Supplementary Information

Zinc agronomic biofortification in wheat and its drivers: A global meta-analysis

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Supplementary Method 1: Detailed information on field trials conducted by ourselves in meta-analysis.

The first trial was a soil Zn application trial, and carried out in 2010–2011 and 2011–2012 in Yongshou County (39°49′N,108°10′E), Shaanxi Province, China. The field trial was arranged in randomized complete blocks, with four replications and 31.5 m^2 plot. Zinc application rates included six levels (0; 2.3; 5.7; 11.4; 22.7; 34.1 kg Zn ha^{-1}), and all the Zn fertilizers were applied to soils before wheat sowing.

The second trial was a multi-site trial of foliar Zn application. It was conducted in 2010–2011 at 31 sites in main wheat-producing areas, containing 14 provinces or cities in China. All field trials adapted randomized complete block designs with four replications and two treatments (i.e., no spray Zn and 1.36 kg Zn ha^{-1} sprayed). The wheat plants were sprayed 750 L ha^{-1} solution with 0.091 g Zn L^{-1} at the early and middle jointing stage, respectively. The control was supplied equal water each time. The plot was 15–20 m^2 and maintained a same size at one site.

The third and fourth trials were based on long-term site-fixed trials of winter wheat planted in soils added different amounts of N fertilizer (0; 80; 160; 240; 320 kg N ha^{-1}) and different amounts of P fertilizer (0; 22; 44; 66; 88 kg P ha^{-1}). The site-fixed trials were initiated in 2004 in Yangling, Shaanxi, China (34°18′ N, 108°05′ E). The third and fourth field trials on Zn fertilizer sprayed on foliage were conducted in 2011–2012 and 2012–2013, and used a split-plot design with N rates or P rates as the main plots and Zn rates (0; 0.85; 1.70; 2.54 kg Zn ha^{-1}) as the subplots. The wheat plants were sprayed three times at the booting stage, once each week. The Zn concentration in the spraying solution was 0.114 g Zn L^{-1}. The control treatments were sprayed equal water each time. For the treatment with 0.85 kg Zn ha^{-1} applied, 750 L ha^{-1} of the Zn solution was sprayed at the first time, and the equal amount of water was sprayed on foliage at the second

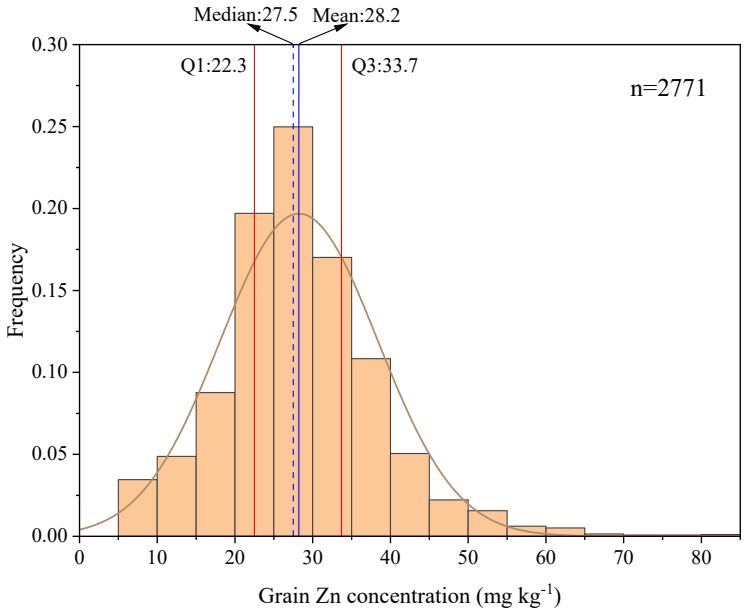
and third time. The treatment applied 1.70 kg Zn ha^{-1} was sprayed with Zn solutions for the first two times, and for the third time, 750 L ha^{-1} of water was sprayed. The treatment applied 2.54 kg Zn ha^{-1} was sprayed with 750 L ha^{-1} of Zn solution each time. The main plot was 40 m^2, and the subplot was 4 m^2.

In all the field trials, the winter wheat cultivar used was widely planted locally. Other management measures, such as other fertilizer applications, irrigation, and weed and pest control, were consistent with the management of local farmers. For all the field trials, measurements of wheat yield, yield components, biomass and Zn concentration in different organs (grain, glume+rachis, stem+leaf) were made, together with soil properties (including available Zn, pH, organic matter, mineral N, available P and available K) before sowing.

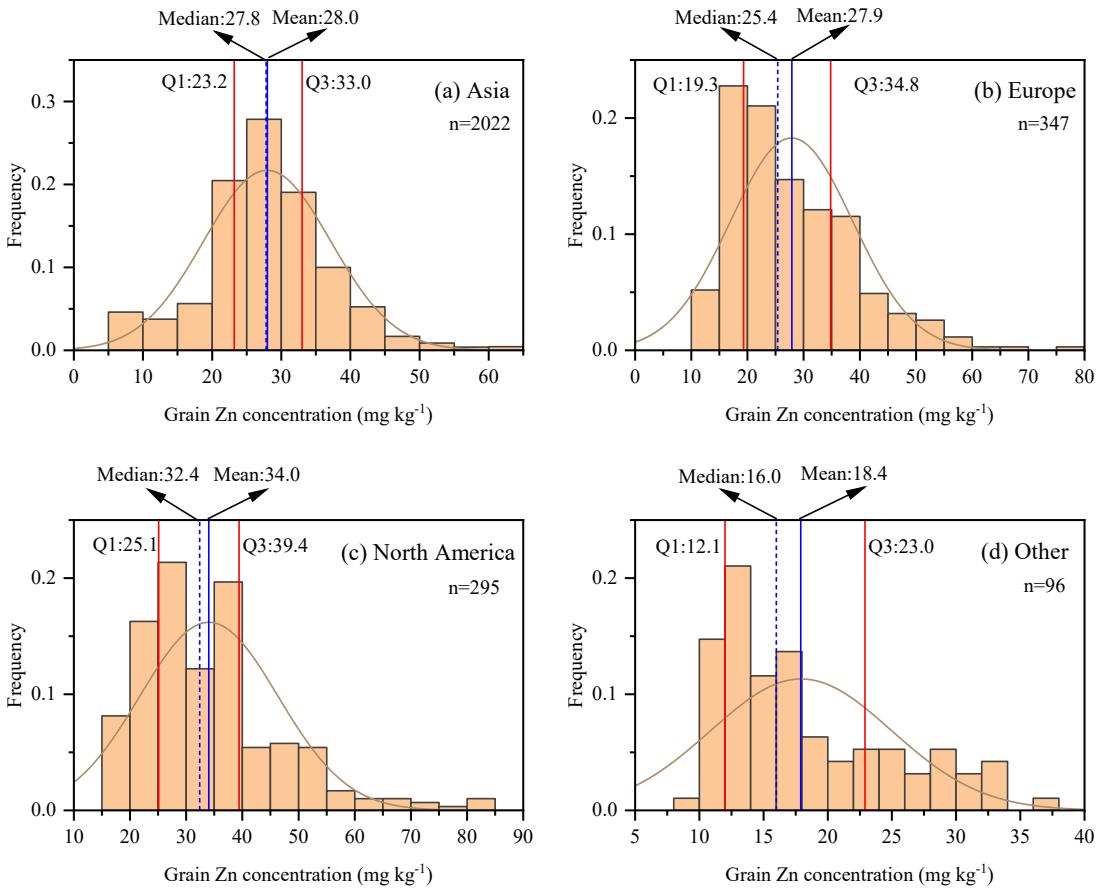
Supplementary Method 2: Detailed information on confirmation trials.

To validate the calculation method on Zn recommended application rate to soil and foliage, we conducted six field trials in the 2022–2023 season in 6 sites in China, including three trials of Zn fertilizer applied to soil and three trials of Zn fertilizers supplied to foliage of wheat. All field trials adapted randomized complete block designs with three replications and two treatments (i.e., no Zn application and Zn applied to soil or foliage). Zinc sulphate was used as Zn fertilizer in the six trials. For soil application trails, all the Zn fertilizers were applied to soils before wheat sowing. In foliar spraying trails, the wheat plants were sprayed Zn solution at flowering and early grain filling stage, respectively. The control was supplied equal water each time. The plot was 96 m^2 for soil application and 222 m^2 for foliage application. In all field trials, the wheat used is a variety widely planted in the local area. Other management measures were consistent with the local farmers' management, such as N, P and K applications, irrigation, and weed and pest control.

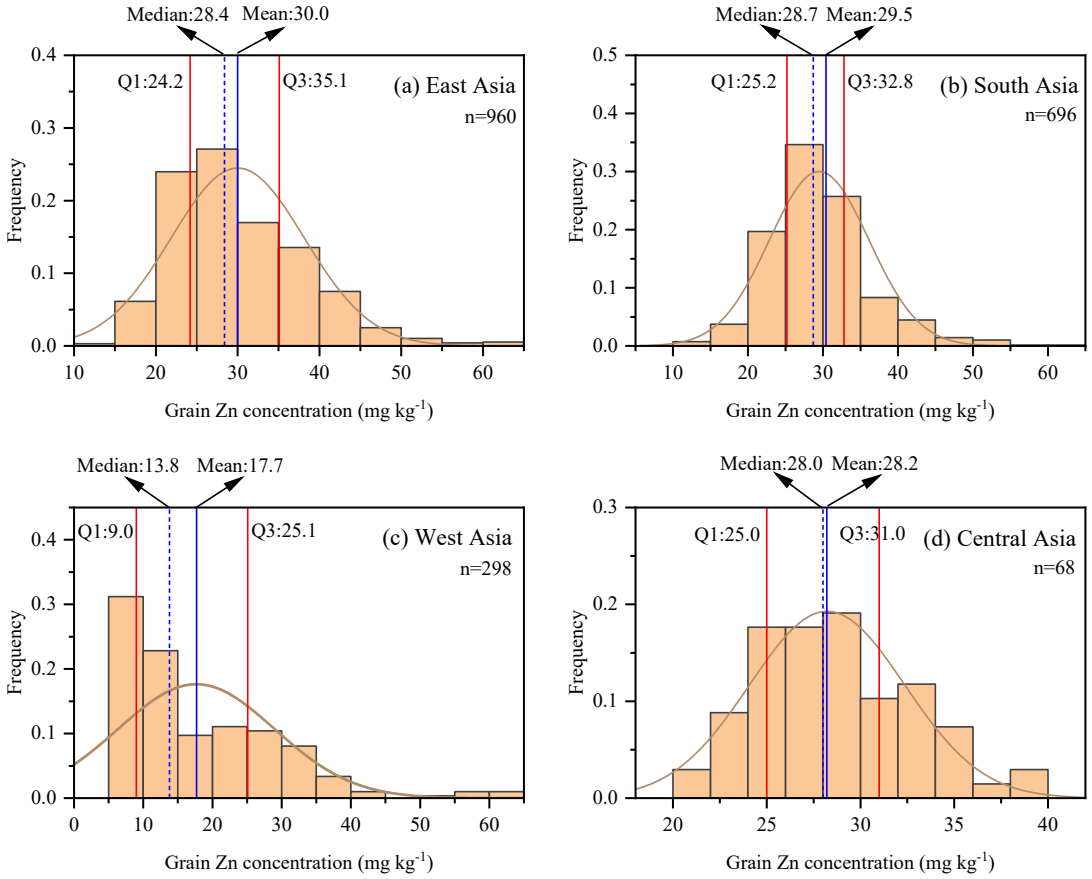
To determine the grain Zn concentration of wheat grown in China, wheat grain samples were randomly collected from 217, 201, 168, 248 and 197 from farmers' fields in the major wheat cropping regions of China in 2018, 2019, 2020, 2021 and 2022, respectively. Each sample contained 100 ears of wheat and was randomly sampled from a 5 × 10 m^2 area in the middle of the farmer's field. The air-dried ears were threshed and divided into grain and glume. The grain was cleaned, dried, crushed and turned into powder for chemical analysis.



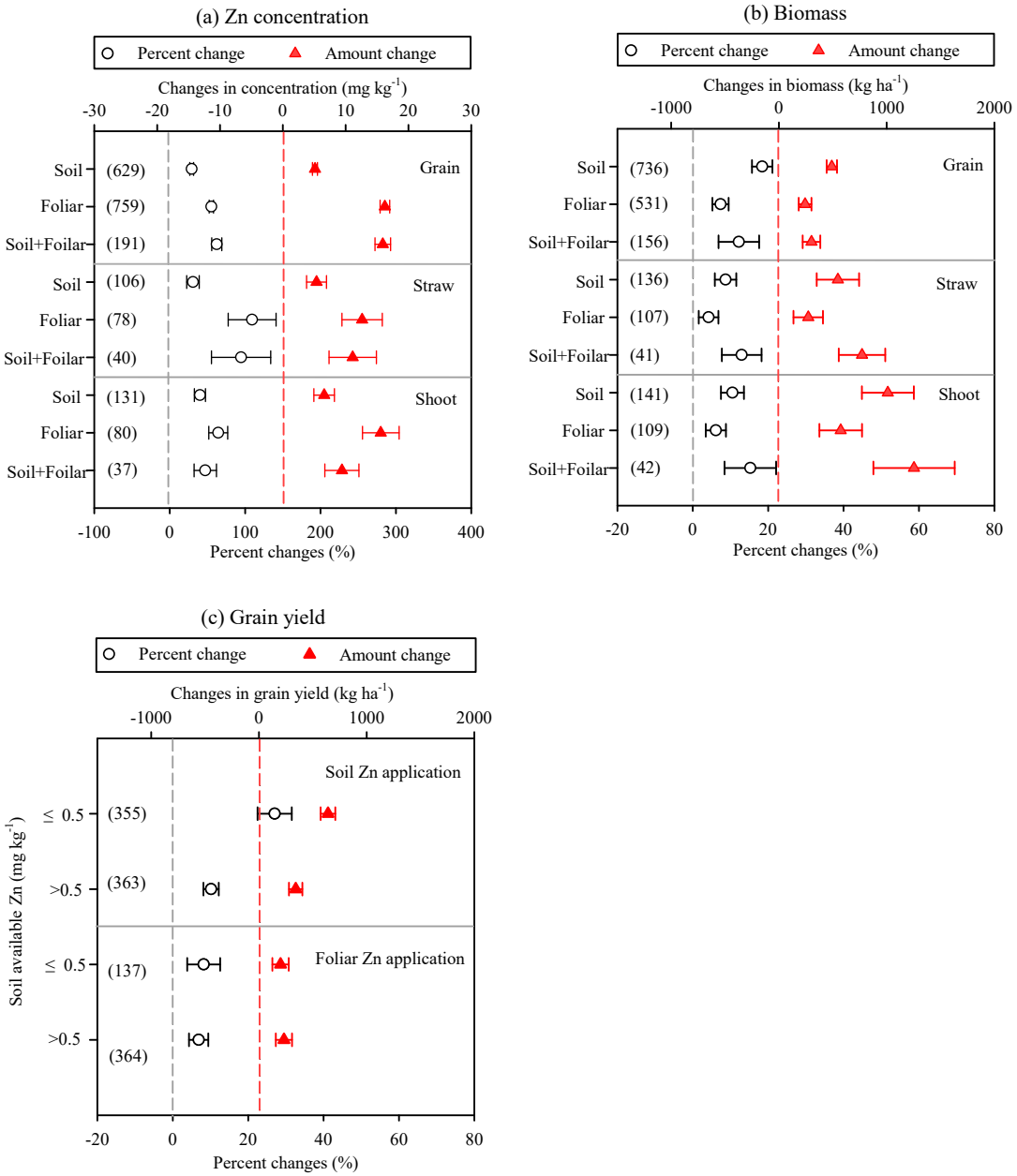
Supplementary Fig. 1 Frequency distribution of grain Zn concentration of global wheat grown without Zn fertilizers applied. Blue solid and dashed lines denote the mean and median of grain Zn concentration, respectively, and red lines indicate the first (Q1) and third (Q3) quartile of grain Zn concentration. Numbers following n represent samples sizes. Source data are provided as a Source Data file.



Supplementary Fig. 2 Frequency distribution of grain Zn concentration of wheat grown without Zn fertilizers applied in different continents. Panel a, b, c, and d present Asia, Europe, North America, and other continents (Africa, Oceania, and South America), respectively. Blue solid and dashed lines denote the mean and median of grain Zn concentration, respectively, and red lines indicate the first (Q1) and third (Q3) quartile of grain Zn concentration. Numbers following n represent samples sizes. Source data are provided as a Source Data file.

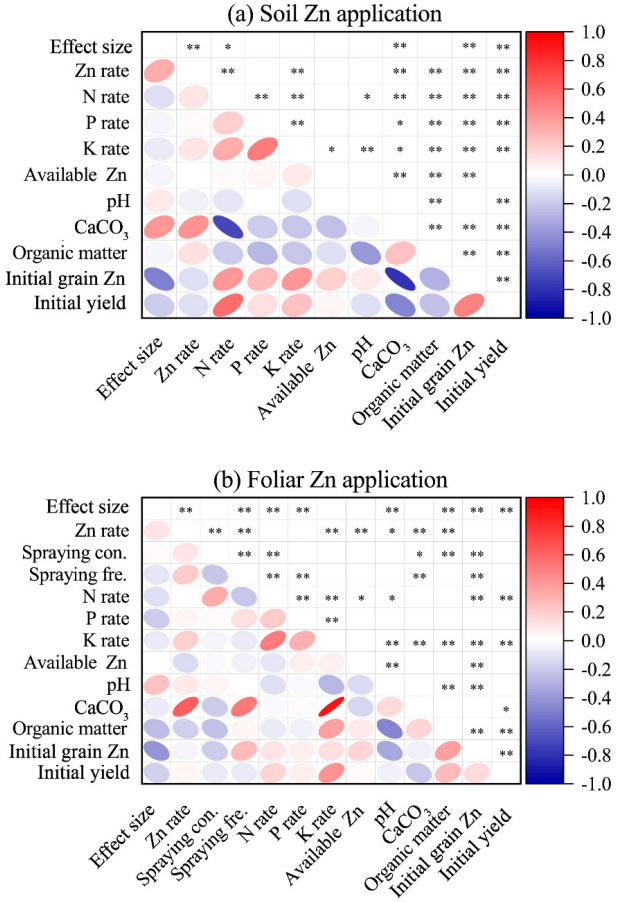


Supplementary Fig. 3 Frequency distribution of grain Zn concentration of wheat grown without Zn fertilizers applied in Asia. Panel a, b, c, and d present East Asia, South Asia, West Asia, and Central Asia, respectively. Blue solid and dashed lines denote the mean and median of grain Zn concentration, respectively, and red lines indicate the first (Q1) and third (Q3) quartile of grain Zn concentration. Numbers following n represent samples sizes. Source data are provided as a Source Data file.

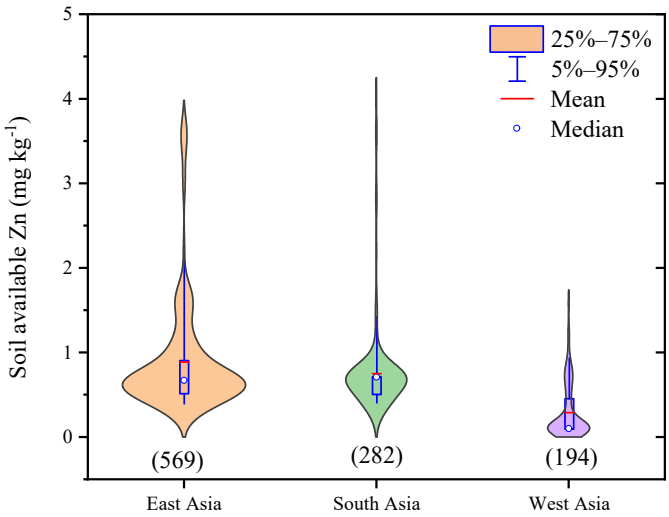


Supplementary Fig. 4 Effects of different forms of Zn application on changes in Zn concentration and biomass in grain, straw and shoot compared to no Zn applied. Panel a and b present Zn fertilizers applied to soil and foliage, respectively. Panel c presents the changes in grain yield of wheat grown in the soil with different available Zn levels. Black and red data points denote the mean of percent change and amount change in grain Zn concentration following Zn application, respectively. Bars indicate 95% confidence intervals (CIs). When the CIs do not intersect with zero, the significant difference are between the Zn application treatment and the control treatment. When the CIs do not overlap with each other, the significant difference are among subgroups.

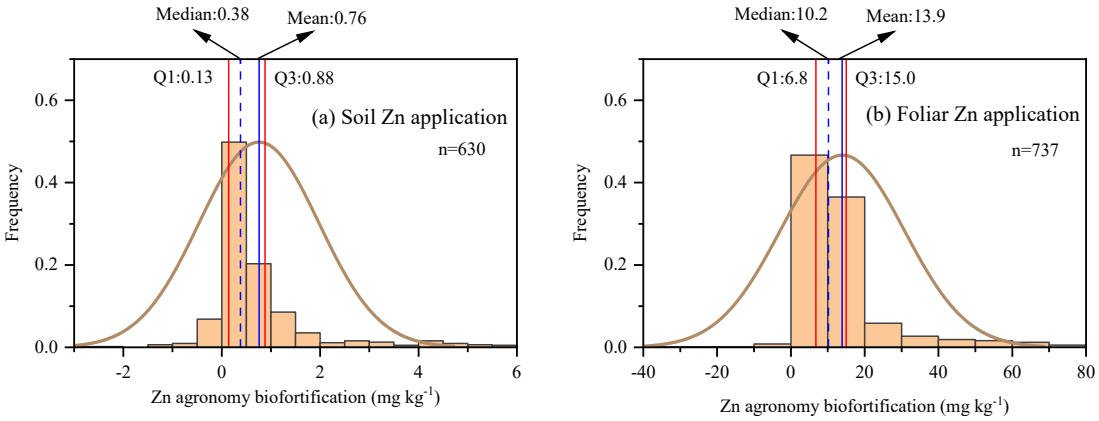
Black and red dash lines denote zero values of percentage and amount, respectively. Numbers in parentheses represent samples sizes. Source data are provided as a Source Data file.



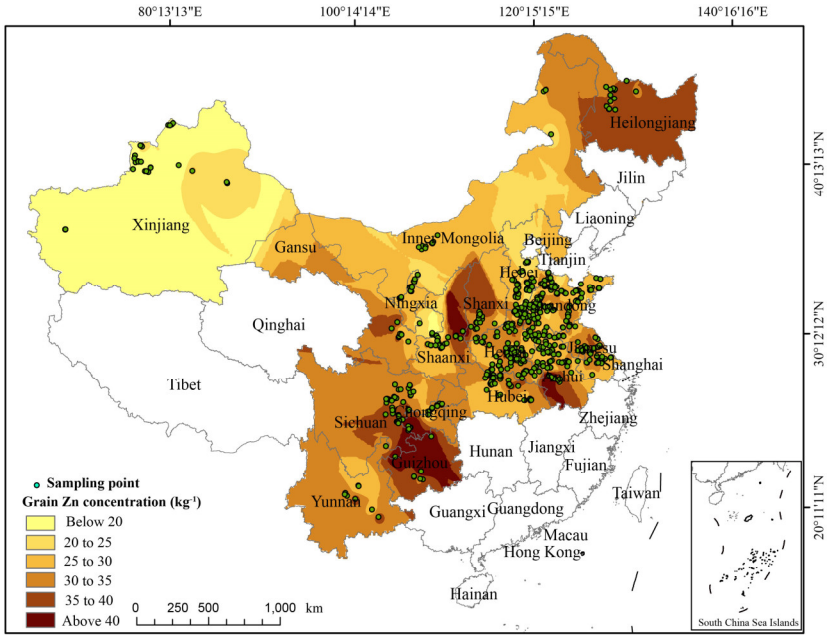
Supplementary Fig. 5 Pearson correlation analysis between fertilizer managements, initial soil properties, wheat varieties and effect size for grain Zn concentration of wheat. Panel a and b present Zn fertilizers applied to soil and foliage, respectively. Spraying fre.: Spraying frequency. Spraying con.: Spraying concentration. * and ** indicate the significance at $P<0.05$ and $P<0.01$ levels, respectively. Source data are provided as a Source Data file.



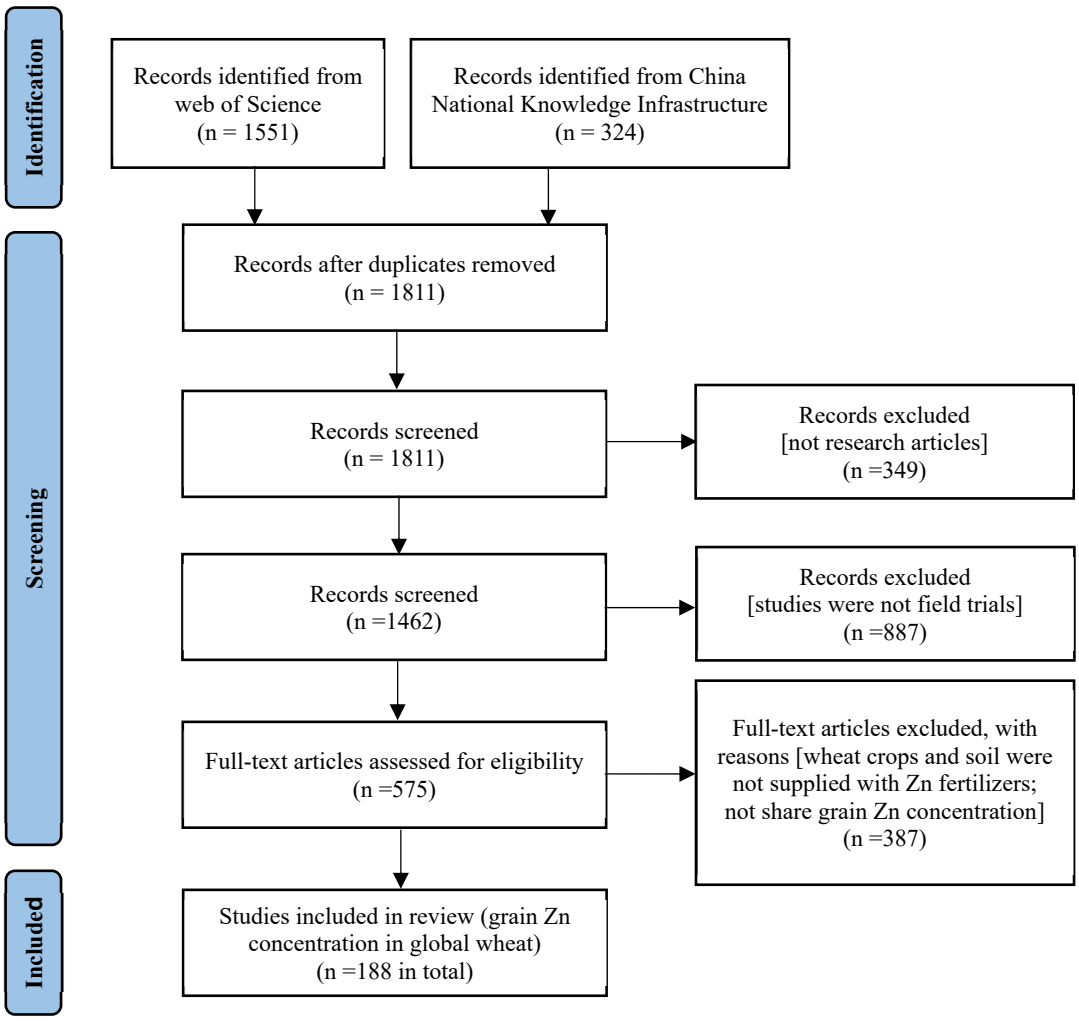
Supplementary Fig. 6 Soil available Zn concentration in East Asia, South Asia and West Asia. The fatter the violin, the larger the sample size. Numbers in parentheses represent samples sizes. Solid lines and white dots in boxes represent the mean and median values. Box bars and boundaries denote the 5th and 95th, and 25th and 75th percentiles of all the analyzed data. Source data are provided as a Source Data file.



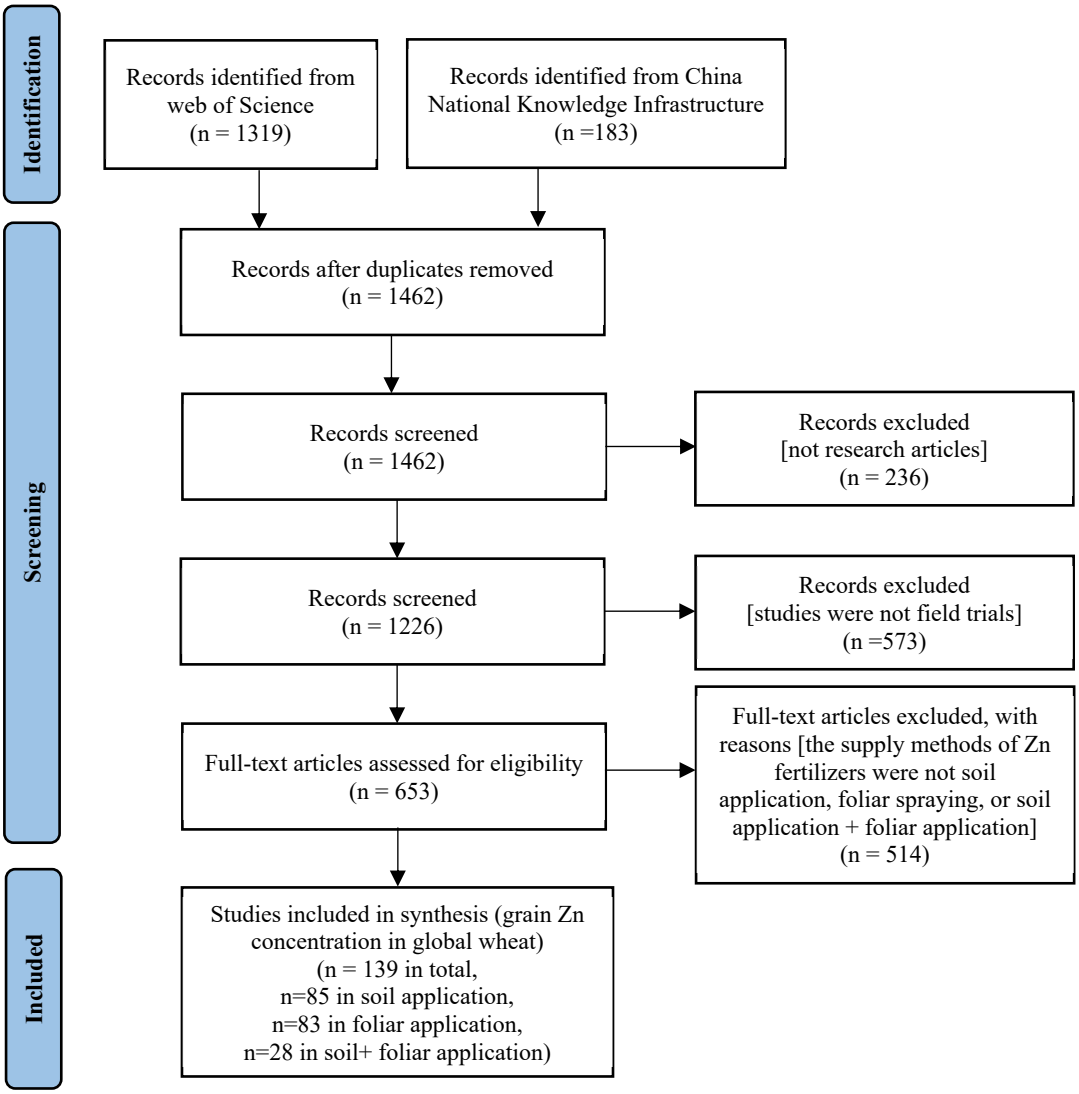
Supplementary Fig. 7 Frequency distribution of Zn agronomy biofortification index of global wheat. Panel a and b present Zn fertilizers applied to soil and foliage, respectively. Blue solid and dashed lines denote the mean and median of grain Zn concentration, respectively, and red lines indicate the first (Q1) and third (Q3) quartile of grain Zn concentration. Numbers following n represent samples sizes. Source data are provided as a Source Data file.



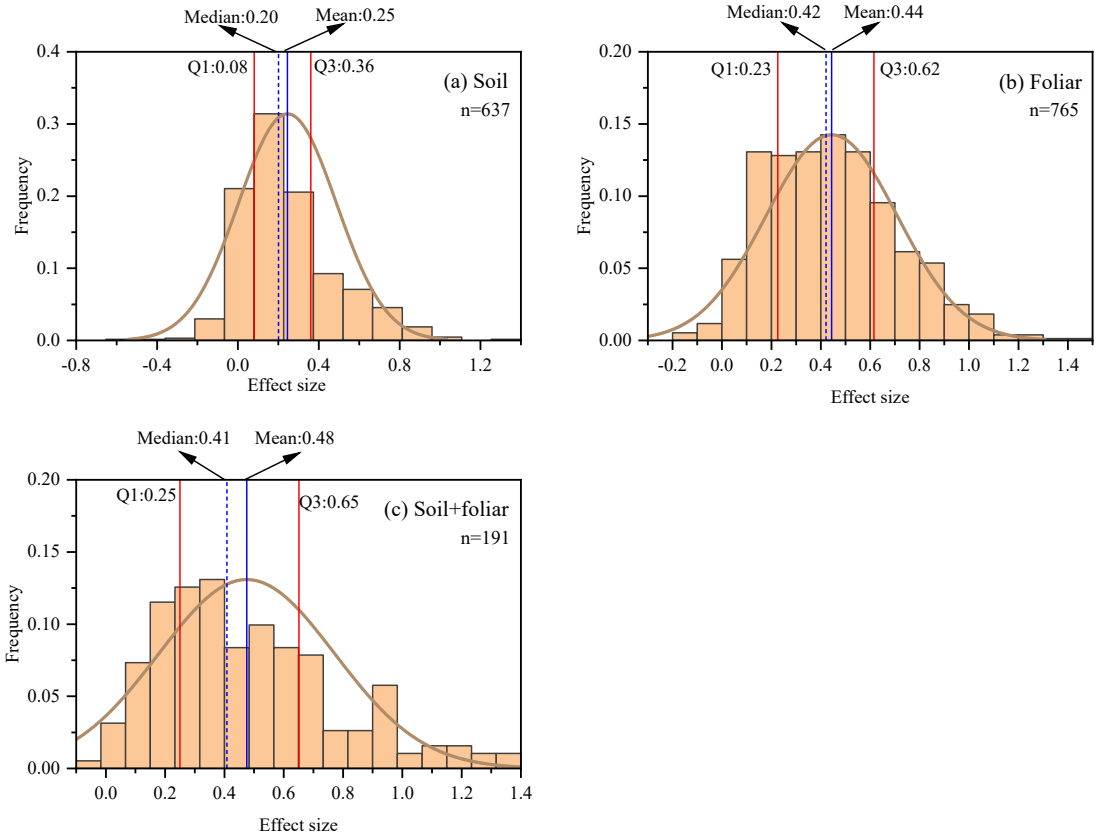
Supplementary Fig. 8 Grain Zn concentration in major wheat cropping regions of China from 2018 to 2022. Green points denote the collection sites of wheat samples. Source data are provided as a Source Data file.



Supplementary Fig. 9 PRISMA diagram showing the process of locating publications included in the present analysis on grain Zn concentration in global wheat grown without Zn fertilizer applied.



Supplementary Fig. 10 PRISMA diagram showing the process of locating publications included in the present meta-analysis.



Supplementary Fig. 11 Frequency distributions of effect sizes for grain Zn concentration of wheat. Panel a, b, and c present Zn fertilizers applied to soil, foliage, and soil+foliage, respectively. Solid lines are the curves fitted by Gaussian (normal) distributions. Numbers following n represent samples sizes. Source data are provided as a Source Data file.

Supplementary Table 1 The Zn recommended rate, initial grain Zn concentration, grain Zn concentration after applying Zn fertilizer from six field experiments conducted in 2022–2023 cropping season in China. The Zn biofortification index is the average of the data from studies published near the experimental sites. Source data are provided as a Source Data file.

Trail site	Fertilization method	Zn recommended rate (kg Zn ha^{-1})	Initial Zn concentration in grain (mg kg^{-1})	Grain Zn concentration after applying Zn (mg kg^{-1})		Zn biofortification index (mg kg^{-1})	nRMSE (%)
				Estimated (Target value)	Measured		
Yongshou, Xianyang, Shaanxi Province	Soil application	16.7	25.6	30	31.0	0.26	3.6
Baixiang, Xingtai, Hebei Province	Soil application	23.1	32.1	40	41.4	0.34	27.6
Zitong, Mianyang, Sichuan Province	Soil application	12.0	31.2	40	41.2	0.74	27.2
Xunyang, Ankang, Shaanxi Province	Foliar application	0.57	32	40	41.5	13.9	27.7
Hemujing, Xinji, Hebei Province	Foliar application	0.58	31.4	40	41.4	14.7	27.7
Liuhe, Nanjing, Jiangsu Province	Foliar application	0.58	33.1	40	41.2	11.9	27.3