Measurement report: Leaf-scale gas exchange of atmospheric reactive trace species (NO₂, NO, O₃) at a northern hardwood forest in Michigan

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Abstract. During the **P**rogram for **R**esearch on **O**xidants: **PH**otochemistry, **E**missions, and **T**ransport (PROPHET) campaign

from July 21 to August 3, 2016, field experiments of leaf-level trace gas exchange of nitric oxide (NO), nitrogen dioxide

10 (NO₂), and ozone (O₃) were conducted for the first time on the native American tree species *Pinus strobus* (eastern white pine),

Acer rubrum (red maple), *Populus grandidentata* (bigtooth aspen), and *Quercus rubra* (red oak) in a temperate hardwood

forest in Michigan, USA. We measured the leaf-level trace gas exchange rates and investigated the existence of an NO₂

compensation point of 1 ppb, hypothesized based on a comparison of a previously observed average diurnal cycle of NOₓ (NO₂

+ NO) concentrations with that simulated using a multi-layer canopy exchange model. Known amounts of trace gases were

15 introduced into a tree branch enclosure and a paired blank reference enclosure. The trace gas concentrations before and after

the enclosures were measured, as well as the enclosed leaf area (single-sided) and gas flow rate to obtain the trace gas fluxes

with respect to leaf surface. There was no detectable NO uptake for all tree types. The foliar NO₂ and O₃ uptake largely

followed a diurnal cycle, correlating with that of the leaf stomatal conductance. NO₂ and O₃ fluxes were driven by their

concentration gradient from ambient to leaf internal space. The NO₂ loss rate at leaf surface, equivalently, the foliar NO₂

20 deposition velocity toward the leaf surface, ranged from 0–3.6 mm s⁻¹ for bigtooth aspen, and 0–0.76 mm s⁻¹ for red oak, both

of which are ~90% of the expected values based on the stomatal conductance of water. The deposition velocity for red maple

and white pine ranged from 0.3–1.6 mm s⁻¹ and from 0.01–1.1 mm s⁻¹, respectively, and were lower than predicted from the

stomatal conductance, implying a mesophyll resistance to the uptake. Additionally, for white pine, the extrapolated velocity at

zero stomatal conductance was 0.4 ± 0.08 mm s⁻¹, indicating a non-stomatal uptake pathway. The NO₂ compensation point

25 was ≤60 ppt for all four tree species and indistinguishable from zero at the 95% confidence level. This agrees with recent

reports for several European and California tree species but contradicts some earlier experimental results where the

compensation points were found to be on the order of 1 ppb or higher. Given that the sampled tree types represent 80-90% of

the total leaf area at this site, these results negate the previously hypothesized important role of a leaf-scale NO₂ compensation

point. Consequently, to reconcile these findings, further detailed comparisons between the observed and the simulated in- and

30 above-canopy NOₓ concentrations, and the leaf- and canopy-scale NOₓ fluxes, using the multi-layer canopy exchange model

with consideration of the leaf-scale NO_x deposition velocities as well as stomatal conductances reported here, are recommended.

1 Introduction

The reactive nitrogen species nitric oxide (NO) and nitrogen dioxide (NO_2) are key components in tropospheric oxidation chemistry, affecting air quality by triggering the production of ground-level ozone, secondary organic aerosol, and acid rain. Forests cover 27% of the world's land surface, and 34% of the land area of the United States (FAO, 2016), and are an important land cover type in the continental cycling of NO and NO_2 (collectively termed NO_x). In remote and relatively unpolluted forests, the main source of NO is biogenic emission from soil microbial nitrification and denitrification processes. Once it escapes the soil, NO is transported through the canopy by turbulent mixing that is coupled to the atmosphere above the forest. During this time, NO participates in chemical reactions with trace species present in ambient air, primarily with ozone to form NO_2. This happens on a relatively short time scale of tens to a few hundred seconds. During daytime, additional reactions may further transform NO_2 to other oxidized nitrogen species, but on a longer time scale (Min et al., 2014). Physical loss pathways of NO_x within the canopy include dry deposition and leaf stomatal and cuticular uptake. The relative differences in the time scales of the turbulent mixing and the chemical and physical sink processes determine the amount of NO_x removed within the canopy. Any NO_x remaining in the ambient air is subject to be transported to the free troposphere above the forest.

The effect of leaf stomatal and cuticular uptake on the release of soil-emitted NO_x through forest canopy to the atmosphere is described using an empirical parameter, the canopy reduction factor (CRF), introduced by Yienger and Levy (1995) for application in large-scale atmospheric chemistry studies that generally rely on the so-called "big-leaf" approach to represent atmosphere-biosphere exchange without considering the process inhomogeneity within the canopy. Based on a parameterization using leaf area index and stomatal area index, it was estimated that 25−55% of soil-emitted NO_x is lost within forest canopies annually or seasonally depending on forest type. Those estimates of the effective release of soil NO_x were further corroborated in a study by Ganzeveld et al. (2002) using, instead of the big-leaf approach, a multi-layer canopy exchange model in a chemistry-climate model. Additionally, by including the influences of wind speed, turbulence, and canopy structure when calculating the CRF, Wang et al. (1998) estimated that up to 70% of NO_x was removed within the canopy in Amazon in April, agreeing with earlier results (Jacob and Wofsy, 1990). Accounting for both forests and other types of ecosystems, Wang et al. (1998) also estimated the global average canopy reduction at 20%, versus 50% by Yienger and Levy (1995). More recently, Delaria et al. (2018) investigated NO_x exchange with the leaves of *Quercus agrifolia* (California live oak) and obtained deposition velocities of NO_2 and NO under light and dark conditions. Implementing these results in a multi-layer single-column model, it was calculated the California oak woodland canopy removes 15−30% of soil-emitted NO_x, a significant amount but lower than the previously estimated numbers (Delaria and Cohen, 2019). Forest canopy plays a significant role in regulating the trace gas compositions in the atmosphere. Direct observations of NO_x exchange with a wide variety of plants and in various ecosystems are necessary to better understand ecosystem impacts on NO_x cycling globally.

In fact, there have been over a dozen field and laboratory studies aimed at understanding leaf-level NO_x uptake conducted from the 1990s to 2009, but primarily on European tree species (Raivonen et al., 2009), and references therein). From direct measurements of foliar NO_x uptake, a reasonably detailed understanding of the gas exchange processes between NO_x and O_3 and plant leaves has been developed. Plants absorb NO_2 and O_3 mainly through leaf stomata, but also by leaf cuticular uptake (Chaparro-Suarez et al., 2011; Coe, 1995; Geßler et al., 2002; Rondón et al., 1993). The uptake efficiency varies across plants and is influenced by environmental conditions. Studies at leaf level and within leaves have found that after entering the stomata, NO_2 is metabolized through dissolution and enzyme-catalyzed reactions (Hu et al., 2014; Nussbaum et al., 1993; Vallano and Sparks, 2008; Weber et al., 1998). Unlike NO_2 and O_3, foliar exchange of NO is small to insignificant (Hereid and Monson, 2001; Rondón et al., 1993), except for herbicide-treated soybeans (Klepper, 1979) and nutrient-fed sugar cane, sunflower, corn, spinach, and tobacco plants (Wildt et al., 1997), where NO emission was observed. Results from (Delaria et al., 2018) are consistent with these earlier findings.

In addition to NO_x and O_3 deposition fluxes, NO_2 compensation points have also been obtained by extrapolating the linear relationship between NO_2 flux and the ambient NO_2 concentration over the leaf surface (Raivonen et al., 2009; Slovik et al., 1996). The compensation point is the specific NO_2 ambient mole fraction or concentration at which NO_2 uptake by the plant leaves or NO_2 flux toward the leaf surface becomes zero. Reported values for this NO_2 compensation point ranged from 0.3 to over 3 ppb, depending on tree type and the conditions under which the measurements were made. The existence of such a point implies that when ambient NO_2 is below these thresholds, for example, in remote, unpolluted forest areas, the soil-emitted NO_x would not be efficiently removed by the forest canopy as necessary for balancing the NO_x budget. Instead, the forest provides an additional atmospheric NO_x source.

This conundrum, discussed by Lerdau et al. (2000), seemed to be resolved in the past decade when additional leaf-scale experiments were made using a new NO_x measurement instrument (Breuninger et al., 2012, 2013; Chaparro-Suarez et al., 2011). The improved NO_2 detection specificity of the instrument prevented artifacts caused by augmentation of the NO_2 signal from other nitrogen compounds such as nitrous acid (HONO), nitric acid (HNO_3) and peroxyacyl nitrates (PANs). These artifacts may have caused an observed reduction of NO_x uptake that led to the conclusion of an (inferred) compensation point. The above work, on several native European trees, showed either a lower NO_2 compensation point than previously measured, at 0.05 to 0.65 ppb, or values not significantly different from zero at the 95% confidence interval, and do not support the possibility of a foliar NO_x source. However, when analyzing the observed NO_x and O_3 concentrations in a North American hardwood forest at the University of Michigan Biological Station (UMBS) research site using a multi-layer canopy exchange model, Seok et al. (2013) found that the best agreement between simulated and measured NO_x concentrations was obtained when a 1 ppb NO_2 compensation point was invoked. Further analysis to assess the sensitivity of the simulated NO_x mixing ratios to the representation of soil NO emission, leaf surface photolysis of nitrate, or advection was not able to reproduce the observations especially regarding the diurnal cycle of NO_x.

In order to verify these findings regarding the potential role of an NO_2 compensation point for the UMBS site, we conducted further field experiments on leaf-level gas exchange in summer 2016. This work is the first direct observation of folia gas

exchange of NO_x and O_3 on mature trees growing naturally in a North American forest. To our knowledge, there has been one early study on young seedlings of several American tree species (Hanson et al., 1989), and one recent study on seedlings of California live oak (Delaria et al., 2018). In this work, we used a branch enclosure technique to measure NO_2, NO, and O_3

100   exchange rates at the leaf surface of four locally dominant tree species, *Pinus strobus* (eastern white pine), *Acer rubrum* (red maple), *Populus grandidentata* (bigtooth aspen), and *Quercus rubra* (red oak). Results obtained from these measurements provide information to reassess the possibility of a foliar NO_2 source and the role of the canopy on NO_x and O_3 cycling at this forest site. In this paper, we use both "uptake" and "foliar deposition" when describing trace gas exchange at the foliar level. Both terms refer to the process of trace gas loss upon contact with the leaf surface; but generally, the subject of "uptake" is the

105   plant whereas the subject of "deposition" is the trace gas.

2 Experiment

2.1 Site description

The experiments were carried out at the Program for Research on Oxidants: PHotochemistry, Emissions, and Transport (PROPHET) research site at UMBS, which occupies about 10,000 acres on the northern tip of the Lower Michigan Peninsula

110   (45.56° N, 84.71° W, Fig. 1). The area was heavily logged until the end of the 19th century. It also experienced several severe wildfires from 1880 to 1920. Natural reforestation started when the location was acquired for the research station in 1909. Today, bigtooth aspen, trembling aspen (*Populus tremuloides*), red maple, red oak, and white pine dominate within about a 1-km radius of the PROPHET site, whereas within a 60-m radius of the site, there are more white pine trees and almost no trembling aspen.

115   The northern part of the peninsula is fairly remote. The air is free from anthropogenic pollutants unless meteorological conditions result in the advection of air masses from surrounding major cities: to the southwest, Chicago, IL and Milwaukee, WI; to the southeast, Detroit, MI; and to the east, Toronto, ON. During the field experiments, about 35% of the time, air masses were coming from these directions. However, since the enclosures were purged with scrubbed ambient air (see below), the direct influence of pollutants on the enclosed plant material was minimal.

120   The enclosure measurements were carried out from July 20 to August 3, 2016. Sky conditions were sunny to mixed sun and clouds most of the time. The average ambient temperature measured in the canopy (~2 m) was 24°C during daylight, and 18°C at night, with maximum and minimum temperatures of 31°C and 12°C, respectively. There were two main rainfall events, with the most recent being two days prior to the start of the enclosure experiments. The average soil temperature was near 19°C through the experiment period, and the soil moisture decreased gradually after the rainfall. These conditions are within the

125   normal ranges for this site in July.

2.2 Methods

Branch enclosure experiments were conducted sequentially on branches of white pine, red maple, bigtooth aspen, and red oak. The estimated ages of the white pine, red maple, and red oak trees were about 15 to 20 years, and the bigtooth aspen, 5–10 years. All tree branches were selected based on their sun exposure, accessibility, and size. The height of the enclosed branches ranged from 3 to 10 m above the ground.

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The enclosure system was composed of three parts: the enclosures, the airflow system, and the trace gas measurement instruments (Fig. 2). The enclosure, essentially a flow chamber, was constructed using a 61 x 91 cm Tedlar bag (Jensen Inert Products, Florida, USA) with three factory-installed 0.95 cm diameter ports to attach tubing and sensor wires. The branch was carefully enclosed by the bag so that it was situated as close to the middle of the bag as possible. The open end of the bag was then closed around and tied onto the main stem of the branch, tight enough to secure the enclosure when it was inflated by the purge air, but also with enough leakage to allow air to escape during purging. Each branch enclosure was paired with an identical enclosure assembly without any plant material as the background reference to account for wall effects and other factors that may affect trace gas concentrations. The reference enclosure was placed adjacent to the branch enclosure but without obstructing the sunlight to the enclosed tree leaves.

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Air delivery and air sample lines, each about 30 m long, were connected to the enclosures and the instruments that were housed in a trailer parked nearby at the site. Between the trailer and the enclosures, the air and sample lines were bundled together and sheathed inside black flexible insulation hoses linked together end to end. The hoses were wrapped in aluminum foil to keep the sample lines from absorbing heat from sunlight.

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Ambient air from outside the trailer and scrubbed free of dust, O_3, and NO_x was used as the purge gas. An oil-free air compressor (Medo USA, now Nitto Kohki USA) was used to pull the ambient air through an organic vapor/acid gas respirator cartridge (Magid, Illinois, USA), which functioned as a dust filter. Downstream of the compressor, the air was further filtered by an ozone scrubber (Thermo Fisher Scientific), activated charcoal, and a NO_x scrubber (Purafil, Inc., Georgia, USA). Polytetrafluoroethylene (PTFE) tubing (12.7 mm OD and 9.52 mm OD) after the filters) was used to carry the air to the enclosure chambers. The tubing was connected to the port on the Tedlar bag at the end near the tip of the enclosed branch, opposite the bag opening. Inside the bag connected to the same port was an air distributor made from a loop of tubing (9.52 mm OD) with pinholes (~1.5 mm diameter) about 1 cm apart drilled along its entire length. This allowed even distribution and mixing of the purge air insider the enclosure. The flow rate of the purge air was maintained at 37 L m^{-1}. The volume of the inflated enclosure was ~57 L, giving the air a residence time of ~1.5 min.

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For the trace gas exchange experiments, known amounts of NO_2, NO, or O_3 were added into the purge air stream. NO_2 and NO were from compressed standard gas cylinders (Scott-Marrin, Inc., California, USA), and O_3 was made in situ using a mercury Pen-Ray lamp O_3 generator (UVP, LLC. California, USA) and compressed zero air. A KOFLO® type mixer was placed just downstream of the trace gas inlet to ensure even mixing of the added trace component with the scrubbed air.

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NO, NO$_2$, O$_3$, CO$_2$, and H$_2$O mixing ratios before and after the enclosures were measured, with the air sample selected using a set of solenoid valves. The concentrations of these gases were calculated using the ideal gas law and the measured air

160 temperature. The sampling time for the branch enclosure was five minutes each, alternating between the enclosure inlet and outlet. The reference enclosure inlet and outlet were sampled once an hour. The environmental conditions were also recorded, including ambient and enclosure temperatures (S-THB, Onset Computer Corp., Massachusetts, USA), leaf temperatures (thermocouple wire sensors, Omega Engineering, Connecticut, USA), relative humidity (S-THB, Onset), leaf wetness (S-LWA, Onset), and photosynthetically active radiation (PAR) (S-LIA, Onset). Standard commercially available instruments

165 were used for O$_3$ (Model 49i, ThermoFisher Scientific, USA) and CO$_2$/H$_2$O (LiCor 840, Li-Cor Corp., Nebraska, USA). NO and NO$_2$ were measured using a home-built chemiluminescence detector that utilizes the light-emitting reaction of NO with O$_3$ (Ryerson et al., 2000).

The NO$_x$ instrument was programmed to run on 5-minute cycles, each with a one-minute measurement of zero air (UHP, Airgas, USA), followed by a 2.5-min measurement of NO, and a 2.5-min measurement of NO$_2$. In NO$_2$ mode, NO$_2$ was first

170 converted to NO, then measured the same as in the NO mode. An LED UV light source (L11921, Hamamatsu Photonics) was used to photolyze NO$_2$ for the conversion. The peak light emission of this LED was at 365 ± 5 nm, matching the absorption peak of NO$_2$ and minimizing the interference from the unwanted photolysis of HONO. A high-concentration (1.5 ppm) NO standard dynamically diluted with ultra-high purity zero air (Airgas, USA) was used to calibrate the NO measurement. For the NO$_2$ calibration, NO in the same diluted standard was partially converted to NO$_2$ by adding a controlled amount of ozone

175 (generated in situ using a Pen-Ray ozone generator and 99.98% oxygen). The instrument calibration runs were initiated automatically about every 7 hours during regular operation. The overall 1σ-precision for a 5-minute measurement cycle was ~2 ppt for NO and ~4 ppt for NO$_2$. The accuracy of the NO/NO$_2$ measurements was ~30 ppt.

Water vapor at the enclosure inlet and outlet was measured using a LiCor 840. The instrument was calibrated using a LiCor dew point generator. The ambient relative humidity results from the Onset sensors were compared with the data from a nearby

180 AmeriFlux tower (within 100 m) (Vogel, 2016a), and the agreement was within 3%. It was noticed that on particularly hot and humid afternoons, there was condensation of water in the sample line leading to the instruments. The condensed water was removed promptly with gentle warming of the affected section of the sample line. The data recorded during these times were excluded.

After installation, each set of branch and reference enclosures was first purged with scrubbed ambient air at least overnight

185 and through the early morning hours (up to ~10:00 local time) to allow the branches to acclimate, and also to reduce the amount of any possible surface-deposited photochemically labile compounds that might interfere with the measurements (Raivonen et al., 2006). The gas exchange experiments were then started and carried out for the following 2 to 4 days. A known amount of the trace gas was introduced into the purge air flow. This included zero concentration, i.e. purging with scrubbed air only between the trace gas additions. The maximum mixing ratios of the trace gases in the purge air were kept within the range of

190 typically observed ambient measurements, i.e. NO$_2$ <1.2 ppb, NO<300 ppt, and O$_3$<60 ppb.

The enclosed leaves were harvested after the completion of the measurements and immediately placed in an oven to be air-dried at 65°C. The leaf area was then measured by forming a monolayer of the dried leaves on graph paper. The enclosed single-sided leaf area for white pine, red maple, bigtooth aspen, and red oak was 0.35, 0.26, 0.11, and 0.44 m^2, respectively.

195   Leaf-level uptake or emission of the trace gas leads to a trace gas concentration difference between the enclosure inlet and outlet. In the enclosure, the flux of the trace gas with respect to the leaf surface is:

$$F_x = \frac{Q}{A}(c_o - c_i) \, , \tag{1}$$

where F_x is the flux of the trace gas x; c_i and c_o are the trace gas concentration measured at the enclosure inlet and outlet, respectively. A negative flux indicates trace gas loss in the enclosure due to uptake. Q is the purge air flow rate. A is the one-sided area of the enclosed leaves, as the stomata, the part of leaf anatomy most relevant to gas exchange, generally are located

200   on the underside of tree leaves (Kirkham, 2014). A resulting flux with a negative sign reflects the loss of the trace gas at the leaf surface, and a positive flux, emission from the foliage. All the trace gas concentration changes through the branch enclosure $(c_o - c_i)$ were corrected against the background obtained from the reference enclosure before the fluxes were determined according to Eq. (1). The detection limit of flux, i.e. the minimum absolute value above which the flux is significantly non-zero (p<0.05, or at the 95% confidence level), was determined using the flux data obtained during the

205   scrubbed air purge at nighttime when no emission from the leaves was expected because the leaf stomata are closed. These detection limits (Table 1) reflect the measurement precision of the instruments, variations of the actual enclosure conditions over time, and fluctuation of the purge air flow rate.

2.3 Tree branch samples

The representative tree species were determined based on the basal and leaf area coverage within the 60 m radius of the research

210   site. Tree branches for the enclosure experiments were selected for their accessibility from the ground. Preferences were given to those with adequate sun exposure and to mature trees whenever possible. Enclosed branches of white pine (*Pinus strobus*) and red maple (*Acer rubrum*) were ~7 m above ground and from trees that were over 10 m in height. The enclosed branches of red oak (*Quercus rubra*, ~6 m) and bigtooth aspen (*Populus grandidentata*, ~4.5 m) were ~3 m above ground.

3. Results

215   We first examine the results obtained when only scrubbed air (without any addition of NO$_x$ or O$_3$) flowed through the enclosures. The NO and NO$_2$ fluxes during the scrubbed air purge are shown in Fig. 3 along with the PAR and leaf temperature measured at the same time. Each data point represents a 5-minute measurement. The data points in gray are within the 95% confidence interval based on the detection limits listed in Table 1. Those outside the confidence interval are marked by black symbols. It is expected that after the plant enclosures are conditioned with the hours-long scrubbed air purge, there will be no

220   signal of NO$_x$ at the enclosure outlet unless there is a source within the enclosure to supply a detectable amount of NO$_x$. Indeed,

for most of the day, there was no detectable amount of NO_x (or O_3) at the enclosure outlet. However, besides a few scattered data points that are outside the confidence interval, there also appears to be some consistent positive fluxes of NO_2 lasting around 30 min or less occurring around noon or early afternoon. Because of the relatively short duration of this NO_2 emission during the brightest time of the day, it is unclear whether the flux was due to emission from leaves, or due to the photolysis of

225 any oxidized nitrogen substrate remaining on the leaf surface even after the initial overnight and morning purging. The mixing ratios corresponding to the observed fluxes were less than 30 ppt in each of the enclosures. Below we present the results from each of the trace gas addition experiments.

3.1 NO₂

Nitrogen dioxide (NO_2) was introduced into the purge air at different concentrations between zero to 40 nmol m^{-3} (~1 ppb).

230 Generally, when NO_2 was added to the enclosure, there was a negative flux, indicating an uptake of the trace gas by the plant material (Fig. 4, top panel). The magnitude of the flux is proportional to the input NO_2 concentration. In addition, when the input concentration was held constant over the course of several hours or overnight, the flux had a diurnal pattern, e.g. bigtooth aspen on July 30 to July 31. It was lowest at night, increased through the morning hours and peaked around midday before diminishing again toward nighttime. This behavior strongly suggests that the NO_2 uptake by these trees is in large part

235 controlled by leaf stomatal aperture and, at the same time, driven by the NO_2 concentration gradient from the air around the leaf surface to the leaf internal space.

It is necessary to point out here that there were a couple of factors that complicated the NO_2 gas exchange experiment. First, it was found that the NO_2 standard used for this experiment contained about 5% NO, which was unavoidably added to the enclosure along with the NO_2. However, the mole fraction of this NO was at most ~50 ppt (~2 nmol m^{-3}), an amount that did

240 not induce any significant NO flux in the enclosure as shown in the NO gas exchange experiment. Second, there was photolysis of NO_2 in the enclosure when it was under relatively intense sunlight. In theory, this effect is removed by the background subtraction using the results from the reference enclosure. However, in practice, although the branch enclosure and the reference enclosure were set up side by side, the amount of sunlight experienced by each chamber often was different due to the enclosure bag sizes and the need to always set the reference on the relatively shaded side of the accessible space to avoid

245 blocking the sunlight to the enclosed branch. This arrangement likely leads to an underestimation and insufficient correction for the photolysis of NO_2 in the branch enclosure, and consequently, an overestimation of the NO_2 uptake flux. When extrapolating the linear relation of NO_2 flux vs. NO_2 concentration to determine the compensation point (see below), this flux overestimation may cause an overestimation of the inferred compensation point. It is possible to use the observed apparent NO flux from the photolysis of NO_2 to correct this error, but, because there was also NO released from the enclosure when only

250 the scrubbed air was added, and it is not well understood and quantified, directly using the NO flux to correct for the photolysis effect may introduce an even larger error. Without any correction, even in the worst case, the maximum amount of NO flux observed was only about 20% of the total NO_2 flux at the same time, from both the photolysis of NO_2 and possible NO emission from the enclosure.

The size of the stomatal aperture, regulated by the plant's need to optimize photosynthesis and simultaneously minimize

255   water loss, can be gauged by stomatal conductance of water using Eq. (2) (Weber and Rennenberg, 1996):

$$g_{H_2O} = F_{H_2O} / (C_{H_2O\_leaf} - C_{H_2O\_enclosure}) , \qquad (2)$$

in which the flux of water (F_{H_2O}) due to plant transpiration is calculated by applying the measured water concentration

difference at the inlet and outlet of the enclosure to Eq. (1). $C_{H_2O\_leaf}$, the water concentration inside the leaf air space, is

calculated using the measured temperature of the enclosed leaves, assuming the air in the leaf internal space is saturated with

260   water vapor. $C_{H_2O\_enclosure}$, the water concentration of the branch enclosure, is evaluated using the measured enclosure relative

humidity and temperature data. The resulting stomatal conductance of water, g_{H_2O}, for the four enclosed branches is shown

in Fig. 5a. The stomatal conductance has a clear diurnal pattern, mainly following the daily cycles of sunlight and

photosynthesis. The magnitude varies from tree to tree. The conductance of the white pine and the red maple branches were

similar, ranging from near zero at night to about 3 mm s^{-1}, while the conductance of red oak was 0 to ~1 mm s^{-1}, and the

265   bigtooth aspen, 0 to ~6 mm s^{-1}.

Knowing the stomatal conductance of water, the expected rate of NO$_2$ deposition through the plant stomata can be calculated.

Across the stomata, the deposition is a diffusion-controlled process (Weber and Rennenberg, 1996; Weber et al., 1998), where

the expected rate is the product of the stomatal conductance of water multiplied by the square root of the ratio of the molecular

weight of water to the molecular weight of NO$_2$:

270     $$g_x = g_{H_2O} \times \sqrt{\frac{MW_{H_2O}}{MW_x}} , \qquad (3)$$

where g_x represents the expected stomatal uptake rate for the trace gas species x (here x = NO$_2$), and MW represents

molecular weight.

From the measurements, the leaf-level NO$_2$ deposition velocity, v_{dNO_2}, is the NO$_2$ flux toward leaf surface normalized to the

corresponding NO$_2$ concentration in the enclosure:

275     $$v_{dNO_2} = F_{NO_2} / c_{o,NO_2} . \qquad (4)$$

If NO$_2$ deposition is exclusively controlled by stomatal uptake, agreement between the measured deposition velocity and the

calculated stomatal uptake rate is expected, i.e. $v_{dNO_2} = g_{NO_2}$. If not, additional factors, such as internal mesophyll resistance

(Gut, 2002; Thoene et al., 1996), or leaf cuticular adsorption (Coe, 1995; Geßler et al., 2002; Rondón et al., 1993), may also

play a role with the former decreases and the latter increases the overall foliar deposition velocity.

280   In Fig. 5b, the measured foliar deposition velocity of NO$_2$ is plotted together with the calculated stomatal uptake rate for

comparison. The agreement is generally good for all experiments, suggesting that the foliar deposition of NO$_2$ for these tree

species is indeed closely related to stomatal aperture. This also suggests that the effects of internal mesophyll resistance and

cuticular uptake of NO$_2$ are relatively minor. The strength of correlation between NO$_2$ deposition and stomatal conductance is

evaluated using the Pearson correlation coefficient, ρ, which has a possible value between -1 to 1, with a value of 0 indicating

285   no correlation and a value that is away from zero indicating increasing positive or negative correlation. In Fig. 6, the foliar

NO$_2$ deposition velocity is plotted against the stomatal conductance for each tree. The correlation coefficient for bigtooth aspen is 0.96 and for red oak is 0.85, both showing a strong positive correlation between the deposition velocity and stomatal conductance. The correlation is also evident but relatively weaker for the white pine ($\rho = 0.73$) and the red maple ($\rho = 0.71$).

The relationship between the deposition velocity and stomatal conductance is also examined using linear regression analysis.
290 If NO$_2$ deposition is entirely controlled by stomata, the deposition rate at zero conductance ($g_{H_2O} = 0$), when the stomata are closed, should be zero; and the slope of the deposition rate vs. stomatal conductance should be equal to $\sqrt{\frac{MW_{H_2O}}{MW_{NO_2}}}$, or 0.62 (recall Eq. (2)). This relationship is shown in Fig. 6 with the solid blue line. The best fit and the 95% confidence bounds are represented by the red solid and dashed lines. Also listed in the figure are the slope (m), the intercept (b), and the r^2 value of each fit. The linear relationship for bigtooth aspen appears to be the tightest, with over 90% of the data variation can be explained by the
295 fit. The intercept is nearly zero, and the slope of 0.56 is close to 0.62, making it reasonable to conclude that for the bigtooth aspen, stomatal uptake dominates NO$_2$ loss at the leaf surface. A similar conclusion can be made for red oak, where r^2 is 0.72, the slope and the intercept are 0.54 and 0.03, respectively.

The red maple is different. The slope of NO$_2$ deposition rate to stomatal conductance is 0.25, far less than 0.62. The data also appear to have more scatter. On the time series plot (Fig. 5a), the stomatal conductance on the morning of July 25 (from 8:30
300 to 12:30) shows high variability that is not reflected by the NO$_2$ deposition rate at the same time. Possibly an unknown measurement issue for water concentration during this time led to this high variability. However, excluding this portion of the observations and using only the data obtained prior to this time window, from 13:00 on July 24 to 8:00 on July 25, resulted in a modestly improved linear fit with a slope still below 0.3. For white pine, the same slope is 0.40, also lower than the expected value of 0.62 based on stomatal-controlled diffusion. These lower than expected slopes imply there may exist mesophyll
305 resistance to NO$_2$ uptake for these tree species. Such resistance to stomatal uptake of NO$_2$ has previously been observed on some trees such as European *Picea abies* (Norway spruce) seedlings (Thoene et al., 1996), and Amazonian *Laetia corymbulosa* and *Pouteria glomerate* (Gut, 2002). However, in a separate study of Norway spruce seedlings (Rondón and Granat, 1994), no evidence of internal resistance to NO$_2$ stomatal uptake was found. Researches on CO$_2$ diffusion and H$_2$O transport into leaf internal spaces have revealed that mesophyll resistance is subject to environmental perturbations, and the responses among
310 and within species can vary (Xiao and Zhu, 2017). It is reasonable to assume that the mesophyll resistance to NO$_2$ uptake may also subject to environmental conditions, and systematic observations under different conditions are needed for obtaining more general conclusions.

The y-intercept of the fitted line accounts for any possible additional foliar deposition when the stomata are closed and consequently the stomatal conductance is zero. For white pine, the intercept is 0.43 ± 0.09, indicating a possible role of wet
315 leaf surfaces and/or cuticular uptake. We first examined the possibility of wet leaf surfaces in the enclosure. We calculated the enclosure dew point using the temperature and relative humidity recordings and compared it to the measured leaf temperature. The leaf temperature was higher than the dew point at all times during the experiments, excluding the possibility of wet leaf surface. Thus, cuticular adsorption remains a possible explanation for this uptake while the stomata are closed. Previous

researches on Norway spruce (Rondón et al., 1993, Geßler et al., 2002) also gave conflicting conclusions on the existence of

320   cuticular uptake of NO_2. As with the mesophyll resistance, further investigations involving experiments over a longer time

duration and under different environmental conditions are necessary to better understand this possible pathway and its

dependencies.

3.1.1 Compensation point of NO_2

To determine at what concentration the NO_2 flux becomes zero, we plot the NO_2 flux vs the enclosure NO_2 concentration in

325   Fig. 7. The stomatal conductance of each data point is represented by the color scale, with cool to warm colors corresponding

to the stomatal conductance from low to high in each enclosure. As expected, the flux increases with increasing NO_2

concentration in the air surrounding the leaves; and at a given concentration, the flux increases with stomatal conductance. For

each enclosure, we selected the data points taken when the stomatal conductance was at least 50−60% of its maximum

measured during the experiments, indicated by the large, warm-colored symbols in Fig. 7. These data were then fit with linear

330   regression for flux vs NO_2 concentration. The intercepts of the best-fit regression line and the zero-flux line, representing the

compensation point, are listed in Table 2. For all four tree types within the range of stomatal conductance considered, the

inferred NO_2 compensation point is well below 100 ppt, and not distinguishable from zero within measurement uncertainties.

3.2 NO

Nitric oxide (NO) was added to the purge air at concentrations up to ~10 nmol m^{-3} (~250 ppt) to the white pine, red maple,

335   and bigtooth aspen enclosures (Fig. 4b). Red oak was not included in this experiment. No significant NO flux toward the leaf

surface was observed. This agrees with observations made on Scots pine (Rondón et al., 1993), corn leaves (Hereid and

Monson, 2001), and *Quercus agrifolia* (Delaria et al., 2018). On the contrary, for the white pine, a positive flux up to 2.7 pmol

m^{-2} s^{-1} from the enclosure was measured when NO was added (Fig. 4b, White Pine), indicating emission from the enclosed

plant material. This flux also appears to increase with the enclosure NO mixing ratio. Although the photolysis of surface

340   deposited nitrogen oxides may cause such positive NO flux, during the scrubbed air purge prior to the addition of NO, there

was no significant NO emission from the pine enclosure. That said, this experiment was done only once in a span of five hours

from late morning to early afternoon. We cannot absolutely rule out possible interference from nitrogen containing chemical

components in the system.

3.3 O_3

345   Up to 2.2 μmol m^{-3} (~55 ppb) of ozone (O_3) was introduced to the enclosures. As in the case of NO_2, there was an O_3 loss

within the enclosure, and it increased with input O_3 concentration (Fig. 4c). Shown in Fig. 5c is the comparison of the measured

foliar O_3 deposition velocity to the expected stomatal uptake rate calculated using leaf stomatal conductance and the square

root of the ratio of the O_3 and H_2O molecular weight, $\sqrt{\frac{MW_{H_2O}}{MW_{O_3}}}$. For the red maple, bigtooth aspen, and red oak, these two

values agree reasonably well, implying that foliar ozone loss is mainly through leaf stomatal and closely related to stomatal

350   conductance. Correlation analyses were not performed here due to the limited number of data points.

For the white pine, the measured leaf-level O_3 deposition velocity is significantly greater than the expected stomatal uptake rate. During the experiment, the input O_3 mole fraction was ~45 ppb, and the outlet O_3 mole fraction from the enclosure was only ~25 ppb. We first examined the enclosure humidity and leaf wetness to look for evidence of water condensation on leaf surface. It is known that a 'wet skin' condition would enhance ozone deposition (Altimir et al., 2004; Zhou et al., 2017). Again,

355   the comparison between leaf temperature and the dew point does not support the possibility of water condensing on the pine needles, as the leaf temperature was consistently higher than the dew point. Another known O_3 loss process is chemical removal by gas-phase reactions with biogenically emitted reactive volatile organic compounds (BVOCs). It could be at least partially responsible for the additional ozone loss besides leaf stomatal uptake because conifers have relatively high emission rates of monoterpenes and sesquiterpenes, both of which are more reactive towards O_3 compared to isoprene, the main component of

360   BVOCs emitted by many deciduous trees. Among typical BVOCs, β-caryophyllene has the fastest reaction rate constant with O_3, on the order of 1×10^{-14} cm^3 molecule^{-1} s^{-1} (Burkholder et al., 2015). The residence time of the gas in the enclosure is 1.5 minutes. Thus, at least 20 ppb of such highly reactive BVOCs would be needed inside the enclosure to account for the additional ozone loss, a condition that is hard to fulfill as it would require unusually high BVOC emission rates. O_3 foliar deposition velocities higher than predicted by stomatal conductance have also been reported for other conifers (Norway spruce,

365   2.5 mm s^{-1} and Scots pine, 3.1 mm s^{-1}, (Rondón et al., 1993). Because leaf-level O_3 removal may involve pathways of stomatal uptake, leaf surface cuticular uptake, gas-phase chemical reactions, as well as chemical reactions at leaf surface (Fares et al., 2010; Jud et al., 2016), the observed results here could be due to one dominant pathway or more probably a combination of these sinks, whose dependencies on environmental conditions remain to be investigated.

4 Discussion

370   ### 4.1 Foliar trace gas exchange: NO₂ and O₃

For two weeks during the summer PROPHET2016 campaign, we examined the leaf-level NO_2, NO, and O_3 gas exchange of four different tree species. This work provided a first insight into the general characteristics of the gas exchange of these North American trees in their natural habitat. The trees used in the enclosure measurement represent 80% of the total leaf area within 1000 m radius of the research site, and 90% within the 60 m radius. It is evident from the results that bidirectional foliar gas

375   exchange depends on the trace gas in question and tree type, and is influenced by a combination of diverse and complex environmental conditions, similar to the findings from previous studies mainly on European tree species and on annual plants (grasses and crops). The foliar uptake rates of NO_2 and O_3 vary from tree to tree and even within the same tree. Leaf stomatal conductance of H_2O emerges as a strong indicator of the uptake efficiency. The foliar NO_2 deposition of bigtooth aspen and red oak is almost entirely controlled by stomatal aperture. For red maple and white pine, the correlation coefficient is over 0.7,

380   even though the measured NO_2 foliar deposition velocity is 40−50% of the predicted stomatal uptake rate. Except for white

pine, the O_3 foliar deposition velocity of red maple, bigtooth aspen, and red oak also covary with stomatal conductance (Fig. 5c). Generally, the leaf-level NO_2 and O_3 deposition velocity can largely be inferred from the stomatal conductance of water only, as also concluded from earlier studies for European tree species (Breuninger et al., 2013; Rondón and Granat, 1994).

Thus, the factors controlling leaf stomatal conductance would in turn greatly influence NO_2 and O_3 deposition to the forest
385   canopy. These factors include PAR level, ambient temperature, moisture, soil conditions, as well as ambient CO_2 (Jarvis, 1976). Further, the capability of the foliar uptake of trace gases would also depend on the intrinsic characteristics of leaf stomata, such as their size and density on the leaf surface, determined by plant species and stage of maturity, and factors such as growth history, leaf age, tree height and the vertical location of the leaf on the tree (Kirkham, 2014; Schäfer et al., 2000; Sparks et al., 2001). In this work, the stomatal conductance of the bigtooth aspen was 3–5 times higher than that of the other
390   trees. To look for potential causes of this large difference, we examined the environmental conditions of the enclosures. The integrated PAR exposure levels were similar. The daily variation of the relative humidity in the bigtooth aspen enclosure was not significantly different from the others. In contrast, the average daily temperature was 19.2°C, cooler than the temperatures (23.9°C, 22.6°C, and 21.6°C) in the other enclosures. The effect of temperature on the stomatal conductance is generally coupled with the effect of water vapor pressure deficit (VDP), with the combined influence possibly leading to an increase in
395   stomatal conductance if the VDP effect is not yet inducing stomatal closure for further temperature increases (Urban et al., 2017a, 2017b). A more likely explanation, without excluding the possible environmental influence, comes from tree species, and leaf position and age. Although many trees have stomata on only the lower leaf surface (abaxial surface), trees that belong to the genus *Populus*, which includes aspen, are an exception. Leaves of these trees have stomata on both sides, a feature that allows increased photosynthetic rate and fast growth (Kirkham, 2014). Additionally, the aspen sampled in this study was
400   smaller and younger than the other three trees. The enclosed branch was close to the top of the tree, with relatively young and developing leaves. This type of leaves generally has relatively high stomatal density compared to more mature leaves located lower on a tree. Likely, the aspen leaves had more stomata per unit area than the other tree leaves sampled in this work, which led to higher stomatal conductance and the higher rates of NO_2 and O_3 uptake through stomata. In future, this possibility should be further verified by comparing the stomatal counts on the sampled leaves, but unfortunately was beyond the scope of this
405   work. Given the large dependence of NO_2 and O_3 uptake on the leaf stomata properties, it is of interest to consider how the factors influencing stomatal conductivity, giving its variability and inhomogeneity, could be better incorporated into models from leaf to canopy scale to improve the canopy-wide understanding and description of NO_x and O_3 dynamics.

To put our measurement results in perspective, we compare our measured daytime maximum foliar deposition velocity of NO_2 with the results from previous studies on American trees (Table 3). Although the development stages of the trees, PAR,
410   humidity and temperature conditions are different, the results are comparable, ranging from 0.76 to 1.6 mm s^{-1} from this work and 0.4–1.8 mm s^{-1} from earlier work. We also compare our results to those of several native European trees - scots pine, evergreen oak, common oak, European beech, and silver birch, measured under the conditions of PAR = 900 μmol m^{-2} s^{-1}, maximum temperature 27.7°C, and relative humidity 31.2–99.9% (Breuninger et al., 2013). The maximum NO_2 deposition

415   rates were ~0.5–1 mm s^{-1} for all but the birch tree, which was ~1.5 mm s^{-1}. These numbers are also fairly similar to those of pine, maple and oak reported here. What stands out but without a direct or closely related comparison is the high rate of trace gas uptake by the aspen leaves. Although the comparisons show reasonable agreement, it is evident that the NO_2 (and O_3) foliar uptake is highly variable depending on a myriad of conditions, both environmental and intrinsic to tree species and its developmental stage. Measurement results and comparisons from different studies are probably also sensitive to experimental protocols and environmental conditions. These factors should be taken into consideration if more comparisons are to be made

420   in the future.

4.1.1 NO_2 compensation point

Measured fluxes of NO_2 toward the leaf surface while the stomatal conductance was at least 50% of the observed maximum value were used to assess the possible existence of an NO_2 compensation point. It would have been indicated by a zero or positive NO_2 flux at significantly non-zero NO_2 concentrations defined by the measurement system detection limit (Table 1).

425   We found no such evidence of an NO_2 compensation point for all the tree species measured in this work. Indeed, this lack of evidence of a compensation point is also supported by the fact that no significant, sustained NO_2 emission was observed while the enclosures were purged with the scrubbed air only. For all four trees in this study, the compensation point, if it exists at all, would be well below 150 ppt. Thus, this finding does not support the existence of a 1 ppb NO_2 compensation point as suggested in the previously mentioned combined NO_x concentration measurement and canopy exchange model study (Seok et al., 2013)

430   to reach the best agreement between the simulated and observed NO_x concentrations above and within the forest canopy at the UMBS site. We would like to point out that the NO_2 flux may reach zero at higher NO_2 concentration if the stomata are not adequately open and the stomatal conductance is lower than the values used above (Fig. 7). However, because here the NO_2 uptake is mainly through stomata, such zero flux at relatively high NO_2 mixing ratio is not indicative of a compensation point; rather, the reduced capability of absorbing NO_2 under the reduced stomatal conductance. Our result agrees with recent reports

435   on several other tree species that an NO_2 compensation point is not observed above the detection limit of the measurement using improved NO_2-specific instruments with minimal interference from other nitrogen compounds (Breuninger et al., 2013; Chaparro-Suarez et al., 2011).

4.2 NO

There was no significant leaf-level deposition of NO for all the tree species studied here. Instead, relatively small NO emissions

440   were detected from white pine when up to ~250 ppt NO was added to the enclosure. We searched for possible errors that might have led to this observation but could not find an obvious explanation. Certainly, additional measurements are necessary to verify this observation. Using the leaf area index of white pine at UMBS, 0.11 m^2/m^2 (Vogel, 2016b) and the maximum measured flux, 2.7 pmol m^{-2} s^{-1}, we estimated the potential canopy-wide NO flux from this emission to be 0.3 pmol m^{-2} s^{-1}, less than 10% of the reported minimum soil NO emission flux of 4–10 pmol m^{-2} s^{-1} at UMBS (Nave et al., 2011).

445   Although this observation seems counterintuitive, in previous publications, emission of NO has been reported from leaves of individual corn plants exposed to 0.1–0.3 ppb NO (Hereid and Monson, 2001), from leaves of California live oak exposed to air containing NO (Delaria et al., 2018), from several nitrate-nourished plant species (Wildt et al., 1997) as well as pesticide-treated soybean leaves (Klepper, 1979). Additionally, recent plant physiological studies have started to reveal the mechanism of plant NO production and its importance for regulating growth and development, immunity, and signaling (Astier et al.,

450   2017; Río, 2015; Yu et al., 2014), as well as for responding to pollutants and stress (Bison et al., 2018; Farnese et al., 2017; Velikova et al., 2008). In light of these advances, more targeted observations of foliar NO exchange probably should be conducted while taking these biological factors into consideration.

5 Summary and Conclusions

Using a branch enclosure technique and with controlled addition of trace gases, we obtained data on NO, NO_2, and O_3 leaf-

455   level gas exchange from field experiments on several native tree species in a northern hardwood forest in Michigan, USA. To our knowledge, this is the first time such experiments have been done on North American tree species in a field study. The results provided a new dataset of NO_x and O_3 leaf-scale fluxes and have allowed comparisons of the gas exchange characteristics of mature trees compared to seedlings of these species in the lab and to mature European tree species in the field. The data also provide information, including an upper bound on NO_2 compensation points for these trees, to models of

460   NO_x and O_3 dynamics at the canopy level, particularly for the forest at the PROPHET research site.

A brief survey of the foliar O_3 loss found that the uptake by the deciduous trees also closely followed stomatal conductance, while the O_3 foliar deposition velocity for white pine was much larger than expected from leaf stomatal uptake alone, a difference that cannot be explained by leaf surface moisture conditions nor solely by gas-phase chemical removal. It is likely that other ozone removal pathways, such as cuticular uptake and the plant response to increased ozone level, were

465   simultaneously responsible for this elevated deposition velocity.

The trace gas exchange characteristics of NO, NO_2, and O_3 at the leaf level varied depending on tree type and environmental conditions. For NO, there was no measurable foliar uptake from any of the trees studied here. On the contrary, there appeared to be a small emission of NO from white pine when NO was added to the enclosure. Leaf-level NO_2 uptake of bigtooth aspen and red oak was mainly through leaf stomata, with the leaf-level deposition velocity of NO_2 closely following predicted values

470   based on the stomatal conductance of water and molecular diffusivity. The stomatal conductance of aspen was ~5 times higher than that of red oak (and thus the foliar NO_2 deposition velocity for aspen). Because stomatal conductance is subject to a variety of factors including those intrinsic to plants, further investigation is needed to determine whether this difference is generally associated with the plant species or is environmentally driven. For white pine and red maple, the foliar NO_2 deposition velocity correlated with stomatal conductance, but there were additional factors that prevented the deposition from

475   increasing as much as expected with the increasing conductance, suggesting the existence of internal mesophyll resistance to

uptake. Furthermore, for white pine, there was foliar NO_2 deposition when stomatal conductance was zero, suggesting a non-stomatal NO_2 loss pathway such as cuticular uptake.

The possible existence of an NO_2 compensation point was inferred by examining the linear relationship between NO_2 flux and ambient NO_2 concentration when the stomata were open, and the stomatal conductance was at least 60% of the maximum
480 measured value. The results showed that the compensation point was ≤ 60 ppt for all trees and was statistically indistinguishable from zero within the measurement sensitivity. This finding does not support the suggested 1 ppb compensation point needed to reconcile the observed and model-simulated NO_x mixing ratios by Seok et al. (2013). Neither does it support any significant foliar NO_2 emission from these tree species at low ambient NO_2 conditions.

It is noteworthy that, beyond the findings in Seok et al. (2013), inclusion of an NO_2 compensation point not only provided
485 the best agreement in terms of NO_x concentrations, but also gave the best agreement between simulated and observed atmosphere-biosphere NO_x fluxes at UMBS in summer 2016 (J. Murphy, personal communications, 2018). Evaluations of these simulations with MLC-CHEM have not yet included a direct comparison with the leaf-scale NO_x and O_3 fluxes reported here. Such a comparison could address both the observed large differences in the magnitude of the stomatal conductance for specific trees and its diurnal cycle, focusing on the early morning onset of stomatal opening and uptake. This would help to
490 establish whether there is a leaf-scale NO_x emission flux due to an NO_2 compensation point, or if a strongly reduced NO_x uptake might partially explain the observed dynamics in the above- and in-canopy NO_x concentrations. This analysis would also benefit from more detailed temporally and vertically resolved NO_x concentration gradient observations compared to the Seok et al. (2013) study, which we measured in conjunction with the leaf-level work described here. This comparison would be an essential next step to potentially reconcile the findings of this study with previous studies of NO_x exchange at the UMBS
495 forest, and will be presented in a follow-up publication.

Our findings confirmed that the main conduit of trace gas foliar uptake is leaf stomata. A thorough grasp of the trace gas uptake efficiency hinges on an understanding of the leaf stomatal properties, which depend on the genetic makeup and developmental stage of the plant, as well as the environmental conditions of sunlight, water vapor, ambient temperature, soil, and nutrients. Meanwhile, the characteristics of additional factors affecting the foliar trace gas exchange, mesophyll resistance,
500 cuticular uptake, and stress responses, are also subject to plant intrinsic and external conditions and remain to be better understood.

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510 *Data availability*. The data from this work are available upon request.

Author contributions. WW, SR, and JH constructed the enclosure chambers, deployed the instruments, and carried out the field experiments. WW performed the data analysis and prepared the manuscript. LG and DH provided extensive comments and suggestions for the manuscript. LG and DH initiated this project based on their previous fieldwork at UMBS and model
515 analysis regarding leaf-level gas exchange.

Competing interests. The authors declare that they have no conflict of interest.

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Figures and Tables
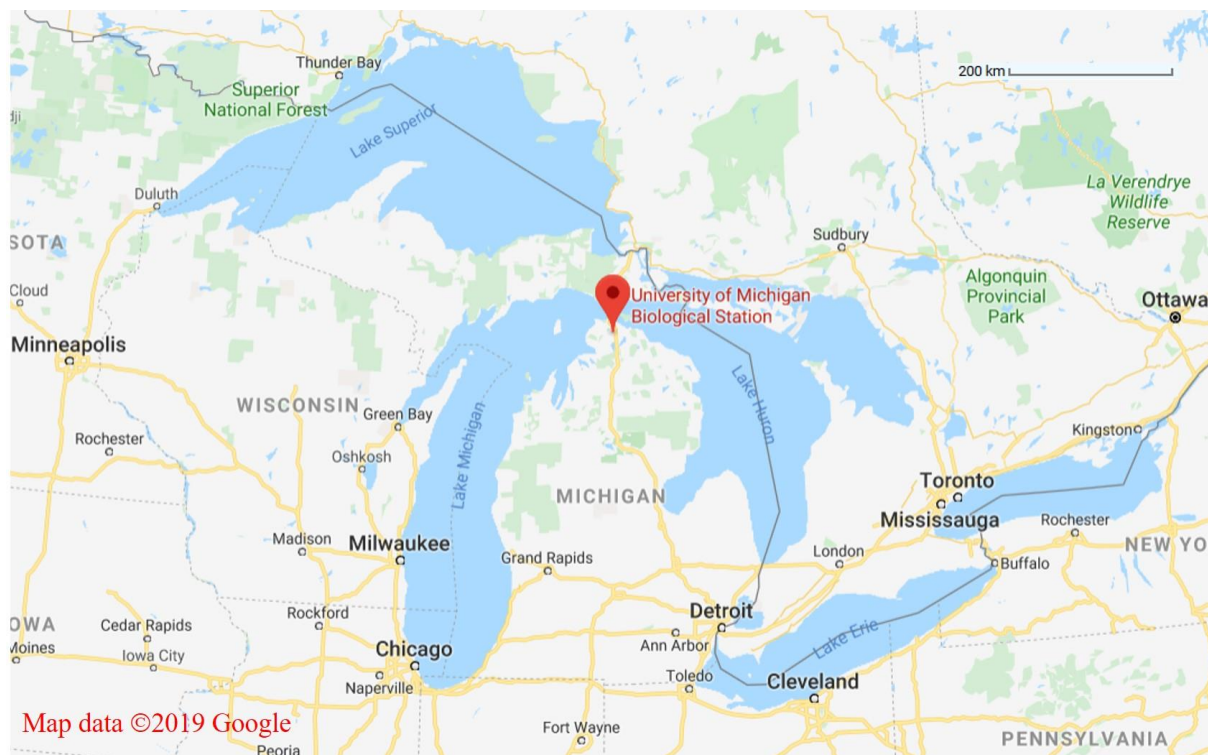
Figure 1. Location of the University of Michigan Biological Station, (45.56°N, 84.71°W), indicated by the red pin on the map. The map scale is shown in the upper right corner. (Map data ©2019 Google).

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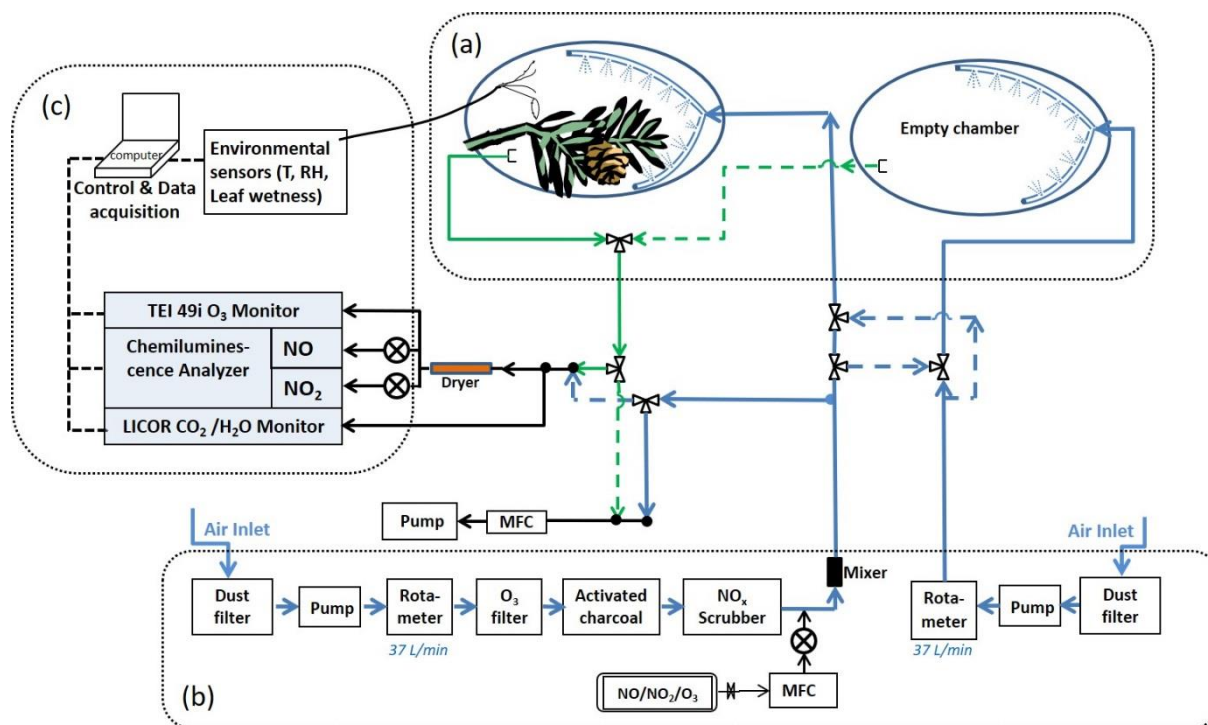
Figure 2. Schematic of the enclosure experiment system. The system is comprised of three main parts as shown in the figure: (a) the enclosures, (b) the purge air flow system, and (c) the trace gas measurement instruments. The blue lines and arrows indicate the air flowing into the enclosures; the green lines and arrows indicate the air flowing out of the enclosures; and the black lines and arrows indicate the air sample flow and the balance flow (to maintain constant flow rates in the enclosures). NO and NO$_2$ gas standards were used for the controlled addition of these trace gases to the input air stream. Controlled O$_3$ addition was done by generating ozone on demand using a pen-ray lamp and ultra-high purity oxygen. See the text for instrument details.

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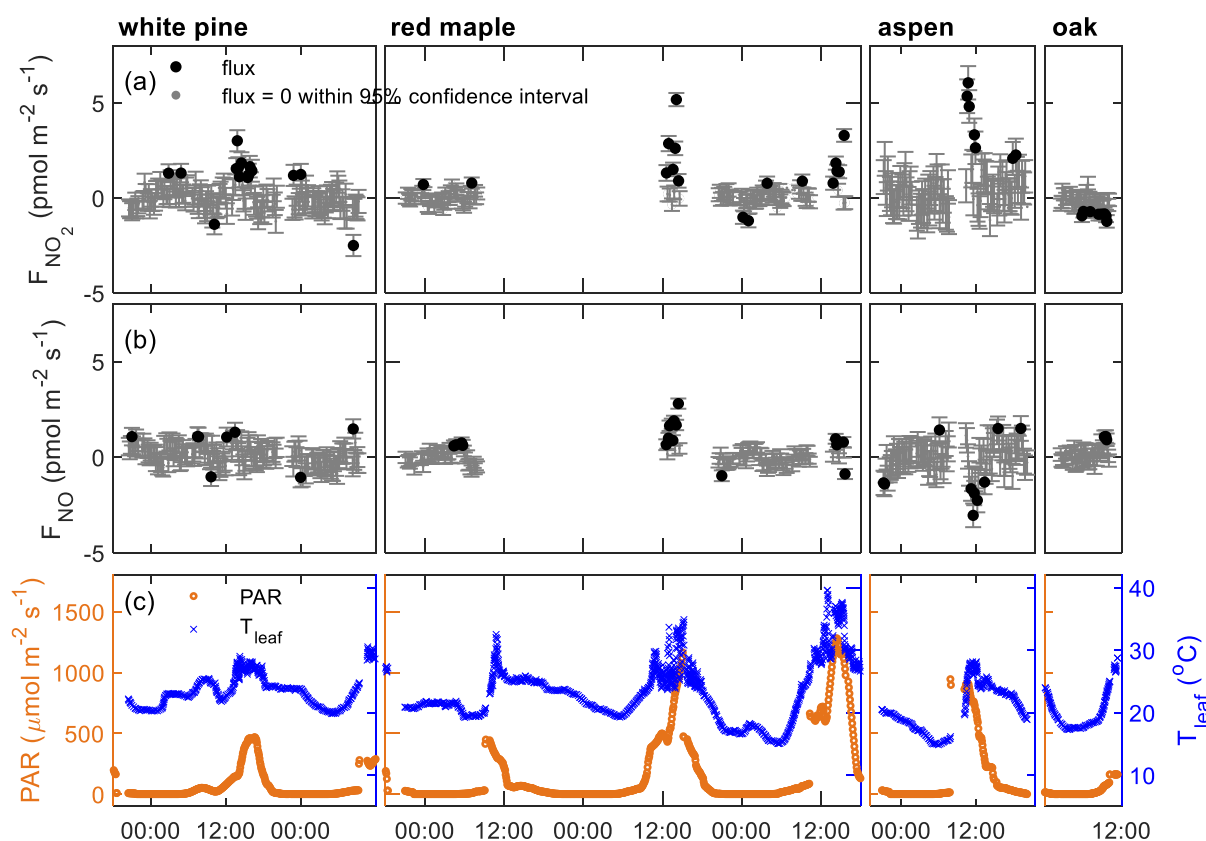
Figure 3. Apparent fluxes of NO₂ and NO when the enclosures were purged with scrubbed air. From left to right each panel corresponds to the enclosure of white pine, red maple, bigtooth aspen, and red oak. From top to bottom: (a) NO₂ flux, (b) NO flux, and (c) PAR (left axis, orange) and temperature of the enclosure leaves (right axis, blue). In panel (a) and (b), fluxes that are indistinguishable from zero within the 95% confidence interval are represented by gray dots; statistically significant fluxes are represented by black dots; error bars represent 1-σ measurements uncertainties propagated through the calculations.
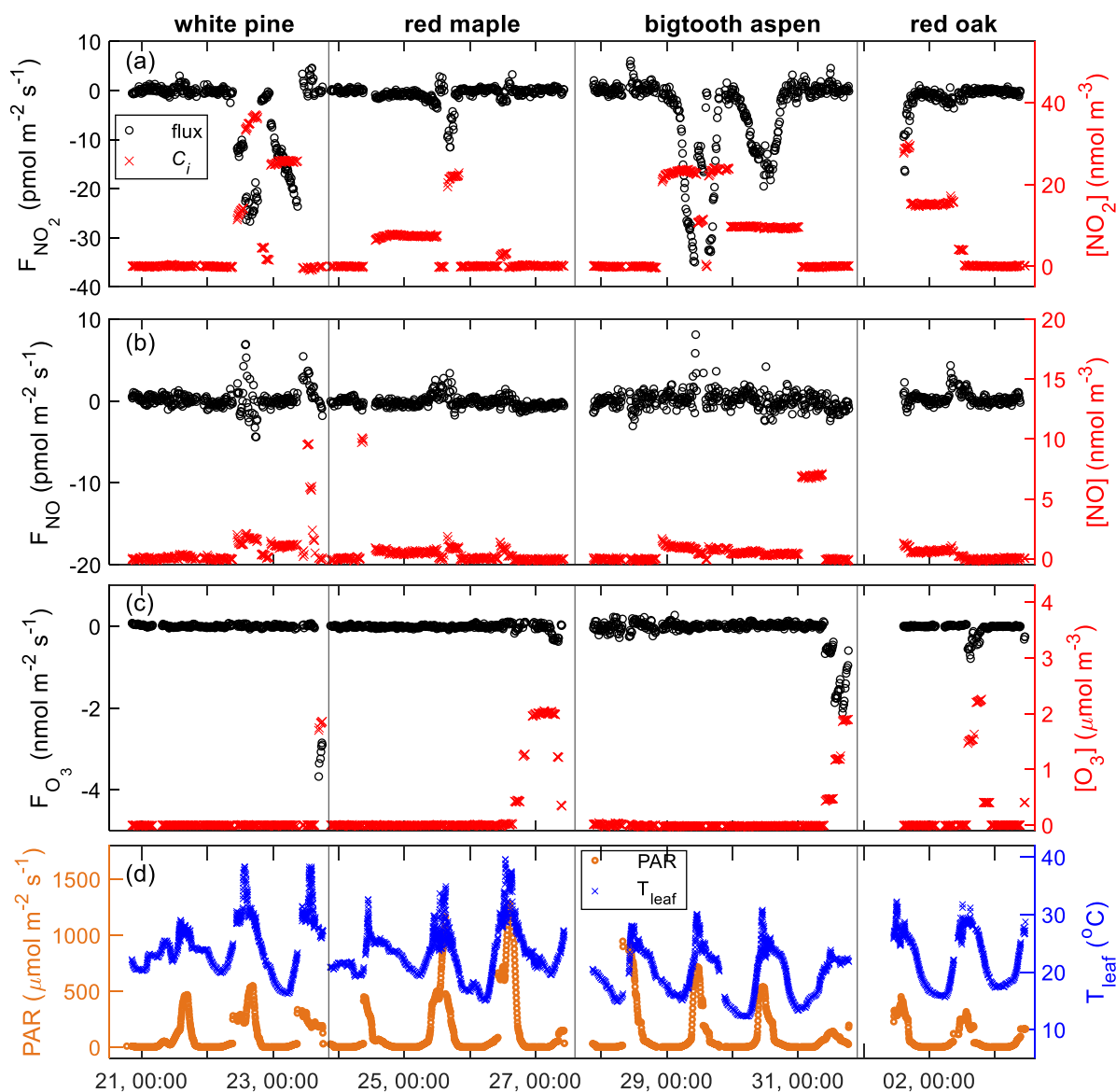
Figure 4. Time series of the enclosure gas exchange experiments from July 21 to August 3, showing the trace gas fluxes (black symbols: o, left axis) and input trace gas concentrations, C_i, (red symbols: x, right axis) of, (a) NO_2, (b) NO, and (c) O_3. Solar irradiation PAR (orange: o, left axis) and temperature of the enclosure leaves (blue x, right axis) are shown in the bottom panel (d). The tree species are labeled for each enclosure period at the top of the figure. The x-axis tick label format is Day, HH:MM.
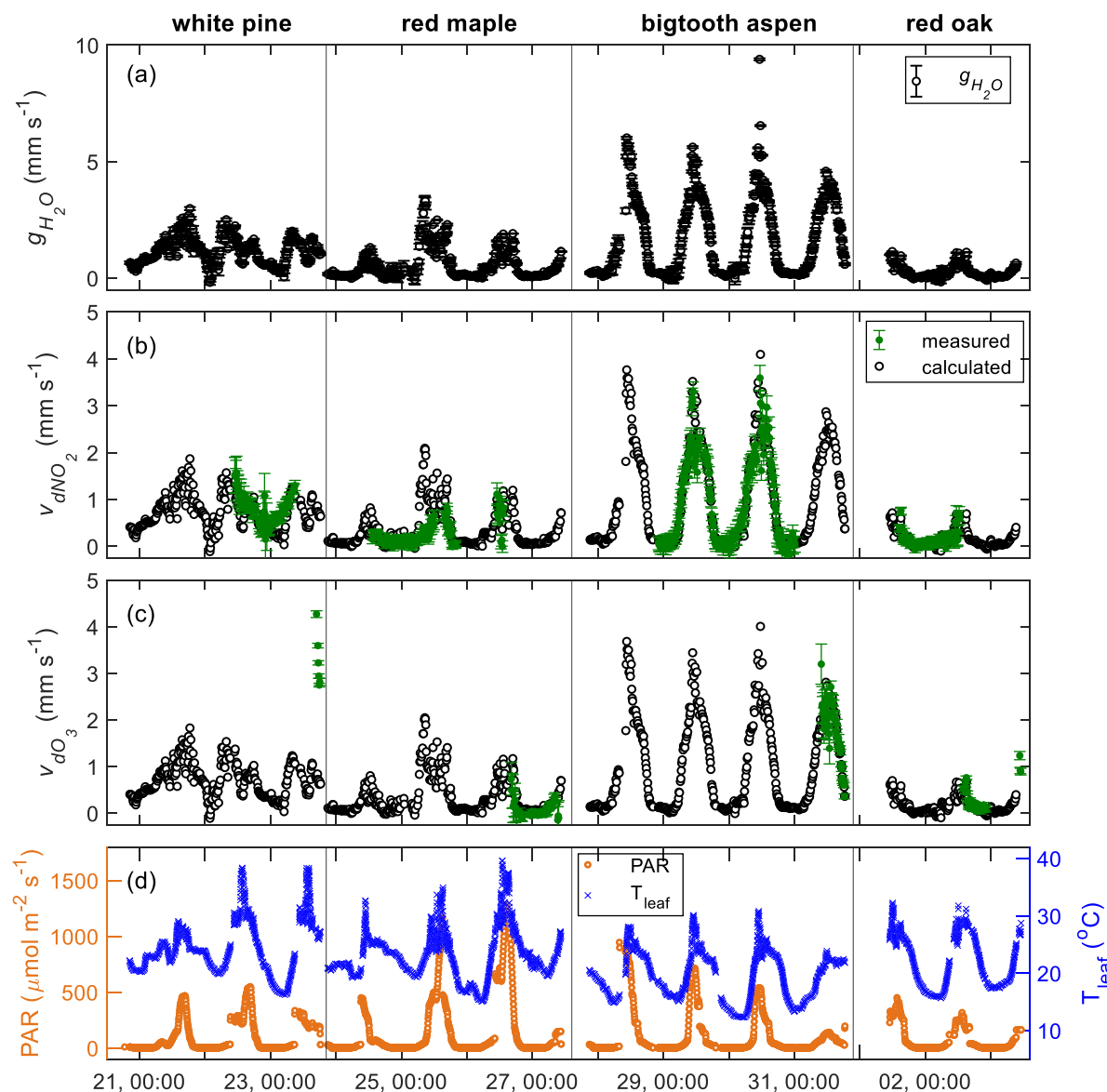
Figure 5. Time series plots of (a) the measured stomatal conductance of water; (b) the measured foliar deposition velocity (green symbol) and the calculated stomatal uptake rate (black symbol) of NO_2; (c) the measured foliar deposition velocity (green symbol) and the calculated stomatal uptake rate (black circle) of O_3; and (d) the corresponding PAR and leaf temperature during the experiments from July 21 to August 3, 2016.
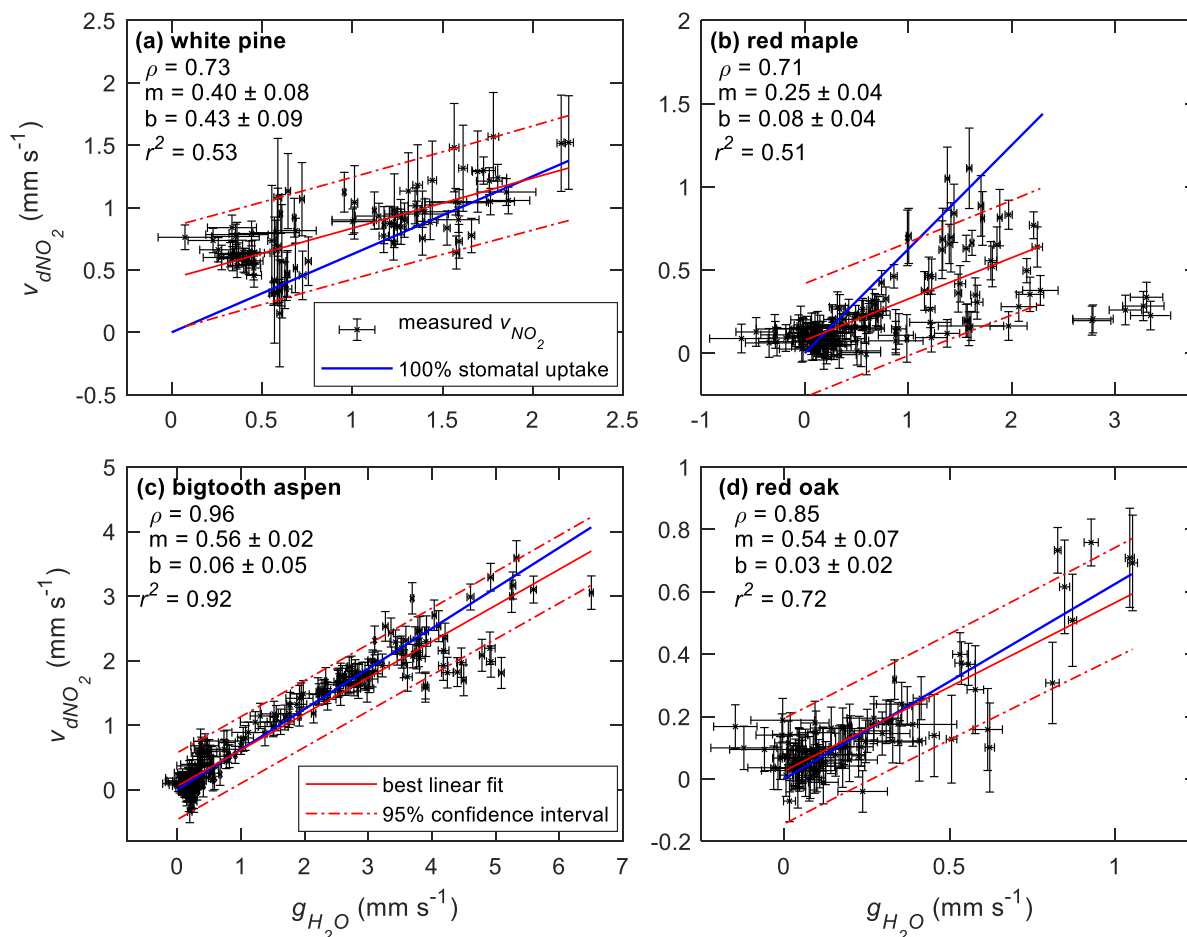
Figure 6: Scatter plots of foliar NO_2 deposition velocity (v_{dNO_2}) vs. stomatal conductance of water (g_{H_2O}) for (a) white pine, (b) red maple, (c) bigtooth aspen, and (d) red oak. The data points and their error bars are represented by the black symbols. The solid and dashed red lines are the best-fit linear regression and the 95% confidence bounds, respectively. The solid blue line shows the relationship between the deposition velocity and the stomatal conductance if NO_2 loss is entirely controlled by the stomata. The slope of the blue line is 0.62, the square root of the ratio of the molecular weight of water to NO_2. Listed in each subplot under the tree name are the Pearson correlation coefficient (ρ), and the slope (m) and intercept (b) of the best fit linear regression line.
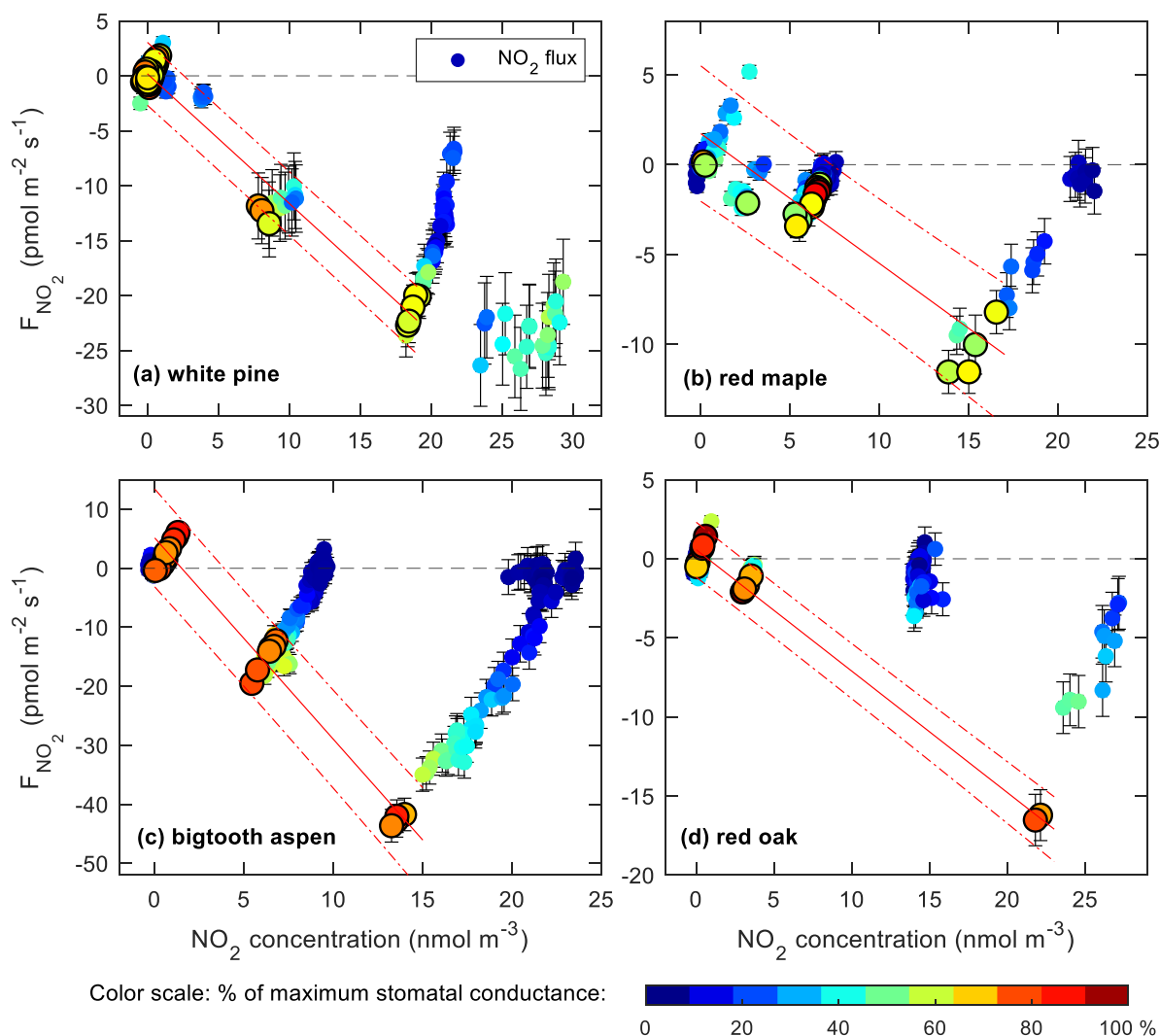
Figure 7. NO₂ flux toward the leaf surface vs. NO₂ concentration in each enclosure. Also shown is the stomatal conductance on a cool–warm color scale with the dark blue representing the lowest values and the red the highest values observed. Flux data with the stomatal conductance 60% of the observed maximum or higher were used for the linear extrapolation to find the compensation point. These data points are shown as larger symbols with a black outline/border. The solid and dashed red lines show the linear fit and 95% confidence bounds. The resulting compensation points are listed in Table 2.

Table 1. Detection limits of the foliar fluxes of NO₂, NO, and O₃. These were determined using the flux data obtained during the nighttime scrubbed air purges when no foliar gas exchange was expected. These limits mainly reflect the measurement precision of the trace gas concentrations at the inlets and outlets of the branch and reference enclosures, variations of the enclosure conditions over time, and the fluctuation of the purge air flow rate.

Detection limit	F_{NO_2} (pmol m^{-2} s^{-1})	F_{NO} (pmol m^{-2} s^{-1})	F_{O_3} (pmol m^{-2} s^{-1})
White pine	1.1	1.0	76.3
Red maple	0.6	0.6	68.0
Bigtooth aspen	2.0	1.3	233
Red oak	0.8	0.8	42.7

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Table 2. Compensation points from the flux vs. concentration linear fits in Fig. 7. The ranges of the stomatal conductance of the data used are also listed.

Tree species	White pine	Red maple	Bigtooth aspen	Red oak
Stomatal conductance (mm s^{-1})	1.8–3.0	1.8–3.5	4.5–6.0	0.8–1.3
Compensation point ± 95% confidence level (ppt)	4 ± 60	60 ± 119	38 ± 59	19 ± 56
Compensation point ± 95% confidence level (nmol m^{-3})	0.2 ± 2.4	2.4 ± 4.9	1.6 ± 2.4	0.8 ± 2.3

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Table 3: Comparison of foliar NO₂ deposition velocity from this work and earlier studies. The maximum velocity measured in each enclosure is listed with the corresponding light, RH, and leaf temperature at the time of the measurement.

Tree species	v_{NO_2} (mm s^{-1})	PAR (μmol m^{-2} s^{-1})	RH (%)	T_leaf (°C)	Source
Pinus strobus (white pine)	1.6	601	67	30	This work
Pinus strobus (white pine, seedling)	0.4	"Adequate to open leaf stomata"	n/a	29.4	Hansen (1989)
Acer rubrum (red maple)	1.1	1200	72	30	This work
Acer rubrum (red maple, seedling)	1.8	"Adequate to open leaf stomata"	n/a	29.4	Hansen (1989)
Quercus rubra (red oak)	0.76	1086	61	28	This work
Quercus agrifolia (California live oak)	1.23	1190	50–65	26	Delaria (2018)
Populus grandidentata (bigtooth aspen)	3.6	850	71	25	This work