Effects of carbon dioxide and ozone on wheat crop yield and grain quality

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Abstract

Atmospheric concentrations of carbon dioxide (CO_2) and ozone (O_3) have steadily increased since the industrial revolution. CO_2 and O_3 directly affect plant physiology, CO_2 being an essential substrate for photosynthesis, while O_3 is an oxidative agent causing damage to plant tissues. Due to strong concerns for future food security, effects on crop production are of particular interest. Wheat is a major food crop globally, being the second most important energy source. Accordingly, the overall aim of this thesis was to explore the general effects of elevated CO_2 and O_3 on wheat crops.

CO_2 and O_3 impacts on wheat crops were systematically reviewed, using meta-analysis to estimate average effects, and deriving response functions to assess the effect size in relation to the concentration of either CO_2 or O_3. The underlying effects of O_3 on grain nutrients was further explored in three experimental studies.

Wheat yield increased by 25% on average under elevated CO_2, but there was no further yield stimulation above 600 ppm. Elevated CO_2 decreased grain protein concentration by 8% on average, but the effect was overestimated in pot grown plants. There was also a CO_2-induced reduction in concentration of other grain nutrients, where CO_2 effects on Fe and S were strongly correlated to the effects on protein but showed no relationship with grain yield stimulation.

O_3-induced reductions in wheat grain yield was shown to be mainly due to a decrease in grain mass, while grain number was only reduced to a small extent. Grain starch concentration was significantly reduced under O_3 exposure, making starch yield the wheat yield variable most strongly affected by O_3. O_3 enhanced concentrations but strongly reduced the yield of important wheat grain nutrients such as protein, P, Mg, K, Ca, Zn and Mg. Both concentration and yield of Cd were reduced by O_3. A comparison among our most important staple crops showed that O_3 promoted a larger protein yield loss in soybean compared to rice and wheat.

O_3 reduced harvest index (HI) for most nutrient elements, but also for Cd, while the total element pool in aboveground biomass was unaffected (except for P). Consequently, the O_3-induced reduction in grain element yield can be explained by lower remobilization rates rather than reduced uptake. There was a strong correlation of element HI when comparing sites and cultivars, indicating that it is primarily element specific and not strongly dependent on growing environment and genotypic differences. An experiment testing the interaction between O_3, heat and drought stress showed that O_3 effects on light saturated photosynthesis, grain mass and several grain nutrient concentrations were reduced under drought. Grain concentrations of protein, Ca and Zn were closely linked to grain yield regardless of O_3, heat and drought stress.

The significant impacts on wheat yield and grain quality suggest that there is a need to incorporate the influence of both CO_2 and O_3 in assessments of current and future global food security, but also account for the modifying effect of soil moisture.

Sammanfattning

Atmosfärens halter av koldioxid (CO_2) och ozon (O_3) har stadigt ökat sedan den industriella revolutionen. CO_2 och O_3 har en direkt inverkan på växters fysiologi. CO_2 är essentiellt för fotosyntesen medan O_3 ger upphov till oxidativ stress som skadar växtens vävnader. Effekter på matgrödor är av särskilt intresse på grund av oro för den globala tillgången och kvalitén på livsmedel. Vete är en av våra viktigaste grödor, då den utgör den näst största energikällan för jordens befolkning. Följaktligen är syftet med denna avhandling att undersöka hur vete generellt påverkas av förhöjda halter av CO_2 och O_3.

De genomsnittliga effekterna av CO_2 och O_3 på vete har systematiskt sammanfattats genom meta-analys, medan responssamband har tagits fram mellan effektstorlek och koncentration av CO_2 och O_3. För att utreda de bakomliggande orsakerna till O_3-effekter på vetekornens innehåll av näringsämnen har observationer från tre olika experiment använts.

Under förhöjda halter av CO_2 ökade veteskörden i genomsnitt med 25%, men tillväxten avtog vid halter över 600 ppm. Samtidigt minskade vetekornens koncentration av protein med 8%, en effekt som dock överskattades i experiment med krukodlade växter. Förhöjda halter av CO_2 ledde också till en nedgång av ett flertal andra näringsämnen, där effekterna på Fe och S var starkt korrelerade med effekterna på protein, medan de inte visade något samband med skördeökningen.

O_3-exponering gav en reducerad skörd, vilket främst berodde på en minskning av den genomsnittliga kornvikten, medan antalet korn bara var måttligt påverkat. Kornens koncentration av stärkelse minskade under förhöjda halter av O_3, vilket medför att den totala mängden stärkelse var den mest negativt påverkade responsvariabeln. O_3-exponering ledde till ökade koncentrationer men minskade mängder av ett flertal näringsämnen i kornet, såsom protein, P, Mg, K, Ca, Zn och Mg. För det giftiga ämnet Cd minskade både koncentration och mängd. En jämförelse av våra främsta matgrödor visade att O_3-exponering leder till en större förlust av proteinmängd hos sojaböna jämfört med ris och vete.

O_3 minskade skördeindex för de flesta näringsämnen och Cd, samtidigt som den totala mängden av näringsämnena förblev oförändrad. Därför kan den O_3-inducerade minskningen av mängden näringsämnen i kornet förklaras av reducerad omfördelning snarare än ett minskat upptag. Skördeindex för både näringsämnen och icke-essentiella ämnen var starkt korrelerade vid jämförelse mellan olika platser och vetesorter, vilket tyder på att skördeindex är ämnesspecifikt och inte starkt beroende av växtförhållanden och genetiska skillnader. Ett interaktionsexperiment visade att O_3-effekterna på fotosyntes, kornvikt och koncentration av ett flertal näringsämnen minskade under torka. Kornens koncentration av protein, Ca och Zn var starkt kopplade till skördens storlek oberoende av O_3-exponering, värme eller torka.

De signifikanta effekterna på veteskörd och kornkvalité visar att det finns ett behov att beakta både CO_2 och O_3 i bedömningar av risker för den globala livsmedelsförsörjningen, men även av att ta hänsyn till betydelsen av vattentillgång.

List of papers

This thesis is based on the following papers, referred in the text by roman numerals, reproduced with permission from respective journal.

I. **Broberg MC**, Högy P, Feng Z, Pleijel H (2019) Effects of elevated CO_2 on wheat yield: non-linear response and relation to site productivity. *Agronomy,* 9, 243

II. **Broberg MC**, Högy P, Pleijel H (2017) CO_2-induced changes in wheat grain composition: meta-analysis and response functions. *Agronomy,* 7, 32

III. **Broberg MC**, Feng Z, Xin Y, Pleijel H (2015) Ozone effects on wheat grain quality – A summary. *Environmental Pollution*, 197, 203-213

IV. **Broberg MC**, Xu Y, Feng Z, Pleijel H (2021) Harvest index and remobilization of 13 elements during wheat grain filling: Experiences from ozone experiments in China and Sweden. *Field Crops Research*, 271, 108259

V. **Broberg MC**, Daun S, Pleijel H (2020) Ozone induced loss of seed protein accumulation is larger in soybean than in wheat and rice. *Agronomy*, 10, 357

VI. **Broberg MC**, Hayes F, Harmens H, Uddling J, Mills G, Pleijel H (2021) Effects of ozone, drought and heat stress on wheat yield and grain quality (manuscript for Environmental and Experimental Botany)

Other scientific papers co-authored by Malin Broberg, not included in this thesis.

Klingberg J, **Broberg MC**, Strandberg B, Thorsson P, Pleijel H (2017) Influence of urban vegetation on air pollution and noise exposure – A case study in Gothenburg, Sweden. *Science of the Total Environment*, 599-600, 1728-1739

Broberg MC, Uddling J, Mills G, Pleijel H (2017) Fertilizer efficiency in wheat is reduced by ozone pollution. *Science of the Total Environment,* 607-608, 876-880

Mills G, Sharps K, Simpson D, Pleijel H, **Broberg MC**, Uddling J, Jamarillo F, Davies WJ, Dentener F, Van den Berg M, Agrawal M, Agrawal SB, Ainsworth EA, Büker P, Emberson L, Feng Z, Harmens H, Hayes F, Kobayashi K, Paoletti E, Van Dingenen R (2018) Ozone pollution will compromise efforts to increase global wheat production. *Global Change Biology*, 24, 3560–3574

Mills G, Sharps K, Simpson D, Pleijel H, Frei M, Burkey K, Emberson L, Uddling J, **Broberg MC**, Feng Z, Kobayashi K, Agrawal M (2018) Closing the global ozone yield gap; Quantification and cobenefits for multistress tolerance. *Global Change Biology*, 24, 4869-4893

Pleijel H, **Broberg MC**, Uddling J, Mills G (2018) Current surface ozone concentrations significantly decrease wheat growth, yield and quality. *Science of the Total Environment*, 613-614, 687-692.

Uddling J, **Broberg MC**, Feng Z, Pleijel H (2018) Crop quality under rising atmospheric CO_2. *Current Opinion in Plant Biology*, 45, 262-267

Pleijel H, **Broberg MC**, Högy P, Uddling J (2019) Nitrogen application is required to realize wheat yield stimulation by elevated CO_2 but will not remove the CO_2-induced reduction in grain protein concentration. *Global Change Biology*, 25, 1868-1876

Pleijel H, **Broberg MC**, Uddling J (2019) Ozone impact on wheat in Europe, Asia and North America – A comparison. *Science of the Total Environment*, 664, 908-914

Pleijel H, **Broberg MC**, Uddling J, Kobayashi K (2021) Letter to the editor regarding Pleijel et al. 2019: Ozone sensitivity of wheat in different continents – An addendum. *Science of the Total Environment*, 773, 146335

Pleijel H, Klingberg J, Nerentorp M, **Broberg MC**, Nyirambangutse B, Munthe J, Wallin G (2021) Mercury accumulation in leaves of different plant types – the significance of tissue age and specific leaf area. *Biogeosciences*, [preprint, under revision]

Abbreviations

A_n	Net photosynthetic rate (μmol CO_2 m^{-2} s^{-1})
CF	Charcoal-filtered
CO_2	Carbon dioxide
FACE	Free-air concentration enrichment
g_s	Stomatal conductance (mol H_2O m^{-2} s^{-1})
GM	Individual grain mass (mg)
GN	Number of grains (m^{-2} or $plant^{-1}$)
GY	Grain yield (g m^{-2} or g $plant^{-1}$)
HI	Harvest index
J_{max}	Maximum rate of photosynthetic electron transport (μmol m^{-2} s^{-1})
LMA	Leaf mass per area
NF	Non-filtered
O_3	Ozone
OTC	Open-top chamber
POD	Phytotoxic ozone dose
TAB	Total aboveground biomass
V_{cmax}	Maximum rate of photosynthetic carboxylation (μmol m^{-2} s^{-1})
VPD	Vapor pressure deficit

Contents

1 Introduction

Atmospheric concentrations of carbon dioxide (CO_2) and ozone (O_3) have steadily increased during the past two centuries due anthropogenic emissions (IPCC, 2013). They are both greenhouse gases contributing to current and future global warming, where changes in temperature and water availability influence plant growth. CO_2 and O_3 also directly affect plant physiology by direct leaf uptake, CO_2 being an essential substrate for photosynthesis, while O_3 is an oxidative agent causing damage to plant tissues. Due to strong concerns for future food security, effects on crop yield production are of particular interest. Accordingly, the overall aim of this thesis is to explore the general effects of elevated CO_2 and O_3 on wheat crops.

1.1 Global trends in CO_2, O_3 and climate

1.1.1 CO_2: past, present and future concentrations

CO_2 is a well-mixed and long-lived greenhouse gas, with a typical atmospheric lifetime of 100 years. Over timescales of thousands to million years atmospheric CO_2 concentration has varied substantially, where the evolution of plant stomata has occurred when CO_2 levels were more than ten times higher than today. Despite this, the overall sensitivity of plants to changes in atmospheric CO_2 is qualitatively similar, due to an adaptive feedback loop that maintains the relative gradient for CO_2 diffusion into the leaf (Franks *et al.*, 2013). Crop breeding has occurred since the first agricultural revolution about 10 000 years ago (Ross-Ibarra *et al.*, 2007) and during this time period CO_2 levels have stayed rather constant around 280 ppm. Since the industrial revolution CO_2 concentrations has risen to the current 417 ppm (Figure 1). Future scenarios predict concentrations either staying close to the current level (RCP2.6) or reaching up to 1313 ppm (RCP8.5) by the year 2100 (IPCC, 2013), where the later scenario seems more likely at present.

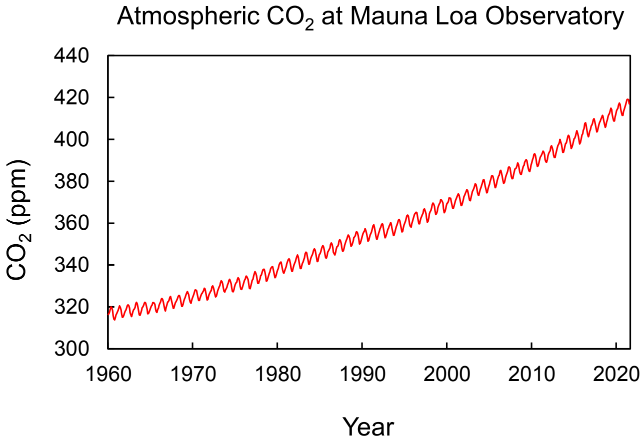

Figure 1. Monthly mean CO_2 concentration measured at Mauna Loa Observatory, Hawaii (data obtained from https://gml.noaa.gov/ccgg/trends/).

1.1.2 O_3: Spatial and temporal variation in O_3, current levels and projections

Tropospheric O_3 is semi-global air pollutant produced through photochemical reactions of nitrogen oxides (NOx) and volatile organic compounds (VOC) (including methane and carbon monoxide). These O_3 precursors are generally emitted from anthropogenic sources, and background average O_3 concentration have risen from pre-industrial levels of about 10 ppb to current 20-35 ppb (Cooper *et al.*, 2014). However, there are both spatial and temporal variations in O_3, and higher concentrations might occur for shorter time-periods, so called O_3 episodes. Figure 2 shows an example of the average diurnal variation over one year and the growing season (May-August). O_3 concentration is strongly linked to the presence of its precursors and the meteorological conditions; hence it mainly peaks during summer when there are high levels of sunlight and high-pressure weather conditions prevail (Pleijel *et al.*, 2007). Future O_3 concentrations will mainly be determined by the emission of its precursors. It is also suggested that global warming may act to decrease background concentration, while high methane (CH_4) levels (as predicted with RCP8.5 scenario) can offset this decrease and by year 2100 raising global average concentration by 8 ppb (25 % of current levels) (IPCC, 2013). On the other hand, higher surface temperatures in polluted areas could trigger regional feedbacks in chemistry and local emissions that will promote an increase in peak levels of O_3 (Ulpiani, 2021).

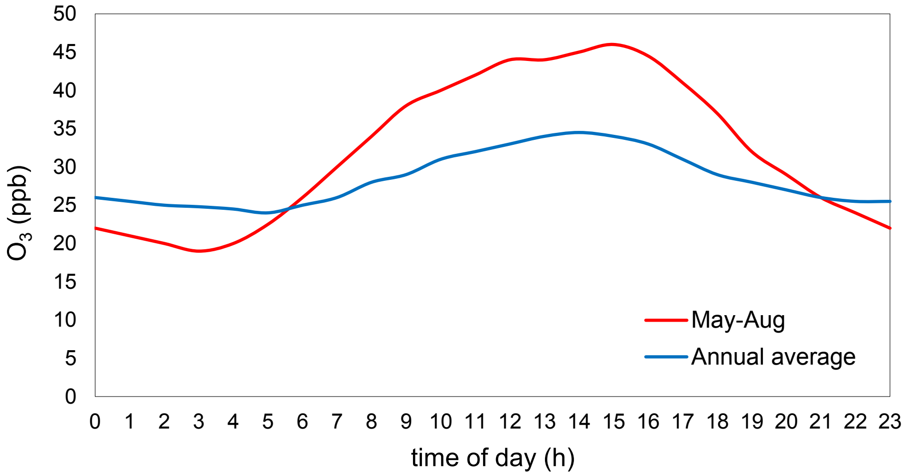

Figure 2. Average O_3 concentration for 40 European monitoring stations in 2007, data from The European Monitoring and Evaluation Programme (EMEP, www.emep.int).

1.1.3 Climate change

Because of anthropogenic emissions of greenhouse gases, an increase in global mean temperature has already been observed during the past century (IPCC, 2021). Depending on future emissions, we are facing global temperatures to rise from 1 to 5.7 degrees at the year 2100, using year 1850-1900 as the reference (IPCC, 2021). However, temperatures are not increasing evenly throughout the globe and over seasons, hence stronger warming is expected at higher latitudes and the frequency and intensity of heatwaves are predicted to increase (IPCC, 2014).

The hydrological cycle will also be affected by global warming and consequently precipitation changes are expected. Precipitation patterns can change in both directions, where some areas are predicted to become wetter while others will be dryer. Like for temperature, the shift in precipitation will probably not change evenly throughout the year. As a consequence of the change in amount and timing of precipitation, it is predicted that the frequency and intensity of both flooding and droughts will increase with climate change (IPCC, 2014). The increase in temperature will also lead to higher evapotranspiration rates, especially during heatwaves, which results in dryer soils even if precipitation remains unchanged.

1.2 Crop responses to elevated CO_2 and O_3

1.2.1 CO_2 responses

Net photosynthesis (A_n) in plants with the C3 photosynthetic pathway, like wheat, is directly stimulated by elevated CO_2, where the carboxylation rate of ribulose-1,5-biphosphate carboxylase-oxygenase (Rubisco) increase while its oxygenation rate is suppressed, resulting in reduced photorespiration (Drake *et al.*, 1997). Under current atmospheric CO_2 concentration and full daylight, C3 plants typically operate under carboxylation-limited conditions. Consequently, we can expect further increase in A_n if CO_2 keeps rising, which has been confirmed in field studies with crops grown under elevated CO_2 (Ainsworth & Long, 2005). Plants with C4 photosynthesis, like maize, has a mechanism of concentrating CO_2 around Rubisco and photosynthesis is generally not limited by carboxylation under current atmospheric CO2 concentration, unless there is drought.

Another direct response to elevated CO_2 is partial stomatal closure, which causes a decrease in stomatal conductance (g_s) in both C3 and C4 plants (Ainsworth & Rogers, 2007). With higher atmospheric concentration of CO_2, a smaller stomatal aperture is required for the plant to obtain the same amount of CO_2 molecules. The decrease in g_s leads to lower transpiration, thus a reduced loss of water. The net effect of stimulated photosynthesis and lower stomatal conductance is an improved water use efficiency at leaf level. Crops like wheat have a deterministic growth pattern, thus the number of leaves is largely pre-determined and consequently not affected much by the growth stimulation of elevated CO_2. Therefore, the total leaf area is only affected to a small extent (Ainsworth & Long, 2005). This means that canopy transpiration generally decreases, leading to higher soil moisture and consequently the growth stimulation by elevated CO_2 is expected to be stronger in dry environments.

Elevated CO_2 also leads to effects on crop quality. A common response is a reduction in nitrogen (N) concentration in plant tissues, including the edible parts of our most important food crops (Taub *et al.*, 2008), and several mechanisms has been suggested to explain the observed pattern. A CO_2-induced increase in carbohydrates has been proposed to cause dilution of N, resulting in a shift in plant stoichiometry with higher concentrations of carbon in relation to N and other nutrients Loladze (2002), hereafter referred to as the "dilution hypothesis". Pleijel and Uddling (2012) found that the dilution hypothesis is likely to exist but cannot fully explain the reduction since the N concentration in wheat grains is reduced by elevated CO_2 also in the absence of yield stimulation.

Down-regulation of Rubisco has been detected in some studies, which is believed to be a way to optimize the utilization of N (Ainsworth & Rogers, 2007). This mechanism offsets the direct stimulation by CO_2 of photosynthesis to some extent, even though the net effect on carbon gain remains positive. Another mechanism was suggested by Bloom *et al.* (2012) who demonstrated that elevated CO_2 inhibits shoot nitrate assimilation in wheat plants. The suppression of photorespiration by CO_2 leads to a reduced malate export from the chloroplasts. Malate generates nicotinamide adenine dinucleotide hydride (NADH) in the cytoplasm, which power the reduction of nitrate to nitrite in the first step of plant nitrate assimilation. In a third mechanism, the CO_2-induced reduction in stomatal conductance leads to a decrease in the transpiration-driven mass flow, which may negatively affect uptake of N and other plant nutrients (K, S, Ca, Mg and Mn) (Houshmandfar *et al.*, 2018). Fourth, the reduction in transpiration also results in higher canopy temperature (Long *et al.*, 2006), which can accelerate plant development and thus reduce the time for N to remobilize in leaves and accumulate in grains (Barnabas *et al.*, 2008).

1.2.2 O_3 responses

O_3 enters the plant through stomata and form reactive oxygen species (ROS) by reaction with biomolecules inside the leaf. The production of ROS triggers metabolically expensive defense mechanisms but also cause visible injury and promotes leaf senescence, consequently leading to less resources are available for growth and seed production (Ainsworth, 2016). Leaf duration in wheat crops has been shown to be significantly reduced by chronic O_3 exposure (Grandjean & Fuhrer, 1989, Ojanperä *et al.*, 1998), resulting in a shorter time for grain filling that results in a lower grain mass and grain yield (Gelang *et al.*, 2000).

The sensitivity to O_3 is partly determined by the rate of stomatal uptake, but can also be dependent on detoxification capacity as well as leaf mass per area (LMA), where leaves with low LMA is more sensitive simply because there is a higher O_3 load per unit leaf mass (Li *et al.*, 2016, Wu *et al.*, 2021). Crop breeding has promoted plants with high stomatal conductance to maximize the capacity of carbon assimilation, which is associated with high yielding varieties. There is also a trade-off for investing assimilated carbon into growth or to use part of its resources for defense compounds, such as antioxidants (Bussotti, 2008). Thus, crop breeding may unintentionally have promoted plants with lower investment in defense capacity (Pleijel *et al.*, 2006). Many of our most important staple crops are also annual plants with a lifetime of a few months that generally allocate a small proportion of resources into defense and have a low LMA (Bussotti, 2008). As a result, crops generally have low O_3 tolerance, where wheat and soybean are among the most O_3 sensitive staple crops, while the negative response of rice and maize is somewhat lower (Mills *et al.*, 2018b).

A number of metrics are used to express the O_3 exposure of plants, such as the O_3 daytime concentration ($[O_3]_{day}$), AOT40 and POD_6. $[O_3]_{day}$ is basically the average O_3 concentration for a specific time-interval, i.e. 7-h seasonal average for 10:00-17:00. AOT40 is the accumulated exposure over a threshold of 40 ppb during daylight hours for a specific time-interval (e.g. growing season) (Fuhrer *et al.*, 1997, Mills *et al.*, 2018b). Since O_3 uptake occurs through stomata, the total dose is strongly linked to environmental factors controlling stomatal conductance, such as soil moisture, temperature, solar radiation, and vapor pressure deficit (VPD). POD_6 (phytotoxic O_3 dose above a threshold of 6 nmol s^{-1} m^{-2} projected leaf area) is a flux-based exposure index and is estimated from O_3 concentrations and g_s which varies among species and depends on different environmental factors (Mills *et al.*, 2011).

1.2.3 Interaction between CO_2, O_3, temperature and water availability

Since both CO_2 and O_3 enters the plant through stomata, environmental factors controlling g_s are very likely to influence the effects of CO_2 and O_3. Dry soils induce low plant water potential and formation of the plant hormone abscisic acid (ABA), which causes stomatal closure and also reduces leaf expansion (Yang *et al.*, 2001). Stomatal conductance is also controlled by vapor pressure deficit (VPD), where stomata progressively close to counteract the increase in transpiration loss of water with rising VPD (Grossiord *et al.*, 2020). Conditions with high VPD usually occur in parallel with heatwaves and droughts, which are favorable also for O_3 formation (Bloomer *et al.*, 2009, Johansson *et al.*, 2020).

Moreover, elevated levels of CO_2 and O_3 also directly reduce g_s (Ainsworth & Long, 2005, Feng *et al.*, 2008), which dampens the negative effect of drought by reducing total water use and consequently increase soil moisture. Elevated CO_2 may also protect the plant from O_3 by partial stomatal closure and reduction in g_s (McKee *et al.*, 1995). On the other hand, lower g_s is associated with reduced transpiration rates that increase leaf temperature, but also may decrease nutrient uptake. The response to elevated CO_2 is also temperature dependent, where the suppression of photorespiration is more pronounced under high temperature, resulting in stronger stimulation of net carbon assimilation. However, the negative effect of CO_2 on N assimilation, as described earlier, will then also increase with temperature (Bloom, 2015b).

Both heat stress and drought can lead to production of ROS inside the leaf, as a consequence of disturbed metabolism (Barnabas *et al.*, 2008). This suggest that the ability of plants to cope with O_3 exposure can be altered by additional stress that interferes with the detoxification capacity. However, the antioxidant defense system may instead be improved if the plant is exposed to heat and drought in early development stages (Almeselmani *et al.*, 2006).

1.3 Food security

The definition of food security, by United Nations' Committee on World Food Security, means that all people, at all times, have physical, social, and economic access to sufficient, safe, and nutritious food that meets their food preferences and dietary needs for an active and healthy life (FAO, 1996). Currently and over the coming decades, climate change and other environmental stressors will have significant impact on global food production and quality, yet with high uncertainties. When assessing the impacts of elevated CO_2 and O_3 on crop yields it is crucial to do it in the light of food security. The food security concept is very broad, as given by its definition, and this thesis will generally focus on the impacts related to having sufficient, nutritious and safe food, by specifically estimating the impacts on grain yield and grain concentrations of nutrients and the toxic element Cadmium (Cd).

1.3.1 Yield production

It is well established that seasonal weather conditions strongly influence crop performance and yield production, where extreme heat and long periods of drought could be detrimental. However, the effects of climate may be significantly modified by CO_2 and O_3. By upscaling the impacts of elevated CO_2 and O_3 at plant level, potential effects on global crop yield production can be estimated for past, current, and future conditions. Several studies have attempted to quantify the global impacts of CO_2 or O_3, both as individual factors but also combined with climate change.

Lobell *et al.* (2011) assessed the impact of climate change on global production of staple crops for the time-period 1980-2008. They estimated a decrease in wheat yield of about 6%, mainly due to increased warming, but when taking rising levels of CO_2 into account the effect was offset by 3%. By simulating 125 years of metrological data, Addy *et al.* (2021) investigated the separate effects of CO_2 and climate on wheat yields for a UK experimental field site, where they concluded that wheat yields would not have increased during this time period without the stimulating effect of CO_2.

Mills *et al.* (2018a) estimated that current O_3 pollution reduce global wheat yields by 9.4%. A similar yield reduction was observed when comparing wheat grown under pre-industrial O_3 concentrations to current ambient levels, using observations from 33 open-top chamber (OTC) experiments (Pleijel *et al.*, 2018). By relating historical yields in the United States to variation in O_3 exposure and climate, McGrath *et al.* (2015) showed that O_3 reduced yields of rainfed soybean and maize by 5% and 10% respectively. The interactive effects of climate and O_3 pollution was explored in a modelling study by Tai *et al.* (2014). Using two emission scenarios for 2050 they showed that the effects of climate change and O_3 varied between continents and cultivars. O_3 exacerbated the effects of climate change, especially for wheat. Under the RCP8.5 scenario the combined effect of O_3 and climate changed reduced the yield of wheat, maize, soybean, and rice by 3.6%, whereas aggressive pollution control (RCP4.5) would instead increase yields by 3.1%.

1.3.2 Nutritional quality

More than two billion people suffer from micronutrient deficiencies, also known as hidden hunger, in particular due to low intake of Iron (Fe) and Zinc (Zn) (FAO, 2014). Nelson *et al.* (2018) argue that the greatest food security challenge in 2050 will be providing nutritious diets rather than adequate calories. The green revolution in the 1960s has contributed to large increases in wheat crop production (Evenson & Gollin, 2003). However, improvement of nutritional quality (other than protein) has received less attention resulting in lower levels of micronutrients in modern wheat cultivars compared to old landraces, which has been assumed to be a dilution effect (Murphy *et al.*, 2008).

In recent years, biofortification of staple crops has emerged as a strategy to deal with micronutrient deficiencies in low- and middle-income countries, especially for Zn, Fe and Se (Gupta *et al.*, 2021, White & Broadley, 2005). Understanding and improving leaf remobilization rates of micronutrients is pivotal to this work, where both genetic controls (Distelfeld *et al.*, 2007, Waters *et al.*, 2009), fertilization rates (Liu *et al.*, 2019, Zheng *et al.*, 2020) and environmental factors (Ercoli *et al.*, 2008) determine how large fraction of the nutrients that are allocated to the edible parts of the crop. In parallel with developments in biofortification projects, it is also important to monitor shifts in both macro- and micronutrient levels that may occur during traditional plant breeding, in order to maintain or improve the content of essential nutrients, as well as avoiding accumulation of toxic compounds.

In addition to the breeding induced decrease in micronutrients, elevated CO_2 also leads to a lowered nutritional quality of wheat grains and is a contributing factor to hidden hunger (Loladze, 2014, Myers *et al.*, 2014). Styczen *et al.* (2020) found that the significant drop in N concentration, observed for Danish grain crops during 1990-2015, can be attributed to both breeding and rising CO_2 levels. In a global assessment by Myers *et al.* (2015) it was estimated that the CO_2-induced reduction of the Zn concentration in staple crops could substantially increase the number of people at risk of Zn deficiency by 138 million in year 2050. A similar study estimated that elevated CO_2 may lead to an increase of 148 million people at risk of protein deficiency (Medek *et al.*, 2017).

1.3.3 Cadmium in food

Cadmium (Cd) is a toxic heavy metal, and consumption of cereals contribute to a major part of human dietary Cd intake (European Food Safety Authority, 2009). It is a non-essential element for the plant and the uptake is known to be dependent on transpiration-driven mass flow (Salt *et al.*, 1995). Accordingly, environmental factors controlling transpiration rates, such as CO_2 and O_3 concentrations (Feng *et al.*, 2008), soil moisture and temperature, can potentially affect the uptake of Cd. Uptake, accumulation and translocation of Cd is also influenced by the type of fertilizer (ammonium or nitrate) and the overall N status of the crop (Yang *et al.*, 2020). Cd is chemically similar to Zn and interactions between these elements occur in plant uptake and transport (White, 2012). Moreover, Cd accumulation was shown to be promoted under low Zn supply while higher supply of Zn inhibited Cd toxicity and enhanced plant growth (Zhao *et al.*, 2011). The amount of Cd ending up in the grain/seed is partly dependent on total plant uptake but also remobilization within the plant during senescence (Yan *et al.*, 2018).

2 Aim and research questions

This PhD thesis aims to assess the effects of elevated levels of CO_2 and O_3 on wheat crops, by examining plant variables related to yield and grain quality. The interactions between O_3, temperature and water availability are also examined. The overall research questions for this thesis are:

- What are the general effects of elevated CO_2 (Paper I and II) and O_3 (Paper III) on wheat crop yield and grain quality based on existing literature?
- How does elevated O_3 affect uptake and allocation of nutrients and non-essential elements? (Paper IV)
- How does the effect of elevated O_3 on wheat protein concentration and yield compared to other staple crops (soybean and rice)? (Paper V)
- What are the interactive effects of O_3, heat and drought stress on yield and grain quality in wheat crops? (Paper VI).

3 Materials and methods

3.1 Systematic reviews of CO_2 and O_3 responses

Paper I, II, III and V are systematic reviews that aims to assess overall effects of CO_2 and O_3 on wheat yield (Paper I, II, and III), and comparison with other staple crops (Paper V), but also deriving functional relationships between response variables and exposure levels of either CO_2 or O_3. The following sections (3.1.1-3.1.3) briefly describe the methods used for these four papers.

3.1.1 Data mining

Web of Science, Scopus and Google Scholar were used to survey all peer-reviewed literature (published from 1980 → year of data analysis) related to crop yield responses to elevated CO_2 and O_3. Paper I and II include responses associated with CO_2 effects on wheat yield and wheat grain quality from various experimental systems, including both potted and field grown plants and different types of open fumigation systems. Data mining for Paper III included data for the effect of elevated O_3 on variables related to wheat yield and grain quality, where only experiments conducted under field conditions (not potted plants) was included but using different fumigation systems. Paper V included O_3 exposure experiments with both field grown and potted plants with wheat, soybean and rice where grain/seed yield was reported together with protein concentration. The difference in criteria for data selection is mainly due to differences in traditions within the research fields. Field experiments have been more commonly used within O_3 research, already since the start in the 1980's, while potted plants have been used to a larger extent to test CO_2 responses.

3.1.2 Meta-analysis

In order to estimate the average effects of elevated CO_2 (Paper I and II) and O_3 (Paper III), meta-analyses were performed using MetaWin (Rosenberg *et al.*, 2000). The natural logarithm of the response ratio was used as the effect size and reported as the percentage change. Average effect size was considered to be significant if the 95% CI did not overlap zero, and for subgroup analysis different groups were considered to be significantly different if their 95% CI did not overlap. For Paper I and II the ambient CO_2 treatment was used as control, while for Paper III both charcoal-filtered (CF) treatment and non-filtered (NF) treatment were used as controls by running two parallel sets of meta-analyses. CF treatments are generally close to pre-industrial levels of O_3, whereas NF treatments represent the current ambient concentrations.

3.1.3 Response-functions

Exposure-response relationships were derived through regression between relative effects of yield variables and CO_2/O_3 concentration (Fuhrer *et al.*, 1997). For CO_2 data, the response was related to the effect estimated at 350 ppm for each experiment, where the yield variable was set to the value 1 on a relative scale with the assumption that there was no effect at 350 ppm. The same approach was used to derive response functions for O_3, but with the effects related to the estimated effect at zero $[O_3]_{day}$ in the individual experiments.

3.2 Experimental data

3.2.1 Element allocation in wheat

Paper IV aimed to determine harvest index and remobilization rates of 13 elements (Ba, Ca, Cd, Cu, K, Mg, Mn, Mo, N, P, Se, Sr, and Zn) in wheat crops grown under O_3 exposure. Data was obtained from experiments in China and Sweden, representing different agro-ecosystems and cultivars under a wide range of O_3 exposure levels.

China

The Chinese experiment was conducted in 2007 in Xiaoji town, Jiangdu county, Jiangsu province, China (32°35'N, 119°42'E). Two modern winter wheat cultivars, Yangmai 16 (Y16) and Yangfumai 2 (Y2) were grown under elevated O_3 in a free air O_3 concentration enrichment (O_3-FACE) system. Three plots were treated with elevated O_3 (E-O_3) and three equally sized plots were maintained at ambient concentration (AA). $[O_3]_{day}$ was estimated for 7h (09:00 to 16:00) and the accumulation period for AOT40 was 49 days. O_3 exposure started at tillering stage and lasted until maturity (final harvest). Plants of each cultivar and O_3 treatment at each plot were harvested at both anthesis and maturity and separated into different plant fractions (leaves, stem, grain, chaff). The experimental system and treatments has been described in detail by Feng *et al.* (2010).

Sweden

The Swedish experiment was conducted in 1997 at the Östad field site, 50 km north-east of Gothenburg, Sweden (57°54'N, 12°24'E). The spring wheat cultivar *Triticum aestivum* L. cv. Dragon was grown in Open top chambers (OTC) and exposed plants to five different levels of O_3; CF, NF, and three treatments with elevated O_3 (NF+, NF++ and NF+++). Each treatment was replicated five times (n=5). $[O_3]_{day}$ was estimated for 7h (09:00 to 16:00) and the accumulation period for AOT40 was 45 days. O_3 exposure started at the onset of anthesis and lasted for 1 month. Shoots were sampled in the early part of the grain filling period and short before final harvest. Four different fractions of the plants, leaves, straw, grain and chaff, were sampled. The experimental system has been described in detail by Gelang *et al.* (2000).

Determination of element harvest index and remobilization

For both experiments, samples of the aboveground plant fractions were analyzed to determine the concentration of Ba, Ca, Cd, Cu, K, Mg, Mn, Mo, N, P, Se, Sr, and Zn. The element pool, being equal to element accumulation, in each plant fraction (leaf, stem, ear, chaff and grain) was determined by multiplying the element concentration with biomass. Element harvest index (HI) was derived by calculating the fraction between grain element pool and total element pool at maturity (harvest). Element pools in different plant fractions were compared for the two developmental stages (anthesis and maturity) to determine if remobilization had occurred.

3.2.2 O_3 interaction with heat and drought stress

Treatments

The experiment that provided data for the study in Paper VI took place at the UK Centre for Ecology & Hydrology air pollution exposure facility in Abergwyngregyn, North Wales (53°23'N, 4°02'W). Four 'solardomes', hemispherical glass domes (3m diameter and 2m high), were used to control O_3 exposure and temperature (Figure 3). Two solardomes were maintained at ambient O_3 concentrations, one with ambient temperature (aO_3 aT) and one with elevated temperature (aO_3 eT). The other two solardomes had elevated levels of O_3, one with ambient temperature (eO_3 aT) and one with elevated temperature (eO_3 eT). In all four solardomes there were two watering regimes, dry (~50% less water given) and well-watered. O_3 treatment started at 20th May and treatments with elevated temperature and reduced water supply started at 10th June (mid-anthesis) and lasted throughout the grain-filling period until final harvest, 15th August. Environmental conditions for each treatment are summarized in Table 1. The spring wheat variety Skyfall were sown in 25 L pots on 23rd February. Seedlings emerged on 15th April and pots were moved into the solardomes on 21st April, prior to O_3 exposure starting.

Figure 3. Solardomes used to control levels of O_3 and temperature at the UK Centre for Ecology & Hydrology air pollution exposure facility in Abergwyngregyn (Wales). Photo by Malin Broberg.

Table 1. Average 7h daytime O_3 concentration ($[O_3]_{7h}$) during fumigation period, temperature (T), relative humidity (RH) and vapor pressure deficit (VPD) from mid anthesis until end of O_3 fumigation (grain filling period).

Treatment	$[O_3]_{7h}$ (ppb)	T	RH (%)	VPD
aO_3 aT	25	20.9	69.4	0.76
eO_3 aT	73	20.9	69.4	0.76
aO_3 eT	22	29.5	45.2	2.26
eO_3 eT	65	29.6	44.1	2.32

Gas exchange measurements

Gas exchange measurement were performed on flag leaves at growth temperature using a LI-6400 photosynthesis system (LICOR, Lincoln, NE, USA). Stomatal conductance of water vapor (g_s) and light-saturated net photosynthesis (A_n) were measured simultaneously, with light set to a photosynthetic photon flux density (PPFD) of 1500 µmol m^{-2} s^{-1} and CO_2 concentration of air entering the leaf cuvette was kept at 400 ppm. The response of A_n to intercellular CO_2 concentration (C_i) was obtained by changing the CO_2 concentration of air entering the leaf cuvette in 12 steps (400, 50, 100, 150, 200, 300, 400, 600, 800, 1000, 1500, 2000 ppm) under a constant PPFD of 1500 µmol m^{-2} s^{-1}. Parametrization of photosynthetic capacities (V_{cmax} and J_{max}) were determined using the photosynthesis model by (Farquhar *et al.*, 1980), with later modification of Rubisco temperature dependencies (Bernacchi *et al.*, 2001).

Yield variables and grain nutrients

For all treatments the following yield variables were determined: grain yield, grain mass, grain number and harvest index. Grains were also analyzed to determine the concentration of Ca, K, Mg, Mn, Mo, N, P, and Zn and nutrient yield was calculated by multiplying grain nutrient concentration with grain yield.

4 Findings and discussion

4.1 CO_2 effects on wheat

4.1.1 Grain yield (Paper I)

Paper I is an updated research synthesis on the CO_2 impacts on wheat crop yield. The average grain yield stimulation of elevated CO_2 was estimated to 25% and the increase could mainly be attributed to an increase in grain number, while grain mass was only stimulated to a small extent. There was a non-linear relationship between grain yield and CO_2 concentration (Figure 4), which suggests that no further yield stimulation can be expected above 600 ppm. The response pattern partly agrees with the earlier review of Amthor (2001) who also found a non-linear relationship but with a maximum yield stimulation at 890 ppm. The two studies differ with respect to the types of experiments included where the Amthor study also included more artificial exposure systems (laboratory chambers, greenhouses, and closed-top chambers) and only one FACE study. Whereas the analysis in Paper I is based on a much larger number of field observations, which can explain the difference in estimated maximum response. In contrast, several other studies have suggested the CO_2 response of wheat grain yield to be linear within the range of CO_2 (350-750 ppm) that are included in the response function for Paper I (Hogy & Fangmeier, 2008, Wang *et al.*, 2013, Ziska & Bunce, 2007). The lack of further response at CO_2 concentrations above 600 ppm could potentially be due to nutrient limitation (Pleijel *et al.*, 2019), where N assimilation can be reduced due to suppression of photorespiration (Bloom *et al.*, 2012). In addition, high CO_2 reduces the transpiration-driven mass flow, which is of importance for some plant nutrients (N, K, S, Ca, Mg, Mn) (Houshmandfar *et al.*, 2018). Lower transpiration rates may also increase canopy temperatures (Long *et al.*, 2006) leading to heat stress and earlier senescence, which are associated with shorter grain filling time (Barnabas *et al.*, 2008).

Furthermore, the findings from Paper I demonstrated that there was no significant difference in grain yield response to elevated CO_2 when comparing exposure systems (FACE vs. OTC), which have been suggested in earlier studies (Long *et al.*, 2006). The potential influence of microclimate, by chamber enclosure, did not affect the estimated CO_2 response of wheat grain yield. Also the comparison of rooting environment (pots vs. field soil) did not reveal any differences in yield response, which is in contrast to (Wang *et al.*, 2013) who found the CO_2 response of grain yield to be significantly larger in potted plants. Hogy and Fangmeier (2008) also showed that the CO_2 effect on wheat grain yield is related to pot size, but that larger pots (>10 l) are comparable to field conditions. Consequently, observations of wheat CO_2 response using OTCs and large pots are still relevant to use for parameterization and validation of crop models that aims to assess future crops yields.

Another novelty of Paper I was the relationship between grain yield CO_2 response (% effect of elevated CO_2) and grain yield in the control treatments (ambient CO_2.), shown in Figure 5. Assuming that grain yield in the control treatment is good a proxy for average productivity of the specific site and year, it can be concluded that the relative stimulating effect of CO_2 is stronger in low productive systems. Separating the data by experimental system (OTC field, OTC pots, FACE, field tunnels) geographical location (continent) or exposure level (above or below 600 ppm) did not influence the relationship. Low productive systems are generally dryer sites where the CO_2 response is expected to be stronger due to the water-saving effect.

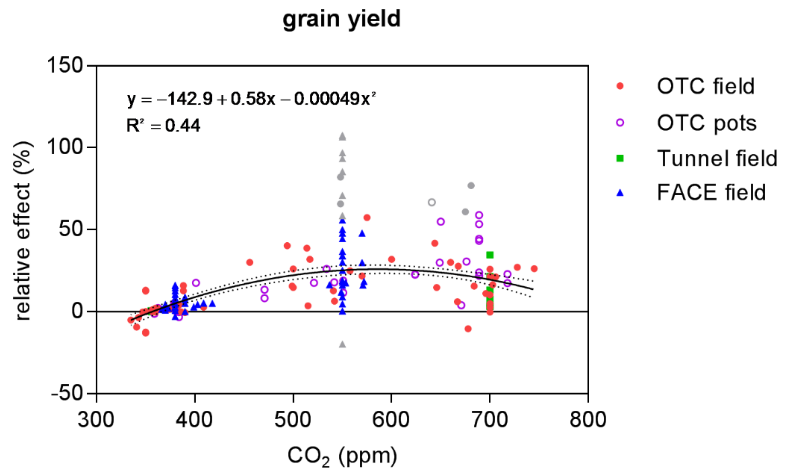

Figure 4. Response function for grain yield (relative to yield at 350 ppm) with CO_2 concentration. Grey markers are statistical outliers excluded from the curve fitting. Dotted line represents the 95 % confidence interval of the regression model.

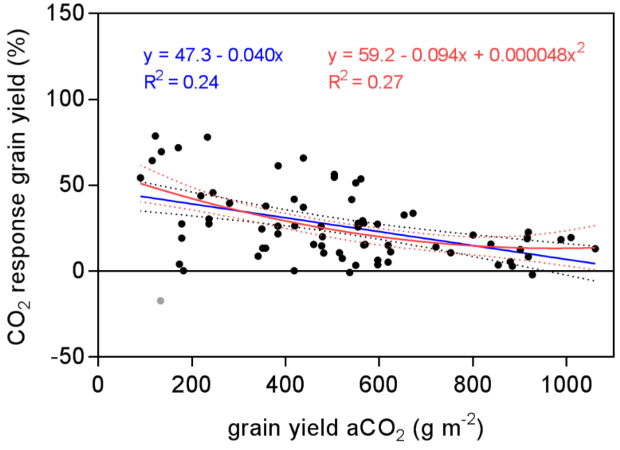

Figure 5. Relationship between relative CO_2 response for grain yield and absolute grain yield in control treatment (aCO_2). Blue line and equation give the linear fitted regression model and the red line and equation is fitted with second order polynomial fit. Grey markers are statistical outliers excluded from the curve fitting. Dotted line represents the 95 % confidence interval of the regression model.

By including a large number of ecologically realistic experiments from many different locations and different wheat cultivars, Paper I provides useful insights to the general response of wheat yield to rising CO_2 that to some extent contrast with previous findings.

4.1.2 Grain nutrients (Paper II)

In Paper II the CO_2-induced reduction in wheat grain N concentration was estimated to approximately 8% (Figure 6), which is in line with previous studies (Myers *et al.*, 2014, Taub *et al.*, 2008). The effect was much more pronounced in potted plants compared to plants rooted in the field, which means that a restricted rooting volume is exaggerating the effects of elevated CO_2, at least for N uptake. At a first sight, the comparison of exposure systems showed a significant difference between N concentration effects in FACE and OTC facilities. However, all FACE observations were based on CO_2 exposure levels below 600 ppm, while about half of the OTCs used higher concentrations. By comparing the FACE observations to a subset of the OTC observation, where elevated CO_2 treatments were below 600 ppm, the difference disappeared. Hence, it can be concluded that exposure system did not significantly influence the CO_2 effect on grain N concentration when comparing similar levels of CO_2 exposure.

A recent study by Bloom and Plant (2021) completely contradicts the findings in Paper I and II, where 35 years of field observations shows that wheat grain yield and N yield has significantly decreased due to rising CO_2, while N concentration has remained constant. These authors argue that less extreme CO_2 enrichment has a different effect on wheat crops, compared to many of the exposure levels included in for example Paper I and II. Suggested mechanisms for their observations are an increase in the Mn to Mg ratio in the chloroplast, which increase photorespiration and nitrate assimilation that offsets the effect of CO_2 on photorespiration described earlier. In addition, elevated CO_2 can increase carbohydrate export from shoots to root, which enhance root nitrate assimilation at the expense of lower grain yield (Bloom *et al.*, 2020). There is however a need to test and confirm these effects and suggested mechanisms in a controlled field environment where CO_2 concentration is the only variable factor.

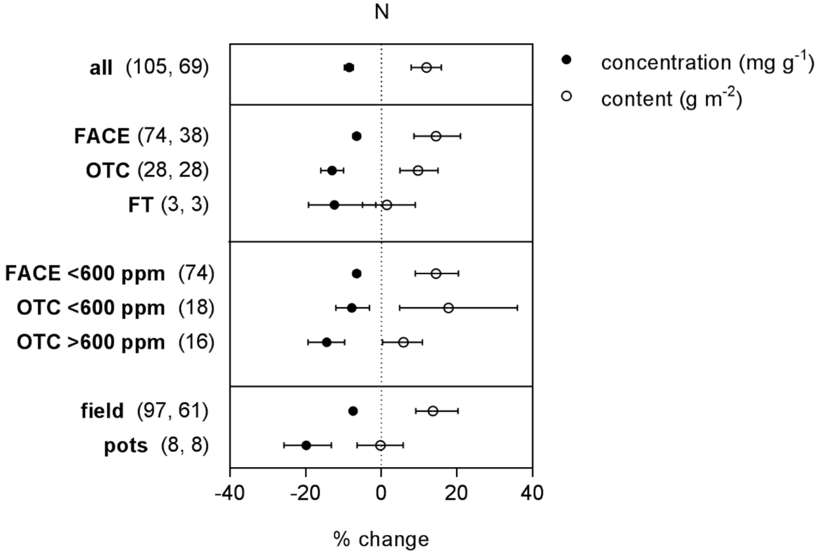

Figure 6. Meta-analysis of elevated CO_2 effects on N concentration and N content using ambient CO_2 treatment as the reference, with subgroup analysis of exposure system, rooting environment, and concentration level of elevated CO_2 treatment. Number of observations pairs are given within brackets.

Along with the reduction in N it was also observed that grain concentration of many nutrient elements significantly decreased under elevated CO_2 (Figure 7), where the magnitude of response varied from non-significant up to 10% reduction. The correlation between relative effect on grain element concentration and grain yield stimulation was tested for all elements end revealed that K, P and Zn showed strong association of effects (Figure 8). Thus, for these elements the growth dilution hypothesis can explain the reduction to a large extent. For the CO_2 effect on N there was no correlation with grain yield stimulation. On the other hand, many elements showed a strong correlation of effects with the CO_2 effects on N, especially Fe (r=0.79) and S (r=0.87). Together with Zn, these were also the elements most strongly affected by elevated CO_2 (Figure 9). The elements that showed little (Cu and Mn) or non-significant (B and K) response to elevated CO_2 also showed a weak correlation with CO_2 effects on N. This suggests that the CO_2 effect on N may play a role also for other mineral elements, either that they are affected by the same mechanisms or possibly element concentrations in the grain are coordinated to the availability of N, which is usually the most limiting element in agriculture. It can be noted that the elements most strongly affected by CO_2 (N, Zn, Fe and S) are also mostly found in proteins, which supports the idea of coordination to N concentration. In addition to the nutrients, also the toxic element Cd was strongly reduced by elevated CO_2, which is an element strongly controlled by transpiration but with no active plant uptake. Thus, both reduction in transpiration driven mass flow and impaired N assimilation are possible explanations for the observed reductions in wheat grain elements.

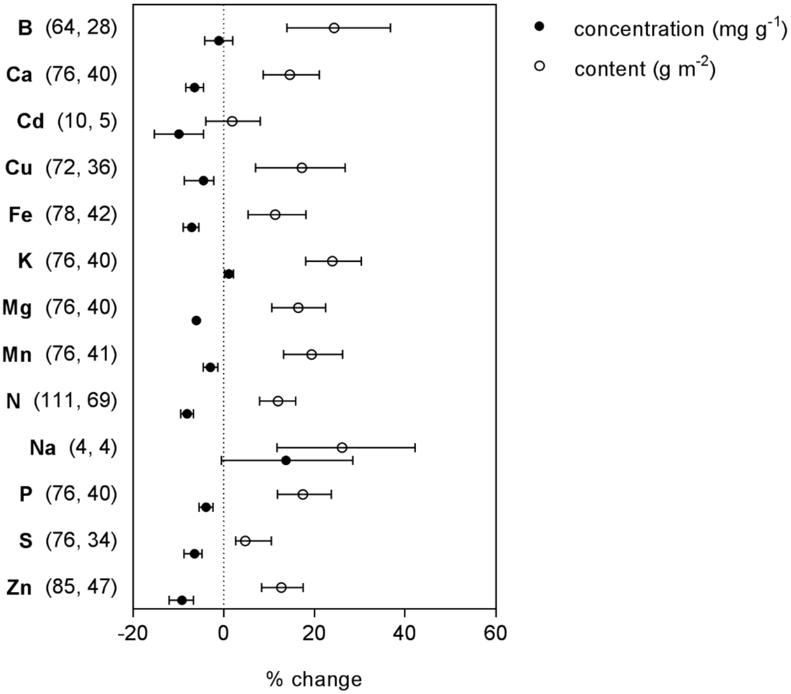

Figure 7. Meta-analysis of elevated CO_2 effects on wheat grain element concentration and element content using ambient CO_2 treatment as the reference. Number of observations pairs are given within brackets.

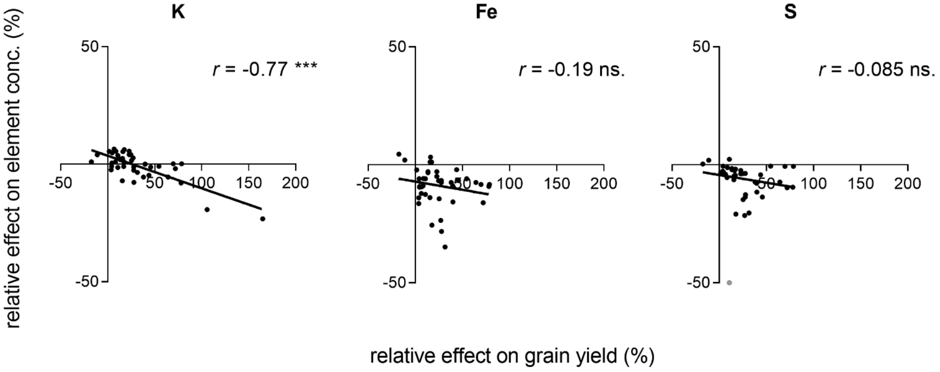

Figure 8. Relative effect of elevated CO_2 on the grain element concentration K, Fe and S vs. the relative effect on grain yield. Black solid lines represent the linear regression model, for which parameters and model performance are presented in Paper II, Table 2.

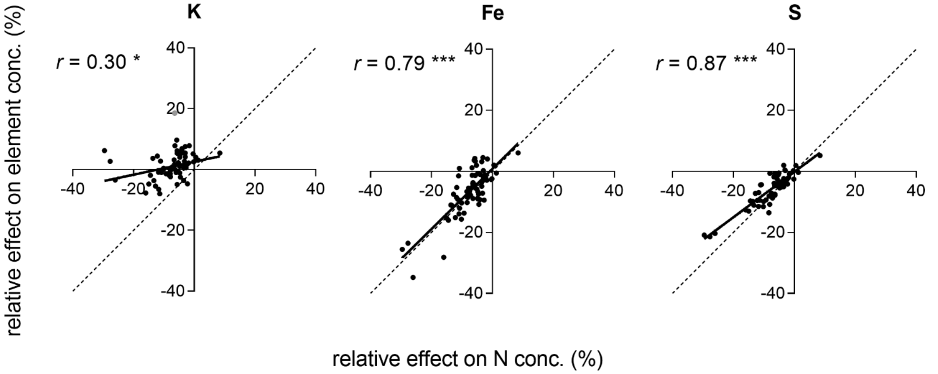

Figure 9. Relative effect of elevated CO_2 on grain element concentration of K, Fe and S vs. the relative effect on grain N concentration. Correlation coefficient (r) and its significance is presented in each plot. Black solid lines represent the linear regression model, for which parameters and model performance are presented in paper II, Table 2. Dashed lines represent the hypothetical situation where the effect of elevate CO_2 on element concentration is equal to the effect on N concentration.

4.2 O_3 effects on wheat

4.2.1 Yield and grain quality (Paper III)

Paper III provides a summary of O_3 effects on wheat yield and grain quality by estimating average effects with meta-analysis and deriving functional relationships between grain quality variables and O_3 exposure. One of the most adverse effect was the negative impact on grain mass, which to a large extent explains the overall reduction in grain yield (Figure 10). A lower grain mass is associated with smaller and malformed grains, this results in lower flour yield per mass unit of grains and consequently reducing the market price. The strong reduction in grain mass agrees with the general post-anthesis senescence promoting effect of O_3 (Grandjean & Fuhrer, 1989), which shortens the grain filling period (Gelang *et al.*, 2000).

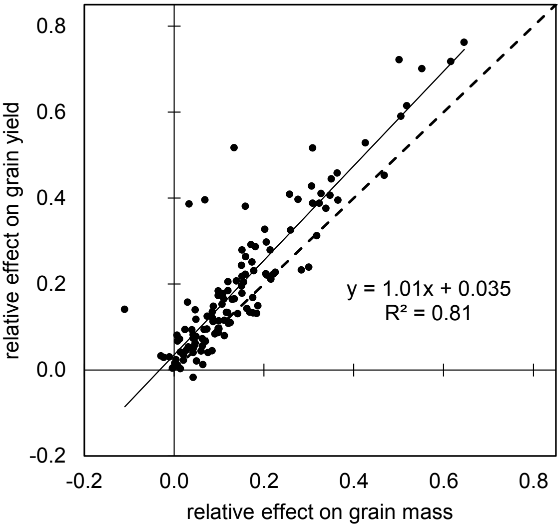

Figure 10. Regression between the O_3 effect on grain yield vs. the corresponding effect on grain mass. The broken line is a 1:1 line that represents the theoretical situation where the O_3 effect on grain yield is entirely explained by effects on grain mass.

There was a significant reduction in starch concentration (Figure 11), which is in line with the observation of reduced grain mass, since for small grains the fraction of starch is likely to be lower. As a result of the O_3-induced reduction in both grain yield and starch concentration, the total amount of starch per unit area (starch yield) is reduced to an even larger extent. The response function for starch yield was not included in Paper III, but presented here in Figure 11, calculated from the data in Paper III but with addition of six data points from Tomer *et al.* (2015). The slope of the response function reveals that this is the wheat yield response variable most strongly affected by O_3 exposure.

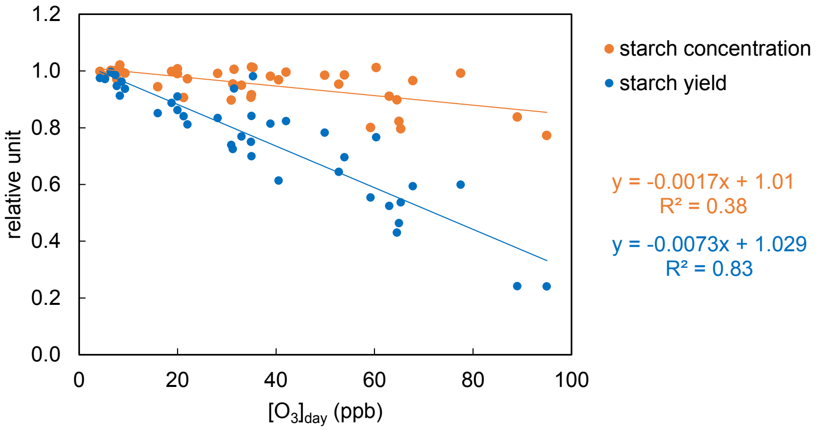

Figure 11. Relationship between relative starch concentration and starch yield with daytime O_3 concentration ($[O_3]_{day}$). Based on data used in Paper III, with addition of observations from Tomer *et al.* (2015).

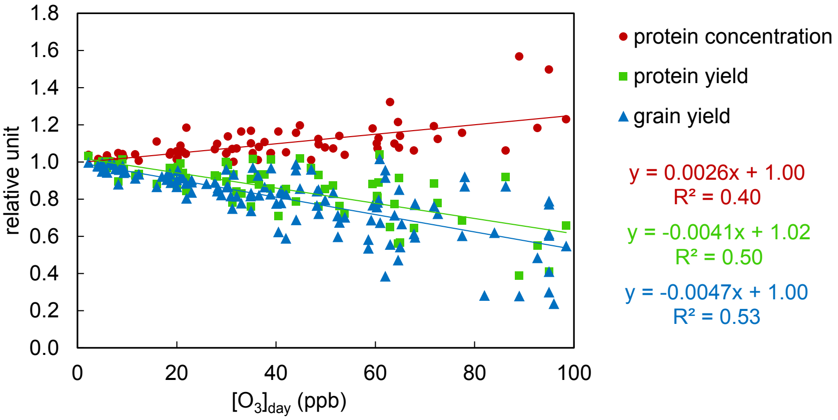

Figure 12. Relationship between relative protein concentration, protein yield and grain yield with daytime O_3 concentration ($[O_3]_{day}$). Based on data used in Paper III.

Grain concentration of protein increased under O_3 exposure while the total amount (protein yield) was reduced (Figure 12). This is a common response among cereals grown under environmental stress (Wang & Frei, 2011). The stress promoted leaf-senescence and shortened grain-filling duration is somewhat compensated by enhanced remobilization of amino acids (Triboi & Triboi-Blondel, 2002). On the other hand, accumulation of starch primarily depends on concurrent carbon fixation during grain filling (Gebbing & Schnyder, 1999) and, consequently, the accumulation of protein is less affected than starch, resulting in higher protein concentration. This line of argument also agrees with the observation of reduced starch concentration.

Nutrient elements responded in a similar way as protein, with higher concentrations but lower yield (Figure 13), and the response pattern is likely to also be an effect of reduced starch accumulation. However, the meta-analysis indicates that the average response of element yield for macronutrients is somewhat stronger than for micronutrients, even though no statistical differences could be detected. In contrast to the nutrients, concentrations of the non-essential elements Cd and Na showed a negative response to O_3. The uptake of these elements is mainly controlled by transpiration, which often is reduced by elevated O_3 (Feng *et al.*, 2008). In addition to the increase in nutrient elements, there was also an overall improvement in baking properties in wheat grown under O_3 exposure, which is a response mainly associated with the change in protein concentration.

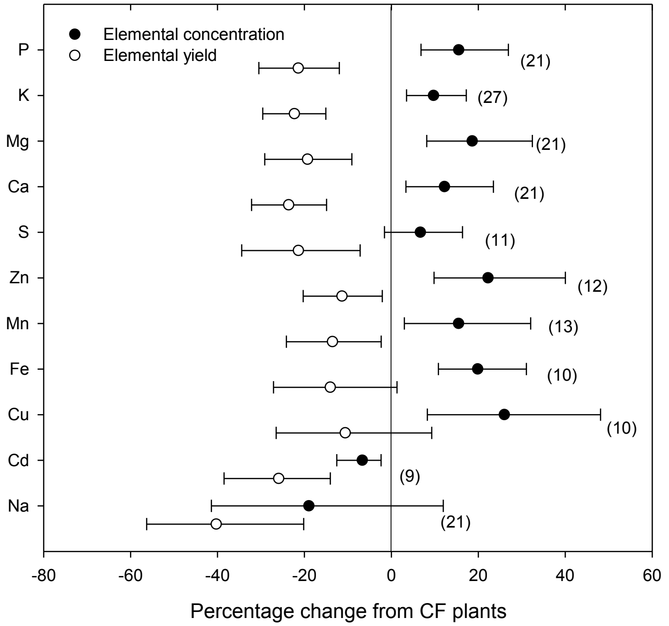

Figure 13. Meta-analysis for O_3 effects on element concentration and element yield using CF as the reference. Numbers within brackets gives the number of observational pairs for each variable.

4.2.2 Element harvest index (Paper IV)

Paper III summarized the overall O_3 effects on grain elements, but the underlying mechanisms of changes in elemental content were not fully understood. The decrease in grain element yield can be a result of reduced uptake from the soil, but also a reduction in remobilization and transport of elements from vegetative to reproductive plant parts during the grain filling period. In Paper IV, element HI was used as an integrative measure of how efficiently elements were allocated to the grain. Element HI for nutrients were significantly reduced by O_3 exposure, while the total element pool in aboveground biomass was unaffected for all nutrient elements, except P. Since O_3 exposure promotes premature senescence and thus shortens the grain filling period, there is also less time for nutrient elements to remobilize and be transported to the grain. Thus it can be concluded that the reduction in element yield, as observed in Paper III, is a result of reduced allocation of nutrients to the grain rather than reduced uptake from the soil, with the exception of P, for which also the total pool in aboveground biomass was reduced by O_3. HI for Cd showed the same response as nutrient elements, while the other non-essential elements (Ba, Se, Sr) was not affected by O_3 exposure.

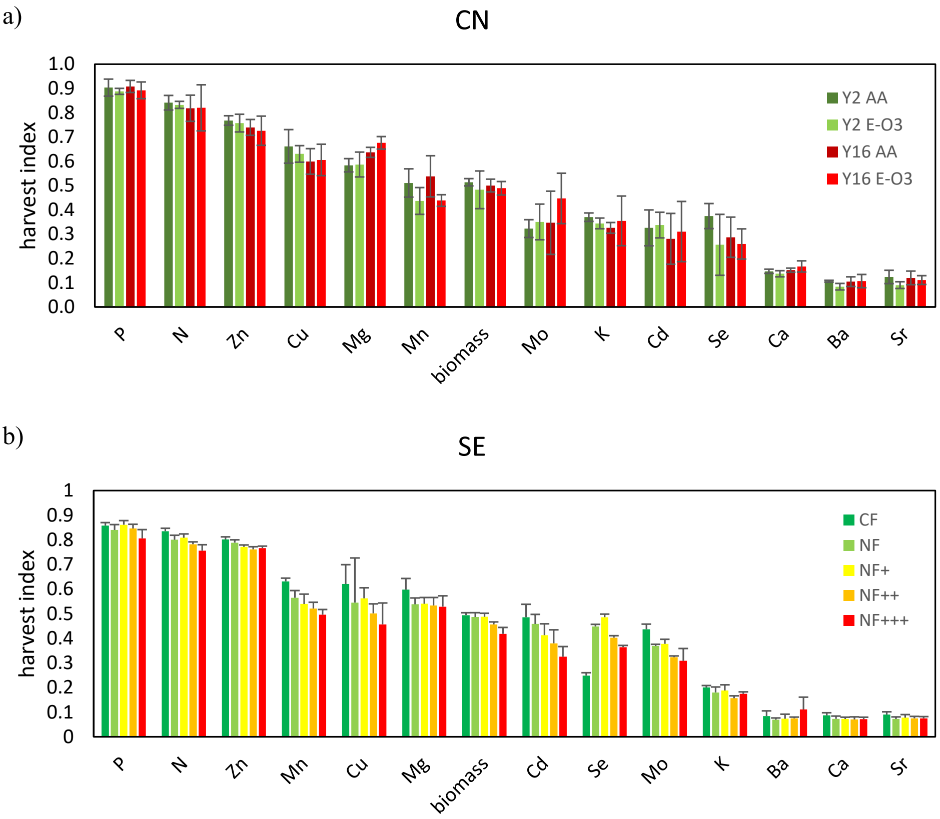

Figure 14. Element and biomass harvest index for a) Chinese (CN) experiment b) Swedish (SE) experiment. Elements are sorted in descending order of average HI for all treatments. Error bars are standard deviation.

In addition to the effects of O_3 on element HI, it can also be concluded that nutrient elements generally are remobilized to a larger extent than non-essentials, resulting in higher element HI (Figure 14a-b). Using observations from similar O_3 treatments in the Chinese and Swedish experiment showed a very strong correlation of element HI (Figure 15). Element HI for the two cultivars in the Chinese experiment were also strongly correlated. The two experimental sites differ in climate, soil type, using different rates of fertilization rates, plant density, wheat type (winter and spring wheat) and fumigation system (FACE and OTC). These factors could potentially influence the availability, uptake, and allocation of plant nutrients, but despite these dissimilarities we still observe a strong correlation of element HI between China and Sweden. This suggest that HI in wheat is element specific and not strongly dependent on geographical location, genotype, or agricultural management.

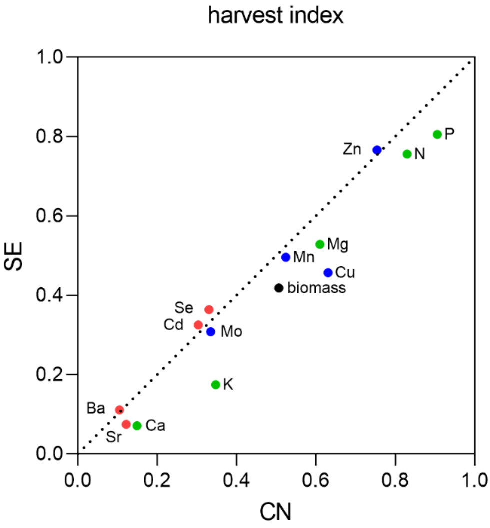

Figure 15. Correlation of element and biomass harvest index in two experimental sites, China (CN) and Sweden (SE). Pearson $r = 0.97$, $p < 0.0001$. CN observations are the average of two cultivars from ambient O_3 treatment. Se observations are NF+++ treatment, which had an O_3 concentration similar to the CN ambient treatment. Marker color denotes element type: green = macronutrient, blue = micronutrient, red = non-essential element.

4.2.3 Effect on protein in wheat compared to soybean and rice (Paper V)

Wheat, soybean, and rice are among the most important staple crops on a global scale. In similarity to wheat, O_3-induced yield reductions in soybean and rice have been observed. The impacts on grain protein concentration has also been reported in individual studies for both crops, while the O_3 impact on protein yield has been given less attention. Paper V aimed to synthesize the O_3 effects on seed protein concentration and seed protein yield in soybean and rice for comparison with the effects on wheat crops (already summarized in Paper III).

Response functions for seed protein concentration with O_3 concentration demonstrated that rice showed a response pattern very similar to wheat (Figure 16b-c), where a positive relationship was observed. It is likely that the mechanism behind the observed pattern is also the same, where O_3 stress reduce grain accumulation of starch to a larger extent than for protein. The effect on starch was not summarized here, but Frei *et al.* (2012) demonstrated that the increase in concentration of both protein and lipids occurred at the expense of reduced starch for rice grown under O_3 exposure. This study was also part of the observations included in the current synthesis.

On the contrary, seed protein concentration for soybean was not affected by O_3 exposure (Figure 16a). Previous studies have observed O_3 to impair the symbiotic N fixation in legumes (Cheng *et al.*, 2011, Hewitt *et al.*, 2016). For legumes like soybean, N fixation is the main source of N, thus N availability becomes closely dependent on plant performance. When grown under environmental stress, such as O_3 exposure, the negative effect in N fixation becomes proportional to the loss in biomass and seed yield, leaving N/protein concentration constant.

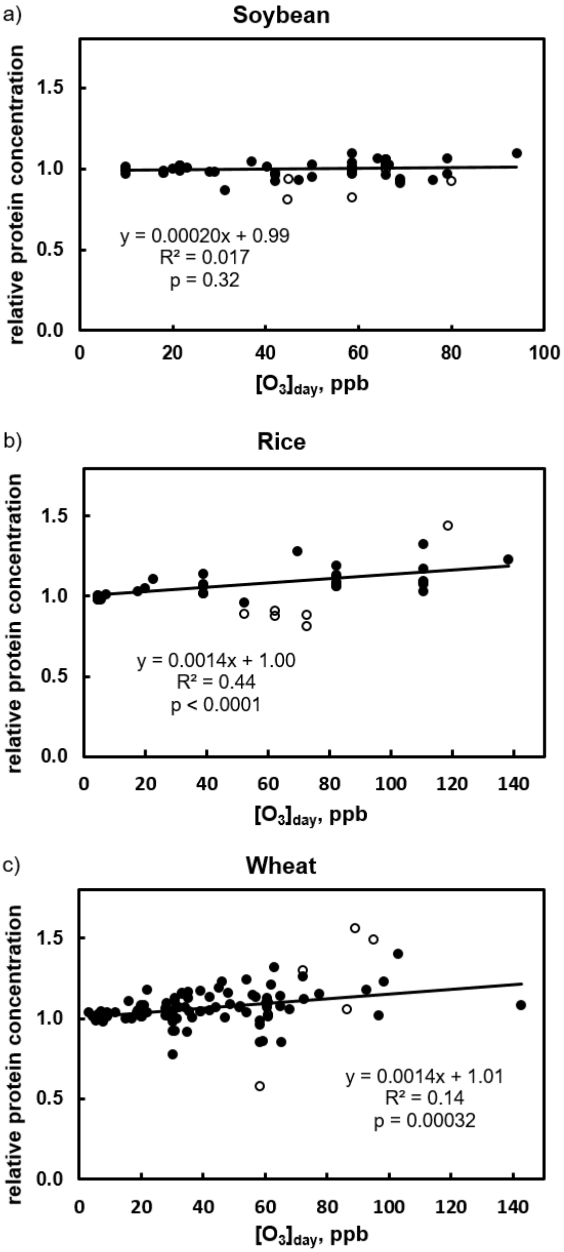

Figure 16. Relationship between relative seed protein concentration and daytime O_3 concentration $[O_3]_{day}$, for a) soybean, b) rice, and c) wheat. Open circles denote data points identified as statistical outliers.

Because of the already established fact that O_3 reduced seed yield of soybean, rice and wheat, together with the current estimated impacts on protein concentration, O_3 effects on protein yield could be assessed and compared for the three crops. Results showed that the O_3-induced loss in protein yield was most pronounced for soybean, followed by wheat and then rice. Since the average protein concentration in soybean is much higher compared to the rice and wheat, the negative effect of O_3 on absolute protein yield per area unit (ton ha^{-1}) is considerably more severe for soybean. This is demonstrated in an example in Figure 17, using global average seed yield (FAO, 2019) for each crop as the reference and three O_3 scenarios (10 ppb = preindustrial, 37 ppb = average of control treatments in the current study, 60 ppb = hypothetical future projection). It becomes clear that the loss of protein accumulation in tons ha^{-1}, is much larger for soybean compared to the cereals.

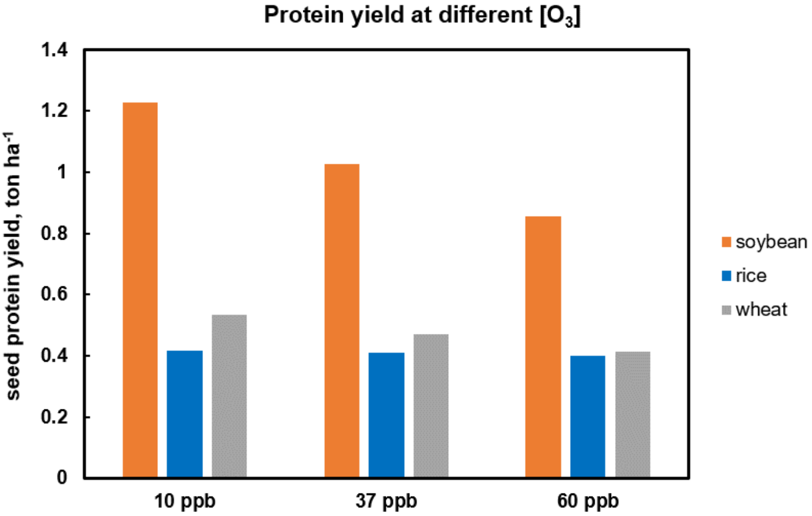

Figure 17. Estimation of typical seed protein yield based on global average seed yield and response functions in Paper V for three O_3 scenarios: 10 ppb = preindustrial, 37 ppb = average of control treatments in Paper V, 60 ppb = hypothetical future projection.

4.3 O_3 interaction with heat and drought stress (Paper VI)

Paper VI explored the interactive effects between O_3, temperature and water supply. O_3, drought and warming individually decreased grain yield, grain mass and harvest index, whereas grain number was only negatively affected by heat and drought stress but not O_3 exposure. In line with previous observations (Khan & Soja, 2003) the current study showed a significant interaction between O_3 and water for grain mass (Figure 18a), where the negative effect of O_3 was reduced by low water supply. However, the protective effect of drought was not observed for grain yield, even though direction of effects were similar but not statistically significant. Since grain yield is the combined result of average grain mass and grain number, there are larger variation among replicates for grain yield compared to grain mass, thus the inconsistence of the results may be due to low statistical power (n=4).

The underlying mechanisms of O_3 and water interaction was explored by measuring leaf gas exchange. A significant interaction was observed for light saturated photosynthesis (A_{sat}), which was clearly impaired by O_3 exposure under well-watered conditions but not affected by under drought (Figure 18b). A similar response was observed when testing the interaction between O_3 and temperature, but with borderline significance (p=0.066). Drought significantly reduced g_s, which suggest that O_3 damage is reduced due to lower stomatal uptake. However, the same response was not detected for elevated temperature where the effect on g_s was non-significant.

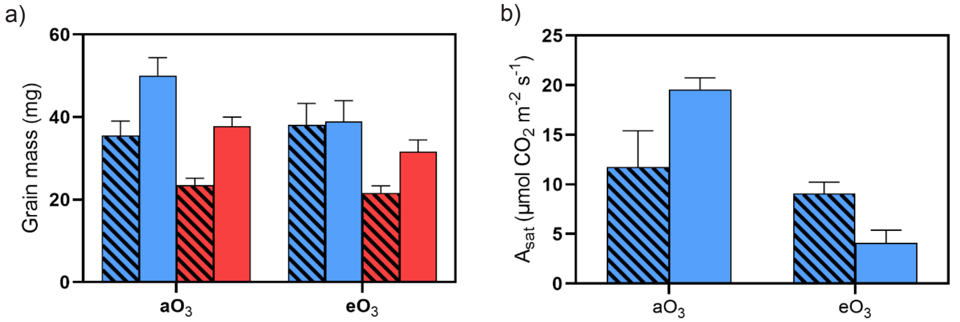

Figure 18. Effects of O_3, warming and low water supply on a) light saturated net photosynthesis (A_{sat}), average of three measurements (day 190, 196, 202), and b) average grain mass at final harvest. Error bars gives the standard deviation. Blue bars = ambient temperature, red bars = elevated temperature, hatched bars = dry (50% less water supply), filled bars = well-watered, aO_3 = ambient O_3, eO_3 = elevated O_3.

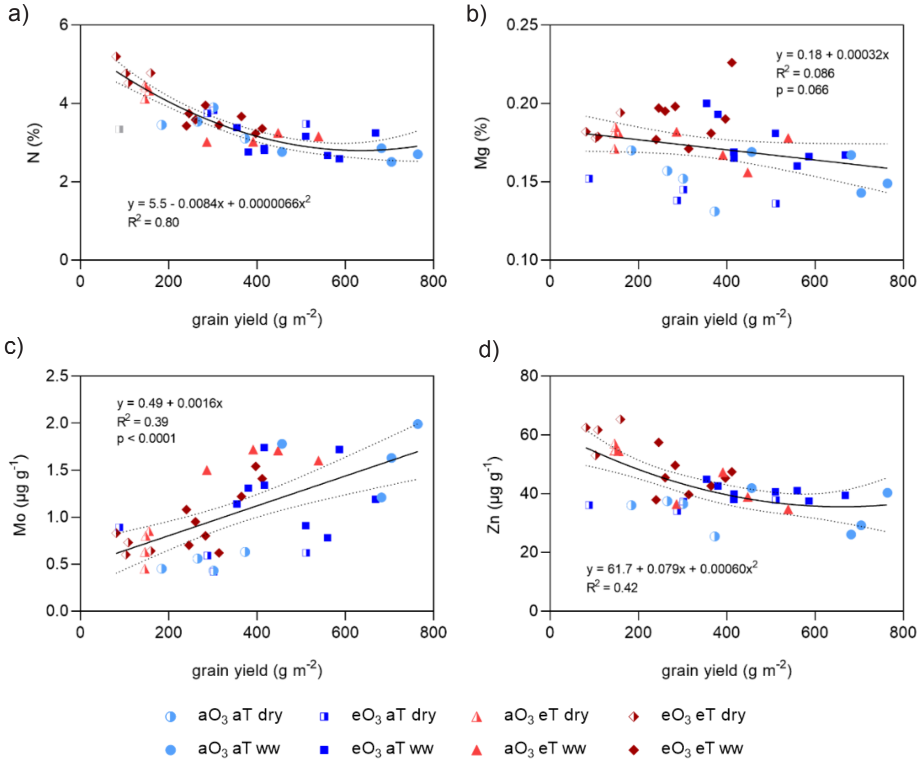

Figure 19. Relationship between grain element concentration and grain yield at final harvest. Linear (y=a+bx) and quadratic ($y=a+bx+cx^2$) regressions were fitted to the observations, with the best fit shown in figures.

Paper VI also investigated how O_3, temperature and water supply affected grain nutrients. Elevated O_3 and temperature mainly increased grain concentrations, and since both stressors act to reduce grain filling time this is likely a result of the "concentration effect" by reduced starch accumulation (Wang & Frei, 2011). Grain concentrations of K and Ca were the only variables where an O_3 and temperature interaction was detected, where the "concentration effect" of O_3 was aggravated by elevated temperature.

The effects of drought were bidirectional. Grain concentrations of K, Ca, and Zn significantly increased under low water supply and the effect was more pronounced under high temperature. For K and Ca there was also an interaction between O_3 and water supply, where the "concentration effect" of O_3 exposure was only present under well-watered conditions, in line with the observations for grain mass. On the contrary, grain concentrations of Mg and Mo were reduced by low water supply, possibly linked to a reduction in transpiration-driven mass flow that limits the total plant uptake of these nutrients to a large extent and consequently overrides the "concentration effect". Grain concentration of Mo was only negatively affected by O_3 under well-watered conditions, which aligns with the O_3 induced reduction of g_s and suggest that Mo uptake is strongly linked to transpiration.

The relationship between grain nutrient concentration and grain yield was derived to test the hypothesis that nutrient concentration follows grain yield regardless of environmental stressor, as a consequence of the "concentration effect". There was an overall negative relationship between nutrient concentrations and grain yield (Figure 19), with Mo as an exception to this pattern and instead showing a positive relationship. N and Ca strongly followed the shifts in grain yield (R^2=0.80), suggesting that the accumulation of these elements is not affected by environmental stress per se, but indirectly change due to the "concentration effect" of reduced starch. For other elements (P, K, Mg, Mn, Mo) slopes were not affected by environmental stress, but the intercept was significantly shifted in many cases and was generally larger for elevated O_3 and high temperature, while the effects of water varied between elements.

From this study it becomes clear that water supply significantly modifies the negative O_3 response of wheat crops. The O_3 metric POD is one way to account for both soil moisture deficit and metrological factors on the stomatal O_3 uptake, which can be linked to the effects on yield and grain quality with response functions. Our study shows that global assessments of O_3 effects on grain quality characteristics, such as grain mass and nutrient concentrations, also needs to be considered in relation to water supply. However, there is a need to validate the results of the current study under realistic field conditions to obtain accurate estimates of the magnitude of agronomic effects. The outcome of Paper II clearly showed that studies using potted plants overestimated the CO_2 effect on grain N concentration, while no difference was observed for grain yield (Paper I). Therefore, the magnitude of effects observed for grain nutrients in Paper VI may not be directly comparable with field conditions, but the direction of change is likely to be the same.

4.4 CO_2 + O_3

4.4.1 Comparing effects

Table 1 qualitatively summarizes the overall effects of elevated CO_2 and O_3 on wheat yield variables and grain quality characteristics, obtained from the meta-analyses and response functions in Paper I, Paper II, Paper III and Paper V. Many of the effects appear as direct opposites when comparing the responses of CO_2 to O_3, but the underlying mechanisms are often different which is crucial when trying to understand how CO_2 and O_3 interact.

The increase in grain yield under elevated CO_2 is mainly due to a larger number of grains, which is a process determined during the vegetative growth phase and not affected by elevated O_3. The yield loss observed under O_3 exposure can instead be attributed to a reduced grain mass, because the grain filling period is shortened due to premature senescence. From the response of total aboveground biomass and harvest index it becomes clear that the positive yield effect of CO_2 is primarily due to an overall growth stimulation, whereas the negative impacts of O_3 is a result of reduced allocation of biomass (and nutrients) to the grains together with a reduction in total aboveground biomass.

Table 2. Direction and magnitude of change under elevated CO_2 or O_3 for each response variable, ↑=large increase, ↗=small increase, →=no change, ↘=small decrease, ↓=large decrease, - = analysis not done.

Response variable		**CO_2**	**O_3**
Grain yield		↑	↓
Total aboveground biomass		↑	↓
Biomass harvest index		↗	↓
Grain number		↑	↘
Grain mass		↗	↓
Starch	concentration	→	↓
	yield	↑	↓
Protein/N	concentration	↓	↑
	yield	↑	↓
	harvest index	-	↓
Nutrients	concentration	↓	↑
	yield	↑	↓
	harvest index	-	↓
Cd	concentration	↓	↓
	yield	↓	↓
	harvest index	-	↓
Baking properties		↓	↑

For grain constituents (starch, protein and nutrients) O_3 effects are also attributed to the shortened grain filling period, where the “concentration effect” on protein and other nutrient elements is a result of reduced starch accumulation. As indicated by the negative impact on nutrient harvest index, the reduction in protein and nutrient yield is a consequence of reduced allocation, whereas total nutrient uptake is not affected to a large extent (shown in Paper IV).

On the other hand, starch concentration does not change significantly under elevated CO_2. Thus, the effects on nutrients cannot simply be explained by a dilution effect. As described earlier, the suggested mechanisms behind the observed effects on protein and other nutrients is probably linked to the plant uptake (Bloom *et al.*, 2012, Houshmandfar *et al.*, 2018), even though the non-significant effect on starch can still contribute to some extent.

Cd is the only response variable where the same effects are observed for both CO_2 and O_3. This is likely linked to the reduction in g_s observed for both elevated CO_2 (Ainsworth & Long, 2005) and O_3 (Feng *et al.*, 2008). No active plant uptake occur for this non-essential and toxic element and uptake is mainly controlled by transpiration rates. Since wheat is used for baking to a large extent, the impact on response variables related to baking properties are also of interest. Elevated CO_2 generally reduced the baking quality while it was improved by O_3 exposure. This response pattern is closely linked to the effects on protein content, where gluten is one of the major protein types in wheat grains and its concentration largely determines the suitability for baking.

In summary, CO_2 effects mainly operate during the vegetative growth phase when seed set is determined and the majority of nutrient uptake occur, while the effects of O_3 develop during the reproductive growth phase when plant resources are transferred from vegetative to reproductive plant parts. Elevated CO_2 promotes higher wheat yield but of lower quality, while the opposite is observed for wheat grown under O_3 exposure.

4.4.2 Interaction

The rising concentrations of CO_2 and O_3 do not occur separately, and the sources of anthropogenic emissions are often common, where burning of fossil fuels produce both CO_2 and O_3 precursors. The interaction of these gases is of great importance to get a more complete understanding of the combined effects of climate change and air pollution on current and future food production. It is well established that increasing CO_2 concentration leads to a reduction in stomatal aperture, which decrease the exchange of gases between air and leaf, including a reduced uptake of O_3.

The idea that elevated CO_2 can offset the negative effects of O_3 is not new but was presented already in the 1980s (Idso, 1989). Since then, several studies have been conducted to test the interactive effects for several crops, including wheat. By studying the antioxidant status McKee *et al.* (1997) demonstrated that stomatal exclusion plays a major role in the protective effect of CO_2 against damage from O_3. Mortensen (1990) showed that the negative effect of O_3 on wheat growth was reduced by elevated CO_2. The same response was observed also for wheat grain yield, harvest index, grain mass and protein concentration (Mishra *et al.*, 2013). Mulchi *et al.* (1995) found that elevated CO_2 protected from O_3 induced yield loss in both soybean and wheat, while the effect on maize was non-significant. On the contrary, a wheat study by Mulholland *et al.* (1998) did not find any significant interaction between CO_2 and O_3 for any growth or yield parameters, even though there were indications that O_3 damage was less severe under elevated CO_2.

Several studies, including Paper VI, have shown that both CO_2 and O_3 interact with climate as well as with each other. A climate chamber study by (Hansen *et al.*, 2019) showed that the relationship between wheat grain yield and O_3 dose was weaker under both elevated CO_2 and high temperature. The influence of temperature can explain some of the scatter observed in the response function for grain yield with O_3 exposure in Figure 12, since observations are from many different locations with varying climate conditions. However, the variation in ambient CO_2 concentrations is rather small (~50ppm) for the dataset included in O_3 response functions of Paper III and is not likely to influence the relationship to a large extent. Another climate chamber experiment tested the interactive effect of CO_2, O_3 and temperature on rapeseed, where it was observed that temperature induced a reduction in oil content which was offset by both CO_2 and O_3 (Namazkar *et al.*, 2016). When all factors were combined the change in oil content was close to zero suggesting a significant interaction between all three factors. There is however a lack of field experiments that firmly evaluate the interactive effects of CO_2 and O_3 in combination with climate change.

4.5 Implications for crop modelling

Several of the findings in this thesis can be applied in crop models that aims to explore the impacts of CO_2 and O_3 on wheat yield and grain quality. The response functions describe the gradual change in each response variable relation to concentrations of CO_2 and O_3 and can consequently be applied to investigate impacts under many different emission scenarios, as long as they are within the current concentration range. The meta-analysis did not detect any differences in CO_2 response for grain yield and protein concentration when comparing observations FACE and OTC. This means that both types of observational data are useful for calibration and validation of crop models. However, the overestimation of CO_2-induced reduction in grain protein in potted plants highlights that studies using pots may not provide reliable estimates of the magnitude of effects for nutrient uptake, even though they may be useful for a mechanistic understanding of processes. Current crop models often consider N dynamics while other nutrients are neglected. Even though the processes of most other nutrients are not capture, the findings in Paper II suggests that CO_2 effects on nutrients strongly correlated to N, such as Fe and S, could be incorporated into crop models through its association with N.

Paper IV provides functional relationships between O_3 exposure and nutrient HI, and also indicates that nutrient HI is very element specific and not strongly dependent on cultivar or agro-environment. This allows for the O_3 effect on nutrient allocation to be incorporated in crop modelling. The findings in Paper VI clearly shows that the modifying effect of soil moisture is crucial to make more accurate estimates of O_3 effects. Response functions using POD as O_3 metric, instead of daytime concentration, is one way to incorporate this into crop models. Since POD is a metric that gives the total dose of O_3 it is probable most suitable to use for response variables where the O_3 effect is directly linked to senescence, whereas it may not be applicable for effects on nutrients that are more strongly controlled by transpiration without further consideration of plant water relations.

5 Conclusions

5.1 CO_2 effects

- 25% increase in wheat grain yield under elevated CO_2, but no further wheat yield stimulation of CO_2 above 600 ppm.
- The relative CO_2 stimulation of grain yield is higher in low productive systems.
- No difference in the response of grain yield or protein concentration when comparing plants grown in OTC and FACE fumigation systems using similar exposure levels.
- Grain protein concentration decreased by 8% on average, but the effect was overestimated in pot grown plants.
- Grain nutrient concentrations of Ca, Cu, Fe, Mg, Mn, P, S and Zn decreased under elevated CO_2 together with the toxic element Cd.
- CO_2 effects on Fe and S were strongly correlated to the effects on N, but showed no relationship with grain yield stimulation.
- Response functions for wheat yield variables provide useful information for the gradual change in each variable in relation to rising CO_2 concentration, whereas the meta-analyses summarize the overall effects observed in CO_2 experiments.

5.2 O_3 effects

- O_3-induced reductions in wheat grain yield is mainly due to a decrease in grain mass, while grain number is only reduced to a small extent.
- Grain starch concentration significantly reduced under O_3 exposure, making starch yield the wheat yield variable most strongly affected by O_3.
- O_3 enhance concentrations but strongly reduced the yield of important wheat grain nutrients such as protein, P, Mg, K, Ca, Zn and Mg. Both concentration and yield of Cd were reduced by O_3.
- O_3 significantly reduced HI for most nutrient elements, but also Cd, while there was no effect on the total pool for any included element except P and Ba. Consequently, the O_3-induced reduction in grain element yield can be explained by lower remobilization rates rather than reduced uptake.
- There was a strong correlation of element HI when comparing sites and cultivars, indicating that element HI for wheat crops are primarily element specific and not strongly dependent on growing environment and genotypic differences.
- Rice yield was less sensitive to O_3 compared to wheat and soybean, whereas grain protein concentration increased equally for rice and wheat but remained unchanged for soybean. Consequently, O_3 promoted a larger protein yield loss in soybean compared to rice and wheat, having large implications for global protein production because of the much higher absolute protein concentration in soybean.
- O_3 effects on A_{sat}, grain mass and grain nutrient concentrations (K, Ca, Mg, Mo) were reduced under drought.
- Grain concentrations of N, Ca and Zn were closely linked to grain yield regardless of O_3, heat and drought stress.

6 Outlook

6.1 Agricultural adaptation

Since cutting emissions is the obvious, but unfortunately very difficult way to avoid the negative effects of elevated CO_2 and O_3, there is a need for agricultural adaptation. There are two major ways of adapting crop production to environmental changes: breeding for more suitable cultivars and changing agricultural management, where a combination of strategies is probably the most successful way (Lobell & Burke, 2010). By having a good understanding of how crops respond to elevated levels of CO_2 and O_3 there is potential to utilize positive effects while avoiding drawbacks.

Several recent studies have been screening existing cultivars to identify traits related to positive CO_2 responses (Ingvordsen *et al.*, 2015, Marcos-Barbero *et al.*, 2021) and O_3 resistance (Betzelberger *et al.*, 2010, Feng *et al.*, 2016). The focus for wheat breeding in relation to CO_2 responses should be to maintain yield stimulation without having significant loss of grain nutrient concentrations. This is something that can only be achieved if CO_2 effects on nutrient uptake mechanisms are fully understood, which is currently not the case. So far, the CO_2 effect on nutrient harvest index, other than for N (Lenka *et al.*, 2021), has not been studied for wheat crops. The O_3-induced reduction in nutrient allocation, observed in Paper IV, can potentially be affected by elevated CO_2 since the rate of remobilization also depends on nutrient concentrations in vegetative plant parts, which is known to be reduced by elevated CO_2.

Breeding for O_3 tolerant crops is not straight forward, since there are often trade-offs between traits related to reduced O_3 uptake or defense and assimilation of carbon that are allocated to grain yield. Lower stomatal conductance leads to reduced O_3 uptake but is also associated with lower carbon assimilation rates, however this is not always the case. Cultivars with high water use efficiency can assimilate a larger amount of carbon per unit water, which means that they also take up less O_3. Thus, this is a trait to aim for when breeding for O_3 tolerance. On the other hand, there are also indications that antioxidant capacity is more strongly correlated to O_3 effects on photosynthesis compared to stomatal conductance (Feng *et al.*, 2016). It has also been shown that there is a substantial natural variation in O_3 tolerance among rice cultivars, where mapping of the genome has identified quantitative trait loci related O_3 induced symptoms, grain yield losses and degradation of straw quality (Frei, 2015).

Since O_3 damage accumulates over the growing season it is also possible to use cultivars with a faster development rate, which is a trait that is variable among existing cultivars, but development rate is also temperature dependent (Barnabas *et al.*, 2008). If aiming for cultivars with faster development rate, it is important to make sure that not only carbon, but also essential nutrient elements are remobilized efficiently. Whether focusing on achieving O_3 tolerance with improved water use efficiency, higher antioxidant defense or faster development rate, it is crucial to keep track of yield stability and interaction with climate conditions. (Mills *et al.*, 2018b) suggested that it would be beneficial to incorporate O_3 in breeding strategies that aims to improve tolerance to multiple stressors, such as heat and drought, where both synergies and trade-offs may occur.

It is also possible that to some extent mitigate the negative effects of CO_2 and O_3 by altering agricultural management practices. The water-saving effect observed under elevated CO_2

suggests that irrigation can be reduced. There may also be shifts in growing areas, where water demanding crops could be grown in areas that were previously too dry. Fertilization is one potential way utilize the positive effect of CO_2, where Pleijel *et al.* (2019) showed that the CO_2-induced stimulation of wheat grain yield increased with N application rates up to ~200 kg ha^{-1}. The same study also revealed the reduction in grain N concentration was not related to N application rates. However, Bloom (2015a) suggested that the type of N fertilizer may be of importance for the CO_2 effect on N concentration in plant tissues, where larger effects are expected when using nitrate as N source compared to ammonium.

There are also potential management strategies that can be applied to mitigate negative impacts of O_3 exposure. Paper VI clearly shows that water availability determines the magnitude of O_3 damage, which is something that could be utilized in agricultural practices. With forecasts of O_3 levels, irrigation can be reduced or completely stopped when O_3 episodes are expected. The seasonality of O_3 exposure also provides the opportunity to adjust the timing of sowing. In that way, the growing season could be shifted a bit to make sure that the most sensitive growth phase, anthesis to maturity, does not overlap too much with the time-period of highest O_3 levels.

6.2 Food security

The significant impacts on wheat yield and grain quality suggest that there is a need to incorporate the influence of both CO_2 and O_3 in assessments of current and future global food production. In previous work, most emphasis has been given to estimating impacts on yield production, being equivalent to calorie supply, whereas shifts in accumulation of nutrients has received less attention. From this thesis it can be concluded that effects of CO_2 and O_3 on wheat yield and grain nutrients operate in different directions and are controlled by different mechanisms. The interaction between CO_2 and O_3 has so far only been considered for growth and yield variables, while the impacts on grain quality has been neglected. Hence, there is a need to better understand the interactive effects of CO_2 and O_3 on nutrient uptake and accumulation under different climate conditions. This can be achieved by performing field experiments together with mechanistic crop modelling. A better understanding of factors controlling uptake and allocation of nutrients are crucial for improving the nutritional quality of wheat and other staple crops, both for traditional breeding but also for biofortification projects that aim to mitigate hidden hunger.

6.3 Knowledge gaps

Several knowledge gaps remain within the current research field. A deeper understanding of how CO_2 and O_3 interacts with climate has already been mentioned. The effects of CO_2 and O_3 may also be modified by other factors, such as weeds, pests, and diseases, which has been poorly considered in a global context. In order to avoid loss of grain nutrients in crops grown under elevated CO_2, there is a need for a more complete mechanistic understanding of processes related to uptake and relocation within the plant. Several likely hypotheses have been tested and gained support but without consensus, thus a larger number of agronomically realistic field observations is required.

Water controls the magnitude of response for both CO_2 and O_3, where dry conditions promote the positive yield response of CO_2 and at the same time reduce the O_3 uptake. Gas exchange measurement may have good precision in moment of data collection but does not fully capture variation during and between days. Crop water-use changes throughout the growing season and it is rather time-consuming and difficult to obtain reliable estimates of total water use. Using isotopic signatures provides a possibility to estimate the integrated response of gas exchange but also N dynamics (Araus *et al.*, 2013, Farquhar & Richards, 1984). Chang-Espino *et al.* (2021) demonstrated that $\delta^{15}N$ and $\delta^{13}C$ were correlated to O_3 effects on wheat grain yield and grain N concentration, suggesting that they are good indicators of chronic O_3 exposure.

Developments can also be made when it comes to incorporating O_3 impacts within crop modelling (Emberson *et al.*, 2018). Mechanistic crop models can provide dynamic assessments of O_3 impacts on crop growth under various scenarios of CO_2 and climate change. However, there are still uncertainties of how different plant processes are affected by O_3 during the growing season. There is a need to better understand the direct impacts of O_3 on carbon assimilation and how strongly this is linked to plant senescence. By synthesizing data from the literature, together with new observations, functional relationships can be derived between net assimilation and chlorophyll content (being a proxy for senescence). A functional relationship between chlorophyll content and O_3 exposure, for a broad range of observational data, would also be useful to better capture how O_3 promotes senescence.

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