RC2

This manuscript presents the development of the DO3SE-CropN model, which simulates reductions in protein and amino acids in wheat subjected to ozone (O_3) stress. The authors effectively incorporate antioxidant processes, thereby increasing the model's applicability for predicting O_3-induced quality losses in crops. Including crucial amino acids, such as lysine and methionine, is essential for assessing the effects on protein quality. Further clarification regarding the specific enhancements to the original model framework and how these modifications rectify limitations in earlier versions would enhance the manuscript.

1. The authors illustrate the model's ability to predict protein quality, successfully simulating lysine and methionine concentrations in wheat grain. However, the underestimation of reductions in amino acid content induced by O_3, particularly for lysine, is significant. The manuscript should discuss potential reasons for these discrepancies, such as limitations in the underlying assumptions of antioxidant pathways.

We thank the reviewer for their suggestion. We agree that the discussion of AA simulations was limited. We have now incorporated an additional section into the discussion, in between previous sections 5.1 and 5.2, to better discuss the reasons for the discrepancy in AA simulations. Some of the differences in simulations do occur due to limitations in our current understanding of O_3's impacts on antioxidants and grain quality which subsequently affects the assumptions made in the model construct and also the calibration. I have discussed this in sections 5.3 and 5.4 and direct the reader here in the new discussion section. However, there is an additional factor affecting the amino acid simulations which was not discussed prior, and this relates to the regressions used to convert grain protein to grain amino acids. The additional section in the discussion is as follows:

"While the grain methionine concentrations were reproduced well, the grain lysine concentrations were overestimated for the elevated O_3 treatment. It is also clear to see that the reduction in concentrations of both lysine and methionine was underestimated by the DO$_3$SE-CropN model. The AA concentrations were calculated using regressions linking protein concentrations to AAs from Liu et al. (2019), which were constructed using data from 48 field experiments from major wheat producing areas in China. Approximately 95% of wheat grown in China is winter wheat (United States Department of Agriculture, 2022), and most of the cultivars used to produce the regressions were winter wheat (Liu et al., 2019). However, the model was parameterised for Indian spring wheat. Given the differences between the growing conditions in India and China, and spring and winter wheat, deviations in simulations of lysine and methionine concentrations from the observed are to be expected. Additionally, Liu et al. (2019) did not include experiments with differing levels of O_3 in their treatments. For lysine, this has culminated in a much better simulation of the AA concentrations under ambient O_3 compared to the elevated treatment. For both lysine and methionine, using the regressions alone to convert grain protein to grain AA concentrations was not sufficient to account for the O_3 effect on grain quality. Additionally, there is currently a knowledge gap (discussed further in section 5.3) relating to our understanding of O_3's effects on both antioxidants and grain quality, which affects not only the construction of the model but also its parameterisation. Suggestions for experiments which could reduce the knowledge gap for both modelling and understanding the effect of under O_3 exposure on grain protein and AAs are discussed in sections 5.3 and 5.4."

2. The study examines the critical issue of O_3 pollution and its impact on food security in India, highlighting the significance of this research given global nutrition challenges. The study could

be improved by addressing potential regional variations in O_3 sensitivity within the context of the model's application to Indian wheat and exploring how this framework may be adapted for other significant wheat-producing regions experiencing comparable environmental stressors.

We agree with the reviewer that such a discussion would add great value to the manuscript. We believe the first comment: "addressing potential regional variations in O_3 sensitivity within the context of the model's application to Indian wheat" fits well with the current discussion section "Further work for understanding O_3 effects on wheat nutrition". Into this section we have added some remarks on the current wheat growing regions in India which are projected to experience the greatest O_3 effect on yield due to having the greatest modelled stomatal O_3 uptake, as these will likely overlap with the regions that will experience the greatest O_3 effect on nutrition. In this, we consider only models that have estimated stomatal O_3 uptake, as concentration-response studies have also been used to predict the spatial impact of O_3 on yields in India, but studies have shown that the areas with the greatest O_3 concentrations do not always overlap with those with the greatest yield losses due to the modifying effect of the environment on O_3 uptake (Pleijel, Danielsson and Broberg, 2022; Emberson et al., 2000). (e.g. If it is hot the stomata are likely closed and not taking up O_3).

The second part of the comment "exploring how this framework may be adapted for other significant wheat-producing regions experiencing comparable environmental stressors" is very interesting. In section 5.2 we had previously written: "The design of the antioxidant equations has several benefits which make it useful for further applications. Firstly, the structure of Eq. 1 means that it could be easily translated to other ROS mediated stressors, provided the corresponding equation parameters are identified, meaning the framework is flexible." Other ROS mediated stressors include high temperature and drought stress, which have been shown to also cause yield and protein reductions. Given that all the stressors cause similar effects on crop yield and quality, and are ROS mediated, it can be assumed that the mechanisms of reductions to yield and quality are similar and could be approximated using the same mechanism. In order to use such a mechanism, we would require a suitable proxy for measuring damage. In this study, we linked accumulated O_3 flux to antioxidant production. However, for drought stress it would not be as simple, due to the fact that N is taken up by the plant dissolved in water. In this case, the crop model would require suitable soil water algorithms to first simulate the effect on nutrient uptake, and to then simulate the effect of drought stress on increasing antioxidant production a suitable metric could be the duration and timing (e.g. pre- or post-anthesis) that the stressor occurs. Similarly, for heat stress it could be timing and duration of the stress. We incorporated these comments as follows:

"The design of the antioxidant equations has several benefits which make it useful for further applications. Firstly, the structure of Eq. 1 means that it could be translated to other stressors provided they have a similar mechanism of damage to O_3, meaning the framework is flexible. Drought and high temperature stress are good candidates for this framework as they are ROS mediated, like O_3, and cause a reduction in both grain yield and protein content (Broberg et al., 2015, 2023; Mariem et al., 2021). The effect of heat stress on antioxidant production, and hence grain quality, could be incorporated by modifying Eq. 1 and Fig. 2 to incorporate the duration (and potentially timing) of the stress as these are the key factors affecting grain yield under heat stress (Balla et al., 2019). For drought stress, the duration of the stress would be useful, but there would need to be an additional effect of drought on reducing nutrient uptake (as this affects grain quality) (Rijal et al., 2020; Faisal et al., 2017). The second benefit of the framework is that it is simple..."

3. The suggestion to combine nitrogen and protein assessments from leaves and stems, along with a deeper exploration of nitrogen allocation to antioxidants, is noteworthy. These efforts are expected to enhance model precision. It would be beneficial for the authors to delineate the types of experimental data required to refine these aspects and to articulate specific hypotheses concerning the influence of antioxidant allocation on grain protein quality under O_3 stress.

We thank the reviewer for this suggestion and agree that this would then help future work to target the remaining uncertainties regarding O_3, antioxidant processes and O_3's effects on crop nutrition. We believe that the reviewers' suggestions best fit in the final section "Further work for understanding O_3 effects on wheat nutrition" In this section we have two bullet points which originally vaguely summarised the kinds of information required to further develop our understanding: "3) To advance the antioxidant equations, and understand O3 effects on grain quality, an experiment measuring N and protein concentrations in the leaf and stem at anthesis, and harvest, should be conducted. The proportion of N associated with antioxidants under the same O3 treatments should also be obtained to improve mechanistic understanding of plant antioxidant response to O3 which can be used to further develop the model. 4) Relationships linking grain protein to grain AA concentrations should be investigated for how they change under the influence of O3. The modified equations could be integrated in the model so improve its ability to simulate AA concentrations under stress, and hence provide more trustworthy estimates of protein quality."

We improve on these two points by referencing the specific type of experiment that is required to obtain such data, which are O_3 exposure experiments. We don't distinguish whether these should be solardome, OTC or FACE as all would provide valuable information. We also specify greater detail on the kinds of data which should be obtained and which research questions the data will help to address. The improved text is as follows:

"3) To advance the antioxidant equations, and understand O3 effects on grain quality, an O3 exposure (e.g. FACE, OTC or solardome) experiment measuring total N and protein content, and N and protein concentrations in the leaf and stem at anthesis, and harvest stages under varying O3 treatments should be conducted. The proportion of N associated with specific antioxidants (such as glutathione and enzymatic antioxidants) under these O3 treatments should also be obtained to improve mechanistic understanding of plant antioxidant response to O3. This can be used to further develop the model, as it is anticipated that increased allocation of N to antioxidant production in leaves and stems under O3 stress reduces the N available for remobilisation to grains during grain filling, leading to a decrease in grain protein concentration and altered amino acid profiles.

4) From the same O3 exposure experiments, measurements of grain protein and AA concentrations for each O3 treatment should be collected to produce relationships linking the two and how the relationship changes under the influence of O3 to verify whether there is a trade-off between stress mitigation and nutritional quality. Such relationships could be integrated in the model to improve its ability to simulate AA concentrations under stress, and hence provide more trustworthy estimates of protein quality."

4. While the model accurately predicts yield loss, the discrepancies in amino acid concentration predictions indicate a need for further calibration. Additional validation steps, such as utilizing independent datasets or conducting field trials, may improve the credibility and generalisability of the model outputs.

We agree completely with the reviewer here. Unfortunately, there is limited availability of such data. To date, the only study that has investigated the effect of O_3 on amino acid concentrations in wheat is that conducted by Dr Durgesh Singh Yadav, who generously provided his data and expertise for the development of the present model. We hope that this study will provide a modelling foundation, and useful suggestions for experimentalists and modellers alike so that in the future we may improve our understanding of O_3's effects on crop nutrition further. We have incorporated remarks to this effect in the main manuscript as others may have similar questions.

We added the following at the beginning of the new section 5.2 (see response to comment 1): "To date, there is only one study (by Yadav et al. (2020)) that has investigated the effect of elevated O_3 on the AA concentrations of wheat . Data from this study was used to calibrate and evaluate the DO_3SE-CropN model, as well as test the framework for the AA simulations." Then at the end of the new section 5.2 we write: "Suggestions for more specific experiments which could reduce the knowledge gap for both modelling and understanding the effect of under O_3 exposure on grain protein and AAs are discussed in sections 5.3, 5.4 and 5.7. Nevertheless, it is clear that additional data on O_3's effect on grain AA would be beneficial for not only model development, but improving confidence for modelling results."

The particular comment addressed in point 3 then link nicely to the additions here as well, as point 3 then provides ideas as to how these particular kinds of experiments would take place.

5. The authors emphasize the model's adaptability in simulating responses to various abiotic stressors. To enhance the manuscript, it would be beneficial to include examples of specific stressors, such as drought and heat, to which this framework could be adapted and discuss any preliminary adaptations made to expand its applicability.

I agree with the reviewer and believe I have now incorporated this in comment 2, where I have included some remarks on how to incorporate drought and heat stress into the framework, with additional remarks for drought which will also affect nutrient uptake

We thank the reviewer for providing such valuable feedback which has greatly improved the strength of the manuscript .
