Modelling the nutritional implications of ozone on wheat protein and amino acids

Jo Cook[1], Durgesh Singh Yadav[2], Felicity Hayes[3], Nathan Booth[1], Sam Bland[4], Pritha Pande[4], Samarthia Thankappan[1], Lisa Emberson[1]

[1]Environment & Geography Dept., University of York, YO10 5DD, UK
[2]Department of Botany, Government Raza P.G. College, Rampur, India
[3]UK Centre for Ecology & Hydrology, Environment Centre Wales, Bangor, Wales, UK
[4]Stockholm Environment Institute at York, Environment & Geography Dept., University of York, YO10 5DD, UK

Correspondence to: Jo Cook (jo.cook@york.ac.uk)

Abstract. Ozone (O_3) pollution reduces wheat yields as well as the protein and micronutrient yield of the crop. O_3 concentrations are particularly high in India, and are set to increase, threatening wheat yields and quality in a country already facing challenges to food security. This study aims to improve the existing DO_3SE-CropN model to simulate the effects of O_3 on Indian wheat quality by incorporating antioxidant processes to simulate protein, and the concentrations of nutritionally relevant amino acids. As a result, the improved model can now capture the decrease in protein concentration that occurs in Indian wheat exposed to elevated O_3. The structure of the modelling framework is transferrable to other abiotic stressors and easily integrable into other crop models, provided they simulate leaf and stem N, demonstrating the flexibility and usefulness of the framework developed in this study. Further, the modelling results can be used to simulate the FAO recommended metric for measuring protein quality, the DIAAS, setting up a foundation for nutrition-based risk assessments of O_3 effects on crops. The resulting model was able to capture grain protein, lysine and methionine concentrations reasonably well. As a proportion of dry matter, the simulated percentages ranged from 0.26% to 0.38% for lysine, and from 0.13% to 0.22% for methionine, while the observed values were 0.16% to 0.38% and 0.14% to 0.22%, respectively. For grain and leaf protein simulations, the interdependence between parameters reduced the accuracy of their respective relative protein loss under O_3 exposure. Additionally, the decrease in lysine and methionine concentrations under O_3 exposure was underestimated by ~10 percentage points for methionine for both cultivars, and by 37 and 19 percentage points for lysine for HUW234 and HD3118 respectively. This underestimation occurs despite simulations of relative yield loss being fairly accurate (average deviation of 2.5 percentage points excluding outliers). To provide further mechanistic understanding of O_3 effects on wheat grain quality, future experiments should measure nitrogen (N) and protein concentrations in leaves and stems, along with the proportion of N associated with antioxidants, which will aid in informing future model development. Additionally, exploring how grain protein relates to amino acid concentrations under O_3 will enhance the model's accuracy in predicting protein quality and provide more reliable estimates of the influence of O_3 on wheat quality. This study builds on the work of Cook et al. (2024) and supports the

second phase of the tropospheric O_3 assessment report (TOAR) by investigating the impacts of tropospheric O_3 on Indian wheat and the potential of this to exacerbate existing malnutrition in India.

1. Introduction

35 A growing body of literature from Europe, China and India has shown that exposure to O_3 reduces wheat protein and micronutrient yields (Broberg et al., 2015; Yadav et al., 2020; Mishra, Rai and Agrawal, 2013; Feng, Kobayashi and Ainsworth, 2008). This is important as cereals often make up the most available protein source per capita and wheat is the dominant dietary cereal globally (Shiferaw et al., 2013). Therefore, any reduction in yield, protein and micronutrient contents caused by O_3 could threaten both food and nutrition security, especially in countries such as India where O_3 concentrations are

40 high and food security is low (FAO et al., 2020; Herforth et al., 2020; Mills et al., 2018b). The first phase of the tropospheric ozone (O_3) assessment report (TOAR) (https://igacproject.org/activities/TOAR/TOAR-I) compiled information on surface O_3 metrics to produce the world's largest database for identification of global distribution and trends of O_3. The second phase of TOAR (https://igacproject.org/activities/TOAR/TOAR-II), for which this paper is a part, expands on the goals of the first phase, to investigate O_3 impacts on human health and vegetation. This study contributes to the second phase of TOAR by

45 examining the impacts of tropospheric O_3 on wheat yield and quality in India, enhancing our understanding of the broader implications for food and nutrition security. Understanding the interplay of different factors affecting O_3 induced reductions in wheat yield and quality will be important for current, and future, food and nutritional security risk assessments.

1.1 Malnutrition and the importance of wheat in India

Malnutrition is prevalent in India with ~40% of the population unable to afford a nutritionally adequate diet, and ~80% unable

50 to afford a healthy diet (FAO et al., 2023). In India, ~35% of children under the age of 5 are affected by stunting and ~20% affected by wasting, with the prevalence of wasting in India being one of the highest in the world (Development Initiatives, 2020). Stunting and wasting occur when an individual does not have sufficient calories or micronutrients in their diet to grow and develop (Gonmei and Toteja, 2018). Wasting and muscle function loss can result from a poor quantity, or quality, of dietary protein (Medek, Schwartz and Myers, 2017). For most Indian states, at least 30% of the population are at risk of protein

55 deficiency, which is of concern for people who are pregnant or in poorer socioeconomic circumstances, who require higher qualities of protein for growth or fighting infections (Swaminathan, Vaz and Kurpad, 2012; Minocha, Thomas and Kurpad, 2017).

In India, cereals are the most available protein source per capita, and are a key dietary protein source (Minocha, Thomas and Kurpad, 2017). Wheat makes up the dominant dietary cereal in the north of India where the majority of the crop is grown

60 (Khatkar, Chaudhary and Dangi, 2015). Globally, India has the greatest area under wheat cultivation, 31.6 million hectares, and produced 109.5 million tonnes of wheat in 2021, second only to the amount of wheat produced by China (Ministry of Agriculture & Farmers Welfare, 2022). As a result, the country is self-sufficient/reliant when it comes to wheat (Tripathi and

Mishra, 2016). Consumption of wheat varies by state with the dominant wheat producing states (Punjab, Rajasthan, Haryana and Madhya Pradesh) consuming the most. Resulting from population growth and increases to income, the total demand for

65 wheat is increasing (Tripathi and Mishra, 2016). However, numerous experimental and modelling studies have shown that O_3 is substantially reducing wheat yields across India (Sharma et al., 2019; Sinha et al., 2015; Mills et al., 2018a; Yadav, Agrawal and Agrawal, 2021; Mishra, Rai and Agrawal, 2013).

1.2 O_3 pollution in India

Ground level O_3 is a secondary pollutant, formed when precursor gases (predominantly volatile organic compounds and nitrous

70 oxides) react in the presence of ultraviolet light (Fowler et al., 2008). Current literature, and the first phase of TOAR, identified that South Asia, and in particular India, experience some of the highest O_3 burdens of any region or country worldwide (Mills et al., 2018b; Emberson, 2020). Geographically, the highest O_3 concentrations in India occur in the northern part of the country and the Indo-Gangetic planes (IGP), where the majority of wheat is grown (Rathore, Gopikrishnan and Kuttippurath, 2023; Lu et al., 2018; Ministry of Agriculture & Farmers Welfare, 2022). In the future, the changing climate will affect O_3

75 concentrations, with climatic conditions across the north of India favouring greater O_3 production (Stevenson et al., 2013; Li et al., 2022a). Comparative to present day, Kumar et al. (2018) showed the greatest increase in O_3 concentrations across India would be during the dry (wheat growing) season across the IGP. This is a critical finding given the majority of wheat is grown in the north of India, across the IGP (Ministry of Agriculture & Farmers Welfare, 2022).

1.3 Effects of O_3 pollution on wheat yields

80 O_3 diffuses into wheat leaves via the stomates and impacts photosynthesis and senescence when antioxidant defences are compromised (Emberson et al., 2018; Tiwari and Agrawal, 2018; Rai and Agrawal, 2012). Accelerated senescence shortens the grain filling period, and the decline in photosynthesis decreases biomass production, ultimately leading to lower crop yields (Tiwari and Agrawal, 2018; Emberson et al., 2018).

Several experimental studies using wheat cultivars commonly grown in India have shown decreases in yield due to elevated

85 O_3 exposure (Yadav, Agrawal and Agrawal, 2021; Pandey et al., 2018; Tomer et al., 2015; Naaz et al., 2022). National estimates of relative yield (RY) loss due to O_3 across India vary between 3.8-41% between studies (Ghude et al., 2014; Lal et al., 2017; Avnery et al., 2011; Van Dingenen et al., 2009; Sinha et al., 2015; Sharma et al., 2019; Droutsas, 2020). The effects of O_3 on wheat yield also vary spatially (Mills et al., 2018a; Droutsas, 2020; Ghude et al., 2014; Lal et al., 2017; Sharma et al., 2019). Mills et al. (2018) found the greatest yield losses across the north of the country as the meteorological conditions are

90 more favourable to O_3 uptake. Lal et al. (2017) also found the greatest wheat yield losses due to O_3 in the north and west of India, where the majority of wheat is grown. Further, Naaz et al. (2022) exposed Indian wheat cultivars to different conditions representing future O_3 and climate scenarios, finding that areas suitable for wheat cultivation will be reduced in the future.

1.4 Effects of O$_3$ pollution on wheat quality

Studies have shown that the starch, protein and micronutrient yield of wheat decreases under elevated O$_3$ exposure (Broberg

95   et al., 2015; Tomer et al., 2015; Piikki et al., 2008). Pre-anthesis, the accumulation of nitrogen (N) in upper plant parts is unaffected by elevated O$_3$ concentrations (Brewster, Fenner and Hayes, 2024). However, after anthesis, the O$_3$ induced acceleration of plant senescence limits the remobilisation of N from the leaves and stem to the grain (Broberg et al., 2017; Brewster, Fenner and Hayes, 2024; Chang-Espino et al., 2021). Brewster, Fenner and Hayes (2024) also suggest that an additional process affects N remobilisation to the grain, as they found an increase in residual N in the flag leaf, despite not

100 detecting a difference in senescence onset. It is possible that the residual N is in the form of antioxidants (for example glutathione) which the plant creates for defence against O$_3$ induced reactive oxygen species (ROS) (Brewster, Fenner and Hayes, 2024; Sarkar and Agrawal, 2010; Yadav et al., 2019). Overall, the reduction in N remobilisation, leads to reduced N deposition to the grain and a reduced grain N, and protein, yield (Broberg et al., 2015; Yadav et al., 2020; Cook et al., 2024). In wheat, since the grain yield is decreased to a greater extent than proteins and micronutrients under increased O$_3$, the

105 concentration of protein and micronutrients in the grains generally increases (Feng and Kobayashi, 2009; Piikki et al., 2008). However, some wheat varieties, particularly Indian wheat, have shown a different pattern, where the protein yield and concentration of the grains decreases under O$_3$ exposure (Baqasi et al., 2018; Yadav et al., 2020; Mishra, Rai and Agrawal, 2013).

Indispensable amino acids (AA's) are most important for nutrition as they cannot be synthesised by the body and must be

110 obtained through diet (Brestenský et al., 2019). Additionally, the quantity of N containing compounds consumed is important for synthesis of dispensable AA (Brestenský et al., 2019). Nevertheless, while dispensable AA can be produced by the body, their consumption is still important for supporting metabolic functions (Brestenský et al., 2019). The production of different proteins in the body requires AA in differing proportions (Shewry and Hey, 2015). The AA that is available in the lowest proportion, the most limiting AA, determines protein production (Elango, Ball and Pencharz, 2008; Shewry and Hey, 2015).

115 Un-utilised AA cannot be stored so if they are not used for protein production they are oxidised (Elango, Ball and Pencharz, 2008; Brestenský et al., 2019). Yadav et al. (2020) looked at the AA profiles of a modern (HD3118), and old (HUW234), wheat cultivar exposed to O$_3$, finding indispensable and dispensable AA decreased under O$_3$ exposure. The effect of O$_3$ on protein quality of wheat is of particular concern given the existing state of malnutrition in India.

1.5 Crop modelling for O$_3$ and nutrition

120 Several crop models have been used to investigate the impacts of O$_3$ pollution on crop yields in a wide range of countries and globally (Nguyen et al., 2024; Guarin et al., 2019, 2024; Schauberger et al., 2019; Tao et al., 2017; Tai et al., 2021; Tian et al., 2015; Zhou et al., 2018; Droutsas, 2020; Xu et al., 2023). Ebi et al. (2021) highlight the usefulness of models for such risk assessments, while stressing that most do not consider aspects relevant for human nutrition in their simulations. Currently, only one model has been developed which captures the effect of O$_3$ on crop nutrition: DO$_3$SE-CropN (Cook et al., 2024).

125   DO₃SE-CropN is built on the existing DO₃SE-Crop model and simulates explicitly the effect of O₃ on reducing the amount of N from the leaves and stems that is available for the grain. From the grain N content (gN m⁻²), grain protein content (gProtein m⁻²) is easily obtained using conversion factors (Mariotti, Tomé and Mirand, 2008).

The DO₃SE-CropN model was originally developed to capture the increase in N concentration (100gN gDM⁻¹) and decrease in N yield (gN m⁻²) that occurs under O₃ exposure in European wheat (Cook et al., 2024). However, Indian wheat experiences

130   a decrease in grain protein concentration as well as a decrease in grain protein yield under elevated O₃ concentrations (Mishra, Rai and Agrawal, 2013; Yadav et al., 2020). In India, the ambient O₃ concentrations are high, leading to ROS production and subsequent yield losses (Tiwari and Agrawal, 2018; Sinha et al., 2015; Sharma et al., 2019). The production of antioxidants by the plant to defend against ROS reduces the proportion of proteins that would otherwise be remobilised to the grain, reducing grain protein (Yadav et al., 2019, 2020). Therefore, to capture the decrease in the protein concentration of Indian wheat under

135   O₃ exposure, the inclusion of antioxidant processes is essential. Further, the inclusion of such processes will improve the wider applicability of the model for simulating O₃ effects on wheat quality for regions with high O₃ concentrations.

Further, to expand the nutritional relevance of the model, it would be useful to simulate the effect of O₃ on protein quality. This can be done through simulating AA concentrations, which can subsequently be used to calculate the recommended metric for measuring protein quality by the FAO: the dietary indispensable AA score (DIAAS). The inclusion of protein quality would

140   allow for risk assessments of O₃ effects on wheat nutrition in addition to yield.

1.6 Aims

In the present study, the DO₃SE-CropN model was further developed and applied with two years of meteorological data. The model was calibrated using phenology, photosynthesis and yield data collected for two cultivars (HUW234 and HD3118) grown under both ambient and elevated O₃ treatments. All data were available from Yadav, Agrawal and Agrawal (2021).

145   Grain quality data were obtained from an experiment on the same cultivars a year prior, however hourly meteorological and O₃ data were not available for this year (Yadav et al., 2020). In the absence of further data, this study assumes that the grain protein concentration and grain protein quality will respond similarly to O₃ between years. The aims of the present study were to use the available data for the following:

1)   Develop a framework to simulate the antioxidant response of wheat under O₃ exposure for incorporation into the
150   existing DO₃SE-CropN model

2)   Develop a method for simulating the impact of O₃ exposure on the protein quality of wheat, focussing on AA essential for human nutrition, for incorporation into the existing DO₃SE-CropN model.


2 Model development

2.1 Integrating antioxidant processes into DO₃SE-CropN

155   The DO₃SE-Crop model is a coupled stomatal conductance-photosynthesis model, which simulates stomatal O₃ uptake and its impact on photosynthesis, recovery from O₃ damage overnight, as well as O₃ induced accelerated crop senescence (Pande et

al., 2024a). Daily photosynthate is partitioned between the leaves, stem, roots and grains according to the plant's growth stage (Pande et al., 2024a; Osborne et al., 2015). Development of DO₃SE-Crop has allowed the O_3 impact on wheat production in China and Europe to be estimated (Pande et al., 2024a; Nguyen et al., 2024). The N module for DO₃SE-Crop, developed by

160   Cook et al. (2024), takes inputs of daily stem and leaf dry matter (DM), as well as the onset of crop senescence, to simulate the N accumulated by the leaf and stem. The remobilisation of N from the leaf and stem to the grain after anthesis is simulated using a sigmoid function. To account for the reduction in N remobilisation under O_3 exposure, a relationship linking accumulated O_3 flux to the minimum N levels in the leaf and stem is incorporated (Cook et al., 2024; Brewster, Fenner and Hayes, 2024). The model allowed the decrease in grain N yield (gN m^{-2}) and increase in grain N concentration (100gN gDM^{-1})

165   of European wheat under O_3 exposure to be simulated (Cook et al., 2024). A full write up of the equations and processes of the DO₃SE-Crop model is given in Pande et al. (2024). Additionally, a full description of the equations and processes of version 1.0 of the N module developed for DO₃SE-Crop is given in (Cook et al., 2024). In this study version 4.39.16 of the DO₃SE-Crop model was used (Bland, 2024), along with version 2.0 of the N module (Cook, 2024).

The first iteration of the N module for DO₃SE-Crop (Cook et al., 2024), did not consider the utilisation of leaf/stem N in

170   creating defence proteins, yet for Indian wheat this may be an important process to explain the decrease in grain protein concentration as well as yield (Yadav et al., 2019, 2020). Here we propose a method by which the leaf and stem N involved in antioxidant production may be quantified (Fig. 1).
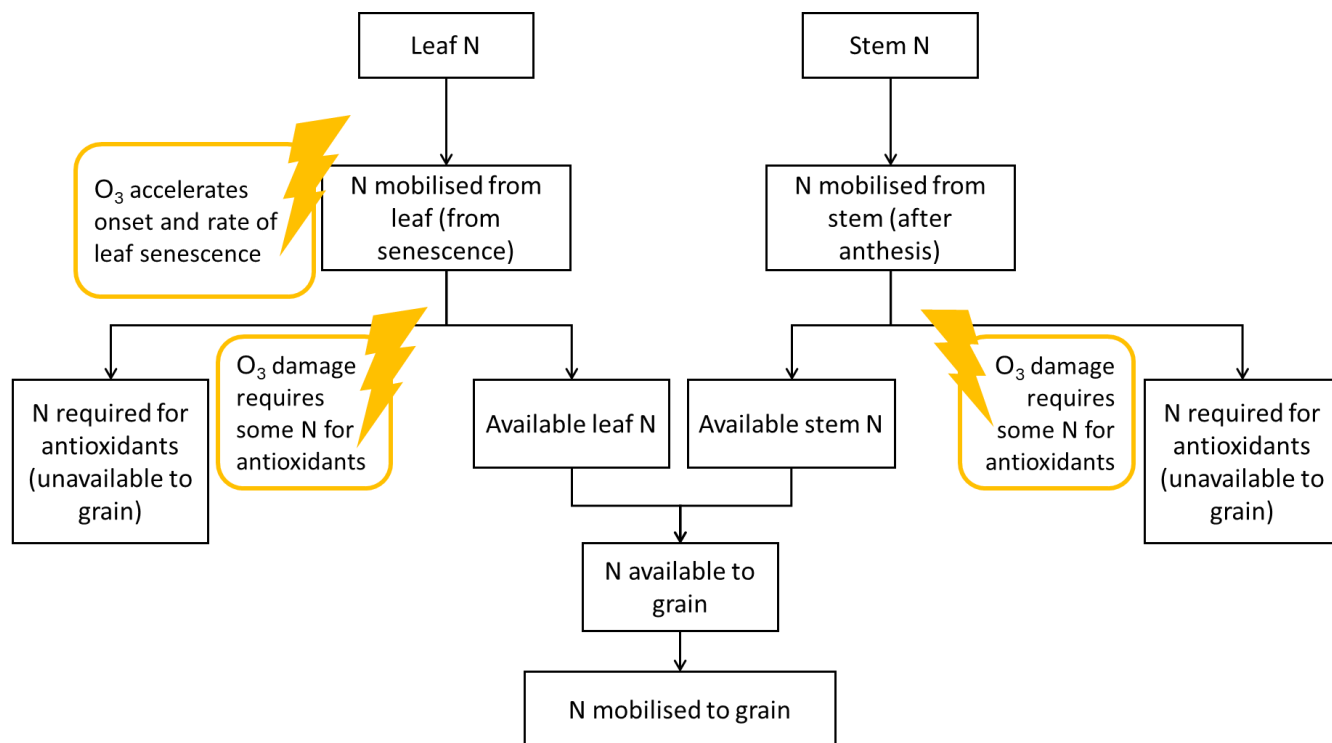
Fig. 1: Diagram of the proposed method for integrating antioxidant response under O₃ exposure into the existing N module for DO₃SE-Crop. The lightning strikes represent where O₃ can interrupt plant N processes. The lightning strikes represent the points where O₃ interacts with the antioxidant processes in the model.

Fig. 1 shows how antioxidant processes can be integrated within the existing DO₃SE-CropN framework. When the leaf senesces, N is released from the leaf. The N module is linked to the existing DO₃SE-Crop model so that increasing stomatal O₃ flux accelerates senescence, which accelerates N release from the leaf. N is released until the minimum leaf N concentration is reached. Previously, the minimum leaf N concentration increased with O₃ concentration to represent the increase in residual N (Cook et al., 2024). Now it is hypothesised that this increase in residual N is due to the leaf and stem using N for antioxidants which remain in the leaf. After a threshold of accumulated O₃ flux has been exceeded, we allocate a proportion of the released N to an antioxidant pool, which means it is unavailable to the grain. Since the stem is also involved in antioxidant response and defence against ROS (Bazargani et al., 2011; Gao et al., 2018; Li et al., 2022b), the same mechanism is used for the stem. We determine the proportion of N that will be allocated to the antioxidant pool using an equation that follows a similar structure as the drought stress factor of Liu et al. (2018), as both O₃ and drought stress are ROS mediated (Khanna-Chopra, 2012). Liu et al. (2018) use their drought stress factor to empirically modify the N:protein conversion factor under drought stress. Here, we introduce this method to the DO₃SE-CropN model via equation (1) as a more mechanistic approach. Instead of modifying the N:protein conversion factor under an abiotic stress, we use the structure of Liu et al.'s (2018) equation to determine N allocation to the antioxidant pool, thereby reducing the N available to the grain, and subsequently affecting grain protein.

The proportion of N allocated to the antioxidant pool, $f_{O_3,Antioxidants}$, takes the following form:

$$f_{O_3,Antioxidants} = \begin{cases} 0, & fst_{acc} < cL_{O_3} \\ \dfrac{fst_{acc} - cL_{O_3}}{a_{part} \times fst_{end} - cL_{O_3}}, & fst_{acc} \geq cL_{O_3} \end{cases} \tag{1}$$

where fst_{acc} is the current stomatal accumulation of O_3 flux in the DO_3SE model, fst_{end} is the stomatal accumulation of O_3 flux when N is only allocated to antioxidant pool and is not available to the grain, cL_{O_3} is the critical level above which O_3 flux starts affecting the onset of senescence in the DO_3SE-Crop model, and a_{part} is a constant modifier that can be calibrated to customise the O_3 effect on antioxidants for each plant part (leaf and stem). a_{part} must be equal to or greater than $\frac{cL_{O_3}}{fst_{end}}$ for the antioxidant factor equation to show a decrease in released N with accumulated O_3 flux. Further, fst_{end} must be greater than cL_{O_3}. Of the N released that day, the proportion available to the grain is $1 - f_{O_3,Antioxidants}$. The cL_{O_3} term was chosen as the O_3 stress factor as if O_3 has exceeded a critical threshold and is affecting senescence onset, we can hypothesise that the allocation of N to antioxidants to protect against O_3 stress will be increased. fst_{end} was incorporated into the equation to allow the end point of the slope to be customised. For varying values of a_{part}, the O_3 stress factor is used to calculate the proportion of N available to the grain as a function of accumulated stomatal O_3 flux according to Fig. 2.
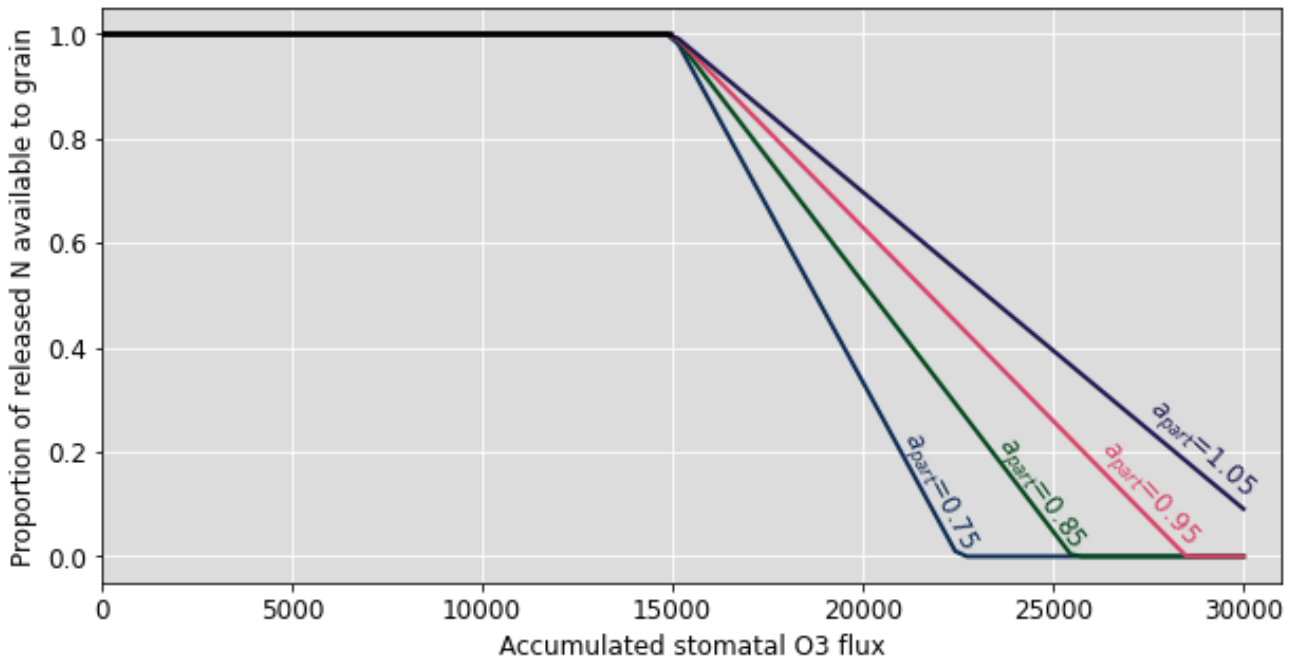

Fig. 2: Proportion of N released from stem or leaf senescence that is available to the grain for varying values of a_{part}. The plot uses fst_{end} = 30000, and cL_{O_3} = 15000.

2.2 Identification of nutritionally relevant AA for O₃ exposed wheat

The quality of a protein depends on the proportions of indispensable and dispensable AA's in the food. While Yadav et al. (2020) found that dispensable AA's were reduced to a greater extent than indispensable AA's under O_3 exposure, the most limiting for protein production were the indispensable AA's lysine and methionine. Additionally, the concentrations of lysine and methionine were reduced under O_3 exposure for both the HD3118 and HUW234 cultivars (Yadav et al., 2020). Therefore, to simulate the protein quality under O_3 exposure, lysine and methionine were focussed on.

2.3 Protein and AA calculations

The DO₃SE-CropN model outputs a grain N yield (gN m^{-2}) and concentration (100gN gDM^{-1}). From the grain N content, the protein content can be calculated by considering a standard N:protein conversion factor. The Jones' factors are commonly used to convert from N to protein, however, these factors vary between foods and within the same food group (Jones, 1941; Mariotti, Tomé and Mirand, 2008). On average for whole wheat, the conversion factor is 5.49, which is used in this study to convert grain N to protein (Mariotti, Tomé and Mirand, 2008). The regressions used to calculate lysine and methionine concentrations of the wheat grain from grain protein percentage are taken from Table 5 of Liu et al. (2019).

2.4 The DIAAS score

The metric recommended by the FAO for evaluating food protein quality is the dietary indispensable AA score (DIAAS), which corrects for the AA digestibility at the end of the small intestine (FAO, 2013). It therefore reflects the fact that the nutritional quality of protein should account for the AA required for metabolism (FAO, 2013). The metric also varies for different age groups which have different protein quality requirements (FAO, 2013). Currently, no crop model has incorporated a nutrition measure such as the DIAAS into their models. Additionally, no model has considered the impact of O_3 pollution on protein quality which is critical for risk assessments of O_3 stress on food and nutrition security.

There are two steps in calculating the DIAAS score. First, the DIAAS reference ratio is calculated for each AA as follows:

$$\genfrac{}{}{0pt}{}{DIAAS}{reference\ ratio} = \frac{True\ ileal\ IAA\ Digestibility \times mg\ of\ AA\ in\ 1g\ of\ the\ dietary\ protein}{mg\ of\ digestible\ indispensible\ AA\ in\ 1g\ of\ the\ dietary\ protein} \qquad (2)$$

where IAA stands for indispensable AA.

Once the AA concentrations have been obtained from grain protein simulations, as detailed in Section 2.3, then Eq. (2) is re-written using the parameters used in the crop modelling as:

$$\genfrac{}{}{0pt}{}{DIAAS}{reference\ ratio} = \frac{True\ ileal\ IAA\ Digestibility \times \dfrac{1000 \times Grain\ AA\ (\%\ in\ DM)}{Grain\ protein\ (\%\ in\ DM)}}{mg\ of\ digestible\ indispensible\ AA\ in\ 1g\ of\ the\ dietary\ protein} \qquad (3)$$

In the second step, the lowest DIAAS reference ratio is selected and used to calculate the DIAAS score as in Eq. (4). The lowest reference ratio is selected as this corresponds to the AA which is most limiting in the food, and is available in the

smallest proportion relative to a person's requirements (Elango, Ball and Pencharz, 2008). The AA with the lowest availability determines protein production, and quality, and the other AA which are in excess of the most limiting one will be oxidised (Elango, Ball and Pencharz, 2008).

$$\frac{DIAAS}{score} = 100 \times lowest(reference\ ratio) \tag{4}$$

235   The true ileal IAA digestibility coefficients for wheat flour required for Eq.(3) can be obtained from Shaheen et al. (2016) for the different AA. Additionally, the mg of digestible indispensable AA in 1g of the dietary protein are tabulated for the different AA and age groups in FAO (2013). There are different requirements for different age groups as adults only require AA for maintenance, whereas children require them for growth and maintenance (Shewry and Hey, 2015).

3. Parameterisation and calibration of DO$_3$SE-CropN model

3.1 Experimental datasets

240

Datasets for training and evaluation of the DO$_3$SE-CropN model were taken from three years of field experiments for wheat harvested between March 2016-2018 at the Botanical Garden, Banaras Hindu University, Varanasi, India using the HUW234 and HD3118 cultivars. The cultivars are both late sown and heat tolerant wheat varieties. For all years, O$_3$ fumigation began 3 days after seed germination, the 13th, 14th and 15th of December respectively for the 2015, 29016 and 2017 sowing on the 14th

245   of December. The wheat was exposed to ambient O$_3$ concentrations and an elevated O$_3$ treatment (ambient + 20 ppb), with the seasonal maximum O$_3$ concentrations ranging from 80-100 ppb, and an average ambient M7 of 48 ppb across 2017 and 2018. Across all experiments the wheat was sown on the 5th of December and harvested on the 30th of March. The wheat was grown in non-filtered open top chambers across all three years. The wheat did not experience any soil water or N stress. For greater detail of the experimental set up and measurements taken, the reader is referred to Yadav et al. (2020) and Yadav et al.

250   (2021). A scaling factor was applied to each AA concentration in Yadav et al. (2020) based on the mean concentration of AA in Siddiqi et al. (2020) to ensure values were consistent with the wider literature on AA concentrations for Indian wheat. The meteorological data for the model input was taken from an onsite weather and O$_3$ monitoring system. The input temperature data were corrected for the heating effect of the open top chambers, with the chambers found to be approximately 2°C warmer than the ambient air (see supplementary information). Due to gaps in the hourly meteorological data, gap filling

255   was performed according to Emberson et al. (2021).

3.2 Model calibration and evaluation

3.2.1 Model calibration

The calibration for DO$_3$SE-CropN is performed sequentially to allow the interactions between parameters at each stage to be limited (Cook et al., 2024). The key parameters calibrated in the DO$_3$SE-CropN model are given in Cook et al. (2024) and the

260   same method of calibration is used in this study. In the present study, there are 3 additional parameters to calibrate based on the newly introduced antioxidant module: fst_{end}, a_{leaf} and, a_{stem}.

The maximum catalytic rate at 25°C ($V_{cmax,25}$) and the maximum rate of electron transport at 25°C ($J_{max,25}$) were fixed at the values provided by Yadav et al. (2020) in their supplementary data. The author's supplementary data on maximum photosynthetic rate were combined with data provided by the authors on maximum stomatal conductance to vary the species-

265   specific sensitivity of stomatal conductance to assimilation rate (m), and the parameter describing variation in stomatal conductance in response to VPD (VPD_0), until a close match between photosynthetic rate and stomatal conductance was achieved (Yadav et al., 2020). Additional data provided by the authors of Yadav et al. (2020) were utilised to calculate the dark respiration rate; allowing calibration of the dark respiration coefficient for all simulations. Subsequently, the parameters controlling biomass accumulation and O₃ damage were calibrated using biomass data provided and assuming a seasonal

270   maximum LAI of 5. The O₃ damage parameters were incorporated at this stage due to high ambient O₃ concentrations which caused an O₃ induced reduction in yield even under the ambient treatment. The parameters controlling leaf and stem N were varied to achieve a close match for the leaf and grain protein simulations, as no stem N data was available for calibration. During this stage of the calibration, the gradient of the equations describing the effect of O₃ on N remobilisation of the leaf and stem were set to 0 to allow the newly developed antioxidant processes to be tested, as it was hypothesised in Cook et al.

275   (2024) that the O₃ impact on N remobilisation occurs due to antioxidant processes. However, as the calibration was performed, the best results were achieved when the new antioxidant processes were used in combination with the previously developed O₃ effect on remobilisation. Therefore, the parameters controlling N remobilisation from the leaf and stem (calibrated in Cook et al. (2024)) were varied as little as possible from their defaults to allow the newly developed antioxidant processes to be parameterised. For a tabulation of parameters calibrated for, and the values they were calibrated to, please refer to the

280   supplementary data.

Model parameters were calibrated using a combination of genetic algorithm and a trial-and-error approach, to minimise the difference between simulated and observed values while also retaining parameterisations that are physiologically realistic for the plant. For further details of the calibration method see Cook et al. (2024).

3.2.2 Model evaluation

285   The input data available for the present study were limited. Initially, the data were split in half with the 2017 data used for model calibration and the 2018 data used for the model evaluation. However, when looking at the results of the evaluation it was clear that the limited input data led to overfitting of the 2017 dataset (see supplementary Fig.'s S8 and S9). Therefore, to focus on the development of the modelling framework, all available data were used for model calibration. The root mean square error (RMSE) and R^2 were used to evaluate the model's suitability at simulating the yields and protein contents of the

290   two cultivars using Scikit-Learn (Pedregosa et al., 2011). Using the R^2 calculation from Pedregosa et al. (2011) can give negative R^2 values, where a negative value means that using the mean of the observed values is a better fit to the data than

using the model. In this paper the units of the RMSE are the same as the units of the model variable, e.g. for yield RMSE is reported in in g m^{-2} and for protein percentage (% or 100gProtein/gDM^{-1}) RMSE is reported as percentage points.

4. Results

295 ### 4.1 Biomass and protein simulations

Overall, the calibrations for grain yield, and leaf and grain protein, were reasonable for both cultivars. The grain yield and RY loss simulations performed better for 2018 than 2017. However, there was little difference in the model's capacity to capture the leaf and grain protein concentrations, and the relative loss in these, under O$_3$ exposure between the years. From Fig. 3a, the grain yield calibration was satisfactory with a RMSE of 141 gm^{-2}, however it is clear that the calibration was able to
300 simulate the grain DM better for 2018 than 2017. The underestimation of the grain DM in the 2017 dataset ranged between 35-46%. Further, the negative R^2 implies that using the mean of the observed data would be a better estimate of grain DM than the model (Pedregosa et al., 2011). The RY loss was captured much better than the grain DM. In Fig. 3b, the model captures the RY loss of cultivar HD3118 well. However, cultivar HUW234 has a large difference in RY loss between the two years which the model was unable to capture. The average deviation of RY loss from the observed value is 2.5 percentage
305 points excluding the HUW234 cultivar for 2018. When this cultivar is included, the deviation increases to 7 percentage points.
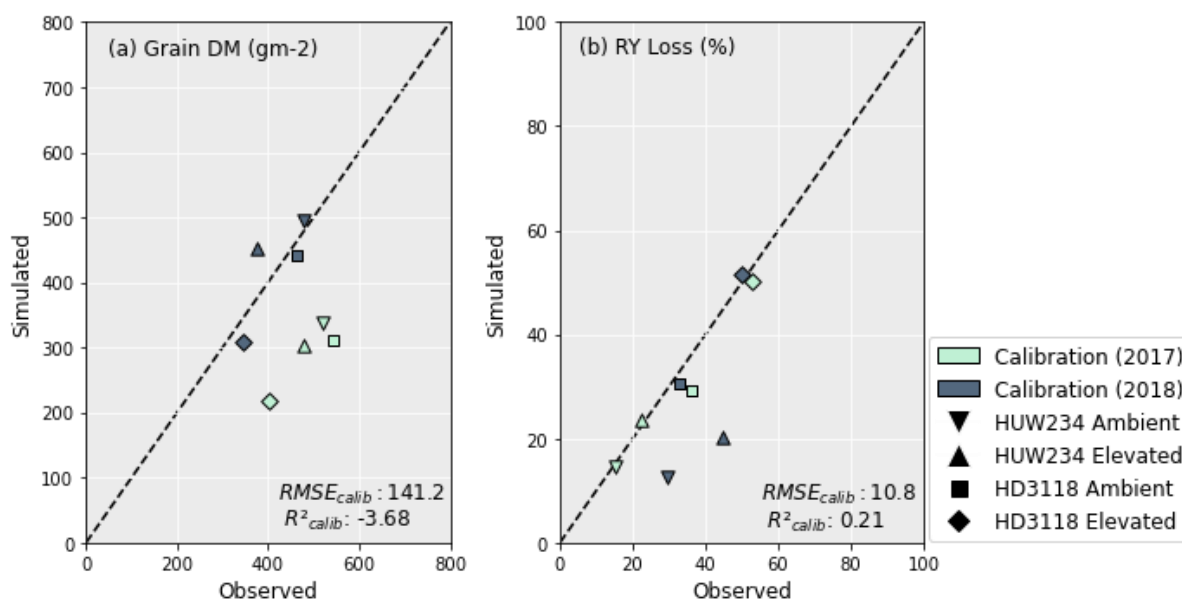

Fig. 3: Calibration of grain DM (a) and RY loss (b) using the DO$_3$SE-Crop model for the Varanasi dataset. RY loss was calculated comparative to preindustrial O$_3$ concentrations (CLRTAP, 2017).

Fig. 4 shows the grain and leaf protein simulations, and the relative protein (RP) loss between the ambient and elevated
310 treatments. The grain protein (Fig. 4a) is captured better for 2017 than 2018, but overall, the results are good, with an R^2 of

0.5 and a RMSE of only 1.3%. The grain RP loss between the ambient and elevated O_3 treatment is slightly overestimated for the HUW234 cultivar, and for the HD3118 cultivar in 2017 it is slightly underestimated, all by ~2.5 percentage points. However, in 2018 the grain RP loss of the HD3118 cultivar was heavily overestimated by ~6.5 percentage points.

The simulations of leaf protein (Fig. 4c) showed a good fit to the experimental data and were closer to the observed values than grain protein simulations, with an R^2 of 0.6 and a RMSE of 0.8%. Nevertheless, the model captured the pattern of the grain protein concentrations under ambient and elevated O_3 concentrations better than the pattern of the leaf protein concentrations (Fig.'s 4a and 4c). The leaf RP loss (Fig. 4d) was not well captured. For the HD3118 cultivar, the leaf RP loss was underestimated, and for the HUW234 cultivar it was overestimated. For the HUW234 cultivar leaf RP loss was overestimated by ~42 percentage points and for the HD3118 cultivar, the RP losses were more variable, with the leaf RP loss underestimated by ~13.5 and ~25 percentage points for 2017 and 2018 respectively.
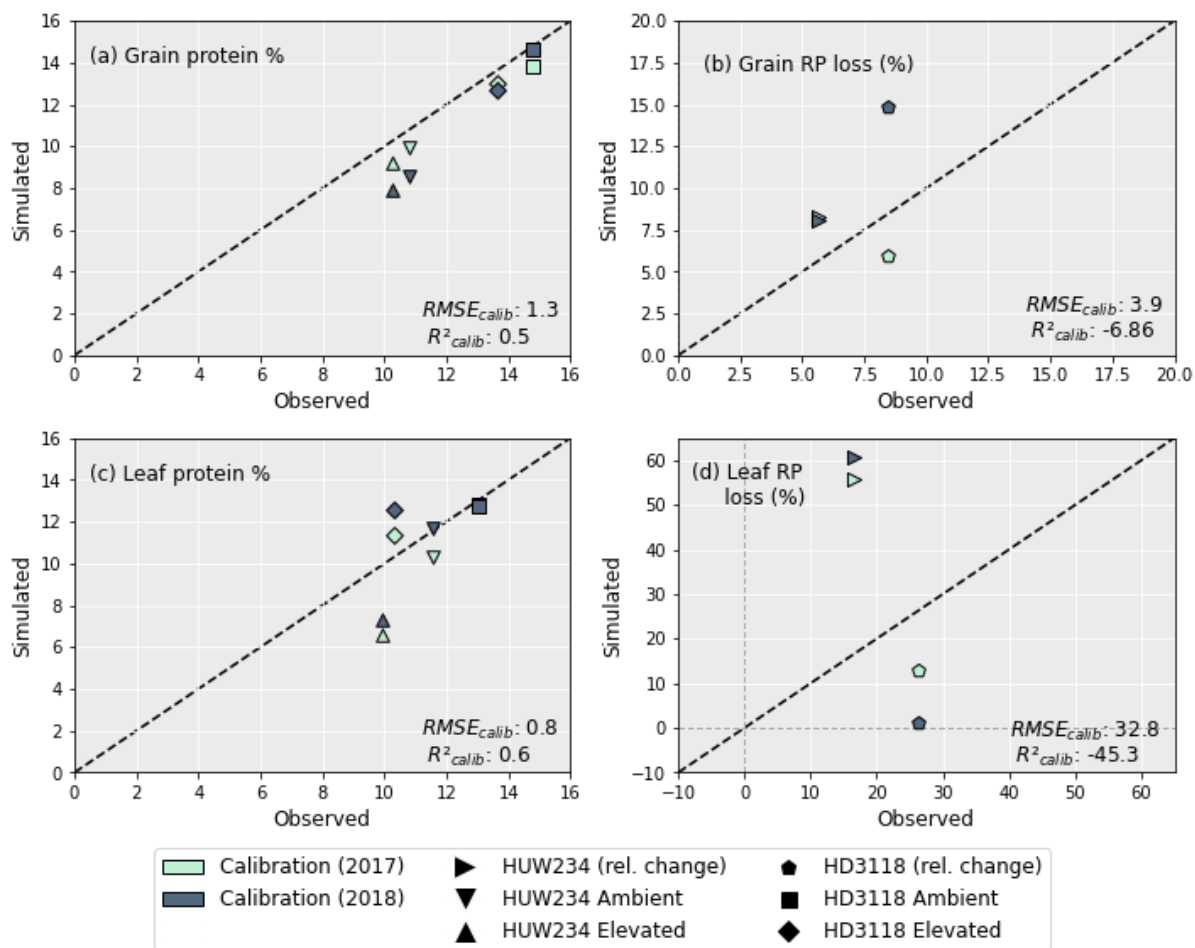

Fig. 4: The concentration of grain (a) and leaf (c) protein of HUW234 and HD3118 cultivars under ambient and elevated O_3. Calibration of the RP loss in grain (b) and leaf (d). In figure (b) the relative change in grain protein for the HUW234 cultivar for the years 2017 and 2018 was almost identical, hence the overlaid points. In figure (c) the leaf protein concentration for HD3118 cultivar

325 **in the ambient treatment was almost identical for 2017 and 2018, giving the overlaid points. The RMSE and R^2 of the calibration are indicated on the plot.**

4.2 AA simulations

Lysine and methionine were the key AAs focussed on as they were found to be the most limiting to protein production under O_3 exposure (Yadav et al., 2020). To calculate their concentrations the grain protein concentrations (Fig. 4) were used along

330 with the regressions from Liu et al. (2019). Fig.'s 5a and 5c show the concentration of methionine in the wheat grains is predicted better than the lysine concentrations, with a higher R^2 of 0.73 (compared with 0.31) and a lower RMSE (0.02 compared with 0.06). However, the decrease in both AA concentrations under O_3 exposure was not captured as well (Fig.'s 5b and 5d). For both lysine and methionine, the decrease in AA under O_3 exposure was heavily underestimated. The decrease in methionine for HUW234 and HD3118 was underestimated by 9 and 10.5 percentage points respectively. The decrease in

335 lysine concentrations were underestimated by 37 and 19 percentage points for HUW234 and HD3118 respectively. The decrease in AA concentration for HUW234 was similar between years for both methionine and lysine, whereas for the HD3118 cultivar, the simulations showed a drastically different decrease in AA concentration between years.
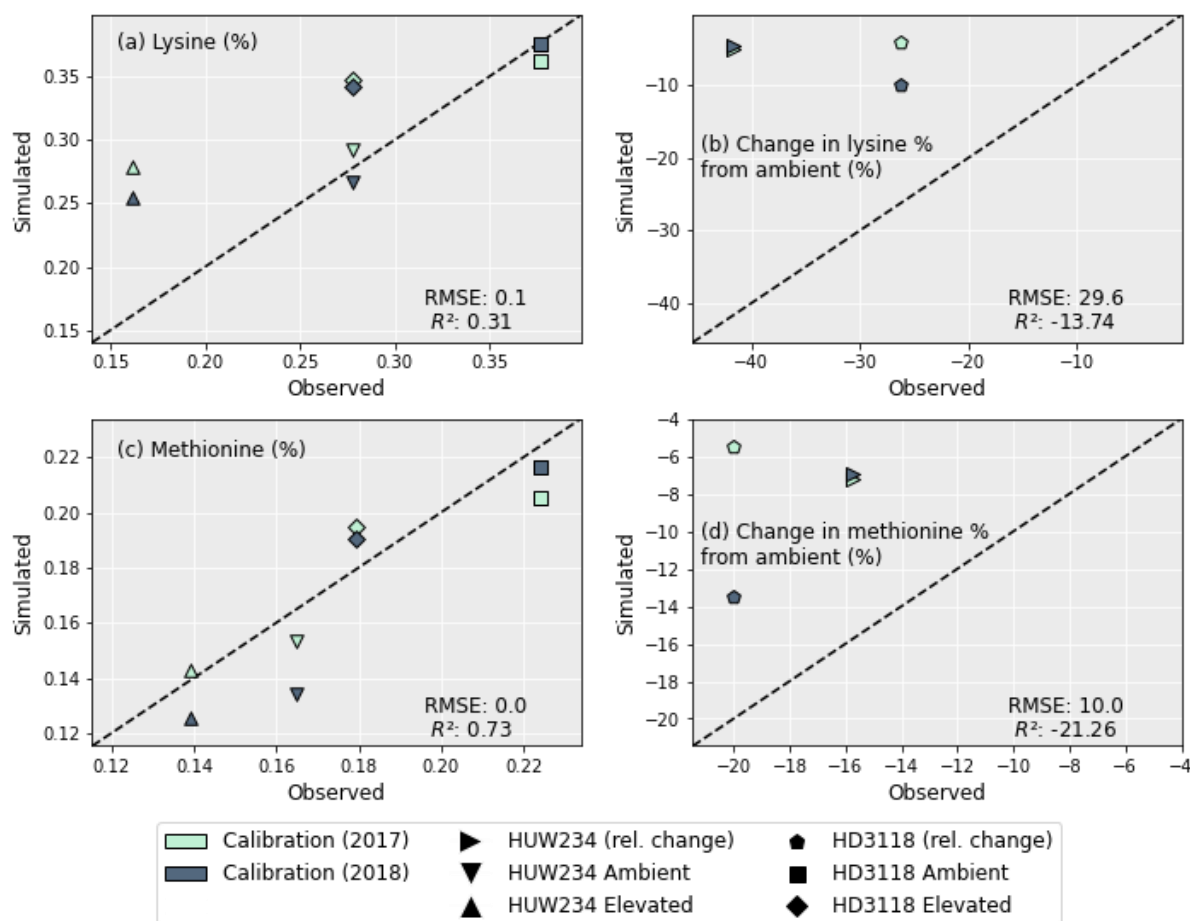
Fig. 5: A comparison of the simulated concentrations of lysine (a) and methionine (c) for the different cultivars and years of
340 **treatment. The change in AA from ambient for lysine (b) and methionine (d) is also shown for both cultivars.**

4.3 DIAAS of the nutritionally relevant AA

Methionine and lysine are the most limiting AA for protein production for the HUW234 and HD3118 cultivars, and experience
a decrease in concentration under O_3 exposure (Yadav et al., 2020). Since the relative impact of O_3 on the AA concentrations
was not captured well, the observed concentrations of the AA were used to calculate the DIAAS score, with the value that
345 would be obtained if using the simulated outputs in brackets. After calculating the reference ratios for lysine and methionine
using Eq. (3), lysine was found to give the lowest reference ratio for all O_3 treatments and cultivars, and was used to calculate
the DIAAS using Eq. (4). **Error! Reference source not found.** shows the results of the DIAAS calculation. Using the observed
data, both cultivars experience a decrease in protein quality under elevated O_3 with the HUW234 cultivar experiencing a greater
reduction than the HD3118 cultivar. Overall, the quality of wheat protein was lower for children aged between 6 months – 3
350 years than for older children and adults (>3 years). When using the simulated outputs to calculate the DIAAS, there is an
increase in protein quality under O_3 exposure. This discrepancy occurs due to the construct of the DIAAS equation. As the

decrease in AA concentrations under O_3 were underestimated by DO$_3$SE-CropN in comparison to the grain protein (see Fig.'s 4 and 5), it led to a greater ratio of grain AA to grain protein (Eq. (3)). The greater ratio under elevated O_3 then led to a higher value DIAAS under the treatment compared to the ambient, though this would not be the case in reality.

355 **Table 1: The DIAAS for the HUW234, and HD3118 cultivars under the two O_3 treatments and for the age categories 6 months – 3 years, and for older children and adults (>3 years). The reduction in DIAAS under O_3 for the HUW234 and HD3118 cultivars was also calculated. The numbers in brackets represent the DIAAS score calculated using model outputs, the average AA and protein concentrations across the 2017 and 2018 simulations were used in the calculation.**

|				DIAAS		
Age category	HUW234 Ambient	HUW234 Elevated	HUW234 rel. change	HD3118 Ambient	HD3118 Elevated	HD3118 rel. change
DIAAS Score (> 3 years)	49.9 (59.1)	31.0 (61.1)	- 37.9% (+3.3%)	49.9 (50.8)	39.9 (52.4)	- 20.1% (+3.2%)
DIAAS Score (6 months - 3 years)	42.0 (49.80)	26.1 (51.5)	- 37.9% (+3.3%)	42.0 (42.8)	33.6 (44.1)	- 20.1% (+3.2%)

4.4 Difference between the 2017 and 2018 simulations

360 After performing the simulations for 2017 and 2018 in section 4.1, it was clear there was a large difference in grain DM for the two years. The reasons for this discrepancy are important to understand since uncertainties on grain DM simulation will compound errors in protein concentration and yield (Cook et al., 2024). To investigate the grain DM discrepancy further, the meteorological variables, stomatal conductance and photosynthetic rate were plotted for both years. The accumulation of biomass each day and the LAI were overlaid to see if there were any differences that could explain the large difference in

365 biomass. The temperature in 2018 was greater at the beginning and end of the growing season compared to 2017 (Fig. 6). The reverse was true for relative humidity (Fig. S2). With relation to the other inputs, air pressure, precipitation and wind speed had negligible differences between the years (Fig.'s S1, S3 and S4). O_3 concentrations were generally greater in 2017 than 2018 (Fig. 7), and photosynthetic photon flux density (PPFD) was greater at the start of the growing season in 2018 (Fig. 8). Daily photosynthetic rate was mostly greater in 2018 than 2017 and showed the same pattern for both cultivars (Fig.'s 9 and

370 S5). The difference in stomatal conductance between the years for both cultivars mimicked the shape of the photosynthetic rate plots (Fig.'s S6 and S7). Given that the O_3 effect is more strongly determined by senescence than the instantaneous impact on photosynthesis (Pande et al., 2024b), and senescence onset did not differ strongly between years (Fig. S11), it is unlikely that the differences in yield were caused by O_3 effects. Instead, it is likely that 2018's higher early-season PPFD and temperatures, along with lower RH, promoted earlier LAI development and increased biomass production in simulations.
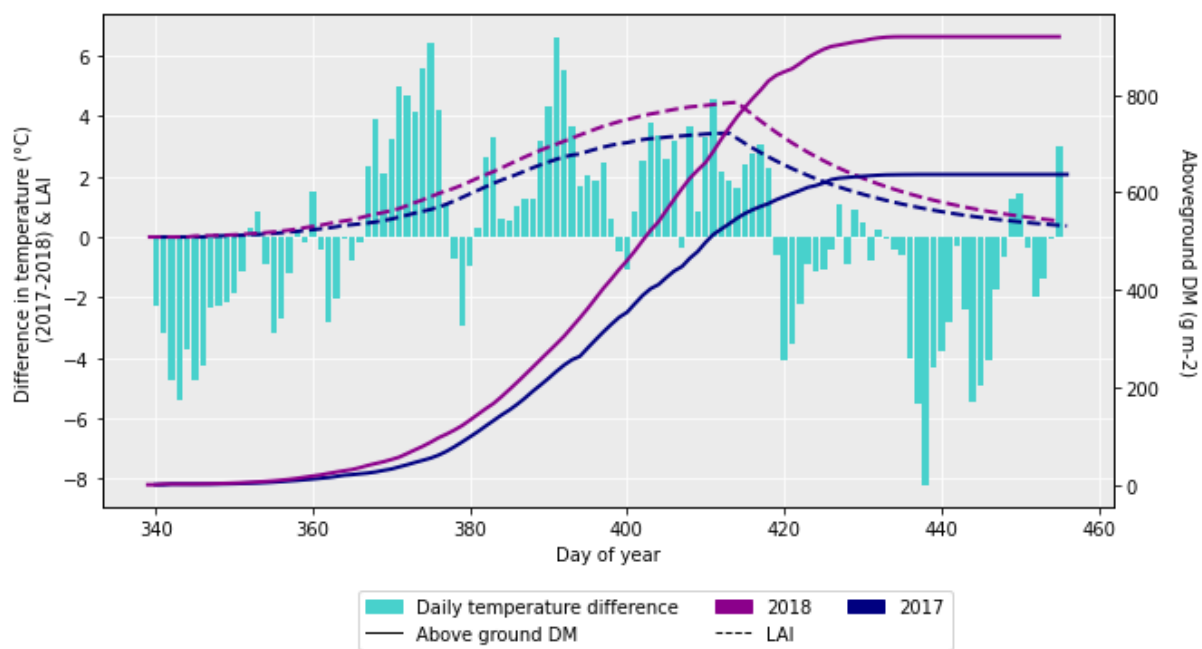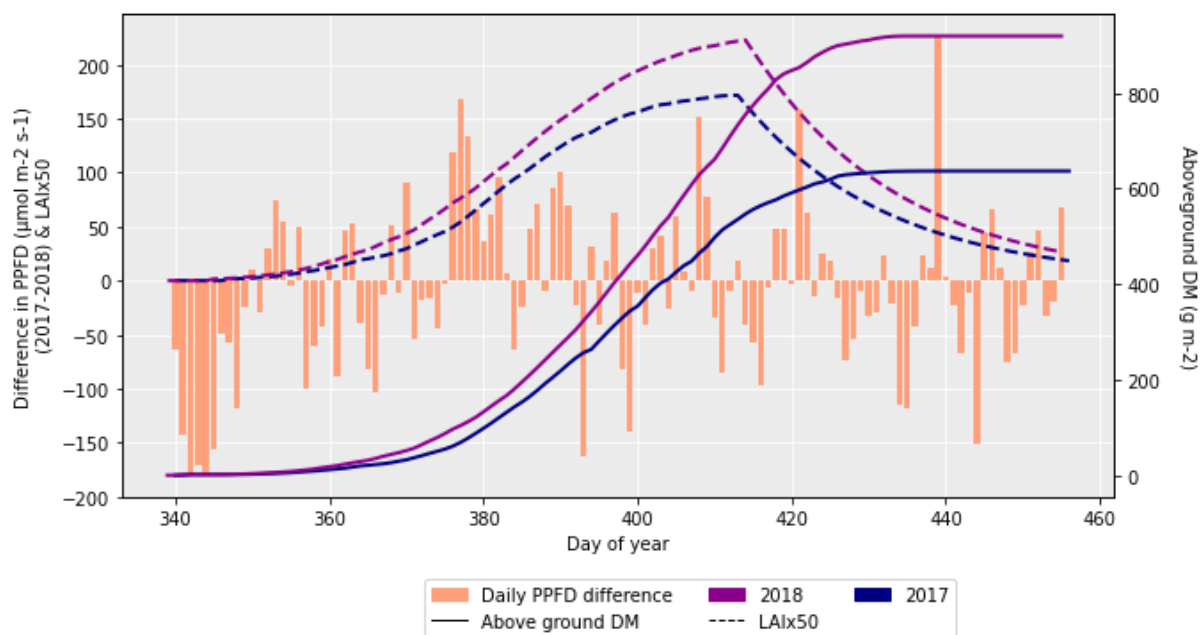
Fig. 6: Plot of the difference in daily temperature between 2017 and 2018, along with the difference in aboveground DM accumulation for the ambient treatment for both years and the LAI profiles. The LAI and aboveground DM profiles are for the HUW234 cultivar.
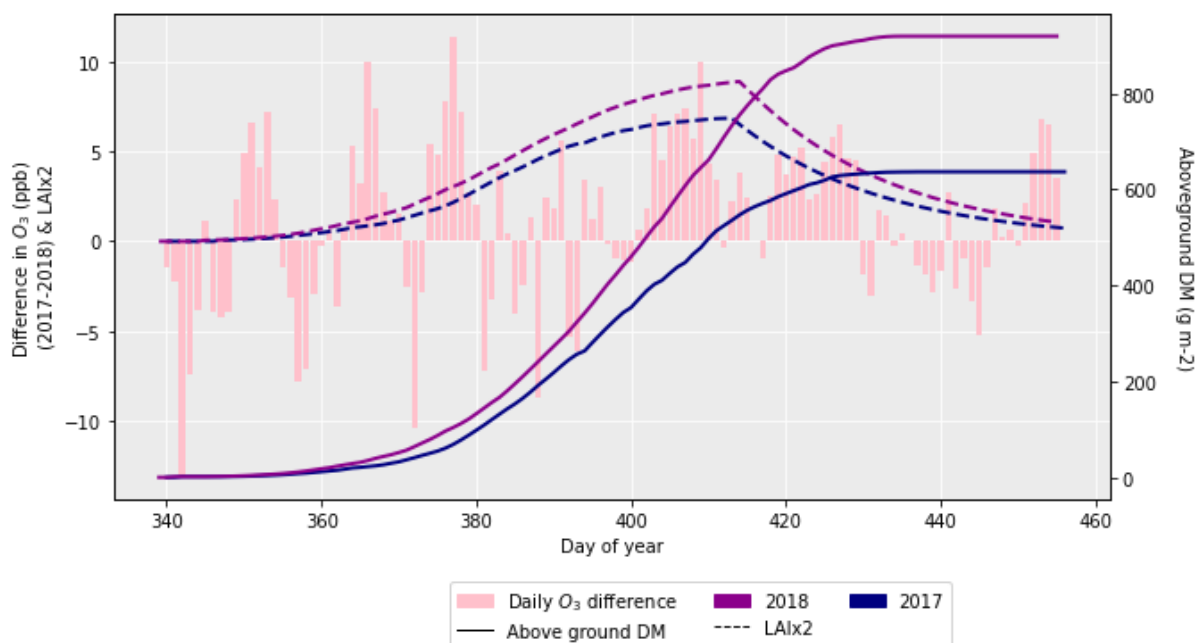
Fig. 7: The difference in daily O₃ between 2017 and 2018 along with the difference in aboveground DM accumulation for the ambient treatment for both years and the LAI profiles. LAI has been multiplied by 2 to easier show the profile. The LAI and aboveground DM profiles are for the HUW234 cultivar.

17

Fig. 8: The difference in daily PPFD between 2017 and 2018 along with the difference in aboveground DM accumulation for the ambient treatment for both years and the LAI profiles. LAI has been multiplied by 50 to more clearly show the profile. The LAI and aboveground DM profiles are for the HUW234 cultivar.
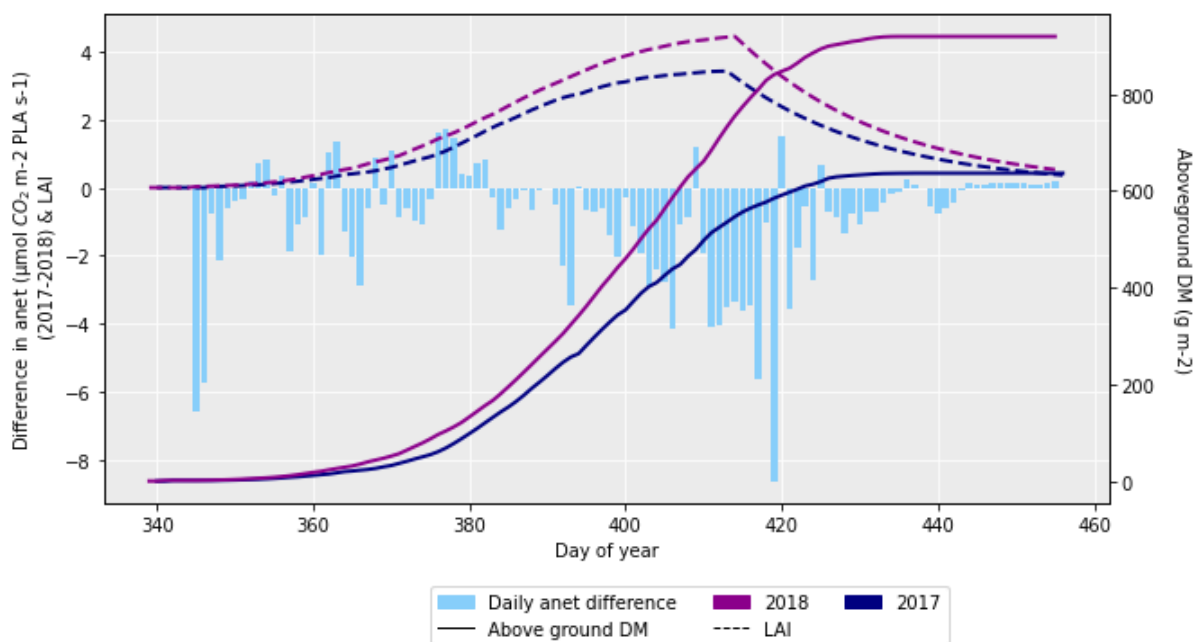
385

Fig. 9: The difference in net photosynthetic rate for 2017 and 2018 along with the difference in aboveground DM accumulation and LAI for the ambient treatment for the 2 years. The LAI and aboveground DM profiles are for the HUW234 cultivar.

5 Discussion

390 We developed the DO$_3$SE-CropN model to address a current limitation in the ability of crop models to assess the effects of O$_3$ stress on not only crop yields, but also quality. We describe the further development and applications of the model to simulate O$_3$ effects on nutritionally important AA based on current understanding of antioxidant processes and implications for N remobilisation. This work is important since currently there are few models that consider protein quality in their simulations. CN-Wheat considers AA from the perspective of being used for leaf, stem and grain protein production (Barillot, Chambon

395 and Andrieu, 2016). They do not explicitly consider the AA of the wheat grains, relevant for nutrition (Barillot, Chambon and Andrieu, 2016). In SiriusQuality, Martre et al. (2006) consider the fractions of N that are split between gliadin, glutenin, albumin-globulin and other proteins in the wheat grains as a measure of wheat quality for bread production, not human nutrition. To our current knowledge, Liu et al. (2019) are the only authors who have extended a crop model to simulate protein quality, in the form of AA, from the perspective of human nutrition. In their work they extended the CERES-Wheat model to

400 simulate lysine and other indispensable AA concentrations. Further, none of these crop models that consider crop quality have incorporated the effects of antioxidants. In our study we extend the equations used by Liu et al. (2019) to produce the first framework by which the effect of O$_3$ on protein quality (through antioxidants, AA and the DIAAS) can be captured.

5.1 Ability of the model to simulate DM and protein

The present model was able to reproduce the observed grain DM for 2018 but underestimated it for 2017 due to differences in

405 meteorology that triggered earlier LAI development, leading to greater photosynthesis and biomass production in the model for 2018. The model was able to capture the RY loss of the HD3118 cultivar well for both years. However, the HUW234 cultivar experienced a large difference in RY loss between the two years, with the model only able to capture the RY loss well for one year. With only 2 years of data, it was not possible to determine which of the observed RY loss is the most common response for HUW234. Data for additional O$_3$ treatments and years are required to develop a more robust model

410 parameterisation for different meteorology's and cultivars.

While the model underestimated the grain DM for 2017 by ~ 40%, there appears to have been no effect of this underestimation on the capacity of the model to capture grain protein concentration (100gProtein gDM^{-1}). A possible reason for this is that the lower photosynthesis in 2017, led to lower simulations of leaf and stem biomass. As a result, the N required by the leaf and stem for growth in the model was reduced, leading to lower N accumulation in these parts. Upon remobilisation to the grains,

415 the reduction in N accumulated by the grains in 2017 compared to 2018, along with the reduced grain DM in 2017, led to similar protein concentrations. The model's ability to reproduce the observed grain protein concentration, despite yield discrepancies, suggests that the underlying N allocation and remobilisation equations reasonably approximate plant processes. This outcome supports the reliability of the equations, though further validation is needed to confirm their accuracy. Future work should focus on improving the model's estimates of protein yield and concentration so that O$_3$ threats to food security

420 can be assessed with greater confidence.

In the model, there was a strong interdependence between the parameters controlling protein accumulation in the leaf and stem with grain protein, which is to be expected as protein remobilisation from the leaf and stem are key contributors to grain protein (Feller and Fischer, 1994; Gaju et al., 2014; Nehe et al., 2020). On calibrating the model, this interdependence meant that any attempt to improve the model's accuracy in capturing the decrease in leaf protein under elevated O_3 resulted in a reduced

425 model accuracy in capturing the decrease in grain protein under elevated O_3, and vice-versa (see Fig.'s 4b and 4d). This meant that there was a trade-off between calibrating leaf and grain RP loss under O_3 exposure. No data was available on stem RP loss, so the accuracy of the stem parameterisation is unclear. Given this study focussed on grain quality, capturing the grain RP loss under O_3 was prioritised over the leaf. If the model is not able to match the decrease in leaf protein with the corresponding decrease in grain protein under O_3 exposure for a given cultivar, then it implies a problem with the

430 parameterisation or model construct. Regarding the parameterisation, leaf DM data were not available which would affect leaf N, and hence protein, accumulation. Therefore, in the future leaf (and stem) DM data at anthesis and harvest would aid in parameterising the equations describing the partitioning of photosynthate each day and could improve simulations of RP loss.

5.2 Modelling antioxidant processes under O_3 exposure

The first iteration of the DO$_3$SE-CropN model simulated the decrease in grain protein yield (gProtein m^{-2}), and increase in

435 grain protein concentration (100gProtein gDM^{-1}) experienced by European and Chinese wheat cultivars (Broberg et al., 2015; Cook et al., 2024). However, Indian wheat has been shown to experience a decrease in both protein yield and concentration under O_3 exposure (Yadav et al., 2020; Mishra, Rai and Agrawal, 2013). Through the incorporation of antioxidant processes, the present model is now able to capture the decrease in protein concentration, and yield, of protein in Indian wheat under O_3 exposure, improving the regional applicability and nutritional relevance of the model.

440 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. Secondly, the modelling framework is simple. It does not require a large number of additional parameters, which reduces the complexity of the modelling process and makes it easier for other modellers to introduce into their models. Thirdly, it is compatible with the structure of other models that simulate plant N. The

445 equations can be used to simply divide leaf and stem N into pools that are accessible or inaccessible (antioxidants) to the grain. Following this, the modeller only needs to ensure that any N remobilised from the leaf and stem to the grain comes from the accessible pool.

It was hypothesised that the introduction of the antioxidant processes would replace the previous O_3 effect on leaf and stem residual N that was parameterised in Cook et al. (2024), as it was previously hypothesised that the increase in residual N

450 occurred as a result of antioxidant production (Brewster, Fenner and Hayes, 2024; Sarkar et al., 2010; Cook et al., 2024). However, during model calibration it was noted that the simulations of leaf and grain protein were improved when both processes were used in combination (see model parameterisation in supplementary information). There are two potential explanations for this: 1) The shape of the antioxidant response to O_3 is such that the two effects working in combination are a

more effective approximation, meaning further data to investigate the effect could provide insight into the truer shape of the
455   response, 2) O_3 has an effect on N remobilisation from the leaf and stem to the grains that is separate to antioxidant production. For example, ROS have been shown to oxidise proteins which would decrease protein concentrations but lead to greater residual N in the leaf and stem (Gill and Tuteja, 2010). Given this, and the previously described trade-off when calibrating leaf and grain RP loss, there is clearly a knowledge gap in our current understanding of antioxidant production and remobilisation of nutrients under O_3 exposure. Therefore, a study with multiple O_3 treatments that identifies the proportion of N in the leaf

460   and stem at anthesis, and the leaf, stem and grains at harvest, as well as the corresponding proportion of proteins, would allow identification of how much N is associated with proteins, and whether this fraction changes under O_3 exposure and affects N remobilisation. Such data would also allow further development of the antioxidant equations in this study, as for simplicity, and lack of data to test a more complex relationship, we have assumed linearity, but this may not be the case. Additionally, identification of the N associated with antioxidants at anthesis and harvest, and how these change under O_3 exposure, would

465   also allow further development of the antioxidant equations. If combined with protein measurements at anthesis and harvest, mechanistic understanding of O_3 impacts on protein, antioxidant processes and grain filling with N could be developed further and used to refine existing model processes.

5.3 Antioxidant processes and grain quality

For consideration of O_3 effects on nutrition, it is important to consider the protein quality, in addition to its concentration.
470   From a dietary perspective, indispensable AA, such as lysine and methionine, are the most important to consider when thinking about protein quality as they cannot be produced by the body and must be obtained through diet (Elango, Ball and Pencharz, 2008; Shewry and Hey, 2015). Lysine and methionine are key as they are the AA available in the lowest quantity in wheat exposed to O_3, and therefore limit the body's capacity to produce proteins from them (Yadav et al., 2020). If a person does not consume enough, or a high enough quality, of protein, then they are at risk of wasting and loss of muscle function (Medek,

475   Schwartz and Myers, 2017). Understanding how O_3 induced changes to wheat protein will affect protein quality, and hence quality of diet, is key in understanding O_3 effects on human nutrition and its potential role exacerbating malnutrition.

The regressions from Liu et al. (2019) were used to simulate grain lysine and methionine concentrations as these were the most limiting for protein production under O_3 exposure (Yadav et al., 2020). However, there is variability in the response of AA in wheat grains under O_3 due to the differential activation of metabolic pathways under stress (Ali et al., 2019; Li et al., 2024;

480   Wang et al., 2018; G A et al., 2024). Yadav et al. (2020) found that while overall protein concentrations decreased under elevated O_3, lysine and methionine concentrations decreased, while grain serine concentrations increased. The responses also differed between cultivars with HUW234 having an increase in threonine, while HD3118 had a decrease (Yadav et al., 2020). During stress conditions, the concentrations of AA vary to enhance plant defence mechanisms against abiotic stressors (Ali et al., 2019; Li et al., 2024; Wang et al., 2018; G A et al., 2024). In HUW234 and HD3118, lysine concentrations decreased under

485   elevated O_3, due to its breakdown for energy production and plant defence (Ali et al., 2019; Yadav et al., 2020). Lysine breakdown produces proline, the concentration of which increased in both cultivars, which has been shown to protect against

ROS-induced oxidative damage (Nayyar and Walia, 2003; Yadav et al., 2020; Yang, Zhao and Liu, 2020). Additionally, the concentration of methionine decreased in both cultivars under elevated O_3 (Yadav et al., 2020). The decrease is likely due to methionine's role as an antioxidant, and that it is very sensitive to oxidation by ROS (Ali et al., 2019). The changes in AA aid

490   in the maintenance of photosynthetic rate and protection of photosynthetic pigments from ROS (Kaur and Kapoor, 2021; Naidu et al., 1991; Simon-Sarkadi and Galiba, 1996). The specific response of an AA to abiotic stress is cultivar specific and depends on the intensity of the stress (Ali et al., 2019). As a result, grain AA concentrations are linked to the stress response of the plant under O_3. Measurements of AA concentrations under multiple O_3 treatments would help to elucidate the shape of the response of AA's to O_3 stress. This is a field which has largely been neglected with only Yadav et al. (2020) having investigated it so

495   far. Such data would allow the effect of O_3 on nutrition to be better understood.

5.4 Protein quality estimates using the DIAAS

Through extending DO₃SE-CropN to simulate the DIAAS, estimates of protein quality are translated into a metric that is commonly used to assess dietary quality in the nutrition field (e.g. Kurpad and Thomas (2020)). Using the observed data, the HUW234 cultivar experienced the greatest loss in protein quality under increased O_3 concentrations, despite showing the

500   smallest RP and RY loss. The reason for this is that HUW234 experienced the greatest decrease in lysine concentrations, and lysine is the most limiting AA in wheat (Meybodi et al., 2019; Siddiqi et al., 2020). The DO₃SE-CropN model was not able to reproduce the reduction in protein quality calculated through the DIAAS as it was not able to reproduce the magnitude of the decrease in protein and lysine concentrations under elevated O_3 for either cultivar (**Error! Reference source not found.**, Fig.'s 5b and 5d). Using the observed data, the calculations of DIAAS were the same for both cultivars due to the scaling factor used

505   for the AA (see section 3.1) but, in reality, the DIAAS would differ between the cultivars. While using the simulations of grain protein and AA's was able to produce a difference in DIAAS between cultivars, it was only able to reproduce the DIAAS calculated from the observed data for the HD3118 cultivar in the ambient O_3 treatment, as the protein and lysine concentrations were captured well for this cultivar and treatment. To develop crop models that use the DIAAS to understand the reduction in protein quality under abiotic stress, the reduction in grain protein and the most limiting AA's for protein production under that

510   stress need to be understood.

5.5 Data requirements for effective model calibration

Initially in this study, the data were split in half, and the 2017 data were used for model calibration and the 2018 for evaluation. However, due to the model overfitting to the 2017 dataset (see supplementary data), the decision was made to utilise all available data for calibration. This allowed the paper to focus on the development of the antioxidant processes and protein

515   quality simulations. Should future work utilise the antioxidant or protein quality framework presented in this work, a thorough model calibration and evaluation is recommended. Calibrations that use data from contrasting growing conditions, such as different growing seasons/years, sowing dates or experimental conditions have been shown to reduce the chance of multiple combinations of parameters giving the same answer (equifinality), reduce model uncertainty, and improve simulation accuracy

520 (He et al., 2017; Zhang et al., 2023). This is likely a result of achieving a truer parametrisation for the cultivar, leading to improved generalisation of the model upon application (Wallach, 2011). Hence, if there are few growing seasons of data available, it would be helpful to have data spanning a range of crop treatments.

5.6 Further work for understanding O3 effects on wheat nutrition

Understanding cultivar-specific responses to increasing O_3 concentrations will be important for food security in order to breed cultivars that can maintain yields and protein quantity, as well as quality, in the future. Additionally, it can be seen in the 525 calculations of the DIAAS score, and reflected in the wider literature, that the quality of protein in wheat is low, even without the impact of O_3, which will exacerbate protein deficiencies in consumers who rely on wheat based diets (Swaminathan, Vaz and Kurpad, 2012). Therefore, to reduce malnutrition, cultivars with a high protein quality, that can maintain yields and protein concentration under O_3 exposure should be investigated for their potential to maintain wheat supply and quality under conditions of elevated O_3 concentration. Additionally, existing barriers to diet diversification need to be overcome, so that 530 individuals may have access to higher quality protein sources (Agrawal et al., 2019).

To develop an understanding of cultivar specific responses to abiotic stress, a modelling approach similar to that used in this study would be useful, as such a model can capture the effect of antioxidant processes under stress on grain quality. To ensure the applicability of the model in addressing this goal there are a few existing barriers identified in this study:

1) Before model application, models need to be thoroughly calibrated and evaluated. To perform a thorough
535   calibration and evaluation, a range of treatments and/or years of data need to be available to provide a set of calibration parameters that are more general for that cultivar and prevent over-fitting. Additionally, obtaining leaf and stem DM anthesis and harvest will aid in parameterising partitioning and remobilisation of photosynthate.

2) Differences in meteorological conditions between the two years of experiments in the present study had a large effect on simulations of grain DM. The effect of meteorology on simulations of photosynthetic processes and
540   biomass production in crop models should be further investigated in the future to ascertain crop model sensitivity to input data choices.

3) To advance the antioxidant equations, and understand O_3 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 O_3 treatments should also be obtained to improve mechanistic
545   understanding of plant antioxidant response to O_3 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 O_3. 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.

Reliable estimates of DIAAS would allow dietary protein quality to be incorporated into O_3 risk assessments. Performing yield 550 and nutrition-based risk assessments utilising AA and DIAAS simulations under future O_3 scenarios would allow for

assessment of which wheat growing areas will experience a decrease in wheat protein quality as well as yield. Such results could then be combined with dietary surveys to evaluate adult's and children's risk of not getting enough, or a high enough quality of, food under increasing O_3.

6. Conclusions

555   In summary, the present study has developed a framework by which the antioxidant response of wheat under O_3 exposure can be incorporated into wheat quality simulations in the existing crop model DO$_3$SE-CropN. The key benefits of the framework are that it is flexible, simple and compatible with other crop models provided they simulate leaf and stem N. The AA's most limiting for human nutrition under O_3 exposure were found to be lysine and methionine. The new modelling framework allowed the effect of high O_3 concentrations leading to a decrease in grain protein, lysine and methionine concentrations of

560   Indian wheat to be simulated. Through calculations of the AA's, the FAO recommended metric for simulating wheat quality, the DIAAS, can be calculated. To improve the present model, we identified key experimental data needed to test and refine model formulations and parametrisations for a wider range of meteorological conditions and wheat cultivars. These include greater calibration data across multiple years and treatments with leaf and stem DM and N measurements, mechanistic understanding of plant antioxidant response, and further development of relationships linking grain protein concentrations to

565   AA concentrations under elevated O_3.

Code availability

An open version of the DO$_3$SE-Crop model, version 4.39.16 as used in the present study can be found at Bland (2024) and version 2.0 of the N module for DO$_3$SE-Crop is found at Cook (2024).

Data availability

570   Data from Yadav et al. (2020) and Yadav, Agrawal and Agrawal (2021) were used in the present study with additional data provided by Durgesh Singh Yadav (durgeshsinghy@gmail.com). Due to data ownership, please contact Durgesh Singh Yadav directly for access to required data.

Author contributions

Conceptualisation: Jo Cook, Lisa Emberson, Felicity Hayes, Samarthia Thankappan, Durgesh Singh Yadav
575   Data curation: Jo Cook, Durgesh Singh Yadav, Nathan Booth
Formal analysis: Jo Cook
Methodology: Jo Cook, Lisa Emberson, Felicity Hayes, Samarthia Thankappan, Durgesh Singh Yadav

Competing interests

The authors declare that they have no conflict of interest.

References

590 Agrawal, S. et al. (2019). Socio-economic patterning of food consumption and dietary diversity among Indian children: evidence from NFHS-4. *European Journal of Clinical Nutrition*, 73 (10), pp.1361–1372. [Online]. Available at: doi:10.1038/s41430-019-0406-0.

Ali, Q. et al. (2019). Role of Amino Acids in Improving Abiotic Stress Tolerance to Plants. In: *Plant Tolerance to Environmental Stress*. pp.175–204. [Online]. Available at: doi:10.1201/9780203705315-12.

595 Avnery, S. et al. (2011). Global crop yield reductions due to surface ozone exposure: 1. Year 2000 crop production losses and economic damage. *Atmospheric Environment*, 45 (13), pp.2284–2296. [Online]. Available at: doi:10.1016/j.atmosenv.2010.11.045.

Baqasi, L. A. et al. (2018). Effects of low concentrations of ozone (O3) on metabolic and physiological attributes in wheat (Triticum aestivum L.) pants. *Biomedical and Pharmacology Journal*, 11 (2), pp.929–934. 600 [Online]. Available at: doi:10.13005/bpj/1450.

Barillot, R., Chambon, C. and Andrieu, B. (2016). CN-Wheat, a functional-structural model of carbon and nitrogen metabolism in wheat culms after anthesis. I. Model description. *Annals of Botany*, 118 (5), pp.997–1013. [Online]. Available at: doi:10.1093/aob/mcw143.

Bazargani, M. M. et al. (2011). A proteomics view on the role of drought-induced senescence and oxidative 605 stress defense in enhanced stem reserves remobilization in wheat. *Journal of Proteomics*, 74 (10), pp.1959–1973. [Online]. Available at: doi:10.1016/j.jprot.2011.05.015.

Brestenský, M. et al. (2019). Dietary Requirements for Proteins and Amino Acids in Human Nutrition. *Current Nutrition & Food Science*, 15, pp.638–645. [Online]. Available at: doi:10.2174/1573401314666180507123506.

610   Brewster, C., Fenner, N. and Hayes, F. (2024). Chronic ozone exposure affects nitrogen remobilization in wheat at key growth stages. *Science of the Total Environment*, 908 (168288). [Online]. Available at: doi:10.1016/j.scitotenv.2023.168288.

Broberg, M. C. et al. (2015). Ozone effects on wheat grain quality - A summary. *Environmental Pollution*, 197, pp.203–213. [Online]. Available at: doi:10.1016/j.envpol.2014.12.009.

615   Broberg, M. C. et al. (2017). Fertilizer efficiency in wheat is reduced by ozone pollution. *Science of the Total Environment*, 607–608, pp.876–880. [Online]. Available at: doi:10.1016/j.scitotenv.2017.07.069.

Chang-Espino, M. et al. (2021). The effect of increased ozone levels on the stable carbon and nitrogen isotopic signature of wheat cultivars and landraces. *Atmosphere*, 12 (7), pp.1–25. [Online]. Available at: doi:10.3390/atmos12070883.

620   CLRTAP. (2017). Chapter 3: Mapping critical levels for vegetation. In: *Manual on methodologies and criteria for modelling and mapping critical loads and levels and air pollution effects, risks and trends*. [Online]. Available   at: https://unece.org/fileadmin/DAM/env/documents/2017/AIR/EMEP/Final__new_Chapter_3_v2__August_2 017_.pdf.

625   Cook, J. et al. (2024). New ozone-nitrogen model shows early senescence onset is the primary cause of ozone-induced reduction in grain quality of wheat. *EGUSphere [preprint]*, (May), pp.1–43. [Online]. Available at: doi:10.5194/egusphere-2024-1311.

Development Initiatives. (2020). *Global Nutrition Report | Country Nutrition Profiles - Global Nutrition Report*. [Online]. Available at: https://globalnutritionreport.org/resources/nutrition-profiles/asia/southern-
630   asia/india/.

Van Dingenen, R. et al. (2009). The global impact of ozone on agricultural crop yields under current and future air quality legislation. *Atmospheric Environment*, 43 (3), pp.604–618. [Online]. Available at: doi:10.1016/j.atmosenv.2008.10.033.

Droutsas, I. (2020). *How do climate, ozone and crops interact to impact on health and nutrition?*

635   Ebi, K. L. et al. (2021). Nutritional quality of crops in a high CO2 world: an agenda for research and technology development. *Environmental Research Letters*, 16 (6), p.064045. [Online]. Available at: doi:10.1088/1748-9326/abfcfa.

Elango, R., Ball, R. O. and Pencharz, P. B. (2008). Indicator amino acid oxidation: Concept and application. *Journal of Nutrition*, 138 (2), pp.243–246. [Online]. Available at: doi:10.1093/jn/138.2.243.

640   Emberson, L. (2020). Effects of ozone on agriculture, forests and grasslands: Improving risk assessment methods for O3. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences*, 378 (2183). [Online]. Available at: doi:10.1098/rsta.2019.0327.

Emberson, L. et al. (2021). *Methodology for gap-filling and standardisation of data for AgMIP Ozone V8*. [Online]. Available at: https://agmipdatahub.files.wordpress.com/2021/10/agmip-gap-filling-protocol_-
645   v8.docx.pdf [Accessed 17 April 2024].

Emberson, L. D. et al. (2018). Ozone effects on crops and consideration in crop models. *European Journal of Agronomy*, 100 (June), pp.19–34. [Online]. Available at: doi:10.1016/j.eja.2018.06.002.

FAO. (2013). *Dietary protein quality evaluation in human nutrition: Report of an FAO Expert Consultation*. Rome.

650 FAO et al. (2020). *The State of Food Security and Nutrition in the World 2020. Transforming food systems for affordable healthy diets*. Rome: FAO. [Online]. Available at: https://www.fao.org/policy-support/tools-and-publications/resources-details/en/c/1298217/.

FAO et al. (2023). *The state of food security and nutrition in the world*. [Online]. Available at: doi:10.1016/S2213-8587(22)00220-0.

655 Feller, U. and Fischer, A. (1994). Nitrogen metabolism in senescing leaves. *Critical Reviews in Plant Sciences*, 13 (3), pp.241–273. [Online]. Available at: doi:10.1080/07352689409701916.

Feng, Z. and Kobayashi, K. (2009). Assessing the impacts of current and future concentrations of surface ozone on crop yield with meta-analysis. *Atmospheric Environment*, 43 (8), pp.1510–1519. [Online]. Available at: doi:10.1016/j.atmosenv.2008.11.033.

660 Feng, Z., Kobayashi, K. and Ainsworth, E. A. (2008). Impact of elevated ozone concentration on growth, physiology, and yield of wheat (Triticum aestivum L.): A meta-analysis. *Global Change Biology*, 14 (11), pp.2696–2708. [Online]. Available at: doi:10.1111/j.1365-2486.2008.01673.x.

Fowler, D. et al. (2008). *Ground-level ozone in the 21st century: future trends, impacts and policy implications*. (October). [Online]. Available at: https://royalsociety.org/news-665 resources/publications/2008/ground-level-ozone/.

G A, N. et al. (2024). Unraveling the effect of drought and heat stresses on grain quality of wheat (Triticum aestivum). *The Indian Journal of Agricultural Sciences*, 94 (5), pp.489–494. [Online]. Available at: doi:10.56093/ijas.v94i5.142783.

Gaju, O. et al. (2014). Nitrogen partitioning and remobilization in relation to leaf senescence, grain yield and 670 grain nitrogen concentration in wheat cultivars. *Field Crops Research*, 155, pp.213–223. [Online]. Available at: doi:10.1016/j.fcr.2013.09.003.

Gao, M. et al. (2018). Photosynthetic and antioxidant response of wheat to di(2-ethylhexyl) phthalate (DEHP) contamination in the soil. *Chemosphere*, 209, pp.258–267. [Online]. Available at: doi:10.1016/j.chemosphere.2018.06.090.

675 Ghude, S. D. et al. (2014). Reductions in India's crop yield due to ozone. *Geophysical Research Letters*, 41 (15), pp.5685–5691. [Online]. Available at: doi:10.1002/2014GL060930.

Gill, S. S. and Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry*, 48 (12), pp.909–930. [Online]. Available at: doi:10.1016/j.plaphy.2010.08.016.

680 Gonmei, Z. and Toteja, G. S. (2018). Micronutrient status of Indian population. *Indian Journal of Medical*

Research, 148 (5), pp.511–521. [Online]. Available at: doi:10.4103/ijmr.IJMR_1768_18.

Guarin, J. R. et al. (2019). Impacts of tropospheric ozone and climate change on Mexico wheat production. *Climatic Change*, 155 (2), pp.157–174. [Online]. Available at: doi:10.1007/s10584-019-02451-4.

Guarin, J. R. et al. (2024). Modeling the effects of tropospheric ozone on the growth and yield of global staple crops with DSSAT v4.8.0. *Geoscientific Model Development*, 17 (7), pp.2547–2567. [Online]. Available at: doi:10.5194/gmd-17-2547-2024.

He, D. et al. (2017). Data requirement for effective calibration of process-based crop models. *Agricultural and Forest Meteorology*, 234–235, pp.136–148. [Online]. Available at: doi:10.1016/j.agrformet.2016.12.015.

Herforth, A. et al. (2020). Cost and affordability of nutritious diets across and within countries. *Background paper for The State of Food Security and Nutrition in the World.*, Rome.

Jones, D. B. (1941). Factors for converting percentages of nitrogen in foods and feeds into percentages of proteins. *United States Department of Agriculture*, Washington, D.C.

Kaur, R. and Kapoor, N. (2021). Role of Amino Acids other than Proline in Abiotic Stress Amelioration in Plants. *Journal of University of Shanghai for Science and Technology*, 23 (2), pp.271–291. [Online]. Available at: doi:10.51201/Jusst12616.

Khanna-Chopra, R. (2012). Leaf senescence and abiotic stresses share reactive oxygen species-mediated chloroplast degradation. *Protoplasma*, 249 (3), pp.469–481. [Online]. Available at: doi:10.1007/s00709-011-0308-z.

Khatkar, B. S., Chaudhary, N. and Dangi, P. (2015). *Production and Consumption of Grains: India*. 2nd ed. Elsevier Ltd. [Online]. Available at: doi:10.1016/B978-0-12-394437-5.00044-9.

Kumar, R. et al. (2018). How Will Air Quality Change in South Asia by 2050? *Journal of Geophysical Research: Atmospheres*, 123 (3), pp.1840–1864. [Online]. Available at: doi:10.1002/2017JD027357.

Kurpad, A. V. and Thomas, T. (2020). Protein Quality and its Food Source in the Diets of Young Indian Children. *Journal of Nutrition*, 150 (6), Oxford University Press., pp.1350–1351. [Online]. Available at: doi:10.1093/jn/nxaa100.

Lal, S. et al. (2017). Loss of crop yields in India due to surface ozone: an estimation based on a network of observations. *Environmental Science and Pollution Research*, 24 (26), pp.20972–20981. [Online]. Available at: doi:10.1007/s11356-017-9729-3.

Li, G. et al. (2024). Amino acid metabolism response to post-anthesis drought stress during critical periods of elite wheat (Triticum aestivum L.) endosperm development. *Environmental and Experimental Botany*, 218 (November 2023). [Online]. Available at: doi:10.1016/j.envexpbot.2023.105577.

Li, H. et al. (2022a). *Climate-driven deterioration of future ozone pollution in Asia predicted by machine learning with multisource data*. 2019 (September), pp.1–40. [Online]. Available at: doi:10.5194/acp-23-1131-2023.

Li, J. et al. (2022b). Effects of light quality on growth, nutritional characteristics, and antioxidant properties

of winter wheat seedlings (Triticum aestivum L.). *Frontiers in Plant Science*, 13 (September), pp.1–15. [Online]. Available at: doi:10.3389/fpls.2022.978468.

Liu, J. et al. (2018). The effects of nitrogen and water stresses on the nitrogen-to-protein conversion factor of winter wheat. *Agricultural Water Management*, 210 (February), pp.217–223. [Online]. Available at: doi:10.1016/j.agwat.2018.07.042.

Liu, J. et al. (2019). Modeling wheat nutritional quality with a modified CERES-wheat model. *European Journal of Agronomy*, 109 (September 2018), p.125901. [Online]. Available at: doi:10.1016/j.eja.2019.03.005.

Lu, X. et al. (2018). Lower tropospheric ozone over India and its linkage to the South Asian monsoon. *Atmospheric Chemistry and Physics*, 18 (5), pp.3101–3118. [Online]. Available at: doi:10.5194/acp-18-3101-2018.

Mariotti, F., Tomé, D. and Mirand, P. P. (2008). Converting nitrogen into protein - Beyond 6.25 and Jones' factors. *Critical Reviews in Food Science and Nutrition*, 48 (2), pp.177–184. [Online]. Available at: doi:10.1080/10408390701279749.

Martre, P. et al. (2006). Modelling protein content and composition in relation to crop nitrogen dynamics for wheat. *European Journal of Agronomy*, 25 (2), pp.138–154. [Online]. Available at: doi:10.1016/j.eja.2006.04.007.

Medek, D. E., Schwartz, J. and Myers, S. S. (2017). Estimated effects of future atmospheric co2 concentrations on protein intake and the risk of protein deficiency by country and region. *Environmental Health Perspectives*, 125 (8), pp.1–8. [Online]. Available at: doi:10.1289/EHP41.

Meybodi, N. M. et al. (2019). Wheat Bread: Potential Approach to Fortify its Lysine Content. *Current Nutrition & Food Science*, 15 (7). [Online]. Available at: doi:10.2174/1573401315666190228125241.

Mills, G. et al. (2018a). Ozone pollution will compromise efforts to increase global wheat production. *Global Change Biology*, 24 (8), pp.3560–3574. [Online]. Available at: doi:10.1111/gcb.14157.

Mills, G. et al. (2018b). Tropospheric Ozone Assessment Report: Present-day ozone distribution and trends relevant to human health. *Elementa: Science of the Anthropocene*, 6 (47). [Online]. Available at: doi:10.1525/elementa.302.

Ministry of Agriculture & Farmers Welfare. (2022). *Agricultural Statistics at a Glance 2021*. New Delhi.

Minocha, S., Thomas, T. and Kurpad, A. V. (2017). Dietary protein and the health-nutrition-agriculture connection in India. *Journal of Nutrition*, 147 (7), pp.1243–1250. [Online]. Available at: doi:10.3945/jn.116.243980.

Mishra, A. K., Rai, R. and Agrawal, S. B. (2013). Individual and interactive effects of elevated carbon dioxide and ozone on tropical wheat (Triticum aestivum L.) cultivars with special emphasis on ROS generation and activation of antioxidant defence system. *Indian Journal of Biochemistry and Biophysics*, 50 (2), pp.139–149. [Online]. Available at: https://pubmed.ncbi.nlm.nih.gov/23720888/.

Naaz, S. et al. (2022). Bioclimatic modeling and FACE study forecast a bleak future for wheat production in India. *Environmental Monitoring and Assessment*, 195 (1). [Online]. Available at: doi:10.1007/s10661-022-10551-5.

Naidu, B. P. et al. (1991). Amino acid and glycine betaine accumulation in cold-stressed wheat seedlings. *Phytochemistry*, 30 (2), pp.407–409. [Online]. Available at: doi:10.1016/0031-9422(91)83693-F.

Nayyar, H. and Walia, D. P. (2003). Water stress induced proline accumulation in contrasting wheat genotypes as affected by calcium and abscisic acid. *Biologia Plantarum*, 46 (2), pp.275–279. [Online]. Available at: doi:10.1023/A:1022867030790.

Nehe, A. S. et al. (2020). Nitrogen partitioning and remobilization in relation to leaf senescence, grain yield and protein concentration in Indian wheat cultivars. *Field Crops Research*, 251 (November 2019), p.107778. [Online]. Available at: doi:10.1016/j.fcr.2020.107778.

Nguyen, T. H. et al. (2024). Assessing the spatio-temporal tropospheric ozone and drought impacts on leaf growth and grain yield of wheat across Europe through crop modeling and remote sensing data. *European Journal of Agronomy*, 153 (September 2023). [Online]. Available at: doi:10.1016/j.eja.2023.127052.

Osborne, T. et al. (2015). JULES-crop: A parametrisation of crops in the Joint UK Land Environment Simulator. *Geoscientific Model Development*, 8 (4), pp.1139–1155. [Online]. Available at: doi:10.5194/gmd-8-1139-2015.

Pande, P. et al. (2024a). Developing the DO3SE-crop model for Xiaoji , China. *EGUsphere [preprint]*. [Online]. Available at: doi:10.5194/egusphere-2024-694.

Pande, P. et al. (2024b). Ozone dose-response relationships for wheat can be derived using photosynthetic-based stomatal conductance models. *Agricultural and Forest Meteorology*, 356 (August 2023), p.110150. [Online]. Available at: doi:10.1016/j.agrformet.2024.110150.

Pandey, A. K. et al. (2018). Effect of elevated ozone and varying levels of soil nitrogen in two wheat (Triticum aestivum L.) cultivars: Growth, gas-exchange, antioxidant status, grain yield and quality. *Ecotoxicology and Environmental Safety*, 158 (April), pp.59–68. [Online]. Available at: doi:10.1016/j.ecoenv.2018.04.014.

Pedregosa, F. et al. (2011). Scikit-learn: Machine Learning in Python Fabian. *Journal of Machine Learning Research*, 12, pp.2825–2830. [Online]. Available at: doi:10.48550/arXiv.1201.0490.

Piikki, K. et al. (2008). The grain quality of spring wheat (Triticum aestivum L.) in relation to elevated ozone uptake and carbon dioxide exposure. *European Journal of Agronomy*, 28 (3), pp.245–254. [Online]. Available at: doi:10.1016/j.eja.2007.07.004.

Rai, R. and Agrawal, M. (2012). Impact of tropospheric ozone on crop plants. *Proceedings of the National Academy of Sciences India Section B - Biological Sciences*, 82 (2), pp.241–257. [Online]. Available at: doi:10.1007/s40011-012-0032-2.

Rathore, A., Gopikrishnan, G. S. and Kuttippurath, J. (2023). Changes in tropospheric ozone over India: Variability, long-term trends and climate forcing. *Atmospheric Environment*, 309 (December 2022), p.119959. [Online]. Available at: doi:10.1016/j.atmosenv.2023.119959.

Sarkar, A. et al. (2010). Investigating the impact of elevated levels of ozone on tropical wheat using integrated phenotypical, physiological, biochemical, and proteomics approaches. *Journal of Proteome Research*, 9 (9), pp.4565–4584. [Online]. Available at: doi:10.1021/pr1002824.

790 Sarkar, A. and Agrawal, S. B. (2010). Elevated ozone and two modern wheat cultivars: An assessment of dose dependent sensitivity with respect to growth, reproductive and yield parameters. *Environmental and Experimental Botany*, 69 (3), pp.328–337. [Online]. Available at: doi:10.1016/j.envexpbot.2010.04.016.

Schauberger, B. et al. (2019). Global historical soybean and wheat yield loss estimates from ozone pollution considering water and temperature as modifying effects. *Agricultural and Forest Meteorology*, 265 (October 795 2018), pp.1–15. [Online]. Available at: doi:10.1016/j.agrformet.2018.11.004.

Shaheen, N. et al. (2016). Amino acid profiles and digestible indispensable amino acid scores of proteins from the prioritized key foods in Bangladesh. *Food Chemistry*, 213, pp.83–89. [Online]. Available at: doi:10.1016/j.foodchem.2016.06.057.

Sharma, A. et al. (2019). Revisiting the crop yield loss in India attributable to ozone. *Atmospheric* 800 *Environment: X*, 1 (December 2018), p.100008. [Online]. Available at: doi:10.1016/j.aeaoa.2019.100008.

Shewry, P. R. and Hey, S. J. (2015). The contribution of wheat to human diet and health. *Food and Energy Security*, 4 (3), pp.178–202. [Online]. Available at: doi:10.1002/FES3.64.

Shiferaw, B. et al. (2013). Crops that feed the world 10. Past successes and future challenges to the role played by wheat in global food security. *Food Security*, 5 (3), pp.291–317. [Online]. Available at: 805 doi:10.1007/s12571-013-0263-y.

Siddiqi, R. A. et al. (2020). Diversity in Grain, Flour, Amino Acid Composition, Protein Profiling, and Proportion of Total Flour Proteins of Different Wheat Cultivars of North India. *Frontiers in Nutrition*, 7. [Online]. Available at: doi:10.3389/fnut.2020.00141.

Simon-Sarkadi, L. and Galiba, G. (1996). Reflection of Environmental Stresses. *Periodica Polytechnica:* 810 *Chemical Engineering*, 40 (1–2), pp.79–86.

Sinha, B. et al. (2015). Assessment of crop yield losses in Punjab and Haryana using 2 years of continuous in situ ozone measurements. *Atmospheric Chemistry and Physics*, 15 (16), pp.9555–9576. [Online]. Available at: doi:10.5194/acp-15-9555-2015.

Stevenson, D. S. et al. (2013). Tropospheric ozone changes, radiative forcing and attribution to emissions in 815 the Atmospheric Chemistry and Climate Model Intercomparison Project (ACCMIP). *Atmospheric Chemistry and Physics*, 13 (6), pp.3063–3085. [Online]. Available at: doi:10.5194/acp-13-3063-2013.

Swaminathan, S., Vaz, M. and Kurpad, A. V. (2012). Protein intakes in India. *British Journal of Nutrition*, 108 (SUPPL. 2), pp.50–58. [Online]. Available at: doi:10.1017/S0007114512002413.

Tai, A. P. K. et al. (2021). Impacts of Surface Ozone Pollution on Global Crop Yields: Comparing Different 820 Ozone Exposure Metrics and Incorporating Co-effects of CO2. *Frontiers in Sustainable Food Systems*, 5 (March), pp.1–18. [Online]. Available at: doi:10.3389/fsufs.2021.534616.

Tao, F. et al. (2017). Effects of climate change , CO2 and O3 on wheat productivity in Eastern China, singly and in combination. *Atmospheric Environment*, 153, pp.182–193. [Online]. Available at: doi:10.1016/j.atmosenv.2017.01.032.

825 Tian, H. et al. (2015). Climate extremes and ozone pollution: a growing threat to China' s food security. *Ecosystem Health and Sustainability*, 2 (1). [Online]. Available at: doi:10.1002/ehs2.1203.

Tiwari, S. and Agrawal, M. (2018). *Tropospheric Ozone and its Impacts on Crop Plants*. Cham, Switzerland: Springer International Publishing. [Online]. Available at: doi:10.1007/978-3-319-71873-6.

Tomer, R. et al. (2015). Impact of elevated ozone on growth, yield and nutritional quality of two wheat 830 species in northern india. *Aerosol and Air Quality Research*, 15 (1), pp.329–340. [Online]. Available at: doi:10.4209/aaqr.2013.12.0354.

Tripathi, A. and Mishra, A. K. (2016). The Wheat Sector in India: Production, Policies and Food Security. In: *The Eurasian Wheat Belt and Food Security: Global and Regional Aspects*. pp.275–296. [Online]. Available at: doi:10.1007/978-3-319-33239-0_17.

835 Wallach, D. (2011). Crop model calibration: A statistical perspective. *Agronomy Journal*, 103 (4), pp.1144–1151. [Online]. Available at: doi:10.2134/agronj2010.0432.

Wang, X. et al. (2018). Metabolic adaptation of wheat grain contributes to a stable filling rate under heat stress. *Journal of Experimental Botany*, 69 (22), pp.5531–5545. [Online]. Available at: doi:10.1093/jxb/ery303.

840 Xu, B. et al. (2023). Impacts of meteorological factors and ozone variation on crop yields in China concerning carbon neutrality objectives in 2060. *Environmental Pollution*, 317 (September 2022). [Online]. Available at: doi:10.1016/j.envpol.2022.120715.

Yadav, D. S. et al. (2019). ROS production and its detoxification in early and late sown cultivars of wheat under future O3 concentration. *Science of the Total Environment*, 659, pp.200–210. [Online]. Available at: 845 doi:10.1016/j.scitotenv.2018.12.352.

Yadav, D. S. et al. (2020). Responses of an old and a modern Indian wheat cultivar to future O3 level: Physiological, yield and grain quality parameters. *Environmental Pollution*, 259. [Online]. Available at: doi:10.1016/j.envpol.2020.113939.

Yadav, D. S., Agrawal, S. B. and Agrawal, M. (2021). Ozone flux-effect relationship for early and late sown 850 Indian wheat cultivars: Growth, biomass, and yield. *Field Crops Research*, 263 (December 2020), p.108076. [Online]. Available at: doi:10.1016/j.fcr.2021.108076.

Yang, Q., Zhao, D. and Liu, Q. (2020). Connections Between Amino Acid Metabolisms in Plants: Lysine as an Example. *Frontiers in Plant Science*, 11 (June), pp.1–8. [Online]. Available at: doi:10.3389/fpls.2020.00928.

855 Zhang, D. et al. (2023). Future climate change impacts on wheat grain yield and protein in the North China Region. *Science of the Total Environment*, 902 (August), p.166147. [Online]. Available at: doi:10.1016/j.scitotenv.2023.166147.

Zhou, S. S. et al. (2018). *Coupling between surface ozone and leaf area index in a chemical transport model : strength of feedback and implications for ozone air quality and vegetation health*. (x), pp.14133–14148.
860   [Online]. Available at: doi:10.5194/acp-18-14133-2018.