nature portfolio

Peer Review File

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

Corresponding Author: Professor Zhaohui Wang

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The manuscript “Zinc biofortification in wheat and its drivers: A meta-analysis- NCOMMS-23-07703” highlights factors governing zinc (Zn) supply in global wheat production systems. Agronomic biofortification of wheat with different micronutrient fertilizers for improved grain micronutrient concentration is an important strategy in alleviating dietary micronutrient deficiencies. The manuscript is supported by a sound methodology and an extensive literature review and data extraction. The meta-analysis was mostly focused on soil and management factors with a limited focus on environmental factors such as agro-ecological region, climatic zone, rainfall etc, factors also known to influence agronomic biofortification and Zn bioavailability. Authors did an incredible job of extracting data from global wheat agronomic biofortification field studies which they supported with their own field data sets. I find the evidence generated confirming background understanding of factors governing Zn supply (i.e. soil pH, organic matter, Zn fertilizer application methods, timing etc), with an additional novel approach on Zn fertilizer management. I particularly found the authors' focus on use of different Zn fertilizer management options based on initial grain Zn concentration novel, and is crucial in guiding ongoing agronomic biofortification interventions in different agro-ecological regions and socio-economic settings. This evidence is supported by well-constructed graphs in the main text and supplementary files. The authors provide a detailed discussion section which ends by highlighting different Zn fertilizer application methods to be used for grain Zn concentrations less than and/ or greater than 30 mg kg-1. This helps in reducing fertilizer costs and potential toxicities from excess fertilizer application. Rates of fertilizer to be applied per litre for foliar fertilization are provided by the authors for additional guidance. I, however, find the discussion rather too long as authors presented commonly known factors limiting or enhancing Zn uptake and concentration. I suggest authors ensure that the novelty of their meta-analyses (presented last in their discussion section) is not hidden among commonly known background information on factors governing Zn supply. The novelty of the analysis could be elaborated on a bit more (instead of a few lines at the end of the discussion), with less emphasis on what we already know (i.e. reduction in costs from co-application of Zn and pesticides which was published a few years back and superiority of soil + foliar versus soil, or foliar applications which most agronomic biofortification studies tend to discuss). I end by emphasising that the need for different Zn application methods for wheat grain with different Zn concentrations is novel and very interesting. This novel message warrants publication of the manuscript in Nature Communications. I however think this message is presented a bit too late in the manuscript. I did not see any study strengths and limitations section or conclusion section, maybe this is per Nature Communications guidelines. I include here and also attach as pdf file, detailed comments for authors to revise and improve clarity of their manuscript. The writing of the manuscript can also be improved in some areas as indicated in my detailed comments.

Detailed Comments:

Zinc biofortification in wheat and its drivers: A meta-analysis- NCOMMS-23-07703

Reviewer’s comments
Title
1. Biofortification is mainly used to describe conventional genetic breeding for crops with higher concentrations of micronutrients. I suggest authors revise title to “Zinc agronomic biofortification in wheat and its drivers: A global meta-analysis”.
2. I suggest global here as authors did not restrict on region when accessing papers from web of science.
3. Also change biofortification to agronomic biofortification throughout the manuscript.

Abstract
1. Line 20-Change grain Zn content to grain Zn concentration and also throughout the manuscript.
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3. Line 43: delete repeated word "in"
4. Line 45: start with "The deficiency in Zn.." or "Zinc deficiency…" instead.
5. Line 49: Wheat-provide Latin or scientific name
6. Line 55 to 57- information on factors governing Zn supply in soils is too limited here. I appreciate factors like pH, and P were mentioned in later paragraph, but I suggest authors also focus on OM and its effect on Zn availability.
7. In the entire introduction, risks of Zn deficiency to human health i.e. physical impairments, stunting are lacking. I suggest authors also focus on this.
8. Authors using ~ to denote range instead of –. Check and change throughout the manuscript.
9. Lines 85 to 87 are not reading well. I suggest revising and check the rest of the manuscript for words which need to be removed of included for sentence flow.
10. Line 97- change in globally wheat production to in global wheat production

11. Aim 1b on target of grain Zn biofortification. This cannot be an aim as target of grain Zn biofortification in wheat is already known (40-60 mg kg-1). See Cakmak, 2008. Revise for clarity.
12. Line 99: As authors mention about soil management, it is important they mention effects of OM on Zn availability as in my earlier comment for the introduction.
Methods
1. Line 512: Revise to read "A search of peer reviewed publication.." and delete "for"
2. 513: Change grain Zn nutrition to grain Zn concentration. Also check throughout manuscript.
3. 518: Change trails to trials
4. Line 519 criteria 2: why were wheat crops and soils without Zn supplied selected? Were authors looking for baseline concentrations? If so, elaborate for clarity as readers will wonder why given this is an agronomic biofortification focused paper.
5. Line 532: As Africa is not mentioned as one of the regions, I am surprised the Zou et al 2012 paper on wheat in Zambia did not come up in your search. I see the data in Figure 1 but Africa is not mentioned here.
6. Line 534: Revise over the in
7. Line 536: What was the form of Zn supplied? Sulphate, oxide,
8. How is line 512 to 513 different from details in Lines 551 to 554? What is Zn nutritional level. I wonder these need to be merged.
9. Line 553: search term Zn biofortification implies breeding for higher micronutrients. I wonder if many papers were excluded because of this term and some on zinc agronomic biofortification not included at all?
10. Line 557: insert "at least"
11. Lines 579-580: which method was used to denote the ≤0.5 mg/kg as deficient. The DTPA and EDTA methods have different critical limits.

Figures
1. Figure 2a: something on top of the right legend in Chinese. Revise.
2. Figure 2 caption: Explain the meaning of CK in the caption/ figure title.
3. Figure 3b: Folial Zn to Foliar Zn
Results
1. Line 108- Revise gain to grain
2. Line 110- The value of 34.0 mg Zn kg-1 in North America, what could be the reason for such a larger value than in other continents? Is it a dilution effect from biofortified grain i.e. Zincol-16 in some countries i.e. Mexico? Would be good if additional details can be provided.
3. Line 114-Similarly, for 17.7 for west Asia. Any explanation how this was much lower than the other Asian regions.
4. Line 115: insert South America, after Mexico
5. Line 116: Revise "the average Zn" to an average Zn, "above the" to above the 40 mg kg-1 threshold, "required by human nutrition" to required for human nutrition.
6. Line 117 to 118- Why the reference of the 64 countries to a mean value of 28.2 mg kg-1. Is it because it is the mean for Central Asia? When authors mention "less than 28.2 mg kg-1", is it not better to make the reference to the critical threshold of 40 mg kg-1?
7. Line 119-122: This sentence will be more relatable if readers could get an understanding of why 28.2 was used as a reference earlier.
8. Line 127: increased by only 29.1%. I suggest delete the word only as 29% is not so little when it comes to grain Zn nutrition. What is the baseline control here that the authors are comparing the increase to. Similarly for foliar values (Line 129-131).
9. Line 133- revise "required by human nutrition" to required for human nutrition
10. Subheading title on line 134: I am not entirely sure if this needs to be revised from "on increase effectiveness" to on increasing effectiveness. I realise later in the manuscript that authors occasionally use the word increase effectiveness (Line 140 etc). It would be good if this term is defined for clarity.
11. Line 140: It would be good if the application rates increasing grain Zn can be mentioned here.
12. Line 154-155: Were SO4 and EDTA applied at the same rate? It is common knowledge that EDTA and any other chelates out-perform sulphates and oxides. Could other factors i.e. rate, environmental factors have influenced this?
13. Line 168: revise "increased the increased percentage"
14. Line 171: K-write in full at start of sentence

15. Line 167-69: Is this implying then that for farmers who cannot afford Zn fertilizer, P application is an alternative to increasing grain Zn by a margin. My understanding is P inhibits Zn uptake. What could be the mechanistic approach here that resulted in P increasing grain Zn in the absence of Zn fertilization?
16. Line 165 to 166: Also explain the term “greatest increased percentage”
17. N,P,K categorization rates are not mentioned in the data categorization section in the Methods but occasionally referred to in Results Lines 170-75. Can these be mentioned in the Methods section.
18. Line 187-188: Again here, I do not understand what the phrase “was the most increase effectiveness” means.
19. Line 195-Here organic matter is in mg kg-1. Revise units.
20. Line 195: the grain Zn concentration be increased by a maximum. This is not clear, I do not understand.
21. Line 181: Revise “When Zn is” to when Zn was. Also check throughout the manuscript for when present tense was used instead of past tense.
22. Line 203-Has a negligible to “had a negligible”

Discussion

1. Line 244- Revise “only by” to “by only”
2. Line 247- Straw is mentioned here and presented in a Figure but not mentioned anywhere in the abstract, introduction, methods. It appears to readers that the main focus is on grain only when other plant tissue data are presented.
3. Line 256- Zn: start sentence with “Zinc” in full and also for K in line 354 and anywhere else in the manuscript.
4. Line 258-Revise was to is.
5. Is Zn only translocated to the grain under conditions of insufficiency in the root system. Is this not a natural mechanistic pathway for Zn which happens towards crop maturity and is very different from Nitrogen which is not translocated as the crop matures. I would like to argue that Zn translocation to the edible portion happens even under conditions of sufficient Zn in the soil.
6. Line 266-Revise the phrase “due to that”
7. Line 303-4: Revise “always that” to “ensuring that”
8. Line 336: Revise “is associated to” to “is associated with”
9. Line 345: insert space between P and ha-1
10. Authors did not clearly mention the mechanism around decreased Zn with increased Nitrogen. They only mentioned the mechanism around increased Zn with N.
11. Line 405: Any suggestions on optimal ranges for soil Zn and pH?
12. Line 418: Revise “having a wide range” to “would have a wide range”
13. Line 493: Revise “can achieve” to “can be achieved by”
14. I found it rather interesting that the authors state methods to be used for grain Zn concentrations < and > 30 mg kg-1. This helps in reducing fertilizer costs and toxicities. Rates of fertilizer per litre provided by the authors provide guidance. I, however, find the discussion rather too long as authors presented commonly known factors limiting or enhancing Zn uptake and presented this important point last (from lines 472). I suggest making sure this point is elaborated on a bit more with less text on what we already know (i.e. reduction in costs from co-application of Zn and pesticides which was published a few years back). The application methods for grain with different Zn concentrations is novel and very interesting. I think this message is presented a bit too late in the manuscript.
15. No conclusion section, Is this per Journal guidelines?

References, Figures, Tables, Supplementary files

1. Check use of brackets on reference 82. Alloway.
2. I did not see reference 91 in the main text.
3. Figure 1 caption: Authors did not mention in the legend what the blue line and number represent. I understand this is mentioned in the main text only.
4. Figure 2 caption: Revise to “Grain Zn concentration of wheat grown with Zn fertilizers applied as soil (a)…
5. Second and 3rd sentence of Figure 2 caption not reading well. Check language on Figure 2a
6. Figure 3b, revise Foliarl
7. Sentence 3 of Figure 3 caption not reading well (Line 898-900)
8. Similarly for line 905-907 of Figure 4
9. Figure 6: I would propose that the x-axis is the same for both soil (currently 0-5) and foliar (currently 0-2.5)
10. First part of line 912 is not reading well. Revise Important of to “Importance of”
11. Figure 6 shows spray timing and Zn rates as important factors in foliar application but Figure 7 indicates Zn rates and initial grain Zn concentration in the far right column. I did not totally understand why.
12. Similarly for the far left column for soil application which shows Zn rate and initial grain Zn concentration as important factors in Figure 7 but initial grain Zn and initial grain yield in Figure 6. Is there a difference between relative importance (Figure 6) and main drivers (Figure 7)?
13. Figure 7b: I can barely see the different shades of green.
14. Figure 7 has 3 captions, a, b, c but in main text authors only write Figure 7 without indicating if 7a, b or c (Line 235-240)
15. Similarly for Figure 2 which has 4 sub Figures (Line 126)

I see no Tables used in the manuscript.

Data sets

They are good to me.

Supplementary files

1. File . Could be important to mention software used for plotting graphs in the “Software and code” section or in main methods section of the manuscript. Also check and correct language in this supplementary file i.e. Line 5 of study description “wheat grain Zn concentration supplied with Zn fertilizers to soil, foliage. Similarly for Research sample section Line 5 “wheat supplied Zn fertilizers to t… “ to read “wheat supplied with Zn fertilizers to t...

2. Sampling strategy section: revise trails to trials
3. These are the few examples I managed to spot.
4. Supplementary file . Screening flow chart box 2 “records after duplicates” Fig. S7. I do not totally understand. Does this mean after duplicates were removed or records including duplicates. It reads incomplete to me. Similarly for Fig. S8. Figure S7 far left bottom box uses “Include” but S8 uses “Included”
5. Supplementary file seems OK. Check Figure captions, language as per my earlier comments.

Reviewer #2

(Remarks to the Author)
This manuscript describes the meta-analysis of the biofortification effect of foliar or soil Zn application, or the combination, on wheat grain Zn concentrations. While the meta-analysis is nice and certainly noteworthy, there is always the risk that the conclusions drawn from the analysis are ambiguous, based on data collected under different conditions, that not always allow direct comparison. In the abstract, the authors indicate that validation experiments have been performed to confirm the findings from the meta-analysis. Much to my surprise, I could not find such validation experiments in the results, but based on the discussion, it appeared that these experiments had been performed already a long time, ~10 years, ago (L. 476-492). Perhaps I misunderstood this completely, but if not, I find it hard to see how such validation experiments could have been performed long before the actual meta-analysis was performed, and still justify the conclusions drawn by the authors. In any case, where one would expect new validation experiments to be performed with the suggested Zn fertilization levels and applications, to confirm the finding derived from the meta-analysis, this is not obvious from section 2.4. Also how the authors come to the final conclusion, as written in L. 232-235, and their recommendations (l. 236-240), or the conception shown in fig 7, is not clear to me.

Furthermore, I have serious concerns about several statements made in the discussion.
E.g., authors suggest that soil used for wheat generally has high pH and high calcium carbonate levels, contributing to Zn availability issues, but I wonder if this is true. There are for sure also many areas which do not suffer from such conditions, on which wheat yield is high, even though this certainly often does not go along with high grain Zn concentrations. Most of the trial sites that provided the data for the meta-analysis, as shown in fig 8, are from areas with such unfavorable conditions, probably as these result in low yield and poor Zn levels, which inspired Zn fertilization trials. However, these do not appear to be representative for all wheat production areas.

The section written in L. 259-271 is puzzling. There will be different degrees of Zn deficiency in wheat production areas. If Zn is limiting, yield will be low, and the grain Zn concentration will likewise be below target. If Zn is not limiting, and neither are other nutrients, additional supply will not be beneficial, biomass production will be high, as will grain Zn concentrations. Between these extremes, there will be levels at which there is enough Zn to support plant biomass production and moderate grain yield, but grain Zn concentration may still be (far) below target. In such situations, foliar application may not spur root system development, and thus not contribute to high grain yield, only to elevate grain Zn concentration. In more Zn limiting conditions soil Zn application may be foremost needed to feed biomass production. Such more elaborate evaluation of current practices may be needed here.

Authors claim that overuse of Zn fertilizers will cause Zn accumulation in the soil, leading to environmental risks (L. 275), but in case of low Zn utilization efficiency, much of the Zn applied is apparently quickly made unavailable to the plants, due to the unfavorable soil properties. This is unlikely to pose much of a risk unless the unavailable Zn is suddenly made available again, which is very unlikely to happen as it will require large-scale acidification of the soil. And even so, soil Zn concentrations up to 100 ppm are still far from acute Zn toxicity levels.

The discussion on the effect of other nutrients, N, P, K, combined with Zn is very useful, but often this discussion not very strong. Plants will need NPK as well as Zn. If one is limiting, adding a lot of the others is unlikely to be very effective. When two are limiting, adding one is also unlikely to be very effective. Plants have normally very effective strategies to acquire essential nutrients, including for N, P, K and Zn. If two are suboptimal, adding one is likely to induce enhanced expression of the genes involved in acquisition of the other, especially if the supply is relatively close to sufficiency. In this respect, increasing N supply is likely to enhance the Zn deficiency response, as described in L. 313-319, but only if Zn supply is only mildly deficient.

It is all about finding optimal supply levels, for all essential nutrients. It is therefore not strange to see that at excessive N supply in Zn deficient conditions, Zn supply will be less effective (with plant growth repressed by high N), unless also N supply is reduced (L. 328-329). Such considerations are not mentioned.

Finally, the genetic component to Zn biofortification is poorly addressed. Based on L. 200-201, wheat varieties were not assessed based on genotype, but on initial grain Zn concentration. That will be a poor predictor of genotype, knowing that Zn fertilisation in the previous generation has such a strong effect on grain Zn concentration. Also since it is well known that there is substantial genetic variation for grain Zn concentration (eg. L 407-408), it seems an omission of the meta-analysis not to properly account for this. L. 419-423 suggest that the analysis identified wheat varieties with different grain Zn concentrations, but it is not clear where this is reported in the manuscript.

In conclusion, the lack of validation experiments performed based on the results of the meta-analysis, and the poor discussion, seriously undermine the significance of the work, which I think is below what is expected for publication.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
Dear Authors,

Thank you for submitting revisions to comments on your manuscript "Zinc agronomic biofortification in wheat and its drivers: A global meta-analysis". I reviewed your responses which addressed most of the comments from the initial review. I am convinced with your responses apart from a few which I indicated below. For your ease, my comments are in a bracket [] and have three *** before the comment.

(2) I, however, find the discussion rather too long as authors presented commonly known factors limiting or enhancing Zn uptake and concentration. I suggest authors ensure that the novelty of their meta-analyses (presented last in their discussion section) is not hidden among commonly known background information on factors governing Zn supply. The novelty of the analysis could be elaborated on a bit more (instead of a few lines at the end of the discussion), with less emphasis on what we already know (i.e. reduction in costs from co-application of Zn and pesticides which was published a few years back and superiority of soil + foliar versus soil, or foliar applications which most agronomic biofortification studies tend to discuss). I end by emphasising that the need for different Zn application methods for wheat grain with different Zn concentrations is novel and very interesting. This novel message warrants publication of the manuscript in Nature Communications. I however think this message is presented a bit too late in the manuscript. I did not see any study strengths and limitations section or conclusion section, maybe this is per Nature Communications guidelines. I include here and also attach as pdf file, detailed comments for authors to revise and improve clarity of their manuscript. The writing of the manuscript can also be improved in some areas as indicated in my detailed comments.
Response: I have shortened the discussion section following your suggestion, and revised the discussion to emphasize the novelty. In addition, the significances and limitations of the study have been added at the end of the discussion. Detailed responses to each suggestion are listed as follows.
****
[***Thank you for considering my suggestions. I read the first sub-section of the authors' discussion. Unfortunately, it is now mainly focusing on China alone (Lines 322-344), and deviates from the title, which is supposed to provide recommendations for global wheat production systems. Can a few sentences be provided to make similar recommendations for other regions. I also do not agree with the sentence that wheat production regions have low organic matter content (Line 312). Can a reference be provided. If authors already provided a reference, can this be provided alongside the soil property instead of placing all the references (6,13,22) at the end of the sentence].

****

5. Line 532: As Africa is not mentioned as one of the regions, I am surprised the Zou et al 2012 paper on wheat in Zambia did not come up in your search. I see the data in Figure 1 but Africa is not mentioned here.
Response: Sorry for the incomplete description. Our search included the paper published by Zou et al. (2012), and Zambia came up in Fig. 8, but we forgot to mention it. The corresponding part has been revised as: "These data were sourced from studies conducted in 19 countries across East, West, and South Asia, North America, South America, Europe, South Africa, and Oceania (Fig. 8)" (Line 496-498).

[***I appreciate authors' responses. Please change South Africa to Southern Africa. South Africa is a country and I think the authors sought to mention the region, which is Southern Africa].

****

For the sake of convenience in calculation, the target of 30 mg kg-1 is utilized for Zn biofortification in the first stage. 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" (Line 257-274).

[***I am not sure if the statement "For the sake of convenience in calculation" reads scientifically sound. One would argue that authors could have used the global mean average of 28.2 mg kg-1 as it is without rounding it up by 6%, which when you are working with micronutrients, can make such a big difference. Please kindly revise the wording].

****

"For each of our studies and publications, we extracted geographic location, available soil Zn, organic matter, $CaCO_3$, 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 yield, biomass, and Zn concentration in grain and straw" (Line 531-535).

[***The word "wheat yield" is misleading. Include the word "grain" if this was wheat grain yield to read "wheat grain yield"].

Reviewer #3

(Remarks to the Author)
(Asked to consider if the response to Reviewer 2 is adequate from the authors.)

Having read the initial reviews, and the author responses, I would support publication of the manuscript. The two major concerns of Reviewer 2, i.e. the lack of recent validation experiments and the quality of the Discussion (also raised by Reviewer 1) have been addressed through the inclusion of new field data from 6 sites in China. The Discussion has been comprehensively edited and reads clearly.

There was one editorial comment:
P12/L279 “A grain Zn concentration in wheat of 40 mg kg-1 is essential for human nutrition.” I would suggest rephrasing to say that “A grain Zn concentration threshold of 40 mg kg-1 has been suggested in terms adequate Zn nutrition”.

I have not scrutinised the Supplementary Materials in detail, and it seems that most of the data are presented to allow someone to reanalyse the data, which is great. But, please can the authors ensure that ALL data are presented (i.e. I could not see the primary data from the 2022-2023 validation trials), and this needs to be checked.

Reviewer #4

(Remarks to the Author)
The manuscript is well-written and addresses an important research topic effectively. The use of meta-analysis and machine learning (ML) modeling is commendable. However, to strengthen the manuscript, consider including a small comparative statement of these two methodologies and providing additional clarity on the methods. Here are some specific suggestions:
1. There is no description of the output variable, specifically the response of grain zinc (Zn) concentration to zinc fertilization. It is unclear what this variable represents and what its unit of measurement is. Additionally, is it similar to Agronomic Nutrient Use Efficiency. Could you clarify these details?
2. The procedure for determining the significance of the Random Forest (RF) model is not clear. It is unclear whether the RF model was compared against a null model, multiple random models generated from permuted data, or any other statistical benchmarks. Could you provide more details on the significance testing approach used for the RF model? Same for the significance of the predictors, how authors approached. Just mentioning the package does not make it clear.
3. Whether the aim of the random forest modelling was to find the important predictors only, which authors has also described in figure 5, where they have compared the effect size from meta-analysis.
In this setting a highlight on how the important predictors screened from RF differ from meta-analysis (screened from total effect size may be?), also in supplementary figure a comparison between the partial dependency plot and figure 5 will be nice. Else if the unit are same, they can be in same figure only.
What is the unit of relative importance? whether it was percentage, in the figure 6, x-axis?
An additional figure with above changes may justify more the use of RF, when authors are already doing the meta-analysis.

Version 2:

Reviewer comments:

Reviewer #4

(Remarks to the Author)
The authors have improved the clarity of the method based on earlier comments.

Authors' Responses to Comments

Reviewer #1:

(1) The manuscript "Zinc biofortification in wheat and its drivers: A meta-analysis- NCOMMS-23-07703" highlights factors governing zinc (Zn) supply in global wheat production systems. Agronomic biofortification of wheat with different micronutrient fertilizers for improved grain micronutrient concentration is an important strategy in alleviating dietary micronutrient deficiencies. The manuscript is supported by a sound methodology and an extensive literature review and data extraction. The meta-analysis was mostly focused on soil and management factors with a limited focus on environmental factors such as agro-ecological region, climatic zone, rainfall etc, factors also known to influence agronomic biofortification and Zn bioavailability. Authors did an incredible job of extracting data from global wheat agronomic biofortification field studies which they supported with their own field data sets. I find the evidence generated confirming background understanding of factors governing Zn supply (i.e. soil pH, organic matter, Zn fertilizer application methods, timing etc), with an additional novel approach on Zn fertilizer management. I particularly found the authors' focus on use of different Zn fertilizer management options based on initial grain Zn concentration novel, and is crucial in guiding ongoing agronomic biofortification interventions in different agro-ecological regions and socio-economic settings. This evidence is supported by well-constructed graphs in the main text and supplementary files. The authors provide a detailed discussion section which ends by highlighting different Zn fertilizer application methods to be used for grain Zn concentrations less than and/ or greater than 30 mg kg-1. This helps in reducing fertilizer costs and potential toxicities from excess fertilizer application. Rates of fertilizer to be applied per litre for foliar fertilization are provided by the authors for additional guidance.

Response: Thank you very much for your positive comments which is very exciting and encouraging.

(2) I, however, find the discussion rather too long as authors presented commonly known factors limiting or enhancing Zn uptake and concentration. I suggest authors ensure that the novelty of their meta-analyses (presented last in their discussion section) is not hidden among commonly

known background information on factors governing Zn supply. The novelty of the analysis could be elaborated on a bit more (instead of a few lines at the end of the discussion), with less emphasis on what we already know (i.e. reduction in costs from co-application of Zn and pesticides which was published a few years back and superiority of soil + foliar versus soil, or foliar applications which most agronomic biofortification studies tend to discuss). I end by emphasising that the need for different Zn application methods for wheat grain with different Zn concentrations is novel and very interesting. This novel message warrants publication of the manuscript in Nature Communications. I however think this message is presented a bit too late in the manuscript. I did not see any study strengths and limitations section or conclusion section, maybe this is per Nature Communications guidelines. I include here and also attach as pdf file, detailed comments for authors to revise and improve clarity of their manuscript. The writing of the manuscript can also be improved in some areas as indicated in my detailed comments.

Response: I have shortened the discussion section following your suggestion, and revised the discussion to emphasize the novelty. In addition, the significances and limitations of the study have been added at the end of the discussion. Detailed responses to each suggestion are listed as follows.

Detailed Comments:

Title

1. Biofortification is mainly used to describe conventional genetic breeding for crops with higher concentrations of micronutrients. I suggest authors revise title to "Zinc agronomic biofortification in wheat and its drivers: A global meta-analysis".

2. I suggest global here as authors did not restrict on region when accessing papers from web of science.

Response: The title has been revised as suggested: "Zinc agronomic biofortification in wheat and its drivers: A global meta-analysis".

3. Also change biofortification to agronomic biofortification throughout the manuscript.

Response: As suggested, "Zn biofortification index" and "Zn biofortification in wheat grain" have been replaced with "Zn agronomic biofortification index" (Line 37, 248, 254, 277, 296, 307, 327, 448, 470, 562, 613, 861 and 862) and "Zn agronomic biofortification in wheat grain (Line 98, 345, 369, 390 and 416)". "Zn biofortification target" remains unchanged, as Zn biofortification strategies include agronomic and genetic biofortification of Zn, and their biofortification target is the same, both of which are to increase the Zn concentration in wheat grains to the 40 mg kg^{-1}.

Abstract

1. Line 20-Change grain Zn content to grain Zn concentration and also throughout the manuscript.

Response: As suggested, we have checked the entire manuscript, and "grain Zn content" has been replaced by "grain Zn concentration". The corresponding sentences have been revised (Line 19-23).

2. Key word: I was going to also suggest Global to denote the scale of data extraction. But if authors choose to put it in the title then this won't be relevant anymore.

Response: Thanks. We have put "Global" in the title, and thus removed it from the keywords (Line 1-2).

3. Line 43: delete repeated word "in"

Response: Suggestion is taken (Line 47).

4. Line 45: start with "The deficiency in Zn." or "Zinc deficiency…" instead.

Response: Suggestion is taken, and "The Zn deficiency" has been replaced by "Zinc deficiency" (Line 49).

5. Line 49: Wheat-provide Latin or scientific name

Response: Suggestion is taken (Line 55-56).

6. Line 55 to 57- information on factors governing Zn supply in soils is too limited here. I appreciate factors like pH, and P were mentioned in later paragraph, but I suggest authors also focus on OM and its effect on Zn availability.

Response: Detailed information on factors governing Zn supply in soils has been added in two places: "Soil Zn availability is influenced by various factors including pH levels, total Zn, $CaCO_3$, available phosphorus (P), organic matter, and moisture content" (Line 64-66).

"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 grains" (Line 76-79).

7. In the entire introduction, risks of Zn deficiency to human health i.e. physical impairments, stunting are lacking. I suggest authors also focus on this.

Response: As suggested, corresponding information has been added as: "Human Zn deficiency can lead to slow physical growth, impaired brain development, compromised immune function, and increased susceptibility to infectious diseases" (Line 51-53).

8. Authors using ~ to denote range instead of –. Check and change throughout the manuscript.

Response: Suggestion is taken with modifications in 7 places: Line 59, 164, 182, 186, 340, 543-546, 553, and 557-560.

9. Lines 85 to 87 are not reading well. I suggest revising and check the rest of the manuscript for words which need to be removed of included for sentence flow.

Response: This sentence has been rewritten (Line 98-103), and we checked the language in throughout the manuscript.

10. Line 97- change in globally wheat production to in global wheat production

Response: Suggestion is taken (Line 109-110).

11. Aim 1b on target of grain Zn biofortific ation. This cannot be an aim as target of grain Zn biofortification in wheat is already known (40-60 mg kg^{-1}). See Cakmak, 2008. Revise for clarity.

Response: Suggestion is taken, and the sentence has been revised as: "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" (Line 109-113).

12. Line 99: As authors mention about soil management, it is important they mention effects of OM on Zn availability as in my earlier comment for the introduction.

Response: More detailed description on the effect of OM on Zn availability has been added in the introduction as: "Soil Zn availability is influenced by various factors including pH levels, total Zn, $CaCO_3$, available phosphorus (P), organic matter, and moisture content" (Line 64-66). "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 grains" (Line 76-79).

Methods

1. Line 512: Revise to read "A search of peer reviewed publication." and delete "for"

Response: Suggestion is taken (Line 475).

2. 513: Change grain Zn nutrition to grain Zn concentration. Also check throughout manuscript.

Response: Suggestion is taken (Line 476). We also corrected throughout the manuscript.

3. 518: Change trails to trials

Response: "trails" has been replaced by "trials (Line 483-486)，and we checked the language in throughout the manuscript.

4. Line 519 criteria 2: why were wheat crops and soils without Zn supplied selected? Were authors looking for baseline concentrations? If so, elaborate for clarity as readers will wonder why given this is an agronomic biofortification focused paper.

Response: The present study established two databases. The first one is used to assess the level of grain Zn concentration in global wheat grown without Zn fertilizers supplied, while the second one is to evaluate the response of grain Zn concentration to Zn fertilization. Considering the relatively limited application of Zn fertilizer in global wheat production, we collected the Zn concentration in wheat grains only without Zn fertilization in the first database.

5. Line 532: As Africa is not mentioned as one of the regions, I am surprised the Zou et al 2012 paper on wheat in Zambia did not come up in your search. I see the data in Figure 1 but Africa is not mentioned here.

Response: Sorry for the incomplete description. Our search included the paper published by Zou et al. (2012), and Zambia came up in Fig. 8, but we forgot to mention it. The corresponding part has been revised as: "These data were sourced from studies conducted in 19 countries across East, West, and South Asia, North America, South America, Europe, South Africa, and Oceania (Fig. 8)" (Line 496-498).

6. Line 534: Revise over the in

Response: Suggestion is taken, and "over the in" has been replaced by "during the" (Line 500).

7. Line 536: What was the form of Zn supplied? Sulphate, oxide,

Response: Zinc sulphate was used as Zn fertilizer in the four trials conducted by us. Detailed information has been added as: "Zinc sulfate was the Zn source applied to the soil or foliage in all four trials" (Line 513-514).

8. How is line 512 to 513 different from details in Lines 551 to 554? What is Zn nutritional level. I wonder these need to be merged.

Response: The scope of the two searches on literatures was different. The keywords used in the first search was "grain zinc concentration" AND "wheat" to assess the level of grain Zn concentration in global wheat in the absence of Zn fertilizers supply. While the formulation of the second search was ("grain zinc concentration" OR "zinc biofortification" OR "zinc nutrition") AND "wheat" AND "zinc application", to evaluate the response of wheat grain Zn concentration to Zn fertilization. In the present study, "grain Zn nutrition" has been replaced by "grain Zn concentration". Corresponding parts have been revised as: "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 /kns/brief/result.aspx?dbprefi x=CJFQ). The search query used was "grain zinc concentration" AND "wheat" " (Line 475-480).

"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" " (Line 518-522).

9. Line 553: search term Zn biofortification implies breeding for higher micronutrients. I wonder if many papers were excluded because of this term and some on zinc agronomic biofortification not included at all?

Response: There are no problems with the search formulation. We are sorry for the unclear description. In addition to "zinc biofortification", the search terms included "grain zinc concentration" and "zinc nutrition". The Boolean operator is OR between the search terms, that is, if one of the search terms is satisfied, the relevant papers on Zn agronomic biofortification can be searched. The corresponding parts have been revised as: "The search formulation was ("grain zinc concentration" OR "zinc biofortification" OR "zinc nutrition) AND "wheat" AND "zinc application" " (Line 520-522).

10. Line 557: insert "at least"

Response: Suggestion is taken (Line 526).

11. Lines 579-580: which method was used to denote the ≤0.5 mg/kg as deficient. The DTPA and EDTA methods have different critical limits.

Response: It is true that the extraction methods used DTPA and EDTA have different critical limits. All the data collected according to the screening criteria in the present study were extracted by DTPA methods. Therefore, available Zn ≤0.5 mg/kg is defined as deficiency of Zn in soil. The corresponding sentence has been revised as: "Initial Zn availability (DTPA-Zn) in soils was categorized as deficient (≤0.5 mg kg^{-1}) and non-deficient (>0.5 mg kg^{-1})" (Line 550-551).

Figures

1. Figure 2a: something on top of the right legend in Chinese. Revise.

Response: Suggestion is taken, and the figure has been corrected for Chinese characters.

2. Figure 2 caption: Explain the meaning of CK in the caption/ figure title.

Response: The CK denotes the wheat grown without Zn fertilizer applied. Detailed description has been added in the title of Figure 2 as: "The CK denotes the wheat grown with no Zn application" (Line 830).

3. Figure 3b: Folial Zn to Foliar Zn

Response: Suggestion is taken.

Results

1. Line 108- Revise gain to grain

Response: Suggestion is taken (Line 119).

2. Line 110- The value of 34.0 mg Zn kg^{-1} in North America, what could be the reason for such a larger value than in other continents? Is it a dilution effect from biofortified grain i.e. Zincol-16 in some countries i.e. Mexico? Would be good if additional details can be provided.

Response: It is indeed due to the Zn-biofortified wheat varieties, such as Zinc Shakti and Zincol2016, developed by the International Maize and Wheat Improvement Center (CIMMYT) and grown in Mexico (Cardoso et al., 2018). Because Pearson analysis showed no correlation between grain yield and grain Zn concentration, indicating that there was no nutrient concentration effect caused by low grain yield. The related information has been added as: "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)[35]" (Line 290-293).

Cardoso, P., et al. Localization and distribution of Zn and Fe in grains of biofortified bread wheat lines through micro- and triaxial-X-ray fluorescence spectrometry. Spectrochimica Acta Part B-Atomic Spectroscopy 141, 70-79 (2018).

3. Line 114-Similarly, for 17.7 for west Asia. Any explanation how this was much lower than

the other Asian regions.

Response: Low availability of Zn in soil results in the low Zn concentration in wheat grain in West Asia. The data from the corresponding literature showed that soil available Zn was only 0.30 mg kg^{-1} in West Asia, but it reached 0.90 and 0.76 mg kg^{-1} in East Asia and South Asia, respectively. The data of soil available Zn in Central Asia was not provided in the corresponding literature. The detailed explanation has been added in the Discussion section as: "Notably, wheat grown in regions characterized by severe soil Zn deficiency exhibits grain Zn concentrations below 20 mg kg^{-1} [6]. 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 30.4 mg kg^{-1} observed in wheat grains from East Asia and South Asia, respectively, where the soil's available Zn content is substantially higher (Fig. S6)" (Line 284-290).

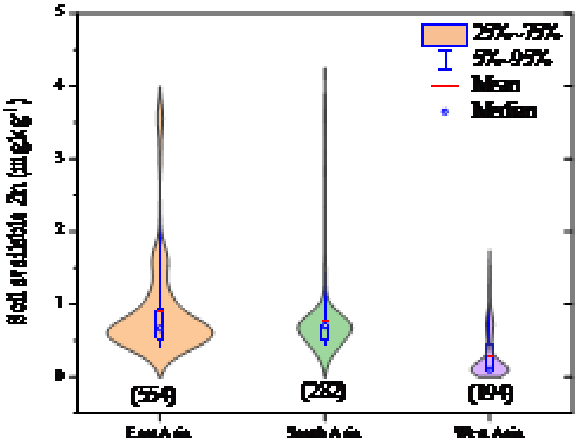

Fig. S6 Soil available Zn concentration in East Asia, South Asia and West Asia. The fatter the violin, the larger the sample size. Numbers in parentheses represent samples sizes. Solid lines and white dots in boxes represent the mean and median values. Box bars and boundaries denote 5th and 95th, and 25th and 75th percentiles of all the analyzed data.

4. Line 115: insert South America, after Mexico

Response: Suggestion is taken (Line 127).

5. Line 116: Revise "the average Zn" to an average Zn, "above the" to above the 40 mg kg^{-1} threshold, "required by human nutrition" to required for human nutrition.

Response: Suggestion is taken (Line 126-128).

6. Line 117 to 118- Why the reference of the 64% countries to a mean value of 28.2 mg kg^{-1}. Is it because it is the mean for Central Asia? When authors mention "less than 28.2 mg kg^{-1} is it not better to make the reference to the critical threshold of 40 mg kg^{-1}?

Response: Here, 28.2 mg kg^{-1} was the mean gain Zn concentration in global wheat, not the mean value in Central Asia. Grain Zn concentration and yield of wheat were limited by the low availability of Zn in soil with severe Zn deficiency. This study by analyzing the data from global wheat Zn fertilizer experiments, demonstrated that application of Zn fertilizers to soils increased the wheat grain yield greatly by 18.4%, but the grain Zn concentration increased only by 5.1 mg kg^{-1} on average across all countries, and the Zn concentration in 87.5% of the grains did not reach the 40 mg kg^{-1} required for human nutrition (Fig. 2d, S4b). In foliar Zn application, the grain Zn concentration increased by 16.2 mg kg^{-1} across all countries, but the increase in yield is very low, only 7.4%. Considering the importance of grain yield in wheat production, this study divided the target of wheat Zn agronomy biofortification into two stages. In the first stage, the wheat grain with very low Zn concentration will increase to the global average Zn concentration, while improving available Zn concentration in soil and achieving a high yield. In the second stage, the Zn concentration in wheat grain will be further increased to 40 mg kg^{-1}. Therefore, this study chose the global average grain Zn concentration as a reference of the 64% of countries. For convenience of calculating the recommended amount of Zn fertilizer, the 30 mg kg^{-1} was used as the target of Zn biofortification in the first stage (i.e., the first biofortification target), and the 40 mg kg^{-1} was chosen as the target of Zn biofortification in the second stage (i.e., the final biofortification target). The corresponding part has been revised as:

"Alarmingly, up to 96% of countries produce wheat with an average grain Zn concentration below the 40 mg kg^{-1} requirement for human nutrition" (Line 129-130).

"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, S4b). 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 10.0%, respectively (Fig. S4c). Given the importance of grain yield in wheat production, this study divides the target for wheat Zn biofortification into two stages (Fig. 7a). 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. For the sake of convenience in calculation, the target of 30 mg kg^{-1} is utilized for Zn biofortification in the first stage. 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}" (Line 257-274).

7. Line 119-122: This sentence will be more relatable if readers could get an understanding of why 28.2 was used as a reference earlier.

Response: Please see the response #6.

8. Line 127: increased by only 29.1%. I suggest delete the word only as 29% is not so little when it comes to grain Zn nutrition. What is the baseline control here that the authors are comparing the increase to. Similarly for foliar values (Line 129-131).

Response: "only" has been deleted. The present study chose the wheat grown without Zn fertilizer supply as the baseline control, to compare with the wheat grown with Zn fertilizer application. The corresponding sentence has been revised as: "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 87.5% of the grains, the Zn concentration did not reach the 40 mg kg^{-1} threshold required for human nutrition (Fig. 2d, S4b)" (Line 134-138).

9. Line 133- revise "required by human nutrition" to required for human nutrition

Response: Suggestion is taken with modifications in 5 places: Line 59, 128, 137, 142, and 251.

10. Subheading title on line 134: I am not entirely sure if this needs to be revised from "on increase effectiveness" to on increasing effectiveness. I realise later in the manuscript that authors occasionally use the word increase effectiveness (Line 140 etc). It would be good if this

term is defined for clarity.

Response: “increase effectiveness” denotes the percentage of increase in grain Zn concentration of wheat following Zn fertilization compared with that without Zn application. In consultation with experts whose native language is English, it is recommended that no changes be made. The corresponding sentences has been revised as: “Effects of different factors on the efficacy of improving grain Zn concentration by Zn fertilization” (Line 143-144).

“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)” (Line 149-152).

11. Line 140: It would be good if the application rates increasing grain Zn can be mentioned here.

Response: As you suggested. The corresponding part has been revised as: “However, it was observed that an increase in the Zn application rate reduced the effectiveness of increasing grain Zn concentration under soil Zn application” (Line 149-150).

12. Line 154-155: Were SO_4 and EDTA applied at the same rate? It is common knowledge that EDTA and any other chelates out-perform sulphates and oxides. Could other factors i.e. rate, environmental factors have influenced this?

Response: The amount of Zn sulfate and Zn-EDTA are different. The literatures in meta-analysis showed that the amount of Zn fertilizer applied in the form of Zn sulfate was greater than that applied in the form of Zn-EDTA. Researchers believe that under the same amount of Zn application, Zn-EDTA increases the Zn concentration in wheat grains more than that with Zn sulfate. The amount of Zn-EDTA application is usually reduced when the experiment is conducted. The present published research suggests that application of Zn fertilizer in the form of sulphates at higher application rates may be more effective than the chelates due to lower cost, as Modaihsh (1997) and Zhao et al. (2020) believed.

Modaihsh, A. S. Foliar application of chelated and non-chelated metals for supplying micronutrients to wheat grown on calcareous soil. Experimental Agriculture **33**, 237-245 (1997).

Zhao, A.Q., Wang, B.N., Tian, X.H. & Yang, X.B. Combined soil and foliar $ZnSO_4$ application improves wheat grain Zn concentration and Zn fractions in a calcareous soil. European Journal of Soil Science **71**, 681-694 (2020).

13. Line 171: K-write in full at start of sentence

Response: "K" has been replaced with "Potassium", and the corresponding sentence has been revised (Line 182).

14. Line 167-69: Is this implying then that for farmers who cannot afford Zn fertilizer, P application is an alternative to increasing grain Zn by a margin. My understanding is P inhibits Zn uptake. What could be the mechanistic approach here that resulted in P increasing grain Zn in the absence of Zn fertilization?

Response: Several studies have confirmed that P fertilization poses a negative influence Zn uptake of cereal crops. However, field studies have shown that an appropriate amount of P application could promote root development and improve root morphology. There is a positive correlation between root biomass, root surface area and root length density with Zn uptake by roots and shoot Zn accumulation in wheat (Zhang et al., 2016). Our previous study based on long-term filed experiments also showed that Zn accumulation in shoot and grain at maturity significantly improved under an appropriate P supply (Hui et al., 2019). This suggests that a medium amount of P application could provide more opportunities for roots to absorb Zn from the soil, because of an improved root morphology. Consequently, the larger increase percentage of grain Zn concentration in wheat from Zn fertilization was a result of an improvement in root morphology under medium P rates. The detailed explanation has been added in Discussion Part as: "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]" (Line 430-433).

55. Hui, X. L. et al. Critical concentration of available soil phosphorus for grain yield and zinc nutrition of winter wheat in a zinc-deficient calcareous soil. Plant Soil **444**, 315–330 (2019).

60. Zhang, W., Liu, D. Y., Liu, Y. M., Cui, Z. L., Chen, X. P. & Zou, C. Q. Zinc uptake and accumulation in winter wheat relative to changes in root morphology and mycorrhizal colonization following varying phosphorus application on calcareous soil. Field Crop. Res. **197**, 74–82 (2016).

15. Line 169 to 170: Also explain the term "greatest increased percentage"

Response: "greatest increased percentage" denotes the percentage of increase in grain Zn concentration that is the greatest. The corresponding sentence has been revised as: "The highest percentage increase in grain Zn concentration was achieved at medium P rates of 26–44 kg P ha^{-1}" (Line 181-182).

16. N, P, K categorization rates are not mentioned in the data categorization section in the Methods but occasionally referred to in Results Lines 170-75. Can these be mentioned in the Methods section.

Response: As suggested, N, P, K categorization rates have been added as: "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" (Line 541-546).

17. Line 187-188: Again here, I do not understand what the phrase "was the most increase effectiveness" means.

Response: The corresponding sentence has been revised as: "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" (Line 198-201).

18. Line 195-Here organic matter is in mg kg^{-1}. Revise units.

Response: Sorry for the mistake. "mg kg^{-1}" has been replaced by "g kg^{-1}". The corresponding sentence has been revised as: "However, at high organic matter content (>20 g kg^{-1}), the percentage improvement in grain Zn concentration could be maximized" (Line 209-210).

19. Line 195: the grain Zn concentration be increased by a maximum. This is not clear, I do not understand.

Response: The corresponding sentence has been modified as: "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 g kg^{-1}), the percentage improvement in grain Zn concentration could be maximized" (Line 207-210).

20. Line 181: Revise "When Zn is" to when Zn was. Also check throughout the manuscript for when present tense was used instead of past tense.

Response: As suggested, we have checked the entire manuscript. "When Zn is" has been replaced with "When Zn was" (Line 192), and "When Zn fertilizer is" has been replaced with "When Zn fertilizer was" (Line 207).

21. Line 203-Has a negligible to "had a negligible"

Response: Suggestion is taken (Line 217).

Discussion

1. Line 244- Revise "only by" to "by only"

6. Line 266-Revise the phrase "due to that"

7. Line 303-4: Revise "always that" to "ensuring that"

8. Line 336: Revise "is associated to" to "is associated with"

9. Line 345: insert space between P and ha^{-1}

13. Line 493: Revise "can achieve" to "can be achieved by"

Response: All suggestions are taken in the revised manuscript.

2. Line 247- Straw is mentioned here and presented in a Figure but not mentioned anywhere in the abstract, introduction, methods. It appears to readers that the main focus is on grain only when other plant tissue data are presented.

Response: This study focused on grains, and straws mentioned here were only used to support the results of grains. This information has been added in Method section: "For each of our studies and publications, we extracted geographic location, available soil Zn, organic matter, $CaCO_3$, 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 yield, biomass, and Zn concentration in grain and straw" (Line 531-535).

3. Line 256- Zn: start sentence with "Zinc" in full and anywhere else in the manuscript.

Response: Suggestion is taken (Line 316).

4. Line 258-Revise was to is.

Response: Suggestion is taken (Line 317).

5. Is Zn only translocated to the grain under conditions of insufficiency in the root system. Is this not a natural mechanistic pathway for Zn which happens towards crop maturity and is very different from Nitrogen which is not translocated as the crop matures. I would like to argue that Zn translocation to the edible portion happens even under conditions of sufficient Zn in the soil.

Response: The remobilization of Zn from vegetative tissues to grains did not occur only under the conditions of insufficiency in the root system. Field experiment has demonstrated that Zn remobilization could happen under conditions of sufficient Zn in the soil, but it decreased with increasing Zn availability in soil when soil available Zn was more than 7.15 mg kg^{-1} (Liu et al. 2019). Furthermore, solution culture experiment showed that up to 100% of the grain Zn came from root uptake during grain-filling stage, when the root uptake could continue (Kutman et al. 2012).

Liu, D. Y., Liu, Y. M., Zhang, W., Chen, X. P. & Zou, C. Q. Zinc uptake, translocation, and remobilization in winter wheat as affected by soil application of Zn fertilizer. Front. Plant Sci. 10, 426 (2019).

Kutman, U., Kutman, B., Ceylan, Y., Ova, E. & Cakmak, I. Contributions of root uptake and remobilization to grain zinc accumulation in wheat depending on post-anthesis zinc availability and nitrogen nutrition. Plan Soil 361, 177–187 (2012).

10. Authors did not clearly mention the mechanism around decreased Zn with increased Nitrogen. They only mentioned the mechanism around increased Zn with N.

Response: The corresponding information has been added as: "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]" (Line 423-430).

11. Line 405: Any suggestions on optimal ranges for soil Zn and pH?

Response: The meta-regression showed that under Zn fertilizer applied to the soil, the effect size of grain Zn concentration was reduced with the increase in soil Zn available levels in a power function fashion. When soil available Zn was 0.5 and 1.0 mg kg^{-1} (i.e., thresholds for Zn deficiency and potential Zn deficiency of soil), the grain Zn concentration increased by 20% and 13% following Zn application, respectively. However, the improvement of wheat grain yield by applying Zn fertilizer mainly occurred in Zn-deficient soil, and was rarely reported in potential Zn-deficient soil. To obtain a substantial improvement in grain Zn concentration and grain yield in wheat production, Zn fertilizers is recommended to apply to soils with available Zn less than 0.5 mg kg^{-1}. The meta-regression showed that the effect size of grain Zn concentration following Zn application to soil increased first, then decreased as soil pH values increased. The percentage of increase in grain Zn concentration had a maximum of 34% at a soil pH of 7.5. When soil pH was 6.4 and 8.6, the grain Zn concentration increased by about 20% following Zn application. If wheat grain Zn concentration increases by more than 20%, Zn fertilizer should be applied to soils with pH between 6.4 and 8.6. The corresponding information has been added as: "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. 6a)" (Line 411-415).

12. Line 418: Revise "having a wide range" to "would have a wide range"

Response: Suggestion is taken (Line 354-355).

14. I found it rather interesting that the authors state methods to be used for grain Zn concentrations < and > 30 mg kg-1. This helps in reducing fertilizer costs and toxicities. Rates of fertilizer per litre provided by the authors provide guidance. I, however, find the discussion rather too long as authors presented commonly known factors limiting or enhancing Zn uptake and presented this important point last (from lines 472). I suggest making sure this point is elaborated on a bit more with less text on what we already know (i.e. reduction in costs from co-application of Zn and pesticides which was published a few years back). The application methods for grain with different Zn concentrations is novel and very interesting. I think this message is presented a bit too late in the manuscript.

Response: As suggested, to emphasize the important point and innovation of the manuscript, the discussion part has been shortened, and the important point has been placed in the first part of the discussion. The corresponding part has been revised (Line 322-344).

15. No conclusion section, Is this per Journal guidelines?

Response: Yes, this is followed the Journal guidelines. However, we added several sentences to highlight the significance of the study in the end of discussion: "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. Additionally, foliar spraying should be conducted during the late wheat growth stages, such as at anthesis and early filling stages" (Line 443-458).

References, Figures, Tables, Supplementary files

1. Check use of brackets on reference 82. Alloway.

2. I did not see reference 91 in the main text.

Response: The reference 82 and 91 have been removed from the revised manuscript.

3. Figure 1 caption: Authors did not mention in the legend what the blue line and number represent. I understand this is mentioned in the main text only.

Response: Blue line and blue number in parentheses denote the average Zn concentration in global wheat. The corresponding part has been revised as: "**Fig. 1** Grain Zn concentration of global wheat (see supplementary materials for the source of the database). Black numbers in parentheses represent sample sizes. Blue line and blue number in parentheses denote the average Zn concentration in global wheat" (Line 823-825).

4. Figure 2 caption: Revise to "Grain Zn concentration of wheat grown with Zn fertilizers applied to soil (a)…

5. Second and 3rd sentence of Figure 2 caption not reading well. Check language on Figure 2a

Response: Suggestions are taken (Line 826-833).

6. Figure 3b, revise Foliarl

Response: Suggestion is taken (Fig. 3b).

7. Sentence 3 of Figure 3 caption not reading well (Line 898-900)

8. Similarly for line 905-907 of Figure 4

Response: Suggestions are taken (Line 837-839 and 844-846).

9. Figure 6: I would propose that the x-axis is the same for both soil (currently 0-5) and foliar (currently 0-2.5)

Response: As suggested, the x-axis of Figure 6b has been set from 0 to 5.

10. First part of line 912 is not reading well. Revise Important of to "Importance of"

Response: Suggestion is taken, and the corresponding sentence has revised as: "**Fig. 6** Importance of factors (fertilization managements, initial soil properties, wheat cultivars) influencing the increase in wheat grain Zn concentration by applying Zn fertilizer to soil (a) and foliage (b)" (Line 852-853).

11. Figure 6 shows spray timing and Zn rates as important factors in foliar application but Figure 7 indicates Zn rates and initial grain Zn concentration in the far right column. I did not totally understand why.

12. Similarly for the far left column for soil application which shows Zn rate and initial grain Zn concentration as important factors in Figure 7 but initial grain Zn and initial grain yield in Figure 6. Is there a difference between relative importance (Figure 6) and main drivers (Figure 7)?

Response: Spraying timing has been added as a main driver for foliar application. The random forest analysis showed that the effect of initial grain yield did not reach significant level on the response of grain Zn concentration to soil Zn application, so the initial grain yield was not listed as the main drivers for soil application. The corresponding Figure has revised.

13. Figure 7b: I can barely see the different shades of green.

Response: We have modified the color in Figure 7b to make them clearly to read in the revised version.

14. Figure 7 has 3 captions, a, b, c but in main text authors only write Figure 7 without indicating if 7a, b or c (Line 235-240)

Response: As suggested, the detailed subgraph information has been added as follows:

"Based on the findings from meta-analysis, random forest analysis, and correlation analysis (Fig. 3a, 6a, S5), we identified the Zn rate and initial grain Zn concentration as essential factors for enhancing Zn levels in wheat grain through soil fertilization (Fig. 7a). 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. 7a, S5)" (Line 235-240).

"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. 7b). 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. 7c)" (Line 339-344).

15. Similarly for Figure 2 which has 4 sub Figures (Line 126)

I see no Tables used in the manuscript.

Response: The subgraph information has been added as follows: "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, S4b). 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 87.5% of the grains, the Zn concentration did not reach the 40 mg kg^{-1} threshold required for human nutrition (Fig. 2d, S4b). 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 63.5% and 60.6% of the wheat grains, respectively, achieved the 40 mg kg^{-1} threshold required for human nutrition" (Line 132-142).

Fig. 8

Supplementary files

1. File. Could be important to mention software used for plotting graphs in the "Software and code" section or in main methods section of the manuscript. Also check and correct language

in this supplementary file i.e. Line 5 of study description "wheat grain Zn concentration supplied with Zn fertilizers to soil, foliage. Similarly for Research sample section Line 5 "wheat supplied Zn fertilizers to t… " to read "wheat supplied with Zn fertilizers to t...

Response: The relevant information on software has been added in the methods section: " ArcGIS 10.8, Origin 2021, and SigmaPlot 12.5 were utilized to create relevant figures in this study" (Line 604-605). The corresponding titles have been modified (Line 969 and 989).

2. Sampling strategy section: revise trails to trials

3. These are the few examples I managed to spot.

Response: "trails" has been replaced by "trials" throughout the manuscript and supplementary materials.

4. Supplementary file . Screening flow chart box 2 "records after duplicates" Fig. S7. I do not totally understand. Does this mean after duplicates were removed or records including duplicates. It reads incomplete to me. Similarly for Fig. S8. Figure S7 far left bottom box uses "Include" but S8 uses "Included"

Response: The box 2 of screening flow chart denotes that records after duplicates were removed in Figure S8 and S9. "include" has been replaced by "included" in Figure S8.

5. Supplementary file seems OK. Check Figure captions, language as per my earlier comments.

Response: The caption of Figure S4 has been revised as follows: "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" (Line 959-962).

Reviewer #2 (Remarks to the Author):

1. This manuscript describes the meta-analysis of the biofortification effect of foliar or soil Zn application, or the combination, on wheat grain Zn concentrations. While the meta-analysis is nice and certainly noteworthy, there is always the risk that the conclusions drawn from the analysis are ambiguous, based on data collected under different conditions, that not always allow direct comparison.

Response: Thanks for your positive comments. In this study, the sample size used for the meta-analysis was large. A total of 83 publications for Zn fertilizer applied to the soil and 82 for foliar application was used for the meta-analysis, including 637 and 765 paired observations for grain Zn concentration of wheat. For improving the reliability of the results of meta-analysis, this study conducted Gaussian distribution test on the analyzed data, and only the data that met the normal distribution or nearly normal distribution were used for the meta-analysis. Furthermore, to determine the influence of different conditions (e.g., various soil properties and soil managements) on the results of meta-analysis, subgroup analysis and meta-regression analysis were also carried out in this study. Results from subgroup analysis and meta-regression analysis showed that Zn rate, soil available Zn, pH, initial grain Zn concentration and spaying Zn timing to foliage produced significant effects on the improvement in grain Zn concentration. Finally, the main factors affecting the improvement in grain Zn concentration by Zn fertilization were determined based on the results of correlation analysis and random forest analysis. We are confident that the conclusions drawn from this analysis are reliable.

2. In the abstract, the authors indicate that validation experiments have been performed to confirm the findings from the meta-analysis. Much to my surprise, I could not find such validation experiments in the results, but based on the discussion, it appeared that these experiments had been performed already a long time, ~10 years, ago (L. 476-492). Perhaps I misunderstood this completely, but if not, I find it hard to see how such validation experiments could have been performed long before the actual meta-analysis was performed, and still justify the conclusions drawn by the authors. In any case, where one would expect new validation experiments to be performed with the suggested Zn fertilization levels and applications, to confirm the finding derived from the meta-analysis, this is not obvious from section 2.4.

Response: To validate the calculation method on Zn recommended application rate, 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. However, the wheat had not yet been harvested when the manuscript was submitted, so no relevant results were provided in the manuscript. Therefore, to verify the feasibility of the method for calculating the Zn recommended rate, we compared the amount of Zn fertilizer obtained by the calculation method with the recommended amount of Zn fertilizer in previous experiments published in the submitted manuscript. The results of validation experiments have been added in the revised manuscript as follows: "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 4.8% to 24.6% (Table S1)" (Line 325-331).

3. Also how the authors come to the final conclusion, as written in L. 232-235, and their recommendations (l. 236-240), or the conception shown in fig 7, is not clear to me.

Response: The corresponding parts have been revised as: "Based on the findings from meta-analysis, random forest analysis, and correlation analysis (Fig. 3a, 6a, S5), we identified the Zn rate and initial grain Zn concentration as essential factors for enhancing Zn levels in wheat grain through soil fertilization (Fig. 7a). 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. 7a, S5). 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:

$$\text{Zn recommended rate } (\text{kg Zn ha}^{-1}) \frac{\text{Zn biofortification target} - \text{initial grain Zn concentration}}{\text{Zn agronomic biofortification index}} \quad 5)$$

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, S4b). 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 10.0%, respectively (Fig. S4c). Given the importance of grain yield in wheat production, this study divides the target for wheat Zn biofortification into two stages (Fig. 7a). 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. For the sake of convenience in calculation, the target of 30 mg kg^{-1} is utilized for Zn biofortification in the first stage. 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}" (Line 235-274).

4. Furthermore, I have serious concerns about several statements made in the discussion.

E.g., authors suggest that soil used for wheat generally has high pH and high calcium carbonate levels, contributing to Zn availability issues, but I wonder if this is true. There are for sure also many areas which do not suffer from such conditions, on which wheat yield is high, even though this certainly often does not go along with high grain Zn concentrations. Most of the trial sites that provided the data for the meta-analysis, as shown in fig 8, are from areas with such unfavorable conditions, probably as these result in low yield and poor Zn levels, which inspired Zn fertilization trials. However, these do not appear to be representative for all wheat production areas.

Response: Indeed, there are many areas that does not suffer from adverse soil conditions and grow wheat with high yields and low Zn concentration in grain. The low Zn concentration in grains in these areas was related to the availability of Zn in soils. This is because the availability of Zn in soil required for high grain Zn concentration in wheat production was higher than that required for high yield (Liu et al., 2016). Furthermore, no matter what the soil conditions, Zn has poor mobility in soil and reaches the rhizosphere of crops mainly by diffusion, which could not make up for the deficiency of Zn in the rhizosphere in time. Therefore, many areas without adverse soil conditions produce wheat with high yield and grain Zn concentration. The area where Zn fertilizer trials were conducted, although not representative of all wheat production areas, is also an area where wheat is widely grown. The corresponding has been revised as: "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 time[8]. Moreover, wheat is widely cultivated in soils characterized by high pH, $CaCO_3$, and low organic matter and moisture levels, which hinder Zn solubility and diffusion to roots, even after Zn application to the soil[6,13,22]. These conditions restrict the amount of Zn available in the rhizosphere for root uptake by wheat crops, resulting in low grain Zn concentrations" (Line 307-315)

Liu, D.Y., et al. Effects of zinc application rate and zinc distribution relative to root distribution on grain yield and grain Zn concentration in wheat. *Plant & Soil* **411**, 1-12 (2016).

5. The section written in L. 259-271 is puzzling. There will be different degrees of Zn deficiency in wheat production areas. If Zn is limiting, yield will be low, and the grain Zn concentration will likewise be below target. If Zn is not limiting, and neither are other nutrients, additional supply will not be beneficial, biomass production will be high, as will grain Zn concentrations.

Between these extremes, there will be levels at which there is enough Zn to support plant biomass production and moderate grain yield, but grain Zn concentration may still be (far) below target. In such situations, foliar application may not spur root system development, and thus not contribute to high grain yield, only to elevate grain Zn concentration. In more Zn limiting conditions soil Zn application may be foremost needed to feed biomass production. Such more elaborate evaluation of current practices may be needed here L. 259-271

Response: As you suggested, the corresponding part has been revised as: "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, S4b). 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 10.0%, respectively (Fig. S4c). Given the importance of grain yield in wheat production, this study divides the target for wheat Zn biofortification into two stages (Fig. 7a). 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. For the sake of convenience in calculation, the target of 30 mg kg^{-1} is utilized for Zn biofortification in the first stage" (Line 257-274).

6. Authors claim that overuse of Zn fertilizers will cause Zn accumulation in the soil, leading to environmental risks (L. 275), but in case of low Zn utilization efficiency, much of the Zn applied is apparently quickly made unavailable to the plants, due to the unfavorable soil properties. This is unlikely to pose much of a risk unless the unavailable Zn is suddenly made available again, which is very unlikely to happen as it will require large-scale acidification of the soil. And even so, soil Zn concentrations up to 100 ppm are still far from acute Zn toxicity levels.

Response: In the case of low utilization of Zn fertilizer, most of the Zn applied to soil is transformed into unavailable fraction for crops. This is not much risk to the crop. However, the total Zn in the soil continues to increase after long-term application of Zn fertilizer. The maximum allowable concentration is 300 mg kg^{-1} in the soil with pH>7.5 according to China Environmental Quality Standard for Soil (CEPA, 1995). The case provided in this study shows that the total Zn concentration in the soil increased from 61.3 to 185.8 mg kg^{-1} following continuous application of 34.1 kg Zn ha^{-1} for 8 years. If the rate of increase in total Zn remains constant when 34.1 kg Zn ha^{-1} is applied to the soil, the total Zn concentration in the soil will exceed 300 mg kg^{-1} in the next 8 years. Therefore, the overuse of Zn fertilizers may cause accumulation of Zn in the soil and pose environmental risks to the soil. The corresponding description has been removed in the revised manuscript according to the first reviewer's suggestions to reduce the discussion part.

CEPA, 1995. Environmental Quality Standard for Soils (GB15618-1995). Chinese Environmental Protection Administration.

7. The discussion on the effect of other nutrients, N, P, K, combined with Zn is very useful, but often this discussion not very strong. Plants will need NPK as well as Zn. If one is limiting, adding a lot of the others is unlikely to be very effective. When two are limiting, adding one is also unlikely to be very effective. Plants have normally very effective strategies to acquire essential nutrients, including for N, P, K and Zn. If two are suboptimal, adding one is likely to induce enhanced expression of the genes involved in acquisition of the other, especially if the supply is relatively close to sufficiency. In this respect, increasing N supply is likely to enhance the Zn deficiency response, as described in L. 313-319, but only if Zn supply is only mildly deficient.

8. It is all about finding optimal supply levels, for all essential nutrients. It is therefore not strange to see that at excessive N supply in Zn deficient conditions, Zn supply will be less effective (with plant growth repressed by high N), unless also N supply is reduced (L. 328-329).

Response: As suggested, the corresponding part has been revised as: "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" (Line 423-433).

9. Finally, the genetic component to Zn biofortification is poorly addressed. Based on L. 200-201, wheat varieties were not assessed based on genotype, but on initial grain Zn concentration. That will be a poor predictor of genotype, knowing that Zn fertilization in the previous generation has such a strong effect on grain Zn concentration. Also since it is well known that there is substantial genetic variation for grain Zn concentration (eg. L 407-408), it seems an omission of the meta-analysis not to properly account for this.

Response: Grain Zn concentration of different wheat varieties is determined by genotype and environmental factors. During the breeding process, wheat trials are often conducted on soil with sufficient Zn, resulting in newly developed varieties unable to realize their high-Zn characteristics when grown under cropping conditions where the availability of Zn in soil is limited and stress factors, such as, heat and drought stress occur. Shuttle breeding, a breeding method in which, wheat breeding material from different generations is alternately grown in two or more different environments, has been recommended in selecting wheat varieties with high grain Zn concentration, so that the newly developed wheat varieties would have a wide range of adaptability under actual different production conditions. This study defined varieties that had high Zn concentration in wheat grains grown in soils with low availability of Zn (i.e., soils were not added Zn) as high-Zn varieties, which meet the current breeding goals for high-Zn varieties and quantify the contribution of wheat varieties to the increase in grain Zn concentration by Zn fertilization. The corresponding parts have been revised as: "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 drought[6]. 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 conditions[40]. 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 varieties[6]. 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" (Line 347-361).

10. L. 419-423 suggest that the analysis identified wheat varieties with different grain Zn concentrations, but it is not clear where this is reported in the manuscript.

Response: In the present study, meta-analysis of data has some wheat varieties with grain Zn concentration higher than the final biofortification target of 40 mg kg^{-1} under Zn-deficient soils and with application of Zn fertilizers. The corresponding sentence has been removed from manuscript according to the first reviewer's suggestions.

11. In conclusion, the lack of validation experiments performed based on the results of the meta-analysis, and the poor discussion, seriously undermine the significance of the work, which I think is below what is expected for publication.

Response: To validate the calculation method on Zn recommended application rate, 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. However, the wheat had not yet been harvested when the manuscript was submitted, so no relevant results were provided in the manuscript. At present, the wheat of the validation experiments has been harvested, and the related work on sample measurements has also been completed. The results of validation experiments have been added in the revised manuscript as follows: "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 4.8% to 24.6% (Table S1)" (Line 327-331).

In addition, as both reviewers suggested, the discussion part has been rewritten. To emphasize the innovation of the manuscript, the discussion part has been shortened as the first reviewer suggested, and the novelty of the analysis has been placed in the first part of the discussion, that is, agronomically biofortifying Zn concentration in wheat grains based on the Zn biofortification target, Zn agronomic biofortification index and initial grain Zn concentration. Secondly, the important factors regulating Zn agronomic biofortification in wheat grain by Zn fertilization were discussed. The significances and limitations of the study were finally added at the end of the discussion. The discussion parts have been revised as Line 276-472.

Authors Responses to Comments

Reviewer #1:

Thank you for submitting revisions to comments on your manuscript "Zinc agronomic biofortification in wheat and its drivers: A global meta-analysis". I reviewed your responses which addressed most of the comments from the initial review. I am convinced with your responses apart from a few which I indicated below. For your ease, my comments are in a bracket [] and have three *** before the comment.

Response: Thank you very much for your positive comments and suggestions which is very exciting and encouraging. We have considered all your suggestions and made improvements in our revised version (R2). Please see details under each comment here below.

1. Comments from the first review: (2) I, however, find the discussion rather too long as authors presented commonly known factors limiting or enhancing Zn uptake and concentration. I suggest authors ensure that the novelty of their meta-analyses (presented last in their discussion section) is not hidden among commonly known background information on factors governing Zn supply. The novelty of the analysis could be elaborated on a bit more (instead of a few lines at the end of the discussion), with less emphasis on what we already know (i.e. reduction in costs from co-application of Zn and pesticides which was published a few years back and superiority of soil + foliar versus soil, or foliar applications which most agronomic biofortification studies tend to discuss). I end by emphasising that the need for different Zn application methods for wheat grain with different Zn concentrations is novel and very interesting. This novel message warrants publication of the manuscript in Nature Communications. I however think this message is presented a bit too late in the manuscript. I did not see any study strengths and limitations section or conclusion section, maybe this is per Nature Communications guidelines. I include here and also attach as pdf file, detailed comments for authors to revise and improve clarity of their manuscript. The writing of the manuscript can also be improved in some areas as indicated in my detailed comments.

Response to the first comments: I have shortened the discussion section following your suggestion, and revised the discussion to emphasize the novelty. In addition, the significances and limitations of the study have been added at the end of the discussion. Detailed responses to each suggestion are listed as follows.

Comments from the second review: [***Thank you for considering my suggestions. I read the first sub-section of the authors' discussion. Unfortunately, it is now mainly focusing on China alone (Lines 322-344), and deviates from the title, which is supposed to provide recommendations for global wheat production systems. Can a few sentences be provided to make similar recommendations for other regions. I also do not agree with the sentence that wheat production regions have low organic matter content (Line 312). Can a reference be provided. If authors already provided a reference, can this be provided alongside the soil property instead of placing all the references (6,13,22) at the end of the sentence].

Response: Suggestions have been taken. The corresponding information on Zn recommended application rates for global wheat production systems has been added as: "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 kg Zn ha^{-1} for soils and 0.75 kg Zn ha^{-1} for foliage (Fig. 8a). Specifically, the average application rates for soils and foliage are 10.4 and 0.80 kg Zn ha^{-1} in Asia, 11.7 and 0.63 kg Zn ha^{-1} in Europe, 7.7 and 0.56 kg Zn ha^{-1} in North America, and 17.7 and 0.70 kg Zn ha^{-1} in other continents (Africa, Oceania, and South America), respectively" (Line 342-351).

Indeed, there are many wheat production regions where soils do not suffer from small organic matter contents. However, there are also many wheat cropping regions where soils contain low organic matter. For instance, Lantican et al. (2002) reported that around 10% of the world's wheat is produced in regions with a Mediterranean-type climate, with predominantly calcareous soils, which have not only high pH and $CaCO_3$ but also small organic matter and moisture contents. The corresponding part has been revised as: "Moreover, there are many wheat production regions with soils that suffer from high pH, $CaCO_3$, and low moisture and organic matter contents[6,12,36], hindering Zn solubility and diffusion to roots[13], even after Zn application to the soil[22]" (Line 321-323).

Additional 3 new references have been added to the list:

6 Cakmak, I. & Kutman, U. B. Agronomic biofortification of cereals with zinc: a review. *European Journal of Soil Science* **69**, 172–180 (2018).

12 Alloway, B. Soil factors associated with zinc deficiency in crops and humans. *Environmental Geochemistry and Health* **31**, 537–548 (2009).

36 Lantican, M., Pingali, P. & Rajaram, S. Are marginal wheat environments catching up? *CYMMIT World Wheat Overview and Outlook Part 2*, 39–44 (2002).

2. Comments from the first review: 5. Line 532: As Africa is not mentioned as one of the regions, I am surprised the Zou et al 2012 paper on wheat in Zambia did not come up in your search. I see the data in Figure 1 but Africa is not mentioned here.

Response to the first comments: Sorry for the incomplete description. Our search included the paper published by Zou et al. (2012), and Zambia came up in Fig. 8, but we forgot to mention it. The corresponding part has been revised as: "These data were sourced from studies conducted in 19 countries across East, West, and South Asia, North America, South America, Europe, South Africa, and Oceania (Fig. 8)" (Line 496-498).

Comments from the second review: [***I appreciate authors' responses. Please change South Africa to Southern Africa. South Africa is a country and I think the authors sought to mention the region, which is Southern Africa].

Response: Thanks for your reminding. "South Africa" has been replaced by "Southern Africa" (Line 522).

3. Comments from the second review: For the sake of convenience in calculation, the target of 30 mg kg^{-1} is utilized for Zn biofortification in the first stage. 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}" (Line 257-274).

[***I am not sure if the statement "For the sake of convenience in calculation" reads scientifically sound. One would argue that authors could have used the global mean average of 28.2 mg kg^{-1} as it is without rounding it up by 6%, which when you are working with micronutrients, can make such a big difference. Please kindly revise the wording]. Response: In this study, optimizing Zn agronomic biofortification measures includes two stages. The first stage aims to enhance soil available Zn levels for mitigating yield losses in wheat grown on severely Zn-deficient soils and elevating grain Zn concentration to match the global average of 28.2 mg kg^{-1}. The second stage focuses on increasing grain Zn concentration for wheat grown on soils without Zn deficiency to 40 mg kg^{-1}, which is the level required for human health. Therefore, in order to achieve high grain yield and facilitate calculation, the grain Zn concentration of 30 mg kg^{-1} is set as the biofortification target for the first stage. The corresponding sentence has been revised as: "In this study, we used Zn concentration of 30 mg kg^{-1} as Zn biofortification in the first stage for targeting high grain yield" (Line 276-278).

4. Comments from the second review: "For each of our studies and publications, we extracted geographic location, available soil Zn, organic matter, $CaCO_3$, 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 yield, biomass, and Zn concentration in grain and straw" (Line 531-535).

[***The word "wheat yield" is misleading. Include the word "grain" if this was wheat grain yield to read "wheat grain yield"].

Response: As suggested, "wheat yield" has been replaced by "wheat grain yield" (Line 561).

Reviewer #3:

1. Having read the initial reviews, and the author responses, I would support publication of the manuscript. The two major concerns of Reviewer 2, i.e. the lack of recent validation experiments and the quality of the Discussion (also raised by Reviewer 1) have been addressed through the inclusion of new field data from 6 sites in China. The Discussion has been comprehensively edited and reads clearly.

Response: Thanks for your positive feedback on our manuscript revision, which is greatly appreciated and exciting.

2. There was one editorial comment:

P12/L279 "A grain Zn concentration in wheat of 40 mg kg^{-1} is essential for human nutrition." I would suggest rephrasing to say that "A grain Zn concentration threshold of 40 mg kg^{-1} has been suggested in terms adequate Zn nutrition".

Response: Suggestions have been taken. The corresponding sentence has been replaced by "A grain Zn concentration threshold of 40 mg kg^{-1} has been suggested in terms adequate Zn nutrition for humans" (Line 287-288).

3. I have not scrutinised the Supplementary Materials in detail, and it seems that most of the data are presented to allow someone to reanalyse the data, which is great. But, please can the authors ensure that ALL data are presented (i.e. I could not see the primary data from the 2022-2023 validation trials), and this needs to be checked.

Response: We have checked all the data and ensured its accuracy in presentation. The data from the 2022-2023 validation trials has been added in Table S1 and the file "Data of validation trials.xlsx".

Reviewer #4 (Remarks to the Author):

The manuscript is well-written and addresses an important research topic effectively. The use of meta-analysis and machine learning (ML) modeling is commendable. However, to strengthen the manuscript, consider including a small comparative statement of these two methodologies and providing additional clarity on the methods. Here are some specific suggestions:

1. There is no description of the output variable, specifically the response of grain zinc (Zn) concentration to zinc fertilization. It is unclear what this variable represents and what its unit of measurement is. Additionally, is it similar to Agronomic Nutrient Use Efficiency. Could you clarify these details?

Response: Thank you very much for your comments and suggestions. The response variable (i.e., the response of grain Zn concentration to zinc fertilization) is the effect size of grain Zn concentration, which is the natural log of response ratio (lnRR) and calculated as follows:

$$\text{Effect size} = \ln\text{RR} = \ln\frac{\overline{X}_t}{\overline{X}_c} \tag{2}$$

where $\overline{X}_t$ and $\overline{X}_c$ are the averages of the grain Zn concentration under the Zn application treatment and the control treatment, respectively. Consequently, the response variable is dimensionless, and differs from agronomic nutrient use efficiency, which refers to the increase in grain yield by applying 1 kg of pure nutrient. The corresponding information has been revised as: "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). Additionally, a regression analysis using random forest (RF) was conducted to identify the primary drivers influencing the effect size of grain Zn concentration" (Line 621-625).

"**Fig. 6** Importance of fertilization managements, initial soil properties and wheat cultivars influencing the effect size of grain Zn concentration in wheat grown with Zn fertilizer applied to soil (a) and foliage (b)" (Line 918-920).

2. The procedure for determining the significance of the Random Forest (RF) model is not clear. It is unclear whether the RF model was compared against a null model, multiple random models generated from permuted data, or any other statistical benchmarks. Could you provide more details on the significance testing approach used for the RF model? Same for the significance of the predictors, how authors approached. Just mentioning the package does not make it clear.

Response: "A3" package in R is an error metrics for predictive models. It can make the accurate assessment of model errors more accessible to a wider audience. Furthermore, a standardized set of reporting features are provided by this package which create consistent outputs for virtually any predictive model. The standard outputs for each model fit provided by the A3 package include: average slope, cross validated R^2, and *P*-values. The cross validated R^2 is robust calculation of R^2 (percent of squared error explained by the model compared to the null model) values adjusting for over-fitting. Consequently, the Random Forest model was compared against a null model. This study use "A3" package to determine the significance of the Random Forest models. The key procedures include: (1) use “model.fn” function (i.e., model.fn=randomForest) to generate Random Forest models; (2) set the parameter of p.acc=0.01 to replace 100 times (1/0.01=100 times) of permutation for the calculation of the *P*-values of Random Forest models; (3) use “model.args” function to pass the parameters to the Random Forest models. The detailed R code for the significance of the Random Forest models has been provided in Supplementary Code 2”. The corresponding information has been revised as: “The significance of the RF models and the R^2 values were determined using the “A3” package in R 4.1.3, which can provide a robust calculation for R^2 values through comparing against a null model. The key procedures include: (1) set the model.fn=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 models” (Line 632-637).

“rfPermute” package can estimate the significance of importance metrics for a Random Forest model by permuting the response variable, produce null distribution of importance metrics for each predictor variable and *P*-value of observed, and provide summary and visualization functions for 'randomForest' results. This study use “rfPermute” package to assess the significance of predictor variables. The key parameters include: (1) set the importance=TRUE to determine the importance of predictor variables that need to be assessed; (2) set ntree=1000 to grow 1000 trees for Random Forest models; (3) set num.rep=100 to replace 100 times of permutation to construct null distribution and calculate *P*-values of predictor variables. The detailed R code for the significance of the Random Forest models has been provided in Supplementary Code 2”. The corresponding information has been revised as: “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 permutation” (Line 637-642).

3. Whether the aim of the random forest modelling was to find the important predictors only, which authors has also described in figure 5, where they have compared the effect size from meta-analysis.

In this setting a highlight on how the important predictors screened from RF differ from meta-analysis (screened from total effect size may be?) also in supplementary figure a comparison between the partial dependency plot and figure 5 will be nice. Else if the unit are same, they can be in same figure only.

What is the unit of relative importance? whether it was percentage, in the figure 6, x-axis? An additional figure with above changes may justify more the use of RF, when authors are already doing the meta-analysis.

Response: The subgroup analysis presented in Figs. 3 and 4 and the meta-regression analysis in Fig. 5 were to determine the factors influencing the effect size of grain Zn concentration and its change rule as the factors changed. The random forest modelling was to assess the importance of various influence factors only. Furthermore, correlation analysis was performed between fertilization management, initial soil properties, wheat varieties and effect size for grain Zn concentration, as presented in Supplementary Fig. 5. Finally, based on the findings from random forest analysis and correlation, we identified essential factors influencing the effect size of grain Zn concentration, that is, the essential factors for enhancing Zn levels in wheat grain through applying Zn fertilizers. The corresponding information has been revised as: "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). Additionally, a regression analysis using random forest (RF) was conducted to identify the primary drivers influencing the effect size of grain Zn concentration" (Line 621-625).

In these random forest models, the increase in node purity (IncNodePurity) of the predictors was employed to gauge the importance of these factors. It was measured by the reduction in the sums of squares of residuals (RSS), representing the impact of each variable on the heterogeneity of observations at each node in a regression tree. Specifically, when a variable is used to split a node, it reduces the heterogeneity of observations within the node (i.e., increases node purity). The corresponding reduction in RSS is the IncNodePurity value for that variable. A larger value indicates that the variable contributes more to the model's predictive accuracy, meaning it ranks higher in terms of variable importance. Therefore, the importance of predictor variables presented in Fig. 6 did not have a unit, and was not percentage. To describe accurately the importance of predictor variables, "Relative importance" has been replaced by "IncNodePurity" Fig. 6. The corresponding information has been revised as: "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 predictors" (Line 625-631).