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The Effects of Gaseous Ozone and Nitric Acid Deposition
on two Crustose Lichen Species From Joshua Tree National Park

A Thesis submitted in partial satisfaction
of the requirements for the degree of

Master of Science

in

Environmental Sciences

by

Elizabeth Curie Hessom

December 2012

Thesis Committee:
Dr. David Parker, Chairperson
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Dr. David Crowley

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The Thesis of Elizabeth Curie Hessom is approved:

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Abstract

Lichens are dependent on atmospheric deposition for much of their water and nutrients, and due to their sensitivity to pollutants, are commonly used as bioindicators for air quality. While studies have focused on epiphytic (tree dwelling) lichens as bioindicators, virtually nothing is known about crustose (rock dwelling) lichens. The atmospheric pollutants ozone (O_3) and nitric acid (HNO_3) are two major pollutants found within the Los Angeles Basin. While recent O_3 research suggests it does not significantly affect lichen growth, HNO_3 appears to be phytotoxic to some lichens. As both of these pollutants are deposited downwind from the L.A. basin into Joshua Tree National Park (JOTR), lichen species located in the park may provide a sensitive indicator of pollution effects. This research studied two lichen species of particular interest from Joshua Tree National Park, *Lobothallia praeradiosa* (Nyl.) Hafellner, and *Acarospora socialis* H. Magn., both of which are crustose species with unknown sensitivities to O_3, as well as hypothesized and unknown sensitivities to nitrogen compounds, respectively. Little research exists for either species, possibly because of the difficulty in working with crustose lichens. This research attempted to expand the background knowledge of these species by exposing them to varying levels of O_3 and HNO_3, to ascertain their physiological responses. Physiological measures of chlorophyll fluorescence, dark respiration, microscopic imaging, and lichen washes (as a proxy for membrane leakage), were measured throughout the exposure period. Results indicated that both species had similar sensitivities to O_3 and HNO_3. Both species registered physical damage during the O_3 fumigation, as well as a decrease in respiration. Neither species showed major

physical damage to HNO_3, but both manifested a decrease in chlorophyll fluorescence, suggesting damage to the photosynthetic systems of the algae symbiont. These results suggest that both of these species could be used as passive bioindicators for O_3, but may not be technically feasible for use as bioindicators of HNO_3 pollution. Overall, this research expanded the background knowledge of these two crustose species, their possible interactions between the fungal and algal components, their susceptibilities to two different pollutants, and their potential use as passive bioindicators for atmospheric pollution.

Introduction

I. Lichens

Lichens are composite organisms consisting of fungi (mycobiont) and algae (photobiont) components living symbiotically. They rely heavily on atmospheric deposition for many nutrients (Purvis, 2000). Because lichens have no root structures, water and nutrients are taken up through the thallus surface (Hauck, 2010). Due to their sensitivity to air pollution, they are often used as ecological bioindicators for air quality (Jovan, 2008; Riddell, 2009). Lichens are poikilohydric in nature, meaning that they are passive water regulators (Nash, 2008); when the lichens are dry, they metabolically shut down (a state of quiescence), during which time they accumulate pollutants on the lichen surface. This can lead to a concentrated dose of the pollutants when the lichen is re-wetted (Riddell, 2009; Nash, 2008). In arid climates, such as Joshua Tree National Park,

this is more likely to occur due to the long gaps between precipitation events (Nash, 2008).

Previous studies have identified the effects of various pollutants, including O_3 and HNO_3, on various species of lichens, including those with epiphytic, foliose, and fruticose morphologies (Riddell, 2009; Van Herk, 1999; Hauck, 2010; Calatayud, 2000). However, crustose species are seldom studied in comparison to the other growth forms, largely due to their small size, and the difficulty in working with an organism that must remain attached to its substrate, making it difficult to transport to laboratory settings. Little is known about atmospheric pollutant effects on crustose lichens.

II. Ozone (O_3)

Ozone is a strong oxidant, and is one of the major gaseous pollutants that make up the tropospheric photochemical smog found within the Los Angeles Basin (Fowler *et al*., 1999). Ozone is formed when combustion processes produce nitrogen dioxide (NO_2), which is then converted to nitrogen oxide (NO) and ground state oxygen radicals (O^3P). The O^3P then reacts with gaseous oxygen (O_2) to form O_3 (Fowler *et al*., 1999). Current O_3 levels in the atmosphere, specifically in the Northern hemisphere, have been increasing, and in the past 30 years the O_3 levels have increased an average of 40 ppb because of increased industrialization and urbanization (Wilkinson and Davies, 2010). As air pollution generated by automobile exhaust leads to continued increases in O_3 in many areas, dry deposition will increase as well (Seinfeld, 2006). Dry deposition of ozone makes up about 18% of the total global ozone sinks, and has a relatively short residence time of 25 days (Seinfeld, 2006). Joshua Tree National Park is shown to experience an

atmospheric concentration of O_3 between 40-70 ppb, having it be higher during the summer months (Allen *et al*., 2009) (Fig. 1). Previous studies indicate that O_3 within southern California, specifically Riverside, follows a diurnal trend (Stripe, 2010 unpublished).

Early studies demonstrating O_3 effects on epiphytic lichens so far generated inconsistent results, with some studies showing declines in lichen vitality, while others did not (Riddell *et al*., 2012). Further research helped demonstrate that in areas with both O_3 and N pollution, there are stronger correlations between lichen community changes and N pollution than there are in response to O_3 (Riddell *et al*., 2012). Earlier studies had difficulty isolating the distinct effects of HNO_3 and O_3 on lichens in areas where both pollutants were present, hence the conflicting early results regarding their effects. However, current studies illustrate that N pollutants seem to have a stronger negative effect on lichens than O_3. Indeed, a current study that fumigated 6 different epiphytic lichen species found that O_3 appeared to have no major negative impact on lichens (Riddell *et al*., 2012; Riddell, 2009). While a strong oxidant, it appears that N pollutants may have a stronger negative impact on lichen physiology than O_3.

III. Nitric Acid (HNO_3)

Nitric acid is a NO_x gas, and one way it can enter the atmosphere is through fossil fuel burning (tailpipe NO_x) (Schlesinger, 1997). Its atmospheric residence time is short, approximately 10 days, so areas downwind of high vehicle traffic areas usually experience deposition (Van Herk, 1999). Joshua Tree National Park is downwind of the Los Angeles Basin, and westerly winds often cause HNO_3 deposition to occur,

predominately on the western side of the park (Allen *et al*., 2006; Allen *et al*., 2009). The park experiences atmospheric concentrations between 0.9 – 9 μg/m^3 of HNO_3, with concentrations being higher during the summer months (Allen *et al*., 2009) (Fig. 2). Nitric acid has a high depositional velocity, and it is highly reactive with most substances (including water). Nitric acid has also been determined to be the largest reservoir of reactive N in the lower troposphere, making it of great interest for studies focusing on atmospheric pollution (Padgett *et al*., 2009). Initial trials conducted for this study showed that NO_x gases exhibit a diurnal trend within Riverside, CA.

Air-borne nitrogen is essential for lichen growth, and can even be a limiting nutrient if not enough is available (Hauck, 2010). However, excessively high levels of atmospheric N pollution can have overall negative effects, depending on the lichen species (Vilsholm *et al*., 2009; Van Herk, 2003; Riddell, 2009). Previous studies have shown HNO_3 to be phytotoxic to the epiphytic lichen *Ramalina menziesii*, even damaging photosystems at concentrations as low as 8 ppb (Riddell, 2009). Nitric acid was found to negatively affect the lichen species *Ramalina menziesii* by decreasing photosynthesis, respiration, chlorophyll content, and increasing potassium leakage, indicating cell damage (Riddell, 2009). Further studies have found correlations relating the decrease in diversity and distributions of epiphytic lichens in relation to increases in NO_x gases (Davies, 2007). Lichen community dynamics also appear to change, related to the presence of N, where more susceptible species become less present (Jovan, 2008; Riddell, 2008; Dahlman *et al*., 2002). However overall, the effect of HNO_3 on crustose

lichens is unstudied, and this research seeks to determine the susceptibility of two crustose species to HNO_3.

IV. Hypotheses and Objectives

To address the current lack of knowledge regarding crustose lichens, two common crustose species from Joshua Tree National Park were selected for studying the effects of O_3 and HNO_3. The two species chosen were *Lobothallia praeradiosa* (Nyl.) Hafellner, and *Acarospora socialis* H. Magn.. Hereafter referred to as their genus names, *Lobothallia* has a *Trebouxia* algae component, and it is predominately found on granite within JOTR (Nash, 2004). Its' sensitivity to O_3 is unknown, however *Lobothallia*'s algal component *Trebouxia* has been found to occur in a number of species that are tolerant to high nitrogen levels (Hauck, 2010). It is believed that *Trebouxia* has a carbon-concentrating mechanism that provides carbon skeletons for ammonia and nitrate assimilation. This enables the algal component to process, and be more tolerant to high nitrogen levels (Hauck, 2010). *Acarospora* on the other hand, has an unknown green algae component, and has unknown sensitivity to O_3 and N compounds (Nash, 2007). Multiple physiological measures will be conducted on both species during the fumigations to test sensitivity. Both species are unstudied, and were chosen in the hopes to expand the research background for them in regards to O_3 and N pollution sensitivity.

The lack of knowledge regarding these two crustose species, *Lobothallia* and *Acarospora*, makes it difficult to predict their responses to the fumigations. Based on previous research however, it is fair to postulate that both species may not be negatively affected by the low and high O_3 fumigation exposures. Based on *Lobothallia*'s possible

nitrophilous response to N pollutants as a result of its' algal component, it is postulated that *Lobothallia* will exhibit a positive response to the HNO_3 fumigation, when compared to *Acaropora*'s response. However, since HNO_3 has been shown to be phytotoxic, it is postulated that at the higher fumigant exposure treatment, both species will exhibit negative physiological responses to HNO_3. This study will expand the current physiological knowledge of these two crustose lichens. At the conclusion of this study we will be able to determine the sensitivities of these two species to O_3 and HNO_3, and recommend whether these lichens should be used as bioindicators.

Materials and Methods

I. Lichen Samples and Field Collection

Lobothallia and *Acarospora* rock samples were collected from Joshua Tree National Park on horizontal rock surfaces using a hammer and chisel to ensure the lichen were still attached to their rock substrate. Samples were transported to the Riverside greenhouse in paper bags on the same day. Samples were collected from two sites within the park, Pine City and Stirrup Tank (Fig. 3). Both sites in the park had moderate to low amounts of deposition occurring during the time of collection to help ensure the lichen samples were as pristine as possible before the fumigation treatments (Fig.1, 2, 3). Samples used in the HNO_3 fumigation were collected in October 2011, and samples used in the O_3 fumigation were collected in January 2012. Both sets of samples were allowed to equilibrate to Riverside greenhouse conditions for at least 3 months before being used in the fumigation experiments. To relate fumigant levels to actual field conditions,

ambient N deposition filter samplers were placed out at each collection site to determine current HNO_3 atmospheric concentrations. The samplers were the same design used by the USDA Forest Service, and consisted of an 8 foot tall t-pole, with holders for 6 HNO_3 (Nylasorb) filters. These samplers were placed out for 2 weeks during the October 2011 collection and the January 2012 collection. After the two weeks, the filters were extracted and placed in 20 mL of ultra-pure water in a 250 mL flask. The filters were covered and shaken for 15 minutes. The samples were then run on a Dionex ICS 2000 (Ion Chromatography system, Dionex, Thermo Scientific, CA, USA) using an AS9HC 2mm column and guard column, detecting HNO_3 (in $\mu g/m^3$). Baseline ambient O_3 field levels were determined by consulting previous data collected in the area (Allen, 2009).

II. Experimental Design

Sixteen lichen samples were placed in each experimental chamber, eight from each species, and from those eight, four from each collection site. The samples were arranged randomly on the bottom of the fumigant chambers. There were ten fumigation chambers in the Riverside greenhouse, organized onto two benches with five chambers on each bench. The chambers on the first bench were used for the O_3 fumigation, while the chambers on the second bench were used for the HNO_3 fumigation (Fig. 4). For each fumigation, one chamber was assigned as a control with no pollutants present within it, two chambers were designated with different pollutant levels (high and low exposures), one chamber was designated as a monitor chamber, and one chamber was left to monitor ambient HNO_3 and O_3 levels in the greenhouse. In the control and the monitor chambers, hobos (Pyle, Brooklyn, NY, USA) were set-up to digitally collect temperature and

humidity every 3 hours. Nylon and Ogawa filter packs were placed in the control and fumigant chambers to measure HNO_3 and O_3 deposition respectively.

After the lichen samples were equilibrated, they were placed within the fumigant chambers. These chambers (as described in Padgett *et al*., 2004; Riddell, 2008) were called continuously stirred tank reactors (CSTRs, Fig. 5). The CSTRs were housed in a climate-controlled greenhouse on the University of California Riverside campus in Riverside, CA. The chambers were 1.35 m (diameter) by 1.35 m (height), clear Teflon cylinders, that had air exchanged through them every 1.5 minutes. Teflon tubing was also used in the pollutant delivery system to the chambers bulk air input. The bulk air input air flow was maintained by a blower that pushed the filtered air into the chambers, and the air exchanges were maintained by an exhaust pump which removed the air and kept the chambers under a slight negative pressure. Once the desired pollutant had entered the chamber, it was circulated throughout the chamber via continuous speed impellers (Dayton, Model 22811A) to ensure even distribution upon the lichen samples on the bottom of the chamber. To mimic light conditions present in Joshua Tree National Park, no shade cloths were used over the CSTRs, and PAR (photosynthetically active radiation) measurements were conducted using a Li-Cor light meter (LI-185B Model, Quantum/Radiometer/Photometer, Lincoln, NE, USA) to ensure that the lichens were being exposed to high levels of PAR. Inside the CSTR chambers, the lichens were exposed to levels up to 1200 $\mu mol/s/m^2$ in October 2011, which is comparable to field conditions (1100 $\mu mol/s/m^2$, October 2011).

The O_3 gas was generated through passing O_2 compressed air through an ozone generator (Superior Electric Co., Bristol, CT, USA). The delivery of the O_3 gas to the CSTRs was controlled through the use of flow meters (Atheson Gas Products Model 602), and was specifically delivered via Teflon tubing to the bulk air flow input of the desired O_3 chambers. Ozone was delivered to the chambers between the hours of 09:00 and 17:00 everyday for the 90 day fumigation to mimic southern California ambient diurnal O_3 patterns. For the high O_3 exposure, a desired level of 120 ppb was delivered into chamber 2 (Fig. 4), where the low O_3 exposure level of 60 ppb was delivered into chamber 3 (Fig. 4). The 90 day O_3 fumigation ran from June 8th, 2012 to September 10th, 2012.

The HNO_3 gas was generated by diluting HNO_3 at a 1:50 ratio with distilled water, and then vaporizing it. The liquid HNO_3 was passed via a pump (Fluid Metering Inc., Oyster Bay NY, USA) into a volatilization chamber, where the liquid HNO_3 entered dropwise into a glass bead filled glass cylinder. The cylinder was submerged within an antifreeze bath heated to ~90° C, which caused the liquid drops of HNO_3 to volatize. The volatized HNO_3 was then transported from the glass chamber via heatless dry air flow into output tubing, and was then transported to flow meters at the input of the bulk air flow for the desired CSTR chambers. The flow meters acted as a method to control the HNO_3 delivery into the bulk air flow input to the CSTRs. Nitric acid was delivered to the chambers between the hours of 09:00 and 17:00 everyday for the 90 day fumigation to mimic southern California ambient diurnal HNO_3 patterns. For the high HNO_3 exposure, a desired level of 20-40 ppb was delivered into chamber 8 (Fig. 4), where the low HNO_3

exposure level of 10-20 ppb was delivered into chamber 9 (Fig. 4). The 90 day HNO_3 fumigation ran from January 27th, 2012 to May 2nd, 2012.

The O_3 and HNO_3 chamber levels were monitored continuously for both 90 day fumigations through a modified Scanivalve system (Scanivalve Corp., San Diego CA, USA), that sampled every chamber for six minutes out of every hour. The O_3 concentration levels were sampled directly from the CSTR chambers and then transmitted to a Dasibi Ozone Monitor (Dasibi Environmental Corp., Model 1003-AH, Glendale, CA, USA). This measured information was then collected by a Campell CR21X datalogger (Campbell Scientific, Inc. Logan Utah, USA), and then stored, making it available for manipulation on an additionally connected computer and monitor. The HNO_3 levels were monitored slightly differently and required air samples from the CSTRs to be converted first into NO by a molybdenum converter ('Molycon' Monitor Labs Inc., Englewood, CO, USA) before they were measured. After this occurred, the NO gas was measured by a nitrogen oxide monitor model 8840 (Monitor Labs, Inc., Englewood, CO, USA), and was similarly recorded like the O_3 data. Nitrous oxide levels were measured due to the fact that all of the NO in the chamber samples was assumed to have had come from the HNO_3 present.

III. Preliminary Studies

The lack of knowledge regarding these two species and crustose lichen in general, made preliminary studies necessary. For the dark respiration and fluorescence protocols, water content of the lichen needed to be determined. To determine water content, 54 individual thalli samples for each species were removed from their rock substrate, in

order to exclude how much water the rock substrate absorbs. These thalli were dried for 24 hours in a 65 °C oven. Dry weights were obtained, and then, wet weights were obtained 0, 10, 15, 30, 60, and 1440 minutes after soaking in deionized water. Water content was determined by the following formula: ((wet weight – dry weight)/wet weight)*100. This data enabled us to determine the water content of the lichen after 30 minutes of wetting.

Environmental conditions of Riverside also were determined. To define what levels of NO_x gases occur within Riverside, CA, NO_x and ammonia (NH_3) levels were measured over 12 days during the summer of 2010 using an EC9842 NH_3 Analyser (Ecotech, Australia), and HTO-1000 TRS/NH_3 Converter (Ecotech, Australia). Averages were taken for each hour over 12 days. To characterize the light conditions, PAR (photosynthetically active radiation) measurements were taken using a Li-Cor light meter (LI-185B Model, Quantum/Radiometer/Photometer, Lincoln, NE, USA) during March 2012-September 2012. Measures were taken within the CSTRs as well as outside of the greenhouse.

To characterize these species further, isotopic analysis was done looking at the $\delta^{18}O$ and $\delta^{15}N$ isotopic ratios (in per mil) from the nitrate (NO_3^-) present. Two samples were selected from each species, one with a large surface area (in cm^2), and one with a small surface area. Three deionized water washes were performed on each sample, and then analyzed using an isotope ratio mass spectrometer, Delta-V advantage system, provided by the Facility for Isotope Ratio Mass Spectrometry (FIRMS) at UC Riverside. Results from this analysis are presented within the appendix (Fig. 1 & 2 – Appendix).

IV. Physiological Measures – Dark Respiration

Dark respiration measurements were taken from the lichen samples on day 0, 30, 60, and 90 of the O_3 and HNO_3 fumigations, and were measured without removing the lichen samples from their rock substrata. Prior to measuring, lichen samples were soaked in deionized water for 30 minutes to rehydrate them to a water content of approximately 50-55%. After wetting, samples were patted dry with a Kimwipe and placed in dark mason jars. Following an adapted soil respiration protocol (Chatterjee, 2011), the dark mason jars were connected individually to a Licor-7000 infrared gas analyzer (LI-COR Environmental, NE, USA) to measure the total released CO_2 during dark respiration from the lichen sample. Measurements were taken 0, 60, and 180 minutes after re-wetting to observe high peaks in respiration at 0 minutes, and to obtain stabilized CO_2 dark respiration measures at 180 minutes (Sundberg, 1999). Lichen temperatures were also recorded using an IR-gun (Raytek, Santa Cruz, CA, USA) at each time interval. Temperature and CO_2 measures were taken in replicates of three for each lichen sample. The CO_2 release data was analyzed in Matlab (The MathWorks Inc., Natick, MA, USA) under a "Simpleflux" program designed by Dr. Darrel Jenerette to calculate the total flux of CO_2 from the lichen sample for each time interval. Lichen surface area (in cm^2), and mason jar volume were taken into account when calculating the fluxes.

V. Physiological Measures – Fluorescence

Chlorophyll fluorescence measurements were taken from the lichen samples on day 0, 30, 60, and 90 of the O_3 and HNO_3 fumigations. Chlorophyll fluorescence uses saturating light pulses to measure the integrity of photosystem II (PSII) and the electron

chain transport system (Riddell, 2009). This was measured using the PAM 2000 fluorometer (MINI-PAM, Walz, Effeltrich, Germany), which measures the amount of fluorescent light emitted by the sample during the episodes of minimal light (F_o) and saturating light pulses (F_m). Based on similar protocols explained in Riddell (2009) and Jensen (2002), lichen samples were soaked in deionized water for 30 minutes to rehydrate them to a water content of approximately 50-55%, while in a dark adaption chamber of approximately 22°C. After the soaking/dark adaption, the lichen samples were patted dry, and measured to determine the photosynthetic yield (Fv/Fm). Lichen temperatures were also recorded using an IR-gun (Raytek, Santa Cruz, CA, USA). Temperature and fluorescence measures were taken in replicates of three for each lichen sample.

VI. Physiological Measures – Lichen Washes

Lichen washes were conducted on day 0 and 90 of the O_3 and HNO_3 fumigations. Lichen washes were conducted on day 0 in order to “clean” the lichen samples before the fumigation treatments, and also to account for what deposition occurred within the field on the samples. Washes were also conducted on day 90 to determine what deposition had occurred, and also to conclude if the lichen thallus experienced damage from the fumigations. Since damaged cell membranes are unable to regulate ion loss, washes were analyzed for cations, specifically looking at potassium, magnesium, and sodium. All of the day 0 and 90 washes were analyzed for cation presence, and for nitrate ions (NO_3^-). Lichen washes were conducted by taking the lichen samples, placing them lichen side down in beakers, and filling the beakers with measured amounts of deionized water in order to ensure that the lichen thallus was completely submerged and washed. The

beakers were then placed on an orbital and allowed to lightly orbit at 80 RPM (rotations per minute) for 30 minutes. Beakers were covered with parafilm to ensure no wash sample came out. The washes were analyzed by a continuous flow analyzer (Alpkem, O.I. Corporation, TX, USA) for NO_3^- and by a Dionex DX-600 ion chromatograph (Dionex, Thermo Scientific, CA, USA) for cations. In addition to the 48 samples that were washed for each fumigation, 12 additional lichen samples were added to the O_3 fumigation (4 samples in control, high and low exposures) that were considered "depositional samples." These were not sampled during the regular intervals, but instead were left in the chambers undisturbed for all 90 days. These "depositional samples" were used to evaluate the long term deposition without disruption. Once the lichen wash data was obtained, it was multiplied by a ratio taking into account the amount of DI water used for the wash, divided over the lichen surface area (final units mg/cm^2).

VII. Physiological Measures – Microscopy Imaging

Lichen microscopic imaging was conducted on day 0 and 90 of the O_3 and HNO_3 fumigations to view external physical responses to the pollutants. The lichens were examined under a dissecting microscope (Nikon SMZ800, Japan), lighted with a Dyna lite 150 Fiber Optic Power Supply (A. G. Heinze precision micro optics, Irvine, CA, USA) and photographed using a PAXcam digital microscope camera (Midwest Information Systems, Inc., Villa Park, IL, USA). Five positions on each lichen sample were randomly selected for imaging on day 0, and images were taken at 1x, 2x, 4x, and 6.3x magnification, totaling in 20 images taken per lichen sample. The same sites on the lichen were found on day 90 and imaged at the same magnifications for comparison. In

addition to the 48 samples that were imaged for each fumigation, 12 additional lichen samples were added to the O_3 fumigation (4 samples in control, high and low exposures) that were considered "depositional samples" and were not sampled during the regular intervals, but instead were left in the chambers undisturbed for all 90 days. These "depositional samples" were used to evaluate the long term deposition without disruption, and were imaged in the same fashion.

VIII. Chamber Filter Analysis

Passive samplers were placed in the control, high and low exposure chambers for both the O_3 and HNO_3 fumigations. Four nylon filters were placed in the four major compass positions within the chambers 8, 9, and 10 (Fig. 4) for the HNO_3 fumigation, and 1 Ogawa filter pack was placed within the chambers 2, 3, and 5 (Fig. 4) for the O_3 fumigation. Filters were left in the chambers undisturbed for all 90 days. At the conclusion of the fumigations, the nylon filters were washed with 20 mL of ultra-pure water in a 250 mL flask and shaken for 15 minutes. The washes were then analyzed for NO_3^- by using a continuous flow analyzer (Alpkem, O.I. Corporation, TX, USA). The Ogawa O_3 filters were processed differently. For O_3, the filter was placed in a 8 mL bottle and 5 mL of ultra-pure water was added. This was then shaken for 15 minutes and then run using the Dionex ICS 2000 (Ion Chromatography system, Dionex, Thermo Scientific, CA, USA) using an AS9HC 2mm column and guard column. This was analyzed for NO_3^-, which is quantitatively formed from NO_2^- and O_3 (how O_3 was determined). Lab blank filters were also taken for comparison.

IX. Statistical Treatment

The dark respiration and chlorophyll fluorescence data for both fumigations was analyzed through 3-way ANOVAs. The ANOVAs were run in Mystat, a student version of Systat (Systat Software, Cranes Software International Ltd., Chicago, IL, USA), and were analyzed using sums of squares Type III-Adjusted. For further analysis, repeated measures ANOVAs were run in SPSS (IBM SPSS Statistics, IBM Corporation, Armonk, NY, USA). The repeated measures ANOVAs were tested for the assumption of sphericity and the degrees of freedom were adjusted if the assumption was not met. Data points that fell outside of the upper and lower outlier boundaries were excluded before the ANOVAs were run. The upper boundary was determined as (UB = median + 1.5* 4th spread), whereas the lower boundary was determined as (LB = median – 1.5* 4th spread). For the lichen wash data and the chamber filter analysis data, student t-tests were run within Microsoft Office Excel (2010 version).

Results

I. Preliminary Studies

Water content did not change significantly between 30, 60, and 1440 minutes after soaking in deionized water (Fig. 6). *Acarospora* exhibits a water content of approximately 50%, whereas *Lobothallia* exhibits a water content of approximately 55% during 30, 60, and 1440 minutes after soaking (Fig. 6). Ambient NO_x levels in Riverside exhibited a diurnal trend, with NO_x levels climbing in the morning, and peaking between 07:00 and 09:00 am at approximately 80 ppb (Fig. 7). PAR levels from March 2012-

September 2012 taken inside the CSTRs ranged from 500 to 1500 µmol photons/m^2/second, whereas PAR outside of the greenhouse ranged from 1000 to 2000 µmol photons/m^2/second (Table 1).

II. Ozone (O_3) Fumigation Results

II-I. Dark Respiration

The *Acarospora* dark respiration 3-way ANOVA showed a significant difference in the fumigant treatments ($p < 0.005$), and the interactions between treatment x site ($p < 0.005$). There were no significant differences present in the remaining variable relations (Fig. 8A). The repeated measures ANOVA supported some of those finding, indicating that there was a significant difference between the sampling days ($p < 0.005$), the fumigant treatments ($p < 0.05$), the sites ($p < 0.05$), with approaching significance between the days x treatment x site interaction ($p = 0.070$). A repeated measure ANOVA between day 0 and day 90 found the sampling days ($p < 0.005$) and fumigant treatments ($p < 0.05$) to be significantly different.

The *Lobothallia* dark respiration 3-way ANOVA showed a significance difference in the fumigant treatments ($p<0.005$), and the interactions between treatment x site ($p < 0.05$). There was approaching significance for the sampling days ($p = 0.091$), and for the sites ($p = 0.097$), however, there were no significant differences present in the remaining variable relations (Fig. 8B). The repeated measure ANOVA supported some of those findings, indicating that there was a significant difference between the sampling days ($p < 0.005$), the fumigant treatments ($p < 0.05$), the sites ($p < 0.05$), and the interaction

between days x treatments x sites ($p < 0.05$). A repeated measure ANOVA just between day 0 and day 90 found only sampling days to be significantly different ($p < 0.005$).

II-II. Chlorophyll fluorescence

Acarospora's 3-way ANOVA on the chlorophyll fluorescence data indicated there was a significant difference between the fumigant treatments ($p < 0.005$), sampling days ($p < 0.005$), sites ($p < 0.05$), treatment x site interaction ($p < 0.005$), day x site interaction ($p < 0.05$), and day x treatment site interaction ($p < 0.005$). There was also approaching significance for the treatment x day interaction ($p = 0.097$) (Fig. 9A). The repeated measures ANOVA however specified that only the sampling days were significant ($p < 0.005$). A repeated measure ANOVA between day 0 and day 90 showed the sampling days ($p < 0.05$), fumigant treatment ($p < 0.005$), sites ($p < 0.005$), and day x treatment x site analysis ($p < 0.005$) were all significant.

Lobothallia's 3-way ANOVA on the chlorophyll fluorescence data indicated there was a significant difference between the fumigant treatments ($p < 0.05$), the sampling days ($p < 0.005$), and the sites ($p < 0.005$). There was also approaching significance in the treatment x site interaction ($p = 0.079$) (Fig. 9B). The repeated measures ANOVA however specified that only the sampling days were significant ($p < 0.005$). A repeated measure ANOVA between day 0 and day 90 found sampling days ($p < 0.05$) to be significantly different as well.

II-III. Lichen Washes

Data for the O_3 fumigation showed that there were significant differences between the washes done on day 0, and day 90 for K ($p < 0.005$) and Na ($p < 0.05$) (Fig. 10A). The

“depositional” lichen wash data showed that there were significant differences between the washes done on day 0, and day 90 for NO_3^- ($p< 0.005$), K ($p< 0.005$), Mg ($p< 0.005$), and Na ($p< 0.05$) (Fig. 10B).

II-IV. Microscopy Imaging

There appeared to be differences in the thallus color of both species for all of the fumigant treatments between day 0 and day 90. From the control fumigation, 7 out of the 8 *Acarospora* samples exhibited a slight to extreme thallus color change (Fig. Fig. 11 A1 & A2), with the majority of the samples coming from the Stirrup Tank site. The *Lobothallia* control samples appeared to be not as affected with 4 out of the 8 exhibiting color changes, split evenly between the two sites. An opposite trend was viewed when examining the samples from the low fumigation. *Lobothallia* seemed to be more affected, with 5 out of the 8 samples experiencing discoloration, the majority of them coming from the Pine City site. *Acarospora* on the other hand only had 3 out of the 8 affected with discoloration, with the majority of them coming from the Pine City site as well. A similar trend was viewed when looking at the samples from the high fumigation. *Lobothallia* seemed to be more affected, with 5 out of the 8 samples experiencing discoloration, with the majority of them coming from the Pine City site. *Acarospora* on the other hand only had 3 out of the 8 affected with discoloration, with the majority of them coming from the Stirrup Tank site. From the depositional lichen samples, *Acarospora* exhibited no thallus color change in any of the samples, whereas *Lobothallia* had 3 samples out of the 6 that exhibited a slight discoloration.

II-V. Chamber Filter Analysis/Recordings of Deposition-Chamber Conditions

Ambient O_3 concentrations were determined after accounting for ambient filter deposition, and converting the NO_3^- concentration to account for O_3. Ogawa filter pack analysis showed the high exposure (chamber 2) yielded an O_3 concentration of 266 ppb/hour, the low exposure (chamber 3) yielded an O_3 concentration 158 ppb/hour, and the control (chamber 5) yielded an O_3 concentration of 57 ppb/hour (Fig. 12). These levels were higher than the 60 ppb/hour (low) and 120 ppb/hour (high) we were aiming for. There were significant differences present between all 3 chambers ($p < 0.005$) (Fig. 12).

Hobo data collected from the control treatment (chamber 5), and the monitor chamber (chamber 4) indicated that temperatures in the chambers reached a maximum of 60.9 °C (141.6 °F), and dropped to a minimum of 16.3 °C (61.3°F) over the entire O_3 fumigation (Table 2). Humidity also peaked at 72.2%, and was lowest at 1.7% (Table 2). Chambers showed no significant differences in environmental conditions. Ozone levels were monitored through the modified Scanivalve system and measured by an ozone monitor. Sums were calculated for each day for each chamber (control, low, and high), after taking into account the ambient O_3 gases present within the greenhouse (Fig. 13A). The highest daily sum for the treatments for the entire fumigation was 1081 ppb, 577 ppb, and 29 ppb (for high, low and control treatments respectively), where the lowest daily sums were 461 ppb, 105 ppb, and 0 ppb (respectively). Sums for O_3 gases up to each sampling day were also totaled (Table 3). Hourly ppb exposures were also determined with the highest ppb/hour exposure for each chamber being 45 ppb/hour, 24

ppb/hour, and 1.2 ppb/hour (for high, low and control treatments respectively), where the lowest daily sums were 19 ppb/hour, 4 ppb/hour, and 0 ppb/hour (respectively).

III. Nitric Acid (HNO_3) Fumigation Results

III-I. Dark Respiration

The *Acarospora* dark respiration 3-way ANOVA showed a significant difference between the fumigant treatments ($p < 0.05$), and the sites ($p < 0.005$) (Fig. 14A). There were also approaching significance for the sampling days ($p = 0.062$), and the interaction between the fumigant treatments x sites ($p = 0.082$). The repeated measures ANOVA helped support these findings by indicating there was a significant difference between the fumigant treatments ($p \leq 0.05$), the sites ($p \leq 0.05$), and the sampling days ($p < 0.005$). A repeated measure ANOVA between day 0 and day 90 found sampling days ($p < 0.005$) and fumigant treatments ($p < 0.05$) to be significantly different.

The *Lobothallia* dark respiration 3-way ANOVA showed a significant difference between the fumigant treatments ($p<0.05$), but none was found between the sampling days, site, or interactions between the 3 variables (Fig. 14B). The repeated measures ANOVA conversely indicated that there was a significant difference between the fumigant treatments ($p < 0.005$), and the sampling days ($p < 0.005$). A repeated measure ANOVA between day 0 and day 90 found sampling days ($p < 0.005$) and fumigant treatments ($p = 0.05$) to be significantly different.

III-II. Chlorophyll fluorescence

Acarospora's 3-way ANOVA on the chlorophyll fluorescence data indicated that there was a significant difference between the fumigant treatments ($p < 0.05$), and the

sampling days ($p < 0.005$) (Fig. 15A). The repeated measures ANOVA specified however that there was only a significant difference between the sampling days ($p < 0.005$). A repeated measure ANOVA between day 0 and day 90 agreed with this, finding only the sampling days to be significant ($p < 0.005$).

Lobothallia's 3-way ANOVA on the chlorophyll fluorescence data indicated that there was a significant difference between the sampling days ($p < 0.005$) (Fig. 15B). The repeated measures ANOVA specified also that there was only a significant difference between the sampling days ($p < 0.005$). A repeated measure ANOVA between day 0 and day 90 found sampling days to be significant ($p < 0.005$) as well.

III-III. Lichen Washes

Data for the HNO_3 fumigation indicated that there were significant differences between the washes done on day 0, and day 90 for NO_3^- ($p < 0.005$), K ($p < 0.005$), Mg ($p < 0.005$), and Na ($p < 0.005$) (Fig. 16).

III-IV. Microscopy Imaging

From all of the treatments, *Lobothallia* lichen samples showed no difference between imaging on day 0 to day 90. *Acarospora* samples from Stirrup Tank additionally showed no significant difference; however *Acarospora* samples from Pine City did exhibit discoloration on the thallus changing from green on day 0 to brown on day 90 (Fig. 11 B1 & B2). This discoloration was present on the Pine City *Acarospora* samples from all three treatments.

III-V. Chamber Filter Analysis/Recordings of Deposition-Chamber Conditions/Field Data

Nitrate deposition from the nylon filters in the control, low, and high treatment chambers were all significantly different from one another ($p \leq 0.05$) (Fig. 17). The control chamber had the least amount of deposition (0.8 ppb/hour), and the high chamber had the highest amount (14.7 ppb/hour), and the low chamber had 12.6 ppb/hour, after accounting for ambient filter deposition in the greenhouse (Fig. 17). These levels were close to the targets of 10-20 ppb/hour for the low treatment, and 20-40 ppb/hour for the high treatment, with the actual high exposure being a little lower than desired.

Passive sampler field data showed that there were no significant differences in the air concentration data between the October 2011, and January 2012 collections, and there were no significant differences between the repeated January 2012 collections (Fig. 18). Sample size was unequal over the collections due to collection error, therefore n = 1, 2, or 3, as specified on the figure (Fig. 18). Higher atmospheric concentrations appeared to be present on the filters collected in January, versus the filters collected in October (Fig. 18).

Hobo data collected from the control treatment (chamber 10), and the monitor chamber (chamber 5) indicated that temperatures in the chambers reached a maximum of 53 °C (127 °F), and dropped to a minimum of 9.9 °C (49°F) over the entire HNO_3 fumigation (Table 2). Humidity also peaked at 69%, and was lowest at 0.7% (Table 2). Chambers showed no significant differences in environmental conditions. Nitric acid levels were monitored through the modified Scanivalve system and measured by a nitrogen oxide monitor, measuring the total NO_x gases in each chamber. Sums were

calculated for each day for each chamber (control, low, and high), after taking into account the ambient NO_x gases present within the greenhouse (Fig. 13B). The highest daily sum for the treatments for the entire fumigation was 634 ppb, 266 ppb, and 167 ppb (for high, low and control treatments respectively), where the lowest daily sums were 35 ppb, 55 ppb, and 0.5 ppb (respectively). Sums for the NO_x gases up to each sampling day were also totaled (Table 3). Hourly ppb exposures were also determined with the highest ppb/hour exposure for each chamber being 26 ppb/hour, 11 ppb/hour, and 6.9 ppb/hour (for high, low and control treatments respectively), where the lowest daily sums were 1.4 ppb/hour, 2.3 ppb/hour, and 0 ppb/hour (respectively).

Discussion

I. Ozone (O_3) Results

The O_3 fumigation data from the repeated measures ANOVA analysis suggests that the fungal component, or the dark respiration measure, was more susceptible to the O_3 exposures than the chlorophyll fluorescence measure, or the algal component. For both species, the dark respiration measure exhibited more significant differences between the experimental factors than the chlorophyll fluorescence measures. The data also suggests that the species have similar susceptibilities to O_3. It was hypothesized that both species would react similarly and not be negatively affected by O_3 based on recent studies (Riddell *et al*., 2012). Instead the data indicated that both species were negatively affected, particularly visible within the imaging results. It was confirmed however that both species had similar reactions, showing almost completely identical ANOVA

analyses results over the entire fumigation. Imaging data confirmed that both species responded negatively to the fumigation, however, there seemed to be more thallus discoloration present on the *Lobothallia* samples than the *Acarospora*. Lichen wash data indicated a significant increase in cations from day 0 to day 90, suggesting thallus damage had also occurred.

A snapshot repeated measures ANOVA analysis was also done only viewing day 0 to day 90. The analysis suggested that both species reacted similarly, showing similar trends in significance for the dark respiration and chlorophyll fluorescence measures. This additional analysis helped confirm the species similar reactions to the O_3 fumigation. The trends visible from the snapshot analysis are also something to note. The dark respiration ANOVA analysis only viewing day 0 to day 90 showed decreasing trends of CO_2 release between the start and the end of the fumigation for both species. This decrease in CO_2 respiration can possibly indicate that more carbon is being taken up via photosynthesis, and that more carbon is being allocated towards lichen growth (Palmqvist, 2000). If this is the case, we would predict that the chlorophyll fluorescence data would exhibit an increasing or unchanging trend, indicating positive PSII health, and therefore positive or nominal photosynthesis occurring. This trend was seen in the *Lobothallia* data and for the majority of the *Acarospora* data; however the control level within the *Acarospora* data showed a downward trend. This suggests that the *Acarospora* control samples were being negatively affected. Reviewing the remainder of the data, there were additional trends suggesting that the controls were also being affected.

Reviewing the O_3 results for both species, it is unclear whether either of these species are appropriate bioindicators for O_3. Both species did exhibit similar downward trends in respiration, showed similar thallus damage occurring, and had an increase in cation presence, indicating cell damage. However, due to the fact that the controls also seemed to be effected throughout the fumigation, makes one question the application of these measures towards O_3 monitoring. It is possible that there was some unintentional bias when the lichen samples were chosen from the field, and the fact that there was no chamber replication for any of the O_3 fumigant levels or the control, makes it difficult to verify the results of this study. Additionally, there were 2 days within the fumigation that had unusually high O_3 levels which may have "shocked" the lichen and increased the possible error within the measures. There was however definite discoloration occurring on the lichen thallus, so there is potential for these species to be used as passive bioindicators for O_3 pollution; however I would recommend a repeated study be done to confirm these findings. If a repeated fumigation were done, I would recommend increasing the chamber replication, not allowing the lichen to equilibrate in the greenhouse for quite as long, and taking measurements from the lichens in the field before the fumigation, to see how they respond under their natural conditions.

II. Nitric Acid (HNO_3) Results

The HNO_3 fumigation data from the repeated measures ANOVA analysis exhibits a similar trend to the O_3, suggesting that the fungal component, or the dark respiration measure, was more susceptible to the HNO_3 exposures than the chlorophyll fluorescence measure, or the algal component. For both species, the dark respiration measure

displayed more significant differences between the experimental factors than the chlorophyll fluorescence measures. The data also helped suggest that the species have similar susceptibilities to HNO_3. It was hypothesized that *Lobothallia* would respond more positively to the HNO_3 exposures than *Acarospora* because of its hypothesized nitrophilic nature. However, the data indicated that both species had similar reactions, showing almost completely identical ANOVA analyses results over the entire fumigation. Imaging data, however, did show some slight discoloration for *Acarospora*, while *Lobothallia* exhibited none, suggesting that *Acarospora* might be slightly more susceptible to HNO_3. Lichen wash data indicated a significant increase in cations from day 0 to day 90, suggesting thallus damage had also occurred.

When a snapshot repeated measures ANOVA analysis was done only viewing day 0 to day 90, both species had similar trends in significance. This further supports the suggestion that both species have similar susceptibilities to HNO_3. The trends visible from the snapshot analysis are also something to note. The dark respiration ANOVA analysis only viewing day 0 to day 90 showed increasing trends of CO_2 release between the start and the end of the fumigation for both species. This increase in CO_2 respiration can indicate that less carbon is being taken up via photosynthesis, and that less carbon is being allocated towards lichen growth (Palmqvist, 2000). If this is the case, we would predict that the chlorophyll fluorescence data would exhibit a downward trend, indicating decreasing PSII health, and therefore less photosynthesis occurring. In fact, a downward trend is exhibited when the chlorophyll fluorescence was analyzed only for day 0 and day 90. This data suggests that the HNO_3 fumigations caused a decrease in the algal

component's PSII health, which in turn lead to an increase in CO_2 dark respiration. Reviewing the remainder of the data however, it appeared that the control samples were reacting in similar ways to the low and high HNO_3 fumigant samples, which can call into question some of the results of this study.

Based on the results, it can be concluded that neither species should be used as a passive bioindicator for HNO_3 atmospheric pollution. Both species exhibited negative changes to the fumigation exposures, however to be used as passive bioindicators, the change would need to register physically on the lichen thallus. *Lobothallia* did not have any significant changes to the lichen thallus, however *Acarospora* did have some thallus discoloration. Though, the *Acarospora* samples that displayed the thallus color change were all from one sampling site (Pine City), the results suggest that the thallus color change may be a result of the site where the sample was collected at. So, although both species did demonstrate negative responses to the HNO_3 fumigation at the chlorophyll fluorescence measurement level, *Lobothallia*'s lack of visual thallus response, and *Acrospora*'s inconsistent visual thallus response are the deciding factors. In addition, because the controls also seemed to be affected throughout the fumigation can question the application of these measures towards HNO_3 monitoring. It is possible that there was some unintentional bias when the lichen samples were chosen from the field, and the fact that there was no chamber replication for any of the HNO_3 fumigant levels or the control, makes it difficult to verify the results of this study. To confirm these results, I would suggest another round of experiments, however based on the collected data; I would conclude that neither species could be used as a passive bioindicator for HNO_3. If a

repeated fumigation were done, I would recommend increasing the chamber replication, not allowing the lichen to equilibrate in the greenhouse for quite as long, and taking measurements from the lichens in the field before the fumigation to see how they respond under their natural conditions.

III. Implications

Overall data from both fumigations suggest that the fungal component is more sensitive to the present atmospheric pollutants than the algal component. This may be due to the fact that the fungal component makes up the majority of the lichen structure, thus more of it is exposed to atmospheric deposition, whereas the algal component is more protected within the fungal structure (Purvis, 2000). It has been postulated that the fungal component acts as a farmer, “cultivating” the selected algal component within its thallus (Piercey-Normore and Deduke, 2011), to receive carbon produced via photosynthesis from the algal component (Purvis, 2000). The algal component in turn receives protection from the fungal component, and often makes up a much smaller percentage of the total lichen structure (<50%), when compared to the fungal contribution (Honegger, 1991). This difference in contribution may account for increased exposure, and the fungal component being more sensitive to the O_3 and HNO_3 fumigant exposures. The imaging data also helps support this finding. As stated, the fungal component makes up the majority of the lichen outer structure. The O_3 respiration data exhibited the strongest decline in dark respiration, and also exhibited the most thallus discoloration. Though it is difficult to determine how much the algal component contributes to the color of the lichen structure, since the fungal component makes up the majority of the structure, we

can hypothesize that the lichen discoloration is also a sign of decreased fungal health. So based on this data, it can be postulated that the fungal component of the crustose growth form of these species seems to be more susceptible to atmospheric deposition than the algal component.

The findings from this research also help expand the current knowledge about how O_3 and HNO_3 affect crustose lichens. This research postulated that both species would not be negatively affected by the O_3 fumigations based on current literature that suggested it did not negatively affect epiphytic lichens (Riddell *et al*., 2012). However, this was not the case, and both species overall responded negatively to the O_3 fumigations. This is in line with previous research which found O_3 to negatively affect lichens in a number of ways, such as; causing shifts in lichen community composition, loss of species (Sigal, 1979; Sigal and Nash, 1983), decrease in gross photosynthesis (Ross and Nash, 1983), electron opacity (Tarhanen, 1997), decrease in photobiont cell integrity, and decreased potential quantum yield values due to stress on PSII (Scheidegger and Schroeter, 1995). Though I suggest in my analysis that a repeated fumigation should be done to verify the O_3 fumigation results, this initial experiment appears to support previous research in finding that O_3 negatively affects lichens, in this case specifically crustose lichens.

We found a similar trend in the HNO_3 results from this study. It was hypothesized within this research that at high exposure levels, both species would be negatively affected. This prediction was based on previous research that found HNO_3 negatively affected the epiphytic lichen species *Ramalina menziesii* by decreasing photosynthesis,

respiration, chlorophyll content, and increasing potassium leakage, indicating cell damage at concentrations as low as 8 ppb (Riddell, 2009). This study helped confirm these findings as both species experienced a decrease in Fv/Fm values, and *Acarospora* exhibited thallus discoloration. A second round of fumigations would help verify these findings. In addition, it seems like HNO_3 does not negatively affect crustose lichens as much as epiphytic lichens. The crustose lichens in the study experienced higher levels than 8 ppb (10-20 ppb for low treatment, and 20-40 for high treatment), and seemed to experience less damage than the epiphytic lichen species *Ramalina menziesii* in the previous study. This may indicate that crustose lichens are hardier and more resistant to damage from atmospheric pollutants than epiphytic lichens.

Lastly, this research revealed how both *Acarospora* and *Lobothallia* had similar sensitivities to O_3 and HNO_3. Initially it was predicted that *Lobothallia* might have a nitrophilous reactivity to HNO_3 because of its *Trebouxia* algal component, which has been found to be an unifying factor in a number of species that are tolerant to high nitrogen levels (Hauck, 2010). However, this research revealed that it was not the case, and that both species had similar sensitivities. *Acarospora*'s algal component is still unknown, however due to its similar reactivity to O_3 and HNO_3, it may be possible to hypothesize that it has the same algal component as *Lobothallia*. Examining *Acarospora* we can tell that is has a green algal component present instead of a cyanobacterium due to its coloring (Purvis, 2000). *Treboxia* is a green algal component, and it happens to be the most common green algal component, occurring in about 40% of all lichens (Rikkinen, 1995). Additionally, since 90% of all known lichens have a green algal component, it is

possible that *Lobothallia* and *Acarospora* may share the same algal component (Rikkinen, 1995). Though we are not able to discern for sure if *Acarospora* has a *Treboxia* algal component, this could be a possible future avenue of research.

IV. Conclusion

This research determined the physiological effects of O_3 and HNO_3 on two common crustose lichen species (*Lobothallia* and *Acarospora*) from Joshua Tree National Park. It was concluded that both species had similar susceptibilities to both pollutants. Either species could be possibly used as a passive bioindicator for O_3 atmospheric pollution, however further studies are recommended to confirm this finding. Additionally, it was determined that neither species would be a good passive bioindicator for HNO_3 atmospheric pollution, however further studies are again recommended to confirm this. This research helped expand the current research on crustose lichen species, and formed postulates for future research involving them. In addition, the work presented here helped expand the current background information on crustose lichens, suggesting that crustose lichens are hardier than epiphytic, and that the fungal component seems to be more sensitive to atmospheric deposition than the algal component. This research illustrates that atmospheric deposition fumigation studies can be done on crustose lichens, despite the difficulties they present. It is in the hopes of the author that this research generates interest to work with crustose lichens, and use them as bioindicators in the future.

Tables and Figures

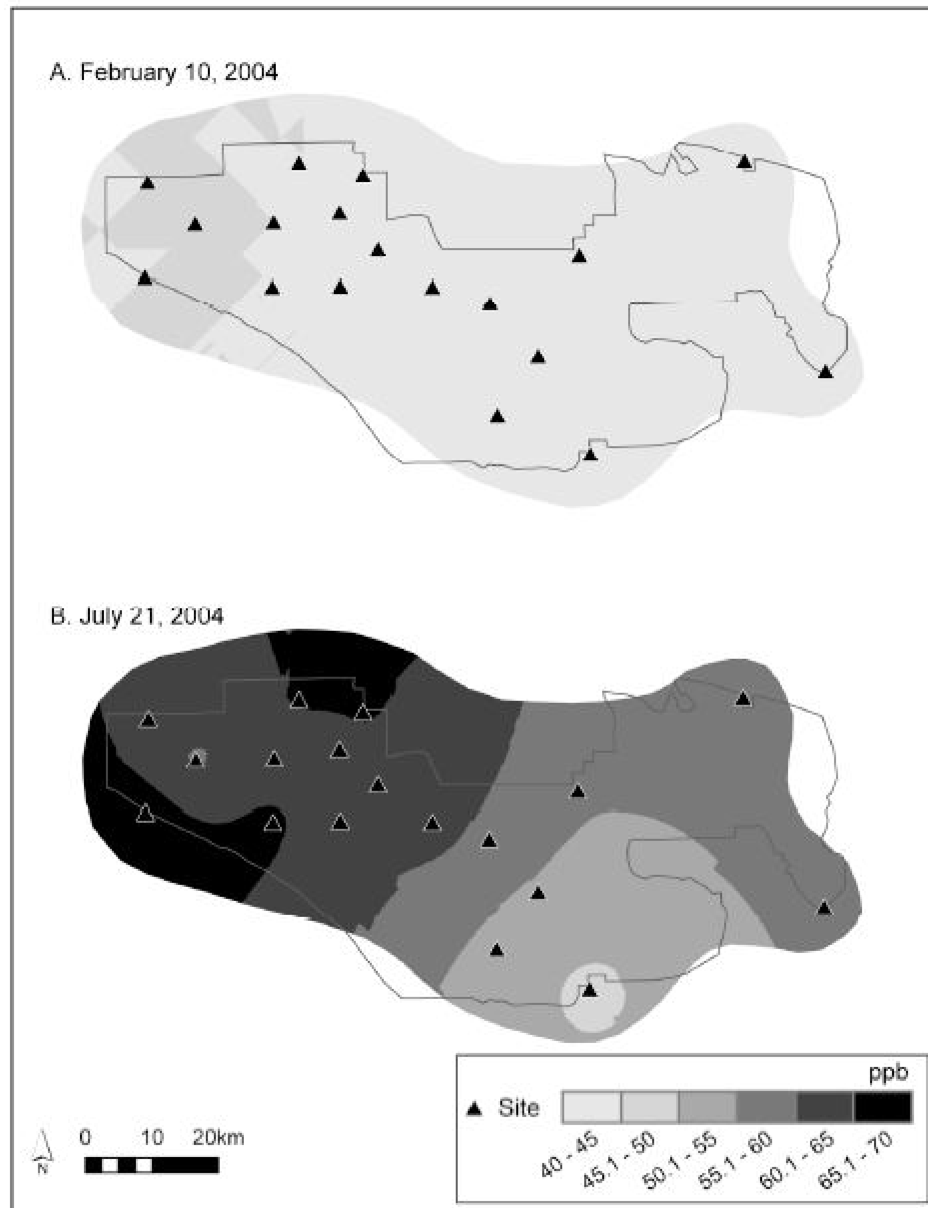


Figure. 1. Two-week-long average ozone (O_3) concentration (ppb) in the atmosphere over Joshua Tree National Park in A) winter and B) summer, 2004 (dates on graph show start of sampling). Figure reproduced from Allen *et al*., 2009.

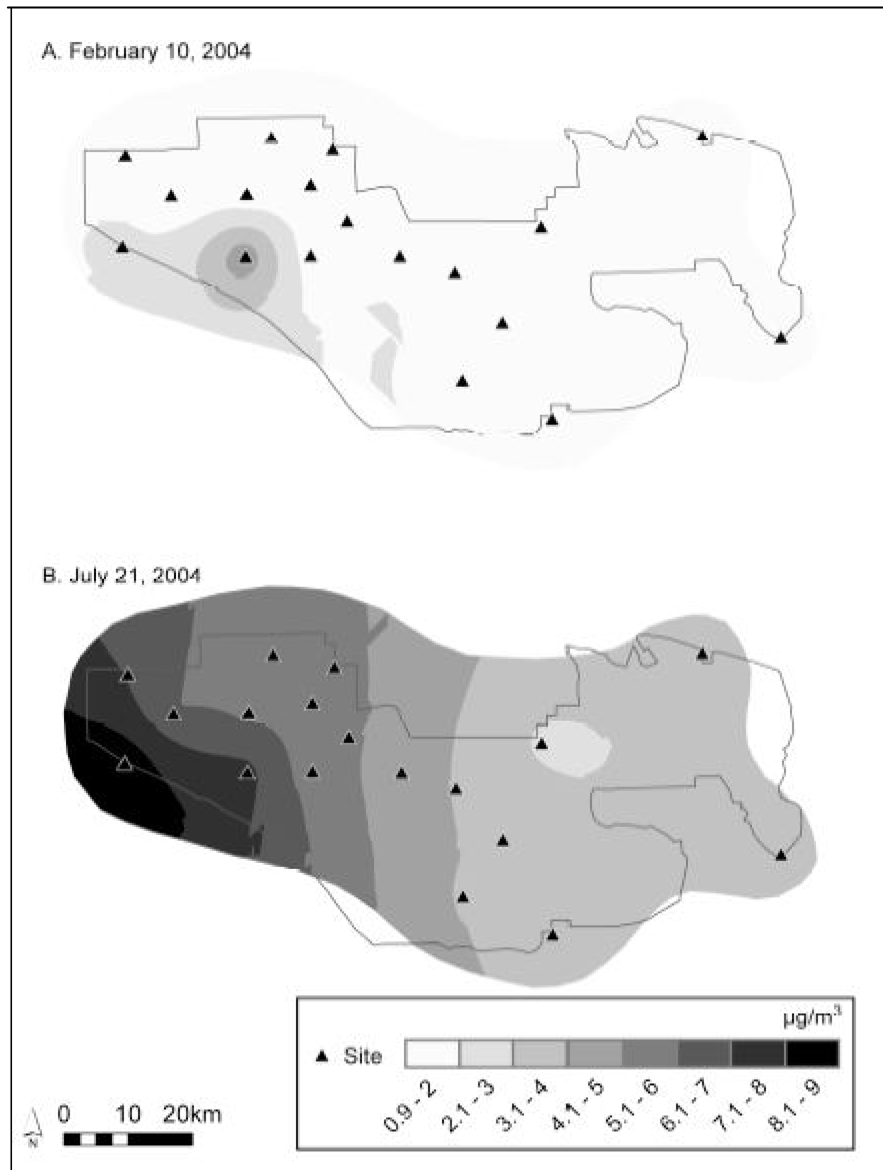


Figure. 2. Two-week-long average nitric acid (HNO_3) concentration (μg/m^3) in the atmosphere over Joshua Tree National Park in A) winter and B) summer, 2004 (dates on graph show start of sampling). Figure reproduced from Allen *et al*., 2009.

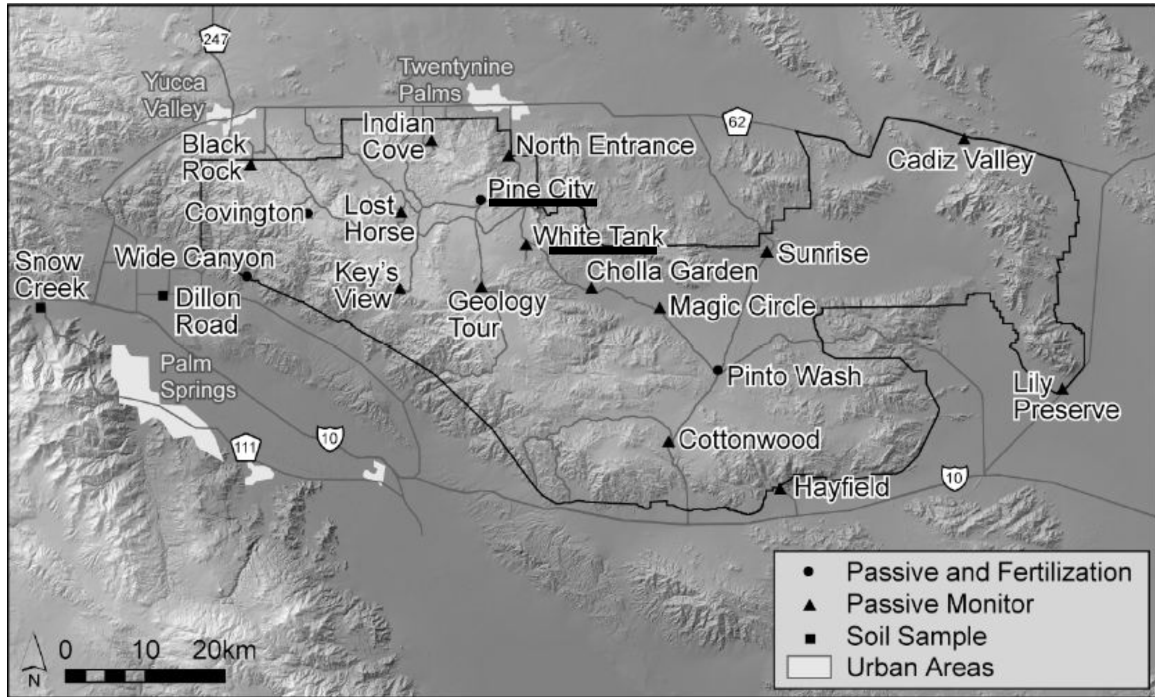


Figure. 3. Map of Joshua Tree National Park from Allen *et al*. (2009), showing different sites within the park. The underlined sites, Pine City, and White Tank (which is very close to Stirrup Tank), were the two sites sampled within this study.

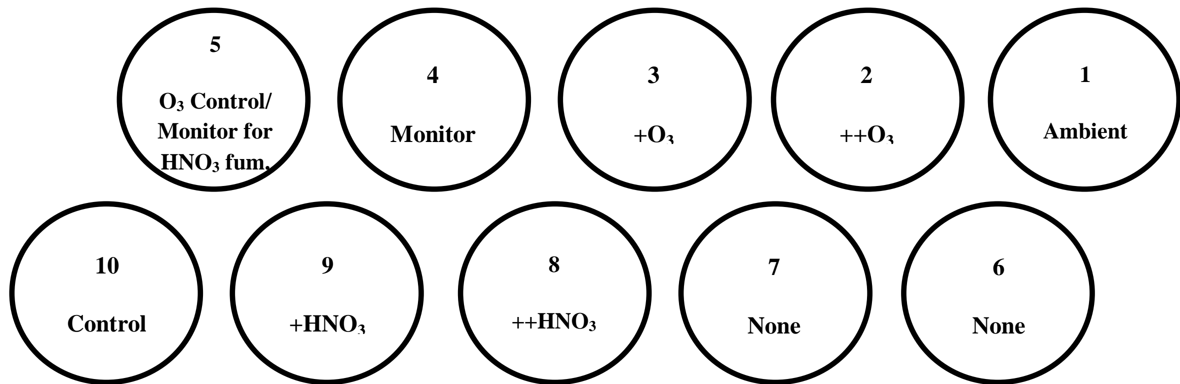


Figure. 4. Set-up of chamber pollutant levels. One plus (+) indicates low levels of pollutants. Two plus (++) indicate high level of pollutants

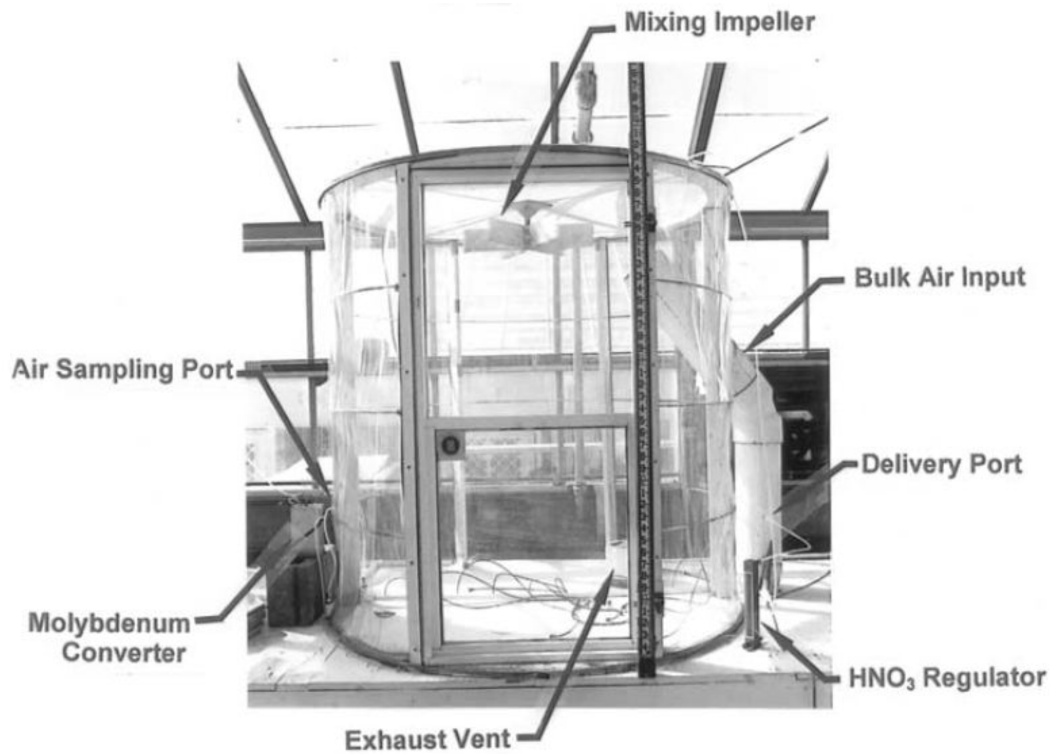


Figure. 5. Continuously stirred tank reactor (CSTR) design taken from Padgett, Bytnerowicz *et al*. (2004). Figure indicates the set-up used in the HNO_3 chambers. A similar set-up is used for O_3 fumigations without the molybdenum converter and HNO_3 regulator.

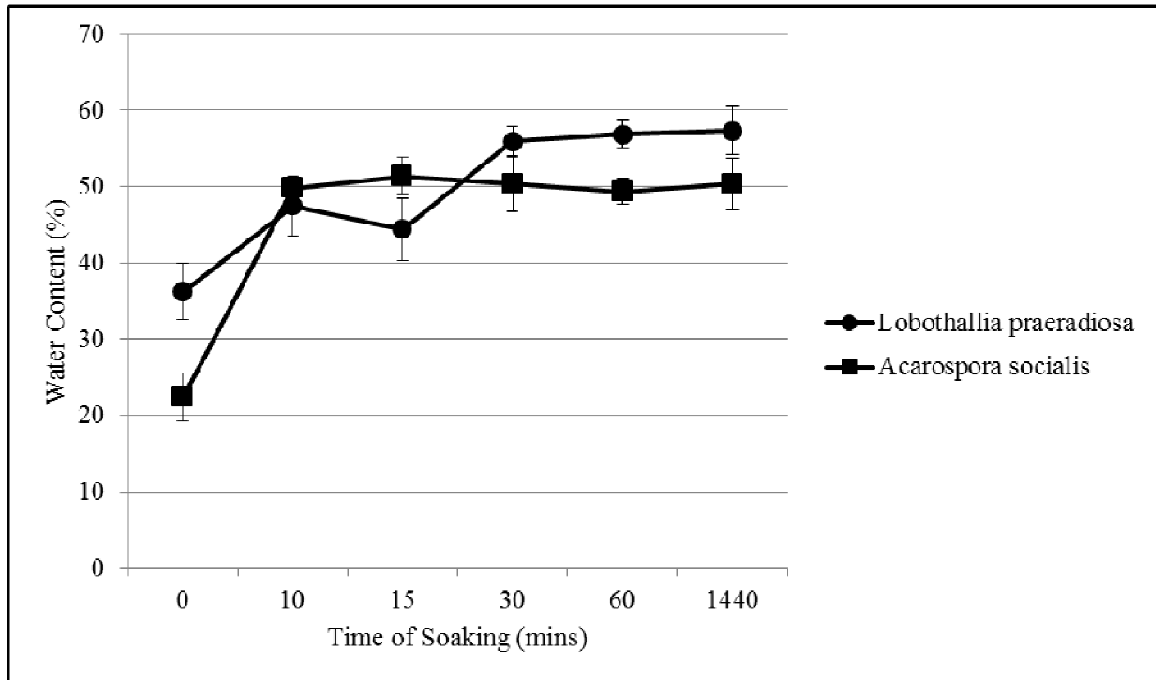


Figure. 6. Water content for each species 0, 10, 15, 30, 60, and 1440 minutes after soaking in DI water (n= 9 for each time interval, standard error presented).

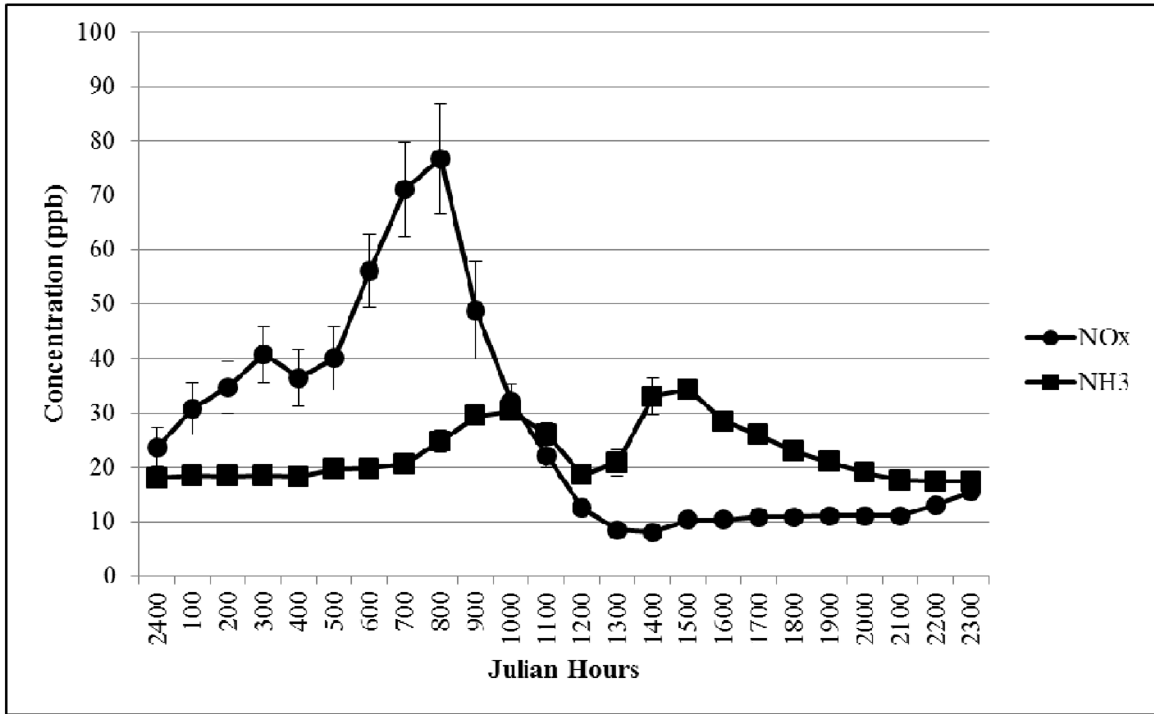


Figure. 7. Averages of NO_x and NH_3 gases in parts per billion (ppb) over 24 hours during summer 2010, in Riverside, CA. (n = 12, standard error presented).

Table 1. Photosynthetic active radiation (PAR) data from March – Sept. 2012 in µmol photons/m^2/second. Measures taken within the continuosly stirred tank reactors (CSTRs) and outside of the greenhouse (GH). Miniumum and maximums are presented.

	Photosynthetic Active Radiation (µmol photons/m^2/second)			
Month (2012)	**Inside CSTRs Min**	**Inside CSTRs Max**	**Outside GH Min**	**Outside GH Max**
March	500	1500	1000	1500
April	800	1200	1700	1900
May	500	1400	1900	2000
June	850	1250	2000	2000
July	800	1000	1900	1900
August	500	900	1800	1850
September	800	1000	1500	1700

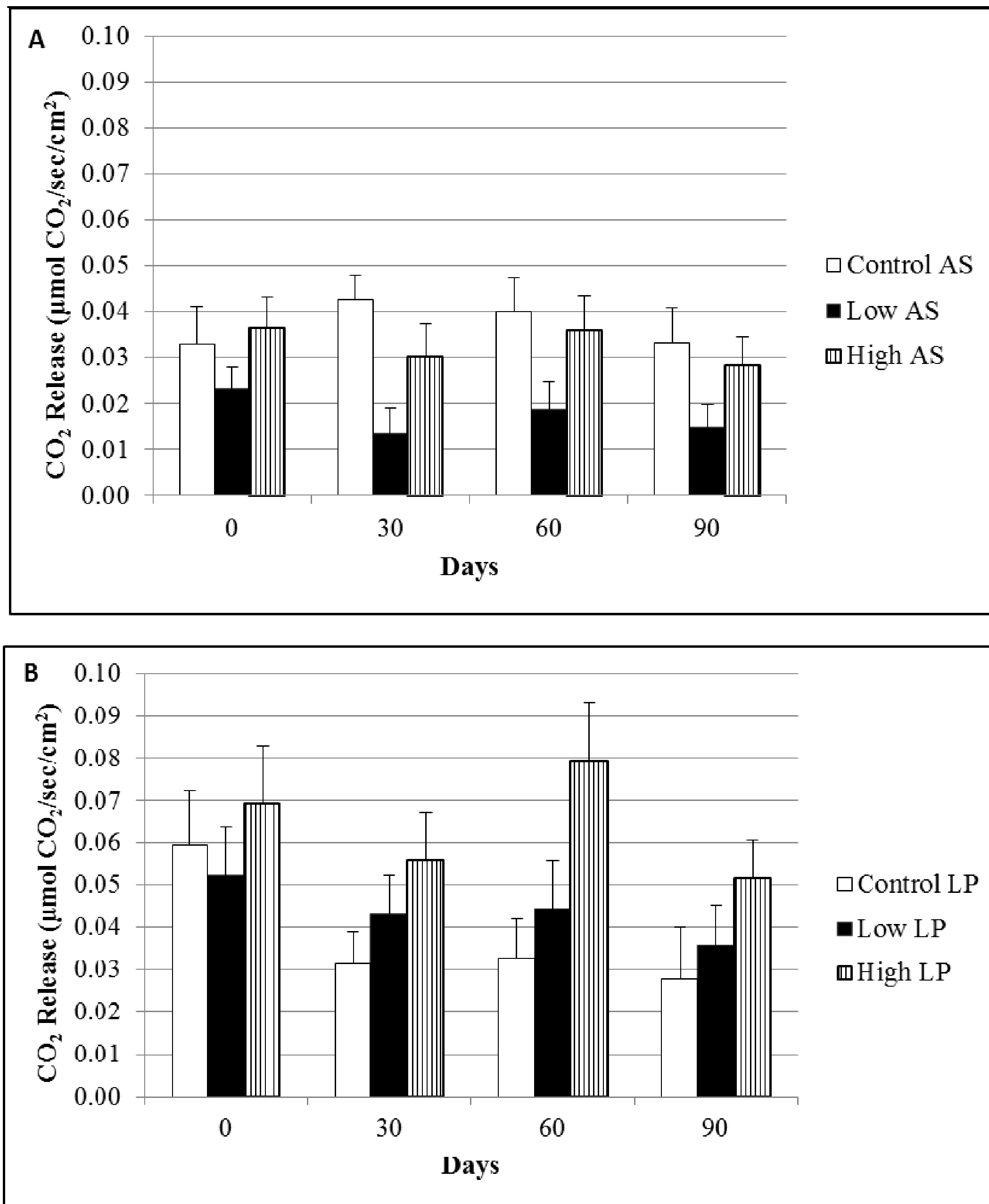


Figure. 8. A) Averages of dark respiration CO_2 release from the O_3 fumigation for *Acarospora*, separated between the fumigant levels and sampling days (standard error presented). B) Averages of dark respiration CO_2 release from the O_3 fumigation for *Lobothallia*, separated between the fumigant levels and sampling days (standard error presented).

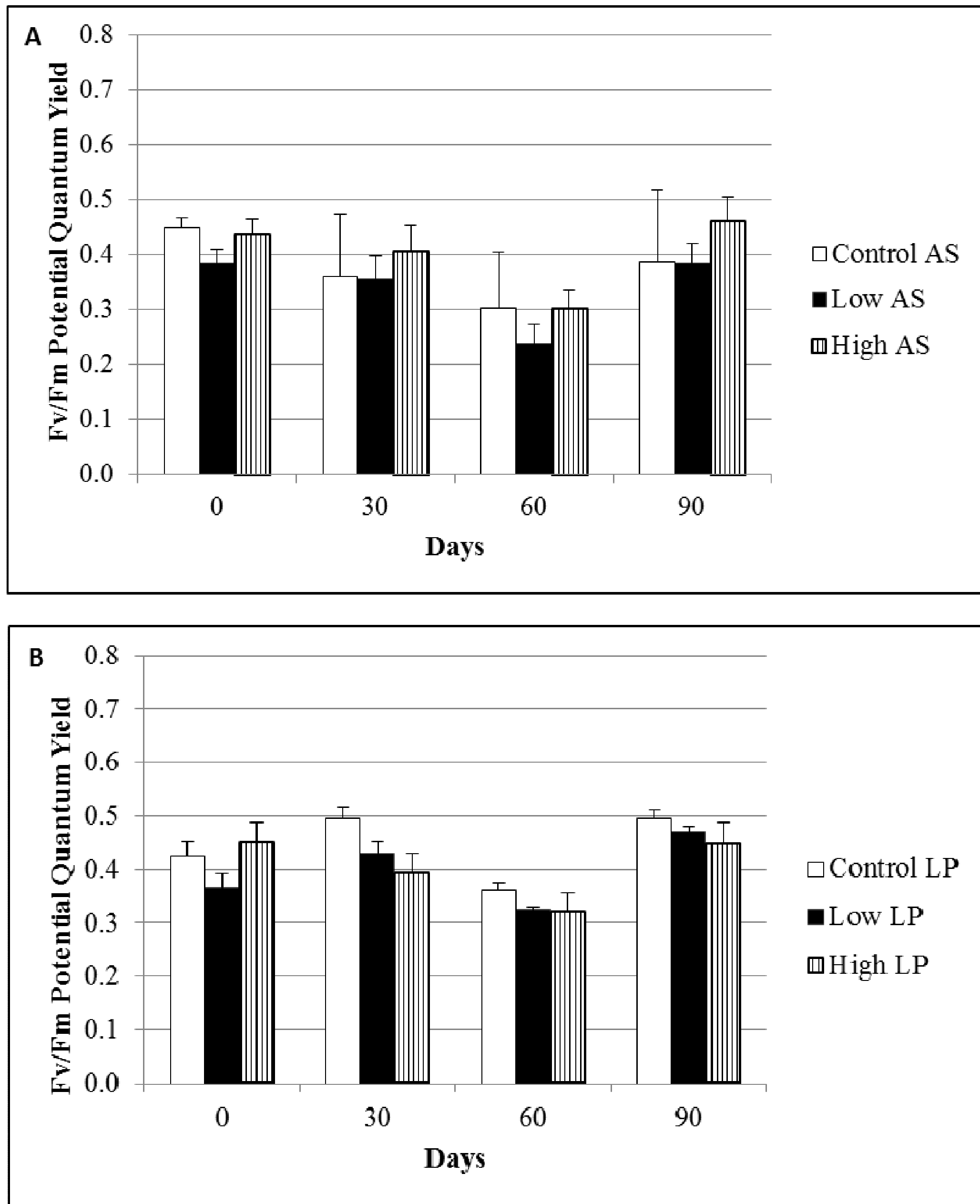
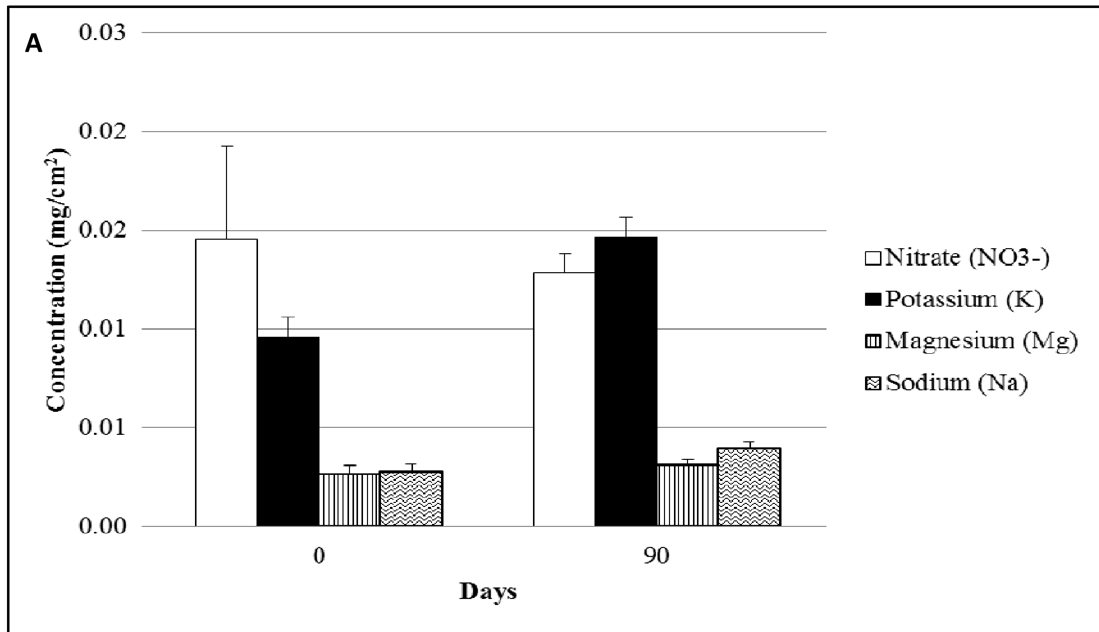


Figure. 9. A) Averages of potential quantum yield from the O_3 fumigation for *Acarospora*, separated between the fumigant levels and sampling days (standard error presented). Measures on day 30, 60, 90 were taken 3 hours after wetting, where measures on day 0 were taken immediately after wetting. B) Averages of potential quantum yield from the O_3 fumigation for *Lobothallia*, separated between the fumigant levels and sampling days (standard error presented). Measures on day 30, 60, 90 were taken 3 hours after wetting, where measures on day 0 were taken immediately after wetting.



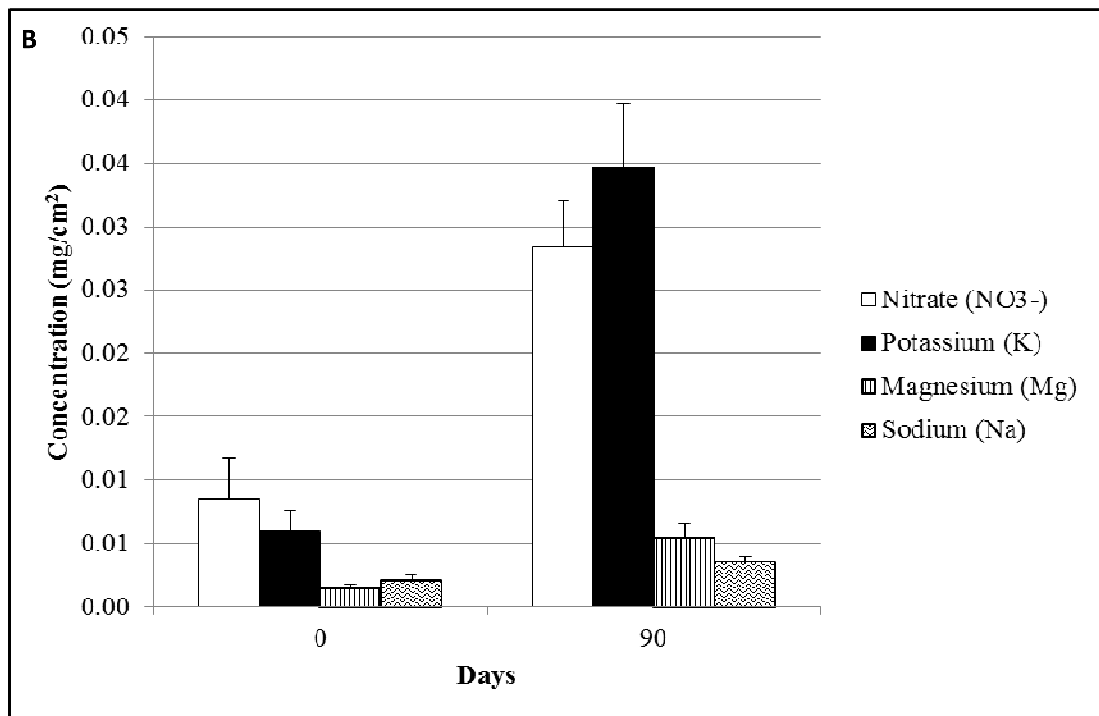


Figure. 10. A) Lichen wash analysis from the O_3 fumigation showing the averages of NO_3^-, K, Mg, and Na in mg/cm^2 between day 0 and day 90 (n= 48, standard error presented). B) Lichen wash analysis from the O_3 fumigation showing the averages of NO_3^-, K, Mg, Na in mg/cm^2 between day 0 and day 90 for the depositional lichen samples (n=12, standard error presented).

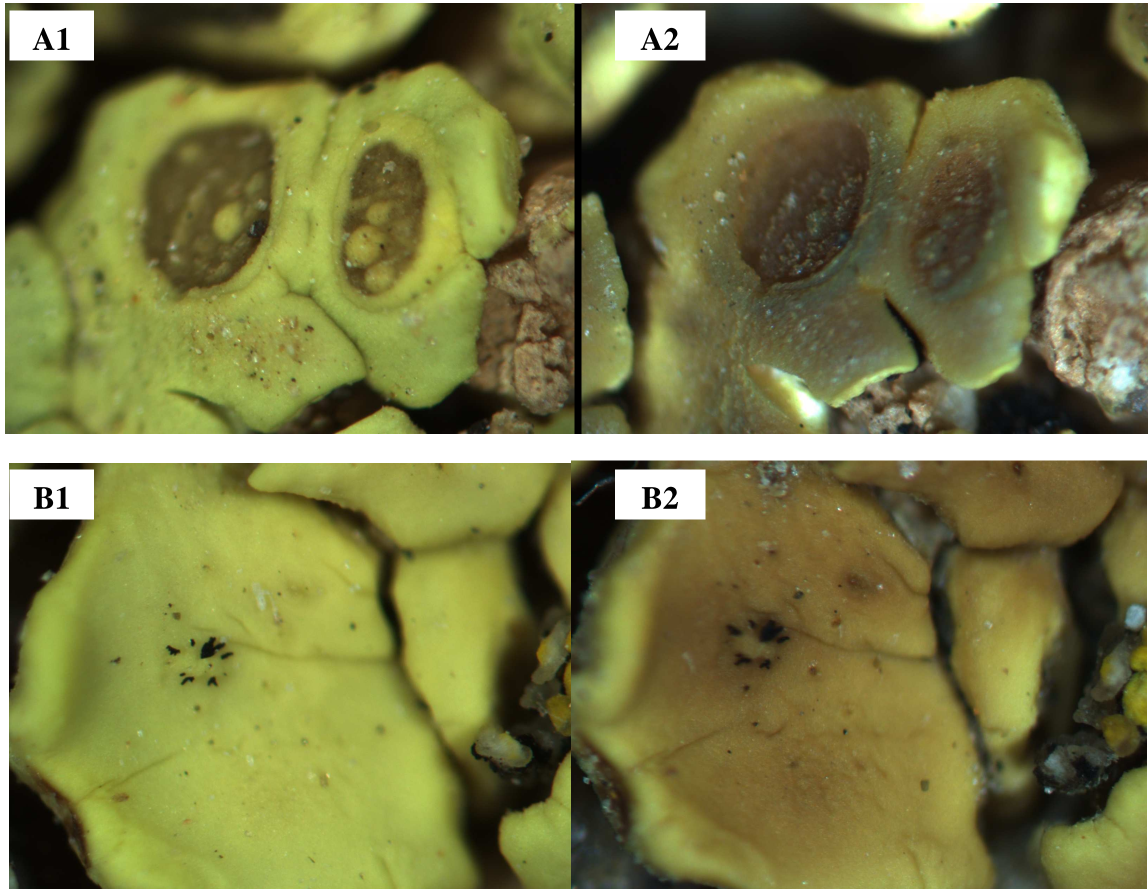


Figure. 11. A) Example of extreme discoloration found on *Acarospora* from the O_3 fumigation. A1) Image of *Acarospora* control sample from Stirrup Tank site on day 0 (magnification 6.3x). A2) Image of same *Acarospora* sample from Stirrup Tank on day 90 (magnification 6.3x). B) Example of discoloration found on *Acarospora* samples within all three HNO_3 treatments. B1) Image of *Acarospora* control sample from Pine City site on day 0 (magnification 6.3x). B2) Image of same *Acarospora* control sample from Pine City on day 90 (magnification 6.3x).

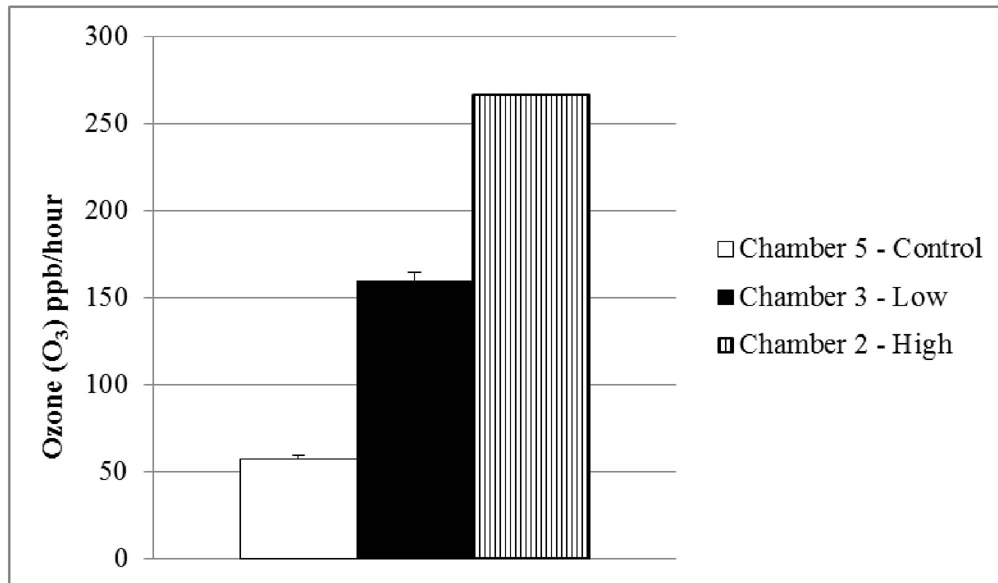
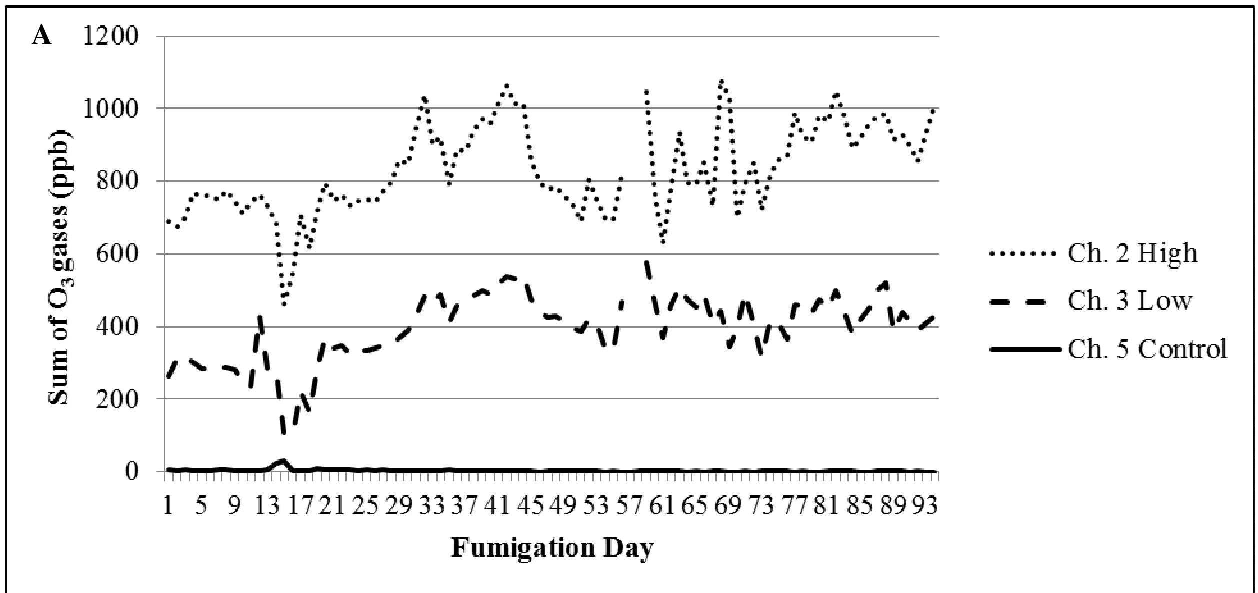


Figure. 12. Averages of Ogawa filter pack analysis (O_3) in parts per billion/hour (ppb/hour) (n= 2, standard error presented). There were significant differences between all three chambers ($p < 0.005$).

Table 2. Temperature and humidity data collected from digital hobos within the continuously stirred tank reactors (CSTRs). Control and monitor chambers for the O_3 fumigation presented (chamber 5, 4) and control and monitor chambers for the HNO_3 fumigation presented (chamber 10, 5).

	Temp. °C (Min)	**Temp. °C (Max)**	**Humidity % (Min)**	**Humidity % (Max)**
Ch. 5 - O_3 Control	17.1	60.9	3	69.9
Ch. 4 - O_3 Monitor	16.3	59.8	1.7	72.2
Ch. 10 - HNO_3 Control	10.1	53.3	0.7	68.6
Ch. 5 - HNO_3 Monitor	9.9	52.8	2.1	69.8



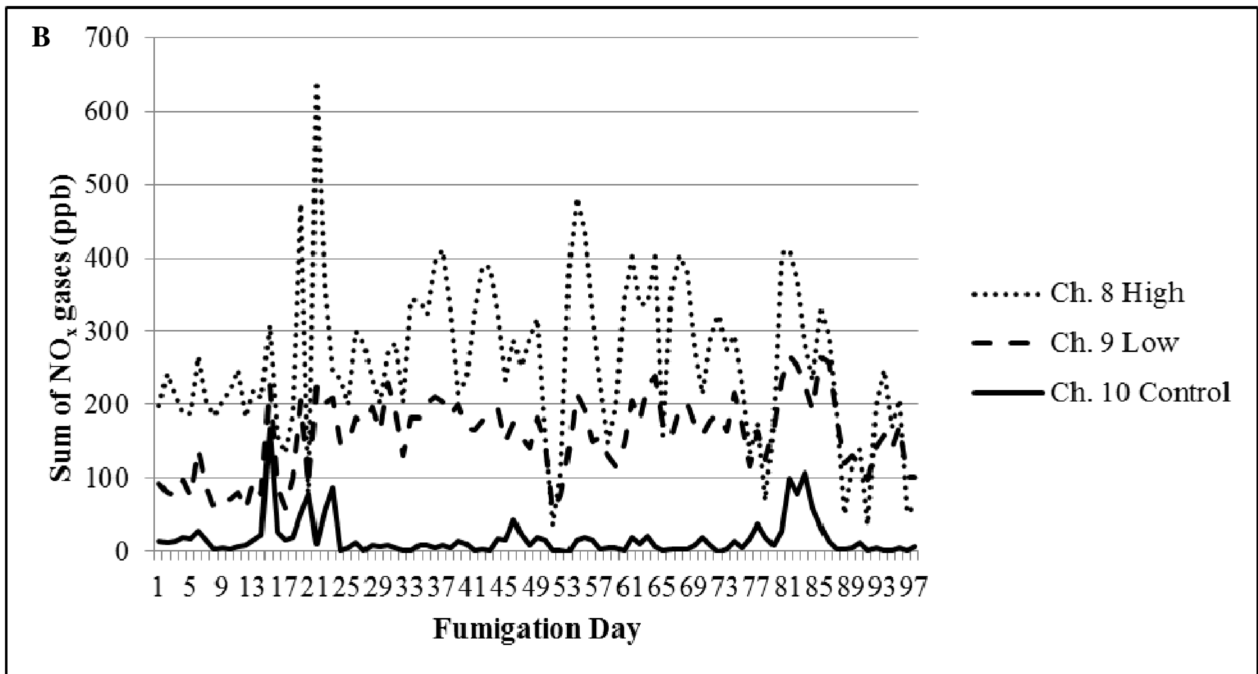


Figure. 13. A) Daily sums of O_3 gases (in parts per billion over 24 hours) for each treatment chamber over the entire O_3 fumigation. Ambient O_3 gases taken into account and subtracted from data. B) Daily sums of NO_x gases (in parts per billion over 24 hours) for each treatment chamber over the entire HNO_3 fumigation. Ambient NO_x gases taken into account and subtracted from data.

Table 3. Sum of O_3 and NO_x gases (dose - in parts per billion), at each sampling day for both fumigations. Chamber 2 (high O_3), chamber 3 (low O_3), chamber 5 (control O_3), chamber 8 (high NO_x), chamber 9 (low NO_x), and chamber 10 (control NO_x) represented.

Chamber	Gases in ppb (sum over time)		
	Sampling Day		
	30	60	90
2-High O_3	21850	32789	30297
3-Low O_3	8822	18461	14709
5-Control O_3	155	34	21
8- High NOx	7256	8729	9017
9-Low NOx	3812	4901	6557
10-Control NOx	746	292	674

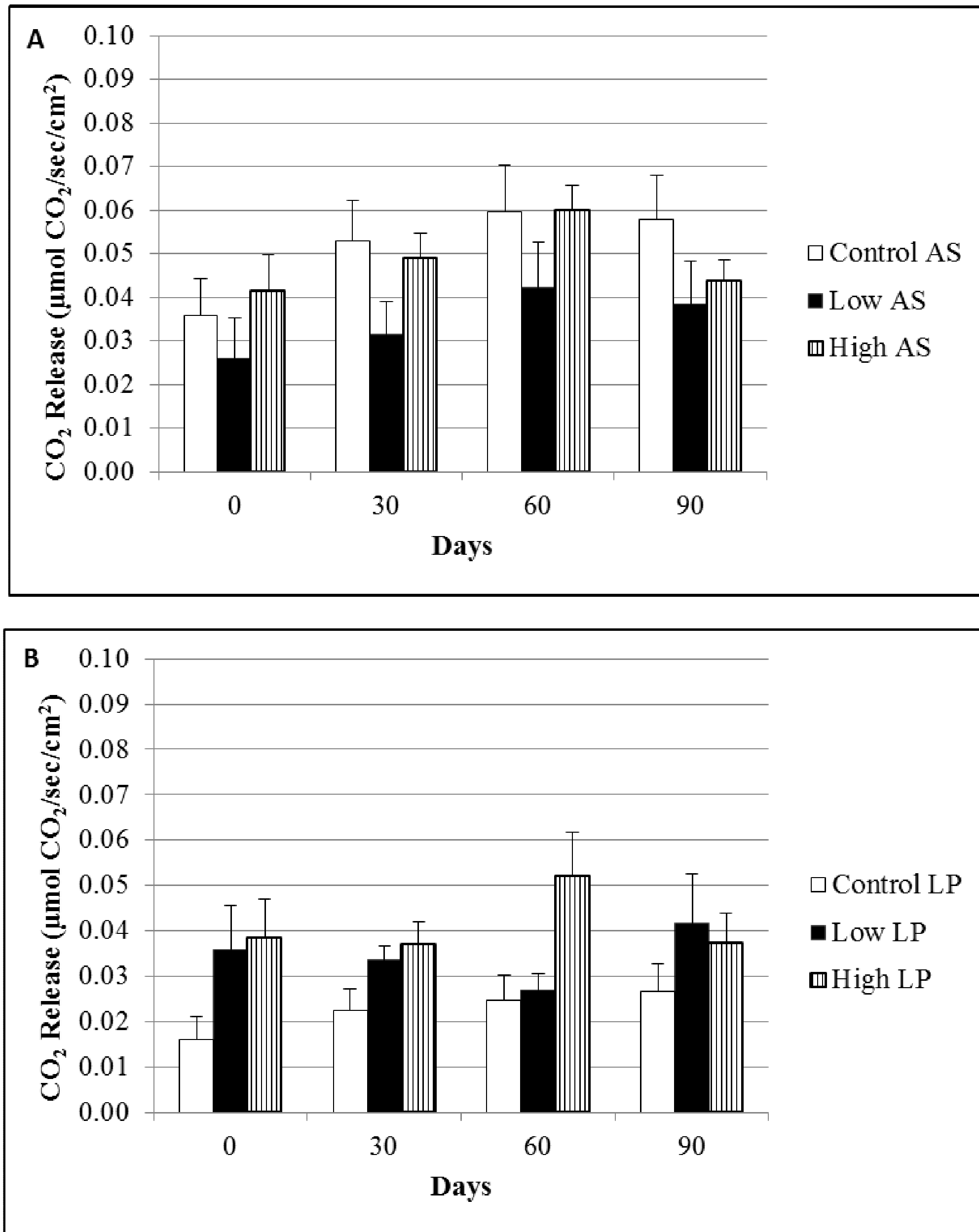
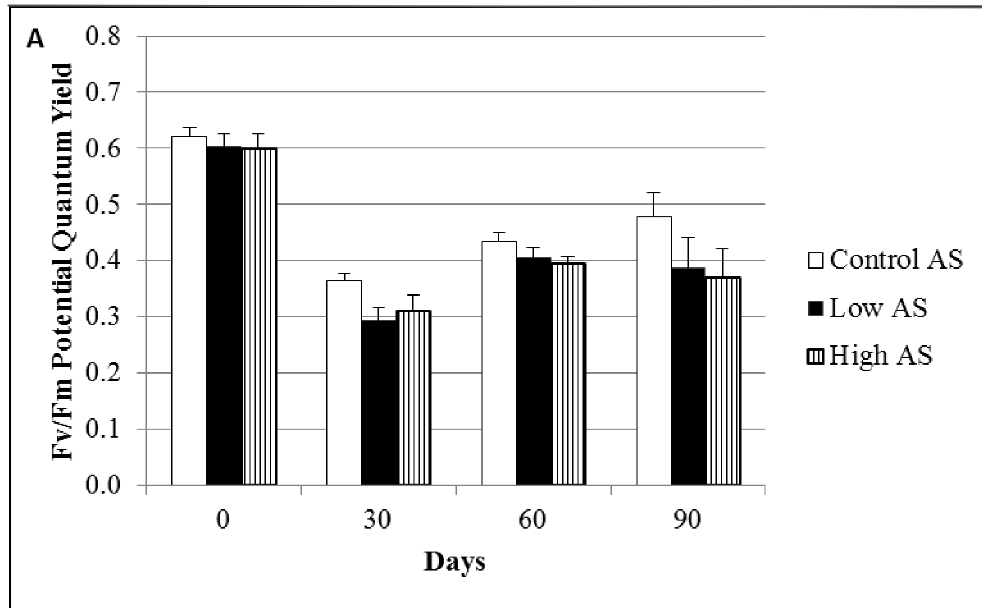


Figure. 14. A) Averages of dark respiration CO_2 release from the HNO_3 fumigation for *Acarospora*, separated between the fumigant levels and sampling days (standard error presented). B) Averages of dark respiration CO_2 release from the HNO_3 fumigation for *Lobothallia*, separated between the fumigant levels and sampling days (standard error presented).



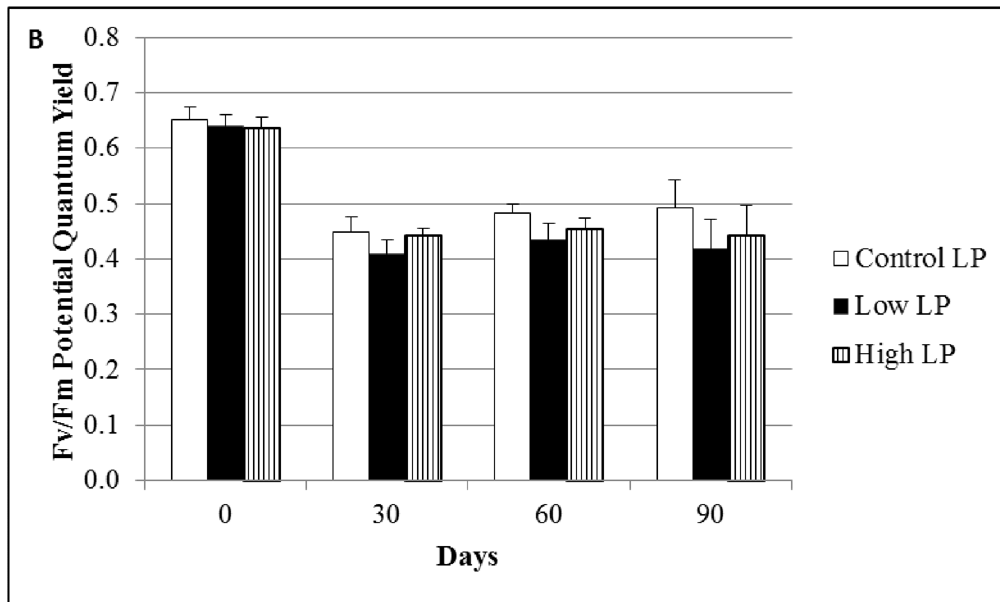


Figure. 15. A) Averages of potential quantum yield from the HNO_3 fumigation for *Acarospora*, separated between the fumigant levels and sampling days (standard error presented). B) Averages of potential quantum yield from the HNO_3 fumigation for *Lobothallia*, separated between the fumigant levels and sampling days (standard error presented).

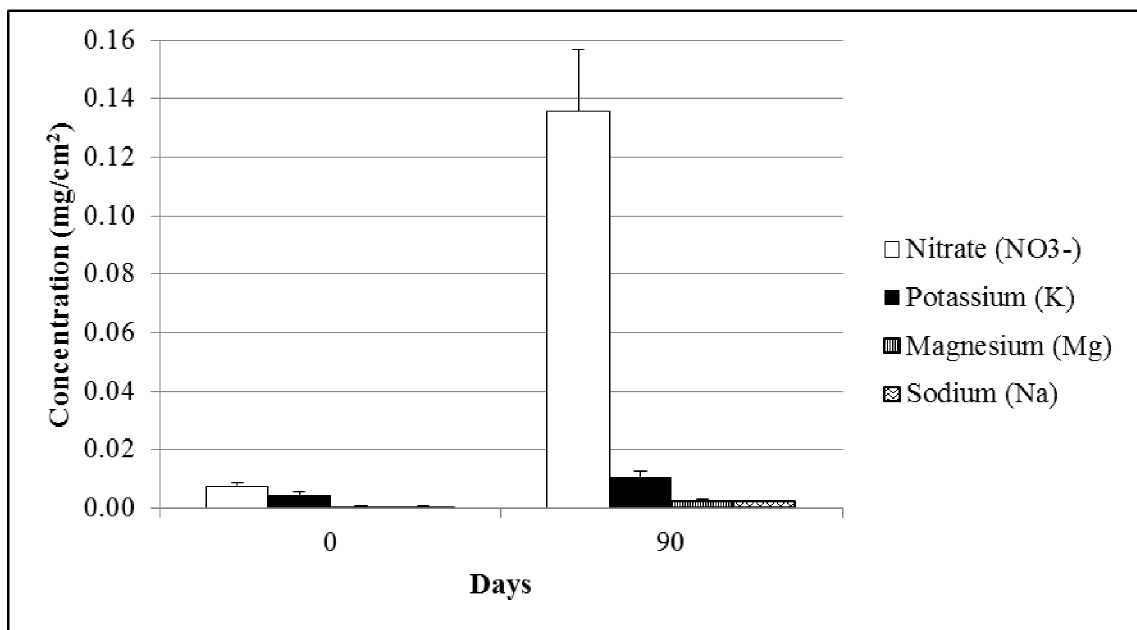


Figure 16. Lichen wash analysis from the HNO_3 fumigation showing the averages of NO_3^-, K, Mg, and Na in mg/cm^2 between day 0 and day 90 (day 0 n= 48 (NO_3^-), n= 39 for cations, and day 90 n= 22 (NO_3^-), n= 20 for cations) (standard error presented).

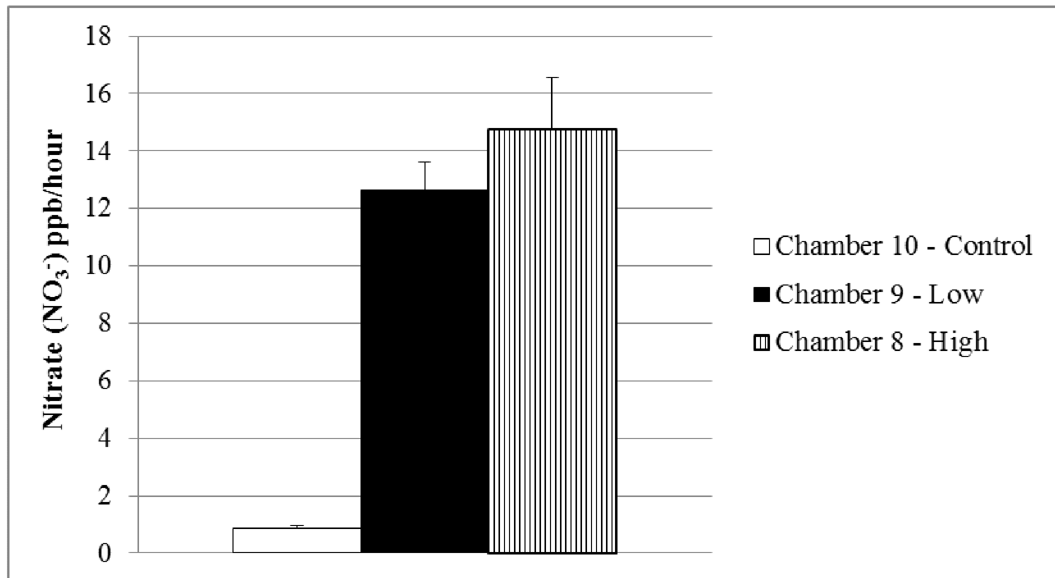
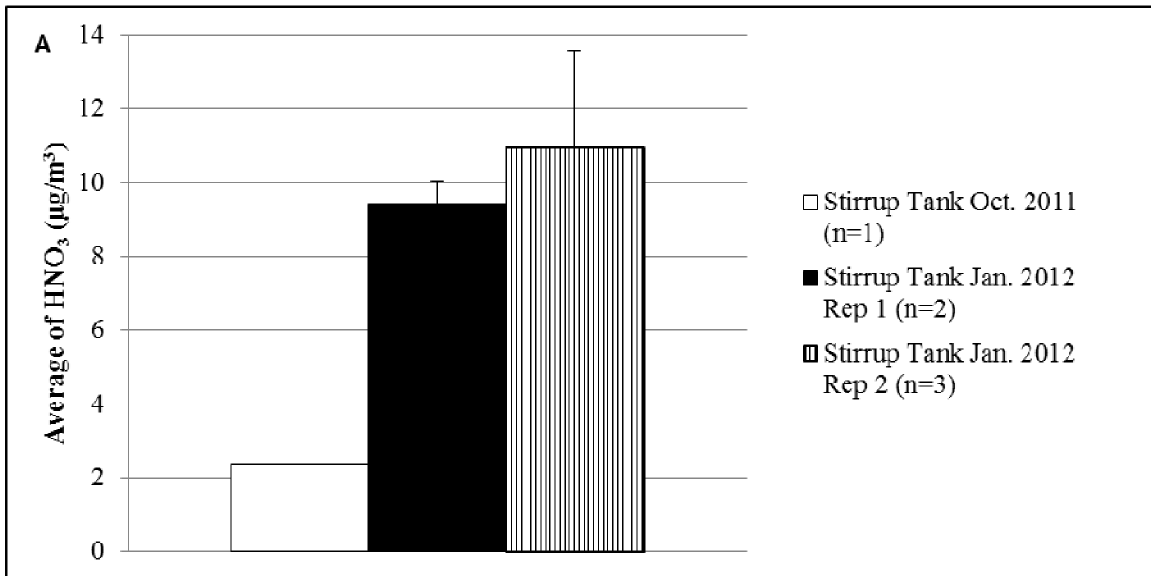


Figure. 17. Averages of nylon filter analysis (NO_3^-) in parts per billion/hour (ppb/hour) (n= 4, standard error presented). There were significant differences between all three chambers ($p \leq 0.05$).



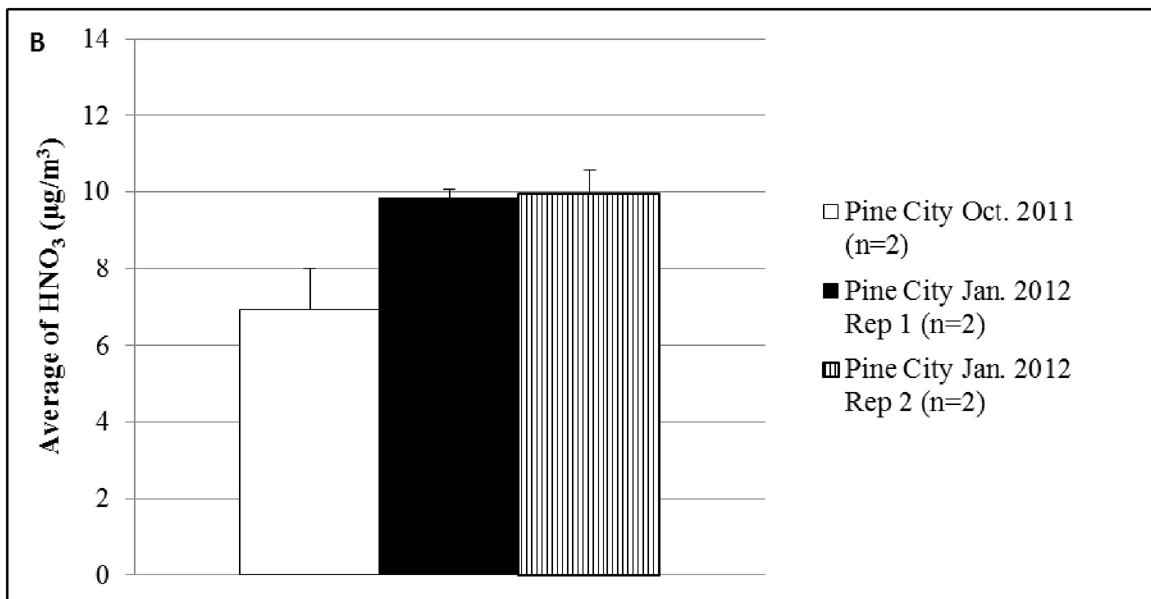


Figure. 18. Passive sampler field data measuring average atmospheric concentrations of HNO_3 (in µg/m^3), for Stirrup Tank (A), and Pine City (B) (n = 1, 2, 3 as specified, standard error presented). Collections took place in October 2011, and Januray 2012. Data from both sites indicated no significant differences between atmospheric concentrations in October and January ($p > 0.05$), and no significant difference between the Januray collections ($p > 0.05$).

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Appendix

Isotopic ratios for both species were determined (Fig. 1-Appendix). When the data was separated by isotope and averaged, $\delta^{18}O$ data shows a significant difference between the two species, and the *Acarospora* replicates (Fig. 2A-Appendix). Data from $\delta^{15}N$ indicated that there were no significant differences between the *Acarospora* samples and the first *Lobothallia* replicate; however there were significant differences between the *Lobothallia* samples and *Acarospora* to the second *Lobothallia* sample (Fig. 2B-Appendix). There were no significant linear relationships when relating the $\delta^{18}O$ and $\delta^{15}N$ values to lichen surface area ($R^2 \leq 0.4$).

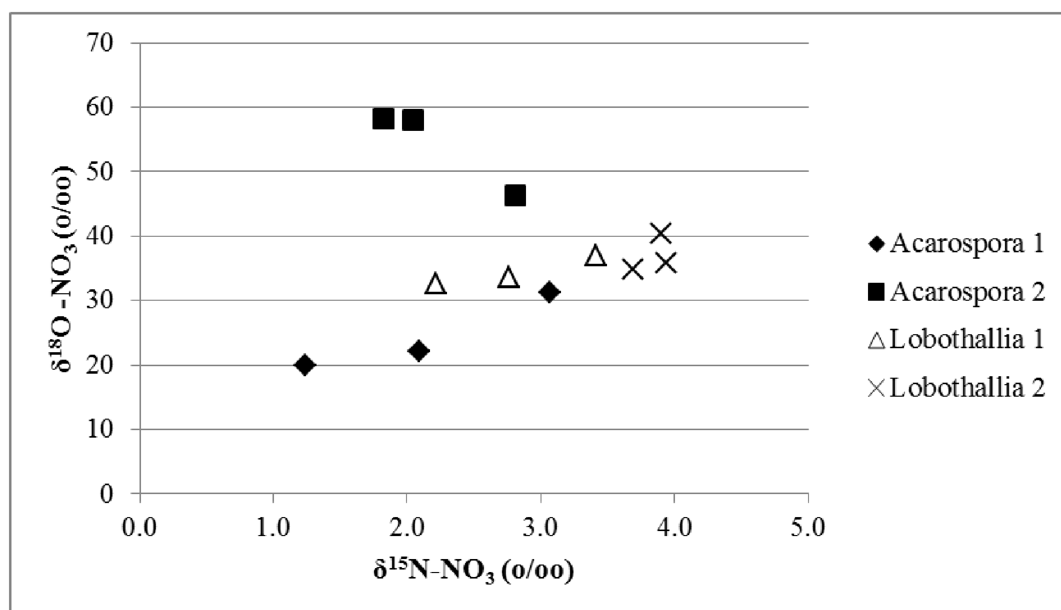

Figure. 1. Isotopic ratios showing the $\delta^{18}O$ and $\delta^{15}N$ isotopic ratios (per mil) from the nitrate (NO_3^-) present in the lichen washes. For 2 samples from each species (3 washes for each).

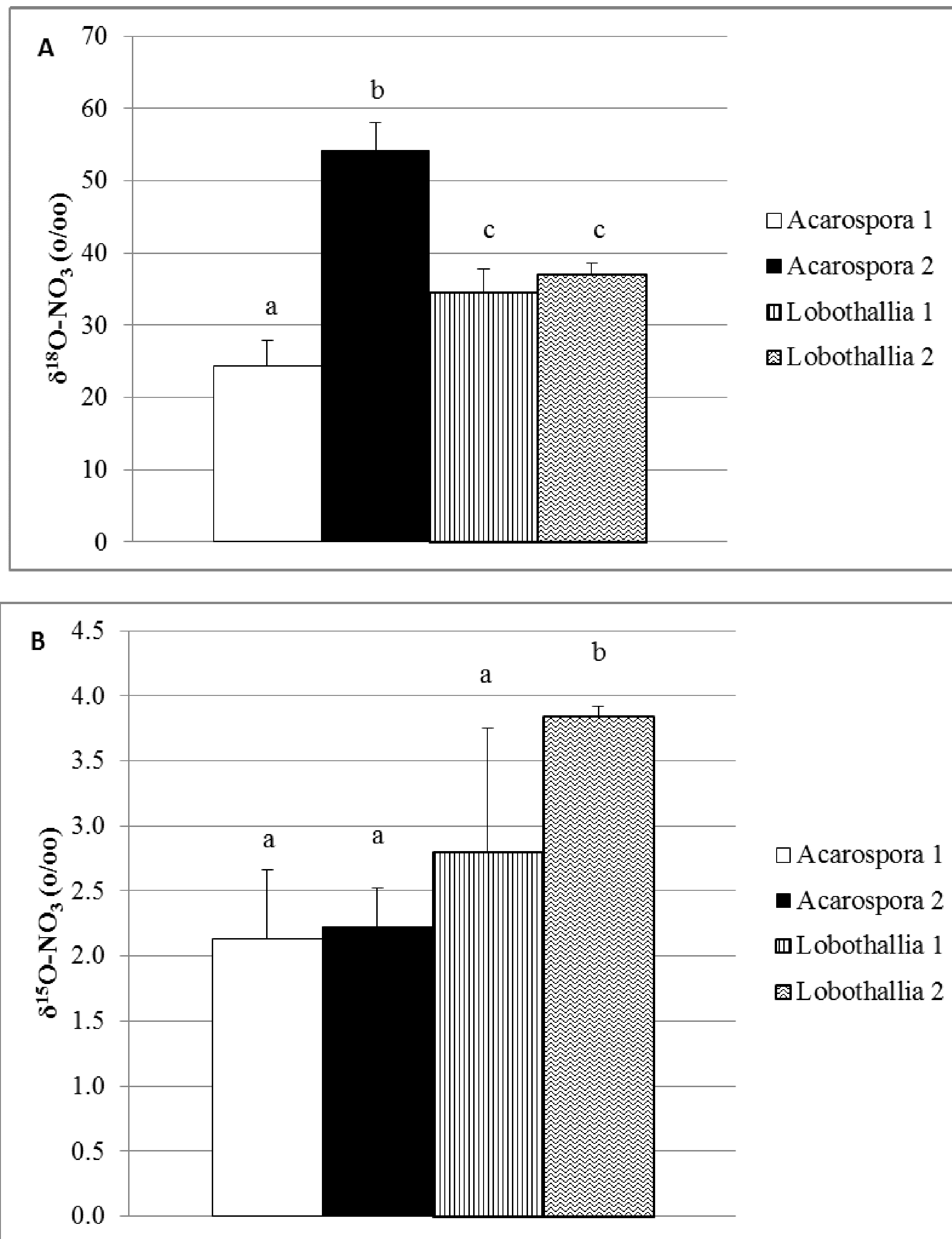

Figure. 2. Averages of $\delta^{18}O$ and $\delta^{15}N$ (per mil) from the nitrate (NO_3^-) present in the lichen washes, separated out by sample (n = 3 washes, standard error presented). A) There is a significant difference between *Acarospora* and *Lobothallia* ($p \leq 0.05$), and there is a significant difference between the two *Acarospora* samples ($p < 0.005$). There is no significant difference between the two *Lobothallia* samples ($p > 0.05$). B) There is no significant difference between the two *Acarospora* samples and the first *Lobothallia* sample ($p > 0.05$). There was a significant difference between the two *Lobothallia* samples ($p < 0.05$), and between *Acarospora* and the 2nd *Lobothallia* sample ($p < 0.05$).