size (s) available for the meta-analysis. Figures on

the left sides of the horizontal bars represent the marginal mean RRs. The RR for Znseed is missing in (c) because the number of observations were too few to estimate the CIs. Explanations for abbreviation of treatments are in Table S6.

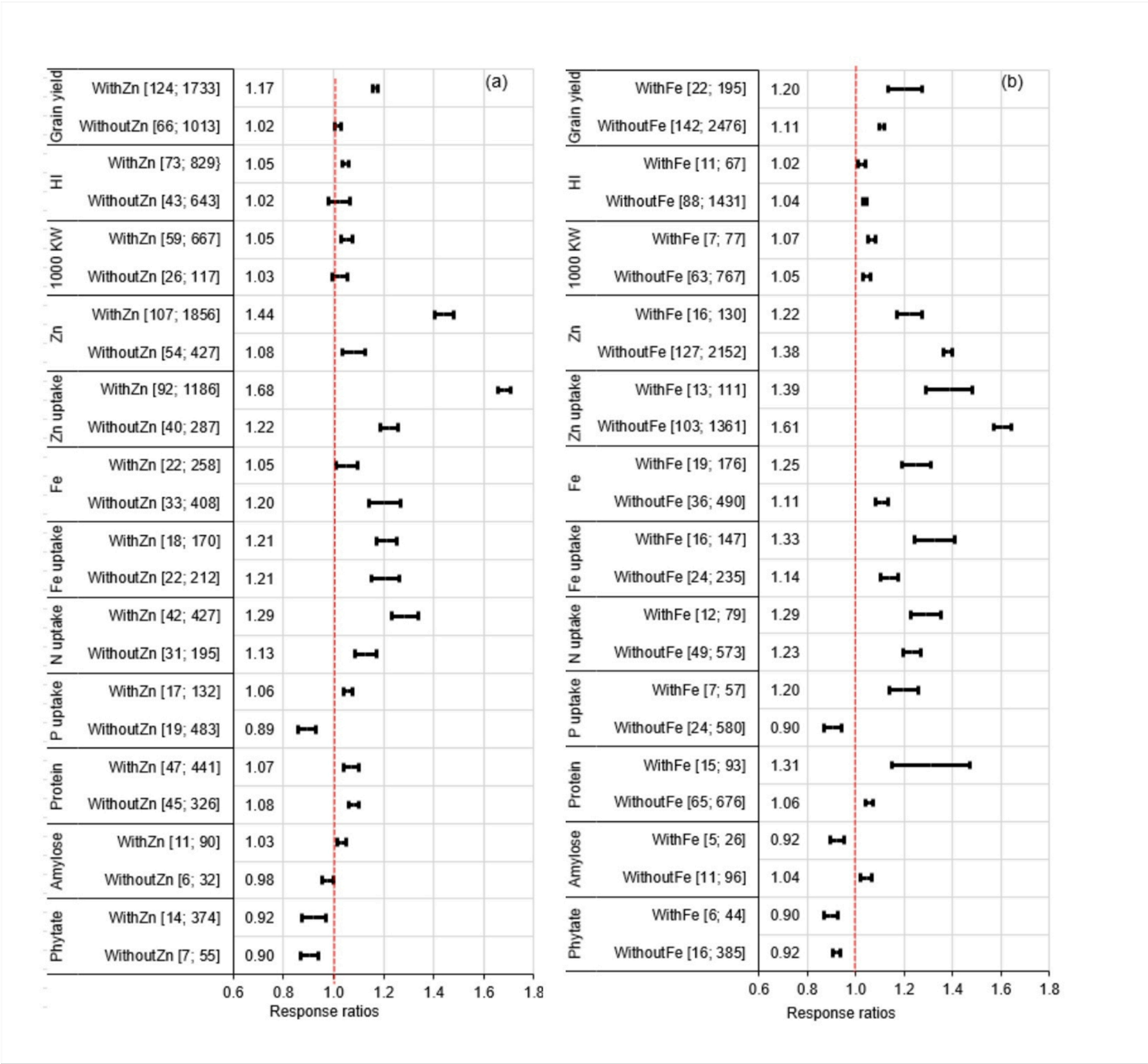


Figure 3

Comparison of Zn fertilization (WithZn) with treatments without Zn (WithoutZn) (a) and with Fe and without Fe (b) in terms of the response ratios of grain yield, harvest index (HI), thousand-grain weight (1000 GW), grain nutrient concentrations and uptake, grain protein, amylose and phytate concentrations, and the benefit-to-cost ratios (BCR). Horizontal bars represent the 95% confidence intervals (CIs) of means. CIs falling above the red line (RR = 1) indicate significant improvement in response to soil application of Zn over the recommended fertilizer. Figures in brackets beside each variable represent the

number of studies (n) and total sample size (s) available for the meta-analysis. Figures on the left side of the horizontal bars represent the marginal means.

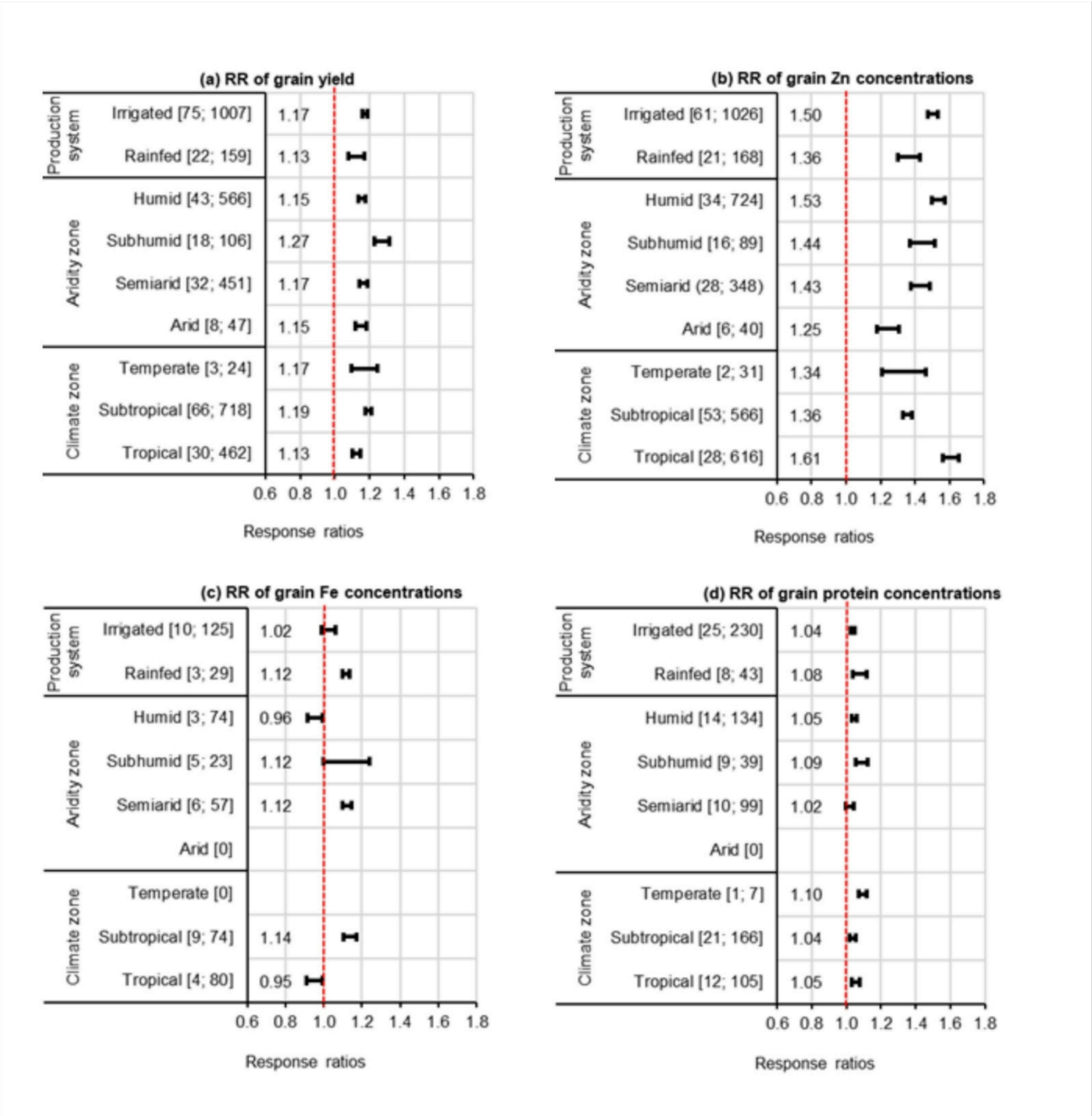


Figure 4

Variations in the response ratios of grain yield, grain Zn, Fe, and protein concentrations with production system (irrigated vs. rainfed), climate zone, and aridity of study site where Zn fertilizer was soil-applied. Horizontal bars represent the 95% confidence intervals (CIs) of means. CIs falling above the red line (RR = 1) indicate significant improvement in response to soil application of Zn over the recommended NPK

fertilizer. Figures in brackets in front of each category represent the number of studies (n) and total sample size (s) available for the meta-analysis. Figures on the left side of the horizontal bars represent the marginal means.

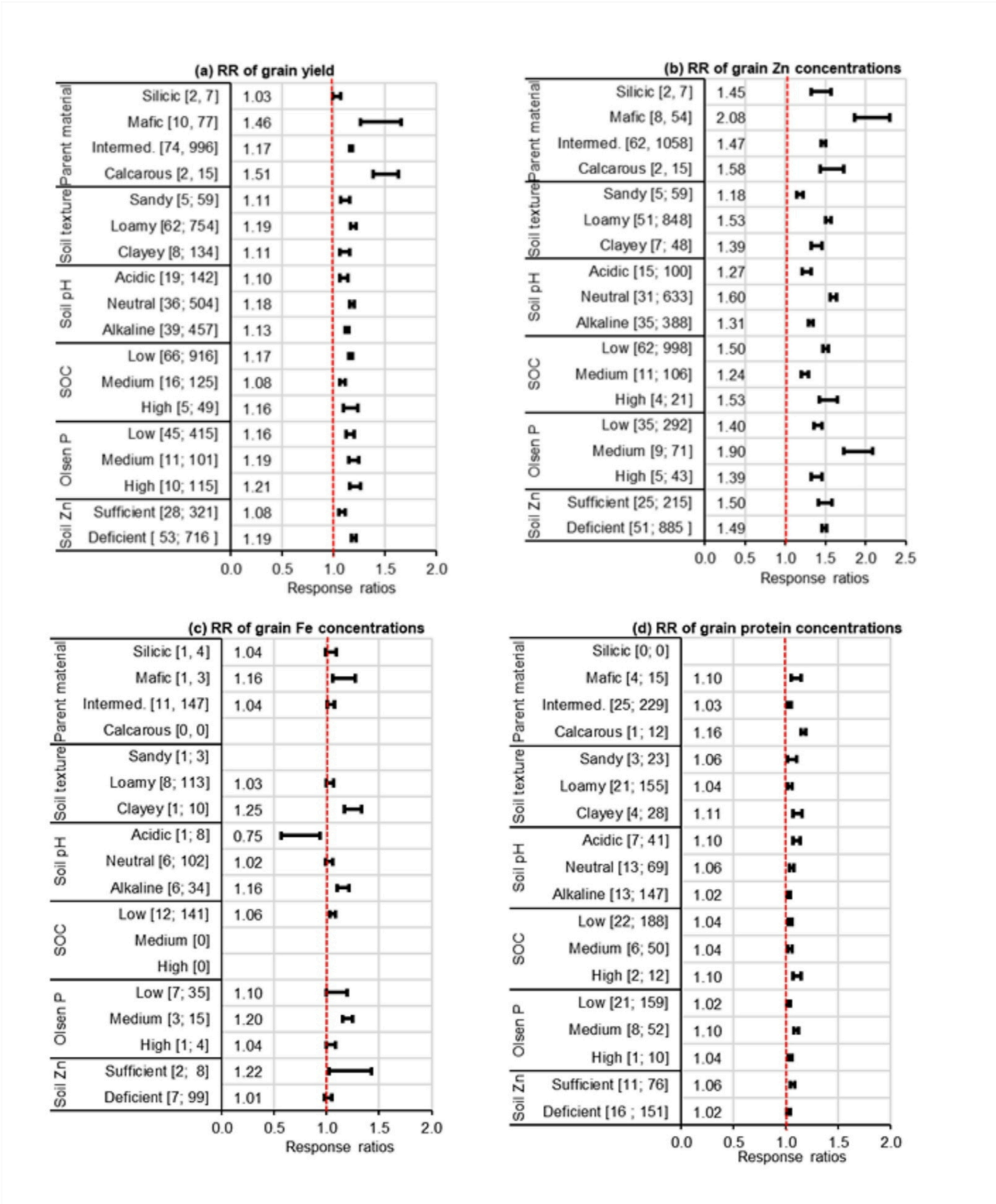


Figure 5

Variations in the response ratios of grain yield, grain Zn, Fe, and protein concentrations with soil parent material, texture, pH, soil organic carbon (SOC), available phosphorus (Olsen P), and soil available Zn

concentrations (DTPA) of the study sites where Zn fertilizer was soil-applied. Horizontal bars represent the 95% confidence intervals (CIs) of means. CIs falling to the right of the red line ($RR = 1$) indicate significant improvement in response to soil application of Zn over the recommended fertilizer. Figures in brackets in front of each category represent the number of studies (n) and total sample size (s) available for the meta-analysis. Figures on the left side of the horizontal bars represent the marginal means.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SUPPLE1.doc](#)
- [SUPPLE2.doc](#)
- [SupplementaryMethods.docx](#)