

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

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Zinc (Zn) deficiency affects 17% of the world's population, particularly those consuming large cereal grains with low Zn concentration and poor bioavailability. Here, we synthesize data from four field trials along with 139 studies from the literature, to evaluate the impact of management practices and soil properties on improving grain Zn concentration in wheat through applying Zn fertilizers. Soil application of Zn fertilizer improves grain Zn concentration by only 29.1%, below the biofortification target of 40 mg kg⁻¹ required for human nutrition. Foliar Zn application and soil+foliar applications increase grain Zn concentration by 55.2% and 62.3%, respectively, surpassing the 40 mg kg⁻¹ threshold. Factors such as the rate of Zn application, initial grain Zn concentration (prior to Zn application), and timing of Zn spraying are identified as principal factors influencing the response of grain Zn concentration to Zn fertilization. In search of desirable grain Zn concentration in wheat while minimizing potential environmental risks, our study proposes a method for assessing rates of Zn fertilizer application based on the Zn biofortification target, the Zn agronomic biofortification index, and the contribution of principal factors influencing the improvement in grain Zn concentration.

Zinc (Zn) deficiency remains a significant issue in human nutrition, affecting 17% of the world's population, with the highest prevalence found in Africa (24%) and Asia (19%)^{1,2}. Zinc deficiency, often termed “hidden hunger”, presents a major challenge in addressing micronutrient deficiencies and achieving the zero hunger and sustainable development goals of the United Nations³. Human Zn deficiency can lead to slow physical growth, impaired brain development, compromised immune function, and increased susceptibility to infectious diseases^{1,4,5}. This deficiency is particularly common in populations consuming large amounts of cereal grains with low Zn concentration and limited animal and plant foods rich in Zn⁶. Wheat (*Triticum aestivum* L.) serves as a staple cereal for 35% of the global population,

providing nearly 20% of protein and dietary energy in developing countries⁷. However, its grain Zn concentration is inherently low, averaging between 28 to 30 mg kg⁻¹, which falls below the 40–60 mg kg⁻¹ threshold required for human nutrition^{6,8–11}. Thus, enhancing grain Zn concentration in wheat presents a critical challenge in addressing Zn deficiency in human nutrition.

The low concentration of Zn in wheat grains primarily stems from the limited availability of Zn in the soil. Globally, over 50% of soils used for wheat cultivation are categorized as Zn deficient⁶. Soil Zn availability is influenced by various factors including pH levels, total Zn, CaCO₃, available phosphorus (P), organic matter, and moisture content^{12,13}. Prioritizing the application of Zn fertilizers to the soil is

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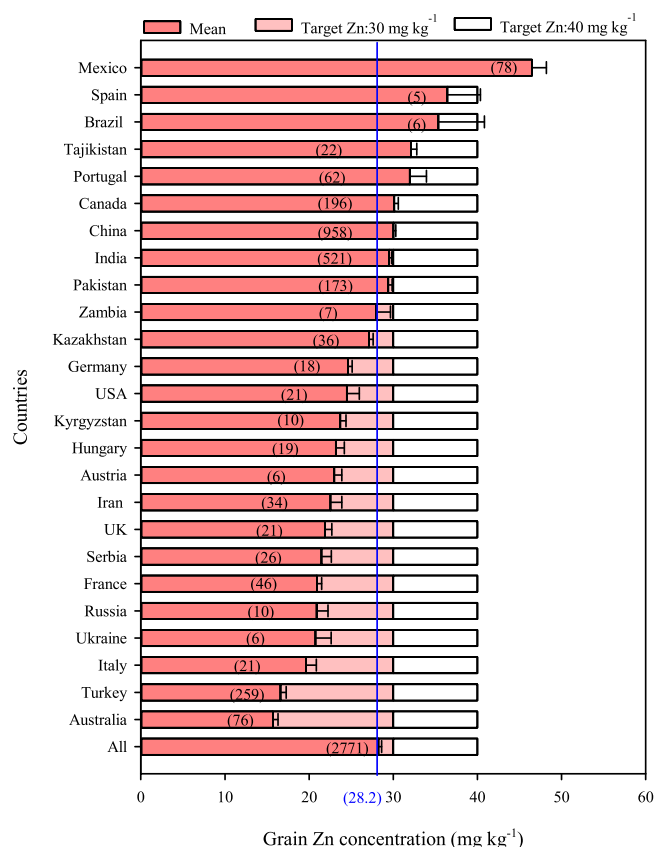


Fig. 1 | Grain Zn concentration of global wheat. Pillars represent the average values, while the bars on pillars represent standard error. Black numbers in parentheses represent sample sizes. Blue line and blue number in parentheses denote the average Zn concentration in global wheat. The Zn biofortification target is abbreviated to target Zn. Source data are provided as a Source data file.

crucial for enhancing soil Zn availability, ensuring sufficient uptake of Zn by the plant's root system^{14–17}. Typically, Zn fertilizers are applied to the soil before sowing, often alongside other fertilizers, a practice readily embraced by wheat farmers. However, the efficacy of increasing grain Zn concentration through soil Zn application is contingent upon other soil fertilization practices and soil properties^{18–21}. For instance, in severely Zn-deficient soils, the application of both Zn and nitrogen (N) fertilizers together could lead to a notable increase, with wheat grain Zn concentration reaching up to 61.2 mg kg⁻¹¹⁹. Furthermore, studies have shown that combining Zn application with phosphorus (P) fertilization could minimize Zn concentration in wheat grains compared to scenarios without Zn and P fertilization, or with only single Zn application²⁰. Integrating Zn application and organic amendments has demonstrated to a greater conversion of applied Zn into a form loosely bound to organic matter, thereby, synergistically improving Zn concentration in wheat grains²¹. In calcareous soils characterized by high pH and CaCO₃ content, along with low moisture and organic matter levels, the solubility and diffusion of Zn in soil are compromised, leading to the retention of applied Zn within the soil and a utilization efficiency of Zn fertilizer of less than 1.5%^{8,17,22}. Moreover, repeated application of Zn at high rates may lead to the accumulation of heavy metals in the soil, posing potential hazards to soil ecology and functionality²³.

The increase in wheat grain Zn concentration resulting from foliar application of Zn fertilizer has ranged from 32% to 137%, significantly surpassing the effects observed with Zn application to the soil²⁴. Utilization efficiency of Zn fertilizer applied via foliar spray has been reported to be 8% to 19% higher compared to soil application^{17,25},

indicating considerable potential for enhancing wheat grain Zn concentration through this method. However, foliage application of Zn fertilizer during the growing season demands substantial labor and has shown limited effectiveness in improving wheat grain yield, leading to its relatively low adoption rate^{26,27}. Field experiments conducted in seven countries have demonstrated that foliar application of Zn combined with common pesticides, such as acetamiprid, imidacloprid has been an effective approach to reduce the cost of application, while improving grain Zn concentration and grain yield in wheat^{28,29}. Timing and concentration of Zn solution during spraying are critical factors influencing Zn agronomic biofortification in wheat grain^{30–32}. Field experiments have observed that late-stage foliage application of Zn tended to improve wheat grain Zn concentration more effectively than early-stage application³³. Optimal Zn concentration in the spraying solution, typically ranging between 0.9 to 1.1 g L⁻¹ of Zn has been associated with achieving the highest Zn concentration and bioavailability in wheat grain and flour³¹.

Globally, the number of studies investigating the response of wheat grain Zn concentration to Zn fertilizer applied to soil and foliage^{12,13,19,20,30,31}, but there remains a challenge in evaluating the contribution of soil properties and fertilization managements to this response. To address this gap, we conducted a meta-analysis synthesizing data from multi-site trials and long-term site-fixed trials, along with data from 139 studies from the literature. Our objectives were to (1) determine global grain Zn concentration levels in wheat production, (2) assess the relationship between soil properties, soil management practices, and the response of grain Zn concentration to Zn fertilization, and (3) propose a method for optimizing Zn fertilizer management in wheat production under varying soil conditions and management models. We anticipate that this method will provide valuable guidance for enriching grain Zn and optimizing Zn fertilization practices in wheat production, thereby improving Zn nutrition in populations relying heavily on wheat-based diets.

In this work, we show that foliar spraying and soil+foliar application of Zn fertilizer increase the grain Zn concentration in wheat more effectively than soil application alone, surpassing the 40 mg kg⁻¹ threshold required for human nutrition. The crucial factors influencing the improvement in grain Zn concentration are the Zn rate, initial Zn concentration, and timing of Zn spraying. To achieve the desired grain Zn concentration and high grain yield, we recommend applying Zn fertilizers to both soil and foliage for wheat with grain Zn concentration below 30 mg kg⁻¹, and for wheat with grain Zn concentration exceeding 30 mg kg⁻¹, only foliar application should be considered. In addition, we propose a method for estimating Zn application rates based on the Zn biofortification target, the Zn agronomic biofortification index, and the contribution of these influential factors.

Results

Grain Zn concentration in global wheat

The average grain Zn concentration in global wheat was found to be 28.2 mg kg⁻¹, with values ranging from 5.0 mg kg⁻¹ to 82.0 mg kg⁻¹ and a median of 27.5 mg kg⁻¹ (Fig. 1 and Supplementary Fig. 1). Specifically, grain Zn concentrations averaged 28.0 mg kg⁻¹ in Asia, 27.9 mg kg⁻¹ in Europe, 34.0 mg kg⁻¹ in North America, and 18.4 mg kg⁻¹ in other continents (Africa, Oceania, and South America) (Supplementary Fig. 2). Within Asia, where the largest sample size and number of studies related to wheat grain Zn were observed, the average Zn concentration was 30.0 mg kg⁻¹ in East Asia, 29.5 mg kg⁻¹ in South Asia, 17.7 mg kg⁻¹ in West Asia, and 28.2 mg kg⁻¹ in Central Asia (Supplementary Fig. 3). Among all countries, Mexico in South America stood out for producing wheat with an average grain Zn concentration exceeding the 40 mg kg⁻¹ threshold required for human nutrition (Fig. 1). Alarmingly, up to 96% of countries produce wheat with an average grain Zn concentration below the 40 mg kg⁻¹ requirement for human nutrition.

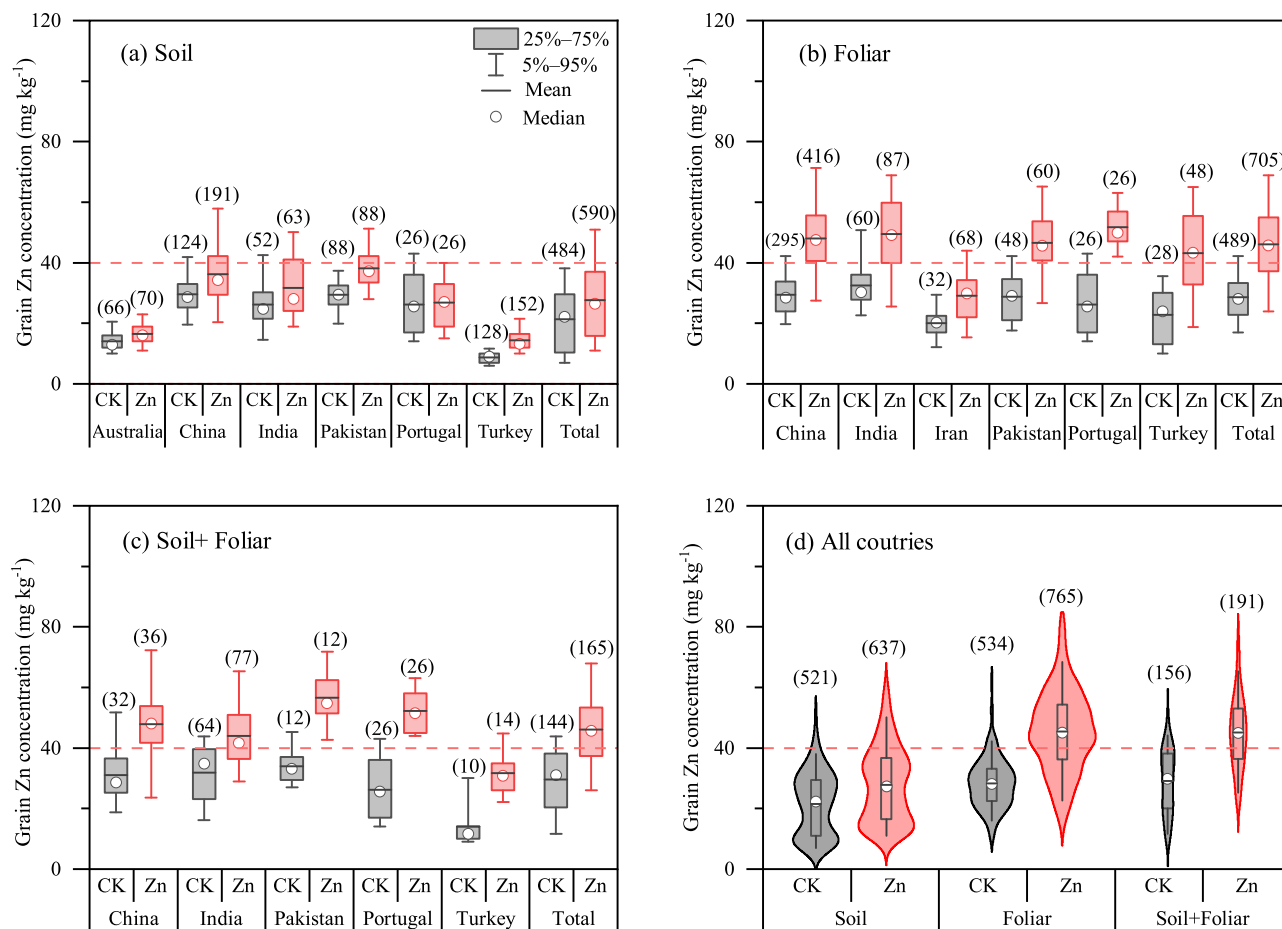


Fig. 2 | Zinc concentration in wheat grains affected by different application forms of Zn fertilizers. Panels (a), (b), and (c) present Zn fertilizers applied to soil, foliage, and soil+foliage in main countries, respectively. Panel (d) presents Zn fertilization in all countries. The total in panels (a), (b), and (c) indicates the Zn concentration in wheat grains from all the major countries, not all the countries. The CK and Zn denote the wheat grown without and with Zn fertilizers applied,

respectively. Numbers in parentheses represent sample sizes. Red dashed lines denote the biofortification target of 40 mg kg^{-1} for grain Zn concentration. 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. The fatter the violin, the larger the sample size. Source data are provided as a Source data file.

Improving Zn concentration in global wheat grains by Zn fertilization

The increase in wheat grain Zn concentration varied depending on the method of Zn fertilizer application, with foliar application and soil+foliar application resulting in a greater increase compared to soil application (Fig. 2a–d, Supplementary Fig. 4a). Soil application, on average across all countries, increased grain Zn concentration by 29.1% (5.1 mg kg^{-1}) compared to the treatment without Zn application. However, in 83.5% of the grains, the Zn concentration did not reach the 40 mg kg^{-1} threshold required for human nutrition (Fig. 2d, Supplementary Fig. 4a). In contrast, foliar application and soil+foliar application increased grain Zn concentration by an average of 55.2% and 62.3% (16.2 mg kg^{-1} and 15.9 mg kg^{-1}), respectively, across all countries. Under foliar application and soil+foliar application, up to 67.3% and 66.0% of the wheat grains, respectively, achieved the 40 mg kg^{-1} threshold required for human nutrition.

Effects of different factors on the efficacy of improving grain Zn concentration by Zn fertilization

Since the application of Zn fertilizer to the soil typically occurs once before the sowing of wheat, the timing and frequency of application were not considered in the subgroup meta-analysis regarding the improvement in wheat grain Zn concentration. However, it was observed that an increase in the Zn application rate reduced the

effectiveness of increasing grain Zn concentration under soil Zn application (i.e., the percentage increase in grain Zn concentration compared to the treatment with no Zn application) (Figs. 3a and 5a). The meta-regression analysis between the effect size of grain Zn concentration and the Zn application rate revealed a linear increase in the effectiveness of grain Zn concentration with increasing Zn application rate (Fig. 5a). In addition, subgroup analysis indicated that the application of ZnSO_4 to soil resulted in a greater increase in grain Zn concentration compared to the application of ZnO (Fig. 3a).

In foliar Zn application, various factors such as spraying rate, concentration, timing, frequency, and fertilizer form significantly influenced the effectiveness of increasing grain Zn concentration (Fig. 3b, c). Analysis revealed a non-linear relationship between the effect size of grain Zn concentration and the Zn application rate, with grain Zn concentration reaching a maximum effectiveness at a Zn rate of 3.2 kg ha^{-1} (Fig. 5b). Similarly, the greatest improvement in wheat grain Zn concentration was observed under medium concentrations of $0.7\text{--}1.1 \text{ g Zn L}^{-1}$ in the spraying solution (Fig. 3c). The application of ZnSO_4 was found to increase grain Zn concentration more effectively than Zn-EDTA . Foliar application of Zn at late growth stages exhibited greater effectiveness in increasing grain Zn concentration compared to early growth stages. The percentage increase in grain Zn concentration was the highest at the flowering stage, followed by the grain-filling stage, heading stage, booting stage, and pre-booting stage.

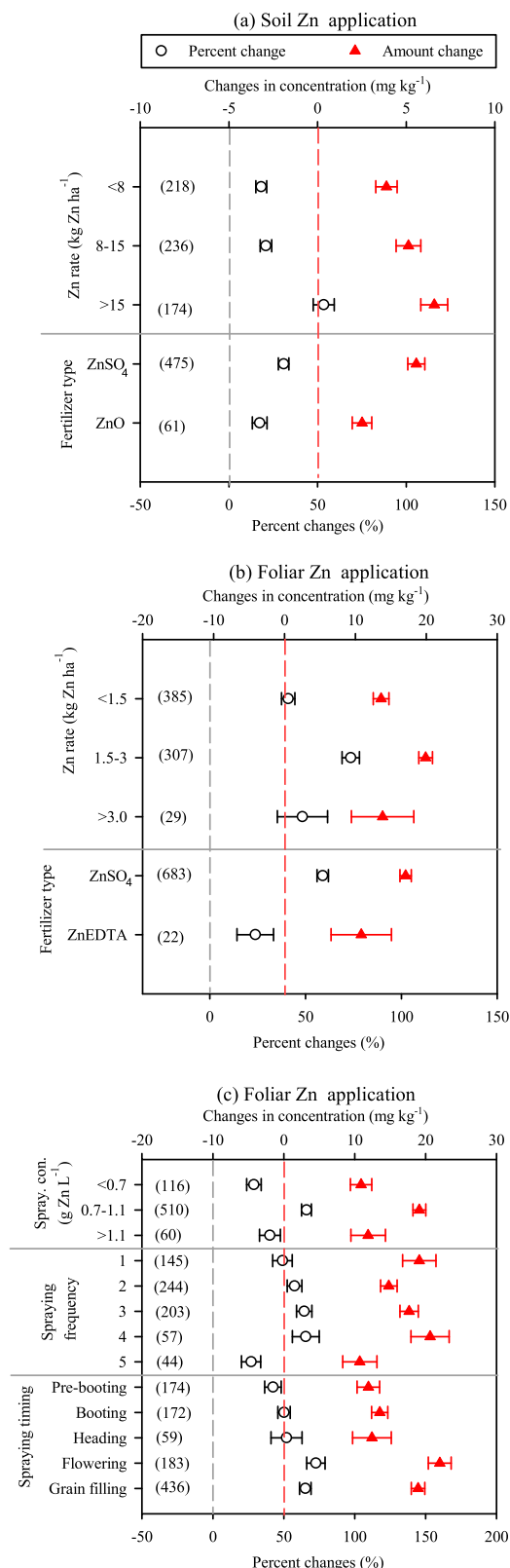


Fig. 3 | Effects of Zn fertilizer managements on the improvement in Zn concentration in wheat grains. Panels (a) and (b) present the amounts and types of Zn fertilizers applied to soil and foliage, respectively. Panel (c) presents the Zn concentration in spraying solution, spraying timing, and spraying frequency to foliage. 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. Dash black and red lines denote zero values of percentage and concentration, respectively. Numbers in parentheses represent sample sizes. Spraying Zn concentration in solution is abbreviated to spray con. Source data are provided as a Source data file.

application rates (Fig. 4a, b). The percentage increase in grain Zn concentration tended to decrease with increasing N rates when Zn fertilizer was applied to both the soil and foliage. Conversely, an increase in P application rates initially led to a rise in the percentage improvement in grain Zn concentration, but then this effect diminished under Zn application to both the soil and foliage. The highest percentage increase in grain Zn concentration was achieved at medium P rates of 26–44 kg P ha^{-1} . Potassium application rates had different effects on the effectiveness of increasing grain Zn concentration under soil and foliar Zn fertilization. Under Zn fertilizer applied to the soil, the improvement in grain Zn concentration initially decreased but then increased with increasing K rates, with the lowest improvement observed at medium K rates of 25–58 kg K ha^{-1} . In contrast, under foliar application, the greatest improvement in grain Zn concentration occurred at medium K rates.

Soil parameters such as available Zn, pH, CaCO_3 , and organic matter content at sowing significantly influenced the effectiveness of increasing grain Zn concentration when Zn was applied to the soil. However, when Zn was applied to the foliage, CaCO_3 had no impact on the effectiveness of increasing grain Zn concentration (Figs. 4c, d and 5c–f). In cases where Zn was applied to the soil, subgroup meta-analysis revealed that the percentage improvement in grain Zn concentration decreased as the availability of Zn in the soil at sowing increased. Conversely, the percentage improvement in grain Zn concentration increased with higher soil pH and CaCO_3 content at sowing (Fig. 4c). Meta-regression analysis showed a non-linear correlation between soil pH at sowing and the efficacy of soil application in improving grain Zn concentration, with the effect size reaching a maximum (i.e., the highest percentage increase in grain Zn concentration following soil application) at a soil pH of 7.5 (Fig. 5e).

Under foliar Zn application, the effectiveness of increasing grain Zn concentration linearly increased with higher soil Zn availability and linearly decreased with increasing soil pH (Fig. 5d, f). Subgroup meta-analysis demonstrated that organic matter content affected the effectiveness of increasing grain Zn concentration differently depending on whether Zn fertilizer was applied to the soil or foliage (Fig. 4c, d). When Zn fertilizer was applied to the soil, increasing soil organic matter at sowing initially reduced the percentage improvement in grain Zn concentration. However, at high organic matter content ($>20 \text{ g kg}^{-1}$), the percentage improvement in grain Zn concentration could be maximized. Under foliar application, the percentage improvement in grain Zn concentration decreased as organic matter content increased.

Wheat varieties were categorized based on their initial grain Zn concentration and grain yield, representing the grain Zn concentration and yield without Zn application. The initial grain Zn concentration significantly influenced the effectiveness of Zn fertilization in increasing grain Zn concentration. However, the initial yield had a negligible impact on the effectiveness of increasing grain Zn concentration, particularly under soil application of Zn fertilizers (Fig. 4e, f). The effectiveness of increasing grain Zn concentration

In addition, the effectiveness of increasing grain Zn concentration initially increased with an increase in spraying frequency, but then decreased. The highest percentage increase in grain Zn concentration was achieved with four sprayings, followed by three, two, one, and five sprayings, respectively.

The effectiveness of Zn application in increasing grain Zn concentration varied significantly with increasing N, P, and potassium (K)

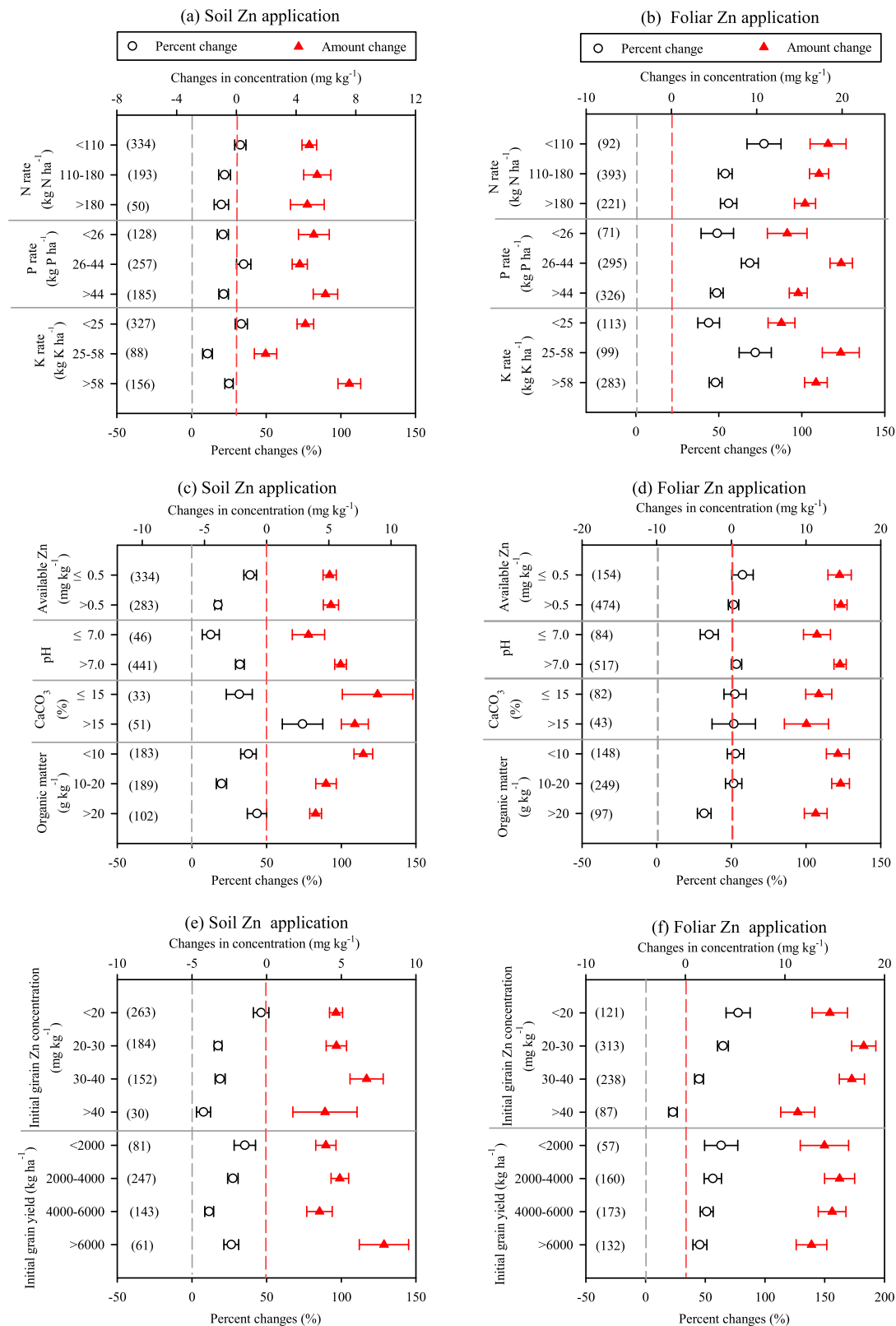


Fig. 4 | Effects of other fertilization management practices, soil properties, and wheat cultivars on the improvement in Zn concentration in wheat grains following Zn fertilization. Panels (a) and (b) present N, P, and K application managements, panels (c) and (d) show soil properties before wheat sowing, and panels (e) and (f) depict wheat cultivars. 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 is 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 concentration, respectively. Numbers in parentheses represent sample sizes. Source data are provided as a Source data file.

decreased with higher initial grain Zn concentration. As demonstrated in the meta-regression analysis, the effect of grain Zn concentration decreased in a power way and linear way, respectively under Zn fertilizer applied to the soil and foliage (Fig. 5g, h).

To identify the primary factors influencing the effectiveness of Zn fertilization for increasing grain Zn concentration, a random forest analysis was performed (Fig. 6a, b). Initial grain Zn concentration emerged as the most crucial factor affecting the effectiveness of grain Zn concentration enhancement through Zn application to the soil, followed by the Zn rate, soil Zn availability, soil pH at sowing, P rate, and K rate. Under foliar Zn application, the application rate proved to be the most significant influencing factor, followed by spraying timing, Zn concentration in the solution used for spraying, and initial Zn concentration in the grain.

Optimizing Zn fertilizer management measures to achieve biofortification target of Zn in wheat grains

Based on the findings from meta-analysis, random forest analysis, and correlation analysis (Figs. 3a, 6a, and Supplementary Fig. 5), we identified the Zn rate and initial grain Zn concentration as essential factors for enhancing Zn levels in wheat grain through soil fertilization (Fig. 7). For Zn applied to foliage, only the Zn rate, spraying timing, and initial grain Zn concentration were considered, as there was a significant positive correlation between the Zn rate and spraying Zn concentration (Fig. 7, Supplementary Fig. 5). Notably, meta-analyses revealed that spraying Zn fertilizer during the late growth stages (i.e., flowering and grain-filling stages) substantially increased grain Zn concentration (Fig. 3c). Assuming foliar application of Zn fertilizer occurs during these late growth stages, the primary factors influencing the increase in grain Zn concentration through foliar application align with those for soil application, namely the Zn rate and initial grain Zn concentration. Consequently, the recommended application rates for Zn fertilizer to soil and foliage are calculated as follows:

$$\begin{aligned} & \text{Zn recommended rate (kg Zn ha}^{-1}\text{)} \\ &= \frac{\text{Zn biofortification target} - \text{initial grain Zn concentration}}{\text{Zn agronomic biofortification index}} \quad (1) \end{aligned}$$

The Zn biofortification target, set at 30 mg kg⁻¹, closely aligns with the average Zn concentration in global wheat grains (referred to as the initial biofortification target), while 40 mg kg⁻¹ is the threshold required for human nutrition (referred to as the final biofortification target). The initial grain Zn concentration (mg kg⁻¹) was determined from wheat samples without Zn application but with sufficient supply of other fertilizers. The Zn agronomic biofortification index was defined as the increase in Zn concentration (mg kg⁻¹) in grain with the application of 1 kg Zn ha⁻¹ to soil or wheat foliage.

This meta-analysis reveals that foliar Zn application can improve grain Zn concentration by up to 55.2%, significantly higher than the increase achieved through soil application (Fig. 2d, Supplementary Fig. 4a). However, foliar Zn spraying resulted in only a modest increase in grain yield for wheat grown in both Zn-deficient and non-deficient soils, with increases of 8.2% and 6.9%, respectively. In contrast, soil application led to more substantial grain yield increases, with enhancements of up to 27.2% and 10.2%, respectively (Supplementary Fig. 4b, c). Given the importance of grain yield in wheat production, this study divides the target for wheat Zn biofortification into two stages (Fig. 7). The first stage aims to enhance soil available Zn levels to mitigate yield losses in wheat grown on severely Zn-deficient soils and elevate grain Zn concentration to match the global average of 28.2 mg kg⁻¹. The second stage targets an increase in grain Zn concentration for wheat grown on soils without Zn deficiency to

40 mg kg⁻¹, the level required for human health. In this study, we used Zn concentration of 30 mg kg⁻¹ as Zn biofortification in the first stage for targeting high grain yield. To attain the final biofortification target and ensure high grain yield, it is suggested that a combination of soil application and foliar application should be employed for wheat with grain Zn levels below 30 mg kg⁻¹, while only foliar application is recommended for wheat with grain Zn levels ranging from 30 mg kg⁻¹ to 40 mg kg⁻¹.

Discussion

Agronomically biofortifying Zn concentration in wheat grains based on the Zn biofortification target, Zn agronomic biofortification index and initial grain Zn concentration

A grain Zn concentration threshold of 40 mg kg⁻¹ has been suggested in terms adequate Zn nutrition for humans⁷. However, our comprehensive global dataset assembled for this study revealed an average Zn concentration in wheat of only 28.2 mg kg⁻¹. Remarkably, wheat cultivated solely in Mexico boasts a grain Zn concentration exceeding 40 mg kg⁻¹. Conversely, up to 96% of countries worldwide produce wheat with grain Zn concentrations below the crucial 40 mg kg⁻¹ threshold. Notably, wheat grown in regions characterized by severe soil Zn deficiency exhibits grain Zn concentrations below 20 mg kg⁻¹⁶. For example, West Asia, where the soil's available Zn concentration is a mere 0.30 mg kg⁻¹, yields wheat grains with Zn concentrations as low as 17.7 mg kg⁻¹, contrasting starkly with the Zn concentrations of 30.0 mg kg⁻¹ and 29.5 mg kg⁻¹ observed in wheat grains from East Asia and South Asia, respectively, where the soil's available Zn concentration is substantially higher (Supplementary Fig. 6). The higher Zn concentration in Mexican wheat grains can be attributed to the cultivation of Zn-biofortified wheat varieties, such as Zinc Shakti and Zincol2016, developed by the International Maize and Wheat Improvement Center (CIMMYT)³⁴. This underscores the critical role of improving soil Zn availability and breeding high-Zn varieties in enhancing Zn concentration in wheat grains to address human Zn deficiency.

The Zn agronomic biofortification index, defined as the increase in grain Zn concentration when 1 kg ha⁻¹ of Zn is supplied to the soil or applied as a foliar spray, serves as a measure of Zn application's efficacy in enhancing grain Zn concentration. Our study revealed that the grain Zn concentration in wheat increased by an average of 0.76 mg kg⁻¹ with soil application of 1 kg Zn ha⁻¹, substantially lower than the increase achieved through foliar application, which averaged 13.9 mg kg⁻¹ globally (Supplementary Fig. 7). This finding underscores the superior efficacy of foliar Zn application in boosting wheat grain Zn concentration compared to soil application. Consistent with these results, meta-analysis data revealed that while soil application of Zn fertilizer led to a mere 29.1% increase in grain Zn concentration, foliar Zn application resulted in a substantially higher increase of 55.2%. This further validates the Zn agronomic biofortification index as a reliable indicator of Zn fertilizer efficacy. The disparity in grain Zn concentration enhancement between soil and foliar application can be attributed to adverse soil properties and Zn mobility in soils and plants. Zinc exhibits limited mobility in soil and reaches the rhizosphere of crops mainly by diffusion, which could not compensate for Zn deficiency in the rhizosphere in time⁸. Moreover, there are many wheat production regions with soils that suffer from high pH, CaCO₃, and low moisture and organic matter contents^{6,12,35}, hindering Zn solubility and diffusion to roots¹³, even after Zn application to the soil¹⁷. These conditions restrict the amount of Zn available in the rhizosphere for root uptake by wheat crops, resulting in low grain Zn concentrations. In contrast, Zn is highly mobile in plant phloem^{36–38}. Up to 91% of the stored Zn in vegetative organs is remobilized into the grain when soil Zn availability is limited during grain filling³⁹. Foliar Zn application can provide a sufficient Zn reservoir within vegetative tissues, facilitating Zn accumulation in the grain^{14,30}. As a result, the enhancement in Zn

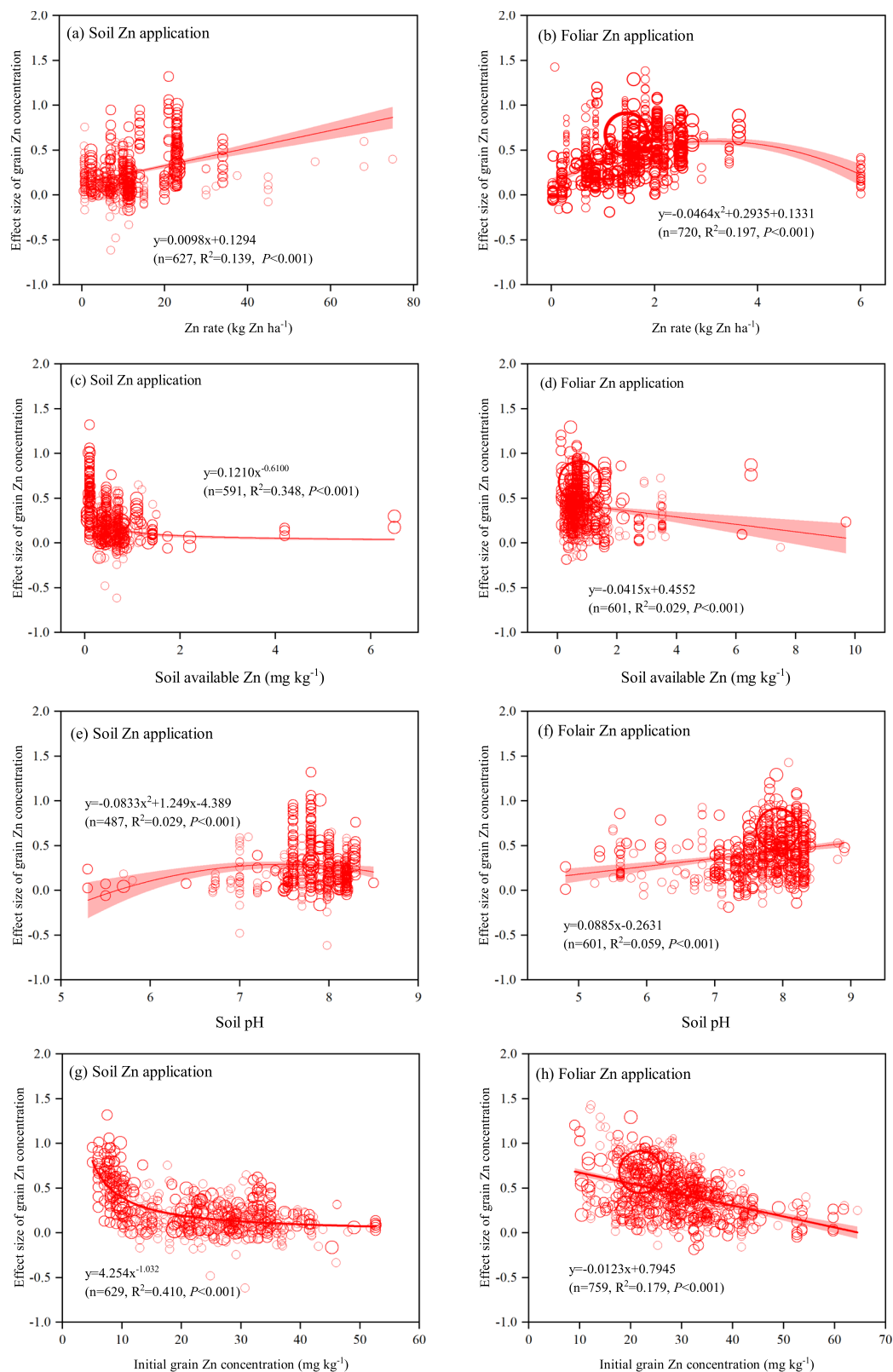


Fig. 5 | Relationships between Zn application rate, soil properties and wheat cultivars and the effect size of wheat grain Zn concentration. Panels (a) and (b) present the relationships between the effect size and Zn application rate, panels (c) and (d) show the relationships between the effect size and available Zn in initial soil, panel (e) and (f) illustrate the relationships between the effect size and pH in initial soil, and panels (g) and (h) depict the relationships between the

effect size and initial grain Zn concentration. Red lines illustrate the linear or non-linear correlations between the target variable and the effect size of grain Zn concentration. Shadows denote the 95% CIs of the regression line. Circle size denotes the weight of each effect size. n in parentheses represents sample sizes. Exact P -values and source data are provided as a Source data file.

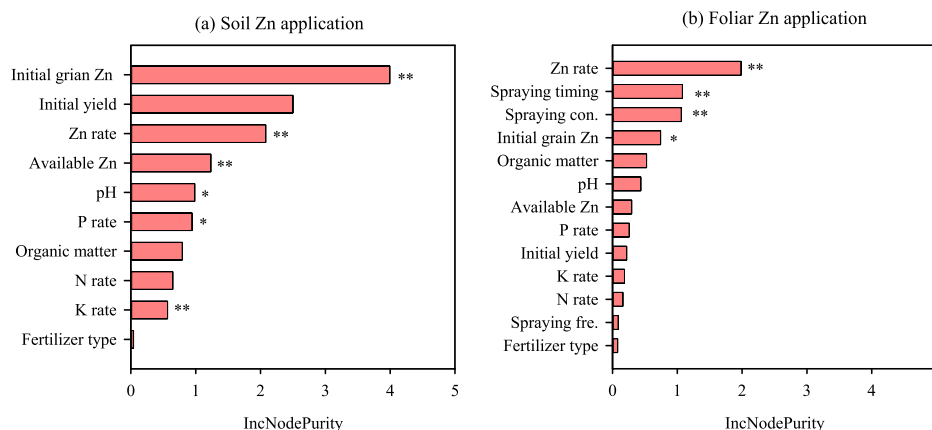


Fig. 6 | Importance of fertilization managements, initial soil properties and wheat cultivars influencing the effect size of grain Zn concentration in wheat. Panels (a) and (b) present Zn fertilizers applied to soil and foliage, respectively. Random forest models are used for regression analyses. Increase in node purity (IncNodePurity) were used to estimate the importance of these predictors, and

higher IncNodePurity values imply greater importance of the corresponding predictors. Spraying con.: spraying concentration of Zn in solution, spraying fre.: spraying frequency. * and ** indicate the importance that is significant at the $P < 0.05$ and $P < 0.01$ level. Exact P -values and source data are provided as a Source data file.

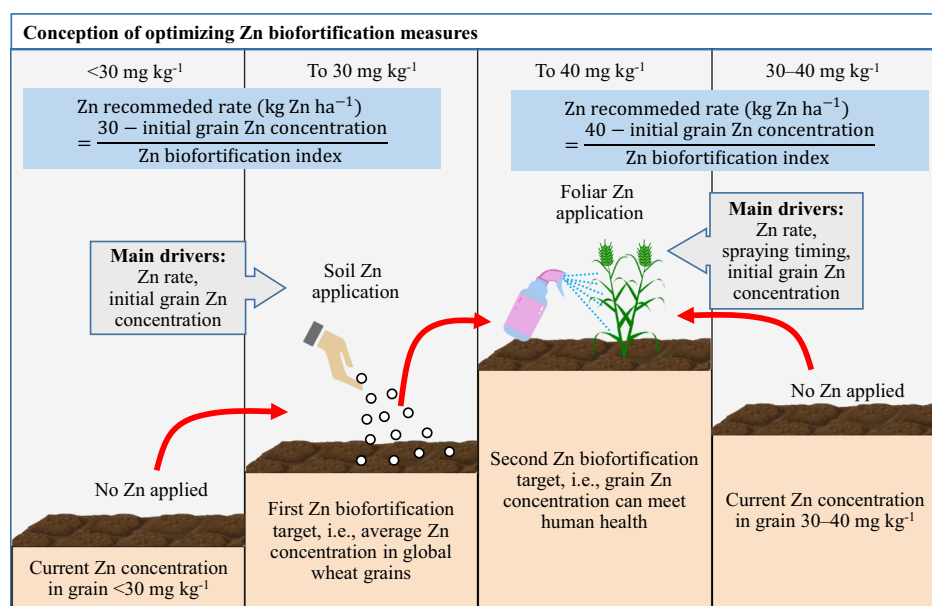


Fig. 7 | Conception of optimizing Zn agronomic biofortification measures in wheat grain. The 30 mg kg^{-1} is the first Zn biofortification target that close to the average Zn concentration in global wheat grains, and the 40 mg kg^{-1} is the final biofortification target that required for human nutrition health. The initial Zn

concentration (mg kg^{-1}) in grain is from wheat without Zn applied but other fertilizers supplied sufficiently. The Zn agronomic biofortification index is the amount of increase in Zn concentration (mg kg^{-1}) in grain with 1 kg Zn ha^{-1} applied to soil or wheat foliage.

concentration and deposition into the grain is more pronounced with foliar Zn application compared to soil application.

In our study, we utilized the gap between the Zn concentration in wheat grains grown without Zn fertilization and the Zn biofortification target (referred to as the concentration gap of Zn in grain) to determine the necessary increase in grain Zn concentration. The recommended Zn fertilizer application rate to achieve the Zn biofortification target in grain was determined by dividing the Zn concentration gap by the Zn agronomic biofortification index. This calculation method was theoretically feasible, and its reliability was verified through six field experiments conducted in China during the 2022–2023 season, where measured grain Zn concentrations were found to slightly exceed the Zn biofortification target, with a normalized root mean square error (nRMSE) ranging from 3.6% to 27.7% (Supplementary Table 1).

To provide evidence-based guidance for policymakers and optimize Zn fertilizer application in global wheat production, this study,

based on investigations of grain Zn concentrations in major wheat cropping regions of China from 2018 to 2022 and an extensive review of literature, determined the recommended Zn application rates for global wheat production. The results indicate that the average recommended Zn application rates are $10.7 \text{ kg Zn ha}^{-1}$ for soils and $0.75 \text{ kg Zn ha}^{-1}$ for foliage (Fig. 8a). Specifically, the average application rates for soils and foliage are 10.4 and $0.80 \text{ kg Zn ha}^{-1}$ in Asia, 11.7 and $0.63 \text{ kg Zn ha}^{-1}$ in Europe, 7.7 and $0.56 \text{ kg Zn ha}^{-1}$ in North America, and 17.7 and $0.70 \text{ kg Zn ha}^{-1}$ in other continents (Africa, Oceania, and South America), respectively. In China, the grain Zn concentration data suggest that a combination of soil and foliar Zn application is necessary to enhance Zn concentration in wheat grains to the 40 mg kg^{-1} threshold required for human health in dryland areas of northwest China (Supplementary Fig. 8). In regions such as northeast and southwest China, as well as the middle and lower reaches of the Yangtze River, foliar Zn application alone is sufficient in most cases.

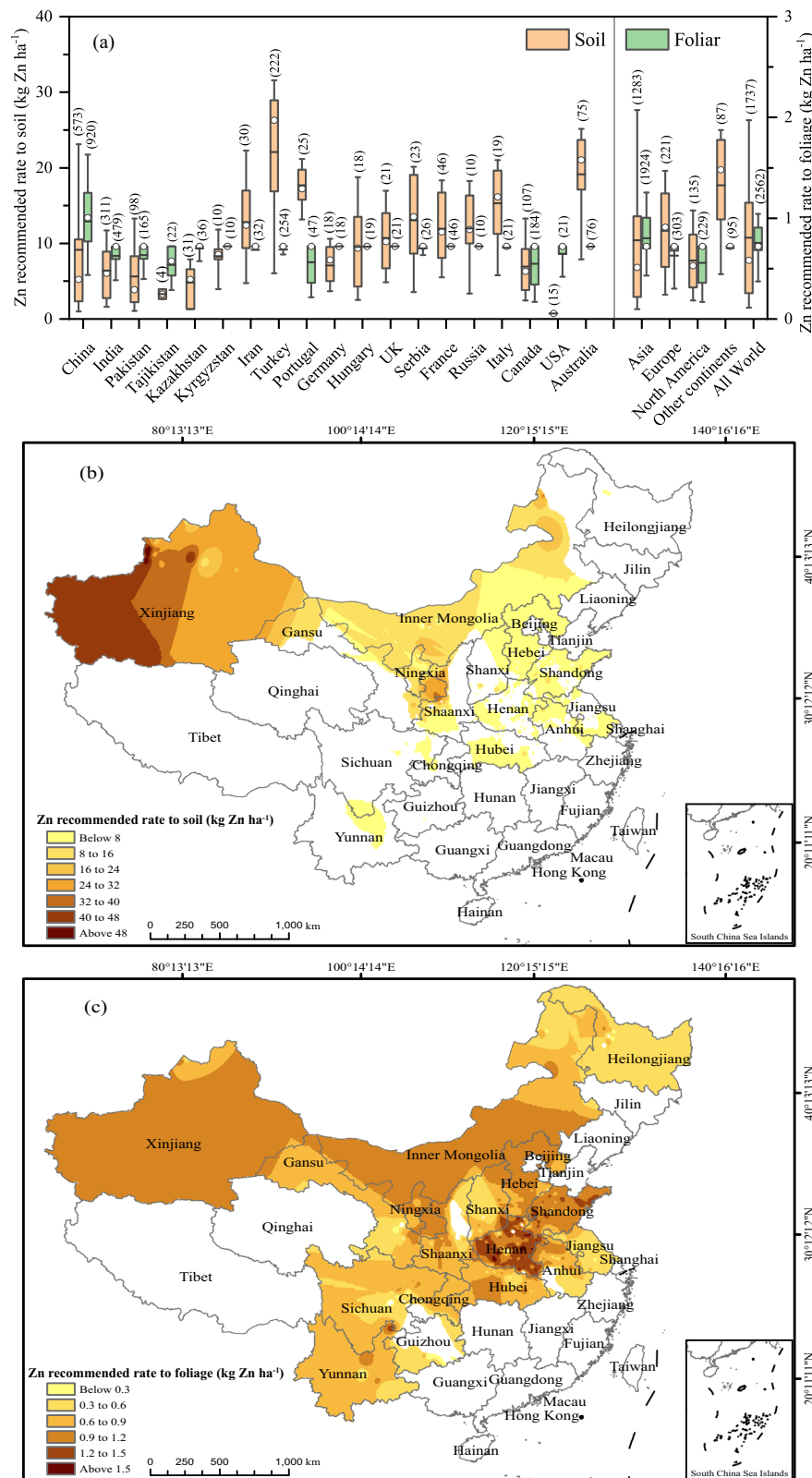


Fig. 8 | Recommended application rates of Zn fertilizer to soil and foliage in wheat production. Panel a presents global wheat, while panels b and c show China's wheat. In panel a, numbers in parentheses represent samples sizes; solid lines and white dots in boxes represent the mean and median values, respectively; box bars and boundaries denote the 10th and 90th, and 25th

and 75th percentiles of all the analyzed data. In panels (a–c), the Zn agronomic biofortification index, used to estimate the Zn recommended rate for soil application and foliar application, is based on literatures and our trials for China and the global average for other countries. Source data are provided as a Source data file.

Recommended Zn fertilizer application rates for soils range between 24–32 and 32–48 kg Zn ha⁻¹ in eastern and western Xinjiang, respectively, while in most other regions, application rates remain below 8 kg Zn ha⁻¹ (Fig. 8b). For foliar application, Zn fertilizer should be supplied at rates between 0.9 and 1.5 kg Zn ha⁻¹ in Xinjiang, Inner Mongolia, and the North China Plain (Fig. 8c).

Wheat cultivars regulating Zn agronomic biofortification in wheat grain by Zn fertilization

Breeding wheat varieties with elevated grain Zn concentration is currently recognized as a sustainable strategy to address Zn deficiency^{40,41}. However, during the breeding process, wheat trials often take place in soils with ample Zn availability, leading to newly developed varieties failing to express their high-Zn traits when cultivated in environments with limited Zn availability and stressors such as heat and drought⁶. Shuttle breeding, a method involving the alternating growth of wheat breeding material across diverse environments, has been advocated for selecting wheat varieties with enhanced grain Zn concentration. This approach ensures that newly developed wheat varieties exhibit adaptability under varied production conditions⁴⁰. In this study, varieties exhibiting high Zn concentration in wheat grains grown in Zn-deficient soils (i.e., soils without Zn supplementation) were defined as high-Zn varieties, aligning with current breeding objectives for high-Zn varieties⁶. Moreover, our meta-analysis and regression analysis with random forest underscored the significance of wheat variety as a key factor influencing the improvement in grain Zn concentration through Zn fertilization. According to our recommended Zn application rate calculation method, high-Zn varieties require lower Zn application rates compared to low-Zn varieties under identical growing conditions. This is because high-Zn varieties facilitate the release of phytosiderophores from roots and possess robust root systems, including a large root surface area, which enhances Zn mobilization in the soil and facilitates Zn uptake by the roots^{42–44}. Therefore, to promote the cultivation of high-Zn varieties in wheat-growing regions with Zn-deficient soils, promoters should advise growers to appropriately reduce the amount of Zn fertilizer applied to the soils.

Spraying timing of Zn fertilizer regulating Zn agronomic biofortification in wheat grain by Zn fertilization

Under foliar Zn application, the timing of spraying significantly influences on the enhancement of wheat grain Zn concentration. Spraying Zn during the late growth stages results in a greater increase in grain Zn concentration compared to spraying during the early growth stages. This amplified effect during the late growth stages is attributed to the heightened remobilization of Zn stored in vegetative organs when root absorption is constrained^{45,46}. Wheat cultivation is prevalent in arid and semiarid regions characterized by dry weather during the late stages of wheat growth, leading to restricted water availability in the topsoil and consequently limited Zn availability. In addition, root activity and growth in wheat declined after the booting stage due to decreased allocation of photo-assimilates to the roots⁴⁷. Given the diminished Zn absorption by roots from the soil during the late crop growth stages, Zn accumulation in wheat grain relies primarily on the remobilization of Zn stored in vegetative organs^{6,19}. It is estimated that up to 96% of the Zn in wheat grain from remobilization of the Zn stored in vegetative organs before flowering⁴⁵. Consequently, foliar Zn applications after wheat flowering are more effective in increasing grain Zn concentration than those made during the early growth stages. In wheat production, it is advisable to integrate Zn application with pesticide application during the late growth period (e.g., anthesis and early filling stages)²⁸. This approach not only boosts Zn uptake in wheat grain but also reduces input cost and enhances growers' net income.

Soil Zn availability and pH regulating Zn agronomic biofortification in wheat grain by Zn fertilization

The percentage increase in grain Zn concentration due to Zn fertilization declined with rising soil Zn availability but improved with higher soil pH levels. Furthermore, the regression analysis using random forest revealed that the impact of soil available Zn and pH on the rise in grain Zn concentration through soil Zn fertilization was more pronounced than that through foliar Zn application. This finding aligns with the observation from Zou et al.¹⁴ that grain Zn concentration in wheat exhibits greater sensitivity to soil conditions under soil Zn fertilization compared to foliar Zn application. The primary factor constraining Zn uptake and accumulation in wheat is the low availability of Zn in the soil^{6,12}. Indeed, in major wheat-producing regions, grain Zn concentration in wheat correlates positively with Zn availability⁴⁸. Soil pH influences Zn solubility in the soil. At high pH values, the solubility of Zn in the soil is reduced by enhancing the absorptive capacity of Zn, due to forming hydrolyzed Zn forms, precipitation of iron oxides, and strong chemisorption with CaCO₃^{12,13,49}. The pH in the 0–1.0 m soil layer is negatively correlated with grain Zn concentration in rainfed wheat cultivation⁵⁰. This implies that the limited increase in grain Zn concentration by Zn fertilization to the soil with high Zn availability may be linked to the already high grain Zn concentration in the absence of Zn application. Conversely, in soils with high pH levels, the significant increase in grain Zn concentration is associated with reduced soil Zn availability, resulting in low grain Zn concentration in the absence of Zn application. To achieve a substantial enhancement in grain Zn concentration in wheat production, Zn fertilizers should be applied to soils with low Zn availability and high pH values. For an increase of more than 20% in wheat grain Zn concentration, Zn fertilizer application is recommended to soils with available Zn levels below 0.5 mg kg⁻¹ and pH levels between 6.4 and 8.6 (Fig. 5c, e).

NPK fertilizer management regulating Zn agronomic biofortification in wheat grain by Zn fertilization

Nitrogen, P and K have been reported to have significant relationships with Zn in crops. The application of N fertilizers is considered a promising strategy for agronomic biofortification of Zn in cereal grain, owing to the synergistic relationship between N and Zn in crops^{51–54}. However, P fertilization has been found to negatively impact the grain Zn concentration of cereal crops, due to the antagonistic interaction between P and Zn in the soil-plant system^{55–57}. In this meta-analysis, it was observed that the percentage improvement in grain Zn concentration due to Zn fertilization decreased with increasing N rates. Conversely, with increasing P rates, the percentage improvement first increased and then decreased, reaching its peak at medium P rates. These findings suggest that agronomic biofortification of grain Zn through increased N application rates should be accompanied by enhanced Zn inputs, particularly in soils with poor Zn availability. Insufficient Zn supply can result in a Zn deficiency response as crop demand for Zn increases^{58,59}. Regarding P fertilization in wheat production, optimizing P management is recommended. Appropriate P application levels can provide more opportunities for roots to absorb Zn from the soil and mitigate P-Zn antagonism by promoting root development and improving root morphology^{55,60}. The effect of K fertilization on grain Zn concentration in wheat varies across published studies^{61–63}. As demonstrated in this study, K application had different effects on the improvement of grain Zn concentration in wheat. When Zn fertilizer was applied to the soil, the improvement in grain Zn concentration was minimal under medium K rates. However, under foliar Zn application, the greatest improvement in grain Zn concentration was observed at medium K rates. Further research is needed to understand the mechanism of K and Zn interaction in soil-plant systems and the relationship between soil K supply and crop Zn uptake and accumulation⁴⁸.

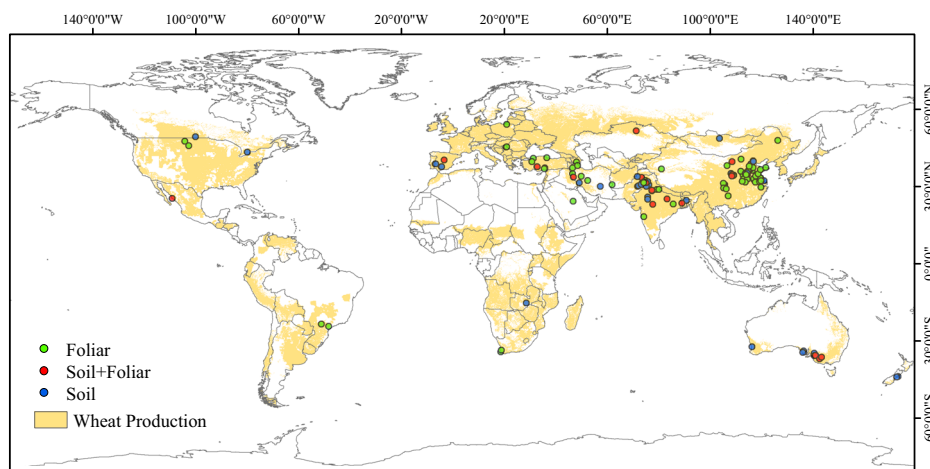


Fig. 9 | Locations of the trial sites in the meta-analysis. Yellow shadings represent the regions of wheat production (obtained from <http://www.earthstat.org/harvested-area-yield-175-crops/>). Blue, green, and red points denote the trial sites

where Zn fertilizers are applied to soil, foliage, and soil+foliage, respectively. Source data are provided as a Source data file.

Significances and limitations of this study

Global data suggest that a slight increase in grain Zn concentration can be achieved by applying Zn fertilizer to the soil. However, foliar spraying and soil+foliar application of Zn fertilizer can elevate grain Zn concentration to the 40 mg kg⁻¹ threshold required for human nutrition. The Zn rate, initial Zn concentration, and timing of Zn spraying were identified as the most crucial factors influencing the improvement in grain Zn concentration. Based on the Zn biofortification target, the Zn agronomic biofortification index, and the contribution of these influential factors, this study proposed a method for estimating Zn application rates. This method enables growers to conveniently determine the optimal Zn application rates based on the Zn concentration in their wheat grains. To achieve the desired grain Zn concentration and high grain yield, soil+foliar application should be considered for wheat with grain Zn concentration below 30 mg kg⁻¹, and for wheat with grain Zn concentration exceeding 30 mg kg⁻¹, only foliar application is recommended. For a more significant increase in grain Zn concentration, Zn fertilizer should be applied to soils with available Zn levels below 0.5 mg kg⁻¹ and pH between 6.4 and 8.6. In addition, foliar spraying should be conducted during the late wheat growth stages, such as at anthesis and early filling stages.

Most of the literature collected in this study did not provide data on soil texture and soil moisture management during the crop growth period, making it impossible to determine their effects on the improvement in grain Zn concentration in wheat through Zn fertilization. Furthermore, with the expansion of agricultural operations, especially in developed countries and China, and the advancement of fertilization technology (e.g., fertilizer and water integration technology and drone-based fertilization technology), farmers' attitudes toward Zn fertilizer application have evolved. Many farmers are now opting to use drones for spraying Zn fertilizer on cereal crops due to reduced labor input costs and improved operational efficiency compared to manual spraying. In addition, Zn-rich grains and their products command higher prices compared to those with low Zn concentration. This shift poses a challenge to our current calculation method for Zn recommended rates. In the future, we need to investigate whether the Zn agronomic biofortification index changes under new fertilization techniques and assess the effectiveness of the calculation method under these conditions.

Methods

Data search and collection

A search of peer-reviewed publications conducted prior to November 2021 aimed to evaluate the grain Zn concentration in global wheat

grown without the application of Zn fertilizers. Data published in English were gathered from the Web of Science (<https://www.webofscience.com/wos/alldb/basic-search>) and the China National Knowledge Infrastructure (<https://kns.cnki.net/>). The search query used was (grain zinc concentration AND wheat). The Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines (Supplementary Fig. 9) were adhered for data collection. The screening criteria for publications were as follows: (1) trials were conducted in field settings, excluding pot trials and solution culture trials; (2) wheat crops and soil were not provided with Zn fertilizers; (3) studies reported grain Zn concentration in wheat. Following these criteria, a total of 188 publications (137 in English and 61 in Chinese), containing 2771 observations from 279 sites, were compiled into the dataset (Supplementary Data 1).

Compiling data of the grain Zn concentration in wheat with Zn application

The database consisted of two components. The first part was from four field trials previously conducted by our team, consisting of one trial in which Zn fertilizer was applied to the soil at the planting of winter wheat, and three trials in which Zn fertilizers were applied to the foliage of winter wheat. The second part of the database was from literature reviewed by peers. A total of 85 publications for Zn fertilizer applied to the soil, 83 for foliar application and 28 for soil+foliar application of Zn fertilizer was retained in the dataset, encompassing 637, 765, and 191 paired observations for grain Zn concentration, respectively (Supplementary Data 2–4). These data were sourced from studies conducted in 19 countries across East, West, and South Asia, North America, South America, Europe, Southern Africa, and Oceania (Fig. 9).

Field trials. The first trial was a soil Zn application trial conducted during the 2010–2011 and 2011–2012 growing seasons in Yongshou County (39°49'N, 108°10'E), Shaanxi Province, China. This field trial comprised six rates of Zn fertilizer (0; 2.3; 5.7; 11.4; 22.7; 34.1 kg Zn ha⁻¹). The second trial was a multi-site trial of foliar Zn application, which included two treatments: no spray and 1.36 kg Zn ha⁻¹ sprayed onto the crop. This multi-site trial conducted during the 2010–2011 season in 31 sites in the main wheat-producing areas, encompassing 14 provinces or cities in China. The third and fourth field trials were long-term site-fixed trials of winter wheat, in which different rates of N fertilizer (0; 80; 160; 240; 320 kg N ha⁻¹) and different rates of P fertilizer (0; 22; 44; 66; 88 kg P ha⁻¹) were applied to the soil, respectively.

The site-fixed trial was initiated in 2004 in Yangling, Shaanxi, China (34°18' N, 108°05' E). The third and fourth field trials on foliar Zn fertilizer were conducted during the 2011–2012 and 2012–2013 growing seasons, employing 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. Zinc sulfate was the Zn source applied to the soil or foliage in all four trials. Further detailed information on the field trials is provided in the Supplementary Method 1. The data from the field trials are presented in the database as an annual average for each treatment.

Data compilation. Peer-reviewed publications on Zn application before November 2021 were also searched on the Web of Science and China National Knowledge Infrastructure to evaluate the response of wheat grain Zn concentration to Zn fertilization. The search formulation was ((grain zinc concentration OR zinc biofortification OR zinc nutrition) AND (wheat) AND (zinc application)). The PRISMA guidelines (Supplementary Fig. 10) were followed for data collection. The criteria for screening publications were as follows: (1) field trials, excluding both pot trials and solution culture trials; (2) the supply methods of Zn fertilizer including soil application, foliar spraying, or soil application + foliar application; (3) studies reporting at least one pair of data with Zn fertilization and without Zn applied (a control treatment), with identical management measures; (4) studies reporting grain Zn concentration in wheat; (5) for data from the same trial reported in multiple publications, we combined the data from relevant publications to obtain a complete dataset.

For each of our studies and publications, we extracted geographic location, available soil Zn, organic matter, CaCO₃, mineral N, P, K and pH, as well as the number of replicates, Zn fertilizer type, application method, rate, timing and frequency of application, and N, P and K fertilizer application rate, together with wheat grain yield, biomass, and Zn concentration in grain and straw. The data presented in figures were extracted using the GetData Graph Digitizer 52.

Data categorization. To investigate the effect of soil properties and fertilization management (explanatory variables) on the response of grain Zn concentration to Zn fertilization (response variables), the extracted data were pre-grouped for each explanatory variable to facilitate statistical analysis. Application rates of Zn, N, P, and K fertilizers were grouped into low, medium, and high categories. For Zn fertilizer, the corresponding rates were categorized as <8, 8–15, and >15 kg Zn ha⁻¹ for soil application, and <1.5, 1.5–3.0, and >3.0 kg Zn ha⁻¹ for foliar application. The corresponding rates for N, P, and K fertilizers were <110, 110–180, and >180 kg ha⁻¹ for N, <26, 26–44, and >44 kg ha⁻¹ for P, and <25, 25–58, and >58 kg ha⁻¹ for K, respectively. Spraying timing of Zn fertilizer included five stages: pre-booting, booting, heading, flowering, and grain-filling. There were 12 types of Zn fertilizers used in the trials; however, for the sake of representativeness of the sample size, only ZnSO₄, ZnO, and Zn-EDTA were analyzed. Initial Zn availability (DTPA-Zn) in soils was categorized as deficient (≤0.5 mg kg⁻¹) and non-deficient (>0.5 mg kg⁻¹)¹². Soil pH value and CaCO₃ content before sowing were divided into ≤7 and >7 for pH, and ≤15% and >15% for CaCO₃. Initial organic matter content was grouped into low (<10 g kg⁻¹), medium (10–20 g kg⁻¹), and high (>20 g kg⁻¹). Sample sizes were small for other initial nutrients (N, P, K) in soil; hence, they were not grouped. Wheat cultivars were classified based on initial grain Zn concentration and grain yield (without Zn application). Regarding Zn concentration, wheat was categorized into four cultivars: very low (<20 mg kg⁻¹), low (20–30 mg kg⁻¹), medium (30–40 mg kg⁻¹), and high (>40 mg kg⁻¹). According to yield levels, there were also four cultivars: very low (<2000 kg ha⁻¹), low (2000–4000 kg ha⁻¹), medium (4000–6000 kg ha⁻¹), and high (>6000 kg ha⁻¹).

Data analysis. The Zn agronomic biofortification index quantifies the increase in grain Zn concentration (mg kg⁻¹) resulting from the application of 1 kg Zn ha⁻¹ to the soil or wheat foliage. Its calculation method

is as follows:

$$\text{Zn agronomic biofortification index} = \frac{\text{GZn}_t - \text{GZn}_c}{\text{Zn rate}} \quad (2)$$

where GZn_t and GZn_c are the grain Zn concentration (mg kg⁻¹) of the Zn application treatment and the control, respectively, and the Zn rate (kg Zn ha⁻¹) indicates the amount of Zn fertilizer applied.

The efficacy of Zn application on concentration and accumulation of Zn in wheat, and grain yield and biomass was evaluated using the effect size, which was the natural log of response ratio (ln RR) and calculated as follows⁶⁴:

$$\text{Effect size} = \ln \text{RR} = \ln \frac{\bar{X}_t}{\bar{X}_c} \quad (3)$$

where \bar{X}_t and \bar{X}_c are the averages of the variable under the Zn application treatment and the control treatment, respectively.

The weight of each effect size was estimated as follows:

$$\text{Weight} = \frac{n_t \times n_c}{n_t + n_c} \quad (4)$$

where n_t and n_c are the numbers of replicates of the Zn application treatment and the control treatment, respectively.

A bootstrapping resampling procedure with 4999 iterations was conducted using R 4.1.3 to estimate the weighted effect sizes and the corresponding confidence intervals (CIs) at a 95% level. To facilitate comprehension and interpretation of the meta-analysis results, the weighted effect sizes and the CIs were exponentially transformed and then presented as the percent change in response variables following Zn fertilization. The calculation equation is as follows:

$$\text{Percent change(\%)} = (e^Y - 1) \times 100 \quad (5)$$

where Y represents the weighted effect sizes or the CIs. If the transformed CIs do not intersect zero, it indicates that the Zn application treatment differs significantly from the control treatment. Likewise, significant differences exist among subgroups if their transformed CIs do not overlap.

The frequency distribution of effect sizes for wheat grain Zn concentration was analyzed using a Gaussian distribution function, indicating that the dataset exhibited homogeneity and conformed to a normal distribution (Supplementary Fig. 11). Subgroup analysis and meta-regression were subsequently performed to determine the factors influencing the response of grain Zn concentration to Zn fertilization (i.e., the effect size of grain Zn concentration). In addition, a regression analysis using random forest (RF) was conducted to identify the primary drivers influencing the effect size of grain Zn concentration. In these RF models, soil properties and management practices were utilized as predictors for the response variable, and the increase in node purity (IncNodePurity) was employed to assess the importance of each factor. The IncNodePurity metric was calculated based on the sums of squares of residuals, reflecting the contribution of each predictor to improving the model's accuracy. Higher IncNodePurity values denote greater importance of the corresponding predictors⁶⁵.

The significance of the RF models and the R² values were determined using the “A3” package in R 4.1.3, which can provide a robust calculation for R² values through comparing against a null model. The key procedures include: (1) set the model.fcn=randomForest to generate RF models; (2) set the p.acc = 0.01 to replace 100 times of permutation; (3) use “model.args” function to pass the parameters to RF models⁶⁶. The significance of each predictor variable was assessed using the “rfPermute” package in R 4.1.3. In order to construct null distribution and calculate P-values, the key parameters that need to be set include: (1) the

importance=TRUE to determine the importance of predictor variables that need to be assessed; (2) the ntree = 1000 to grow 1000 trees for Random Forest models; (3) the num.rep=100 to replace 100 times of permutation⁶⁶. The R code for the meta-analysis and random forest analysis is provided in the Supplementary Software. Correlation analyses were conducted using SPSS Statistics 26.0 to examine the relationships among the variables. ArcGIS 10.8, Origin 2021, and SigmaPlot 12.5 were utilized to create relevant figures in this study.

Validation trials

Based on the findings from meta-analysis and regression analysis, this study proposes a calculation method for determining the recommended application rate of Zn to soil and foliage. To validate this method, six field trials were conducted during the 2022–2023 season at six sites in China. These trials consisted of three trials involving the application of Zn fertilizer to soil and three trials involving the application of Zn fertilizers to the foliage of wheat. Zinc agronomic biofortification indexes were calculated for each site based on studies published near the experimental sites. Additional detailed information regarding the field trials can be found in the Supplementary Method 2 and Supplementary Data 5. The normalized Root Mean Square Error (nRMSE) was utilized to quantify the relative difference between estimated and reported data, defined as follows:

$$\text{nRMSE} = \sqrt{\frac{\sum_{i=1}^n (E_i - M_i)^2}{n}} \times \frac{100}{M} \quad (6)$$

where M is the mean value, M_i and E_i are the reported data and estimated data, respectively, n denotes the numbers of replicates confirmation trials.

Furthermore, in order to assess the Zn concentration in wheat grains across China, our team collected 1031 wheat grain samples from farmers' fields in the primary wheat cropping regions of the country over a five-year period from 2018 to 2022 (Supplementary Data 6). Details regarding the sample collection process can be found in the Supplementary Method 2. Utilizing the grain Zn concentration data obtained, this study estimated Zn application rates using the calculation method for recommended Zn rates. These findings aim to offer guidance for wheat production practices.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

All data generated in this study are provided in the Supplementary Information files. Source data are provided with this paper.

Code availability

The code for data analysis that supports the findings of this study is provided with this paper as Supplementary Software 1 and 2.

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Author contributions

Z.H.W. designed the research. X.L.H. and L.C.L. collected samples and data, performed data analysis, created the figures, and wrote the first draft of the manuscript. L.C.L., Y.L.C., and J.A.P. contributed substantially to revisions.

Competing interests

The authors declare no competing interests.

Additional information

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