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# Ozone impacts on vegetation in a nitrogen enriched and changing climate

**Gina Mills[1*], Harry Harmens[1], Serena Wagg[2], Katrina Sharps[1], Felicity Hayes[1]**

**David Fowler[3], Mark Sutton[3] and Bill Davies[4]**

[1] Centre for Ecology and Hydrology, Environment Centre Wales, Deiniol Road, Bangor, LL57 2UW, UK

[2] School of Biological Sciences, Bangor University, Deiniol Road, Bangor, Gwynedd, LL57 2UW, UK

[3] Centre for Ecology and Hydrology, Bush estate, Penicuik, Midlothian, EH26 0QB, UK

[4] Lancaster Environment Centre, Lancaster University, Lancaster, LA1 4YQ, UK

* for correspondence: gmi@ceh.ac.uk

## Abstract

This paper provides a process-oriented perspective on the combined effects of ozone ($O_3$), climate change and/or nitrogen (N) on vegetation. Whereas increasing $CO_2$ in controlled environments or open-top chambers often ameliorates effects of $O_3$ on leaf physiology, growth and C allocation, this is less likely in the field. Combined responses to elevated temperature and $O_3$ have rarely been studied even though some critical growth stages such as seed initiation are sensitive to both. Under $O_3$ exposure, many species have smaller roots, thereby enhancing drought sensitivity. Of the 68 species assessed for stomatal responses to ozone, 22.5% were unaffected, 33.5% had sluggish or increased opening and 44% stomatal closure. The beneficial effect of N on root development was lost at higher $O_3$ treatments whilst the effects of increasing $O_3$ on root biomass became more pronounced as N increased. Both responses to gradual changes in pollutants and climate and those under extreme weather events require further study.

## Capsule

A process-oriented perspective on the combined effects of ozone, climate change and/or nitrogen on vegetation

## Highlights

- $CO_2$ amelioration of $O_3$ effects on leaf physiology are less likely in the field
- Both extremes of temperature and $O_3$ impact on critical growth stages.
- Many species are more sensitive to drought as a result of exposure to $O_3$ pollution.
- The beneficial effect of N on root development is lost at higher $O_3$ treatments

- The effects of $O_3$ on root biomass are higher at high than low N.

**Key words**

Ozone, nitrogen, climate change, drought, vegetation

## Introduction

Air quality impacts on vegetation are frequently considered as effects of single stressors working in isolation from other air pollutants, climate and soil modifying factors. In this review, we bring together current knowledge on how these other factors impact on the responses of vegetation to tropospheric ozone ($O_3$), classed by many as the most damaging air pollutant to vegetation (Ashmore, 2005; Karnosky et al., 2007), and predict from a process-perspective what the impacts might be for vegetation growing in a future nitrogen (N) polluted and changing climate. Tropospheric or “ground-level” $O_3$ is a secondary air pollutant formed and destroyed by a series of complex photochemical reactions involving nitrogen oxides (NOx), methane ($CH_4$), carbon monoxide (CO) and non-methane volatile organic carbons (NMVOC) (Avnery et al., 2011; Royal Society, 2008). Ozone concentrations are usually highest in rural and upland areas downwind of major conurbations, where many vulnerable ecosystems provide essential services to humankind. Ozone and its precursors are also transported around the world in air masses, adding to background concentrations across the continents (Royal Society, 2008). Uptake of $O_3$ by sensitive plant species alters leaf physiology (increased respiration, reduced photosynthesis, stomatal functioning, enhanced senescence), reduces growth (both above– and below-ground and altering C allocation), and alters phenology (e.g. timing and number of flowers) (for further details see review by Ainsworth et al., 2012). In isolation, these process-effects may be sufficient to impact on key ecosystem services including crop yield, C sequestration by trees and grasslands, water provision and biodiversity (e.g. Harmens and Mills, 2012; McLaughlin et al., 2007a; Mills et al., 2013; Sun et al., 2012). As well as responding to $O_3$, under open field conditions, vegetation is frequently also exposed to atmospheric N inputs in oxidised (e.g. $NO_2$) or reduced (e.g. $NH_3$) form (see van der Berg et al., this issue), to climate extremes such as drought, heat stress and/or flooding, and to nutrient stress. We consider here whether we can reach a consensus on the direction of change of such impacts and what the implications might be for vegetation.

Ozone uptake is via the stomatal pores in the leaf surface and thus any plant, pollutant, climatic or soil factor that influences stomatal functioning will modify the amount of $O_3$ taken up by the plant and subsequent effects. Models of stomatal uptake or "flux" have been instrumental in improving our understanding of the areas and vegetation types at greatest risk of damage from the pollutant. For example, in the last 15 years, European scientists have developed models of $O_3$ flux – the so-called $DO_3SE$ model (Emberson et al., 2000; LRTAP, 2014), applied the model to show areas of greatest risk (e.g. Simpson et al., 2007) and shown that field evidence provided greater support for the application of this biologically more meaningful risk assessment method than one based on the atmospheric concentration of $O_3$ above the canopy (Mills et al., 2011). Effects of climate change on stomatal $O_3$ flux can be either direct – e.g. temperature, $CO_2$ and humidity effects on stomatal conductance or indirect via an influence on soil water potential (SWP) and plant development (Harmens et al., 2007; Vandermeiren et al., 2009). In addition, $O_3$ itself can, for example, modify the responses of plants to naturally occurring environmental stresses such as drought (e.g. Mills et al., 2009; Wilkinson and Davies, 2009, 2010) via effects on stomatal control and plant development (canopy and roots), and can feedback to global warming by reducing the C sink strength (e.g. Sitch et al., 2007). We first review current predictions of future $O_3$, N deposition and physical climates and then consider how these combined effects might occur in the two-factor combinations in which they are usually studied (e.g. $CO_2$ and $O_3$, drought and $O_3$, N and $O_3$). We then speculate on how all might interact together in a future $O_3$, N and $CO_2$-enriched climate, focussing on responses to subtle long-term changes as well as to the extreme climatic and pollutant events predicted to occur with greater frequency in the coming decades (IPCC, 2013).

## Trends and future projections

Over the past 150 years, increasing anthropogenic emissions of $O_3$ precursors and increased stratospheric mixing of $O_3$ into the lower layers of the atmosphere have led to an increase in the global average $O_3$ concentration from 20-30 ppb to 30 – 50 ppb, with significant spatial and temporal variability (IPCC, 2013). With the global population estimated to exceed 9 billion by 2050, associated increased demand for resources such as fossil fuels, energy production, transport and agriculture is likely to further increase $O_3$ precursor emissions and reactive nitrogen (Nr, all N compounds excluding $N_2$, and including $NH_3$, $NO_3$, $NO_x$ and $N_2O$) in the environment (Oltmans et al., 2006; Winiwarter et al., 2011). Future $O_3$ and Nr trends will not only depend on anthropogenic emission levels of precursors, but also on trends in temperature, humidity and solar radiation. For example, a multi-model study of impacts of climate change alone on $O_3$ concentrations in Europe predicts increases in the mean $O_3$ concentration in the range 0.9 to 3.6 ppb for 2040-49 climates compared to 2000-09 climates, if precursor emissions remain constant (Langner et al., 2012). Despite overall rises in pollutant levels globally, there have been some (partial) success stories in reducing emissions in some regions of the world leading to large spatial heterogeneity in predictions of future pollutant concentrations. Here, we consider some of the changes that have occurred in recent decades, and using the latest modelling, speculate on future $O_3$ and Nr concentrations and the feedbacks to climate of these short-lived climate forcers (SLCFs). Further details on ozone and reactive nitrogen chemistry and trends can be found in recent reviews by Monks et al. (2014) and Fowler et al. (2015) respectively.

*Ozone*

In assessing all available data from ground-level monitoring stations in the Northern Hemisphere, Cooper et al. (2014) showed that the ozone concentrations have been rising by 1 – 5 ppbv per decade since the start of comparable records in the 1950s to 1970s. Set against these rising background $O_3$ concentrations, regional controls on precursor emissions have led to reductions in peak $O_3$ concentrations in North America and Europe over recent decades. For example, in the once highly polluted California South Coast Air Basin (SoCAB), the annual 8h mean $O_3$ concentration has declined between 1973 to 2010, from over 300 ppb to approx. 100 ppb (Pollack et al., 2013) and declines in peak $O_3$ concentration of ca. 30 ppb from 1990 – 2006 have been reported in many parts of Europe (Simpson et al., 2014; Tørseth et al., 2012). Even so, potentially plant-damaging $O_3$ episodes are still occurring in the USA and Europe when climatic conditions conducive to $O_3$ formation (hot, dry and sunny days) coincide with precursor abundance. For example, in 2006, two significant $O_3$ episodes occurred in Europe between 17 – 22 July and 25 – 28 July with $O_3$ concentrations in excess of 90 ppb experienced in many countries (EEA, 2007). Often used as an example of a future climate year for Europe, these high episodes in 2006 suggest that the immediate threat caused by $O_3$ episodes will remain under climate change.

In estimating future regionally averaged $O_3$ concentration from 14 global transport models for a range of Representative Transport Pathways (RCP) emission scenarios without changes in climate, Wild et al. (2012) predicted very small decreases in global surface $O_3$ in 2050 relative to 2000 of 2 ± 0.5 ppb for RCP2.6, 0.8 ± 0.54 ppb for RCP4.5 and 0.4 ± 0.2 ppb for RCP6.0 and an increase of 1.5 ± 0.5 ppb for the most pessimistic scenario, RCP8.5. Predictions to 2050 are similar for North America and Europe with a decrease of 2 – 4 ppb for RCPs 4.5 and 6.0, whilst in Asia, $O_3$ concentrations for these RCPs range from little net change (RCP6.0) to a 3 ppb increase and 2.5 ppb decrease for RCP4.5 in south and east Asia, respectively (Wild et al., 2012, reproduced in Figure 1). Over 75% of these predicted changes in $O_3$ can be attributed to changes in methane abundance – a clear target for international pollutant emission control negotiations (HTAP, 2010).

Having a dual role as both a pollutant and an SLCF, increasing $O_3$ concentrations have been, and will in the future continue to be, influential in global warming. As well as directly influencing radiative forcing as a greenhouse gas (Shindell et al., 2013), $O_3$ also suppresses the global land carbon sink by reducing photosynthesis and carbon sequestration thereby increasing the $CO_2$ concentration in the atmosphere and indirectly increasing radiative forcing (Collins et al., 2010; Sitch et al., 2007). Global warming alone would lead to increases in $O_3$ production (Langner et al., 2012; Rasmussen et al., 2013), potentially negating beneficial effects of controls of precursor emissions. This so-called "climate penalty" arises from increased BVOC emissions (contributing, for example, to 8% of predicted increases in $O_3$ in China by 2050 (Wang et al., 2013)), accelerated photochemical reactions leading to more $O_3$ formation and increased stagnation of air masses (for further information, see Rasmussen et al., 2013). The climate effect of ozone is higher in NOx-saturated areas such as the SoCAB than in $NO_x$-limited regions of California such as the San Joaquim valley (Rasmussen et al., 2013). Similar differences in magnitude and direction of the $O_3$ -climate penalty are predicted at the regional scale, for example, modelling by Wang et al. (2013), suggested a climate-change benefit under present day emissions for 2050 of a 5 ppb reduction in $O_3$ in the less populated west and a climate penalty of a 3 ppb increase in the more densely populated eastern provinces of China for the IPCC A1B scenario. Adding domestic and hemispheric emission projections as well as climate change for 2050, Wang et al. (2013) predicted similar increases overall in $O_3$ concentration of 9 and 10.3 ppb for east and west China respectively. Their modelling suggests large differences in attribution, with domestic and global emissions contributing 45 and 47% respectively of increases in Eastern China, but 17 and 87% in Western China.

*Reactive nitrogen*

Over the 100 years since the introduction of the Haber-Bosch process for synthetic fertilizer production and exacerbated by increased industrialisation and population, global anthropogenic sources of Nr species have increased five-fold to 120 Tg N y-1 in 2005 (Erisman et al., 2011). Despite this, in some regions of the world, emission control measures in recent decades have been successful in reducing Nr release into the environment. For example, UK emissions of $NO_x$ fell by 58% between 1970 and 2010 (RoTAP, 2012), with the largest decreases due to reductions in coal-based energy production and the introduction of three-way catalysts into petrol cars in 1992. However, over the same time period there has been only a small fall (15%) in emissions of ammonia, with agricultural sources including livestock and fertilizer application responsible for about 90% of emissions. Averaged between 2000 and 2008, the annual total deposition of N in the UK was 363 Gg-N $y^{-1}$ and was roughly evenly split between oxidised (178) and reduced (175) forms (RoTAP, 2012). In contrast, in China, $NO_x$ and $NH_3$ emissions increased by 2 – 3 fold over the period 1980 to 2010, with a decrease in the ratio of $NH_3$-N to $NO_x$-N in emissions from about 4 to 2.5 (Figure 2, reproduced from Liu et al., 2013). Future projections for Nr in the environment suggest that in Europe, $NO_x$ emissions are likely to continue to fall as legislation for controls continues to be implemented (Engardt and Langner, 2013; Simpson et al., 2014). Controls on ammonia are much harder to achieve, with European emissions predicted to stabilise at current levels in the 2020s and 2030s (Winiwarter et al., 2011). Globally, the highest rises in fertilizer use are predicted for Latin America and South Asia between 2006 and 2050 (Sutton and Bleeker, 2013), with large regional increases in Nr in the environment predicted in these regions by 2100 under most RCP scenarios, including more than a two-fold increase in South Asia for RCP2.6 (Lamarque et al., 2013).

### *Carbon dioxide*

Future increases in $CO_2$ concentration could potentially significantly modify plant responses to $O_3$ via effects on stomatal conductance, photosynthesis and carbon allocation (see later). The rapid increase in ambient $CO_2$ concentration from ca. 300 ppm in 1950 to > 390 ppm by 2011 is predicted to continue for the pessimistic RCP8.5 scenario, reaching 936 ppm by the year 2100 (IPCC, 2013). Even with the most ambitious RCP2.6 scenario, $CO_2$ concentrations are predicted to continue to rise to 421 ppm by the end of the century, with intermediate concentrations of 630 and 800 ppm predicted for RCP4.5 and RCP6.0 respectively.

### *Climate*

The mean global surface temperature is predicted to increase by 0.3 to 0.7 °C by 2016 – 2035 relative to 1986 – 2005 (IPCC, 2013). By the end of the century, there is high confidence that the temperature rise is likely to exceed 1.5 °C for all RCPs and 2 °C for RCP6.0 and RCP8.5. Extremes of temperature, including more frequent hot and fewer cold temperatures are “virtually certain” and a higher frequency and longer duration of heat waves is “highly likely”. Rainfall is predicted to decrease in the drier mid-latitude and sub-tropical regions and increase, especially under the more extreme RCP8.5 scenario, in the wetter mid-latitude regions. As with temperature, the frequency and duration of extreme rainfall events and droughts are very likely to increase. Thus, to understand the potential implications for effects of $O_3$ in a future climate, we need to consider both effects of gradual underlying trends such as increasing temperature together with effects under the extreme climatic conditions that are likely to occur more frequently in coming decades.

## Implications of climate change for effects of ozone on vegetation

The interactive effects of $O_3$ with $CO_2$, temperature, air and soil moisture and light on vegetation, are important but not well understood, particularly not under field conditions (Ainsworth et al., 2012). Although recently discussed in the 5th Assessment Report of the IPCC (2014), major current projections of global food production under atmospheric change scenarios do not always account for the damaging effect of rising $O_3$. Similarly, many coupled climate-carbon models have currently neglected the impacts of changing ground-level $O_3$ concentrations on carbon cycling (Sitch et al., 2007). We consider here, from a process perspective, the potential direction of interactions.

### *Interactions between elevated $CO_2$ and* $O_3$

Recent field studies using Free Air $CO_2$ Enrichment (FACE) systems indicate that the positive effect of elevated $CO_2$ on plant growth and C sequestration might have been overestimated in previous studies using more controlled environments, smaller scale and/or shorter time periods (Ainsworth, 2008; Bernacchi et al., 2006; Long et al., 2005). The actual increase in plant biomass, and its capacity to act as a C sink under elevated $CO_2$ were smaller than predicted, partly due to accompanying $O_3$ pollution, and partly due to accompanying increases in global temperature and drought frequency. Given that elevated $O_3$ and $CO_2$ often affect plant physiology, community responses and soil processes in opposite directions (Ainsworth et al., 2012; Fuhrer, 2009), the direction of combined responses to both gases is thus finely balanced dependent on their relative concentrations and limiting effects of other stressors such as elevated temperature.

Ozone generally reduces photosynthesis per unit leaf area (Ainsworth, 2008; Ainsworth et al., 2012; Wittig et al., 2007), either directly via effects on the photosynthetic machinery such as a reduction in Rubisco and chlorophyll content (Dizengremel, 2001; Fiscus et al., 2005; Kobayakawa and Imai, 2011; Wittig et al., 2009), and/or indirectly by reducing the aperture of the stomatal leaf pores (Evans et al., 2005; Overmyer et al., 2008; Wittig et al., 2009), resulting in a reduction in whole plant photosynthesis. Despite the often reported decline in stomatal conductance at elevated $O_3$, stimulation of stomatal opening (Mills et al., 2009) and the induction of stomatal sluggishness (Paoletti and Grulke, 2010) have also been observed (discussed later). On the other hand, elevated $CO_2$ reduces stomatal conductance (Curtis and Wang, 1998; Drake et al., 1997; Kim et al., 2010; Morgan, 2003) and therefore the uptake of $O_3$ and its damaging impacts on plants (Fiscus et al., 1997; Harmens et al., 2007; McKee et al., 1997). Indeed, meta-analysis provided supportive evidence that elevated $CO_2$ counteracts the impacts of $O_3$ on stomatal conductance and light-saturated photosynthesis in boreal and temperate forests (Wittig et al., 2007). Taking this effect into account, Klingberg et al. (2011) concluded that despite substantially increased future $O_3$ concentrations in central and southern Europe, the flux-based risk of $O_3$ damage to vegetation was unchanged or decreased at sites across Europe under rising $CO_2$ concentrations. However, more complex interactions have been reported between elevated $CO_2$ and $O_3$ in field-exposed northern hardwood forests: both gases stimulated leaf-level stomatal conductance whereas the combined gases did not affect leaf-level stomatal conductance differently to the control (Uddling et al., 2010).

In addition to stomatal responses, compensatory interactions between $O_3$ and $CO_2$ have been demonstrated directly at the level of the photosynthetic machinery (e.g. Kobayakawa and Imai, 2011), such that $O_3$ and $CO_2$ effectively compensate for one another's effects on C fixation at the level of leaf physiology (e.g. Gray et al., 2010; Wittig et al., 2007, 2009). Once inside the leaf, $O_3$ induces oxidative stress through the production of reactive oxygen species (ROS) such as hydrogen peroxide, superoxide, and hydroxyl radicals (reviewed in Fiscus et al., 2005). Plants have a limited ability to detoxify ROS by "mopping up" or scavenging them via antioxidants such as ascorbic acid, flavonoids and phenolics or enzymes such as superoxide dismutase, catalase or peroxidases (Blokhina et al., 2003). Unscavenged ROS induce cell death, accelerated senescence and abscission, and evidence is growing that these processes are in part mediated by the plant hormones ethylene, jasmonic acid and salicylic acid (see Fiscus et al., 2005; Kangasjarvi et al., 2005 for reviews). Accelerated senescence will lead to a reduction in stomatal conductance (Emberson et al., 2000) and a down-regulation of photosynthetic genes and an up-regulation of genes involved in programmed cell death. As a consequence, whole plant photosynthesis will be reduced due to leaf damage, early senescence and abscission, and total green leaf area and leaf area index are reduced by ozone (Ainsworth, 2008; Morgan et al., 2003; Wittig et al., 2009). Elevated atmospheric $CO_2$ and increased C supply *per se* can either accelerate leaf development and senescence (Ludewig and Sonnewald, 2000; Pourtau et al., 2004) or delay senescence induced by $O_3$ by acting directly at the level of gene expression associated with senescence (e.g. Gray et al. (2010) and Kontunen-Soppela et al. (2010a,b)).

As more C is required for secondary plant metabolism to detoxify $O_3$ and/or repair $O_3$-induced cell damage in leaves (Betzelberger et al., 2010), less C will be available for plant growth (Dizengremel, 2001), and allocation to roots and soil. Indeed, a significant decline in the root to shoot ratio has been observed at elevated compared to either current ambient or pre-industrial $O_3$ levels (Wittig et al., 2009). Hence, root biomass appears to be more sensitive to adverse effects of $O_3$ than shoot biomass. In addition, increased ethylene formation, a common response to $O_3$ pollution, tends to reduce root growth directly (Wilkinson and Davies, 2010). As a consequence, total C allocation to the soil is likely to be reduced. Contrasting responses have been reported for the impact of elevated $CO_2$ on C allocation to roots, as increases, decreases and no changes in root-shoot ratio have been found (e.g. Kimball et al., 2007; Maroco et al., 2002). Competition between species is likely to affect the response of individual species (Kozovits et al., 2005). Although high $CO_2$ alleviates the effect of $O_3$ in reducing below ground root biomass in woody species, this seems not to be the case in herbaceous species (Wang and Taub, 2010). The latter authors concluded that elevated $CO_2$ has less pronounced effects on the root mass fraction than other environmental factors such as soil nutrient and water status. Dieleman et al. (2010) showed that elevated $CO_2$ induces a C allocation shift towards below-ground biomass compartments. However, the increased soil C inputs were offset by increased heterotrophic respiration, such that soil C content was not affected by elevated $CO_2$. Indeed, elevated $CO_2$-induced increases in the amount of soil organic matter in less stable pools such as microbial biomass, seem to reduce the capacity of the soil as a sink for C by making less C available to the more stable pools (Carney et al., 2007; Cheng et al., 2011; Hofmockel et al., 2011). Ozone increased the amount of C entering more stable pools in the study by Hofmockel et al. (2011) in a northern European hardwood forest, but not in other studies with a crop ecosystem (Cheng et al., 2011) or beech (Esperschutz et al., 2009). Nikolova et al. (2010) showed that the impact of $O_3$ on soil respiration might be dependent on soil water availability. In addition, Dieleman et al. (2010) showed that the effect of elevated $CO_2$ on fine root biomass and -production and on microbial activity increased with increasing soil nitrogen concentration, while the effect on soil C content decreased with increasing soil nitrogen concentration.

In summary, whereas increasing $O_3$ and $CO_2$ are frequently reported from controlled environment experiments as having opposite effects on leaf physiology, growth and C allocation, the evidence from field-based experiments does not fully support that they have compensatory effects when co-occurring.

*Ozone impacts in a warmer climate*

The combined effects of ozone and increases in temperature have attracted little study. In one of the very few field experiments investigating combined effects of ozone and global warming, Kasurinen et al., (2012) used infrared heaters to raise the temperature by 0.6 to 1 °C for three growing seasons and raised the seasonal mean ozone concentration by 5 ppb over young silver birch trees. Elevated temperature increased above- and below-ground growth and soil respiration rates in silver birch. These effects were modified by atmospheric $O_3$ concentration, with variation in response amongst genotypes. For example, soil respiration was increased in elevated temperature (T), elevated $O_3$ and combined $O_3$ and T in the gt14 genotype, but $O_3$ either partly (gt12) or totally (gt25) alleviated temperature effects on soil respiration, or had a synergistic interaction with temperature (gt15).

The complexity of the potential interactions between global warming and ozone impacts on vegetation is illustrated by effects on the canopy uptake of $O_3$. When considered as a single factor, increased temperature in temperate climates is likely to increase stomatal uptake of $O_3$ providing the optimum temperature for stomatal conductance has not been reached (Figure 3, reproduced from Pleijel et al., 2007). However, the response to warming will also be affected by the following indirect effects of increased warming: greater tropospheric $O_3$ formation increasing the atmospheric concentration, an increase in vapour pressure deficit, a decrease in soil water potential (soils will dry out faster due to enhanced soil evaporation and enhanced canopy transpiration), changes in seasonal patterns in the occurrence of peak episodes of $O_3$ and earlier and enhanced plant development, resulting in a forward shift of the period within the year when plants are absorbing $O_3$. In addition, a reduction in stomatal conductance due to $O_3$ will lead to an increase in leaf temperature, therefore exaggerating the impact of global warming on leaf processes (e.g. Bernacchi et al., 2011). Global warming will also affect photosynthesis and plant and soil respiration.

It is also surprising that few studies have explicitly studied combined effects of ozone and extreme heat events, even though these frequently coincide and may do so even more often in a future more variable climate. Their combined effects potentially could be particularly detrimental for many crops if they coincide with critical developmental stages of vegetation, e.g. spikelet development and grain filling stages in cereal crops which are both heat- (Asseng et al., 2015) and ozone- (e.g. Soja et al., 2000) sensitive.

### *Interactions between ozone and drought*

Since $O_3$ episodes frequently co-occur with climatic conditions associated with drought and an increased frequency of drought is predicted for the future particularly in drier areas of the world (IPCC, 2013), it is important to understand how vegetation will respond to their combined effects. It has been widely reported that drought-induced stomatal closure will limit $O_3$ uptake and damage (e.g. Fagnano et al., 2009; Fuhrer, 2009). However, several studies have shown that drought does not always reduce $O_3$-induced damage to plants in sensitive species (e.g. Mills et al., 2009; McLaughlin et al., 2007a; Robinson et al., 1998; Wagg et al., 2012; Wilkinson and Davies, 2009, 2010), and that the genetic variability in $O_3$ sensitivity may be related to the extent to which $O_3$

reduces the sensitivity of stomatal closure to soil drying. Such effects have been attributed to reduced response to drought signals such as abscisic acid in the presence of ozone-induced stress ethylene emission (Mills et al., 2009; Wilkinson and Davies 2009, 2010). This reduced stomatal closing response to $O_3$ will directly increase plant water loss, and therefore increase vulnerability to the drought episode, particularly when combined with a reduced root biomass, often seen as a response to $O_3$ (e.g. Grantz et al., 2006; Hayes et al., 2012; Wyness et al., 2011). This might eventually cause secondary reductions in C sequestration (Wilkinson and Davies, 2009, 2010), particularly if the vulnerable plants begin to experience additional/subsequent stresses such as wind, biotic attack, high light/VPD or flood/storm conditions (Wilkinson and Davies, 2010). As indicated below, a growing number of species exhibit $O_3$ -induced stomatal opening either in the presence or absence of soil drying, that is genotype-dependent (Wagg et al., 2013; Wilkinson and Davies, 2010). Such effects, leading initially to enhanced nutrient flow and increases in leaf surface area are not sustainable as leaf tissue eventually becomes water-stressed, resulting in a reduction in photosynthesis and premature senescence (Wilkinson and Davies, 2010).

Mills et al. (2013) conducted a comprehensive review of the published literature on $O_3$ effects on stomatal functioning to determine if generalisations can be made based on species, genotype, duration and intensity of $O_3$ exposure, climatic conditions and soil water availability. The results are summarised in Table 1 and reproduced in full in the Supplementary Information. Of the 68 species examined (including trees, crops and (semi-)natural grassland species), 22% showed no change in stomatal conductance, 10% showed a slowed (sluggish) stomatal response to elevated $O_3$, 23.5% showed an increased stomatal opening under elevated $O_3$ and 44% displayed stomatal closure in response to $O_3$. Tree species were the most adversely affected with 73% of species showing an altered stomatal response, with 13 species showing stomatal opening and 15 showing stomatal closure in response to $O_3$. Crops tended to respond to $O_3$ stress with stomatal closure (occurring in 75% of the species), whilst increased, or “sluggish” stomatal response was only reported in 19% of the species. For the 8 grassland species included, responses were more or less evenly spread across the four categories of stomatal response. However, the proportions of species showing different responses should be interpreted with some caution as there were large variations in exposure methods, the magnitude and duration of $O_3$ exposure, climatic conditions (i.e. differences in light, temperature, soil moisture etc.), as well as inherent differences in response of species/genotypes to elevated $O_3$. No clear patterns emerged for the $O_3$ concentration range for the different responses, except perhaps the tendency for stomatal opening to occur at lower concentrations. Further analysis is needed to understand the complexities of $O_3$ exposure duration, concentration and climate effects in the stomatal responses to $O_3$.

Extensive measurements of a Southern Appalachian forest in the USA by McLaughlin et al. (2007a, b) provide field evidence to support the concept of $O_3$-induced increases in transpiration. These authors document an almost linear increase in average daily sap flows and enhancement of the amplitude of daily water–loss from native trees with increasing $O_3$ exposure, suggesting an $O_3$-induced disruption to the whole-tree water balance, not only as a result of increased day-time transpiration but also due to increased night-time stomatal conductance. In a more recent study of the same Appalachian region, Sun et al. (2012) report reduced late season stream-flow from six forested watersheds in the South Eastern United States by as much as 23% based on analysis of 18-26 year data records. These effects were attributed to increased transpiration inferred by an $O_3$-induced loss of stomatal control and increased sap flow (Figure 4) alongside $O_3$-induced secondary changes in root biomass and associated altered moisture holding capacity of soils. Sun et al. (2012) suggest that loss of stomatal sensitivity will not only increase drought severity in the region, thus affecting ecosystem hydrology and productivity, but it will also have negative implications for flow-dependent aquatic biota.

## Modifying effect of reactive nitrogen (Nr) on the responses of vegetation to ozone

Wet and dry deposition of Nr bring added nutrients to plants and soils which can be growth promoting or inhibiting depending on current soil nutrient and pH status, climatic conditions, species-sensitivity, form, extent and duration of Nr (Dise et al., 2011). Deposition rates to forests are significantly higher than to shorter vegetation such as shrubs and grassland, with forest canopies being efficient at capturing gaseous and particulate input whilst wet deposition dominates for shorter canopies. In Europe, deposition to forests and shorter vegetation can be as high as 60 kg N and 30 kg N ha$^{-1}$ y$^{-1}$ respectively in central and western regions, but is much lower at 5 and 3 kg N ha$^{-1}$ y$^{-1}$ respectively in more remote northern areas (Butterbach Bahl et al., 2011). A recent meta-analysis of effects of N addition experiments provided some insight into the implications for roots for vegetation growing in a range of ecosystems: for the 74 available datasets, a mean of 110 kg N ha$^{-1}$N (range 10 to 250) resulted in a 20% increase in root biomass. For application rates of < 100 kg N ha$^{-1}$ y$^{-1}$ there was ca. an 18% decrease in the C: N ratio and a 10% increase in the respiration of fine roots, with fungal colonization effects only becoming significant at higher deposition rates (Li et al., 2015). If at high enough concentrations, effects of short-term gaseous exposures can be directly toxic to plant foliage, with lichens and bryophytes being particularly sensitive to dry-deposited N in the form of $NH_3$ (Dise et al., 2011). More usually, effects of Nr are considered as a long-term threat to vegetation. Over the first few years, enhanced N deposition increases availability of inorganic N in the upper layers of the soil leading to increased plant growth in N limited soils and invasion of more productive species (Dise et al., 2011). Over the longer term, litter production increases and N cycling in the ecosystem is accelerated, leading to a decline in species diversity as N-exploiters dominate (e.g. Henrys et al., 2011; Phoenix et al., 2012).

This longer-term effect of Nr deposition leads to practical difficulties when investigating experimentally how a future N enriched climate might modify $O_3$ effects. Most research funding cycles are relatively short, often over a maximum of three years, and thus combined $O_3$ and N experiments are usually short-term and frequently involve relatively high N deposition rates in order to induce an effect over a time period that is often only one growing season or less. A recent meta-analysis by Yendrek et al. (2013) comparing responses to $O_3$ under limiting N with those under sufficient N, indicated that negative effects of $O_3$ on leaf area, above ground and root biomass were partially mitigated by the presence of sufficient N, although many of these effects were not significant. We attempted to conduct a different analysis for this review, looking specifically at effects of enhanced N deposition consistent with current and projected future deposition rates, on the growth responses to $O_3$. Unfortunately we were hampered by many factors: a lack of consistency of methodology; inconsistency in reporting of key information on treatment conditions; use of very short experimental periods (frequently < 9 weeks); use of treatments outside the projected ambient range ($O_3$ 24h mean > 100 ppb, > 80 kg N ha$^{-1}$ y$^{-1}$); use of controlled environments that are inconsistent with the field conditions we were most interested in; and use of N treatments that were negatively influenced by shortages of other nutrients. After an extensive search for papers, of the 93 selected as potentially useful from a Web of Science search conducted in August 2014, only four contained sufficiently comparable data with roughly similar in-study variances for analysis of combined data. These are outlined in Table 2 and provided a total of 51 data points for analysis, including data for three tree and two grassland species. Details on data preparation and statistical analysis using general linear mixed effect models within R are provided in the Supplementary Information. Ozone treatments were split to represent implementation of precursor emissions controls (24h mean <35 ppb, “low”); business as usual legislation (24h mean of 40-55ppb, “medium”) and worse-case scenario without further controls (24h mean of 60 – 90 ppb, “high”).

Statistical analysis of the combined data from the four papers revealed that $O_3$ concentration did not modify the root biomass response to N for the “low” and “medium” treatment ranges (i.e. treatment effects were additive, Figure 5); these datasets were combined for subsequent analysis. Under the “high” $O_3$ treatment range, the root response to added N evident under low/medium $O_3$ was lost resulting in a significant $O_3$ x N interaction for the combined dataset (p= 0.025, Figure 5(a)). It was also evident that proportionately, the negative effect of $O_3$ on root biomass was more pronounced at higher added N than low added N. For example, there was a greater difference in relative biomass between the 60 – 90 ppb and < 30 ppb ozone categories of 0.46 for an N treatment rate currently experienced in central European forests of 60 kg N $ha^{-1}$ $y^{-1}$ than that an N application rate representative of pristine environments (0.29 for 10 kg N $ha^{-1}$ $y^{-1}$). Effects on above ground biomass were dominated by the N treatment rate, with no significant $O_3$ effect or $O_3$ x N interaction (Figure 5(b)). Four important messages emerged from this analysis: (i) the beneficial effect of N fertilisation on root development expected for this realistic N addition range is lost at higher $O_3$ treatments; (ii) the proportionate effects of increasing $O_3$ on root biomass become more pronounced at higher N treatments; (iii) interactions are apparent in roots rather than shoots and (iv) generalisations on responses need to take into account the relative concentrations/deposition rates and deposition history of both pollutants.

Measurements conducted by Yamaguchi et al., (2007), one of the contributory studies to the above analysis, provided some insight into the dynamics of the relative contributions of each pollutant over the growing season. In May and July of the second exposure season, increasing N treatment (0, 20 and 50 kg of added N $ha^{-1}$ $y^{-1}$) increased photosynthesis (A) in *Fagus crenata*, with increasing activity and concentration of RuBisCo measured in July, thereby increasing N content per unit leaf area ($N_{area}$). In late season (September), $N_{area}$ continued to increase with increasing N, but with no effects on Pn, the photosynthetic N use efficiency (PNUE) declined. In contrast, increasing $O_3$ (1.5 x and 2 x ambient $O_3$) had no significant effects on all five parameters early in the season but decreased A and PNUE in July and September. Interactions between $O_3$ and N were dynamic – changing throughout the growing season. Initially in May, the only interaction present was a loss of the beneficial effect of increasing N on $N_{area}$ with increasing $O_3$. By July, this interaction was evident in the photosynthetic rate and activity and content of RuBisCo, with negative effects of increasing $O_3$ being more pronounced at the highest N treatment resulting in a decreased PNUE. By September, the negative effects of $O_3$ on A and PNUE, and the positive effects on $N_{area}$ and stomatal conductance (g) were evident in all three N treatments, indicating that the $O_3$ effect was over-riding the N effect by the end of the growing season. These results illustrate a potential shift occurring during the season, possibly due to the cumulative effects of both pollutants.

Many studies have shown that increasing N fertilization increases stomatal conductance as photosynthesis and leaf growth are enhanced (e.g. Azuchi et al., 2014; Yamaguchi et al., 2007). The net effects of such an enhancement could be to steadily increase $O_3$ uptake until a tipping point is reached whereby $O_3$ detoxification and respiration repair processes are no longer sufficient to provide protection against this powerful oxidant, leading to a shift in C allocation in favour of shoots and away from roots as illustrated in Figure 5(a).

Only one experiment has studied the long-term effects of combinations of $O_3$ and Nr on biodiversity and plant processes under field conditions. The Alp Flix experiment (Volk et al., 2003) in Switzerland exposed *Geo-Montani-Nardetum* pasture monoliths for seven years to three $O_3$ levels and five N deposition rates at an alpine site 2000m a.s.l. with high annual precipitation of 1200mm, low mean temperature of +2.8 °C, low background N deposition of ca. 4 kg N $ha^{-1}$ $y^{-1}$ and growing season mean $O_3$ concentration of 45 – 47 ppb (Bassin et al., 2013). Under these high $O_3$ /low N and climatically challenging conditions, added N caused large changes in the community composition, with sedges becoming particularly dominant, whilst added $O_3$ at 1.2 and 1.6 x ambient had no effect on functional group composition and few effects on productivity; there were no significant $O_3$ x N interactions (Bassin et al., 2013 and references therein). The lack of sensitivity to $O_3$ was attributed to enhanced levels of anti-oxidants for tolerance of UV radiation at high altitude, continual exposure to high background rather than peak $O_3$ and enhanced natural resilience in this long-lived, slow-growing community. In contrast, addition of Nr to this nutrient-limited vegetation had a much larger effect than $O_3$, with Nr being the dominant pollutant in this instance, thereby emphasising the importance of previous pollution history in interpretation of interactions.

Analysis of national species distribution data in relation to drivers of change provide field evidence of long-term effects of pollutants. For example, species shifts in favour of those with high Ellenberg N together with reductions in low N species have been correlated with increasing N deposition (Henrys et al., 2011) and changes in species richness and habitat species composition have been found along N gradients in the UK (Field et al., 2014, Southon et al., 2014). In one such study, $O_3$ was included for the first time as a modifying factor influencing plant communities in UK acid grasslands (Payne et al., 2011). Redundancy analysis identified current total inorganic N deposition, mean annual potential evapotranspiration and $O_3$ exposure as the top three most important environmental variables explaining species cover. Further analysis revealed that whereas N is a key driver of species richness, $O_3$ was a key driver of species compositional change but not species richness. In a biodiversity context, the two pollutants appear to be working in distinct but different ways.

As with $CO_2$, one would expect that increasing Nr would at least in part ameliorate the effects of $O_3$ as the two pollutants affect plants in opposing ways. This may well be the case under nutrient-poor conditions where N-induced additional growth offers some protection. Where high Nr inputs combine with high $O_3$, the $O_3$ effect is more likely to dominate, with effects being especially pronounced in the roots. Such an interaction is particularly relevant close to point sources for Nr.

## Conclusions

From the review and analyses presented here it is apparent that it is not always straight forward to predict the direction of $O_3$ effect once one or more interacting factors are included. There is evidence of tipping points occurring where there is a shift from one factor being dominant to another. This shift can be dynamic, changing during the growing season, for example, for the tree species *Fagus crenata* where initially the N effect was dominant and then the $O_3$ effect became dominant later in the season (Yamaguchi et al., 2007). It is also dependant on the relative concentrations/values and exposure duration of the combining stresses as well as the relative sensitivity of the species studied. For example, the ameliorating effects of elevated $CO_2$ on the negative impacts of ozone are less pronounced for rice under field conditions than expected from shorter-term controlled environment studies (Ainsworth, 2008).

This study has also shown that there are two types of interactions that need to be considered differently for assessing current and future effects of combined stressors: (i) Responses to gradual long-term changes in climate, background $O_3$ and Nr typically included in regional or global modelling. Here, the evidence base is growing for inclusion of multiple factors but more experimental work is needed to fully understand the dynamics of interactions. (ii) Responses to extreme climatic and pollutant conditions, likely to become more frequent in the coming decades. Here, effects on vegetation appear to depend on previous exposure history, with, for example chronic exposure to $O_3$ prior to drought likely to reduce the ability of plants to control water loss thereby exacerbating the effects of drought, whilst exposure to a relative high $O_3$ episode with no prior exposure might induce stomatal closure offering some protection against drought. Although heat, drought and $O_3$ stress frequently occur together, there has been surprisingly little study so far of their combined effects, nor has the added effect of Nr been studied under these conditions.

This partially quantitative study has been hampered by a lack of consistency in reporting of experimental conditions and results across studies. There is merit in establishing a quality assurance code for data from air quality exposure experiments, with a minimum set and form of parameters for reporting. It is clear from this study that new sources of effects data are needed for the development, parameterisation and testing of multi-factor models capable of predicting future multi-stress impacts at a range of geographical- and time-scales.

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**Table 1** Summary of effects of $O_3$ on stomatal conductance in trees, crops and grassland species (data obtained from 49 peer reviewed publications, for details, see Supplementary Information). Reproduced from (Mills et al., 2013).

| | **Total number** | **No effect** | **Increased opening** | **Sluggish control** | **Stomatal Closing** |
|---|---|---|---|---|---|
| Crops (no. of species) | 16 | 1 | 1 | 2 | 12 |
| Crops (no. of experiments) | 22 | 2 | 1 | 2 | 17 |
| Trees (no. of species) | 44 | 12 | 13 | 4 | 15 |
| Trees (no. of experiments) | 60 | 12 | 17 | 10 | 21 |
| Grasslands (no. of species) | 8 | 2 | 2 | 1 | 3 |
| Grasslands (no. of expts.) | 11 | 2 | 5 | 1 | 3 |
| Total (no. of species) | 68 | 15 | 16 | 7 | 30 |
| Total (no. of experiments) | 93 | 16 | 23 | 13 | 41 |
| Ozone range (25th to 75th percentile), ppb | | 35 – 80 | 50 – 90 | 70 – 120 | 59 – 100 |
| **Mean ozone concentration, ppb** | | **59** | **67** | **91** | **89** |

**Table 2**: Brief description of data sources for ozone and nitrogen interaction studies included in Figure 5.

| **Publication** | **Species** | **Brief description** |
|---|---|---|
| Watanabe et al., 2008 | Evergreen broadleaf *(Castanopsis siebaldii)* | Two year old seedlings monitored over two growing seasons (2004 and 2005) in open top chambers; 3 x $O_3$ (max double ambient), 3 x N (max 50 kg N $ha^{-1}$ $y^{-1}$). |
| Yamaguchi et al., 2007 | Decidous broadleaf (*Fagus crenata*) | Two year old seedlings monitored over two growing seasons (2004 and 2005) in open top chambers; 3 x $O_3$ (max double ambient), 3 x N (max 50 kg N $ha^{-1}$ $y^{-1}$). |
| Wyness et al., 2011 | Forb (*Ranunculus acris*) and grass (*Dactylis glomerata*) | 8 x $O_3$ treatments (max ambient +72 ppb), 2 x N (max 75 kg N $ha^{-1}$ $y^{-1}$), solardomes |
| Thomas et al., 2005 | Evergreen needle (*Picea abies*) | two-year old seedlings, 2 x $O_3$ (max ca 40 ppb), 4 x N (max 80 kg N $ha^{-1}$ $y^{-1}$); outdoor exposure |

## Figure legends

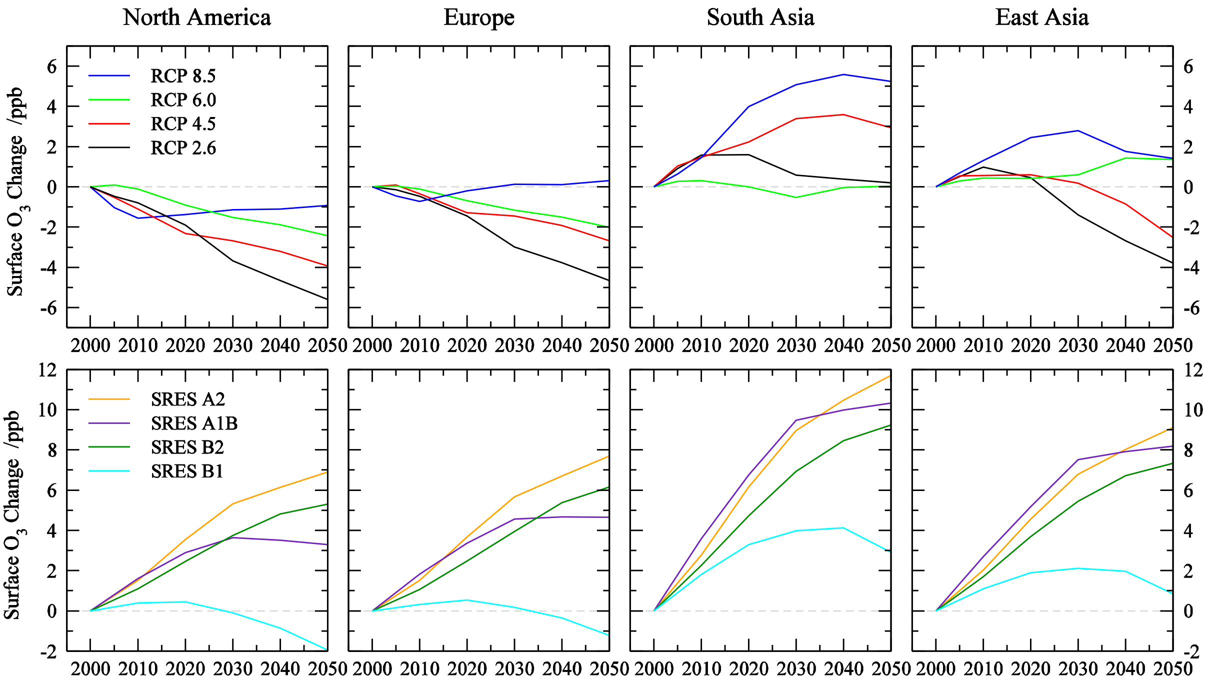
**Figure 1**: Model ensemble annual regional mean surface $O_3$ changes over the four HTAP regions from the parameterization following (a) the different RCP precursor emission pathways and (b) the SRES scenarios. The y-axis spans an $O_3$ change of 14 ppb in each case to allow direct comparison of the magnitude of $O_3$ changes. Reproduced from Wild et al., 2012.

**Figure 2:** Trends in (a), $NH_3$ and $NO_x$ emissions and ratios of $NH_3$-N to $NO_x$-N emission in China; (b) number of domestic animals (expressed as livestock units) and N fertilizer consumption; (c) number of motor vehicles and coal consumption. Reproduced from Liu et al., 2013.

**Figure 3:** Parameterisation for the effects of temperature on stomatal conductance (g) of wheat and potato for the stomatal flux algorithm used by the LRTAP Convention (2014). Data represent the short-term response of stomatal conductance to temperature and are expressed relative to a maximum stomatal conductance of 450 mmol $O_3$ $m^{-2}$ PLA $s^{-1}$ for wheat and 750 mmol $O_3$ $m^{-2}$ PLA $s^{-1}$ for potato. Reproduced from Pleijel et al., 2007.

**Figure 4** Empirical models of annual variations in later season streamflow were significantly improved for the 94ha Walker Branch catchment, Tennessee USA when climate and ozone were included ($R^2 = 0.78$) compared to a climate only model ($R^2 = 0.51$). Re-drawn from Sun et al. (2012).

**Figure 5:** The modifying effects of N addition on the response of (a) shoot and (b) root biomass to ozone, categorised by 24h mean as: −●− < 35 ppb, - ○ - 40 – 55 ppb, and ··◊·· 60 – 90 ppb. The combined datasets are from the papers listed in Table 2, and further details on statistical analysis can be found in the Supplementary Information. Relative effect was calculated as the treatment biomass/control biomass.



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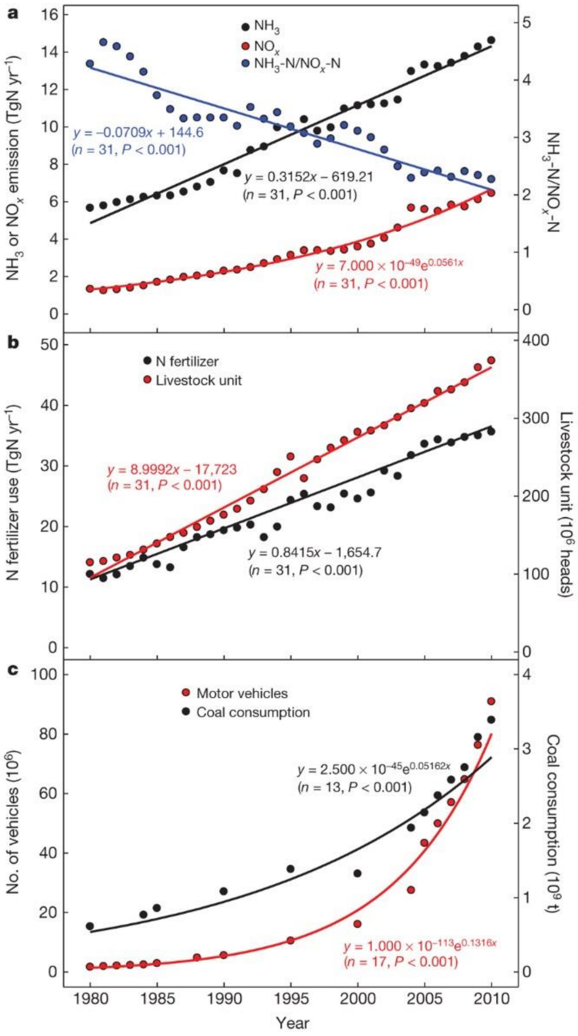

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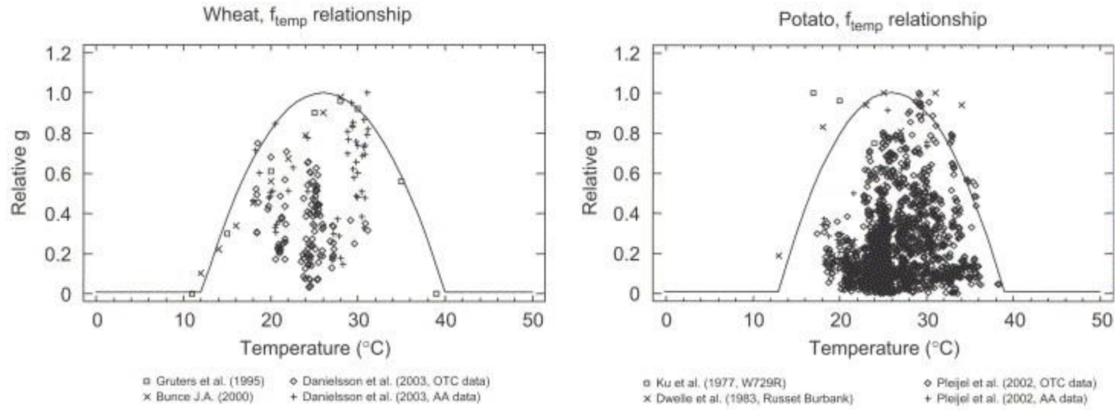

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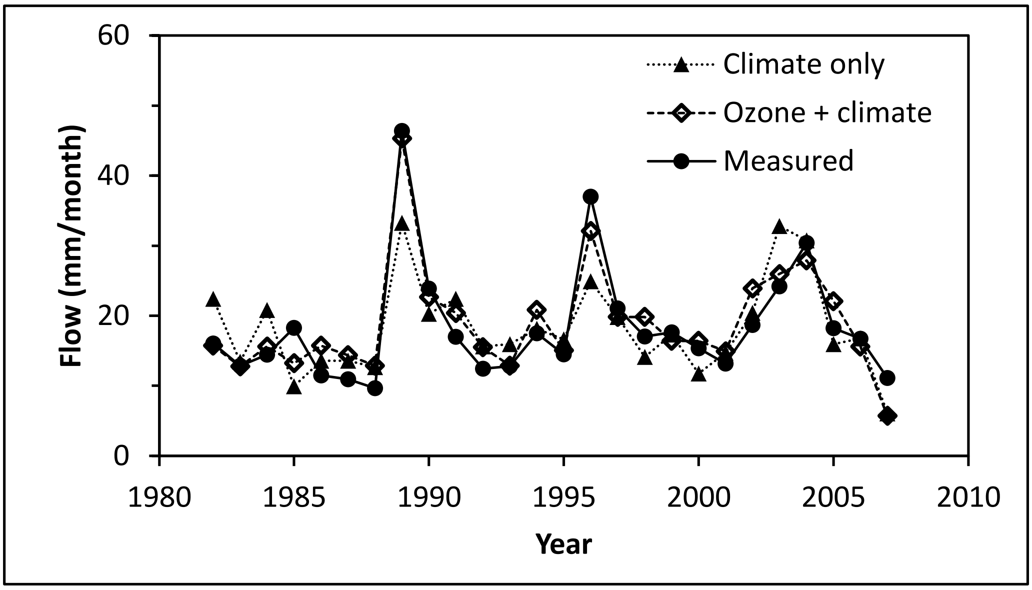

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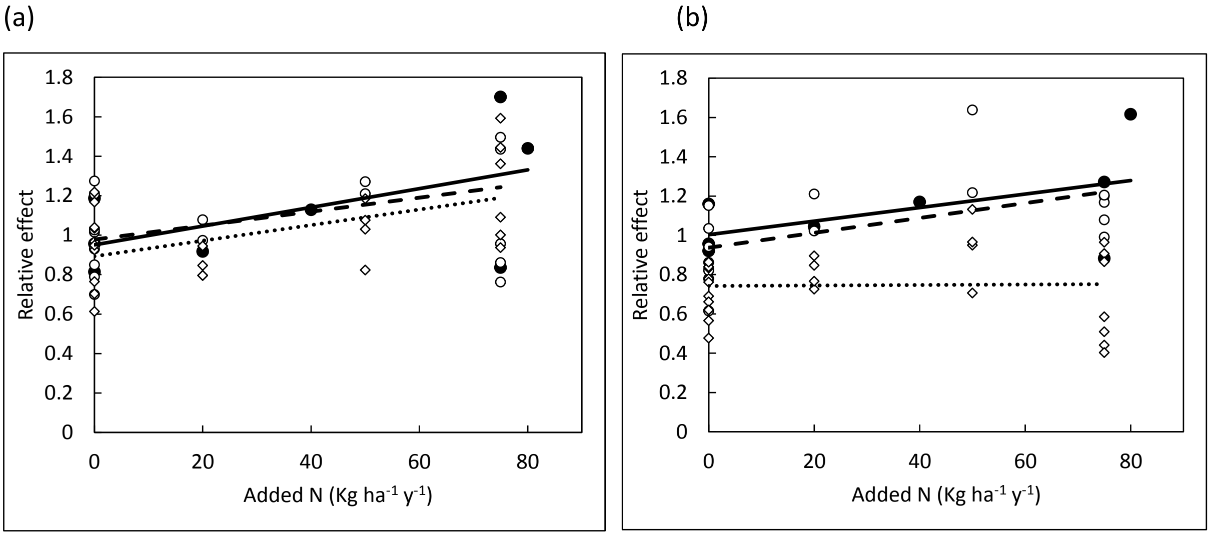

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# Ozone impacts on vegetation in a nitrogen enriched and changing climate

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# Supplementary Information

## (1) Data sources on ozone effects on stomatal conductance provided in Table 1

### Trees and woody species

| **Author** | **Species** | **Length of exposure** | **Ozone concentration** | **Environment** | **Stomatal response** | **Additional treatment** |
|---|---|---|---|---|---|---|
| Freer-Smith & Dobson 1989 | *Sikka spruce* | 2 days | 80 ppb | chambers | increased gs | N/A |
| Gregg et al. 2006 | *Populus deltoides* | 11 weeks | 98 ppb | open top chamber | increased gs | N/A |
| Mclaughlin et al. 2007 | *forest trees* | 23 yrs | natural flunctuating | natural | increased gs | N/A |
| Onandia et al. 2011 | *Betula papyrifera* | 10 years | 1.5 x ambient | Aspen FACE | increased gs | CO2 |
| Paakkonen et al. 1998 | *Betula pendula* | 11 weeks | 1.5 x ambient | open field | increased gs | well-watered |
| Pearson & Mansfield 1993 | *Fagus sylvaticum L* | 18.2 weeks | 60 - 120 ppb | Solardomes | increased gs | red.-watered |
| Pearson & Mansfield 1993 | *Fagus sylvaticum* L | 18.2 weeks | 60 - 120 ppb | Solardomes | increased gs | well-watered |
| Roberts et al. 1990 | *Liriodendron tulipifers* | 14 weeks | 50 ppb | chambers | increased gs | N/A |
| Sun et al. 2012 | *Betula papyrifera* March. | 12.8 weeks | 80 ppb | Aspen FACE | increased gs | N/A |
| Sun et al. 2012 | forest trees | 18-24yr | natural flunctuating | natural | increased gs | N/A |
| Sun et al. 2012 | *Populus tremuloides* Michx. | 12.8 weeks | 80 ppb | Aspen FACE | increased gs | N/A |
| Uddling et al. 2009 | *Betula papyrifers* | 2 growing seasons | ambient + 35% | Aspen FACE | increased gs | N/A |
| Uddling et al. 2009 | *Populus tremuloides* | 2 growing seasons | ambient + 35% | Aspen FACE | increased gs | N/A |
| Zhang et al. 2013 | *Elaeocarpus balansae* | 3 weeks | ambient | open-air | increased gs | N/A |
| Zhang et al. 2013 | *Ficus microcarpa* | 3 weeks | ambient | open-air | increased gs | N/A |
| Zhang et al. 2013 | *Manglietia glauca* | 3 weeks | ambient | open-air | increased gs | N/A |
| Tjoelker et al. 1995 | *Acer saccharum* Marsh. | 9.7 weeks | ambient x2 (approx 95 ppb) | free air exposure system | initial increase | N/A |
| Hoshika et al. 2012a | *Betula ermanii* | 12 weeks | 60 ppb | open top chamber | no change | N/A |
| Hoshika et al. 2012a | *Betula maximowicziana* | 12 weeks | 60 ppb | open top chamber | no change | N/A |
| Hoshika et al. 2012a | *Betula platyphylla* var. *japonica* | 12 weeks | 60 ppb | open top chamber | no change | N/A |
| Samuelson 1994 | *Acer rubrum* | 20 weeks | ambient x2 (60 - 120 ppb) | open-top chambers | no change | N/A |
| samuelson 1994 | *Prunus serotina* | 20 weeks | ambient x2 (60 - 120 ppb) | open-top chambers | no change | N/A |
| Zhang et al. 2013 | *Aporusa dioica* | 3 weeks | ambient | open-air | no change | N/A |
| Zhang et al. 2013 | *Cinnamomum camphora* | 3 weeks | ambient | open-air | no change | N/A |
| Zhang et al. 2013 | *Litsea glutinosa* | 3 weeks | ambient | open-air | no change | N/A |
| Zhang et al. 2013 | *Sapium discolor* | 3 weeks | ambient | open-air | no change | N/A |
| Zhang et al. 2013 | *Schmima superba* | 3 weeks | ambient | open-air | no change | N/A |
| Zhang et al. 2013 | *Toxicodendron succedaneum* | 3 weeks | ambient | open-air | no change | N/A |
| Hanson et al. 1994 | *Quercus rubra* L | 2 growing seasons | 2x ambient | large open-top chambers | reduced gs | N/A |
| Hartikainen et al. 2012 | *Betula pendula* Roth | 2 growing seasons | ambient x 1.4 | open field | reduced gs | incr. temp. |
| Hoshika et al. 2012b | *Fagus crenata* | 12 weeks | 56.7 ±10.5 ppb | free air exposure system | reduced gs | N/A |
| Lombardozzi et al. 2012 | *Liriodendron tulipifers* | 12 weeks | ambient + 70 ppb (100ppb appro | open-top chambers | reduced gs | N/A |
| Matyssek et al. 1991 | *Betula pendula* | 5 weeks | 100 ppb | field chambers | reduced gs | N/A |
| Minnocci et al. 1999 | *Olea europaea* L. cv Frantoio | 17.1 weeks | 100 ppb | chambers | reduced gs | N/A |
| Minnocci et al. 1999 | *Olea europaea* L. cv Moraiolo | 17.1 weeks | 100 ppb | chambers | reduced gs | N/A |
| Novak et al. 2005 | *Fraxinus excelsior* | 20 weeks | 40.6 ppb | chambers | reduced gs | N/A |
| Novak et al. 2005 | *Populus nigra* L | 20 weeks | 40.6 ppb | chambers | reduced gs | N/A |
| Novak et al. 2005 | *Viburnum lantana* | 20 weeks | 40.6 ppb | chambers | reduced gs | N/A |
| Paakkonen et al. 1998 | *Betula pendula* | 11 weeks | 1.5 x ambient | open field | reduced gs | red.-watered |
| Pollastrini et al. 2010 | *Fagus sylvatiucm* L. | 24 weeks | 150 ppb | open-top chambers | reduced gs | red.-watered |
| Pollastrini et al. 2010 | *Populus maximowiczii* | 24 weeks | 150 ppb | open-top chambers | reduced gs | red.-watered |
| Pollastrini et al. 2010 | *Quercus robur* L | 24 weeks | 150 ppb | open-top chambers | reduced gs | red.-watered |
| Roberts. 1990 | *Liriodendron tulipifers* | 14 weeks | 200 ppb | chambers | reduced gs | N/A |
| Shan et al. 1996 | *Pinus armandi* Franch | 14 weeks | 300 ppb | field chamber | reduced gs | N/A |
| Tjoelker et al. 1991 | *Liriodendron tulipifers L.* | 18 weeks | ambient + 60 ppb (approx 108 pp | open-top field chambers | reduced gs | N/A |
| Tjoelker et al. 1991 | *Pinus taeda* L. | 18 weeks | ambient + 60 ppb (approx 108 pp | open-top field chambers | reduced gs | N/A |
| Vandermeiren et al. 2002 | *Solanum tuberosun* cv. Bintje | I growing season | ambient x 2 | open-top chambers | reduced gs | N/A |
| Wullschleger et al. 1996 | *Quercus rubra L* | I growing season | ambient x2 | single tree chamber | reduced gs | nitrogen |
| Zhang et al. 2013 | *Schefflera octophylla* | 3 weeks | ambient | open-air | reduced gs | N/A |
| Dumont et al. 2013 | *Populus deltoides x Populus nigra* | 3 weeks | 120 ppb | growth chambers | slowed response | blue light |
| Dumont et al. 2013 | *Populus deltoides x Populus nigra* | 3 weeks | 120 ppb | growth chambers | slowed response | red light |
| Dumont et al. 2013 | *Populus deltoides x Populus nigra* | 3 weeks | 120 ppb | growth chambers | slowed response | CO2 |
| Dumont et al. 2013 | *Populus deltoides x Populus nigra* | 3 weeks | 120 ppb | growth chambers | slowed response | VPD |
| Grulke et al. 2007b | *Quercus douglasii* | 4 weeks | 70 ppb | open top chamber | slowed response | light |
| Grulke et al. 2007b | *Quercus kelloggii* | 4 weeks | 70 ppb | open top chamber | slowed response | light |
| Paoletti & Grulke 2010 | *Quercus douglasii* | 8 weeks | 70 ppb | open top chamber | slowed response | light |
| Paoletti & Grulke 2010 | *Quercus kelloggii* | 8 weeks | 70 ppb | open top chamber | slowed response | light |
| Paoletti 2005 | *Arbutus unedo* | 13 weeks | 110 ppb | greenhouse | slowed response | red.-watered |
| Paoletti 2005 | *Arbutus unedo* | 13 weeks | 110 ppb | greenhouse | slowed response | reduced light |

## Grasslands

| | | | | | | |
|---|---|---|---|---|---|---|
| Hayes et al. 2012 | *Dactylis glomerata* | 20 weeks | 70 -90 ppb | Solardomes | increased gs | red.-watered |
| Mills et al. 2009 | *Dactylis glomerata* | 20 weeks | 101.3 ppb | Solardomes | increased gs | N/A |
| Mills et al. 2009 | *Leontodon huspidus* | 20 weeks | 101.3 ppb | Solardomes | increased gs | N/A |
| Wagg et al. 2012 | *Dactylis glomerata* | 20 weeks | 70-90 ppb | Solardomes | increased gs | red.-watered |
| Wilkinson & Davies 2009 | *Leontodon huspidus* | 5 weeks | 70 ppb | growth cabinets | increased gs | red.-watered |
| Jaggi et al. 2005 | *Trifolium prantense* | 2 growing seasons | ambient x 1.5 (2002) ambient x1. | free- air fumigation system | No change | red.-watered |
| Wagg et al. 2012 | *Ranulculus acris* | 20 weeks | 70-90 ppb | Solardomes | No change | red.-watered |
| Zhang et al. 2012 | *Chionanthus retusus* Lindl. & Paxt. | 9.3 weeks | 70 ppb | open top chambers | No change | N/A |
| Jaggi et al. 2005 | *Holcus lanatus* L. | 2 growing seasons | ambient x 1.5 (2002) ambient x1. | free- air fumigation system | reduced gs | red.-watered |
| Jaggi et al. 2005 | *Plantago lanceolata* L. | 2 growing seasons | ambient x 1.5 (2002) ambient x1. | free- air fumigation system | reduced gs | red.-watered |
| Neufeld et al. 1012 | *Rudbeckia laciniata* L | 16 weeks | natural fluctating | open field | reduced gs | N/A |
| Reiling and Davison 1995 | *Plantago major* L. (28 populations) | 3 weeks | 70 ppb | small chambers | reduced gs | N/A |
| Zhang et al. 2012 | *Cornus alba L* | 9.3 weeks | 70 ppb | open top chambers | reduced gs | N/A |
| Zhang et al. 2012 | *Euonymus bungeanus* Maxim | 9.3 weeks | 70 ppb | open top chambers | reduced gs | N/A |
| Zhang et al. 2012 | *Photinia x fraseri* | 9.3 weeks | 70 ppb | open top chambers | reduced gs | N/A |
| Zouzoulas et al. 2009 | *Gossypium allegria* | 23 weeks | 100 ppb | closed chambers | reduced gs | N/A |
| Zouzoulas et al. 2009 | *Gossypium romanos* | 23 weeks | 100 ppb | closed chambers | reduced gs | N/A |
| Grulke et al. 2007a | *Rudbeckia laciniata var. digitata* | 7 days | fluctuating ambient (25-80 ppb) | open air | slowed respsone | dynamic light |

## Crops

| **Author** | **Species** | **Length of exposure** | **Ozone concentration** | **Environment** | **Stomatal response** | **Additional treatment** |
|---|---|---|---|---|---|---|
| Hassan et al. 1994 | *Raphanus sativus* L. cv Baladey | 8 days | 80 ppb | chambers | increased gs | N/A |
| Bernacchi et al. 2006 | *Glycine max* L | 3 years | 1.23x ambient | SoyFACE | no change | N/A |
| Betzelberger et al. 2012 | *Glycine max* | 2 growing seasons | 38-120 ppb | open- field | no change | N/A |
| Biswas et al. 2011 | *Triticum aestivum* L cv. Xiaoyan 22 | 3 weeks | 83 ppb | open top chambers | no change | red.-watered |
| Flowers et al. 2007 | *Phaseolus vulgaris L* | 9 weeks | 60 ppb | field chambers | no change | N/A |
| Biswas et al. 2011 | *Triticum aestivum* L cv. Xiaoyan 22 | 3 weeks | 83 ppb | open top chambers | reduced gs | well-watered |
| Biswas et al. 2011 | *Triticum turgidum* ssp. *durum* | 3 weeks | 83 ppb | open top chambers | reduced gs | well-watered |
| Fernandez-bayon et al. 1993 | *Citrullus lanatus* | 3 weeks | 70 ppb | chambers | reduced gs | N/A |
| Fernandez-bayon et al. 1993 | *Cucumis melo* | 3 weeks | 70 ppb | chambers | reduced gs | N/A |
| Grantz et al. 2003 | *Cucumis melo* cv. Ambrosia Hybrid | 5 weeks | 90ppb | open top chambers | reduced gs | N/A |
| Grantz et al. 2003 | *Cucumis melo* cv. Ambrosia Hybrid | 5 weeks | 143 ppb | open top chambers | reduced gs | N/A |
| Grantz et al. 2003 | *Gossypium barbadense* L | 6 weeks | 90ppb | open top chambers | reduced gs | N/A |
| Grantz et al. 2003 | *Gossypium barbadense* L | 6 weeks | 143 ppb | open top chambers | reduced gs | N/A |
| Grulke et al. 2007 | *Phaseolus vulgaris L* | I hr | 120-250 ppb | curvette | reduced gs | N/A |
| Hassan et al. 1994 | *Brassica rapa* L. cv. Sultani | 8 days | 80 ppb | chambers | reduced gs | N/A |
| Tiwari & Agrawal. 2011 | *Raphanus sativus L.* | 12 weeks | 40.8 ppb | open- top chmabers | reduced gs | N/A |
| Tiwari & Agrawal. 2011 | *Solanum melongena* | 12 weeks | 40.8 ppb | open- top chmabers | reduced gs | N/A |
| VanLoocke et al. 2012 | *Glycine max* L | 1 season | 70.8 ppb | SoyFACE | reduced gs | N/A |
| Biswas et al. 2011 | *Triticum turgidum* ssp. *durum* | 3 weeks | 83 ppb | open top chambers | slowed response | red.-watered |
| Paoletti & Grulke 2010 | Phaseolus vulgaris L | 4 weeks | 70 ppb | open top chambers | slowed response | light |

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## (2) Analysis of data on O3 * N interactions (Figure 6 and Table 2)

## Data collection

Data on the combined effects of ozone and nitrogen on tree biomass (roots, shoots and total) were gathered by searching the peer-reviewed literature using the Web of Science (Thomson-ISI, Philadelphia, PA, USA). From a total of 904 articles containing the chosen keywords, 93 were potentially relevant. Journal articles were excluded if: (1) Nitrogen treatments were applied only once; (2) Nitrogen treatments exceeded 80 kg ha$^{-1}$ yr$^{-1}$; (3) Mean 24 hour ozone exposure was >100ppb; (4) Ozone exposure period was <9 weeks. After applying these exclusion criteria, a total of four peer-reviewed articles published between 2005 and 2011 were included in the analysis (Table 2, main paper). Experiments were carried out in either Open Top Chambers (OTCs) or free air systems. A database was created, including the mean 24 hour ozone concentration (ppb) for each study, nitrogen treatment, species, experimental parameter (root biomass, shoot biomass, total biomass) and mean values per treatment. If the 24 hour mean ozone concentration was not reported, this was calculated from the 7 hour daylight mean ozone, using an equation derived from the ICP Vegetation bio-monitoring dataset, which took into account the daily profile of the hourly ozone concentrations in Europe.

Data was extracted from graphs using the GetData Graph Digitiser, version 2.26 (http://getdata-graph-digitizer.com). For each study, the relative treatment effect was calculated (treatment biomass/control biomass). Across all studies, control treatments had a mean 24 hour mean ozone concentration of 19.4 ppb and a mean nitrogen addition of 2.75 kg ha$^{-1}$ yr$^{-1}$. Ozone treatments were split into three categories for the 24h mean : low (<35ppb), medium (40-55ppb) and high (60-95ppb).

### Statistical methodology

A series of general linear mixed effect models were run using the package ‘lme4’ in the statistical program R (R Core Team, 2014), containing relative effect as the response variable and an interaction between the nitrogen (continuous) and ozone (factor) predictor variables. Response variables were transformed (square root) where necessary to ensure normality of residuals. A random effect of species was included to account for any variation in the data due to species specific responses to the treatments. A fifth data set from Jones et al. (2010) was removed prior to analysis as the variance in biomass was much smaller than for all other included species. A set of models was run for each parameter of interest (above ground biomass; below ground biomass; total biomass). Model selection was carried out by examining the change in Akaike’s Information Criterion (AIC) on removal of terms from the global model, following Burnham and Anderson (2002). For each biomass parameter, p-values were then provided for the variables in the top model using the R package ‘lmerTest’ (Table 2). As model results indicated there was no difference in the slope for the interaction between nitrogen and ozone at <35ppb and 40-55ppb for relative root biomass, these ozone categories were combined in the final model set.

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