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Road Salt Effects on Leaf-Level Isoprene Emission

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Abstract

Road salt accumulation in the environment influences plants, soils, and surrounding ecosystem. From the use of chloride-based deicers, [Na^+] and [Cl^-] can be found in leaf tissue and have been shown to affect plant function negatively through reduced photosynthetic activity. Photosynthetic activity is an important plant function that drives plant health and is also linked to isoprene emissions. Certain plant genera emit isoprene, a biogenic volatile organic compound (BVOC) which produces tropospheric ozone and reduces regional air quality. Isoprene is emitted into the atmosphere by photosynthetic activity from leaves. Isoprene emissions from these plants are an indicator of plant health and function despite reducing regional air quality. Past research has shown that isoprene emissions are connected to photosynthetic activity. Salt stress reduces photosynthetic activity in leaves; however, it is not fully understood what effect road salts have on leaf-level isoprene emission. The purpose of this research is to determine if road salts suppress leaf-level isoprene emissions. We hypothesize [Na^+] from road salt will suppress leaf-level isoprene emissions since salt stress can act like drought stress, and drought stress can suppress isoprene emissions. This project consisted of field measurements and two growth chamber experiments. For the field measurements, ten suitable field sites from Cook and Lake County Forest Preserves were determined. Five sites had relatively lower salinity index [Na^+] (*non-road*) and the other five sites had relatively higher salinity index and were more exposed to road salt (*near-road*). Leaf-level isoprene emission and photosynthesis rates from the ten selected field sites were measured in situ to test for a difference between the *non-road* and *near-road* sites. There was no significant between the sites for photosynthetic rates and isoprene. In the laboratory, velvet bean plants (*Mucuna pruriens*) grown from seed were salt-stressed in growth chambers. Leaf-level isoprene emissions and photosynthetic rates were used to test for a difference between treated and control pot. There was no significant difference between the treated and control pots for photosynthetic rates and isoprene. Unfortunately, COVID-19 limited some of the tests in the field and lab measurements leaf and soil [Na^+] and [Cl^-], not allowing us to test the hypothesis fully. With the results we completed, we failed to reject the null hypothesis and observed no impact of road salt application on photosynthetic or isoprene emission rates.

Systematic Literature Review

The scope of the literature review covered isoprene and road salts. Because there is a gap in the literature on what effect road salts have on isoprene emissions, the literature review synthesized three different research categories. The first category was **road salts**, which included road salts pertaining to soil, paved surfaces, and salt accumulation in roadside plants. An additional term used for road salts was deicers. Deicers are a broad term for chloride-based salts, such as MgCl, NaCl, and CaCl, which are important for understanding the different types of road salts used as deicers (Tiwari and Rachlin 2018). The second category was **isoprene**, which included isoprene emissions, photosynthesis, drought stress, and isoprene emitting plants. The third category had articles that discussed concepts from both research categories were called **isoprene/road salts** and discussed photosynthesis pertaining to road salts and plant function. For the literature review, I used the search engines Web of Science, Elsevier, Google Scholar, and Science Direct to find journal articles and books and other DePaul University research resources. I also analyzed the citations from the selected literature for relevant authors and/or papers.

I used two different search terms for the road salts category: "road salts and soil" and "road salts and paved surfaces." Salt accumulation was a common problem addressed in the literature and did not require an individual search term. "Road salts and soil" was a combined search term because extensive research on soils was present and too broad for the literature review. Combining "road salts and soil" was a narrow yet flexible search term with the area of interest for this project. The second search term, "road salts and paved surfaces," was also a combined search term that was narrow yet flexible for isolating literature material that emphasized roadsides, highways, and any other type of impervious surface. From the generated articles, I synthesized select articles that addressed three or more of the following criteria:

1. Road salt interactions in the soil
2. Included paved, impervious, or cemented surfaces as an experimental parameter or type of environment
3. Addressed roadside vegetation
4. Experiments focusing on the distance from the point of contamination
5. Discussed the consequences of excessive salt concentration in soil

Criteria (1) and (3) focused on the interaction between road salts and soil near paved surfaces, which was important in understanding how salts accumulated in tree leaves and roadside plants from the surrounding soil (Cunningham et al. 2008). This literature also explained what and how the effects occurred in soil from excessive road salt concentrations. Criteria (2) and (4) helped refine the literature review to impervious surfaces like roadside or highways and helped develop the methods we proposed for this project. Criteria (5) discussed the effects or consequences of salt accumulation in soil, and it was important to understand how and why road salt is considered an environmental contaminant. With these search terms and selection criteria, research materials like review articles and original research articles were the predominant literature forms. For the road salts category, two review articles, one technical report, and five original research articles were selected.

The search terms in the isoprene category were: "isoprene emissions and photosynthesis "and "isoprene emissions and drought stress." Adding the keywords "isoprene emissions" to photosynthesis and drought stress specified the research literature scope. Because road salts and isoprene emissions were not explicitly discussed together in the literature, I used photosynthesis to draw out connections between road salt and isoprene emissions. Some articles from this category overlap with the road salts category, and these articles are considered part of the isoprene/road salts category. Photosynthesis was the plant mechanism that linked isoprene emissions and road salts together, which developed the project's hypothesis. The second search term, "isoprene emissions and drought stress," was used to fill in gaps and understand the type of stress road salts have on roadside vegetation. Several studies have interrogated the connection between drought stress and isoprene emissions and suggest that salt stress acts like drought stress for photosynthesis because both use osmotic effects as the mechanism that affects plant function and response to plant emissions (Chaves et al. 2009; Potosnak et al. 2014; Zheng et al. 2017; Loreto et al. 2003; Munns 2002; Munns and Tester 2008). Therefore, salt stress in plants and drought stress with isoprene emissions were key concepts that connected the gap existing in the research literature used to form the critical experiment (**Figure 1**). Grey literature published on the Morton Arboretum website that focused on the regional tree community was used as a reference for forming the experiment through species-specific plant responses to environmental stressors. From the articles generated from the isoprene and isoprene/road salts category, I selected articles that addressed three or more of the following criteria:

1. How does the plant response to abiotic stress condition (e.g. drought, water stress, salt exposure) affect isoprene emissions
2. How does drought stress and or salt stress affect photosynthetic activity in plant leaves
3. What gap in the literature does this literature fill in

4. What methods did the researchers use to collect [Na^+] and [Cl^-] from plant tissue, how was it related to photosynthetic activity, and was stress concentration included?

Criteria (1) helped determine cause and effect from existing literature. Criteria (2) and (4) were used to develop the methods of this experiment. Criteria (3) was used to develop an effective methodology and include significant elements from other experiments and researchers. With these search terms, the predominant forms of literature were original research articles. For the isoprene category, one review article and seven original research articles were selected. For the isoprene/road salt category, one review article and one original research article were selected.

Introduction

Road salt is a common deicer used to maintain roadways and areas associated with automobile traffic during the winter season. The most commonly used road salt is sodium chloride (NaCl), while magnesium chloride ($MgCl_2$) and calcium chloride ($CaCl_2$) are used as alternatives depending on the temperature. NaCl is an effective road salt for temperatures above -12˚C (10˚F), while $MgCl_2$ and $CaCl_2$ are used for temperatures below -21˚C (-5.8˚F) (Tiwari and Rachlin 2018). Road salt is not a US EPA controlled substance (Cunningham et al. 2008) and salt application is not tested or controlled by the EPA. This leads to detectable salt ion concentrations in soil due to its repeated applications and runoff from precipitation and snowmelt. Soils nearest to roads and paved surfaces have the highest concentrations of salts. Soils located farther away from a paved roadway have lower salt concentrations; however, salts are still present in these soils. Long-term studies conducted by Cunningham et al. (2008) found a significant decrease in Mg^{2+} ~90 meters away from a paved surface. Equiza et al. (2017) also observed a decline in Na^+ with a greater distance away from the main road. Accumulation of NaCl can negatively affect the soil quality by changing the abiotic conditions of the soil. Salt accumulation has been shown to reduce water permeability, increase soil alkalinity, cause poor soil aeration, change soil structure, and increase soil electrical conductivity (Fay and Shi 2012; Bäckström et al. 2004; Equiza et al. 2017). Changes in the abiotic conditions from salts influence the soil and the surrounding environment. The salts are stored for a short period in roadside soils (Tiwari and Rachlin 2018) until infiltrating water moves the salt ions through the soil profile. The ions can be absorbed by the root of roadside vegetation (Tiwari and Rachlin 2008; Equiza et al. 2017; Laffray et al. 2018) and uptake from the roots result in [Na^+] accumulation in tree leaves.

Soil salinity and salt stress in leaves are therefore linked. Soil electrical conductivity is a common measure of salt ions in soils that can detect various salts used in deicers. Equiza et al. (2017) found that with higher soil electrical conductivity exceeding 2 dS m^{-1}, leaf chlorophyll concentrations are reduced, and the leaves have higher [Na^+] in elms, ash, pine, and spruce trees located on urban roadside sites. Soil salinity was the main factor for leaf chlorophyll reduction in trees, which causes reduced photosynthetic activity. This reduction in chlorophyll concentrations can be used as a proxy measurement for the reduced photosynthesis. Munns and Tester(2008) found that long-term salt stress in plants decreases stomatal response and causes a reduction in photosynthetic rates over time (Munns and Tester 2008). Reduced photosynthetic rate caused by salt-stressed plants has been attributed to two hypotheses. Salt stress can: (1) directly limit CO_2 diffusion between stomata and the mesophyll, affecting the photosynthetic activity, or (2) indirectly affect plant photosynthesis in which plant tissues not responsible for photosynthetic activity are causing reduced photosynthetic rates (Chaves et al. 2009). Loreto et al. (2003) came to a similar conclusion to Chaves et al. (2009), however, they did not associate salt stress with photosynthetic activity directly. They associated salt stress only with a reduction of stomatal and mesophyll conductance. [Na^+] is a large ion and in leaf tissue where gas exchange occurs can lower CO_2 diffusion rates (Equiza et al. 2017; Munns 2002; Munns and Tester 2008). The outcome of salt accumulated in plant tissue from both hypotheses is reduced photosynthetic rates, however, the mechanism by which salt stress affects processes related to photosynthesis has not been experimentally confirmed. A related process is isoprene emission, a stress compound and trace gas emitted by some plants.

Isoprene (2-methyl-1, 3-butadience) is a biogenic volatile organic compound (BVOC) emitted by certain plant genera. Isoprene synthesis and its emission is species-specific, and the production of isoprene depends on light exposure, elevated temperature, and rate of photosynthesis (Fall and Monson 1992; Geron et al. 2001). Photosynthetic activity in the plant tissue initiates isoprene synthesis and the molecule is emitted into the atmosphere through the stomatal opening. Because isoprene is emitted during high-temperature extremes and with the leaf exposed to full sun or light, it is hypothesized to be a mechanism to protect the structures responsible for photosynthesis during high-temperature extremes (Sharkey et al. 2007; Vickers et al. 2009). Once emitted from the plant, isoprene molecules lead to the production of the tropospheric ozone through interaction with nitrogen oxides in the atmosphere. Isoprene emissions have a negative impact on regional air quality (Geron et al. 2001). Since isoprene emission is tied to photosynthetic processes, salt stress could hypothetically reduce isoprene emissions thereby reducing tropospheric ozone formation.

Photosynthetic activity in plants has a direct effect on isoprene synthesis and emission rates. Environmental stressors, specifically drought, reduce photosynthetic rates. The effects of drought stress on isoprene emissions have been previously studied and have shown a reduced photosynthetic activity associated with drought (Chaves et al. 2009; Potosnak et al. 2014; and Zheng et al. 2017). Severe drought has complex effects on photosynthetic rates and isoprene emissions. These effects have been modeled in one study (Potosnak et al. 2014) in which the author found an initial increase in isoprene emissions followed by a decrease during drought as photosynthetic activity decreased. Their finding demonstrates that drought stress ultimately decreases isoprene emissions and photosynthetic activity over time. In a study using remote sensing, Zheng et al. (2017) also showed a negative plant isoprene emission response during a severe drought in 2012; photosynthetic activity and isoprene emissions were reduced over a 3-month drought. Drought stress and salt stress in plants have a similar underlying mechanism, an osmotic effect, that causes reduced photosynthetic activity. Osmotic effect in plants controls the flow and absorption of water from the soil through the roots (Munns 2002; Munns and Tester 2008). The osmotic effect is responsible for inducing salt stress and drought stress in plants by causing dehydration in the plant tissue. Because drought stress and salt stress have a similar effect on photosynthesis, excessive salt concentrations could affect isoprene emissions. In salt-stressed environments, isoprene emitting plants accumulate salts from the soil that flow into the roots then plant tissue and decrease stomatal conductance in leaves (Munns and Tester 2008). Secondly, salt molecules stored in the leaf tissue limit the diffusion rate of carbon dioxide through the stomata, reducing photosynthetic rates, and thus isoprene synthesis and emissions. Therefore, increased sodium concentrations from road salts in isoprene emitting plants could reduce leaf-level photosynthetic activity and hence isoprene emissions.

Research Objective

Justification and Motivation

Due to the accumulation of road salts in the soil, high soil salinity has been observed well after the wintertime. Road salt accumulation in plant tissue has been shown to reduce photosynthetic activity in leaves by limiting stomatal conductance. Isoprene is a plant volatile emission produced via the photosynthetic pathway and its production is independent of stomatal closure in leaves. Once emitted, isoprene reacts with oxides of nitrogen to produce ozone, an air pollutant. There is no evidence in the literature of what effect road salts have on leaf-level isoprene emissions. Studies on drought stress have found reduced stomatal conductance decreases photosynthetic activity and isoprene emissions. Salt stress is analogous to drought stress for photosynthesis, and so salt stress could reduce leaf-level isoprene emissions. If road salt accumulation in soils reduces isoprene emissions, then road salt application has the potential to improve regional air quality. However, this will be accompanied by reductions in photosynthesis, which would negatively impact plant function.

Research Question and Hypothesis

We hypothesize [Na^+] from road salt will suppress leaf-level isoprene emissions.

H_o: [Na^+] does not affect leaf-level isoprene emissions
H_A: [Na^+] suppresses leaf-level isoprene emissions

Because of the pandemic, no ion measurements were conducted. Only soil salinity, as estimated by soil conductivity, was measured. Therefore, the hypothesis was only tested for soil salinity and not leaf chemistry.

Research Methods

This experiment used field measurements and laboratory experiments (Table 1). For the field measurements, an initial 25 sites were selected by tree species present and proximity (*near-road*) or lack of proximity (*non-road*) to presumably-salt treated roads. Of these 25 sites, 15 were *near-road* sites and 10 were *non-road* sites. The 25 sites were screened for soil salinity using a soil conductivity proxy with a field-deployed instrument (POGO TurfPro). For the *near-road* and *non-road* site categories, the 5 highest and 5 lowest soil salinity sites were selected for further analysis. Leaf-level isoprene emissions, photosynthesis, and laboratory-based soil salinity were measured for three trees at each of these 10 sites. Leaf and soil [Na^+] and [Cl^-] were intended to be measured but that was not possible due to COVID19. For the lab measurements, velvet beans seeds (*Mucuna pruriens*) were raised in a growth chamber. The laboratory experiment employed two sequential methods with varied salt application and measurement frequency: salt accumulation and prolonged salt stress. The velvet bean plants were salt-stressed with NaCl-spiked Hoagland solution and leaf-level isoprene emissions and photosynthesis were measured over three months for the two sequential experiments. Leaf [Na^+] and [Cl^-] were also intended to be measured in the laboratory experiments but again this not possible due to COVID19.

Field Site Selection

European buckthorn is the most abundant invasive woody species in the Chicagoland area and is often found on forest edges like roadsides and recreational trails (Kua et al. 2020). European buckthorn (*Rhamnus cathartica*) is also a strong isoprene emitter and was selected as the tree species used for the field measurements. First to find European buckthorn trees at sites suitable for testing the hypothesis, we used google maps to identify forest preserves in Cook and Lake counties that were located near a major roadway. We identified 5 preserves in Lake County and 5 preserves in Cook County. After the preserves were identified, each preserve was visited in person looking for suitable *non-road* or *near-road* sites. Each site was classified as *non-road* or *near-road* qualitatively. The *near-road* sites were identified as containing groups of three individual European buckthorn trees on an edge near a paved and presumably salted road. The *non-road* sites were identified as three edge trees (for example, trees along an edge with turf grass or walking path) that are distant from a road and were presumably not exposed to road salt. *Non-road* sites were further than 100 m from a road and *near-road* were within 15 m of a road. We identified 15 *non-road* and 15 *near-road* candidate sites within the preserves. After this step, sites that were not suitable due to lack of access, canopy cover, and gravel were removed and 25 candidate sites were selected for soil salinity screening (15 *near-road* and 10 *non-road*). The 25 candidate sites were located within four forest preserves in Lake and Cook County, Illinois (**Figure 3 and Table 2**). To find sites that were most impacted by road salt application (*near-road*) or not (*non-road*), we surveyed the 25 candidate sites and measured for soil salinity index with a POGO TurfPro field instrument. The distance from each site to the nearest road and the latitude and longitude of each tree were also measured. Next, soil properties (**Figure 2**) were measured at all three trees at each site using a POGO TurfPro . The goal was to determine 5 *near-road* and 5 *non-road* sites that had the largest difference in field-measured soil salinity index (**Table 2**).

At each tree using a meter tape, a 1-meter line was laid out at the base of the tree trunk facing the road. From the end of the transect away from the tree, a 2-meter perpendicular transect was measured (**Figure 2**). A POGO TurfPro was used to measure volumetric soil moisture content (%), electrical conductivity (dS/m), temperature (˚C), and salinity index (dS/m) at distances of -1.0 m, -0.5 m, 0 m, 0.5 m, and 1.0 m relative to the center of the perpendicular transect. This preliminary step was used to test sites for soil salinity index and sort the sites from relativity low to relativity high average salinity index. Salinity index is a derived quantity: the POGO electrical conductivity reading is divided by POGO volumetric soil moisture reading. For each site, the soil points were averaged. There were 15 *near-road* sites and 10 *non-road* sites with initially 75 total trees (**Table 2 and Table 3**). Because of construction, site 22 had only two trees instead of three at the end of field site selection, so there are only 74 total trees (**Figure 3**). We selected the 5 relatively highest soil salinity index sites for *near-road* sites, which had an average POGO salinity index greater than 1.06 dS/m based on our field screening. The cutoff point was arbitrary and based on our observed measurements. Relativity lower salinity index sites were selected for *non-road* sites, which had an average POGO salinity index less than 1.05 dS/m (**Table 2**). After this step, the highest 5 *near-road* and the lowest 5 *non-road* sites were selected for further soil and leaf testing.

<u>Field Measurements</u>

After the 5 *near-road* and 5 *non-road* sites were selected for further analysis, each tree from the site was revisited for soil and leaf testing. In July 2019 and August 2019, soil samples were collected to ~8 cm at each soil point (15 soil points) with a trowel until one soil bag (~500 g total) was full for one composite soil sample per tree. Soil electrical conductivity and pH were measured following the method of (Carter and Gregorich 2008) using a Hanna Instruments pocket pH/EC tester. Electrical conductivity and pH are good indicators of salts in soil. If the soil conductivity is non-saline (0.0 dS/m-2.0 dS/m) or slightly saline (2.2 dS/m-4.0 dS/m), that site is considered low. If the soil conductivity is strongly saline (8.1 dS/m-16.0 dS/m) or very strongly saline (> 16.0 dS/m), the site is considered high. In the lab, the soil samples were oven-dried for 12 hours, ground, and sieved. The same trees were measured for photosynthesis (μmol m^{-2} s^{-1}) and leaf-level isoprene (nmol m^{-2} s^{-1}).

Along with soil testing, leaf measurements were conducted on the European buckthorn trees in July and August of 2019 at the field sites. Leaf-level isoprene emissions and photosynthetic rates from one leaf on the 3 trees was collected with a Licor LI-6400 leaf-gas exchange system (LI-6400, LI-COR Biosciences, Lincoln, NE). The leaf was placed in the cuvette (2 x 3 cm) for 15 minutes to acclimate to the controlled conditions (Figure Supplement 1). The Licor conditions were set to a leaf temperature of 30 ˚C, photosynthetically active radiation (PAR) of 1000 µmol/m^2/sec, and CO_2 control reference of 400 ppm. These are standard specifications for replicating the conditions that have long been agreed upon by the scientific community (Sharkey et al. 2007). After 15 minutes, the photosynthetic rate and leaf temperature was recorded. The dynamic system had a continuous flow rate of ~750 mL/min and 1 liter of air was collected directly from the leaf exhaust outlet with an inflatable sample bag (Sample Pro, model 236–001, SKC Inc., Eighty-Four, PA). The air samples were analyzed for isoprene in the lab within 8 hours of the collection using a gas chromatograph with a flame ionization detector (GC/FID).

Lab Measurements

Field measurements can reveal real-world impacts of road salt under outdoor, urban conditions. For lab measurements, we wanted to reduce environmental variation and identify if there was a measurable effect between road salts and isoprene emissions. The treatment in the lab measurements was application of a NaCl solution and a control. In the DePaul University growth chambers, a controlled experiment was conducted for 24 weeks using velvet bean plants (*Mucuna pruriens*). Velvet beans are a strong isoprene emitters that grow quickly. Sixteen plants were grown from seeds in the greenhouse with 3.87 L of quartz sand as the substrate. Hoagland solution was used to provide nutrients and the treatment of sodium chloride. Two methods were used sequentially to test the impact of salts: salt accumulation and prolonged salt stress. In the salt accumulation experiment, on 10/21/2019 16 plants were placed in the two growth chambers. The plants were randomized and evenly distributed by salt treatment in two growth chambers set at 22˚C with 60% relative humidity (RH) for nighttime conditions (0:00 – 6:00 and 20:00 – 24:00) and 26˚C with 60% RH for daytime conditions (6:00 – 20:00), reflecting typical temperate growing conditions. Atmospheric CO_2 was set to 450 ppm for the entire time. The salt accumulation experiment tested for the effects of salt accumulation with 8 plants received 0 M NaCl Hoagland solution and the other 8 plants received up to 0.175M NaCl Hoagland solution following the procedure of Loreto et al. (2003) (**Figure 4**). For the treated pots, 0.175M NaCl solution is a concentration of NaCl that causes salt stress in plants, while 0 M NaCl is the control group (Loreto et al. 2003; Munns 2002). In the treated pots, NaCl solution was added three or more times a week starting from 0.025 M NaCl ending at 0.175 M for six weeks on 12/19/2019 and were measured after salt accumulated in the pots (**Table Supplement 2**). The 16 pots were measured for leaf-level isoprene emissions and photosynthesis with a Licor LI-6400 leaf-gas exchange system from mature leaves, which accumulate more salt, (Munns 2002; Munns and Tester 2008; Loreto et al. 2003) in the same method as field measurements. Leaf-level isoprene emissions and photosynthetic rates were measure once a month. The maximum estimate of added NaCl was 8 grams per treated pot based on the concentration of the solution and the total amount applied.

Due to plant mortality on 1/10/2020, the experiment was adjusted to continue working with the surviving control plants. The second method, the prolonged salt stress experiment, tested for periodic salt applications and started with the 8 control plants from the salt accumulation experiment (**Figure 4**). Four treated and four control plants were then selected and the NaCl solution was added as a treatment starting at 0.150 M and finishing at 0.175 M of NaCl after five weeks. The maximum estimate of added NaCl was 1.89 grams per treated pot. The pots were sampled for leaf-level isoprene and photosynthesis approximately weekly. The prolonged salt stress experiment ended on 3/14/2020 due to the start of the pandemic (**Table Supplement 2**).

Table 1 documents the changes and pending tests that needed to be changed because of COVID19. We could not finish the soil chemistry test and leaf chemistry test for field measurements and lab measurements because of lack of access to equipment. A partial soil analysis was completed. We did not conduct any leaf chemistry analysis. In the future, we will consider completing these tests.

Statistical Methods

To analyze the data collected from the field measurements, the 15 soil measurements from each site were averaged. The field site selection and field measurements variables were combined and tested to determine if there was a significant difference between the *near-road* and *non-road* sites using a t-test (n = 5). A Pearson Correlation was conducted to test for significant correlations and confounding variables. There were six variables from our methodology and field measurement samples were further analyzed with MANOVA, bar plots with standard error bars (t-tests), and linear regression: POGO volumetric soil moisture content (PSM), POGO electrical conductivity (PEC), POGO salinity index (PSI), Hanna electrical conductivity (HEC), Licor photosynthetic rate (LP) and isoprene (IP). For the lab measurements, t-tests and linear models (repeated measures) were used to test the treatment effect on isoprene and photosynthesis during the salt accumulation and prolonged salt stress experiments. The treatment in each experiment was application of a NaCl solution. Salt accumulation had two measurement time periods of photosynthesis and isoprene emission and prolonged salt stress had similar measurements weekly.

Results

Variables measured with our field-site selection methodology were compared for differences between *near-road* and *non-road* sites: POGO volumetric soil moisture content (PSM), POGO electrical conductivity (PEC), and POGO salinity index (PSI). POGO soil temperature (PT) was also measured but assumed to be unaffected by the treatment *a priori* and subsequently was determined to be not statistically different. POGO volumetric soil moisture and POGO electrical conductivity are independent variables and are used to compute the salinity index. The average POGO volumetric soil moisture (Figure 5, top) for *non-road* and *near-road* sites was not significantly different (**p-value = 0.68**) and there was no *a priori* reason to assume that soil moisture would vary by proximity to the road. POGO volumetric soil moisture was therefore not a confounding variable. The average POGO electrical conductivity from field site selection (Figure 5, bottom) for *non-road* and *near-road* sites was not significantly different (**p-value = 0.11**). While somewhat counter-intuitive, POGO electrical conductivity depends on both soil moisture and salinity and therefore is highly variable. POGO salinity index from field site selection (Figure 6, top) was statistically different (**p-value = 0.006**), which follows from the fact that we used that variable to select the 5 *near-road* and 5 *non-road* sites. The salinity was further examined with a lab-based method after site selection. Measurements of the saturated-paste salinity technique using a Hanna electrical conductivity (HEC) probe were also used to compare the soil electrical conductivity between *near-road* and *non-road* sites. The average HEC soil salinity for (Figure 6, bottom) the *non-road* and *near-road* sites was statistically significantly different (**p-value = 0.003**). *Non-road* had a lower electrical conductivity than the *near-road* sites, as expected. Using a linear model (Figure 7), there was a positive relationship between soil HEC soil salinity and the POGO salinity index from field site selection methodology (**R^2= 0.95, p-value=1.565 x 10^{-7}**). This confirmed the validity of the POGO site selection method and the utility of using the POGO to identify near-road sites in the field without laboratory measurements. The saturated paste soil pH from the Hanna probe (**p-value = 0.98**) was also not statistically different between the *near-road* and *non-road* sites.

Leaf-level Field Measurements

Leaf-level physiology measurements were conducted and results were tested against site type (*near-road/non-road*) and versus soil properties. Licor photosynthetic rate (LP) and isoprene (IP) were the leaf-level measurements. Isoprene emissions and photosynthetic rates from the field measurements were compared using a t-test. The average photosynthetic rates (Figure 8, top) were not statistically significantly different (**p-value = 0.44**) between the *non-road* and *near-road* sites. The average isoprene emission rates (Figure 8, bottom) were also not statistically significantly different (**p-value = 0.61**) between the *non-road* and *near-road* sites. Isoprene emission rates have a much smaller range for *non-road* (27.52 -33.73 nmol m^{-2} s^{-1}) compared to the *near-road* sites.

Soil moisture content affects photosynthetic rates from leaves and thus isoprene emissions. The volumetric soil moisture content measured by the POGO instrument from both *near-road* and *non-road* sites ranged from 20.52% - 44.83%, which spans relatively dry conditions to near-field capacity saturation (Ghosh et al. 2017). Field measurements of isoprene and photosynthesis were compared with POGO volumetric soil moisture content using linear regression. There is a positive relationship between photosynthetic rates and volumetric soil moisture content across all the sites (Figure 9, top) which was statistically significant (**R^2= 0.58, p-value = 0.01**). There is a negative relationship between isoprene emissions and volumetric soil moisture content (Figure 9, bottom) which was not statistically significant (**R^2= 0.23, p-value = 0.15**). Our results support that soil moisture content has an effect on photosynthetic rates but soil moisture does not significantly affect isoprene emissions.

Measurements of saturated-paste Hanna electrical conductivity conducted in the lab were compared with isoprene and photosynthesis using linear regression. While there is a negative relationship between photosynthetic rate and Hanna electrical conductivity (Figure 10, top), it was not statistically significant (**R^2= 0.10, p-value = 0.35**) across both the *near-road* and *non-road* sites. There is a positive relationship between isoprene emissions and saturated-paste electrical conductivity (Figure 10, bottom) but again it was not statistically significant (**R^2= 0.05, p-value = 0.55**). The effect of approximity to the road (*near-road* versus *non-road*) on electrical conductivity was not significant and did not have the hypothesized effect on isoprene emissions and photosynthetic rates.

Lab Measurements

Each plant in the salt accumulation experiment was measured twice overall: once per month. The data were aggregated by month to test the effects of NaCl accumulation on isoprene and photosynthetic rates. Photosynthetic rates (Figure 11, top) were not statistically significant (**R^2= 0.27, p-value=0.38, t-test**) between the control and treated pots. The average photosynthetic rates for the control pots in December 2019 pots were higher than in January 2020. Average photosynthetic rates for the treated pots in December 2019 pots were lower than January 2020 pots. Isoprene emissions (Figure 11, bottom) were not statistically different (**R^2= 0.09, p-value=0.82, t-test**) between the control and treated pots. The average isoprene emissions for the control pots in December 2019 pots were higher than January 2020 pots. Average isoprene emissions for the treated pots in December 2019 pots were higher than January 2020 pots. Over time in the growth chambers, the control pots had decreased photosynthetic rates and isoprene emissions. The treated pots had an increase in photosynthetic rates but decrease in isoprene emissions. Since there were variations in time, we also considered a repeated measures test, but treatment differences remained not significant.

In the prolonged salt stress experiment, each pot was measured once a week for five weeks and the data were aggregated by sample date. Using a linear model with time as the repeated measure, the statistical significance was determined between the treated and control plants for photosynthesis and isoprene with periodic salt applications. In the prolonged salt stress experiment, the treated and control pots with respect to photosynthetic rates (Figure 12, top) were not statistically significantly different (**p-value = 0.545**). With respect to isoprene emissions between the treated and control pots (Figure 12,bottom) again the treatment was not statistically significantly different (**p-value = 0.249**). Our results indicate that photosynthesis and isoprene were not statistically impacted by the salt treatment in both the salt accmulation and prolonged salt stress experiments, and thus our results do not support our hypothesis that NaCl decreases isoprene emission.

Discussion

We failed to reject the null hypothesis (H_o: [Na^+] does not affect leaf-level isoprene emissions) and did not observe a decrease in isoprene emissions with higher salt concentrations in any of our experiments. From the field and lab-grown plant experiments, our analysis and models show that isoprene emissions were highly variable, and trends for isoprene emissions were not consistent with salt content (field-measured) or salt application (lab treatment). Despite our field sites having distinct *non-road* and *near-road* salt contents (determined via electrical conductivity), isoprene emissions showed no treatment effect. Isoprene emissions for *near-road* sites were not statistically significantly different or lower than the *non-road* sites. From field measurements, soil moisture and photosynthesis had a positive and significant relationship (Figure 9, top), as expected. Isoprene emission had an insignificant negative relationship to soil moisture (Figure 9, bottom). Similarly, for the velvet bean from the salt accumulation and prolonged salt stress experiments, there were no statistically significant or lessened isoprene emission rates from the treated pots compared to control pots. The pots did vary greatly from week to week, and this large variability could have obscured a treatment effect.

Time of soil sample collections and certain soil properties were important factors in field measurements that affected our results. We collected our soil samples in summer when soil salinity from road salt application did not represent the maximum road salt content. The ideal season to collect soil salinity measurements for road salt application was after winter and at the start of spring (Bonn et al. 2019) before the salts entered waterways and plants. This is consistent with relatively low soil salinities in our *near-road* sites. Soils with electrical conductivities that are considered slightly-saline (2.2 dS/m-4.0 dS/m, Munns and Tester 2008). Our *non-road* (0.88-1.13 dS/m) and *near-road* (0.90-1.52 dS/m) field sites were all in the non-saline category (Figure 6, bottom, 0.0 dS/m-2.0 dS/m). Using another literature criteria, again the low salinity present in our *near-road* sites may have been too low to cause salt stress in the plants. Soil electrical conductivities exceeding 2 dS m^{-1} are above the tolerance level for salt-sensitive plants (Equiza et al. 2017; Munns 2002; Munns and Tester 2008). Our sites never exceeded 2 dS m^{-}, and therefore no reductions in photosynthetic rates should have been expected.

An additional soil property is soil pH; we considered this an important variable in the methodology and the field measurements because soil pH is affected by soil electrical conductivity. Our soil pH (6.70 – 7.47) was neutral for all sites and did not differ between the *non-road* and *near-road* field sites. Due to COVID19, leaf and soil ion testing were not possible, and we used our lab soil measurements to confirm that each site has salts in the soil. Without leaf and soil ion testing, we could not confirm if chloride-based deicers were used and if [Na^+] and [Cl^-] were related to other measured variables. We could not test our hypothesis fully, and we may consider completing this part of the experiment in the future.

Field samples and testing had many factors that we could not control, and in lab measurements we reduced many of the factors to isolate NaCl impacts. Lab measurements differed from field measurements because we used NaCl solution as a treatment, unlike field measurements, which relied on the assumed application of road salt. We salt-stressed the velvet beans and controlled for other variables. The treated pots showed salt stress symptoms: leaf wilting, leaf browning, and recovery after water application (Munns 2002; Munns and Tester 2008 and Baraldi et al. 2019). We expected to see a decreased photosynthetic rate and isoprene emissions. However, we experienced limitations with the sampling frequency and sample size with lab measurements. Fungus formed in the soil and near the stem, which was one cause of plant mortality in the salt accumulation experiment. Another cause could have been very high salt concentrations which crossed the salt stress threshold for velvet bean plants too quickly. Despite these limitations, our field and lab measurements provided insights that other similar studies have observed and discussed, as discussed below.

Salt and drought stress have overlapping outcomes like photosynthetic reduction, but both stressors have different mechanisms and processes in plants, which could result in different isoprene emission rates. Drought and salt-stressed plants are both affected by the osmotic effect, which causes dehydration in plant organs. However, in prolonged salt-stressed plants, two additional physiological responses that differentiate salt stress from drought stress are hyper-ionic and hyper-osmotic effects (Chaves et al. 2009; Munns 2002). With salt stress, the higher concentrations of dissolved salt ions (hyper-ionic), higher osmotic pressure (hyper-osmotic), and dehydration from the osmotic effect may not induce isoprene emissions in a similar way to drought stress. Isoprene production and emission are linked to photosynthesis and environmental stress. Elevated temperatures are one factor that induces isoprene emission (Sharkey et al. 2007). Temperatures 30˚C and above do trigger isoprene synthesis and are hypothesized to protect plant function during heat stress (Vickers et al. 2009). Isoprene emissions and photosynthesis relationships could differ in response to drought and salt stress. Isoprene emission and photosynthesis are a coupled photochemical response in salt stress, but drought stress can also be decoupled (Chaves et al. 2009; Potosnak et al. 2014; Loreto et al. 2003 and Zheng et al. 2017). Isoprene emissions from salt stress may be dependent on photosynthesis, while isoprene emissions from drought stress can be independent of photosynthesis.

Understanding how photosynthesis is affected by salt and drought provides insights into why isoprene emissions respond to stress differently. Salt and drought stress reduces photosynthetic rates by restrictions of CO_2 diffusion in the chloroplast, limiting stomatal opening and CO_2 transport from and to the mesophyll (Chaves et al. 2009; Munns 2002). Thus, stress has a direct effect on plant photosynthetic rates. Plants experiencing more than one type of stress can have various effects on isoprene emissions. Indirect stress like fungal infestations (Niinemets, 2010), as we observed in the salt accumulation experiment from lab measurements, was a biotic stress caused by salt accumulation in the sand that was one cause of plant death. Many stressors could affect photochemical responses or cause death (Baraldi et al. 2019). For example, water stress can occur in the plant during periods of drought or salt stress, but water stress affects photosynthesis primarily. Photosynthesis is more significantly affected by water stress than isoprene emissions (Centritto et al. 2011; Flexas et al. 2013; Saleem et al. 2018 and Bonn et al. 2019) because water stress impairs carbon metabolism by limiting carbon dioxide diffusion from the stoma through the mesophyll and isoprene emissions can rely on stored sugars (Centritto et al. 2011). Our experiment also found statistical significance in field measurements of this relationship (Figure 8 and Figure 9). Volumetric soil moisture had a positive effect on photosynthesis. Sites with higher soil moisture had higher photosynthetic rates in Figure 9. For isoprene, the non-road sites were at field capacity as near-road sites varied, suggesting soil properties like nitrogen (Yuan et al. 2020) or soil type (Table Supplement 1) from these sites could affect isoprene emission (Bonn et al. 2019). Water content and photosynthetic rates have a linear relationship, and water stress affects photosynthetic rates in plants but does not affect isoprene emissions. Isoprene emissions are more resistant to water stress.

Salt stress could reduce isoprene emissions due to direct physiological changes in the plant tissue. Many studies on salt stress and BVOC emissions have observed a reduction in isoprene emissions along with photosynthetic rates (Bonn et al. 2019; Brilli et al. 2013; Flexas et al. 2013; Loreto et al. 2003; and Saleem et al. 2018). Studies similar to ours (Saleem et al. 2018; Bonn et al. 2019; Baraldi et al. 2019 and Brilli et al. 2013) conducted experiments on isoprene emissions and photosynthesis in either water-stress, drought, and/or salt-stressed environments over time using NaCl. Each experiment had a control group and used the same techniques for isoprene measurement and a temperature of 30˚C to standardize emissions. With drought stress, isoprene emissions were higher, with reduced photosynthetic rates, transpiration, and stomatal conductance. In salt-stressed plants, they observed reduced isoprene emissions compared to emission from drought-stressed plants, while transpiration, photosynthetic rates, and stomatal conductance were again reduced as in drought-stressed plants. Isoprene emissions could be a temperature-induced response rather than water stress and salt stress-induced response. Brilli et al.(2013) found that leaf temperatures ranging from 40˚C to 45˚C were the optimum conditions for induced isoprene emissions in water-stressed conditions. In the water-stressed conditions, isoprene emissions were inhibited from 30˚C to 39˚C and again at 50˚C. In standard isoprene emission measurements, 30˚C is most often used for field and lab collections. These findings suggest that induced isoprene emission can occur at higher temperatures in either drought or salt stress, and 40˚C to 45˚C could be the temperature where photosynthesis and isoprene emission decouple. This temperature modification could explain why photosynthesis and isoprene are coupled in salt stress and decoupled in drought stress (Baraldi et al. 2019 and Bonn et al. 2009). In the future, similar studies should increase the temperature to determine the temperature at which isoprene emission decouples from photosynthesis to understand how drought stress affects air quality.

In conclusion, isoprene emissions can be reduced due to salt stress. However, our results did not confirm this effect. Salt stress affects the plants differently and has long-term negative effects on plants and soil quality, which can affect air quality. Photosynthesis is coupled with isoprene emissions in salt stress, and the decreases in plant function are attributed to salt ion toxicity. Changes in soil chemistry influence biospheric interactions between soils and the atmosphere. Salt accumulation in soils has complex effects on greenhouse gas emissions from different soil types, and gas emissions from the soil have the potential to be a major source of regional air pollutants. CO_2 and N_2O emissions from saline-sodic soils have an adverse effect on air quality (Ghosh et al. 2017). Drought stress does not have a similar isoprene emission effect compared with rates due to salt stress. Drought stress decouples photosynthesis from isoprene emissions and induces isoprene emissions, which negatively affects regional air quality by leading to tropospheric ozone production. Future studies should investigate why and how drought stress decouples photosynthesis from isoprene emissions to understand how climate change will affect air quality in areas affected by drought.

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Table 1. The red text indicates experimental protocols that were planned but were unable to be completed due to the COVID19 pandemic.

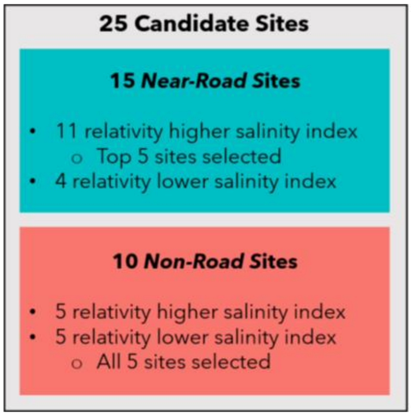
Field Site Selection	**Field Measurements**	**Lab Measurements**
Completed: Identified higher salinity index and lower salinity index sites with strong isoprene emitting trees species • Completed spreadsheet	Completed: **Field** – Collected soil, measured isoprene/photosynthesis, and collected leaves from 10 sites • Tested soil for electrical conductivity and pH • Analyzed leaf isoprene • Analyzed photosynthesis • Completed spreadsheet ** There are 30 dried leaf and 30 soil samples from the field*	Completed: **Lab** – Growth chamber plants salt-stressed, with collected isoprene/photosynthesis and collected leaves • Analyzed isoprene/photosynthesis • But, data quality is suspect, should repeat ** From the salt accumulation experiment, there are 19 samples of dried leaves from the growth chambers.* ** From the prolonged salt stress experiment, there are 40 samples of dried leaves from the growth chambers.* ** There are 16 bags of dried leaves and stems from the pots in the growth chambers.*

This phase is complete.	COVID19 limitations:
	
	• Measure leaves for $[Na^+]$ and $[Cl^-]$
	  ○ Dried, but not ground
	• Measure soil for $[Na^+]$ and $[Cl^-]$
	  ○ Soil is dry and ground
		COVID19 limitations:
		
		• Measure leaves for $[Na^+]$ and $[Cl^-]$
		  ○ All leaves are air-dried
		  ○ All plant material from the pots are air-dried

Table 2. The ten *near-road* and *non-road* sites were selected based on the average Pogo Salinity index from field site selection. Sites with an average POGO salinity index less than 1.05 dS/m were categorized as low. Sites with an average POGO salinity index greater than 1.06 dS/m were categorized as high. Relatively lower salinity index non-road sites and relatively higher salinity index near-road sites were picked for field measurements.

Site				
Site	Type	Average Pogo Salinity Index	Site_Type	Selected
20	Non-Road	0.74	Low	**Yes**
15	Non-Road	0.82	Low	**Yes**
4	Near-Road	0.84	Low	No
8	Non-Road	0.86	Low	**Yes**
16	Near-Road	0.91	Low	No
3	Non-Road	0.91	Low	**Yes**
9	Non-Road	0.97	Low	**Yes**
1	Near-Road	1.02	Low	No
5	Near-Road	1.02	Low	No
21	Non-Road	1.08	High	No
18	Near-Road	1.08	High	No
25	Near-Road	1.08	High	No
11	Non-Road	1.09	High	No
14	Near-Road	1.14	High	No
2	Near-Road	1.14	High	**Yes**
19	Near-Road	1.14	High	No
17	Near-Road	1.15	High	No
12	Non-Road	1.15	High	No
6	Non-Road	1.18	High	No
22	Near-Road	1.21	High	No
24	Non-Road	1.30	High	No
23	Near-Road	1.37	High	**Yes**
13	Near-Road	1.44	High	**Yes**
10	Near-Road	1.49	High	**Yes**
7	Near-Road	1.76	High	**Yes**

Table 3. From the methodolgy, our results from field site selection confirmed the 10 sites selected were had 2 levels of salt exposure.



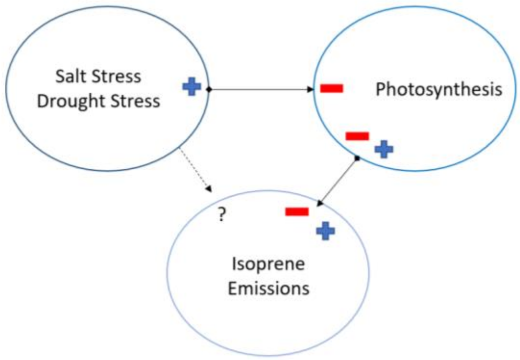


Figure 1. Soil salinity or drought exposure are environmental stressors that can reduce photosynthetic activity in plants. Increase or decreases in photosynthetic activity have the same effect on isoprene emissions, a stress compound. Isoprene emissions are plant emissions linked to photosynthesis and depend on photosynthesis occurring in the leaf from the plant. During events of higher temperatures and light exposure, if photosynthetic rates increase isoprene emissions increase. If photosynthetic rates are decreasing, isoprene emissions decrease.

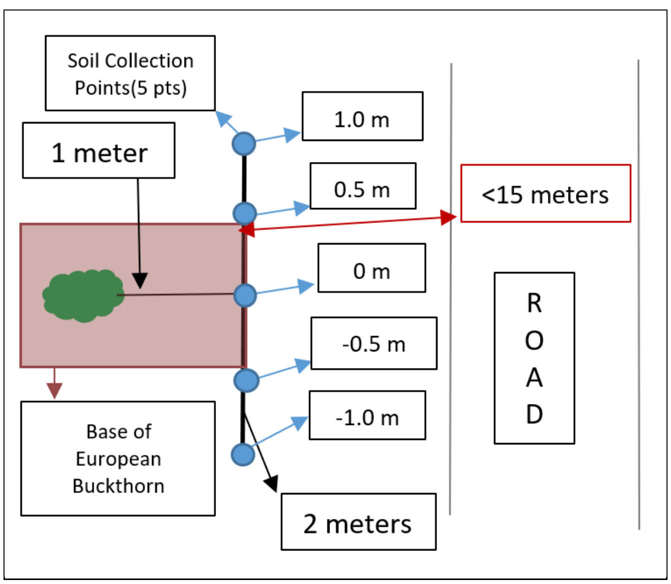


Figure 2. In field site selection, at each tree, this sample protocol was used to collect a soil sample. Cracks in pavement indicated that road salts were applied.

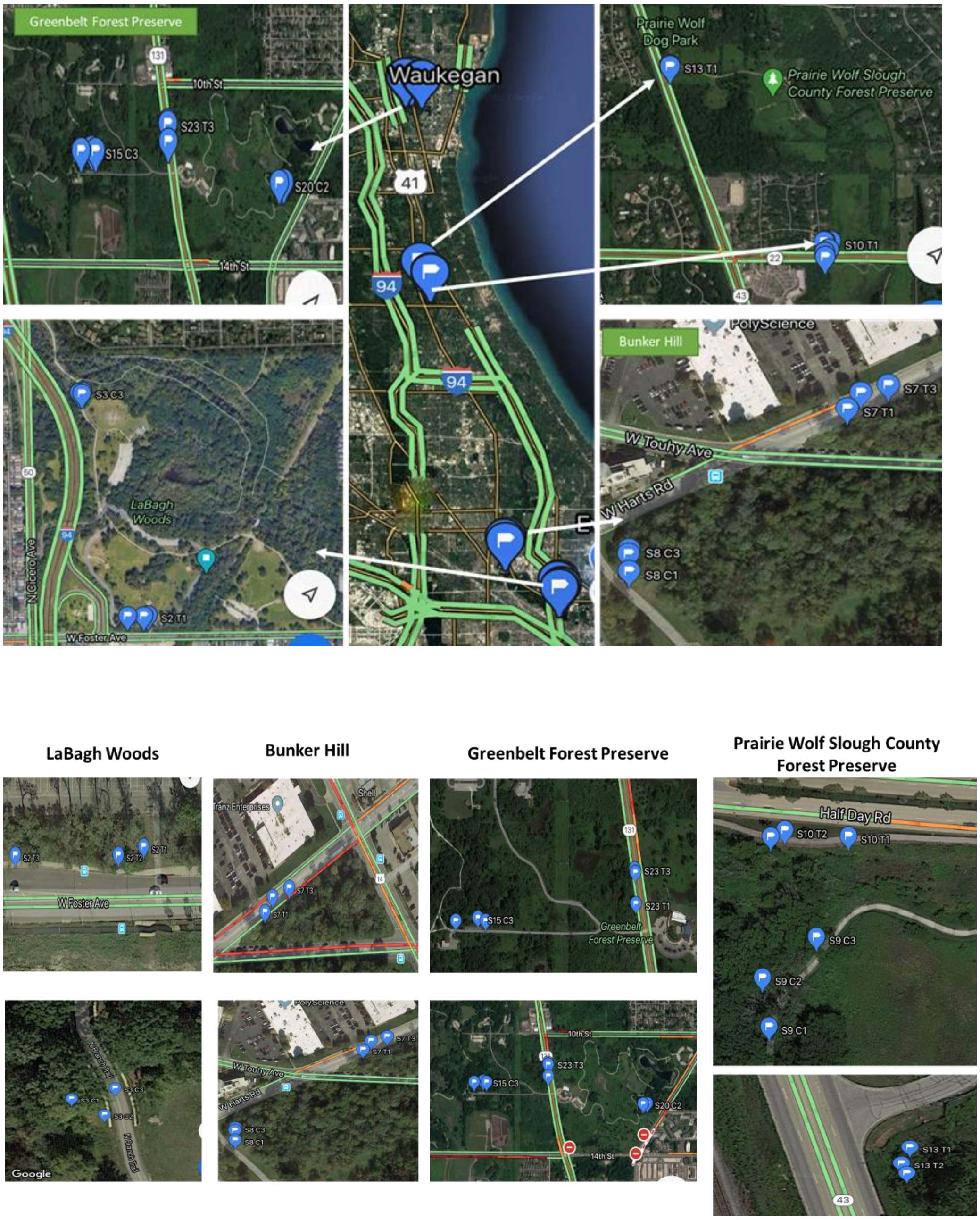


Figure 3. After field site selection and in field measurements, 5 *near-road* and 5 *non-road* were selected for sampling. Each location was identified by site number (S#), treatment (T or C), and tree number (1-3) at the site (top). At each site, the three European buckthorn trees were marked by GPS. *Near-road* sites were located near roadways as *non-road* sites were located away from roadways (bottom).

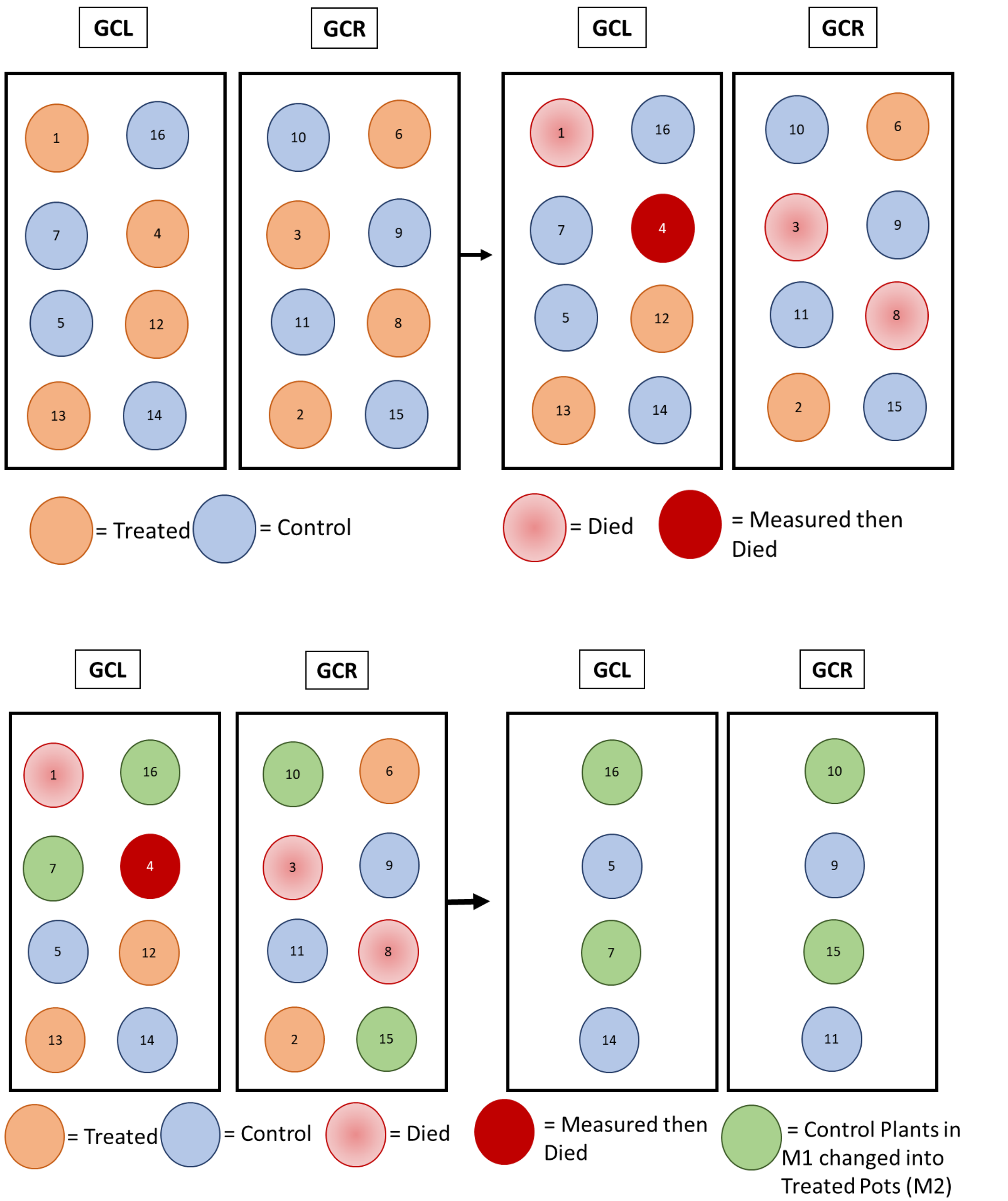


Figure 4. In growth chamber left (GCL) and growth chamber right (GCR) salt accumulation transitioned to prolonged salt stress in lab measurements. In salt accumulation, the treated pots experienced salt stress then died with 0.175M NaCl solution. To prevent additional plant mortality, in prolonged salt stress the NaCl solution was added incrementally to the pots. Two control pots from salt accumulation (M1) were changed to treated pots for prolonged salt stress (M2). The key differences in prolonged salt stress and salt accumulation were sample size and sampling frequencies.

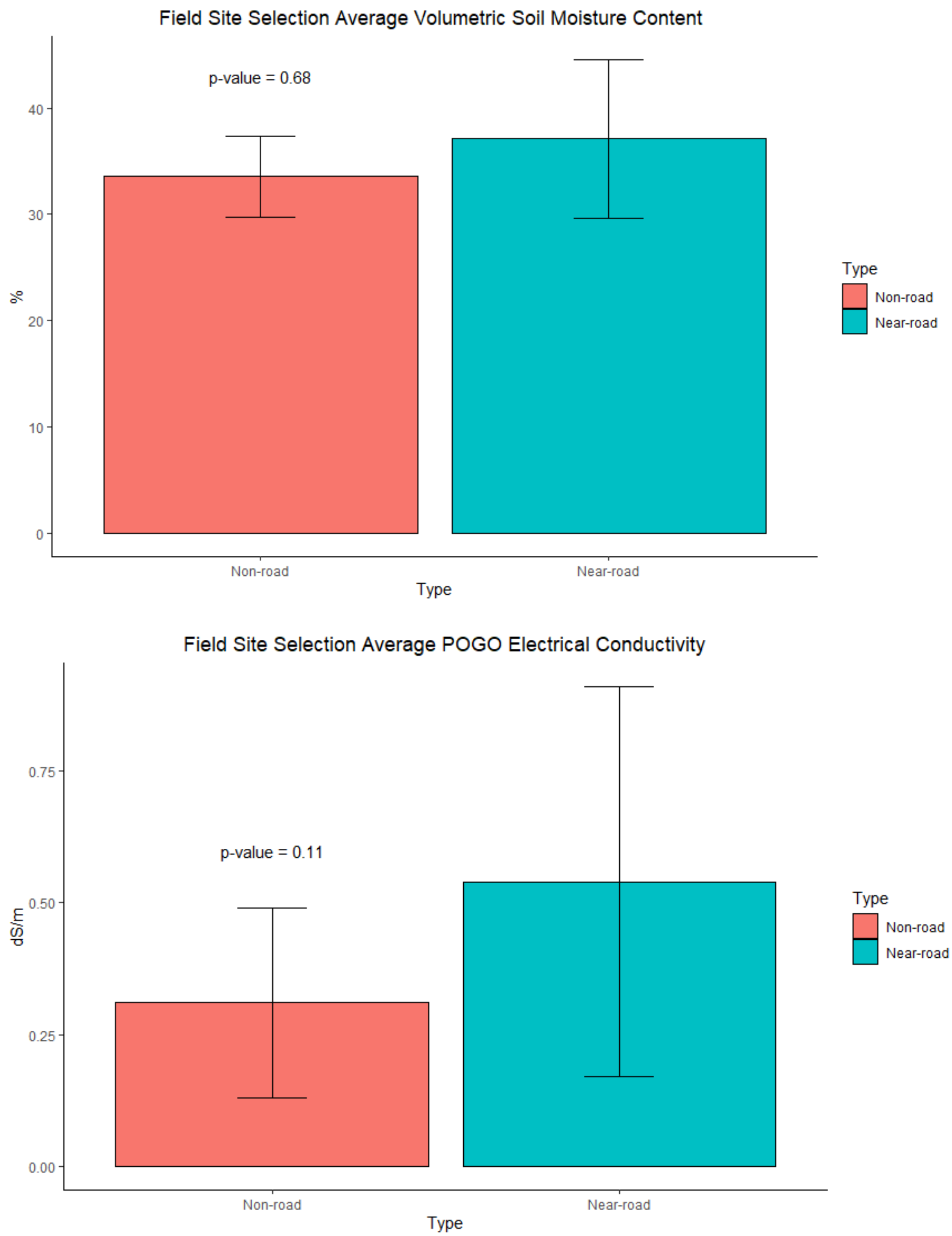


Figure 5. The POGO electrical conductivity (bottom) divided by the POGO volumetric soil moisture content (top) was calculated to give the POGO salinity index (Figure 5, top). Volumetric soil moisture content is an important measure of electrical conductivity but for statistically insignificant for the experiment.

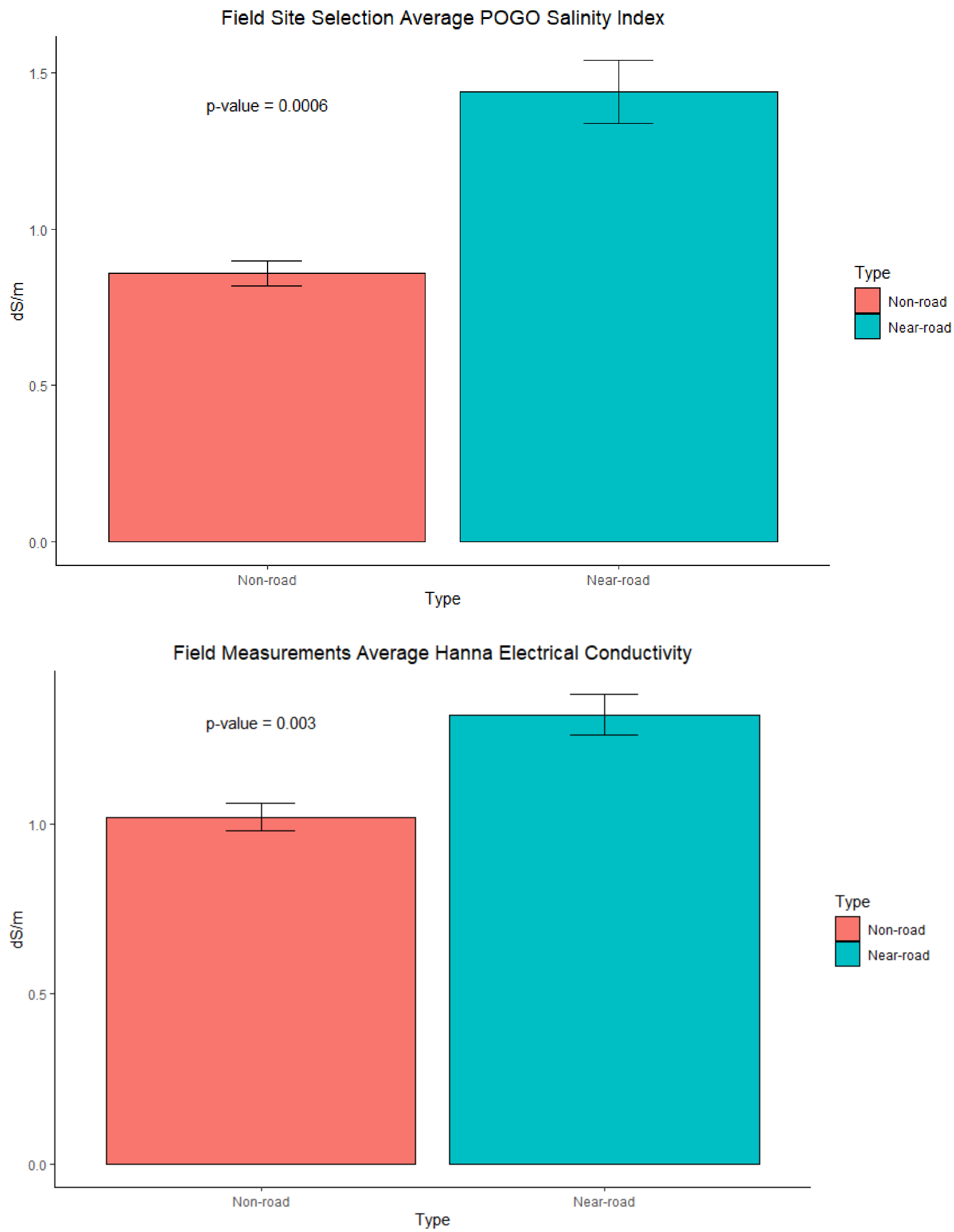


Figure 6. POGO salinity index (top) calculated lower values for salt concentrations from the *non-road* and *near-road* sites than the Hanna electrical conductivity (bottom).

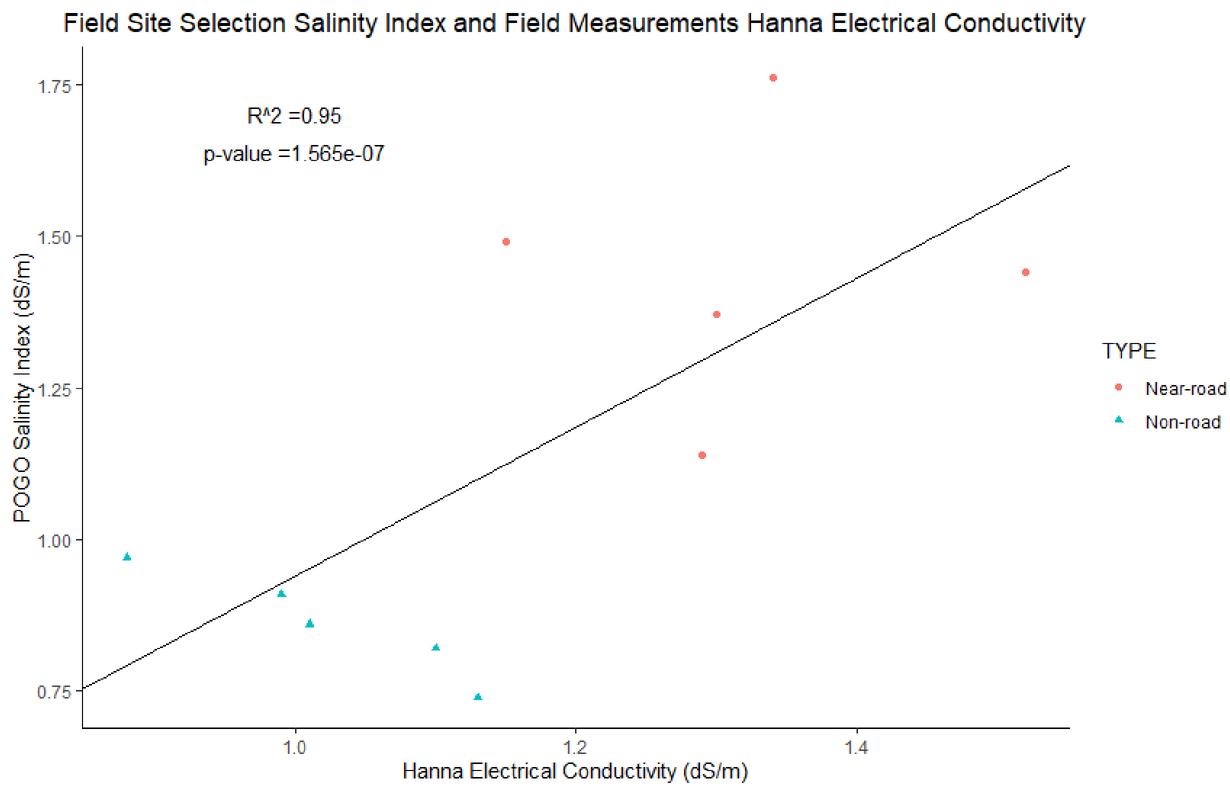
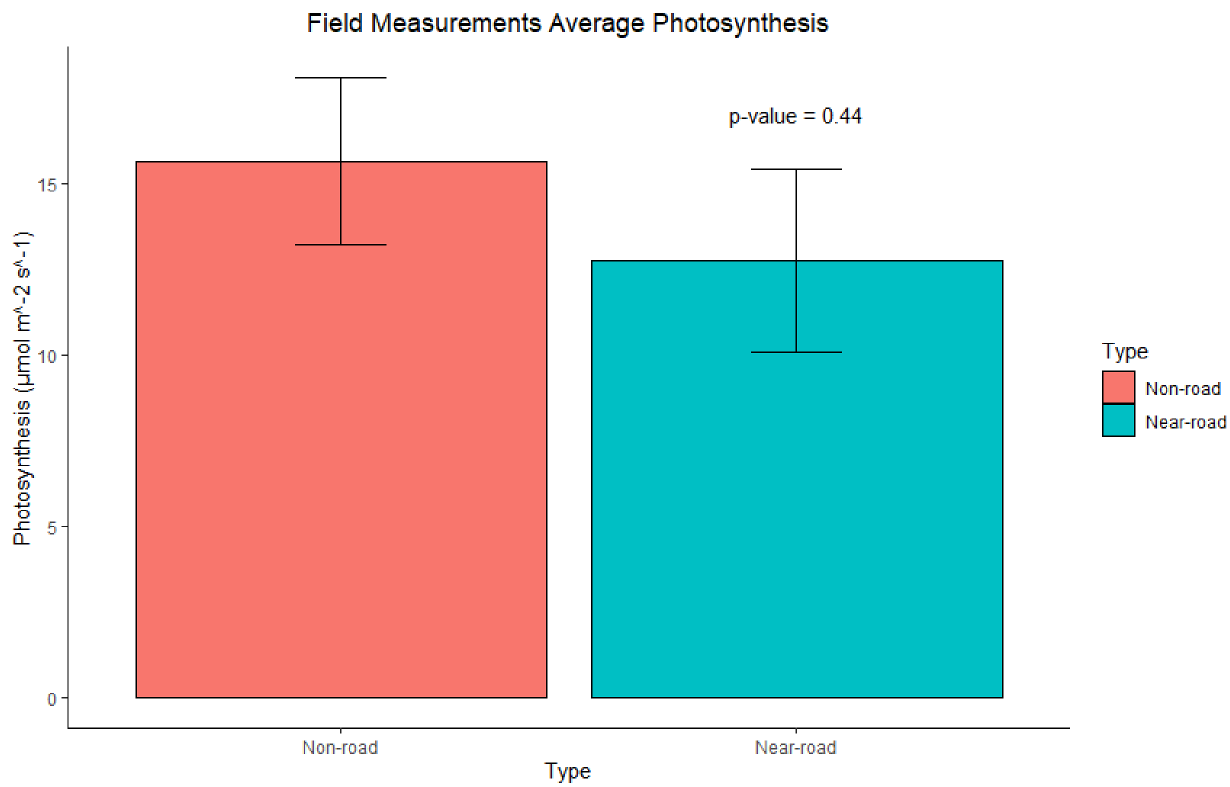


Figure 7. POGO salinity index was used in field site selection to determine field measurements sites. In field measurements using a saturated paste and the Hanna probe, the *non-road* and *near-road* sites were discrete. The POGO salinity index used the site volumetric soil moisture content which was lower soil moisture than the Hanna.



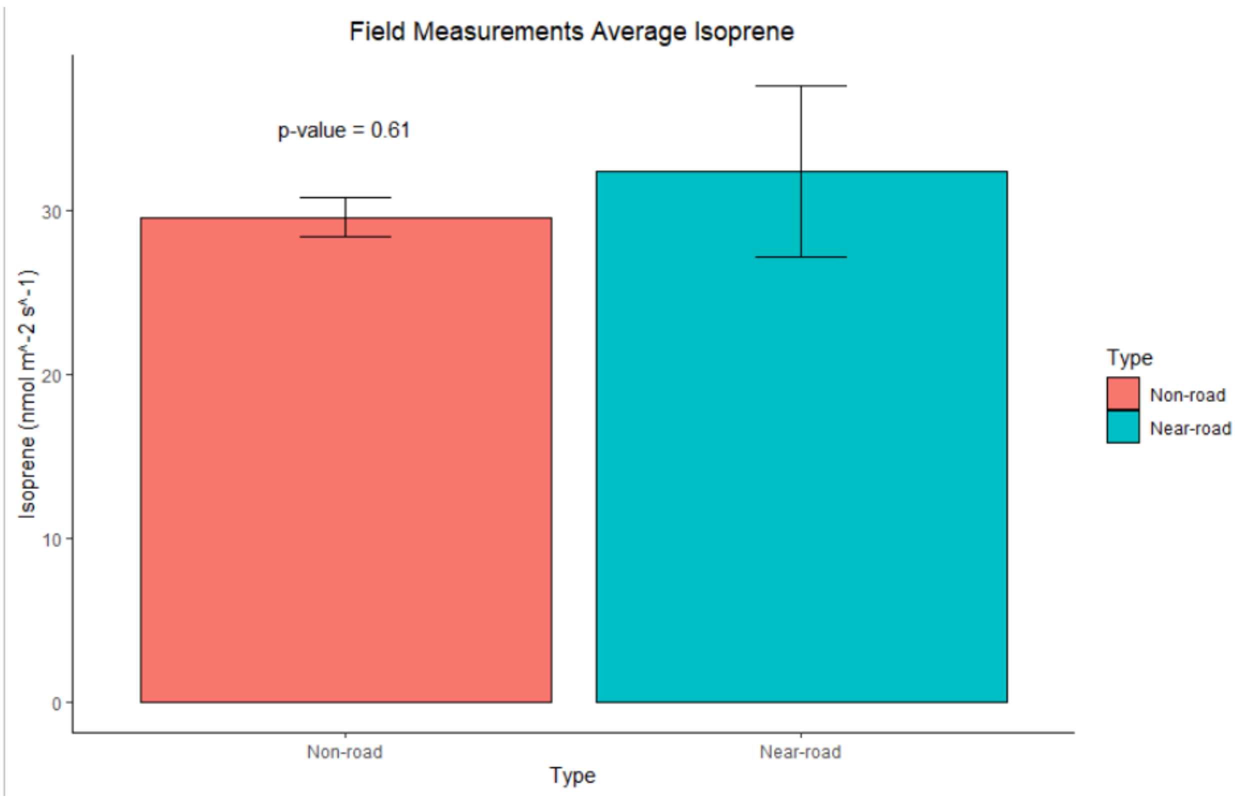


Figure 8. In field measurements, photosynthetic rates (top) and isoprene (bottom) were not significant between *non-road* and *near-road* sites.

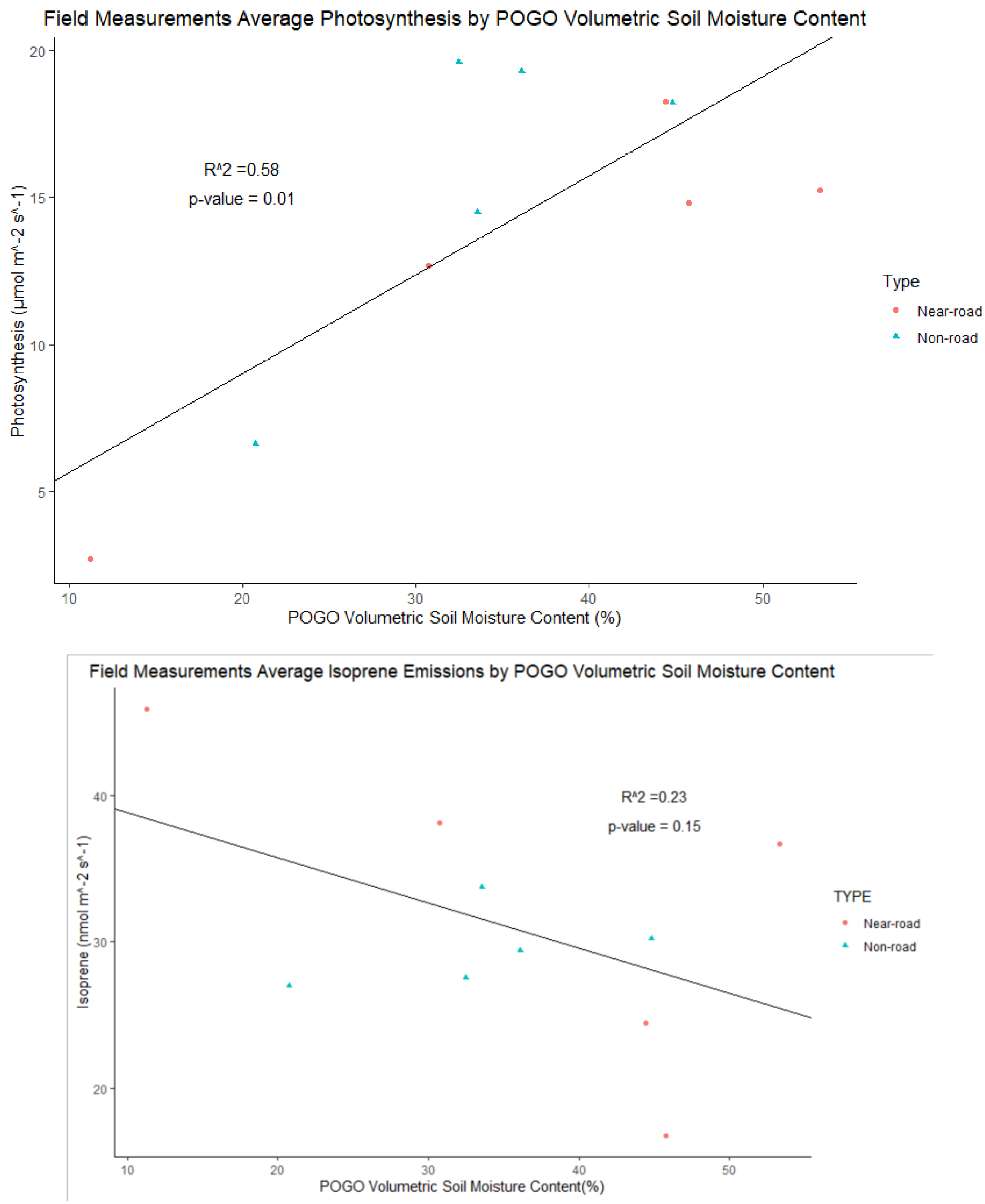


Figure 9. POGO volumetric soil moisture content was an important factor for photosynthesis (top) than isoprene emissions (bottom) between *non-road* and *near-road* sites.

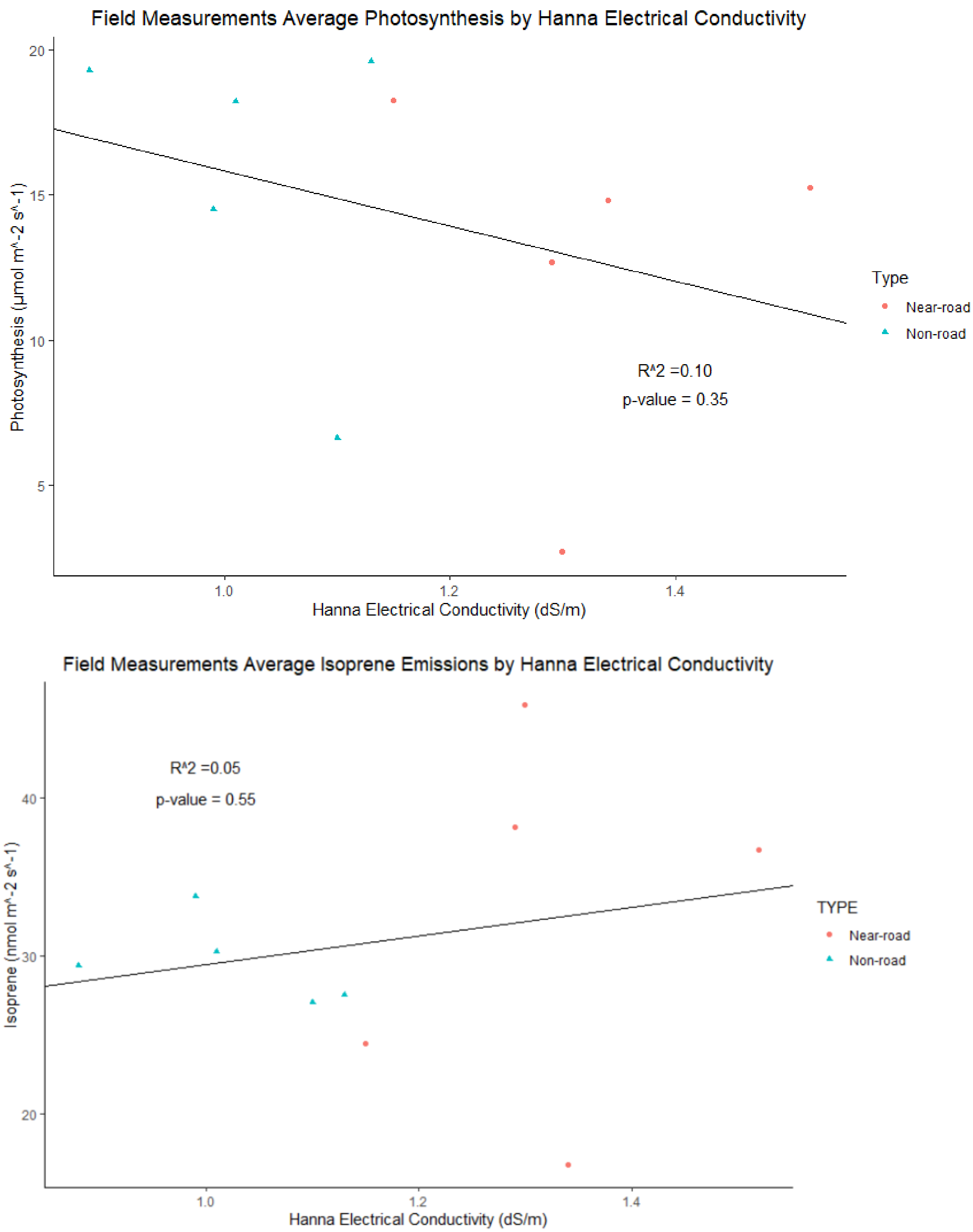
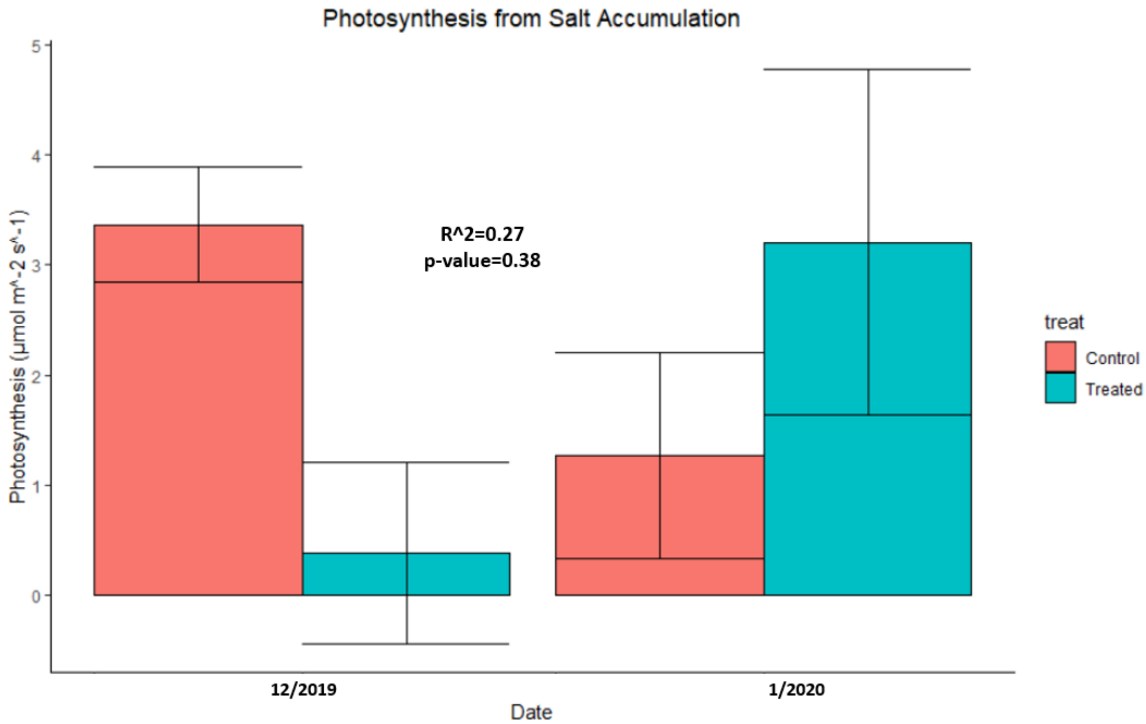


Figure 10. Photosynthesis (top) decreased as isoprene (bottom) increased with increasing electrical conductivity for control and treated pots.



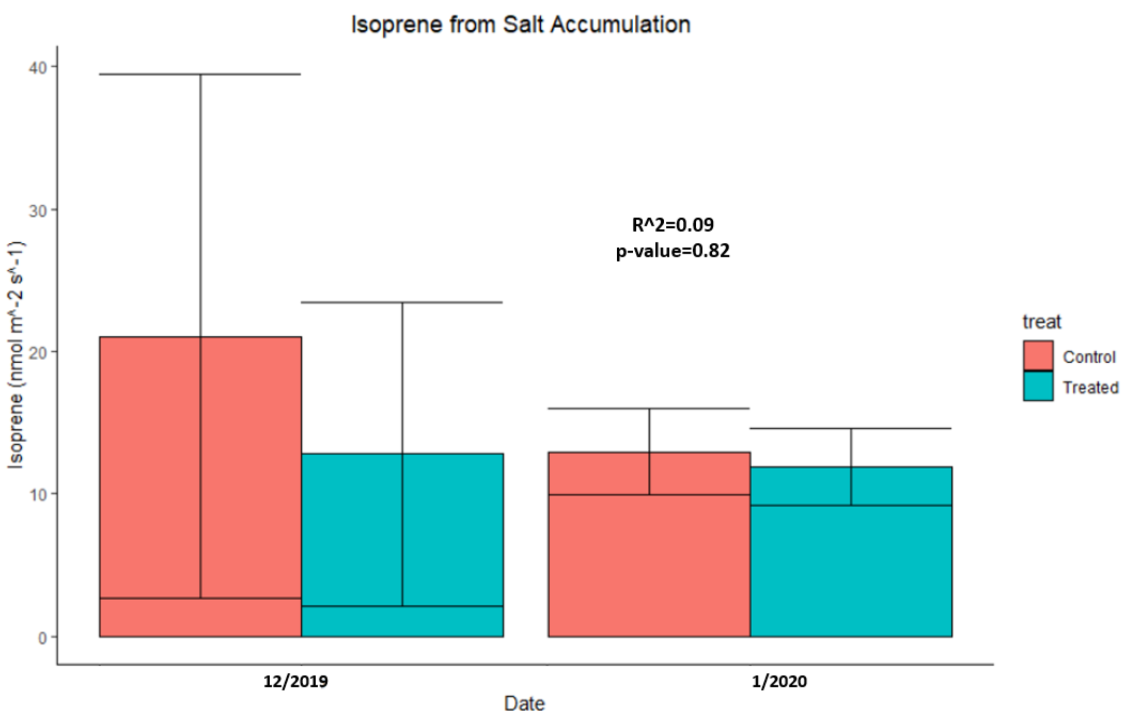


Figure 11. From the salt accumulation experiment, photosynthetic rates (top) and isoprene emissions (bottom) were not significant between the treated and control pots.

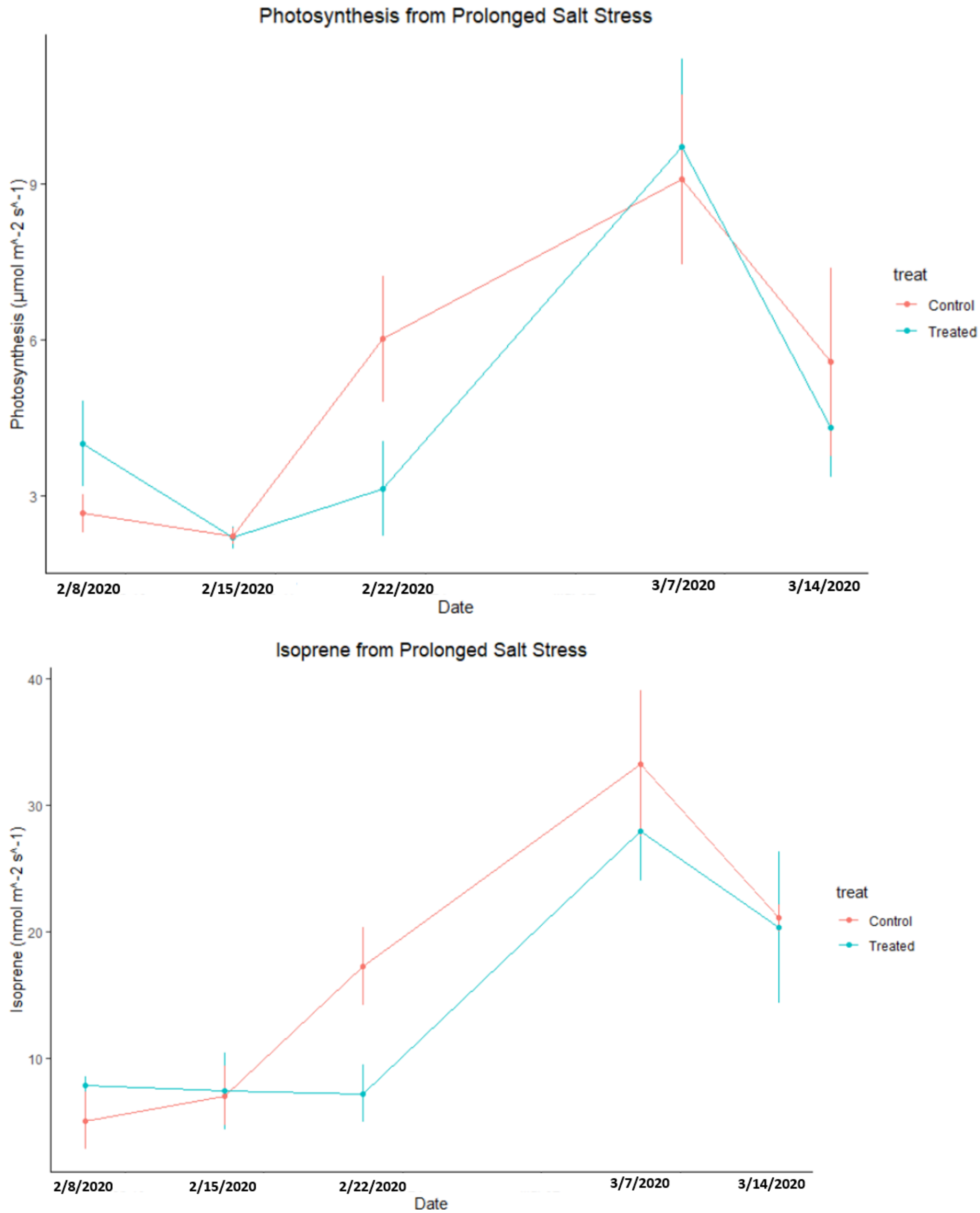


Figure 12. For five weeks from the prolonged salt stress experiment, photosynthesis (top) and isoprene (bottom) was measured with 0.150 M to 0.175 M NaCl solution. We did not observe a significant effect between control and treated pots.

Supplement

Table Supplement 1. This table summarizes at each location the selected sites.

Near-road Sites				
Site #	County	Preserve Name	Locations	Descriptions
Site 2	Cook County, IL	LaBagh Woods Forest Preserve	N 41.97545 W -87.744417	This site is on a sidewalk lined with trees on West Foster Ave near the highway entrance. Soil type is 192A-Del Rey silt loam (Web Soil Survey, USDA).
Site 7	Cook County, IL	Bunker Hill Forest Preserve	N 42.01241 W -87.79038	This site is located on a sidewalk lined with trees surrounded by three major roads. West Touhy Ave, Gross Point Road, and North Caldwell form a triangle with a sidewalk perimeter and woodland in the center. The trees measured here face Gross Point Road near west Touhy Ave. Soil type is 329A-Will silty clay loam (Web Soil Survey, USDA).
Site 10	Lake County, IL	Prairie Wolf Slough County Forest Preserve	N 42.199731 W -87.856885	This site is located on a wooded sidewalk near Half Day Road. Soil type is 153A—Pella silty clay loam (Web Soil Survey, USDA).
Site 13	Lake County, IL	Prairie Wolf Dog Park	N 42.207007 W-87.866146	It is on the roadside of South Waukegan Road lined by trees. Soil type is 298A—Beecher silt loam (Web Soil Survey, USDA).
Site 23	Lake County, IL	Greenbelt Forest Preserve	N 42.337826 W -87.8779329	This site was on the edge of Green Bay Road, west of the Greenbelt Cultural Center. Soil type is 232A—Ashkum silty clay loam and 298B—Beecher silt loam (Web Soil Survey, USDA).

Non-road Sites				
Site #	County	Preserve Name	Locations	Descriptions
Site 3	Cook County, IL	LaBagh Woods Forest Preserve	N 41.981033 W -87.746417	The is site is inside the preserve near the North Branch Trail. Soil type is 192A-Del Rey silt loam (Web Soil Survey, USDA).
Site 8	Cook County, IL	Bunker Hill Forest Preserve	N 42.011051 W -87.792367	This site is located inside the preserve near West Hart Road. Soil type is 329A- Will silty clay loam (Web Soil Survey, USDA).
Site 9	Lake County, IL	Prairie Wolf Slough County Forest Preserve	N 42.199202 W-87.857161	This site is inside the recreational woodland trail near Half Day Road. Soil type is 153A—Pella silty clay loam (Web Soil Survey, USDA).
Site 15	Lake County, IL	Greenbelt Forest Preserve	N 42.336523 W -87.864762	This site was located inside the preserve, west of the Greenbelt Cultural Center. It was surrounded by a recreational trail in the woods. Soil type is 530B—Ozaukee silt loam (Web Soil Survey, USDA).
Site 20	Lake County, IL	Greenbelt Forest Preserve	N 42.336523 W -87.864762	This site was inside the recreational prairie trail near West Dugdale Road. Soil type is 530B—Ozaukee silt loam and 530C2—Ozaukee silt loam (Web Soil Survey, USDA).

Table Supplement 2. Lab measurements started on 10/3/2019 and ended on 3/14/2020. Two methods were used for lab measurements: salt accumulation and prolonged salt stress. This table provides the details and the timeline for the 24 weeks.

<table>
<tr><th>Date</th><th>Lab Measurements Timeline</th><th>Notes</th></tr>
<tr><td>10/3/2019</td><td>Seed germination started</td><td>For weeks 1-3, the seeds germinated.</td></tr>
<tr><td>10/21/2019</td><td>Plants entered growth chambers</td><td>Experimental pots were prepared and placed in the growth chambers on 10/21/2019.
For weeks 4 and 5, the plants adjusted to the chamber environment.
On 11/6/2019, 50 mL of salt treatment started with salt accumulation and was added for six weeks (weeks 6 – 12) more than three times a week to induce salt stress.
<table>
<tr><th>Date Started</th><th># of Treatment</th><th>NaCl Concentration</th></tr>
<tr><td>10/19/2019</td><td>NA</td><td>0 M</td></tr>
<tr><td>11/6/2019</td><td>5</td><td>0.025 M</td></tr>
<tr><td>11/14/2019</td><td>5</td><td>0.075 M</td></tr>
<tr><td>11/20/2019</td><td>5</td><td>0.1 M</td></tr>
<tr><td>11/25/2019</td><td>5</td><td>0.125 M</td></tr>
<tr><td>12/3/2019</td><td>3</td><td>0.15 M</td></tr>
<tr><td>12/9/2019</td><td>4</td><td>0.175 M</td></tr>
</table></td></tr>
<tr><td>12/19/2019</td><td>Lab measurements of salt accumulation experiment ended</td><td>Salt treatment stopped due to plant mortality on week 12.
The pots were sampled for isoprene and photosynthesis in December and January (weeks 11- 15).</td></tr>
<tr><td>1/10/2020</td><td>Lab measurements of prolonged salt stress experiment started</td><td>After sampling for 5 weeks, the plants were observed and under-recovery. Hoagland solution and DI water were added during weeks 16- 18.
On 2/5/2020 (week 19), 50 mL of salt treatment started the prolonged salt stress experiment.
<table>
<tr><th>Date Started</th><th># of Treatments</th><th>NaCl Concentrations</th></tr>
<tr><td>2/5/2020</td><td>2</td><td>0.15 M</td></tr>
<tr><td>2/19/2020</td><td>2</td><td>0.175 M</td></tr>
</table></td></tr>
<tr><td>3/14/2020</td><td>Lab measurements of prolonged salt stress experiment ended</td><td>For weeks 19 - 24, salt treatment was added and sampled once a week.
The last sample date was 3/14/2020.</td></tr>
</table>

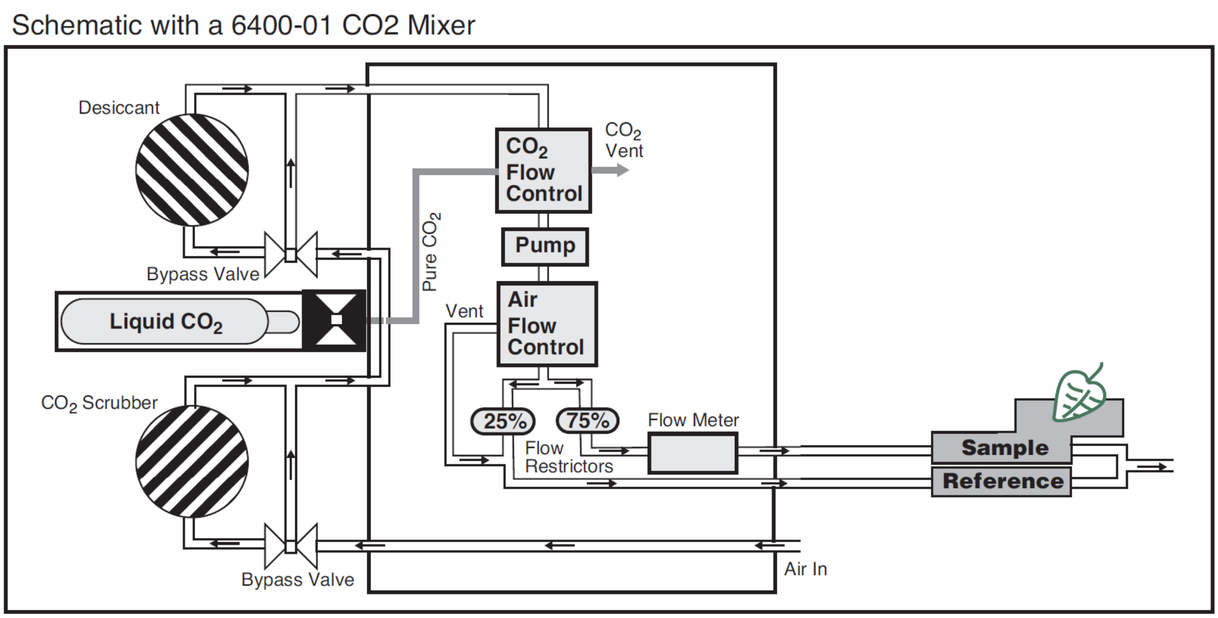


Figure 1-2. LI-6400XT flow schematic, with and without a 6400-01 CO_2 mixer.

Figure Supplement 1. The Licor is in the field with a leaf placed inside the cuvette (top) with the diagram from the LI-6400XT instruction manual (bottom, v6.2, Licor Incorporated, Lincoln, NE, USA).

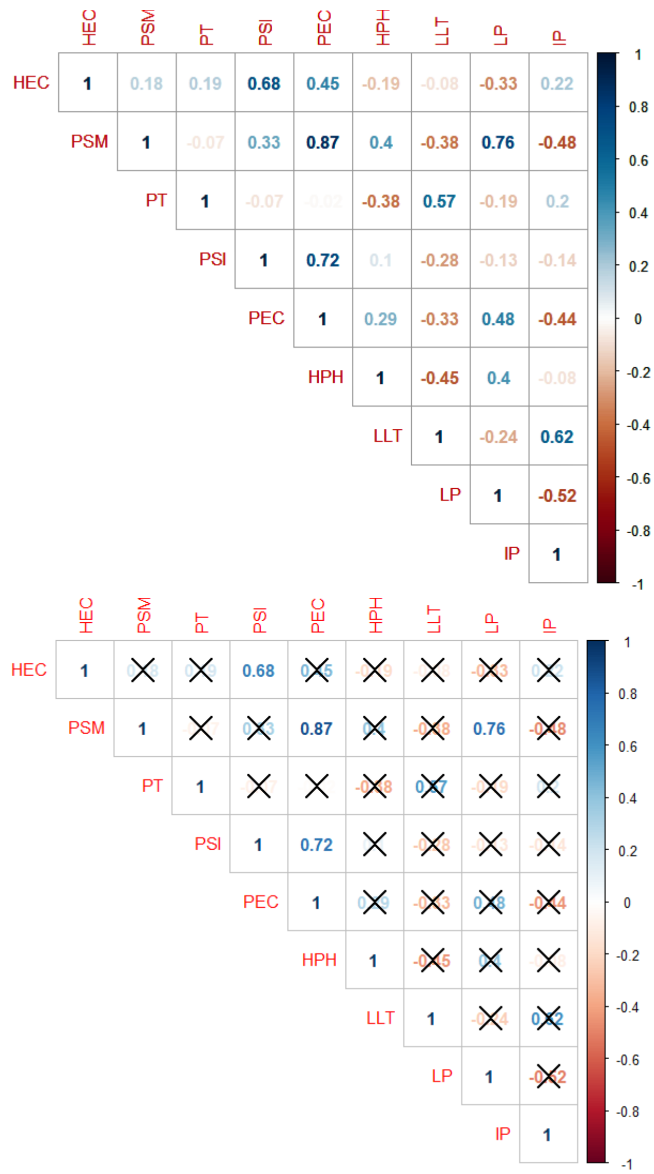


Figure Supplement 2. Using a Pearson Correlation for field site selection and field measurements data, the correlation coefficient (r^2) inside the boxes and significance value (p-value) on the right side of the plot (top) were used to see which relationships correlated to each other. With an alpha value less than 0.05, four correlations were found (bottom). The relationships between POGO electrical conductivity (PEC), POGO salinity index (PSI) and POGO volumetric soil moisture content (PSM) were expected. PEC with PSI (p-value = 0.02) and PSM (p-value = 0.01) were significant. PSM with photosynthesis (LP) was significant (p-value = 0.01) but isoprene (IP) was insignificant (p-value = 0.16). PSI with Hanna electrical conductivity (HEC) was significant (p-value = 0.03).