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Interactive effects of O_3 exposure on California black oak (*Quercus kelloggii* Newb.) seedlings with and without N amendment

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Short term O_3 exposure reduced carbon acquisition and water use efficiency in Quercus kelloggii seedlings; nitrogen amendment reduced sensitivity to ozone.

Abstract

We examined the short-term separate and combined effects of simulated nitrogen (N) deposition (fertilization) and ozone (O_3) exposure on California black oak seedlings (*Quercus kelloggii* Newb.), an ecologically important tree of the San Bernardino Mountains downwind of Los Angeles. Realistic concentrations of O_3 were found to cause statistically and biologically significant negative effects on plant health, including lowered photosynthetic ability, lowered water use efficiency, and increased leaf chlorosis and necrosis. When subjected to abrupt changes in light levels, O_3-exposed plants showed both a slower and smaller response than O_3-free plants. Fertilized plants exhibited a significantly greater pre- to post-treatment decline in A at saturated [CO_2] and a significantly lower level of post-treatment chlorosis than unfertilized plants. Fertilization tended to reduce plant sensitivity to O_3.

Keywords: N deposition; Ozone exposure; California black oak; *Quercus kelloggii*

1. Introduction

Industry, agriculture, and transportation in Los Angeles, California, generate large amounts of nitrogenous compounds which are transported by the prevailing winds into the neighboring San Gabriel and San Bernardino Mountains. Ecosystems in these mountains experience some of the highest rates of nitrogen (N) deposition in the country (Fenn et al., 2003), with deposition rates ranging from 4 to 40 kg N ha^{-1} $year^{-1}$ (Fenn et al., 2000; Fenn and Bytnerowicz, 1997). The western end of the range currently exhibits symptoms of nitrogen saturation (Fenn et al., 1996). Much of the nitrogen is produced in the form of NO_2, which in the presence of O_2 and ultraviolet light is converted into ozone (O_3), a phytotoxic oxidant (Stockwell et al., 1997; Heath and Taylor, 1997). There is a well-documented gradient of O_3 levels across the San Bernardino Mountains, with the western end suffering from extremely high O_3 exposure, and levels at the eastern end near global background (Bytnerowicz et al., 2007). During a typical year, sites at the western end have 8-h average O_3 levels of 120–130 ppb, and yearly maximum 1-h concentrations in the range 160–180 ppb (e.g. Crestline site, CA ARB, 2007).

The independent effects of N and O_3 at the plant level are generally well understood. Responses to N deposition are known primarily from studies of N amendment, where common effects include increased photosynthetic capacity (e.g. cork oak, Maroco et al., 2002; hybrid poplar, Cooke et al., 2005; aspen, Coleman et al., 1998; birch, Wendler and Millard, 1996) and increased leaf chlorophyll content (Cooke et al., 2005; Coleman et al., 1998). However, there are relatively few studies on plant response to anthropogenic N deposition. A study of mature oaks at a high-O_3, nitrogen-saturated site

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found a complex response to long-term nitrogen amendment that was strongly influenced by environmental conditions (Grulke et al., 2005). In a near-average precipitation year, fertilized plants had greater stomatal conductance (gs), and lower water use efficiency (WUE) than unfertilized plants; and in a drought year, fertilized trees showed greater levels of foliar chlorosis and began senescing earlier than unfertilized plants (Grulke et al., 2005). In general, plant exposure to moderate O_3 concentrations reduces chlorophyll content and increases chlorotic mottling of foliage, accelerates foliar senescence, reduces stomatal conductance (gs), lowers plant photosynthetic capacity, and sometimes increases susceptibility to insects and disease (Karnosky et al., 2003; Heath and Taylor, 1997).

The effects of N deposition and O_3 exposure in combination appear complex. In general, plants grown with low levels of N were more resistant to O_3 damage than those grown with higher levels of N (e.g. in aspen, Pell et al., 1995; wheat, Cardoso-Vilhena and Barnes, 2001). However, a few studies have shown an increase in O_3 resistance with increased N or increased overall nutrient levels (e.g. in birch, Pääkkönen and Holopainen, 1995; plantain, Whitfield et al., 1998).

Some studies have found more complex responses. For example, a study of aspen (*Populus tremuloides*) found a significant O_3-induced reduction in plant biomass at optimum nitrogen levels, but no such reduction at sub-optimal or excess nitrogen levels (Pell et al., 1995). Also, a study of birch (*Betula pendula*) found that plants grown with low levels of nutrients responded to O_3 by producing increased levels of antioxidants and retaining their leaves for a longer period of time, while plants grown with high levels of nutrients responded with earlier leaf abscission and an extended period of new leaf growth (Maurer and Matyssek, 1997a,b). In this study, both low-nutrient and high-nutrient plants responded to ozone exposure by making changes to optimize their growth, but the differing availability of nutrients resulted in different strategic choices (Maurer and Matyssek, 1997b).

Since NO_2 emissions and O_3 production are chemically linked, nitrogen deposition and O_3 exposure generally co-occur. Despite this fact, relatively few studies have investigated the interactive effects of N and O_3 on plants, and those effects are still poorly understood. To date, much of the work in the western US has focused on yellow pines, and other species remain relatively little studied. For example, the California black oak (*Quercus kelloggii* Newb.) is an ecologically important tree that is common in the oak woodlands and mixed conifer zone of the San Bernardino Mountains of California, yet there has been only one previous study of the effects of N and O_3 on California black oaks (Grulke et al., 2005). This was a study of mature oaks at a high-O_3, nitrogen-saturated site (described earlier).

The purpose of the present study was to retest the field observations of Grulke et al. (2005) in a controlled experiment, and to help fill these knowledge gaps. Specifically, to understand the effects of N, O_3, and the combination of N and O_3 on photosynthesis, stomatal behavior, and foliage condition in California black oak seedlings. Gas exchange measurements were used to understand the effects of these pollutants on stomatal behavior and photosynthetic capacity, and visual observations were used to assess leaf-level effects, such as leaf chlorosis and necrosis.

2. Materials and methods

2.1. Plant material

In the fall of 2004, 94 California black oak seedlings, 2–3 years in age, were purchased from a grower in Lassen County, CA. The closest air pollution monitoring site maintained by the California Air Resources Board (CA ARB) is at Manzanita Lake in Lassen National Park, just outside the western border of Lassen County. At this site, the average of daily 8-h O_3 averages from 2003 to 2006 was 69 ppb (CA ARB, 2007). As this site was directly downwind of a point-source in Burney, CA, O_3 levels further east (in Lassen County) were likely considerably lower than this, and were probably close to that of the global background of 20–45 ppb (Vingarzan, 2004). Thus, the seedlings obtained from this region and used in this experiment had experienced relatively little selection pressure for ozone-tolerance.

The purchased seedlings were delivered in the fall of 2004. In mid-January 2005, the roots were rinsed free of potting mix and placed in plastic bags, and the plants were placed in a refrigerator for 1 month. In mid-February, the seedlings were replanted in 9.5-l pots with native soil (B1 and B2 mixed) which had been sieved to pass a 5-mm screen. The soil came from a site whose B1 horizon contained an average of 0.02% soil N, a level which was at the very low end for the region (Grulke et al., 1998). About 3 cm of coarse gravel was placed in the bottom of each pot for drainage, and 3–5 cm of peat/perlite mix (Sunshine Mix #2, Sun Gro Horticulture) was placed on top of the soil. Ten bamboo sticks were inserted into the soil of each pot to provide aeration to roots in the loamy-clay soil. Reflective bubble pack was wrapped around the sides of the pots to reduce direct radiative heating of the pots. From mid-February through mid-July, the seedlings were grown in an O_3-free greenhouse at the USFS Fire Lab (Riverside, California), and watered to field capacity 2× per week.

Despite efforts to create a uniform growing medium, later observation showed a range of soil compaction and rates of water infiltration. A post-experiment comparison of slow-draining plants (unusually low rate of water infiltration) and outlier plants (having at least one unusually large/small measured value) found little overlap between the two groups. The few slow-draining plants that were also outliers were mostly identified as outliers for different reasons. Thus, the variation in soil texture did not have a systematic effect on experimental results.

2.2. Treatment groups

This experiment used a two-factor crossed design, with two levels of N (unfertilized (0 kg ha^{-1}); fertilized (50 kg ha^{-1})) and three levels of O_3 (O_3-free (0 ppb); moderate-O_3 (75 ppb); high-O_3 (150 ppb)), for a total of six different treatments. N levels were chosen to mimic an unpolluted site (unfertilized) and a highly polluted site (fertilized). O_3 levels were chosen to span most of the observed range. The high-O_3 level (150 ppb 8 h day^{-1}) was comparable to a highly polluted site at the western end of the San Bernardino mountains, as 150 ppb was modestly higher than typical 8-h averages (120–130 ppb) and modestly lower than 1-h maximum levels (160–180 ppb) (CA ARB, 2007) Thus, although the details of the exposure regime were different, the high-O_3 treatment was qualitatively similar to highly polluted sites in the San Bernardino Mountains.

As the experimental plants varied in morphology, an attempt was made to create treatment groups with similar distributions of plant morphologies. For each plant, five morphological characteristics were recorded: age, height, basal diameter, leaf color, and leaf size. Using these five characteristics, a cluster analysis was performed on the plants (flexible beta method, $\beta = -0.25$, Euclidean metric; PC-ORD version 4.39; McCune and Mefford, 1999). The resulting dendrogram was used to assign plants to treatment groups such that each treatment group had a similar distribution of morphologies (similar distribution of plants from each clade identified by the dendrogram).

After the pre-treatment measurements were completed, plants were randomly placed in one of six O_3 exposure chambers (described later). Two chambers were set to each O_3 level. Every 7–10 days, all the plants were removed from the growth chambers, each chamber was randomly reassigned a new O_3 level, and the plants were placed back into a random chamber of the appropriate ozone level. This allowed us to ignore positional effects, and treat each plant as an independent experimental unit.

2.3. Fertilization

Time constraints dictated that fertilization be done just prior to the first gas exchange measurements. In mid-June, nitrogen fertilizer (slow-release urea, "Blue chip") was applied to plants in fertilized treatment groups at a rate of 50 kg ha^{-1}. The expectation was that this would have no effect on pre-treatment measurements, due to the delay time between fertilization and plant response.

2.4. Ozone fumigation

The ozone exposure chambers were custom-built continuously stirred tank reactors (originally described in Padgett et al., 2004). Each chamber received a steady flow of activated-charcoal-filtered air which was delivered to and carried away from the chambers by a pair of large PVC pipes. The system produced O_3 from pure O_2 gas via spark discharge. After production, the O_3 was split into six separate flows, each of which was routed through an independently adjustable needle valve (Model FM-1050 Series, Tube 603, Matheson Gas Products) and then delivered to the air-inflow pipe at the top of one of the elevated O_3 chambers. An impeller at the top of each chamber ensured good mixing of the air within each chamber. Sampling tubes from each chamber were sequentially sampled by an O_3 monitor (model 1008-AH, Dasibi, Inc.), so that the level in each chamber was measured every 30 min.

The O_3 production and distribution system ran from 0800 to 1700 h daily, from August 18 to November 10. In the mornings, O_3 levels in the exposure chambers were reduced due to a combination of high humidity, NO from nearby rush-hour traffic, and adsorption of O_3 onto system surfaces. After a 1–2 h long morning ramp-up, O_3 levels reached their target maxima (0 ppb, 75 ppb, 150 ppb), and remained there throughout the day. After system shutdown at 1700, O_3 levels quickly dropped to 0 ppb over approximately 10 min. Equipment problems and electrical outages resulted in the O_3 production system being off for a total of approximately 10 days distributed throughout the exposure period.

2.5. Gas exchange measurements

Leaf-level gas exchange was measured pre- and post-treatment on a subset of the plants in each of the six treatments (Model LI-6400, LiCor Instr., Lincoln, NE). The gas exchange systems were regularly calibrated against a NIST-certified source. Leaves chosen for gas exchange measurements were representative of the whole canopy to the extent practical without excessive bending of plant parts. In many cases, the leaf that was chosen for pre-treatment measurement had, by the time of post-treatment measurements, senesced to a different degree than the majority of other leaves. In such cases, a new leaf was chosen for post-treatment measurement. Irradiance within the cuvette was supplied by a blue + red LED light source. Cuvette temperature and humidity were largely uncontrolled (and uncontrollable) as the range of temperature and humidity in the greenhouse exceeded the LI-COR's compensation ability. Pre-treatment leaf temperatures ranged from 22.5 to 27.2 °C, while leaf vapor pressure deficit (VPD) ranged from 1.0 to 2.1 kPa. Post-treatment leaf temperatures ranged from 23 to 32 °C, while leaf VPD ranged from 1.0 to 4.0 kPa.

2.5.1. Sunfleck response

Response to abrupt changes in light ("sunfleck response") was measured to better understand stomatal behavior and photosynthetic responsiveness. Pre-treatment, sunfleck response was measured for a random subset of 36 plants from all six treatments. Post-treatment, sunfleck response was measured for a random subset of 18 plants from three treatments: O_3-free/unfertilized, moderate-O_3/fertilized, and moderate-O_3/unfertilized. The remaining three treatment groups were unmeasured post-treatment due to premature senescence in the majority of the plants. In each measurement, the selected leaf was placed in the LI-COR cuvette under an irradiance of 1400 μmol photons m^{-2} s^{-1} (high light), and allowed to equilibrate for approximately 8 min, at which time the light was reduced to 200 μmol photons m^{-2} s^{-1} (low light). Leaves were then alternately exposed to high and low light for 2–4 high/low cycles, allowing for equilibration at each light level before proceeding to the next. Equilibration of a particular gas-exchange parameter to a given light level was defined as an average rate of change of <5% of that parameter over the preceding 10 min. Data were recorded at 1-min intervals.

2.5.2. Ambient-light/low-light measurements

This was a post-treatment, modified "sunfleck" response. It was intended to gather the same type of information using a quicker methodology, resulting in a lower level of detail but a greatly increased sample size. Post-treatment, ambient-light to low-light (AL/LL) measurements were taken on ca. 50 plants from among all six treatment groups. Light and humidity within the cuvette were set as close to ambient as possible, and the leaf was inserted in the cuvette. Ambient light levels were 700–1000 μmol photons m^{-2} s^{-1}. After several minutes of equilibration time, leaf gas-exchange parameters were recorded. This ambient-light measurement was an approximation of the leaf behavior under growth chamber environmental conditions. Afterwards, light levels were lowered to 200 μmol photons m^{-2} s^{-1} and leaf behavior was recorded at 1-min intervals until stomata reached equilibrium.

2.5.3. A–C_i response

A–C_i responses were recorded for ca. 50 plants pre- and post-treatment. Measurements were conducted using an automated A–C_i measurement program. Light levels were set to 1800 μmol photons m^{-2} s^{-1} as previous experiments had shown that 90% light saturation for greenhouse grown CA black oak trees occurs around 800 μmol photons m^{-2} s^{-1} (E. Paoletti, unpublished data). Subsequently, gs and A were measured at ambient CO_2 concentration (375 ppm) and then at 275, 125, 375, 675, 975, 1275, and 1675 ppm, allowing for equilibration at each CO_2 level (<0.5% CV).

2.6. Foliage condition

On the last day of O_3 exposure, levels of chlorosis, bronzing, necrosis, and new growth in attached foliage were recorded. Chlorosis ratings followed the method described in Miller et al. (1996). Bronzing was recorded as a presence/absence of either marginal bronzing or uniform bronzing. Necrosis was recorded as the percentage of attached foliar area showing necrosis. As the number of leaves on each plant at the beginning of the ozone exposure was not recorded, unattached leaves were not included in the assessment of necrosis. Thus, the recorded proportion of necrotic attached leaf area is an underestimate of the total proportion of necrotic leaf area. New growth was recorded on a scale from 0 to 3: no new growth, 0; a single new branch with unusually small leaves, 1; a single new branch with normal-sized leaves, 2; multiple new branches with normal-sized leaves, 3.

2.7. Data analysis

Data analysis was carried out using R version 2.4.0 (R Development Core Team, 2006), an open-source statistical package similar to S+.

Upon examination of the ratio-scale data, most sampling distributions appeared to be non-normal, and several were demonstrably so (Shapiro–Wilk test, $p < 0.05$). Therefore, the analysis utilized the non-parametric Kruskal–Wallis test and Steele's non-parametric multiple contrast test (Steele, 1960). Steele's test is similar to the Kruskal–Wallis test in that it is a one-factor test, but rather than testing for a difference among a number of treatment groups, Steele's test evaluates the null hypothesis of no difference among two different sets of treatment groups. This test allows groups to be combined in biologically meaningful ways as, for example, those having the same level of O_3 exposure but different levels of fertilization. This allowed testing of slightly different hypotheses than the Kruskal–Wallis test, and often increased the power of the statistical analysis by allowing the

inclusion of larger numbers of experimental units in each test. Non-parametric 95% confidence intervals for the difference of two medians were calculated using the wilcox.test function in R. Nominal-scale data were tested using Fisher's exact test.

Because O_3 exposure did not begin until after the pre-treatment measurements, pre-treatment data from plants with similar fertilization levels but different O_3 levels were pooled together. In cases where there were no significant differences between fertilized and unfertilized treatment groups, the data were further pooled together into a single pre-treatment group.

3. Results

3.1. Sunfleck and ambient-light/low-light response

Responses to changing light level varied both quantitatively and qualitatively. Following an abrupt change in light, gs and *A* of some plants exhibited a smooth, monotonic transition from one equilibrium level to the next (Fig. 1a). In other plants, gs and *A* exhibited an over- or undershoot (Fig. 1b). In extracting quantitative information from these curves, efforts were made to be as conservative as possible. Any equilibration times or levels that were unclear (due to premature change in light levels, excessive variation of environmental conditions, etc.) were excluded from the analysis. Relevant summary statistics from sunfleck response data are described below, while summary statistics from ambient-light/low-light response data are given in Table 1.

Post-treatment, the time required for *A* to equilibrate after an abrupt low-light to high-light (LL/HL) change was significantly greater in moderate-O_3/fertilized plants (31 ± SE 2 min) than in O_3-free/fertilized plants (14 ± SE 5 min) (Kruskal–Wallis, $p = 0.0495$). Further, the time required for *A* to equilibrate after an abrupt LL/HL change was significantly greater in post-treatment moderate-O_3/fertilized plants (31 ± SE 2 min) than in pre-treatment plants as a whole (9 ± SE 1 min) (Kruskal–Wallis, $p = 0.006$). There were no statistically significant differences in either low-light *A* or high-light *A* among treatment groups in pre-treatment measurements or in post-treatment measurements. Additionally, there were no statistically significant differences between pre- and post-treatment low-light *A*, or between pre- and post-treatment high-light *A* in any treatment group.

Post-treatment ambient-light/low-light (AL/LL) data showed a trend of decreasing ambient-light *A* (AL *A*), and low-light *A* (LL *A*), with increasing O_3. The size of the *A* response (ΔA = AL *A* − LL *A*) also tended to decrease with increasing O_3. AL *A* was significantly smaller in high-O_3 plants than in O_3-free plants (pooled N levels, Steele's test, $p = 0.02$). LL *A* and ΔA were both significantly smaller in moderate-O_3 plants than in O_3-free plants (pooled N levels, Steele's test, $p = 0.045$ and 0.01 respectively).

Water use efficiency (WUE) was calculated as *A*/*E*. WUE at ambient-light (AL WUE), at low-light (LL WUE), and the size of the WUE response (ΔWUE = AL WUE − LL WUE), all tended to decrease with increasing O_3 exposure. AL WUE and LL WUE were both significantly smaller in moderate-O_3 plants than in O_3-free plants (pooled N levels, Steele's test,

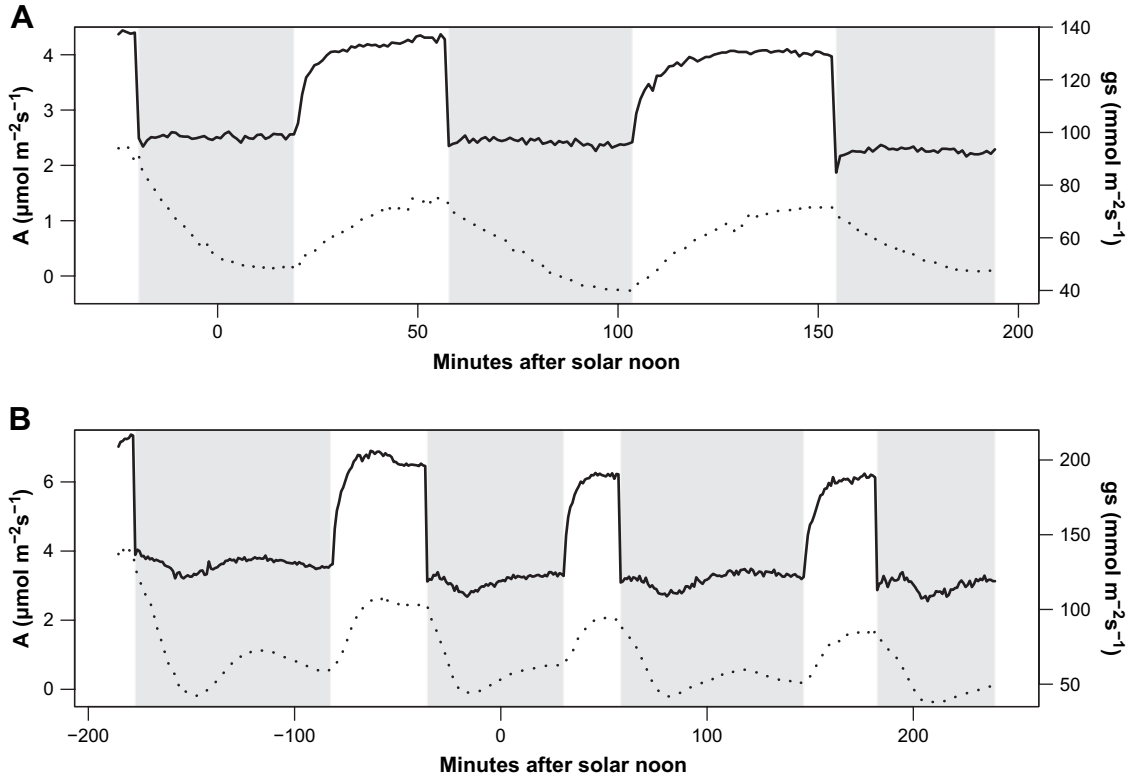

Fig. 1. Examples of two types of sunfleck response: smooth/monotonic (A), overshoot/ringing (B). Solid lines show *A* (left scale); dotted lines show gs (right scale). White/grey regions indicate periods of exposure to high/low light.

Table 1
Summary statistics from ambient-light/low-light (AL/LL) measurements

	0 ppb	75 ppb	150 ppb	C	F
AL *A*	4.4 ± 0.9 A	2.5 ± 0.4 AB	2.3 ± 0.3 B	2.9 ± 0.6	2.8 ± 0.3
LL *A*	2.4 ± 0.5 A	1.5 ± 0.3 B	1.6 ± 0.2 AB	1.7 ± 0.3	1.8 ± 0.2
Δ*A*	1.9 ± 0.4 A	0.8 ± 0.1 B	0.8 ± 0.1 B	1.2 ± 0.3	1.0 ± 0.1
AL WUE	1.8 ± 0.3 A	1.2 ± 0.2 B	1.6 ± 0.4 AB	1.4 ± 0.2	1.6 ± 0.3
LL WUE	1.5 ± 0.3 A	0.9 ± 0.2 B	1.5 ± 0.4 AB	1.2 ± 0.2	1.3 ± 0.3
ΔWUE	0.5 ± 0.1 A	0.3 ± 0.1 AB	0.1 ± 0.1 B	0.3 ± 0.1	0.2 ± 0.1
AL gs	78 ± 29 A	51 ± 9 A	43 ± 8 A	60 ± 14	50 ± 8
LL gs	55 ± 20 A	43 ± 6 A	30 ± 4 A	43 ± 10	41 ± 6
Δgs	22 ± 10 A	9 ± 3 A	12 ± 7 A	17 ± 6	9 ± 3
Δt_{gs}	15 ± 5 A	10 ± 2 A	8 ± 3 A	12 ± 3	9 ± 2

Numbers are mean ± 1 SE. The first three columns report results by O_3 level. Each one summarizes results from both treatment groups with the given O_3 level (e.g. the "0 ppb" column includes all plants in both the O_3-free/unfertilized treatment and the O_3-free/fertilized treatment). The latter two columns report results by fertilization level (C is control/unfertilized, F is fertilized). Each one of these columns summarizes results from all three treatment groups with the given N level (e.g. the "C" column includes all plants in the O_3-free/unfertilized, moderate-O_3/unfertilized, and high-O_3/unfertilized treatments). Rows give *A*, WUE and gs under ambient-light (AL) and low-light (LL); the size of change in *A*, WUE and gs following an abrupt change from ambient-light to low-light (Δ*A*, ΔWUE, Δgs); and the time required for stomatal equilibration following an abrupt change in light levels (Δt_{gs}). Units: *A*, μmol CO_2 m^{-2} s^{-1}; WUE, mmol CO_2 mol^{-1} H_2O; gs, mmol H_2O m^{-2} s^{-1}; Δ*t*, minutes. Entries within a row that do not share a letter were significantly different (Steele's test, $p < 0.05$). There were no statistically significant differences between N treatments in this data, therefore no letters were used in the latter two columns.

$p = 0.04$ and 0.04, respectively). ΔWUE was significantly smaller in high-O_3 plants than in O_3-free plants (pooled N levels, Steele's test, $p = 0.01$).

Post-treatment ambient-light gs (AL gs), and low-light gs (LL gs), both tended to decrease with increasing O_3. The size of the gs response (Δgs = AL gs −LL gs), and ambient/low-light gs equilibration time (Δt_{gs}) also tended to decrease with increasing to O_3. None of these differences were statistically significant.

Water use efficiency at saturating light and ambient CO_2 (WUE) was calculated from the first point of each $A-C_i$ response curve at ambient CO_2 concentration (data in Table 2). The majority of plants in all treatment groups showed a decline in WUE from pre- to post-treatment period. In general, the size of the decline increased with increasing O_3 and fertilization (Table 2). The same-plant relative change (decline) in WUE was significantly greater in moderate- and high-O_3 plants than in ozone-free plants (pooled N levels, Steele's test, $p = 0.03$ and 0.048, respectively).

3.2. $A-C_i$ response

The majority of plants in all treatment groups exhibited a decline in carboxylation efficiency (CE) and P_{max} (net carbon uptake under saturating light and CO_2) from pre- to post-treatment, although the magnitude of the decline varied. There was a tendency towards greater decline in both CE and P_{max} with increasing ozone and with fertilization (Table 2). The same-plant relative decline in both CE and P_{max} was significantly greater in high-O_3 plants than in O_3-free plants (pooled N levels, Steele's test, $p = 0.01$ and 0.01, respectively). Also, the relative decline in P_{max} was significantly greater in fertilized plants than unfertilized plants (pooled O_3 levels, Steele's test, $p = 0.046$). Differences in relative decline in CE between fertilized and unfertilized plants were not statistically significant.

3.3. Foliage condition

Two types of bronzing were observed: uniform bronzing (UB), and marginal bronzing (MB). Total rate of occurrence of bronzing (sum of UB and MB rates) was dependent on the treatment group (Fisher's exact test; $p = 0.03$). MB was observed only in moderate-O_3 treatments, and its rate of occurrence was found to be dependent on O_3 level (Fisher's exact test; $p = 0.03$). There were trends towards greater numbers of wholly defoliated trees in treatments with higher O_3 levels, and greater rates of occurrence of new growth in fertilized treatments, but neither trend was statistically significant. Data for bronzing, defoliation, and new growth are summarized in Table 3.

Attached leaves of high-ozone plants had a significantly greater proportion of necrotic area than either moderate-O_3 or O_3-free plants (pooled N levels, Steele's test; $p = 0.002$ and 0.001 respectively). Although there was a slight trend towards greater necrosis in fertilized plants, it was not statistically significant.

At the end of the ozone exposure, 40 plants were suffering from foliar chlorosis. The majority of these (36 plants) exhibited a uniform leaf-color change. Marginal chlorosis was observed in two plants, veinal chlorosis in one, and interveinal

Table 2
Summary statistics from $A-C_i$ measurements

	0 ppb	75 ppb	150 ppb	C	F
Rel. ΔWUE (%)	−25 ± 13* A	−23 ± 14 A	−73 ± 6* B	−14 ± 14 X	−52 ± 8* X
Rel. ΔCE (%)	5 ± 21* A	−41 ± 13 AB	−83 ± 5* B	2 ± 22 X	−53 ± 12* X
Rel. P_{max} (%)	2 ± 18* A	−28 ± 16 AB	− 79 ± 9* B	6 ± 19 X	−55 ± 10* Y

Numbers are mean ± 1 SE. The first three columns report results by O_3 level, while the latter two columns report results by fertilization level (C is control/unfertilized, F is fertilized). Rows give same-plant pre- to post-treatment relative change in WUE, CE, and P_{max}. WUE is under saturating light and ambient CO_2. P_{max} is *A* under saturating light and CO_2. Relative change is calculated as a percentage: 100 × (post-treatment − pre-treatment)/pre-treatment. Entries within a row that do not share a letter were significantly different (Steele's test, $p < 0.05$). An asterisk (*) indicates the mean and SE values in that cell were calculated after removing either one or two large outlier that had a misleading effect on these numbers (statistical tests still used the full data set).

Table 3
End of treatment foliage condition

	0 ppb	75 ppb	150 ppb	C	F
UB/MB/total	2/0/20	3/3/19	4/0/20	5/2/29	4/1/30
Defoliated/total	0/20	1/20	3/22	2/32	2/30
New growth/total	4/20	8/21	7/22	8/32	11/31
Necrotic area (%)	9 ± 5 A	12 ± 4 A	44 ± 8 B	21 ± 6 X	23 ± 6 X
Level of chlorosis (%)	10 ± 4 A	11 ± 3 AB	16 ± 4 B	19 ± 3 X	8 ± 2 Y

The first three columns report results by O_3 level, while the latter two columns report results by fertilization level (C is control/unfertilized, F is fertilized). The first three rows give rates of bronzing, defoliation, and regrowth. Values are number of plants. UB, uniform bronzing across the lamina; MB, marginal bronzing. The latter two rows give necrotic area in attached foliage and level of foliar chlorosis (in percent). Values are mean ± 1 SE. Entries within a row that do not share a letter were significantly different (Steele's test, $p < 0.05$).

chlorosis in one. The level of foliar chlorosis increased with increasing levels of O_3, but the only significant difference was between high-O_3 and O_3-free plants (pooled N levels, Steele's test, $p = 0.03$). Fertilized plants showed significantly lower levels of foliar chlorosis than unfertilized plants (pooled O_3 levels, Steele's test, $p = 0.002$). Summary statistics for necrosis and chlorosis are given in Table 3.

3.4. Relative sizes of O_3-induced response

Non-parametric 95% confidence intervals were calculated for the size of O_3-induced responses as the difference between the median O_3-free and median high-O_3 measurements for each N level, and each parameter from the AL/LL and $A-C_i$ measurements. Pairs of confidence intervals were compared to examine whether the O_3-induced difference in a given parameter was greater in fertilized or unfertilized plants. In seven of the ten pairs of intervals derived from ambient/low measurements, the median size of the O_3-induced response was greater in unfertilized plants than in fertilized plants (Table 1). In all three pairs of intervals derived from $A-C_i$ responses, the median size of the O_3-induced response was greater in unfertilized plants than in fertilized plants (Table 2). However, as corresponding pairs of confidence intervals invariably overlapped, none of these differences were statistically significant.

4. Discussion

Exposure to realistic levels of atmospheric O_3 produced clear negative health effects in California black oak seedlings. Ozone reduced the ability of plants to function in a steady-state environment, as evidenced by significant declines in WUE, CE, P_{max}, and ambient A. Additionally, O_3 reduced the ability of plants to adapt to the changing light levels often found in natural environments. Following a change from high-light to low-light, A of moderate-O_3 plants was significantly slower to equilibrate than that of O_3-free plants. Also, following an ambient-light to low-light change, plants exposed to higher levels of O_3 showed significantly smaller changes in A and WUE than those exposed to lower levels of O_3. Thus, ozone-exposed seedlings showed both a slower and smaller response to changes in light than their O_3-free counterparts. Ozone exposure also produced significant visible injury, as levels of chlorosis and necrosis both significantly increased with increasing O_3 exposure. These effects are consistent with those observed in similar studies of other species (e.g. Karnosky et al., 2003). On a larger scale, such effects may eventually lead to changes in plant community structure, with O_3-sensitive species giving way to O_3-resistant ones (Miller et al., 1989).

N fertilization appears to have little short-term effect on California black oak seedlings, and those effects are mixed. Compared to unfertilized plants, fertilized plants generally had greater declines in pre- to post-treatment WUE, CE, and P_{max}; although only the difference in P_{max} was statistically significant. Other gas-exchange measurements showed no discernible effect of fertilization. However, fertilized plants showed a significantly reduced level of leaf chlorosis, as well as a non-statistically significant trend towards greater stimulation of growth of dormant buds (leaf and branch). The small negative N-induced effects on photosynthetic ability are in strong contrast with the majority of current literature, where positive effects of N fertilization on photosynthesis are almost universally observed. It may be that the duration of this experiment was too short for positive effects of N addition to become apparent, however this seems unlikely. Other studies have observed significant effects of N on photosynthesis after similar or shorter periods of time: hybrid poplar, <7 days (Cooke et al., 2005); hybrid poplar, <90 days (Ibrahim et al., 1998); *Populus tremuloides*, <80 days (Coleman et al., 1998); *Quercus robur*, <77 days (Welander and Ottoson 2000).

The non-statistically significant trend of greater pre- to post-treatment decline in WUE with fertilization that was observed in this experiment agrees with the significant declines in WUE observed in fertilized (N amended vs background N) mature California black oaks by Grulke et al. (2005). The observed decline in chlorosis with fertilization is different from the results of Grulke et al. (2005), who found that N addition significantly increased foliar chlorosis in a drought year, but had no significant effect on chlorosis in a year of near-average precipitation. As the Grulke et al. (2005) study found that the effect of N on chlorosis varied with water availability, it seems reasonable that the well-watered seedlings in this study should have behaved differently than the more-or-less drought stressed trees of their study.

The non-statistically significant trend towards greater stimulation of growth of dormant buds in fertilized plants is interesting in the context of the detailed study of Maurer and Matyssek (1997a,b). Gas exchange parameters of these new growth leaves were not measured in this study, and it is possible that the photosynthetic ability of new leaves could have offset the relatively poor performance of the older leaves, such that the overall performance of the fertilized plants matched or exceeded that of the corresponding unfertilized plants (e.g. Maurer and Matyssek, 1997b). Thus, nitrogen fertilization may not categorically help or hinder ozone response

in California black oaks, but rather *change* the response (Maurer and Matyssek, 1997b).

When the size of the O_3-induced response in fertilized plants was compared to the size of the O_3-induced response in unfertilized plants, most comparisons found a smaller response size in fertilized plants than in unfertilized plants. Although none of the differences were statistically significant, the observed trend supports the idea that N fertilization reduces plant sensitivity to O_3 exposure.

5. Conclusion

Short-term exposure to realistic levels of O_3 caused negative health effects in greenhouse-grown California black oak seedlings. Effects included lowered photosynthetic ability, increased foliar chlorosis and necrosis, and sluggish stomata. Simulated N-deposition at realistic levels produced small effects of mixed character. Statistically significant effects of N-amendment were lowered maximum net carbon uptake (P_{max}) and reduced chlorosis. Although the interactive effect of O_3 and N was unclear, there was a non-statistically significant trend towards reduced O_3 sensitivity in fertilized plants.

Many of the negative O_3-induced effects were apparent in both the moderate-O_3 and high-O_3 treatments. As these two treatments span most of the range of O_3 levels observed in the San Bernardino mountains, and it seems reasonable to expect that native California black oaks in this locale may be suffering many of these same symptoms. In the long-term, this may lead to reduced competitiveness, and to changes in the plant community structure (Miller et al., 1989).

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