2010

Nitrogen Dioxide in the Urban Forest: Exposure and Uptake

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NITROGEN DIOXIDE IN THE URBAN FOREST: EXPOSURE AND UPTAKE

A Thesis Presented

by

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Submitted to the Graduate School of the
University of Massachusetts Amherst in partial fulfillment
of the requirements for the degree of

MASTER OF SCIENCE

September 2010

Plant and Soil Science

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ACKNOWLEDGMENTS

Many people have helped to make this research possible. First and foremost, I thank my advisor, Dr. William J. Manning, and the other members of my committee, Dr. Allen V. Barker and Dr. Michelle DaCosta, for their guidance and support. For their assistance in the field and lab, I thank Tom Langelier, Jennifer Albertine, and Lindsey Hoffman. I thank Dena Vallano of Cornell University for sharing advice and data from her dissertation work. I thank the people of the Massachusetts Department of Environmental Protection, and in particular Mark Ducomb, for providing real-time monitoring data and access to their monitoring site in Springfield. I thank Springfield College and Springfield Museums for granting access to their grounds and for assistance in setting up equipment and the Springfield Department of Parks and Recreation, particularly Deryk Roach, for granting access to Forest Park and the Springfield Visitor Center and for their support and enthusiasm for my work. This work was funded by a McIntire Stennis research grant from the Massachusetts Agricultural Experiment Station, University of Massachusetts, Amherst.

ABSTRACT

NITROGEN DIOXIDE IN THE URBAN FOREST: EXPOSURE AND UPTAKE

SEPTEMBER 2010

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It is accepted widely that trees are useful for improving air quality, particularly in polluted urban environments. Most evidence for this apparent axiom comes from complex models; however, little effort has been made to validate these models using data collected under ambient conditions in the field. Overall there is a need to understand better the urban environment in terms of meteorology and pollution and their respective variations over multiple spatial and temporal scales. There is a particular need to document the environmental conditions of the urban forest with respect to water relations among soil, plant, and atmosphere and with respect to pollution levels in and around tree canopies. There is also a need to develop techniques for quantifying foliar uptake of air pollution by trees under ambient urban conditions.

As a step toward improving our understanding of the urban environment, nitrogen dioxide (NO_2) levels were measured inside and adjacent to canopies of urban trees in Springfield, MA, over two growing seasons with the hypothesis that if trees are a useful sink for NO_2 there should be a downward NO_2 gradient moving from outside to inside of the tree canopy. Nitrogen dioxide levels were consistently and significantly higher inside tree canopies compared to levels outside the same canopies. During the second growing

season, ozone (O_3), temperature, and relative humidity (RH) were also measured using samplers co-located with the NO_2 samplers. Ozone levels were significantly lower inside the canopy whereas temperatures were slightly, but significantly, higher inside the canopy, and RH was not significantly different between inner and outer canopy locations. Overall, these results appear to corroborate theoretical models predicting elevated NO_2 and depressed O_3 levels inside tree canopies based on photochemistry, but put into question the mechanisms involved in generating these levels.

In a separate study, the use of a common urban street tree (red maple, *Acer rubrum*) as a tool for measuring NO_2 uptake under field conditions was evaluated using a model that has previously been applied only to potted herbaceous plants and potted coniferous trees. Using potted saplings of *A. rubrum* located at locations with high or low NO_2 levels in Springfield, MA, and Amherst, MA, we measured ^{15}N stable isotope signatures ($\delta^{15}N$) and total N (%N) of leaves throughout the growing season. Overall, there was not a significant difference in leaf $\delta^{15}N$ or %N change between sites over the course of the season. Changes in leaf $\delta^{15}N$ were likely the result of input from N sources in the nutrient solution whereas changes in leaf %N over the course of the season followed a natural seasonal decline reported elsewhere in the literature. The study highlights the difficulties in applying this particular model to deciduous trees and suggests work needed to overcome these challenges.

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INTRODUCTION

CHAPTER 1

THE URBAN ENVIRONMENT AND THE ROLE OF THE URBAN FOREST

1.1 Urbanization and Urban Metabolism

The global population is currently estimated at 6.8 billion people (U.S. Census Bureau 2010) with more than half of them living in urban areas[1] (Bettencourt et al. 2007; Crane and Kinzig 2005; Parrish and Zhu 2009). The trend of global urbanization will continue; it is expected that future population growth will occur in cities with estimates of urban population growth ranging from 1.75 to 4.9 billion people by 2030 (Mcdonald et al. 2008; Patel and Burke 2009). With half of the world's population living in a combined area estimated at 0.3 to 2% of the Earth's land surface (Bettencourt et al. 2007; Crane and Kinzig 2005), city life has the possibility of being highly efficient and sustainable (Kaye et al. 2006). However, today's cities are far from sustainable, requiring vast inputs in the form of water, food, and fuel, and producing enormous quantities of heat, waste, and pollution (Crane and Kinzig 2005; Decker et al. 2000). Although more than half of the global population live in cities, we are only just beginning to understand the complex dynamics of the urban ecosystem (Decker et al. 2000; Kaye et al. 2006).

It has been suggested that urban ecosystems have a biogeochemistry distinct from that of agricultural or unmanaged ecosystems (Kaye et al. 2006). In response to increasing urbanization, Wolman (1965) was the first to apply an ecosystem framework to cities, drawing upon the traditional ecological concept of metabolism (i.e. Odum

[1] For purposes herein, urban areas are defined as built environments with high population densities, generally removed from the source of production of raw materials necessary to sustain such population densities (i.e. cities).

1971). Urban metabolism has been variously defined as the flow of energy and materials through the urban ecosystem (Kennedy et al. 2007; Wolman 1965). Although Wolman first described urban metabolism and its usefulness in understanding the urban ecosystem and creating sustainable cities 65 years ago, few comprehensive efforts have been made to quantify the metabolism of urban cities (Decker et al. 2000; Kennedy et al. 2007). Fewer efforts have been made to apply this concept in sustainable urban design.

Using this framework, Decker et al. (2000) divided the flow of energy and material through the urban ecosystem into inputs and outputs, with energy and material being stored or transformed, or both, in between. The flow of energy and materials through the urban ecosystem increases as cities grow, and the transformation of food, fuel, and water results in waste products of heat, landfill, sewage, and air pollution (Decker et al. 2000; Kaye et al. 2006; Kennedy et al. 2007; Mayer 1999). The particular concern here will be on the emissions of air pollution.

1.2 Urban Forest: Definition and Role in Urban Metabolism

The urban forest is defined as all trees within and associated with an urban area, including the planted landscape, remnants of original forest, and new introductions of invasive or otherwise opportunistic tree species (Gerhold 2007; McPherson 2006; Nowak et al. 2005). Components of the urban forest include parks and greenways (both planted and residual forest), trees planted in parking lots and along streets and sidewalks, and so called “weed-trees” that often sprout in derelict lots and other unmaintained areas. With over half of the world’s population living in cities and this

number projected to increase rapidly, urban forests may be the primary means by which people experience nature.

It has been suggested that the urban forest plays an essential role in urban metabolism, particularly with respect to creating sustainable cities (Manning 2008). Much work has been done to describe and quantify the role trees play in urban ecosystems—a body of work based largely in models (Dwyer et al. 1992; McPherson and Simpson 2002; Nowak et al. 2002a). The benefits attributed to urban forests are wide ranging and include climate moderation, reductions in energy use (and associated CO_2 production), improved air quality, reduced water runoff and flooding (and associated discharge of untreated wastewater), noise reduction, increased wildlife habitat, improvements in human health and general sense of well being, reductions in crime, and increased property values (Nowak and Dwyer 2007). Some of these benefits are based on actual measurements and observations (e.g. Freer-Smith et al. 2005; Streiling and Matzarakis 2003), but most are based on large-scale modeling (e.g. McPherson and Simpson 2003; Nowak et al. 2000), and thus many of these benefits are theoretical (Manning 2008). While the theoretical data provided by such models may ultimately be correct, such information may be unintentionally misleading if the process by which trees provide such services is not understood.

1.3 Air Pollution Removal by the Urban Forest

In terms of urban metabolism, the transformation of fuel into energy and by-products of heat and air pollution in the form of particulates, aerosols, and oxidized gases is a major component of the urban ecosystem (Decker et al. 2000). It is accepted widely that trees clean the air and that the urban forests can have a significant impact on

urban air quality (Manning 2008). A number of complex models have been employed to estimate the magnitude of this impact (e.g. McPherson and Simpson 2002; Shan et al. 2007). The most notable of these models is the Urban Forest Effects (UFORE) model created by the U.S. Forest Service (Nowak and Crane 2000). The UFORE model has been applied to a number of cities with great success, suggesting that trees in these cities remove significant quantities of air pollutants (Escobedo and Chacalo 2008; Nowak et al. 1999, 2002b). Results from such studies have led the U.S. EPA to grant State Implementation Plan credits for tree planting as pollution reduction strategy for attainment of National Ambient Air Quality Standards under the Clean Air Act (Nowak 2005).

The UFORE model for dry deposition of air pollution, termed pollutant flux (F), is a function of the deposition velocity (V_d) of the pollutant and its concentration (C):

$$F\ (g\ m^{-2}\ s^{-1}) = V_d\ (m\ s^{-1}) \times C\ (g\ m^{-3})$$

where V_d is the inverse of the sum of the aerodynamic (R_a), quasi-laminar boundary layer (R_b), and canopy (R_c) resistances:

$$V_d = (R_a + R_b + R_c)^{-1}$$

The model is based in large part on the model created by Baldocchi et al. (1987). R_a and R_b are well rooted in physics; they are based on physical and chemical properties of the gas in question and its interaction with the physical environment. Inputs for R_a and R_b are well established in the literature. R_c is based on plant biology; its inputs vary by species and environment in complex ways and are not well established. Baldocchi et al. (1987) define R_c as an inverse function of canopy stomatal resistance (R_s), canopy mesophyll resistance (R_m), soil resistance (R_{soil}) and cuticle resistance ($R_{cuticle}$):

$$1/R_c = [1/(R_s + R_m) + 1/R_{soil} + 1/R_{cuticle}]$$

R_s is based largely on the model created by Jarvis (1976), with the assumption that temperature, vapor pressure deficit, and leaf water potential are constant with height in the canopy (Baldocchi et al. 1987). R_m is based on several studies that suggest mesophyll resistance is determined by the surface area of the mesophyll and the solubility of the gas (Hill 1971; Hosker and Lindberg 1981; O'Dell et al. 1977)[2]. At the time of publication, $R_{cuticle}$ and R_{soil} were based solely on published values. Inputs for R_c in UFORE include photosynthetically active radiation (PAR), air temperature, wind speed, carbon dioxide concentration (generally set at 360 ppm), and absolute humidity (Nowak and Crane 2000). These inputs primarily concern R_s, and it is not clear how R_m, R_{soil}, or $R_{cuticle}$ are accounted for in the model.

Implicit in modeling of gas uptake by UFORE is that stomates are open and leaves are actively taking up gases during daylight hours throughout the leaf-on period; little attention is paid to the multitude of environmental and physiological factors affecting stomatal conductance, carbon-fixation, or the metabolism of nitrogen and other nutrients that plants may acquire from air pollution (e.g. sulfur from SO_2). As such, predictions of pollutant uptake based on this and other models may have a large error component, particularly when modeling the uptake of gaseous pollutants which is largely under stomatal control (Fowler 2002). Sources of error for gas uptake include (1) elements of tree exposure such as spatial and temporal heterogeneity of air pollutants, canopy structure, and variations in boundary layer resistance, and (2) elements of tree uptake such as vapor pressure deficit, soil moisture status, leaf

[2] It has since been established that mesophyll resistance is significantly more complicated than presented in the UFORE model. See discussion in section 2.3.

nutritional status, endogenously controlled seasonal changes in stomatal conductance during the leaf-on period, vertical changes in stomatal conductance within the tree canopy, PAR levels, and variations in air temperature, wind speed and wind direction, and humidity (Escobedo and Nowak 2009; Eller and Sparks 2006; Fowler 2002; Hanson and Lindberg 1991; Parkhurst 1994; Scott et al. 1998; Ramge et al. 1993). Only PAR, temperature, wind speed, and humidity are included in UFORE calculations of R_c (Nowak and Crane 2000).

Urban environments are a complex composite of street canyons, pockets of open areas such as parking lots or green spaces, and residential areas, all with varied topography and vegetation. Associated with this complex structural environment are large variations in usage, traffic, and microclimate at both temporal and spatial scales. As such, the modeling of urban environments in terms of meteorology and pollution levels is a formidable task. Canopy resistances (R_c) used in UFORE are based on models derived from relatively uniform forest settings (Baldocchi et al. 1987; Baldocchi 1988) and may not accurately describe R_c for trees growing in complex urban environments. Furthermore, calculations of R_c are based on meteorological data taken from a limited number of sites within a city, in some cases from a single meteorological station, and may not accurately represent the wide variation of microclimates found within a city.

Air pollution monitoring relies on a variety of expensive instruments that require regular maintenance by qualified technicians, making it a very costly endeavor. As such, few urban areas have more than one or two air pollution monitoring stations (Wallace et al. 2009). In order to measure ambient conditions, such monitoring stations

are often placed in open lots, away from the immediate influence of emission sources such as automobiles. Measurements from such sites are not biologically relevant in that human exposure largely occurs on sidewalks, often within a meter or less of the source of pollution (i.e. automobile exhaust). Likewise, such measurements may not accurately represent pollution levels experienced by trees throughout the city (i.e. *C* in the UFORE model). Little is known about the microclimate or pollution levels at street level where urban trees experience air pollution. Such information is critical to the success of uptake models such as UFORE. Recent work examining pollution dynamics at this scale suggests that trees may potentially exacerbate air pollution levels at street level by limiting airflow and trapping pollutants within the street canyon (Gormke and Ruck 2009), highlighting the need to better understand the environment, in particular the atmospheric environment, immediately in and around urban tree canopies.

CHAPTER 2

URBAN AIR POLLUTION, NITROGEN DIOXIDE, AND PLANT UPTAKE

2.1 Urban Air Pollutants: Overview

Urban areas are major sources of air pollution. Poor air quality has been linked to short- and long-term human health problems including increased rates of premature mortality as well as cardiovascular and respiratory illnesses (Chen et al. 2008). These problems are more severe for certain subpopulations including infants and young children, the elderly, and socioeconomically disadvantaged groups. Many urban air pollutants, such as carbon dioxide (CO_2) and ozone (O_3), are potent greenhouse gasses. A number of these air pollutants, such as O_3, are created in urban centers but are carried downwind where they can have negative impacts on rural communities and natural areas (Gregg et al. 2003).

The U.S. EPA currently regulates six major, or criteria, air pollutants of concern in urban areas: carbon monoxide (CO), lead (Pb), oxides of nitrogen (NO_x), tropospheric (or ground-level) O_3, particulate matter (PM_{10}, $PM_{2.5}$), and sulfur dioxide (SO_2) (U.S. EPA 2009). These six pollutants are in all urban areas and have been demonstrated to affect human and environmental health detrimentally. Of these pollutants, O_3, PM_{10}, and $PM_{2.5}$ are considered to pose the most significant threats to human health. Of the six criteria pollutants, O_3 is the only one not directly emitted; rather O_3 is produced from the interaction of volatile organic compounds (VOCs), NO_x, and sunlight in the photochemical oxidant cycle (Anderson 1983).

Emission sources of the remaining five criteria pollutants can be categorized broadly into automobile traffic, industry, energy production, and domestic fuel (Mayer

1999). Most studies point to automobile traffic as the most significant emission source in major urban areas of the world, with overall emissions predicted to increase dramatically as the global vehicle fleet increases in response to increasing urbanization (Mage et al. 1996; Mayer 1999; Parrish and Zhu 2009). Combustion processes, particularly those from automobile traffic, are the major anthropogenic emission source for NO_x and other precursors of O_3 formation (Anderson 1983; Fowler et al. 1998; Parrish and Zhu 2009).

Because of the close tie between automobile traffic and air pollution emissions in urban areas, the distribution of NO_x and other air pollutants varies greatly over space and time and is dependent on factors such as traffic density and patterns, individual driving habits, and the ratio of passenger vehicles to trucks and other heavy vehicles (Mayer 1999). The dispersion of air pollutants within the urban environment is dependent upon regional, local, and micro-scale meteorological and ambient weather conditions, particularly wind speed, wind direction, and turbulence (Mayer 1999). These conditions, in turn, are affected greatly by topography and urban structures. A number of authors have modeled and/or measured air pollutant dispersal in relation to emission sources and climatic factors within the urban environment (Costabile et al. 2006; Gilbert et al. 2003; Gormke and Ruck 2009; Grawe et al. 2007; Tsai and Chen 2004; Xie et al. 2003; Xie et al. 2006). These authors have found that pollutant dispersal in the urban environment is determined by emission sources in relation to diurnal, seasonal, and spatial variations in sunlight and air temperature, above-roof wind speed and direction, aspect ratios of street canyon height to canyon width, up-wind pollutant concentrations, and vertical and horizontal position within the street canyon,

and relation to street intersections, alleyways, etc, and obstacles within the street canyon.

2.2 Nitrogen Dioxide: Chemical Nature, Production, Importance in Urban Atmospheric Chemistry

Oxides of nitrogen (NO_x) include nitric oxide (NO) and nitrogen dioxide (NO_2). They are sometimes referred to as a larger group of nitrogen oxides known as total reactive nitrogen oxides (NO_y), which includes NO, NO_2, nitrous oxide (N_2O), nitric acid (HNO_3), nitrous acid (HNO_2), peroxyacetyl nitrate (PAN), organic nitrates, and other forms of oxidized nitrogen (Weller et al. 2002). Nitric oxide is a very unstable free radical that is not thought to deposit readily to surfaces in significant amounts (Horii et al. 2004); thus, of the two NO_x species, NO_2 is of primary concern in deposition studies. Nitrogen dioxide (NO_2) is a free radical, a potent oxidant, and a principal component of urban air pollution (Jacobson 2002). It is produced by the oxidation of nitric oxide (NO)—formed by the oxidation of atmospheric N_2 at high temperatures in combustion processes in energy production and the burning of fossil fuels in automobiles—by tropospheric ozone (O_3). The oxidation of NO to NO_2 by O_3 occurs rapidly and with near completeness[3]. As such the U.S. EPA uses NO_2 levels as an overall indicator of the atmospheric NO_x status (U.S. EPA 2010).

Although NO_2 at high concentrations can be toxic to humans, at ambient levels it is thought to pose little risk; rather, it is the role of NO_2 in the photochemical oxidant cycle which is of most concern to human health (Jacobson 2002). In the presence of strong sunlight, NO_2 is photolyzed into NO and a ground state oxygen atom $O(^3P)$

which reacts with atmospheric oxygen (O_2) to create O_3 (Anderson 1983; Fowler et al. 1998). The cycle, starting from emission of NO, occurs as the following set of reactions:

$$NO + O_3 \rightarrow NO_2 + O_2$$

$$NO_2 + hv\ (\lambda < 410\ nm) \rightarrow NO + O(^3P)$$

$$O(^3P) + O_2 \rightarrow O_3$$

Under natural conditions (i.e. in a forest setting with ambient O_3 and low-level emission of NO from soil), a quasi-equilibrium is established and there is no net increase in O_3. However, with increased emission of NO from the burning of fossil fuels by automobiles, NO_2 is allowed to build up at night when photolysis does not occur[4]. This excess NO_2 is photolyzed the following morning, leading to an overall increase in O_3. The cycle is further complicated by the addition of peroxy radicals (RO_2; formed by the oxidation of small hydrocarbons by hydroxyl radicals) which can react with NO to form NO_2 without consuming O_3, thus resulting in a further buildup of O_3 (Fowler et al. 1998). In this respect, NO_2 also plays an important role in the regulation of atmospheric hydroxyl radicals and other HO_x species, important acid rain precursors. It is thought that reductions of NO_2 are likely to result in reductions of O_3 under certain conditions (Steadman 2004; Wellburn 1998). In reality the interconversions between oxidized forms of nitrogen in the atmosphere are more complex than presented here, particularly for polluted environments (Figure 2.1).

In addition to its importance for atmospheric chemistry, NO_2 has been shown to have detrimental effects on plant growth. Because of its reactivity with other

[3] Sparks (2009) reports an atmospheric lifetime for NO of 57-600 seconds.
[4] Sparks (2009) reports an atmospheric lifetime for NO_2 of 143 seconds during daylight hours and 7 hours during the night.

components of the troposphere, NO_2 is a pollutant of primary importance near its source of production (Gilbert et al. 2003) such as urban areas with high automobile traffic. As such, it has great importance for the health and longevity of urban plantings exposed to high concentrations of automobile exhaust. Despite its generally accepted phytotoxicity, a number of authors have demonstrated the ability of plants to take up atmospheric NO_2 and incorporate it into different nitrogen pools within the plant (e.g. Segschneider et al. 1995; Takahashi et al. 2003; Vallano and Sparks 2007). Demonstrated NO_2 uptake suggests the possibility for the use of NO_2 as an alternative fertilizer and in turn the use of plants, particularly trees, for air pollution control (Wellburn 1998).

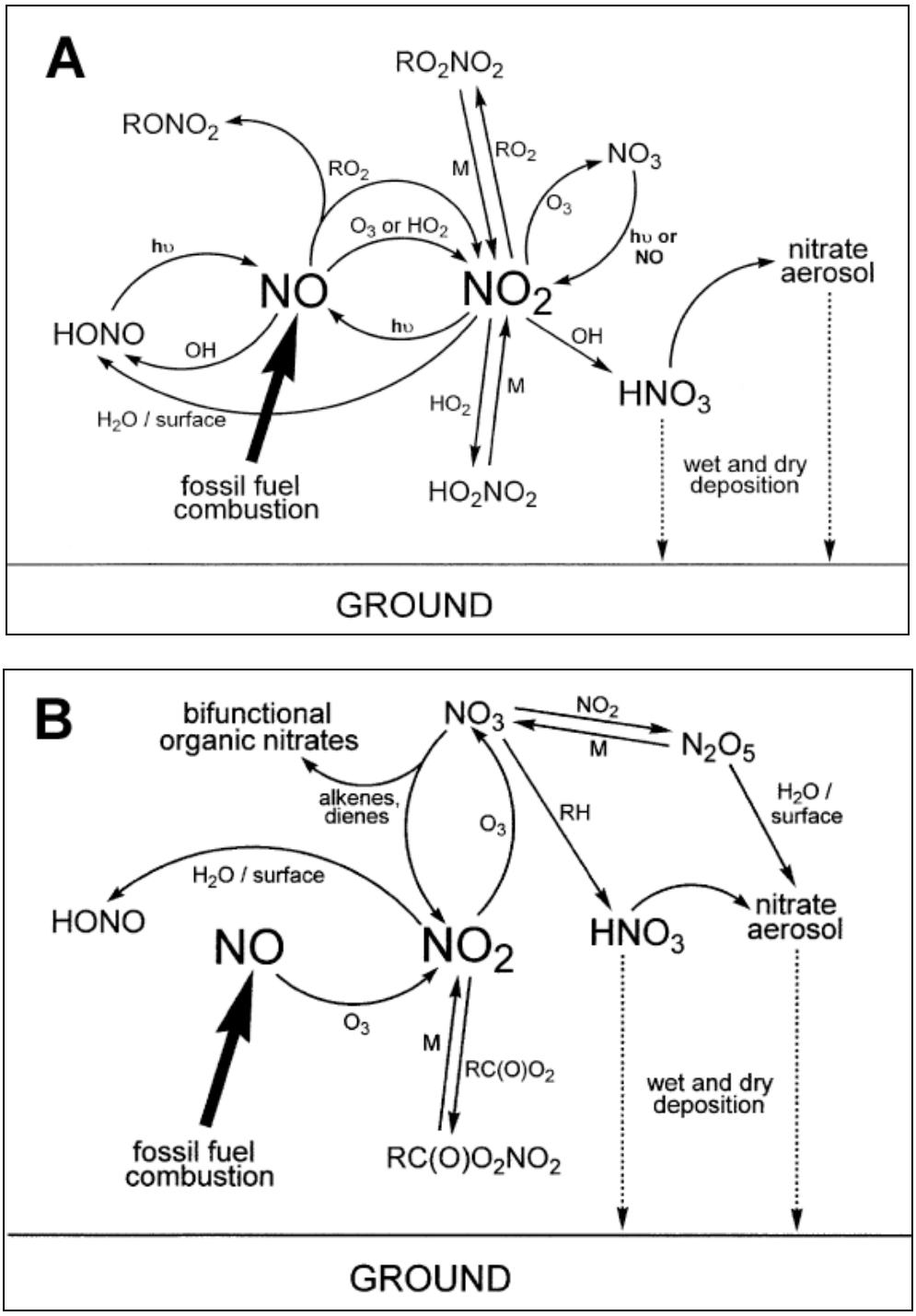

Figure 2.1 Interconversions of oxidized nitrogen during the day (A) and at night (B). Reproduced from Jenkin and Clemitshaw (2000).

2.3 Plant Uptake and Metabolism of Nitrogen Dioxide

Dry deposition of NO_2 to plants occurs by adsorption to leaf surfaces, absorption by root surfaces via the air-soil-root pathway, and stomatal uptake to the apoplast—the latter being the primary means of deposition (Wellburn 1990). As the interface between the leaf interior and the atmospheric environment (Parkhurst 1994), the apoplast is of great importance in plant metabolism (Sattelmacher 2001), and thus uptake via the stomata is not only the primary means of NO_2 deposition but also the most physiologically important exposure route. Although some authors have noted large deposition rates to leaf surfaces (Geβler et al. 2002; Theone et al. 1991), which has been attributed to the presence of chemolithoautotrophic bacteria on leaf surfaces (Papen et al. 2002), the focus remains on stomatal uptake as the uptake route of primary importance.

A great variety of factors have been shown to affect stomatal uptake of NO_2, particularly those that affect stomatal aperture and conductance such as quality and intensity of light, temperature, relative humidity, soil water-status, transpiration rate, canopy height and vertical position within the canopy, whole-plant N-status, and pollutant concentrations (Geβler et al. 2002; Theone et al. 1991; Wellburn 1990). In addition to stomatal aperture, basic leaf morphological considerations such as stomatal frequency and distribution and total leaf surface area also will affect uptake (Wellburn 1990). Furthermore, before a gas reaches the stomata, it must pass through a laminar boundary layer at the leaf surface, and the thickness and relative turbulence of this layer likely plays an important role in determining deposition rates (Parkhurst 1994).

In his seminal review of atmospheric NO_x and plant growth, Wellburn (1990) uses the term *mesophyll resistance* to encapsulate the factors influencing NO_2 uptake between the stomates and the final sites of reaction within the symplast. Influences of mesophyll resistance include the apoplast-atmosphere NO_2 gradient, the presence and concentration of chemolithoautotrophic bacteria within the apoplast, the solubility and disproportionation rate of NO_2 in the aqueous phase of the apoplast (as determined by temperature, pH, and NO_2^-, NO_3^-, and other solute concentrations in the apoplastic water), antioxidant and radical-scavenging enzyme activity, nitrate and nitrite reductase activities, solubility of NO_2 through the plasma membrane, rate of assimilation into amino and nucleic acids, N_2O and NO_2 emission rates, and total leaf N (Geβler et al. 2000, 2002; Hereid and Monson 2001; Sparks 2009; Sparks et al. 2001; Weber and Rennenberg 1996; Wellburn 1990)[5] .

Upon entry into the leaf NO_2 first dissolves into the aqueous phase of the apoplast (Wellburn 1990). Here two reactions may occur: reduction by antioxidants such as ascorbic acid to produce nitrous acid (HNO_2) and dehydroascorbate (Ramge et al. 1993) or dissociation to produce nitrate (NO_3^-) and nitrite (NO_2^-) and protons (H^+; Stulen et al. 1998; Wellburn 1990). The latter reaction is irreversible and dependent upon the concentration of NO_2^- and NO_3^- in the apoplastic water (Sparks 2009). Unreacted NO_2 or NO_2^-, both powerful oxidants, may initiate hydrogen abstraction with components of the mesophyll and thereby initiate the production of free radicals and free radical chain reactions (Ramge 1993; Sparks et al. 2001). Although it is not clear from the literature, it appears that under all but extremely high concentrations, NO_2 is

[5] It should be noted that Wellburn's mesophyll resistance is much more comprehensive than the mesophyll resistance (R_m) employed by Baldocchi (1987), which is even further simplified by the UFORE model (Nowak and Crane 2000).

fully reacted upon by antioxidants or is fully dissociated, leaving only its reaction products, NO_3^-, NO_2^-, and H^+ to cross the plasma membrane. After entering the cytosol, NO_3^- is reduced to NO_2^- by nitrate reductase (Stulen et al. 1998; Wellburn 1990). Nitrite produced in this reaction and from the original dissociation then moves to the plastids where it is reduced to ammonium (NH_4^+) by nitrite reductase and is assimilated into amino acids (Stulen et al. 1998). Figure 2.2 illustrates the chain of events involved in the uptake and assimilation of NO, NO_2, and NH_3. Morikawa et al. (1998, 2003, 2004) and Takahashi et al. (2001, 2003, 2005a) recently have done much work on NO_2 metabolism and on the genetic control of NO_2 uptake. They have reported growth stimulation from NO_2 fumigation separate from the fertilizer effects described by most authors (Adam et al. 2008, Takahashi et al. 2005b) as well as the formation of an unidentified form of nitrogen following fumigation, suggesting a novel pathway for NO_2 metabolism (Kawamura et al. 2002; Morikawa et al. 2004, 2005).

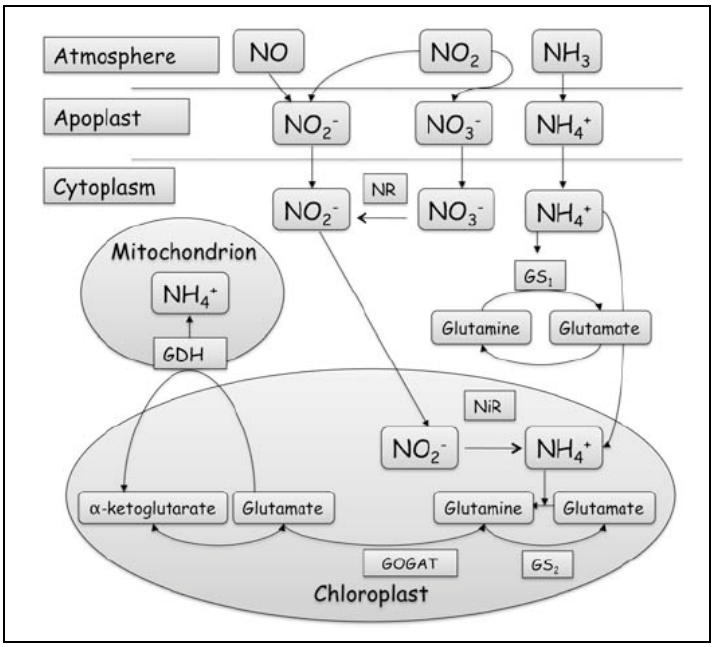

Figure 2.2 Schematic depicting the biochemical processes involved in the uptake and assimilation of NO, NO_2, and NH_3. Reproduced from Sparks (2009).

2.4 Laboratory and Field Methods for Measuring Nitrogen Dioxide Uptake

While it has been clearly demonstrated that plants are able to assimilate N derived from atmospheric NO_2, the quantification of this process, particularly under field conditions, remains challenging (Sparks 2009). Such quantification is necessary to validate models such as UFORE (Nowak and Crane 2000) which suggest that plants, particularly trees, are a substantial sink for gases in the urban environment such as NO_2. From an ecological standpoint, it is also important to differentiate between direct foliar uptake and deposition to leaf surfaces, bark, or soil (Sparks 2009). Foliar uptake represents a direct addition to plant N metabolism and thus may have short and long term consequences for plant growth quite separate from those of N deposition to leaf surfaces, bark, or soil. Deposition to the latter surfaces is subject to ecological processing and may return to the atmosphere as a gas via microbial processes or

volatilization or be leached from the system and transported to other areas (Sparks 2009; Vallano and Sparks 2007). The affects of chronic N addition to ecosystems are well documented (e.g. Galloway et al. 1995; Vitousek et al. 1997); however, most studies consider only total N deposition and do not separate effects between foliar and other deposition routes. Little is known about the long term effects of N deposition via foliar uptake of NO_2 and other reactive N gases (Sparks 2009).

A number of laboratory and field studies have been undertaken in an attempt to quantify foliar uptake of NO_2; most can be lumped into either micrometeorological or dynamic chamber studies, or some combination of the two (Fowler et al. 1989; Sparks 2009). Micrometeorological methods rely largely on the physical and chemical properties of the gas in question and its interaction with the environment (i.e. R_a and R_b in the UFORE model; Nowak and Crane 2000) and the assumption that uptake responds linearly to stomatal conductance with minimal internal resistance (R_c in the UFORE model; Nowak and Crane 2000). Micrometeorological models have been tested against flux measurements in the field with some success; however, such measurements do not differentiate between deposition to surfaces (e.g. leaves, bark, soil, etc.), stomatal uptake, and chemical destruction, and are influenced greatly by local meteorological conditions (Sparks 2009). Further, such methods are of limited usefulness over varied terrain (e.g. in the urban environment) (Rondón and Granat 1994). Most micrometeorological field studies rely on flux measurements of pollutants solely *over* forest and crop canopies (e.g. Duyzer et al. 1995; Hargreaves et al. 1992). Few studies have actually taken measurements of NO_x flux from within the canopy (e.g. Joss and Graber 1996). No studies have taken measurements from within urban tree canopies.

Unfortunately, many authors confuse micrometeorological *estimates* of pollutant flux with *actual measurements* of stomatal uptake (e.g. Rondón and Granat 1994).

Dynamic chamber studies have been employed in the laboratory and in the field using whole plant (enclosed and open-top), branch/twig, and leaf chambers (Hanson and Lindberg 1991). Early chamber studies employed a mass balance technique in which pollutant concentrations measured at the chamber inlet are compared to those at the chamber outlet (e.g. Rogers et al. 1977). Coupled with foliar extraction techniques to determine the fraction of reactive N deposited to the leaf surface, the mass balance technique provided a reasonable estimate of plant uptake. However, this method was limited by the precision of available monitoring equipment at the time and can be complicated by pollutant deposition to and subsequent volatilization from the chamber walls and other non-plant surfaces within the chamber (Rondón and Granat 1994). In mass-balance studies employing chambers exposed to ultraviolet light, the photolysis of NO_2 can cause further discrepancies between concentrations measured at the intake and concentrations measured at the outlet (Segschneider et al. 1995). Other chamber studies employed micrometeorological methods (see Hanson and Lindberg 1991), which serve only as rough estimates of pollutant uptake for the reasons described above.

The best available data for the quantification of NO_2 uptake comes from chamber fumigation studies using ^{15}N labeled NO_2 (e.g. Vallano and Sparks 2008). Such studies offer the most direct means of measuring NO_2 uptake and assimilation into plant tissues. Some studies employ a dilution method in which ^{15}N is supplied to the roots in the form of N fertilizer at relatively high enrichment rates (> 95%) after which plants are fumigated with ^{15}N-free NO_2, and the dilution of initial ^{15}N concentrations is

presumed to represent NO_2 uptake (e.g. Okano et al. 1986, 1988). Most studies, however, employ a direct fumigation technique in which plants are fumigated with varying concentrations of ^{15}N-enriched NO_2 and the increase in ^{15}N from initial levels is presumed to be due to NO_2 uptake (e.g. Latus et al. 1990). Early studies employed unrealistically high concentrations of NO_2, on the order of parts per million (Hanson and Lindberg 1991). It is from these studies that reports of phytotoxicity occur (Wellburn 1990). Ambient levels of NO_2 range from about 4 ppb in rural areas to 40 ppb in heavily polluted urban areas (Segschneider et al. 1995). Fumigations studies at these levels generally report a fertilizer effect for NO_2 uptake (Wellburn 1990).

At either high (ppm) or low (ppb) levels, ^{15}N fumigation studies offer a powerful tool for determining the mechanisms involved in NO_2 uptake and metabolism and identifying the eventual fate of NO_2 derived N in the overall plant N pool. In addition to confirming plant uptake of NO_2, such studies have shown that NO_2 derived N is rapidly (within minutes) assimilated into the plant N pool (Wellburn 1990), with highest concentrations found in the soluble protein fraction within 24 hours after fumigation (Möcker et al. 1998; Segschneider et al. 1995). Studies also have shown that while NO_2-derived N is transported to all portions of the plant and that there is little change in overall N distribution within the plant, the majority of NO_2-derived N remains in the leaves (Möcker et al. 1998; Segschneider et al. 1995; Vallano and Sparks 2007).

Most ^{15}N fumigation studies have been conducted on herbaceous species (Hanson and Lindberg 1991; Wellburn 1990), likely due to their ease of use in chamber systems. However, some studies have been conducted using woody plants. The work by Morikawa et al. (1998) and Takahashi et al. (2003, 2005a) is perhaps the most

extensive in terms of the number of woody plant taxa assessed. Using a NO_2 at 100 ppb (highly polluted urban centers may range from 40-60 ppb) they determined NO_2 uptake ability in 70 woody plant taxa (Takahashi et al. 2005a). They found a more than 120-fold difference in NO_2-derived N assimilation among the woody taxa that they studied. Comparing NO_2-derived N assimilation at 100 ppb to assimilation at 4000 ppb (Morikawa et al. 1998), they divided species into four groups based on assimilation capability of and resistance to NO_2 at high and low concentrations: (1) species with high assimilation capability and high resistance to NO_2 phytotoxicity, (2) species with high assimilation capability but low resistance to phytotoxicity, (3) species with low assimilation capability but high resistance to phytotoxicity, and (4) species with low assimilation capability and low resistance to phytotoxicity. Overall, they found that NO_2-derived nitrogen assimilation was higher in deciduous trees than in evergreens. Among deciduous species, broadleaved trees overall had higher NO_2 assimilation capability compared to deciduous conifers. They attributed this difference to general patterns reported by other authors for deciduous broadleaved trees including higher total leaf nitrogen content, higher net photosynthesis, larger specific leaf area, and higher relative growth rates (Takahashi et al. 2005).

Although chamber studies have proven useful for elucidating the mechanisms involved in NO_2 uptake and for quantifying NO_2 uptake, such enclosures invariably differ from the natural environment (Sparks 2009). As such, results from chamber studies may not be applicable to plants growing under ambient conditions in the natural environment. This problem necessitates other means of measuring NO_2 uptake under ambient conditions. The majority of field studies documenting NO_2 deposition to forest

and crop canopies rely on micrometeorological methods (Duyzer et al. 1995; Hesterberg et al. 1996; Horri et al. 2004, 2006; Joss and Graber 1996; Munger et al. 1996; Walton et al. 1997), which are only rough estimates of uptake and have significant sources of error for the reasons outlined above. Through-fall measurements are another way researchers have attempted to quantify atmospheric N deposition to tree canopies under ambient conditions (e.g. Lovett and Lindberg 1993). However, most such studies do not differentiate between wet and dry deposition or between atmospheric reactive N sources and ultimately only provide an estimate of canopy *retention*, not necessarily canopy uptake (Sparks 2009). Such studies also do not account for gaseous losses from the canopy.

The use of stable isotopes, particularly ^{13}C, ^{15}N, and ^{18}O, in ecological and ecophysiological studies has increased over the last several decades (Dawson and Siegwolf 2007). Recently there have been a number of studies employing ^{15}N signatures either alone or in combination with measurements of leaf nitrate reductase activity (NRA) and measurements of total leaf N to document NO_2 uptake under ambient conditions in the field (e.g. Ammann et al. 1999; Marsh et al. 2004). Stable isotopes are subject to fractionation processes associated with biological (e.g. chemical reactions involved in metabolism) and physical processes (e.g. diffusion). It has been shown that N sources can significantly vary in isotopic composition. With an understanding of the fractionation processes involved in N metabolism and the isotopic composition of N sources, it may be possible to determine the contribution of various N sources to the N metabolism of plants, particularly if there is a large difference in isotopic composition among N sources (Vallano and Sparks 2007).

Field studies assessing NO_2 uptake using ^{15}N stable isotopes rely on the generally enriched ^{15}N signature of NO_2 from car exhaust (Ammann et al. 1999). Because it is difficult to assess fully the range of N inputs to any given ecosystem, such studies often make use of transects along NO_2 gradients from sources such as major roads or highways. It has been shown that NO_2 levels drop rapidly with increasing distance from major sources of traffic, reaching near background levels within 200 m downwind (Gilbert et al. 2003). Plants growing along such a gradient are likely to have significantly different NO_2 exposures, but few other differences in environmental conditions. A number of studies have relied on such gradients and the enriched ^{15}N signature of automobile exhaust to demonstrate NO_2 uptake based on increased ^{15}N levels in tissues from plants growing nearer to the freeway. Such studies have been conducted using natural vegetation (Marsh et al. 2004; Pearson et al. 2000) as well as potted plants (Laffray et al. 2010) or both (Ammann et al. 1999). Several studies have also used ^{15}N stable isotope evidence from tree rings to demonstrate long-term trends in NO_x deposition in relation to anthropogenic emission sources (Saurer et al. 2004; Savard et al. 2009). Ammann et al. (1999) used ^{15}N data from needles of potted and naturally grown *Picea abies* (Norway spruce) along a gradient from a major highway to quantify NO_2 uptake and used this data to verify estimates of uptake from a micrometeorological based model. Despite the robustness of this tool for determining the validity of uptake models under field conditions, the study by Ammann et al. (1999) appears to be the only one of its kind.

CHAPTER 3

SUMMARY AND PROPOSED RESEARCH

3.1 Summary

There is a growing trend of urbanization among the global population, a trend that is predicted to continue indefinitely. Despite being home to such a large portion of the human population, we know comparatively little about the urban ecosystem. Understanding how urban centers function, particularly in terms of material flow and transformation, is vital to the long term sustainability of cities. It has been suggested that trees play an important role in the urban ecosystem; however, most of the benefits assigned to the urban forest remain theoretical. Little data are available to validate such claims, particularly the claim that trees improve urban air quality.

A number of complex models have been created to estimate the magnitude of air pollution removal by urban forests. These models rely largely on the physical properties of air pollutants. Biological inputs for such models are limited in scope and are based on models created largely from laboratory experiments and limited field measurements. Models based on models give rise to greatly compounded error, yet such models have been widely accepted among the scientific community. Results from such models has led government entities such as the EPA to sanction wide-scale tree planting as an air pollution reduction strategy. However, little effort has been made to validate these models using data collected under ambient conditions in the field. There is a real need to validate these models.

Nitrogen dioxide is a signature urban pollutant and is involved in the photochemical oxidation cycle that produces ozone. Ozone can accumulate above

background levels and adversely affect human and plant health. It has been suggested that reducing NO_2 will lead to reductions in O_3. Nitrogen dioxide is also an important source of added N to many ecosystems with both beneficial and detrimental effects on ecosystem function. It has been suggested that trees are an important sink for NO_2 in urban environments. Laboratory experiments using ^{15}N labeled NO_2 have shown that plants are capable of incorporating NO_2-derived N into their overall N pool and that uptake of NO_2 is primarily under stomatal control. Despite the robust potential of ^{15}N stable isotope techniques for quantifying NO_2 uptake under ambient field conditions, most studies documenting NO_2 deposition to urban forests rely on micrometeorological methods, which are only estimates of deposition. Further, these methods do not distinguish between actual uptake at the leaf level and deposition to plant surfaces and soil. Leaf uptake and deposition to other plant surfaces and soil have very different ecological implications, and it is important to be able to distinguish between them. A number of studies have demonstrated the usefulness of the ^{15}N stable isotope in plant biomonitoring studies; however, few studies have attempted to actually quantify plant uptake of NO_2 using the ^{15}N stable isotope under ambient field conditions.

Overall there is a need to understand better the urban environment in terms of micrometeorology and pollution and their respective variations over multiple spatial and temporal scales. We have good information for these factors at large spatial scales and over long time periods; however, these scales are not necessarily relevant to human health or the question of tree uptake of gaseous pollutants. There is a particular need to document better the environmental conditions of the urban forest with respect to water relations among soil, plant, and atmosphere and with respect to pollution levels in and

around tree canopies. Finally, there is a need to improve techniques for quantifying foliar uptake of air pollution by trees under ambient urban conditions.

3.2 Proposed Research

3.2.1 Characterization of Nitrogen Dioxide Levels Inside Urban Tree Canopies

It is accepted widely that NO_2 uptake is largely under stomatal control and that uptake rates are a function of external NO_2 concentration and stomatal aperture. However, plant uptake models rely on measurements of NO_2 concentration from municipal air pollution monitoring stations, of which there are generally only one or two per city located well removed from immediate sources of pollution. Instead, it seems measurements are best if made from directly adjacent to or even within the plant canopy where leaf exposure and uptake actually occur. A number of studies have measured NO_2 levels above forest canopies, but only one study has measured levels from within the canopy. No studies have measured NO_2 levels from inside isolated urban trees, which make up a large portion of the urban forest. Understanding the canopy dynamics of isolated urban trees is an important, but missing, component of the modeling of air pollution removal by urban trees. As an attempt to address this oversight, I proposed to measure NO_2 levels inside and directly adjacent to isolated urban trees in Springfield, MA. I hypothesized that if trees are indeed useful for removing NO_2 from the atmosphere and thus acting as an NO_2 sink, there should be a diffusion gradient between the external atmosphere and the internal tree canopy with greater concentrations in the atmosphere relative to the canopy. In other words, NO_2

levels should be lower inside the tree canopy compared to levels directly outside of the tree canopy.

3.2.2 Use of Potted Trees as a Model for Nitrogen Dioxide Uptake

The use of the ^{15}N stable isotope in biomonitoring studies promises to be a robust tool for quantifying NO_2 uptake in the field. Several recent studies have used the ^{15}N stable isotope and potted herbaceous plants to demonstrate NO_2 uptake in the field (e.g. Laffray et al. 2010). If this model can be applied successfully to potted trees, it would offer one of the best means available for validating uptake models under ambient urban conditions. Ammann et al. (1999) successfully employed this model using potted specimens of Norway spruce (*Picea abies* (L.) Karst.). However, Norway spruce is not a species widely used in urban plantings. I proposed to test this model using a common street tree, red maple (*Acer rubrum* L. (Aceraceae)), under field conditions in Springfield, MA. No one has reported results from such a study, and the question here is largely one of feasibility.

PART II

RESEARCH

CHAPTER 4

NITROGEN DIOXIDE LEVELS INSIDE URBAN TREE CANOPIES

4.1 Materials and Methods

4.1.1 Study Sites

Springfield is the third largest city in Massachusetts with a population of approximately 150,000 and a population density of approximately 1828 km^{-2}. The city is bounded by the Connecticut River and Interstate 91 (I-91) on the west and by suburban and rural areas to the north, east, and south. The prevalent wind direction is from the southwest (A. Sorensen, Massachusetts Department of Environmental Protection, Lawrence, MA, pers. comm.), resulting in the transport of vehicular emissions from I-91 directly into the downtown area. Study sites within Springfield were chosen based on (1) proximity to I-91 and other sources of automobile traffic, (2) occurrence of similarly sized study specimens, and (3) accessibility and security. After an extensive survey of the city for suitable areas, the following study sites were chosen.

Picknelly Field (PF). Picknelly Field is a small baseball diamond located on the western edge of Forest Park, upslope from State Route 5 (Columbus Avenue) and approximately 0.5 km northeast of I-91. Traffic is high and often becomes backed up near this site during commuting hours. At Picknelly Field is a row of seven equally sized (approximately 8-m tall) *Platanus hybrida* Brot. (Platanaceae) (London planetree) running west to east. The trees are located in a large patch of grass between a paved parking lot and the western entrance to the Park.

Visitor Center (VC). The Springfield Visitor Center is located at the southwestern edge of the business district in Springfield, approximately 0.1 km southwest of I-91. The site is on the northeastern side of the Amtrak lines and the southwestern side of I-91 between an on-ramp and off-ramp on West Columbus Avenue. At this site are 15 equally sized (approximately 8-m tall) specimens of *P. hybrida* located in a large grass-covered area between the parking lot and West Columbus Avenue. There are also 12 equally sized (approximately 8-m tall) specimens of *Acer rubrum* located in a narrow planting strip between the parking lot and the Amtrak lines.

Springfield College (SC). Springfield College is located approximately 3 km northeast of I-91 in a relatively quiet residential area of Springfield. Six equally sized (approximately 8-m tall) specimens of *A. rubrum* occur in a small lawn area between the northeast side of a dormitory building and a parking lot. In addition to being the farthest site downwind of I-91, the planting is protected from the prevailing wind by the dormitory building.

Springfield Museums (SM). The Springfield Museums is a grouping of buildings located at a slightly elevated site approximately 0.5 km northeast of I-91 in the middle of the downtown area. Three equally sized (approximately 8-m tall) specimens of *A. rubrum* are located within the grounds of the Museums: one next to Chestnut Street, a busy street running north-south, one adjacent to the Museums parking lot, and one adjacent to Edwards Street, a side street running along the northern edge of the Museums.

4.1.2 Experimental Setup

In 2008, NO_2 levels were measured inside and outside the canopy of three individuals of *A. rubrum* (one tree each at SC, VC, and SM) and two individuals of *P. hybrida* (one at PF and one at VC). Sampling was conducted using Ogawa passive samplers (Ogawa and Company USA, Inc., Pompano Beach, Florida). One passive sampler was placed 3 m from the ground on a pole 30 to 45 cm from the edge of the tree canopy, and another sampler was attached to the leading shoot inside the canopy at the same height—in all cases the sampler was near the base of the canopy (Figure 4.1). Samplers were placed inside opaque rain shelters (Ogawa and Company), which serve primarily to keep the sampler dry but also to reduce the influence of light and wind on the samplers (Krupa and Legge 2000).

Based on the results from 2008, measurements were expanded in 2009 to include O_3 (also using Ogawa passive samplers with rain shelters), temperature, and relative humidity (RH). The latter two variables were measured using temperature-RH data loggers inside solar-radiation shields (Hobo Pro v2, Onset Computer Corporation, Bourne, Massachusetts) co-located with the passive samplers inside and outside of each tree canopy (Figure 4.2). Temperature and RH were recorded at five minute intervals and averaged over the length of the passive sampler exposure periods. Because of the increased value of the sampling setup in 2009, a site with good security was needed. The Springfield Museums provided the best security and the added benefit of being located in the heart of the downtown area. As such, measurements in 2009 were taken from the three specimens of *A. rubrum* located at the Springfield Museums, one of which was also used in 2008 for NO_2 sampling.

Passive samplers were replaced approximately every two weeks during the summer, from 10 June through 19 August 2008 (NO_2 only) and from 10 June through 23 August 2009 (NO_2 and O_3), for a total of five sets of samples per tree, per season. Samples were analyzed at the Research Triangle Institute (Research Triangle Park, NC) using standard colorimetric methods (Ogawa, 2001, 2006).

4.1.3 Statistical Analysis

Because of complex nesting created by the experimental setup, only data from *A. rubrum* were subjected to statistical analysis. Data were analyzed as five biweekly averages for each tree within the two study years by analysis of variance (ANOVA) using a mixed linear model (PROC MIXED procedure) in SAS 9.1 (SAS Institute, Cary, North Carolina). For analysis of differences in NO_2 levels, independent variables were year, tree (nested within year), sample period (1-5, corresponding to the five sequential two-week sample periods used in both years), and sampler location (inner, outer). Tree was treated as a random variable, and year, sample period, and sampler location as fixed variables. A similar model was used for analysis of differences in O_3, temperature, and RH values with the omission of year as an independent variable. Correlations between the average differences between inner and outer values for NO_2, O_3, temperature, and RH were performed using linear regression (PROC REG procedure) in SAS 9.1.

Figure 4.1 Locations of inner and outer NO_2 samplers at a typical site in 2008.

Figure 4.2. Setup of O_3 and NO_2 passive samplers inside rain shelters and temperature/RH logger inside solar radiation shield (left to right, respectively within each image) for inner (A) and outer (B) canopy locations.

4.2 Results

Measured NO_2 levels were higher inside the canopy of each tree for all sample periods in both years (Figure 4.3). Results from the ANOVA are presented in Table 4.1. The ANOVA indicated a highly significant effect ($P \leq 0.0001$) for sampler location (inner vs. outer) on NO_2 levels. Average seasonal inner canopy NO_2 levels by tree ranged from 6 to 84% (0.30-3.90 ppb) greater than outer levels, with an average of 35% greater. There was not a significant effect for year ($P=0.40$) or sample period ($P=0.72$) or their interaction ($P=0.32$). The interaction between year and sampler location was highly significant ($P=0.006$), with a smaller average difference in 2009 (1.48 ppb) than in 2008 (2.97 ppb) (Figure 4.4). A partition of the interaction revealed a significant effect for sampler location on NO_2 levels in either year, indicating that difference between inner and outer canopy levels was significant in both years.

Measured O_3 levels were lower inside the canopy of all three trees over each sampling period in 2009 (Figure 4.5). The ANOVA indicated an overall significant effect ($P=0.02$) for sampler location (inner vs. outer) on O_3 levels (Table 4.2). Average seasonal inner canopy O_3 levels by tree ranged from 6 to 46% (1.01-10.60 ppb) lower than outer levels, with an average of 20% lower (Figure 4.6). There was a highly significant effect for sample period on ozone levels ($P \leq 0.001$) and a significant effect for the interaction between sample period and sampler location ($P=0.01$). A partition of the interaction by date indicated that the difference between inner and outer O_3 levels was highly significant ($P \leq 0.001$) for all sample periods except the first ($P=0.11$) in which the average difference between inner and outer O_3 levels was only 1.34 ppb (Figure 4.5).

Results from the ANOVA of RH and temperature are presented in Tables 4.3 and 4.4, respectively. There was a highly significant ($P \leq 0.001$) effect for sample period for RH and temperature. There was not a significant effect for sampler location or the interaction between sampler location and sample period on RH suggesting that differences in RH between inner and outer canopy locations were negligible. There was a significant effect ($P=0.035$) for sampler location on temperature, but not for the interaction of sample period and sampler location. On average, temperatures inside the canopy were 0.07˚C higher than temperatures outside the canopy (Figure 4.7). Regression analyses at linear, quadratic, and cubic levels revealed no significant correlations between average NO_2 differences and average O_3 differences or between average NO_2 or O_3 differences and average temperature or RH differences (data not presented).

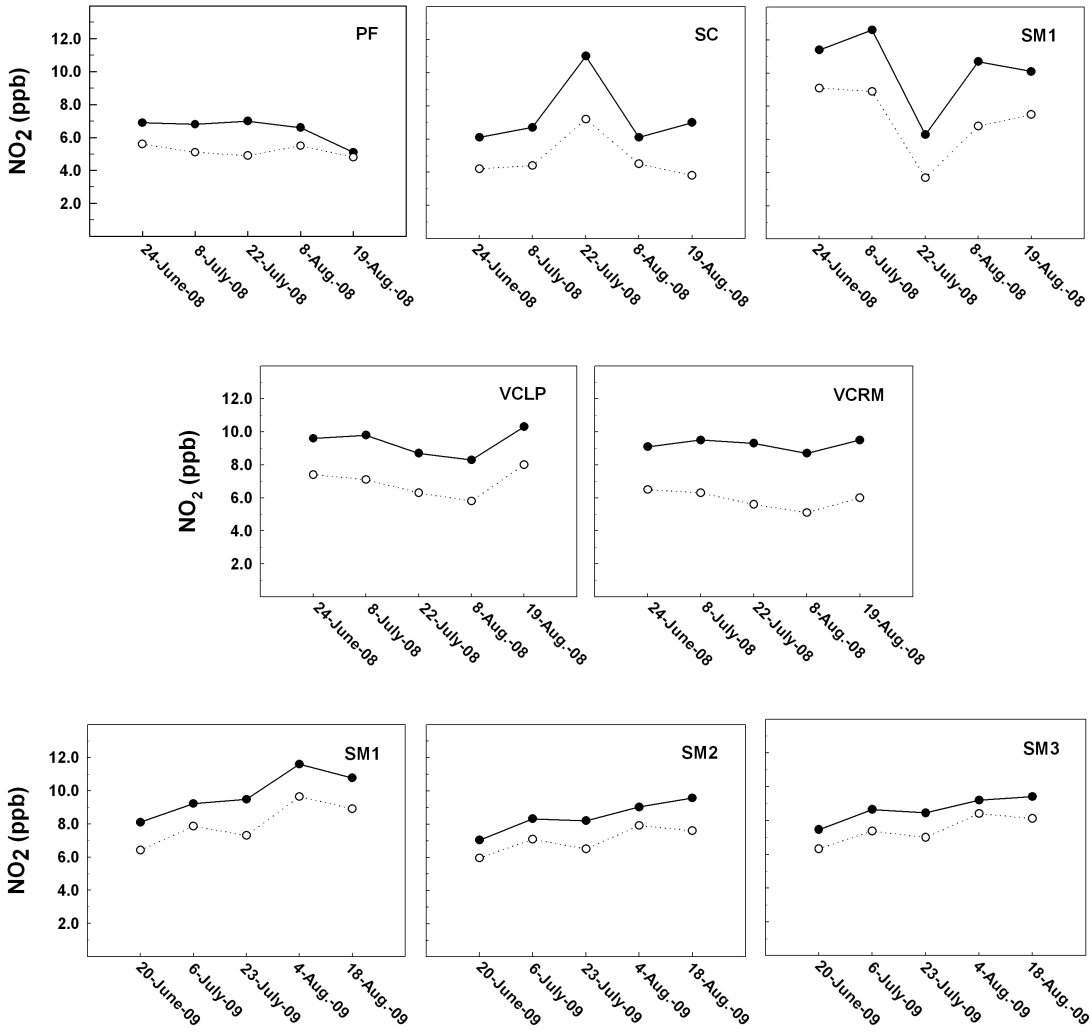

Figure 4.3 Seasonal trends in NO_2 concentrations at inner (solid line) and outer (dashed line) canopy locations for each study tree in 2008 (PF = Picknelly Field, *P. hybrida*; SC = Springfield College, *A. rubrum*; SM1 = Springfield Museums, *A. rubrum*; VCLP = Visitor Center, *P. hybrida*; VCRM = Visitor Center, *A. rubrum*) and 2009 (SM 1-3 = Springfield Museums, *A. rubrum* numbers 1-3).

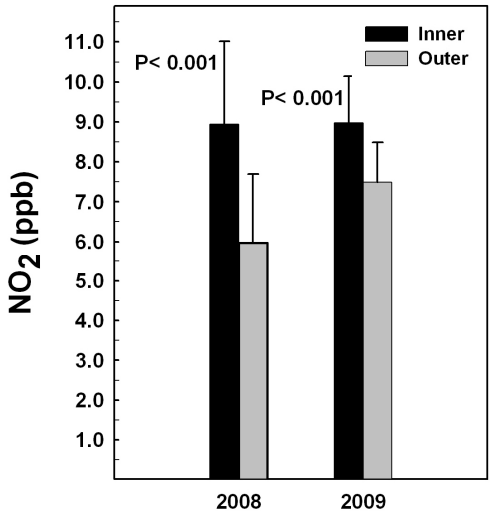

Figure 4.4 Average inner and outer NO_2 concentrations in 2008 and 2009 (*Acer rubrum* only). Differences between inner and outer concentrations were highly significant ($P \leq 0.001$) in either year. Error bars indicate standard deviation.

Table 4.1 Results of ANOVA for data presented in Figures 4.3 and 4.4 determining the significance of effects of year (Y), sample period (P), sampler location (L), tree (T), and their interactions on NO_2 concentrations. NT indicates no appropriate test for F value.

Source	df	SS	MS	F	P
Y	1	9.04	9.04	0.86	0.406
P	4	7.93	1.98	0.51	0.726
L	1	74.14	74.14	245.80	**<0.001**
T(Y)	4	42.04	10.51	**NT**	
Y × P	4	19.44	4.86	1.26	0.326
Y × L	1	8.28	8.28	27.47	**0.006**
Y_1 × L	1	66.00	66.00	218.81	**<0.001**
Y_2 × L	1	16.42	16.42	54.46	**0.001**
P × L	4	1.10	0.28	1.81	0.176
P × T(Y)	16	61.77	3.86	**NT**	
L x T(Y)	4	1.20	0.30	**NT**	
Y × P × L	4	0.33	0.08	0.56	0.698
P × L × T(Y)	16	2.43	0.15	**NT**	

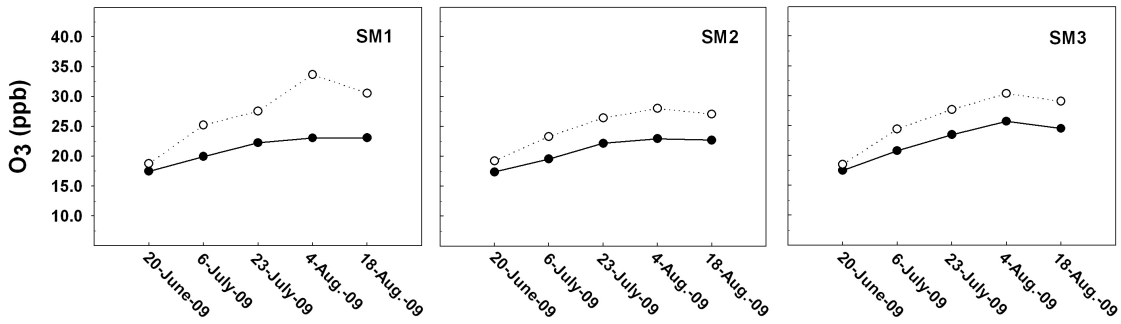

Figure 4.5 Seasonal trends in O_3 concentrations at inner (solid line) and outer canopy (dashed line) locations for each study tree in 2009 (SM 1-3 = Springfield Museums, *A. rubrum* numbers 1-3).

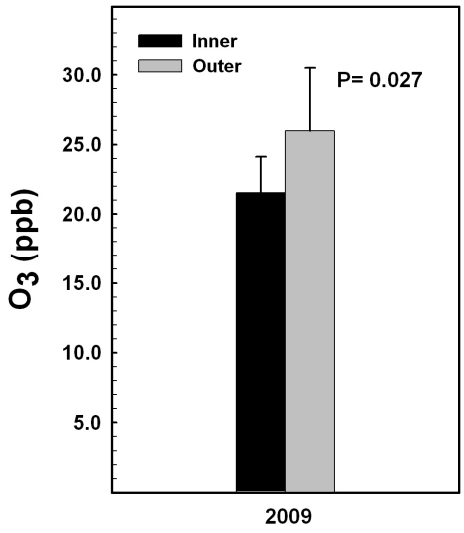

Figure 4.6 Average inner and outer O_3 concentrations in 2009. Differences between inner and outer concentrations were significant (*P*=0.027). Error bars indicate standard deviation.

Table 4.2 Results of ANOVA for data presented in Figures 4.5 and 4.6 determining the significance of effects of sample period (P), sampler location (L), tree (T), and their interactions on O_3 concentrations. NT indicates no appropriate test for F values.

Source	df	SS	MS	F	P
P	4	324.18	81.05	90.55	**<0.001**
L	1	150.08	150.08	35.07	**0.027**
T	2	11.27	5.64	**NT**	
P × L	4	24.38	6.10	7.00	**0.010**
P_1 × L	1	2.66	2.66	3.06	0.118
P_2 × L	1	26.46	26.46	30.34	**<0.001**
P_3 × L	1	31.28	31.28	35.87	**<0.001**
P_4 × L	1	68.68	68.68	78.75	**<0.001**
P_5 × L	1	45.37	45.37	52.03	**<0.001**
P × T	8	7.16	0.90	**NT**	
L × T	2	8.56	4.28	**NT**	
P × T × L	8	6.97	0.87	**NT**	

Table 4.3 Results of ANOVA determining the significance of effects of sampler location (L), sample period (P), and their interaction on relative humidity (data not presented). NT indicates no appropriate test for F value.

Source	df	SS	MS	F	P
P	4	634.34	158.58	613.92	**<0.001**
L	1	2.25	2.25	2.00	0.292
T	2	34.05	17.02	**NT**	
P × L	4	0.22	0.05	0.56	0.698
P × T	8	2.06	0.25	**NT**	
L × T	2	2.25	1.12	**NT**	
P × T × L	8	0.80	0.10	**NT**	

Table 4.4 Results of ANOVA for data presented in Figure 4.7 determining the significance of effects of sampler location (L), sample period (P), and their interaction on temperature. NT indicates no appropriate test for F value.

Source	df	SS	MS	F	P
P	4	88.379	22.094	1256.65	**<0.001**
L	1	0.038	0.038	26.45	**0.035**
T	2	0.290	0.145	**NT**	
P × L	4	0.019	0.004	1.10	0.418
P × T	8	0.140	0.017	**NT**	
L × T	2	0.002	0.001	**NT**	
P × T × L	8	0.035	0.004	**NT**	

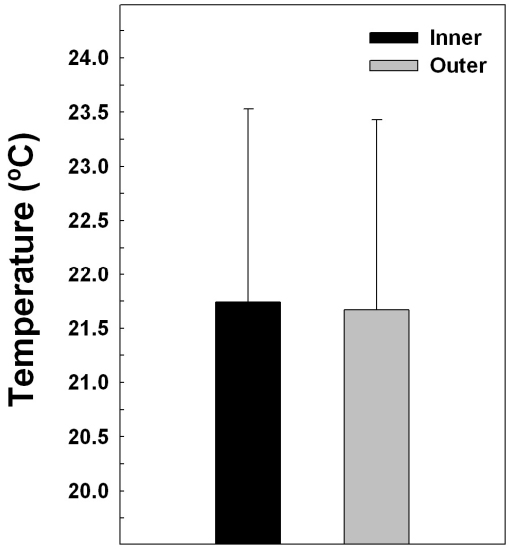

Figure 4.7 Average temperature at inner and outer canopy locations in 2009. Error bars indicate standard deviation.

4.3 Discussion

Although there are some limitations involved in the use of passive samplers, numerous studies have shown strong agreement between passive samplers and continuous monitoring (e.g. Sather et al. 2007). Temperature and RH affect reaction rates for the types of passive samplers used in our study and are taken into consideration in the calculations involved in the passive sampler analysis (Ogawa 2001, 2006). Thus, the significant differences in temperature in 2009 between inner and outer canopy locations should not have affected the results of the passive sampling. Consistently higher NO_2 levels inside tree canopies in 2009 (Figure 4.4) despite negligible differences in RH between inner and outer canopy locations suggests that RH had little to no effect on NO_2 levels. Although there was a small but significant difference in

temperature between inner and outer canopy locations, the lack of any significant correlation between the difference in temperature and the differences in NO_2 or O_3 suggests that temperature had little to do with differences in NO_2 or O_3.

Although inner canopy NO_2 and O_3 values have not been reported previously, Fowler (2002) predicted that NO_2 levels inside canopies would be higher than levels outside canopies, and vice versa for O_3 levels, based on NO_x-O_3 chemistry (Figure 2.1). He suggested that NO released from soils would be rapidly oxidized by O_3 inside the canopy to form NO_2. The difference between the rate of NO emission and subsequent oxidation to NO_2 and the rate of leaf uptake of NO_2 resulting in an "effective" canopy compensation point. Under this model, when ambient NO_2 levels are high, canopies will act as NO_2 sinks; when ambient levels are low, canopies will act as indirect sources of NO_2. Taken alone, consistently elevated NO_2 levels inside the canopy suggest the possibility of a longer residence time inside the canopy due to decreased airflow. However, consistently elevated NO_2 levels combined with consistently depressed O_3 levels inside the canopy suggest a role for the photochemical oxidant cycle. Yet there was not a significant correlation between average NO_2 differences and average O_3 differences, suggesting that the differences in concentrations may have been established independently. The data presented here appear to support Fowler's general predictions, but do not provide any insight into the mechanisms behind the differences found between inner and outer canopy levels.

Fowler's (2002) canopy-level compensation point model is based on studies using flux measurements above agricultural crops and forest canopies (e.g. Duyzer et al. 1995; Hargreaves et al. 1992). Nitric oxide production from agricultural and forest soils

is well documented; however, emissions from urban soils are less well documented. Soils around urban trees, particularly street trees, are not intensively managed; most urban trees never receive additional water or nutrients after establishment. It is likely that the lack of soil disturbance and low water and nutrient status of soils in urban tree plantings results in lower NO emission compared to emissions from agricultural or forest soils. Further, urban trees often do not form closed canopies with neighboring trees, allowing NO emitted from soils under the tree canopy to rise into the atmosphere without necessarily being processed by the canopy.

Given my initial results, it seems likely that factors in addition to temperature, RH, and soil NO emission may play a role in creating higher levels of NO_2 inside urban tree canopies. The role of sunlight in the photochemical oxidant cycle may be one possible factor (Anderson 1983). Decreased airflow inside the canopy may also play a role. These findings suggest additional complications in the modeling of NO_2 and O_3 uptake by trees. Further, they suggest that current models may be insufficient for describing exposure to and uptake of NO_2 and O_3 by urban trees, particularly those not forming a closed canopy with neighboring trees. More work is needed to document environmental factors affecting urban trees in order to make more accurate predictions of their ability to remove pollutants such as NO_2 and O_3 from the urban atmosphere.

CHAPTER 5

POTTED TREES AS A MODEL FOR NITROGEN DIOXIDE UPTAKE

5.1 Materials and Methods

5.1.1 Study Sites

Sites for this study were chosen based on predicted NO_2 levels (high vs. low), accessibility, and security, the latter being the primary factor due to the value of equipment and trees being left in the field. Four sites were chosen, two in high traffic areas of Springfield (Springfield Visitor Center, Liberty Street DEP site), one in a large urban park in Springfield (Forest Park Tree Nursery), and one in a rural setting in Amherst, Massachusetts.

Springfield Visitor Center (VC). See description above, section 4.1.1. The proximity of this site to I-91 and other sources of automobile traffic make it a high pollution site.

Liberty Street DEP Site (LIB). The Massachusetts Department of Environmental Protection (DEP) maintains an air pollution monitoring site in a large paved lot adjacent to the Registry of Motor Vehicles on Liberty Street in Springfield. The DEP actively monitors levels for a variety of air pollutants at this site, including NO_2 and O_3. The site has full sun exposure and little nearby vegetation. High automobile traffic adjacent to the site makes it a high-pollution site.

Forest Park Tree Nursery (FP). Forest Park is a large urban park located on 237 hectares at the southern edge of Springfield. The City of Springfield formerly maintained a large tree nursery at the edge of the park. Currently the tree nursery is

used only as a dump site for wood chips and other plant debris. Other than the occasional service vehicle or pedestrian, the site receives little traffic. The site is surrounded by mature trees, which serve to block wind from most directions. Combined with little nearby vehicular traffic, this site has relatively low air pollution levels.

Amherst (AMH). This site is located at Montague Field, in a rural setting at the northern edge of the Amherst campus of the University of Massachusetts. The site is used as an environmental monitoring facility for the Air Pollution Research Group in the Department of Plant, Soil, and Insect Sciences. The site also is used as an active ozone monitoring station for the DEP. The site is in an open field setting, surrounded by trees on three sides and a large hay field on the fourth side. The site is well removed from any sources of automobile traffic making it a low pollution site.

5.1.2 Experimental Setup

Thirty-six specimens of *Acer rubrum* L. (Aceraceae) (red maple) in 3-gallon pots (average height 1.4 m) were purchased from Bigelow Nurseries (Northborough, MA) in March, 2009. The potted trees were maintained outdoors in a holding area until April, at which time the trees were removed from their pots, the soil was washed from the roots to remove any residual slow-release fertilizer, and the trees were repotted in larger pots (5-gallon) using a soilless potting media (Metro-Mix 300, Sun Gro Horticulture Canada Ltd., Bellevue, WA). The repotted trees were then returned to the holding area until just after bud break in mid-May at which time they were taken to one of the four study sites where they remained until the end of the study in September. Nine individuals of *A. rubrum* were located at each of the four study sites. Trees were

watered to saturation with tap water one to two times weekly, depending on weather conditions, and were fertilized monthly with equal amounts of a modified Hoagland solution (see Appendix).

Average NO_2 levels were measured at each site using Ogawa passive samplers (Ogawa and Company USA, Inc., Pompano Beach, FL). Samplers were replaced approximately every two weeks during the study period, for a total of seven two-week sample periods. Samples were analyzed at the Research Triangle Institute (Research Triangle Park, NC) using standard colorimetric methods (Ogawa, 2001, 2006). Measurements of temperature, photosynthetically active radiation (PAR), and relative humidity (RH) were made at each site using data loggers in solar radiation shelters (temperature and RH only) (HOBO, Onset Computer Corporation, Bourne, MA). Temperature, PAR, and RH were recorded at 20 minute intervals. Vapor pressure deficit (VPD) was calculated following Method 1 in Howell and Dusek (1995). Because of the strong correlation between NO_2 uptake and stomatal conductance (Wellburn 1990), only meteorological data from daylight hours (approximately 05:00-18:00), when plants are actively transpiring, were used for analysis. Daytime meteorological data and NO_2 concentrations were averaged over the periods corresponding to leaf sampling dates for statistical analysis.

Leaf samples consisting of 15 to 20 fully expanded leaves were collected from each tree at the beginning of the study and at (approximately) monthly intervals thereafter, for a total of four sets of samples from each tree over the study period. Leaves were dried in a forced-draft oven at 75˚C for 72 hours after which they were crushed by hand and sent to the Stable Isotope Laboratory at Cornell University (Ithaca,

NY) for analysis of total N (%N) and ^{15}N enrichment ($\delta^{15}N$). Analyses were performed on a Thermo Delta V isotope ratio mass spectrometer (Thermo Fisher Scientific, Waltham, MA) connected to a NC2500 elemental analyzer (CE Instruments, Wigan, UK). In-house standards, verified against international reference material from the International Atomic Energy Association, were run after every tenth sample, and values were corrected using a two-point normalization based on in-house standards. $\delta^{15}N$ is generally expressed as the ratio of $^{15}N/^{14}N$ in the sample normalized by the $^{15}N/^{14}N$ ratio of the standard (atmospheric N_2) using the following equation:

$$\delta^{15}N = (R^{15/14}\,\text{sample}/R^{15/14}\,\text{standard}) \times 1000\ (‰)$$

where $R^{15/14}$ is the atomic ratio of ^{15}N to ^{14}N and units are expressed as per mil (‰) (Vallano and Sparks 2007).

To determine the effect of washing leaves to rid them of surface contaminates on leaf %N and $\delta^{15}N$ values, fully expanded leaf samples were collected from five specimens of *A. rubrum* growing in tree pits in a sidewalk along a busy street in Springfield. Leaves were collected in August, after a full season of exposure to dust and other airborne contaminants. Five to ten leaves were collected from limbs on each of the north, south, east, and west sides and from the middle of each tree canopy. Leaves were transported to the laboratory where half of the leaves in each sample were rinsed for thirty seconds with running tap water and for another thirty seconds with running distilled water. Samples were then dried in a forced-draft oven at 75˚C for 72 hours after which they were hand crushed and sent to the Cornell Stable Isotope Laboratory for determination of %N and $\delta^{15}N$ as outlined above.

5.1.3 Statistical Analysis

To determine the significance of differences in leaf %N and $\delta^{15}N$ between sites, data were analyzed by analysis of variance (ANOVA). Dependent variables were leaf %N and $\delta^{15}N$; independent variables were site, tree (nested within site), and sample period. Tree was treated as a random variable; site and sample period were treated as fixed variables. A similar model was used to determine the significance of differences in meteorological measurements and NO_2 concentrations between sites. Meteorological and gas data were treated as dependent variables, and independent variables were site and sample period. Site was treated as a fixed variable and sample period as a random variable. Range tests were performed using Tukey's HSD. Data for the washing experiment were also analyzed by ANOVA. Dependent variables were leaf %N and $\delta^{15}N$, and independent variables were tree and washing treatment. Tree was treated as a random variable; washing treatment was fixed. Correlations of meteorological data and NO_2 concentrations with changes in leaf %N and $\delta^{15}N$ were determined using regression analysis. All analyses were performed in SAS 9.1 (SAS Institute, Cary, NC) using the general linear model (PROC GLM) with appropriate test statements for ANOVA and a general regression model (PROC REG) for correlation analyses.

5.2 Results

5.2.1 Leaf ^{15}N Enrichment

Results for the ANOVA testing the significance of differences in leaf $\delta^{15}N$ between the four sites are presented Table 5.1. Differences between sites (P=0.63; Figure 5.1) and individual site by date interactions (P=0.27; Figure 5.2) were not

significant, whereas differences between dates were significant (P=0.004; Figure 5.3). An ANOVA of initial leaf $\delta^{15}N$ revealed no significant differences between sites (P=0.75; data not presented). Analysis of variance of overall change in $\delta^{15}N$ showed no significant differences between sites (Table 5.2; P=0.83) including when lumped by city (Table 5.3; Springfield/Amherst; P=0.39) or NO_2 level (Table 5.4; High = Liberty St. and Visitor Center; Low = Forest Park and Amherst; P=0.88). Overall, $\delta^{15}N$ became enriched at all sites (Figure 5.1), with the lowest mean increase at the Amherst site (0.11 ± 0.16 ‰) and the highest mean increase at the Forest Park site (0.23 ± 0.39 ‰). Figure 5.3 shows the average change in $\delta^{15}N$ within each site for the four sample dates. Overall there appears to be little consistency in trends within or among sites. An ANOVA of initial $\delta^{15}N$ values among sites indicated no significant differences between sites (Table 5.5). Regression analyses through the quadratic level revealed no significant correlations between average change in leaf $\delta^{15}N$ and average meteorological values or NO_2 levels at each site (Table 5.6).

Table 5.1 Results of ANOVA for data presented in Figure 5.2 determining the significance of differences in $\delta^{15}N$ based on the independent variables sample date (D), site (S), tree (T), and their interactions. NT indicates no appropriate test for F value.

Source	df	SS	MS	F	P
D	3	0.58	0.19	4.71	**0.004**
S	3	1.37	0.45	0.58	0.630
T(S)	32	25.17	0.78	**NT**	
D × S	9	0.46	0.05	1.25	0.277
D × T(S)	93	3.87	0.41	**NT**	

Table 5.2 Results of ANOVA for data presented in Figure 5.1 testing significance of change in $\delta^{15}N$ from the beginning to the end of the study period based on the independent variables site (S) and tree (T). NT indicates no appropriate test for F value.

Source	df	SS	MS	F	P
S	3	0.063	0.021	0.29	0.834
T(S)	32	2.373	0.074	NT	

Table 5.3 Results of ANOVA testing significance of change in $\delta^{15}N$ from the beginning to the end of the study period when grouped by city (C) and tree nested within city [T(C)]. NT indicates no appropriate test for F value.

Source	df	SS	MS	F	P
C	1	0.051	0.051	0.73	0.399
T(C)	34	2.385	0.070	NT	

Table 5.4 Results of ANOVA testing significance of change in $\delta^{15}N$ from the beginning to the end of the study period when grouped by NO_2 level (N) and tree nested within NO_2 level [T(N)]. NT indicates no appropriate test for F value.

Source	df	SS	MS	F	P
N	1	0.001	0.001	0.02	0.884
T(N)	34	2.435	0.071	NT	

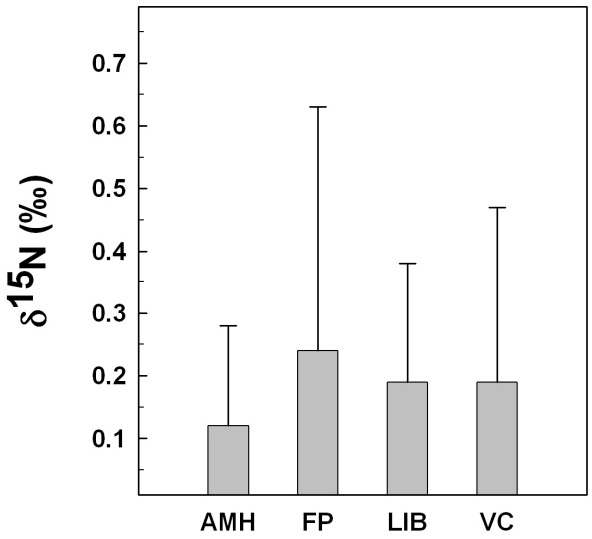

Figure 5.1 Change in $\delta^{15}N$ from the beginning to the end of the study for each site. Differences among sites were not significant. Error bars indicate standard deviation.

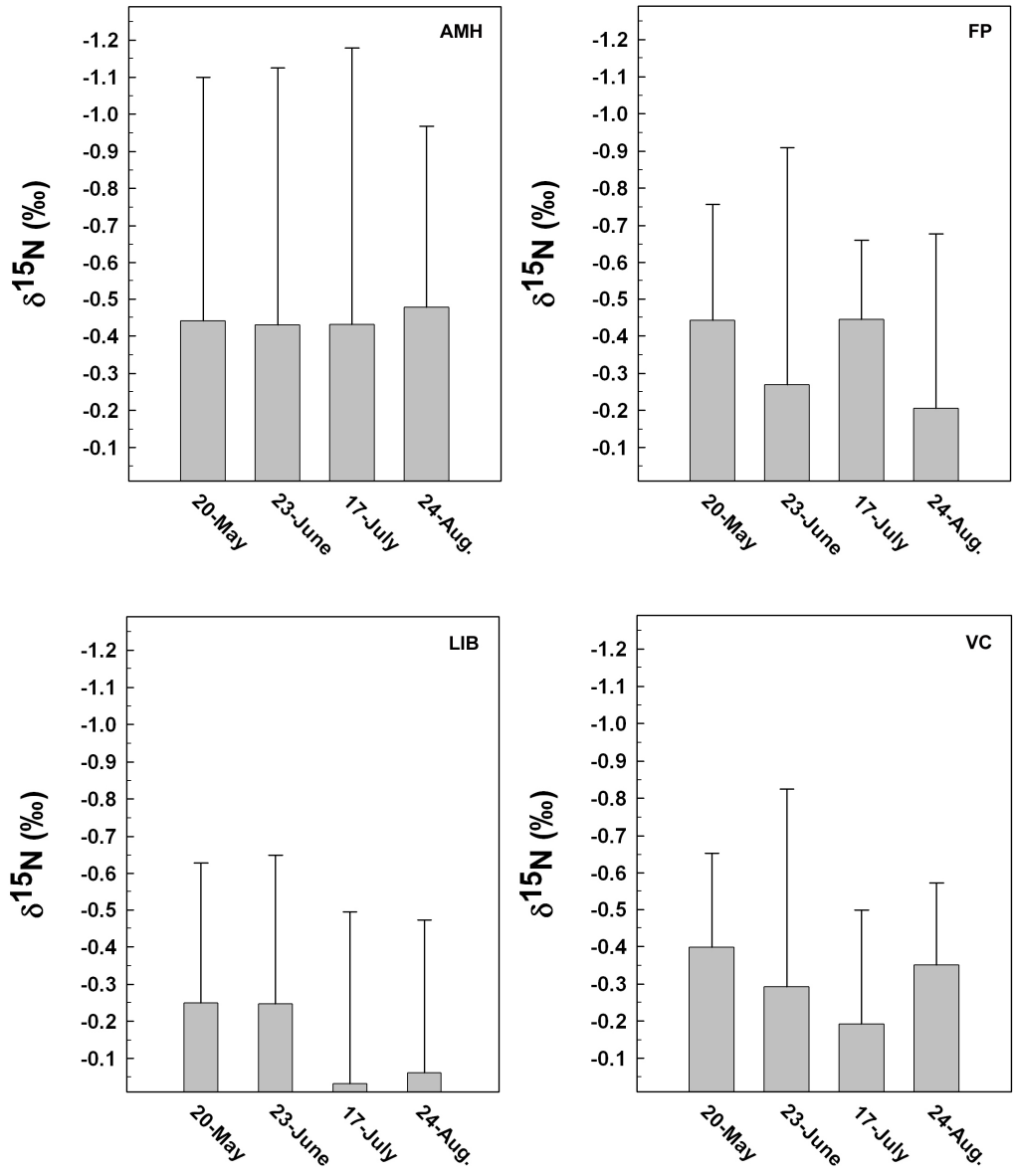

Figure 5.2 Average $\delta^{15}N$ at each sample date within the four sites. Amherst = AMH, Forest Park = FP, Liberty St. = LIB, Visitor Center = VC. The site × date interaction was not significant nor were the individual site or date treatments significant. Error bars indicate standard deviation.

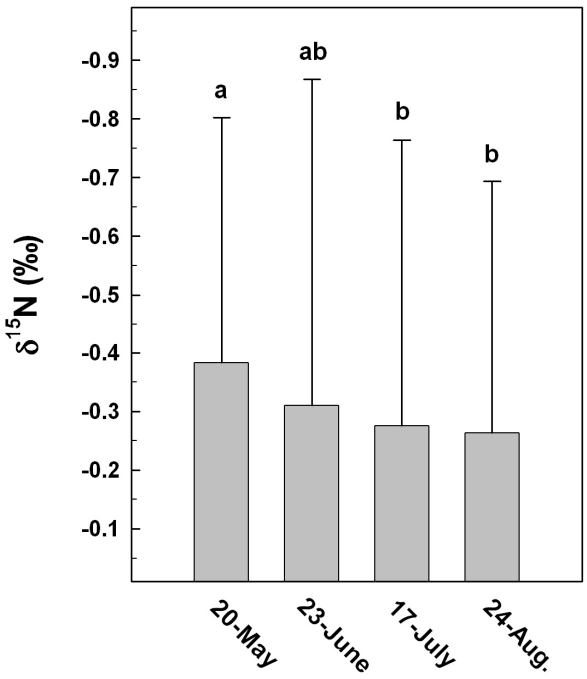

Figure 5.3 Average $\delta^{15}N$ among all sites by date. Dates with different letters are significantly different (*P*=0.05) based on Tukey's HSD. Error bars indicate standard deviation.

Table 5.5 Results of ANOVA testing the significance of initial differences in $\delta^{15}N$ among sites based on the variables site (S) and tree within site [T(S)]. NT indicates no appropriate test for F value.

Source	df	SS	MS	F	P
S	3	0.222	0.074	0.40	0.754
T(S)	32	5.931	0.185	NT	

Table 5.6 Results of regression analysis determining the significance of correlations between average change in $\delta^{15}N$ and average meteorological values (temperature, RH, VPD, PAR) and NO_2 levels among sites.

Variable: Temperature

Order	Source	df	SS	MS	F	P	R^2
Linear	Model	1	0.0000	0.0000	0.00	1.000	0.00
	Error	2	0.0070	0.0035			
	Total	3	0.0070				
Quadratic	Model	2	0.0003	0.0001	0.03	0.974	0.05
	Error	1	0.0067	0.0067			
	Total	3	0.0070				

Variable: RH

Order	Source	df	SS	MS	F	P	R^2
Linear	Model	1	0.0000	0.0000	0.00	1.000	0.00
	Error	2	0.0070	0.0035			
	Total	3	0.0070				
Quadratic	Model	2	0.0056	0.0028	1.95	0.451	0.80
	Error	1	0.0014	0.0014			
	Total	3	0.0070				

Variable: VPD

Order	Source	df	SS	MS	F	P	R^2
Linear	Model	1	0.0000	0.0000	0.00	1.000	0.00
	Error	2	0.0070	0.0035			
	Total	3	0.0070				
Quadratic	Model	2	0.0022	0.0011	0.23	0.828	0.31
	Error	1	0.0048	0.0048			
	Total	3	0.0070				

Variable: PAR

Order	Source	df	SS	MS	F	P	R^2
Linear	Model	1	0.0056	0.0056	7.85	0.107	0.79
	Error	2	0.0014	0.0007			
	Total	3	0.0070				
Quadratic	Model	2	0.0065	0.0032	5.94	0.278	0.92
	Error	1	0.0005	0.0005			
	Total	3	0.0070				

Variable: NO_2

Order	Source	df	SS	MS	F	P	R^2
Linear	Model	1	0.0009	0.0009	0.31	0.634	0.13
	Error	2	0.0061	0.0030			
	Total	3	0.0070				
Quadratic	Model	2	0.0062	0.0031	3.98	0.334	0.88
	Error	1	0.0007	0.0007			
	Total	3	0.0070				

5.2.2 Total Leaf Nitrogen

Results for the ANOVA of differences in leaf %N are presented in Table 5.7. Overall, results for leaf %N were similar to those of $\delta^{15}N$, with significant differences among dates ($P<0.001$; Figure 5.4), but not among sites ($P=0.51$; Figure 5.5) or the interaction between sites and dates ($P=0.99$; Figure 5.6). An ANOVA of initial leaf %N revealed no significant differences among sites ($P=0.91$). An ANOVA of change in %N showed no significant differences among sites ($P=0.88$; Table 5.8) including when grouped by city ($P=0.88$; Table 5.9) or NO_2 levels ($P=0.59$; Table 5.10). Figure 5.5 shows the average decrease in leaf %N for each site and Figure 5.6 shows the average decrease in total leaf %N for the four sample dates within each site. Overall the decline in leaf %N throughout the study period was very even among sites. An ANOVA of initial %N values among sites indicated no significant differences between sites ($P=0.91$; Table 5.11). Regression analysis revealed no significant correlations between average change in leaf %N and average meteorological values or NO_2 levels at each site (Table 5.12).

Table 5.7 Results of ANOVA for data presented in Figures 5.4, 5.5, and 5.6 testing the significance of differences in total leaf nitrogen based on the independent variables sample date [D], site [S], tree nested within site [T(S)], and their individual interactions. NT indicates no appropriate test for F value.

Source	df	SS	MS	F	P
D	3	54.04	18.01	286.99	**<0.001**
S	3	0.64	0.21	0.77	0.518
T(S)	32	8.84	0.27	**NT**	
D × S	9	0.12	0.01	0.23	0.990
D × T(S)	93	5.83	0.06	**NT**	

Table 5.8 Results of ANOVA testing significance of change in total leaf nitrogen from the beginning to the end of the study period based on the independent variables site (S) and tree within site [T(S)]. NT indicates no appropriate test for F value.

Source	df	SS	MS	F	P
S	3	0.109	0.036	0.21	0.887
T(S)	32	5.534	0.172	**NT**	

Table 5.9 Results of ANOVA testing significance of change in total leaf nitrogen from the beginning to the end of the study period when grouped by city (C). NT indicates no appropriate test for F value.

Source	df	SS	MS	F	P
C	1	0.003	0.003	0.02	0.884
T(C)	34	5.640	0.165	**NT**	

Table 5.10 Results of ANOVA testing significance of change in total leaf nitrogen from the beginning to the end of the study period when grouped by NO_2 levels (N). NT indicates no appropriate test for F value.

Source	df	SS	MS	F	P
N	1	0.048	0.048	0.29	0.591
T(N)	34	5.595	0.160	**NT**	

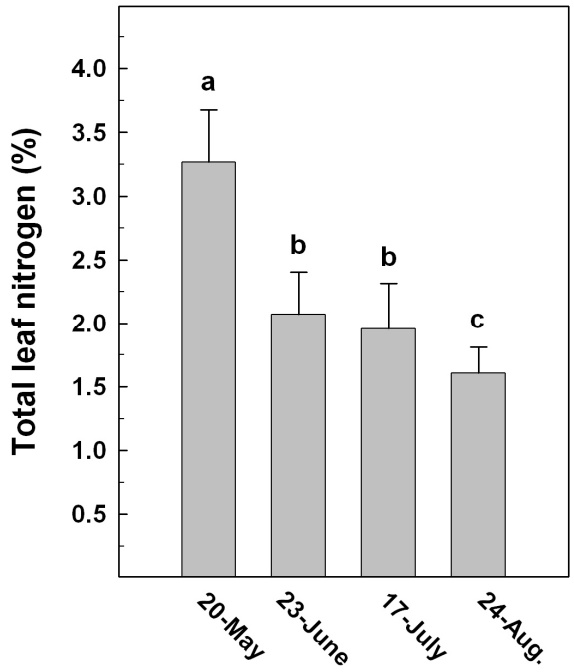

Figure 5.4 Average total leaf nitrogen among sites by date. Dates with different letters are significantly different (*P*=0.05) based on Tukey's HSD. Error bars indicate standard deviation.

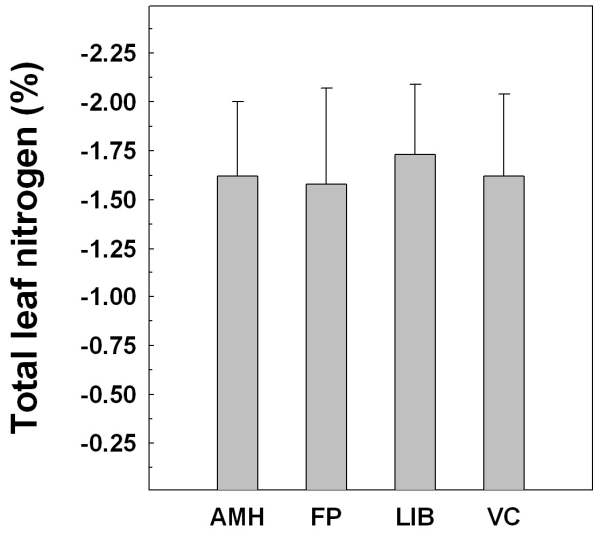

Figure 5.5 Average change in total leaf nitrogen for each site. Differences between sites were not significant. Error bars indicate standard deviation.

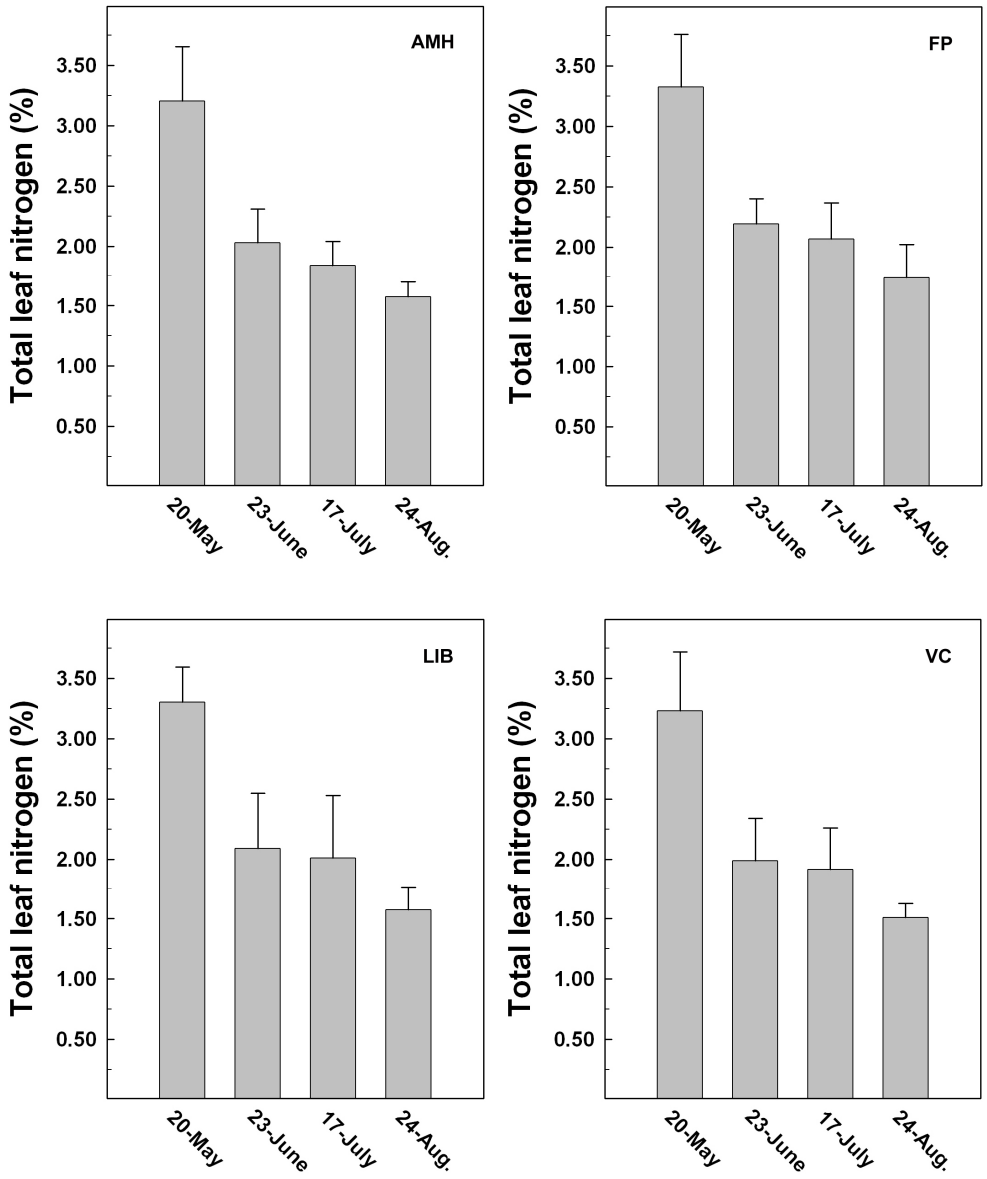

Figure 5.6 Average total leaf nitrogen at each sample date within the four sites. Differences between sites and interaction between site and date were not significant. Error bars indicate standard deviation.

Table 5.11 Results of ANOVA testing the significance of initial differences in total leaf N among sites. NT indicates no appropriate test for F value.

Source	df	SS	MS	F	P
S	3	0.088	0.029	0.16	0.919
T(S)	32	5.765	0.180	**NT**	

Table 5.12 Results of regression analysis determining the significance of correlations between average change in total leaf nitrogen and average meteorological values (temperature, RH, VPD, PAR) or NO_2 levels among sites.

Variable: Temperature

Order	Source	df	SS	MS	F	P	R^2
Linear	Model	1	0.0094	0.0094	6.21	0.130	0.75
	Error	2	0.0030	0.0015			
	Total	3	0.0124				
Quadratic	Model	2	0.0112	0.0056	4.62	0.312	0.90
	Error	1	0.0012	0.0012			
	Total	3	0.0124				

Variable: RH

Order	Source	df	SS	MS	F	P	R^2
Linear	Model	1	0.0068	0.0068	2.46	0.257	0.55
	Error	2	0.0056	0.0028			
	Total	3	0.1240				
Quadratic	Model	2	0.0071	0.0035	0.66	0.655	0.57
	Error	1	0.0053	0.0053			
	Total	3	0.0124				

Variable: VPD

Order	Source	df	SS	MS	F	P	R^2
Linear	Model	1	0.0081	0.0081	3.75	0.192	0.65
	Error	2	0.0043	0.0021			
	Total	3	0.0124				
Quadratic	Model	2	0.0097	0.0048	1.80	0.465	0.78
	Error	1	0.0027	0.0027			
	Total	3	0.0124				

Variable: PAR

Order	Source	df	SS	MS	F	P	R^2
Linear	Model	1	0.0038	0.0038	0.89	0.445	0.30
	Error	2	0.0086	0.0043			
	Total	3	0.0124				
Quadratic	Model	2	0.0052	0.0026	0.36	0.760	0.42
	Error	1	0.0072	0.0072			
	Total	3	0.0124				

Variable NO_2

Order	Source	df	SS	MS	F	P	R^2
Linear	Model	1	0.0037	0.0037	0.85	0.453	0.29
	Error	2	0.0087	0.0043			
	Total	3	0.0124				
Quadratic	Model	2	0.0038	0.0019	0.22	0.831	0.30
	Error	1	0.0086	0.0086			
	Total	3	0.0124				

5.2.3 Meteorological Data and NO_2 Concentrations

Results for the ANOVA testing the significance of differences in meteorological data and in NO_2 concentrations among sites are presented in Table 5.13. Figure 5.7 shows the mean values for meteorological data and NO_2 concentrations by site. There were significant differences among sites in temperature (P=0.003), RH (P<0.001), VPD (P<0.001), and NO_2 (P<0.001). Differences in PAR were not significant. Differences determined by Tukey's HSD showed that the two high NO_2 sites (LIB, VC) and the two low NO_2 sites (AMH, FP) tended to group together in terms of meteorological values, particularly in terms of RH and VPD, and to a lesser extent temperature. Relative humidity was significantly higher and VPD significantly lower at AMH and FP. Temperature was lowest at AMH and highest at LIB with intermediate levels at FP and VC. Nitrogen dioxide levels were similar between LIB and VC but not between AMH and FP, with NO_2 levels at FP were intermediate between those at LIB/VC and AMH. Although regression analysis did not reveal a significant correlation between average NO_2 levels determined by passive sampling at LIB and NO_2 levels monitored by the DEP at the same site over the same time periods, an ANOVA indicated that the values were not significantly different (P=0.86).

Table 5.13 Results of ANOVA for data presented in Figure 5.7 determining the significance of differences among sites in temperature, relative humidity (RH), vapor pressure deficit (VPD), photosynthetically active radiation (PAR), and NO_2 based on site (S), sample period (P) and the interaction between the two. NT indicates no appropriate test for F value.

Temperature

Source	df	SS	MS	F	P
S	3	4.78	1.59	14.30	**0.003**
P	2	51.00	25.50	**NT**	
S × P	6	0.66	0.11	**NT**	

RH

Source	df	SS	MS	F	P
S	3	377.82	125.94	27.55	**<0.001**
P	2	53.79	26.89	**NT**	
S × P	6	27.42	4.57	**NT**	

VPD

Source	df	SS	MS	F	P
S	3	0.24	0.08	21.32	**0.001**
P	2	0.00	0.00	**NT**	
S × P	6	0.02	0.00	**NT**	

PAR

Source	df	SS	MS	F	P
S	3	65355.94	21785.31	2.11	0.200
P	2	42582.18	21291.09	**NT**	
S × P	6	61955.58	10325.93	**NT**	

NO_2

Source	df	SS	MS	F	P
S	3	171.11	57.03	101.65	**<0.001**
P	2	4.80	2.40	**NT**	
S × P	6	3.36	0.56	**NT**	

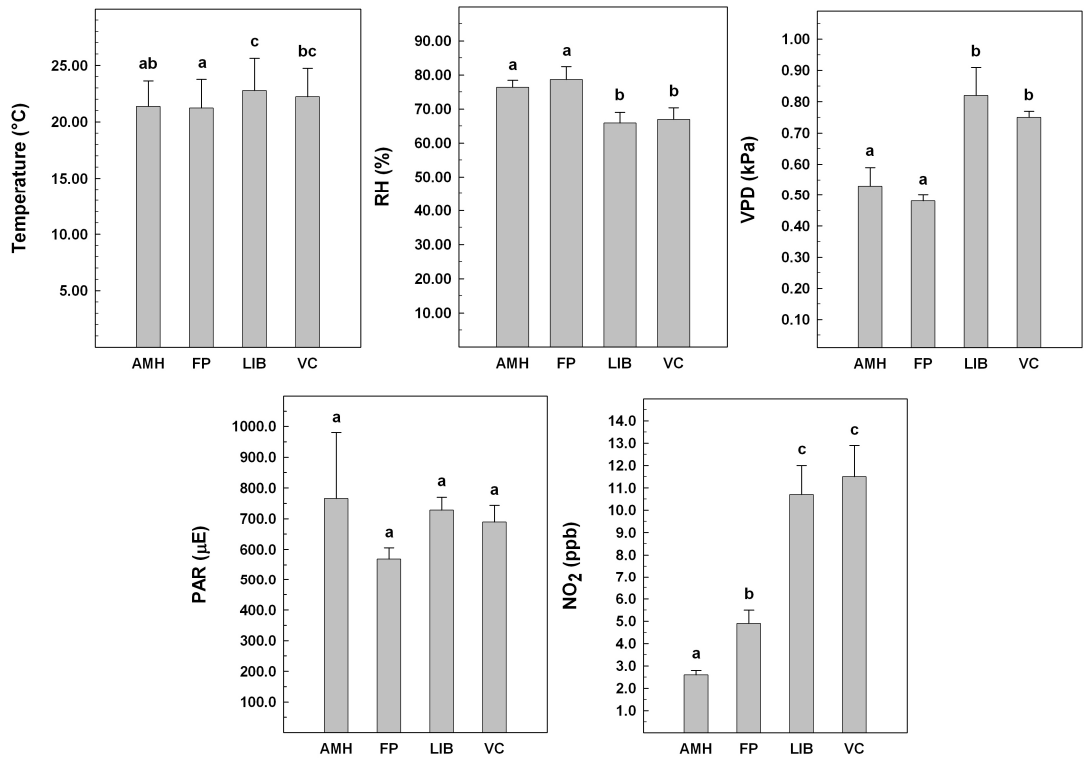

Figure 5.7 Mean values for meteorological data and NO_2 concentrations at each site. Means with different letters are significantly different (P=0.05) by Tukey's HSD. Error bars indicate standard deviation.

5.2.4 Leaf Washing Experiment

Results for the ANOVA determining the significance of the effect of washing leaves on $\delta^{15}N$ and %N are presented in Tables 5.14 and 5.15, respectively. Washing leaves had a small but significant effect (P=0.01) on $\delta^{15}N$, with an average decrease in $\delta^{15}N$ of 0.06‰ (Figure 5.8). Washing did not significantly alter leaf %N (Figure 5.9).

Table 5.14 Results of ANOVA for data presented in Figure 5.8 determining the significance of differences in $\delta^{15}N$ based on independent variables washing treatment (W), tree (T), and the interaction between the two. NT indicates no appropriate test for F value.

Source	df	SS	MS	F	P
W	1	0.055	0.055	20.36	0.010
T	4	19.320	4.831	NT	
W × T	4	0.010	0.002	NT	

Table 5.15 Results of ANOVA for data presented in Figure 5.9 determining the significance of differences in total leaf nitrogen based on independent variables washing treatment (W), tree (T), and the interaction between the two. NT indicates no appropriate test for F value.

Source	df	SS	MS	F	P
W	1	0.008	0.008	0.28	0.626
T	4	0.971	0.242	NT	
W × T	4	0.129	0.032	NT	

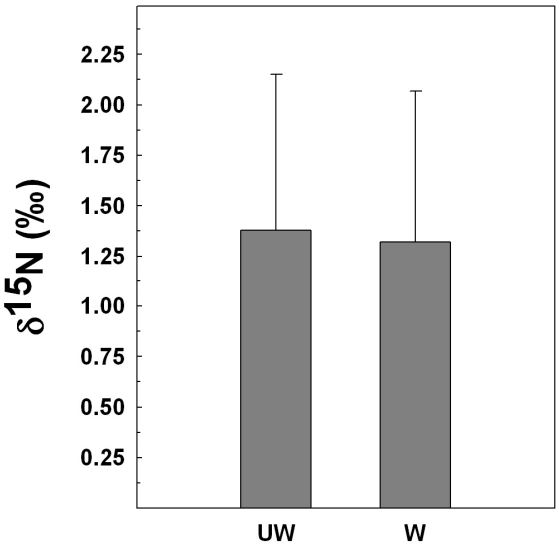

Figure 5.8 Average $\delta^{15}N$ values for washed (W) and unwashed (UW) leaf samples. Differences between washed and unwashed samples were significantly different (*P*=0.01). Error bars indicate standard deviation.

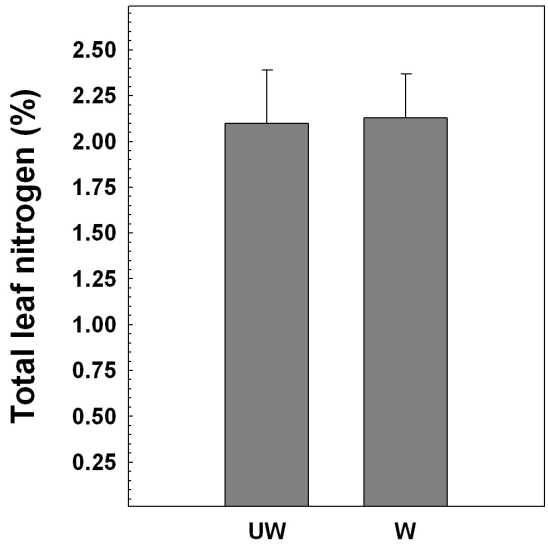

Figure 5.9 Average total leaf nitrogen values for washed (W) and unwashed (UW) leaf samples. Differences between washed and unwashed samples were not significantly different (*P*=0.62). Error bars indicate standard error.

5.3 Discussion

A number of studies using either natural vegetation or potted plants have demonstrated NO_2 uptake using a combination of measurements of leaf %N, $\delta^{15}N$, and nitrate reductase activity (NRA) from mosses, herbaceous plants, and trees growing along a NO_2 gradient (Ammann et al. 1999; Laffray et al. 2010; Marsh et al. 2004; Pearson et al. 2000). Similar results have also been achieved using $\delta^{15}N$ in tree rings from trees growing along pollution gradients (Saurer et al. 2004; Savard et al. 2009). Using potted purple moorgrass (*Molinia caerulea* Moench.) along a NO_2 gradient from a major freeway in France, Laffray et al. (2010) found a highly significant negative correlation between both leaf %N and $\delta^{15}N$ and distance from the freeway. Nitrogen dioxide levels in their study ranged from 19 ppb near the freeway (6-20 m from the freeway) to 3 ppb at their most distant sites (400-500 m from the freeway). Using potted *Picea abies* along a NO_2 gradient from a major freeway in Switzerland, Ammann

et al. (1999) estimated a 25% contribution of NO_2-derived N to overall N metabolism based on an average enrichment in $\delta^{15}N$ of 2‰ after a single season of growth in trees closest to the freeway. Nitrogen dioxide levels ranged from 20 ppb near the freeway (5 m away from the freeway) to 5 ppb at the site furthest from the freeway (980 m away from the freeway). Using natural vegetation along a NO_2 gradient from a major freeway in the UK, Marsh et al. (2004) found significant increases in leaf $\delta^{15}N$ and NRA in several tree species (*Acer pseudoplatanus*, *Betula pendula*, *Crataegus monogyna*, *Quercus* spp.), growing near the freeway compared to the same species growing further away. Although they did not present values for other species, they found $\delta^{15}N$ enrichment of approximately 3‰ for *C. monogyna*. Marsh et al. (2004) suggested that leaf NRA might be a reliable indicator of NO_2 uptake because NRA is substrate inducible. They noted that this phenomenon may be especially true for fast-growing "pioneer" species because of their generally higher NRA and preference for shoot assimilation of N over root assimilation.

Overall, differences in NO_2 levels among sites in the current study (Figure 5.7) were not evident in measurements of leaf $\delta^{15}N$ or %N (Figures 5.1, 5.5), suggesting that either 1) the trees used in this study were not incorporating NO_2-derived nitrogen into leaves or 2) the experimental system was not effective in demonstrating NO_2 uptake. It is not possible to empirically determine which is the case, and it is possible to pose a strong argument for either.

The overall significant decline in leaf %N over the course of the season (Figure 5.4) is consistent with previous work showing a seasonal decline in leaf %N for *A. rubrum* grown under natural forested conditions (Reich et al. 1991). Trends in leaf %N

in previous studies of NO_2 uptake are inconsistent. Marsh et al. (2004) evaluated the use of leaf %N as a tree leaf biomarker for NO_2 uptake along a downwind gradient from a highway and found no correlation between %N in tree leaves and NO_2 exposure whereas Laffray et al. (2010) found a highly significant negative correlation between leaf %N and distance from the freeway (used as a proxy for NO_2 levels). This discrepancy may be due to the difference in study species and experimental setup (i.e. natural woody vegetation vs. potted herbaceous plants). The results presented here for %N corroborate the results of Marsh et al. (2004), showing little difference between sites in overall change in leaf %N. Furthermore, the consistent trends in leaf %N among sites (Figure 5.6) suggest little variation in overall N uptake among sites whereas the inconsistent trends in $\delta^{15}N$ among sites suggest large variation in ^{15}N discrimination.

The overall significant increase in leaf $\delta^{15}N$ (Figure 5.3) is likely the result of enrichment from the KNO_3 used in the nutrient solution. Isotopic analysis of the N sources used in the nutrient solution indicated a $\delta^{15}N$ of 12.93‰ for KNO_3 and -0.736‰ for $CaNO_3$. Ideally the $\delta^{15}N$ of the nutrient solution would have been as close to zero as possible; however, it is difficult to find commercially available ^{15}N-depleted N sources suitable for use in nutrient solutions. Due to a delay in receiving the initial isotopic analysis results from the Cornell Stable Isotope Lab, it was not possible to adjust the N sources in the nutrient solution prior to the first fertilizing date, and for consistency the same nutrient solution throughout the experiment. It is possible that the high ^{15}N enrichment of the nutrient solution combined with the inherent variability of ^{15}N fractionation events masked any differences between sites that could have been attributed to NO_2 uptake. Ammann et al. (1999) appears to be the only other similar

NO_2 uptake study in which potted trees were used; however, there is no mention of fertilizing having taken place during the study. Given the inherently slow growth of conifers and the perennial nature of their needles it may be possible to conduct such a study without the complication of additional fertilizing. This is likely also true for studies such as that of Laffray et al. (2010) in which quick-growing herbaceous plants are used over a single season. However, this is not possible with fast growing, woody species such as *A. rubrum*. Because of some evidence suggesting NO_2 uptake is reduced in plants supplied with high NO_3^- to the roots (Vallano and Sparks 2007) and the fact that urban trees generally are not fertilized after establishment, trees in this study were purposefully minimally fertilized. As a result, the trees began to show signs of nutrient deficiency (in the form of chlorosis) toward the end of the study period. Had the trees received no additional fertilizer as in the case of Ammann et al. (1999), they surely would have suffered severe nutrient deficiency.

In theory, if the contribution of ^{15}N enriched NO_2 to leaf N metabolism was greater than the inherent variation in fractionation events associated with the uptake of N from the soil, this would appear as a greater relative increase in $\delta^{15}N$ among sites with greater NO_2 exposure and uptake. It has been shown that NO_2 uptake is largely under the control of stomatal conductance (g_s) with minimal internal resistance (Wellburn 1990). Thus sites with higher NO_2 concentrations and higher rates of g_s should have relatively higher NO_2 uptake rates and subsequently greater increases in $\delta^{15}N$. A number of studies have shown strong positive correlations between environmental factors such as photosynthetic photon flux denisty (PPFD; measured as PAR in this study), air temperature, RH, or VPD and g_s in trees, including *A. rubrum*

grown under field conditions or in containers (Augé et al. 2000; Bovard et al. 2005; Johnson et al. 2001; Short et al. 1999). Of these variables, PPFD (PAR) and VPD have shown the strongest correlations with g_s, leading to their use as a proxy for g_s. In the current study, there were no significant differences in PAR among sites; however, there was a clear separation in VPD between sites, with significantly higher VPD at LIB and VC compared to AMH and FP (Figure 5.7). Likewise, NO_2 concentrations were highest at LIB and VC. Using VPD as a proxy for g_s, LIB and VC presumably had both the highest exposure to (as determined by significantly higher NO_2 levels) and uptake of (as determined by significantly higher VPD) NO_2 yet did not have the greatest increases in $\delta^{15}N$ (Figure 5.1). Bovard et al. (2005) noted that of a number northern hardwood trees examined, *A. rubrum* showed a relatively high degree of stomatal closure in response to decreasing soil water availability. Although soil moisture availability was not measured during the current study, it was observed that pots at LIB and VC tended to dry out quicker than pots at FP or AMH, which may have limited NO_2 uptake due to earlier onset of reduced g_s at LIB and VC.

The lack of any significant differences in leaf $\delta^{15}N$ change between sites and the lack of any significant correlation between changes in $\delta^{15}N$ and environmental variables makes it difficult to support any conclusions regarding trends in ^{15}N enrichment. It is likely that the variation seen in $\delta^{15}N$ among sites is due to inherent variation in ^{15}N discrimination in the uptake and assimilation of NO_3^- from the nutrient solution. This conclusion is supported by the consistent trends in leaf %N among sites (Figures 5.5, 5.6) which suggest little variation in overall N supplied to the trees. The significant difference in $\delta^{15}N$ between washed and unwashed leaf samples (P=0.01; Figure 5.8) is

likely an additional source of variation; however, considering the large initial differences in leaf $\delta^{15}N$, it seems unlikely that the average 0.06‰ difference between washed and unwashed samples would have a significant impact on overall change in $\delta^{15}N$. Additional sources of possible variation in ^{15}N inputs include wet and dry deposition of NO_2 and other forms of reactive gaseous or particulate N to the soil and stomatal uptake of other forms of gaseous reactive N. Furthermore, a more complicated analysis may be required to determine the significance of correlations between interrelated variables such as temperature, PAR, RH, and VPD. Bassow and Bazzaz (1998) employed path analysis for this purpose; however, this type of analysis is beyond the scope of the current study given its preliminary nature.

As a model for determining NO_2 uptake, this technique is applied easily in laboratory fumigation studies using ^{15}N labeled fertilizers or fumigants (e.g. Takahashi et al. 2005a); however, applying this technique in the field can be difficult. In the laboratory it is possible to limit N inputs and their availability to roots and shoots, as well as to eliminate the influence of microbial processes and other soil-related fractionation processes. A variety of N inputs are available to plants grown in the field including reduced, oxidized, and organic forms, each with their own unique $\delta^{15}N$. These inputs are subject to a number of fractionation events as they cycle through air, soil, water, and the plant (Vallano and Sparks 2007). Atmospheric N inputs can be taken up directly by leaves via dry deposition, in which case fractionation events are limited to those involved in stomatal uptake, diffusion through the apoplast, and N metabolism in the leaf (Dawson et al. 2002; Evans 2001). Atmospheric N inputs can also be wet-deposited to soil where they may be processed by microbes before being

taken up by plant roots, in which case fractionation events include those from microbial metabolism, diffusion through soil, uptake at the root-soil interface, and N metabolism in the roots (Dawson et al. 2002; Evans 2001). Without a thorough understanding of the fractionation processes involved in each step of the soil-microbe-root pathway, it is difficult to determine with any certainty the influence of atmospherically derived N in plant metabolism (Vallano and Sparks 2007). Although our knowledge of stable isotopes and their use as indicators of ecological change is increasing, the quantification of fractionation processes and their overall contribution to stable isotope signatures remains challenging (Dawson et al. 2002; Dawson and Siegwolf 2007).

Ammann et al. (1999) have shown that it is possible to estimate NO_2 uptake using potted evergreen trees, getting results which suggests that this technique may be successfully applied to other trees. Evergreen trees are not commonly used urban plantings, particularly along urban streets, and in order to estimate NO_2 uptake by urban trees the model must be applied to more commonly used deciduous trees such as *A. rubrum*. The current study highlights the difficulties involved in using fast-growing, deciduous species such as *A. rubrum* for this type of study, namely the issues involved in overcoming variability in ^{15}N input from the fertilizer source as well as accounting for natural variation in ^{15}N fractionation events involved with NO_2 uptake and N metabolism. The successful employment of this type of model using common urban trees would bring us one step closer to true field validation of current, widely employed uptake models.

PART III

CONCLUSION

CHAPTER 6

CONCLUDING REMARKS

Given the ever-increasing proportion of the global population living urban environments and the rising trend in automobile use in these areas, urban centers will only increase in their importance for atmospheric chemistry and air quality at both local and global scales. We have increasingly become reliant on models to determine the complex trends in atmospheric pollutants and their interactions with plants and animals. It is essential that such models be validated using real data given their importance in shaping policy decisions regarding air pollution. Validating such models is particularly important if we are to use trees as an effective air pollution control method. The data generated from the canopy NO_2 and O_3 measurement study (Chapter 4) offer a useful contribution to our understanding of the urban environment, in particular the atmospheric environment experienced by urban trees. More data like these, data based on actual measurements as opposed to estimates of flux, need to be generated in the effort to characterize the urban environment and to validate current models.

The use of ^{15}N stable isotope signatures offers one of the most promising tools for quantifying uptake of NO_2 under ambient conditions. The model employed here (Chapter 5) has been used successfully with potted herbaceous plants as well as potted conifer saplings. Although studies of this nature offer useful information for understanding uptake under ambient conditions, they are not necessarily relevant to the question of tree uptake of NO_2 in the urban environment. For this, tree species more commonly used in the urban environment are necessary. *Acer rubrum* is one of the most widely planted urban trees in North America and as such is an ideal candidate for

this type of work. Although the data collected here were inconclusive, the study highlights some of the difficulties involved in employing this model with deciduous trees. In particular it highlights the need to overcome additional ^{15}N inputs such as those from N sources in the fertilizer and the need to better document the natural variation in ^{15}N fractionation events involved in NO_2 uptake and N metabolism. With a better understanding of these events, it may eventually be possible to use ^{15}N stable isotope signatures to determine NO_2 uptake rates in trees grown in-situ. Such data would be extremely useful in shaping policy regarding the use of trees for pollution control.

APPENDIX

NUTRIENT SOLUTION

Half-strength modified Hoagland Solution.

Compound	Molecular weight	[Stock mM]	[Stock g L^{-1}]	Volume Stock (mL) per L
Macronutrients				
KNO_3	101.10	1000	101.10	3.0
$Ca(NO_3)_2 \bullet 4H_2O$	236.16	1000	236.16	2.0
KH_2PO_4	136.09	500	68.05	2.0
$MgSO_4 \bullet 7H_20$	246.47	500	123.24	1.0
Micronutrients				
KCl	74.55	25	1.86	2.0
H_3BO_3	61.83	12.5	0.77	
$MnCl_2$	125.85	14.5	1.82	
$ZnSO_4 \bullet 7H_20$	287.54	1.0	0.29	
$CuSO_4 \bullet 5H_2O$	249.68	0.25	0.06	
H_2MoO_4 (85% MoO_3)	161.97	0.25	0.04	
NaFe EDDHA			16.70	0.5

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