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Abstract

Six plant species of Yaupon, Eastern red cedar, American holly, Arizona cypress, Arborvitae and Roughleaf dogwood were utilized to determine their effectiveness in the removal of atmospheric ammonia. All species were exposed to three ammonia levels (1, 5 and 10 ppm) in an environmental chamber. Foliar ammonia content was quantified using an enzymatic technique. The effects of exposure to ammonia on the physiological responses (e.g. photosynthetic activity, stomatal conductance, and transpiration rate) of plants in ambient condition were also determined using an open design photosynthetic gas exchange system. Foliar ammonia content was significantly different among the six plant species (p<0.0001) with Eastern red cedar exhibiting the highest content. The physiological responses differed significantly depending on the plant species and the ammonia treatment level. The photosynthetic response of plants to the presence of ammonia was mixed. At low exposure level, all species except Arborvitae had decreased photosynthetic activity, reducing by as much as 44.5% for Yaupon. At the highest concentration, however, Yaupon's photosynthetic activity improved by about 10%. Exposure to ammonia caused increased stomatal conductance and transpiration rate on American holly and Arizona cypress, making them more susceptible to water loss.

Keywords: enzymatic technique, foliar ammonia, physiological response, stomatal conductance, transpiration

1. Introduction

Highly variable combinations of multiple pollutants in ambient atmosphere can pose negative effects on vegetation (Krupa & Legge, 2009) through transfer by the combined forces of diffusion and flowing air movement (Khan & Abbasi, 1999). Vegetation constantly exposed to the atmospheric pollutants may absorb, accumulate and integrate pollutants impinging on the foliar surfaces. Effects are primarily reflected in the plant physiology on major system and organs of plants constantly exposed to the atmosphere where continuous exchange of gases in and out of the environment occurs (Radhapriya et al., 2012).A number of agricultural chemicals adversely affect growth and development of plants (Furlan et al., 1999).In poultry houses, ammonia (NH_3) and dust were the primary contaminants of concern (Adrizal et al., 2008).

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Ammonia is a colorless gas at room temperature but becomes a liquid when compressed. It easily dissolves in water and has a pungent and suffocating odor with an odor threshold of 5 ppm. It reacts with strong oxidizers, acids, halogens, bleach, salts of silver, zinc, copper, and other heavy metals which makes it corrosive to copper and galvanized surfaces. A major use of it includes refrigeration, plastic and textiles processing but aqueous solution are commonly used as household cleaning agents (Salocks & Kaley, 2003). In the atmosphere, it is the third most abundant nitrogen form which can increase more rapidly due to various natural and anthropogenic sources (Wollenweber & Raven, 1993). Agriculture is a major source of NH_3 emissions mainly due to livestock management and fertilizer application, while processing plants, power plants, traffic, human excreta and other sources only play a minor role (Fangmeier et al., 1994).

Serious dieback of forests and decline in the number of plant species are observed in the Netherlands where intensive animal breeding is concentrated (Roelofs, 1986). According to Van Hove et al. (1990), NH_3 is toxic in plants since it functions as an electron acceptor causing uncoupling of electron transport in the membrane and saturating membrane lipids causing membrane dysfunction. In general, it is the assimilation capacity of the plant species that determines the degree of injury. If the assimilation capacity is not sufficient to detoxify NH_y, acute (visible) injuries may occur (Fangmeier et al., 1994). However, the sensitivity of terrestrial plants to NH_3 exposure varies; the capacity of some plants to detoxify NH_3 upon absorption is entirely dependent on the availability of carbohydrate (Dueck et al., 1998).

In a study by Dueck et al. (1998), Scots pine enclosed in a chamber with NH_3 had lower needle water potential, increasing the drought sensitivity of the plant. In another study, honey locust responded differently in the presence of increased NH_3 compared to other plant species; its color was enhanced, lower damage values were recorded, and significant increase of nitrogen in the leaves were measured that indicated greater metabolism or detoxification of the absorbed NH_3 compared to red cedar, hybrid poplar and reed canary grass (Adrizal et al., 2008). Wollenweber and Raven (1993) reported that increasing atmospheric NH_3 concentrations increased the growth rate of perennial ryegrass (*Loliumperenne)* but not its nitrogen acquisition per unit dry matter.

There have been several studies on deposition of air pollutants on vegetation but very limited studies have determined the effectiveness of trees on mitigating air emissions from poultry buildings. The objective of this study was to compare the effectiveness of several species of trees for the removal of atmospheric NH_3, which is one of the major pollutants emitted from poultry buildings. Technologies have been developed and adopted to mitigate and reduce the environmental impact of NH_3 emissions from animal facilities in general. However, limitations on widespread use of control technologies in animal buildings exist because of issues on cost effectiveness and performance of such technologies especially under harsh environmental conditions. Tree species that have higher foliar ammonia content might be effective in withstanding the poultry farms' environmental conditions and can potentially benefit confined animal feeding operation's owners and managers in deciding an alternative environmental control efforts for mitigating the impact of air emissions from their facilities.

2. Materials and Methods

Exposure to Ammonia.

Seedlings of six different plant species comprised of 12-months-old *Ilex vomitoria (evergreen shrub)*, 14-months-old *Juniperusvirginiana (evergreen conifer)*, 10-months-old *Ilex opaca (broad-leaved evergreen)*, 12-months-old *Cupressusarizonica (evergreen conifer)*, 12-months-old *Thijaplicata x standishii* (evergreen conifer) and 15-months-old *Cornusdrumondii (deciduous shrub)*, which were moderately to fast growing trees were selected as test species for the experiment. The seedlings (20-50 cm in height) were obtained from commercial sources. They were selected for uniform sizes and individually planted into one-gallon polypropylene pots. Seedlings were housed and allowed to adjust to greenhouse conditions for four months prior to the start of ammonia exposure measurements. The planting medium consisted of a potting soil mixture with slow release of macro and micro mineral nutrients (Hyponex by Scotts© Potting Soil). All plants were watered to field capacity as needed before exposure to different ammonia levels to minimize stress due to greenhouse condition. Throughout the experiment, each pot was watered twice per week or whenever the soil volumetric water content, measured with a soil moisture meter (Field Scout TDR 100/200, Spectrum Technologies, Plainfield, IL), decreased to less than 10%. During the exposure, plant pots were sealed with Parafilm sheets to avoid NH_3 deposition on the soil which might affect apoplastic NH_4^+ assimilation through the roots.

An environmental chamber (82-liter capacity) that was used in the experiment was made up of Plexiglas material (40.64 cm x 30.48 cm x 66.04 cm) (Figure 1) was designed systematically and constructed to ensure the containment of NH_3 and prevent the escape of the gas into the surrounding.

The chamber was housed inside the greenhouse to reflect ideal ambient conditions and primarily to ensure stomatal opening during the experiment. Several holes were drilled into the walls of the chamber to serve as inlets and pathways for NH_3 injection point and an outlet for an NH_3 detector tube to measure the concentrations inside. Inlets were covered with rubber stopper and supplemented with Parafilm tape to prevent wall losses. Inner walls and other supplementary instruments were coated with Teflon material to avoid ammonia reaction with the surface. Air circulation in the chamber was facilitated by an axial fan mounted through a 2.54-cm stainless steel rod attached inside the chamber. The fan was kept in continuous operation to reduce space variability of gas concentration, reduce the boundary resistance layer of the leaf and, to avoid a critical wind velocity (0.3 – 0.4 m s^{-1}) (Van Hove et.al., 1989).
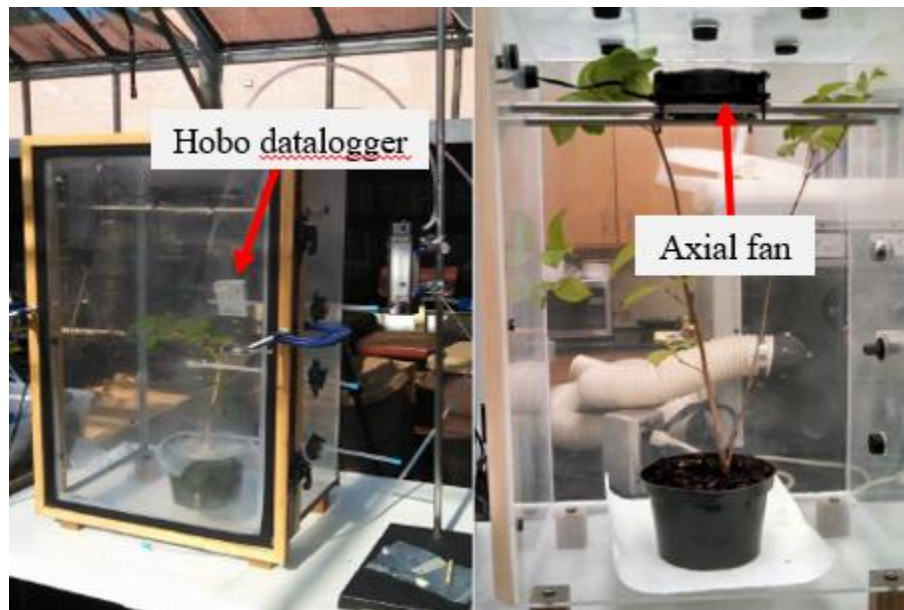
Figure 1. A Plexiglas environmental control chamber showing the locations of an axial fan, a hobo datalogger, and multiple plugged openings to provide access for NH_3 injection and measurement.

Preliminary experiment was performed to ensure leaf damage will be prevented due to air velocity and the velocity was monitored using an air velocity meter (Model 9545-A, TSI Inc., Shoreview, MN). Air velocity measurements were done at 12.7 cm and 38.10 cm above the surface floor of the environmental chamber; these heights were chosen since they covered the range of seedlings heights that were measured from the pots up to the leaf foliage surface. Temperature, relative humidity and light intensity were also monitored throughout the experiment using Hobo Data loggers (Hobo U-12 Logger, Onset Computer Corporation, Bourne, MA). The chamber was tested for air leakage using the Nextteq Irritant Smoke Tube Kit (P/N 9501, Nextteq LLC, Tampa, FL). The kit was used to generate smoke inside the chamber and after about 1 min, an axial fan mounted inside the chamber was turned on and allowed to run for about 15 min. Visual inspections did not indicate any leak.

The seedlings were exposed to three concentrations of ammonia (low = 1 ppm, medium = 5 ppm, and high = 10 ppm) for an hour at a constant gas flow rate of 0.03 m^3·h^{-1} (0.5 lpm). The maximum NH_3 concentration of 10 ppm was chosen since it was the typical concentration measured at the exhausts of poultry buildings at Stephen F. Austin State University's Broiler Research Center where the same plants species were used for a separate study. Ammonia was supplied to the chamber as a compressed gas from 1R (29.50 liters internal volume) cylinder size (Matheson Tri-Gas Company) using polytetrafluoroethylene tubing. The delivery of gas into the chamber was controlled by a stainless steel regulator and metered by a glass tube flow meter (Series GC, Key Instruments, Hatfield, PA). Ammonia concentration inside the chamber was measured using NH_3 passive dosimeter tubes (Ammonia 2/a, Drager Safety, Atlanta, GA) prior to every start of a new exposure experiment and after the designated sampling duration.

The exposure period of one hour was used to achieve a threshold (0.397 to 0.864 ppm) that has been found by Krupa & Legge(2009) to cause damage to some terrestrial plants exposed to NH_3 in an environmentally controlled chamber. A total of 108 seedlings of all six plant species were exposed to three levels of ammonia for the foliar ammonia measurements. Eighteen seedlings per species were subjected to a complete balanced-randomized design in the greenhouse benches. Two plants were used per exposure to three treatment levels in order to collect enough samples for enzymatic analysis. Exposure to NH_3 was replicated three times resulting in nine separate runs for each plant species. After each exposure, fresh plant foliage were randomly collected from each exposed species and control (not exposed to ammonia) species and subjected to laboratory analysis at the Environmental Assessment Laboratory for foliar ammonia content. Foliar ammonia was determined through a modified enzymatic technique (Kun & Kearney, 1974).

Foliar Ammonia Quantification.

Leaf samples were thoroughly washed with water, 0.1 M HCl, and 0.2% detergent solution to remove waxy/greasy coating on the leaf surfaces. Samples were then dried with tissue paper before drying in the oven at 70°C for 48 hours to remove moisture, render plant tissues inactive, and stop enzymatic reactions. Drying plant material was necessary to reduce risk of degradation and other chemical changes during storage (Marur&Sudek, 1995). After drying, the leaf samples were brittle enough to be grounded with cooled mortar and pestle (Mattson et. al., 2009) to a powdery consistency and stored until ready for extraction process. Oven-dried leaf samples (200 mg) were suspended in 10% Trichloroacetic acid solution to deproteinize (Ali &Lovatt, 1995) and stored at -20°C to prevent degradation of leaf samples especially when analysis wasn't performed immediately. Leaf samples underwent centrifuge processing at 4100 revolutions per minute (rpm) at 4°C for 10 minutes to liberate NH_3 from the extract. The centrifuge duration of 10 minutes was pre-determined during the initial experiment on leaf samples taken from all six plant species. The homogenate (extracts separated from the solid particles) were neutralized using 2 M of $KHCO_3$ to attain dynamic equilibrium between NH_3 and NH_4. The amount of $KHCO_3$ required to entirely neutralize the plant extract was determined in a separate experiment. Neutralized extract sample was then prepared for enzymatic analysis. The following reagents were pipetted into the sample cell (1 cm diameter of sample cell light path): (a)200μL of 0.5M tris-HCl buffer (pH 8); (b) 100μL of 0.1M 2-oxoglutarate solution (pH 7.4);(c) 200μL of 8mM β-NADH solution; (d) 2000μL of distilled water; and(e) 100μL of neutral extract sample. The first absorbance (A_1) of the solution was recorded after two minutes in the spectrophotometer (DR 3900 Benchtop Spectrophotometer, Hach Company, Loveland, CO). The 20μL of glutamate dehydrogenase (GLDH) enzyme which was commercially purchased was added to the solution to start the reaction, and the absorbance (A_2) was recorded immediately. The calibration curve was generated using NH_3 standard dilution to produce six ammonia concentrations. Absorbance was measured using the same enzymatic procedures. Foliar ammonia content was then calculated using Equation 1. In Equation 1, A_1 and A_2were the measured absorbance before and after the enzyme was added, respectively; V_1 was the sample volume (ml); V_2 was the total volume (ml); ε was the extinction coefficient of NADH; and F was the dilution factor and was equal to 1 when the sample was not diluted.

$$Foliar\, ammonia = [A_1 - A_2] \times \frac{V_1}{V_2 \times \epsilon} \times F \qquad \text{Equation 1}$$

Physiological Response Measurement.

An open design of photosynthetic gas exchange system (Model 6400, LI-COR Instruments, Lincoln, NE) was used to determine the effect of NH_3 on the physiological responses of plants in ambient conditions. As air is continuously passed through the leaf chamber of this instrument, rate of carbon dioxide (CO_2) uptake and water loss were used to determine the changes on the physiological responses of plants. Differences in CO_2 and H_2O in an air stream flowing to the leaf cuvette (reference cell) were compared with the air stream flowing out of the sample. The net CO_2 assimilation rates or net rate of photosynthesis (*A*), transpiration rate (*E*), stomatal conductance (*g_s*) were obtained before and immediately after exposure to NH_3 on the youngest fully expanded leaf (YFLs), which was mostly the 3rd leaf from the top of non-stressed (field water capacity) individual plants. All measurements utilized the constant and controlled value of the leaf temperature of 25°C (Watanabe et al., 2012). When it was evident that the 3rd leaf had signs of senescence (desiccation), the next younger leaf was considered and subjected to measurements instead. For visible injuries on the plants, the effects of NH_3 exposure were categorized into three types:

(1) Collapse of leaf tissue with the development of necrotic patterns, (2) yellowing or other color changes, and (3) alterations in growth or premature loss of foliage (Sikora & Chappelka, 2004).

During the study, no noticeable changes in the plants were observed after the exposure. Hence, a short exposure to NH_3 for one hour was determined irrelevant to initiate such injuries mentioned and was understood as not applicable to this study. All measured values were expressed on a leaf-area basis by taking the average areas of leaf images scanned five times on a leaf-area meter (Model CI-202, CID Bio-Science Inc., Camas, WA). For conifer plants, needles were separated individually and laid flat on the leaf area meter. Prior to exposure inside the chamber, specific part of the foliage was marked and carefully identified so troubles on locating the identified part after exposure were avoided.

Statistical Analysis

The effectiveness of various tree species on foliar NH_3 uptake was determined with two-way analysis of variance (ANOVA) procedure using SAS statistical software (Version 9.2, SAS, Cary, NC). Tukey's test was performed after significant ANOVA results to compare the differences of means. A repeated measures analysis was used to determine the differences on the physiological responses of the six plant species before and after exposure to three ammonia levels.

3. Results and Discussion

Environmental Conditions

Temperature, relative humidity and light intensity were closely monitored throughout the experiment with the Hobo Datalogger. Temperature within the chamber was higher than the outside temperature by about $0.1°C - 6.4°C$ during the experiments.   The relative humidity inside the chamber during the measurements ranged from about 66% to 86% while the light intensity was from approximately 210 lum/ft² to 1128 lum/ft².
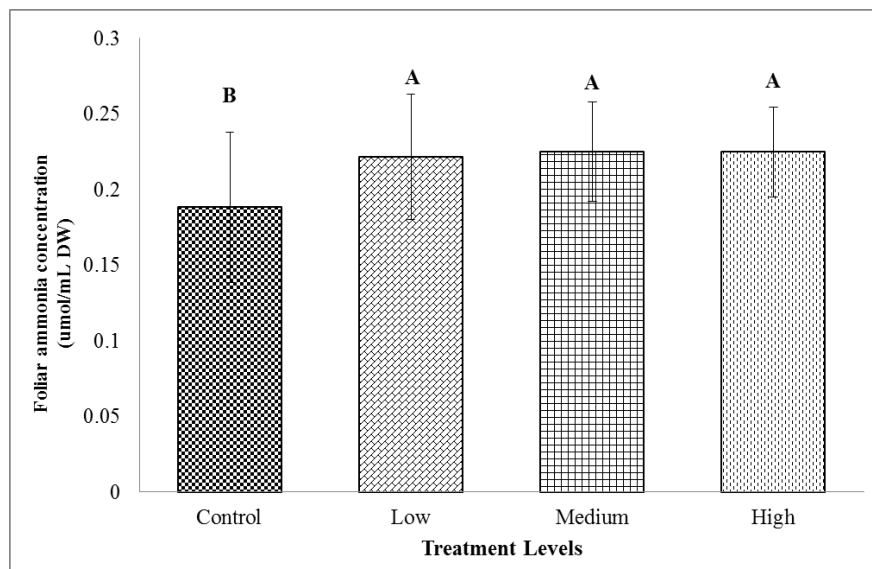
Foliar NH_3 content

The pooled mean of the foliar NH_3 concentrations (μmol NH_3/ml of leaf extracts from dry weight leaf samples) of six plant species are shown in Figure 2. Results showed a significant statistical difference ($p<0.0001$) on foliar NH_3 content of all six species of plants. Eastern red cedar had the highest foliar NH_3content (0.26 μmol/mL) followed by Arizona cypress (0.24 μmol/mL), American holly (0.23 μmol/mL), Roughleaf dogwoods (0.22 μmol/mL), Arborvitae (0.19 μmol/mL) and Yaupon (0. 14 μmol/mL)

Adrizal et al. (2008) found higher capacity of deciduous trees over evergreens (e.g. hybrid poplar, honey locust, and reed canary grass) to incorporate NH_3 into their tissue and plant foliage to trap approximately 30% of NH_3 discharged from the exhaust fans of poultry and livestock barns. Adriaenssens et al. (2010) found that NH_3 uptake was always higher for deciduous species than for pine species (e.g. potted silver birch, European beech, pedunculated oak and Scots pine saplings). Results of this study however did not follow the same conclusions reached by Adrizal et al. (2008) and Adriaenssens et al. (2010). Eastern red cedar which is a conifer yielded the highest NH_3 content compared to Roughleaf dogwood plants. Adrizal et al. (2008) mentioned that leaf surface area between broad-leaf and needle leaf can be a factor for this higher efficiency of conifers to absorb NH_3-N. Adriaenssens et al. (2010) related this to lower N demand of conifers because of lengthy needle retention and efficient internal N recycling as an attributing factor. For plants measured with lower foliar NH_3 content (e.g. Yaupon, Arborvitae), assimilation rate might have been limited due to compensation capacity of plants. Langford and Fehsenfeld (1992) reported that in montane-subalpine forest in Colorado, trees became either a source or sink depending on the atmospheric conditions. In a laboratory experiment, increased temperature caused the plant to switch from being a strong sink for atmospheric NH_3 to being a significant source (Husted & Schjoerring, 1996).
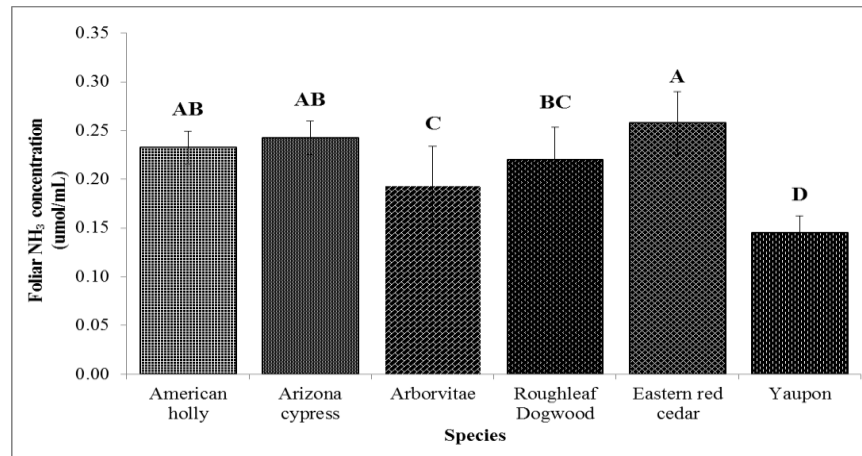
Figure 2. Comparison of means of foliar NH₃ content of the six plant species among all treatment levels. Error bars represent standard errors. Groups with the same letter are not statistically different at the 5% level.

Figure 3 shows significant statistical difference (p=0.0006) of treatment levels on the foliar NH₃ content of the plants as assimilated by the leaves. Ammonia concentration of plants species not exposed to NH₃ (0.19 umol/mL) were significantly lower when compared to plants exposed at three treatment levels (e.g. 0.221 umol/mL, 0.224 umol/mL, 0.225 umol/mL). Both species x treatment interaction significantly influenced the foliar NH₃ content (p=0.0137).Regardless of treatment levels, exposure toNH₃resulted in increased foliar NH₃ content of plants. Obvious increased on foliar NH₃ content on control and plants subjected to treatment was observed on Yaupon, Arborvitae and Roughleaf dogwood plants (Figure 4). Treatment levels also affected the foliar ammonia concentration as plants not exposed to ammonia were analyzed with lower ammonia content (p<0.0001) than those exposed at various concentration.

Figure 3. Tukey's test for thedifferences on foliar NH₃ content among treatment levels. Groups with the same letter are not statistically different at the 5% level.
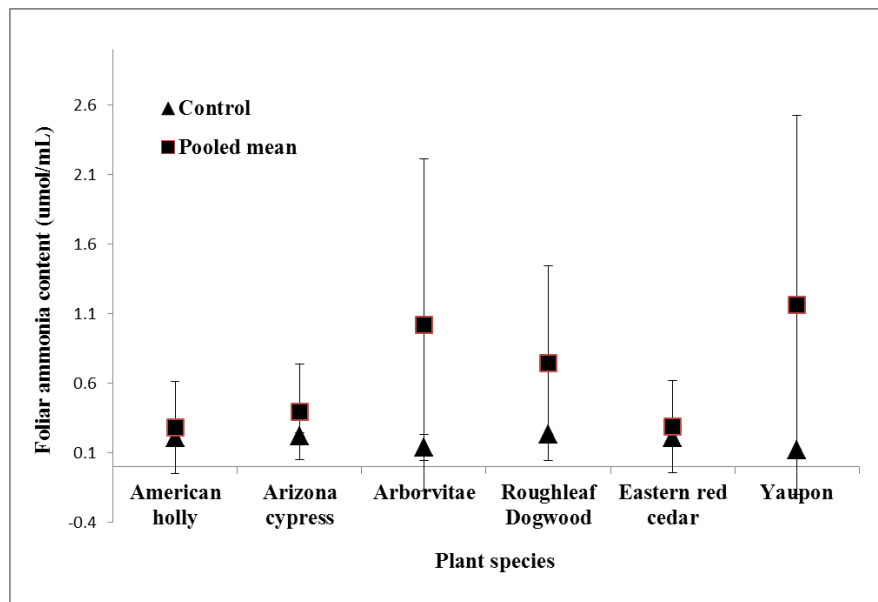
Figure 4. Pooled foliar ammonia content at three treatment levels in comparison to control plants. Error bars represent standard errors.

Physiological response: Net photosynthetic rate, stomatal conductance, and transpiration rate

Leaf photosynthesis (A_n) varied with species type ranging from 2 to 17 μmol $CO_2m^{-2}s^{-1}$ for conifers, 3-22 μmol$CO_2m^{-2}s^{-1}$ for evergreen broad-leaved plants and highest for deciduous broad-leaved plants at 3-27 μmol $CO_2m^{-2}s^{-1}$ (Raghavendra, 1991).Unlike Adriaenssens et al.(2010) study, the results showed high A_n on most conifer and evergreen plants (e.g. Arizona cypress, American holly) than deciduous plant species (e.g. Roughleaf dogwood) used for the study. This high $A_n$might indicate, according to Raghavendra (1991), that evergreen needle-leaf trees are efficient in light capture, thus reducing energy waste especially during light saturation.

Change in the photosynthetic rate of plant species was evident even when exposed to the low level of treatment (Figure 5a). The response of plants to the presence of NH3is seen as either an increase or decrease in their photosynthetic rate. Interactions of species vs. treatment levels (p<0.0001) and species vs. exposure (p=0.0056) have a significant difference in plants response (A_n) at three treatment levels. At low NH_3 treatment level, all four species of Yaupon (-44.53%), American holly (-18.62%), Eastern red cedar (-28.79%) and Roughleaf dogwood (-16.99%) have an observable decrease in their photosynthetic rate after exposure (Figure 5a).Both for Arborvitae (4.14%) and Arizona cypress (3.01%), photosynthetic rate was observed to increase, though the increase was observably higher on Arborvitae. At medium treatment level (Figure 5b), Yaupon (61.12%) and American holly (13.86%) still responded the same as it was in the low treatment level with a decrease in their photosynthetic rate. Both Arizona cypress (-26.55%) and Arborvitae (-1.95%) with higher photosynthetic value after exposure to low treatment level showed a decrease in their uptake rate when exposed to a higher concentration. On the other hand, Eastern red cedar (130.57%) and Roughleaf dogwood (102.03%) showed increased in their photosynthetic rate after medium treatment level exposure. At the highest treatment level of exposure (Figure 5c), plant species were observed to have higher photosynthetic values after exposure except for American holly (-25.05%), Eastern red cedar (-4.4%) and Roughleaf dogwood (-8.26%). Yaupon's change in its photosynthetic rate increased (9.66%) after exposure to high concentration of NH_3. Arizona cypress' photosynthetic rate did not start to increase (6.47%) until the high treatment level was reached while Arborvitae showed a slight increase of0.74%. All other three species had lower photosynthetic rates.

American holly plants' A_n decreased after exposure to NH_3 at all treatment levels while five other plants have inconsistent response at different treatment levels. Eastern red cedar which is a conifer and deciduous broad-leaf Roughleaf dogwood plant both responded with decreased A_n at low and high treatment level. Regardless of the response, Eastern red cedar's A_n values still at the expected range of 2-17 μmol $CO_2m^{-2}s^{-1}$ but an alarming indicator for Roughleaf dogwood with all values at three treatment levels falling below its A_n. Pearson and Stewart (1993) mentioned that NH_3does not adversely affect photosynthesis, in fact, it increases CO_2 uptake.

A study by Van Hove et al.(1990) supported Pearson and Steward (1993)'s findings in which there was an increased CO_2 assimilation in poplar leaves after exposure to144 ppb of $NH_3$for 6 to 8 weeks. The increased assimilation of NH_3 by plants can also be associated with the required carbon skeletons from carbohydrates synthesized during photosynthesis, which is an essential component for amino acids production (Massad et al., 2008).Increased values of A_n of some plants after NH_3 exposure can have a good effect as photosynthesis is the only requirement for growth of plants, particularly on productivity. However, as stomata constantly take in more CO_2, it also lets in more NH_3 inside making it vulnerable to other pollutants in real field conditions (Raghavendra, 1991).

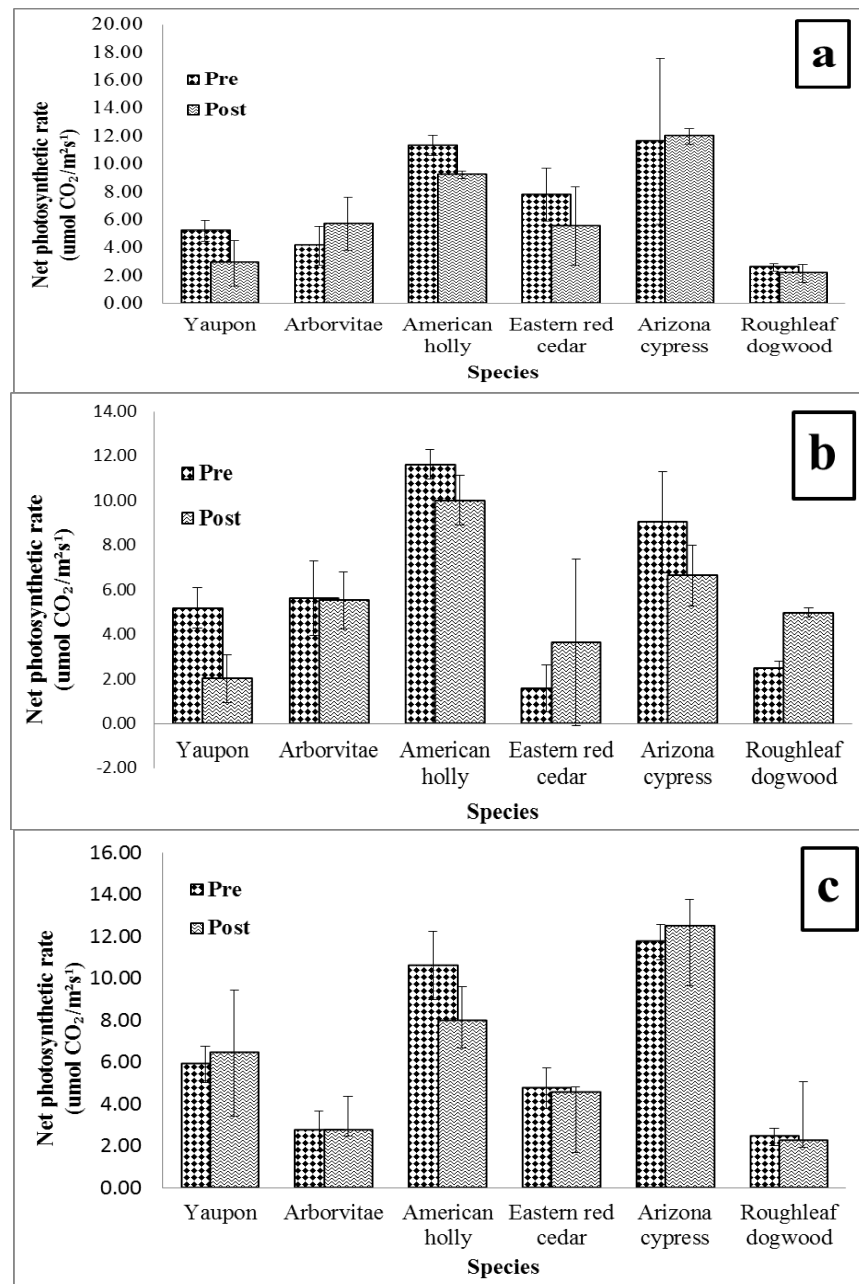**Figure 5. Comparison of net photosynthetic rate of six plant species measured using LiCOR 6400 prior to and after exposure to NH_3 at (a) low, (b) medium, and (c) high treatment levels. Error bars represent standard errors.**

Stomatal conductance of the lower surface of the leaf has a representative conductance value of 0.02-0.12 mol H_2O m^{-2}s^{-1} for trees and 0.08-0.40 mol H_2O m^{-2}s^{-1} for crops. For open and large area stomata, representative conductance value can be as high as 0.76 mol H_2O m^{-2}s^{-1}but only 0.07 mol H_2O m^{2}s^1 for small open stomata.

All throughout the leaf parts, open stomata of trees and xerophytes can be in the range of 0.04 to 0.16 mol H_2O $m^{-2}s^{-1}$(Raghavendra, 1991). Figure 6(a, b, c) shows the stomatal conductance before and after exposure to NH_3 at all treatment levels. As observed in net photosynthetic response of plants, there wasno consistent pattern or trend of increasing or decreasing response on stomatal conductance of plants at increasing treatment levels.Yet, species vs. treatment levels and species vs. exposure interactions both have a significant statistical difference ($p<0.0001$) on plants response. As a result, all three factors significantly ($p=0.03$) affected the stomatal response of plants.

Stomatal conductance (g_s) of Arizona cypress and American holly plants were unaffected by the concentration level, i.e. no matter what the treatment level was, the response was constant. Both plants responded and exhibited increased stomatal conductance at all treatment levels which could reflect higher intake of NH_3 into the leaf. Van Hove andBossen (1994) had the same observation on Douglas fir plants when exposed to low concentrations of NH_3 but with control on light intensity. However, the interference and effect of light on plants in this study has been considered and the amount of light waskept constant (1200 umol $CO_2m^{-2}s^{-1}$) in the leaf-gas exchange system. Hanstein et al. (1999) also observed g_s to increase with increasing NH_3 concentration (0 - 30 nmol/mol of air) on three native meadow grasses species and poplar leaves after exposure at 144 ppb for 6 to 8 weeks (Van Hove et al., 1990).

Evergreen conifer needles have generally lower g_s than do broadleaf plants (Matyssek et al., 1995), therefore explaining the lower stomatal conductance of Yaupon and Arborvitae which were evergreen and conifer. Despite the decreased response of plants at some treatment levels (e.g. Arbovitae, Yaupon),g_s values were representative values for both large and small open stomata.
Even If $g_s$on the lower leaf surface were to be reflected, regulated $g_s$values still fell within the expected range (e.g. 0.02-0.12 mol H_2O $m^{-2}s^{-1}$ for trees) but not for Roughleaf dogwood after exposure at high treatment level (0.01 mol H_2O $m^{-2}s^{-1}$).

Transpiration rate (E) after exposure to NH_3treatment levels showed either an increased or decreased response of plants (Figure 7a, b, c). Transpiration rate values were low at 0.8 mmolm^{-2}s^{-1} if photosynthetic photon flux density (PPFD) or light energy for plants was low although it can gradually increase if PPFD became medium (2.5 mmolm^{-2}s^{-1}) and high (5 mmolm^{-2}s^{-1}). Interactions of species vs. treatment levels and species vs. exposure yielded significant statistical difference ($p<0.0001$) on transpiration rate of plants but not on all three factors ($p=0.07$).

Usually, increased E values are dictated by absence of water stress in the plants and decreased E when water supplies in intercellular surfaces are limited (Raghavendra, 1991). In this study, the amount of water vapor delivered into the leaf's intercellular surface was controlled. Despite lower E values per unit of leaf surface for loblolly pine than broad-leaf species, E values per seedlings of similar plant size was greater for pine because of its greater total leaf surface area (Kramer, 2012) but this was not observed in one out of three conifers used in this experiment. Eastern red cedar constantly resulted in decreased E values at all treatment levels of NH_3(9.50 to 2.97 mmolm^{-2}s^{-1}). However, the response was opposite for the American holly plants. A leaf with greater surface area transpires more water compared to plants with less surface area; however, leaf structure and stomatal aperture also determine the amount of water that escapes from the plant (Devlin, 1975) which could be another factor that caused American holly's E values to increase. In general, the control on ΔE rests with stomata and the function of stomatal closure highly influenced E status especially by mid-day when stomata closed as a response to increasing temperature. In a study on two species of olive trees, Lo Bianco and Avellone (2014) observed E values ranging from 0.3 to 1.6 mmolm^{-2}s^{-1}throughout the sampling duration (08:00-19:00 hours) with peak measurements at noon in *Biancolilla*species. *Cerasuola* species was observed with 0.3 to 1.2 mmolm^{-2}s^{-1}E values but had a different peak time (13:30 hour). However, since light intensity was taken into account to play a major role in plants E,an established photo synthetically active radiation (PAR) of 1200 μmol m^2s^1 was set constant and applied for all the gas-exchange measurements on all plants. Even if the response was inconsistent for Arizona cypress and Arborvitae, results showed higher E values than high PPFD at 5 mmolm^{-2}s$^{-1,}$ specifically for Arizona cypress as reflected at all treatment levels.

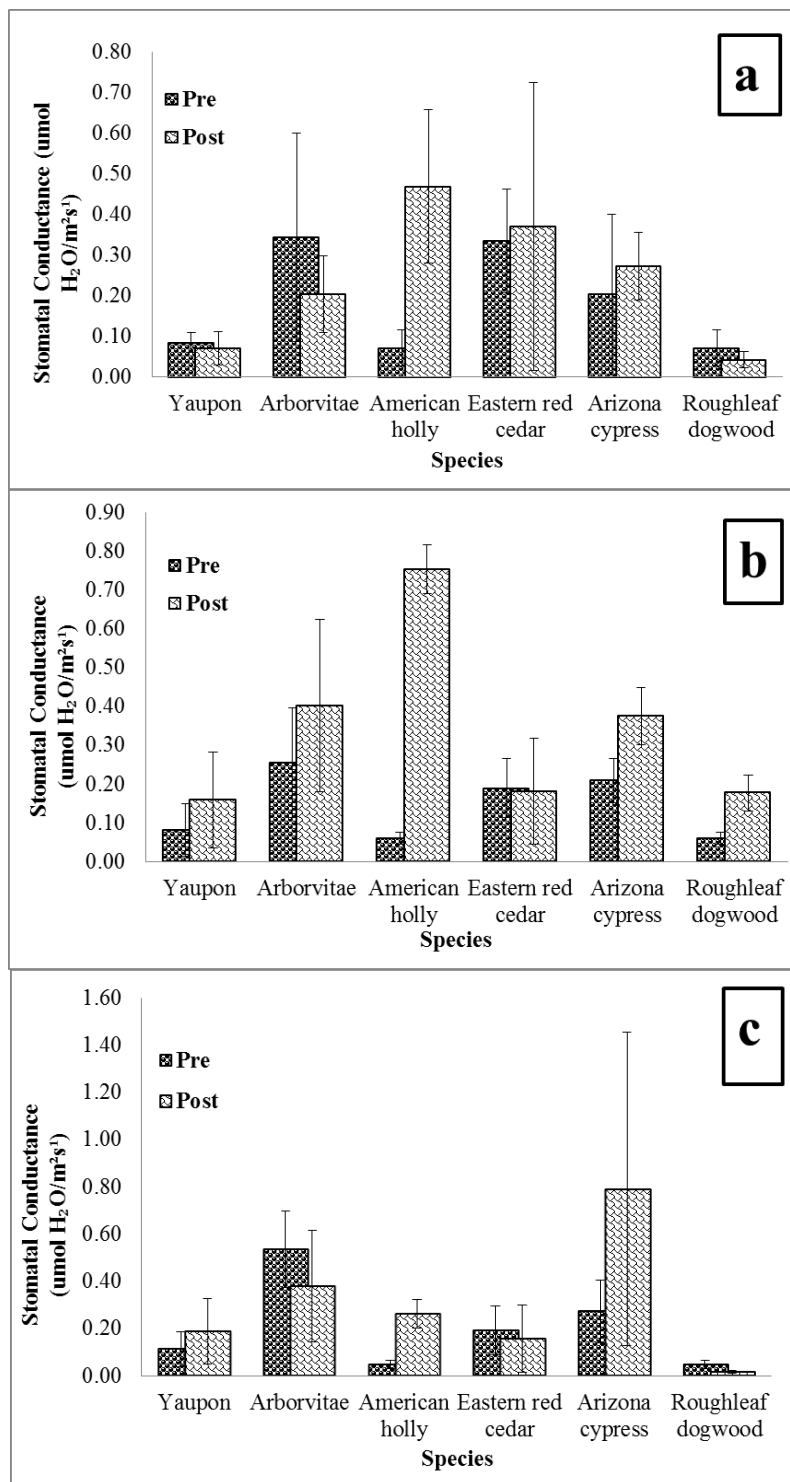**Figure 6. Comparison of stomatal conductance of six plant species measured using LiCOR 6400 prior to and after exposure to NH₃ at (a) low, (b) medium, and (c) high treatment levels. Error bars represent standard errors.**
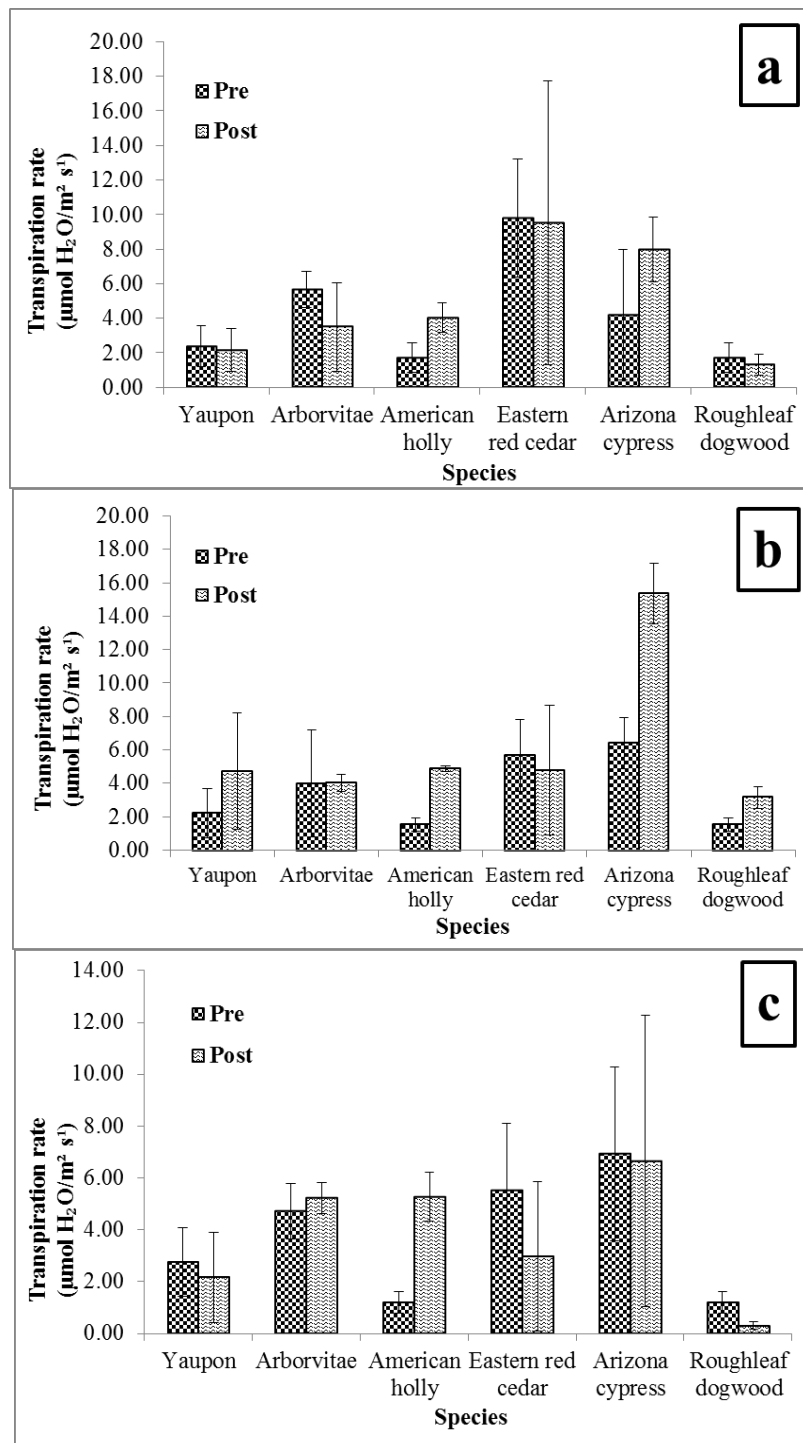
Figure 7. Comparison of transpiration rate of six plant species measured using LiCOR 6400 prior to and after exposure to NH₃ at (a) low, (b) medium, and (c) high treatment levels. Error bars represent standard errors.

4. Conclusion

Based on NH_3 assimilation, Eastern red cedar can assimilate NH_3 better than the other five species of Arizona cypress, American holly, Roughleaf dogwood, Arborvitae and Yaupon that were used in this study. Therefore, considering only the foliar uptake, Eastern red cedar will be the best option for shelterbelt design to remove NH_3 emitted from confined animal buildings.

Exposure to NH_3 significantly lowered the net photosynthetic rates of the three plants analyzed with higher NH_3 content (Eastern red cedar, Arizona cypress and American holly). Yet, all values were still within the range expected for conifers (2 - 17 μmol CO_2 $m^{-2}s^{-1}$) and evergreen plants (3 - 22 μmol CO_2 $m^{-2}s^{-1}$). Therefore, photosynthetic rate, which is essential to plant metabolism (Fitter&Hay,2002), will not be limited. Exposure to NH_3 caused increased stomatal conductance and transpiration rate on American holly and Arizona cypress. In dry and drought conditions and when NH_3 concentration is elevated, both plants will be at risk for higher potential for water loss as water relations will be altered and recovery from water loss will be difficult. Therefore, under these conditions, American holly and Arizona cypress will not be good choices for a shelterbelt to control NH_3 emissions from animal facilities.

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