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Effects of ground-level ozone on vegetation modified by nitrogen and components of climate change: a literature study

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Effects of ground-level ozone on vegetation modified by nitrogen and components of climate change: a literature study

by

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Abstract: Effects of ground-level ozone on vegetation modified by nitrogen and components of climate change: a literature study

Tropospheric ozone (O_3) is considered as the most significant phytotoxic air pollutant. It is well established that elevated O_3 concentrations negatively impact plant growth, development and productivity, as well as species composition and biodiversity. In the context of the Convention on Long-Range Transboundary Air Pollution (CLRTAP) critical levels for O_3 to protect vegetation have been derived for different types of vegetation and are continuously developed on the basis of present scientific knowledge. The aim is to estimate the O_3risk for vegetation in the current and future pollution situation in Europe as a basis for mitigation measures in the European air pollution control policy. The derivation of O_3 critical levels is based on many years of research on the impact of O_3 on vegetation by means of experiments in which plants were exposed to different levels of O_3, mostly under otherwise optimal growth conditions. However, it is well known that the effects of O_3 in the field can be significantly modified by a number of other environmental and anthropogenic factors. That means that any assessment of the impacts of O_3 exposure in a future climate needs to consider possible interactions with nitrogen deposition and factors of climate change. The present study summarises the current knowledge of how climate change factors, including temperature and drought stress, N input, and elevated CO_2 influence or alter growth, yield and gas exchange responses to O_3.

Kurzbeschreibung: Auswirkungen von bodennahem Ozon auf die Vegetation in Kombination mit Stickstoff und Komponenten des Klimawandels: eine Literaturstudie

Troposphärisches Ozon (O_3) gilt als der bedeutendste phytotoxische Luftschadstoff. Erhöhte O_3-Konzentrationen können das Pflanzenwachstum, die Entwicklung und Produktivität sowie die Artenzusammensetzung und die biologische Vielfalt negativ beeinflussen. Im Rahmen des Übereinkommens über weiträumige, grenzüberschreitende Luftverunreinigungen wurden kritische Schwellenwerte (Critical Levels) für O_3 zum Schutz der Vegetation für verschiedene Vegetationstypen abgeleitet und sie werden auf der Grundlage der vorliegenden wissenschaftlichen Erkenntnisse fortlaufend weiterentwickelt. Ziel ist es, das O_3-Risiko für die Vegetation in der gegenwärtigen und zukünftigen Belastungssituation in Europa als Grundlage für Minderungsmaßnahmen in der europäischen Luftreinhaltepolitik abzuschätzen. Die Ableitung kritischer O_3-Werte basiert auf langjährigen Untersuchungen zum Einfluss von O_3 auf die Vegetation v.a. durch Experimente, bei denen Pflanzen unterschiedlichen O_3-Konzentrationen ausgesetzt waren, meist unter ansonsten optimalen Wachstumsbedingungen. Es ist jedoch bekannt, dass die Auswirkungen von O_3 unter Freilandbedingungen durch eine Reihe anderer Faktoren erheblich verändert werden können. Eine Bewertung der Auswirkungen von O_3-Belastungen in einem zukünftigen Klima muss daher mögliche Wechselwirkungen insbesondere mit erhöhter Stickstoffdeposition und Faktoren des Klimawandels berücksichtigen. Die vorliegende Studie fasst das aktuelle Wissen darüber zusammen, wie Faktoren des Klimawandels wie Temperatur- und Trockenstress, N-Eintrag und erhöhte CO_2-Konzentrationen O_3-Effekte auf das Wachstum, den Ertrag und den Gaswechsel beeinflussen oder verändern.

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A	photosynthetic rate
A_{sat}	light-saturated photosynthesis
AOT40	Accumulated Ozone Exposure over a threshold of 40 ppb
CF	charcoal-filtered air
CL	critical level
CLRTAP	Convention on Long-Range Transboundary Air Pollution
CO_2	carbon dioxide
FACE	free air carbon dioxide enrichment
GPP	Gross primary production
g_s	stomatal conductance
IPCC	Intergovernmental Panel on Climate Change
LAI	leaf-area index
NCER	Net Carbon Exchange Rate
NEP	net ecosystem production
NF	non-filtered air
NPP	net primary production
O_3	ozone
OTC	open-top chambers
PI_{total}	performance index for photosynthetic efficiency
P_n	net photosynthetic rate
POD_y	phytotoxic O_3 dose above an hourly threshold y
ppb	parts per billion
ppm	parts per million
RMF	root mass fraction
Rubisco	Ribulose-1,5-bisphosphate carboxylase
V_{cmax}	maximum carboxylation rate catalysed by Rubisco
ROS	reactive oxygen species

Summary

Tropospheric ozone (O_3) is considered the most significant phytotoxic air pollutant and future trend projections predict an increase in background O_3 concentrations. Current and future levels of O_3 have been estimated to potentially reduce crop yield and timber production by 25% and more. To protect vegetation from O_3 impacts *critical levels* for O_3 have been derived for different types of vegetation (Convention on Long-Range Transboundary Air Pollution, CLRTAP). These are based on a great number of experiments in which plants were exposed to different levels of O_3, mostly under optimal growth conditions and thus disregard any kind of modification of the response to O_3 by other environmental and anthropogenic factors. The present study summarises the current knowledge of how nitrogen deposition and climate change factors, including elevated CO_2, increased temperature, and drought stress influence or alter the plant response to O_3. Taking into account 315 publications, information on main interactive effects on 65 plant species is presented in tabular form or as percentage derivation from the control treatment for crops, grassland, and woody species separately. For graphical analysis effects of O_3 exposures under normal growth conditions are compared to effects caused by the O_3 exposure in combination with one of these modifying factors. At this, we will focus on the parameters like yield, growth, photosynthesis and on stomatal conductance due to its importance for plant's water balance and stomatal uptake of O_3.

The modifying factor nitrogen (N)

As a fertiliser and because of its capability to reduce stomatal conductance additional N supply was thought to counteract adverse O_3 effects.

- Experimental data on exposures to elevated O_3 and N, singly and in combination, are available for six crop species, 26 grassland or semi-natural herbaceous species, and 25 tree species (incl. poplar hybrids).
- For crops different response patterns are described: adverse effects caused by O_3 can either be amplified or ameliorated by N supply, or both factors can cause negative effects which are additive in their combination. In summary, the interactive effect of O_3 and N is likely to depend on exposure conditions, the plant cultivar and its sensitivity to O_3.
- N supply is indicated to partially counterbalance the adverse growth effects of O_3 in grassland species. Depending on the level of elevated O_3 and N this may result in a more or less complete suppression of growth effects caused by O_3 or a predominance of the fertilising effect of N. For clover, there are also some indications of an exacerbation of O_3 effects.
- Mitigation of O_3 effects by addition of N is also shown for several tree species (*Betula pendula*, *Fraxinus ornus*, *Populus* spec., *Quercus ilex*, *Larix kaempheri*, *Picea abies*, *Pinus densiflora*, and *Pinus taeda*) and refers to impacts on growth, senescence, and gas exchange.
- The responsiveness of stomatal conductance and net photosynthesis to O_3 with or without N addition varies between single studies and species. Both mitigation and exacerbation of detrimental O_3 effects mediated by additional N supply have been observed.
- At the community level, the negative impact of atmospheric N deposition manifests in increase in soil N, which is promoted by the accelerated production and abscission of N-rich foliage by elevated levels of O_3.

The modifying factor carbon dioxide (CO_2)

Future levels of CO_2 (550 to 800 ppm) are estimated to cause growth and yield stimulations by 30% and even more in ambient or sub-ambient levels of O_3. This effect goes along with an increase in net photosynthesis and thus O_3 and CO_2 are known to affect plant growth and physiology in opposite ways. In addition, CO_2 is evidenced to have a direct effect on stomatal conductance and in this way on the O_3 flux into the leaves. This suggests that enhancement of CO_2 levels may counteract adverse effect of O_3.

- Data on the single and interactive effects of elevated O_3 and CO_2 are available for 12 crop species, 10 grassland species, 18 broad-leaved species and 4 coniferous species. The most frequently examined species are soybean, wheat, potato, rice, and poplar.
- In almost all experiments with crops (94% of all records) CO_2 is proven to counteract the detrimental effects of O_3 on yield, above-ground biomass and even crop quality. Thus, simultaneous exposure to O_3 and elevated CO_2 (≤ 600 ppm and > 600 ppm) provide complete protection from O_3-induced yield reduction in 62 and 70% of all records, respectively. Averaged over crop species, a mean yield reduction of 23% compared to the reference treatments (control) is changed into an increase in yield by 6.5% if CO_2 concentration is elevated at the same time.
- With the exception of two Japanese FACE studies, all other O_3 exposures of trees to O_3 resulted in reductions of total biomass which amounted to 28.7% on average while the effect of both gases combined was a stimulation of growth by 10%. Mitigation effects on O_3 impacts on growth are shown for seven species (*Betula pubescens*, *Fagus sylvatica, F. crenata, Fraxinus excelsior, Quercus petraea, Q. rubra,* and *Q. mongolica* var. *crispula*).
- On native herbaceous plants the occurrence of visible leaf injury was frequently reduced by CO_2 and this is evidenced for *Phleum pratense, Centaurea jacea* and species of the *Trifolium* genus. CO_2 only slightly ameliorates the negative effects of O_3 on flowering or biomass production of grassland species.
- In terms of the photosynthetic rate, the predominant response to elevated O_3 is a reduction, irrespective of the exposure conditions. When CO_2 concentrations are elevated simultaneously, there is a shift towards the positive range. One exception is given by a poplar clone tolerant to O_3 for which an exacerbation of negative O_3 effects on photosynthesis was detected.
- Within the experimental data that we compiled for crops, the change in stomatal conductance caused by elevated CO_2 ranges from +10% to –59%. A decrease in stomatal conductance induced by O_3 appears to be strengthened by elevated concentrations of CO_2, thus in most records stomatal conductance is reduced more by the combined treatment. Overall, for trees the number of studies investigating the interactive effects of O_3 and CO_2 on stomatal conductance is quite low, hence the type and magnitude of the modifying influence of increasing CO_2 concentrations on the O_3 flux into leaves of woody plants seem to be uncertain.

The modifying factor soil water moisture

Soil water-deficit reduces the photosynthetic activity, plant productivity, and crop yield and is limiting the O_3 flux into plants by regulating stomatal aperture.

- There are only 19 studies published since 1990 and some information is provided by publications from the late 1980s. The most frequently studied species are soybean, cotton, and wheat. Overall, the effects of the treatment with O_3 plus drought are in the same range as those with O_3 alone.
- The drought treatment is shown to significantly reduce the expression of O_3-specific symptoms on several grassland species.
- Data are available for 30 woody species and the graphical analysis does not display a clear trend of a modifying effect in the O_3 response by drought stress for any of the species.
- The predominant response to O_3 and drought is an additive effect on growth and gas exchange parameters whereby severe drought impacts can dominate or mask the O_3 effect in the combined treatment. Although there is some evidence for mitigation effects, drought appeared to be the most limiting factor with no statistically significant O_3 x drought interaction being detected.
- A comparison of the O_3 effect on stomatal conductance measured in drought-stressed trees with that measured in well-watered plants showed that there is a clear tendency towards more negative values, which indicates a reduced stomatal O_3 uptake in drought-stressed plants.

The modifying factor temperature

Two-factorial studies in which O_3 concentration and air temperature are manipulated separately are rare, but temperature has been used frequently as a co-factor that was changed along with other climate factors.

- There are some indications that elevated temperature (+5°C) may enhance O_3 damage on crops and increasing the O_3 concentration may exacerbate temperature effects, respectively. *Raphanus sativus* and *Glycine max* have been shown to respond to O_3 with growth stimulations under warming climate conditions.
- By means of free-air exposure studies, it was shown that even a moderate increase in temperature and O_3 may be effective to cause notable changes in growth, gas exchange, and leaf anatomy. A counteractive effect of increased temperature on the impacts caused by O_3 has been evidenced for all species tested: *Populus tremula, Betula pendula, Pinus sylvestris,* and *Picea abies*.

Conclusion

The present study shows that along with O_3 a simultaneous exposure to environmental factors, which in general have a positive effect on plant growth and photosynthesis (N, CO_2 or temperature), has the ability to reduce adverse O_3 effects or even cause a net positive effect. The extent of this net effect seems to depend on the concentration of O_3, the sensitivity of the plant species, the concentration of CO_2 or the amount of N, respectively, and the response parameter considered. If, in addition to elevated O_3, plants are simultaneously exposed to drought stress, they are impaired more severely.

This conclusion changes when interactive effects are considered in terms of increasing O_3 levels in an environment where these second factors have already changed. Under conditions of elevated CO_2, the impact of O_3 is often reduced relative to the O_3 effect in ambient CO_2, while under conditions of increased N availability or soil moisture deficit no clear trend for a modification of the O_3 effect can be detected.

Zusammenfassung

Troposphärisches Ozon (O_3) gilt als der bedeutendste phytotoxische Luftschadstoff und Trendprognosen sagen einen weiteren Anstieg der O_3-Hintergrundkonzentrationen voraus. Sowohl für die aktuell vorherrschenden als auch für die zu erwartenden Konzentrationen wird der durch O_3 verursachte Verlust von Ernteertrag und Holzproduktion auf 25 % und darüber hinaus geschätzt. Zum Schutz der Vegetation vor dem schädigenden Einfluss von O_3 wurden *critical levels* für verschiedene Vegetationstypen erarbeitet (Convention on Long-Range Transboundary Air Pollution, CLRTAP). Als Basis dafür diente eine Vielzahl von Experimenten, in denen Pflanzen unterschiedlichen O_3-Konzentrationen ausgesetzt waren, wobei sonst zumeist optimale Wachstumsbedingungen vorherrschten und jegliche Art von Einflüssen durch Umwelt- und anthropogenen Faktoren vernachlässigt wurden, die eine modifizierenden Einfluss auf die O_3-Wirkung haben können. In der vorliegenden Studie wird der derzeitige Wissensstand darüber zusammengefasst, wie die Reaktion der Pflanzen auf O_3 durch erhöhte Stickstoffdeposition und Faktoren der Klimaänderung, wie erhöhte CO_2-Konzentration und Temperatur oder Trockenstress, verändert wird. Unter Einbeziehung von 315 Publikationen werden dazu wesentliche Auswirkungen dieser Wechselwirkungen in tabellarischer Form zusammengestellt und - wenn möglich - prozentuale Abweichungen von der jeweiligen Kontrollbehandlung berechnet. Dies geschieht getrennt nach Kulturpflanzen, Grünlandarten und Gehölzpflanzen und berücksichtigt insgesamt 65 verschiedene Pflanzenarten. In einer graphischen Auswertung werden die Auswirkungen einer O_3-Behandlung unter normalen Wachstumsbedingungen mit jenen verglichen, die unter gleichzeitigem Einfluss eines der genannten Faktoren auftreten. Hierbei liegt der Schwerpunkt auf Parametern wie Ertrag, Wachstum, Photosynthese und, wegen der Bedeutung für Wasserhaushalt und stomatäre O_3-Aufnahme, auf der stomatären Leitfähigkeit.

Der Faktor Stickstoff (N)

Wegen seiner generellen Düngewirkung und aufgrund seiner Fähigkeit, die stomatäre Leitfähigkeit zu senken, geht man davon aus, dass ein zusätzliches Angebot an N Beeinträchtigungen der Pflanzen durch O_3 entgegenwirken kann.

- Es liegen Daten aus Experimenten mit Expositionen gegenüber erhöhten N-Gaben und erhöhten O_3-Konzentrationen - jeweils einzeln und in Kombination - für sechs Kulturpflanzenarten, 26 Grünland- oder krautigen Arten der natürlichen Vegetation und 25 Baumarten vor (inkl. Pappelhybride).
- Bei den Kulturpflanzen lassen sich unterschiedliche Reaktionsmuster erkennen: Entweder werden schädigende O_3-Wirkungen durch das zusätzliche N-Angebot verstärkt oder vermindert oder aber beide Faktoren haben einen schädigen Einfluss, der sich in der Kombination addiert. Insgesamt scheint die Art der Wechselwirkung zwischen O_3 und N von den Expositionsbedingungen, der Pflanzensorte und deren Empfindlichkeit gegenüber O_3 abhängig zu sein.
- Untersuchungen mit Grünlandarten deuten darauf hin, dass eine N-Gabe den nachteiligen O_3-Auswirkungen auf das Wachstum teilweise entgegenwirkt, was je nach Höhe der N-Gabe und der O_3-Belastung einen mehr oder weniger kompletten Schutz vor O_3-bedingten Wachstumsminderungen oder aber ein Überwiegen der Düngewirkung von N zur Folge haben kann.

- Abschwächungen der O_3-Wirkungen durch N-Gaben wurden auch für mehrere Baumarten nachgewiesen (*Betula pendula*, *Fraxinus ornus*, *Populus* spec., *Quercus ilex*, *Larix kaempheri*, *Picea abies*, *Pinus densiflora* und *Pinus taeda*), was für Einflüsse auf Wachstum, Seneszenz und Gaswechsel zutrifft.
- Dir Reaktionsfähigkeit der stomatären Leitfähigkeit und der Netto-Photosyntheserate auf O_3 mit und ohne zusätzliche N-Versorgung variiert deutlich zwischen einzelnen Studien und Arten. Es sind sowohl abschwächende wie auch verstärkende Effekte von N auf die O_3-Wirkung beobachtet worden.
- Auf Eben der Pflanzengemeinschaften manifestieren sich die negativen Auswirkungen der atmosphärischen N-Deposition in einem Anstieg der N-Menge im Boden, die durch die beschleunigte Produktion und den Abwurf von N-reichem Laub bei erhöhter O_3-Belastung gefördert wird.

Der Faktor Kohlendioxid (CO_2)

Es wird geschätzt, dass CO_2 in künftig zu erwartenden Konzentrationen (550 bis 800 ppm) Wachstums- und Ertragsstimulierungen von 30 % und sogar noch mehr verursacht, wobei dieser Effekt mit einer Zunahme der Netto-Photosyntheserate einhergeht. Damit stellen O_3 und CO_2 Faktoren dar, die das Wachstum und die Physiologie der Pflanzen auf entgegengesetzte Weise beeinflussen können. Darüber hinaus hat CO_2 nachweislich einen direkten Einfluss auf die stomatäre Leitfähigkeit und somit auf die O_3-Aufnahme in die Blätter. Diese beiden Tatsachen deuten darauf hin, dass eine Erhöhung der CO_2-Konzentration einer schädigenden Wirkung von O_3 entgegenwirken kann.

- Für 12 Kulturpflanzen-, 10 Grünland-, 18 Laubbaum- und 4 Nadelbaumarten liegen Daten zur Einzel- und Kombinationswirkung von O_3 und CO_2 vor. Die am häufigsten untersuchten Arten sind Sojabohne, Weizen, Kartoffel, Reis und Pappel.
- In fast allen Versuchen mit Nutzpflanzen (94% aller Einträge) wird nachgewiesen, dass CO_2 den schädlichen Auswirkungen von O_3 auf den Ertrag, die oberirdische Biomasse und sogar die Qualität der Pflanzen entgegenwirkt. So bietet die gleichzeitige Exposition gegenüber O_3 und CO_2 (≤ 600 ppm und > 600 ppm) in 62 bzw. 70 % aller Funde einen vollständigen Schutz vor O_3-induzierter Ertragsreduzierung. Gemittelt über alle Daten zu Kulturpflanzen wird durch die gleichzeitige Erhöhung der CO_2-Konzentration eine O_3-bedingte Ertragssenkung von 23 % in eine Ertragssteigerung von 6,5 % umgewandelt.
- Mit Ausnahme zweier japanischer FACE-Studien bewirken alle anderen O_3-Behandlungen bei Bäumen eine Wachstumsminderung, die im Mittel 28,7 % beträgt, wohingegen eine Behandlung mit beiden Gasen in erhöhten Konzentrationen eine Wachstumsstimulierung um 10 % bewirkt. Eine abschwächende Wirkung wurde für sieben Arten festgestellt (*Betula pubescens, Fagus sylvatica, F. crenata Fraxinus excelsior, Quercus petraea, Q. rubra* und *Q. mongolica* var. *crispula*).
- Bei Vertretern der natürlichen Vegetation wird häufig eine Verminderung O_3-bedingter Blattschäden beschrieben, wie z.B. für *Phleum pratense, Centaurea jacea* und Arten der

Gattung *Trifolium*. Allerdings verbessert CO_2 nur geringfügig die O_3-Auswirkungen auf Blühverhalten und Biomasseproduktion von Grünlandarten.

- Hinsichtlich der Photosyntheserate kann ungeachtet der Expositionsbedingungen davon ausgegangen werden, dass die vorherrschende Reaktion auf O_3 in einer Reduzierung besteht. Wird aber gleichzeitig mit O_3 auch die CO_2-Konzentration erhöht, resultiert eine eher positive Änderung gegenüber der Kontrolle. Eine Ausnahme bildet dabei ein O_3-toleranter Pappel-Genotyp, bei dem eine Verstärkung der negativen Auswirkungen von O_3 auf die Photosynthese festgestellt wurde.
- Bei den für Kulturpflanzen zusammengestellten Wirkungen beläuft sich die durch CO_2 verursachte Veränderung der stomatären Leitfähigkeit auf +10 % bis -59 %. Eine O_3-induzierte Abnahme scheint durch erhöhte CO_2-Konzentrationen verstärkt zu werden, wodurch der Parameter stomatäre Leitfähigkeit am stärksten durch beide Gase in Kombination gesenkt wird. Insgesamt gibt es nur wenige Studien, die die Wechselwirkung zwischen O_3 und CO_2 auf die stomatäre Leitfähigkeit von Bäumen untersuchen, so dass Art und Ausmaß des modifizierenden Einflusses von CO_2 auf den O_3-Fluss in die Blätter eher ungewiss sind.

Der Faktor Bodenfeuchte

Wasserdefizit im Boden reduziert allgemein die Produktivität und den Ertrag von Pflanzen, was sich auch in der Absenkung der Photosyntheserate niederschlägt. Durch Wassermangel wird über die Stomataregulierung der O_3-Fluss in die Pflanzen limitiert.

- Lediglich 19 Publikationen wurden gefunden, die nach 1990 veröffentlicht wurden, dafür gibt es mehr Informationen aus Arbeiten aus den späten 1980er Jahren. Die am häufigsten untersuchten Kulturpflanzenarten sind Sojabohne, Baumwolle und Weizen. Insgesamt hat sich gezeigt, dass sich die kombinierte Wirkung von O_3 und Trockenstress von den O_3-Effekten allein kaum unterscheidet.
- Für Grünlandarten wurde vereinzelt eine signifikante Reduzierung der sichtbaren O_3-bedingten Blattschädigung durch Trockenstress nachgewiesen.
- Für 30 Baumarten liegen Daten vor, für die die graphische Analyse keinen klaren Trend eines modifizierenden Einflusses der O_3-Auswirkungen durch Trockenstress erkennen lässt.
- Die Wirkung von O_3 und Trockenstress auf Wachstum und Gaswechsel ist überwiegend additiv wobei starke Auswirkungen des Trockenstress die O_3-Auswirkungen in der kombinierten Behandlung dominieren oder diese überdecken können. Obwohl es auch Hinweise dafür gibt, dass durch Trockenheit O_3-Effekte abgemildert werden, scheint in den hier betrachteten Experimenten der Trockenstress der limitierende Faktor zu sein, so dass statistisch signifikante Wechselwirkungen nicht nachgewiesen werden konnten.
- Wenn man jedoch den O_3-Effekt auf die stomatäre Leitfähigkeit gemessen in trocken-gestressten Bäumen mit dem in gut bewässerten Pflanzen vergleicht, gibt es eine klare Tendenz zu negativeren Werten, was auf eine verminderte O_3-Aufnahme unter Trockenstress hinweist.

Der Faktor Temperatur

Es gibt kaum zwei-faktorielle Untersuchungen, in denen O_3-Konzentration und Lufttemperatur unabhängig voneinander variiert wurden. Allerdings wurde der Faktor Temperatur manchmal als Co-Faktor eingesetzt und gemeinsam mit anderen Klimafaktoren experimentell verändert.

- Es gibt einig Hinweise darauf, dass Temperaturerhöhungen um 5°C den schädigenden Einfluss von O_3 auf den Ertrag von Kulturpflanzen noch verstärken können bzw. steigende O_3-Konzentrationen Temperatureffekte intensivieren können. Für *Raphanus sativus* und *Glycine max* wird gezeigt, dass O_3 eine wachstumsstimulierende Wirkung ausüben kann, wenn die Exposition gegenüber O_3 unter erhöhten Temperaturen stattfindet.
- In mehreren Studien mit einer kammerlosen O_3-Exposition wurde deutlich, dass selbst geringe Änderungen in O_3-Konzentration und Temperatur deutliche Änderungen in Wachstum, Gaswechsel und Blattanatomie bewirken können. Es wurde außerdem gezeigt, dass bei allen untersuchten Arten (*Populus tremula, Betula pendula, Pinus sylvestris* und *Picea abies*) bereits eine Temperaturerhöhung um 1 bis 1,5°C den O_3-Wirkungen durch mäßig erhöhte Konzentrationen entgegenwirken kann.

Schlussfolgerung

Die vorliegende Studie zeigt, dass eine simultane Exposition von O_3 mit jenen Umweltfaktoren, die generell einen positiven Einfluss auf Wachstum und Photosynthese haben (N, CO_2 oder Temperatur), nachteilige Einzelwirkungen von O_3 mindern oder gemeinsam einen positiven Effekt ausüben können. Die Höhe dieses Nettoeffektes hängt von dem Ausmaß des Ozonstress, der Empfindlichkeit des Untersuchungsobjektes, dem Ausmaß, in dem der Umweltfaktor erhöht wurde, und von dem untersuchten Parameter ab. Wenn Pflanzen jedoch zusätzlich zu O_3 Trockenstress ausgesetzt sind, werden sie stärker beeinträchtigt als durch O_3 allein.

Eine andere Schlussfolgerung ergibt sich, wenn man die beobachteten Wirkungen beider Faktoren nicht auf eine „stressfreie“ Umwelt bezieht (zumeist die Kontrollvarianten in den Experimenten), sondern die Wirkung erhöhter O_3-Konzentrationen unter Umweltbedingungen betrachtet, in denen der zweite Umweltfaktor bereits in verändertem Maß vorliegt: Unter Bedingungen mit erhöhter CO_2-Konzentration ist die schädigende Wirkung von O_3 verglichen mit dem O_3-Effekt bei aktueller CO_2-Konzentration geringer. Unter Bedingungen erhöhter N-Verfügbarkeit oder verstärktem Bodenwasserdefizit kann hingegen kein einheitlicher Trend einer modifizierenden Wirkung aufgezeigt werden.

1 Introduction

Tropospheric ozone (O_3) is considered as the most significant phytotoxic air pollutant. It is well established that elevated O_3 concentrations negatively impact plant growth, development and productivity, as well as species composition and biodiversity (Agathokleous et al., 2020; Ainsworth et al., 2012; Fuhrer et al., 2016). A recent review by Bergmann et al. (2017) showed that more than half of the crop, wild plant and woody plant species examined must be classified as O_3-sensitive in terms of visible injury, or growth and biomass reduction. In the context of the Convention on Long-Range Transboundary Air Pollution (CLRTAP) critical levels for O_3 to protect vegetation have been derived for different types of vegetation and are continuously developed on the basis of present scientific knowledge (CLRTAP 2017). The aim is to estimate the O_3 risk for vegetation in the current and future pollution situation in Europe as a basis for mitigation measures in the European air pollution control policy. The derivation of O_3 critical levels is based on many years of research on transport processes of O_3 into the plants and their physiological responses, as well as on experiments in which plants were exposed to different levels of O_3, mostly under otherwise optimal growth conditions. However, it is well known that the effects of O_3 in the field can be significantly modified by a number of other environmental and anthropogenic factors (Weigel et al., 2015). In particular, any assessment of the impacts of O_3 exposure in a future climate needs to consider possible interactions with nitrogen deposition and factors of climate change (e.g. drought, temperature, increased CO_2 concentration). These factors may either exacerbate or negate the effects of O_3, but our understanding how O_3 interacts with other stressors is still limited and the available information was not systematically reviewed so far. The present study summarises the current knowledge of how climate change factors, including temperature and drought stress, N input, and elevated CO_2 influence or alter growth, yield and gas exchange responses to O_3. The study aims to improve our understanding of the role of these interactions in modifying O_3-induced plant responses. This can help improve existing risk assessment methods by taking into account the effects of multiple stresses and climate change.

1.1 Impact of O_3 on plants and plant communities

1.1.1 A brief overview on the mode of action of O_3

Deposition of O_3 to vegetation can take place in two ways: as non-stomatal and stomatal deposition. The former includes deposition to stems and cuticles as well as to soil and other external surfaces and is assumed to play a minor role compared to the uptake through the stomata (Kerstiens and Lendzian, 1989). Stomatal aperture or stomatal conductance (g_s), respectively, is considered as a key factor determining the O_3 uptake into leaves which is the initial process for subsequent plant injury. Thus, any environmental change that is able to modify the O_3 flux directly interferes with the stress potential posed by elevated O_3 concentrations. Besides endogenous signals, environmental factors such as CO_2 concentration, light, and water status also control the stomatal aperture and thus the dry deposition of O_3 to vegetation.

After entering the plant interior through the stomata, O_3 reacts with the apoplastic fluid to generate reactive oxygen species (ROS). Their subsequent effects on the plant include effects on structure and function of the cell membrane and changes of cell metabolism, which finally result in the induction of tissue damage observable as chlorotic or necrotic spots or lesions on the upper leaf surface. As visible leaf injuries have been observed on a wide range of plant species including trees, crops and species of semi-natural vegetation (reviewed e.g. by Bergmann et al., 2017 or Agathokleous et al., 2015) they are used as a common tool for monitoring O_3 impacts (Flagler, 1998; Innes et al., 2001; Mills and Harmens, 2011).

At chronic exposure to O_3, a reduction in overall plant vigour, photosynthesis, productivity or plant development occurs (Ashmore, 2005; Cho et al., 2011; Dizengremel et al., 2013; Matyssek et al., 2010). In the past decades effects of elevated O_3 have been thoroughly investigated within crops (reviewed by e.g. Booker et al., 2009; Fiscus et al., 2005; Mills and Harmens, 2011), trees (reviewed e.g. by Gomez-Garay et al., 2013; Matyssek et al., 2013; Percy et al., 2003), and natural or semi-natural vegetation (reviewed by e.g. Ashmore, 2005; Davison and Barnes, 1998; Fuhrer, 1997).

In addition to quantifying the O_3 impacts on plant's productivity, researchers were also interested in the differential sensitivity among plant species, vegetation types, plant families or genotypes and their causes. In our previous literature study, we analysed studies reporting on 53 crop species, 164 woody species, and 298 herbaceous and pasture plant species and came to the conclusion that forbs and deciduous trees tend to be more sensitive than grasses and evergreen trees (Bergmann et al., 2015). A further evaluation of this data set showed that species, which belong to the family of Myrtaceae, Salicaceae and Betulaceae seemed to be most sensitive whereas those of the Boraginaceae and Brassicacea family responded less sensitive to O_3 (Bergmann et al., 2017). Recently, Agathokleous et al. (2020) compiled available rankings of O_3 sensitivity. They concluded that shoot growth of annuals seems to be more affected than those of perennial plants.

1.1.2 Methods to study effects of O_3 and climate factors on plants

Experimental techniques to control and to modify exposure of plants to O_3 range from controlled environmental chambers (phytotrons), greenhouse chambers, field chambers up to open-air O_3 exposure systems. Controlled environmental chambers and exposure chambers within a greenhouse have widely been used for assessing physiological and biochemical effects of O_3. To overcome limitations posed by artificial climate conditions, field-based open-top chambers (OTC) have been developed (Heagle et al., 1973) representing one of the most widely used exposure system up to now all over the world (e.g. Calvete-Sogo et al., 2016; Guo et al., 2016; Rämö et al., 2006; Wang et al., 2020). In UK, however, field exposure chambers constructed as hemi-spherical glasshouses (solardomes) have been used frequently (Mills et al., 2009). These two facilities allowed to either exclude pollutants (CF = charcoal-filtered air), use ambient concentrations (NF = non-filtered air), or add gaseous pollutants to CF or NF air. It is important to note that chamber effects have been observed on plants grown in OTCs related to morphological traits of test plants and their responsiveness to O_3 (Grünhage and Jäger, 2003; Sallas et al., 2001; Utriainen et al., 2000) which generally restricts an extrapolation of the observed responses in chambers to field conditions.

In the late 1990s, the requirement to grow plants in a more realistic ambient environment and to investigate established ecosystems led to the design of free-air exposure systems (Percy et al., 2010) such as a modification of the circular free air carbon dioxide enrichment system (FACE, Miglietta et al., 2001). Since then, this facility has been used in experimental investigations of crops like soybean (Morgan et al., 2004), forest tree ecosystems (Karnosky et al., 2005), or grassland (Volk et al., 2003). In Germany, a unique free air O_3 fumigation system has been built within a mature stand of beech and spruce (Karnosky et al., 2007; Pretzsch and Dieler, 2011; Werner and Fabian, 2002).

Regarding interactive effects of other climate factors, all these field exposure facilities offer the opportunity to elevate CO_2 concentrations at the same time, but field exposure facilities are generally not suitable for examining effects of increasing temperatures. However, there was an open-air exposure field system at Kuopio, Finland, equipped with infrared heaters to increase the air temperature over a full season by 0.8 to 1.3°C (Riikonen et al., 2009).

Finally, there is one more method to assess effects of ambient O_3 levels on plants, which is represented by the application of chemical protectants to avoid O_3 impacts. The most commonly used chemical is EDU (Manning et al., 2011) and its usage recently has been reviewed by Feng et al. (2010).

In conclusion, the experimental designs mentioned here provide a number of methods which are used in the past depending on the respective question to be clarified. However, a compromise must always be made between minimal changes in environmental conditions and maximum reproducibility and controllability. For example, in order to study O_3 effects combined with increasing temperatures, the most commonly used exposure chambers were phytotrons. Overall, any experimental finding must be seen in the context of the experimental design used.

1.1.3 Quantitative analyses of the impact of O_3 on vegetation

Today, a number of quantitative meta-analyses assessing the effect on O_3 on various types of vegetation is available including crops (Feng and Kobayashi, 2009; Feng et al., 2008; Feng et al., 2009; Morgan et al., 2003; Peng et al., 2018; Wang et al., 2012), trees (Feng et al., 2019; Wittig et al., 2007; Wittig et al., 2009), and natural vegetation (Hayes et al., 2007; Laurence and Andersen, 2003). In addition to diverse growth and yield studies, allocation and reproductive traits play an important role in describing the response of ecosystems to O_3. Across all vegetation types and irrespective of their agricultural usage, Leisner and Ainsworth (2012) analysed the effect of O_3 on various sexual reproduction processes. They report on an average decrease in most reproductive parameters ranging from 13% at 40 – 70 ppb O_3 to 29% at 70 – 100 ppb O_3 and 52% at O_3 > 100 ppb. Fruit weight or seed yield have been proving to be most sensitive parameters in indeterminate plants, whereas flower number was most affected in determinate plants. Biomass allocation has been analysed by two meta-analyses: Wang and Taub (2010) estimated a reduction in allocation to roots by 6.7% in woody plants (average on approx. 150 species) and by 6.7% in herbaceous plants (average on more than 200 species). Grantz et al. (2006) found a reduction in the root/shoot allometric coefficient of 60 predominantly grassland species by 5.6% on average indicating a disturbed allocation of assimilates to the roots due to O_3.

1.1.3.1 Agricultural and horticultural crop plants

As a result of decades of air pollution research, an enormous number of studies on the effect of O_3 on plants are available. In our literature review we collected data on 54 crop species represented by 350 genotypes, for which data on chronical exposures under non-artificial environmental conditions are described (Bergmann et al., 2017). In summary, about 90% of species and 83% of all cultivars experienced a significant growth effect. Mills et al. (2007) analysed over 700 published studies on full season exposures of 19 field grown European agricultural and horticultural crops and derived yield-response functions related to accumulated AOT40 doses for O_3 (Accumulated Ozone Exposure over a threshold of 40 ppb). Based on this analysis, critical levels for a 5% yield reduction (AOT40, 3 months) for each species were established which range from 83.3 ppm h for resistant crop species (barley) to 8.6 - 20.0 ppm h for moderately sensitive species (sugar beet, oilseed rape, potato, tobacco, rice, maize, grape and broccoli) and 3.0 - 6.0 ppm h for sensitive species (pulses, cotton, wheat, soybean, lettuce, and tomato).

Table 1 Mean relative O_3 effects on crops as shown by recent quantitative meta-analysis

O_3 exposure conditions and mean effects of O_3 (% change from control) at current or future (= elevated) levels of O_3 on growth and yield; ABM = above-ground biomass, n. spec. = not specified, ref. = references

species	no. of studies	duration	control	elevated O_3	yield [%]	ABM [%]	ref.
current levels							
15 crops[1]	50[2]	season	EDU	30-85 ppb[3]	-15.0		[1]
barley	81[2]	>10 days	<26 ppb	41-49 ppb[3]	-8.9		[2]
bean	81[2]	>10 days	<26 ppb	41-49 ppb[3]	-19.0		[2]
bean	50[2]	season	EDU	30-85 ppb[3]	-9.2		[1]
potato	81[2]	>10 days	<26 ppb	41-49 ppb[3]	-5.3		[2]
potato	50[2]	season	EDU	30-85 ppb[3]	-13.5		[1]
rice	81[2]	>10 days	<26 ppb	41-49 ppb[3]	-17.5		[2]
soybean	81[2]	>10 days	<26 ppb	41-49 ppb[3]	-7.7		[2]
soybean	50[2]	season	EDU	30-85 ppb[3]	-19.4		[1]
soybean	53	≥7 days	≤25 ppb	<60 ppb[3]	-10.0	-10.0	[3]
wheat	81[2]	>10 days	<26 ppb	41-49 ppb[3]	-9.7		[2]
wheat	53	≥10 days	CF	42-46 ppb[4]	-17.2	-15.8	[4]
wheat	53	≥10 days	CF	67-69 ppb[4]	-24.9	-20.6	[4]
wheat	50[2]	season	EDU	30-85 ppb[3]	-19.5		[1]
wheat	39	>10 days	AA	51 ppb[3]	-17.0		[5]
future levels							
bean	81[2]	>10 days	<26 ppb	63 ppb[3]	-41.4		[2]
rice	12	n. spec.	CF	62 ppb[3]	-14.0	-16.0	[6]
soybean	81[2]	>10 days	<26 ppb	63 ppb[3]	-21.6		[2]
soybean	53	≥7 days	≤25 ppb	70 ppb[3]	-24.0	-38.0	[3]
wheat	81[2]	>10 days	<26 ppb	63 ppb[3]	-21.1		[2]
wheat	53	≥10 days	CF	97 ppb[4]	-49.5		[4]
wheat	39	>10 days	AA	75 ppb[3]	-24.0		[5]
wheat	39	>10 days	AA	96 ppb[3]	-40.0		[5]
wheat	39	>10 days	AA	161 ppb[3]	-64.0		[5]

[1] *Arachis hypogaea, Beta vulgaris, Brassica rapa, Echinacea purpurea, Glycine max, Lycopersicon esculentum, Nicotiana tabacum, Oryza sativa, Phaseolus vulgaris, Raphanus sativus, Rudbeckia laciniata, Solanum tuberosum, Triticum aestivum, Vicia faba,* and *Vigna radiata*

[2] no. of references used in the whole study

[1] Feng et al. (2010), [2] Feng and Kobayashi (2009), [3] Morgan et al. (2003), [4] Feng et al. (2008), [5] Feng et al. (2009), [6] Ainsworth (2008)

Meta-analyses have been proven to be a suitable approach to quantify the extent of yield reduction caused by either current or elevated O_3 levels. In Table 1 mean percentage growth and yield changes calculated for major food crops and trees are summarised which result from recent meta-analyses each based on a number of 12 up to 263 published studies with single species up to 15 species being involved. Hence, an exposure to current O_3 concentrations (i.e. average concentrations below 50 ppb) is shown to reduce yield of barley by approx. 9%, potato by 5 to 13.5%, bean by 9 to 19%, soybean by 7 to 19.5%, wheat by 9 to 19.5%, and rice by 17% (Feng and Kobayashi, 2009; Feng et al., 2008; Feng et al., 2010; Morgan et al., 2003) which corresponds to an average yield reduction of 15% on the basis of 15 food crops (Feng et al., 2010). Based on data of the O_3 pollution situation in China, Feng et al. (2020) estimated mean relative yield losses for maize and reported on continuously rising losses from 8.2% in 2014 up to 13.4% in 2017. Assuming that O_3 continues to rise in the future, a number of studies has been performed with average O_3 concentrations up to 70 ppb. Meta-analyses of these experiments calculated yield reductions up to 14% for rice, 24% for soybean, 29% for wheat and 21% for beans under elevated O_3 (Ainsworth, 2008; Feng and Kobayashi, 2009; Feng et al., 2008; Morgan et al., 2003). Assuming a 25% increase in O_3 concentration Scheelbeek et al. (2018) estimated a mean yield reduction for all vegetables and legumes combined of 8.9%.

Recently, Hayes et al. (2019) studied the sensitivity of African staple food crops to episodic O_3 regime because these crops usually have been under-represented in risk analyses so far. In response to elevated levels of O_3 (45.8 ppb, weekly mean) cultivars of wheat and bean experienced severe yield reductions up to 50 and 75%, respectively, while there was no effect detected on cultivars of pearl millet (*Pennisetum glaucum*) and an increase by up to 32% on cultivars of finger millet (*Eleusine coracana*) was found.

Avnery et al. (2011a) calculated global yield reductions due to O_3 exposure in the base year 2000 for three key crops. Yield losses are estimated to range from 3.9 to 15% for wheat, 8.5 to 14% for soybean, and 2.2 to 5.5% for maize depending on the metric used. Simulation of the potential global risk in the near future (year 2030, A2 scenario, IPCC) yield losses may rise to 5.4 to 26% for wheat, 15 to 19% for soybean, and 4.4 to 8.7% for maize (Avnery et al., 2011b). An increase in O_3 concentrations by 20% would result in yield losses relative to today's yields of 5 to 12% for these three crops (Long et al., 2005).

1.1.3.2 Woody plants

Because exposure of mature trees to O_3 is an experimental challenge primarily seedlings or young trees have been used in experimental studies so far. However, during the past twenty years there is a number of publications reporting on experiments under more realistic conditions including mature trees. In our literature review we compiled a list of 165 woody species from 142 references (Bergmann et al., 2017). Only six of these studies reported on mature trees whereas all other investigations are based on experiments with cuttings or seedlings (one to eight-year-old). About 80% of these species responded to O_3 in terms of visible injuries and even 62% showed significant growth reductions. Differences in the O_3 sensitivity between coniferous and broadleaved species are minimal with a slight tendency to more sensitive responses of deciduous versus evergreen species. For several angiosperm trees, Wittig et al. (2009) calculated a mean reduction in total biomass by 15% due to O_3 exposures of 59 ppb on average compared to ambient air and by 23% due to exposures to 75 ppb compared to CF, whereas in gymnosperm trees growth impairments did not exceed 6% even if the O_3 levels were elevated up to 92 ppb. Across all studies current O_3 exposure is reducing light-saturated photosynthesis (A_{sat}) and stomatal conductance (g_s) by14% and 16%, respectively, in angiosperm trees, whereas in gymnosperm trees significant changes in A_{sat} and g_s are more likely to occur under elevated O_3 levels (Wittig et al., 2007).

Averaged over 23 studies using a mean exposure to O_3 of 15 and 17 ppm h per season (AOT40), respectively, Oksanen et al. (2009) estimated a loss in stem biomass by 10% for birch and 14% for aspen, while root biomass was reduced by 19% and 24%, respectively. These results are similar to the calculations of Wittig et al. (2009) who found a 7% reduction in total biomass for birch by moderate and 20% by elevated O_3 exposures. For aspen the corresponding data are 4% and 22 to 27% (Feng et al., 2019; Wittig et al., 2009, see Table 2). Total biomass of *Prunus* species was reduced by 23 to 27% due to O_3 at approx. 60 ppb irrespective of the reference used, while *Liriodendron* showed an indifferent response with an average growth induction by 1 to 23% (Wittig et al., 2009). However, the authors concluded that root-to-shoot ratio indicated a greater sensitivity to O_3 than shoot production.

Karlsson et al. (2009) reviewed two Swedish long-term OTC exposure experiments on Norway spruce (*Picea abies*) which clearly showed that under elevated O_3 photosynthetic capacity was reduced in older spruce shoots but may be increased in current-year shoots. After three years of exposure the stem volume was decreased by 8%.

Table 2 Mean relative O_3 effects on trees as shown by recent quantitative meta-analysis

O_3 exposure conditions and mean effects of O_3 (% change from control) at current or future (= elevated) levels on growth and yield, n.s. = not significant, n. spec. = not specified, ref. = references, stem diam. = stem diameter; n. spec. = not specified

genera	duration	control	elevated O_3	height [%]	stem mass [%]	stem diam. [%]	total mass [%]	leaf mass [%]	ref.
current levels									
15 trees1)	≥7 days	CF	38-44 ppb6)	n.s.		-5	-7	-5.0 n.s.	[1]
7 trees2)	1 year	EDU	30-85 ppb6)		-17	-17			[2]
Betula	≥7 days	AA	46 ppb6)				-7		[1]
Picea	≥7 days	AA	49 ppb6)				+5		[1]
Pinus	≥7 days	CF	45 ppb6)				-6		[1]
Populus	≥30 days	CF	40-50 ppb6)	-5	-21	-18	-4	-16	[3]
future levels									
15 trees1)	≥7 days	CF	81-97ppb6)	-9		-10	-17	-15	[1]
11 angiosperms3)	≥7 days	CF	74 -75 ppb6)				-23	-16	[1]
8 angiosperms4)	≥7 days	AA	59 ppb6)				-15		[1]
4 gymnosperms5)	≥7 days	CF	92 ppb6)				-6	n.s.	[1]
Pinus and Picea	≥7 days	AA	57 ppb6)				-1		[1]
Betula	n. spec.	n. spec.	15 ppm.h7)	n.s.	-10				[4]
Betula	≥7 days	CF	84 ppb6)				-20		[1]
Liriodendron	≥7 days	CF	83 ppb6)				+1		[1]
Liriodendron	≥7 days	AA	79 ppb6)				+23		[1]

genera	duration	control	elevated O_3	height [%]	stem mass [%]	stem diam. [%]	total mass [%]	leaf mass [%]	ref.
Picea	≥7 days	CF	92 ppb[6)]				-2		[1]
Pinus	≥7 days	AA	59 ppb[6)]				-4		[1]
Populus	≥30 days	CF	83-106 ppb[6)]	-17	-25	-14	-24	-24	[3]
Populus	≥7 days	CF	74 ppb[6)]				-27		[1]
Populus	≥7 days	AA	60 ppb[6)]				-22		[1]
Populus	n. spec.	n. spec.	17 ppm.h[7)]	-14	-17				[4]
Prunus	≥7 days	CF	60 ppb[6)]				-27		[1]
Prunus	≥7 days	AA	57 ppb[6)]				-24		[1]
Quercus	≥7 days	AA	58 ppb[6)]				-23		[1]

[1)] *Acer, Alnus, Betula, Fagus, Fraxinus, Liquidambar, Liriodendron, Quercus, Platanus, Populus* and *Prunus, Abies, Picea, Pinus* and *Sequoiadendron*

[2)] *Fraxinus excelsior, Hibiscus syriacus, Pinus taeda, Populus euramericana, Populus deltoides x maximowiczii, Populus nigra,* and *Prunus serotina*

[3)] *Acer, Alnus, Betula, Fagus, Fraxinus, Liquidambar, Liriodendron, Quercus, Platanus, Populus* and *Prunus*

[4)] *Acer, Betula, Fagus, Fraxinus, Liriodendron, Populus, Prunus and Quercus*

[5)] *Abies, Picea, Pinus* and *Sequoiadendron*

[6)] 24h mean

[7)] AOT40

[1] Wittig et al. (2009) [2] Feng et al. (2010), [3] Feng et al. (2019), [4] Oksanen et al. (2009)

1.1.3.3 Native herbaceous and grassland plants

In their meta-analysis Hayes et al. (2007) described the relative sensitivity to O_3 for 83 native species from existing publications and concluded that about one-third of these species experienced significant biomass reductions of about more than10%. In our literature review we compiled data about the O_3 sensitivity of 293 native herbaceous species. Approximately one half of all species tested were found to express visible injury symptoms or changes in biomass production, at least in one experiment (Bergmann et al., 2017). In terms of growth effects, the proportion of sensitive species was higher for herbs than for grasses and annuals and biennials seemed to be more sensitive than perennials.

Van Goethem et al. (2013) presented an approach to consider a cumulative stressor-response distribution for O_3 exposure on natural vegetation named 'Species Sensitivity Distributions', SSD. Their findings evidenced that up to 20% of the species are at risk of a 10% reduction in biomass due to ambient O_3 with annual grassland species being more sensitive than perennial ones. In addition, Hayes et al. (2007) described a trend that high leaf N concentrations are indicating higher sensitivity to O_3. Species with higher leaf N concentration were more sensitive to O_3 than those with lower leaf N concentration.

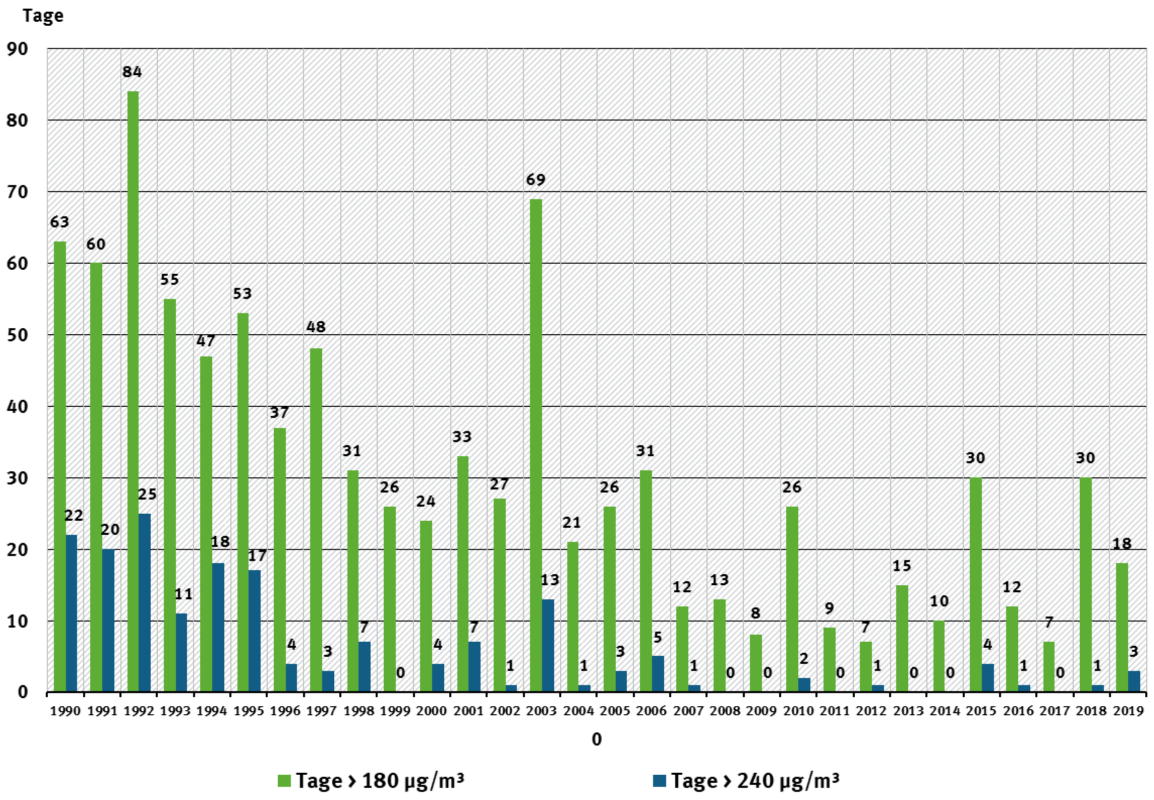
1.2 Trends in nitrogen deposition and climate change factors

1.2.1 Ozone (O_3)

Changes in atmospheric chemistry have affected the lifetimes of many greenhouse gases with tropospheric ozone (O_3) being the third most important greenhouse gas after carbon dioxide (CO_2) and methane (CH_4) (Ehhalt et al., 2001). Ozone in the troposphere is formed by sunlight driven photochemical reactions of precursors such as nitrogen oxides, volatile organic compounds (VOCs), carbon monoxide and CH_4. Since the pre-industrial era the global annual average background O_3 concentrations have doubled to values between ca. 20 – 45 ppb depending on the geographical location (Vingarzan, 2004; Wittig et al., 2007) and are predicted to increase 50 and 20% by 2050 (Ehhalt et al., 2001). During the second half of the past century the rates of increases ranged between 1 - 5 ppb per decade (Cooper et al., 2014). Because of its rapid chemical turnover its concentrations may vary from less than 10 ppb over oceans to more than 100 ppb downwind of polluted metropolitan regions. Schultz et al. (2017) presented maps of monthly mean gridded daytime O_3 averages in January and July between 2010 and 2014**.** These results figure out that for the Northern Hemisphere between 30°N and 50°N, monthly daytime average O_3 concentrations exceeded 40 ppb in large areas with values noticed in the Mediterranean region and W-USA being greater than 55 ppb. As a result of legislation and control of precursor emissions (nitrogen oxides, carbon monoxide, VOCs), O_3 concentrations decreased in recent decades in parts of Europe, which is particularly true for peak values (Ronan et al. 2020). However, future trend projections predict changes by −4 to +10 ppb by 2100 depending on the underlying scenario (Jacob and Winner, 2009; Prather et al., 2003), i.e. future O_3 concentrations will only decrease with the most optimistic low emission scenario RCP 2.6 (Eyring et al. 2013).

Figure 1 **Exceedance of hourly mean O_3 concentrations of 180 µg m^{-3} or 240 µg m^{-3} in Germany**

Number of calendar days from 1990 to 2019, data evaluation of the German Environment Agency based on data of the monitoring network of the German federal government and states.



Source: UBA (German Environment Agency)

A similar trend of a decrease in O_3 peak concentrations can also be observed in Germany over the past two decades (Figure 1). However, due to the predominant effect of regional weather conditions, level and frequency of short-term peak concentrations depend significantly on the course of the summer. For example, higher O_3 levels are episodically reached in years with long-lasting periods of sunny weather such as during the extraordinary summer in 2003, but also during the summers of 2015 and 2018 in which exceedances of 180 µg m^{-3} (equivalent to 90 ppb) were observed on 30 days (Figure 1).

In their recent 'Tropospheric Ozone Assessment Report' (TOAR) Mills et al. (2018) reported on present-day global distribution of ozone and its relevance for vegetation. They have shown that critical levels for O_3 were extensively exceeded in many regions in America, Asia and Europe.

1.2.2 Nitrogen deposition

Deposition of nitrogenous air pollutants both oxidized (NO_x) and reduced (NH_y) compounds have greatly increased during the past century. As a result, the availability of nitrogen (N) in the soil is gradually increased (Bobbink, 1998). Excessive N inputs to sensitive ecosystems result in changes to structure and function, and plants become more susceptible to other stress factors such as O_3 or drought. A comprehensive European dataset on atmospheric gases and aerosols is recently presented by Tang et al. (2020) demonstrating that "...*gas-phase NH_3 and aerosol NH_4NO_3 were the dominant species in the total inorganic gas and aerosol species … with largest concentrations measured at cropland sites in intensively managed agricultural areas…, and smallest at remote semi-natural and forest sites* …".

Critical loads for eutrophication by nitrogen (N) have been defined within the UNECE Convention on Long Range Transboundary Air Pollution. A critical load is defined as a "quantitative estimate of an exposure to one or more pollutants below which significant harmful effects on specified sensitive elements of the environment do not occur according to present knowledge" (Nilsson and Grennfelt 1988). For Germany, there is considerable regional variation in deposition with highest deposition on forest ecosystems or near the main agricultural areas. However, nitrogen deposition shows a decrease in Germany in the recent decade, which primarily can be attributed to a decrease in oxidized nitrogen deposition. Nevertheless, about 70% of the total area in the ecosystems considered still remains at risk from exceeding the critical load (Schaap et al. 2018).

1.2.3 Carbon dioxide (CO_2), temperature, and drought

Today, our atmosphere has the highest concentrations of CO_2 since the past 420,000 years and they are projected to increase by the end of the century up to 540 to 960 ppm depending on the emissions scenario considered (IPCC 2013). While the CO_2 concentration was around 280 - 290 ppm up to the end of the 19th century, global CO_2 concentration increased rapidly since then and has currently already reached 410 ppm. Anthropogenic CO_2 emissions are considered to be the dominant factor determining atmospheric CO_2 concentrations throughout the 21st century.

The global rise in atmospheric CO_2 is the main driver of climate change. Concomitant with this, global mean temperatures are projected to increase between 1 and 5° C, and heat waves and drought are likely to become more frequent in the future (IPCC 2013). In Germany, mean air temperature already increased by 1.5° from 1881 to 2019 (DWD 2020). Nine of the 10 warmest years in Germany were within the last 20 years. The increase in mean air temperature is also associated with a higher number of "hot days", i.e. days when temperatures are above 30°C. Since 1951 the number of hot days increased from around three to currently around ten days per year (DWD 2020). In recent years, higher temperatures combined with lower precipitation resulted in drier soils during the growing season in many regions of Germany compared to the long-term average, causing drought stress to plants.

1.3 Methods of this study

The aim of this literature study is to compile available data on crop, grassland, and woody plants which have been subjected to exposures of different levels of O_3 in two-factorial experiments in combination with treatments where one of the following environmental factor was manipulated: N, CO_2, temperature, or soil moisture.

Literature search has been done using Web of Science™ by Clarivate Analytics (Web of Science Core Collection, Biological Abstracts, CAB Abstracts®, KCI-Korean Journal Database, Russian Science Citation Index, SciELO Citation Index). Only those studies that met the following experimental requirements were considered for this evaluation:

- a 'control treatment' had been used for both factors with the O_3 control being either charcoal filtered air (CF) with or without low levels of O_3 or non-filtered air (NF)
- in the 'elevated treatment' O_3 and the second climate factor had been added singly and in combination to the same control treatment
- in controlled environment chambers O_3 exposure pattern is adapted to the photoperiod and O_3 concentration did not exceed 100 ppb (8h mean)
- chronic exposures lasted for more than 1 week
- publication was not older than 1990

With regard to the parameters examined, we had a clear focus on data about growth, yield, and gas exchange (photosynthesis and stomatal conductance). For this reason, a lot of papers have been excluded, especially for trees.

The main objective was to give an indication of the extent of the modifying effect on the response to O_3. For this purpose, it is necessary to compare treatment means measured for the treatments with the elevated climate factor singly and in combination with the control treatment for both low O_3 and low conditions for CO_2, N, temperature or no soil moisture deficit. In many publications, however, the effect of O_3 was often averaged over all treatments with the second factor and vice versa. Therefore, if these effects were not given directly, we have taken treatment means from figures and tables i.e. treatment means for O_3 and the second factor singly and in combination. If data were available for different developmental stages those data of the longest exposure period were taken. For photosynthesis and stomatal conductance percentage changes derived from measurements at different stages or leaf ages either have been averaged whenever possible or the stage of the highest physiological activity was chosen. We then calculated the appropriate effect that means the percentage deviation from control (see Table 3).

For each climate factor, the following data have been compiled in separate files for further evaluation:

- species, cultivar, age (trees)
- kind of exposure facility and experimental site
- 'control treatment' for O_3 and the second factor
- 'elevated treatment' for O_3 and the second factor
- index for O_3 concentration or dose

- single and combined effects of O_3 and the second climate factor on yield and/or plant biomass (total, shoot and/or root), photosynthesis (P_n, A or A_{max}), and stomatal conductance (g_s) as the percentage deviation from the control (control for both O_3 and the second factor, chapter 3 to chapter 4, see Table 3)
- O_3 effect under elevated (=future) levels of the second factors (i.e. effects of are related to a reference treatment (control) of low O_3 but high levels of nitrogen, CO_2, or soil moisture deficit, respectively, chapter 6, see Table 3).

Table 3 Calculation of effects

treatment	O_3	CO_2	N	drought	effect of O_3 at normal conditions for the 2nd factor	effect of O_3 + 2nd factor	effect of O_3 under change conditions for the 2nd factor
control	low	ambient	low	well-watered	✓	✓	
elevated CO_2/N/drought	low	elevated	high	water-stressed			✓
elevated O_3	elevated	ambient	low	well-watered	✓		
elevated O_3 +elevated CO_2/N/drought	elevated	elevated	high	water-stressed		✓	✓

Regardless of whether these effects were indicated to be statistically significant, the resulting range of percentage changes was illustrated graphically. In all figures shown below, each data point represents a survey taken from the literature and recalculated if necessary.

1.4 References

Agathokleous, E., Feng, Z., Oksanen, E., Sicard, P., Wang, Q., Saitanis, C. J., Araminiene, V., Blande, J. D., Hayes, F., Calatayud, V., et al. (2020). Ozone affects plant, insect, and soil microbial communities: A threat to terrestrial ecosystems and biodiversity. Science Advances 6, eabc1176.

Agathokleous, E., Saitanis, C. J., and Koike, T. (2015). Tropospheric O-3, the nightmare of wild plants: a review study. Journal of Agricultural Meteorology 71, 142-152.

Ainsworth, E. A. (2008). Rice production in a changing climate: a meta-analysis of responses to elevated carbon dioxide and elevated ozone concentration. Global Change Biology 14, 1642-1650.

Ainsworth, E. A., Yendrek, C. R., Sitch, S., Collins, W. J., and Emberson, L. D. (2012). The Effects of Tropospheric Ozone on Net Primary Productivity and Implications for Climate Change. Annual Review of Plant Biology 63, 637-661.

Ashmore, M. R. (2005). Assessing the future global impacts of ozone on vegetation. Plant Cell and Environment 28, 949-964.

Avnery, S., Mauzerall, D. L., Liu, J., and Horowitz, L. W. (2011a). Global crop yield reductions due to surface ozone exposure: 1. Year 2000 crop production losses and economic damage. Atmospheric Environment 45, 2284-2296.

Avnery, S., Mauzerall, D. L., Liu, J., and Horowitz, L. W. (2011b). Global crop yield reductions due to surface ozone exposure: 2. Year 2030 potential crop production losses and economic damage under two scenarios of O-3 pollution. Atmospheric Environment 45, 2297-2309.

Bergmann, E., Bender, J., and Weigel, H. J. (2015). Assessment of the impacts of ozone on biodiversity in terrestrial ecosystems: Literature review and analysis of methods and uncertainties in current risk assessment approaches. Part II: Literature review of the current state of knowledge on the impact of ozone on biodiversity in terrestrial ecosystems. Umweltbundesamt, Texte 71/2015, http://www.umweltbundesamt.de/en/publikationen/assessment-of-theimpacts-of-ozone-on-biodiversity

Bergmann, E., Bender, J., and Weigel, H. J. (2017). Impact of tropospheric ozone on terrestrial biodiversity: A literature analysis to identify ozone sensitive taxa. Journal of Applied Botany and Food Quality 90, 23.

Bobbink, R. (1998). Impacts of tropospheric ozone and airborne nitrogenous pollutants on natural and seminatural ecosystems: a commentary. New Phytologist 139, 161-168.

Booker, F., Muntifering, R., McGrath, M., Burkey, K., Decoteau, D., Fiscus, E., Manning, W., Krupa, S., Chappelka, A., and Grantz, D. (2009). The ozone component of global change: Potential effects on agricultural and horticultural plant yield, product quality and interactions with invasive species. Journal of Integrative Plant Biology 51, 337-351.

Calvete-Sogo, H., González-Fernández, I., Sanz, J., Elvira, S., Alonso, R., García-Gómez, H., Ibáñez-Ruiz, M. A., and Bermejo-Bermejo, V. (2016). Heterogeneous responses to ozone and nitrogen alter the species composition of Mediterranean annual pastures. Oecologia, 1-13.

Cho, K., Tiwari, S., Agrawal, S. B., Torres, N. L., Agrawal, M., Sarkar, A., Shibato, J., Agrawal, G. K., Kubo, A., and Rakwal, R. (2011). Tropospheric Ozone and Plants: Absorption, Responses, and Consequences. *In* "Reviews of Environmental Contamination and Toxicology Vol. 212" (D. M. Whitacre, ed.), Vol. 212, pp. 61-111.

CLRTAP (2017). Manual on methodologies and criteria for modelling and mapping critical loads and levels and air pollution effects, risks and trends. Chapter 3: Mapping critical levels for vegetation. https://icpvegetation.ceh.ac.uk

Cooper, O. R., Parrish, D. D., Ziemke, J., Balashov, N. V., Cupeiro, M., Galbally, I. E., Gilge, S., Horowitz, L., Jensen, N. R., Lamarque, J.-F., Naik, V., Oltmans, S. J., Schwab, J., Shindell, D. T., Thompson, A. M., Thouret, V., Wang, Y., and Zbinden, R. M. (2014). Global distribution and trends of tropospheric ozone: An observation-based review. Elementa-Science of the Anthropocene 2.

Davison, A. W., and Barnes, J. D. (1998). Effects of ozone on wild plants. New Phytologist 139, 135-151.

Dizengremel, P., Jolivet, I., Tuzet, A., Ranieri, A., and LeThiec, D. (2013). Integrative leaf-level phytotoxic ozone dose assessment and forest risk modelling. *In* "Climate Change, Air Pollution and Global Challenges, Vol 13. Developments in Environmental Science" (R. Matyssek, N. Clarke, P. Cudlin, T. N. Mikkelsen, J. P. Tuovinen, G. Wieser and E. Paoletti, eds.), pp. 267–282. Elsevier.

DWD (2020). https://www.dwd.de/DE/klimaumwelt/klimawandel/klimawandel_node.htm (Zugriff 17.09.2020)

Ehhalt, D., Prather, M., Dentener, F., Derwent, R., Dlugokencky, E., Holland, E., Isaksen, I., Katima, J., Kirchhoff, V., Matson, P., Midgley, P., and Wang, M. (2001). Atmospheric Chemistry and Greenhouse Gases. *In* "Climate Change 2001: Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change" (J. T. Houghton, Y. Ding, D. J. Griggs, M. Noguer, van der Linden, X. Dai, K. Maskell and C. A. Johnson, eds.), pp. 239-287. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Eyring, V., Arblaster, J.M., Cionni, I., Sedlacek,J., Perlwitz, J. et al. (2013) Long-term ozone changes and associated climate impacts in CMIP5 simulations. J Geophys Res: Atmospheres 118, 5029-5060.

Feng, Z., Hu, T., Tai, A. P. K., and Calatayud, V. (2020). Yield and economic losses in maize caused by ambient ozone in the North China Plain (2014–2017). Science of the Total Environment 722, 137958.

Feng, Z. 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, 1510-1519.

Feng, Z. 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, 2696-2708.

Feng, Z. Z., Kobayashi, K., Wang, X. K., and Feng, Z. W. (2009). A meta-analysis of responses of wheat yield formation to elevated ozone concentration. Chinese Science Bulletin 54, 249-255.

Feng, Z. Z., Shang, B., Gao, F., and Calatayud, V. (2019). Current ambient and elevated ozone effects on poplar: A global meta-analysis and response relationships. Science of the Total Environment 654, 832-840.

Feng, Z. Z., Wang, S. G., Szantoic, Z., Chen, S. A., and Wang, X. K. (2010). Protection of plants from ambient ozone by applications of ethylenediurea (EDU): A meta-analytic review. Environmental Pollution 158, 3236-3242.

Fiscus, E. L., Booker, F. L., and Burkey, K. O. (2005). Crop responses to ozone: uptake, modes of action, carbon assimilation and partitioning. Plant Cell and Environment 28, 997-1011.

Flagler, R. B., ed. (1998). "Recognition of air pollution injury to vegetation: a pictorial atlas." Air & Waste Management Association.

Fuhrer, J. (1997). Ozone sensitivity of managed pastures. *In* "Ecological Issues and Environmental Impact Assessment" (P. N. Cheremisinoff, ed.), pp. 681-706. Gulf Professional Publishing.

Fuhrer, J., Val Martin, M., Mills, G., Heald, C. L., Harmens, H., Hayes, F., Sharps, K., Bender, J., and Ashmore, M. R. (2016). Current and future ozone risks to global terrestrial biodiversity and ecosystem processes. Ecology and Evolution 6, 8785-8799.

Gomez-Garay, A., Calderon Guerrero, C., Saiz de Omenaca, J. A., and Manzanera, J. A. (2013). Ozone effects on forests. African Journal of Biotechnology 12, 320-326.

Grantz, D. A., Gunn, S., and Vu, H. B. (2006). O-3 impacts on plant development: a meta-analysis of root/shoot allocation and growth. Plant Cell and Environment 29, 1193-1209.

Grünhage, L., and Jäger, H. J. (2003). From critical levels to critical loads for ozone: a discussion of a new experimental and modelling approach for establishing flux-response relationships for agricultural crops and native plant species. Environmental Pollution 125, 99-110.

Guo, Z. W., Zhuang, M. H., Li, Y. C., Chen, S. L., and Yang, Q. P. (2016). Adaptability of Indocalamus decorus to climate change based on physiological and biochemical responses to elevated carbon dioxide and ozone. Iforest-Biogeosciences and Forestry 9, 311-317.

Hayes, F., Jones, M. L. M., Mills, G., and Ashmore, M. (2007). Meta-analysis of the relative sensitivity of semi-natural vegetation species to ozone. Environmental Pollution 146, 754-762.

Hayes, F., Sharps, K., Harmens, H., Roberts, I., and Mills, G. (2019). Tropospheric ozone pollution reduces the yield of African crops. Journal of Agronomy and Crop Science.

Heagle, A. S., Body, D. E., and Heck, W. W. (1973). An Open-Top Field Chamber to Assess the Impact of Air Pollution on Plants. Journal of Environmental Quality 2, 365-368.

Hunova, I., Kurfurst, P., and Balakova, L. (2019). Areas under high ozone and nitrogen loads are spatially disjunct in Czech forests. Science of the Total Environment 656, 567-575.

IPCC (2013). Climate Change 2013. The Physical Science Basis. www.ipcc.ch (Zugriff 17.07.2020)

Innes, J., Skelly, J., and Schaub, H. (2001). "Ozone and broadleaved species - A guide to the identification of ozone-induced foliar injury," Haupt, Birmensdorf, Eidgenössische Foschungsanstalt WSL. Bern, Stuttgart, Wien.

Jacob, D. J., and Winner, D. A. (2009). Effect of climate change on air quality. Atmospheric Environment 43, 51-63.

Karlsson, P. E., Pleijel, H., Danielsson, H., Karlsson, G. P., Piikki, K., and Uddling, J. (2009). Evidence for Impacts of Near-ambient Ozone Concentrations on Vegetation in Southern Sweden. Ambio 38, 425-431.

Karnosky, D. F., Pregitzer, K. S., Zak, D. R., Kubiske, M. E., Hendrey, G. R., Weinstein, D., Nosal, M., and Percy, K. E. (2005). Scaling ozone responses of forest trees to the ecosystem level in a changing climate. Plant Cell and Environment 28, 965-981.

Karnosky, D. F., Werner, H., Holopainen, T., Percy, K., Oksanen, T., Oksanen, E., Heerdt, C., Fabian, P., Nagy, J., Heilman, W., Cox, R., Nelson, N., and Matyssek, R. (2007). Free-air exposure systems to scale up ozone research to mature trees. Plant Biology 9, 181-190.

Kerstiens, G., and Lendzian, K. J. (1989). Interactions between ozone and plant cuticles .1. Ozone deposition and permeability. New Phytologist 112, 13-19.

Laurence, J. A., and Andersen, C. P. (2003). Ozone and natural systems: understanding exposure, response, and risk. Environment International 29, 155-160.

Leisner, C. P., and Ainsworth, E. A. (2012). Quantifying the effects of ozone on plant reproductive growth and development. Global Change Biology 18, 606-616.

Long, S. P., Ainsworth, E. A., Leakey, A. D. B., and Morgan, P. B. (2005). Global food insecurity. Treatment of major food crops with elevated carbon dioxide or ozone under large-scale fully open-air conditions suggests recent models may have overestimated future yields. Philosophical Transactions of the Royal Society B-Biological Sciences 360, 2011-2020.

Manning, W. J., Paoletti, E., Sandermann, H., and Ernst, D. (2011). Ethylenediurea (EDU): A research tool for assessment and verification of the effects of ground level ozone on plants under natural conditions. Environmental Pollution 159, 3283-3293.

Matyssek, R., Clarke, N., Cudlin, P., Mikkelsen, T. N., Tuovinen, J. P., Wieser, G., and Paoletti, E., eds. (2013). "Climate Change, Air Pollution and Global Challenges." Elsevier.

Matyssek, R., Wieser, G., Ceulemans, R., Rennenberg, H., Pretzsch, H., Haberer, K., Loew, M., Nunn, A. J., Werner, H., Wipfler, P., et al. (2010). Enhanced ozone strongly reduces carbon sink strength of adult beech (Fagus sylvatica) - Resume from the free-air fumigation study at Kranzberg Forest. Environmental Pollution 158, 2527-2532.

Miglietta, F., Peressotti, A., Vaccari, F. P., Zaldei, A., deAngelis, P., and Scarascia-Mugnozza, G. (2001). Free-air CO2 enrichment (FACE) of a poplar plantation: the POPFACE fumigation system. New Phytologist 150, 465-476.

Mills, G., Buse, A., Gimeno, B., Bermejo, V., Holland, M., Emberson, L., and Pleijel, H. (2007). A synthesis of AOT40-based response functions and critical levels of ozone for agricultural and horticultural crops. Atmospheric Environment 41, 2630-2643.

Mills, G., and Harmens, H., eds. (2011). "Ozone pollution: a hidden threat to food security. Report prepared by the ICP Vegetation." Centre for Ecology and Hydrology, Bangor.

Mills, G., Hayes, F., Wilkinson, S., and Davies, W. J. (2009). Chronic exposure to increasing background ozone impairs stomatal functioning in grassland species. Global Change Biology 15, 1522-1533.

Mills, G., Pleijel, H., Malley, C. S., Sinha, B., Cooper, O. R., Schultz, M. G., Neufeld, H. S., Simpson, D., Sharps, K., Feng, Z. Z., Gerosa, G., Harmens, H., Kobayashi, K., Saxena, P., Paoletti, E., Sinha, V., and Xu, X. B. (2018).

Tropospheric Ozone Assessment Report: Present-day tropospheric ozone distribution and trends relevant to vegetation. Elementa-Science of the Anthropocene 6, 46.

Morgan, P. B., Ainsworth, E. A., and Long, S. P. (2003). How does elevated ozone impact soybean? A meta-analysis of photosynthesis, growth and yield. Plant Cell and Environment 26, 1317-1328.

Morgan, P. B., Bernacchi, C. J., Ort, D. R., and Long, S. P. (2004). An in vivo analysis of the effect of season-long open-air elevation of ozone to anticipated 2050 levels on photosynthesis in soybean. Plant Physiology 135, 2348-2357.

Nilsson J, Grennfelt P. (1988). Critical loads for sulphur and nitrogen. Report from a workshop held at Skokloster, Sweden, 19-24 March, 1988. Copenhagen: Nordic Council of Ministers.

Oksanen, E., Manninen, S., Vapaavuori, E., and Holopainen, T. (2009). Near-ambient ozone concentrations reduce the vigor of Betula and Populus species in Finland. Ambio 38, 413-417.

Peng, B., Wang, Y. X., Zhu, J. G., Wang, Y. L., and Yang, L. X. (2018). Effects of Ozone Stress on Rice Growth and Yield Formation under Different Planting Densities - A Face Study. International Journal of Agriculture and Biology 20, 2599-2605.

Percy, K. E., Legge, A. H., and Krupa, S. V. (2003). Tropospheric ozone: A continuing threat to global forests? *In* "Air Pollution, Global Change and Forests in the New Millennium" (D. F. Karnosky, K. E. Percy, A. H. Chappelka, C. Simpson and J. Pikkarainen, eds.), Vol. Volume 3, pp. 85-118. Elsevier.

Percy, K. E., Matyssek, R., and King, J. S. (2010). Facing the Future: Evidence from Joint Aspen FACE, SoyFACE and SFB 607 Meeting. Environmental Pollution 158, 955-958.

Prather, M., Gauss, M., Berntsen, T., Isaksen, I., Sundet, J., Bey, I., Brasseur, G., Dentener, F., Derwent, R., Stevenson, D., et al. (2003). Fresh air in the 21st century? Geophysical Research Letters 30.

Prentice, I. C., Farquhar, G. D., Fashan, M. J. R., Goulden, M. L., Heimann, M., Jaramillo, V. J., Kheshgi, H. S., Le Quere, C., Scholes, R. J., and Wallace, D. W. R. (2001). The Carbon Cycle and Atmospheric Carbon Dioxide. *In* "Climate Change 2001: Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change" (J. T. Houghton, Y. Ding, D. J. Griggs, M. Noguer, van der Linden, X. Dai, K. Maskell and C. A. Johnson, eds.), pp. 183-238. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Pretzsch, H., and Dieler, J. (2011). The dependency of the size-growth relationship of Norway spruce (Picea abies L. Karst.) and European beech (Fagus sylvatica L.) in forest stands on long-term site conditions, drought events, and ozone stress. Trees-Structure and Function 25, 355-369.

Rämö, K., Kanerva, T., Nikula, S., Ojanperä, K., and Manninen, S. (2006). Influences of elevated ozone and carbon dioxide in growth responses of lowland hay meadow mesocosms. Environmental Pollution 144, 101-111.

Riikonen, J., Maenpaa, M., Alavillamo, M., Silfver, T., and Oksanen, E. (2009). Interactive effect of elevated temperature and O-3 on antioxidant capacity and gas exchange in Betula pendula saplings. Planta 230, 419-427.

Ronan, A.C., Ducker, J.A., Schnell, J.L., Holmes, C.D. (2020). Have improvements in ozone air quality reduced ozone uptake into plants? Elem Sci Anth 8.

Sallas, L., Kainulainen, P., Utriainen, J., Holopainen, T., and Holopainen, J. K. (2001). The influence of elevated O-3 and CO2 concentrations on secondary metabolites of Scots pine (Pinus sylvestris L.) seedlings. Global Change Biology 7, 303-311.

Schaap, M., Hendriks, C., Kranenburg, R., Kuenen, J., Segers, A., Schlutow, A., Nagel, H., Ritter, A., Banzhaf, S. (2018). PINETI-3: Modellierung atmosphärischer Stoffeinträge von 2000 bis 2015 zur Bewertung der ökosystem-spezifischen Gefährdung von Biodiversität durch Luftschadstoffe in Deutschland. Umweltbundesamt Deutschlane, Texte 79/2018.

Scheelbeek, P. F. D., Bird, F. A., Tuomisto, H. L., Green, R., Harris, F. B., Joy, E. J. M., Chalabi, Z., Allen, E., Haines, A., and Dangour, A. D. (2018). Effect of environmental changes on vegetable and legume yields and nutritional quality. Proceedings of the National Academy of Sciences of the United States of America 115, 6804-6809.

Schultz, M. G., Schroder, S., Lyapina, O., Cooper, O. R., Galbally, I., Petropavlovskikh, I., von Schneidemesser, E., Tanimoto, H., Elshorbany, Y., Naja, M., et al. (2017). Tropospheric Ozone Assessment Report: Database and metrics data of global surface ozone observations. Elementa-Science of the Anthropocene 5, 26.

Tang, Y. S., Flechard, C. R., Dämmgen, U., Vidic, S., Djuricic, V., Mitosinkova, M., Uggerud, H. T., Sanz, M. J., Simmons, I., Dragosits, U., et al. (2020). Pan-European rural atmospheric monitoring network shows dominance of NH3 gas and NH4NO3 aerosol in inorganic pollution load. Atmos. Chem. Phys. Discuss. 2020, 1-61.

Utriainen, J., Janhunen, S., Helmisaari, H. S., and Holopainen, T. (2000). Biomass allocation, needle structural characteristics and nutrient composition in Scots pine seedlings exposed to elevated CO2 and O-3 concentrations. Trees-Structure and Function 14, 475-484.

van Goethem, T. M. W. J., Azevedo, L. B., van Zelm, R., Hayes, F., Ashmore, M. R., and Huijbregts, M. A. J. (2013). Plant Species Sensitivity Distributions for ozone exposure. Environmental Pollution 178, 1-6.

Vingarzan, R. (2004). A review of surface ozone background levels and trends. Atmospheric Environment 38, 3431-3442.

Volk, M., Geissmann, M., Blatter, A., Contat, F., and Fuhrer, J. (2003). Design and performance of a free-air exposure system to study long-term effects of ozone on grasslands. Atmospheric Environment 37, 1341-1350.

Wang, X. Z., and Taub, D. R. (2010). Interactive effects of elevated carbon dioxide and environmental stresses on root mass fraction in plants: a meta-analytical synthesis using pairwise techniques. Oecologia 163, 1-11.

Wang, Y., Hu, Z., Shang, D., Xue, Y., Islam, A. R. M. T., and Chen, S. (2020). Effects of warming and elevated O-3 concentrations on N2O emission and soil nitrification and denitrification rates in a wheat-soybean rotation cropland. Environmental Pollution 257.

Wang, Y. X., Yang, L. X., Han, Y., Zhu, J. G., Kobayashi, K., Tang, H. Y., and Wang, Y. L. (2012). The impact of elevated tropospheric ozone on grain quality of hybrid rice: A free-air gas concentration enrichment (FACE) experiment. Field Crops Research 129, 81-89.

Weigel, H. J., Bergmann, E., and Bender, J. (2015). Plant-Mediated Ecosystem Effects of Tropospheric Ozone. *In* "Progress in Botany" (U. Lüttge and W. Beyschlag, eds.), Vol. 76, pp. 395-438. Springer International Publishing.

Werner, H., and Fabian, P. (2002). Free-air fumigation of mature trees. Environmental Science and Pollution Research 9, 117-121.

Wittig, V. E., Ainsworth, E. A., and Long, S. P. (2007). To what extent do current and projected increases in surface ozone affect photosynthesis and stomatal conductance of trees? A meta-analytic review of the last 3 decades of experiments. Plant Cell and Environment 30, 1150-1162.

Wittig, V. E., Ainsworth, E. A., Naidu, S. L., Karnosky, D. F., and Long, S. P. (2009). Quantifying the impact of current and future tropospheric ozone on tree biomass, growth, physiology and biochemistry: a quantitative meta-analysis. Global Change Biology 15, 396-424.

2 The modifying factor nitrogen (N)

2.1 N deposition and its impact on vegetation

In the North-eastern US forest detrimental responses in terms of growth reduction and crown dieback due to critical N load exceedance have been observed for a number of tree species with *Abies balsamea, Picea rubens, Populus tremuloides,* and *Betula papyifera* being the most responsive (Duarte et al., 2013). A recent analysis of forest volume increment data across 23 European countries revealed that "*N deposition is at least as important as climate to modulate forest growth at continental scale in Europe, with a potential negative effect at sites with high N deposition, most pronounced for beech forests*" (Etzold et al., 2020).

There is strong evidence that N deposition is a serious threat to terrestrial biodiversity (Dise et al., 2011). Exceedance of critical N loads has been related to a loss in plant species richness in a broad range of European ecosystems. Species and communities that are adapted to low N availability such as grassland, heathland, peatland, forest, and arctic/montane ecosystems are particularly at risk (Bobbink, 1998). Moreover, N deposition may reduce C-sequestration which in turn implies changes in N-sequestration. Therefore, the interaction between O_3 concentrations and N-cycling are manifold and are related to N availability and N-uptake (Simpson et al., 2014).

Gaseous ammonia (NH_3) can be particularly harmful to vegetation and may impact vegetation by modifying the susceptibility to other stresses. Plants are known to be either a source or a sink of ammonia (NH_3, Massad et al., 2010) and emitted NH_3 returns to the biosphere as dry or wet deposition (NH_4+, Flechard et al., 2013). Because of its presence in the apoplast there is an exchange of NH_3 between leaf and atmosphere. That atmospheric NH_3 concentration for which the plant-atmosphere exchange is at equilibrium is defined as the NH_3 stomatal compensation point (χ_s). Xu et al. (2018) presented leaf-scale measurements of χ_s for hybrid poplar treated with urea and/or elevated O_3 and showed that it was significantly reduced by elevated O_3.

2.2 Interactive effects of O_3 and N supply on plants and plant communities

Data on experimental exposures to elevated N and O_3, singly and in combination, were available for six crop species (10 references), 26 grassland or semi-natural herbaceous species (10 references), and 25 tree species (incl. poplar hybrids, 34 references). Across all experiments the control treatment for N varied greatly: low N supply ranged from 0 to 120 kg N ha^{-1} for crops, 0 to 10 kg N ha^{-1} for grassland, and 0 -60 kg N ha^{-1} for trees, while high N treatments ranged from 50 -436 , 15-75 , and 20- 150 (406) kg N ha^{-1}, respectively. The N regime was usually adjusted by varying the amount of urea or NH_4NO_3 applied to the soil. We found only three studies on experimental variation of NH_3 deposition on trees (Dueck et al., 1998; Perezsoba et al., 1995; Vanhove and Bossen, 1994) and one on crops (beans, Tonneijck and van Dijk, 1998) – all associated with the research team in the Netherlands.

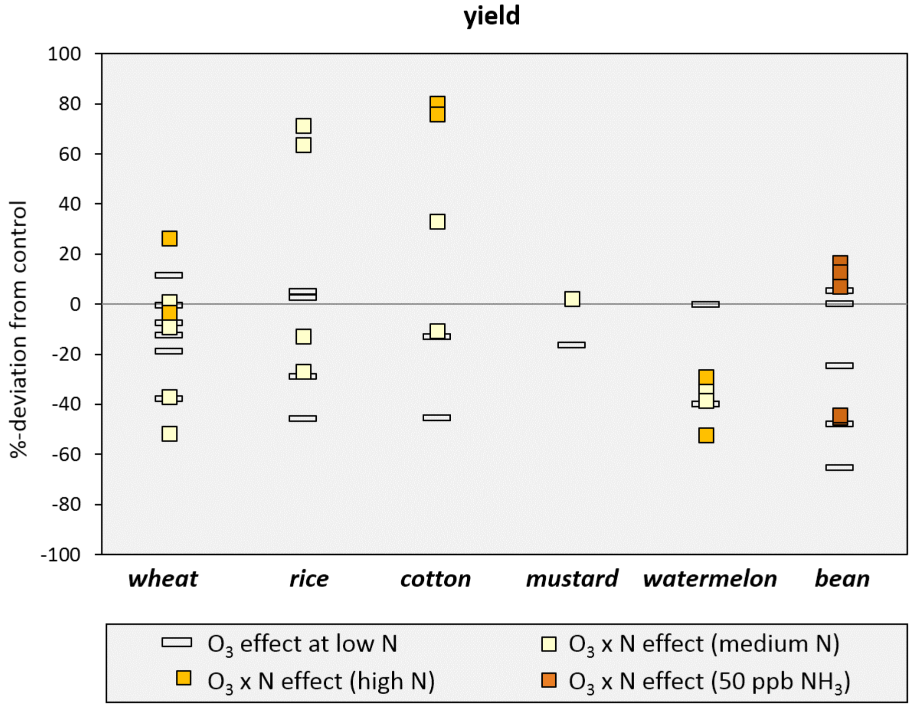
2.2.1 Crops

For crops, N application is a general characteristic of cultivation practice and knowledge about the specific N demand and optimal N fertilisation rate are fundamental for plant growth. Thus, the control N treatment was rather a recommended or low fertilisation than no N supply. Contrary to our expectation, there were only 10 references we became aware of reporting on findings about the interactive effect of elevated O_3 and N supply on yield, growth, or gas exchange on crops.

Most of the research has been done on *wheat* cultivars. Under the experimental conditions used, wheat cultivars responded very differently to the N application ranging from a decrease in yield to an increase by up to 23% under low O_3. Similarly, the effect of elevated O_3 exposures showed an indifferent picture Figure 2: *(i)* both factors caused negative effects which were additive in their combination (Pandey et al., 2018), *(ii)* the negative effect caused by O_3 was amplified by N supply (Brewster et al., 2018; Pandey et al., 2018), *(iii)* the negative effect caused by O_3 was ameliorated by N supply (Chen et al., 2011; Luo et al., 2013), or *(iv)* the interactive effect reflected the growth stimulating effect of N fertilisation when the cultivar showed less sensitivity to O_3 (Fangmeier et al., 1996). In summary, the interactive effects seem to depend on exposure conditions, the plant cultivar and its sensitivity to O_3. For example, Pandey et al. (2018) compared the response of two wheat cultivars differing in their O_3 susceptibility and concluded that addition of N did not provide full protection against O_3 impacts and that the response of antioxidants varied between the cultivars. However, high levels of N were shown to be able to protect wheat plants from visible leaf injuries when exposed to O_3 for five days in climate chambers (vonTiedemann, 1996).

Figure 2 **Effects of elevated O_3 on yield of six crop species interacting with different levels of N supply**

The O_3 effect is expressed as % deviation from control (CF with low N). For classification of N level, experimental conditions and references see Table 22, chapter A.1.1.



Source: own illustration, Thünen Institute

Broberg et al. (2017) investigated the N fertiliser efficiency in wheat by analysing a large number of data available from open-top chamber field experiments. They clearly evidenced a negative effect of O_3 on the N efficiency with respect to both grain and protein yield. Thus, O_3 may increase the risk for nitrate leaching by reducing the translocation of N to the grains which becomes more evident when N fertilisation would be increased in order to counteract O_3-induced yield losses. Moreover, in wheat plants treated with ^{15}N-urea it has been shown that elevated O_3 may decrease mineral nitrogen and fixed ammonium derived from rhizodeposition, thus (Cao et al., 2018). Both studies have shown that exposure to elevated O_3 may affect soil N cycling in an agro-ecosystem.

Increased N supply could mitigate O_3-induced reduction of growth, yield, and net photosynthetic rate in two rice cultivars (Luo et al., 2013; Tatsumi et al., 2019) thus the sensitivity of rice growth to O_3 appears to be dependent on soil N levels. Heagle et al. (1999) have shown that N nutrition is especially important in cotton cultivation, where doubling the N supply resulted in 1.6 times higher yield mass. In the combined treatment yield reductions up to 45.5% caused by O_3 have been reversed into increases up to 80%. In O_3 sensitive mustard plants, the application of 1.5 times of the recommended NPK provided protection against yield loss and detrimental effects on gas exchange due to ambient O_3 (Singh et al., 2009). Comparing three tropical varieties of mustard Singh et al. (2011) figured out, that this was in common with the maintenance of higher levels of pigments and proteins in the combined treatment and could not be attributed to a stimulation of the antioxidant defence system. A contrary observation was made by Calatayud et al. (2006) on watermelons which were grown for two consecutive years in open-top chambers. Here, high N concentrations enhanced the detrimental effects of ozone on the maximum efficiency of photosystem II and lipid peroxidation. As a result, O_3-induced yield reduction was more pronounced in plants with higher N supply.

Tonneijck and van Dijk (1998) investigated the long-term effect of elevated O_3 on beans at two levels of atmospheric NH_3 dry deposition (21 and 45 ppb), a pollutant which coincide with high exposures to O_3 and markedly contributes to the total N-deposition in the Netherlands. The treatment with high NH_3 did not represent a phytotoxic level but rather a fertilisation level, since there was a general growth stimulation effect, independently from the O_3 treatment, i.e. adverse effects of O_3 on biomass and pod yield did not depend on the NH_3 level.

2.2.2 Grassland species

Most natural and semi-natural grassland communities are adapted to a limitation in nutrient availability. Limitations by nitrogen and soil water are considered the main factors driving the functional response at the community level (plant strategy selection, Pérez-Ramos et al., 2012). Species diversity and species evenness are strongly determined by N mineralisation and to a lesser extent by total soil N and extractable P, respectively. Total aboveground biomass of the vegetation is determined by total soil N (Aerts et al., 2003). As a consequence, elevated levels of N deposition pose a threat to the species composition and ecological integrity of the ecosystem.

2.2.2.1 Intact plant community level

In 2000 a Swiss research team established a free-air O_3 exposure experiment to study the long-term impact of elevated O_3 and N deposition on a mature, extensively managed grassland (species-rich Geo-Montani-Nardetum pasture) located at a high plateau in the central Alps (Alp Flix, Switzerland, 2000 m asl). O_3 was added to ambient air to gain 1.2 x ambient and 1.6 x ambient concentrations and total N loads range from 4 to 54 kg N ha^{-1} yr^{-1} (Bassin et al., 2007). Three years of exposure to elevated O_3 affected neither the above-ground productivity nor the species composition, whereas N treatments above 10 kg N ha^{-1} yr^{-1} were sufficient to affect the composition of functional groups (Bassin et al., 2007). This observation was evident even after seven years of exposure (Bassin et al., 2013) while N addition caused strong changes in the fractional biomass of functional groups and slightly reduced the Shannon diversity. Although the O_3-induced acceleration of leaf senescence was counteracted by N supply, no interaction of O_3 and N has been detected. Volk et al. (2011) determined the ecosystem-level CO_2 exchange for a 10-day period, the gross primary production (GPP), ecosystem respiration rates (R_{eco}), and seasonal net ecosystem production (NEP). Seasonal mean rates of both ecosystem R_{eco} and GPP decreased by approx. 8% due to the exposure to 1.6 x ambient O_3. However, calculated NEP indicated an unaltered CO_2-C balance of the growing season and there was no interaction between O_3 and N.

In semi-improved grassland in North Wales free-air exposure to O_3 resulted in a reduction of net ecosystem CO_2 exchange and contributed to accelerated rates of soil N cycling, while application of N significantly increased ecosystem respiration during the growing season (Wang et al., 2019).

In a recent study, Hayes et al. (2019) tested the O_3 sensitivity of semi-natural grassland mesocosms collected in the UK along an N gradient to assess whether long-term N deposition may have altered the sensitivity to O_3 of sand dune grassland communities. Mesocosms from N-polluted sites contained fewer O_3-sensitive forbs and sedges, a higher proportion of comparatively O_3-tolerant grasses and thus exhibited a higher level of resilience to O_3 damage. Conversely, mesocosms from 'clean' habitats were most vulnerable to O_3. This study clearly indicates that long-term deposition of higher N levels may interact with O_3 apart from a combined action.

Table 4 Single and interactive effects of O_3 and N on intact plant communities

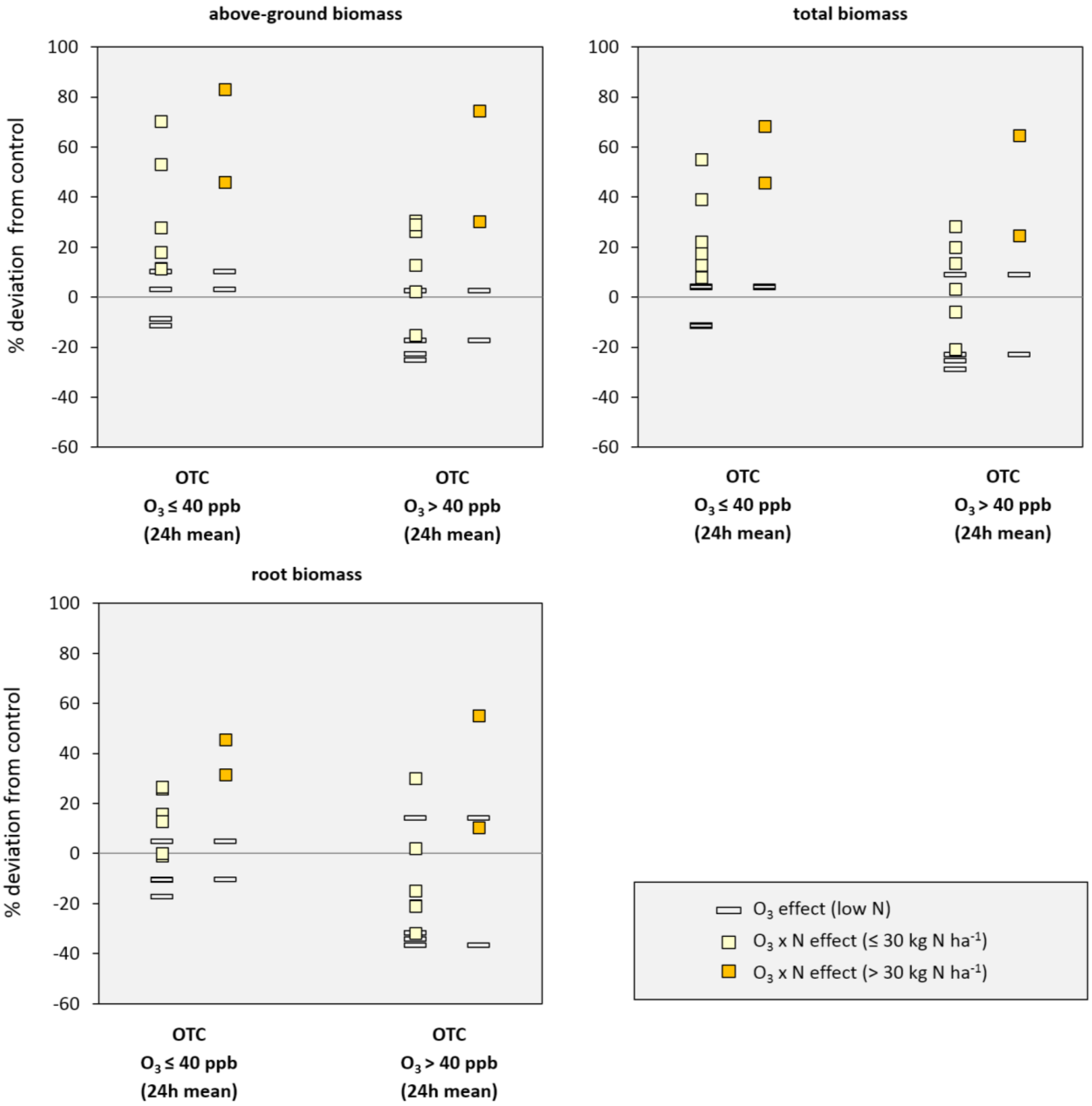
vegetation	facility	low N	elevated N	N supply	O_3 contr.	elevated O_3	N effect	O_3 effect	interactive effect	reference
dune grasslands	free-air	gradient of N deposition	gradient of N deposition	gradient of N deposition	AA	36 ppb, 49 ppb (24h mean)	number of vascular plants declined with increasing N deposition, most pronounced for forbs	the highest O_3 treatment damaged individuals from all the 20 target species	the mean proportion of O_3 damaged leaves declined with increasing N deposition	Hayes et al. (2019)
Cynosurus cristatus-Centaurea nigra grassland	free-air	soil N	100 kg N ha^{-1}	NH_4NO_3	AA	55 ppb (24h mean)	N fertilization significantly increased ecosystem respiration	no effect on aboveground biomass, reduced net C uptake	none	Wang et al. (2019)
species-rich Geo-Montani-Nardetum pasture	free-air	4 kg N ha^{-1}	9 kg N ha^{-1} 14 kg N ha^{-1} 29 kg N ha^{-1} 54 kg N ha^{-1}	NH_4NO_3	AA	51 ppb, 74 ppb (24h mean)	N affected the abundance of the most frequent species	no effect on biomass, number of species, nor the Shannon diversity index, increase in relative proportion of dead biomass	accelerated leaf senescence in the highest O_3 treatment was counteracted by N, no interactive effects for growth/ species abundance	Bassin et al. (2007, 2013)

2.2.2.2 Single plant level

There were 10 publications reporting on interactive effects of O_3 and N which provide information about the response of 23 grassland species at a single species or plant level (Table 5 to Table 7). If possible, percentage deviation from control have been calculated and shown in graphs (Figure 3).

Figure 3 **Effects of O_3 on growth parameters of five grassland species interacting with different levels of N**

The species are *Bromus hordeaceus, Ornithopus compressus, Trifolium cherleri, T. striatum,* and *T. subterraneum*. The O_3-effect is expressed as percentage deviation from control (CF with low N); for exposure conditions and references see Table 23, chapter A.1.1.



Source: own illustration, Thünen Institute

Table 5 Single and interactive effects of O_3 and N on grasses

species	facility	low N	elevated N	N supply	O_3 contr.	elevated O_3	N effect	O_3 effect	interactive effect	reference
Briza maxima	OTC	soil N	20 kg N ha^{-1}, 40 kg N ha^{-1}	NH_4NO_3	CF	34 ppb, 45 ppb, 50 ppb (12h mean)		foliar damage, increased senescence	interaction for senescent biomass	Calvete-Sogo et al. (2016)
Bromus hordeaceus	OTC	7.5 kg N ha^{-1}	22.5 kg N ha^{-1}, 45.0 kg N ha^{-1}	NPK and NH_4NO_3	CF	≈35 ppb$^{1)}$ ≈55 ppb$^{2)}$ (24h mean)		no effects on biomass, increase in senescence	medium O_3 effect on senescence was mitigated by N	Sanz et al. (2015)
Carex arenaria	solar-domes	12 kg N ha^{-1}	100 kg N ha^{-1}	NH_4NO_3	AA +20 ppb	32 ppb -83 ppb (24h mean)	growth stimulation, increase in leaf number	increase in senescence, reduction in root biomass	O_3 effect on senescence was reduced by N	Jones et al. (2010)
Carex sempervirens	free air	4 kg N ha^{-1}	9 kg N ha^{-1}, 14 kg N ha^{-1}, 29 kg N ha^{-1}, 54 kg N ha^{-1}	NH_4NO_3	AA	51 ppb, 74 ppb (24h mean)	reduction in SLA, increase in leaf weight, increase in chlorophyll conc.		none	Bassin et al. (2009, 2017)
Cynosurus echinatus	OTC	soil N	20 kg N ha^{-1}, 40 kg N ha^{-1}	NH_4NO_3	CF	34 ppb, 45 ppb, 50 ppb (12h mean)	none	none	none	Calvete-Sogo et al. (2016)
Dactylis glomerata	solar-domes	soil N	75 kg N ha^{-1}	NH_4NO_3	AA +20 ppb	32 ppb -83 ppb (24h mean)	increase in biomass and seed mass, no effect on A_{max}	increase in senescent leaf biomass, no effect on A_{max}	significant interaction on root biomass	Wyness et al. (2011)
Festuca spp.	free air	4 kg N ha^{-1}	9 kg N ha^{-1}, 14 kg N ha^{-1}, 29 kg N ha^{-1}, 54 kg N ha^{-1}	NH_4NO_3	AA	51 ppb, 74 ppb (24h mean)	shoot biomass was increased, increase in chlorophyll conc.	root and total biomass were reduced (*Festuca rubra*)	none	Bassin et al. (2009, 2017)
Nardus stricta	free air	4 kg N ha^{-1}	9 kg N ha^{-1}, 14 kg N ha^{-1}, 29 kg N ha^{-1}, 54 kg N ha^{-1}	NH_4NO_3	AA	51 ppb, 74 ppb (24h mean)	increase in chlorophyll conc. and leaf weight	none	interaction for chlorophyll conc.	Bassin et al. (2009, 2017)

species	facility	low N	elevated N	N supply	O_3 contr.	elevated O_3	N effect	O_3 effect	interactive effect	reference
Helictotrichon versicolor	free air	4 kg N ha^{-1}	9 kg N ha^{-1}, 14 kg N ha^{-1}, 29 kg N ha^{-1}, 54 kg N ha^{-1}	NH_4NO_3	AA	51 ppb, 74 ppb (24h mean)	increase in SLA	none	none	Bassin et al. (2009, 2017)

[1] AOT40: 221-1198 ppb h

[2] AOT40: 6032-10891 ppb h

Table 6 Single and interactive effects of O_3 and N on herbs

species	facility	low N	elevated N	N supply	O_3 contr.	elevated O_3	N effect	O_3 effect	interactive effect	reference
Arnica montana	free air	4 kg N ha^{-1}	9 kg N ha^{-1}, 14 kg N ha^{-1}, 29 kg N ha^{-1}, 54 kg N ha^{-1}	NH_4NO_3	AA	51 ppb, 74 ppb (24h mean)		reduction in chlorophyll conc.		Bassin et al. (2009)
Gentiana acaulis	free air	4 kg N ha^{-1}	9 kg N ha^{-1}, 14 kg N ha^{-1}, 29 kg N ha^{-1}, 54 kg N ha^{-1}	NH_4NO_3	AA	51 ppb, 74 ppb (24h mean)	increase in chlorophyll conc.	none	none	Bassin et al. (2009, 2017)
Leontodon helveticus	free air	4 kg N ha^{-1}	9 kg N ha^{-1}, 14 kg N ha^{-1}, 29 kg N ha^{-1}, 54 kg N ha^{-1}	NH_4NO_3	AA	51 ppb, 74 ppb (24h mean)	increase in chlorophyll conc. and SLA	none	none	Bassin et al. (2009, 2017)
Ligusticum mutellina	free air	4 kg N ha^{-1}	9 kg N ha^{-1}, 14 kg N ha^{-1}, 29 kg N ha^{-1}, 54 kg N ha^{-1}	NH_4NO_3	AA	51 ppb, 74 ppb (24h mean)	increase in chlorophyll conc.	none	interaction for chlorophyll conc.	Bassin et al. (2009)
Plantago major	contr. environ.	low	high	Chemopak Formula 2	CF	70 ppb (7h mean)	increase in total and seed mass	effects on physiology and biomass	none	Whitfield et al. (1998)

species	**facility**	**low N**	**elevated N**	**N supply**	**O_3 contr.**	**elevated O_3**	**N effect**	**O_3 effect**	**interactive effect**	**reference**
Potentilla aurea	free air	4 kg N ha^{-1}	9 kg N ha^{-1}, 14 kg N ha^{-1}, 29 kg N ha^{-1}, 54 kg N ha^{-1}	NH_4NO_3	AA	51 ppb, 74 ppb (24h mean)	increase in chlorophyll conc. and leaf weight	none	none	Bassin et al. (2009)
Ranunculus acris	solar-domes	soil N	75 kg N ha^{-1}	NH_4NO_3	AA +20 ppb	32 ppb -83 ppb (24h mean)	none	reduced root biomass	none	Wyness et al. (2011)
Ranunculus villarsii	free air	4 kg N ha^{-1}	9 kg N ha^{-1}, 14 kg N ha^{-1}, 29 kg N ha^{-1}, 54 kg N ha^{-1}	NH_4NO_3	AA	51 ppb, 74 ppb (24h mean)	increase in chlorophyll conc.	reduction in chlorophyll conc.	interaction for chlorophyll conc.	Bassin et al. (2009)
Silene gallica	OTC	soil N	20 kg N ha^{-1}, 40 kg N ha^{-1}	NH_4NO_3	CF	34 ppb, -50 ppb (12h mean)	none	increased senescence	no interaction	Calvete-Sogo et al. (2016)

Table 7 **Single and interactive effects of O_3 and N on legumes**

species	**facility**	**low N**	**elevated N**	**N supply**	**O_3 contr.**	**elevated O_3**	**N effect**	**O_3 effect**	**interactive effect**	**reference**
Ornithopus compressus	OTC	soil N	20 kg N ha^{-1}, 40 kg N ha^{-1}	NH_4NO_3	CF	34 ppb, 45 ppb, 50 ppb (12h mean)	none	Increase in green and total biomass	none	Calvete-Sogo et al. (2016)
Ornithopus compressus	OTC	soil N	40 kg N ha^{-1}	NH_4NO_3	CF	26 ppb (24h mean)	no effects on gas exchange	increase in the stomatal conductance	none	Llusia, J. et al. (2014)
Trifolium alpinum	free air	4 kg N ha^{-1}	9 kg N ha^{-1}, 14 kg N ha^{-1}, 29 kg N ha^{-1}, 54 kg N ha^{-1}	NH_4NO_3	AA	51 ppb, 74 ppb (24h mean)		reduction in shoot and root biomass		Bassin et al. (2009, 2017)

species	**facility**	**low N**	**elevated N**	**N supply**	**O_3 contr.**	**elevated O_3**	**N effect**	**O_3 effect**	**interactive effect**	**reference**
Trifolium cherleri	OTC	soil N	20 kg N ha^{-1}, 40 kg N ha^{-1}	NH_4NO_3	CF	34 ppb, 45 ppb, 50 ppb (12h mean)		foliar damage, linear decrease in green biomass	no interaction leaf injury	Calvete-Sogo et al. (2016)
Trifolium cherleri	OTC	5 kg N ha^{-1}	30 kg N ha^{-1}	NPK and NH_4NO_3	CF	≈55 ppb1) (24h mean)	N stimulated all biomass parameters	foliar damage, accelerated senescence and growth reduction		Sanz. et al. (2014)
Trifolium striatum	OTC	soil N	20 kg N ha^{-1}, 40 kg N ha^{-1}	NH_4NO_3	CF	34 ppb, 45 ppb, 50 ppb (12h mean)		foliar damage, decrease in green and root biomass	enhancement in O_3-induced root reduction	Calvete-Sogo et al. (2016)
Trifolium subterraneum	OTC	soil N	40 kg N ha^{-1}	NH_4NO_3	CF	26 ppb, 30 ppb (24h mean)	no effect on g_s	g_s increased with O_3 concentration	no interaction g_s	Llusia et al. (2014)
Trifolium subterraneum	OTC	10 kg N ha^{-1}	30 kg N ha^{-1}, 60 kg N ha^{-1}	NPK and NH_4NO_3	CF	34 ppb, 56 ppb (24h mean)	Increase in shoot and root biomass,	reduced total aerial and flower biomass and seed production	N mitigated effects induced by moderate O_3	Sanz. et al. (2007)
Trifolium subterraneum	OTC	5 kg N ha^{-1}	15 kg N ha^{-1}, 30 kg N ha^{-1}	NPK and NH_4NO_3	CF	34 ppb, 56 ppb (24h mean)	biomass and green biomass was positively affected	increase in senescence and foliar injury, reduced biomass	none	Sanz et al. (2005)

$^{1)}$AOT40: 6032-10891 ppb h

At low levels of O_3, the predominant effect of N treatment was a general stimulation of growth and an increase in the chlorophyll content. Six of these species responded to O_3 with accelerated senescence or loss in chlorophyll content and for 10 species growth changes have been recorded in one study at least (Table 5 to Table 7): In Spain, the response of three annual clover species occurring in Mediterranean (dehesa) grasslands has been studied frequently: *Trifolium subterraneum, T. striatum,* and *T. cherleri*. Exposure to elevated O_3 caused an increase in senescent biomass and a reduction in above-ground biomass, seed production (for *T. striatum*), or nutritive quality (*T. cherleri*) and O_3 effects were more adverse in the root (Sanz et al., 2007; Sanz et al., 2014; Sanz et al., 2005)**.** N supply partially counterbalanced the adverse effects of O_3 but in some cases an exacerbation of the O_3 effects has been observed as N further enhanced the root reduction (*T. striatum,* Calvete-Sogo et al., 2016; Sanz et al., 2007) or increase in senescent biomass (*T. cherleri,* Sanz et al., 2014) induced by O_3.

Comparing the response of six species representative of annual Mediterranean pastures Calvete-Sogo et al. (2016) concluded that O_3 sensitive legumes did not respond to N, whereas O_3 tolerant grasses were most responsive to N. For example, *Bromus hordeaceus* responded to O_3 with increased senescence but showed some positive responses related to root growth (Sanz et al., 2015). By contrast, root growth of the subalpine plants *Festuca rubra* and *Trifolium alpinum* (Bassin et al., 2017) or the sedge *Carex arenaria* (Jones et al., 2010) was decreased by elevated O_3 and extra N input did not influence growth response to O_3, while for the mesotrophic grassland species *Dactylis glomerata* a loss in biomass was more pronounced under high N (Wyness et al., 2011).

In summary, for five species statistically significant interactions between O_3 and N were apparent and adverse effects of O_3 were partly mitigated by N. This pattern could be seen for senescent biomass on *Briza maxima* (Calvete-Sogo et al., 2016), *Bromus hordeaceus* (Sanz et al., 2015), and *Carex arenaria* (Jones et al., 2010) and for root biomass on *Dactylis glomerata* (Wyness et al., 2011), *Plantago major* (Whitfield et al., 1998), and *Trifolium striatum* (Calvete-Sogo et al., 2016; Sanz et al., 2007). The results clearly indicate that for grassland species additional N supply may counteract adverse growth effects and that depending on the level of elevated O_3 and N this may result in a more or less complete suppression of growth depressions caused by O_3 or a predominance of the fertilising effect of N. The latter became more evident for shoot than for root biomass (Figure 3). Moreover, in their experiments using a mixture of six Mediterranean species Calvete-Sogo et al. (2016) were able to show that both tropospheric O_3 and N deposition may alter the competitive relationships among species and carbon cycling in grass communities.

2.2.3 Woody plants

2.2.3.1 O_3 response as a function of foliar N-content

Some studies provide information about the relationship between foliar N content and O_3 response of Japanese trees without having varied the N supply. In response to elevated O_3, foliar N content was shown to be decreased in the deciduous larch (*Larix kaempferi*) and to a lesser extent in the broad-leaved Siebold's beech (*Fagus crenata*) whereas it was not decreased in the evergreen fir (*Abies sachalinensis*) and the Japanese oak (*Quercus mongolica* var. *crispula,* Sugai et al., 2019a; Watanabe et al., 2018). A decline in photosynthetic nitrogen-use efficiency was observed in both coniferous species (Sugai et al., 2019a), and O_3-induced reductions of the maximum rates of carboxylation (V_{cmax}) have been shown for the both broad-leaved species beech and oak; moreover, in beech leaves this reduction was more obvious with increasing foliar N content (Watanabe et al., 2018). The authors concluded that needle N dynamics may have a determining influence on the sensitivity of photosynthesis to O_3.

2.2.3.2 O_3 exposure studies with varied amounts of complete fertiliser

The importance of the nutrient supply for the nature and severity of the O_3 response has been tested on cloned cuttings of *Betula pendula* by varying all micro- und macronutrient. It has been shown that most biochemical effects induced by exposure to elevated O_3 only became obvious in older leaves of plants with low nutrient supply (Landolt et al., 1997). Although fertilisation seemed to mitigate O_3 impacts it was not sufficient to maintain a high photosynthetic capacity in leaves. Moreover, nutrition did not affect the O_3 uptake into leaves (Matyssek et al., 1997; Maurer and Matyssek, 1997; Maurer et al., 1997). In contrast, fertilisation was found to enhance physiological damage and growth reductions on *Platanus occidentalis* caused by exposure to elevated O_3 (Han et al., 2009).

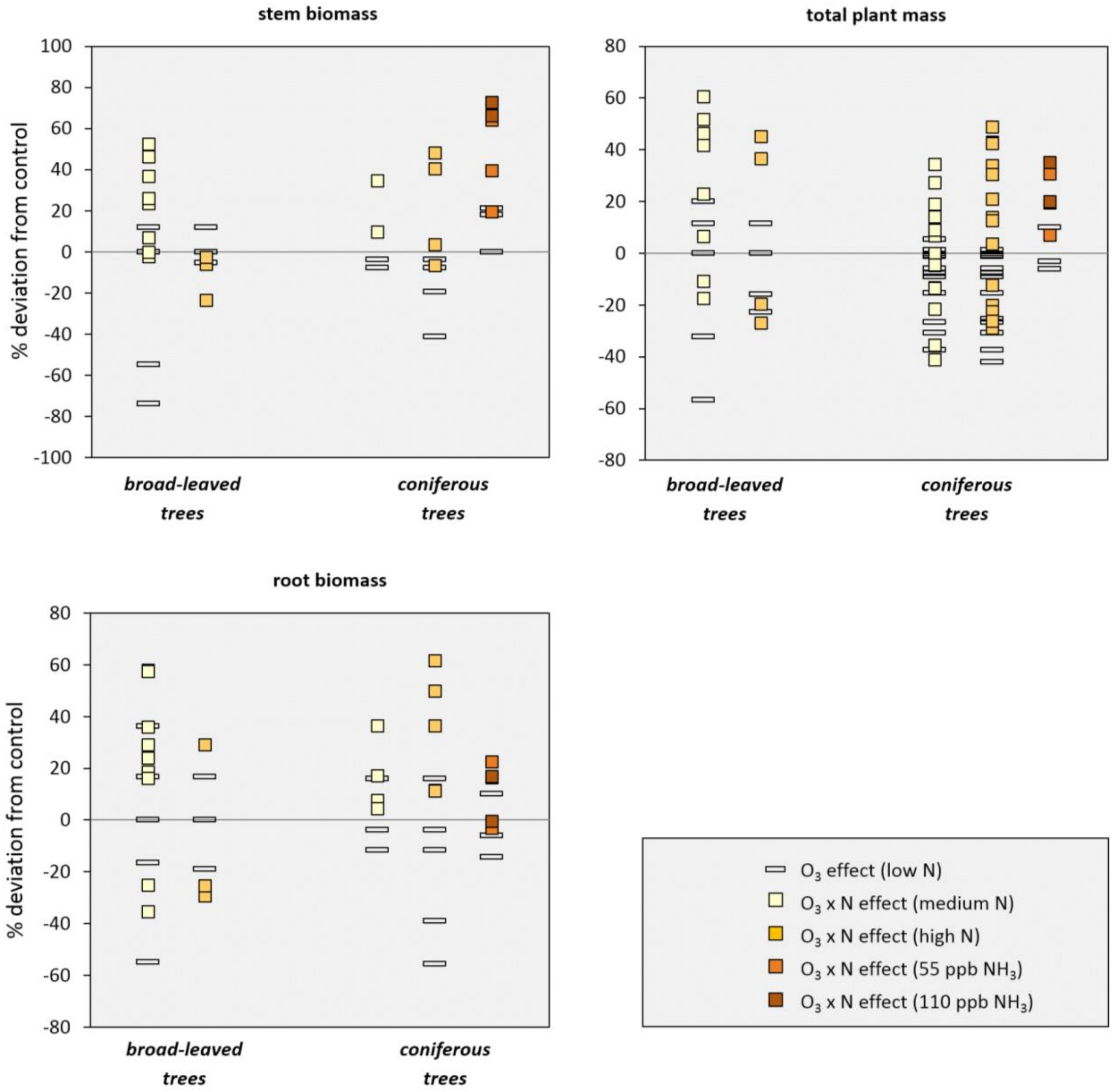
2.2.3.3 O_3 exposure studies with varied amounts of N supply

In contrast to crops, the main objective of studies on trees was to investigate the ecological effect of an additional N load originating from N deposition, whereby the control treatment for N differ considerably in terms of quantity. In some experiments, soil was used for the low N treatment without further N addition, while in others N amounts ranged from about 5 up to 60 kg N ha^{-1} yr^{-1}, with or without including N deficit treatments as well.

In Table 8 and Table 9 data on interactive effects of N and O_3 are compiled for broad-leaved and coniferous trees, respectively, encompassing 21 species in total. Within the very most studies exposure to O_3 lasted one season (up to three seasons) and nitrogen supply was varied by addition of different amounts of NH_4NO_3 or urea to the soil, while three Dutch studies examined the effects of dry NH_3 deposition on coniferous trees (Dueck et al., 1998; Perezsoba et al., 1995; Vanhove and Bossen, 1994). In addition to the frequently observed growth stimulations caused by additional N, increases in net photosynthesis, stomatal conductance, chlorophyll content, and carbohydrate concentrations or reduced accumulation of reactive oxygen species have been reported (see tables). As much as experimental conditions differ, the amplitude of changes in growth parameters induced by the combined treatment with N and O_3 relative to the control varied markedly (Figure 4).

Figure 4 Effects of O_3 on growth parameters of broad-leaved and coniferous trees interacting with different levels of N

The species are *Cryptomeria japonica, Larix gmelinii var. japonica x L. kaempferi, Larix kaempferi, Liriodendron tulipifera, Picea abies, Pinus densiflora, P. sylvestris, P. taeda, Populus tremuloides,* and *Populus* hybrids. The O_3-effect is expressed as percentage deviation from control (CF or NF with low N); medium N: 10-40 kg N ha^{-1}, 96 µg N g^{-1} soil, or 50 mg l^{-1} soil and high N: 50-80 kg N ha^{-1}, 172 µg N g^{-1} soil, 315 mg l^{-1} soil; for exposure conditions and references see Table 24, chapter A.1.1



Source: own illustration, Thünen Institute

Table 8 **Single and interactive effects of O_3 and N on broad-leaved woody plants**

conc. = concentration, sig. significant(ly), neg. negative(ly), g_s = stomatal conductance, photosynthetic parameters: P_n = A = net photosynthetic rate, A_{total} = total net photosynthetic rate, A_{350} = net photosynthetic rate at 350 µmol mol^{-1} CO_2, A_{380} = net photosynthetic rate at 380 µmol mol^{-1} CO_2,, A_{max} or P_N or P_{nmax} = net photosynthetic rate at 1,600-1,500 µmol mol^{-1} CO_2, BVOC = Biogenic Volatile Organic Compounds, WUE = Water Use Efficiency, contr. env. = controlled environment.

species	age/ duration	N control	elevated N	N supply	facility	O_3 contr.	elevated O_3	N effect	O_3 effect	interactive effect	reference
Betula pendula	3-yr-old 2 seasons	10 kg N h^{-1}	30 kg N ha^{-1}, 50 kg N ha^{-1}, 70 kg N ha^{-1}	NH_4NO_3	solar-domes	CF	40 ppb - 68 ppb (24 h mean)	increased chlorophyll content, stimulated photosynthetic capacity	decreased chlorophyll content, reduced photosynthetic capacity	no interactive effects	Harmens et al. (2017)
Betula pendula	3-yr-old 2 seasons	10 kg N h^{-1}	30 kg N ha^{-1}, 50 kg N ha^{-1}, 70 kg N ha^{-1}	NH_4NO_3	solar-domes	CF	40 ppb - 68 ppb (24 h mean)	increased g_s, stimulated growth	no effect on biomass and g_s	biomass showed a clear decline with increasing O_3 only under high N	Dai et al. (2019)
Betula pendula	3-yr-old 2 seasons	10 kg N h^{-1}	30 kg N ha^{-1}, 50 kg N ha^{-1}, 70 kg N ha^{-1}	NH_4NO_3	solar-domes	CF+	46 ppb 66 ppb (24 h mean)	increased total leaf area per plant	decreased total leaf area, increased BVOC emission	N ameliorated the negative effects of O_3 on leaf area, effects on BVOC emission were additive	Carriero et al. (2016)
Betula pendula	2-yr-old 2 seasons	37 kg N h^{-1}	75 kg N ha^{-1}, 150 kg N ha^{-1}	NPK and NH_4NO_3	free-air	AA	9.2 -18 µl l^{-1}h (AOT40)	increased growth and shoot/root ratio, retarded senescence	reduced the mean leaf size and the leaf area, accelerated senescence	N supply can confer greater tolerance to O_3	Pääkkönen & Holopainen, (1995)
Carpinus betulus	3-yr-old 2 seasons	soil N	70 kg N ha^{-1}	NH_4NO_3	OTC	CF	31-43 ppb (24h mean)	increased biomass	no neg. effects on biomass, increase in stem biomass	under high N linear decrease in biomass with increasing POD	Marzuoli et al. (2018)
Fagus crenata	2-yr-old 2 seasons	0 kg N h^{-1}	20 kg N ha^{-1}, 50 kg N ha^{-1}	NH_4NO_3	OTC	CF	42-43 ppb 63-64 ppb	increase in all growth	reduction in all growth	The O_3 -induced reduction in	Yamaguchi et al. (2007)

species	**age/ duration**	**N control**	**elevated N**	**N supply**	**facility**	**O_3 contr.**	**elevated O_3**	**N effect**	**O_3 effect**	**interactive effect**	**reference**
							83-84 ppb (24 h mean)	parameters, A_{380} and g_s	parameters and root shoot ratio, reduced A_{380} and increased g_s	growth and effects on gas exchange were greater in the N treated plants	
Fraxinus ornus	2-yr-old 10 days	0 kg N h^{-1}	20 kg N ha^{-1}	NH_4NO_3	contr. env.	CF	87 ppb (5 h mean)	increase in P_N and g_s and WUE	decrease in P_N and g_s	decrease in P_N and g_s less pronounced in O_3+N than in O_3 alone	Fusaro et al. (2017)
Liriodendron tulipifera	1-yr-old 18 weeks	58 µg $g^{-1\#}$	96 µg $g^{-1\#}$, 172 µg $g^{-1\#}$	NH_4NO_3	OTC	CF	56 ppb, 108 ppb (7h mean)	no effects on growth, stimulated photosynthesis and increased WUE	no effects on growth	no effects on growth	Tjoelker & Luxmoore (1991)
Populus deltoides cv. 55/56 x P. deltoides cv. Imperial	cuttings 1 season	0 kg N h^{-1}	50 kg N ha^{-1}	urea	OTC	CF	80.6 ppb (24h mean)	increase in leaf and aboveground biomass	reduced biomass of fine, roots, above-ground biomass, total biomass	interactive effects: O_3 decreased growth more severe under high N	Li et al. (2019)
Populus deltoides cv. 55/56 x P. deltoides cv. Imperial	cuttings 1 season	0 kg N h^{-1}	50 kg N ha^{-1}	urea	OTC	CF	80.6 ppb (24h mean)	increased A_{sat}	reduced A_{sat}, reduction in chlorophyll content	no sign. interactive effects for A_{sat} and g_s,	Xu et al. (2018)
Populus maximoviczii × P. berolinensis, Oxford clone	cuttings 1 season	0 kg N h^{-1}	80 kg N ha^{-1}	NH_4NO_3	free-air	AA	51.6 ppb, 66.7 ppb (24h mean)	reduced reactive oxygen species accumulation	visible injury, altered membrane integrity, oxidative damage	N reduced the O_3 sensitivity (mitigation effect)	Podda et al. (2019)
Liriodendron tulipifera	1-yr-old 18 weeks	58 µg $g^{-1\#}$	96 µg $g^{-1\#}$, 172 µg $g^{-1\#}$	NH_4NO_3	OTC	CF	56 ppb, 108 ppb (7h mean)	no effects on growth, increase in P_N and WUE	no effects on growth	no effects on growth	Tjoelker & Luxmoore (1991)

species	**age/ duration**	**N control**	**elevated N**	**N supply**	**facility**	**O_3 contr.**	**elevated O_3**	**N effect**	**O_3 effect**	**interactive effect**	**reference**
Populus deltoides cv. 55/56 x P. deltoides cv. Imperial	cuttings 1 season	0 kg N h^{-1}	50 kg N ha^{-1}	urea	OTC	CF	80.6 ppb (24h mean)	increase in leaf and aboveground biomass	reduced biomass of fine, roots, above-ground biomass, total biomass	interactive effects: O_3 decreased growth more severe under high N	Li et al. (2019)
Populus deltoides cv. 55/56 x P. deltoides cv. Imperial	cuttings 1 season	0 kg N h^{-1}	50 kg N ha^{-1}	urea	OTC	CF	80.6 ppb (24h mean)	increased A_{sat}	reduced A_{sat}, reduction in chlorophyll content	no sign. interactive effects for A_{sat} and g_s,	Xu et al. (2018)
Populus maximoviczii × P. berolinensis, Oxford clone	cuttings 1 season	0 kg N h^{-1}	80 kg N ha^{-1}	NH_4NO_3	free-air	AA	51.6 ppb, 66.7 ppb (24h mean)	reduced reactive oxygen species accumulation	visible injury, altered membrane integrity, oxidative damage	N reduced the O_3 sensitivity (mitigation effect)	Podda et al. (2019)
Liriodendron tulipifera	1-yr-old 18 weeks	58 µg g$^{-1\#}$	96 µg g$^{-1\#}$, 172 µg g$^{-1\#}$	NH_4NO_3	OTC	CF	56 ppb, 108 ppb (7h mean)	no effects on growth, increase in P_N and WUE	no effects on growth	no effects on growth	Tjoelker & Luxmoore (1991)
Populus deltoides cv. 55/56 x P. deltoides cv. Imperial	cuttings 1 season	0 kg N h^{-1}	50 kg N ha^{-1}	urea	OTC	CF	80.6 ppb (24h mean)	increase in leaf and aboveground biomass	reduced biomass of fine, roots, above-ground biomass, total biomass	interactive effects: O_3 decreased growth more severe under high N	Li et al. (2019)
Populus deltoides cv. 55/56 x P. deltoides cv. Imperial	cuttings 1 season	0 kg N h^{-1}	50 kg N ha^{-1}	urea	OTC	CF	80.6 ppb (24h mean)	increased A_{sat}	reduced A_{sat}, reduction in chlorophyll content	no sign. interactive effects for A_{sat} and g_s,	Xu et al. (2018)
Populus maximoviczii × P. berolinensis, Oxford clone	cuttings 1 season	0 kg N h^{-1}	80 kg N ha^{-1}	NH_4NO_3	free-air	AA	51.6 ppb, 66.7 ppb (24h mean)	reduced reactive oxygen species accumulation	visible injury, altered membrane integrity, oxidative damage	N reduced the O_3 sensitivity (mitigation effect)	Podda et al. (2019)

species	**age/ duration**	**N control**	**elevated N**	**N supply**	**facility**	**O_3 contr.**	**elevated O_3**	**N effect**	**O_3 effect**	**interactive effect**	**reference**
Liriodendron tulipifera	1-yr-old 18 weeks	58 µg g$^{-1\#}$	96 µg g$^{-1\#}$, 172 µg g$^{-1\#}$	NH_4NO_3	OTC	CF	56 ppb, 108 ppb (7h mean)	no effects on growth, stimulated photosynthesis and increased WUE	no effects on growth	no effects on growth	Tjoelker & Luxmoore (1991)
Populus deltoides cv. 55/56 x P. deltoides cv. Imperial	cuttings 1 season	0 kg N h^{-1}	50 kg N ha^{-1}	urea	OTC	CF	80.6 ppb (24h mean)	increase in leaf and aboveground biomass	reduced biomass of fine, roots, above-ground biomass, total biomass	interactive effects: O_3 decreased growth more severe under high N	Li et al. (2019)
Populus deltoides cv. 55/56 x P. deltoides cv. Imperial	cuttings 1 season	0 kg N h^{-1}	50 kg N ha^{-1}	urea	OTC	CF	80.6 ppb (24h mean)	increased A_{sat}	reduced A_{sat}, reduction in chlorophyll content	no sign. interactive effects for A_{sat} and g_s,	Xu et al. (2018)
Populus tremula	1-yr-old 2 seasons	up to 60 kg N h^{-1}	78 -140 kg N ha^{-1}	NPK +NH_4NO_3	free-air	AA	≈ 33 ppb (24h mean)	increases in P_N and growth	decreased P_N	N increased root mass in control plants but not in O_3-treated plants	Häikiö et al. (2007)
Populus tremula x P. tremuloides	1-yr-old 2 seasons	up to 60 kg N h^{-1}	78 -140 kg N ha^{-1}	NPK +NH_4NO_3	free-air	AA	≈ 33 ppb (24h mean)	increases in P_N	decreased P_N	no interactive effects	Häikiö et al. (2007)
Populus tremuloides	cuttings 2 seasons	0 kg N h^{-1}	80 kg N ha^{-1}	NH_4NO_3	FACE	AA	52 ppb, 67 ppb (24h mean)		increased senescence, sluggish closure of stomata at night	night-time stomatal opening was limited by N treatments in August	Hoshika et al. (2019)
Populus tremuloides	5-wks-old 12 weeks	3.5 g NPK l$^{-1\#}$	175 % 250 %	NPK	OTC	CF	73 ppb (9h mean)	maximum growth stimulation obtained at 175 % N fertilisation	reduced growth	reduced growth at high N, O_3-treated seedlings had greater shoot/root	Pell et al. (1995)

species	**age/ duration**	**N control**	**elevated N**	**N supply**	**facility**	**O_3 contr.**	**elevated O_3**	**N effect**	**O_3 effect**	**interactive effect**	**reference**
										ratios in each N treatment	
Populus tremuloides	cuttings 3 months	0 kg N h^{-1}	10 kg N ha^{-1}, 20 kg N ha^{-1}, 40 kg N ha^{-1}	nitric acid	OTC	CF	NF, CF+80 ppb (6h/day, 3d/week)	stimulated growth	foliar injury, trend towards growth reductions	O_3 effect was reversed in all N treatments	Karnosky et al. (1992)
Populus x euramericana	cuttings 16 weeks	105 mg N $l^{-1\#}$	315 mg N $l^{-1\#}$, 630 mg N $l^{-1\#}$	urea	contr. envir.	CF	60 ppb (24h mean)	reduced root/wood ratio, increase in chlorophyll and A_{amb}	reduced total biomass, reduced root/wood ratio, reduced A_{amb}	O_3-effects on growth and senescence decreased with increasing N	Schmutz et al. (1995)
Quercus ilex	2-yr-old 10 days	0 kg N h^{-1}	20 kg N ha^{-1}	NH_4NO_3	contr. envir.	CF	87 ppb (5 h mean)	increase in P_n and g_s	decrease in P_n and g_s	O_3-effects on P_n and g_s was less in N-treated plants	Fusaro et al. (2017)
Quercus robur	saplings 2 seasons	soil N	70 kg N ha^{-1}	NH_4NO_3	OTC	CF	31-43 ppb (24h mean)	stimulated growth	reduction in root biomass, number of leaves, g_s and V_{max}	N mitigated the O_3 impact on physiology and root biomass, opposite effect for the stem biomass	Marzuoli et al. (2016)

[#] related to volume or mass of growing medium

Table 9 **Single and interactive effects of O_3 and N on coniferous trees**

conc. = concentration, sig. significant(ly), neg. negative(ly), g_s = stomatal conductance, photosynthetic parameters: P_N = A = net photosynthetic rate, A_{total} = total net photosynthetic rate, A_{350} = net photosynthetic rate at 350 µmol mol^{-1} CO_2, A_{380} = net photosynthetic rate at 380 µmol mol^{-1} CO_2,, A_{max} = P_{nmax} = net photosynthetic rate at 1,600-1,500 µmol mol^{-1} CO_2, contr. env. = controlled environment, contr. = control.

species	**age/ duration**	**N control**	**elevated N**	**N supply**	**facility**	**O_3 contr.**	**elevated O_3**	**N effect**	**O_3 effect**	**interactive effect**	**reference**
Cryptomeria japonica	2-yr-old 2 seasons	0 kg N ha^{-1}	20 kg N ha^{-1}, 50 kg N ha^{-1}	NH_4NO_3	OTC	CF	42-43 ppb, 63-64 ppb, 83-85 ppb (24h mean)	stimulated growth (dry mass, height, and stem diameter) increased A_{total}	reduced total plant mass reduced, no effect on A_{total}	no sign interactive effects for growth and A_{total}	Watanabe et al. (2006)
Larix gmelinii var. japonica x L. kaempferi	1-yr-old 2 seasons	5.5 kg N ha^{-1}	50 kg N ha^{-1}	$(NH_4)_2SO_4$	OTC	NF	59.6 -64.3 ppb (24h mean)	increase in whole plant growth, increased A_{max}	no effect on growth, no effect on A_{max}, decrease in A_{380}	N increased O_3 sensitivity to some extent	Sugai et al. (2019b)
Larix kaempferi	1-yr-old 2 seasons	5.5 kg N ha^{-1}	50 kg N ha^{-1}	$(NH_4)_2SO_4$	OTC	NF	59.6 -64.3 ppb (24h mean)	minor effects: increased needle mass, decreased stem mass, increase in A_{380}	decreased growth, decreases in g_s and A_{max}	N mitigated the neg. effects of O_3 on total biomass	Sugai et al. (2019b)
Larix kaempferi	3-yr-old 2 seasons	0 kg N ha^{-1}	20 kg N ha^{-1}, 50 kg N ha^{-1}	NH_4NO_3	OTC	CF	42-43 ppb, 63-64 ppb, 83-85 ppb (24h mean)	no significant effects	reduced A_{total} reduction in stem diameter increment	O_3 effects in low N treatment but no effect in the highest N treatment	Watanabe et al. (2006)
Picea abies	2-yr-old 3 seasons	0 kg N ha^{-1}	20 kg N ha^{-1}, 40 kg N ha^{-1}, 80 kg N ha^{-1}	NH_4NO_3	free-air	CF!	35-47 ppb (daylight h mean)	stimulated growth, increased conc. of starch and soluble carbohydrate	negative effect on annual shoot elongation, reduction in starch conc.	antagonistic effect of O_3 and N on conc. of starch concentrations	Thomas et al. (2005)
Picea abies	4-yr-old 2 seasons	143 mg NH_4NO_3 l^{-1}	286 mg NH_4NO_3 l^{-1}	NH_4NO_3	free-air	AA	33.4 -35.6 ppb (24h mean)	stimulated growth increased conc. of chlorophyll a and b and carotenoids	increase in g_s	variable impact of O_3 on growth and g_s in all N treatments	Utriainen & Holopainen (2001a)

species	age/ duration	N control	elevated N	N supply	facility	O_3 contr.	elevated O_3	N effect	O_3 effect	interactive effect	reference
Picea rubens	saplings 4 months	3 g plant^{-1}	6 g plant^{-1}, 11 g plant^{-1}	NPK and NH_4NO_3	OTC	NF	88 ppb (12 h mean)	increased shoot length and conc. of sugars, decreased conc. of starch	minor effects	none	Amundson et al. (1995)
Pinus densiflora	1-yr-old 1 season	0 kg N ha^{-1}	135 kg N ha^{-1}, 405 kg N ha^{-1}	NH_4NO_3	sun-lit chambers	CF	60 ppb (8h mean)	reduction in weight of total plant fine roots, reduction in A_{350} and g_s	reduction in weight of total plant fine roots, reduction in A_{350} but no effect on g_s	the combined effects of O_3 and excess N were additive on biomass and photosynthesis	Nakaji & Izuta (2001)
Pinus densiflora	2-yr-old 2 seasons	0 kg N ha^{-1}	20 kg N ha^{-1}, 50 kg N ha^{-1}	NH_4NO_3	OTC	CF	42-43 ppb, 63-64 ppb, 83-85 ppb (24h mean)	no sign. effects on growth and A_{total}	reduction in A_{total}, height, and stem diameter	no sign. interactive effects	Watanabe et al. (2006)
Pinus densiflora	1-yr-old 8weeks	1.5 mM	4.5 mM	NH_4NO_3	OTC	CF	120 ppb (3h/day)	stimulated net photosynthesis	no effect on biomass, increases in shoot/root ratio and foliar sucrose content, decreased content of starch in roots	O_3 decreased stimulatory N effects, N increased resistance to O_3 by re-allocation of carbohydrates	Bak and Lee (2001)
Pinus sylvestris	1-yr-old 3 seasons	basic kg N ha^{-1}	120 kg N ha^{-1}	NPK+N	free-air	AA	35-42 ppb (14h mean)	increased the concentrations of some flavonoids	phenolic metabolites increased in current year stems, decreased in stems from the previous years	none	Ghimire et al. (2019)
Pinus sylvestris	3-yr-old 3 seasons	143 mg NH_4NO_3 l^{-1}	286 mg NH_4NO_3 l^{-1}	NH_4NO_{3-}	free-air	AA	35.6 -36.3 ppb (24h mean)	increased root, shoot, and whole-plant biomass	growth reductions and increased structural damage to needles	effects of elevated O_3 were most evident in the high N treatment	Utriainen & Holopainen (2001b)

species	**age/ duration**	**N control**	**elevated N**	**N supply**	**facility**	**O_3 contr.**	**elevated O_3**	**N effect**	**O_3 effect**	**interactive effect**	**reference**
Pinus sylvestris	4-yr-old 2 seasons	0 ppb (24h mean)	55, 110 ppb (24h mean)	NH_3	OTC	CF	36-40 ppb, 56-60 ppb (9h mean)	increased needle and stem biomass	reduction in needle biomass	no statistically sign. interactive effects	Dueck et al. (1998)
Pinus sylvestris	3-yr-old 11 weeks	0 ppb	57 ppb	NH_3	contr. env.	CF	55 ppb (9h mean)	gaseous NH_3 decreased mycorrhizal infection, no effects on biomass	no growth effects, decreased mycorrhizal infection	combined exposure lessened the reducing effects of single exposures to NH_3 and O_3	Perezsoba et al. (1995)
Pinus taeda	1-yr-old 18 weeks	58 µg g^{-1}	96,172 µg g^{-1}	NH_4NO_3	OTC	CF	56 ppb, 108 ppb (7h mean)	stimulated P_n rates, current-year needle biomass showed the largest response	decreased shoot weight more than root weight, resulting in higher root/shoot ratio	elevated O_3 lessened the growth stimulating effect by N on current-year needle growth more than additive	Tjoelker & Luxmoore (1991)
Pseudotsuga menziesii	3-yr-old 5 months	15 µg m^{-3}	49-54 µg m^{-3}	NH_3	contr. env.	CF	39-42 ppb (8h mean)	increase in P_n and chlorophyll content during the first half of experimental time	decrease in chlorophyll content and P_N, increased transpiration rate in the dark	no interactive effect on transpiration rate in the dark	Vanhove & Bossen (1994)

[#] related to volume or mass of growing medium

There is a trend that N may reduce the adverse effect of elevated O_3 and in some cases a shifting towards a fertilising effect could be noticed. This observation also applies to the exposure to gaseous NH_3. Moreover, exposure to elevated NH_3 was found to increase the susceptibility to drought and decreased the mycorrhizal infection of *Pinus sylvestris* and O_3 appeared to have an ameliorative effect on both effects (Dueck et al., 1998; Perezsoba et al., 1995). In seedlings of *Pseudotsuga menziesii* an increase in the transpiration rate in the dark was induced by both elevated NH_3 and O_3 although this did not occur when plants were exposed to elevated concentrations of both gases in combination (Vanhove and Bossen, 1994).

Mitigation of O_3 effects by addition of N was shown for *Betula pendula* (Pääkkönen and Holopainen, 1995), *Fraxinus ornus* (Fusaro et al., 2017), *Populus spec*. (Hoshika et al., 2019; Karnosky et al., 1992; Podda et al., 2019; Schmutz et al., 1995), *Quercus ilex* (Fusaro et al., 2017), *Larix kaempheri* (Sugai et al., 2019b; Watanabe et al., 2006), *Picea abies* (Thomas et al., 2005), *Pinus densiflora* (Bak and Lee, 2001), and *Pinus taeda* (Tjoelker and Luxmoore, 1991), and refers to impacts on growth, senescence, and gas exchange. Since both factors affect plants' growth and integrity in opposite ways, the mitigation of adverse effects of elevated O_3 can be seen as a result of their additive effects. If the growth stimulating effect of N prevails, the O_3 effect will be masked as shown in Figure 4 or for example by Karnosky et al. (1992), Thomas et al. (2005) and Tjoelker and Luxmoore (1991).

Most authors intended to identify, whether there is a statistically significant interaction between N and O_3 which is defined as an effect different from the additive effect. For example, for *Pinus sylvestris* Perezsoba et al. (1995) reported on a reduction in mycorrhizal infection induced by both NH_3 and O_3 when applied singly but the combined exposure lessened this effect. In summary, statistically significant interactions have hardly been detected. However, some publications report on an exacerbation of the adverse effect of elevated O_3 on growth by additional N supply. This was evident for broad-leaved trees (Table 8) as *Betula pendula* (Dai et al., 2019), *Carpinus betulus* (Marzuoli et al., 2018), *Fagus crenata* (Yamaguchi et al., 2007), *Populus hybrids* (Li et al., 2019; Zhang et al., 2018b), and *Quercus robur* (Marzuoli et al., 2016) and coniferous trees (Table 9) such as *Larix* hybrid (Sugai et al., 2019b), *Pinus densiflora* (Nakaji and Izuta, 2001), and *Pinus sylvestris* (Utriainen and Holopainen, 2001b). However, the direction of the response appeared to be determined by the amount of extra N (Zhang et al., 2018a; Zhang et al., 2018b), plant species (Dai et al., 2019; Fusaro et al., 2017; Sugai et al., 2019b; Watanabe et al., 2006), population (Häikiö et al., 2007) or the plant organ considered (Marzuoli et al., 2016).

At the community level, the negative impact of atmospheric N deposition manifests in increase in soil N, which is promoted by the accelerated production and abscission of N-rich foliage by elevated levels of O_3 (Harmens et al., 2017; Takemoto et al., 2001) and thus may facilitate nitrophilous plants.

2.2.4 Mechanisms and relevance of leaf traits

2.2.4.1 Gas exchange

Stomatal conductance is considered as a key factor in determining the O_3 flux into leaves and thus the stress potential of an external O_3 concentration (see chapter 1.1.1).

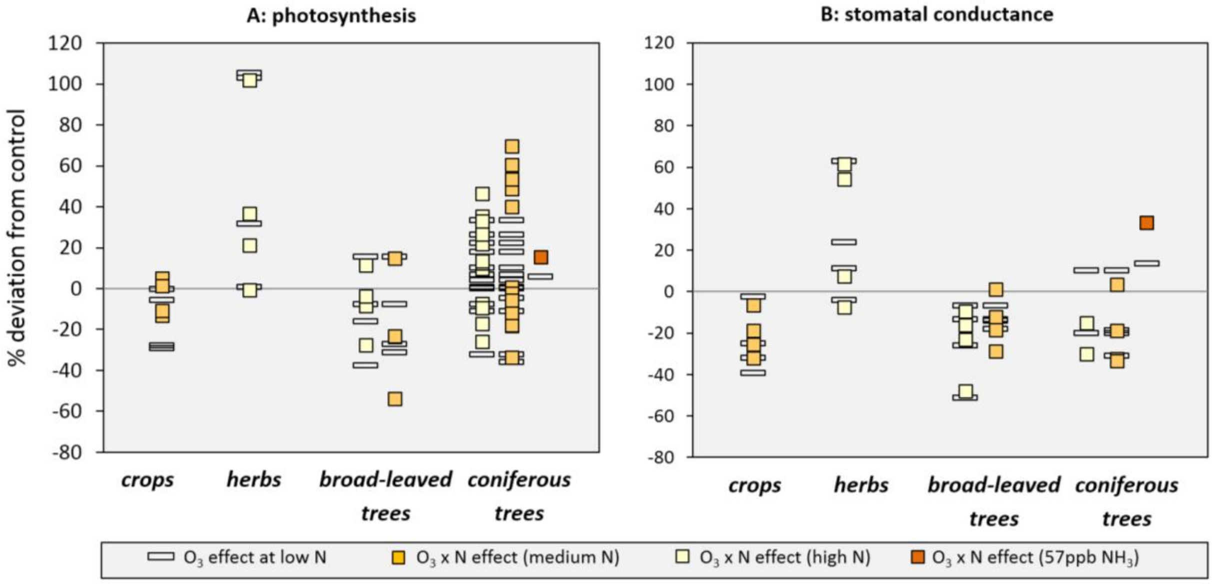
For two Indian wheat cultivars there was an interaction between O_3 × N × cultivar for most of the gas exchange parameters (Pandey et al., 2018): in the O_3 susceptible cultivar, stomatal conductance g_s was reduced by both N and O_3 whereas in the more tolerant species reduction in net photosynthesis and stomatal conductance were more pronounced under high N. This corresponds to the findings of Cardoso-Vilhena and Barnes (2001) who described a depression in net photosynthesis and relative growth rate under high N but not in the treatment with low N.

They attributed these findings to a lower cumulative O_3 uptake in the low N treatment. In a free-air exposure experiment, Chen et al. (2011) cultivated winter wheat at two levels of O_3 (ambient and 1.5 x ambient) and two levels of N supply (210 and 250 kg N ha^{-1}). These O_3 exposure levels have proven to be sufficient to induce significant reductions in net photosynthetic rate, stomatal conductance, content of chlorophyll, and soluble protein in the filling stage as well as biomass and yield at the final developmental stage. Enhancement of N supply above the recommended amount, however, mitigated O_3-induced effects on growth and gas exchange.

Reduction in stomatal conductance due to additional N supply has been described for wheat (Pandey et al., 2018), *Plantago major* (Whitfield et al., 1998), *Pinus densiflora* (Nakaji and Izuta, 2001) and *Picea abies*, (Utriainen and Holopainen, 2001a) but the opposite effect has been shown for *Betula pendula* (Dai et al., 2019) *and Quercus robur* (Marzuoli et al., 2016), where N seemed to weakly contrast the negative effect of O_3. Moreover, responsiveness of stomatal conductance to O_3 with or without N addition varied between single studies and species. For example, in a wheat cultivar susceptible to O_3 stomatal conductance g_s was reduced by both N and O_3 (Pandey et al., 2018) whereas it was increased by O_3 especially und high N in *Trifolium striatum* (Llusia et al., 2014) and *Picea abies* (Utriainen and Holopainen, 2001a). The disparity in effects is reflected by Figure 5 B.

Figure 5 Effects of O_3 on gas exchange parameters interacting with different levels of N

Species are *Cryptomeria japonica, Fraxinus ornus, Larix gmelinii* var. *japonica x L. kaempferi, Larix kaempferi, Liriodendron tulipifera, Pinus densiflora, P. sylvestris, P. taeda, Populus hybrid,* and *Quercus ilex.* The O_3-effect is expressed as percentage deviation from control (CF or NF with low N); for exposure conditions and references see Table 24, chapter A.1.1



Source: own illustration, Thünen Institute

Transpiration in the dark is mentioned to influence water use in plants. Nocturnal stomatal conductance was shown to be increased by O_3 in Douglas fir (Vanhove and Bossen, 1994) and hybrid poplar (Hoshika et al., 2019) and was proposed to reflect the need to improve N acquisition to cope with O_3 stress (Hoshika et al., 2019). In firs, a higher night-time stomatal conductance was also induced by NH_3 but not when plants were exposed to both gases simultaneously (Vanhove and Bossen, 1994). Moreover, the mitigation of detrimental O_3 effects mediated by additional N supply has been shown at the photosynthetic level for *Populus tremuloides* (Zhang et al., 2018b) and *Quercus kelloggii* (Handley and Grulke, 2008), while N may exacerbated O_3-induced reductions in net photosynthesis in *Fagus crenata* (Yamaguchi et al., 2007) or dark respiration in a *Populus* hybrid (Zhang et al., 2018b). A compilation of all available data on crops, herbs, and trees in Figure 5 A does not indicate a clear trend.

2.2.4.2 Allocation processes

Plants of hybrid poplar grown under N limitation have been shown to respond to elevated O_3 by re-allocating N from roots or older leaves to young developing leaves and consequently exhibited accelerated senescence (Bielenberg et al., 2001; Pell et al., 1995; Schmutz et al., 1995; Tjoelker and Luxmoore, 1991). This compensatory response is proposed to be capable to avoid losses in biomass when growth is restricted due to N limitation but is thought to be suppressed by additional N supply. As a result, adverse growth effects due to O_3 became more evident when N supply increased (e.g. Pell et al., 1995; Sanz et al., 2007). A second compensatory response to O_3 which has been observed frequently was to increase shoot/root ratio which was enhanced by high N supply indicating a higher priority of roots for carbon allocation at the expense of foliar biomass (Pääkkönen and Holopainen, 1995; Pell et al., 1995; Tjoelker and Luxmoore, 1991). On the other hand, Bak and Lee (2001) reported on a reduced responsiveness to O_3 of *Pinus densiflora* caused by N addition via re-allocation of increased carbohydrates.

Bassin et al. (2009) hypothesised that the interacting effect of N may be mediated by modifying plants' functional traits. However, the magnitude of the response to both O_3 and N could not be related to species-specific traits such as specific leaf area (SLA) on subalpine plants. In some studies on trees, it has been demonstrated that addition of N may enhance the constitutive level of antioxidant activity which is thought to provide a potential protection against O_3 related impacts. Depending on the plant species' strategy several mechanisms are hypothesised which for example involve an enzymatic antioxidant response or the stimulation of the emission of volatile organic compounds (Fusaro et al., 2017; Podda et al., 2019).

2.3 Interactive effects of atmospheric NO_2 and O_3

In addition to the modifying effect of enhanced N-inputs and the resulting increase in the availability of N in the soil, respectively, to plant responses to O_3, plants are directly exposed to gaseous mixtures of O_3 and NO_2. Because both O_3 and NO_2 are constituents of photochemical smog this combination must be regarded as one of the most common under field conditions. Interactive effects of O_3 and NO_2 have been studied rather intensively between c. 1980 and 2000 when higher atmospheric concentrations of both O_3 *and* NO_2 prevailed over large regions across Europe. However, the majority of these studies are not very useful for impact assessments of present-day pollution climates because of the unrealistic exposure levels and the atypical exposure profiles used. For example, a simultaneous occurrence of O_3 and NO_2 is rather unusual, i.e. concentrations of NO_2 and O_3 vary during the day in patterns that normally result in sequential exposures. Research on the combined effects of O_3 and NO_2 were reviewed in detail by Davison & Barnes (1998), Fangmeier et al. (2002) and Fangmeier & Bender (2002), and the conclusions made there are still relevant:

1. Short-term exposures to relatively high concentrations of both pollutants may result in synergistic (more-than-additive) effects on plants.
2. More realistic studies using sequential exposures of near-ambient concentrations of O_3 and NO_2 showed either no significant interactions (i.e. additive effects predominate) or the mode of interaction was antagonistic, i.e. the presence of NO_2 reduced the negative effects of O_3, suggesting that plants were able to utilize the additional nitrogen source.

Nitrogen dioxide concentrations have decreased significantly in recent decades in Germany and the trend in reduction over the last ten years continues. From 2000 through 2019, the average annual concentration at all monitoring stations in rural areas was about 10 µg/m^3 (Umweltbundesamt 2020). The critical level for nitrogen oxides (NO and NO_2 added, expressed as NO_2) for protection of vegetation in Europe was set at 30 µg/m^3 as annual mean (CLRTAP 2017). Against this background, direct phytotoxicity of gaseous NO_2 seems rather unlikely. The evidence is sufficient to conclude that NO_2 in combination with O_3 mitigates rather than exacerbates the negative effects of O_3.

Major results: O_3 x N

Since both factors are known to affect plant growth in the opposite way it is assumed that in combination either adverse O_3 effects are mitigated or the stimulating effect of N is weakened.

For the majority of the 37 different species tested a modification of the response to O_3 by enhanced N availability has been reported.

The nature and extent of this modifying effect varies greatly and seems to depend on the amount of N supply and plant strategy.

It frequently has been shown that additional N supply may counteract adverse O_3 effects. But there is also abundant evidence that it may enhance the adverse effect of elevated O_3 on photosynthesis, growth, reproduction, and yield, i.e. high N input can make plants more susceptible to O_3.

The resulting effect is partly attributed to an interference with plant's internal compensatory mechanisms and allocation processes induced by O_3.

By inducing accelerated senescence and leaf abscission O_3 itself may influence N cycling in the soil-plant system with consequences for the N availability.

2.4 References

Aerts, R., De Caluwe, H., and Beltman, B. (2003). Is the relation between nutrient supply and biodiversity co-determined by the type of nutrient limitation? Oikos 101, 489-498.

Amundson, R. G., Kohut, R. J., and Laurence, J. A. (1995). Influence of foliar n on foliar soluble sugars and starch of red spruce saplings exposed to ambient and elevated ozone. Tree Physiology 15, 167-174.

Arbaugh, M., Bytnerowicz, A., Grulke, N., Fenn, M., Poth, M., Temple, P., and Miller, P. (2003). Photochemical smog effects in mixed conifer forests along a natural gradient of ozone and nitrogen deposition in the San Bernardino Mountains. Environment International 29, 401-406.

Bak, J. H., and Lee, K. J. (2001). Effects of nitrogen and phosphorus fertilization on the growth, carbohydrate contents and photosynthesis of Pinus densiflora seedlings exposed to ozone in an open-top chamber. Journal of Korean Forestry Society 90, 306-313.

Barnes, J.D. & A.R. Wellburn 1998. Air pollutant combinations. In: DeKok, L.J. & I. Stulen (eds.), Responses of plant metabolism to air pollution and global change, pp. 147-164. Backhuys Publishers, Leiden.

Bassin, S., Blanke, V., Volk, M., and Fuhrer, J. (2017). Ozone and Nitrogen Effects on Juvenile Subalpine Plants: Complex Interactions with Species and Colonization by Arbuscular Mycorrhizal Fungi (AMF). Water Air and Soil Pollution 228.

Bassin, S., Volk, M., and Fuhrer, J. (2013). Species Composition of Subalpine Grassland is Sensitive to Nitrogen Deposition, but Not to Ozone, After Seven Years of Treatment. Ecosystems 16, 1105-1117.

Bassin, S., Volk, M., Suter, M., Buchmann, N., and Fuhrer, J. (2007). Nitrogen deposition but not ozone affects productivity and community composition of subalpine grassland after 3 yr of treatment. New Phytologist 175, 523-534.

Bassin, S., Werner, R. A., Sorgel, K., Volk, M., Buchmann, N., and Fuhrer, J. (2009). Effects of combined ozone and nitrogen deposition on the in situ properties of eleven key plant species of a subalpine pasture. Oecologia 158, 747-756.

Bielenberg, D. G., Lynch, J. R., and Pell, E. J. (2001). A decline in nitrogen availability affects plant responses to ozone. New Phytologist 151, 413-425.

Bobbink, R. (1998). Impacts of tropospheric ozone and airborne nitrogenous pollutants on natural and seminatural ecosystems: a commentary. New Phytologist 139, 161-168.

Braun, S., Achermann, B., De Marco, A., Pleijel, H., Karlsson, P. E., Rihm, B., Schindler, C., and Paoletti, E. (2017a). Epidemiological analysis of ozone and nitrogen impacts on vegetation - Critical evaluation and recommendations. Science of the Total Environment 603, 785-792.

Braun, S., Rihm, B., Schindler, C., and Fluckiger, W. (1999). Growth of mature beech in relation to ozone and nitrogen deposition: An epidemiological approach. Water Air and Soil Pollution 116, 357-364.

Braun, S., Schindler, C., and Rihm, B. (2017b). Growth trends of beech and Norway spruce in Switzerland: The role of nitrogen deposition, ozone, mineral nutrition and climate. Science of the Total Environment 599, 637-646.

Brewster, C., Stevens, C., and McAinsh, M. (2018). Wheat's wild relatives vary in their response to nitrogen and ozone. Annals of Applied Biology 173, 154-163.

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

Bycenkiene, S., Plauskaite, K., Augustaitiene, I., Augustaitis, A., Baumgarten, M., Girgzdiene, R., Jasineviciene, D., Kliucius, A., Marozas, V., Matyssek, R., Mozgeris, G., Pivoras, G., and Ulevicius, V. (2018). Integrated impact of sulphur and nitrogen deposition and ozone on forest ecosystems in Lithuania, 1995-2015. Proceedings of the Estonian Academy of Sciences 67, 315-324.

Bytnerowicz, A. (2002). Physiological/ecological interactions between ozone and nitrogen deposition in forest ecosystems. Phyton-Annales Rei Botanicae 42, 13-28.

Calatayud, A., Pomares, F., and Barreno, E. (2006). Interactions between nitrogen fertilization and ozone in watermelon cultivar Reina de Corazones in open-top chambers. Effects on chlorophyll alpha fluorescence, lipid peroxidation, and yield. Photosynthetica 44, 93-101.

Calvete-Sogo, H., González-Fernández, I., Sanz, J., Elvira, S., Alonso, R., García-Gómez, H., Ibáñez-Ruiz, M. A., and Bermejo-Bermejo, V. (2016). Heterogeneous responses to ozone and nitrogen alter the species composition of Mediterranean annual pastures. Oecologia, 1-13.

Cao, Y. H., Shi, Y., Sun, X. C., Lu, C. Y., and Liu, X. Y. (2018). Effects of elevated ozone on the contribution of nitrogen rhizodeposition by spring wheat to different soil N pools. Plant and Soil 425, 321-333.

Cardoso-Vilhena, J., and Barnes, J. (2001). Does nitrogen supply affect the response of wheat (Triticum aestivum cv. Hanno) to the combination of elevated CO2 and O-3? Journal of Experimental Botany 52, 1901-1911.

Carriero, G., Brunetti, C., Fares, S., Hayes, F., Hoshika, Y., Mills, G., Tattini, M., and Paoletti, E. (2016). BVOC responses to realistic nitrogen fertilization and ozone exposure in silver birch. Environmental Pollution 213, 988-995.

Chen, J., Zeng, Q., Zhu, J.-G., Liu, G., Cao, J.-L., Xie, Z.-B., Tang, H.-Y., and Kazuhiko, K. (2011). Nitrogen supply mitigates the effects of elevated O-3 on photosynthesis and yield in wheat. Chinese Journal of Plant Ecology 35, 523-530.

CLRTAP 2017. Manual on methodologies and criteria for modelling and mapping critical loads and levels and air pollution effects, risks and trends. Chapter 3: Mapping critical levels for vegetation. http://icpvegetation.ac.uk

Dai, L. L., Hayes, F., Sharps, K., Harmens, H., and Mills, G. (2019). Nitrogen availability does not affect ozone flux-effect relationships for biomass in birch (Betula pendula) saplings. Science of the Total Environment 660, 1038-1046.

Davison, A. W., and Barnes, J. D. (1998). Effects of ozone on wild plants. New Phytologist 139, 135-151.

Dise, N. B., Ashmore, M., Belyazid, S., Bleeker, A., Bobbink, R., de Vries, W., Erisman, J. W., Spranger, T., Stevens, C. J., and van den Bergen, L. (2011). Nitrogen as a threat to European terrestrial biodiversity. *In* "The European Nitrogen Assessment - Sources, Effects and Policy Perspecives" (M. A. Sutton, C. M. Howard, J. W. Erisman, G. Billen, A. Bleeker, P. Grennfelt, H. van Grinsven and B. Grizzeftti, eds.), pp. 463 -494. Cambridge University Press.

Duarte, N., Pardo, L. H., and Robin-Abbott, M. J. (2013). Susceptibility of Forests in the Northeastern USA to Nitrogen and Sulfur Deposition: Critical Load Exceedance and Forest Health. Water Air and Soil Pollution 224.

Dueck, T. A., Zuin, A., and Elderson, J. (1998). Influence of ammonia and ozone on growth and drought sensitivity of Pinus sylvestris. Atmospheric Environment 32, 545-550.

Eatough Jones, M., and Paine, T. D. (2002). Changes in insect herbivore communities along an ozone and nitrogen deposition gradient in the San Bernardino Mountains. Ecological Society of America Annual Meeting Abstracts 87, 345-345.

Etzold, S., Ferretti, M., Reinds, G. J., Solberg, S., Gessler, A., Waldner, P., Schaub, M., Simpson, D., Benham, S., Hansen, K., et al. (2020). Nitrogen deposition is the most important environmental driver of growth of pure, even-aged and managed European forests. Forest Ecology and Management 458.

Fangmeier, A., and Bender, J. (2002). Air pollutant combinations - Significance for future impact assessments on vegetation. Phyton-Annales Rei Botanicae 42, 65-71.

Fangmeier, A., Bender, J., Weigel, H.J. & H.J. Jäger (2002). Effects of pollutant mixtures. In: Bell, J.N.B. & M. Treshow (eds.), Air pollution and plant life, pp. 251-272. John Wiley & Sons, Chichester.

Fangmeier, A., Gruters, U., Hertstein, U., SandhageHofmann, A., Vermehren, B., and Jäger, H. J. (1996). Effects of elevated CO2, nitrogen supply and tropospheric ozone on spring wheat .1. Growth and yield. Environmental Pollution 91, 381-390.

Feng, Z. Z., Shang, B., Li, Z. Z., Calatayud, V., and Agathokleous, E. (2019). Ozone will remain a threat for plants independently of nitrogen load. Functional Ecology 33, 1854-1870.

Fenn, M. E., Poth, M. A., Bytnerowicz, A., Sickman, J. O., and Takemoto, B. K. (2003). "Effects of ozone, nitrogen deposition, and other stressors on montane ecosystems in the Sierra Nevada."

Flechard, C. R., Massad, R. S., Loubet, B., Personne, E., Simpson, D., Bash, J. O., Cooter, E. J., Nemitz, E., and Sutton, M. A. (2013). Advances in understanding, models and parameterizations of biosphere-atmosphere ammonia exchange. Biogeosciences 10, 5183-5225.

Fusaro, L., Palma, A., Salvatori, E., Basile, A., Maresca, V., Karam, E. A., and Manes, F. (2017). Functional indicators of response mechanisms to nitrogen deposition, ozone, and their interaction in two Mediterranean tree species. Plos One 12.

Ghimire, R. P., Kasurinen, A., Haikio, E., Holopainen, J. K., Julkunen-Tiitto, R., Holopainen, T., and Kivimaenpaa, M. (2019). Combined effects of elevated ozone, temperature, and nitrogen on stem phenolic concentrations of Scots pine (Pinus sylvestris) seedlings. Canadian Journal of Forest Research 49, 246-255.

Grulke, N. E., and Balduman, L. (1999). Deciduous conifers: High N deposition and O-3 exposure effects on growth and biomass allocation in ponderosa pine. Water Air and Soil Pollution 116, 235-248.

Grulke, N. E., Andersen, C. P., Fenn, M. E., and Miller, P. R. (1998). Ozone exposure and nitrogen deposition lowers root biomass of ponderosa pine in the San Bernardino Mountains, California. Environmental Pollution 103, 63-73.

Häikiö, E., Freiwald, V., Silfver, T., Beuker, E., Holopainen, T., and Oksanen, E. (2007). Impacts of elevated ozone and nitrogen on growth and photosynthesis of European aspen (Populus tremula) and hybrid aspen (P. tremula × Populus tremuloides) clones. Canadian Journal of Forest Research 37, 2326-2336.

Han, S., Kim, P.-G., Lee, J., and Kim, P. (2009). Effects of Fertilization on Physiological Parameters in American Sycamore (Platanus occidentalis) during Ozone Stress and Recovery Phase. Journal of Ecology and Environment 32, 149-158.

Handley, T., and Grulke, N. E. (2008). Interactive effects of O3 exposure on California black oak (Quercus kelloggii Newb.) seedlings with and without N amendment. Environmental Pollution 156, 53-60.

Harmens, H., Hayes, F., Sharps, K., Mills, G., and Calatayud, V. (2017). Leaf traits and photosynthetic responses of Betula pendula saplings to a range of ground-level ozone concentrations at a range of nitrogen loads. Journal of Plant Physiology 211, 42-52.

Hayes, F., Lloyd, B., Mills, G., Jones, L., Dore, A. J., Carnell, E., Vieno, M., Dise, N., and Fenner, N. (2019). Impact of long-term nitrogen deposition on the response of dune grassland ecosystems to elevated summer ozone. Environmental Pollution 253, 821-830.

Heagle, A. S., Miller, J. E., Booker, F. L., and Pursley, W. A. (1999). Ozone stress, carbon dioxide enrichment, and nitrogen fertility interactions in cotton. Crop Science 39, 731-741.

Hoshika, Y., De Carlo, A., Baraldi, R., Neri, L., Carrari, E., Agathokleous, E., Zhang, L., Fares, S., and Paoletti, E. (2019). Ozone-induced impairment of night-time stomatal closure in O-3-sensitive poplar clone is affected by nitrogen but not by phosphorus enrichment. Science of the Total Environment 692, 713-722.

Jones, M. L. M., Hodges, G., and Mills, G. (2010). Nitrogen mediates above-ground effects of ozone but not below-ground effects in a rhizomatous sedge. Environmental Pollution 158, 559-565.

Karnosky, D. F., Witter, J. A., Gagnon, Z. E., and Reed, D. D. (1992). Effects of genotype on the response of Populus-tremuloides Michx to ozone and nitrogen deposition. Water Air and Soil Pollution 62, 189-199.

Landolt, W., GunthardtGoerg, M. S., Pfenninger, I., Einig, W., Hampp, R., Maurer, S., and Matyssek, R. (1997). Effect of fertilization on ozone-induced changes in the metabolism of birch (Betula pendula) leaves. New Phytologist 137, 389-397.

Li, P., Yin, R. B., Shang, B., Agathokleous, E., Zhou, H. M., and Feng, Z. Z. (2020). Interactive effects of ozone exposure and nitrogen addition on tree root traits and biomass allocation pattern: An experimental case study and a literature meta-analysis. Science of the Total Environment 710.

Li, P., Zhou, H. M., Xu, Y. S., Shang, B., and Feng, Z. Z. (2019). The effects of elevated ozone on the accumulation and allocation of poplar biomass depend strongly on water and nitrogen availability. Science of the Total Environment 665, 929-936.

Llusia, J., Bermejo-Bermejo, V., Calvete-Sogo, H., and Penuelas, J. (2014). Decreased rates of terpene emissions in Ornithopus compressus L. and Trifolium striatum L. by ozone exposure and nitrogen fertilization. Environmental Pollution 194, 69-77.

Luo, K., Zhu, J., Liu, G., Tang, H., Li, C., and Zeng, Q. (2013). Responses of Dry Matter Production and Distribution in Rice (Oryza saliva L.) to Ozone and High Nitrogen Supply. Chinese Journal of Applied and Environmental Biology 19, 286-292.

Marzuoli, R., Monga, R., Finco, A., and Gerosa, G. (2016). Biomass and physiological responses of Quercus robur (L.) young trees during 2 years of treatments with different levels of ozone and nitrogen wet deposition. Trees-Structure and Function 30, 1995-2010.

Marzuoli, R., Monga, R., Finco, A., Chiesa, M., and Gerosa, G. (2018). Increased nitrogen wet deposition triggers negative effects of ozone on the biomass production of Carpinus betulus L. young trees. Environmental and Experimental Botany 152, 128-136.

Massad, R. S., Tuzet, A., Loubet, B., Perrier, A., and Cellier, P. (2010). Model of stomatal ammonia compensation point (STAMP) in relation to the plant nitrogen and carbon metabolisms and environmental conditions. Ecological Modelling 221, 479-494.

Matyssek, R., Maurer, S., Gunthardt-Goerg, M. S., Landolt, W., Saurer, M., and Polle, A. (1997). Nutrition determines the 'strategy' of Betula pendula for coping with ozone stress. Phyton-Annales Rei Botanicae 37, 157-167.

Maurer, S., and Matyssek, R. (1997). Nutrition and the ozone sensitivity of birch (Betula pendula) .2. Carbon balance, water-use efficiency and nutritional statue of the whole plant. Trees-Structure and Function 12, 11-20.

Maurer, S., Matyssek, R., GunthardtGoerg, M. S., Landolt, W., and Einig, W. (1997). Nutrition and the ozone sensitivity of birch (Betula pendula) .1. Responses at the leaf level. Trees-Structure and Function 12, 1-10.

Nakaji, T., and Izuta, T. (2001). Effects of ozone and/or excess soil nitrogen on growth, needle gas exchange rates and rubisco contents of Pinus densiflora seedlings. Water Air and Soil Pollution 130, 971-976.

Pääkkönen, E., and Holopainen, T. (1995). Influence of nitrogen supply on the response of clones of birch (Betula-pendula Roth) to ozone. New Phytologist 129, 595-603.

Pandey, A. K., Ghosh, A., Agrawal, M., and Agrawal, S. B. (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, 59-68.

Pell, E. J., Sinn, J. P., and Johansen, C. V. (1995). Nitrogen supply as a limiting factor determining the sensitivity of Populus-tremuloides Michx to ozone stress. New Phytologist 130, 437-446.

Pérez-Ramos, I. M., Roumet, C., Cruz, P., Blanchard, A., Autran, P., and Garnier, E. (2012). Evidence for a ‘plant community economics spectrum’ driven by nutrient and water limitations in a Mediterranean rangeland of southern France. Journal of Ecology 100, 1315-1327.

Perezsoba, M., Dueck, T. A., Puppi, G., and Kuiper, P. J. C. (1995). Interactions of elevated CO2, NH3 and O-3 on mycorrhizal infection, gas-exchange and n-metabolism in saplings of scots pine. Plant and Soil 176, 107-116.

Podda, A., Pisuttu, C., Hoshika, Y., Pellegrini, E., Carrari, E., Lorenzini, G., Nali, C., Cotrozzi, L., Zhang, L., Baraldi, R., Neri, L., and Paoletti, E. (2019). Can nutrient fertilization mitigate the effects of ozone exposure on an ozone-sensitive poplar clone? Science of the Total Environment 657, 340-350.

Sanz, J., Bermejo, V., Gimeno, B. S., Elvira, S., and Alonso, R. (2007). Ozone sensitivity of the Mediterranean terophyte Trifolium striatum is modulated by soil nitrogen content. Atmospheric Environment 41, 8952-8962.

Sanz, J., Calvete-Sogo, H., Gonzalez-Fernandez, I., Lin, J., Garcia-Gomez, H., Muntifering, R., Alonso, R., and Bermejo-Bermejo, V. (2015). Foliar senescence is the most sensitive response to ozone in Bromus hordeaceus and is modulated by nitrogen input. Grass and Forage Science 70, 71-84.

Sanz, J., Gonzalez-Fernandez, I., Calvete-Sogo, H., Lin, J. S., Alonso, R., Muntifering, R., and Bermejo, V. (2014). Ozone and nitrogen effects on yield and nutritive quality of the annual legume Trifolium cherleri. Atmospheric Environment 94, 765-772.

Sanz, J., Muntifering, R. B., Bermejo, V., Gimeno, B. S., and Elvira, S. (2005). Ozone and increased nitrogen supply effects on the yield and nutritive quality of Trifolium subterraneum. Atmospheric Environment 39, 5899-5907.

Schmutz, P., Bucher, J. B., GunthardtGoerg, M. S., Tarjan, D., and Landolt, W. (1995). Response of poplar to ozone alone and in combination with NO2 at different nitrogen fertilization levels. Phyton-Annales Rei Botanicae 35, 269-289.

Simpson, D., Arneth, A., Mills, G., Solberg, S., and Uddling, J. (2014). Ozone - the persistent menace: interactions with the N cycle and climate change. Current Opinion in Environmental Sustainability 9-10, 9-19.

Singh, P., Agrawal, M., and Agrawal, S. B. (2009). Evaluation of physiological, growth and yield responses of a tropical oil crop (Brassica campestris L. var. Kranti) under ambient ozone pollution at varying NPK levels. Environmental Pollution 157, 871-880.

Singh, P., Agrawal, M., and Agrawal, S. B. (2011). Differences in Ozone Sensitivity at Different NPK Levels of Three Tropical Varieties of Mustard (Brassica campestris L.): Photosynthetic Pigments, Metabolites, and Antioxidants. Water Air and Soil Pollution 214, 435-450.

Sugai, T., Kitao, M., Watanabe, T., and Koike, T. (2019a). Can needle nitrogen content explain the interspecific difference in ozone sensitivities of photosynthesis between Japanese larch (Larix kaempferi) and Sakhalin fir (Abies sachalinensis)? Photosynthetica 57, 540-547.

Sugai, T., Watanabe, T., Kita, K., and Koike, T. (2019b). Nitrogen loading increases the ozone sensitivity of larch seedlings with higher sensitivity to nitrogen loading. Science of the Total Environment 663, 587-595.

Takemoto, B. K., Bytnerowicz, A., and Fenn, M. E. (2001). Current and future effects of ozone and atmospheric nitrogen deposition on California's mixed conifer forests. Forest Ecology and Management 144, 159-173.

Tatsumi, K., Abiko, T., Kinose, Y., Inagaki, S., and Izuta, T. (2019). Effects of ozone on the growth and yield of rice (Oryza sativa L.) under different nitrogen fertilization regimes. Environmental Science and Pollution Research 26, 32103-32113.

Thomas, V. F. D., Braun, S., and Fluckiger, W. (2005). Effects of simultaneous ozone exposure and nitrogen loads on carbohydrate concentrations, biomass, and growth of young spruce trees (Picea abies). Environmental Pollution 137, 507-516.

Thomas, V. F. D., Hiltbrunner, E., Braun, S., and Fluckiger, W. (2002). Changes in root starch contents of mature beech (Fagus sylvatica L.) along an ozone and nitrogen gradient in Switzerland. Phyton-Annales Rei Botanicae 42, 223-228.

Tjoelker, M. G., and Luxmoore, R. J. (1991). Soil-nitrogen and chronic ozone stress influence physiology, growth and nutrient status of Pinus-taeda-L and Liriodendron-tulipifera-L seedlings. New Phytologist 119, 69-81.

Tonneijck, A. E. G., and van Dijk, C. J. (1998). Responses of bean (Phaseolus vulgaris L. cv. Pros) to chronic ozone exposure at two levels of atmospheric ammonia. Environmental Pollution 99, 45-51.

Umweltbundesamt 2020. Air quality 2019. Preliminary evaluation. www.umweltbundesamt.de/publikationen/air-quality-2019

Utriainen, J., and Holopainen, T. (2001a). Influence of nitrogen and phosphorus availability and ozone stress on Norway spruce seedlings. Tree Physiology 21, 447-456.

Utriainen, J., and Holopainen, T. (2001b). Nitrogen availability modifies the ozone responses of Scots pine seedlings exposed in an open-field system. Tree Physiology 21, 1205-1213.

Vanhove, L. W. A., and Bossen, M. E. (1994). Physiological-effects of 5 months exposure to low concentrations of O-3 and NH3 on douglas-fir (Pseudotsuga-menziesii). Physiologia Plantarum 92, 140-148.

Volin, J. C., and Reich, P. B. (1996). Interaction of elevated CO2 and O-3 on growth, photosynthesis and respiration of three perennial species grown in low and high nitrogen. Physiologia Plantarum 97, 674-684.

Volk, M., Obrist, D., Novak, K., Giger, R., Bassin, S., and Fuhrer, J. (2011). Subalpine grassland carbon dioxide fluxes indicate substantial carbon losses under increased nitrogen deposition, but not at elevated ozone concentration. Global Change Biology 17, 366-376.

vonTiedemann, A. (1996). Single and combined effects of nitrogen fertilization and ozone on fungal leaf diseases on wheat. Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz 103, 409-419.

Wang, J., Hayes, F., Chadwick, D. R., Hill, P. W., Mills, G., and Jones, D. L. (2019). Short-term responses of greenhouse gas emissions and ecosystem carbon fluxes to elevated ozone and N fertilization in a temperate grassland. Atmospheric Environment 211, 204-213.

Watanabe, M., Hoshika, Y., Inada, N., and Koike, T. (2018). Photosynthetic activity in relation to a gradient of leaf nitrogen content within a canopy of Siebold's beech and Japanese oak saplings under elevated ozone. Science of the Total Environment 636, 1455-1462.

Watanabe, M., Yamaguchi, M., Iwasaki, M., Matsuo, N., Naba, J., Tabe, C., Matsumura, H., Kohno, Y., and Izuta, T. (2006). Effects of ozone and/or nitrogen load on the growth of Larix kaempferi, Pinus densiflora and Cryptomeria japonica seedlings. Journal of Japan Society for Atmospheric Environment / Taiki Kankyo Gakkaishi 41, 320-334.

Watanabe, M., Yamaguchi, M., Matsumura, H., Kohno, Y., and Izuta, T. (2012). Risk assessment of ozone impact on Fagus crenata in Japan: consideration of atmospheric nitrogen deposition. European Journal of Forest Research 131, 475-484.

Whitfield, C. P., Davison, A. W., and Ashenden, T. W. (1998). The effects of nutrient limitation on the response of Plantago major to ozone. New Phytologist 140, 219-230.

Wyness, K., Mills, G., Jones, L., Barnes, J. D., and Jones, D. L. (2011). Enhanced nitrogen deposition exacerbates the negative effect of increasing background ozone in Dactylis glomerata, but not Ranunculus acris. Environmental Pollution 159, 2493-2499.

Xu, W., Shang, B., Xu, Y. S., Yuan, X. Y., Dore, A. J., Zhao, Y. H., Massad, R. S., and Feng, Z. Z. (2018). Effects of elevated ozone concentration and nitrogen addition on ammonia stomatal compensation point in a poplar clone. Environmental Pollution 238, 760-770.

Yamaguchi, M., Watanabe, M., Iwasaki, M., Tabe, C., Matsumura, H., Kohno, Y., and Izuta, T. (2007). Growth and photosynthetic responses of Fagus crenata seedlings to O-3 under different nitrogen loads. Trees-Structure and Function 21, 707-718.

Zhang, L., Hoshika, Y., Carrari, E., Badea, O., and Paoletti, E. (2018a). Ozone risk assessment is affected by nutrient availability: Evidence from a simulation experiment under free air controlled exposure (FACE). Environmental Pollution 238, 812-822.

Zhang, L., Hoshika, Y., Carrari, E., Cotrozzi, L., Pellegrini, E., and Paoletti, E. (2018b). Effects of nitrogen and phosphorus imbalance on photosynthetic traits of poplar Oxford clone under ozone pollution. Journal of Plant Research 131, 915-924.

3 The modifying factor carbon dioxide (CO_2)

3.1 Effects of elevated CO_2 on plants

During the past decades, numerous experimental studies on herbaceous and woody plants have clearly demonstrated that plant growth is generally stimulated when atmospheric CO_2 concentration increase. This effect on growth parameters goes along with an increase in net photosynthesis. Because the enzyme Ribulose-1,5-bisphosphate carboxylase (Rubisco) is not saturated by present atmospheric concentration of CO_2, the velocity of carboxylation is increased by elevated CO_2. Moreover, CO_2 is competitively inhibiting the oxygenation reaction of this enzyme which lowers the rate of photorespiration. For wheat, for example, Wang et al. (2013) estimated an increase in photosynthesis by about 33% and a decline in total activity of the enzyme Rubisco by 26%, while aboveground biomass increased by 28%.

3.1.1 Quantitative effects on growth and gas exchange

By means of meta-analyses, Jablonski et al. (2002) and Wang et al. (2015) estimated an increase in seed mass by 25 and 29%, respectively due to elevated CO_2 (500 – 800 ppm) across a number of crop and wild species (Table 10). Reproductive allocation in response to enhanced CO_2 is shown to be more pronounced in crops than in wild plants, although stimulation in vegetative growth has been evidenced for both groups. For a 250 ppm increase in CO_2 concentration an average increase in yield of 22.0% has been calculated for a group of vegetables and legumes whereat leafy vegetables are shown to be less responsive than legumes (9.0% vs. 28.1%, Scheelbeek et al., 2018). Quantitative meta-analyses ascertained relative yield effects of elevated CO_2 (~450-800 ppm) of +24% for wheat (Wang et al., 2013) and +23% for rice (Ainsworth, 2008). Effects on wild plant species alone, however, amounted to less than 20% with grasses being less responsive Wang et al. (2015).

As shown by recent meta-analyses reduction in stomatal conductance (g_s) is in the same range for wheat (23% Wang et al., 2013), rice (25%, Ainsworth, 2008) and soybean (27%, (Morgan et al., 2003). In the long term, however, this increase is often compensated by downregulation of photosynthetic capacity (Long et al., 2004) and this effect may be amplified by experimental setups that represent any kind of enclosure (Ainsworth and Long, 2005). To avoid these chamber effects, the free-air CO_2 enrichment (FACE) technology has been established since the late 1990s. Analysing recent FACE experiments Ainsworth and Long (2005) estimated an increase in photosynthesis by 47% for trees and concluded that trees are more responsive to elevated CO_2 than herbaceous species. Increase in stem biomass was estimated to amount to 36 and 39% (Table 11) and effects on stomatal conductance indicated that deciduous trees are more responsive than coniferous trees (Medlyn et al., 2001).

3.1.2 Face versus chamber experiments

Ainsworth and Long (2005) analysed data from 120 articles describing effects on biomass production and physiology derived from 12 large-scale FACE experiments which were able to confirm some results from previous chamber experiments. Free-air experiments with soybean, however, showed smaller increases in yield than anticipated from studies in chambers (Morgan et al., 2005). Marked differences have also been demonstrated for wheat (44% less yield increase, Wang et al., 2013) and rice (9% less yield increases and 38% lower stimulation of A_{sat}, Ainsworth, 2008) when free-air CO_2 enrichment were compared to chamber studies.

Table 10 Mean relative effects of elevated CO_2 on crop and herbaceous wild plants

Mean CO_2 effects (% change from control) on yield, growth, and physiology as shown by recent quantitative meta-analysis. Yield* means yield or seed mass, ABM = above-ground biomass, A_{sat} = light saturated photosynthetic rate, g_s = stomatal conductance, ref. = reference.

species	no. of studies	elevated CO_2	yield* [%]	ABM [%]	Asat [%]	g_s [%]	ref.
79 crop and wild species	129	500-800 ppm	+25				[1]
98 herbaceous crop and wild species	$223^{2)}$	550-750 $ppm^{3)}$	+29	+30			[2]
perennial crops	$223^{2)}$	550-750 $ppm^{3)}$	+48	+30			[2]
2 crops	$124^{2)}$	475-600 ppm			+36		[6]
legumes and $vegetables^{1)}$	35	ambient+250 ppm	+22				[3]
leafy vegetables	7	ambient+250 ppm	+9				[3]
legumes	7	ambient+250 ppm	+28				[3]
6 legumes	$124^{2)}$	475-600 ppm			+21		[6]
rice	12	365-627 ppm	+23			-25	[4]
wheat	59	450-800 ppm	+24	+28	+33	-23	[5]
perennial wild species	$223^{2)}$	550-750 $ppm^{3)}$	+10	+19			[2]
wild grasses	$223^{2)}$	550-750 $ppm^{3)}$	+1	+9			[2]
5 forbs	$124^{2)}$	475-600 ppm			+15		[6]
5 grasses	$124^{2)}$	475-600 ppm			+36		[6]

$^{1)}$ tomatoes, cucumbers, peppers, avocados, courgettes, pumpkins, and aubergines

$^{2)}$ no. of references used in the whole study

$^{3)}$ range of CO_2 conc. in the majority of experiments, total range was 500 to 1000 ppm

[1] Jablonski et al. (2002), [2] Wang et al. (2015), [3] Scheelbeek et al. (2018), [4] Ainsworth (2008), [5] Wang et al. (2013), [6] Ainsworth and Long (2005).

Table 11 Mean relative effects of elevated CO_2 on woody plant species

Mean CO_2 effects (% change from control) on total and stem biomass, and gas exchange as shown by recent quantitative meta-analysis. P_n= net photosynthetic rate, A_{sat} = light saturated photosynthetic rate, g_s = stomatal conductance, ref. = reference.

species	no. of studies	elevated CO_2	stem [%]	total [%]	P_n or A_{sat} [%]	g_s [%]	ref.
12 tree species	$124^{1)}$	475-600 ppm			+47		[6]
8 tree species	25	not specified	+36		+58		[7]
25 broad-leaved trees	29	600-800 ppm	+39	+29	+31	-10	[8]
12 tree species	13	700 ppm				-21	[9]

$^{1)}$ no. of references used in the whole study

[6] Ainsworth and Long (2005), [7] de Dios et al. (2016), [8] Curtis and Wang (1998), [9] Medlyn et al. (2001)

3.2 Interactive effects of O_3 and CO_2 on plants and plant communities

The current trend and future changes in atmospheric chemistry are characterised by the co-occurrence of elevated concentrations of both O_3 and CO_2. The two gases are known to affect plant physiology in the opposite way. In addition, CO_2 has been evidenced to have a direct effect on stomatal conductance and in this way on the O_3 flux into the leaves. Thus, there is great agreement that plant growth response to future levels of O_3 is supposed to be modified by elevated CO_2 and vice versa. There is also growing evidence that elevated concentrations of CO_2 may ameliorate or even prevent adverse effects of O_3 on plants. However, the extent and kind of protection is thought to depend on the plant species and the concentration of the interacting gases.

Today, a lot of information about the interactive effects of O_3 and CO_2 on plants is available. In addition to a number of single experiments, European multi-site studies ("European Stress Physiology and Climate Experiment -*ESPACE*-wheat" and "Changing climate and potential impact on potato yield and quality - *CHIP*") and especially the North-American long-term experiments '*Aspen-FACE*' or '*Soy-FACE*' contribute especially to this knowledge.

Our literature research resulted in 223 references reporting on the interactive effects of O_3 and CO_2. About one half of the studies has been published within the first decade of this century and the majority of studies (80%) has been performed by the use of free-air or open top chamber exposure facilities, including 56 publications on the Aspen-FACE study and 13 publications on the Soy-FACE in the USA. The parameters on which these near-ambient studies focussed on are growth or yield, gas exchange or photosynthesis, and injury or litter production. Only those studies that met the above-mentioned criteria for experimental setup (chapter 1.3) are incorporated.

Overall, information is available for 14 crops, 29 tree species, and 22 native herbaceous and grassland species. The list of species include eight European crops which are also grown in Germany (*Triticum aestivum, Solanum tuberosum, Hordeum vulgare, Lycopersicon esculentum, Phaseolus vulgaris, Raphanus sativus, Zea mays,* and *Brassica napus*), 8 tree species (*Picea abies, Betula pendula, Pinus sylvestris, Fagus sylvatica, Quercus petraea, Betula pubescens, Fraxinus excelsior,* and *Populus tremula*), two aquatic plants (*Lemna minor* and *Spirodela polyrhiza*), one understory plant (*Fragaria vesca*) and 15 grassland species (*Agrostis capillaris, Anthoxanthum odoratum, Campanula rotundifolia, Ranunculus acris, Trifolium medium, Vicia cracca, Phleum pratense, Trifolium pratense, Trifolium repens, Centaurea jacea, Festuca pratensis, Koeleria macrantha, Lathyrus pratensis, Lolium perenne,* and *Medicago sativa*) which all are native to Germany.

The aim of this chapter is to compile data available for crop, grassland, and woody plants which have been subjected to studies with both gases in combination and to give an indication of the extent of the modifying effect of CO_2 on the plant response to O_3.

3.2.1 Crops

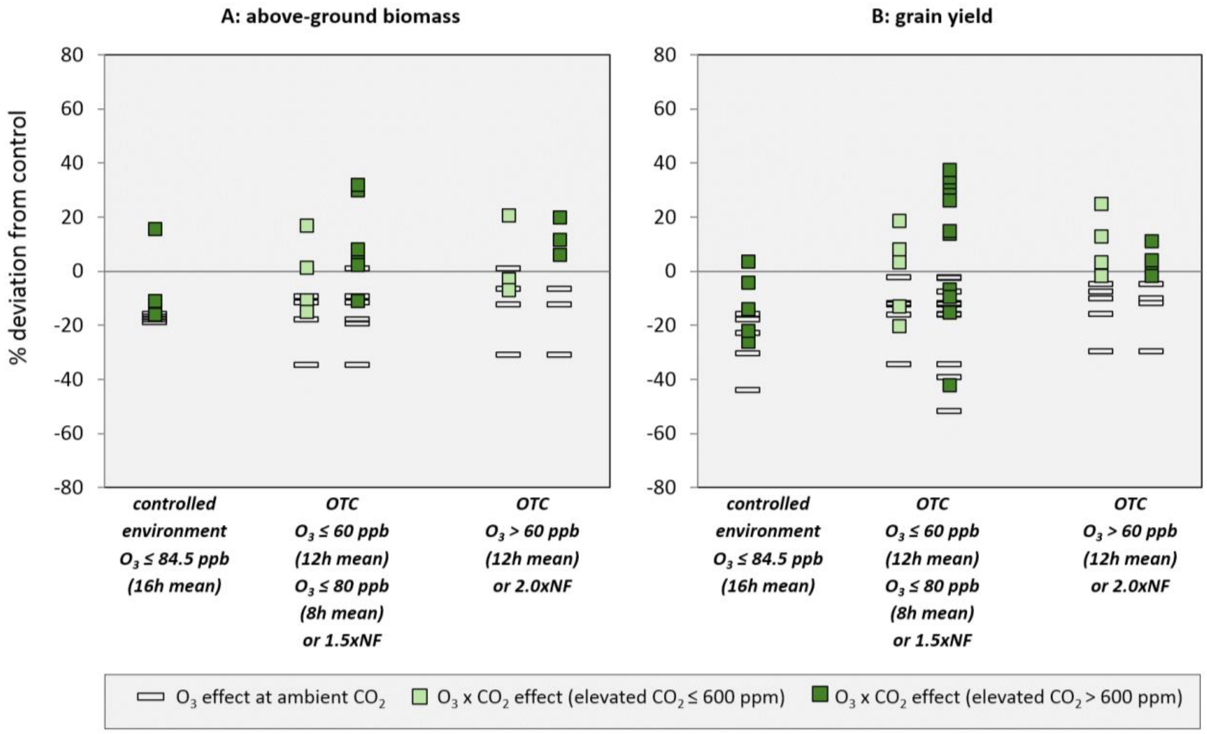
The most frequently studied crop species are soybean (*Glycine max),* wheat (*Triticum aestivum*), potato (*Solanum tuberosum*), and rice (*Oryza sativa*), with wheat being represented by the highest number of different cultivars (29). In total, 113 publications were considered here and data from 44 of them have been used for the comparative analysis.

3.2.1.1 Growth and yield

Irrespective of the sensitivity of the cultivar tested and the concentration used within the studies, O_3 generally affected yield and vegetative growth of crops negatively (see also Table 1, chapter 1.1.3.1). In the studies considered in this chapter, average yield reduction was about 23% for soybean (n=6), 44% for snap bean (n=5), 17% for potato (n=10), and 19% for wheat (n=22). On the opposite, exposures to elevated CO_2 resulted in increases in yield which amounted to 14% under moderate levels of CO_2 (≤ 600 ppm; n=30) and 26% under high levels of CO_2 (> 600 ppm, n=19) when averaged over all crop species and cultivars. For wheat, for example, significant increases in total grain yield by 10 to 32% under moderate (510 – 550 ppm) and 26 to 66% under high CO_2 levels (680 – 700 ppm) have been reported (Bender et al., 1999; Donnelly et al., 1999; Fangmeier et al., 1996; Mishra et al., 2013; Mulholland et al., 1997; Pleijel et al., 2000).

Figure 6 **Effects of O_3 on above ground biomass (A) and grain yield (B) of wheat (*Triticum aestivum*) interacting with different levels of CO_2**

Effect is expressed as percentage deviation from control (CF or NF under ambient CO_2); for exposure conditions and references see Table 25; chapter A.1.2



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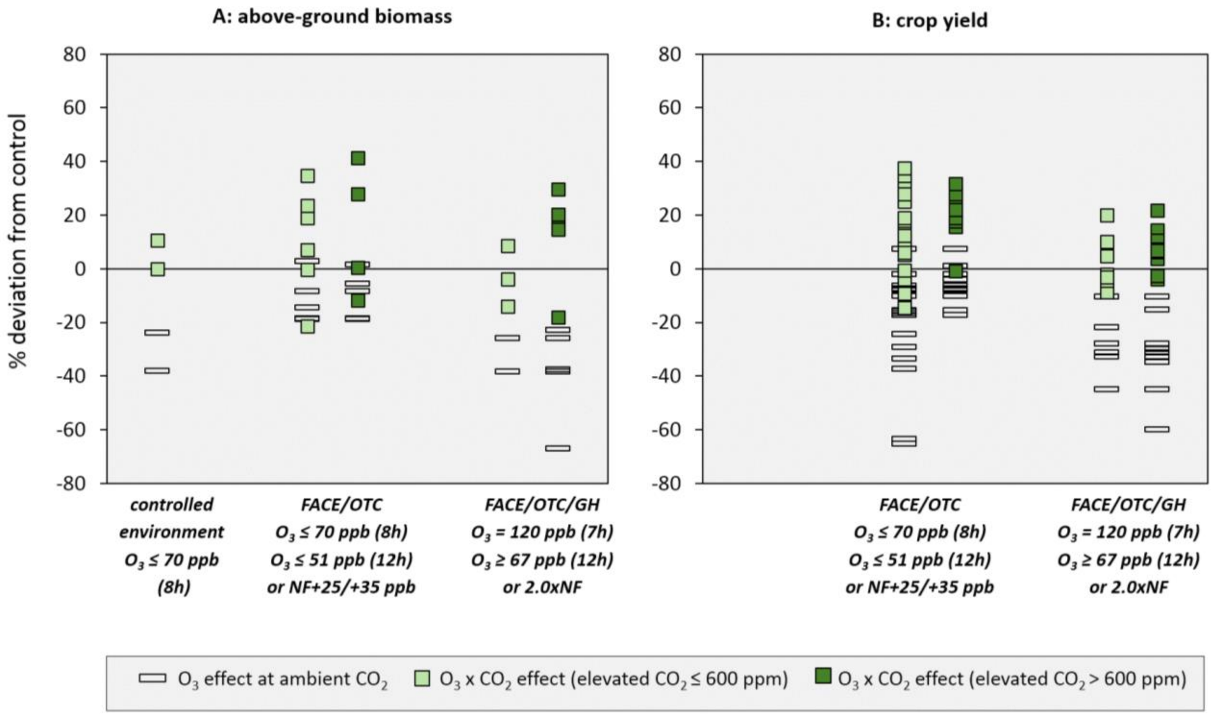
In Figure 6 and Figure 7 the effects of O_3 on growth and yield are shown for wheat and other crops under different levels of CO_2. In almost all experiments (94%) in which the interactive effect of O_3 and CO_2 was examined CO_2 was proven to counteract the detrimental effects of O_3 on yield and above-ground biomass. As a result, depending on exposure conditions and plant species elevated CO_2 either mitigated the extent of growth and yield losses caused by O_3 or even provided complete protection. Thus, simultaneous exposure to O_3 and elevated CO_2 (≤ 600 ppm and > 600 ppm) provided complete protection from O_3-caused yield reduction in 62 and 70% of all records, respectively. Under moderately elevated levels of O_3 further growth stimulation up to 25 and 37% were detected, respectively. In wheat, for example the combined treatment with elevated O_3 and CO_2 concentrations above 600 ppm led to an increase in yield and shoot biomass compared to the control treatment in about 40% and 50% of all records, respectively (Figure 6). In Figure 7 the O_3 effects on growth and yield are shown for 13 crop species under different levels of CO_2. Again, for most records, growth depressions have been turned to growth stimulations when CO_2 was enhanced simultaneously, and this effect became more obvious the lower the O_3 levels or the higher the CO_2 levels. Averaged over all crop species shown in Figure 7 a mean yield loss of 23% caused by elevated O_3 alone was changed into an increase in yield of 8 and 15% if CO_2 concentration was elevated at the same time by less or more than 600 ppm, respectively.

Figure 7 **Effects of O_3 on above ground biomass (A) and yield (B) of different crop species interacting with different levels of CO_2**

The species included are *A. hypogaea, B. campestris, C. arietinum, G. hirsutum, G. max, H. vulgare, L. esculentum, O. sativa, P. vulgaris, R. sativus, S. tuberosum, and Z. mays*. The O_3-effect is expressed as percentage deviation from control (CF or NF under ambient CO_2); for exposure conditions and references see Table 26; chapter A.1.2



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In wheat, the effect of O_3 on total yield was partly associated with a higher 1000-grain weight, a higher number of ears bearing tillers and with an increased number of grains per ear (Donnelly et al., 1999; Ewert and Pleijel, 1999; Mulholland et al., 1997). Since above ground-biomass was stimulated to a similar extent, the harvest index remained unaffected (Donnelly et al., 1999; Pleijel et al., 2000). Another characteristic of vegetative growth under the influence of elevated CO_2 was an increase in the duration of green leaf area and in the number of tillers (Ewert and Pleijel, 1999; Mulholland et al., 1997), a phenomenon that has also been observed in experiments carried out in controlled environment chambers (Balaguer et al., 1995; Barnes et al., 1995a).

Protection against O_3-caused yield loss as indicated by statistically significant interactions between CO_2 and O_3 has also been shown for parameters like ears per unit area and for the 1000-grain weight (Donnelly et al., 1999; Pleijel et al., 2000). As the effect of O_3 on grain yield is cultivar-specific the evidence of significant interactions between CO_2 and O_3 depends on how sensitive a cultivar responded to O_3 (Bender et al., 1999; Biswas et al., 2013; Mishra et al., 2013). Elevated CO_2 was also shown to provide protection against O_3-induced foliar injury and premature senescence during early vegetative growth or the negative effect of O_3 on leaf area duration in wheat plants grown in OTCs (Ewert and Pleijel, 1999; Heagle et al., 2000; Mulholland et al., 1997) or in controlled environment chambers (von Tiedemann and Firsching, 2000).

There were only a few studies reporting on yield and biomass response of soybean to the interactive effect of CO_2 and O_3. Exposure to elevated CO_2 was shown to moderate effects imposed by elevated O_3 on yield, stem biomass, root biomass, and leaf area. High CO_2 concentrations (≥700 ppm) either completely ameliorated adverse O_3 impacts (Heagle et al., 1998; Miller et al., 1998; Reinert and Ho, 1995) or stimulated growth and yield exceeding beyond compensation of O_3 impacts (Zhao et al., 2005). Under greenhouse conditions, CO_2 (450 - 650 ppm) did not affect biomass partitioning among leaves, stems, and roots of soybean but protected roots from growth reductions caused by high O_3 concentrations (120 ppb for 6h per day, Reinert and Ho, 1995). Morgan et al. (2003) assessed the response to O_3 and CO_2 of soybean by means of meta-analysis and calculated that decreases in seed yield caused by elevated O_3 in current ambient concentrations of CO_2 may be reduced by 50% when growing under elevated CO_2 concentrations. Elevated CO_2 alone is known to change the leaf-area index (LAI), while high O_3 concentrations may reduce LAI. As part of the free-air concentration enrichment '*SoyFACE*', studies used large plots (20 m diameter) of soybean that have been exposed to approx. 550 ppm CO_2 and 1.2 times ambient O_3 singly or in combination. Relative to ambient air, exposure to CO_2 delayed senescence and increased leaf-area duration, thus affecting LAI by altering the rate of senescence (Dermody et al., 2004; Dermody et al., 2006).

Within the cooperative programme 'Changing Climate and Potential Impacts on Potato Yield and Quality' (CHIP) effects of season-long elevated CO_2 (550 and 680 ppm) and/or O_3 concentrations (65 ppb) on field-grown potato (*Solanum tuberosum* cv. Bintje) have been investigated at eight European sites (Donnelly et al., 2001a). Elevated CO_2 was shown to reduce visible injury caused by O_3 by 10% (De Temmerman et al., 2002). A growth analysis demonstrated that elevated CO_2 induced a premature senescence and increased tuber yield by about 17%, while O_3 induced a faster reduction of LAI during crop senescence and yield losses by about 5%. Although the combined effects on the chlorophyll content were additive, the decline of LAI due to elevated O_3 was retarded by elevated CO_2 and the stimulatory effect of CO_2 on tuber yield exceeded the detrimental O_3 effect (Bindi et al., 2002; Craigon et al., 2002; Donnelly et al., 2001b; Hacour et al., 2002; Lawson et al., 2001).

Comparing the response of two potato cultivars differing in their sensitivity to O_3, Heagle et al. (2003) observed that elevated CO_2 may moderate O_3-induced growth reductions in the sensitive cultivar during vegetative growth but did not protect any cultivar from yield reductions at the final harvest. However, both O_3 and CO_2 caused visible injury, especially in the sensitive cultivar. A sensitive tropical potato cultivar responded to O_3 (50 and 70 ppb) with significant yield reductions by 34% and 65%, respectively, when grown in ambient CO_2 while in elevated CO_2 (570 ppm) the net growth effect was positive (Kumari and Agrawal, 2014).

In their meta-analysis Ainsworth (2008) reviewed the response of rice to elevated CO_2 and O_3 and presented a mean increase in yield by 23% caused by elevated CO_2 and a mean yield reduction by 14% caused by O_3 (62 ppb vs. CF) and noted that there have been too few studies on field grown rice plants investigating the interaction of CO_2 and O_3. However, some recent studies using exposure periods of one or two weeks up to a full life-cycle exposure documented that elevated CO_2 ameliorated adverse effects caused by O_3 in terms of growth and yield (Imai and Ookoshi, 2011; Ishioh et al., 2005; Phothi et al., 2016; Reid and Fiscus, 2008; Wang et al., 2014). For example, in controlled environment chambers O_3 (40 and 70 ppb) induced marked reductions in total biomass by 25%and 42% on Thai jasmine rice when applied for 28 days at tillering stage. Elevated CO_2 (700 ppm) reduced this negative effect of O_3 at both exposure levels and provided complete protection from the treatment with 40 ppb of O_3 (Phothi et al., 2016).

Increasing CO_2 concentrations were also demonstrated to counteract growth reductions in cotton plants due to O_3. Heagle et al. (1999) used exposures in open-top chambers to study the interactive effects of 3 different concentrations of CO_2 and four concentrations of O_3 and found highest values for above-ground biomass in the combination of moderate O_3 and high CO_2 treatments. Under near-ambient concentrations, highest yield was obtained by combining NF air with ambient CO_2 levels and CF air with elevated CO_2 levels (500 ppm) (Zakaria et al., 1994).

Pod yield of a highly sensitive selection of snap bean (*Phaseolus vulgaris*) was depressed by 63% (8h mean of 59 ppb O_3, FACE, Burkey et al., 2012) up to 75% (12h mean of 72 ppb O_3, OTCs, Heagle et al., 2002), while tolerant genotypes showed minor changes in yield. Depending on the severity of the O_3 stress exposure to elevated CO_2 ameliorated the yield loss to different degrees. Similar mitigation effects have been reported for growth parameters of tomato (Hao et al., 2000; Mortensen, 1992; Olszyk and Wise, 1997; Reinert et al., 1997), corn (Bhatia et al., 2013), mustard (Singh et al., 2013), peanut (Burkey et al., 2007), and chickpea (Singh et al., 2017). However, there can be differences between the plant organs in response to the gas mixture. For example, in radish, CO_2-enrichment completely alleviated the negative effects of O_3 on shoot biomass, but not on biomass of roots and hypocotyls (Barnes and Pfirrmann, 1992).

3.2.1.2 Crop quality

Interactive effects of CO_2 and O_3 on crop quality have been examined for example in the course of the CHIP experiment. Overall, elevated O_3 induced minor changes in tuber quality in terms of the concentration of micro- and macronutrients and the glycoalkaloid content with no O_3 x CO_2 interactions detected (Donnelly et al., 2001b; Fangmeier et al., 2002), while in single experiments increases in nutrient concentrations or decreases in starch content have been described. In this case simultaneous CO_2-enhancement did generally prevent the O_3 induced increases in nutrient concentrations or improved tuber quality (Piikki et al., 2007; Vorne et al., 2002) a finding which is consistent with that of Kumari and Agrawal (2014). Moreover, for other crops e.g. rice (Wang et al., 2014) or mustard (Singh et al., 2013) elevated CO_2 was found to counteract adverse effects on crop quality.

3.2.1.3 Physiological and biochemical aspects

In order to understand the mechanisms underlying the mitigation effect of CO_2 the antioxidant capacity of plants has frequently been examined. In soybean plants, CO_2 and O_3 have the opposite effect on the superoxide generating rate (Zhao et al., 2005) but both have the ability to increase the total ascorbic acid concentration (Booker and Fiscus, 2005). Under controlled environment conditions elevated O_3 concentrations (90 ppb for 6 hours per day) increased the total antioxidant capacity of plants (Gillespie et al., 2011). An additional exposure to an acute O_3 stress (200 ppb for 4 h) was applied to induce an immediate transcriptional response. Growth at elevated CO_2 (650 ppm), however, decreased the total antioxidant capacity, increased the response of antioxidant enzymes to acute O_3 stress, but dampened and delayed the transcriptional response under both controlled environment and field conditions (Gillespie et al., 2011; Gillespie et al., 2012). In paddy rice O_3 induced a reduction in the ascorbic acid content and changes in its redox state were partially ameliorated by elevated CO_2 (Imai and Kobori, 2008; Kobayakawa and Imai, 2013). In potato plants, increased enzyme activities indicating oxidative stress in response to elevated O_3 have been observed in both levels of CO_2, although alterations were alleviated by elevated CO_2 (Kumari et al., 2015), and it is suggested that enhancing the antioxidative property of leaves is only one additional factor in the protective action of CO_2. Hence, the mechanisms by which a mitigation of adverse O_3 effects may be attained seem to be more complex than shifting the antioxidant capacity of plants.

A lot of research has been done to investigate the interactive effects of O_3 and CO_2 by means of gas exchange measurements. Stimulation of photosynthetic rate by elevated concentrations of CO_2 has been evidenced for wheat grown in OTC, while it was negatively affected by O_3 especially late in the season due to premature senescence (Mishra et al., 2013; Rudorff et al., 1996). Similarly, the maximum photochemical efficiency was stimulated by CO_2 and reduced by O_3 (Biswas et al., 2013; Mishra et al., 2013). Studies in growth chambers revealed an increase in net CO_2 assimilation rate by up to 47%, when CO_2 concentration was doubled and a decrease by elevated O_3 by up to 20%. In their meta-analysis Feng et al. (2008) evaluated results from 53 studies using combined exposures of wheat in OTCs, growth chambers, or greenhouses and concluded that for most parameters CO_2 significantly ameliorated adverse effects of O_3. In terms of numbers this means that both the impacts on the Rubisco-catalysed carboxylation (V_{cmax}) and on stomatal conductance caused by O_3 have been reduced by approx. 50% on average. As a result, reduction of leaf photosynthetic rate induced by elevated O_3 was lessened by 79% under elevated CO_2. Other biochemical parameters have been investigated mainly under conditions of controlled environment. Previous studies have shown that O_3-induced a decrease in levels of pigments and proteins such as Rubisco as well as their oxidative damage were absent when wheat plants were exposed simultaneously to elevated CO_2 (Rao et al., 1995) even if both gases independently caused a loss in of Rubisco protein (McKee et al., 1995).

There is proven evidence that elevated CO_2 may mitigate O_3-effects on photosynthesis and assimilation-related parameters in soybean (*Glycine max*). Reduction in net photosynthesis induced by elevated O_3 (73 ppb, 12h mean) did not occur when CO_2 concentrations were elevated simultaneously up to 726 ppm (Booker et al., 1997). However, using free-exposure systems ('*SoyFACE*') Bernacchi et al. (2006) mentioned that future concentrations of both CO_2 and O_3 are likely to have smaller effects on photosynthesis than expected. Mean photosynthetic rate of newly expanded leaves was 24% higher at elevated CO_2 (550 ppm), while it remained statistically unaffected by increased O_3 concentrations (1.25xAA), irrespective of the CO_2 treatment.

Both elevated CO_2 and O_3 were shown to decrease the Rubisco content, its activity and the Rubisco carboxylation efficiency although elevated CO_2 had no significant effect on Rubisco pool size (Reid and Fiscus, 1998; Reid et al., 1998, 1999). For example, during the growing season, O_3 and CO_2 decreased initial activity by 14-64% and 14-34% per unit leaf area, respectively, while the effect of both gases is in the range of the CO_2 effect, on average. Reid and Fiscus (1998) examined the Rubisco carboxylation efficiency and observed a decline for plants grown in elevated CO_2 and/or O_3 during reproductive growth to the same extent. Both CO_2 and O_3 have been shown to decrease the activity of photorespiration-related enzymes at late growth stages, thus it was concluded that suppression of photorespiration might be involved in the mechanism by which CO_2 may promote plant's productivity (Booker et al., 1997). Results of meta-analysis confirmed a mean stimulation of soybean leaf assimilation rate by CO_2 by about 39% despite an 11% decrease in Rubisco activity (Ainsworth et al., 2002) and enhancement of CO_2 concentrations significantly lessened the impact of O_3 on photosynthesis (Morgan et al., 2003).

Compared to the control, elevated CO_2 increased the net photosynthetic rate of rice by about 18% on average and was able to protect from moderate decrease due to O_3 during vegetative growth and reduce decreases during grain-filling stage which were more severe (Shao et al., 2014). A similar amelioration of deleterious effects of O_3 on leaf photosynthesis of rice cultivars by simultaneously increased CO_2 concentrations was observed elsewhere (Imai and Kobori, 2008; Ishioh et al., 2005; Kobayakawa and Imai, 2011). At tuber initiation, a 40% increase in photosynthetic rate of potato plants was measured in response to elevated CO_2 and in the combined treatment the decline induced by O_3 was retarded (Vandermeiren et al., 2002).

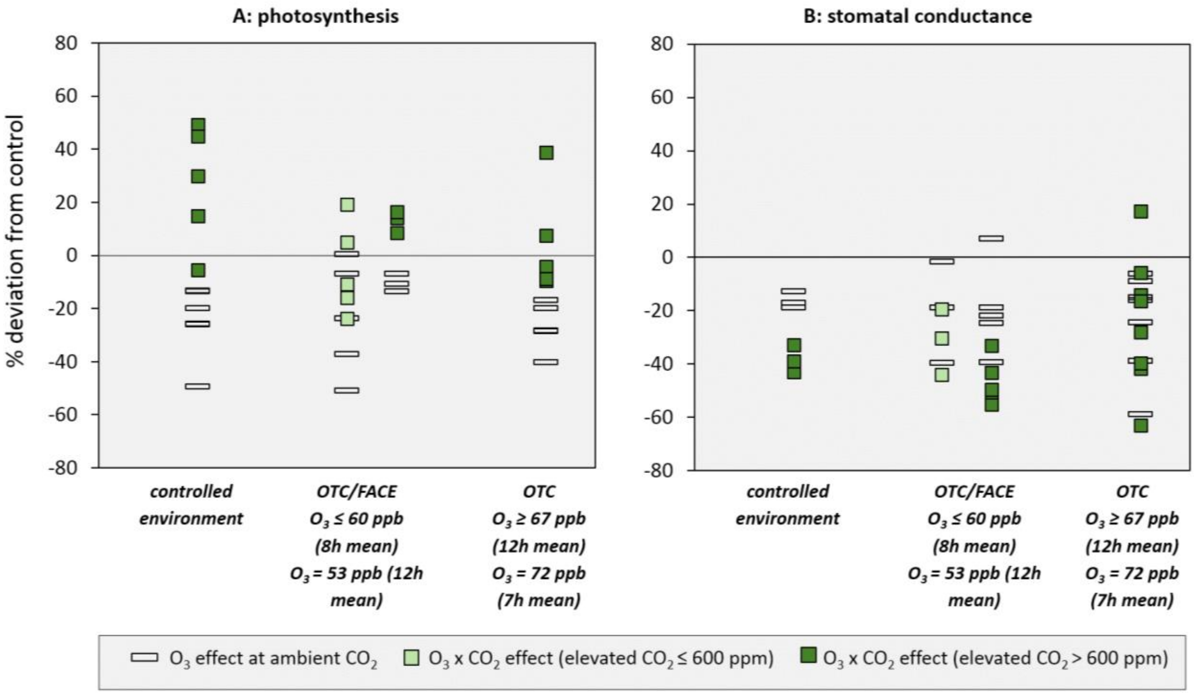
In Figure 8 A we have plotted all available data on photosynthesis referring to nine different crop species that we could deduce from the literature. All effects are related to the control treatment for O_3 and ambient levels of CO_2. Irrespective of the exposure conditions, the predominant response to elevated O_3 was a reduction in photosynthesis with the exception of *Glycine max* exposed to 1.2x AA (FACE). When CO_2 concentrations had been enhanced simultaneously, there was a shift toward the positive range.

Because of its implication for the plant's water balance and the stomatal uptake of O_3, stomatal aperture and transpiration rate have been measured frequently. There is broad evidence that enhancement of CO_2 concentrations may cause a reduction in stomatal conductance. For soybean, for example, this decrease was estimated to be 40% compared to ambient CO_2 (Ainsworth et al., 2002). Within the experimental data on which Figure 8B is based on (19 records), the change in stomatal conductance range from +10% to –59% yielding an average of -24%.

Increasing O_3 levels may also cause a decrease in stomatal conductance, depending on concentration and species. In wheat, for example, O_3 was known to reduce the stomatal conductance and to cause a partial stomatal closure (Balaguer et al., 1995; Barnes et al., 1995a; Cardoso-Vilhena et al., 2004; von Tiedemann and Firsching, 2000). McKee et al. (1995) attributed the stomatal closure largely to a reduction in photo-assimilation. Similarly, on rice it was observed that the potential of O_3 to decrease the stomatal conductance is larger than that for CO_2 (Shao et al., 2014) and the effects of an acute exposure to O_3 (100 ppb) may be accelerated by elevated CO_2 (Imai and Kobori, 2008).

Figure 8 Effects of O_3 on photosynthesis (A) and stomatal conductance (B) of crop species interacting with different levels of CO_2

The species included are Oryza sativa, Triticum aestivum, Raphanus sativus, Glycine max, Solanum tuberosum, Brassica juncea, Zea mays. Gossypium hirsutum, and Phaseolus vulgaris. The O_3-effect is expressed as percentage deviation from control (CF or NF under ambient CO_2); for exposure conditions and references see Table 25 and Table 26; chapter A.1.2



Source: own illustration, Thünen Institute

As both gases may affect the stomatal aperture in the same direction the response pattern of the combined treatment is quite different from that found for photosynthesis (Figure 8 B). Here, O_3 induced a decrease in stomatal conductance by 22% on average and this effect appears to be strengthened by elevated concentrations of CO_2. In fact, this response pattern has been detected for potatoes (Finnan et al., 2002; Lawson et al., 2002) and was shown for soybean by means of meta-analysis (Morgan et al., 2003) but in contrast in wheat plants CO_2 appeared to ameliorate decreases in stomatal conductance due to O_3 (Feng et al., 2008).

Some studies considered the effect of elevated CO_2 on water balance and O_3 uptake in order to understand mechanisms of the protection provided by CO_2. Most authors concluded that reducing stomatal conductance plays a major role in the protective effect of elevated CO_2 against detrimental O_3 impacts via an exclusion of the pollutant from the leaf interior i.e. reducing the O_3 flux into the leaves on wheat and other crops (Cardoso-Vilhena et al., 2004; Cardoso-Vilhena and Barnes, 2001; Kumari et al., 2015; McKee et al., 1997b; McKee et al., 1995). CO_2 was estimated to reduce the cumulative O_3 uptake in wheat by up to 35% (Cardoso-Vilhena et al., 2004) or the midday O_3 uptake in soybean by 41% (Booker and Fiscus, 2005) and thus protected against adverse effects of O_3. However, within some experiments CO_2 enrichment did not afforded protection against O_3 although both gases had a significant additive effect on stomatal conductance that would reduce the effective O_3 doses by 20% to 30% (Balaguer et al., 1995; Barnes et al., 1995a).

3.2.2 Native herbaceous and grassland species

Growth responses of grassland species to elevated concentrations of O_3 and CO_2 have been investigated for example by exposing mixed cultures composed of up to seven species established from seeds. Enhancement of CO_2 concentrations (740 ppm) caused an increase in total weight of a mixture of three grasses (*Phleum pratense, Lolium perenne,* and *Festuca pratensis*) by about 30% after four weeks, whereas elevated O_3 (50 ppb) reduced biomass by about 20%. Furthermore, as a result of its higher tolerance to O_3 the relative dry weight distribution of *L. perenne* was enhanced by O_3 (Mortensen, 1997). CO_2 enrichment counteracted both of these O_3 effects to some extent. In a separate experiment it has been proven that the severity of visible leaf injuries on *P. pratense* caused by O_3 (59 ppb; 8h mean) could be decreased by increasing the CO_2 concentration to 700 ppm (Mortensen, 1999).

Within a Finish open top chamber study, repeated season-long exposures to moderately elevated concentrations of O_3 (40-50 ppb) and CO_2 (+100 ppm) were used to evaluate the response of large grassland mesocosms consisting of seven species. O_3 was shown to reduce the above-ground biomass of the community (up to 40%) and the overall number of produced flowers or to delay flowering with the most responsive species being *Campanula rotundifolia, Fragaria vesca*, *Trifolium medium* and *Vicia cracca* (Rämö et al., 2006a; Rämö et al., 2007). Overall, the treatments caused comparatively few and temporary changes in the relative biomass of different species. However, CO_2 only slightly ameliorated the negative effects of O_3 on flowering or biomass production. Under same experimental conditions, monocultures of *Agrostis capillaris* showed marked losses in both root and shoot biomass in response to both gases singly and in combination, while exposure of *Lathyrus pratensis* led to growth stimulations caused by all combinations of CO_2 or O_3 treatment (Manninen et al., 2010). Individuals of *Centaurea jacea* expressed O_3 specific light brown flecks at elevated O_3 and it was found that CO_2 ameliorated the severity of this kind of visible injury (Rämö et al., 2006b).

All other studies on grassland species that we found refer to clover species and had been done in the USA. Two clones of *Trifolium repens* which clearly differ in their sensitivity to O_3 in terms of visible injury and growth reduction frequently have been utilised pairwise for biomonitoring studies. CO_2 enhancement was shown to suppress adverse effects of O_3 in terms of both foliar injury and growth reduction (Heagle et al., 1993); in case of foliar injury this effect was at least transitorily, but only the highest CO_2 concentration provided full protection from O_3 damage. Awmack et al. (2007) assessed the understory plants of poplar/aspen/birch stands at the aspen-FACE site. Neither for *Trifolium pratense* nor for *T. repens* any changes in abundance that correlated with the O_3 treatment have been detected underneath the trees. Moreover, the response of nine different cultivars of *T. pratense* was quite different than expected: CO_2 considerably reduced shoot and root biomass and this effect even exceeded that of O_3. In contrast, there were no changes in biomass when both gases were enhanced simultaneously. This response to CO_2 is suggested to be a result of the growth stimulating effect on trees which entails increased shading and thus indirect effects may have dominated the response of understory plants.

There are two more records of experimental exposures of grass species, however, in these controlled environment studies high O_3 levels (92-94 ppb; 24h mean) had been applicated continuously (Volin and Reich, 1996; Volin et al., 1998). Under these extreme conditions, the importance of physiological traits and photosynthetic pathway was examined by comparing two C-3 grasses (*Agropyron smithii* and *Koeleria cristata*) with two C-4 grasses (*Bouteloua curtipendula* and *Schizachyrium scoparium*). This study clearly supports the hypothesis that species with low stomatal conductance, such as the C-4 species, were less susceptible to O_3. Nevertheless, for all species tested reductions in photosynthesis and growth induced by O_3 did not occur under conditions of CO_2 enrichment.

3.2.3 Woody plants

The literature search with the subject woody species resulted in a notably number of publications related to the long-term Aspen FACE project in Rhinelander, Wisconsin, USA established in 1997. Free-air CO_2 enrichment (FACE) technology was used to expose either pure stands of trembling aspen (*Populus tremuloides*) or stands of aspen in mixture with paper birch (*Betula papyrifera*) or sugar maple (*Acer saccharum*) to a factorial combination of elevated CO_2 (510 - 548 ppm) and elevated O_3 (32 – 38 ppb, 24h mean) singly and in combination. A comprehensive representation of the amount of data collected in this project since 1997 cannot be provided in this review. However, since the focus of this review is on growth and gas exchange, these data are included here and shown separately in Table 12.

Overall, we have selected 29 papers out of more than 130 publications providing information on the response of trees to O_3 and CO_2 referring to growth or gas exchange parameters under chronic exposure to both gases. These publications report on 18 broad-leaved species and 4 coniferous species, 7 of which are native to Europe (*Betula pendula*, *B. pubescens*, *Fagus sylvatica*, *Fraxinus excelsior*, *Picea abies*, *Pinus sylvestris*, and *Quercus petraea*). With the exception of two Finnish studies (Kasurinen et al., 1999; Kellomaki and Wang, 1998), all others have been performed by using young seedlings or cuttings. An overview on interactive effects on tree species is given in Table 13 and Table 14.

The most frequently examined tree species were *Populus tremuloides* and *Populus* hybrids. There is great agreement within the studies that CO_2 and O_3 may affect young poplar trees in the opposite direction which has been shown for biomass production (Dickson et al., 1998; Isebrands et al., 2001; King et al., 2005; Talhelm et al., 2012) and photosynthesis (Noormets et al., 2010; Noormets et al., 2001). Enhancement of CO_2 levels was found to counteract the adverse effects of O_3 and this effect has been proven for different exposure systems: in controlled environment studies (Volin et al., 1998), in OTC studies (Dickson et al., 2001; Dickson et al., 1998) and in the course of the '*aspenFACE*' experiment (Karnosky et al., 1999; King et al., 2005;

Noormets et al., 2010; Noormets et al., 2001; Talhelm et al., 2012). During six years of exposure, for example, aspen stands responded to elevated CO_2 with an average increase in biomass production up to 71% while elevated O_3 induced a decrease in annual biomass up to 29%. Relative to the ambient air treatment the combined treatment resulted in an increase in total biomass (up to 24%, King et al., 2005) or in stem biomass, which was not significantly different from the control (Isebrands et al., 2001). These effects on growth are shown to be accompanied by a reduction in further O_3-induced effects, visible injuries (Karnosky et al., 1999) and inhibition of photosynthesis (Noormets et al., 2001).

Investigations on *P. tremuloides* often included several different genotypes. Averaged over five different clones, Dickson et al. (1998) estimated growth stimulation in the range of 23 to 36% in response to CO_2 (510 ppm) and growth reductions in the range of 45 to 55% in response to elevated O_3 (48 ppb; 24h mean) while biomass in the combined treatment have been equalled to those in the CF control. However, the single genotypes were affected differently by O_3 as fast-growing clones have been affected more and genotypes showed differences in the protective effect by CO_2. This finding is in accordance with those from the '*aspenFACE*' project, where all the five clones tested exhibited increased growth with elevated CO_2 but differed significantly in their response to O_3 (Isebrands et al., 2001). The most prominent aspen clones are clone-259 (sensitive to O_3) and clone-216 (tolerant to O_3). For the sensitive clone, there is some evidence that adverse O_3 effects on photosynthesis may be ameliorated (Noormets et al., 2010; Noormets et al., 2001) whereas others did not describe such effects (Dickson et al., 2001; Kull et al., 1996). For the tolerant clone, however, an exacerbation of negative O_3 effects on photosynthesis was detected (Kull et al., 1996; Noormets et al., 2010) and this clone appeared to become more sensitive to O_3 with elevated CO_2 (Kull et al., 1996).

Similar differential responses to O_3 and CO_2 were described for clones of *Betula pendula*: the clone being more sensitive to O_3 was less responsive to elevated CO_2 and vice versa. Total dry mass, for example, was reduced by O_3 (-26%) only in clone 4 and was increased by CO_2 (+40%) only in clone 80. The combined treatment, however, resulted in growth stimulations in both clones (+11% and +49%, respectively) thus revealing a significant interaction between O_3 and CO_2 (Riikonen et al., 2004). Elevated O_3 did not affect gas exchange parameters in any of the clones indicating that young silver birches were quite tolerant to O_3. Moreover, changes induced by CO_2 remained unaffected by additional O_3 (Riikonen et al., 2005). Vapaavuori et al. (2009) reviewed all findings concerning these two birch clones and concluded that elevated CO_2 masked most of the negative O_3 effects at least partly due to stomatal closure. Although cellular level symptoms of oxidative stress were observed sometimes in the combined treatment, responses to both elevated O_3 and elevated CO_2 mostly resembled that one of elevated CO_2 alone. With respect to genotypic variation de Dios et al. (2016) concluded from their meta-analysis that in general elevated CO_2 might overcome the negative effect of high O_3 exposures among genotypes while there was a lack of interactions between genotype and environment in response to elevated CO_2.

Mitigation effects on O_3 impacts on growth or photosynthesis by CO_2 have also been shown for European tree species as *Betula pubescens* (Mortensen, 1995), *Fagus sylvatica* (Grams et al., 1999), *Fraxinus excelsior* and *Quercus petraea* (Broadmeadow and Jackson, 2000), *Quercus rubra* (Volin et al., 1998), or Asian species as *Fagus crenata* (Tobita et al., 2019) and *Quercus mongolica* var. *crispula* (Kitao et al., 2015, Table 13). In two species *Acer saccharum* (Noble et al., 1992) and *Quercus serrata* (Kitao et al., 2015), however, O_3 had a positive effect on biomass due to preferable carbon partitioning into leaves at elevated O_3 alone and in combination with elevated CO_2 yielding biomasses higher than at elevated CO_2 alone.

Table 12 **Single and interactive effects of O_3 and CO_2 on broad-leaved trees investigated in the course of the '*aspenFACE*' experiment**

The long-term experiment was established in 1997 and run under continuous exposure to ambient air, elevated CO_2 (510 to 548 ppm; 24h mean) and/or elevated O_3 (40 to 55 ppb; 12h mean or 32 to 38 ppb; 24h mean). "period" means period of observation, P_{max} = maximum photosynthesis.

species/ genotype	**period**	**CO_2 effect**	**O_3 effect**	**interactive effect**	**reference**
Populus tremuloides alone or in mixture with *Acer saccharum* or *Betula papyrifera*	1998 -2003	overall, increased biomass production by 31 to 71%; mixed communities more responsive than pure aspen stands	overall, decreased annual biomass production 9 to 29%; the aspen community was most responsive	CO_2 provided some protection against the effects of O_3; in the mixed plots biomass was stimulated by 8 to 24%	King et al. (2005)
Populus tremuloides alone or in mixture with *Acer saccharum* or *Betula papyrifera*	2002 -2008	increase in leaf mass by 36% (averaged over all O_3 treatments)	decrease in leaf mass by 13% (averaged over all CO_2 treatments)	leaf mass in the combined treatment was similar to ambient air	Talhelm et al. (2012)
Betula papyrifera in mixture with *P. tremuloides*	2004 -2005	leaf-level and canopy conductance were increased	leaf-level conductance was increased by O_3, canopy conductance was not affected	statistically significant interaction for sap flux	Uddling et al. (2009)
Populus tremuloides 5 clones	1998 -2000	growth stimulation	growth reductions for most clones, one clone showed an increase	clones responded differently to the combined treatment	Isebrands et al. (2001)
Populus tremuloides 5 clones	1998		occurrence of visible injury	decrease of O_3-induced injuries (31 to 96%)	Karnosky et al. (1999)
Populus tremuloides 5 clones	1998 -1999	increase in biomass of fine-roots, dead roots, and in larger-diameter roots	no effect on fine-root and larger-diameter root biomass	increase in fine-root biomass was lowered, no effect on larger-diameter roots	King et al. (2001)
Populus tremuloides clone 216 (tolerant)	1998 -1999	increase in photo-synthesis, decrease in leaf area	inhibition of photosynthesis	CO_2 counteracted the adverse effect on photosynthesis	Noormets et al. (2001)
Populus tremuloides clone 216 (tolerant)	2000	increased leaf N and chlorophyll content, no effect on photosynthetic parameters	suppression of all photosynthetic parameters and chlorophyll content	greater suppression of P_{max}	Noormets et al. (2010)
Populus tremuloides clone 259 (sensitive)	1998 -1999	increase in light-saturated photo-synthesis	inhibition of photosynthesis	amelioration of the O_3 effect at the plant level was the result of increased total leaf area at elevated O_3	Noormets et al. (2001)
Populus tremuloides clone 259 (sensitive)	2000	increase in capacities of electron transport	decrease in chlorophyll content, P_{max} and carboxylation efficiency	effect on chlorophyll and carboxylation efficiency was not modified by CO_2, mitigation of effects on P_{max}	Noormets et al. (2010)

Table 13 Single and interactive effects of O_3 and CO_2 on broad-leaved trees

O_3 contr. = control treatment O_3; AA = ambient air, NF = non-filtered air, CF = charcoal filtered air, P_n, A_{growth} = photosynthetic rate at growth conditions, g_s = stomatal conductance.

***species*/genotype**	**age/duration**	**facility**	**high CO_2**	**O_3 contr.**	**high O_3**	**CO_2 effect**	**O_3 effect**	**interactive effect**	**reference**
Acer saccharum	2-yr-old seedlings/ 1 season	OTC	650 ppm	NF	104 ppb (24h mean)	increase in biomass, newly formed leaves	episodic growth (a second flush of leaves)	more plants showed episodic growth	Gaucher et al. (2005)
Betula pendula clone 4 (tolerant)	7-yr-old trees/ 3 seasons	OTC	650 -730 ppm	NF	23-30 ppb$^{1)}$ (24h mean)	no effects	decrease in dry mass of roots and branches, earlier leaf abscission	elevated CO_2 mitigated O_3 effects	Riikonen et al. (2004)
Betula pendula clone 4 (tolerant)	7-yr-old trees/ 3 seasons	OTC	650 -730 ppm	NF	23-30 ppb$^{1)}$ (24h mean)	increase in photosynthesis, slight decreases in the amount and activity of Rubisco	no sign. effect on gas exchange and biochemistry, decrease in chlorophyll content	ameliorative effect on chlorophyll content, additive effect on the activity of Rubisco	Riikonen et al. (2005)
Betula pendula clone 80 (sensitive)	7-yr-old trees/ 3 seasons	OTC	650 -730 ppm	NF	23-30 ppb$^{1)}$ (24h mean)	increase in growth and leaf area	no effects	effects similar to CO_2 alone	Riikonen et al. (2004)
Betula pendula clone 80 (sensitive)	7-yr-old trees/ 3 seasons	OTC	650 -730 ppm	NF	23-30 ppb$^{1)}$ (24h mean)	increase in photosynthetic rate, decreases in the amount and activity of Rubisco	no effect on gas exchange, decrease in and the proportion of Rubisco	effects similar to CO_2 alone	Riikonen et al. (2005)
Betula pubescens	6-wk-old/ 5 weeks	GH	560 ppm	7ppb	62 ppb (8h mean)	growth stimulation	growth reduction	ameliorative effect on growth, leaf size was stimulated more by elevated CO_2 at high O_3 levels.	Mortensen (1995)
Fagus crenata	current year seedlings/ 2 seasons	FACE	546 -562 ppm	AA	33-36 ppb$^{2)}$ (24h mean)	total plant biomass increased, biomass allocation to roots decreased	biomass allocation to roots decreased, A_{growth} was reduced in older leaves	mitigation of O_3 impacts on net CO_2 assimilation, total biomass was largest in the combined treatment	Tobita et al. (2019)

***species*/genotype**	**age/duration**	**facility**	**high CO_2**	**O_3 contr.**	**high O_3**	**CO_2 effect**	**O_3 effect**	**interactive effect**	**reference**
Fagus sylvatica	2-yr-old/ 1 season	contr. environ.	660 ppm	NF	2 x NF	due to acclimation to elevated CO_2 no effect on photosynthesis	no effect in the early season, after onset of leaf necrosis decline in photosynthetic performance	mitigation of adverse O_3 effects	Grams et al. (1999)
Fraxinus excelsior	1-yr-old/ 3 seasons	OTC	690 -710 ppm	NF	39-52 ppb (24h mean)	after the third year the stimulation effect disappeared, and a neg. effect results	growth reduction	depression of O_3 effect, CO_2 effect dominates	Broadmeadow and Jackson (2000)
Populus hybrids 5 clones	cuttings/ 1 season	OTC	510 ppm	CF	48 ppb (24h mean)	growth stimulation in the range of 23 - 36 %; allometric response differs among clones	growth reductions in the range of 45 - 55 %, allometric response differs among clones, fastest growing clones most responsive	effect of O_3 was counteracted by CO_2	Dickson et al. (1998)
Populus tremuloides clone 216 (tolerant)			+150 ppm	CF	2x NF	not determined	decreased photosynthetic rate and stomatal conductance	CO_2 exacerbated the negative effect of O_3 on photosynthesis	Kull et al. (1996)
Populus tremuloides clone 216 (tolerant)	cuttings/ 1 season	OTC	500 ppm	CF	30-41 ppb (24h mean)	not determined	moderate growth reductions	effect of O_3 was counteracted by CO_2	Dickson et al. (2001)
Populus tremuloides clone 259 (sensitive)			+150 ppm	CF	2 x NF	not determined	decrease in photosynthetic rate and stomatal conductance	no amelioration effect	Kull et al. (1996)
Populus tremuloides clone 259 (sensitive)	cuttings/ 1 season	OTC	500 ppm	CF	30-41 ppb (24h mean)	not determined	decreased shoot and root biomass, shift in carbon allocation from shoots to roots	O_3 effect was not modified by CO_2	Dickson et al. (2001)
Quercus mongolica var. crispula	1-yr-old/ 2 seasons	FACE	550 ppm	AA	36-41 ppb (24h mean)	growth and P_n moderately enhanced	no sign. effects on growth, P_n and g_s reduced	over-compensation: significant growth enhancement in the combined treatment	Kitao et al. (2015)

species/genotype	age/duration	facility	high CO_2	O_3 contr.	high O_3	CO_2 effect	O_3 effect	interactive effect	reference
Quercus petraea	2-yr-old/ 1 season	OTC	700 ppm	NF	48 ppm.h (AOT40)	reduced g_s	growth reduction, increased g_s, reduced A_{max}	A_{max} similar to O_3 alone, ameliorative effects on growth due to reduction g_s	Broadmeadow et al. (1999)
Quercus petraea	1-yr-old/ 3 seasons	OTC	690 -710 ppm	NF	39-52 ppb (24h mean)	enhanced C partitioning to the shoots and leaves , effect disappeared after the 3rd year	marked growth reductions	mitigation effect	(Broadmeadow and Jackson, 2000)
Quercus serrata	1-yr-old/ 2 seasons	FACE	550 ppm	AA	36-41 ppb (24h mean)	stimulation of growth and photosynthesis	pos. effect on growth due to preferable biomass partitioning into leaves, no effects on gas exchange	over-compensation: significant growth enhancement in the combined treatment	Kitao et al. (2015)

[1)]24h-mean calc. from AOT0, AOT40: 20.6-30.9 ppm h
[2)]see Hiraoka et al. (2017)

Table 14 Single and interactive effects of O_3 and CO_2 on coniferous trees.

O_3 contr. = control treatment O_3; AA = ambient air, NF = non-filtered air, CF = charcoal filtered air, P_n, A_{growth} = photosynthetic rate und growth conditions, g_s = stomatal conductance; sign. = statistically significant.

species/ genotype	age/ duration	facility	high CO_2	O_3 contr.	high O_3	CO_2 effect	O_3 effect	interactive effect	reference
Cryptomeria japonica 12 clones	2-yr-old/ 2 seasons	FACE	550 ppm	AA	33-36 ppb (24h mean)	increase in growth and photosynthetic rate	no neg. effect on growth and photosynthesis	no sign. interaction	Hiraoka et al. (2017)
Picea abies	5-yr-old/ 1 season	contr. environ.	750 ppm	CF(+)	75 ppb (24h mean)	increase in photosynthesis in current year needles	increase in photosynthesis in current year needles, decrease in the previous year needles	additive in current year needles	Barnes et al. (1995b)

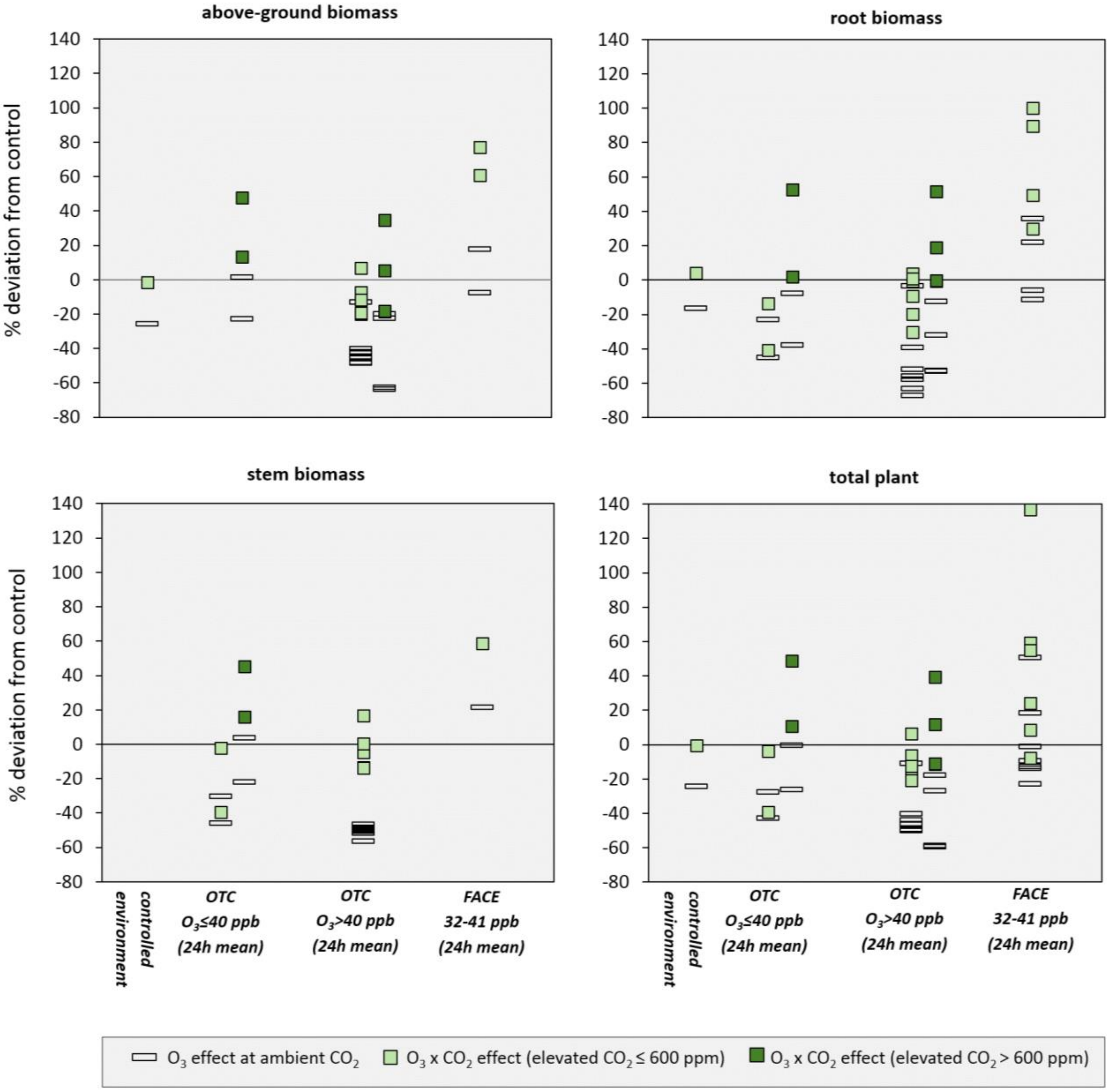
species/ genotype	**age/ duration**	**facility**	**high CO_2**	**O_3 contr.**	**high O_3**	**CO_2 effect**	**O_3 effect**	**interactive effect**	**reference**
Picea abies clone 3663	4-yr-old cuttings/ 1 season	contr. environ.	750 ppm	20 ppb	80 ppb (10 h mean)	reduction in carboxylation efficiency and maximum photosynthetic capacity	transient decrease of both apparent carboxylation efficiency and maximum photosynthetic capacity	lowest values of gas exchange parameters and chlorophyll contents	Lippert et al. (1997)
Pinus ponderosa	1-yr-old seedlings/ 3 seasons	sunlit contr. environ.	722 ppm	?	6.4-17.3 ppm h (AOT40)	reduced canopy conductance when trees were physiologically most active	reductions in canopy conductance after 40 days of exposure to episodic peak O_3	reduced g is less than the sum of the individual effects of these gases	Lee et al. (2009)
Pinus sylvestris	1-yr-old/ 3 seasons	OTC	690 -710 ppm	NF	39-52 ppb (24h mean)	growth stimulation, enhanced leaf production	growth reduction	mitigation of O_3 effect, CO_2 effect dominates	Broadmeadow and Jackson (2000)
Pinus sylvestris	3-yr-old seedlings/ 2 seasons	OTC	590 ppm	NF	42-47 ppb$^{1)}$ (24h mean)	growth stimulation, accumulation of starch	increases in yellowing and chlorotic mottling, decrease in total plant biomass	despite decreases in O_3 -induced visible injuries by CO_2, no mitigation effect	Utriainen et al. (2000)
Pinus sylvestris naturally established	14- to 24-yr-old trees/ 3 seasons	OTC	645 ppm	NF	2xAA 49 ppb$^{2)}$ (24h mean)	no sign. effects on roots, no allocative effects	no sign. effects on roots, initial stimulation of mycorrhiza	CO_2 inhibited the transient stimulating effect on mycorrhiza, increase of old mycorrhizas	Kasurinen et al. (1999)
Pinus sylvestris naturally established	30-yr-old trees/ 3 seasons	OTC	649 ppm		69 ppb (12h mean)	increased maximum specific growth rates, reduced apparent respiration rates	reduced specific growth rates in the early stage of needle expansion, increased apparent respiration rates in the late stage	CO_2 seemed not to modify the effect of elevated O_3	Kellomaki and Wang (1998)

$^{1)}$ calculated from AOT0, AOT40: 33.3-39.9 ppm h

$^{2)}$ calculated from AOT0, see (Palomaki et al., 1996)

Figure 9 **Effects of O_3 on growth parameters of woody species interacting with different levels of CO_2**

The species are *Betula pendula, B. pubescens, Cryptomeria japonica, Fagus crenata, Fraxinus excelsior, Pinus sylvestris, Populus hybrids, Populus tremuloides, Quercus mongolica* var. *crispula, Q. serrata* and *Q. petraea*). The O_3-effect is expressed as percentage deviation from control (CF or NF under ambient CO_2); for exposure conditions and references see Table 27, chapter A.1.2.



Source: own illustration, Thünen Institute

For coniferous trees, the response pattern appeared to be more inconsistent. Under controlled environment for *Picea abies* an increase in photosynthesis in current year needles (Barnes et al., 1995b) and a reduction in carboxylation efficiency and maximum photosynthetic capacity (Lippert et al., 1997) was observed in response to both elevated CO_2 and elevated O_3, and in their combination CO_2 and O_3 affected these parameters additively. For seedlings of *Pinus sylvestris* Broadmeadow and Jackson (2000) observed an effective mitigation of growth impacts caused by O_3 when CO_2 levels were enhanced up to about 700 ppm whereas in the studies of Utriainen et al. (2000) elevated CO_2 levels of 590 ppm reduced O_3-induced visible injuries but were not able to counteract growth reductions. In Finland 15 to 30-year aged trees of *P. sylvestris,* which have been naturally established, were exposed in open top chambers to doubled concentrations of O_3 and CO_2. Elevated O_3 had no or only minor effects on growth parameters during three years of exposure and CO_2 seemed not to modify these effects (Kasurinen et al., 1999; Kellomaki and Wang, 1998).

In Figure 9 the effects of O_3 at ambient CO_2 on growth parameters of tree species are plotted together with those under elevated CO_2. With the exception of two Japanese FACE studies (Hiraoka et al., 2017 and Kitao et al., 2015), all other O_3 treatments resulted in reductions of total biomass which amounted to 28.7% on average while the effect of both gases combined was a stimulation of growth by 10%. However, the (not significant) increasing effect of O_3 on total biomass of *Cryptomeria japonica* and *Quercus mongolica* var. *crispula* was markedly amplified by elevated CO_2.

Wang and Taub (2010) analysed the growth response in detail and estimated the fraction of root to total biomass (RMF= root mass fraction). O_3 was shown to impact the root production in woody plants which was reflected in a reduction in RMF by about 7% at ambient CO_2 on average and this effect was alleviated by enhanced concentrations of O_3. This finding corresponds to the trend shown in Figure 9, which indicates a shift from a negative effect on root biomass in ambient CO_2 (-28% on average) towards a positive range (+15% on average).

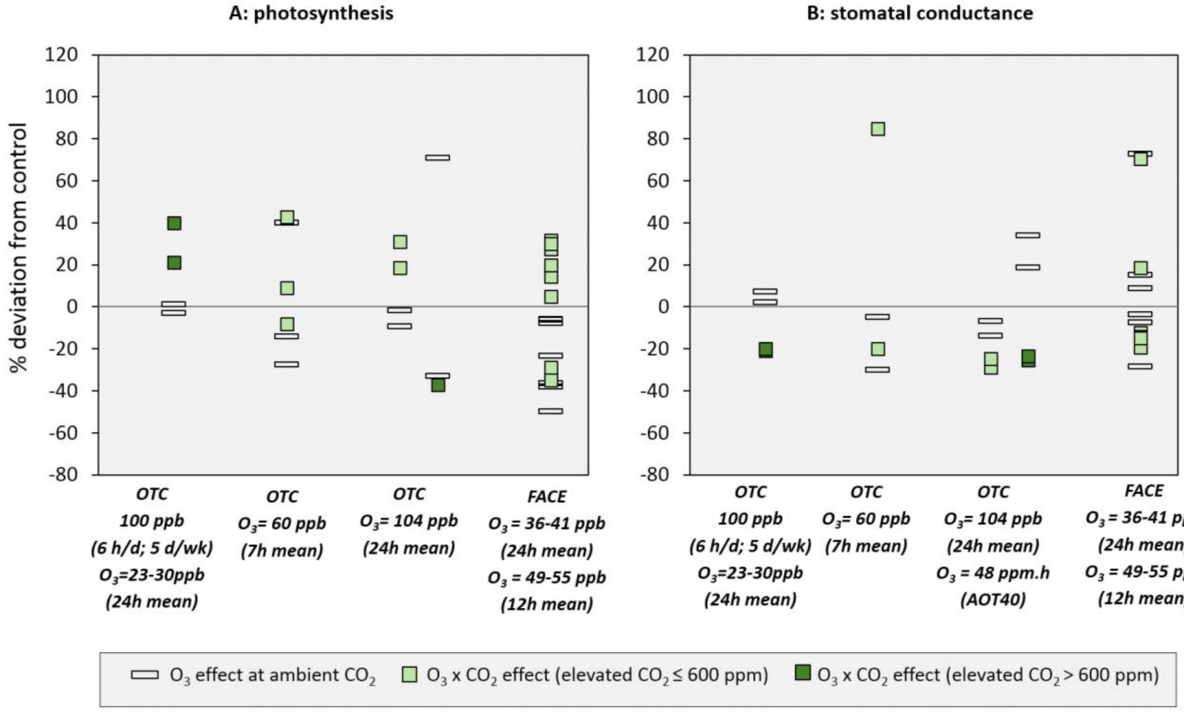
3.2.3.1 Gas exchange and stomatal uptake of O_3

In their review on stomatal responses to elevated CO_2 and O_3, Paoletti and Grulke (2005) stated that both an exposure to elevated CO_2 and a depression of photosynthesis induced by short-term elevated O_3 are known to increase the substomatal CO_2 concentration (C_i) and by this means reduces stomatal conductance and, concomitantly, the potential O_3 flux into leaves. However, this initial response can change over time as regulation processes take effect.

The differential response of photosynthesis and stomatal conductance to elevated O_3 singly and in combination with elevated CO_2 is shown in Figure 10. In our data compilation there is a trend towards a shift of the O_3 effect on photosynthesis from a decrease (-18% on average) at ambient CO_2 to an increase (+15% on average) in combination with elevated CO_2 whereas stomatal conductance was reduced more by the combined treatment in most records. However, there were two studies presenting results which go beyond the range shown here. Due to newly formed leaves, seedlings of *Acer saccharum* responded to high O_3 exposures (three times ambient concentrations) with an increase in biomass, which was accompanied by a clear but not significant increase in photosynthesis (Gaucher et al., 2005). The absence of negative effects of O_3 was attributed to the low stomatal conductance of this species resulting in a low O_3 uptake and its potential to recover from oxidative stress during periods of low O_3 concentrations. As a result, growth stimulating effects of enhanced CO_2 concentrations were not weakened by O_3 (Gaucher et al., 2005; Noble et al., 1992). In the course of the '*aspenFACE*' study values of stomatal conductance measured on aspen and birch after six years of exposure to CO_2 and/or O_3 enrichment were consistently higher than those of the control treatment (+15 to +19% in aspen and +70 to +73% in birch, Uddling et al., 2009). Furthermore, in this study the stand canopy conductance was estimated from stand sap flux in pure aspen and mixed aspen-birch communities. The results clearly show that an initial stomatal closure may be completely offset by long-term cumulative exposures to elevated CO_2 and O_3 to effects of these gases. Lee et al. (2009) reported on a very similar observation for their long-term exposure of *Pinus ponderosa* in outdoor sunlit-controlled environment. Here, canopy conductance was reduced by CO_2 or O_3 in the early season but not in August and the reduction in conductance in the combined treatment is less than the sum of the individual effects of these gases.

Figure 10 **Effects of O_3 on photosynthesis (A) and stomatal conductance (B) of tree species interacting with different levels of CO_2**

The species are *Acer saccharum, Betula papyrifera, Betula pendula, Cryptomeria japonica, Fagus crenata, Eucalyptus globulus, E. grandis, E. deglupta* x *E. camaldulensis, Populus tremuloides, Quercus mongolica* var. *crispula,* and *Q. serrata*. The O_3-effect is expressed as percentage deviation from control (CF or NF under ambient CO_2); parameters of photosynthesis are either A, A_{max}, A_{sat} or NCER; for exposure conditions and references see Table 27, chapter A.1.2



Source: own illustration, Thünen Institute

In this context Paoletti and Grulke (2005) argue that on the one hand, stomatal response is 10 - 100 times more slowly to changes in environmental conditions than does the photosynthetic apparatus and on the other hand, increasing atmospheric CO_2 concentrations may lead to increasing reduction in stomatal conductance and, in agreement with photosynthetic down-regulation, this acclimation may lessen over time at increased CO_2 concentrations.

Overall, the number of studies investigating the interactive effects of O_3 and CO_2 on stomatal conductance is quite low, hence the type and magnitude of the modifying influence of increasing CO_2 concentrations on the O_3 flux into leaves of woody plants remain uncertain.

3.2.3.2 Other parameters

Other reviews and meta-analyses cover other parameters such as secondary metabolites and performance of insect herbivores. Valkama et al. (2007) indicated that effects of elevated O_3 tends to increase tree foliage quality for herbivores, but elevated CO_2 may alleviate these effects of O_3. Finally, there is great interest in determining the consequences of enhanced CO_2 and O_3 concentrations on ecosystem effects like N cycling and carbon storage. After 11 years of exposure of stands within the '*aspenFACE*' study ecosystem C content was enhanced by 11% by elevated CO_2 whereas it was decreased by 9% by elevated O_3 (Talhelm et al., 2014). Thus, the authors concluded that the long-term effect on elevated O_3 on net primary production (NPP) will be smaller than expected. There is some evidence that both CO_2 and O_3 have the potential to change litter production rates via affecting C assimilation and leaf senescence and this will significantly alter litter and chemical inputs into the soil (Kasurinen et al., 2007; Liu et al., 2005, 2007).

Kubiske et al. (2007) attended to shifts in the community composition and ascertained that elevated O_3 hastened the conversion of aspen stands favouring paper birch and sugar maple, whereas the presence of elevated CO_2 delayed it.

Major results: O_3 x CO_2

For O_3-sensitive crop and tree species the predominant effect of simultaneously enhanced concentrations of CO_2 is to mitigate adverse effects of O_3 on growth and photosynthesis, while O_3 is limiting some positive growth responses to elevated CO_2.

The protective effect of elevated CO_2 on O_3-induced injuries becomes less pronounced the greater the O_3 sensitivity of a species.

Depending on the concentrations used in the studies, the combined treatment may even result in a growth stimulation compared to the control with low O_3 at ambient CO_2.

Since CO_2 and O_3 generally affect physiology and biomass accumulation in an opposite way, the ameliorative effect of the gases in combination may exist even without a statistically significant interaction.

Decreasing the stomatal conductance plays a major role by which CO_2 attained a mitigation of adverse O_3-effects but enhancing the antioxidative capacity of foliage may be an additional factor.

Modifying effects of CO_2 on plant's response to O_3 may also apply to edaphic processes, trophic systems, and species composition dynamics.

3.3 References

Barnes, J. D., Pfirrmann, T., Steiner, K., Lutz, C., Busch, U., Kuchenhoff, H., and Payer, H. D. (1995). Effects of elevated CO2, elevated O-3 and potassium deficiency on Norway spruce Picea abies (L) Karst : Seasonal changes in photosynthesis and non-structural carbohydrate content. Plant Cell and Environment 18, 1345-1357.

Broadmeadow, M. S. J., and Jackson, S. B. (2000). Growth responses of Quercus petraea, Fraxinus excelsior and Pinus sylvestris to elevated carbon dioxide, ozone and water supply. New Phytologist 146, 437-451.

de Dios, V. R., Mereed, T. E., Ferrio, J. P., Tissue, D. T., and Voltas, J. (2016). Intraspecific variation in juvenile tree growth under elevated CO2 alone and with O-3: a meta-analysis. Tree Physiology 36, 682-693.

Dickson, R. E., Coleman, M. D., Pechter, P., and Karnosky, D. (2001). Growth and crown architecture of two aspen genotypes exposed to interacting ozone and carbon dioxide. Environmental Pollution 115, 319-334.

Dickson, R. E., Coleman, M. D., Riemenschneider, D. E., Isebrands, J. G., Hogan, G. D., and Karnosky, D. F. (1998). Growth of five hybrid poplar genotypes exposed to interacting elevated CO2 and O-3. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere 28, 1706-1716.

Gaucher, C., Dizengremel, P., Mauffette, Y., and Chevrier, N. (2005). Response of Acer saccharum seedlings to elevated O-3 and CO2 concentrations. Phytoprotection 86, 7-17.

Grams, T. E. E., Anegg, S., Haberle, K. H., Langebartels, C., and Matyssek, R. (1999). Interactions of chronic exposure to elevated CO2 and O-3 levels in the photosynthetic light and dark reactions of European beech (Fagus sylvatica). New Phytologist 144, 95-107.

Hiraoka, Y., Iki, T., Nose, M., Tobita, H., Yazaki, K., Watanabe, A., Fujisawa, Y., and Kitao, M. (2017). Species characteristics and intraspecific variation in growth and photosynthesis of Cryptomeria japonica under elevated O-3 and CO2. Tree Physiology 37, 733-743.

Isebrands, J. G., McDonald, E. P., Kruger, E., Hendrey, G., Percy, K., Pregitzer, K., Sober, J., and Karnosky, D. F. (2001). Growth responses of Populus tremuloides clones to interacting elevated carbon dioxide and tropospheric ozone. Environmental Pollution 115, 359-371.

Kasurinen, A., Helmisaari, H. S., and Holopainen, T. (1999). The influence of elevated CO2 and O-3 on fine roots and mycorrhizas of naturally growing young Scots pine trees during three exposure years. Global Change Biology 5, 771-780.

Kasurinen, A., Peltonen, P., Holopainen, J. K., Vapaavuori, E., and T., H. (2007). Leaf Litter Under Changing Climate: Will Increasing Levels of CO2 and O3 Affect Decomposition and Nutrient Cycling Processes? *In* "Dynamic Soil, Dynamic Plant Global Science Books", Vol. 1, pp. 58-67.

Kellomaki, S., and Wang, K. Y. (1998). Growth, respiration and nitrogen content in needles of Scots pine exposed to elevated ozone and carbon dioxide in the field. Environmental Pollution 101, 263-274.

Kitao, M., Komatsu, M., Yazaki, K., Kitaoka, S., and Tobita, H. (2015). Growth overcompensation against O-3 exposure in two Japanese oak species, Quercus mongolica var. crispula and Quercus serrata, grown under elevated CO2. Environmental Pollution 206, 133-141.

Kubiske, M. E., Quinn, V. S., Marquardt, P. E., and Karnosky, D. F. (2007). Effects of elevated atmospheric CO2 and/or O-3 on intra- and interspecific competitive ability of aspen. Plant Biology 9, 342-355.

Kull, O., Sober, A., Coleman, M. D., Dickson, R. E., Isebrands, J. G., Gagnon, Z., and Karnosky, D. F. (1996). Photosynthetic responses of aspen clones to simultaneous exposures of ozone and CO2. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere 26, 639-648.

Lee, E. H., Tingey, D. T., Waschmann, R. S., Phillips, D. L., Olszyk, D. M., Johnson, M. G., and Hogsett, W. E. (2009). Seasonal and long-term effects of CO2 and O-3 on water loss in ponderosa pine and their interaction with climate and soil moisture. Tree Physiology 29, 1381-1393.

Lippert, M., Steiner, K., Pfirrmann, T., and Payer, H. D. (1997). Assessing the impact of elevated O-3 and CO2 on gas exchange characteristics of differently K supplied clonal Norway spruce trees during exposure and the following season. Trees-Structure and Function 11, 306-315.

Liu, L. L., King, J. S., and Giardina, C. P. (2005). Effects of elevated concentrations of atmospheric CO2 and tropospheric O-3 on leaf litter production and chemistry in trembling aspen and paper birch communities. Tree Physiology 25, 1511-1522.

Liu, L. L., King, J. S., and Giardina, C. P. (2007). Effects of elevated atmospheric CO2 and tropospheric O-3 on nutrient dynamics: decomposition of leaf litter in trembling aspen and paper birch communities. Plant and Soil 299, 65-82.

Mortensen, L. M. (1995). Effect of carbon-dioxide concentration on biomass production and partitioning in Betula-pubescens Ehrh seedlings at different ozone and temperature regimes. Environmental Pollution 87, 337-343.

Noble, R., Jensen, K. F., Ruff, B. S., and Loats, K. (1992). Response of Acer saccharum seedlings to elevated carbon-dioxide and ozone. Ohio Journal of Science 92, 60-62.

Noormets, A., Kull, O., Sober, A., Kubiske, M. E., and Karnosky, D. F. (2010). Elevated CO2 response of photosynthesis depends on ozone concentration in aspen. Environmental Pollution 158, 992-999.

Noormets, A., McDonald, E. P., Dickson, R. E., Kruger, E. L., Sober, A., Isebrands, J. G., and Karnosky, D. F. (2001). The effect of elevated carbon dioxide and ozone on leaf- and branch-level photosynthesis and potential plant-level carbon gain in aspen. Trees-Structure and Function 15, 262-270.

Paoletti, E., and Grulke, N. E. (2005). Does living in elevated CO2 ameliorate tree response to ozone? A review on stomatal responses. Environmental Pollution 137, 483-493.

Riikonen, J., Holopainen, T., Oksanen, E., and Vapaavuori, E. (2005). Leaf photosynthetic characteristics of silver birch during three years of exposure to elevated concentrations of CO2 and O3 in the field. Tree Physiology 25, 621-632.

Riikonen, J., Lindsberg, M.-M., Holopainen, T., Oksanen, E., Lappi, J., Peltonen, P., and Vapaavuori, E. (2004). Silver birch and climate change: variable growth and carbon allocation responses to elevated concentrations of carbon dioxide and ozone. Tree Physiology 24, 1227-1237.

Talhelm, A. F., Pregitzer, K. S., Kubiske, M. E., Zak, D. R., Campany, C. E., Burton, A. J., Dickson, R. E., Hendrey, G. R., Isebrands, J. G., Lewin, K. F., Nagy, J., and Karnosky, D. F. (2014). Elevated carbon dioxide and ozone alter productivity and ecosystem carbon content in northern temperate forests. Global Change Biology 20, 2492-2504.

Tobita, H., Komatsu, M., Harayama, H., Yazaki, K., Kitaoka, S., and Kitao, M. (2019). Effects of Combined CO2 and O3 Exposures on Net CO2 Assimilation and Biomass Allocation in Seedlings of the Late-Successional Fagus Crenata Climate 7, 117.

Uddling, J., Teclaw, R. M., Pregitzer, K. S., and Ellsworth, D. S. (2009). Leaf and canopy conductance in aspen and aspen-birch forests under free-air enrichment of carbon dioxide and ozone. Tree Physiology 29, 1367-1380.

Utriainen, J., Janhunen, S., Helmisaari, H. S., and Holopainen, T. (2000). Biomass allocation, needle structural characteristics and nutrient composition in Scots pine seedlings exposed to elevated CO2 and O-3 concentrations. Trees-Structure and Function 14, 475-484.

Valkama, E., Koricheva, J., and Oksanen, E. (2007). Effects of elevated O-3, alone and in combination with elevated CO2, on tree leaf chemistry and insect herbivore performance: a meta-analysis. Global Change Biology 13, 184-201.

Vapaavuori, E., Holopainen, J. K., Holopainen, T., Julkunen-Tiitto, R., Kaakinen, S., Kasurinen, A., Kontunen-Soppela, S., Kostiainen, K., Oksanen, E., Peltonen, P., Riikonen, J., and Tulva, I. (2009). Rising Atmospheric CO2 Concentration Partially Masks the Negative Effects of Elevated O-3 in Silver Birch (Betula pendula Roth). Ambio 38, 418-424.

Volin, J. C., Reich, P. B., and Givnish, T. J. (1998). Elevated carbon dioxide ameliorates the effects of ozone on photosynthesis and growth: species respond similarly regardless of photosynthetic pathway or plant functional group. New Phytologist 138, 315-325.

Wang, X. Z., and Taub, D. R. (2010). Interactive effects of elevated carbon dioxide and environmental stresses on root mass fraction in plants: a meta-analytical synthesis using pairwise techniques. Oecologia 163, 1-11.

4 The modifying factor soil water

4.1 Effects of increasing soil water deficit on plants

Increasing soil moisture deficit is affecting the soil-plant interaction in many ecosystems. It is generally known that drought poses a global threat to agricultural productivity with higher risk for south-eastern Europe as well as northern and southern Africa (Meza et al., 2020). In addition, it is considered the main driver of forest dieback (Sangüesa-Barreda et al., 2015; Vicente-Serrano et al., 2020) and drought-related effects are clearly evidenced for grassland ecosystems in recent years (Lei et al., 2020; Wellstein et al., 2017). Schuldt et al. (2020) assessed the impact of the severe 2018 summer drought and heatwave on Central European forests and concluded that "*most tree species in temperate forests of Austria, Germany and Switzerland showed severe signs of drought stress*". Effects of drought are thought to be usually temporary as most environmental systems show strong resistance and resilience to drought events (Vicente-Serrano et al., 2020). However, periods of water deficit in plants and elevated O_3 often coincide in time.

Drought is a plant stress that occurs when water supply from the soil water is unable to meet the water demand (Swann, 2018). Soil water-deficit reduces plant productivity and crop yield, which is shown e.g. in terms of delayed emergence, reduction in number of tillers and seeds, or seed size and early leaf senescence (Donaldson, 1996) and is attributable to decreases in photosynthetic rate (Lawlor and Tezara, 2009) and Rubisco activity (Zhou et al., 2014). With regard to biomass allocation, it has been shown that drought may increase the fraction of root biomass to the cost of shoots, while roots of herbaceous plants are more sensitive than woody plants (Eziz et al., 2017). Grass species from temperate systems were shown to respond to drought by decreasing SLA while grasses in Mediterranean systems increased their SLA (Wellstein et al., 2017).

Using climate, water, and crop yield models, crop yields are predicted to be differently affected in various areas depending on the latitude of the area and the irrigation application (Kang et al., 2009). Daryanto et al. (2017) summarised the results of numerous independent field experiments and indicated that legumes and root crops are more sensitive to drought than cereals, and drought stress occurring in the reproductive phase affects crops more than in their vegetative phase (Daryanto et al., 2017; Sarto et al., 2017; Wang et al., 2017; Zhang et al., 2018). With their morphological plasticity and their ability to modify physiological processes, plants have the ability to cope with heat and drought stresses, but it costs in terms of overall performance and results in a reduction in biomass accumulation and yield (Tiwari et al., 2018). For wheat and rice, for example, yield loss due to drought has been estimated to amount to 27.5% and 25.4%, respectively (Zhang et al., 2018). According to the review of Scheelbeek et al. (2018) yield changes for all vegetables and legumes combined is amounted to −34.7% for a 50% reduction in water availability.

Irrespective of the ecosystem considered, the decline in photosynthetic activity and plant productivity subsequently has an impact on the ecosystem carbon cycling. A multinational team of researchers assessed changes in primary productivity during 2003, a year with extreme climate anomaly in Europe manifested in July temperatures up to 6°C above long-term means and annual precipitation deficits up to 50% below the average (Ciais et al., 2005). They estimated a 30% reduction in gross primary productivity over Europe being responsible for the loss of 500 million tonnes of carbon from the forests and fields during July and August 2003.

A key function of all vascular plants, however, is the capability to control transpirational water loss by regulating stomatal aperture i.e. depressing stomatal conductance under drought conditions. Along with stomatal conductance, CO_2 assimilation rate decreased because of a restricted accumulation of the carbon dioxide. For example, reduction of stomatal conductance was found to amount to 44% for peas and 14 to 90% for tomato (Nemeskeri and Helyes, 2019).

However, stomatal aperture is regulated also by endogenous signals, such as abscisic acid and reactive oxygen species and these are shown to be accumulated in response to both high O_3 concentrations and soil drought (Gao et al., 2017b; Qi et al., 2018). By inducing stomatal closure, soil water-deficit has the ability to protect plants against O_3 flux into the leaves. O_3 by itself is known to induce stomatal sluggishness revealing no protection of drought (Wu, 2017). For Europe Anav et al. (2018) estimated a reduction in dry deposition of O_3 by about 10% as a result of stomatal regulation in response to soil water limitation. The fact that soil water deficit is limiting the O_3 flux into plants is taken into account by implementing soil water deficit and its influence on stomatal conductance into O_3 deposition models for risk assessments e.g. within the EMEP (European Monitoring and Evaluation Programme). It has been clearly demonstrated that modelling methods that relate g_s directly to soil water content and potential are suitable to be used to assess the potential risk posed by O_3 to forest trees (DO3SE, Bueker et al., 2012) and crops (CRO3PS, Grünhage et al., 2012).

4.2 Interactive effects of O_3 and soil moisture deficit on plants

In most scientific reports on the interactive effects of soil moisture and O_3 the drought stress treatments used in the experiment was poorly defined; most authors described the treatment as well-watered and water-stressed, only. However, the stress posed by those treatments was usually high enough to severely impair growth and physiology of tested plants.

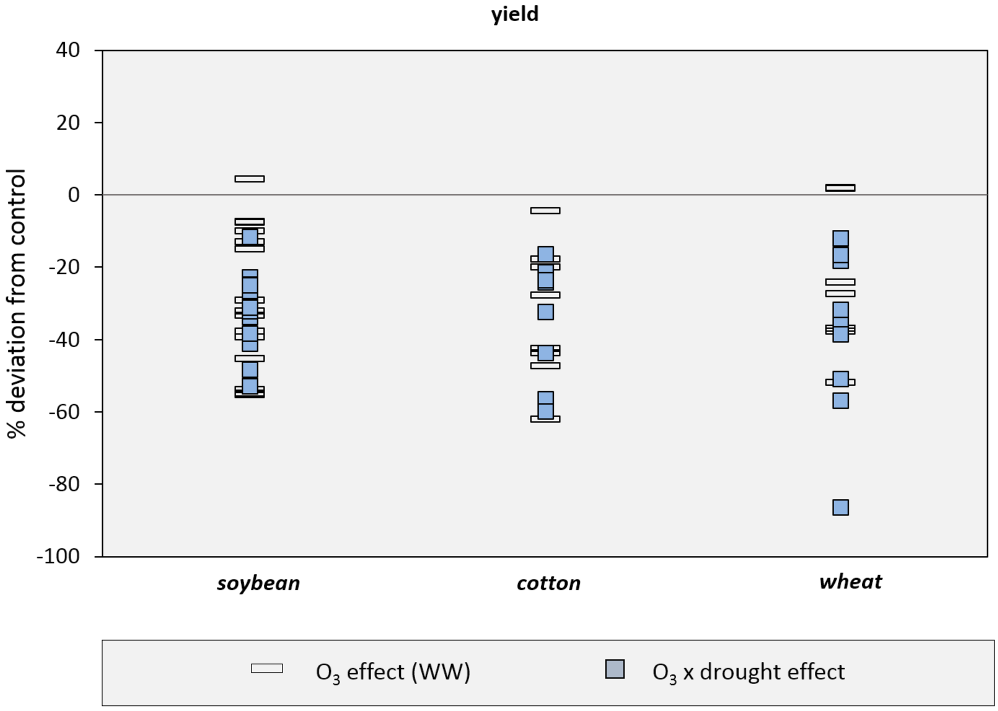
4.2.1 Crops

Contrary to our expectations, we found very few publications published during the past 30 years, which report on the response of crops to O_3 and/or drought. Besides reviews and reports on modelling, there were only 19 papers published since 1990. For this reason, we extended the period of the date of publication for this vegetation type and included 12 further publications from the late 1980s. The most frequently studied species were soybean (*Glycine max*), cotton (*Gossypium hirsutum)***,** and wheat (*Triticum aestivum*), others were canola (*Brassica napus*), alfalfa (*Medicago sativa*), tomato (*Lycopersicon esculentum*), radish (*Raphanus sativus*), and bush bean (*Phaseolus vulgaris*). It is particularly worth mentioning here that there are only four European experiments and that two of them are based on African cultivars.

In Figure 11 the effects of the combined treatment with O_3 plus drought-stress on the three crop species are plotted together with the O_3 effect on well-watered plants. The different treatments with O_3 used herein induced yield reductions by about 30% on average, when plants were well watered. This effect decreased to 26%, 15% and 9% for soybean, cotton and wheat, respectively, when both control and O_3 treated plants have been subjected to drought stress. However, if the question is examined what changes both stressors produce simultaneously the corresponding values change to about 35% for all three species. This means that on average, an additional stress by drought may slightly increase the response to O_3, although the drought effect in the absence of O_3 have the potential to reduce the yield by 12%, 22% and 34%, respectively.

Figure 11 Effects of O_3 on the yield of three crop species interacting with soil moisture

The O_3 effect is expressed as percent deviation from control (CF or NF, WW); WW=well-watered, WS water-stressed, for exposure conditions and references see Table 28, chapter A.1.3



Source: own illustration, Thünen Institute

From the data compiled here it could be concluded that the effect of the combined treatment with elevated O_3 and drought on soybean is additive or less than additive if the impairment by O_3 is more severe. Referring to experimental data from 1979–1983, King and Nelson (1987) predicted that the O_3 impact on soybean yield was reduced by soil-moisture stress by 19%. When exposed to different levels of elevated O_3 (59 ppb and 85 ppb, 12h mean) soybean cv. Young showed yield reduction by 15% and 33% whereas drought did not affect yield response (Miller et al., 1989). However, an O_3 x drought interaction could be found for net carbon exchange rate (NCER, Vozzo et al., 1995) and leaflet concentrations of total soluble carbohydrates (Miller et al., 1995) indicating that water deficit suppressed the response to O_3 in the highest O_3 treatment. With similar findings for yield response (Heggestad and Lee, 1990) and by implementation of data into a Weibull model, the same mean yield loss (15%) was predicted for the cultivar Williams under both well-watered and drought conditions for this cultivar (Heggestad and Lesser, 1990). Heggestad and Lee (1990) reported on how shoot and root biomass was affected by O_3 and drought. Shoot biomass of the cv. Williams was generally more affected by O_3 than yield and roots responded less and, in their combination, both stressors induced less than additive responses. Moreover, this cultivar showed a clear trend to increase its root density in response to drought stress (Heggestad et al., 1988). Cotton responded relatively sensitive to elevated O_3 as even ambient levels of O_3 were shown to reduce yield by up to 20%, however, the additional drought stress did not further modify the yield response to elevated O_3 (Miller et al., 1988; Temple et al., 1985), while doubling the O_3 concentration affected stomatal conductance less in drought stressed plants (Temple, 1986).

On average, water stressed wheat plants responded to elevated O_3 with yield reductions in the same range as well watered plants and the lack of an O_3-drought interaction is indicated for all estimated parameters for example by Fangmeier et al. (1994). However, under conditions of severe water deficit e.g. 35% water capacity O_3 appeared to stimulate yield compared to the control (Hatata et al., 2013; Khan and Soja, 2003). By comparing three wheat species with contrasting sensitivity, Biswas and Jiang (2011) demonstrated that there was no cross-tolerance of the two stressors when applied separately while in the combined treatment the O_3 tolerance of the drought-sensitive cultivar could be completely lost. They concluded that sensitivity to drought is associated with the O_3 tolerance of wheat species.

Recently, the emphasis has been on African cultivars and climate conditions. As a measure of the photosynthetic efficiency, the performance index (PI_{total}) was estimated by Maliba et al. (2019) for wheat plants exposed to drought and O_3 in South Africa. PI_{total} was shown to decrease in response to both stressors with a dominating effect of drought. For a Kenyan wheat cultivar Harmens et al. (2019) described, that a drought-induced stimulation of 1000-grain weight was able to compensate for yield reductions caused by O_3. Moreover, a delay in adverse effects of elevated O_3 on flag leaf photosynthesis has been observed and was attributed to a reduction in stomatal conductance and consequently in the O_3 uptake triggered by reduced soil moisture.

Yield and photosynthesis of an Egyptian cultivar of tomato (*Lycopersicon esculentum*) responded additively to O_3 and drought, while drought stress alone decreased g_s and O_3 caused an increase in g_s in both well-watered water-stressed plants (Hassan et al., 1999). A similar response has been reported for canola (*Brassica napus*) showing a stimulation of g_s by O_3 in water-stressed plants. Simultaneously, above ground biomass of canola plants, which is highly sensitive to both stressors, was less impaired by O_3 under drought-stress (Maliba et al., 2018, 2019).

In alfalfa (*Medicago sativa*) water use efficiency (WUE) was shown to be significantly reduced by O_3 while yield reductions in response to elevated O_3 (2 x NF) amounted to about 25% in well-watered plots and to about 18% in plots which received 30% less irrigation. The effects of O_3 on WUE were attributed to premature senescence and abscission of older alfalfa leaves (Temple and Benoit, 1988; Temple et al., 1988).

4.2.2 Grassland species

There is some information from Swiss studies on species representative of permanent grassland exposed in open-top chambers (CF or elevated O_3 up to 2 x ambient $O_3 \triangleq 43$ ppb, 24h mean) during episodes of reduced water supply. The drought treatment has been shown to significantly reduce the expression of O_3-specific symptoms on *T. repens, T. pratensis, R. obtusifolius, and Trisetum flavescens* and to a lesser extent that on *Bromus erectus, Lolium perenne, Festuca rubra, Onobrychis sativa,* and *Tragopogon orientalis* (Bungener et al., 1999a). Moreover, this effect was found to be associated with a reduction in stomatal conductance during the dry episodes. During different re-growth periods, sporadic increases (*Centaurea jacea, Lychnis flos-cuculi,* and *Silene dioica*) or decreases (*Chrysanthemum leucanthemum, Trifolium pratense,* and *T. repens*) in relative growth induced by elevated O_3 were detected. However, in *Plantago lanceolata, Knautia arvensis,* and *T. repens***,** an O_3 x irrigation interaction was observed (Bungener et al., 1999b). Nussbaum et al. (2000) performed replacement-series of grassland species, which are all proven to be sensitive to O_3. Total dry weight of the plant mixture was reduced when *Trisetum flavescens* grew in competition with either *Centaurea jacea* (slightly) or *T. pratense* (significantly) while the competitive ratio of the grass decreased in competition with the knapweed and increased in competition with the clover. Both effects were less pronounced when grown under reduced soil moisture. This observation could be related to reductions in specific leaf conductance: in well-watered plants, O_3 reduced stomatal conductance by about

30% in *T. pratense* and *C. jacea* whereas there was no significant effect in the dry treatment in any species.

In UK, the temperate grassland species *Dactylis glomerata* (in competition with *Ranunculus acris*) has been exposed to eight levels of O_3 with mean concentrations up to 89.5 ppb in combination with different watering regimes (Hayes et al., 2012; Wagg et al., 2012). Both species expressed visible symptoms of leaf injuries and senescence with increasing O_3 concentrations but there was no protective effect provided by soil-moisture deficit. With regard to below ground competition, O_3 caused an increase in the ratio between *R. acris* and *D. glomerata* roots with increasing O_3; by contrast, in combination with drought stress a decrease in the ratio has been observed (Wagg et al., 2012). Furthermore, there was a significant interaction between O_3 level and soil moisture after exposure for 19 weeks, which became obvious as an increase in the stomatal conductance with increasing O_3 in drought treated plants only. As a result, in the highest O_3 exposure the difference between the two water regimes disappeared, i.e. stomata became less responsive to soil moisture deficit and remained more open. Moreover, under reduced water availability the standard flux model underestimated ozone fluxes in *D. glomerata* by 30 - 40% indicating that O_3 effects may be even greater than previously predicted in the drier areas of the world (Hayes et al., 2012).

Further findings on grassland species come from studies outside Europe. Replacement series of two North American prairie grasses, *Andropogon gerardii* and *Sorghastrum nutans*, together with an annual Asian grass, *Setaria faberi*, were exposed to O_3 up to 108 ppb (7h mean) in combination with soil moisture deficit (Endress and Iverson, 1999). Reduction in biomass by O_3 was significant only in the case of *S. faberi* while *S. nutans* showed a significant O_3 x irrigation interaction. However, under conditions of elevated O_3 and soil moisture deficit, *S. nutans* was the most competitive species and *A. gerardii* was least competitive.

4.2.3 Woody plants

In total, we have found 63 publications, 29 of them have been published in the first decade of this century. Out of these, 35 provide data on growth and gas exchange and were included in this analysis, which thus encompassed information about 30 different woody species (Table 15 and Table 16). Five of them are introduced to Germany and 11 of them are native to Germany: *Alnus glutinosa, Betula pendula, Corylus avellana, Fagus sylvatica, Fraxinus excelsior, Quercus petraea, Quercus robur, Picea abies, Pinus sylvestris*, and the threatened species *Quercus pubescens.* In contrast to the modifying factors described above, the graphical analysis of Figure 12 does not display a clear trend of a modifying effect in the O_3 response by drought stress for any of the species.

Table 15 Single and interactive effects of O_3 and drought stress on broad-leaved trees

Exposure conditions and effects; O_3 contr. = control treatment O_3; AA = ambient air, NF = non filtered air, CF = charcoal filtered air, WW = well-watered, P_n, A, A_{380} = parameters of photosynthetic rate, g_s = stomatal conductance, season = exposure duration of more than 3 months, V_{cmax} = maximum carboxylation rate, ET evapotranspiration, pF value (soil moisture tension) leaf water potential, (VPD) vapour pressure deficit

species/ genotype	**age/duration**	**facility**	**drought treatment**	**O_3 contr.**	**elevated O_3**	**drought effect**	**O_3 effect**	**interactive effect**	**reference**
Acer rubrum	1-yr-old/ 2 seasons	OTC	-0.142 MPa (2nd year)	CF	41-42 ppb (24h mean)	reduction in g_s and P_n	reduction in g_s and P_n	drought stressed plants responded less to O_3	Schaub et al. (2003)
Acer truncatum	1-yr-old seedlings/ 2 years	OTC	60-70% of WW	NF	102-141 ppb	reduction in growth, A and g_s	reduction in growth, A and g_s	mitigation effect on foliar injury, O_3-induced reductions in $A_{sat,}$ but g_s and growth were enhanced by drought	Li et al. (2015a)
Alnus glutinosa	2-yr-old seedlings/ 2 seasons	solar-domes	45% of WW	32 ppb	74 ppb (24h mean)	growth reduction	growth reduction	no sign. Interaction, additive effect by both stressors	Hayes et al. (2015)
Betula pendula	2-yr-old seedlings/ 2 seasons	solar-domes	45% of WW	32 ppb	74 ppb (24h mean)	growth reduction	no sign. growth reductions	none	Hayes et al. (2015)
Betula pendula clone KL-5-M (sensitive)	1-yr-old saplings/ 3 months	free-air	not specified	AA	37 ppb (24h mean)	reduced stomatal density and g_s	leaf injuries, no growth effects, reduced mesophyll starch content	slight reduction in leaf injuries, additive effects reducing leaf number, leaf area, and starch formation.	Pääkkönen et al. (1998a)
Betula pendula clone KL-5-M (sensitive)	--	free-air	not specified	AA	42 ppb (24h mean)	reduction in g_s	no effect on $g_{s,}$ increased stomata density, growth reduction, leaf injuries	drought stressed plants responded to O_3 with reduction in stomata density and increase in g_s, growth was more affected	Pääkkönen et al. (1998c,d)
Betula pendula clone KL-5-M (sensitive)	2-yr-old saplings/ 6 weeks	contr. environ.	50% of WW	CF	100 ppb (12h mean)	reduced RGR, development of leaf injuries	occurrence of leaf injuries, reduced RGR and g_s	O_3 effects on g_s were smaller in drought stressed plants, severity of symptoms was enhanced	Pääkkönen et al. (1998b)

species/ **genotype**	**age/duration**	**facility**	**drought treatment**	**O_3 contr.**	**elevated O_3**	**drought effect**	**O_3 effect**	**interactive effect**	**reference**
Betula pendula clone KL-2-M (tolerant)	--	free-air	not specified	AA	42 ppb (24h mean)	reduction in g_s	leaf injuries	additive effects on g_s	Pääkkönen et al. (1998d)
Betula pendula clone KL-2-M (tolerant)	2-yr-old saplings/ 6 weeks	contr. environ.	50% of WW	CF	100 ppb (12h mean)	reduced RGR, development of leaf injuries, highly sensitive to drought	no growth response leaf injuries, increase in g_s	severity of injury was reduced by drought, in drought stressed plants g_s declined in response to O_3	Pääkkönen et al. (1998b)
Ceratonia siliqua	1-yr-old seedlings/ 2 years	OTC	50% of WW	CF	37 ppb (24h mean)	reduction in growth, g_s, and P_n	no effects on biomass	none	Ribas et al. (2005)
Ceratonia siliqua	1-yr-old seedlings/ 2 years	OTC	50% of WW	CF	57 ppb (24h mean)	reduction in growth, g_s, and P_n	reduction in P_n and growth, increase in g_s	no negative effects of O_3 in drought treated plants	Ribas et al. (2005)
Corylus avellana	2-yr-old seedlings/ 2 seasons	solar-domes	45% of WW	32 ppb	74 ppb (24h mean)		no sign. growth reductions	none	Hayes et al. (2015)
Fagus crenata	4-yr-old seedlings/ 1 season	sunlit growth chamb.	70% of WW	CF	60 ppb (7h mean)	reduction in transpiration rate, leaf water potential, stem and whole plant mass	reduction in photosynthesis and leaf, stem, root and whole plant biomass	additive effects on dry matter, gas exchange and water potential	Yonekura et al. (2001)
Fagus sylvatica	1 season				20 to 48 ppb (8h mean)	reduction in root and leaf biomass, A and g_s	reduced root growth, no effect on gas exchange	root biomass, A and g_s were not reduced by water deficit at high O_3	Davidson et al. (1992)
Fagus sylvatica	3-yr-old/ season	solar-domes	not specified	CF	60-120 ppb (6h mean)	reduction in g_s	reduction in g_s	O_3 mitigated the decrease in g_s due to drought stress	Pearson & Mansfield (1993)

***species/* genotype**	**age/duration**	**facility**	**drought treatment**	**O_3 contr.**	**elevated O_3**	**drought effect**	**O_3 effect**	**interactive effect**	**reference**
Fagus sylvatica	8-yr-old trees/ 3 months	OTC	not specified	CF	NF, NF+25 ppb, NF+50 ppb[1]	reduction in g_s and A	reduction in g_s, chlorophyll content and A	loss of chlorophyll content by elevated O_3 did not occur under drought stress	Le Thiec et al. (1994)
Fagus sylvatica	5-yr-old trees/ 3 seasons	OTC	not specified	CF	NF+50 ppb	slight effect on A, reduced stem diameter	reduction in A and growth	protective effect of drought on symptom appearance	Dixon et al. (1998)
Fagus sylvatica	1-yr-old seedlings/ 2 seasons	OTC	>50% field capacity	CF	25-27ppb.h (AOT40)	growth reduction	increase in growth	no effect of additional drought stress	Pollastrini et al. (2010)
Fagus sylvatica	2-yr-old seedlings/ 2 seasons	solar-domes	45% of WW	32 ppb	74 ppb (24h mean)		growth reduction	increase in growth in response to O_3 in the drought treatment	Hayes et al. (2015)
Fraxinus americana	1-year-old/ 2 seasons	OTC	-0.142 MPa (2nd year)	CF	41-42 ppb (24h mean)	reduction in g_s and P_n	reduction in g_s and P_n	non irrigated plants and well-watered responded similarly	Schaub et al. (2003)
Fraxinus excelsior	2-yr-old saplings/ season	OTC	3 drought episodes	CF	8-h-episodes of 150 ppb	plants acclimated to drought stress	no effects	reduction in growth and g_s in the combined treatment, drought reduced O_3 uptake	Reiner et al. (1996)
Fraxinus excelsior	1-yr-old seedlings/ 3 seasons	OTC	20-200 kPa (VPD?)	NF	39-52 ppb (24h mean)	growth reductions	reduced root biomass	additive effect of both stressors	Broadmeadow & Jackson (2000)
Fraxinus excelsior	2-yr-old seedlings/ 2 seasons	solar-domes	45% of WW	32 ppb	74 ppb (24h mean)	growth reductions	no sign. growth reductions	none	Hayes et al. (2015)
Liriodendron tulipifera	1-yr-old/	GH	not specified		50-200 ppb (8h/d - 3d/w)	increase in P_n, decline in xylem water-potential	no effect on g_s and P_n	greater impact on xylem water-potential	Roberts (1990)

species/ genotype	**age/duration**	**facility**	**drought treatment**	**O_3 contr.**	**elevated O_3**	**drought effect**	**O_3 effect**	**interactive effect**	**reference**
Poplar maximowiczii × P. berolinensis Oxford clone	1-yr-old seedlings/ 2 seasons	OTC	>50% field capacity	CF	25-27 ppb.h (AOT40, 6 months)	growth reduction	growth reduction	growth reduction more pronounced in drought treated plants	Pollastrini et al. (2010)
Poplar maximowiczii × P. berolinensis Oxford clone	cuttings/ 3 months	OTC	not specified	CF	50 ppb (24h mean)	reduction in height, diameter and stem mass	reduced P_n, reduced biomass of roots and leaves	drought masked the effect of O_3 by reducing stomatal uptake	Pollastrini et al. (2013)
P. deltoides cv. 55/56 x cv. Imperial	cuttings/ 1 season	OTC	40% of WW	CF	51 ppb, 78 ppb (24h mean)	increased chlorophyll and carotenoid contents, reduced growth	pigment degradation, impairment of photosynthesis	Impairment of photosynthesis and growth by O_3 were reduced by water stress	Gao et al. (2017a)
P. deltoides cv. 55/56 x cv. Imperial	cuttings/ 1 season	OTC	40% of WW	CF	80.6 ppb (10h mean)	growth reduction	reduced whole plant biomass	no sign O_3 effect on drought stressed plants	Li et al. (2019)
P. deltoides cv. 55/56 x cv. Imperial	cuttings/ 1 season	OTC	40% of WW	CF	80.6 ppb (10h mean)	growth reduction, increased A_{sat}	growth reduction	drought stress protected the saplings from O_3 damage	Shang et al. (2019)
Populus deltoides cv. 55/56 *x x P. deltoides* cv. Imperial (clone 546)	cuttings/ 1 season	OTC	40% of WW	CF	80.6 ppb (10h mean)	growth reduction, increased A_{sat}, increased WUE	accelerated leaf senescence, reduced photosynthetic capacity and WUE	drought prevented O_3-induced decreases in leaf mass per area and increases in leaf loss	Xu et al. (2020)
Populus tremuloides	seedlings/ 3 months	OTC	-0.5 MPa (1st half of the exp.)	CF	73 ppb (8h mean)	production of fewer and smaller leaves, enhanced P_n	decrease in P_n and V_{cmax} for older leaves, increase for younger leaves senescence	effects of exposure to drought and O_3 together were additive for P_n, but less than additive for leaf area	Greitner et al. (1994)
Prunus serotina	1-yr-old/ 2 seasons	OTC	-0.142 MPa (2nd year)	CF	41-42 ppb (24h mean)	reduction in g_s and P_n	visible injuries, no response on gas exchange	significantly less injury development	Schaub et al. (2003)

***species/* genotype**	**age/duration**	**facility**	**drought treatment**	**O_3 contr.**	**elevated O_3**	**drought effect**	**O_3 effect**	**interactive effect**	**reference**
Quercus cerris	3-yr-old saplings/ 3 months	contr. environ.	30% of effective ET	CF	80-100 ppb (5h mean)	marked reductions in growth, A and g_s	reductions in A_{380} and g_s, no growth effects	drought effect dominated	Cotrozzi et al. (2016)
Quercus ilex	3-yr-old saplings/ 3 months	contr. environ.	30% of effective ET	CF	80-100 ppb (5h mean)	marked reductions in growth A and g_s	reductions in A_{380} and g_s, no neg. growth effects	drought effect dominated, O_3 had a pos. effect on gas exchange in drought stressed plants	Cotrozzi et al. (2016)
Quercus ilex	1-yr-old seedlings/ 2 years	OTC	50% of WW	CF	37 ppb, 57 ppb (24h mean)	reduction in growth, g_s and P_n	reduced biomass	drought did not change the response to high O_3, additive response	Ribas et al. (2005)
Quercus petraea	1-yr-old seedlings/ 3 seasons	OTC	20-200 kPa???	NF	39-52 ppb (24h? mean)	reduction in growth and g_s	marked growth reductions	drought reduced the impact of O_3 on growth and physiology	Broadmeadow et al. (1999) Broadmeadow & Jackson (2000)
Quercus pubescens	3-yr-old saplings/ 3 months	contr. environ.	30% of effective ET	CF	80-100 ppb (5h mean)	marked reductions in growth A and g_s	growth reductions	O_3 is effective in WW and drought stressed plants	Cotrozzi et al. (2016)
Quercus robur	1-yr-old seedlings/ 2 seasons	OTC	>50% field capacity	CF	(25-27ppb.h) (AOT40, 6 months)	growth reduction	increased growth	growth was most reduced in the combined treatment	Pollastrini et al. (2010)
Quercus robur	2-yr-old seedlings/ 2 seasons	solar-domes	45% of WW	32 ppb	74 ppb (24h mean)	growth reduction	no sign. growth reductions	drought effect dominated	Hayes et al. (2015)

Table 16 Single and interactive effects of O_3 and drought stress on coniferous trees

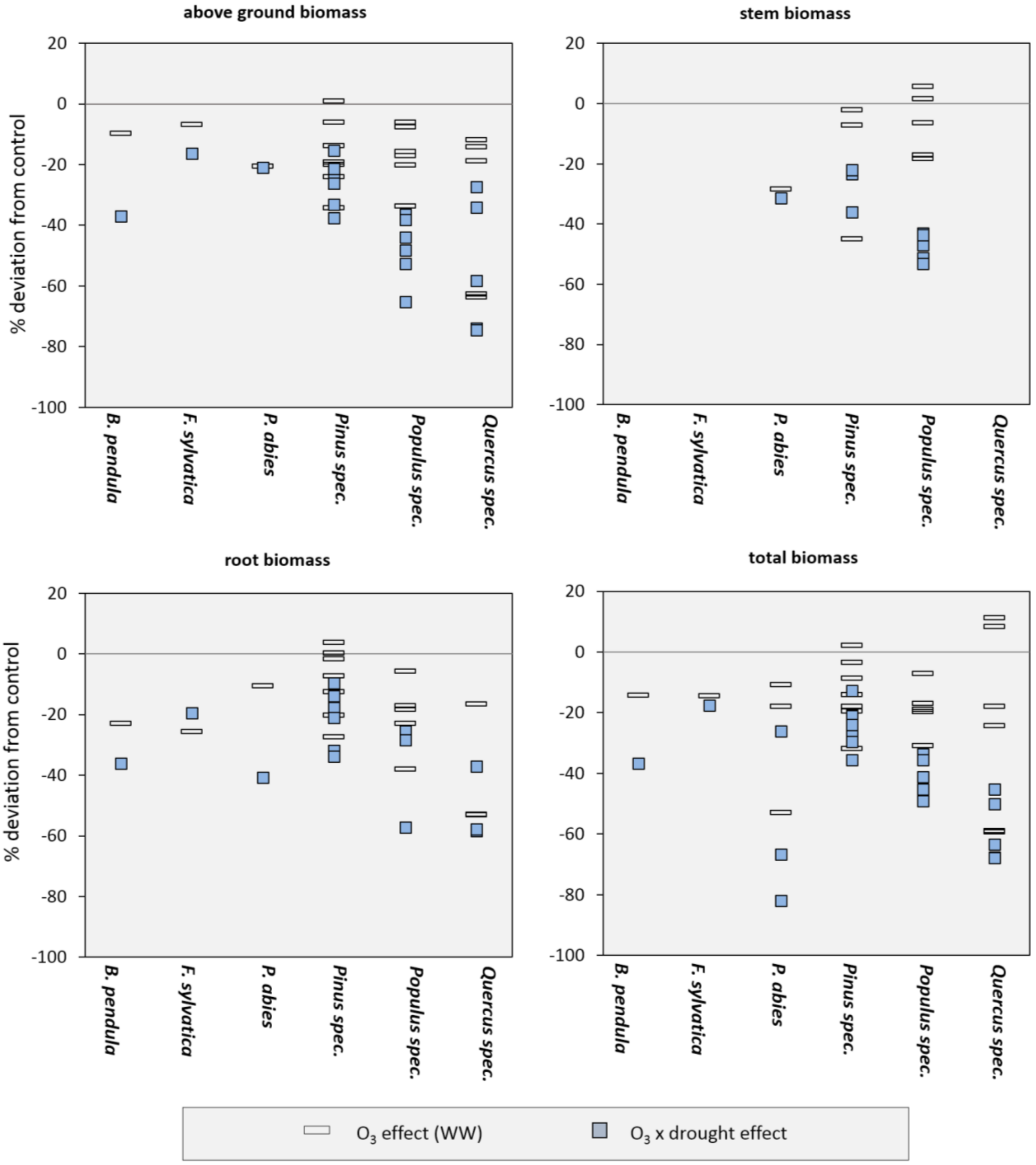
O_3 contr. = control treatment O_3; AA = ambient air, NF = non filtered air, CF = charcoal filtered air, WW = well-watered, P_n, A, A_{380} = parameters of photosynthetic rate, g_s = stomatal conductance, season = exposure duration of more than 3 months, ET evapotranspiration, pF value (soil moisture tension) leaf water potential, (VPD) vapour pressure deficit

species/ genotype	**age/ duration**	**facility**	**drought treatment**	**O_3 contr.**	**high O_3**	**drought effect**	**O_3 effect**	**interactive effect**	**reference**
Abies veitchii	6-yr-old seedlings/ 3 months	sunlit growth chamb.	1.8<pF<2.5, 2.5<pF<3.0.	CF	50 ppb (24h mean)	growth reduction	reduction in biomass of all organs but stems	no significant interactions between O_3 and drought stress	Feng & Shimizu (2005)
Picea abies	grafted plants/ 3 season	OTC	not specified	CF	34 ppb (24h mean)	growth reduction	reduction in total tree mass, notably stem biomass	regarding water stressed plant growth reduction is smaller, additive effect on roots but not on shoot	Karlsson et al. (1995)
Picea abies	grafted plants/ 4 season	OTC	not specified	CF	33-40 ppb (24h mean)	growth reduction	slight growth reduction in the 4th season	none	Karlsson et al. (2002)
Picea abies genotype Istebna	8-yr-old trees/ 3 months	OTC	not specified	CF	NF, NF+25 NF+50	reduction in A and g_s	minor O_3 effects	none	Le Thiec et al. (1994)
Picea abies genotype Istebna	5-yr-old trees/ 3 seasons	OTC	not specified	CF	NF+50 ppb	growth reduction	minor growth effects	no protective effect	Dixon et al. (1998)
Picea abies genotype Gerardmer	8-yr-old trees/ 3 months	OTC	not specified	CF	NF, NF+25 NF+50	reduction in A and g_s	minor O_3 effects	none	Le Thiec et al. (1994)
Picea abies genotype Gerardmer	5-yr-old trees/ 3 seasons	OTC	not specified	CF	NH+50 ppb	severe growth reduction	growth reduction	no protective effect	Dixon et al. (1998)
Picea abies clone C77-0051 slower-growing	1-yr-old cuttings/ season	OTC	not specified	CF	29 ppb, 33.4 ppb (24h mean)	--	stimulated the rate of biomass increase	none	Karlsson et al. (1997)

species/ genotype	**age/ duration**	**facility**	**drought treatment**	**O_3 contr.**	**high O_3**	**drought effect**	**O_3 effect**	**interactive effect**	**reference**
Picea abies clone C77-0068 faster-growing	1-yr-old cuttings/ season	OTC	not specified	CF	29 ppb, 33.4 ppb (24h mean)	--	reduced the rate of total and root biomass increase	no effect on root growth in drought stressed plants = protection to some extent	Karlsson et al. (1997)
Picea abies clone C77-0068 faster-growing	3-yr-old/ 5 seasons	OTC	not specified	CF	25-40 ppb (24h mean)	decrease in stem volume	reduction in chlorophyll conc. and stem growth	stem length most reduced in the combined treatment no other interactions	Wallin et al. (2002)
Pinus halepensis	2-yr-old seedlings/ 4 seasons	OTC	not specified	CF	80 ppb (10h mean) (54.2-70.0 ppm.h, AOT40)	reduction in CO_2 assimilation, g_s, and plant growth	reduction in gas exchange rates, activity of Rubisco and shoot biomass	impact of O_3 on assimilation tend to be counteracted by drought	Inclan et al. (1998, 2005)
Pinus ponderosa	2-yr-old seedlings/ 3 seasons	OTC	not specified	CF	62 ppb, 95 ppb (12h mean)	growth reductions	growth reduction, loss in 2-yr-old needles, increase in current yr needles	Drought-stressed seedlings were partially protected from O_3 damage by decreased g_s	Beyers et al. (1992)
Pinus ponderosa 18 half-sib families + 1 full-sib family	2-yr-old seedlings/ 3 seasons	OTC	not specified	CF	54 ppb, 87 ppb (24h mean)	growth reduction	loss of needles, compensated by increased growth of current yr needles and stems and fine feeder roots	drought stressed trees averaged half the needle loss of WW trees and showed no reduction in radial growth in response to O_3	Temple et al. (1993)
Pinus sylvestris	1-yr-old seedlings/ 3 seasons	OTC	?	NF	39-52 ppb (24h mean)	minor growth reductions	reduced biomass	none	Broadmeadow & Jackson (2000)
Pinus taeda 3 full-sib families	seedlings/ 12 weeks	GH	>-1.0 MPa	CF	50 ppb, 100 ppb (5h/day)	growth reduction	growth reduction, visible injuries	drought lessened the intensity of O_3-induced symptoms	Meier et al. (1990)

Figure 12 **Effects of O_3 on growth parameters of different tree taxa interacting with soil water**

O_3 effect is expressed as % deviation from control (CF or NF, WW), for exposure conditions and references see Table 29, chapter A.1.3



Source: own illustration, Thünen Institute

4.2.3.1 Broad-leaved trees

The compilation of effects on broad-leaved trees induced by experimentally modified drought conditions and O_3 levels (Table 15) clearly indicated a strong response to drought in terms of reductions in growth, stomatal conductance, and net photosynthesis. In some cases, soil moisture deficit caused leaf injuries *Betula pendula* (Pääkkönen et al., 1998b). Other than the detrimental effects, an increase in pigment contents (Gao et al., 2017a) or an increase in P_n has been observed in poplar clone *P. deltoides* cv. '55/56'×P. *deltoides* cv. 'Imperial' (Roberts, 1990;

Shang et al., 2019; Xu et al., 2020). The prevailing response to O_3 and drought is an additive effect on growth and gas exchange parameters whereby severe drought impacts could dominate or mask the O_3 effect in the combined treatment e.g. in species of the *Quercus* genera (Cotrozzi et al., 2016; Hayes et al., 2015).

In some species soil moisture deficit protected young tree plants from the development of O_3 symptoms, which was shown for *Acer truncatum* (Li et al., 2015), *Betula pendula* (Pääkkönen et al., 1998a; Pääkkönen et al., 1998b), *Prunus serotina* (Schaub et al., 2003), and *Fagus sylvatica* (Dixon et al., 1998). However, this protective effect is not necessarily related to all changes observed within the respective experiment. In *A. truncatum*, for example, reductions in gas exchange and growth induced by high O_3 exposures (102-141 ppb) were enhanced (Li et al., 2015).

Nevertheless, there is some evidence for mitigation effects provided by drought. When exposed to non-filtered air, drought stressed plants of *Acer rubrum* showed less reductions in P_n and g_s (Schaub et al., 2003). In carob (*Ceratonia siliqua*), a flowering evergreen tree of the legume family, reductions in growth and photosynthesis have been suppressed by the drought treatment (Ribas et al., 2005). For a Chinese poplar clone, significant reductions in growth and photosynthesis have been described in response to O_3, whereas drought reduced growth and stomatal conductance but increased photosynthesis. Drought-stressed trees, however, showed significant less response to O_3 (Gao et al., 2017a; Li et al., 2019; Shang et al., 2019) although O_3 decreased photosynthetic biochemistry more than stomatal conductance (Xu et al., 2020). On the opposite, growth reductions on further poplar hybrids were more pronounced in the combined treatment with O_3 and drought (*P. maximowiczii* × *P. berolinensis*, Pollastrini et al., 2010) or depend on the genotype (*Populus nigra* x *P. deltoids,* Dusart et al., 2019). For *P. tremuloides*, the effects of prior exposure to drought and O_3 together were additive for the photosynthetic rate (Greitner et al., 1994). In summary, the growth response of all poplar trees as shown in Figure 12 indicated that both stressors together exacerbate the adverse effects observed with O_3 alone.

A lot of research has been done on *Betula pendula* revealing divergent results which range from additive effects with no statistically interaction (Hayes et al., 2015; Pääkkönen et al., 1998a; Pääkkönen et al., 1998d) to a negative interaction in which effects of O_3 and drought were shown to mitigate each other (Pääkkönen et al., 1998b). A sensitive birch clone was shown to respond to O_3 under drought stress with a reduction in stomata density and an increase in g_s, while growth was more affected than in well-watered saplings (Pääkkönen et al., 1998c; Pääkkönen et al., 1998d).

Fagus sylvatica is known to be sensitive to both stressors and reports on the interactive effect of O_3 and drought on this species are mainly indicating a more or less protective effect of drought on the O_3 response (Table 15). Hayes et al. (2015), for example, reported that a 15% growth reduction in well-watered plants has been reversed into a growth increase due to high O_3 levels (72 ppb, 24h mean), when plants received only 55% less water. This finding is assumed to result from changes in hormonal signalling controlling stomatal opening. Root biomass measured in the subsequent spring was reduced by O_3 in well-watered plants but increased in drought treated. Simultaneously, photosynthesis and stomatal conductance increased with increasing O_3 levels in those plants which experienced water deficit (Davidson et al., 1992). Pearson and Mansfield (1993) observed that in the combined treatment the regulation of stomatal conductance was disturbed, thus the coincidence of both stress factors may exacerbate the mechanism to control the water economy of beech trees. On the other hand, it has been indicated that drought-stressed trees were able to support higher rates of photosynthesis under elevated O_3 (Le Thiec et al., 1994). In the course of the "Kranzberg Ozone Fumigation Experiment" mature beech trees (27 m tall) have been exposed to ambient or twice ambient O_3 concentrations by means of a free-air canopy exposure (Werner and Fabian, 2002). The extraordinary drought during the summer of 2003 followed by a year with average moisture conditions allowed studying how the O_3 response interfered with the drought episode. Under drought stress in 2003, O_3 reduced the slope of the size-growth relationship (annual diameter increment over the initial diameter) while A_{max} and g_s were reduced by drought rather than by O_3 (Löw et al., 2006; Pretzsch and Dieler, 2011). A decline in photosynthesis has been observed in ambient air but was absent in the enhanced O_3 treatment and lowering of the stomatal conductance as a consequence of the continuing drought caused a distinct change in the relationship between AOT40 and the cumulative O_3 uptake into leaves. Hence, drought was supposed to protect adult beech trees from the impact of O_3 by stomatal closure (Matyssek et al., 2006).

Quercus robur and *Q. petraea* are widespread distributed in almost all of Europe. In *Q. petraea*, O_3 markedly reduced growth and physiology and limited water supply has been shown to reduce the impact of O_3 (Broadmeadow et al., 1999; Broadmeadow and Jackson, 2000) while *Q. robur* responded less or not sensitive to O_3 and drought dominated the effect within the combined treatment (Hayes et al., 2015; Pollastrini et al., 2010). *Quercus ilex, Quercus pubescens,* and *Quercus cerris*, however, are native to southern Europe and the Mediterranean region. Cotrozzi et al. (2016) used exposures in controlled environment to examine the response to O_3 and drought of these oak species differing in their life-form and traits (drought tolerant, evergreen or deciduous). During the three-month exposure, all species experienced severe impairments on growth and physiology due to the drought treatment and *Q. pubescens* responded most sensitive to elevated O_3. The authors concluded that high biochemical plasticity and an evergreen habitus are likely to increase the tolerance to O_3 when combined with drought stress. In a free-air exposure experiment, stomatal O_3 flux has been estimated and dose-response relationships have been derived for these oak species three (Hoshika et al., 2018). The result was that water availability can significantly affect O_3 risk assessment but the POD_y metric allows the effects of O_3 and the soil water availability to be reconciled.

Experiments on *Fraxinus excelsior* and *F. americana* essentially revealed same responses to O_3 of well-watered or drought-stressed plants (Broadmeadow and Jackson, 2000; Hayes et al., 2015; Schaub et al., 2003). Nevertheless, an episodic exposure to O_3 (8h episodes of 150 ppb) did not affect ash saplings showing a reduced O_3 uptake in response to concomitant drought episodes (Reiner et al., 1996).

4.2.3.2 Coniferous trees

In most experimental studies both O_3 and drought had a detrimental impact on photosynthesis, stomatal conductance, root and aboveground biomass. Drought appeared to be the most limiting factor having a predominant effect in the combined treatment with no statistically significant O_3 x drought interaction being detected (Table 16, Figure 12). This was especially shown for *Abies veitchii* (Feng and Shimizu, 2005), *Picea abies* (Dixon et al., 1998; Karlsson et al., 2002; Karlsson et al., 1995; Le Thiec et al., 1994) and *Pinus sylvestris* (Broadmeadow and Jackson, 2000). However, for two clones of *P. abies* Karlsson et al. (1997) reported on differential growth responses to O_3 which are expressed in a growth stimulation in slower-growing clone and a reduction in the rate of biomass increase in the faster-growing clone. For this sensitive clone, an O_3 x drought interaction has been described as root growth was not affected in drought-stressed plants (Karlsson et al., 1997) and stem length was most reduced in the combined treatment in the long-term experiment of Wallin et al. (2002). Clonal spruce cuttings were exposed for four seasons to O_3 in open-top chambers and subjected to different periods of drought. Measurements of gas exchange suggested that coupling between the stomatal conductance and the photosynthetic rate was changed as stomatal limitation of net photosynthesis was increased by drought in the CF treatment, while it was unaffected in the high O_3 treatment (56 to 70 ppb, 24h mean, Wallin and Skärby, 1992).

For three species of the *Pinus* genus, a partial protection from O_3 damage by drought was mentioned: *P. taeda* (Meier et al., 1990), *P. ponderosa* (Beyers et al., 1992; Temple et al., 1993), and *P. halepensis* (Inclan et al., 1998; Inclan et al., 2005; Le Thiec and Manninen, 2003).

In seedlings of *P. taeda*, O_3 and drought alone reduced growth and carbohydrate content but under conditions of soil moisture deficit the intensity of O_3 symptoms was lessened (Meier et al., 1990). However, Lee et al. (1990) presented an experiment with seedlings predisposed to elevated O_3 prior to drought cycles and observed that plants displayed enhanced net photosynthesis rate in response to drought only when pre-exposed to O_3.

At a moderately-polluted site in the Sierra Nevada of California seedlings of *P. ponderosa* have been studied over three years under different levels of O_3 and two soil moisture conditions. After three years of exposure well-watered trees exposed to high levels of O_3 (87 ppb, 24h mean) reduced radial stem growth and lost 70% and 48% of 2-year-old and 1-year old needles, respectively, and compensated for these losses by increased growth of current-year needles and stem growth (Temple et al., 1993). This was also reflected in higher N concentrations and higher photosynthetic capacity of current-year needles treated with O_3 (Beyers et al., 1992; Temple and Riechers, 1995). Plants grown under drought stress conditions lost significantly less needles, showed no reduction in radial growth and did not exhibit the same degree of photosynthetic compensation in response to O_3. Thus, it could be concluded that drought partially protected from O_3 impacts by decreased stomata conductance (Beyers et al., 1992; Temple et al., 1993). Independently from the O_3 treatment, drought stressed trees averaged 10% higher N in current-year needles than well-watered seedlings. Thus O_3-injured Ponderosa pine seedlings increased resorption of N from older needles and increased partitioning of N to current-year foliage.

The third pine species for which interactive effects of O_3 and drought were evidenced is *P. halepensis*. Since many years an accelerated decline of Aleppo pine forests has been observed in Mediterranean areas (Gerant et al., 1996; Wellburn et al., 1996) and drought and elevated O_3 are suspected to cause this decline as high levels of O_3 impair the ability of the pine trees to withstand severe water stress. In fact, it was shown that recovery from drought stress was delayed under elevated O_3 (Alonso et al., 2001; Inclan et al., 1998). When exposed to O_3 episodes after a drought period CO_2 assimilation was still further depressed. In addition, levels of total phenols, glutathione, ascorbate, and glutathione reductase were significantly reduced, indicating a susceptibility of Aleppo pines to photoinhibition (Wellburn et al., 1996). In a long-term open top chamber experiment both O_3 and drought were shown to reduce photosynthesis, stomatal conductance, root and aboveground biomass and drought tended to counteract the O_3 effects on CO_2 assimilation and root growth (Inclan et al., 1998; Inclan et al., 2005). The authors discussed the ability for internal resource allocation and compensation mechanisms for *P. halepensis* to cope with the stress. Moreover, drought was shown to protect Aleppo pine to some extent from symptom expression and reductions in stem and branch biomass (Le Thiec and Manninen, 2003).

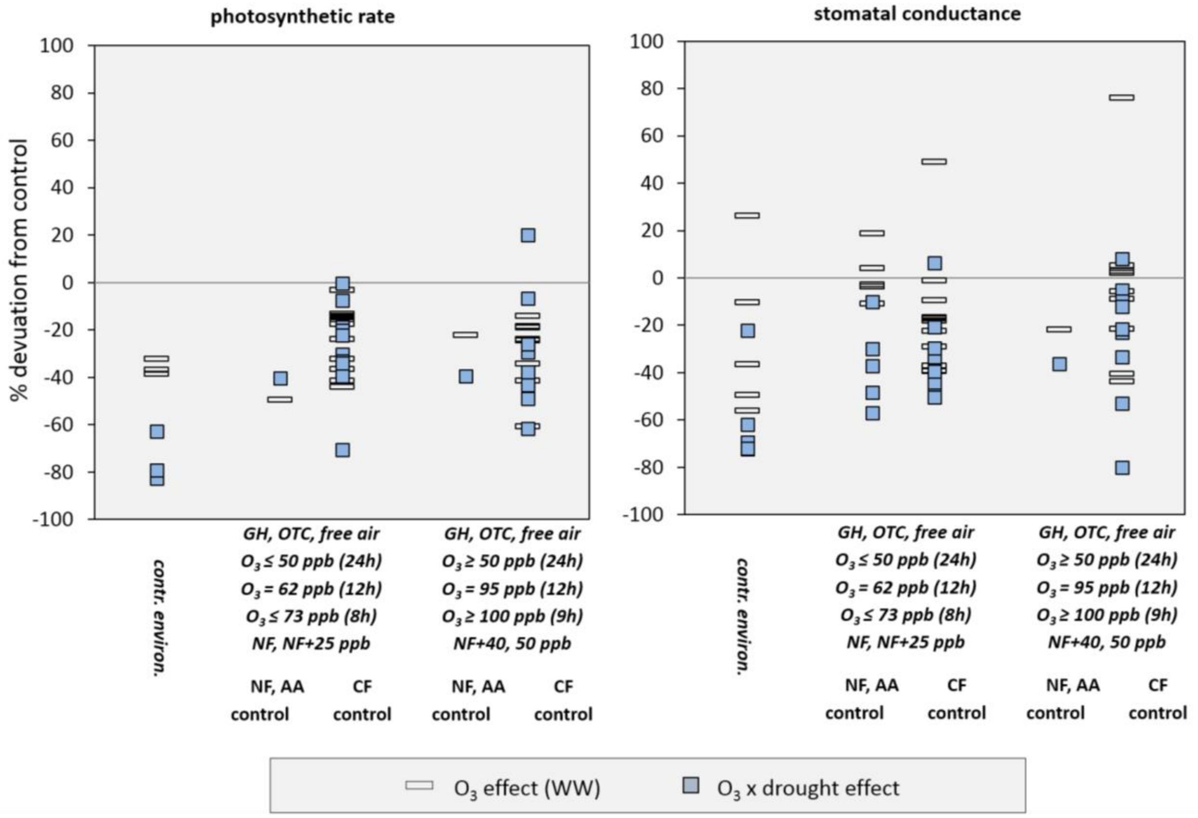
4.2.3.3 Photosynthesis and stomatal conductance

Since plants are used to control transpirational water loss by decreasing stomatal conductance under drought conditions, soil water limitation is thought to have the capability to mitigate O_3 impacts on plants in particular. Under control conditions for O_3, the drought treatment applied in the experiments analysed here caused distinct reductions in stomatal conductance in any case (-15 to -90%, -40% on average, n = 27). Averaged over all experiments, elevated O_3 reduced g_s in well-watered plants to a lesser extent, but in combination both stressors had a less than additive effect, which resulted in values for g_s similar to those caused by drought stress alone.

In Figure 13 O_3 and O_3 x drought effects on gas exchange are plotted for 16 tree species (21 records). Reductions in g_s greater than those caused by O_3 alone seem to be more apparent for moderately elevated O_3 stress relative to ambient O_3 (NF and AA), however, compared to the O_3 effect in well-watered plants there is a clear trend to more negative values indicating a reduced stomatal O_3 uptake in drought stressed woody plants.

Figure 13 Effects of O_3 on photosynthesis and stomatal conductance interacting with soil moisture

The species *Acer rubrum, A. truncatum, Betula pendula, Ceratonia siliqua, Fagus crenata, Fraxinus americana, Picea abies, Pinus halepensis, P. ponderosa, Populus* hybrid*, Prunus serotina, Quercus cerris, Q. ilex, Q. petraea* and *Q. pubescens,* the O_3 effect is expressed as % deviation from control (CF or NF, WW), data are grouped according to the O_3 level and within this group differentiated according to the reference treatment used as control (left NF or AA, right CF), for exposure conditions and references see Table 29, chapter A.1.3



Source: own illustration, Thünen Institute

4.2.3.4 Monitoring studies

The findings presented in Table 15 and Table 16 applied exclusively to one- to five (or eight)-year old seedlings or cuttings under (semi-) controlled conditions. The above mentioned free-air canopy exposure facility at Kranzberg forest (Werner and Fabian, 2002) installed in a mixed stand of tall beech and spruce trees is unique. As a consequence, monitoring studies along gradients are the only appropriate method to examine the response of mature trees without using such complex exposure systems. For example, a reduction of annual primary growth of 40 year old beech trees analysed in a forest near Oxford was demonstrated to be associated with O_3 doses (AOT40) below the critical level, however, in one compartment, the response was significantly correlated with soil moisture deficit (Stribley and Ashmore, 2002). In east Tennessee, USA empirically modelled streamflow patterns over a 23-year observation period suggested that current ambient O_3 exposures may exacerbate the frequency and level of drought impacts on forest growth (McLaughlin et al., 2007).

Most information is available about Ponderosa pine (*Pinus ponderosa*) trees. Physiological measurements on 40-yr-old trees along an O_3 concentration gradient grown in the central Sierra Nevada, California, USA, illustrated that stomatal conductance strongly decreased over the growing season with decreasing the soil water content. They further demonstrated that periods of maximum O_3 uptake did not correspond to periods of peak O_3 concentrations (Panek, 2004). Net assimilation rate generally declined with O_3 exposure. The effect of late summer drought stress was statistically significant only at the moderate pollution site but drought and O_3 combined were assumed to reduce the rate of gross photosynthesis in a synergistic manner (Grulke and Preisler, 2003; Grulke et al., 2002).

Major results: O_3 x drought

Under dry conditions, stomatal conductance and thus O_3 uptake is generally lower. Therefore, it is assumed that plants are partially protected from O_3 impacts.

For crops, the effect of both stressors combined on growth appears to be additive or less than additive; drought may therefore slightly enhance the effects of O_3.

Although there is some evidence of a protective effect of drought from growth reductions caused by O_3, there are also reports on a more adverse effect caused by both stressors together compared to O_3 alone.

In grassland species, symptoms of O_3-induced leaf injury were often less severe when plants were grown under soil moisture deficit.

The available information on O_3 and drought stress experiments comes mainly from studies with young trees or seedlings. On average, both root and shoot biomass was affected more severe by the combined treatment of O_3 and drought stress, although drought partly protected young trees from adverse O_3 effects in some studies.

The damage currently observed in forests in Germany and Europe is mainly due to periods of drought in recent years. The extent to which O_3 plays an additional role in this damage cannot be estimated so far.

4.3 References

Alonso, R., Elvira, S., Castillo, F. J., and Gimeno, B. S. (2001). Interactive effects of ozone and drought stress on pigments and activities of antioxidative enzymes in Pinus halepensis. *Plant Cell and Environment* 24, 905-916.

Anav, A., Proietti, C., Menut, L., Carnicelli, S., De Marco, A., et al. (2018). Sensitivity of stomatal conductance to soil moisture: implications for tropospheric ozone. *Atmospheric Chemistry and Physics* 18, 5747-5763.

Beyers, J. L., Riechers, G. H., and Temple, P. J. (1992). Effects of long-term ozone exposure and drought on the photosynthetic capacity of ponderosa pine (Pinus ponderosa Laws). *New Phytologist* 122, 81-90.

Biswas, D. K., and Jiang, G. M. (2011). Differential drought-induced modulation of ozone tolerance in winter wheat species. *Journal of Experimental Botany* 62, 4153-4162.

Broadmeadow, M. S. J., Heath, J., and Randle, T. J. (1999). Environmental limitations to O-3 uptake - Some key results from young trees growing at elevated CO2 concentrations. *Water Air and Soil Pollution* 116, 299-310.

Broadmeadow, M. S. J., and Jackson, S. B. (2000). Growth responses of Quercus petraea, Fraxinus excelsior and Pinus sylvestris to elevated carbon dioxide, ozone and water supply. *New Phytologist* 146, 437-451.

Bueker, P., Morrissey, T., Briolat, A., Falk, R., Simpson, D., et al. (2012). DO3SE modelling of soil moisture to determine ozone flux to forest trees. *Atmospheric Chemistry and Physics* 12, 5537-5562.

Bungener, P., Balls, G. R., Nussbaum, S., Geissmann, M., Grub, A., et al. (1999a). Leaf injury characteristics of grassland species exposed to ozone in relation to soil moisture condition and vapour pressure deficit. *New Phytologist* 142, 271-282.

Bungener, P., Nussbaum, S., Grub, A., and Fuhrer, J. (1999b). Growth response of grassland species to ozone in relation to soil moisture condition and plant strategy. *New Phytologist* 142, 283-293.

Ciais, P., Reichstein, M., Viovy, N., Granier, A., Ogée, J., et al. (2005). Europe-wide reduction in primary productivity caused by the heat and drought in 2003. *Nature* 437, 529-533.

Cotrozzi, L., Remorini, D., Pellegrini, E., Landi, M., Massai, R., et al. (2016). Variations in physiological and biochemical traits of oak seedlings grown under drought and ozone stress. *Physiologia Plantarum* 157, 69-84.

Daryanto, S., Wang, L. X., and Jacinthe, P. A. (2017). Global synthesis of drought effects on cereal, legume, tuber and root crops production: A review. *Agricultural Water Management* 179, 18-33.

Davidson, S. R., Ashmore, M. R., and Garretty, C. (1992). Effects of ozone and water deficit on the growth and physiology of Fagus-sylvatica. *Forest Ecology and Management* 51, 187-193.

Dixon, M., Le Thiec, D., and Garrec, J. P. (1998). Reactions of Norway spruce and beech trees to 2 years of ozone exposure and episodic drought. *Environmental and Experimental Botany* 40, 77-91.

Donaldson, E. (1996). Crop traits for water stress tolerance. *American Journal of Alternative Agriculture* 11, 89-94.

Dusart, N., Vaultier, M.-N., Olry, J.-C., Bure, C., Gerard, J., et al. (2019). Altered stomatal dynamics of two Euramerican poplar genotypes submitted to successive ozone exposure and water deficit. *Environmental Pollution* 252, 1687-1697.

Emberson, L. D., Buker, P., and Ashmore, M. R. (2007). Assessing the risk caused by ground level ozone to European forest trees: A case study in pine, beech and oak across different climate regions. *Environmental Pollution* 147, 454-466.

Endress, A. G., and Iverson, L. R. (1999). Drought and ozone stress effects on competition among selected prairie grass species and giant foxtail. *HortTechnology* 9, 227-234

Eziz, A., Yan, Z., Tian, D., Han, W., Tang, Z., et al. (2017). Drought effect on plant biomass allocation: A meta-analysis. *Ecology and Evolution* 7, 11002-11010.

Fangmeier, A., Brockerhoff, U., Gruters, U., and Jager, H. J. (1994). Growth and yield responses of spring wheat (Triticum-aestivum L cv Turbo) grown in open-top chambers to ozone and water-stress. *Environmental Pollution* 83, 317-325.

Feng, Y. W., and Shimizu, H. (2005). Effects of ozone and/or water stress on the growth of Abies veitchii seedlings. *Phyton-Annales Rei Botanicae* 45, 591-594

Gao, F., Catalayud, V., Paoletti, E., Hoshika, Y., and Feng, Z. (2017a). Water stress mitigates the negative effects of ozone on photosynthesis and biomass in poplar plants. *Environmental Pollution* 230, 268-279.

Gao, F., Li, P., and Feng, Z.-Z. (2017b). Interactive effects of ozone and drought stress on plants: A review. *Chinese Journal of Plant Ecology* 41, 252-268.

Gerant, D., Podor, M., Grieu, P., Afif, D., Cornu, S., et al. (1996). Carbon metabolism enzyme activities and carbon partitioning in Pinus halepensis Mill exposed to mild drought and ozone. *Journal of Plant Physiology* 148, 142-147

Greitner, C. S., Pell, E. J., and Winner, W. E. (1994). Analysis of aspen foliage exposed to multiple stresses - ozone, nitrogen deficiency and drought. *New Phytologist* 127, 579-589.

Grulke, N. E., and Preisler, H. K. (2003). A statistical approach to the estimation of ozone uptake and evaluation of the effects of water stress and ozone on photosynthesis in a Mediterranean climate pine species. *Monti e Boschi* 54, 41-46

Grulke, N. E., Preisler, H. K., Rose, C., Kirsch, J., and Balduman, L. (2002). O-3 uptake and drought stress effects on carbon acquisition of ponderosa pine in natural stands. *New Phytologist* 154, 621-631.

Grünhage, L., Pleijel, H., Mills, G., Bender, J., Danielsson, H., et al. (2012). Updated stomatal flux and flux-effect models for wheat for quantifying effects of ozone on grain yield, grain mass and protein yield. *Environmental Pollution* 165, 147-157.

Harmens, H., Hayes, F., Sharps, K., Radbourne, A., and Mills, G. (2019). Can Reduced Irrigation Mitigate Ozone Impacts on an Ozone-Sensitive African Wheat Variety? *Plants-Basel* 8, 220.

Hassan, I. A., Bender, J., and Weigel, H. J. (1999). Effects of ozone and drought stress on growth, yield and physiology of tomatoes (Lycopersicon esculentum Mill. cv. Baladey). *Gartenbauwissenschaft* 64, 152-157

Hatata, M. M., Badar, R. H., Ibrahim, M. M., and Hassan, I. A. (2013). Respective and interactive effects of O3 and CO2 and drought stress on photosynthesis, stomatal conductance, antioxidative ability and yield of wheat plants. *Current World Environment* 8, 409-417.

Hayes, F., Wagg, S., Mills, G., Wilkinson, S., and Davies, W. (2012). Ozone effects in a drier climate: implications for stomatal fluxes of reduced stomatal sensitivity to soil drying in a typical grassland species. *Global Change Biology* 18, 948-959.

Hayes, F., Williamson, J., and Mills, G. (2015). Species-Specific Responses to Ozone and Drought in Six Deciduous Trees. *Water Air and Soil Pollution* 226, 13.

Heggestad, H. E., Anderson, E. L., Gish, T. J., and Lee, E. H. (1988). Effects of ozone and soil water deficit on roots and shoots of field-grown soybeans. *Environmental Pollution* 50, 259-278.

Heggestad, H. E., Gish, T. J., Lee, E. H., Bennett, J. H., and Douglass, L. W. (1985). Interaction of soil-moisture stress and ambient ozone on growth and yields of soybeans. *Phytopathology* 75, 472-477.

Heggestad, H. E., and Lee, E. H. (1990). Soybean root distribution, top growth and yield responses to ambient ozone and soil moisture stress when grown in soil columns in greenhouse. *Environmental Pollution* 65, 195-207.

Heggestad, H. E., and Lesser, V. M. (1990). Effects of ozone, sulfur dioxide, soil water deficit, and cultivar on yields of soybean. *Journal of Environmental Quality* 19, 488-495

Hoshika, Y., Moura, B., and Paoletti, E. (2018). Ozone risk assessment in three oak species as affected by soil water availability. Environmental Science and Pollution Research 25, 8125-8136.

Inclan, R., Alonso, R., Pujadas, M., Teres, J., and Gimeno, B. S. (1998). Ozone and drought stress: Interactive effects on gas exchange in Aleppo pine (Pinus halepensis Mill.). *Chemosphere* 36, 685-690.

Inclan, R., Gimeno, B. S., Dizengremel, P., and Sanchez, M. (2005). Compensation processes of Aleppo pine (Pinus halepensis Mill.) to ozone exposure and drought stress. *Environmental Pollution* 137, 517-524.

Kang, Y., Khan, S., and Ma, X. (2009). Climate change impacts on crop yield, crop water productivity and food security – A review. *Progress in Natural Science* 19, 1665-1674.

Karlsson, P. E., Medin, E. L., Sellden, G., Wallin, G., Ottosson, S., et al. (2002). Impact of ozone and reduced water supply on the biomass accumulation of Norway spruce saplings. *Environmental Pollution* 119, 237-244.

Karlsson, P. E., Medin, E. L., Wallin, G., Sellden, G., and Skarby, L. (1997). Effects of ozone and drought stress on the physiology and growth of two clones of Norway spruce (Picea abies). *New Phytologist* 136, 265-275.

Karlsson, P. E., Medin, E. L., Wickstrom, H., Sellden, G., Wallin, G., et al. (1995). Ozone and drought stress - Interactive effects on the growth and physiology of Norway spruce (Picea abies (L) Karst). *Water Air and Soil Pollution* 85, 1325-1330.

Khan, S., and Soja, G. (2003). Yield responses of wheat to ozone exposure as modified by drought-induced differences in ozone uptake. *Water Air and Soil Pollution* 147, 299-315.

King, D. A., and Nelson, W. L. (1987). Assessing the impacts of soil-moisture stress on regional soybean yield and its sensitivity to ozone. *Agriculture Ecosystems & Environment* 20, 23-35.

Lawlor, D. W., and Tezara, W. (2009). Causes of decreased photosynthetic rate and metabolic capacity in water-deficient leaf cells: a critical evaluation of mechanisms and integration of processes. *Annals of Botany* 103, 561-579.

Le Thiec, D., Dixon, M., and Garrec, J. P. (1994). The effects of slightly elevated ozone concentrations and mild drought stress on the physiology and growth of Norway spruce, Picea-abies (l) Karst and beech, Fagus-sylvatica L., in open-top chambers. *New Phytologist* 128, 671-678

Le Thiec, D., and Manninen, S. (2003). Ozone and water deficit reduced growth of Aleppo pine seedlings. *Plant Physiology and Biochemistry* 41, 55-63.

Lee, W. S., Chevone, B. I., and Seiler, J. R. (1990). Growth and gas exchange of loblolly pine seedlings as influenced by drought and air pollutants. *Water, Air, and Soil Pollution* 51, 105-116.

Lei, T., Feng, J., Zheng, C., Li, S., Wang, Y., et al. (2020). Review of drought impacts on carbon cycling in grassland ecosystems. *Frontiers of Earth Science*.14, 462-478.

Li, L., Manning, W. J., Tong, L., and Wang, X. K. (2015). Chronic drought stress reduced but not protected Shantung maple (Acer truncatum Bunge) from adverse effects of ozone (O-3) on growth and physiology in the suburb of Beijing, China. *Environmental Pollution* 201, 34-41.

Li, P., Zhou, H. M., Xu, Y. S., Shang, B., and Feng, Z. Z. (2019). The effects of elevated ozone on the accumulation and allocation of poplar biomass depend strongly on water and nitrogen availability. *Science of the Total Environment* 665, 929-936.

Löw, M., Herbinger, K., Nunn, A. J., Häberle, K. H., Leuchner, M., et al. (2006). Extraordinary drought of 2003 overrules ozone impact on adult beech trees (Fagus sylvatica). *Trees* 20, 539-548.

Maliba, B. G., Inbaraj, P. M., and Berner, J. M. (2018). The effect of ozone and drought on the photosynthetic performance of canola. *Journal of Integrative Agriculture* 17, 1137-1144.

Maliba, B. G., Inbaraj, P. M., and Berner, J. M. (2019). Photosynthetic Responses of Canola and Wheat to Elevated Levels of CO2, O-3 and Water Deficit in Open-Top Chambers. *Plants-Basel* 8, 171.

Matyssek, R., Le Thiec, D., Low, M., Dizengremel, P., Nunn, A. J., et al. (2006). Interactions between drought and O-3 stress in forest trees. *Plant Biology* 8, 11-17.

McLaughlin, S. B., Wullschleger, S. D., Sun, G., and Nosal, M. (2007). Interactive effects of ozone and climate on water use, soil moisture content and streamflow in a southern Appalachian forest in the USA. *New Phytologist* 174, 125-136.

Meier, S., Grand, L. F., Schoeneberger, M. M., Reinert, R. A., and Bruck, R. I. (1990). Growth, ectomycorrhizae and nonstructural carbohydrates of Loblolly-pine seedlings exposed to ozone and soil-water deficit. *Environmental Pollution* 64, 11-27.

Meza, I., Siebert, S., Döll, P., Kusche, J., Herbert, C., et al. (2020). Global-scale drought risk assessment for agricultural systems. *Nat. Hazards Earth Syst. Sci.* 20, 695-712.

Miller, J. E., Heagle, A. S., Vozzo, S. F., Philbeck, R. B., and Heck, W. W. (1989). Effects of ozone and water-stress, separately and in combination, on soybean yield. *Journal of Environmental Quality* 18, 330-336.

Miller, J. E., Patterson, R. P., Heagle, A. S., Pursley, W. A., and Heck, W. W. (1988). Growth of cotton under chronic ozone stress at 2 levels of soil-moisture. *Journal of Environmental Quality* 17, 635-643.

Miller, J. E., Vozzo, S. F., Patterson, R. P., Pursley, W. A., and Heagle, A. S. (1995). Effects of ozone and water-deficit on field-grown soybean .2. Leaflet nonstructural carbohydrates. *Journal of Environmental Quality* 24, 670-677

Nemeskeri, E., and Helyes, L. (2019). Physiological Responses of Selected Vegetable Crop Species to Water Stress. *Agronomy-Basel* 9, 447.

Nussbaum, S., Bungener, P., Geissmann, M., and Fuhrer, J. (2000). Plant-plant interactions and soil moisture might be important in determining ozone impacts on grasslands. *New Phytologist* 147, 327-335.

Pääkkönen, E., Gunthardt-Goerg, M. S., and Holopainen, T. (1998a). Responses of leaf processes in a sensitive birch (Betula pendula Roth) clone to ozone combined with drought. *Annals of Botany* 82, 49-59.

Pääkkönen, E., Seppänen, S., Holopainen, T., Kokko, H., Kärenlampi, S., et al. (1998b). Induction of genes for the stress proteins PR-10 and PAL in relation to growth, visible injuries and stomatal conductance in birch (Betula pendula) clones exposed to ozone and/or drought. *New Phytologist* 138, 295-305.

Pääkkönen, E., Vahala, J., Holopainen, T., and Kärenlampi, L. (1998c). Physiological and ultrastructural responses of birch clones exposed to ozone and drought stress. *Chemosphere* 36, 679-684.

Pääkkönen, E., Vahala, J., Pohjolai, M., Holopainen, T., and Kärenlampi, L. (1998d). Physiological, stomatal and ultrastructural ozone responses in birch (Betula pendula Roth.) are modified by water stress. *Plant Cell and Environment* 21, 671-684.

Panek, J. A. (2004). Ozone uptake, water loss and carbon exchange dynamics in annually drought-stressed Pinus ponderosa forests: measured trends and parameters for uptake modeling. *Tree Physiology* 24, 277-290

Pearson, M., and Mansfield, T. M. (1993). Interacting Effects of Ozone and Water Stress on the Stomatal Resistance of Beech (Fagus sylvatica L.). *New Phytologist* 123, 351-358

Pollastrini, M., Desotgiu, R., Cascio, C., Bussotti, F., Cherubini, P., et al. (2010). Growth and physiological responses to ozone and mild drought stress of tree species with different ecological requirements. *Trees-Structure and Function* 24, 695-704.

Pretzsch, H., and Dieler, J. (2011). The dependency of the size-growth relationship of Norway spruce (Picea abies L. Karst.) and European beech (Fagus sylvatica L.) in forest stands on long-term site conditions, drought events, and ozone stress. *Trees-Structure and Function* 25, 355-369.

Qi, J. S., Song, C. P., Wang, B. S., Zhou, J. M., Kangasjarvi, J., et al. (2018). Reactive oxygen species signaling and stomatal movement in plant responses to drought stress and pathogen attack. *Journal of Integrative Plant Biology* 60, 805-826.

Reiner, S., Wiltshire, J. J. J., Wright, C. J., and Colls, J. J. (1996). The impact of ozone and drought on the water relations of ash trees (Fraxinus excelsior L). *Journal of Plant Physiology* 148, 166-171

Ribas, A., Penuelas, J., Elvira, S., and Gimeno, B. S. (2005). Contrasting effects of ozone under different water supplies in two Mediterranean tree species. *Atmospheric Environment* 39, 685-693.

Roberts, B. R. (1990). Physiological Response of Yellow-Poplar Seedlings to Simulated Acid Rain, Ozone Fumigation and Drought. *Forest Ecology and Management* 31, 215

Sangüesa-Barreda, G., Camarero, J. J., Oliva, J., Montes, F., and Gazol, A. (2015). Past logging, drought and pathogens interact and contribute to forest dieback. *Agricultural and Forest Meteorology* 208, 85-94.

Sarto, M. V. M., Sarto, J. R. W., Rampim, L., Rosset, J. S., Bassegio, D., et al. (2017). Wheat phenology and yield under drought: a review. *Australian Journal of Crop Science* 11, 941-946.

Schaub, M., Skelly, J. M., Steiner, K. C., Davis, D. D., Pennypacker, S. P., et al. (2003). Physiological and foliar injury responses of Prunus serotina, Fraxinus americana, and Acer rubrum seedlings to varying soil moisture and ozone. *Environmental Pollution* 124, 307-320.

Scheelbeek, P. F. D., Bird, F. A., Tuomisto, H. L., Green, R., Harris, F. B., Joy, E. J. M., Chalabi, Z., Allen, E., Haines, A., and Dangour, A. D. (2018). Effect of environmental changes on vegetable and legume yields and nutritional quality. Proceedings of the National Academy of Sciences of the United States of America 115, 6804-6809.

Schuldt, B., Buras, A., Arend, M., Vitasse, Y., Beierkuhnlein, C., et al. (2020). A first assessment of the impact of the extreme 2018 summer drought on Central European forests. *Basic and Applied Ecology* 45, 86-103.

Shang, B., Yuan, X. Y., Li, P., Xu, Y. S., and Feng, Z. Z. (2019). Effects of elevated ozone and water deficit on poplar saplings: Changes in carbon and nitrogen stocks and their allocation to different organs. *Forest Ecology and Management* 441, 89-98.

Stribley, G. H., and Ashmore, M. R. (2002). Quantitative changes in twig growth pattern of young woodland beech (Fagus sylvatica L.) in relation to climate and ozone pollution over 10 years. *Forest Ecology and Management* 157, 191-204.

Swann, A. L. S. (2018). Plants and Drought in a Changing Climate. *Current Climate Change Reports* 4, 192-201.

Temple, P. J. (1986). Stomatal conductance and transpirational responses of field-grown cotton to ozone. *Plant, Cell & Environment* 9, 315-321.

Temple, P. J., and Benoit, L. F. (1988). Effects of Ozone and Water Stress on Canopy Temperature, Water Use, and Water Use Efficiency of Alfalfa. *Agronomy Journal* 80, 439-447.

Temple, P. J., Benoit, L. F., Lennox, R. W., Reagan, C. A., and Taylor, O. C. (1988). Combined effects of ozone and water-stress on alfalfa growth and yield. *Journal of Environmental Quality* 17, 108-113.

Temple, P. J., and Riechers, G. H. (1995). Nitrogen allocation in Ponderosa pine-seedlings exposed to interacting ozone and drought stresses. *New Phytologist* 130, 97-104.

Temple, P. J., Riechers, G. H., Miller, P. R., and Lennox, R. W. (1993). Growth-responses of ponderosa pine to long-term exposure to ozone, wet and dry acidic deposition, and drought. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere* 23, 59-66.

Temple, P. J., Taylor, O. C., and Benoit, L. F. (1985). Cotton yield responses to ozone as mediated by soil-moisture and evapotranspiration. *Journal of Environmental Quality* 14, 55-60.

Tiwari, G. S., Pareek, N., and Raverkar, K. P. (2018). Increased heat and drought stress under climate change and their impact on physiological growth and development of crops: A review. *Indian Journal of Agricultural Sciences* 88, 10-17

Vicente-Serrano, S. M., Quiring, S. M., Pena-Gallardo, M., Yuan, S., and Dominguez-Castro, F. (2020). A review of environmental droughts: Increased risk under global warming? *Earth-Science Reviews* 201.

Vozzo, S. F., Miller, J. E., Pursley, W. A., and Heagle, A. S. (1995). Atmospheric pollutants and trace gases - effects of ozone and water-deficit on field-grown soybean .1. Leaf gas-exchange. *Journal of Environmental Quality* 24, 663-670

Wagg, S., Mills, G., Hayes, F., Wilkinson, S., Cooper, D., et al. (2012). Reduced soil water availability did not protect two competing grassland species from the negative effects of increasing background ozone. *Environmental Pollution* 165, 91-99.

Wallin, G., Karlsson, P. E., Sellden, G., Ottosson, S., Medin, E. L., et al. (2002). Impact of four years exposure to different levels of ozone, phosphorus and drought on chlorophyll, mineral nutrients, and stem volume of Norway spruce, Picea abies. *Physiologia Plantarum* 114, 192-206.

Wallin, G., and Skärby, L. (1992). The influence of ozone on the stomatal and nonstomatal limitation of photosynthesis in norway spruce, Picea-abies (l) Karst, exposed to soil-moisture deficit. *Trees-Structure and Function* 6, 128-136

Wang, J. Y., Xiong, Y. C., Li, F. M., Siddique, K. H. M., and Turner, N. C. (2017). Effects of Drought Stress on Morphophysiological Traits, Biochemical Characteristics, Yield, and Yield Components in Different Ploidy Wheat: A Meta-Analysis. *In* "Advances in Agronomy, Vol 143" (D. L. Sparks, ed.), Vol. 143, pp. 139-173. Elsevier Academic Press Inc, San Diego.

Wellburn, F. A. M., Lau, K. K., Milling, P. M. K., and Wellburn, A. R. (1996). Drought and air pollution affect nitrogen cycling and free radical scavenging in Pinus halepensis (Mill). *Journal of Experimental Botany* 47, 1361-1367.

Wellstein, C., Poschlod, P., Gohlke, A., Chelli, S., Campetella, G., et al. (2017). Effects of extreme drought on specific leaf area of grassland species: A meta-analysis of experimental studies in temperate and sub-Mediterranean systems. *Global Change Biology* 23, 2473-2481.

Werner, H., and Fabian, P. (2002). Free-air fumigation of mature trees. *Environmental Science and Pollution Research* 9, 117-121.

Wu, R.-j. (2017). Research advance in interactive effects of ozone concentration and soil water deficit on plants. *Shengtaixue Zazhi* 36, 846-853.

Xu, Y., Feng, Z., Shang, B., Yuan, X., and Tarvainen, L. (2020). Limited water availability did not protect poplar saplings from water use efficiency reduction under elevated ozone. *Forest Ecology and Management* 462, 117999.

Zhang, J. M., Zhang, S. Q., Cheng, M., Jiang, H., Zhang, X. Y., et al. (2018). Effect of Drought on Agronomic Traits of Rice and Wheat: A Meta-Analysis. *International Journal of Environmental Research and Public Health* 15.

Zhou, S. X., Medlyn, B., Sabate, S., Sperlich, D., and Prentice, I. C. (2014). Short-term water stress impacts on stomatal, mesophyll and biochemical limitations to photosynthesis differ consistently among tree species from contrasting climates. *Tree Physiology* 34, 1035-1046.

5 The modifying factor temperature

5.1 Effects on increasing temperature on vegetation

The consideration of air temperature and plant water status as co-factors in the estimation of (global) yield losses due to O_3 is clearly emphasised by the study of Schauberger et al. (2019). Tai and Martin (2017) predicted that O_3 pollution in combination with future warming will cause an average decline in US wheat, maize and soybean yield by 13%, 43% and 28%, respectively, and a smaller decline for Europe. According to the review of Scheelbeek et al. (2018), yield changes for all vegetables and legumes combined is amounted −31.5% on average for a 4 °C increase in temperature if baseline temperature is above 20 °C. Ainsworth et al. (2012) draw the attention to an indirect effect of increasing temperatures, namely the fact that with increasing temperatures O_3 concentrations will rise and will likely increase O_3 doses taken up by plants.

5.2 Interactive effects of O_3 and temperature on plants

5.2.1 Crops

There are hardly any publications known to us in which temperature and O_3 concentration were experimentally varied in such a way that knowledge about their effects singly and in combination could be obtained. Moreover, all the studies that used temperature as a co-factor were conducted in controlled environment exposure chambers.

For example, an episodic application of O_3 (80-100 ppb; 16h mean) to modern spring wheat varieties (KWS Bittern and Lennox) and a landrace variety (Lantvete) caused a loss in grain yield by 18, 16, and 44%, respectively. A concomitant increase in temperature by 5 °C resulted in a yield loss of 32, 22, and 36%, respectively (Hansen et al., 2019). Photosynthesis and biomass of Asian leaf mustard (*Brassica juncea*) was decreased significantly by O_3 (100 ppb, 8h mean) by 30% and 50%, respectively, while reduction in g_s was not significant (Lee et al., 2020). Raising the temperature by 5°C did amplify the symptom expression and resulted in the most detrimental effects on growth and gas exchange while g_s was significantly increased by about 48%. Furthermore, significant reductions in levels of carotenoids and ascorbic acid were observed when compared to the exposure to O_3 at the control regime for temperature. This result suggested that elevated temperature (+5°C) may enhance O_3 damage to *B. juncea*.

There are only two other publications reporting on O_3 x temperature interactions on crop plants. These, however, only consider the effects of increased root temperatures, while air temperature remained unchanged. For the sake of completeness, they should be mentioned here. *Brassica rapa* plants were treated with O_3 in an episodic pattern that averaged 63 ppb over the course of the experiment. Elevated O_3 reduced both total plant weight and fruit number by about 65%, when root temperature was 13°C and by about 56%, when root temperature was 18°C (Kleier et al., 1998). Radish (*Raphanus sativus*) plants subjected to the same experimental conditions responded to O_3 at 13°C root temperature by decreasing total biomass by 24% whereas O_3 had no effect on total biomass when root temperature was 18°C (Kleier et al., 2001).

The majority of studies which addressed the subject of the O_3 x temperature interaction used a different approach in which several climate factors were often changed at the same time, so that the modifying effect of temperature on the effect of O_3 cannot be clearly deduced from such multifactor treatments. Nevertheless, in Table 17 and Table 18 those interactive effects are compiled focussing on either any modification of the response to O_3 by increased temperature or on the response to O_3 under conditions of elevated CO_2 and temperature.

In four different cultivars of rape (*Brassica napus*) elevated air temperature (+5°C) caused a significant reduction in yield parameters, while enhanced levels of O_3 (48 ppb, daylight hour mean) had no effect on any of the cultivars (Frenck et al., 2011). However, under conditions of elevated CO_2, increasing the O_3 concentration exacerbated the temperature effect. Simultaneously, the oil content was decreased by elevated temperatures and O_3 but under conditions of elevated CO_2 and temperature additional O_3 had no effect on oil content (Namazkar et al., 2016). Barley (*Hordeum vulgare*) was grown in different climatic environments with elevated CO_2, O_3 (60/90 vs 20ppb) and temperature (24°C/19°C vs. 19°C/12°C day/night) as single factors and in combination. Net CO_2 assimilation rate was positively stimulated by all climate factors tested and highest values have been measured in the combination of all three, i.e. O_3 enhanced both photosynthetic rate and g_s by about 35% under control conditions for CO_2 and temperature and by 28% under elevated temperatures and CO_2 concentrations (Mikkelsen et al., 2015). There were two more studies using short term exposures to 40 and 80 ppb (7h day^{-1}) separately under either of 21C/14°C (day/night) and ambient concentrations of CO_2 representing present climate conditions or 25°C/16°C and doubled CO_2 concentrations (warming climate conditions). Both crop species radish (*Raphanus sativus*) and soybean (*Glycine max*) have been indicated to respond to O_3 with distinct growth reductions under lower temperatures and CO_2, whereas under warming climate conditions they accumulated significantly greater amount of shoot biomass in response to O_3 (Ramaskeviciene et al., 2008; Sakalauskaite et al., 2008).

Two genotypes of snap bean (*Phaseolus vulgaris*) have frequently been used as a tool for active biomonitoring of ambient O_3 with one genotype (S156) is showing a greater yield decline than the R123 genotype, reflected as declining S156/R123 yield ratios with increasing levels of O_3 (Burkey et al., 2005). During the growing seasons of 2012 and 2013, Agathokleous et al. (2017) conducted an O_3 biomonitoring study in an urban area in Athens, Greece. Differences in climatic conditions between the two years indicated that air temperature may have a greater impact on yield than O_3, thus, increased temperatures during reproductive growth may confound the O_3 effect detected in the field. The authors could further demonstrate that air temperature actually affected the S156/R123 yield ratios in the absence of O_3.

Table 17 Effects of O_3 on crop plants modified by increased temperature

Exposure facility was either controlled environment or biomonitoring as indicated. Arrows indicate a general trend of response.

***species*/cultivar**	**treatment**	**O_3 effect**		**interactive effect**		**reference**
Phaseolus vulgaris	biomonitoring x different years	decline in of S156/R123 yield ratio	↓	confounded interpretation of O_3 effects on S156/R123 yield ratio		Agathokleous et al. (2017)
Brassica juncea	O_3 (100 ppb, 8h mean) x temp. (+5°C)	leaf injury, reduction in photosynthesis and biomass	↓	amplification of leaf damage and impacts on growth and gas exchange	↓↓	Lee et al. (2020)
Brassica rapa	O_3 (63 ppb, 24h mean) x root temp. (+5°C)	growth and yield reduction	↓	total plant weight and fruit number has been reduced slightly less	↓	Kleier et al. (1998)
Raphanus sativus	O_3 (63 ppb, 24h mean) x root temp. (+5°C)	growth reduction	↓	protection against a reduction in total plant weight	±	Kleier et al. (2001).
Triticum aestivum cv. KWS Bittern	O_3 (80-100 ppb, 16h mean) x temp. (+5°C)	growth and yield reduction	↓	response of shoot biomass not modified impairment of yield increased	↓↓	Hansen et al. (2019)
Triticum aestivum cv. Landvete	O_3 (80-100 ppb, 16h mean) x temp. (+5°C)	growth and yield reduction	↓	reduction in shoot biomass was increased	↓↓	Hansen et al. (2019)
Triticum aestivum cv. Lennox	O_3 (80-100 ppb, 16h mean) x temp. (+5°C)	growth and yield reduction	↓	reduction in yield and shoot biomass was slightly increased	↓↓	Hansen et al. (2019)

Table 18 Effects of O_3 and interactive effects of increased temperature on crop plants under conditions of elevated CO_2

Exposure facility was either controlled environment or biomonitoring as indicated. Arrows indicate a general trend of response.

***species*/cultivar**	**treatment**	**O_3 effect**		**interactive effect**		**reference**
Triticum aestivum cv. KWS Bittern	O_3 (80-100 ppb, 16h mean) x CO_2 (700 ppm) x temp. (+5°C)	growth and yield reduction	↓	no negativeO_3 effect on yield and shoot growth	±	Hansen et al. (2019

***species*/cultivar**	**treatment**	**O_3 effect**		**interactive effect**		**reference**
Triticum aestivum cv. Landvete	O_3 (80-100 ppb, 16h mean) x CO_2 (700 ppm) x temp. (+5°C)	growth and yield reduction	↓	no negativeO_3 effect on yield and increase in shoot growth	±	Hansen et al. (2019
Triticum aestivum cv. Lennox	O_3 (80-100 ppb, 16h mean) x CO_2 (700 ppm) x temp. (+5°C)	growth and yield reduction	↓	same response of yield to O_3 but increase in shoot	↓	Hansen et al. (2019
Brassica napus cv. Bolero	O_3 (48 ppb, 16h mean) x CO_2 (650 ppm) x temp. (+5 °C)	yield reduction	↓	yield reduction was less severe	↓	Frenck et al. (2011)
Brassica napus cv. Mozart	O_3 (48 ppb, 16h mean) x CO_2 (650 ppm) x temp. (+5 °C)	slight yield reduction	↓	increase in yield	↑	Frenck et al. (2011)
Brassica napus cv. Mary	O_3 (48 ppb, 16h mean) x CO_2 (650 ppm) x temp. (+5 °C)	no effect on yield	±	no changes	±	Frenck et al. (2011)
Brassica napus cv. Tanto	O_3 (48 ppb, 16h mean) x CO_2 (650 ppm) x temp. (+5 °C)	no effect on yield	±	severe yield reduction	↓	Frenck et al. (2011)
Brassica napus	O_3 (52 ppb, 16h mean) x CO_2 (650 ppm) x temp. (+5 °C)	decrease in total oil content	↓	protection against a reduction in oil content	±	Namazkar et al. (2016)
Hordeum vulgare	O_3 (50-90 ppb, 24h mean) x CO_2 (700 ppm) x (Temp. (+5°C)	increase in photosynthetic rate and g_s	↑	increase in photosynthetic rate and g_s in the same range	↑	Mikkelsen et al. (2015)
Raphanus sativus	O_3 (80 ppb, 7h day-1) x CO_2 (700 ppm) x temp. (+4°C)	growth reduction	↓	increase in growth	↑	Sakalauskaite et al. (2008)
Glycine max	O_3 (80 ppb, 7h day-1) x CO_2 (700 ppm) x temp. (+4°C)	minor growth effects	±	increase in growth	↑	Ramaskeviciene et al. (2008)

5.2.2 Grassland and native herbaceous plants

Mortensen and Nilsen (1992) studied the response of several Scandinavian wild plant species to elevated O_3 (80 ppb, 8h mean) under two temperature regimes (15°C/11°C and 20°C/16 °C; 12h/12h) using growth chambers within a greenhouse. No interaction with temperature was detected for *Alchemilla alpine, Poa alpine vivipara,* and *Saxifraga cespitosa* (not responsive to O_3) and *Polygonum viviparum and Silene acaulis* (responsive to O_3). For *P. pratense*, however, there was a significant interaction between O_3 and temperature: at low temperatures dry weight decreased by 78% due to O_3 and by 46% due to increased temperatures. Relative to the control at low temperatures the concomitant effect of O_3 and increased temperature was a decrease in weight by 20%. A short-term exposure to O_3 (7 days) of plants pre-conditioned to different temperature regimes could support this finding as increasing the temperature during exposure significantly decreased the amount of leaf injury induced by O_3 (Mortensen, 1999).

There was one further study on a native herbaceous plant species from Lithuania. Under low temperatures (21°C/14°C) total biomass of *Chenopodium album* increased by about 70% when exposed to moderately enhanced concentrations of O_3 (40 ppb, not significant) but only by 35% when exposure occurred at higher temperatures (25°C/16°C), while for green biomass changes were +25% and -0.7%, respectively (Romaneckiene et al., 2008).

5.2.3 Woody plants

The majority of publications on studies with trees (15) come from Finland, where a series of free-air exposure studies was carried out between 2007 and 2013. They all focus on physiological and biochemical aspects and considered broad-leaved (*Populus tremula* and *Betula pendula*) and coniferous tress (*Pinus sylvestris* and *Picea abies*). While O_3 concentrations have been elevated to gain 1.3 to 1.5 times the ambient concentration 14 h day^{-1} infrared heaters were used to increase the air temperature by 1 to 1.3°C (24 h day^{-1}). An overview on the main effects of these experiments is shown in Table 19 and Table 20.

It was found that growth responses to these moderately enhanced concentrations of O_3 either did not occur in *P. abies* (Kivimaenpaa et al., 2013), *B. pendula* (Maenpaa et al., 2011; Riikonen et al., 2009), *P. tremula* (Maenpaa et al., 2011)) or were evident as total growth reductions in *P. sylvestris* (Kivimaenpaa et al., 2017; Rasheed et al., 2017) or a stem growth reduction in *B. pendula* (Kasurinen et al., 2012), while increased temperatures, with the exception of *P. abies* (Kivimaenpaa et al., 2013; Kivimaenpaa et al., 2017; Riikonen et al., 2012), always affected growth parameters and photosynthetic rate positively. Thus, as a result for these parameters a counteractive effect of both factors in their combination has been observed for each of the other tree species (Kasurinen et al., 2012; Kivimaenpaa et al., 2017; Maenpaa et al., 2011; Rasheed et al., 2017; Riikonen et al., 2009). On silver birch (*B. pendula*), for example, O_3 decreased leaf biomass by 27% at ambient temperature whereas it was doubled by increased temperature (Kasurinen et al., 2012). In the combined treatment, however, O_3 reduced the temperature effect, but as a result leaf biomass is about 50% higher than under ambient conditions of O_3 and temperature. This study also demonstrated that both temperature and O_3 effects were modified by the genotype with stem growth reductions caused by O_3 were more severe in the fastest growing clones. The counteractive effect has also been shown to be associated with a reduction in the redox state of ascorbate in the apoplast (Riikonen et al., 2009).

Table 19 Results from the Kuopio free-air exposure study on broad-leaved trees

VOCs = volatile organic compounds, P_n = net photosynthesis rate, g_s = stomatal conductance, temp. = temperature, LT50 = the temperature of 50% lethality of needles in autumn freezing stress resistance FSR= freezing stress resistance

species	**age/duration**	**temp. effect**	**O_3 effect**	**O_3 x temp.**	**reference**
Betula pendula	4 clones potted micro-propagated saplings 1 season	increase in leaf area, effect on P_n depended on leaf position	decrease in P_n and g_s, no growth effect	no interaction for growth, effect on P_n was less and effect on g_s was more severe at higher temp.	Maenpaa et al. (2011)
Betula pendula	4 clones potted micro-propagated saplings 2 seasons	decrease in g_s, increase in P_n and emission of total monoterpenes and green leaf volatiles	no effect on g_s, decrease in P_n and emission of VOCs	none	Hartikainen et al. (2012)
Betula pendula	4 clones potted micro-propagated saplings 2 seasons	did not alter the freezing stress resistance of buds	genotype-specific alterations in carbohydrate metabolism in buds	no clear pattern of an O_3 x temp. interactive effect	Riikonen et al. (2013)
Betula pendula	4 clones potted micro-propagated saplings 2 seasons	increase in leaf area, P_n, redox state of ascorbate and total antioxidant capacity in the apoplast	no effect on leaf area, but P_n was slightly and g_s significantly reduced	effects on leaf area and P_n were counteractive; O_3 reduced the redox state of ascorbate in the combined treatment	Riikonen et al. (2009)
Betula pendula	4 clones potted micro-propagated saplings 2 seasons	increase in above- and below-ground biomass and soil respiration rates, delayed senescence	accelerated leaf senescence, stem growth reduction, increase in mycorrhizal root growth	O_3 reduced the temperature effect, interaction on leaf biomass	Kasurinen et al. (2012)
Betula pendula	2 clones ground-grown micro-propagated saplings 2 seasons	decrease in litter content of N, C and bacterial DNA	increase in litter content of N and bacterial and fungal DNA	warming and O_3 both had only weak or no effects on litter mass loss	Kasurinen et al. (2017)
Populus tremula	2 clones potted micro-propagated saplings 2 seasons	increase in emissions of total monoterpenes and green leaf volatiles	minor effects on VOC emission, leaves exposed to elevated O_3 had thicker adaxial epidermis	increased temp. reduced the impact of O_3 on leaf structure	Hartikainen et al. (2009)
Populus tremula	4 clones potted micro-propagated saplings 1 season	increase in leaf area	decrease in P_n and g_s, no effect on growth	interaction for P_n, effect on P_n was less and effect on g_s was more severe at higher temp.	Maenpaa et al. (2011)

Table 20 Results from the Kuopio free-air exposure study on coniferous trees.

VOCs = volatile organic compounds, P_n = net photosynthesis rate, g_s = stomatal conductance, temp. = temperature, LT_{50} = the temperature of 50% lethality of needles in autumn conc. = concentration

species	age/duration	temp. effect	O_3 effect	O_3 x temp.	reference
Picea abies	ground-grown 3-yr-old seedlings 2 seasons	reduced stem diameter, decrease in P_n and g_s, change in the profile of emitted terpenoids	no effects on growth and gas exchange, doubling of total terpenoid emissions	none	Kivimaenpaa et al. (2013)
Picea abies	ground-grown 3-yr-old seedlings 2 seasons	increased cellular damage in early winter and visible damage in spring	O_3 reduced winter hardiness	damage by increased temp. was enhanced by O_3	Kivimaenpaa et al. (2014)
Picea abies	ground-grown 3-yr-old seedlings 2 seasons	reduction in growth, P_n and g_s, epidermis and hypodermis were thinner	reduction in P_n and g_s, thickening of epi-and hypo-dermis	positive effect on P_n and g_s in combination	Kivimaenpaa et al. (2017)
Picea abies	ground-grown 3-yr-old seedlings 2 seasons	reduced P_n, changes in needle chemical quality, no effect on its sensitivity to autumn and spring frosts	reduction in g_s and P_n, in total concentration of soluble sugars, trend for reduction of LT_{50}	season x temp. x O_3 interaction for g_s elevated temp. responses on g_s were similar in both O_3 treatments	Riikonen et al. (2012)
Pinus sylvestris	seedlings 2 seasons	effects of herbivory on emission rate of monoterpenes emission from pine sawfly-fed branches was reduced	effects of herbivory on emission rate of monoterpenes emission from pine sawfly-fed branches was enhanced	effect of herbivory on systemic emission of sesquiterpenes was enhanced by both factors in combination	Ghimire et al. (2017)
Pinus sylvestris	potted 1-yr-old seedlings 3season	warming reduced the conc. of phenolic defence compounds	reduction of phenolics and several flavonoids in previous-years stems, increase in current-year stems	strength of defence response may increase under the combined exposure	Ghimire et al. (2019)
Pinus sylvestris	potted 1-yr-old seedlings 3season	increase in VOC emissions	increase in VOC emissions	no interaction	Kivimaenpaa et al. (2016)
Pinus sylvestris	potted 1-yr-old seedlings 3season	increase in shoot and stem growth	growth reduction, and lower stomatal density and conductance	decrease in stomatal density only in ambient temp	Kivimaenpaa et al. (2017)
Pinus sylvestris	potted 1-yr-old seedlings 3season	increase in shoot and root biomass and in current year aboveground biomass	current-year aboveground biomass was reduced	O_3 counteracted the temp. effect, growth allocation was not equally increased in the combined exposures	Rasheed et al. (2017)

Net photosynthesis was reduced by elevated O_3 in both broad-leaved species silver birch (*B. pendula*) and European aspen (*P. tremula*) and elevated temperature was shown to counteract the O_3 effect on photosynthesis with the result that in aspen differences to control were only significant at ambient temperatures (Maenpaa et al., 2011). In addition, both elevated temperature and O_3 reduced stomatal conductance with temperature being the most effective factor but in the combined treatment the effect was less than additive and the O_3 x temperature interaction was significant. However, compared to control, the effect of O_3 on g_s was more severe at higher temperatures. Kivimaenpaa et al. (2017) studied the response of gas exchange in the coniferous species (*Pinus sylvestris* and *Picea abies*). O_3 caused a lower stomatal conductance in both species, but also decreased stomatal density in pine**.** There were significant interactive effects on stomatal response in both species: elevated O_3 and temperature alone decreased g_s in spruce, but in the combined treatment highest values have been measured, while the effect on stomatal density in pine was only apparent at ambient temperatures.

Other publications reported on changes in leaf structural characteristics due to elevated O_3 which also could be counteracted by increased temperatures in aspen (Hartikainen et al., 2009) and spruce (Kivimaenpaa et al., 2017). There were some parameters more for which a different response pattern has been described. In spruce, increased temperature had an adverse effect on cellular damage in autumn and spring and this damage could be intensified by elevated O_3 (Kivimaenpaa et al., 2014). In a similar way, increased temperature and elevated O_3 have been demonstrated to reduce the concentration of phenolic defence compounds and both factors strengthened the defence response (Ghimire et al., 2019). Herbivory stress is known to increase systemic emissions of volatile organic compounds (VOCs) and this effect may be enhanced by elevated O_3 or temperature alone or in combination. This effect took place without any O_3 x temperature interaction in *P. sylvestris* (Ghimire et al., 2017; Kivimaenpaa et al., 2016) and *P. abies* (Kivimaenpaa et al., 2013), while in *B. pendula* and *P. tremula* emission of terpenes mainly showed an effect of increased temperature (Hartikainen et al., 2009; Hartikainen et al., 2012).

In conclusion, these studies showed that even a moderate elevation in temperature and O_3 was efficient enough to cause notable changes in growth, gas exchange, leaf anatomy, and VOC emission and O_3 effects on growth and gas exchange may be counteracted by higher temperatures whereas in both coniferous species chemical composition or VOC emission may be mutually reinforced by temperature and O_3.

There were only four more studies on the interactive effects of temperature and O_3 on woody plants. On seedlings of *Betula pubescens*, elevated O_3 (45 to 80 ppb, 8h mean) induced leaf injury, when exposed in growth chambers within a greenhouse. The amount of injury increased progressively with decreasing temperature and was correlated with decreases in leaf diffusion resistance and plant growth rate (Mortensen, 1993; Mortensen, 1999; Mortensen and Nilsen, 1992). A recent Chinese study reported on the response of *Ginkgo biloba* and *Populus alba* to increased temperature (+2°C) and twofold ambient O_3 in open top chambers (Xu et al., 2020). Elevated O_3 decreased growth, physiological parameters (photosynthetic rate, stomatal conductance, and water use efficiency) and increased oxidative stress. These adverse effects have been mitigated by increased temperature and resulted in significant O_3 x temperature interactions.

Major results: O_3 x temperature

Experimental data on the interactive effect of O_3 and temperature are only available for a small number of species.

O_3 x temperature interactions were often studied in combination with other additional climate factors which makes a separate consideration of the sole influence of temperature on the O_3 effects difficult.

For crops, there are indications that under conditions of elevated CO_2 effects of O_3 may be mitigated by increasing the air temperature.

Most studies on trees showed that adverse effects of O_3 were mitigated by increased temperature. The free-air experiment in Kuopio, Finland, evidenced this temperature effect on the O_3 impacts for four tree species.

5.3 References

Agathokleous, E., Saitanis, C. J., Burkey, K. O., Ntatsi, G., Vougeleka, V., Mashaheet, A. M., and Pallides, A. (2017). Application and further characterization of the snap bean S156/R123 ozone biomonitoring system in relation to ambient air temperature. Science of the Total Environment 580, 1046-1055.

Burkey, K. O., Miller, J. E., and Fiscus, E. L. (2005). Assessment of ambient ozone effects on vegetation using snap bean as a bioindicator species. Journal of Environmental Quality 34, 1081-1086.

Frenck, G., van der Linden, L., Mikkelsen, T. N., Brix, H., and Jorgensen, R. B. (2011). Increased CO2 does not compensate for negative effects on yield caused by higher temperature and O-3 in Brassica napus L. European Journal of Agronomy 35, 127-134.

Ghimire, R. P., Kasurinen, A., Haikio, E., Holopainen, J. K., Julkunen-Tiitto, R., Holopainen, T., and Kivimaenpaa, M. (2019). Combined effects of elevated ozone, temperature, and nitrogen on stem phenolic concentrations of Scots pine (Pinus sylvestris) seedlings. Canadian Journal of Forest Research 49, 246-255.

Ghimire, R. P., Kivimaenpaa, M., Kasurinen, A., Haikio, E., Holopainen, T., and Holopainen, J. K. (2017). Herbivore-induced BVOC emissions of Scots pine under warming, elevated ozone and increased nitrogen availability in an open-field exposure. Agricultural and Forest Meteorology 242, 21-32.

Hansen, E. M. O., Hauggaard-Nielsen, H., Launay, M., Rose, P., and Mikkelse, T. N. (2019). The impact of ozone exposure, temperature and CO2 on the growth and yield of three spring wheat varieties. Environmental and Experimental Botany 168.

Hartikainen, K., Nerg, A.-M., Kivimaenpaa, M., Kontunen-Soppela, S., Maenpaa, M., Oksanen, E., Rousi, M., and Holopainen, T. (2009). Emissions of volatile organic compounds and leaf structural characteristics of European aspen (Populus tremula) grown under elevated ozone and temperature. Tree Physiology 29, 1163-1173.

Hartikainen, K., Riikonen, J., Nerg, A.-M., Kivimaenpaa, M., Ahonen, V., Tervahauta, A., Karenlampi, S., Maenpaa, M., Rousi, M., Kontunen-Soppela, S., Oksanen, E., and Holopainen, T. (2012). Impact of elevated temperature and ozone on the emission of volatile organic compounds and gas exchange of silver birch (Betula pendula Roth). Environmental and Experimental Botany 84, 33-43.

Kasurinen, A., Biasi, C., Holopainen, T., Rousi, M., Maenpaa, M., and Oksanen, E. (2012). Interactive effects of elevated ozone and temperature on carbon allocation of silver birch (Betula pendula) genotypes in an open-air field exposure. Tree Physiology 32, 737-751.

Kasurinen, A., Silfver, T., Rousi, M., and Mikola, J. (2017). Warming and ozone exposure effects on silver birch (Betula pendula Roth) leaf litter quality, microbial growth and decomposition. Plant and Soil 414, 127-142.

Kivimaenpaa, M., Ghimire, R. P., Sutinen, S., Haikio, E., Kasurinen, A., Holopainen, T., and Holopainen, J. K. (2016). Increases in volatile organic compound emissions of Scots pine in response to elevated ozone and warming are modified by herbivory and soil nitrogen availability. European Journal of Forest Research 135, 343-360.

Kivimaenpaa, M., Riikonen, J., Ahonen, V., Tervahauta, A., and Holopainen, T. (2013). Sensitivity of Norway spruce physiology and terpenoid emission dynamics to elevated ozone and elevated temperature under open-field exposure. Environmental and Experimental Botany 90, 32-42.

Kivimaenpaa, M., Riikonen, J., Sutinen, S., and Holopainen, T. (2014). Cell structural changes in the mesophyll of Norway spruce needles by elevated ozone and elevated temperature in open-field exposure during cold acclimation. Tree Physiology 34, 389-403.

Kivimaenpaa, M., Sutinen, S., Valolahti, H., Haikio, E., Riikonen, J., Kasurinen, A., Ghimire, R. P., Holopainen, J. K., and Holopainen, T. (2017). Warming and elevated ozone differently modify needle anatomy of Norway spruce (Picea abies) and Scots pine (Pinus sylvestris). Canadian Journal of Forest Research 47, 488-499.

Kleier, C., Farnsworth, B., and Winner, W. (1998). Biomass, reproductive output, and physiological responses of rapid-cycling Brassica (Brassica rapa) to ozone and modified root temperature. New Phytologist 139, 657-664.

Kleier, C., Farnsworth, B., and Winner, W. (2001). Photosynthesis and biomass allocation of radish cv. "Cherry Belle" in response to root temperature and ozone. Environmental Pollution 111, 127-133.

Lee, J. K., Woo, S. Y., Kwak, M. J., Park, S. H., Kim, H. D., Lim, Y. J., Park, J. H., and Lee, K. A. (2020). Effects of Elevated Temperature and Ozone in Brassica juncea L.: Growth, Physiology, and ROS Accumulation. Forests 11.

Maenpaa, M., Riikonen, J., Kontunen-Soppela, S., Rousi, M., and Oksanen, E. (2011). Vertical profiles reveal impact of ozone and temperature on carbon assimilation of Betula pendula and Populus tremula. Tree Physiology 31, 808-818.

Mikkelsen, B. L., Jorgensen, R. B., and Lyngkjaer, M. F. (2015a). Complex interplay of future climate levels of CO2, ozone and temperature on susceptibility to fungal diseases in barley. Plant Pathology 64, 319-327.

Mikkelsen, B. L., Olsen, C. E., and Lyngkjaer, M. F. (2015b). Accumulation of secondary metabolites in healthy and diseased barley, grown under future climate levels of CO2, ozone and temperature. Phytochemistry 118, 162-173.

Mortensen, L. M. (1993). Effects of ozone on young plants of Betula pubescens Ehrh. at different temperature and light levels. Norwegian Journal of Agricultural Sciences 7, 293-300.

Mortensen, L. M. (1999). Foliar injuries caused by ozone in Betula pubescens Ehrh. and Phleum pratense L. as influenced by climatic conditions before and during O-3 exposure. Acta Agriculturae Scandinavica Section B-Soil and Plant Science 49, 44-49.

Mortensen, L. M., and Nilsen, J. (1992). Effects of Ozone and Temperature on Growth of Several Wild Plant Species. Norwegian Journal of Agricultural Sciences 6, 195-204.

Namazkar, S., Stockmarr, A., Frenck, G., Egsgaard, H., Terkelsen, T., Mikkelsen, T. N., Ingvordsen, C. H., and Jorgensen, R. B. (2016). Concurrent elevation of CO2, O-3 and temperature severely affects oil quality and quantity in rapeseed. Journal of Experimental Botany 67, 4117-4125.

Ramaskeviciene, A., Juozaityte, R., Sliesaravicius, A., Venskutoniene, E., and Zilenaite, L. (2008). Ozone influence on photosynthesis pigment system and growth of Soya (Glycine max (L.) Merr.) under warming climate conditions. Sodininkyste ir Darzininkyste 27, 187-197.

Rasheed, M. U., Kasurinen, A., Kivimaenpaa, M., Ghimire, R. P., Haikio, E., Mpamah, P., Holopainen, J. K., and Holopainen, T. (2017). The responses of shoot-root-rhizosphere continuum to simultaneous fertilizer addition, warming, ozone and herbivory in young Scots pine seedlings in a high latitude field experiment. Soil Biology & Biochemistry 114, 279-294.

Riikonen, J., Kontunen-Soppela, S., Ossipov, V., Tervahauta, A., Tuomainen, M., Oksanen, E., Vapaavuori, E., Heinonen, J., and Kivimaenpaa, M. (2012). Needle metabolome, freezing tolerance and gas exchange in Norway spruce seedlings exposed to elevated temperature and ozone concentration. Tree Physiology 32, 1102-1112.

Riikonen, J., Kontunen-Soppela, S., Vapaavuori, E., Tervahauta, A., Tuomainen, M., and Oksanen, E. (2013). Carbohydrate concentrations and freezing stress resistance of silver birch buds grown under elevated temperature and ozone. Tree Physiology 33, 311-319.

Riikonen, J., Maenpaa, M., Alavillamo, M., Silfver, T., and Oksanen, E. (2009). Interactive effect of elevated temperature and O-3 on antioxidant capacity and gas exchange in Betula pendula saplings. Planta 230, 419-427.

Romaneckiene, R., Pilipavicius, V., and Romaneckas, K. (2008). The influence of ozone and uv-b radiation on fat-hen growth in different temperature conditions. Zemdirbyste-Agriculture 95, 122-132.

Sakalauskaite, J., Brazaityte, A., Urbonaviciute, A., Samuoliene, G., Sabajeviene, G., Sakalauskiene, S., and Duchovskis, P. (2008). Radish response to distinct ozone exposure and to its interaction with elevated CO2 concentration and temperature. Sodininkyste ir Darzininkyste 27, 151-160.

Schauberger, B., Rolinski, S., Schaphoff, S., and Mueller, C. (2019). Global historical soybean and wheat yield loss estimates from ozone pollution considering water and temperature as modifying effects. Agricultural and Forest Meteorology 265, 1-15.

Scheelbeek, P. F. D., Bird, F. A., Tuomisto, H. L., Green, R., Harris, F. B., Joy, E. J. M., Chalabi, Z., Allen, E., Haines, A., and Dangour, A. D. (2018). Effect of environmental changes on vegetable and legume yields and nutritional quality. Proceedings of the National Academy of Sciences of the United States of America 115, 6804-6809.

Tai, A. P. K., and Martin, M. V. (2017). Impacts of ozone air pollution and temperature extremes on crop yields: Spatial variability, adaptation and implications for future food security. Atmospheric Environment 169, 11-21.

Xu, S., Wang, Y., Zhang, W., Li, B., Du, Z., He, X., Chen, W., Zhang, Y., Li, Y., Li, M., and Schaub, M. (2020). Experimental warming alleviates the adverse effects from tropospheric ozone on two urban tree species. Environmental Pollution, 115289.

6 O_3 effects under conditions of high N deposition or in a changed climate

Combination studies have usually been designed as two-(or multi-) factorial experiments with different levels of each factor which allows to partition between main effects of O_3 and the corresponding factor on response variables and their interaction. In the graphical analyses presented in the previous chapters, the effects of O_3 singly and in combination with a second factor were expressed relative to a control treatment where both factors represent a non-stressed situation, i.e. the reference treatments in the studies usually were designed with low O_3 levels, low N input, ambient CO_2, or well-watered conditions, respectively (see Table 3 chapter 1.3). This should answer the question whether and how the effect of elevated O_3 on a plant receptor is changed if an additional climate factor occurs to an increased extent at the same time. By this means, it could be shown that an increase in the concentration of CO_2 or in the amount of N, i.e. of those factors, which in general have a positive effect on plant growth and photosynthesis, has the ability to reduce the extent of adverse O_3 effects or even cause a net positive effect. The value of this net effect seems to depend on the concentration of O_3, the sensitivity of target plants, the concentration of CO_2 or the amount of N, respectively and the response parameter considered. Thus, the analysis shown above describe how plant growth is altered when O_3 and a second factor are changed simultaneously and in principle represent one method for estimating the modifying effect of these climate factors in terms of the assessment of the phytotoxic potential of a certain O_3 situation.

Table 21 **Results for linear regressions of combined effects as a function of the O_3 effect alone**

The data refer to the below following figures as indicated. The regression was calculated independently of the level of elevated CO_2 and elevated N, respectively. **A**: x-axis: O_3 as percentage deviation from control; y-axis: O_3 + 2nd factor as percentage deviation from control + 2nd factor. **B**: x-axis: O_3 as percentage deviation from control; y-axis: O_3 + 2nd factor as percentage deviation from control (**B is not shown in the figures**); ABM = above-ground biomass, P_n = photosynthetic rate, g_s = stomatal conductance.

factor	vegetation type	para-meter	A: slope	A: intercept	A: R²	B: slope	B: intercept	B: R²
N	all types	ABM	**0.137**	**-9.89**	**0.0307**	-0.089	26.9	0.0033
N	all types	root	**0.260**	**-6.43**	**0.1259**	0.543	20.9	0.1156
N	all types	total	**0.245**	**-7.40**	**0.0847**	0.338	18.1	0.0491
N	all types	P_n	**0.219**	**-3.08**	**0.0936**	0.590	5.8	0.3236
N	all types	g_s	**0.677**	**-2.66**	**0.3224**	0.865	-1.4	0.5872
CO_2	crops	yield	**0.353**	**-0.15**	**0.1403**	0.776	17.5	0.2724
CO_2	crops	ABM	**-0.024**	**-3,56**	**0.0004**	0.771	16.0	0.2826
CO_2	crops	P_n	**0.090**	**-7.19**	**0.0083**	0.359	18.0	0.0746
CO_2	crops	g_s	**0.291**	**-5.22**	**0.0530**	0.285	-26.7	0.0558
CO_2	trees	root	**0.626**	**16.69**	**0.3159**	1.019	43.8	0.5957
CO_2	trees	ABM	**0.522**	**7.40**	**0.2393**	1.057	40.7	0.6204

factor	vegetation type	para-meter	A: slope	A: intercept	A: R^2	B: slope	B: intercept	B: R^2
CO_2	trees	P_n	**0.409**	**-4.88**	**0.3635**	1.103	30.9	0.6542
CO_2	trees	g_s	**0.155**	**3.15**	**0.1511**	1.121	-5.3	0.6198
drought	all types	ABM	**0.883**	**7.06**	**0.5033**	0.637	-25.1	0.5809
drought	all types	root	**0.805**	**3.11**	**0.3281**	0.899	-2.4	0.1535
drought	all types	total	**0.753**	**4.14**	**0.3855**	0.536	-29.8	0.3304
drought	all types	P_n	**0.647**	**7.41**	**0.1753**	0.434	-23.7	0.1173
drought	all types	g_s	**0.236**	**8.00**	**0.0583**	0.430	-28.5	0.3289

The aim of this chapter is to consider the question of how the effect of O_3 is likely to change under conditions of elevated N, CO_2, and drought stress, respectively. For this purpose we have recalculated the effects of the combined treatment relative to a reference treatment with low O_3 levels (usually the control treatment for O_3 in the studies) but with high levels of N, CO_2, or with drought stress (see Table 3 chapter 1.3). In the following figures, each data point represents a survey taken from the literature. This effect is indicated as the O_3 effect at elevated CO_2/N/drought and was plotted against the O_3 effect without any second factor.

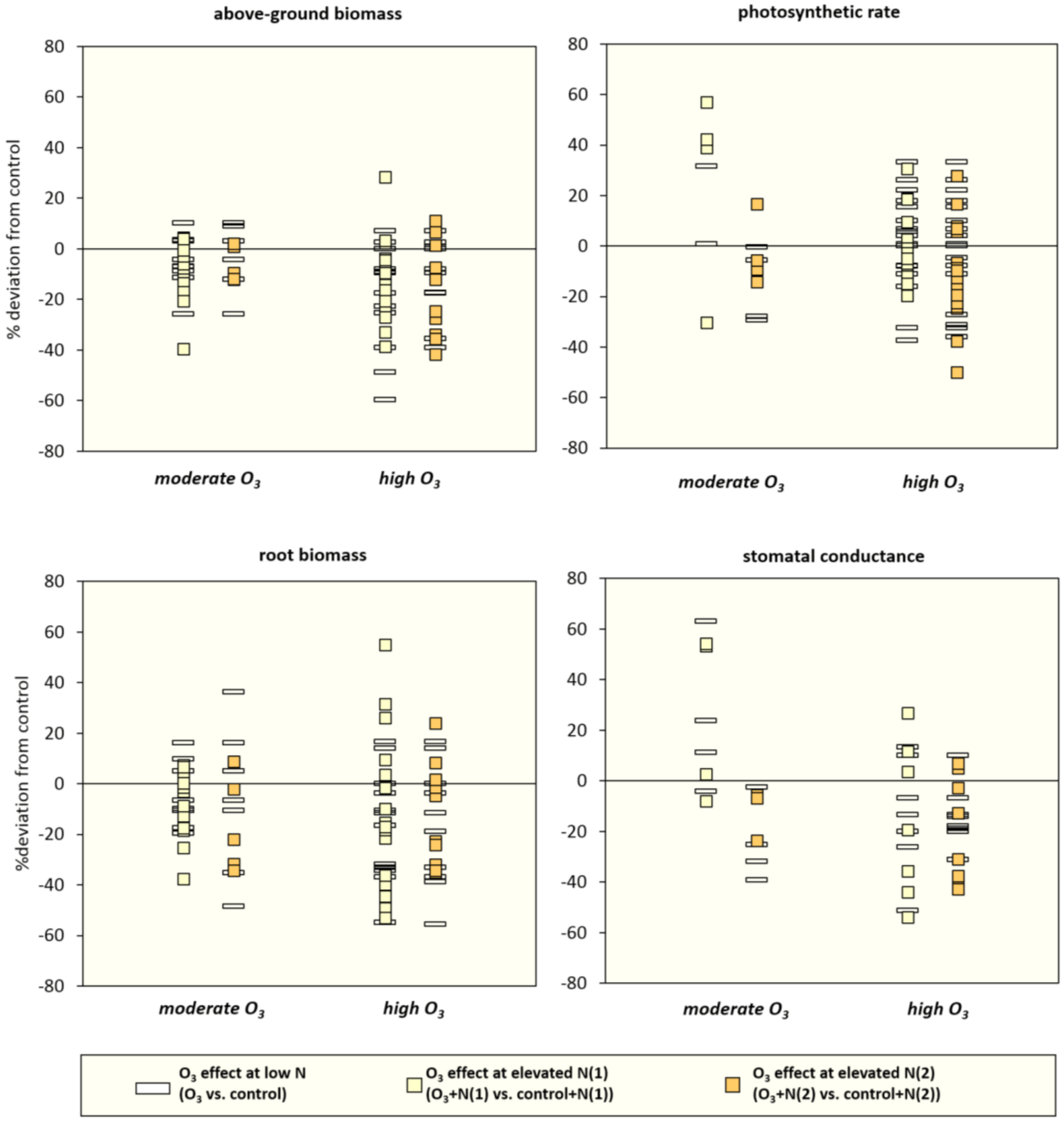
6.1 Nitrogen (N)

Feng et al. (2019) conducted a meta-analysis on semi-natural vegetation and concluded that the negative effect on biomasses is not mitigated by additional N supply. At first view, this contradicts the results of our analyses presented in chapter 2.2. However, when the data that we have compiled for this analysis are expressed relative to the other reference treatment (control for O_3 at elevated N), this discrepancy seem to disappear. For all parameters considered here, effects estimated under additional N supply are in the same range as those under conditions of low N (Figure 14). Moreover, there is no clear relationship between O_3 effects under low and enhanced N supply (Table 21). The lack of correlation between the O_3 effects under different conditions for N indicates some kind of modification by N. However, a mitigation effect could not be detected, probably because of the great variance of experimental conditions and the combined consideration of all vegetation types. For gas exchange parameters, both effects are related more closely and the slope of the regression curve for stomatal conductance is the highest.

To illustrate the importance of the choice of the reference treatment, in Figure 15 the effects obtained by calculation with both reference treatments are compared. In terms of above-ground biomass there was no correlation between the combined effect and the single O_3 effect, but the effect based on the reference treatment with low N (A) reflects the fertilising effect of N by the shift of data to the positive range. The effect of the combined treatment relative on the reference treatment with additional N supply (B) revealed effects in the same range as estimated for O_3 alone, a result that agrees with that of Feng et al. (2019). However, the large scatter of data in the figures shown in this study may reflect the broad range of vegetation types included. Interestingly, for the parameter stomatal conductance there is a greater correlation between the combined and the single effect of O_3 indicating an influence of O_3 independent of the N availability.

Figure 14 The modifying impact of elevated N on the effect of O_3 on growth and gas exchange parameters

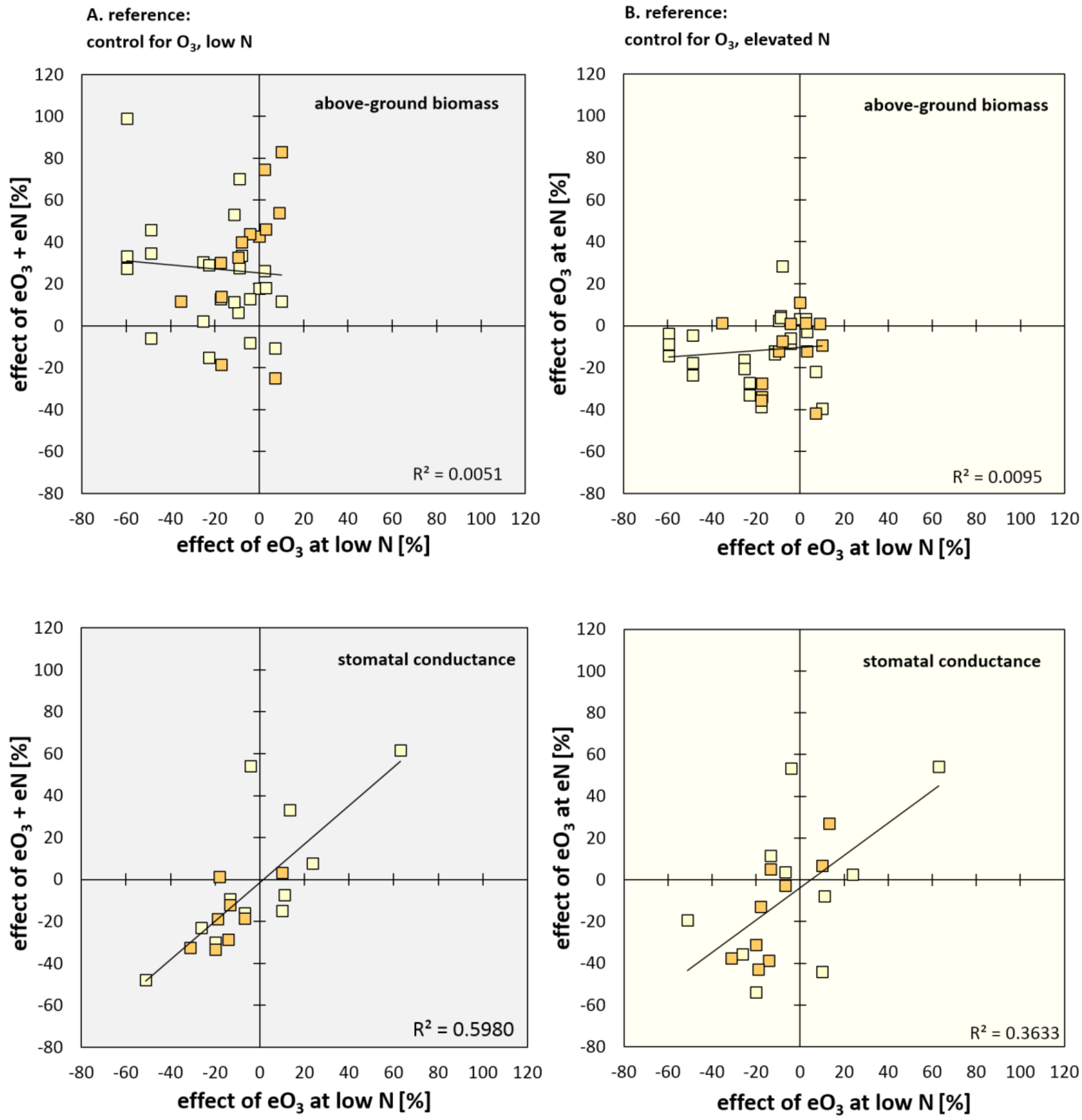
The effect of O_3 under conditions of elevated N (O_3 + N treatment as percentage deviation from the control + N) is compared to the effect of O_3 alone (low N), data of all vegetation types are shown together; moderate O_3 = O_3 concentration ≤ 40 ppb (24h mean) or ≤ 70 ppb (8h mean); high O_3 = O_3 concentration > 40 ppb (24h mean) or > 70 ppb (8h mean); eO_3 = elevated O_3, eN = elevated N. For exposure conditions and references see Table 22 to Table 24, chapter A.1.1.



Source: own illustration, Thünen Institute

Figure 15 **The modifying impact of elevated N on the effect of O_3 by comparing effects obtained by using different reference treatments**

The data for A are corresponding to the data shown in chapter 2.2, the data for B are corresponding to Figure 14; only non-crop plants are included, for symbols see Figure 14.



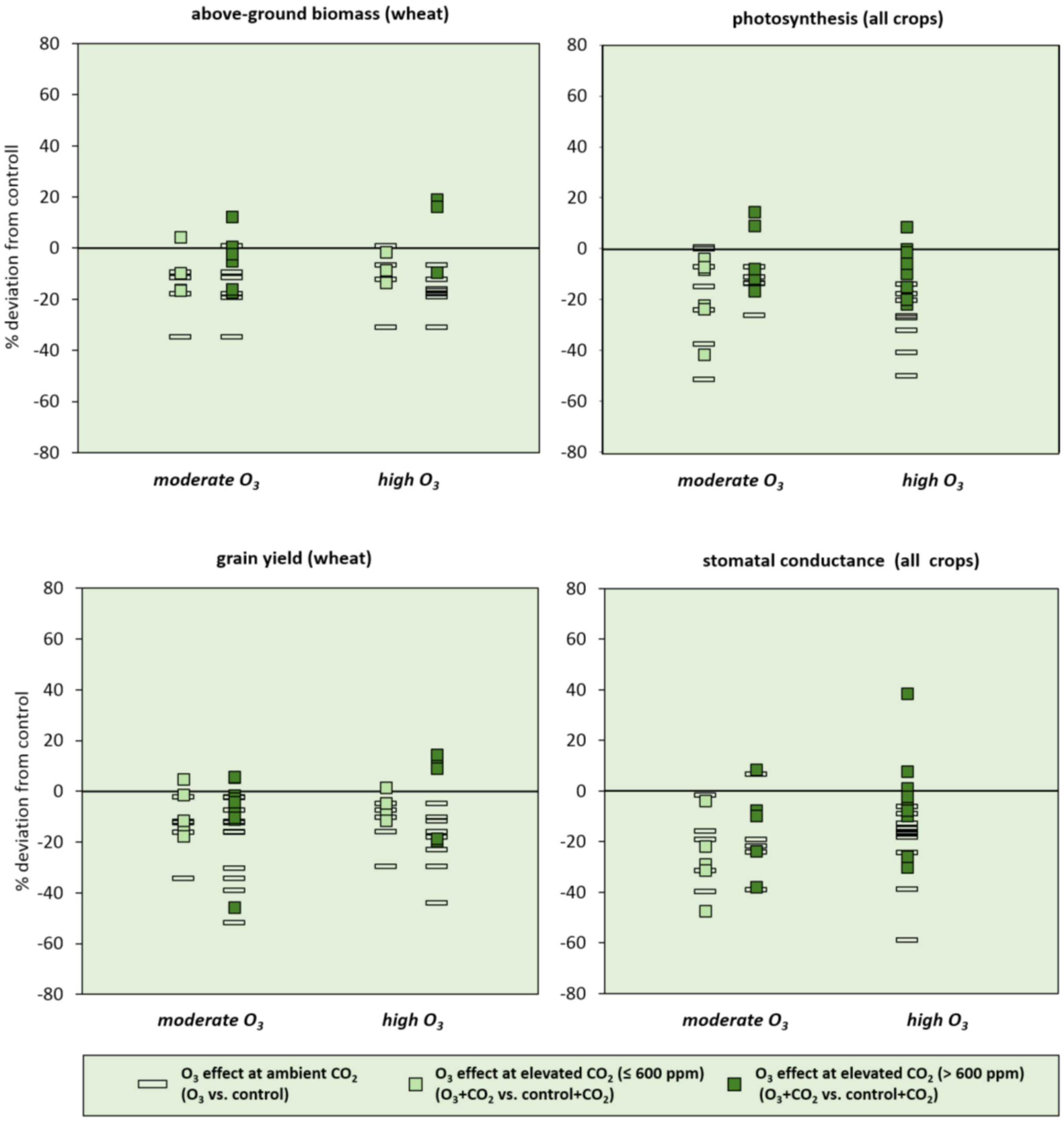
Source: own illustration, Thünen Institute

6.2 Carbon dioxide (CO_2)

For the crop data shown in Figure 16, there is no correlation between the O_3 effect at ambient and at elevated CO_2, however, for some parameters of woody plants there is a poor relationship. In general, regressions with the effect of O_3 plus elevated CO_2 yielded higher values for R^2, slope and intercept that means values are shifted to the positive range (Table 21). For gas exchange parameters a similar pattern was found.

Figure 16 The modifying impact of elevated CO_2 on the effect of O_3 on growth and gas exchange parameters of wheat and other crops

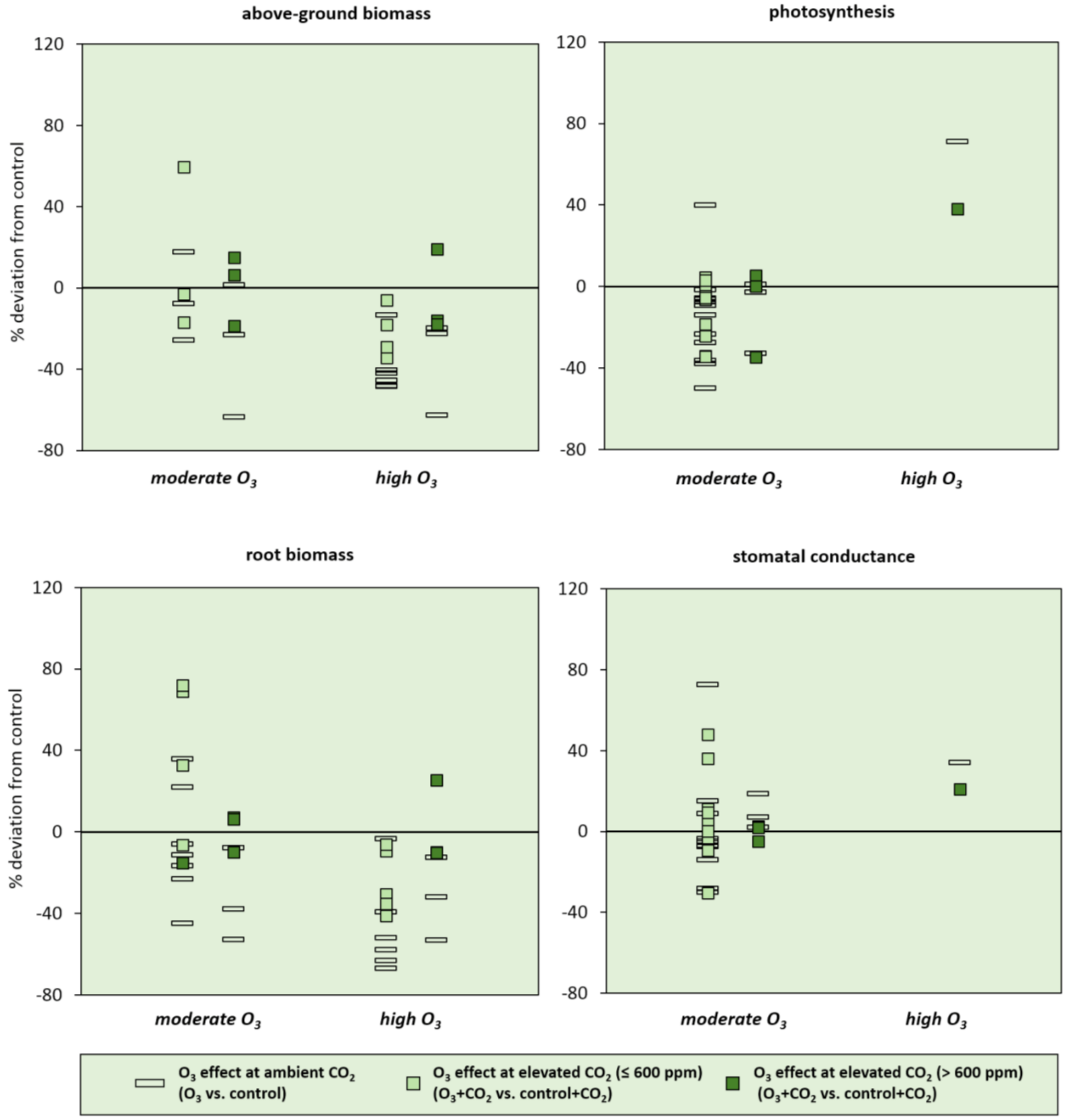
The effect of O_3 under conditions of elevated CO_2 (O_3 + CO_2 treatment as percentage deviation from the control + CO_2) is compared to the effect of O_3 alone (ambient CO_2); moderate O_3 = O_3 concentration ≤ 40 ppb (24h mean) or ≤ 70 ppb (8h mean); high O_3 = O_3 concentration > 40 ppb (24h mean) or > 70 ppb (8h mean); eO_3 = elevated O_3, eCO_2 = elevated CO_2. For exposure conditions and references see Table 25 and Table 26 chapter A.1.2



Source: own illustration, Thünen Institute

Figure 17 **The modifying impact of elevated CO_2 on the effect of O_3 on biomass and gas exchange parameters of woody plants**

The effect of O_3 under conditions of elevated CO_2 (O_3 + CO_2 treatment as percentage deviation from the control + CO_2) is compared to the effect of O_3 alone (ambient CO_2); moderate O_3 = O_3 concentration ≤ 40 ppb (24h mean) or ≤ 70 ppb (8h mean); high O_3 = O_3 concentration > 40 ppb (24h mean) or > 70 ppb (8h mean); eO_3 = elevated O_3, eCO_2 = elevated CO_2. For exposure conditions and references see Table 27, chapter A.1.2.



Source: own illustration, Thünen Institute

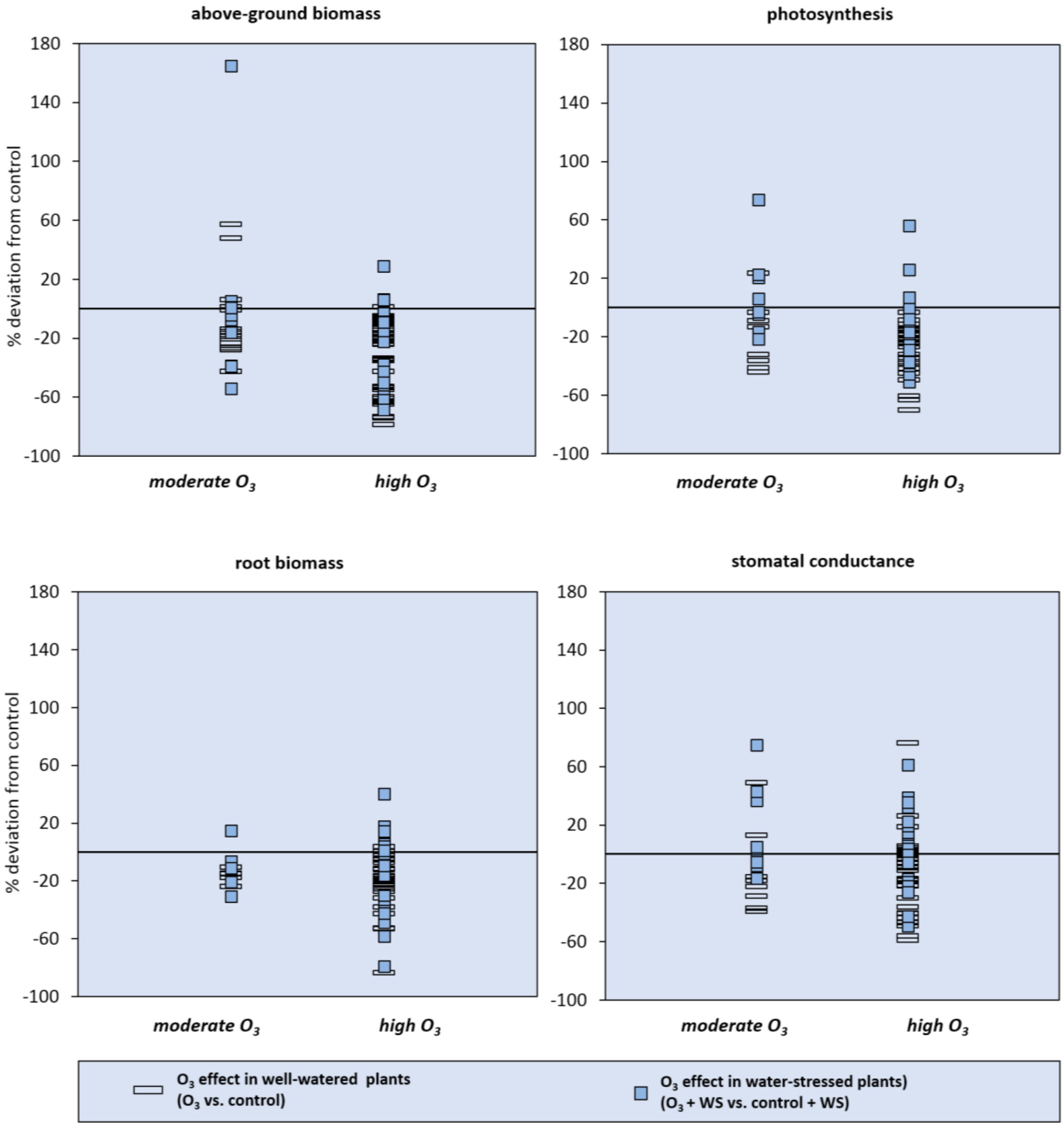
Effects of O_3 obtained under conditions of elevated CO_2 appear to be less severe or sometimes even absent (Figure 16 and Figure 17). Thus, the percentage changes due to O_3 calculated here tend to be less negative than at ambient CO_2 but less positive than those shown in chapter 3.

6.3 Soil water deficit

In the following figures data from experiments with O_3 and soil moisture deficit from all vegetation types are combined. Because the intensity of drought stress set within the experiments was often not specified, no differentiation was made here.

Figure 18 The modifying impact of increasing soil moisture deficit on the effect of O_3 on growth and gas exchange parameters

The effect of O_3 in water-stressed plants (O_3 + WS treatment as percentage deviation from the control + WS) is compared to the effect of O_3 alone (WW); moderate O_3 = O_3 concentration ≤ 40 ppb (24h mean) or ≤ 70 ppb (8h mean); high O_3 = O_3 concentration > 40 ppb (24h mean) or > 70 ppb (8h mean); eO_3 = elevated O_3, WW = well-watered, WS = water-stressed. For exposure conditions and references see Table 28 and Table 29, chapter A.1.3., Data of all vegetation types are shown together.



Source: own illustration, Thünen Institute

In water-stressed plants in the majority of cases similar O_3 effects have been estimated when compared to the O_3 effect in well-watered plants (Figure 18), which is reflected by the best values for regression with respect to growth parameters (Table 21) where the best correlation between O_3 effects in well-watered and water-stressed plants was calculated for growth and photosynthesis but not for stomatal conductance. Different than expected this analysis is not able to confirm a mitigation of the O_3 effect by drought; however, in the case of stomatal conductance an interaction is likely.

6.4 Reference

Feng, Z. Z., Shang, B., Li, Z. Z., Calatayud, V., and Agathokleous, E. (2019). Ozone will remain a threat for plants independently of nitrogen load. Functional Ecology 33, 1854-1870.

7 Conclusion

For the climate factors under consideration here, clear modifications of the response to O_3 can be identified for all types of vegetation, i.e. crops, grassland and wood species. Some of them are indicated by an altered O_3 effect, which may occur without the evidence of a statistically significant interaction. This applies in particular to elevated concentrations of CO_2, where a trend towards a shift in the net effect can be seen when both gases are combined.

A modification of the response to O_3 by increasing the N availability has been reported for the majority of the species tested. As both factors affect plant growth in the opposite way the resulting response is predicted to be opposed. In fact, the nature and the extent of this influence vary greatly and appear to depend on the amount of N added and the plant strategy. In addition, there is abundant evidence that an additional supply of N may enhance the adverse effect of elevated O_3 on photosynthesis, growth, reproduction, and yield. This is partly attributed to an interference with plant's internal compensatory mechanisms and allocation processes induced by O_3. Moreover, by inducing accelerated senescence and leaf abscission, O_3 itself may influence N cycling in the soil-plant system with consequences for the N availability.

Drought stress is predicted to reduce the dose of O_3 absorbed via stomatal uptake. In fact, both a protective and an exacerbating effect have been observed. However, there is little data on the modifying effect of increased temperature on plant responses to O_3. Hence, there is a need to further investigate the modifying influence of the factors drought and temperature, which are usually associated with each other.

Compared to ambient concentrations of CO_2, the impact of O_3 is often reduced when assessed under conditions of elevated CO_2, while under conditions of increased N availability or soil moisture deficit no clear trend for a modification of the O_3 effect can be detected, i.e. adverse effects of O_3 can be affected by N or drought in different ways. This may be due to the fact that timing of N application or timing of drought episodes in the studies under consideration was quite variable and that was also true for the amount of N or water chosen for the control treatment.

Finally, any changes, which are graded from weak mitigation to complete masking of the O_3 impact by the fertilising effect of N or CO_2, should not be neglected in future risk assessments. Especially the N availability has been proven to be a factor for which a modifying effect of the response to O_3 is rather likely and should be considered in current estimates of O_3 effects.

A Appendix

A.1 Exposure conditions

A.1.1 Chapter 2: Nitrogen (N)

Table 22 Exposure conditions and references referred to Figure 2, Figure 14 and Figure 15 (crops, chapter 2.2.1)

Contr. = control treatment, contr. environ. = controlled environment, GH = green house, OTC = open top chamber, FACE = free air carbon dioxide enrichment, CF = charcoal filtered, NF = non filtered, AA = ambient air, exposure duration was three weeks (Brewster et al.,2018) or life span, (1) = moderate N, (2) high N.

***species*/cultivar**	**facility**	**O_3 contr.**	**elevated O_3**	**N control**	**elevated N**	**N supply**	**reference**
Brassica campestris cv. Kranti	OTC	CF	47 ppb (12 h mean)	80 kg N ha^{-1}	120 kg N ha^{-1}(1)	urea	Singh et al. (2009). Evaluation of physiological, growth and yield responses of a tropical oil crop (Brassica campestris L. var. Kranti) … ozone pollution at varying NPK levels. In "Environmental Pollution", Vol. 157, pp. 871-880.
Citrillus lanants cv. Reina de Corazones	OTC	CF	5.1-6.2 ppm h, 36-33 ppm h (AOT40)	140 kg N ha^{-1}	280 kg N ha^{-1}(1) 436 kg N ha^{-1}(2)	NH_4NO_3	Calatayud et al. (2006). Interactions between nitrogen fertilization and ozone in watermelon cultivar …. Effects on chlorophyll alpha fluorescence, lipid peroxidation, and yield. In "Photosynthetica", Vol. 44, pp. 93-101.
Gossypium hirsutum cv. Deltapine 51	OTC	CF	51 ppb, 71-78 ppb (12 h mean)	0.52 g L^{-1} soil	1.02 g L^{-1} soil(1) 2.04 g L^{-1} soil(2)	urea form-aldehyd	Heagle et al. (1999). Ozone stress, carbon dioxide enrichment, and nitrogen fertility interactions in cotton. In "Crop Science", Vol. 39, pp. 731-741.
Oryza sativa cv. Koshihikari	GH	CF	26.6 ppb 38.0 ppb (12 h mean)	0 g pot^{-1}	0.3 g pot^{-1}(1)	NPK (5%N)	Tatsumi et al. (2019). Effects of ozone on the growth and yield of rice (Oryza sativa L.) under different nitrogen fertilization regimes. In "Environmental Science and Pollution Research", Vol. 26, pp. 32103-32113.
Oryza sativa cv. Shanyou 63 cv. Yangdao 6	free air	--	--	200 kg N ha^{-1}	250 kg N ha^{-1}(2)	--	Luo et al. (2013). Responses of Dry Matter Production and Distribution in Rice (Oryza saliva L.) to Ozone and High Nitrogen Supply. In "Chinese Journal of Applied and Environmental Biology", Vol. 19, pp. 286-292.

***species*/cultivar**	**facility**	**O_3 contr.**	**elevated O_3**	**N control**	**elevated N**	**N supply**	**reference**
Phaseolus vulgaris cv. Pros	OTC	CF	16 ppb -70 ppb (9 h mean)	21 ppb (24h)	44-47 ppb (1) (24h mean)	NH_3	Tonneijck and van Dijk (1998). Responses of bean (Phaseolus vulgaris L. cv. Pros) to chronic ozone exposure at two levels of atmospheric ammonia. In "Environmental Pollution", Vol. 99, pp. 45-51.
Triticum aestivum	free air	AA	1.5 NF	210 kg N ha^{-1}	250 kg N ha^{-1}(2)	--	Chen et al. (2011). Nitrogen supply mitigates the effects of elevated O-3 on photosynthesis and yield in wheat. In "Chinese Journal of Plant Ecology", Vol. 35, pp. 523-530.
Triticum aestivum cv. HD2967 cv. Sonalika	OTC	NF	60 ppb (8 h mean)	120 kg N ha^{-1}	180 kg N ha^{-1}(1)	urea	Pandey et al. (2018). Effect of elevated ozone and varying levels of soil nitrogen in two wheat (Triticum aestivum L.) cultivars:… In "Ecotoxicology and Environmental Safety", Vol. 158, pp. 59-68.
Triticum aestivum cv. Minaret	OTC	NF	64-72 ppb (12 h mean)	150 kg N ha^{-1}	270 kg N ha^{-1}(1)	NH_4NO_3	Fangmeier et al. (1996). Effects of elevated CO2, nitrogen supply and tropospheric ozone on spring wheat .1. Growth and yield. In "Environmental Pollution", Vol. 91, pp. 381-390.
Triticum aestivum cv. Cadenza *Triticum dicoccoides* *Triticum monococcum*	closed sunlit chambers	CF	113 ppb (12 h mean)	low nutrient compost	50 kg N ha^{-1}(1)	NH_4NO_3	Brewster et al. (2018). Wheat's wild relatives vary in their response to nitrogen and ozone. In "Annals of Applied Biology", Vol. 173, pp. 154-163.

Table 23 Exposure conditions and references referred to Figure 3, Figure 14 and Figure 15 (grassland species, chapter 2.2.2)

Contr. = control treatment, contr. environ. = controlled environment, GH = green house, OTC = open top chamber, FACE = free air carbon dioxide enrichment, CF = charcoal filtered, NF = non filtered, AA = ambient air

***species*/cultivar**	**facility**	**O_3 contr.**	**elevated O_3**	**N control**	**elevated N**	**N supply**	**reference**
Bromus hordeaceus	OTC	CF	≈35 ppb, ≈55 ppb (24h mean)	7.2 kg N ha^{-1}	23 kg N ha^{-1}, 45 kg N ha^{-1}	N fertiliser	Sanz et al. (2015). Foliar senescence is the most sensitive response to ozone in Bromus hordeaceus and is modulated by nitrogen input. In "Grass and Forage Science", Vol. 70, pp. 71-84.
Plantago major genotype LH genotype Bush	contr. environ.	CF	70 ppb (7h mean)	low	high	Chemopak Formula 2	Whitfield et al. (1998). The effects of nutrient limitation on the response of Plantago major to ozone. In "New Phytologist", Vol. 140, pp. 219-230
Trifolium cherleri	OTC	CF	≈35 ppb, ≈55 ppb (24h mean)	5 kg N ha^{-1}	15 kg N ha^{-1}, 30 kg N ha^{-1}	N fertiliser	Sanz et al. (2014). Ozone and nitrogen effects on yield and nutritive quality of the annual legume Trifolium cherleri. In "Atmospheric Environment", Vol. 94, pp. 765-772.)
Trifolium striatum	OTC	CF	≈35 ppb, ≈55 ppb (24h mean))	10 kg N ha^{-1}	30 kg N ha^{-1}, 60 kg N ha^{-1}	N fertiliser	Sanz et al. (2007). Ozone sensitivity of the Mediterranean terophyte Trifolium striatum is modulated by soil nitrogen content. In "Atmospheric Environment", Vol. 41, pp. 8952-8962.
Trifolium subterraneum	OTC	CF	34.4 ppb 56.4 ppb (24h mean)	5 kg N ha^{-1}	15 kg N ha^{-1}, 30 kg N ha^{-1}	N fertiliser	Sanz et al. (2005). Ozone and increased nitrogen supply effects on the yield and nutritive quality of Trifolium subterraneum. In "Atmospheric Environment", Vol. 39, pp. 5899-5907.

Table 24 Exposure conditions and references referred to Figure 4, Figure 5, Figure 14 and Figure 15 (woody species, 2.2.3)

Contr. = control treatment, contr. environ. = controlled environment, GH = green house, OTC = open top chamber, FACE = free air carbon dioxide enrichment, CF = charcoal filtered, NF = non filtered, AA = ambient air

species/ genotype	**age/ duration**	**facility**	**O_3 contr.**	**elevated O_3**	**N control**	**elevated N**	**N supply**	**reference**
Fraxinus ornus	2-yr-old seedlings 10 days	contr. environ.	CF	87 ppb (5 h mean)	0 kg N ha^{-1}	20 kg N ha^{-1}	NH_4NO_3	Fusaro et al. (2017). Functional indicators of response mechanisms to nitrogen deposition, ozone, and their interaction in two Mediterranean tree species. In "Plos One", Vol. 12.
Liriodendron tulipifera	1-yr-old seedlings 18 weeks	OTC	CF	56 ppb, 108 ppb (7h mean)	58 µg g^{-1}	96 µg g^{-1}, 172 µg g^{-1}	NH_4NO_3	Tjoelker and Luxmoore (1991). Soil-nitrogen and chronic ozone stress influence physiology, growth and nutrient status of Pinus-taeda-l and Liriodendron-tulipifera-l seedlings. In "New Phytologist", Vol. 119, pp. 69-81.
Populus deltoides cv. 55/56 *x P. deltoides* cv. Imperial	cuttings 1 season	OTC	CF	80.6 ppb (24h mean)	0 kg N ha^{-1}	50 kg N ha^{-1}	urea	Li et al. (2019). The effects of elevated ozone on the accumulation and allocation of poplar biomass depend strongly on water and nitrogen availability. In "Science of the Total Environment", Vol. 665, pp. 929-936.
Populus maximoviczii × P. berolinensis Oxford clone	cuttings 1 season	free-air	AA	51.6 ppb, 66.7 ppb (24h mean)	0 kg N ha^{-1}	80 kg N ha^{-1}	NH_4NO_3	Zhang et al. (2018). Effects of nitrogen and phosphorus imbalance on photosynthetic traits of poplar Oxford clone under ozone pollution. In "Journal of Plant Research", Vol. 131, pp. 915-924.
Populus tremuloides clone 253 clone 259	cuttings 3 months	OTC	CF	CF+80 ppb (6h/day, 3d/week)	0 kg N ha^{-1}	10 kg N ha^{-1}, 20 kg N ha^{-1}, 40 kg N ha^{-1}	nitric acid	Karnosky et al. (1992). Effects of genotype on the response of Populus-tremuloides Michx to ozone and nitrogen deposition. In "Water Air and Soil Pollution", Vol. 62, pp. 189-199.
Populus x euramericana	cuttings 12 weeks	contr. environ.	CF	60 ppb (24h mean)	105 mg N l^{-1} soil	315 mg N l^{-1} soil	urea	GunthardtGoerg et al. (1996). Leaf and stem structure of poplar (Populus x euramericana) as influenced by O-3, NO2,…. In "Canadian Journal of Forest Research", Vol. 26, pp. 649-657.
Quercus ilex	2-yr-old seedlings 10 days	contr. environ.	CF	87 ppb (5 h mean)	0 kg N ha^{-1}	20 kg N ha^{-1}	NH_4NO_3	Fusaro et al. (2017). Functional indicators of response mechanisms to nitrogen deposition, ozone, and their interaction in two Mediterranean tree species. In "Plos One", Vol. 12.

species/ genotype	**age/ duration**	**facility**	**O_3 contr.**	**elevated O_3**	**N control**	**elevated N**	**N supply**	**reference**
Cryptomeria japonica	2-yr-old seedlings 2 seasons	OTC	CF	42-43 ppb, 63-64 ppb, 83-85 ppb (24h mean)	0 kg N ha^{-1}	20 kg N ha^{-1}, 50 kg N ha^{-1}	NH_4NO_3	Watanabe et al. (2006). Effects of ozone and/or nitrogen load on the growth of Larix kaempferi, Pinus densiflora and Cryptomeria japonica seedlings. In "Journal of Japan Society for Atmospheric Environment / Taiki Kankyo Gakkaishi", Vol. 41, pp. 320-334.
Larix gmelinii var. japonica x L. kaempferi	1-yr-old s seedlings 2 seasons	OTC	NF	59.6 - 64.3 ppb (24h mean)	5.5 kg N ha^{-1}	50 kg N ha^{-1}	$(NH_4)_2SO_4$	Sugai et al. (2019). Nitrogen loading increases the ozone sensitivity of larch seedlings with higher sensitivity to nitrogen loading. In "Science of the Total Environment", Vol. 663, pp. 587-595.
Larix kaempferi	3-yr-old seedlings 2 seasons	OTC	CF	42-43 ppb, 63-64 ppb, 83-85 ppb (24h mean)	0 kg N ha^{-1}	20 kg N ha^{-1}, 50 kg N ha^{-1}	NH_4NO_3	Watanabe et al. (2006). Effects of ozone and/or nitrogen load on the growth of Larix kaempferi, Pinus densiflora and Cryptomeria japonica seedlings. In "Journal of Japan Society for Atmospheric Environment / Taiki Kankyo Gakkaishi", Vol. 41, pp. 320-334.
Larix kaempferi	1-yr-old seedlings 2 seasons	OTC	NF	59.6 - 64.3 ppb (24h mean)	5.5 kg N ha^{-1}	50 kg N ha^{-1}	$(NH_4)_2SO_4$	Sugai et al. (2019). Nitrogen loading increases the ozone sensitivity of larch seedlings with higher sensitivity to nitrogen loading. In "Science of the Total Environment", Vol. 663, pp. 587-595.
Picea abies	2-yr-old saplings 3 seasons	free-air	CF!	35-47 ppb (daylight h)	0 kg N ha^{-1}	20 kg N ha^{-1}, 40 kg N ha^{-1}, 80 kg N ha^{-1}	NH_4NO_3	Thomas et al. (2005). Effects of simultaneous ozone exposure and nitrogen loads on carbohydrate concentrations, biomass, and growth of young spruce trees (Picea abies). In "Environmental Pollution", Vol. 137, pp. 507-516.
Pinus densiflora	2-yr-old seedlings 2 seasons	OTC	CF	42-43 ppb, 63-64 ppb, 83-85 ppb (24h mean)	0 kg N ha^{-1}	20 kg N ha^{-1}, 50 kg N ha^{-1}	NH_4NO_3	Watanabe et al. (2006). Effects of ozone and/or nitrogen load on the growth of Larix kaempferi, Pinus densiflora and Cryptomeria japonica seedlings. In "Journal of Japan Society for Atmospheric Environment / Taiki Kankyo Gakkaishi", Vol. 41, pp. 320-334.
Pinus densiflora	1-yr-old seedlings 8 weeks	OTC	CF	120 ppb (3h/day)	1.5 mM	4.5 mM	NH_4NO_3	Bak and Lee (2001). Effects of nitrogen and phosphorus fertilization on the growth, carbohydrate contents and photosynthesis of Pinus densiflora seedlings exposed to ozone in an open-top chamber. In "Journal of Korean Forestry Society", Vol. 90, pp. 306-313.

species/ genotype	**age/ duration**	**facility**	**O_3 contr.**	**elevated O_3**	**N control**	**elevated N**	**N supply**	**reference**
Pinus sylvestris	4-yr-old seedlings 2 seasons	OTC	CF	36-40 ppb, 56-60 ppb (9h mean)	0 ppb (24h mean)	55 ppb, 110 ppb (24h mean)	NH_3	Dueck et al. (1998). Influence of ammonia and ozone on growth and drought sensitivity of Pinus sylvestris. In "Atmospheric Environment", Vol. 32, pp. 545-550.
Pinus sylvestris	3-yr-old seedlings 11 weeks	contr. environ.	CF	55 ppb (9h mean)	0 ppb	57 ppb	NH_3	Perezsoba et al. (1995). Interactions of elevated CO2, NH3 and O-3 on mycorrhizal infection, gas-exchange and n-metabolism in saplings of scots pine. In "Plant and Soil", Vol. 176, pp. 107-116.
Pinus sylvestris	3-yr-old seedlings 3 seasons	free-air	AA	35.6 -36.3 ppb (24h mean)	143 mg NH_4NO_3 l^{-1}	286 mg NH_4NO_3 l^{-1}	NH_4NO_3	Utriainen and Holopainen (2001). Nitrogen availability modifies the ozone responses of Scots pine seedlings exposed in an open-field system. In "Tree Physiology", Vol. 21, pp. 1205-1213.
Pinus taeda	1-yr-old seedlings 18 weeks	OTC	CF	56 ppb, 108 ppb (7h mean)	58 µg g^{-1}	96 µg g^{-1}, 172 µg g^{-1}	NH_4NO_3	Tjoelker and Luxmoore (1991). Soil-nitrogen and chronic ozone stress influence physiology, growth and nutrient status of Pinus-taeda-l and Liriodendron-tulipifera-l seedlings. In "New Phytologist", Vol. 119, pp. 69-81.

A.1.2 Chapter 3: Carbon Dioxide (CO_2)

Table 25 Exposure conditions and references referred to Figure 6, Figure 8 and Figure 16 (wheat, chapter 3.2.1)

contr. environ. = controlled environment, GH = green house, OTC = open top chamber, FACE = free air carbon dioxide enrichment, CF = charcoal filtered, NF = non filtered, AA = ambient air

***species*/cultivar**	**facility**	**conditions**	**contr. O_3**	**elevated O_3**	**elevated CO_2**	**reference**
Triticum aestivum	OTC	ground	NF	60 ppb (7h mean)	550 ppm	Lu et al. (2016). Effects of elevated O-3 and CO2 on the relative contribution of carbohydrates to soil organic matter in an agricultural soil. In "Soil & Tillage Research", Vol. 159, pp. 47-55.
Triticum aestivum 5 cv. (averaged)	contr. environ.	pots 6 weeks	CF	75 ppb (4h mean)	700 ppm	Barnes et al. (1995). Effects of elevated CO2 and/or O3 on growth, development and physiology of wheat (Triticum aestivum L.). In "Global Change Biology", Vol. 1, pp. 129-142.
Triticum aestivum cv. Hanno	contr. environ.	30 days	CF	75 ppb (7h mean)	700 ppm	Balaguer et al. (1995). Production and utilization of assimilates in wheat (Triticum aestivum L.) leaves exposed to elevated O3 and/or CO2. In "New Phytologist", Vol. 129, pp. 557-568.
Triticum aestivum cv. Minaret	OTC	field grown season	NF(1xCF)	23 - 85 ppb (12h mean)	520 ppm 680 ppm	Bender et al. (1999). Growth and yield responses of spring wheat to increasing carbon dioxide, ozone and physiological stresses: a statistical analysis 'ESPACE-wheat' results. In "European Journal of Agronomy", Vol. 10, pp. 185-195.
Triticum aestivum cv. Minaret	OTC	ground	NF	47-53 ppb (24h mean)	510 ppm 680 ppm	Donnelly et al. (1999). Does elevated CO2 protect grain yield of wheat from the effects of ozone stress? In "Zeitschrift Fur Naturforschung C-a Journal of Biosciences", Vol. 54, pp. 802-811.
Triticum aestivum cv. Minaret	OTC	pots	NF	28-56 ppb (24h mean)	520 ppm 680 ppm	Fangmeier et al. (1996). Effects of elevated CO2, nitrogen supply and tropospheric ozone on spring wheat .1. Growth and yield. In "Environmental Pollution", Vol. 91, pp. 381-390.
Triticum aestivum cv. Minaret	OTC	field grown season	NF	60 ppb (7h mean)	550 ppm 680 ppm	Mulholland et al. (1997). Effects of elevated carbon dioxide and ozone on the growth and yield of spring wheat (Triticum aestivum L). In "Journal of Experimental Botany", Vol. 48, pp. 113-122.
Triticum aestivum cv. Beijing 6 cv. Zhongmai 9	OTC	pots 3 weeks	CF	72 ppb (7h mean)	714 ppm	Biswas et al. (2013). Modification of photosynthesis and growth responses to elevated CO2 by ozone in two cultivars of winter wheat with different years of release. In "Journal of Experimental Botany", Vol. 64, pp. 1485-1496.

***species*/cultivar**	**facility**	**conditions**	**contr. O_3**	**elevated O_3**	**elevated CO_2**	**reference**
Triticum aestivum cv. KWS Bittern cv. Lennox cv. Lantvete	contr. environ.		CF	83-85 ppb (16h mean)	700 ppm	Hansen et al. (2019). The impact of ozone exposure, temperature and CO2 on the growth and yield of three spring wheat varieties. In "Environmental and Experimental Botany", Vol. 168.
Triticum aestivum cv. Coker 9835 cv. Coker 9904	OTC	pots	CF	45 ppb, 90 ppb (12h mean)	540 ppm, 700 ppm	Heagle et al. (2000). Growth and yield responses of winter wheat to mixtures of ozone and carbon dioxide. In "Crop Science", Vol. 40, pp. 1656-1664.
Triticum aestivum cv. Wembley	contr. environ.		< 5 ppb	60 ppb	700 ppm	McKee et al. (1997). Will elevated CO2 concentrations protect the yield of wheat from O-3 damage? In "Plant Cell and Environment", Vol. 20, pp. 77-84.
Triticum aestivum cv. K-9107 cv. HUW-37	OTC	field grown life cycle	NF	NF+10 ppb (4h) ≈ 52-60 ppb	700 ppm (4h) ≈543-550ppm	Mishra et al. (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. In "Indian Journal of Biochemistry & Biophysics", Vol. 50, pp. 139-149.
Triticum aestivum cv. Dragon	OTC	ground season	NF	1.5xNF 2.0xNF	680 ppm	Pleijel et al. (2000). Effects of elevated carbon dioxide, ozone and water availability on spring wheat growth and yield. In "Physiologia Plantarum", Vol. 108, pp. 61-70.
Triticum aestivum cv. Turbo	contr. environ.	11 weeks	CF	62 ppb (7h mean)	610-615 ppm	von Tiedemann and Firsching (2000). Interactive effects of elevated ozone and carbon dioxide on growth and yield of leaf rust-infected versus non-infected wheat. In "Environmental Pollution", Vol. 108, pp. 357-363.
Triticum aestivum cv. Nandu	OTC	pots 8 weeks	NF	53 ppb (12h mean)	720 ppm	Hudak et al. (1999). Interactive effects of elevated CO2, O-3, and soil water deficit on spring wheat (Triticum aestivum L-cv. Nandu). In "Agronomie", Vol. 19, pp. 677-687.

Table 26 Exposure conditions and references referred to Figure 7, Figure 8 and Figure 16 (crops, chapter 3.2.1)

contr. environ. = controlled environment, GH = green house, OTC = open top chamber, FACE = free air carbon dioxide enrichment, CF = charcoal filtered, NF = non filtered, AA = ambient air

***species*/cultivar**	**facility**	**conditions**	**control O_3**	**elevated O_3**	**elevated CO_2**	**reference**
Arachis hypogaea	OTC	4 months	CF	46 ppb, 75 ppb (12h mean)	548 ppm, 730 ppm	Burkey et al. (2007). Elevated carbon dioxide and ozone effects on peanut: II. Seed yield and quality. In "Crop Science", Vol. 47, pp. 1488-1497.
Brassica juncea cv. Pusa Tarak (EJ-13)	OTC	ground	NF	NF+25 ppb NF+35 ppb (8h mean)	500 ppm	Singh et al. (2013). Synergistic action of tropospheric ozone and carbon dioxide on yield and nutritional quality of Indian mustard (Brassica juncea (L.) Czern.). In "Environmental Monitoring and Assessment", Vol. 185, pp. 6517-6529.
Cicer arietinum cv. Pusa 5023-kabuli type	FACE	ground 14 weeks	AA	70 ppb (12h mean)	550 ppm	Singh et al. (2017). Effect of elevated ozone, carbon dioxide and their interaction on growth, biomass and water use efficiency of chickpea (Cicer arietinum L.). In "Journal of Agrometeorology", Vol. 19, pp. 301-305.
Glycine max cv. Clark	OTC		CF	40.3 ppb, 66.4 ppb (7h mean)	+150 ppm	Mulchi et al. (1992). Growth and physiological-characteristics of soybean in open-top chambers in response to ozone and increased atmospheric CO2. In "Agriculture Ecosystems & Environment", Vol. 38, pp. 107-118.
Glycine max cv. Pioneer 93B15	FACE	15 weeks	AA	1.25xAA	550 ppm	Bernacchi et al. (2006). Hourly and seasonal variation in photosynthesis and stomatal conductance …. In "Plant Cell and Environment", Vol. 29, pp. 2077-2090.
Glycine max cv. Essex	OTC	pots 14 weeks	CF	67-79 ppb (12h mean)	700 ppm	Booker and Fiscus (2005). The role of ozone flux and antioxidants in the suppression of ozone injury by elevated CO2 in soybean. In "Journal of Experimental Botany", Vol. 56, pp. 2139-2151.
Glycine max cv. Essex	OTC	pots 15 weeks	CF	73 ppb (12h mean)	726 ppm	Booker et al. (1997). Photosynthesis and photorespiration in soybean Glycine max (L.) Merr. chronically exposed to elevated carbon dioxide and ozone. In "Journal of Experimental Botany", Vol. 48, pp. 1843-1852.
Glycine max cv. Essex	OTC	pots life cycle	CF	70-73 ppb (12h mean)	714-726 ppm	Booker et al. (2004). Combined effects of elevated atmospheric carbon dioxide and ozone on soybean whole-plant water use. In "Environmental Management", Vol. 33, pp. S355-S362.
Glycine max cv. Essex, cv. Holladay cv. Northrup, cv. King 6955	OTC	pots life cycle	CF	42-50 ppb, 69-79 ppb (12h mean)	480 ppm, 600 ppm, 715 ppm	Heagle et al. (1998). Influence of ozone stress on soybean response to carbon dioxide enrichment: III. Yield and seed quality. In "Crop Science", Vol. 38, pp. 128-134.

***species*/cultivar**	**facility**	**conditions**	**control O_3**	**elevated O_3**	**elevated CO_2**	**reference**
Glycine max cv. Essex	OTC	pots life cycle	CF	50 ppb, 79 ppb (12h mean)	482 ppm, 599 ppm, 713 ppm	Miller et al. (1998). Influence of ozone stress on soybean response to carbon dioxide enrichment: II. Biomass and development. In "Crop Science", Vol. 38, pp. 122-128.
Glycine max cv. Essex	OTC	pots 15 weeks	CF	74 ppbm (12h mean)	727 ppm	Reid and Fiscus (1998). Effects of elevated CO2 and/or ozone on limitations to CO2 assimilation in soybean…. In "Journal of Experimental Botany", Vol. 49, pp. 885-895.
Glycine max cv. Zhonghuang-14	OTC	pots 10 weeks	AA	120 ppb (7h mean)	700 ppm	Zhao et al. (2005). Respective and interactive effects of doubled CO2 and O-3 concentration on membrane lipid peroxidation and antioxidative ability of soybean. *In* "Science in China Series C-Life Sciences", Vol. 48, pp. 136-141.
Gossypium hirsutum cv. Deltapine 51	OTC	pots 17 weeks	CF	51 ppb, 71-78 ppb (12h mean)	537-547 ppm, 700-724 ppm	Heagle et al. (1999). Ozone stress, carbon dioxide enrichment, and nitrogen fertility interactions in cotton. *In* "Crop Science", Vol. 39, pp. 731-741.
Lycopersicon esculentum cv. flacca	GH	4 weeks	CF	60-70 ppb (24h? mean)	664-672 ppm	Olszyk and Wise (1997). Interactive effects of elevated CO2 and O3 on rice and flacca tomato. *In* "Agriculture, Ecosystems & Environment", Vol. 66, pp. 1-10.
Lycopersicon esculentum cv. Tiny Tim	GH	13 weeks	CF	80 ppb (6h mean)	525 ppm, 675 ppm	Reinert et al. (1997). Growth and fruiting of tomato as influenced by elevated carbon dioxide and ozone. *In* "New Phytologist", Vol. 137, pp. 411-420.
Oryza sativa cv. IR 74	GH	pots 4 weeks	CF	60-70 ppb (24h? mean)	664-705 ppm	Olszyk and Wise (1997). Interactive effects of elevated CO2 and O3 on rice and flacca tomato. *In* "Agriculture, Ecosystems & Environment", Vol. 66, pp. 1-10.
Oryza sativa cv. Khao Dawk Mali 105	contr. environ.	pots 4 weeks	CF	40 ppb, 70 ppb (8h mean)	700 ppm	Phothi et al. (2016). Combining effects of ozone and carbon dioxide application on photosynthesis of Thai jasmine rice (Oryza sativa L.) cultivar Khao Dawk Mali 105. *In* "Australian Journal of Crop Science", Vol. 10, pp. 591-597.
Oryza sativa cv. hybrid Lemont/Qi Gui Zao	OTC	pots	CF	73-77 ppb (12h mean)	701-712 ppm	Reid and Fiscus (2008). Ozone and density affect the response of biomass and seed yield to elevated CO2 in rice. *In* "Global Change Biology", Vol. 14, pp. 60-76.
Oryza sativa cv. Shanyou 63	sunlit chamber			1.6xAA	+200 ppm	Shao et al. (2014). Impact of elevated atmospheric carbon dioxide and ozone concentrations on leaf photosynthesis of 'Shanyou 63' hybrid rice. *In* "Zhongguo Shengtai Nongye Xuebao / Chinese Journal of Eco-Agriculture", Vol. 22, pp. 422-429.

***species*/cultivar**	**facility**	**conditions**	**control O_3**	**elevated O_3**	**elevated CO_2**	**reference**
Phaseolus vulgaris cv. Tenderette	OTC	pots	CF	72 ppb (12h mean)	697 ppm	Heagle et al. (2002). Growth and yield responses of snap bean to mixtures of carbon dioxide and ozone. *In* "Journal of Environmental Quality", Vol. 31, pp. 2008-2014.
Phaseolus vulgaris genotype R123 (tol) genotype R331 (tol) genotype S156 (sens)	FACE	ground	AA	59 ppb (8h mean)	550 ppm	Burkey et al. (2012). Field assessment of a snap bean ozone bioindicator system under elevated ozone and carbon dioxide in a free air system. *In* "Environmental Pollution", Vol. 166, pp. 167-171.
Raphanus sativus cv. Cherry Belle	contr. environ.	pots 27 days	CF+20	67-70 ppb (12h mean)	765 ppm	Barnes and Pfirrmann (1992). The influence of CO2 and O3, singly and in combination, on gas-exchange, growth and nutrient status of radish (Raphanus sativus L.). *In* "New Phytologist", Vol. 121, pp. 403-412.
Solanum tuberosum cv. Bintje	OTC	ground/pots 1 season	NF	60 ppb (8h mean)	680 ppm	Craigon et al. (2002). Growth and marketable-yield responses of potato to increased CO2 and ozone. *In* "European Journal of Agronomy", Vol. 17, pp. 273-289.
Solanum tuberosum cv. Bintje	OTC	ground/(large pots) season	NF	60 ppb (8h mean)	550 ppm, 680 ppm	Donnelly et al. (2001). Elevated CO2 increases biomass and tuber yield in potato even at high ozone concentrations. In "New Phytologist", Vol. 149, pp. 265-274.
Solanum tuberosum cv. Bintje	OTC	ground/pots season	NF	60 ppb (8h mean)	680 ppm	Finnan et al. (2002). The effects of elevated concentrations of carbon dioxide and ozone on potato (Solanum tuberosum L.) yield. In "Agriculture, Ecosystems & Environment", Vol. 88, pp. 11-22.
Solanum tuberosum cv. Bintje	OTC	ground	NF	48-52 ppb (8h mean)	556-570 ppm, 660-685 ppm	Lawson et al. (2002). Impact of elevated CO2 and O-3 on gas exchange parameters and epidermal characteristics in potato (Solanum tuberosum L.). In "Journal of Experimental Botany", Vol. 53, pp. 737-746; Lawson et al. (2001). Effects of elevated carbon dioxide and ozone on the growth and yield of potatoes (Solanum tuberosum) grown in open-top chambers. In "Environmental Pollution", Vol. 111, pp. 479-491.
Solanum tuberosum cv. Dark Red Norland cv. Superior	OTC	pots 11 weeks	CF	45 ppb, 80 ppb (12h mean)	540 ppm, 715 ppm	Heagle et al. (2003). Growth and yield responses of potato to mixtures of carbon dioxide and ozone. In "Journal of Environmental Quality", Vol. 32, pp. 1603-1610.
Solanum tuberosum	OTC	ground	CF	50 ppb, 70 ppb (8h mean)	570 ppm	Kumari and Agrawal (2014). Growth, yield and quality attributes of a tropical potato variety (…) under ambient and elevated carbon dioxide and ozone and

***species*/cultivar**	**facility**	**conditions**	**control O_3**	**elevated O_3**	**elevated CO_2**	**reference**
						their interactions. In "Ecotoxicology and Environmental Safety", Vol. 101, pp. 146-156.
Zea mays cv. HQPM-1	OTC	ground	NF	NF+25 ppb (8h), NF+35 ppb (8h)	500 ppm	Bhatia et al. (2013). Effect of elevated ozone and carbon dioxide interaction on growth and yield of maize. In "Maydica", Vol. 58, pp. 291-298.

Table 27 Exposure conditions and references referred to Figure 9, Figure 10 and Figure 17 (woody plants; chapter 3.2.3)

Contr. = control treatment, contr. environ. = controlled environment, chamb. = chamber, OTC = open top chamber, FACE = free air carbon dioxide enrichment, CF = charcoal filtered, NF = non filtered, AA = ambient air

***species*/genotype**	**age/duration**	**facility**	**contr. O_3**	**elevated O_3**	**elevated CO_2**	**reference**
Acer saccharum	2-yr-old seedlings/ season	OTC	NF	104 ppb (24h mean)	650 ppm	Gaucher et al. (2005). Response of Acer saccharum seedlings to elevated O-3 and CO2 concentrations. In "Phytoprotection", Vol. 86, pp. 7-17.
Betula papyrifera	after 6 seasons	aspenFACE	AA	49-55 ppb (12h mean)	530 ppm	Uddling et al. (2009). Leaf and canopy conductance in aspen and aspen-birch forests under free-air enrichment of carbon dioxide and ozone. In "Tree Physiology", Vol. 29, pp. 1367-1380.
Betula pendula clone 4 (tolerant) clone 80 (sensitive)	7-yr-old trees/ 3 seasons	OTC	NF	20.6-30.9 ppm h (AOT40)	650 -730 ppm	Riikonen et al. (2004). Silver birch and climate change: variable growth and carbon allocation responses to elevated concentrations of carbon dioxide and ozone. In "Tree Physiology", Vol. 24, pp. 1227-1237. Riikonen et al. (2005). Leaf photosynthetic characteristics of silver birch during three years of exposure to elevated concentrations of CO2 and O3 in the field. In "Tree Physiology", Vol. 25, pp. 621-632.
Betula pubescens	6-wk-old/ 5 weeks	GH	7 ppb	62 ppb (8h mean)	560 ppm	Mortensen (1995). Effect of carbon-dioxide concentration on biomass production and partitioning in Betula-pubescens ... at different ozone and temperature regimes. In "Environmental Pollution", Vol. 87, pp. 337-343.
Cryptomeria japonica 12 clones	2-yr-old/ 2 seasons	FACE	AA	33-36 ppb (24h mean)	550 ppm	Hiraoka et al. (2017). Species characteristics and intraspecific variation in growth and photosynthesis of Cryptomeria japonica under elevated O-3 and CO2. In "Tree Physiology", Vol. 37, pp. 733-743.

***species*/genotype**	**age/duration**	**facility**	**contr. O_3**	**elevated O_3**	**elevated CO_2**	**reference**
Eucalyptus globulus *E. grandis* *E. deglupta x E. camaldulensis*	seedlings/ 5 months	OTC	NF	60 ppb (7h mean)	600 ppm	Novriyanti et al. (2020). Elevated CO2 offsets the alteration of foliar chemicals (n-icosane, geranyl acetate, and elixene) induced by elevated O-3 in three taxa of O-3-tolerant eucalypts. In "Journal of Forestry Research".
Fagus crenata	current yr seedlings/ 2 seasons	FACE	AA	62-65 ppb (10h mean)	546 -562 ppm	Tobita et al. (2019). Effects of Combined CO2 and O3 Exposures on Net CO2 Assimilation and Biomass Allocation in Seedlings of the Late-Successional Fagus Crenata In "Climate", Vol. 7, pp. 117.)
Fraxinus excelsior	1-yr-old/ 2 seasons	OTC	NF	39-52 ppb (24h mean)	690 -710 ppm	Broadmeadow and Jackson (2000). Growth responses of Quercus petraea, Fraxinus excelsior and Pinus sylvestris to elevated carbon dioxide, ozone and water supply. In "New Phytologist", Vol. 146, pp. 437-451.
Pinus sylvestris	1-yr-old 3 years	OTC	NF	39-52 ppb (24h mean)	690 -710 ppm	Broadmeadow and Jackson (2000). Growth responses of Quercus petraea, Fraxinus excelsior and Pinus sylvestris to elevated carbon dioxide, ozone and water supply. In "New Phytologist", Vol. 146, pp. 437-451.
Pinus sylvestris	3-yr-old seedlings/ 2 seasons	OTC	NF	33.3-39.9 ppm h (AOT40)	590 ppm	Utriainen et al. (2000). Biomass allocation, needle structural characteristics and nutrient composition in Scots pine seedlings exposed to elevated CO2 and O-3 conc... In "Trees-Structure and Function", Vol. 14, pp. 475-484.
Populus deltoides Bartr. x P. nigra DN-33, DN-34, DN-70, DN-74, NM-6	cuttings/ 1 season	OTC	CF	48 ppb (24h mean)	510 ppm	Dickson et al. (1998). Growth of five hybrid poplar genotypes exposed to interacting elevated CO2 and O-3. In "Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere", Vol. 28, pp. 1706-1716.
Populus tremuloides	1-yr-old/ 6 seasons	aspenFACE	AA	49-55 ppb (12h mean)	528 -548 ppm	King et al. (2005). Tropospheric O-3 compromises net primary production in young stands of trembling aspen, paper birch and sugar maple in response to elevated atmospheric CO2. In "New Phytologist", Vol. 168, pp. 623-635., Uddling et al. (2009). Leaf and canopy conductance in aspen and aspen-birch forests under free-air enrichment of carbon dioxide and ozone. In "Tree Physiology", Vol. 29, pp. 1367-1380.
Populus tremuloides clone 259 (sensitive) clone 216 (tolerant)	3-yr-old cuttings/ 1 season	aspenFACE	AA	49-55 ppb (12h mean)	530 -548 ppm	Noormets et al. (2010). Elevated CO2 response of photosynthesis depends on ozone concentration in aspen. In "Environmental Pollution", Vol. 158, pp. 992-999; Noormets et al. (2001). The effect of elevated carbon dioxide and ozone on leaf- and branch-level photosynthesis and potential plant-level carbon gain in aspen. In "Trees-Structure and Function", Vol. 15, pp. 262-270.

***species*/genotype**	**age/duration**	**facility**	**contr. O_3**	**elevated O_3**	**elevated CO_2**	**reference**
Populus tremuloides clone 259 (sensitive) clone 216 (tolerant)	cuttings/ 1 season	OTC	CF	100 ppb (6 h/d; 5d/wk)	+150 ppm	Kull et al. (1996). Photosynthetic responses of aspen clones to simultaneous exposures of ozone and CO2. In "Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere", Vol. 26, pp. 639-648.
Populus tremuloides clone 259 (sensitive) clone 216 (tolerant)	Cuttings/ 1 season	OTC	CF	30-41 ppb (24h mean)	500 ppm	Dickson et al. (2001). Growth and crown architecture of two aspen genotypes exposed to interacting ozone and carbon dioxide. In "Environmental Pollution", Vol. 115, pp. 319-334.
Quercus mongolica var. crispula	1-yr-old 2 seasons	FACE	AA	36-41 ppb (24h mean)	550 ppm	Kitao et al. (2015). Growth overcompensation against O-3 exposure in two Japanese oak species, Quercus mongolica var. crispula and Quercus serrata, grown under elevated CO2. In "Environmental Pollution", Vol. 206, pp. 133-141.
Quercus petraea	1-yr-old/ 2 / 3 seasons	OTC	NF	39-52 ppb (24h mean)/ 48 ppm.h (AOT40)	690 -710 ppm	Broadmeadow et al. (1999). Environmental limitations to O-3 uptake - Some key results from young trees growing at elevated CO2 concentrations. In "Water Air and Soil Pollution", Vol. 116, pp. 299-310; Broadmeadow and Jackson (2000). Growth responses of Quercus petraea, Fraxinus excelsior and Pinus sylvestris to elevated carbon dioxide, ozone and water supply. In "New Phytologist", Vol. 146, pp. 437-451.
Quercus serrata	1-yr-old/ 2 seasons	FACE	AA	36-41 ppb (24h mean)	550 ppm	Kitao et al. (2015). Growth overcompensation against O-3 exposure in two Japanese oak species, Quercus mongolica var. crispula and Quercus serrata, grown under elevated CO2. In "Environmental Pollution", Vol. 206, pp. 133-141.

A.1.3 Chapter 4: Soil water deficit

Table 28 Exposure conditions and references referred to Figure 11 and Figure 18 (crops, chapter 4.2.1)

contr. environ. = controlled environment, GH = green house, OTC = open top chamber, CF = charcoal filtered, NF = non filtered, SWC soil water content

species/ cultivar	**facility**	**H_2O control**	**drought treatment**	**O_3 contr.**	**elevated O_3**	**reference**
Brassica napus cv. Rainbow	OTC	WW	WS	CF	80 ppb (9h mean)	Maliba et al. (2019). Photosynthetic Responses of Canola and Wheat to Elevated Levels of CO2, O-3 and Water Deficit in Open-Top Chambers. In "Plants-Basel", Vol. 8.
Glycine max cv. Corsoy cv. Williams	OTC	WW	WS	CF	56-53 ppb (7h mean)	Heggestad et al. (1985). Interaction of soil-moisture stress and ambient ozone on growth and yields of soybeans. In "Phytopathology", Vol. 75, pp. 472-477.
Glycine max cv. Corsoy cv. Williams	OTC	>-0.05 MPa	-0.45 to -0.32 MPa	CF	52 ppb, 79 ppb, 107 ppb, 132 ppb (7h mean)	Heggestad et al. (1988). Effects of ozone and soil water deficit on roots and shoots of field-grown soybeans. In "Environmental Pollution", Vol. 50, pp. 259-278.
Glycine max cv. Young	OTC	WW	WS	CF	36 - 39 ppb, 57 - 61 ppb, 71 - 72 ppb, 83 - 87ppb (12h mean)	Miller et al. (1989). Effects of ozone and water-stress, separately and in combination, on soybean yield. In "Journal of Environmental Quality", Vol. 18, pp. 330-336.
Glycine max cv. Williams	GH	WW	–0·45 M Pa	CF	39 ppb (7h mean)	Heggestad and Lee (1990). Soybean root distribution, top growth and yield responses to ambient ozone and soil moisture stress when grown in soil columns in greenhouse. In "Environmental Pollution", Vol. 65, pp. 195-207.
Gossypium hirsutum cv. Acala SJ-2	OTC	WW	WS	CF	44-71 ppb, 65-101 ppb, 77-125 ppb, 92-155 ppb (7h mean)	Temple et al. (1985). Cotton yield responses to ozone as mediated by soil-moisture and evapotranspiration. In "Journal of Environmental Quality", Vol. 14, pp. 55-60.
Gossypium hirsutum cv. McNair-325	OTC	>-0.1 MPa	>-0.30 MPa	CF	41 ppb, 51 ppb, 61 ppb,	Miller et al. (1988). Growth of cotton under chronic ozone stress at 2 levels of soil-moisture. In "Journal of Environmental Quality", Vol. 17, pp. 635-643.

species/ **cultivar**	**facility**	**H_2O control**	**drought treatment**	**O_3 contr.**	**elevated O_3**	**reference**
					73 ppb (12h mean)	
Gossypium hirsutum cv.S-6	GH	WW	WS	CF	59 ppb, 114 ppb (12h mean)	Grantz et al. (2016). Diel trends in stomatal response to ozone and water deficit: a unique relationship of midday values to growth and allometry in Pima cotton? In "Plant Biology", Vol. 18, pp. 37-46.
Triticum aestivum	OTC	WW	0.5 MP	CF	78 ppb (7h mean)	Hatata et al. (2013). Respective and interactive effects of O3 and CO2 and drought stress on photosynthesis, stomatal conductance, antioxidative ability and yield of wheat plants. In "Current World Environment", Vol. 8, pp. 409-417.
Triticum aestivum cv. Korongo	solar-domes	WW	WS	19-25 ppb	45 ppb (24h mean)	Harmens et al. (2019). Can Reduced Irrigation Mitigate Ozone Impacts on an Ozone-Sensitive African Wheat Variety? In "Plants-Basel", Vol. 8.
Triticum aestivum cv. Perlo	GH	75% SWC	35% SWC 45% SWC 60% SWC	NF≈CF	80ppb (8h mean)	Khan and Soja (2003). Yield responses of wheat to ozone exposure as modified by drought-induced differences in ozone uptake. In "Water Air and Soil Pollution", Vol. 147, pp. 299-315.
Triticum aestivum cv. Turbo	OTC	WW	WS	CF	17.3 ppb, 20.6 ppb, 24.5 ppb (24h mean)	Fangmeier et al. (1994). Growth and yield responses of spring wheat (Triticum-aestivum L cv turbo) grown in open-top chambers to ozone and water-stress. In "Environmental Pollution", Vol. 83, pp. 317-325.
Triticum aestivum cv. SST875	OTC	WW	WS	CF	80 ppb (9h mean)	Maliba et al. (2019). Photosynthetic Responses of Canola and Wheat to Elevated Levels of CO2, O-3 and Water Deficit in Open-Top Chambers. In "Plants-Basel", Vol. 8.
Triticum aestivum cv. Xiaoyan 22 *Triticum turgidum ssp. durum*	OTC	88% SWC	42% SWC	CF	83 ppb (7h mean)	Biswas and Jiang (2011). Differential drought-induced modulation of ozone tolerance in winter wheat species. In "Journal of Experimental Botany", Vol. 62, pp. 4153-4162.

Table 29 Exposure conditions and references referred to Figure 12, Figure 13 and Figure 18 (woody plants, chapter 4.2.3)

contr. environ. = controlled environment, chamb. = chamber, OTC = open top chamber, CF = charcoal filtered, NF = non filtered, AA, = ambient air, FC = field capacity, EP = effective evapotranspiration

species/ **genotype**	**age/ duration**	**facility**	**H_2O control**	**drought treatment**	**O_3 control**	**elevated O_3**	**reference**
Acer rubrum	1-year-old 2 seasons	OTC	-0.026 MPa	-0.142 MPa (2nd year)	CF	41-42 ppb (24h mean)	Schaub et al. (2003). Physiological and foliar injury responses of Prunus serotina, Fraxinus americana, and Acer rubrum seedlings to varying soil moisture and ozone. In "Environmental Pollution", Vol. 124, pp. 307-320.
Acer truncatum	1-yr-old seedlings 2 years	OTC	75% FC	60-70% f WW	NF	102-141 ppb	Li et al. (2015). Chronic drought stress reduced but not protected Shantung maple (Acer truncatum Bunge) from adverse effects of ozone (O-3) on growth and physiology in the suburb of Beijing, China. In "Environmental Pollution", Vol. 201, pp. 34-41.
Alnus glutinosa	2-yr-old seedlings 2 seasons	solar-domes	WW	45% WW	32 ppb	74 ppb (24h mean)	Hayes et al. (2015). Species-Specific Responses to Ozone and Drought in Six Deciduous Trees. In "Water Air and Soil Pollution", Vol. 226, pp. 13.
Betula pendula	2-yr-old seedlings 2 seasons	solar-domes	WW	45% WW	32 ppb	74 ppb (24h mean)	Hayes et al. (2015). Species-Specific Responses to Ozone and Drought in Six Deciduous Trees. In "Water Air and Soil Pollution", Vol. 226, pp. 13.
Betula pendula clone KL-5-M (sensitive)	1-yr-old saplings three months	free-air			AA	37-42 ppb (24h mean)	Pääkkönen et al. (1998). Responses of leaf processes in a sensitive birch (Betula pendula Roth) clone to ozone combined with drought. In "Annals of Botany", Vol. 82, pp. 49-59. Pääkkönen et al. (1998). Physiological and ultrastructural responses of birch clones exposed to ozone and drought stress. In "Chemosphere", Vol. 36, pp. 679-684.
Betula pendula clone KL-2-M (tolerant) clone KL-5-M (sensitive)		free-air			AA	42 ppb (24h mean)	Pääkkönen et al. (1998). Physiological, stomatal and ultrastructural ozone responses in birch (Betula pendula Roth.) are modified by water stress. In "Plant Cell and Environment", Vol. 21, pp. 671-684.
Betula pendula clone KL-2-M (tolerant) clone KL-5-M (sensitive)	2-yr-old saplings 43 days	contr. environ.	WW	50% WW	CF	100 ppb (12h mean)	Pääkkönen et al. (1998). Induction of genes for the stress proteins PR-10 and PAL in relation to growth, visible injuries and stomatal conductance in birch (Betula pendula) clones exposed to ozone and/or drought. In "New Phytologist", Vol. 138, pp. 295-305.

species/ genotype	**age/ duration**	**facility**	**H_2O control**	**drought treatment**	**O_3 control**	**elevated O_3**	**reference**
Ceratonia siliqua	1-yr-old seedlings 2 years	OTC	WW	50% WW	CF	37 ppb, 57 ppb (24h mean)	Ribas et al. (2005). Contrasting effects of ozone under different water supplies in two Mediterranean tree species. In "Atmospheric Environment", Vol. 39, pp. 685-693.
Corylus avellana	2-yr-old seedlings 2 seasons	Solar-domes	WW	0,45	32 ppb	74 ppb (24h mean)	Hayes et al. (2015). Species-Specific Responses to Ozone and Drought in Six Deciduous Trees. In "Water Air and Soil Pollution", Vol. 226, pp. 13.
Fagus crenata	4-yr-old seedlings 156 days	sunlit chamb.	WW	70% WW	CF	60 ppb (7h mean)	Yonekura et al. (2001). Effects of ozone and/or soil water stress on growth and photosynthesis of Fagus crenata seedlings. In "Water Air and Soil Pollution", Vol. 130, pp. 965-970.
Fagus sylvatica	2-yr-old seedlings 2 seasons	solar-domes	WW	45% WW	32 ppb	74 ppb (24h mean)	Hayes et al. (2015). Species-Specific Responses to Ozone and Drought in Six Deciduous Trees. In "Water Air and Soil Pollution", Vol. 226, pp. 13.
Fagus sylvatica	8-yr-old trees 3 months	OTC			CF	NF, NF+25, NF+50 (continously)	Le Thiec et al. (1994). The effects of slightly elevated ozone concentrations and mild drought stress on the physiology and growth of Norway spruce, Picea-abies (l) Karst and beech, Fagus-sylvatica L., …. In "New Phytologist", Vol. 128, pp. 671-678.
Fagus sylvatica	3-yr-old 128 d	solar-domes			CF	60-120 ppb (6h mean)	Pearson and Mansfield (1993). Interacting Effects of Ozone and Water Stress on the Stomatal Resistance of Beech (Fagus sylvatica L.). In "New Phytologist", Vol. 123, pp. 351-358.
Fraxinus americana	1-yr-old 2 seasons	OTC	-0.026 MPa	-0.142 MPa (2nd year)	CF	41-42 ppb (24h mean)	Schaub et al. (2003). Physiological and foliar injury responses of Prunus serotina, Fraxinus americana, and Acer rubrum seedlings to varying soil moisture and ozone. In "Environmental Pollution", Vol. 124, pp. 307-320.
Fraxinus excelsior	1-yr-old seedlings 3 seasons	OTC	150 kPa	10 kPa	NF	39-52 ppb (24h? mean)	Broadmeadow and Jackson (2000). Growth responses of Quercus petraea, Fraxinus excelsior and Pinus sylvestris to elevated carbon dioxide, ozone and water supply. In "New Phytologist", Vol. 146, pp. 437-451.
Fraxinus excelsior	2-yr-old seedlings 2 seasons	solar-domes	WW	45% WW	32 ppb	74 ppb (24h mean)	Hayes et al. (2015). Species-Specific Responses to Ozone and Drought in Six Deciduous Trees. In "Water Air and Soil Pollution", Vol. 226, pp. 13.

species/ genotype	**age/ duration**	**facility**	**H_2O control**	**drought treatment**	**O_3 control**	**elevated O_3**	**reference**
Picea abies genotype Istebna genotype Gerardmer	5-yr-old trees 5 seasons	OTC	WW	WS	CF	NH+50 ppb	Dixon et al. (1998). Reactions of Norway spruce and beech trees to 2 years of ozone exposure and episodic drought. In "Environmental and Experimental Botany", Vol. 40, pp. 77-91.
Picea abies	grafted plants 3 season	OTC	WW	WS	CF	34 ppb (24h mean)	Karlsson, P. E., Medin, E. L., Wickstrom, H., Sellden, G., Wallin, G., Ottosson, S., and Skärby, L. (1995). Ozone and drought stress - Interactive effects on the growth and physiology of Norway spruce (Picea abies (L) Karst). Water Air and Soil Pollution 85, 1325-1330.
Picea abies genotype Istebna	8-yr-old trees 3 months	OTC	WW	WS	CF	NF, NF+25, NF+50 (continuously)	Le Thiec et al. (1994). The effects of slightly elevated ozone concentrations and mild drought stress on the physiology and growth of Norway spruce, Picea-abies (l) Karst and beech, Fagus-sylvatica L., …. In "New Phytologist", Vol. 128, pp. 671-678.
Pinus halepensis	2-yr-old seedlings 2 seasons	OTC	WW	-2 MPa	CF	90-100 ppb (24h mean)	Le Thiec and Manninen (2003). Ozone and water deficit reduced growth of Aleppo pine seedlings. In "Plant Physiology and Biochemistry", Vol. 41, pp. 55-63.
Pinus halepensis	2-yr-old seedlings 4 seasons	OTC	WW	WS	CF	NF+40; 54.2-70.0 ppm.h (AOT40)	Inclan et al. (2005). Compensation processes of Aleppo pine (Pinus halepensis Mill.) to ozone exposure and drought stress. In "Environmental Pollution", Vol. 137, pp. 517-524.
Pinus ponderosa	2-yr-old seedlings 3 seasons	OTC	WW	WS	CF	62 ppb 95 ppb (12h mean)	Beyers et al. (1992). Effects of long-term ozone exposure and drought on the photosynthetic capacity of ponderosa pine (Pinus ponderosa Laws). In "New Phytologist", Vol. 122, pp. 81-90.
Pinus ponderosa	2-yr-old seedlings 3 seasons	OTC	WW	WS	CF	54 ppb 87 ppb (24h mean)	Temple et al. (1993). Growth-responses of ponderosa pine to long-term exposure to ozone, wet and dry acidic deposition, and drought. In "Canadian Journal of Forest Research- ", Vol. 23, pp. 59-66.
Pinus sylvestris	1-yr-old seedlings 3 seasons	OTC	150 kPa	10 kPa	NF	39-52 ppb (24h? mean)	Broadmeadow and Jackson (2000). Growth responses of Quercus petraea, Fraxinus excelsior and Pinus sylvestris to elevated carbon dioxide, ozone and water supply. In "New Phytologist", Vol. 146, pp. 437-451.

species/ **genotype**	**age/ duration**	**facility**	**H_2O control**	**drought treatment**	**O_3 control**	**elevated O_3**	**reference**
Poplar maximowiczii × P. berolinensis	1-yr-old seedlings 2 seasons	OTC	100% FC	>50% FC	CF	NF(25-27ppb.h) (AOT40, 6 months)	Pollastrini et al. (2010). Growth and physiological responses to ozone and mild drought stress of tree species with different ecological requirements. In "Trees-Structure and Function", Vol. 24, pp. 695-704.
Poplar maximowiczii × P. berolinensis	cuttings 3 months	OTC	WW	WS	CF	50 ppb (24h mean)	Pollastrini et al. (2013). Intra-annual Pattern of Photosynthesis, Growth and Stable Isotope Partitioning in a Poplar Clone Subjected to Ozone and Water Stress. In "Water Air and Soil Pollution", Vol. 224.
Populus deltoides cv. 55/56 *x P. deltoides* cv. Imperial clone 546	cuttings 1 season	OTC	WW	40% WW	CF	80.6 ppb (10h mean)	Li et al. (2019). The effects of elevated ozone on the accumulation and allocation of poplar biomass depend strongly on water and nitrogen availability. In "Science of the Total Environment", Vol. 665, pp. 929-936.
Populus deltoides cv. 55/56 *x P. deltoides* cv. Imperial clone 546	cuttings 105 days	OTC	WW	40% WW	CF	80.6 ppb (10h mean)	Shang et al. (2019). Effects of elevated ozone and water deficit on poplar saplings: Changes in carbon and nitrogen stocks and their allocation to different organs. In "Forest Ecology and Management", Vol. 441, pp. 89-98.
Populus deltoides cv. 55/56 x *P. deltoides* cv. Imperial clone 546		OTC	WW	40% WW	CF	80.6 ppb (10h mean)	Xu et al. (2020). Limited water availability did not protect poplar saplings from water use efficiency reduction under elevated ozone. In "Forest Ecology and Management", Vol. 462, pp. 117999.
Populus deltoides cv. 55/56 *x P.* deltoides cv. Imperial clone 546	cuttings 1 season	OTC	WW	40% irrigation	CF	51 ppb, 78 ppb (24h? mean)	Gao et al. (2017). Water stress mitigates the negative effects of ozone on photosynthesis and biomass in poplar plants. In "Environmental Pollution", Vol. 230, pp. 268-279.
Populus tremuloides	seedlings 12 weeks	OTC	-2 bars	-5 bars	CF	73 ppb (8h mean)	Greitner et al. (1994). Analysis of aspen foliage exposed to multiple stresses - ozone, nitrogen deficiency and drought. In "New Phytologist", Vol. 127, pp. 579-589.
Prunus serotina	1-yr-old 2 seasons	OTC	-0.026 MPa	-0.142 Mpa (2nd year)	CF	41-42 ppb (24h mean)	Schaub et al. (2003). Physiological and foliar injury responses of Prunus serotina, Fraxinus americana, and Acer rubrum seedlings to varying soil moisture and ozone. In "Environmental Pollution", Vol. 124, pp. 307-320.

***species/* genotype**	**age/ duration**	**facility**	**H_2O control**	**drought treatment**	**O_3 control**	**elevated O_3**	**reference**
Quercus cerris *Quercus ilex* *Quercus pubescens*	3-yr-old saplings 11 weeks	contr. environ.	100% EP	30% EP	CF	80-100 ppb (5h mean)	Cotrozzi et al. (2016). Variations in physiological and biochemical traits of oak seedlings grown under drought and ozone stress. In "Physiologia Plantarum", Vol. 157, pp. 69-84.
Quercus ilex	1-yr-old seedlings 2 years	OTC	WW	50% WW	CF	37 ppb, 57 ppb (24h mean)	Ribas et al. (2005). Contrasting effects of ozone under different water supplies in two Mediterranean tree species. In "Atmospheric Environment", Vol. 39, pp. 685-693.
Quercus petraea	1-yr-old seedlings 3 seasons	OTC	150kPa	10 kPa	NF	39-52 ppb (24h? mean)	Broadmeadow and Jackson (2000). Growth responses of Quercus petraea, Fraxinus excelsior and Pinus sylvestris to elevated carbon dioxide, ozone and water supply. In "New Phytologist", Vol. 146, pp. 437-451.
Quercus petraea		OTC	150kPa	10 kPa	NF	48 ppm.h (AOT40)	Broadmeadow et al. (1999). Environmental limitations to O-3 uptake - Some key results from young trees growing at elevated CO2 concentrations. In "Water Air and Soil Pollution", Vol. 116, pp. 299-310.
Quercus robur	2-yr-old seedlings 2 seasons	solar-domes	WW	45% WW	32 ppb	74 ppb (24h mean)	Hayes et al. (2015). Species-Specific Responses to Ozone and Drought in Six Deciduous Trees. In "Water Air and Soil Pollution", Vol. 226, pp. 13.