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California Native and Invasive Plants as Biological Sensors for Nitrogen Pollution

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by

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Dedication

This Thesis is dedicated to my loving family and friends.

To my husband Marcus, this would not have been possible without you by my side. I love you more than you will ever know!

To Dad, Grama, Debbi, Bill, and all of our extended family and friends, your support and love during this time was invaluable in helping me achieve all that I ever hoped for.

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Chapter I

1. Introduction

1.1 Sources of Atmospheric Nitrogen to Ecosystems

Nitrogen, an important and abundant element, is required for life on earth. As a component of DNA, proteins, and other living building blocks, it is also a limiting nutrient for primary production in ecosystems. The majority of the Earth's atmosphere is comprised of nitrogen, as N_2, however this inert gaseous form is not readily utilized by biological processes and must be transformed into usable, or reactive, nitrogen compounds. Humans contribute to nitrogen emissions and consequent deposition through fossil fuel burning, industrial activities and through agricultural practices (Nicolas Gruber & James N. Galloway, 2008 and Galloway et al. 2008). Natural sources of reactive atmospheric nitrogen include biological emissions from microorganisms and lighting fixation (Borucki and Chameides, 1984)

Fossil fuel burning and the application of fertilizers for modern agriculture have greatly increased the availability of reactive, gaseous nitrogen in the atmosphere. Fossil fuel burning, resulting from industrial and transportation sector pollution emissions, occurs when nitrous oxides (NO_x) are emitted from combustion engines. Additionally, the use of fossil fuels in industrial practices such as coal energy production and the creation of synthetic fertilizers contribute reactive nitrogen species to the atmosphere, mostly in the forms of N_2O and NH_3, respectively (Vitousek et al., 1997). However, the

greatest contributor of anthropogenically released nitrogen emissions is from agricultural practices (Mosier et al. 1998). The addition of commercial fertilizers to farmed soils releases nitrogen emissions to the atmosphere. This pathway includes microbial metabolism of the fertilized nitrogen through nitrification and resulting denitrification, resulting in mainly N_2O reactive gas emissions and in the volatilization of ammonia from decaying plant and animal wastes and manures (Mosier et al. 1998)

Biological nitrogen fixation is accomplished by bacteria and archaea microorganisms, independently, or symbiotically, with plants. This process converts N_2 to ammonia and into nitrogen containing proteins. These usable forms of nitrogen are readily metabolized forms for plant growth and subsequent trophic cycling. Decay of plant and animal matter also results in the production of ammonia (NH_3) which is further transformed to nitrate (NO_3) by nitrifying bacteria, and then to NO, HONO, and N_2O gaseous emissions during intermediate denitrification processes (Oswald R et al. 2013 and EPA 2010). These emissions account for gaseous release of nitrogen that then can be taken up by plant stomata, deposited onto plant surfaces via dry deposition, dissolved in rain (mainly NO_3 and NH_4) and consequently rained out as part of storm events (Sutton et al., 1994). Additionally, storm events and cloud activity can further add to atmospheric contributions of reactive nitrogen by lightning. This occurs through high energy splitting of N_2 into elemental N and subsequent combination with oxygen resulting in gaseous nitrogen oxides, such as NO_3 (Borucki and Chameides, 1984).

Excess nitrogen, in the form of nitrogen deposition, can have profoundly negative effects on ecosystems. Nitrogen fertilization is detrimental in that this provides large amounts of a macro nutrient that is otherwise limited in terrestrial ecosystems. The addition of this limited nutrient can cause critical load responses from microbes, algae, and plants and cause nitrogen saturation in ecosystems (Bytnerowicz and Fenn, 1996). Critical loads, or the highest level of pollution, which will not harm sensitive elements of ecosystems are identified by ecosystem responses to excess pollution. Exceeding critical loads in ecosystems can cause vegetation-type conversions, invasive species proliferation, pollution runoff in watersheds and subsequent eutrophication in aquatic systems, among other detrimental effects (Galloway et al. 2008).

1.2 Coastal Sage Scrub Decline

In Southern California, California coastal sage scrub plant assemblages have declined due to urbanization, pollution, and the invasion of exotic species (Allen et al., 1998). Riversidian California coastal sage scrub includes species such as California Buckwheat (*Eriogonum fasciculatum*), California sagebrush (*Artemisia californica*), brittlebush (*Encelia farinosa*), and other assorted sage species (*Salvia* sp.). Endangered species such as the California gnatcatcher, Stephen's kangaroo rat, and quino checkerspot butterfly are dependent on dense, highly diverse coastal sage scrub stands (Minnich and Dezzani, 1998, M.E. Fenn, et al. 2002, Wood et al. 2006). One factor in the decline of coastal sage scrub is the significant increase of nitrogen emissions due to anthropogenic activities. Though NOx emissions have decreased in Southern California since 2005,

nitrogen pollution emissions continue to artificially fertilize CSS from fossil fuel combustion and industrial processes (Brioude et al., 2013). As nitrogen enters CSS habitat through dry deposition, it artificially fertilizes the otherwise nitrogen limited ecosystem (Fenn et al., 1998). This can cause CSS decline through stimulation of invasive, annual Mediterranean plants which readily consume the excess available nitrogen to out-compete slower growing native plants. The result is fragmented CSS habitats and significant losses of CSS diversity (Cox et al. 2014)

Due to the ecological importance of coastal sage scrub and similar semi-arid plant communities, critical loads have been calculated for coastal sage scrub and other ecosystems of the western United States (Fenn et al. 2010, Cox et al. 2014). However, these loads are based upon imperfect measurements of nitrogen exposure. New approaches, such as the Integrated Total Nitrogen Input Method (ITNI), are needed to better assess the risks to these ecosystems including the role excess nitrogen deposition plays in continued and sustained invasion from Mediterranean exotics.

1.3 Integrated Total Nitrogen Input (ITNI) Method

The principal underlying the ITNI method is relatively simple. A closed plant-liquid-sand system (PLS system) is created in a greenhouse and the system is isotopically enriched with ^{15}N-labelled nitrogen. Next the PLS system is placed into the ambient environment where it isotopically equilibrates with atmospheric N. To determine the total amount of nitrogen input from wet, dry and gaseous deposition, the ITNI method utilizes the concept of isotope dilution (Russow et al 2001). Since the ^{15}N content of atmospheric

N is significantly less than the ^{15}N content of the PLS system, the concentration of ^{15}N gradually declines in the plants, sand and liquid. At the time of harvest, the sand, plant parts, and nutrient liquid are all sampled for nitrogen quantity and isotope abundances; these measurements are used to determine how much the ^{15}N tracer had been diluted and how much nitrogen has been gained which are used to calculate the amount of incoming nitrogen deposition from the natural field conditions. Previously, multiple methods were required to quantify all pathways of N deposition to a system (wet, dry and gaseous), but the ITNI method can theoretically resolve this problem with isotope ratio measurements. Moreover, the ITNI method also accounts for nitrogen directly taken up through leaf stomata, an important process that is neglected by traditional deposition collectors (He et al., 2010).

The PLS system contains a plant growing in a nitrogen-free soil composed of silica sand and watered by a liquid reservoir containing plant nutrients and ^{15}N-labelled NO_3^- or NH_4^+. In most studies, a hydroponic system is used to transport the nutrient liquid from the vessel to the plants, and allow drainage back into the liquid reservoir. Once grown and labeled with ^{15}N, the entire PLS system can be transported to the field and exposed for periods of weeks to months. At the end of field exposure, the entire plant and all of the sand and liquid system parts are harvested for isotope ratio and elemental analysis.

ITNI was originally utilized to assess nitrogen deposition in agriculturally influenced ecosystems (Bohme et al., 2003, He et al., 2007, He et al., 2010, Melhert et

al., 1995 and Russow et al., 2005, Weigel et al., 2000). Agricultural plants and cultivated crop species such as corn, rye, and wheat among others, have traditionally been utilized as ITNI study species. Additionally, the ecosystems under investigation were heavily influenced by applied nitrogen fertilizer. The latest investigation into ITNI, as of 2014, has been in peat bogs of Germany (Hurkuck, 2014). This investigation is considered semi-natural, the peat bogs in question have been drained due to surrounding agricultural and livestock operations and therefore represent an ecosystem that is highly influenced by agricultural processes and is altered significantly by human intervention (drainage) (Hurkuck, 2014). To date, no investigators have used the ITNI method to measure N-deposition in arid or semi-arid regions**.**

1.4. Objectives

The application of the critical load concept in semi-arid ecosystems is hampered by uncertainties in estimating dry and gaseous nitrogen deposition (Fenn and Poth 2004, Fenn et al. 2005). Coastal sage scrub habitat is especially prone to underestimations of N loading because of arid conditions and the difficulty involved in measuring dry deposition and gas exchange. Since rainfall is usually a minor component of nitrogen loading in coastal sage scrub habitats, new methods of measuring nitrogen deposition must be employed. Underestimation of critical loads can misinform environmental policy and regulation with potentially negative effects on ecosystem restoration efforts. Therefore, my overall goal is to use the ITNI method to better assess and account for all

nitrogen uptake pathways by coastal sage scrub and provide a more accurate measurement of atmospheric nitrogen deposition in arid and semiarid ecosystems.

The main objective of this chapter was to test the concept of isotope dilution and nitrogen deposition measurement using the ITNI system. It was intended that the ITNI treatments identify the movement and level of equilibrium of ^{15}N in the module system. A specific objective was to test the suitability of the ITNI method in a non-agricultural setting, in which we investigated Coastal Sage Scrub habitats and utilized non-agricultural species such as common California native and Mediterranean invasive plants. By employing coastal sage scrub species in the ITNI method, we can demonstrate the potential to determine species-specific nitrogen deposition rates which would be useful in plant community landscape deposition mosaics of non-agriculturally influenced systems.

2. Methods

2.1 Study and Acquisition Sites

Our two ITNI study sites were selected based on previous nitrogen deposition data produced by traditional measurement techniques (Fenn, et al. 2010). Both ITNI sites lie along a well-described nitrogen deposition gradient in southern California (Figure 1.1). My main study site, denoted as “Riverside”, is located at the United States Department of Agriculture Forest Service Pacific Southwest Research Station situated 0.6 miles south of UC Riverside. This location is representative of an urban area receiving elevated levels of nitrogen deposition. ITNI modules were installed in a fenced work-yard containing native and invasive plant species, including California buckwheat,

California sagebrush, summer mustard (short-podded mustard), red brome, and ripgut brome among others. Using conventional methods for deposition monitoring, previous studies have estimated annual N deposition at Riverside to be 14 kg N ha^{-1} yr^{-1} (Fenn et al., 2010, Pardo et al., 2011).

Motte Rimrock Reserve, denoted as "Motte," is located in the city of Perris, California. Perris is a thirty two square mile city of approximately 70,000 residents. Motte Reserve is managed by the University of California Natural Reserve System (UCNRS) which offers protected habitat for California native animal and plant species. Motte contains Riversidian coastal sage scrub (CSS) and riparian woodlands in addition to native and invasive grasslands. Representative Riversidian species include California buckwheat, California sagebrush, brittlebush, white sage, and black sage. Summer mustard, red brome and ripgut brome are representative Mediterranean invasive plants at the site. Motte, with its buffer of protected habitat surrounded by rural and suburban style developments, represents a moderate nitrogen deposition site (Figure 1.1). ITNI modules were placed in the middle of the reserve, in an open field adjacent to the reserve manager's office and dormitory. This site was selected for its lack of obstructing structures or trees and its visibility by Reserve Staff to protect valuable ITNI components. Using conventional methods for deposition monitoring, previous studies have estimated annual N deposition at Motte Reserve to be 12.1 kg N ha^{-1} yr^{-1} (Fenn et al. 2010, Pardo et al., 2011).

2.2 Plant Propagation

Seeds of invasive plants were harvested from Sycamore Canyon Wilderness Park, an open-area preserve in the city of Riverside. Vegetation at Sycamore Canyon consists mainly of Mediterranean invasive plants such as, ripgut, red brome, summer mustard, Russian thistle, among others, with small pockets of native species such as California buckwheat and California sagebrush. Sycamore Canyon was chosen as a seed collection site because of its proximity to both the ITNI study sites. Additionally, ecotypes present this Sycamore Canyon were representative of species adapted to both experimental sites.

Invasive species used in the ITNI experiments included *Bromus rubens* (Red Brome) in Deployment 1 and *Hirschfeldia incana* (Summer Mustard) in Deployments 2 and 3. *Bromus rubens* and *Hirschfeldia incana* seeds were harvested from mature plants within Sycamore Canyon during September 2013. Approximately 600 seeds from *Bromus rubens* were carefully removed with nitrile gloves and placed into a labeled paper bag. *Hirschfeldia incana* seeds were harvested by selecting senescing mature plants and removing twigs that contained seed pods. The twigs were placed into a labeled paper bag, where the seed pods were broken open by gloved hand to expose the seeds. Seeds of California buckwheat (*Eriogonum fasciculatum foliolosum*), the native plant used in the ITNI experiment, were acquired from S&S Seeds (Carpinteria, California). The California Buckwheat seeds were shipped in one-ounce sealed plastic bags and, upon arrival, were transferred into a paper bag for storage.

Since California buckwheat is a winter germinating species, seeds were stored in a refrigerator for two weeks to increase germination success by breaking any dormancy induced by warm weather conditions. After refrigeration, the seeds were transferred into a petri dish for sepal removal. California buckwheat seeds, or kerns, are actually covered by sepals, or leftover petals from the flowering body that senesce around the seed. The function of the sepal is to protect the seed until germination conditions are correct for growth. To further induce germination, the sepals were removed from the seeds using tweezers. Seeds were propped up on the petri dish, blunt side down, and pushed with the tweezers until the seed was extracted from the sepal material. Successfully extracted kerns were checked for viability by examining for breaks in the seed coat and using a light source to verify that the endosperm inside the seed was still viable. Seeds with breaks in the seed coat and those that did not have a plump endosperm (ones that were shriveled or missing entirely) were discarded. Those that passed the viability inspection were placed in another petri dish for germination.

A 1 mg/ml solution of gibberellic acid was used to increase the germination rate of California buckwheat, which typically exhibits less than a 65% success rate (Montalvo, 2012). The seeds were placed in a petri dish with a single paper towel folded into the bottom of the dish. Approximately 100 seeds were placed on top of the towel and then the gibberellic acid solution was pipetted on top of the seeds and left to soak in the solution overnight. After twenty four hours, the seeds were rinsed with DIW to remove any remaining gibberellic acid and then transferred onto water-dampened paper towel. Approximately 50 seeds were transferred onto each petri dish, using tweezers to place

seeds equidistant apart along the entire bottom petri dish. After seeds were evenly distributed, a second paper towel was folded to the shape of the petri and gently laid on top of the seeds. This towel was also damped with Nanopure water without leaving standing water in the petri dish. Once this was complete, the lids were loosely placed on the dishes to help maintain moisture and placed on an east-facing windowsill to provide sunlight. Water was added to the dishes every morning to ensure proper moisture in the dish for germination. On cloudy days a 60W soft-light desk lamp was used to provide additional lighting.

California buckwheat seeds typically took about 3 weeks to fully germinate into small seedlings while summer mustard germinated in about 3 days. Therefore, native seedlings were always started a month prior to an ITNI deployment to improve seedling survival and viability. For Deployment 1, red brome was sown directly into the modules and therefore there was no pre-ITNI germination period for these seedlings. Red brome was sown directly into modules because the time to maturity, and subsequent senescence (about 60 days) was much shorter than the other species used in the ITNI modules.

2.3 ITNI Module Construction

ITNI modules were constructed from General Hydroponics Water Farm Modules (Item GHWFM), obtained from Discount Hydroponics in Riverside, California. Each module utilized a two reservoir system in which a solid growth medium, in this case sand, was placed in a small reservoir with a perforated bottom. To prevent loss of sand, the bottom of the sand reservoir was lined with 80 µm Nitex fabric. The sand reservoir

drained into a lower liquid reservoir that contained the liquid growth media and isotope tracer (Figure 1.2). Extending through both reservoirs was a tube system consisting of a water lift (3/4" rigid, HDPE plastic) and an air-line (¼ inch HDPE flexible tube). These tubes joined in the liquid reservoir via a junction piece at the bottom of the liquid reservoir. At the top of the tube system, a watering ring delivered liquid throughout the upper sand reservoir. Water lift was generated when air was pumped into the bottom of the water lift tube (Figure 1.2). A single DC-powered DC-20 model 12 Volt air compressor, controlled by a Hydrofarm 7 Day Dual Outlet Digital Timer, provided compressed air to the ITNI modules. The pump was powered by AC power at Riverside, and at Motte we used a deep-cycle lead-acid battery (DieHard Marine RV Battery 24M, 500 cold cranking amps) that was kept charged by a 30 Watt, 12 Volt, Unlimited Solar Off-Grid Solar Panel.

2.4 ITNI Assembly

To discourage microbial growth, the ITNI module components were rinsed with a dilute bleach solution, scrubbed with a bottle brush and then rinsed copiously with deionized water. The liquid reservoirs were labeled with volume markings, which were used in conjunction with a clear level-tube connected to the front of the liquid reservoirs to maintain a constant liquid level in the modules. To protect this tubing, and prevent nitrogen deposition into this extra orifice, a plastic sheath was used to cover the tube when not in use for adjusting and checking water levels.

All ITNI modules were assembled in a greenhouse to prevent contamination from prolonged outdoor exposure before deployment. The liquid reservoir was filled with nine liters of deionized water. The air lift, water lift tubing, and watering ring were assembled with all junctions connected. Nitex was laid in the sand reservoir and was marked where the opening for the air and water lift was present. At this mark a small X was cut into the Nitex to allow the air and water lift to perforate, but not allow extra space for sand to escape into the liquid reservoir. The sand reservoir was stacked into the liquid reservoir container. The assembled air and water lift was slid through the hole in the Nitex, about half way up the shaft of the assembly. This assembled piece was then installed, with the air and water lift junction piece passing through the opening in the sand reservoir, and the assembly resting with the Nitex on the perforated sand reservoir bottom. This action left the watering ring just below the height of the sand reservoir when assembled. Once the ITNI hardware components were in place, #16 Silver Sand (P.W. Gillibrand Co, Simi Valley CA) was added to the sand reservoirs. Each module was filled to two inches below the top of the sand reservoir to reduce sand losses to wind and by transport. The watering ring was adjusted to sit just a millimeter or so above the sand; this was done to prevent any "splattering," of nutrient solution from bubbles in the water lift and to prevent interference by birds drinking water from under the ring (adapted after Deployment 1).

The sand used in the ITNI modules was composed of sub-angular quartz grains with an effective size of 0.5 to 0.7 mm and is a type commonly used in sand-blasting operations. The #16 Silver Sand was baked at 150 to 200 °C before commercial

packaging, resulting in a moisture content of < 0.1% by weight and an essentially sterile media. Elemental analysis of the packaged sand (via high-temperature combustion in a Thermo Flash EA) yielded levels of total carbon and nitrogen below the detection limit, indicating that there was essentially no organic matter or nitrogen to account for in the sand media at the start of the ITNI experiments.

2.5 Isotopic Labeling, Deployment, and Sampling

The ^{15}N labelled tracer used in the ITNI experiments was made by dissolving ACS grade KNO_3 and 98 Atom Percent ^{15}N-KNO_3 (Sigma Aldrich, USA) in 18 megaohm deionized water in a 1-liter volumetric flask to achieve an isotopic composition of 1.01 AP ^{15}N, and a nitrate concentration of 360 millimoles per liter. The concentration of nitrate was adapted from similar hydroponic experiments conducted in Fiest and Parker 2001. The 1.01 AP ^{15}N tracer abundance produced a strong isotopic enrichment in the ITNI system, but was still within the range of ^{15}N concentrations that could be accurately measured on our existing isotope ratio mass spectrometer. Other macro and micro nutrients were added to the KNO_3 solution to essentially produce a ^{15}N-enriched Hoagland solution (adapted from Parker et al. 1999).

During ITNI deployment, seedlings were gently planted into the sand reservoirs of the ITNI modules, with any green, aerial leaves or stems resting above the sand surface. Then, each module's liquid reservoir was dosed with 50 milliliters of the ^{15}N-enriched Hoagland solution resulting in an initial isotopic enrichment of 1.01 ^{15}N AP, an initial nitrate concentration of 2 millimoles per liter and an initial concentration of 0.475

g/L of standard Hoagland solution. Following dosing, the air lift systems were immediately engaged to circulate the nutrient solution within each ITNI module. The ITNI modules were stored for two weeks in a climate controlled greenhouse to ensure thorough distribution of ^{15}N tracer into the different PLS components before deployment at the two field sites.

Beginning with the second ITNI deployment, the modules' liquid reservoirs were wrapped in aluminum foil to discourage algal growth. During all deployments, the modules were spaced equidistant apart from one another surrounding a storage bin, in a square pattern (Figure 1.3). This storage bin housed the air compressor, the timer for the air compressor and air-line manifolds used to distribute compressed air to the air lift systems in the ITNI modules. At Motte reserve, since there were no electrical hookups for the pump and timer, a solar panel and accompanying deep cycle marine battery and solar controller were utilized as an energy source for the air lift system (Figure 1.4). Modules were watered 4 to 5 times throughout the day for approximately 1-2 minutes duration, depending on the season and transpiration needs of the plants.

The ITNI modules were "shuffled" every two weeks while deployed to reduce confounding influences from a singular deployment location. Deionized water was added to the ITNI modules every 2-3 days to maintain the liquid reservoir volume at 9 liters, but no additional spikes of isotopically-labelled Hoagland's solution were made.

The modules were exposed in the field during the following time periods: Deployment 1: March 8, 2013 to May 20, 2013, Deployment 2: May 28, 2013 to August

12, 2013 and Deployment 3: November 15, 2013 to March 24, 2014. At the end of a deployment the modules were disconnected from the air lift system and immediately returned to the greenhouse for sample processing. Above-ground plant material from each module was individually clipped above the sand surface and placed into paper bags. Plant material bags were dried at 60°C for 2 days to drive off moisture and prevent microbial decay of the plant matter. The dried plants were then weighed for total aboveground biomass.

We collected solution from the liquid reservoirs at the end of the deployments by passing the water through a 0.45 membrane filter using a filter holder and syringe. These 125 milliliter aliquots were put into clean HDPE bottles and stored in a freezer at -20 °C until analyzed for nitrate and ammonium concentration and the stable isotope composition of nitrate. A second 125 aliquot of unfiltered water was collected from each module and stored frozen for later determination of total nitrogen. Total nitrogen (TN) was analyzed using a persulfate digestion and EPA method 353.2 (Table 1.4). Total nitrogen measured by this technique includes several forms of N: i) particulate nitrogen (PN), ii) dissolved inorganic N (DIN: sum of NO_3^-+NO_2^- and NH_4^+) and iii) dissolved organic N (DON). Because there is no currently available method for measuring the AP ^{15}N of TN, we could not include its mass and isotopic composition in the deposition computations. However, we did evaluate the possible error introduced into the ITNI deposition measurements by computing the mass of unmeasured N (mg) in the liquid reservoirs:

$$Unmeasured\ N = TN - DIN = PN + DON \qquad \textbf{Equation 1.1}$$

We then compared the mass of the unmeasured N (which was most likely DON) to the total mass of N in the ITNI module at the end of the deployment. If unmeasured N was small relative to the total N in the module(less than a few percent), than neglecting it in the ITNI calculations likely produced little bias.

At the end of the deployments, the sand in each module was passed through a ¼ inch metal screen to separate out the plant roots which were dried and weighed as previously described. The sifted sand from each module was individually weighed, thoroughly-mixed and then duplicate subsamples (5 grams wet weight) were collected from each module for measurement of KCl extractable nitrogen (Maynard et al. 2007). Note: We decided against measuring nitrogen content and stable isotope composition of the sand directly using the elemental analyzer (EA) inlet to the mass spectrometer. First, the N-content of the sand was very low, necessitating that relatively large samples (>25 mg) be analyzed in the EA, and which proved problematic to combust properly. Second, the spatial heterogeneity of N-content in the sand was fairly large so that many, 25 mg replicates would be required to get a representative sample of the sand's N-content and $\delta^{15}N$ for the ITNI computation. KCL extracts allowed us to base our estimates of N-content and isotope composition on much larger subsamples of the sand (i.e., 5000 mg).

Water samples from the modules and KCl extracts from the sand were analyzed for nitrogen concentration and ^{15}N abundance. Inorganic nitrogen concentrations were measured on a discrete analyzer (AQ2; Seal Analytical, Inc.) using the following

methods: nitrate+ nitrite = EPA 353.2 and ammonium = EPA 350.1. Ammonium levels in the water and KCl extracts were below the detection limit so we confined isotopic measurements to nitrate only. The $\delta^{15}N$ and $\delta^{18}O$ of nitrate+nitrite were measured using the microbial denitrifier method (Coplen et al., 2012) at the Facility for Isotope Ratio Mass Spectrometry at UC Riverside. $\delta^{15}N$ and $\delta^{18}O$ values were measured using a Thermo Delta V isotope ratio mass spectrometer (IRMS; Thermo Fisher Scientific Inc.). Three reference standards, USGS-32, USGS-34 and USGS-35, were used for calibration. Isotopic abundances are expressed in standard delta notation relative to VSMOW for oxygen and relative to atmospheric N_2 for nitrogen.

The nitrogen content and stable isotope composition of above- and below-ground plant material were analyzed using a Costech elemental analyzer connected to the Thermo Delta V IRMS. Prior to analysis, the plant materials were ground and homogenized using a Pyrex-glass mortar and pestle. The $\delta^{15}N$ values for the plant materials are expressed in standard delta notation relative to atmospheric N_2.

2.6 Treatments Used in the ITNI Deployments

As our study was a proof-of-concept investigation for the use of the ITNI method in arid and semi-arid regions, we employed a variety of treatments in the three deployments (Tables 1.1 through 1.3). During Deployment 1 we created 3 treatments at both Motte and Riverside: i) modules spiked with ^{15}N-enriched Hoagland solution that contained water, sand and Red Brome plants, ii) control modules spiked with ^{15}N-enriched Hoagland solution that contained water and sand only (no plant) and iii) control modules containing water and sand that were spiked with ^{15}N-enriched nitrate only (no

other nutrients were added). The design of the Deployment 1 allowed us to: i) compare deposition rates to Red Brome at Motte and Riverside, ii) observe a complete ITNI module in relation to a module without a plant and iii) to compare the behavior of the ^{15}N spike in plant-free modules containing complete Hoagland solution versus ones containing only potassium nitrate.

In Deployment 2 we created 2 main treatments at both Motte and Riverside: i) modules spiked with ^{15}N-enriched Hoagland solution that contained water, sand and summer mustard (invasive plant), and ii) modules spiked with ^{15}N-enriched Hoagland solution that contained water, sand and California buckwheat (native plant). A single Hoagland control was also run at each site, but the data were not used to compute N deposition rates. The design of the Deployment 2 allowed us to compare deposition rates for both an invasive and native plant at a single site and between sites. Based on the results from Deployment 1, all of the modules in Deployment 2 were wrapped with aluminum foil to reduce light-levels and algal growth in the liquid reservoirs.

Prior to Deployment 3 we had trouble growing seedlings of California buckwheat so we focused this experiment on summer mustard. Two treatments were created and deployed at Riverside only: i) modules spiked with ^{15}N-enriched Hoagland solution that contained water, sand and summer mustard (invasive plant), and ii) control modules containing water and sand that were spiked with ^{15}N-enriched Hoagland solution (no plant). A single California buckwheat module was created and operated, but the data from this module was not included in the figures and data analyses for Deployment 3.

The design of Deployment 3 allowed us to measure N-deposition at Riverside and to further assess the fate of the ^{15}N tracer in modules not containing plants.

2.7 Computation of Nitrogen Recovery and N Deposition

Careful measurements of the mass of N in the ITNI modules at the start (beginning with the planting of the seed in greenhouse) and end of an experiment (when the modules were broken down) allowed us to compute the recovery of N in the experiments. Recoveries that exceed 100% could potentially result from additions of atmospheric N to a module or errors in computing N masses, while recoveries less than 100% could indicate losses of N (and ^{15}N tracer) caused by liquid leaks, microbial denitrification, seed dispersal and insect or animal herbivory. At the beginning of the experiment, each module contained 252 mg of N. The vast majority of the starting N mass was contributed by the ^{15}N-enriched Hoagland solution (the total N content of the seeds in a single ITNI module was <0.5 mg). Recovery (*R*) was computed using the following equation:

$$R = \frac{^{15}N\ (L+PA+PB+S)+^{14}N\ (L+PA+PB+S)}{252\ mg\ N\ module^{-1}} \qquad \textbf{Equation 1.2}$$

Where L is the mass of N in the liquid reservoir at the end of the field deployment, PA is the mass of N in the above-ground plant biomass at the end of the deployment, PB is the mass of N in the below-ground plant biomass at the end of the deployment and S is the mass of N in the sand at the end of the deployment. In modules with plants, the vast majority of N ended up in the plants with lesser amounts in the sand and very little N in

the liquid (Tables 1.1 through 1.3). In control modules without plants, most of the N remained in the liquid reservoir with modest amounts in the sand (Tables 1.1 through 1.3).

The N mass (*N*) and isotope values of the PLS components (*a*), determined at the end of the field deployment, were used to compute N deposition to each module (i.e., deposition to both the plant *and* the sand surface) using the following equation from Russow et al. 2001:

$$AdN = N_s \times \left(1 - \frac{a'_S}{a'_T}\right) - N_o \qquad \textbf{Equation 1.3}$$

Where AdN is atmospherically derived nitrogen input to the module (mg); a'_s is the mass-weighted excess ^{15}N abundance (i.e., $a' = a - 0.366$; 0.366 is the ^{15}N abundance in the atmospheric N_2 baseline) of the PLS system at the end of the field deployment; a'_T is the excess ^{15}N abundance (i.e., $a' = a - 0.366$) of the original tracer added to the PLS system; N_o is the original N mass (mg) in the seeds or seedlings at the start of the ambient exposure; and N_s is the mass of N in the PLS system at the end of the field deployment (mg). The structure of this equation precludes the separate computation of N deposition to the plant and sand because atmospheric deposition to the sand is ultimately taken up by the plant through the root system just as atmospheric nitrogen is taken up through the stomata on the plant surface (He et al., 2010).

We computed AdN using three values for a'_S and N_s: i) using the mass-weighted average atom% ^{15}N and total N mass of the PLS system at the end of the field

deployment (denoted "Module Average" in tables and figures), ii) using the atom% ^{15}N and N mass of the plant (sum of above- and below-ground biomass and denoted "Plant Only") and iii) using the atom% ^{15}N and N mass of the above-ground portion of the plant (denoted "Aboveground"). Since the vast majority of a module's N resides in plant biomass, using the plant biomass and $\delta^{15}N$ value might prove to be an easier approach to computing deposition, since one would not have to extract and measure N mass and $\delta^{15}N$ of the sand and liquid. Similarly, we were interested to see if measurements of N mass and $\delta^{15}N$ of just the aboveground plant biomass would yield an accurate estimate of atmospheric deposition assuming that there was isotopic equilibria between the plant roots and aboveground biomass.

3. Results

3.1 Deployment 1

Detailed ITNI module specifics for each Deployment (1-3) are addressed in Tables 1.1, 1.2, and 1.3. Mean values of treatments will be addressed in this section and in the accompanying tables.

During Deployment 1, all module treatments recovered less than 100% of the tracer spike for both Riverside and Motte Reserve (Figure 1.5). At Riverside, the Hoagland Control treatment recovered the most tracer spike among all treatments at 91%, and the N-Only Control treatment recovered the most at Motte Reserve with 76% ($p<0.001$). Motte Hoagland Control treatment had a tracer spike recovery of 53%

($p<0.001$). At both sites, the lowest tracer spike recovery occurred in the Invasive Plant treatments at 66% and 16% ($p<0.001$), for Riverside and Motte respectively. Motte Invasive Plant treatments yielded the overall least tracer recovery due to herbivory and interference by rabbits and birds.

Hoagland Control and N-Only Control modules showed incomplete reduction of the initial tracer spike concentration of 28 mg $N\text{-}NO_3$/L within the liquid portion of the ITNI module while levels in the Invasive Plant modules decreased to the detection limit (Figure 1.6). This depletion had a very clear response at Mott with nitrogen concentrations of 11.6 and 19.6 mg $N\text{-}NO_3$/L, for Hoagland Control and N-Only Control treatments ($p=0.008$). This response was less clear at Riverside, where Hoagland Control and N-Only Control treatments resulted in 17.2 and 22.0 mg $N\text{-}NO_3$/L ($p=0.111$).

Modest depletion of the initial tracer spike was noted in the liquid reservoirs of the in the Hoagland Control treatments at Riverside and Motte (0.88 and 0.98 AP ^{15}N, respectively) (Figure 1.7). The tracer in N-Only Control treatments was also depleted from the initial tracer spike, however the treatments were also significantly lower than the Hoagland Control modules for both Riverside and Motte ($p=0.005$ and $p<0.001$). In the Invasive Plant modules, AP ^{15}N levels approached the natural abundance level of 0.366 indicating little of the original tracer remained in the liquid reservoirs of modules containing plants.

Unmeasured N (~sum of PN and DON; Equation 1.1) in the Invasive Plant treatments averaged 1.3% of the mass of nitrogen in the modules at harvest (Table 1.4).

The control at Riverside, which contained only water, had a similar unmeasured N: 1.4%. However, the Motte Control had 11% unmeasured. Riverside Nitrogen Only Controls had an average of 41.6% unmeasured N, while Motte Nitrogen Only Controls had 14.3%. Hoagland Controls at Riverside and Motte averaged 21.8% and 17.4% unmeasured N, respectively.

The Module Average method of calculating nitrogen deposition for Riverside and Motte yielded deposition rates of 5.9 kg/ha and 3.5 kg/ha for the deployment period, respectively (Table 1.5). Nitrogen deposition calculated via only plant material, resulted in a lower deposition rate (4.5 kg/ha), and lower still with the aboveground plant matter only (3.2 kg/ha). However, owing to relatively large variability among replicates, there were no statistical differences among the three methods at Riverside ($p=0.445$). At Motte, N deposition was significantly different among all three computation methods ($p<0.001$): Module Average (3.5 kg/ha)>Plant (1.8 kg/ha)>Aboveground (0.5 kg/ha).

3.2 Deployment 2

In contrast to Deployment 1, nitrogen recovery during Deployment 2 was generally higher and in some modules exceeded 100% (Figure 1.8). At Riverside, Invasive Plant treatments recovered between 74.6% and 190.1% of the added nitrogen. At Motte higher N recoveries relative to Deployment 1 were likely a result of the installation of a rabbit fence to reduce herbivory, resulting in recoveries between 106.8% and 253.5%. Native Plant modules had significantly ($p<0.05$) lower N recovery than Invasive Plant modules at both Motte and Riverside. Recoveries in Native Plant modules

ranged from 89.7% to 101.7% at Riverside and 61.3%-94.5% at Motte. Invasive Plant modules had average recoveries of 125% and 167% at Riverside and Motte respectively. Riverside Native Plant treatments recovered on average 96% of the nitrogen tracer spike, and Motte Native Plant modules recovered 78%. In Invasive Plant modules at both sites, the aboveground plant material held the majority of the total nitrogen within the plant (Table 1.1).

The Native Plant treatments at both sites exhibited less variable, smaller ratios of aboveground to belowground N-biomass than the Invasive Plant treatments (Figure 1.9). Motte Native Plant modules showed a strong positive relationship with aboveground biomass of nitrogen being greater than belowground biomass (R^2=0.883). Riverside Native Plants exhibited a weaker relationship between aboveground and belowground plant material (R^2=0.200); this weaker relationship might be a result of rabbit grazing of aboveground plant biomass at Riverside. Though there were no obvious signs of herbivory of these plants; it is possible since the modules were exposed in an area were rabbits have been present in the past.

Invasive Plant modules at both Riverside and Motte exhibited greater aboveground nitrogen biomass than nitrogen mass in belowground plant biomass (Figure 1.9). Belowground nitrogen biomass ranged from <5 to 25 mg, while the aboveground biomass ranged from about 150 to 450 mg nitrogen. The invasive plants at both sites had roots with much smaller overall biomass than that of the aboveground plant biomass.

Riverside and Motte Native Plant treatments showed relatively strong positive relations between above and belowground AP ^{15}N (Figure 1.10). At Riverside, the California buckwheat plants had less tracer in the aboveground portion of the plant than in the root system ($p=0.01$). At Motte, the California buckwheat plants had similar amounts of tracer in the aboveground portion of the plant relative to the root system ($p=0.51$). In the case of Invasive Plant modules, we observed no correlation between the tracer content of above and belowground plant parts at Riverside ($R^2=0.0015$), while at Motte we observed an inverse relationship between above and belowground tracer concentrations ($R^2=0.520$)

At Riverside there was a clear pattern of higher N deposition measured by the Module Average method, however, it was difficult to detect statistically significant differences among the three computational methods of determining deposition rates (Table 1.6). In the case of Riverside Native Plant modules, the Module Average approach yielded significantly higher N deposition than the Aboveground only approach. The Module Average method yielded deployment nitrogen deposition rates of 8.6 kg N ha^{-1} and 9.8 kg N ha^{-1} for the Native Plant and Invasive Plant treatments, respectively. For the Module Average and Plant computation methods we noted higher apparent N deposition for Invasive Plant modules relative to the Native Plant modules, however these differences were not statistically significant. We observed significantly higher N deposition for the Invasive Plant treatment computed using the aboveground biomass method relative to the Native Plant Treatment ($P<0.01$).

Unmeasured N followed similar patterns to patterns observed during Deployment 1 (Table 1.4). In Invasive and Native Plant treatments at both Riverside and Motte, unmeasured N averaged less than 1% of the total mass of nitrogen in the modules at harvest.

At Motte we noted similar trends in N deposition across the three computation methods and between the Native Plant and Invasive Plant treatments (Module Average>Plant>Aboveground and Invasive Plant>Native Plant), but owing to high variability among the replicate modules, none of these differences is statistically significant (Table 1.6), The Module Average method yielded deployment nitrogen deposition rates of 4.9 kg N ha^{-1} and 7.5 kg N ha^{-1} for the Native Plant and Invasive Plant treatments, respectively.

3.3 Deployment 3

Invasive Plant and Hoagland Control treatments had similar nitrogen tracer recovery at Riverside to that of Deployment 1, perhaps due to the lack of foil installation until the 2nd week of exposure, to be discussed further in the following Discussion Section (Figure 1.11). Invasive Plant modules had N recoveries that averaged 46% and Hoagland Control modules had average N recovery of 53%. The Native Plant module had N recovery of 77%.

In the Riverside Invasive Plant treatment, above and belowground nitrogen amounts varied substantially, with no correlation (Figure 1.12). There did not appear to be a relationship between above and belowground N biomass (R^2=0.0078). Overall, the

Invasive Plant treatments had 2-4 times less aboveground nitrogen biomass as compared to those in Deployment 2.

Belowground AP ^{15}N varied only 0.03 AP ^{15}N in Invasive Plant treatments modules, while aboveground varied by about 0.09 AP ^{15}N (Figure 1.13). There was no significant correlation between the tracer concentrations in the above and belowground plant parts, although this could be an artifact of relatively constrained variation of AP ^{15}N in the belowground biomass.

Deployment 3 experienced similar patterns of unmeasured N as observed in Deployment 1 and Deployment 2 (Table 1.4). In Invasive treatments at Riverside, unmeasured N accounted for less than 1% of the total mass of nitrogen in the modules at harvest. The Hoagland Control at Riverside, had a similar unmeasured N to Deployment 1 at 21.7%.

For Deployment 3, N deposition rates were significantly different ($P<0.05$) among all three computational methods and were 3.8, 2.9 and 1.9 kg N ha^{-1} for the Module Average, Plant and Above Only methods, respectively (Table 1.7). N deposition measured using the Hoagland Control modules was 1.5 kg N ha^{-1} and was significantly lower than N deposition in the Invasive Plant module ($p<0.001$).

4. Discussion

4.1 Deployment 1

4.1.1 Operational Errors

Deployment 1 incurred two experimental errors with the treatments at Riverside, the first being the Nitrogen Only treatment. This treatment was shown to be statistically different from other treatments; however, this was due to the accidental and unknown supplement of additional ^{14}N-KNO_3 to all Nitrogen Only modules, after the deployment had begun. This error highly diluted the ^{15}N tracer, which resulted in overestimated nitrogen deposition to the modules by mimicking an unrealistically high nitrogen deposition rate. Accidental additions were also made to some Complete Nutrient and Invasive Plant modules, but the effected modules were removed from analysis (2 and 3 modules, respectively). This error was also evident in TN analyses where Nitrogen Only Controls at Riverside exhibited unmeasured N on the order of 41.6%, while Motte measured an average of 14.3% unmeasured N.

The other error during Deployment 1 was the placement of the watering ring, approximately 2 inches above the sand surface, which resulted in minor splashing of nutrient solution along the inside surfaces of the ITNI module (not on the sand surface). This resulted in some visual salt accumulation on the sand reservoir surfaces that would represent lost ^{15}N and N mass leading to errors in the nitrogen deposition rate.

4.1.2 Biological Interferences

Riverside and Motte experienced different biological interferences during Deployment 1. These interferences later prompted changes to the ITNI design with subsequent deployments to prevent as much interference as possible without compromising ITNI function.

An internal biological interference was that of microbes in the ITNI experimental setup. This would be the case as Complete Nutrient treatments recovered less than 100% (Figure 1.5), although no plant components were available to be eaten, destroyed, or lost, in this treatment. The only significant pathway for nitrogen loss would have been through microbial growth (although some nitrogen was lost from water splash – see above). This is because the addition of both nitrogen in a consumable form of KNO_3 and a nitrogen-free Hoagland solution to the ITNI modules, would supply all other macro and micro nutrients necessary for microbial (and plant) growth. We attempted to avoid this through dilute bleach rinses of modules before preparation and through baked sand additions to the modules. However, over time, and during prep, microbes could enter the ITNI systems through many pathways including dust deposition, and introduction of microbes from other species "visiting" the ITNI modules such as rabbits, birds, and insects. Upon harvest, biofilms were noted on the in-side surfaces of the modules, but could not be accurately quantified. ; leading to the belief that nitrogen was most likely immobilized by algae in the liquid reservoir. The presence of algae and microorganisms in the nutrient liquid were supported by TN analyses (Table 1.4). Module treatments, such as Nitrogen

Only Control and Hoagland Control, contained significant amounts of unmeasured N (Equation 1.1) in their liquid reservoirs at harvest. However, Invasive treatments had very low levels of unmeasured N in the liquid reservoir. This suggests that by having the plant in the module, the nitrogen was taken up too quickly for microbes to fully utilize the nutrient media. Lastly, looking at the water-only Control (no nutrients or tracer) at both Riverside and Motte, it is apparent that biological interference from more than just microorganisms was present at Motte, with the Riverside Control containing only 1.4% unmeasured N, while Motte had 11%. The source of this additional biological interference from rabbits and birds will be discussed in the following paragraphs.

Herbivory was present at both Riverside and Motte. Riverside modules experienced herbivory from what appeared to be aphids (family *Aphididae*). The aphid-like insects fixed to the *Bromus rubens* along the stems of the invasive grass. The insects were noted, in high densities (enough to cover >50% of the stem surface area) during the 6^{th} week of exposure. This herbivory would result in the removal of both ^{14}N and ^{15}N through the xylem of the plant tissue.

Motte, on the other hand, experienced plant tissue consumption and subsequent removal from the ITNI system, by rabbits. Modules containing invasive plants at Motte had bite marks after two weeks of exposure and were eventually grazed down to the base of the plant above the sand surface. Though they were not observed directly, rabbits were suspected to be the largest contributor to this loss because of identifying teeth marks on the plant surfaces and their scat and footprints discovered on the module sand surface.

Rabbit interference had a significant effect on ITNI function, since all plant material above the plant surface was consumed. This could lead to two, somewhat compensating errors. First, since the plant material was more enriched with ^{15}N than other module components, this would increase the magnitude of the quantity inside the parentheses of Equation 1.3 leading to an overestimation of N-deposition for Invasive modules at Motte (Table 1.1). However, loss of plant biomass also reduced the value of N_s in Equation 1.3 results in an underestimate of N deposition. Based the relatively low rate of N deposition for these modules, we suspect that the N biomass effect overwhelmed the isotope effect leading to unrealistically low estimates for N deposition in the rabbit-grazed modules (Table 1.4). The removal of aboveground plant material might have contributed as much as 40% error to Motte Invasive modules, based on the proportion of aboveground biomass available at Riverside.

The last observed biological interference to the ITNI systems was bird visitation. Since Deployment 1 was exposed during the months of March and May, they were most likely prime targets for bird interference due to spring migration. When ITNI modules were serviced, it was noted that birds were congregating around the modules and resting on the watering ring, above the sand surface. Bird scat was noted in modules, and promptly removed to prevent it entering the ITNI system. N inputs to the modules from animal waste will increase the Ns term and decrease the a'_s term in Equation 1.3 leading to overestimated N deposition. Lastly, birds were also observed drinking from the watering ring apparatus that was installed approximately 2 inches from the surface of the sand, resulting in removal of nitrogen from the system. Since the watering ring

functioned similar to a drip system, it is estimated that birds could have removed approximately 5 ml of water during each watering session. Based off a 9 L reservoir, this would contribute an approximate 11% error in the recovery of N over the course of the deployment.

4.2 Deployment 2

4.2.1 ITNI Improvements

Deployment 2 incorporated the lessons learned from Deployment 1. This included the addition of aluminum foil on the outside of the module, the addition of a fence around the exposure area, and modified heights of the module watering rings. These improvements reduced the unintended losses and gains of nitrogen experienced in Deployment 1.

Foil was wrapped around the liquid reservoir portion of the ITNI to reduce light levels in the liquid reservoirs in order to prevent algal growth. At harvest, the modules showed little or no evidence of biofilms in the liquid reservoir. This improvement may help explain the higher nitrogen recoveries in Deployment 2 relative to Deployment 1 (Figure 1.8). Lower algal or microbial N uptake also contributed to more accurate estimates of average AP ^{15}N levels in the modules.

Modules at Motte were fenced-in to prevent consumption of plants by rabbits. However, our first attempt at fencing (week 1), was not adequate to fully deter rabbits from borrowing or climbing into the modules. Wildlife cameras installed at the site

during week 2 confirmed this. Changes to the fence design led to the complete exclusion of rabbits by week 4. However, during the fencing trial, 2 modules with native plants and 3 modules of invasive plants had evidence of grazing by rabbits and were therefore removed from the experiment.

During Deployment 2 the watering rings were repositioned to lie completely flush with the surface of the sand in order to deter birds. Wildlife cameras confirmed that the lack of access to free-flowing water from the ITNI system eliminated the visits from birds and reduced the overall population of birds in the adjacent area. We are confident that the ITNI N deposition values for Deployment 2 are not significantly affected by rabbit grazing or bird visitation.

4.2.2 Movements of Tracer Within the PLS System

During Deployment 2, we noted that the plant pool of nitrogen was substantially more enriched with ^{15}N than the sand and water despite the fact that most of the N in the modules resided in the plant tissues (Table 1.2). This is most likely attributed to exponential plant growth during the first few weeks of the deployment when there was abundant ^{15}N in the liquid reservoir to fuel plant growth. This physiological process would promote the rapid transfer of nitrogen from the liquid reservoir and into the plant, leaving the nutrient solution and sand depleted in ^{15}N tracer and N mass. After this initial plant growth-spurt fueled by Hoagland solution, we speculate that plant growth slowed because it was dependent on atmospheric N inputs. Interestingly, atmospheric N inputs had a larger impact on the AP ^{15}N of the sand and liquid since these pools were small (in

some cases almost completely diluting the ^{15}N tracer), while the plant pool of N was strongly buffered against changes in AP ^{15}N owing to its large size.

The growth and reproductive habits of the plants selected in this study might help explain why we observed lower N deposition rates when using only the plant in the ITNI computations or when we used only the aboveground plant biomass. The majority of nitrogen in the Invasive Plant modules was in the aboveground plant material (Figure 1.9). This was most likely due to the annual life history of the invasive plants, leading them to maximize aboveground, seed producing organs, as opposed to putting growth into belowground roots. We believe that ample water and nutrients, mixed with the short-lived, annual lifestyle of *H. incana*, led these plants to produce greater aboveground biomass as compared to naturalized, or wild, *H. incana* found at the field sites. Conversely, native *E. fasciculatum* specimens showed more belowground root material than that of the invasive species. This is due to the perennial habit of the native, where a taproot and established root system would be necessary for survival. Because perennials such as *E. fascicultum* do not respond as quickly to increased nutrient availability, the species would not develop the same amount of biomass as that of *H. incana* during the exposure period. Overall, we observed complex movements of tracer within the individual PLS systems which may explain why we computed substantially different N deposition rates using subcomponents of the modules than we computed with the module-average approach.

4.3 Deployment 3

Since Deployment 3 had aluminum foil installed 2 weeks after the exposure had begun, some nitrogen may have been lost to algal or microbial biofilms. In a few Hoagland Controls, there were also very small weeds (species unknown, too small to identify) that were also present. The small seedlings were included in the module calculations as they utilized module nitrogen during exposure, possibly adding a small source of error to deposition rates. Additionally, the prolonged time to senescence, 130 days, might have lowered the apparent N deposition rate as the winter season is not a time in which *H. incana* readily grows; instead *H. incana* sprouts with the last spring rains and grows primarily in the summer season. Because *H. incana* was grown out of season, we speculate that the plants were transpiring less water and therefore assimilating less gaseous N than plants exposed during the summer deployments (Deployments 1 and 2), and therefore growing much slower. This is confirmed by the fact that Deployment 3 took 130 days for *H. incana* to reach senescence, but in Deployments 1 and 2 the species took approximately 70 days to senescence. I also noted that invasive treatments seemed to have smaller aboveground plant structures than in previous deployments.

The AP ^{15}N of belowground biomass had a relatively narrow compared to that of the aboveground plant material (Figure 1.13). While this might be a physiological artifact, we speculate that nitrogen settling on the aboveground plant surface directly from the atmosphere may have contributed to greater isotopic dilution and variability in the aboveground biomass. Since belowground plant material is not exposed to direct

nitrogen deposition, the roots did not experience the same depletion of the ^{15}N tracer. In contrast, stomatal uptake of atmospheric N, and the subsequent allocation of this N to the root system, would be the major pathway in which root tissues would become depleted of ^{15}N.

The Control treatments, lacking a plant component but containing all other ITNI parts, resembled a passive nitrogen deposition collector, because there was no plant component in which to actively uptake gaseous nitrogen or deposition. Modules with plants had higher apparent N deposition because of active absorption of atmospheric N through leaf stomates and because of the larger surface area of plant canopies relative to the sand surface in the Control modules

4.4 Difference Among the ITNI Deposition Rates

In all deployments, and across all treatments, ITNI modules exhibited the following pattern of depositions rates using the different computation methods: Module Average> Plant> Aboveground. This finding is explained by two factors. First, the plant is more isotopically enriched than the liquid and sand components of the ITNI modules so that the portion of Equation 1.3 in parentheses was lower when computed using only the plant than when it was computed using the module average; this smaller number was then multiplied by N_s to yield a smaller apparent N deposition. Secondly, the N_s value for the plant and the aboveground plant are lower than the N_s for the entire module, further decreasing the value of N deposition in Equation 1.3. Thus our hope that the PLS

would reach an internal isotopic equilibrium so that selecting only plant tissue for ITNI calculation could result in accurate N deposition failed.

The high levels of tracer in the plant occurred because of early exponential growth when ^{15}N tracer was abundant in the liquid reservoir. As the Deployment continued, the plant remained isotopically enriched compared to the other components of the module. The sand and liquid reservoirs, because of their small size, experienced higher levels of isotope dilution. This leaves the plant more enriched as the exposure continues as the plant does not appear to exude the ^{15}N-labelled material back into the liquid and sand after it has been allocated to plant tissues. Therefore, the different computational methods of Plant and Aboveground are not helpful in determining the nitrogen deposition experienced by the modules. Module Averages are the only way to compute nitrogen deposition to ITNI modules.

Module Average nitrogen deposition rates were greatest in Deployment 2, compared to Deployments 1 and 3, perhaps due to improved nitrogen recovery at harvest. As a consequence of calculating nitrogen deposition rate via the module average ITNI method, the rate is dependent on nitrogen recovery as represented by N_s. As N_s decreases, the nitrogen deposition rate experienced by the module also decreases, therefore underestimating true nitrogen deposition to the module over the exposure period. Since Deployments 1 and 3 lacked a foil barrier to deter algal/microbial growth in the module during the exponential growth of the plant, they recovered less nitrogen at harvest as a result.

4.5 Comparison to Other ITNI Studies

He et al., 2010, stated that plant detrital material was neglected in their ITNI measurements. In contrast, I included plant detritus during the harvest of the plants. Though the plant detritus was not actively taking up gaseous nitrogen, it derived from the ITNI plant and still experienced dry deposition to surfaces and therefore was included in the sampling for the respective modules. Plant detritus was most likely a conundrum for He et al. as the agricultural species that they utilized had plant organs that senesced during exposure. This senescence led to the volatilization of NH_3, however our modules were harvested as soon as the plants senescenced, therefore reducing this nitrogen loss. In the case of *E. fasciculatum* treatments, there was no plant detritus to include, further supporting our suggestion that perennials, instead of agricultural or annual species, should be used as ITNI study specimens.

Extrapolation methods were introduced by Russow and Bohme 2005 in which the plant density of the module was modified to match the field density of the same species. To correct for this in our own study, *Bromus rubens*, *Hirschfeldia incana*, and *Eriogonum fasciculatum* were sown in the ITNI modules at field density (*B. rubens,* Wu and Jain, 1979) or very close to that of field density when sown a single seedling to a single module (*H. incana* and *E. fasciculatum*). Therefore, only the exposed sand surface was necessary for extrapolating ITNI calculations to the hectare level.

Similarly to He et al. 2010, our study also experienced lower nitrogen deposition rates for species grown outside of their traditional growing season. He et al. contributed

this finding to less active uptake of gaseous nitrogen as compared to normal growing conditions. However, they found that despite this, the ITNI method produced higher deposition rates than traditional methods and concluded that this excess nitrogen deposition was active uptake by the plant. According to the National Atmospheric Deposition Program, total nitrogen deposition for our study area ranged from 12 kg ha^{-1} yr^{-1} (Motte) to 18- >20 kg ha^{-1} yr^{-1} (Riverside) (NADP 2012). When extrapolated to an entire year, the ITNI method estimated total deposition to be approximately 38 ha^{-1} yr^{-1}, also exceeding NADP's measurements. Since our study site was not dominated by wet deposition, as was He et al.'s, we attributed this excess deposition (as compared to NADP models) to the active uptake of gaseous nitrogen by the plant and improvements upon the shortcomings in dry deposition measurements in the region (discussed further in Chapter II).

4.6 Suggestions for Future Work

Future ITNI experiments in arid and semi-arid regions should utilize a single or a very few, representative short-lived perennial species. We make this suggestion based on the considerable variability in biomass among individual specimens in the annual invasive treatments. If a short-lived perennial was used instead of an annual, we suspect there would be less influence from early senescence and less influence from a life history that promotes quick growth. Annual life history traits can increase the chance of biomass loss due to seeding events, flowering, pollen release, etc. By working with a perennial plant, ITNI operators can still grow a plant with enough aerial biomass to actively uptake

nitrogen, but the plant is less likely to senesce or seed during the exposure period. Additionally, we suggest picking one, or just a few representative species, for the ITNI measurements across regions. Interspecies differences in life history, physiology, and aerial plant parts would interfere with a direct comparison across a region. However, if a single species were utilized across all habitats in question, spatial patterns in N deposition would be easier to detect.

We also suggest that ITNI modules contain the same improvements as I have noted, such as the addition of a mechanism to prevent herbivory bird visitation (fencing and applying water directly to the sand surface) and covering the modules to prevent light from entering the liquid reservoir to deter algal/microbial growth in the PLS system.

5. Tables

Table 1.1. Summary of nitrogen mass and ^{15}N measurements from Deployment 1 using *Bromus rubens.*

			LIQUID		PLANT				SAND		TOTAL	
Location	**ITNI Module**	**Treatment**	**mg N**	**AP ^{15}N**	**mg N Above**	**AP ^{15}N Above**	**mg N Below**	**AP ^{15}N Below**	**mg N (NO_3 extractable)**	**AP ^{15}N (NO_3 extractable)**	**mg N**	**AP ^{15}N**
Riverside	C	No Plant/No Nutrients	1.27	0.38					10	0.37	12	0.37
Riverside	P1	Invasive Plant	1.24	0.38	151	0.66	9	0.57	29	0.64	190	0.65
Riverside	P2	Invasive Plant	1.20	0.38	88	0.88	25	0.82	14	0.74	128	0.85
Riverside	P3	Invasive Plant	1.22	0.37	86	0.86	16	0.74	11	0.42	115	0.79
Riverside	P4	Invasive Plant	1.29	0.37	82	0.91	13	0.84	14	0.74	111	0.87
Riverside	P5	Invasive Plant	1.11	0.38	193	0.65	61	0.64	27	0.68	282	0.65
Riverside	H1	Hoagland Control	221	0.67					65	0.67	286	0.67
Riverside	H2	Hoagland Control	124	0.98					33	0.93	157	0.97
Riverside	H3	Hoagland Control	271	0.68					66	0.68	337	0.68
Riverside	H4	Hoagland Control	100	0.99					29	0.88	129	0.97
Riverside	H5	Hoagland Control	198	0.68					38	0.68	235	0.68
Riverside	N1	N-Only Control	170	0.48					51	0.64	221	0.52
Riverside	N2	N-Only Control	184	0.62					28	0.74	212	0.64
Riverside	N3	N-Only Control	170	0.50					32	0.64	202	0.52
Riverside	N4	N-Only Control	147	0.44					45	0.61	192	0.48
Riverside	N5	N-Only Control	150	0.41					50	0.56	200	0.45
Motte	C	No Plant/No Nutrients	1.68	0.37					14	0.37	15	0.37
Motte	P1	Invasive Plant	1.38	0.41	11	0.80	10	0.67	15	0.63	38	0.73
Motte	P2	Invasive Plant	1.33	0.38	8	0.77	13	0.72	10	0.57	33	0.73
Motte	P3	Invasive Plant	1.26	0.39	7	0.87	13	0.71	11	0.58	32	0.75
Motte	P4	Invasive Plant	1.29	0.37	16	0.82	13	0.68	14	0.58	43	0.75
Motte	H1	Hoagland Control	108	0.96					40	0.81	148	0.92
Motte	H2	Hoagland Control	101	0.99					44	0.89	145	0.96
Motte	H3	Hoagland Control	100	0.98					43	0.84	144	0.94
Motte	H4	Hoagland Control	52	0.99					39	0.85	91	0.93
Motte	H5	Hoagland Control	102	0.99					38	0.90	141	0.96
Motte	N1	N-Only Control	150	0.94					53	0.91	203	0.93
Motte	N2	N-Only Control	148	0.92					35	0.89	184	0.92
Motte	N3	N-Only Control	156	0.91					18	0.83	174	0.90
Motte	N4	N-Only Control	143	0.93					55	0.89	197	0.92
Motte	N5	N-Only Control	145	0.88					53	0.85	198	0.87

Table 1.2. Summary of nitrogen mass and ^{15}N measurements from Deployment 2 using *Hirschfeldia incana*.

			LIQUID		PLANT				SAND		TOTAL	
Location	**ITNI Module**	**Treatment**	**mg N**	**AP ^{15}N**	**mg N Above**	**AP ^{15}N Above**	**mg N Below**	**AP ^{15}N Below**	**mg N (NO_3 extractable)**	**AP ^{15}N (NO_3 extractable)**	**mg N**	**AP ^{15}N**
Riverside	Ef1	Native Plant	0.14	0.84	178	0.92	30.9	0.94	19.3	0.76	228	0.91
Riverside	Ef2	Native Plant	0.16	0.84	184	0.88	67.6	0.90	15.8	0.77	267	0.88
Riverside	Ef3	Native Plant	0.38	0.90	147	0.91	77.8	0.94	17.2	0.81	243	0.91
Riverside	Ef4	Native Plant	0.24	0.90	151	0.90	59.6	0.91	15.1	0.79	226	0.89
Riverside	Ef5	Native Plant	0.47	0.50	178	0.89	67.9	0.88	10.4	0.63	256	0.88
Riverside	Ef7	Native Plant	0.24	0.80	179	0.89	44.3	0.87	7.6	0.75	231	0.88
Riverside	Hi2	Invasive Plant	0.46	0.39	173	0.83	13.5	0.92	12.3	0.69	200	0.83
Riverside	Hi3	Invasive Plant	0.48	0.72	168	0.85	12.9	0.93	7.1	0.68	188	0.85
Riverside^	Hi4	Invasive Plant	0.39	0.40	446	0.91	18.6	0.92	14.6	0.65	479	0.91
Riverside^	Hi5	Invasive Plant	0.47	0.38	432	0.91	24.7	0.91	6.4	0.56	464	0.90
Riverside	Hi6	Invasive Plant	0.43	0.61	213	0.90	12.9	0.93	10.4	0.66	237	0.89
Riverside	Hi7	Invasive Plant	0.30	0.82	301	0.91	14.7	0.94	9.2	0.67	325	0.90
Riverside	C	Hoagland Control	84.8	0.99	----------				9.9	0.75	95	0.97
Motte	Ef4	Native Plant	0.43	0.75	132	0.96	24.1	0.94	23.9	0.98	180	0.96
Motte	Ef5	Native Plant	0.27	0.62	148	0.87	33.1	0.84	30.9	0.96	212	0.88
Motte	Ef6	Native Plant	0.45	0.41	164	0.91	58.1	0.93	15.8	0.96	238	0.92
Motte	Ef7	Native Plant	0.24	0.93	105	0.98	13.6	0.96	35.8	1.01	155	0.98
Motte	Hi1	Invasive Plant	0.46	0.43	241	0.89	2.3	0.93	25.3	0.87	269	0.89
Motte^	Hi3	Invasive Plant	0.36	0.37	412	0.93	25.5	0.93	22.3	0.91	460	0.93
Motte	Hi4	Invasive Plant	0.05	0.82	308	0.95	3.4	0.93	22.4	0.87	334	0.95
Motte^	Hi5	Invasive Plant	0.41	0.72	447	0.94	6.8	0.93	21.6	0.87	476	0.94
Motte	Hi6	Invasive Plant	0.37	0.62	323	0.97	13.4	0.90	22.0	0.83	358	0.96
Motte^	Hi7	Invasive Plant	0.38	0.52	609	0.95	10.1	0.92	19.7	0.87	639	0.95
Motte	C	Hoagland Control	96.10	0.99	----------				115.4	1.04	211	1.02

^ *Data from these modules were not used in deposition computations due to likely errors in plant mass measurements (see text)*

Table 1.3. Summary of nitrogen mass and ^{15}N measurements from Deployment 3 using *Hirschfeldia incana*.

			LIQUID		PLANT				SAND		TOTAL	
Location	**ITNI Module**	**Treatment**	**mg N**	**AP ^{15}N**	**mg N Above**	**AP ^{15}N Above**	**mg N Below**	**AP ^{15}N Below**	**mg N (NO_3 extractable)**	**AP ^{15}N (NO_3 extractable)**	**mg N**	**AP ^{15}N**
Riverside	Ef1	Native Plant	1.84	0.50	159.2	0.94	37.7	0.92	4.19	0.54	203	0.56
Riverside	Hi3	Invasive Plant	1.66	0.52	75.9	0.91	43.4	0.94	13.46	0.76	134	0.53
Riverside	Hi4	Invasive Plant	2.90	0.66	27.3	0.85	52.6	0.93	26.1	0.83	109	0.51
Riverside	Hi5	Invasive Plant	1.71	0.49	46.8	0.88	54.4	0.94	0.92	0.48	104	0.54
Riverside	Hi6	Invasive Plant	1.95	0.42	69.8	0.88	43.1	0.94	1.33	0.38	116	0.52
Riverside	Hi7	Invasive Plant	2.48	0.44	33.6	0.87	52.5	0.94	0.91	0.47	89	0.53
Riverside	Hi8	Invasive Plant	3.18	0.44	61.8	0.91	62.2	0.96	0.56	0.40	128	0.56
Riverside	Hi9	Invasive Plant	3.71	0.86	40.2	0.92	9.5	0.94	28.0	0.83	82	0.52
Riverside	Hi10	Invasive Plant	3.38	0.39	127.9	0.93	38.5	0.93	1.39	0.54	171	0.55
Riverside	C11	Hoagland Control	125	0.98	----------	----------	----------	----------	1.16	0.58	126	0.61
Riverside	C12	Hoagland Control	126	0.98	----------	----------	----------	----------	0.67	0.66	127	0.62
Riverside	C13	Hoagland Control	115	0.97	----------	----------	----------	----------	4.15	0.68	119	0.59
Riverside	C14	Hoagland Control	99.8	0.97	0.16	----------	----------	----------	0.05	0.71	100	0.60
Riverside	C15	Hoagland Control	118	0.97	----------	----------	----------	----------	2.12	0.62	120	0.59
Riverside	C16	Hoagland Control	103	0.96	0.08	----------	----------	----------	0.34	0.51	103	0.59

Table 1.4. Summary of total dissolved nitrogen analyses for all Deployments.

Deployment	Site	Treatment	Sample ID	uM	mg-TN	mg-N-NO_3	mg DON	Total Module N	% DON + PN in Liquid
1	Riverside	Control	Riv C	11	1.4	1.3	0.2	12	1.4%
1	Riverside	Invasive	RP1	36	4.5	1.2	3.3	190	1.7%
1	Riverside	Invasive	RP2	35	4.4	1.2	3.2	128	2.4%
1	Riverside	Invasive	RP3	17	2.2	1.2	1.0	115	0.8%
1	Riverside	Invasive	RP4	17	2.1	1.3	0.8	111	0.7%
1	Riverside	Invasive	RP5	26	3.3	1.1	2.2	282	0.8%
1	Riverside	N-Only Ctrl	RN1	2372	299	170	129	221	37%
1	Riverside	N-Only Ctrl	RN2	2651	334	184	150	212	42%
1	Riverside	N-Only Ctrl	RN3	2654	334	170	164	202	45%
1	Riverside	N-Only Ctrl	RN4	2620	330	147	183	192	49%
1	Riverside	N-Only Ctrl	RN5	2028	256	150	106	200	35%
1	Riverside	Hoagland Ctrl	RH1	2553	322	221	101	286	26%
1	Riverside	Hoagland Ctrl	RH2	1276	161	124	37	157	19%
1	Riverside	Hoagland Ctrl	RH3	2680	338	271	67	337	17%
1	Riverside	Hoagland Ctrl	RH4	992	125	100	25	129	16%
1	Riverside	Hoagland Ctrl	RH5	2408	303	198	106	235	31%
1	Motte	Control	Mot C	28	3.5	1.7	1.8	15	11%
1	Motte	N-Only Ctrl	MN1	1488	187	150	38	203	16%
1	Motte	N-Only Ctrl	MN2	1360	171	148	23	184	11%
1	Motte	N-Only Ctrl	MN3	1517	191	156	35	174	17%
1	Motte	N-Only Ctrl	MN4	1257	158	143	15	197	7.3%
1	Motte	N-Only Ctrl	MN5	1545	195	145	50	198	20%
1	Motte	Hoagland Ctrl	MH1	975	123	108	15	148	9.2%
1	Motte	Hoagland Ctrl	MH2	1035	130	101	30	145	17%
1	Motte	Hoagland Ctrl	MH3	1085	137	100	36	144	20%
1	Motte	Hoagland Ctrl	MH4	603	76	52	24	91	21%
1	Motte	Hoagland Ctrl	MH5	1099	138	102	36	141	20%
2	Riverside	Control	RC	1068	135	85	50	95	34%
2	Riverside	Native Plant	R1EF	17	2.1	0.1	2.0	228	0.9%
2	Riverside	Native Plant	R2EF	19	2.4	0.2	2.3	267	0.8%
2	Riverside	Native Plant	R3EF	15	1.9	0.4	1.5	243	0.6%
2	Riverside	Native Plant	R4EF	13	1.6	0.2	1.4	226	0.6%
2	Riverside	Native Plant	R5EF	8.9	1.1	0.5	0.6	256	0.2%
2	Riverside	Native Plant	R7EF	21	2.7	0.2	2.4	231	1.0%
2	Riverside	Invasive Plant	R2HI	8.8	1.1	0.5	0.7	200	0.3%
2	Riverside	Invasive Plant	R3HI	13	1.7	0.5	1.2	188	0.6%
2	Riverside	Invasive Plant	R4HI	10	1.3	0.4	0.9	479	0.2%
2	Riverside	Invasive Plant	R5HI	4.1	0.5	0.5	0.1	464	0.0%
2	Riverside	Invasive Plant	R6HI	8.6	1.1	0.4	0.7	237	0.3%
2	Riverside	Invasive Plant	R7HI	28	3.5	0.3	3.2	325	1.0%
2	Motte	Control	MC	701	88	96	-7.8	95	-9.0%
2	Motte	Native Plant	M4EF	8.9	1.1	0.4	0.7	180	0.4%
2	Motte	Native Plant	M5EF	7.1	0.9	0.3	0.6	212	0.3%
2	Motte	Native Plant	M6EF	1.4	0.2	0.5	-0.3	238	-0.1%
2	Motte	Native Plant	M7EF	1.7	0.2	0.2	0.0	155	0.0%
2	Motte	Invasive Plant	M1HI	3.6	0.5	0.5	0.0	269	0.0%
2	Motte	Invasive Plant	M3HI	2.6	0.3	0.4	0.0	460	0.0%
2	Motte	Invasive Plant	M4HI	7.9	1.0	0.0	0.9	334	0.3%
2	Motte	Invasive Plant	M5HI	7.9	1.0	0.4	0.6	476	0.1%
2	Motte	Invasive Plant	M6HI	0.5	0.1	0.4	-0.3	358	-0.1%
2	Motte	Invasive Plant	M7HI	3.6	0.5	0.4	0.1	639	0.0%
3	Riverside	Native Plant	R1Ef	23	2.9	1.8	1.1	203	0.5%
3	Riverside	Invasive Plant	R3Hi	17	2.1	1.7	0.4	134	0.3%
3	Riverside	Invasive Plant	R4Hi	31	3.9	2.9	1.0	109	0.9%
3	Riverside	Invasive Plant	R5Hi	18	2.3	1.7	0.6	104	0.5%
3	Riverside	Invasive Plant	R6Hi	19	2.5	2.0	0.5	116	0.4%
3	Riverside	Invasive Plant	R7Hi	23	2.9	2.5	0.4	89	0.5%
3	Riverside	Invasive Plant	R8Hi	32	4.1	3.2	0.9	128	0.7%
3	Riverside	Invasive Plant	R9Hi	34	4.3	3.7	0.6	82	0.8%
3	Riverside	Invasive Plant	R10Hi	39	4.9	3.4	1.6	171	0.9%
3	Riverside	Hoagland Ctrl	R11C	1214	153	125	28	126	18%
3	Riverside	Hoagland Ctrl	R12C	1323	167	126	40	127	24%
3	Riverside	Hoagland Ctrl	R13C	1249	157	115	42	119	26%
3	Riverside	Hoagland Ctrl	R14C	1026	129	100	30	100	23%
3	Riverside	Hoagland Ctrl	R15C	1160	146	118	28	120	19%
3	Riverside	Hoagland Ctrl	R16C	1023	129	103	26	103	20%

Table 1.5. Summary of N deposition rates computed for plant-containing ITNI modules during Deployment 1. Deposition was computed three ways: i) using N mass and the weighted average AP ^{15}N for all module components ("Module Average"), ii) using the N mass and the AP ^{15}N for the plant only ("Plant") and iii) using the N mass and the AP ^{15}N of the aboveground plant biomass only ("Above Ground"). Mean values for each treatment ± standard errors are shown and n is the number of replicate modules.

	Table 1.4 Deployment 1 Nitrogen Deposition				
		Module Average	Plant	Above Ground	
	Riverside	5.9 ± 0.64	4.5 ± 1.09	3.2 ± 0.72	
		(n= 3)	(n= 3)	(n= 3)	
	Motte*	3.5 ± 0.2	1.8 ± 0.1	0.5 ± 0.1	
		(n= 4)	(n= 4)	(n= 4)	
Deposition rates are reported as kg N/ha/Deployment 1. Deployment 1 was exposed from March 8 – May 20, 2013 (74 days).*Motte modules had evidence of rabbit grazing, so deposition measurements are likely compromised.					

Table 1.6. Summary of N deposition rates computed for plant-containing ITNI modules during Deployment 2. Deposition was computed three ways: i) using N mass and the weighted average AP ^{15}N for all module components ("Module Average"), ii) using the N mass and the AP ^{15}N for the plant only ("Plant") and iii) using the N mass and the AP ^{15}N of the aboveground plant biomass only ("Above Ground"). Mean values for each treatment ± standard errors are shown and n is the number of replicate modules.

Riverside		Module Average	Plant	Above Ground
	Native	8.6 ± 0.63	7.5 ± 0.65	5.7 ± 0.43
		(n= 6)	(n= 6)	(n= 6)
	Invasive	9.8 ± 0.55	8.8 ± 0.52	8.5 ± 0.51
		(n= 4)	(n= 4)	(n= 4)
Motte				
	Native	4.9 ± 1.59	4.6 ± 0.52	3.6 ± 0.52
		(n= 4)	(n= 4)	(n= 4)
	Invasive	7.5 ± 1.07	6.4 ± 0.60	6.2 ± 1.37
		(n= 3)	(n= 3)	(n= 3)
All data reported as kg N/ha/Deployment 2. Deployment 2 was exposed from May 28 – August 12, 2013 (77 days).				

Table 1.7. Summary of N deposition rates computed for plant-containing ITNI modules and Hoagland Control modules during Deployment 3. Deposition was computed three ways: i) using N mass and the weighted average AP ^{15}N for all module components ("Module Average"), ii) using the N mass and the AP ^{15}N for the plant only ("Plant") and iii) using the N mass and the AP ^{15}N of the aboveground plant biomass only ("Above Ground"). Mean values for each treatment ± standard errors are shown and n is the number of replicate modules.

Riverside		Module Average	Plant	Above Ground
	Native	5.2	4.4	3.4
		(n= 1)	(n= 1)	(n= 1)
	Invasive	3.8 ± 0.24	2.9 ± 0.29	1.9 ± 0.24
		(n= 8)	(n= 8)	(n= 8)
	Hoagland Control	1.5 ± 0.12	----------	----------
		(n= 6)		

All data reported in kg N/ha/Deployment 2. Deployment 3 was exposed from November 15, 2013 – March 24, 2014 (130 days). Control treatments contained no plants, but all other ITNI components including nutrient solution and nitrogen spike.

6. Figures

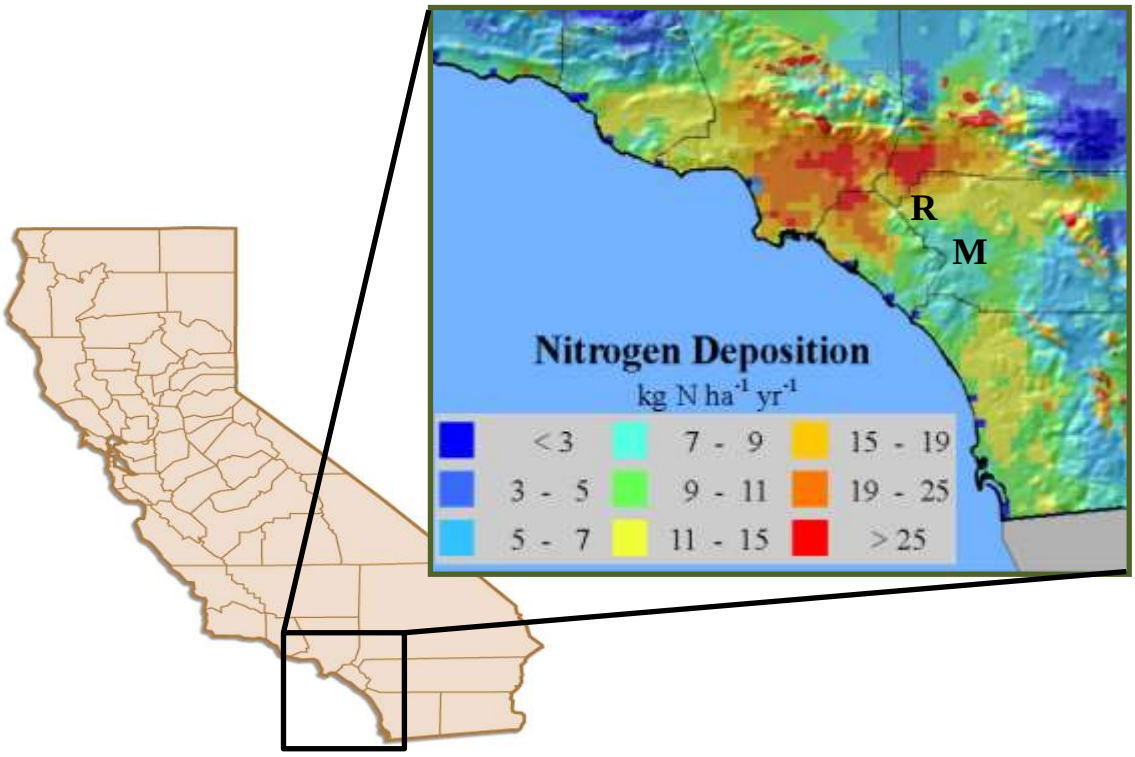


Figure 1.1. Nitrogen Deposition gradient in Southern California Los Angeles Air Basin. Riverside denoted as "R" and Motte denoted as "M". Map adapted from Fenn et al. 2010.

Figure 1.2. ITNI Assembly Diagram and Picture.

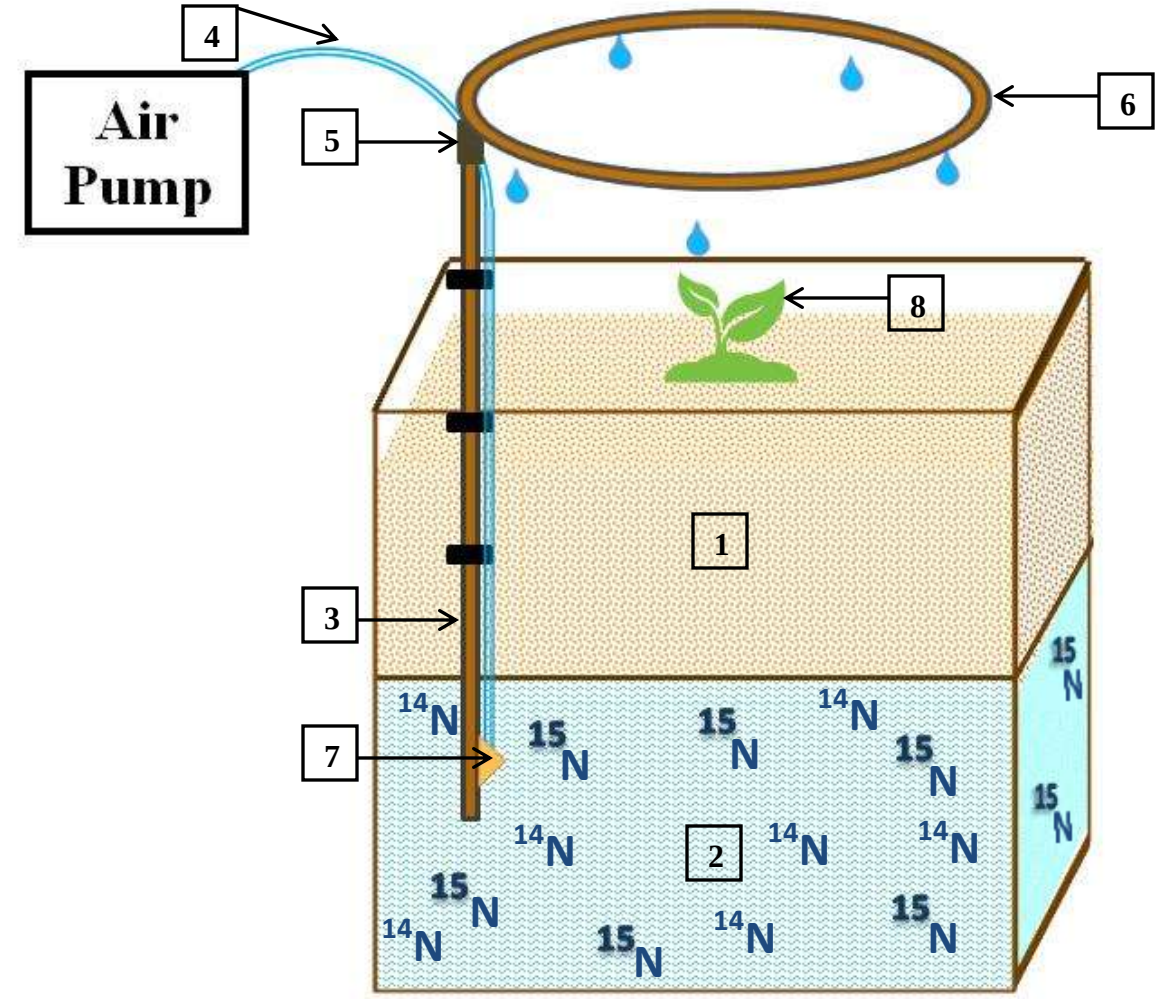


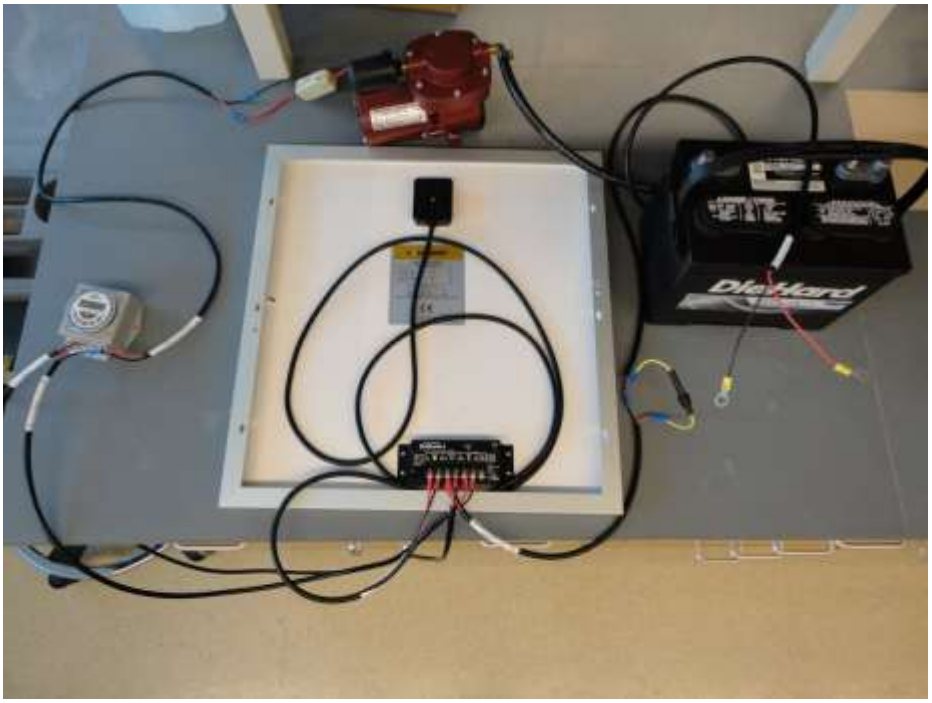
Diagram: 1. Sand reservoir, 2. Liquid reservoir, 3. Water lift, 4. Ai lift, 5. T-junction, 6. Watering ring, 7. Air lift junction, 8. Plant species. Picture shows an ITNI module in use.

Figure 1.3. ITNI field deployment at Motte.

ITNI modules at Motte Rimrock Reserve in Perris, California during Deployment 2. Modules were arranged in a square formation and randomized every couple weeks. The green box in the middle of the modules housed the pump, manifolds, battery, and was topped off by a solar panel. The cinder blocks and posts held up rabbit fencing to deter herbivory during the exposure period.

Figure 1.4. ITNI pump system setup.

Riverside Pump System: battery, pump, timer, manifolds, air lift tubing



Motte Pump System: Battery, solar panel, pump, timer (not pictured: manifolds and air lift tubing)

Figure 1.5. Recovery of nitrogen in Deployment 1.

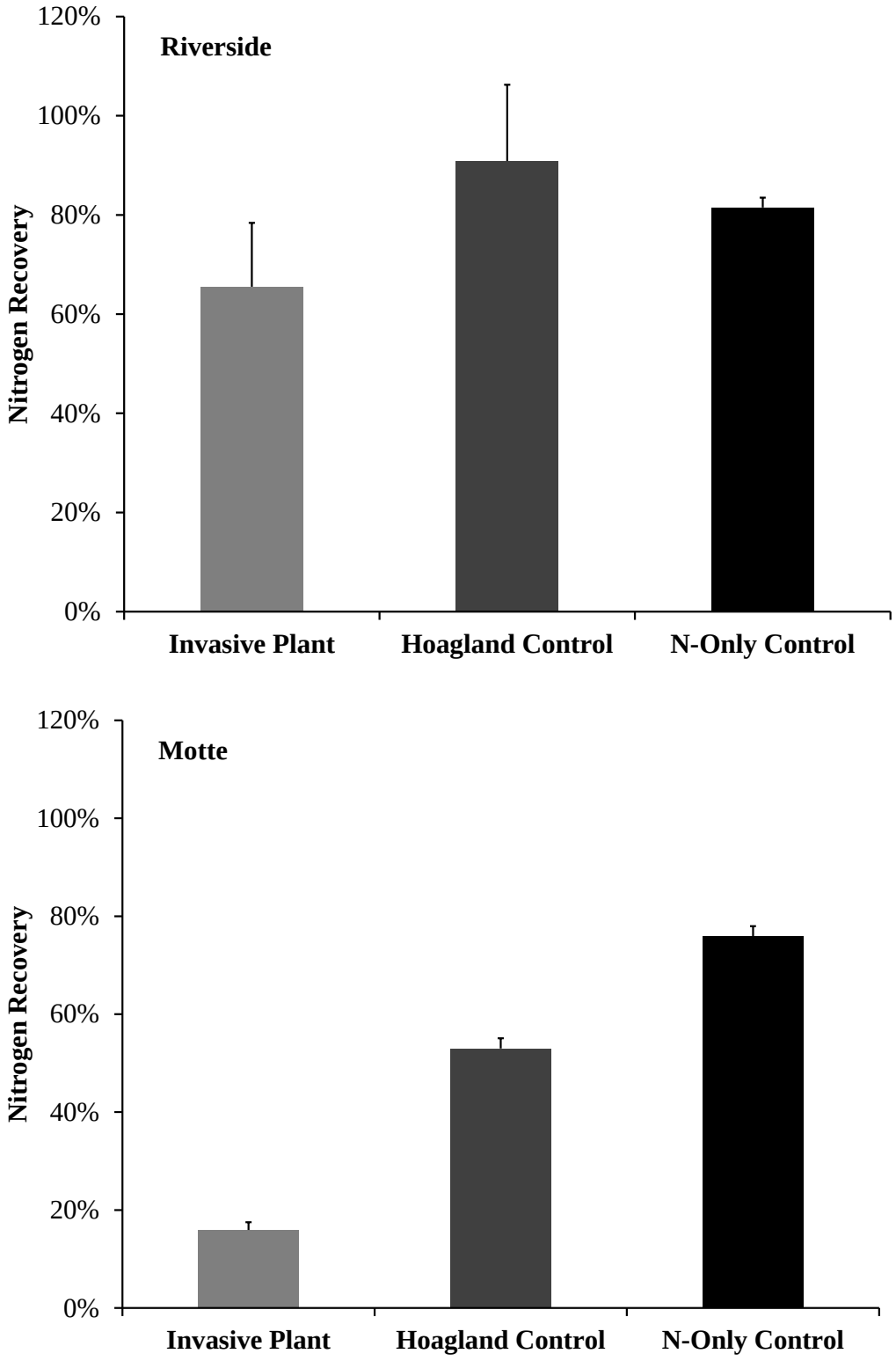


Figure 1.6. Average nitrate concentrations in the liquid reservoirs for the three treatment types during Deployment 1. Error bars denote standard errors.

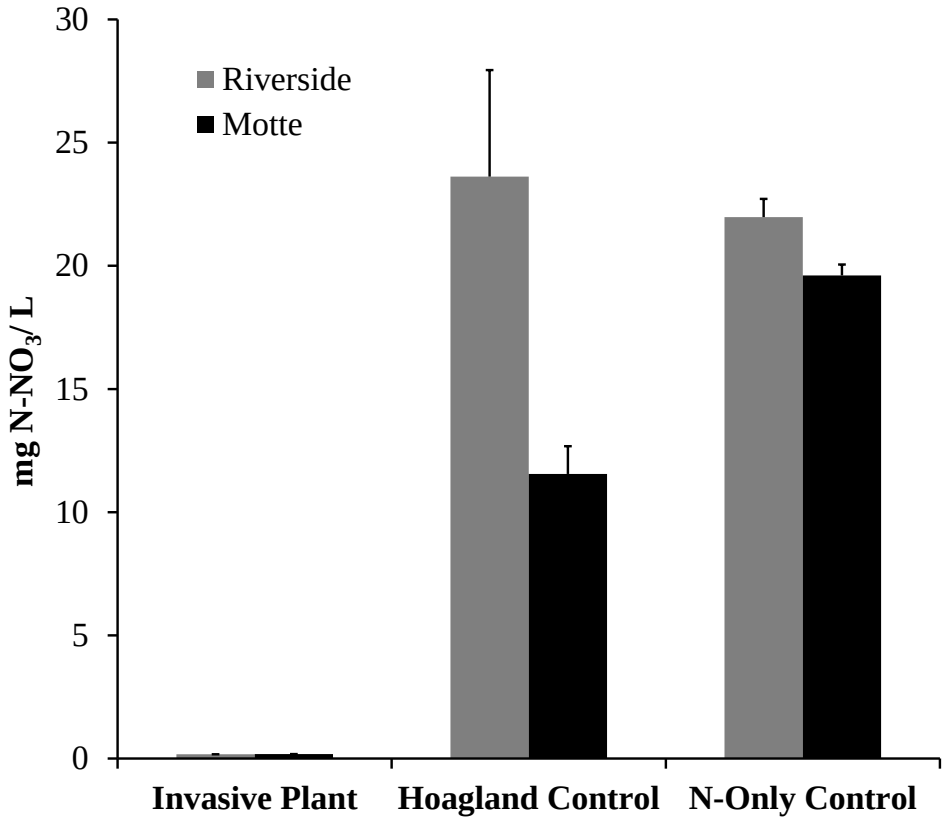


Figure 1.7. Average ^{15}N concentrations in the liquid reservoirs for the three treatment types during Deployment 1. Error bars denote standard errors.

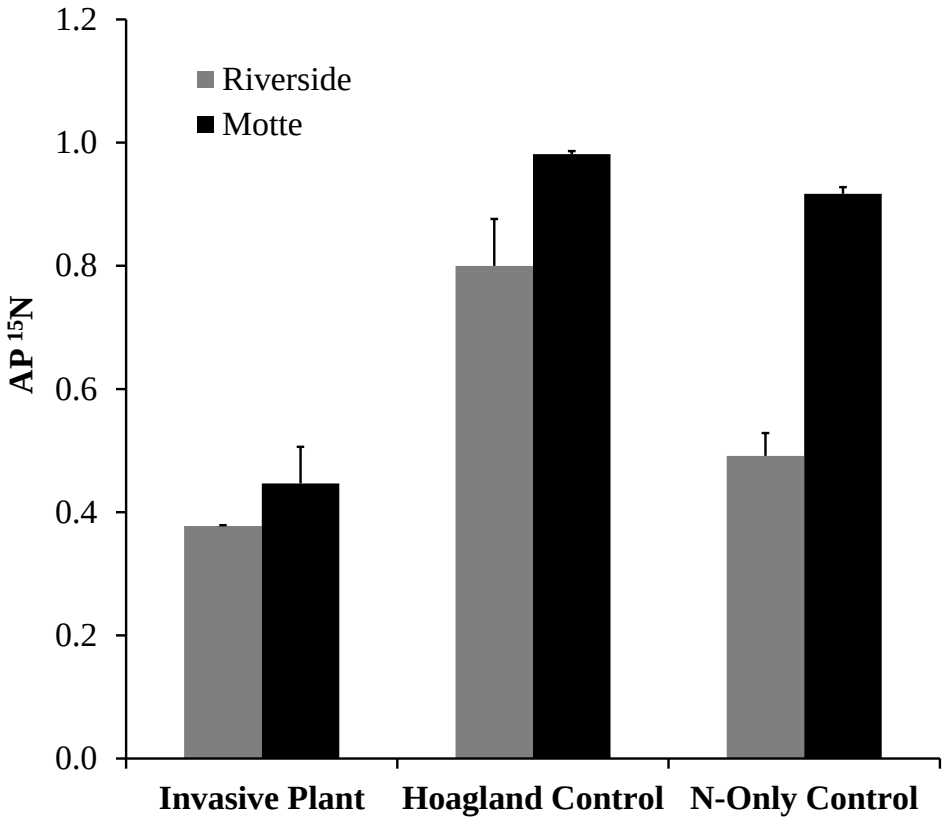


Figure 1.8. Recovery of nitrogen in Deployment 1.

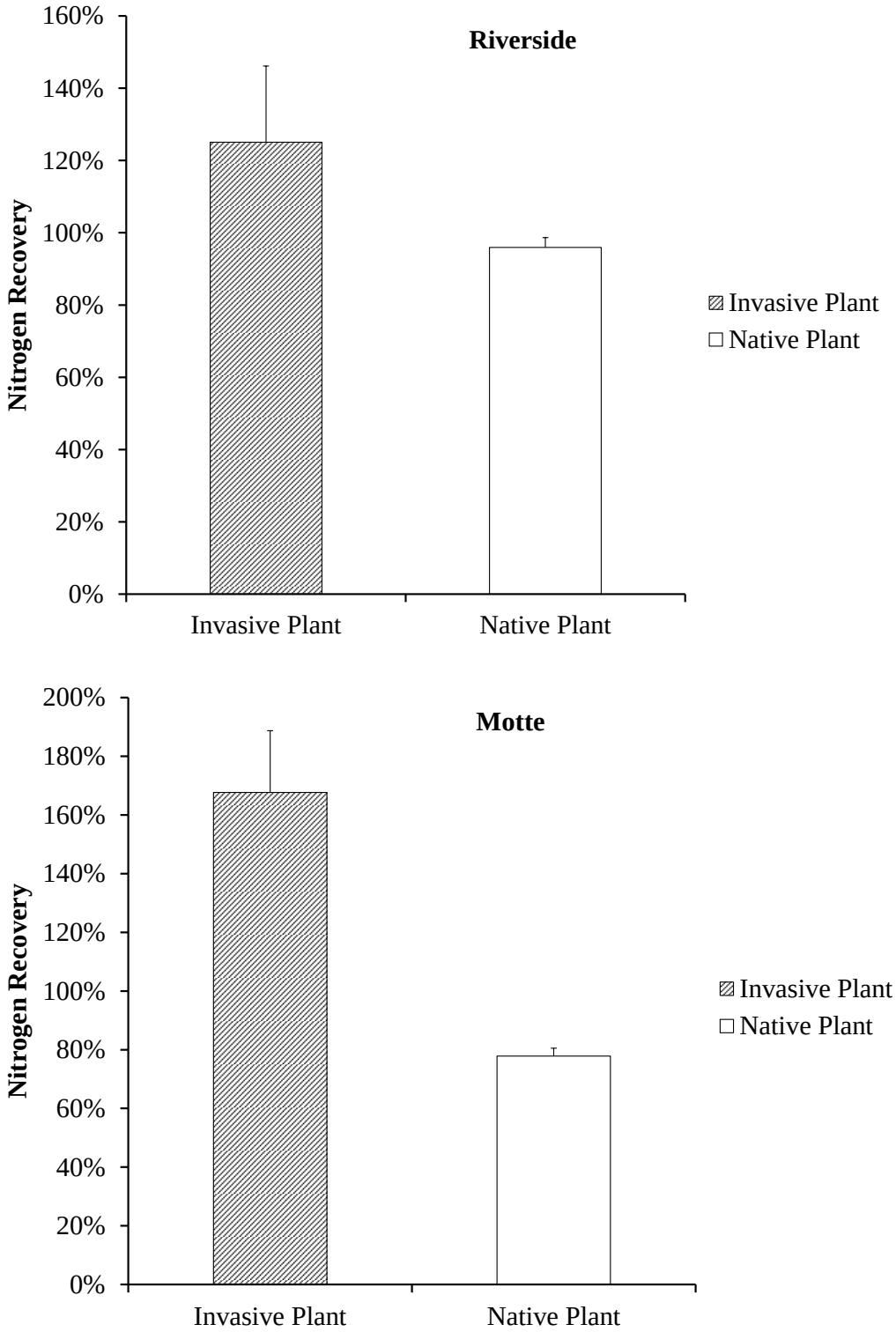


Figure 1.9. Comparison of nitrogen mass in above vs. belowground biomass at the end of Deployment 2.

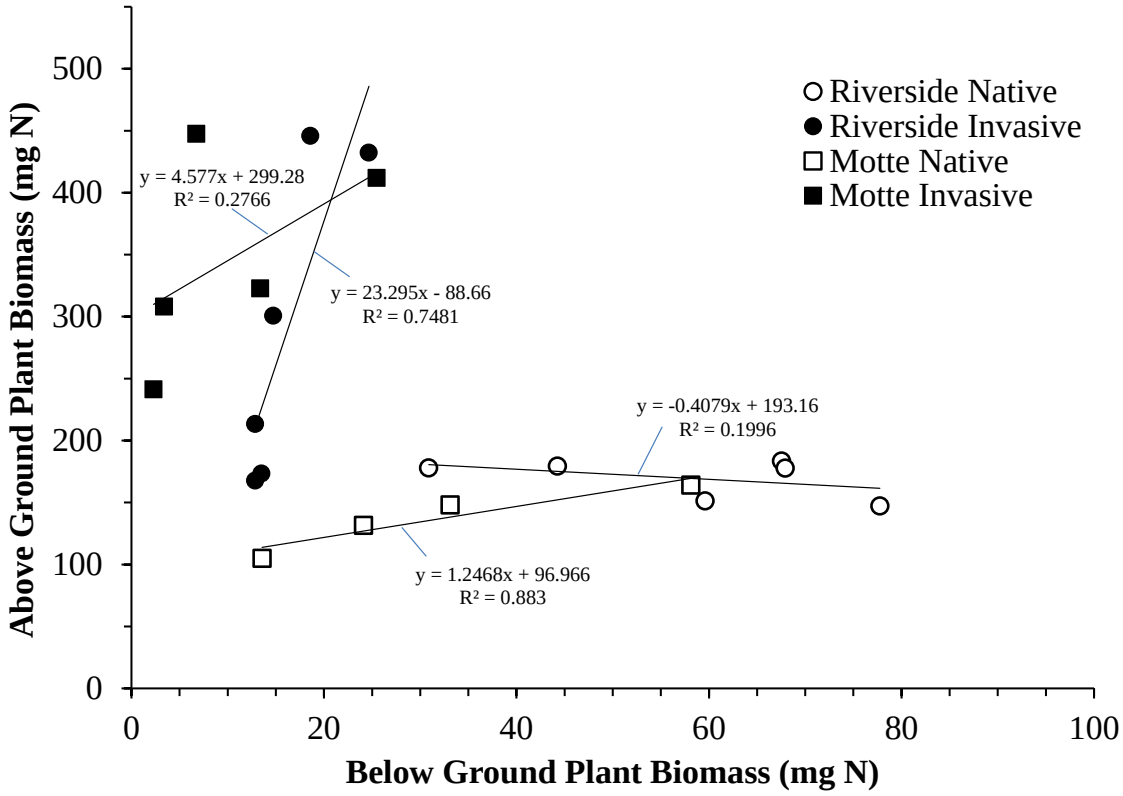


Figure 1.10. Comparison of AP ^{15}N in above vs. belowground plant biomass at the end of Deployment 2.

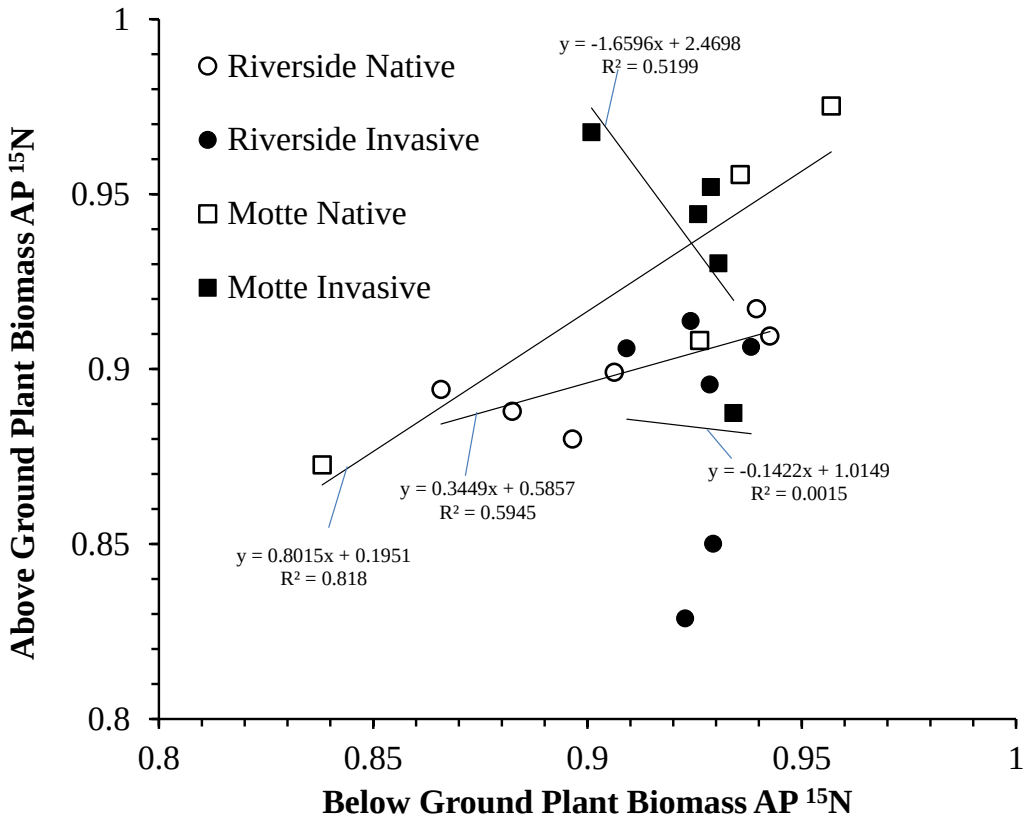


Figure 1.11. Recovery of nitrogen during Deployment 3.

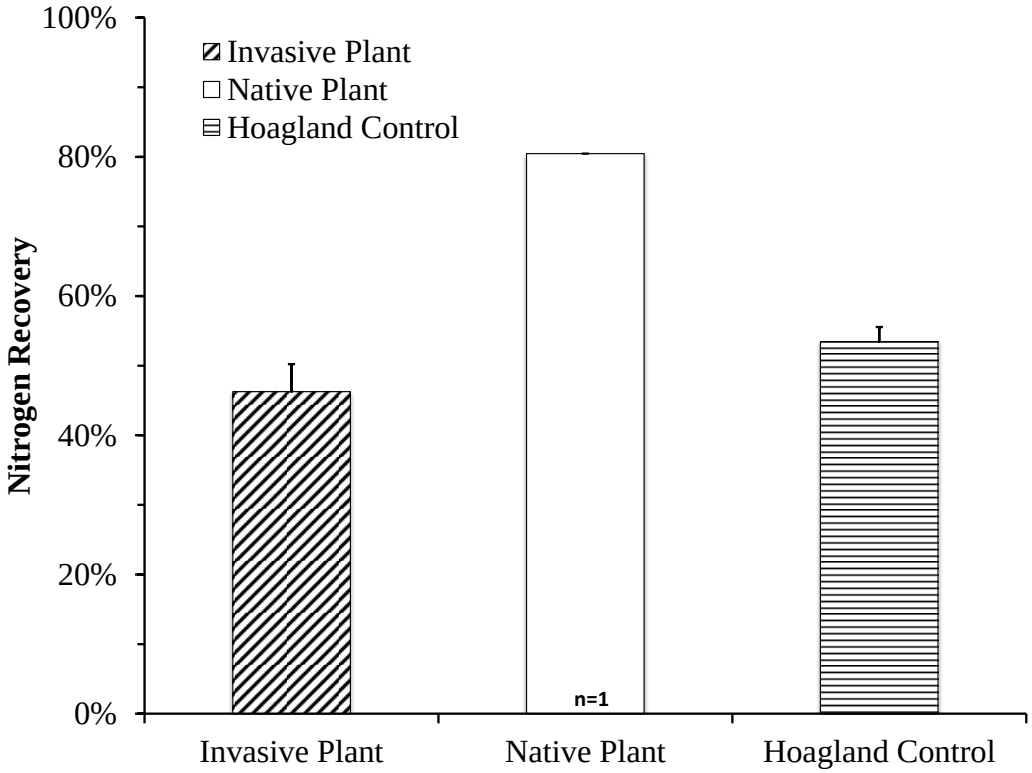


Figure 1.12. Comparison of nitrogen content in above vs. belowground biomass at the end of Deployment 3.

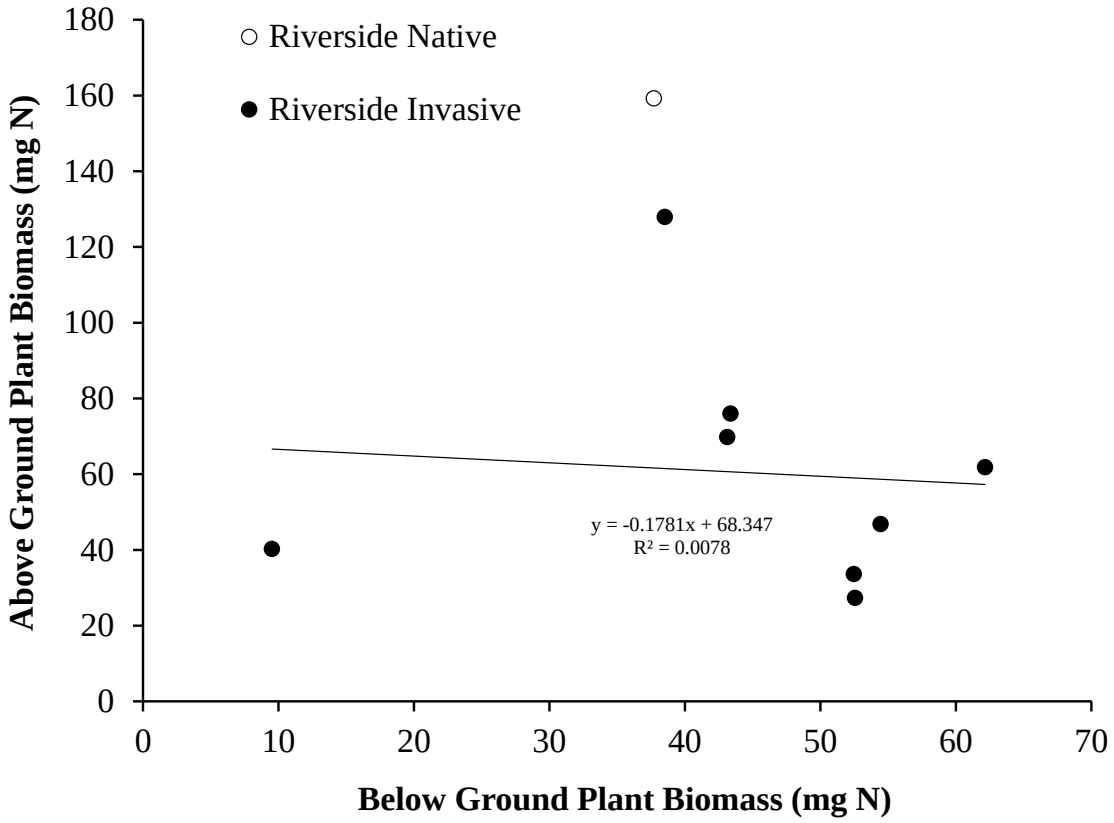
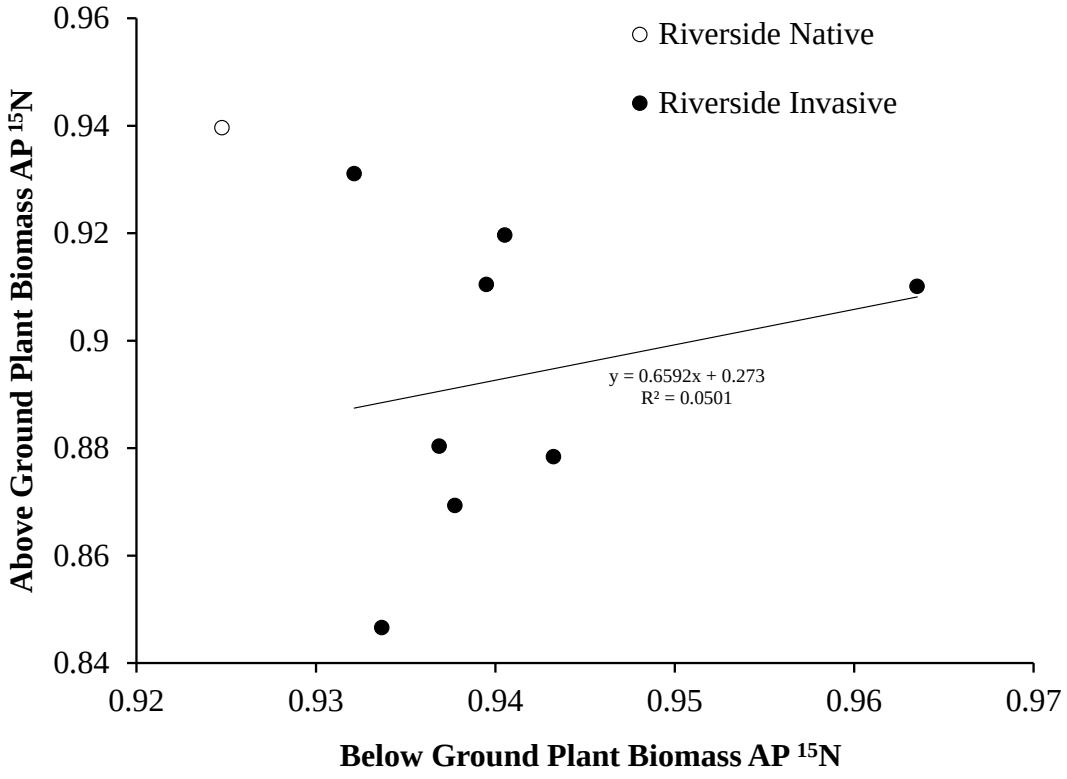


Figure 1.13. Deployment 3 above- and belowground Atom Percent ^{15}N.



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Chapter II

1. Introduction

1.1 Effects of Nitrogen Deposition

Nitrogen deposition, resulting from industrial, transportation, and urban sectors, can be harmful to sensitive ecosystems due to artificial fertilization (Allen et al 1998, Fenn et al. 2010). The burning of fossil fuels releases airborne nitrogen species which result in atmospheric concentrations of nitrogen that are highest in urbanized areas and elevated in the resulting surrounding regions (Fenn et al. 2010). Nitrogen deposition, in the form of inorganic compounds such as NO_2, NH_3, NO_x, and HNO_3, enters ecosystems through atmospheric deposition to plant and soil surfaces. Nitrogen fertilization of polluted ecosystems can occur through stomatal uptake of aerial deposited nitrogen on plant surfaces, incorporation of nitrogen deposition into soils through precipitation events, and lastly runoff of these surfaces into aquatic ecosystems. Nitrogen causes nitrogen saturation in ecosystems when loading rates cross certain thresholds (Bytnerowicz and Fenn, 1996). Threshold responses are those in which a critical load has been reached for a particular pollutant, or the maximum amount of the pollutant that can be added before an ecological response is clear. Examples include algal blooms or acidification in aquatic ecosystems, species type conversions in terrestrial plant communities, including invasion of introduced or exotic species (Jaworski et al., 1997, Allen et al 1998, Paerl et al., 2002, Cox ex al. 2014).

1.2 Nitrogen Deposition Measurement

Nitrogen can be deposited from the atmosphere in liquid, solid and gaseous deposition making it challenging to accurately quantify all N inputs to natural ecosystems (Lovett 1994). Wet deposition is relatively easy to quantify, however, in arid or semi-arid regions, there is a lack of consistent rain for wet deposition measurements. Gaseous and dry deposition often dominates N deposition in dry regions (Fenn et al., 2010). Current methods of measuring dryfall include the use of passively adsorbing filters to determine atmospheric concentrations of nitrogen species and the use of the atmospheric concentration data with atmospheric models that predict the rate at which various N species deposit to surfaces (Zhang et al., 2003). This approach is often referred to as the inferential method. Other methods attempt to collect both wet and dry deposition by placing rain collectors below plant canopies to collect throughfall (direct precipitation + dry particles on leaf surfaces) or by the use of ion-exchange resins (IER) which will adsorb inorganic N ions in both wet and dry deposition (Fenn and Poth 2004).

However, these methods fail to account for gaseous uptake of nitrogen by plant stomata, and may underestimate the true amount of N deposition occurring in terrestrial ecosystems (Fenn et al., 1998). Because of the multiple forms of N present in the atmosphere, it is currently necessary to employ several separate techniques, including rain collectors, IER collector and the inferential method, in an attempt to account for all forms of N deposition in arid regions. This poses a problem in that a total analysis of all nitrogen species is often not practical due to the equipment requirements and the high cost associated with operating the equipment and analyzing the samples. Additionally,

while each method attempts to quantify a different pool of nitrogen deposition, their sum may miss important components of atmospheric deposition and some of the methods have relatively large sources of error. For example, wet deposition collects only nitrogen that was present in the atmosphere prior to the rain event. The inferential method, utilizing filter pads as collection interfaces, only measures atmospheric concentrations of specific nitrogen species over an exposure period. To calculate deposition, concentrations must be combined with deposition velocities, which vary depending on wind speed, relative humidity, and surface characteristics (Zhang 2002). Ion exchange resins only collect ionic nitrogen species that fall in wet deposition or that settle as dry deposition on the inlet funnel to the collector; this dry deposition must be washed into the resin by natural rainfall if it is to be adsorbed by the resin which further complicates their use and interpretation in arid environments.

The National Atmospheric Deposition Program (NADP), which measured precipitation and deposition chemistry across the United States with a network of measurement sites, utilized wet deposition measurements to create total nitrogen deposition estimates based on precipitation-weighted mean concentrations computed on a weekly time-step. These gauges, measuring only bulk (wet) deposition, in an arid region such as Southern California, chronically underestimate true nitrogen deposition occurring mostly as dry deposition. For example, based on National Atmospheric Deposition Program/NTN data for the Tanbark Flat site (located 47 km northwest of Riverside), total wet deposition of inorganic N for 2011, 2012 and 2013 was 1.46, 1.76 and 1.29 kg N ha yr^{-1}, respectively (http://nadp.sws.uiuc.edu/data/ntn/; accessed December 18, 2014). In

contrast, estimates released by NADP that include modeled dry deposition for the Inland Empire in 2012 varied between 10-12 kg N ha^{-1} yr^{-1} in rural areas and upwards of 18->20 kg N ha^{-1} yr^{-1} in urban areas (such as Riverside) (NADP 2012). The NADP network limits estimates to >20 kg N ha^{-1} yr^{-1}, and did not supply additional resolution in calculations exceeding this amount. Riverside, in previous years, was calculated to have between 16-18 kg N ha^{-1} yr^{-1} from 2008-2011, with 2012 exceeding 20 kg N ha^{-1} yr^{-1}. However, years prior to that, 2000-2006, recorded rates of 10-12 kg N ha^{-1} yr^{-1} for the Riverside area (noting however that these estimations were determined at a more coarse resolution than those conducted after 2006.)

Therefore, to properly address N critical loads in sensitive ecosystems, a better method of measuring nitrogen deposition must be developed for arid and semi-arid regions where wet deposition is a very small component of N loading. This new method should require less specialized field instrumentation and be easily implemented on a larger scale, preferably at a lower cost, for deployment and analysis.

1.3 Integrated Total Nitrogen Input Method

The Integrated Total Nitrogen Input method may provide a simpler, less expensive and more accurate estimate of nitrogen deposition in semi-arid and arid regions where dryfall is the dominant pathway for nitrogen deposition (Bytnerowicz and Fenn 1996). ITNI utilizes a Plant-Liquid-Substrate (PLS) system consisting of a plant, liquid reservoir, and sand reservoir, similar to that of a hydroponic environment (Bohme et al. 2003, He et al., 2010, Melhert et al., 1995 and Russow et al., 2005). The PLS is a self-

contained unit that is isotopically enriched with ^{15}N, but is open to the ambient atmosphere, allowing for the collection of all forms of atmospheric N (wet, dry and gaseous deposition) on the surfaces of the sand and plant (Weigel et al, 2000). The rate of N deposition in the PLS can be determined by the degree to which the ^{15}N tracer is diluted during exposure to the ambient atmosphere which contains N with baseline levels of ^{15}N; this approach is referred to as isotope dilution. The rate of nitrogen deposition can be determined once the module is harvested for all PLS components and analyzed for the nitrogen content and isotopic abundance.

ITNI may provide a better approach to the measurement of nitrogen deposition because: i) it does not require specialized equipment like rain collectors, IER collectors or passive samplers, ii) it integrates all forms of N deposition (wet, dry and gaseous), and iii) the rate of N deposition is inferred from measurements of ^{15}N and N mass using commercially available and inexpensive measurements made by isotope ratio mass spectrometers. Additionally, ITNI modules can be set up, maintained and sampled by scientists or citizen scientists with little training and for a cost that is less than that to deploy and analyze traditional methods such as passive inferential collectors. Lastly, by using plants as a collection interface, estimates of nitrogen deposition to ecosystems can be more accurately measured than by passive or non-natural surface collections.

1.4 Objectives

The main objective for Chapter II is to compare N deposition rates by the ITNI method to traditional nitrogen deposition methods including a rainfall collector, the inferential method, and ion exchange resin collectors. Simultaneous measurements made

with multiple methods will allow better understanding of the accuracy of the ITNI method in southern California and help us validate the method for use in other arid and semi-arid regions where wet deposition in a minor component of atmospheric N loading.

2. Methods

2.1 Study sites

All atmospheric deposition measurements were conducted in the vicinity of the United States Department of Agriculture Forest Service, Pacific Southwest Research Station (PSRS) in Riverside, California. The study site is representative of high N-deposition sites in Southern California and has average annual N deposition of 14 kg N ha^{-1} yr^{-1} (Pardo et al., 2011). We selected this site due to the availability of traditional atmospheric deposition measurements and a nearby climate station operated by the California Irrigation Management Information System (CIMIS station no. 44). The ITNI modules and a rain collectors were installed side-by-side within a work yard at the PSRS which was open to the atmosphere and free from interfering buildings or vegetation. For the inferential method, sampling posts were installed 50 meters northwest of the INTI modules, where these measurements have been traditionally made by the US Forest Service. The ion exchange resin collectors needed to be exposed to the open sky and below plant canopies (throughfall), so a larger area with mature vegetation was needed. We utilized a place denoted as "Coyote Hill", 1 kilometer northeast of the PSRS, as the ion exchange resin sampling location. This location has mature stands of coastal sage scrub (CSS) vegetation and was easily accessible from the UCR campus.

2.2 Rainfall Collection

Rain was collected via an ADS 00-120 wet deposition sampler manufactured by N-Con Systems and is the same model used by the US National Atmospheric Deposition Network (NADP; Figure 2.1). The sample is comprised of a stand, HDPE bucket, moving-arm bucket lid, splash shield, and rain sensor, and automatically collected rain during storm events and closed the sampling bucket during periods of no precipitation. The buckets were scrubbed with a nylon brush and 18 MΩ deionized water before installation into the rain collector apparatus. Once installed, the rotating arm and cover kept the bucket sealed until rain was sensed via the rain sensor. When a storm arrived, the arm would slowly open, exposing the sampling bucket for rain collection. When rain ceased, the cover was moved back into a closed position to prevent contamination of the rain sample and discourage evaporation.

Rainfall was collected on an event basis and most samples were picked up within 24 hours of the end of precipitation. When the bucket was picked up, we measured the volume of water in the sample and recorded the amount of rain in a plastic gauge installed near the rain collector. The volume of rain was converted to depth of precipitation and compared to the rain gauge reading to assess the operation of the rain collector. Rain samples were collected using a syringe and a filter cartridge containing a 0.4µm polycarbonate filter (Whatman). Samples were filtered into HDPE sample bottles, and immediately frozen for future analysis. The date of the storm, date of filtration, and details of the storm, such as wind conditions and a rain gauge reading were also recorded.

Samples were later thawed and analyzed for dissolved inorganic N on a Seal Analytical, Inc. AQ2 discrete analyzer for nitrate plus nitrite, EPA 353.2 Ver 2 (1993), and ammonium, EPA 350.1, Ver 2 (1993). CIMIS station no. 44 data was used to supplement rain depths when the rain gauge reading was not available in the field (due to being knocked over, etc.). Nitrogen loading for rain was calculated via Equation 1.

$$D\ (m) \times \left(\left[\frac{mg\ NO_3\ N}{L}\right] + \left[\frac{mg\ NH_4\ N}{L}\right]\right) \times 10 = loading\ \frac{kg\ N}{ha} \qquad \text{(Equation 2.1)}$$

where D is the depth of rain in meters.

Uncertainty in rain deposition was estimate by propagation of error methods and computation of root mean square errors (RMS). The RMS error for rainfall N deposition included errors in rainfall depth (estimated to be 10%), errors in chemical concentrations derived from duplicate samples analyzed on the AQ2 Discrete analyzer (10%) and a sampling error derived from bootstrapping (16%).

2.3 Ion Exchange Resin Method

Ion exchange resin collectors were constructed according to Fenn and Poth (2004). In brief, ion exchange resin collectors were comprised of a PVC tube filled with Amberlite IRN 150 mixed-bed ion exchange resin and plugged with polyester fiber (Fenn, 2013). IER tubes, when installed in the field, were accompanied by a funnel to facilitate deposition collection and an additional PVC pipe that protected the sampler from solar radiation. Exposed for six months at a time alongside field blanks, IERs were collected and capped off for protection against contamination during storage prior to

extraction. The resin in the IERs was extracted with 1 N potassium iodide (Fenn et al., 2013). The nitrogen species and sulfate were analyzed via colorimetric and ion chromatography methods (Fenn et al, 2006).

Deposition of nitrogen measured by IER collectors was computed using an equation from Fenn et al., (2013)

$$\left(W * \left(\frac{1}{1118.34}\right)\right) * N_x * F * 0.9746 = N_x Deposition \frac{kg}{ha} per\ exposure$$

Equation (2.2)

where W is the weight of the salt extractant in grams. Since 1N KI was used to extract the resin, a specific density of 1.118 g/ml was also utilized to determine the volume of the extractant. N_x, is the concentration of nitrate or ammonium in parts per million, and F is a factor to convert molecular weights (i.e., weight of NO_3^-) into weight of N. Therefore, F is 0.777 for NH_4-N and 0.226 for NO_3-N. A scaling factor of 0.9746 was used to obtain deposition in kg N ha^{-1}.

2.4 Inferential Method

The inferential method estimates deposition rates of pollutant species from atmospheric concentration measurements and deposition velocities (Lovett 1994, Schmitt 2005). This is achieved by collecting gaseous N species using passive samplers and incorporating compound-specific, deposition velocities taking into account settling surfaces, wind speed, relative humidity, and other climate variables (Zhang et al., 2003). To estimate appropriate deposition velocities, land use types and vegetative dominance

are used to select the most applicable velocity for the atmospheric measurement site. We utilized the deposition velocities established by Zhang et al., 2003, for deciduous shrub vegetative assemblages, the option closest to CSS. Inferential passive samplers were deployed at the same intervals as ITNI exposures.

In the inferential method, concentrations of NO_2, NH_3, and NO_x were measured using Ogawa collection pads and special nylon filters were utilized to measure HNO_3 (Bytnerowicz et al. 2005). Ogawa passive sampling gas collection pads and dual inlet passive sampler plastic housings were used. The sampler was comprised of protective and ventilation components, contained end caps, screens, and Teflon components on each side of a barrel shaped housing, resulting in two, unconnected, collection inlets on a single sampler (Ogawa USA, 2014). Ogawa passive samplers were installed in "bell" shaped housing pieces to prevent contamination from high winds, UV radiation, rain, and animals. Ogawa collection pads and HNO_3 filters were deployed on a 10ft foot wooden post at PSRS (Figure 2.2). HNO_3 filters were installed on the bottom of petri dish lids using a Teflon ring and affixed with Velcro to the wooden posts. The petri dish housing was sufficient to prevent rainfall contamination of HNO_3 filters, but did not obstruct atmospheric concentration measurements during exposure. Exposed Ogawa pads were removed from their protective housings, extracted with 8 milliliters of deionized water and shaken for approximately 15 minutes. The resulting extract was analyzed in a TrAAcs Technicon autoanalyzer for NO_x, NO_2, and NH_3. HNO_3 filters were extracted with 20 milliliters of deionized water, shaken for 15 minutes, and then analyzed on a Dionex ICS2000 ion chromatograph for nitrate and sulfate (sulfate data not reported).

Weather data from CIMIS station no. 44 data was used to help select appropriate deposition velocities for the inferential method based on dry day, dry night, rain day, rain night, and dew night conditions. Additionally, deposition velocities were adapted from Zhang et al. 2002 for each nitrogen species under each applicable condition. Atmospheric concentrations of NO_2, NH_3, and HNO_3 were summed across all forms and conditions to approximate total nitrogen deposition. To determine each species deposition rate during exposure, the total concentration measured was multiplied by the appropriate deposition velocity determined by the number of hours under those different atmospheric conditions (dry days, rain nights, etc.), and summed across the deployment period, as detailed below:

$$\sum_{N=NO_2,NH_3,HNO_3}^{M} V_d^M [N] D^M \times 0.00864 =$$

$$Nitrogen\ species\ specific\ deposition\ in\ kg\ ha^{-1}\ per\ exposure \qquad \text{(Equation 2.3)}$$

where $V^M{}_d$ represents the deposition velocity in cm s^{-1}. The V_d modifier, *M*, varied as: *dd*: dry day, *dn*: dry night, *rd*: rain day, *rn*: rain night, and *dw*: dew night (5 unique conditions). *N* is the concentration of the nitrogen species of interest, NO_2, NH_3, or HNO_3, in µg m^{-3}. *D* is the number of days (24 hours) for each of the conditions (M= *dd*, *dn*, *rd*, etc.) The terms "night" and "day" are each 12 hour intervals of a 24 hour day.

Depositions for each species are then summed to create a total deposition per exposure in kg N ha^{-1} during the deployment period:

$$\left(NO_2^D \times \left(\frac{14}{46}\right)\right) + \left(NH_3^D \times \left(\frac{14}{17}\right)\right) + \left(HNO_3^D \times \left(\frac{14}{63}\right)\right)$$

$$= total\ nitrogen\ deposition\ in\ kg\ ha^{-1}\ per\ exposure$$

(Equation 2.4)

Where *D* is the deposition rate calculated for each respective nitrogen species in Equation 2.4. Note: NO flux was not included in our deposition estimates as it not directly measured (it is a function of NO_2 and NO_x measurements), and generally has a low concentration compared to other deposited nitrogen species, and is confounded by soil emissions of NO (Lovett 1994 and Zhang et al., 2009).

2.5 Integrated Total Nitrogen Input Method

The Integrated Total Nitrogen Input method utilized a PLS system whose design and construction are fully described in Chapter 1. Briefly, the PLS system consisted of a plant, liquid reservoir, and sand reservoir that was isotopically enriched with ^{15}N-labelled Hoagland solution (adapted from Parker et al. 1999) to 1.01 AP ^{15}N and a nitrate concentration of 18 mM N-KNO_3 in the liquid reservoir. The sand reservoir was comprised of pre-baked #16 silver (silica) sand. The individual ITNI modules were designed so that the plant grew in the sand reservoir, which was stacked on top of the liquid reservoir. An air lift system, powered by a small air compressor, moved nutrient solution from the liquid reservoir up through a watering ring that distributed the solution to the sand surface. Excess liquid in the sand reservoir drained directly back into the liquid reservoir (Figure 2.3).

ITNI modules were assembled in the greenhouse to prevent premature exposure to ambient nitrogen deposition. Germinated seeds were sown into the sand reservoir and the 9 liter liquid reservoir filled with ^{15}N-labelled Hoagland solution. The modules were kept in the greenhouse for about 2 weeks to allow the seedlings to grow into viable young plants for ambient exposure. Ambient exposure with ITNI modules occurred during the following periods: Deployment 1: March 8, 2013 to May 20, 2013 (74 days); Deployment 2: May 28, 2013 to August 12, 2013 (77 days); and Deployment 3: November 15, 2013 to March 24, 2014 (130 days).

At the end of the exposure, all components of the PLS system were destructively harvested and sampled for nitrogen content and ^{15}N abundance. Aboveground plant tissues were collected, bagged, and dried at 60˚C for 48 hours, and total biomass measured on an analytical balance. Aboveground plant tissues were ground and homogenized using a 10 oz. glass mortar and pestle in preparation for isotopic and elemental analysis. The belowground plant tissues were harvested from the sand reservoir, sieved on a ¼" mesh screen, dried and weighed for total biomass and ground with glass mortar and pestle. Sieved sand was added back into the sand reservoir. The nitrogen content and ^{15}N isotopic abundance of all plant samples were determine on a Thermo Delta-V isotope ratio mass spectrometer equipped with an elemental analyzer inlet. A weighted average of the above and belowground tissues abundance was calculated to represent total ^{15}N abundance for the entire plant component.

At harvest, the volume of the liquid reservoir was measured and a subsample was collected for measurements of nitrogen content and ^{15}N abundance. Liquid samples for the determination of nitrate+nitrite and ammonium were filtered through a 0.4 µm polycarbonate membrane filter and into clean HDPE bottles and stored frozen until analyzed on an AQ2 discrete analyzer. Unfiltered samples were collected for total nitrogen, and stored frozen until digested in persulfate oxidizing reagent and analyzed on an AQ2 discrete analyzer. The nutrient analyses demonstrated that ammonium was below detection and that almost all of the total nitrogen existed in the form of nitrate. The isotopic composition of the nitrate was then measured on the mass spectrometer using the denitrifier method (Coplen et al., 2012).

The sand matrix was homogenized by hand and 5 gram subsamples were collected and extracted with 2M KCl (Maynard, et al., 2007) and stored frozen until analyzed. Nitrate concentrations were determined on the AQ2 discrete analyzer, and ^{15}N abundance was determined using the microbial denitrifier method using modifications recommended by Bell and Sickman (2014).

The results from the isotopic and elemental analyses were incorporated into the following equation from Russow et al. 2001:

$$AdN = N_s \times \left(1 - \frac{a'_S}{a'_T}\right) - N_o \qquad \text{(Equation 2.5)}$$

Where AdN is atmospherically derived nitrogen input to the module (mg); a'_s is the mass-weighted excess ^{15}N abundance of the PLS system at the end of the field

deployment (i.e., $a' = a$ - 0.366; 0.366 is the ^{15}N abundance in the atmospheric N_2 baseline); a'_T is the excess ^{15}N abundance (i.e., $a' = a$ - 0.366) of the original tracer added to the PLS system; N_o is the original N mass (mg) in the seeds or seedlings at the start of the ambient exposure; and N_s is the total mass of N in the PLS system at the end of the field deployment (mg).

3. Results

3.1 Wet Deposition

Measurable rainfall events took place from March 8, 2013 to May 7, 2013 for Deployment 1, June 30, 2013 to July 26, 2013 for Deployment 2, and November 21, 2013 to March 1, 2014 for Deployment 3 (Table 2.1). The largest rain event totaled 38.1 mm of rain depth via rain gauge and 2398 milliliters of rain collected in the rain bucket, during Deployment 3, on November 21, 2013. The smallest event was that of July 11, 2013 with 1.27 mm of rain and only 18 mm collected by the rain bucket.

The warmest, overall, events were those in Deployment 2 with an average air temperature of 24.0 °C. Air temperatures during Deployment 1 and 3 rain events were on average, 12.4 °C and 11.3 °C, respectively. NO_3 and NH_4 rain chemistry seemed to track average air temperature and amounts of daily rainfall for rain events during Deployments 1, 2, and 3 (Figure 2.4). Smaller rain events (< 5 mm rainfall) had higher concentrations of NO_3 and NH_4 (> 1 mg N L^{-1}), conversely, larger events (> 5 mm rainfall) within this timeframe yielded lower concentrations of NO_3 and NH_4 (< 1 mg N L^{-1}). NO_3 and NH_4

concentrations were highly correlated (R^2= 0.9467, Figure 2.5). Despite differences in rain volume and nitrogen concentrations, the loading of N by rainfall was similar among the deployments (Table 2.1).

3.2 Ion Exchange Resin Deposition

IER exposure from June 6, 2013 to November 18, 2013 had nitrogen loading of 3.58 and 3.11 kg ha^{-1} for throughfall and bulk measurements, respectively (Table 2.2). IER exposure from November 11, 2012 to June 13, 2013 had a total nitrogen loading of 2.36 kg ha^{-1} for throughfall and 1.83 kg ha^{-1} for bulk collectors. The last exposure from November 18, 2013 to June 6, 2014 had 1.79 kg ha^{-1} and 2.50 kg ha^{-1} of nitrogen loading for throughfall and bulk collectors, respectively.

With the IER method, the average ammonium fraction of nitrogen always exceeded that of the nitrate portion. This trend is also seen in the raw data, with ammonium either being larger or equal to nitrate in every collector (Table 2.2).

3.3 Inferential Method

No inferential measurements were made for Deployment 1. For Deployment 2, there were a total of 2 rain days and 0.5 rain nights, with the remaining 24-hour days being comprised of dry days and dry nights (Table 2.3). During Deployment 3, there were 4 rain days, 2.5 rain nights, and 1.5 dew nights over the 130 day exposure. Nitric acid was the least concentrated of the atmospheric nitrogen species across Deployments 2 and 3. The largest concentrations of nitrogen species during these deployments were

observed for NO_2 and NO (Figure 2.6). Total nitrogen deposition, as measured by the inferential method was 5.9 kg ha^{-1} (7.7 kg N ha^{-1} yr^{-1}) and 8.4 kg N ha^{-1} (10.9 kg N ha^{-1} yr^{-1}) during Deployment 2 and 3, respectively (Table 2.3).

3.4 Integrated Total Nitrogen Input Method

ITNI Deployment 1, from March 8 – May 20, 2013, measured 5.9 kg N ha^{-1} $D1^{-1}$ (Table 2.4). Deployment 2, from May 28 – August 12, 2013, had a deposition rate of 9.8 kg N ha^{-1} $D2^{-1}$. Deployment 3, exposed from November 15, 2013 – March 24, 2014, yielded 3.8 kg N ha^{-1} $D3^{-1}$. Summing across all three deployments yielded deposition of 29.3 kg N ha^{-1} for the 281 days of deployment between March 8, 2013 and March 24, 2014 (382 days). To estimate annual N deposition using the ITNI method we took two approaches. In the first approach we multiplied the sum of deposition for the three deployments, 29.3 kg N ha^{-1}, by 365/281 to yield an annual deposition rate of 38.1 kg N ha^{-1} yr^{-1}. This computation essentially computed an average daily N deposition rate and scaled this rate to an entire year. In the second approach, we linearly interpolated the average daily N deposition for the three deployments to fill in gaps in the data record. Using this approach, annual N deposition at Riverside was 38.4 kg N ha^{-1} yr^{-1}.

4. Discussion

4.1 Method Intercomparison

Deployment 1 involved deposition measurements by ITNI, rain collector, and bulk and throughfall IER collectors (Figure 2.7). No inferential measurements were made

during Deployment 1. The IER bulk and throughfall collectors measured total deposition of 0.62 kg N ha^{-1} and 0.80 kg N ha^{-1}, respectively, during the 74 day exposure period which was far below the amount recorded by the ITNI modules with invasive plants. Extrapolating the IER data to an entire year (i.e., kg N ha^{-1} x 365/74) yields yearly nitrogen loads of 3.09 kg N ha^{-1} and 3.98 kg N ha^{-1}, higher than wet-deposition measurements from NADP for 2011-2013 (annual average = 1.5 kg N ha^{-1}), but well below the average rate of 14 kg N $ha^{-1}yr^{-1}$ for Riverside that includes dry deposition (Fenn et al. 2010).

All methodologies, including the inferential method, were used in Deployment 2 (Figure 2.8). ITNI and inferential+rain had comparable deposition rates, whereas, again due to few and small rain events, the IER's data were much too low (1.34 kg N ha^{-1} and 1.46 kg N ha^{-1} for bulk and throughfall, respectively). Inferential measurements yielded a deposition rate of 5.78 kg N ha^{-1} for the 77 day deployment period, with an added 0.32 kg N ha^{-1} from wet deposition (rain) for a total of 6.1 kg N ha^{-1}. ITNI produced a deposition rate that was 50% higher, 9.8 kg N ha^{-1}.

In Deployment 3 the inferential method yielded deposition of 8.30 kg N ha^{-1} during the 130 day exposure period, and rainfall contributed 0.40 kg N ha^{-1} for a total of 8.7 kg N ha^{-1} for the deployment. In contrast, the ITNI method determined a deposition rate of 3.8 kg N ha^{-1} for the deployment which was more similar to the bulk and throughfall IER collectors (2.33 and 1.20 kg N ha^{-1}, respectively). I hypothesize that ITNI deposition was lower than the sum of inferential+rain because the plant that I used, summer mustard (*H. incana*), was growing outside of its normal growing season and,

therefore, the plant was not a taking up atmospheric nitrogen as rapidly. Normally, this species can mature and senesce with a couple of months, but during Deployment 3 it took 130 days to reach signs of senescence as compared to 77 days during the summer months. Because *H. incana* was not growing at its normal rate we suspect that its rate of gaseous N uptake was lower. This can be corroborated by the single native plant, *E. fasciculatum,* which is adapted to winter-time growth, and which produced a higher N deposition rate of 5.2 kg N ha^{-1} during Deployment 3.

Combining N deposition for Deployments 2 and 3 (207 days), yields 14.1 kg N ha^{-1} for the inferential+rain method and 13.6 kg N ha^{-1} for the ITNI method utilizing invasive plants (Figure 2.10). During the summer growing season, ITNI measured more deposition than the inferential+rain method, we suspect, because of active N assimilation by the plant. One implication for this finding is that the inferential method can underestimate the true deposition to invasive species like summer mustard in southern California. Conversely, ITNI deposition was lower in the winter because summer mustard was growing outside of its normal growing season. The chemical substrates on the Ogawa filters and HNO_3 filters are insensitive to climatic changes, in contrast to plant phenology, and will likely underestimate N deposition to plants during their active growing season, but will overestimate N deposition to plants during the plant dormant season.

4.2 Advantages of the ITNI Method

This experiment was designed to better understand how ITNI measurements of nitrogen deposition compare to those of traditional methods such as the inferential method, rainfall collection, and IER method. In my study, ITNI measurements were comparable to that of the inferential method plus wet deposition over longer periods. However, since my study sites in southern California were dominated by dry, arid conditions, wet deposition alone is not an accurate method of estimating nitrogen deposition. Additionally, IER collectors typically underestimate nitrogen deposition as they rely on natural rainfall for measurement (Fenn and Poth 2002).

ITNI may be a better option for measuring nitrogen deposition under several conditions in which inferential or ion exchange resin collection would be impractical. ITNI is most advantageous in conditions which there is little rain (<100 mm of rainfall). This is because the ITNI module allows for all deposition types to enter the system-gaseous via plant active uptake and assimilation, dry deposition on plant and sand surfaces, and wet deposition with entry to the system via sand reservoir percolation. Additionally, this is the only method that utilizes an actively assimilating plant as a biological sensor for such measurements, better representing nitrogen deposition as it is experienced by plants under field conditions. The ITNI module also has the potential to be deployed over larger areas through collaboration between research scientists and citizen scientists. Such volunteers could adopt ITNI modules on their property, maintain water levels in the modules and insure that the water systems are working. At the end of

a deployment, the research scientists would harvest the ITNI modules. Being freed from operating the ITNI modules, the research scientists could devote their time and effort to preparing modules for deployment and performing the chemical and isotopic analyses. The inferential method however, might not be ideal for citizen scientists due to the necessity for repeated, careful removal of sampling filters and pads; the ITNI method requires sampling only at the end of a deployment. ITNI would also provide a more interesting and involving platform for citizen scientists as opposed to the more technical, and less engaging inferential pads or filters.

Measurement of N deposition via the ITNI method costs less than the inferential method, even for short deployments (Table 2.6). The overall cost for a single ITNI deployment is approximately $2300, compared to an inferential method deployment cost of $5500. To include wet deposition an additional $4300 rain collector system is needed, bringing the total cost to more than $10,000. Also, analytical costs are lower for ITNI than with the inferential and rainfall method, since the ITNI module only requires elemental and isotopic measurements of three nitrogen pools, whereas multiple nitrogen forms must be extracted from the Ogawa and HNO_3 filters and analyzed by multiple analytical methods. Isotopic and elemental analysis of plant material costs $8 per module with an additional cost of about $40 for the microbial denitrifier method analysis of nitrate in the liquid reservoir and in the KCl soil extract. This is compared to the inferential method that costs upwards of $40 per analyte (Diane Alexander, conversation). The ITNI modules are also relatively cheap to construct because the main apparatus can be purchased as hydroponic modules at gardening stores.

4.3 ITNI Suggestions for Future Work

Future ITNI experiments should consider the use of perennials rather than annuals in the ITNI modules. Because ITNI provides an unhindered source of nitrogen for the plants (no competition from other plant species, decreased assimilation from microbes, and readily available forms of nitrogen as NO_3 in solution), I observed quicker maturation times of the annual species in the ITNI modules versus those in natural conditions in the same area. Annual plants tend to reproduce as quickly as possible and then die-off; this type of phenology is not ideal for the ITNI method because the plant can reach senescence too early. Shorter lived perennials, however, have life histories and reproductive strategies that are less dependent on immediate fluxes of nutrients, and therefore might not seed as quickly and would grow in a more consistent manner than annuals.

My study shows that herbivory is a constant threat when ITNI modules are deployed in the field, thus, it would be advantageous to select plant species that are not overly attractive to consumers. In most field settings there will be some type of herbivore, so fencing will be required. However, care must be taken in designing fencing and the smallest fence possible for deterrence should be used. In no case should the fence extend above an angle of 45 degrees above the plants at maturity as this could interfere with nitrogen settling patterns (Mark Fenn, conversation).

Lastly, shrub species may be a better candidate for ITNI than grass species. Grasses can produce greater variability in above-ground biomass among replicates and

require multiple seeds for germination. In contrast, a single shrub can be used in an ITNI module and can produce less variability in aboveground biomass between replicate modules. For example, California buckwheat (*Eriogonum fasiculatum foliolosum*) produced more uniform aboveground plant than the summer mustard. Lastly, I would suggest a single, easily grown species be used as a proxy in urban environments that lack appropriate plant communities for plant species selection (for example, a cultivated rosebush species that can grow in all urban neighborhoods of Los Angeles County, etc.). The ideal species for ITNI would reach maturity slowly, produce few seeds, grow during any season of the year, have few predators in the survey area, and have an aboveground structure that is similar to native species in the study area.

Loss of the N from the ITNI modules should be further studied. Gaseous N losses can occur in the form of N_2O (via denitrification) and NO_x (via nitrification). Because of the well-oxygenated conditions in the ITNI module, denitrification is unlikely and we did not detect any suggestion of denitrification in the $\delta^{15}N$ and $\delta^{18}O$ measurements of nitrate in the liquid reservoir or in the KCl extracts from the sand (Coplen 2012). We made measurements of NO_x emissions near the middle and end of INTI exposures, but did not detect any measurable emission, however, to fully rule out gaseous loss of NO_x, measurements should also be made at the beginning of the deployment when there is great N available to microbial populations.

5. Tables

Table 2.1. Rain Event Descriptions for Rain Collector at USDA USFS Pacific Southwest Research Station, in Riverside, CA.

Deployment	Rain Event Date	Volume (ml)	Rain Gauge (mm)	CIMIS St. 44 Reported Rain (mm)	Depth of Rainfall (m)	Average Air Temp C°	$NO3^-$ mg N L^{-1}	$NH4^+$ mg N L^{-1}	$NO3^-$ +$NH4^+$ mg N L^{-1}	Loading kg N ha^{-1}	Total Loading per Deployment kg N ha^{-1}
1	8-Mar-2013	700	-------	11.9	0.0119	8.5	0.49	0.59	1.08	0.128	0.308
	9-Mar-2013	390	5.08	2.1	0.0051	10.9	0.41	0.44	0.85	0.043	
	6-May-2013	308	4.57	4.9	0.0046	14.7	0.43	0.87	1.30	0.059	
	7-May-2013	230	3.81	1.4	0.0038	15.3	0.60	1.45	2.04	0.078	
2	30-Jun-2013	34	-------	0.4	0.0004	28.1	3.17	4.76	7.93	0.032	0.315
	11-Jul-2013	18	1.27	0.2	0.0013	23.2	2.53	4.59	7.12	0.090	
	20-Jul-2013	310	4.57	7.3	0.0046	21.0	1.04	1.81	2.85	0.130	
	26-Jul-2013	100	1.27	0.9	0.0013	23.5	1.96	2.98	4.94	0.063	
3	21-Nov-2013	2398	38.10	29.4	0.0381	13.6	0.22	0.28	0.50	0.190	0.397
	7-Dec-2013	625	9.40	8	0.0094	7.6	0.21	0.25	0.46	0.044	
	6-Feb-2014	202	3.81	2.5	0.0038	10.3	0.74	2.14	2.88	0.110	
	27-Feb-2014	28	1.27	26.1	0.0013	13.2	0.55	1.06	1.61	0.020	
	1-Mar-2014	825*	-------	11.8	0.0118	11.8	0.09	0.19	0.28	0.034	

Table 2.2. Ion Exchange Resin Collector Data 2012-2014

			Throughfall			Bulk/Open		
Deploy.	**Date Installed**	**Date Removed**	**Avg. NH_4- N kg ha^{-1}**	**Avg. NO_3- N kg ha^{-1}**	**Total Loading kg N ha^{-1}**	**Avg. NH_4- N kg ha^{-1}**	**Avg. NO_3- N kg ha^{-1}**	**Total Loading kg N ha^{-1}**
1	11-Nov-12	6-Jun-13	1.59	0.76	2.36	1.27	0.56	1.83
1, 2	6-Jun-13	18-Nov-13	2.15	1.44	3.58	1.87	1.24	3.11
3	18-Nov-13	6-Jun-14	1.24	0.55	1.79	1.98	0.52	2.50

Table 2.3. Inferential Method Passive Data. Riverside: CIMIS St. 44, Motte: CIMIS St. 240.

Site	Deploy.	Exposure Start Date	# Elapsed Days (24hr)	CIMIS: Dry Days (24 hrs)	CIMIS: Dry Nights (24 hrs)	CIMIS: Rain Days (24 hrs)	CIMIS: Rain Nights (24 hrs)	CIMIS: Dew Nights (24 hrs)	NO2 µg/m3	NO µg/m3	HNO3 µg/m3	NH3 µg/m3	N kg ha^{-1}	Total Deposition N kg ha^{-1}
Riverside	2	28-May-2013	15.1	7.5	7.5	0	0	0	16.6	4.7	3.4	9.9	0.9	5.9
		12-Jun-2013	13.2	6.6	6.6	0	0	0	16.2	7.5	3.3	9.6	0.8	
		25-Jun-2013	14.1	6.5	7.0	0.5	0	0	24.2	7.1	12.9	10.9	1.4	
		9-Jul-2013	21.7	9.3	10.3	1.5	0.5	0	22.5	4.5	14.2	9.9	2.2	
		31-Jul-2013	12.1	6.0	6.0	0	0	0	19.4	8.1	4.0	8.0	0.7	
	3	15-Nov-2013	16.7	6.9	7.4	1.5	0.5	0.5	33.8	11.0	2.1	4.6	0.9	8.4
		2-Dec-2013	14.0	6.5	7.0	0.5	0	0	40.0	32.3	2.1	4.5	0.8	
		16-Dec-2013	14.1	6.5	6.5	0.5	0	0.5	44.4	32.8	2.6	5.4	0.9	
		30-Dec-2013	14.0	7.0	7.0	0	0	0	60.0	44.2	1.9	9.4	1.3	
		13-Jan-2014	14.9	7.5	7.5	0	0	0	46.1	38.4	1.7	5.6	1.0	
		28-Jan-2014	14.0	6.5	6.5	0.5	0.5	0	32.9	11.8	1.1	8.2	0.9	
		11-Feb-2014	13.0	6.5	6.5	0	0	0	51.5	33.4	2.4	11.8	1.2	
		24-Feb-2014	28.1	13.0	12.0	1.0	1.5	0.5	24.8	-1.7	1.9	4.2	1.2	

Table 2.4 ITNI Total Deployment Deposition

		Liquid	Plant		Sand	Total	kg N ha^{-1} D^{-1}	
Deploy. #	**ITNI Module**	**AP ^{15}N**	**AP ^{15}N Above**	**AP ^{15}N Below**	**AP ^{15}N (NO_3 extractable)**	**AP ^{15}N**	**Module Average**	
1	P2	0.38	0.88	0.82	0.74	0.85	6.1	
	P3	0.37	0.86	0.74	0.42	0.79	7.3	
	P4	0.37	0.91	0.84	0.74	0.87	4.4	
							5.9	**D1 Average**
2	Hi2	0.39	0.83	0.92	0.69	0.91	10.9	
	Hi3	0.72	0.85	0.93	0.68	0.92	9.0	
	Hi6	0.61	0.90	0.93	0.66	0.92	8.8	
	Hi7	0.82	0.91	0.94	0.67	0.93	10.6	
							9.8	**D2 Average**
3	Hi3	0.52	0.91	0.94	0.76	0.90	4.4	
	Hi4	0.66	0.85	0.93	0.83	0.88	4.2	
	Hi5	0.49	0.88	0.94	0.48	0.90	3.4	
	Hi6	0.42	0.88	0.94	0.38	0.89	4.3	
	Hi7	0.44	0.87	0.94	0.47	0.89	3.1	
	Hi8	0.44	0.91	0.96	0.40	0.92	3.4	
	Hi9	0.86	0.92	0.94	0.83	0.89	2.9	
	Hi10	0.39	0.93	0.93	0.54	0.92	4.8	
							3.8	**D3 Average**
					Total 'yearly' deposition:		19.6	

kg N ha^{-1} D1-3 $^{-1}$

Table 2.5. Cost comparison between the Inferential and ITNI methods for N deposition monitoring.

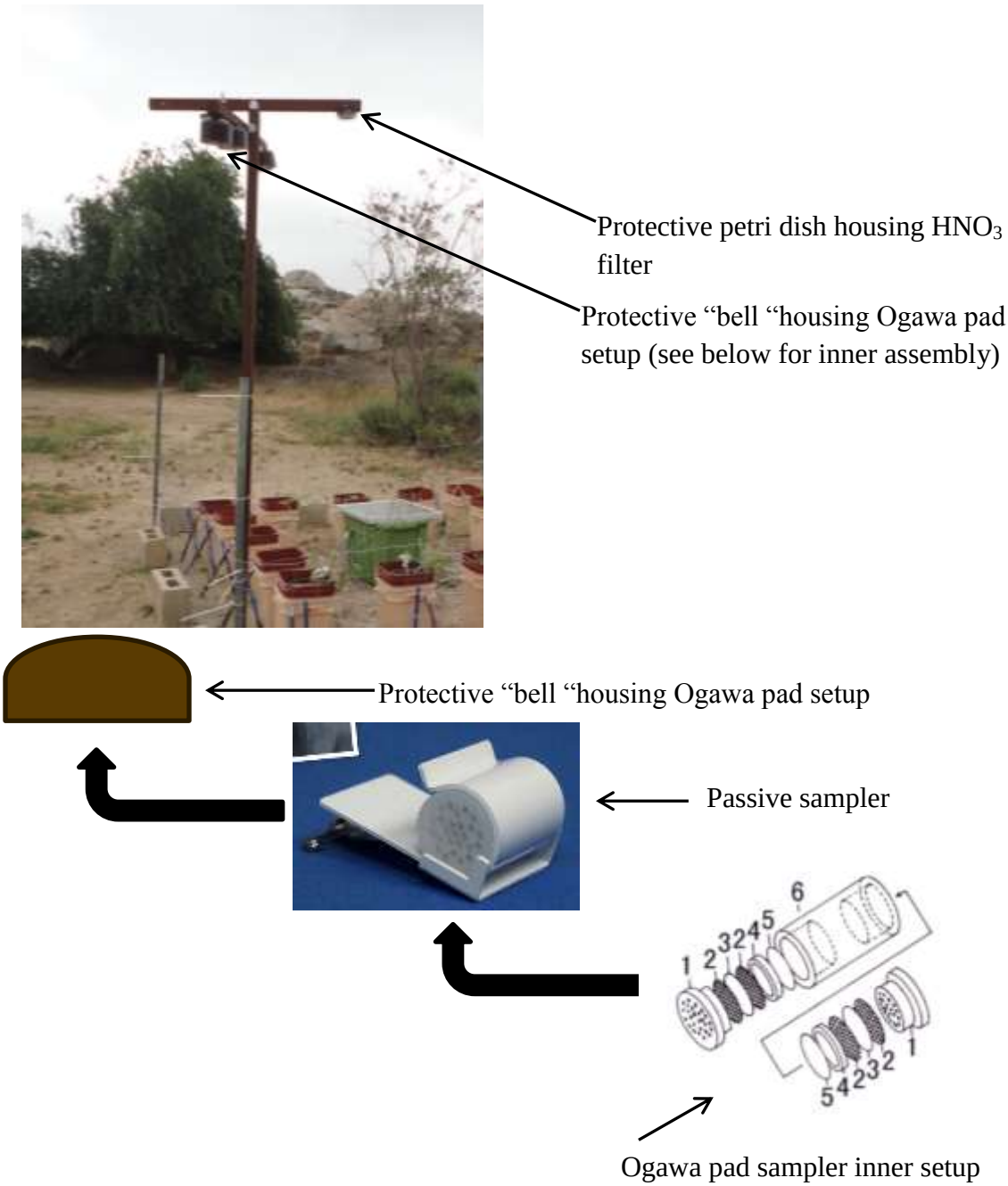
TRADITIONAL				One site only			
CONSUMABLES				**10 WEEK Exposure**		**20 WEEK Exposure**	
Ogawa Pads	Part #	#/unit	Cost $	# of units needed	10 week cost	# of units needed	20 week cost
NH3	PS-154	40	$112.00	2	$224.00	3	$336.00
NO2	PS-134	40	$112.00	2	$224.00	3	$336.00
NOx	PS-124	40	$112.00	2	$224.00	3	$336.00
HNO3 filters							
47 mm	66509	100	$314.69	1	$234.20	2	$468.40
Zefluor, 2 um, 47mm	P5PJ047	50	$234.20	2	$468.40	2	$468.40
Petris			$20.00	1	$20.00	1	$20.00
OVERHEAD							
Wooden Post		1	$25.00	1	$25.00	1	$25.00
Sampler and Shelter							
"Bell" + metal clip	PS-106	1	$28.50	6	$171.00	6	$171.00
Ogawa Passive Sampler	PS-100	1	$72.00	6	$432.00	6	$432.00
Rain Collector		1	$4,350.00	1	$4,350.00	1	$4,350.00
				grand total:	$6,373		$6,942.80

ITNI				One site only			
				10 WEEK Exposure		**20 WEEK Exposure**	
CONSUMABLES	Part #	#/unit	Cost $	# of units needed	10 week cost	# of units needed	20 week cost
plant	seeds	500	0-4	16	$64.00	32	$64.00
15N- 98 AP Sigma Aldrich		250 mg	$30.00	1	$30.00	2	$60.00
N-Free Hoagland Powder		100g	$75.00	1	$75.00	2	$150.00
nitex screen >80 µm		30 squares	$150.00	1	$150.00	2	$300.00
OVERHEAD							
Tubing: air pump		1	$5.00	16	$80.00	32	$160.00
Pipe: air lift		1	$5.00	16	$80.00	32	$160.00
Tubing: water ring		1	$5.00	16	$80.00	32	$160.00
pump			$300.00	1	$300.00	1	$300.00
pump housing			$7.00	1	$7.00	1	$7.00
manifold			$50.00	1	$50.00	1	$50.00
Hydroponic Farm Module		1	$45.00	16	$720.00	32	$1,440.00
OR							
bucket: sand reservoir		1	$5.10	16	$81.60	32	$163.20
bucket: liquid reservoir		1	$5.25	16	$84.00	32	$168.00
			Grand Total:	**Modules**	$1,636.00		$1,411.00
				Buckets	$1,081.60		$1,742.20

6. Figures

Figure 2.1. The ADS 00-120 wet deposition only, bucket sampler by N-Con Systems.

Figure 2.2. The inferential collector and a detail of the Ogawa filter housing and sampling pads.



Ogawa Passive sampler and Ogawa pad sampler inner setup pictures are from OgawaUSA.com

Figure 2.3. The ITNI modules deployed at Riverside, California. Modules with plants are invasive plant treatments and the other modules are controls. The green tote houses the air compressor and battery. Air distribution lines connect to the air-lift system in each module.

Figure 2.4. Rainfall depth, daily average air temperature on days with rainfall, and the concentration of $NO_3^-+NH_4^+$ in individual rain events at Riverside.

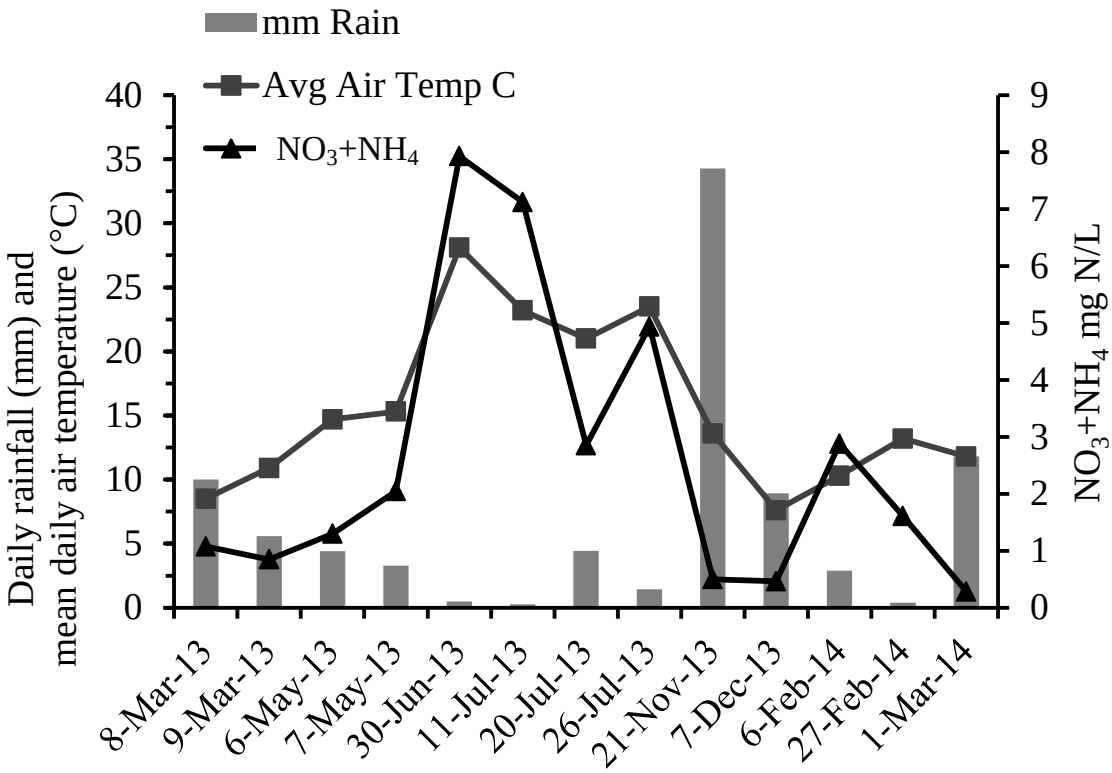


Figure 2.5. Comparison of NO_3^- and NH_4^+ concentrations in individual rain events at Riverside.

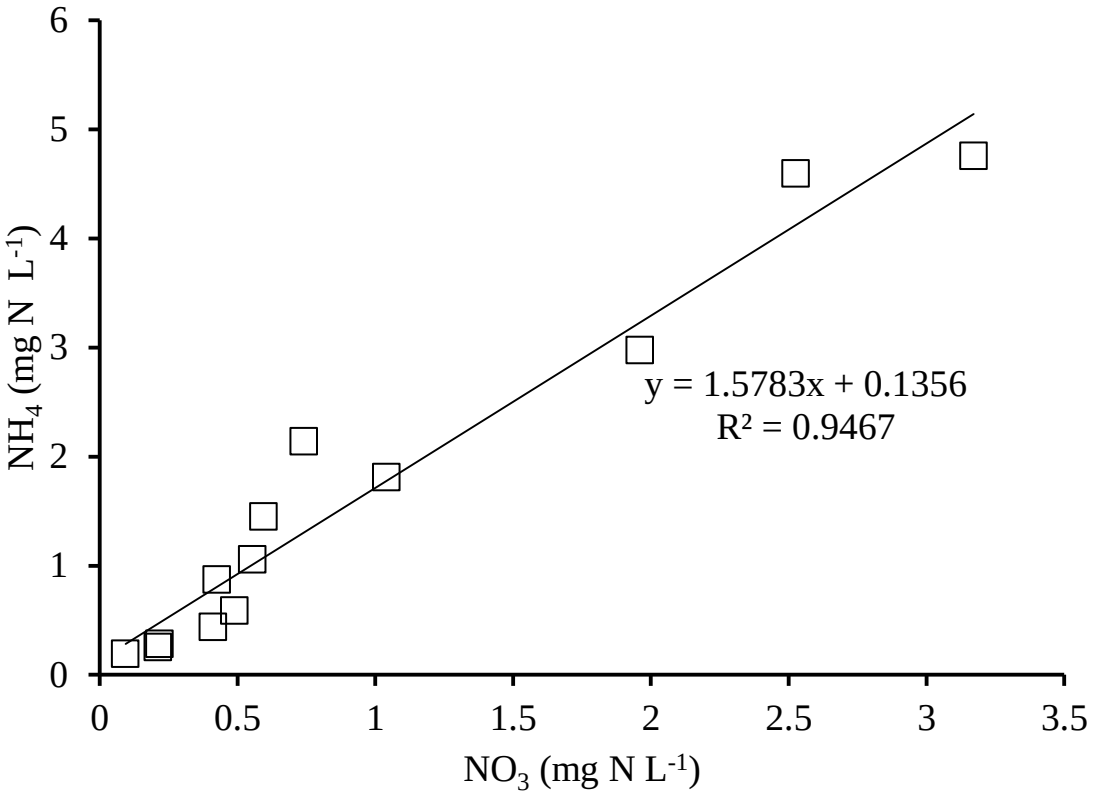
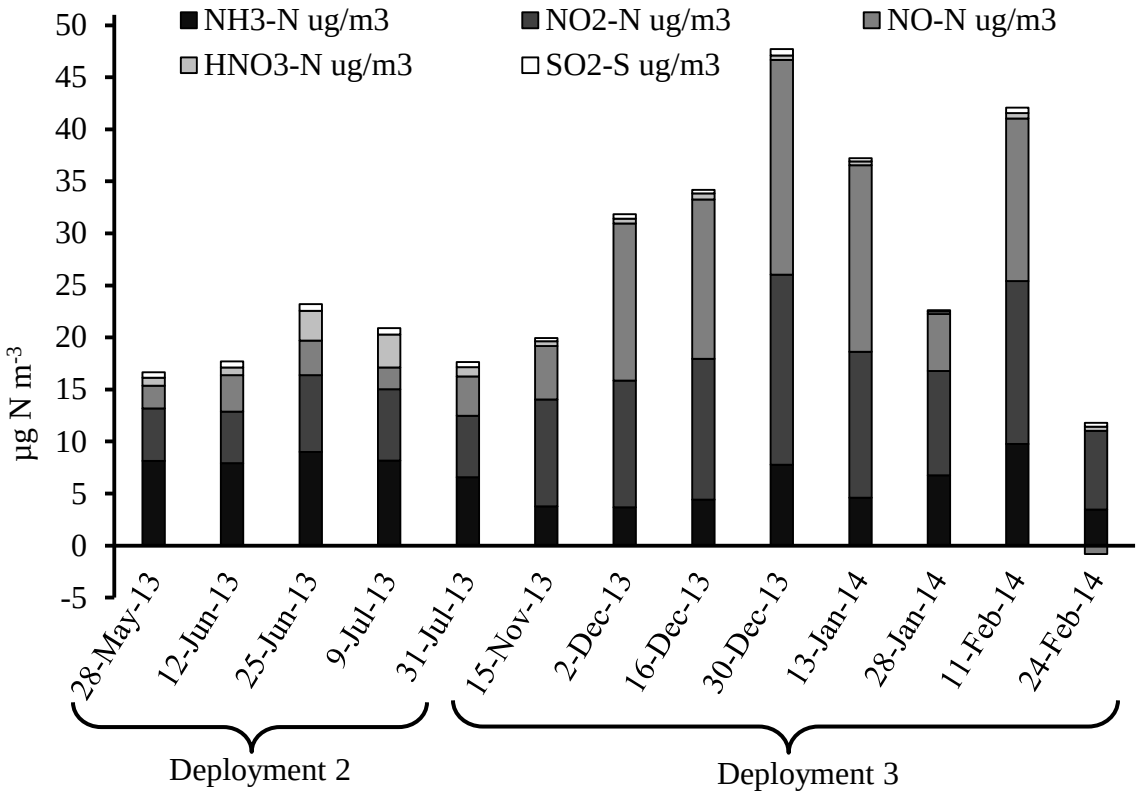


Figure 2.6. Atmospheric concentrations of gaseous nitrogen species measured using the inferential method during Deployments 2 and 3 at Riverside.



Deployment 2 exposed May 28 – August 12, 2013 and Deployment 3 exposed November 15, 2013 – March 24, 2014. Each exposure is labeled above by the day it was deployed. The length of exposure for each was approximately 2 weeks.

Figure 2.7. Comparison among the deposition methods for Deployment 1. No inferential measurements were made during Deployment 1.

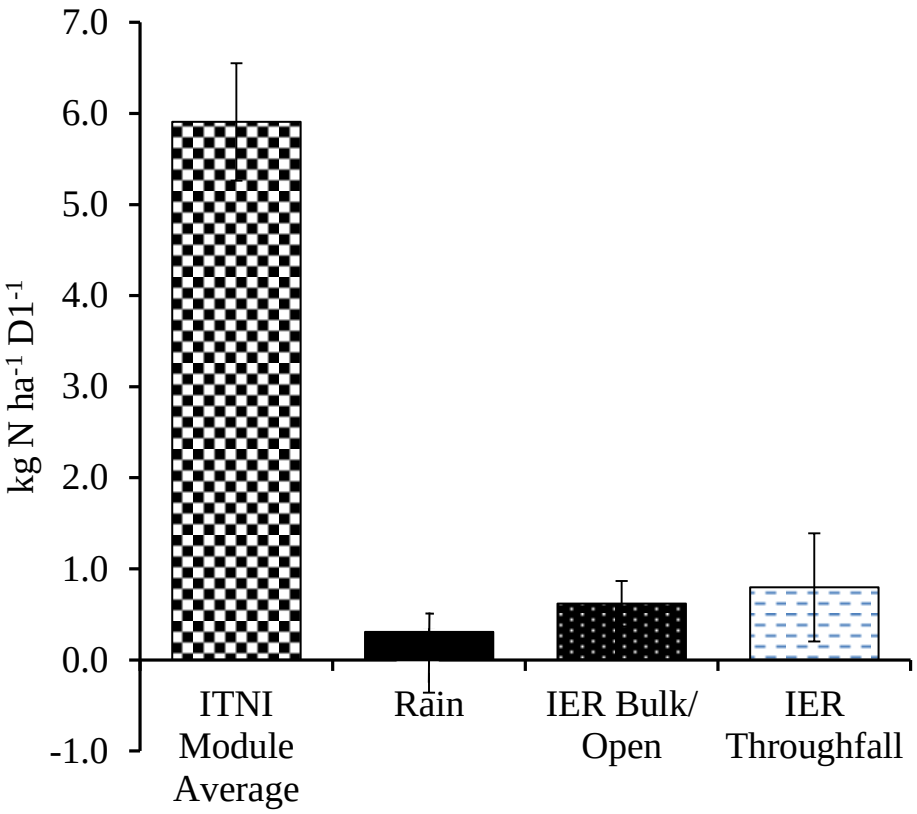


Figure 2.8. Comparison among the deposition methods for Deployment 2.

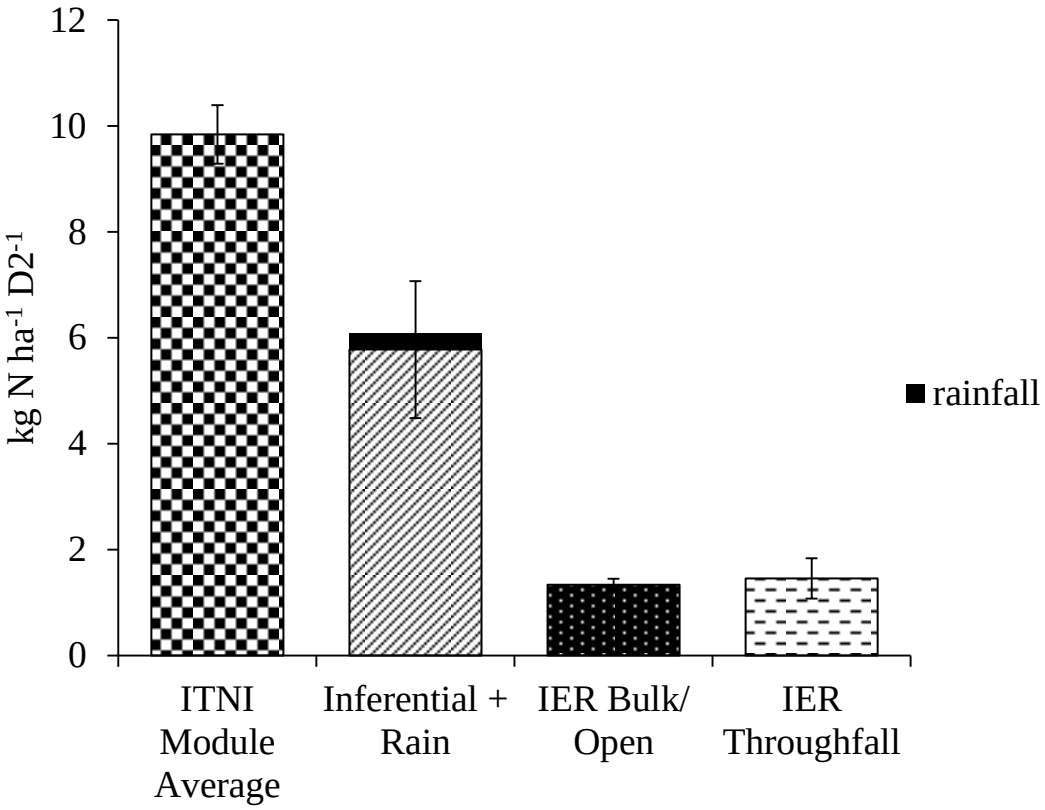


Figure 2.9. Comparison among the deposition methods for Deployment 3.

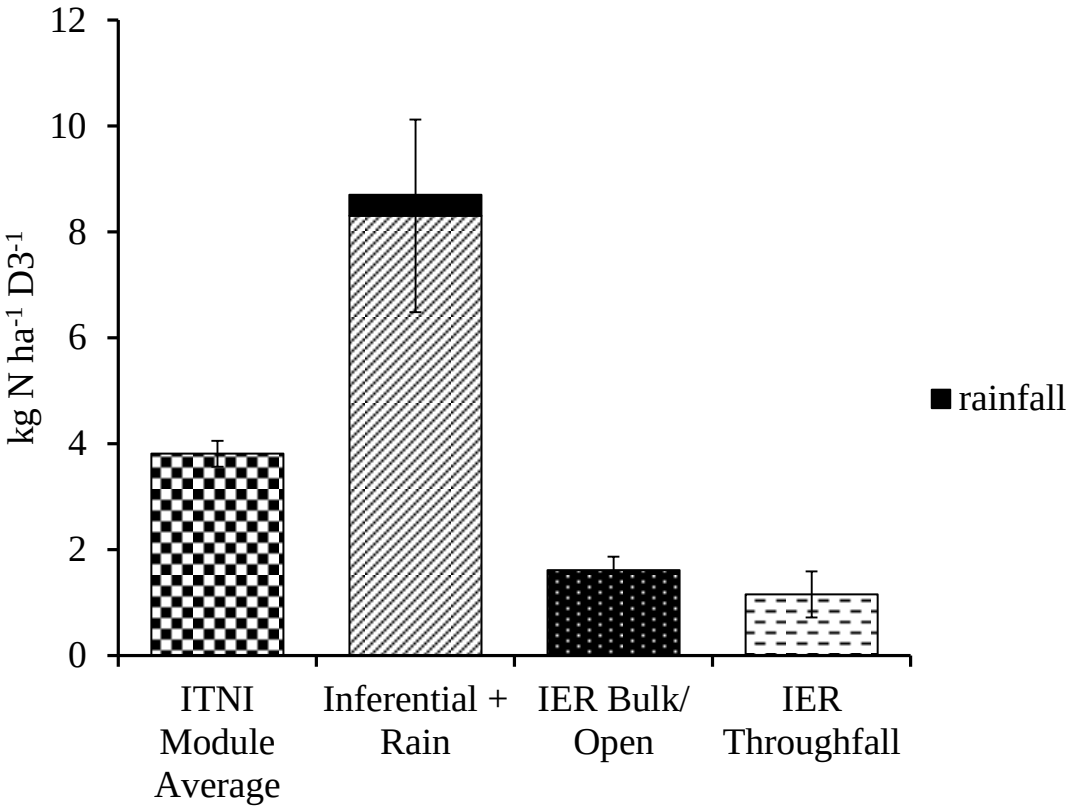
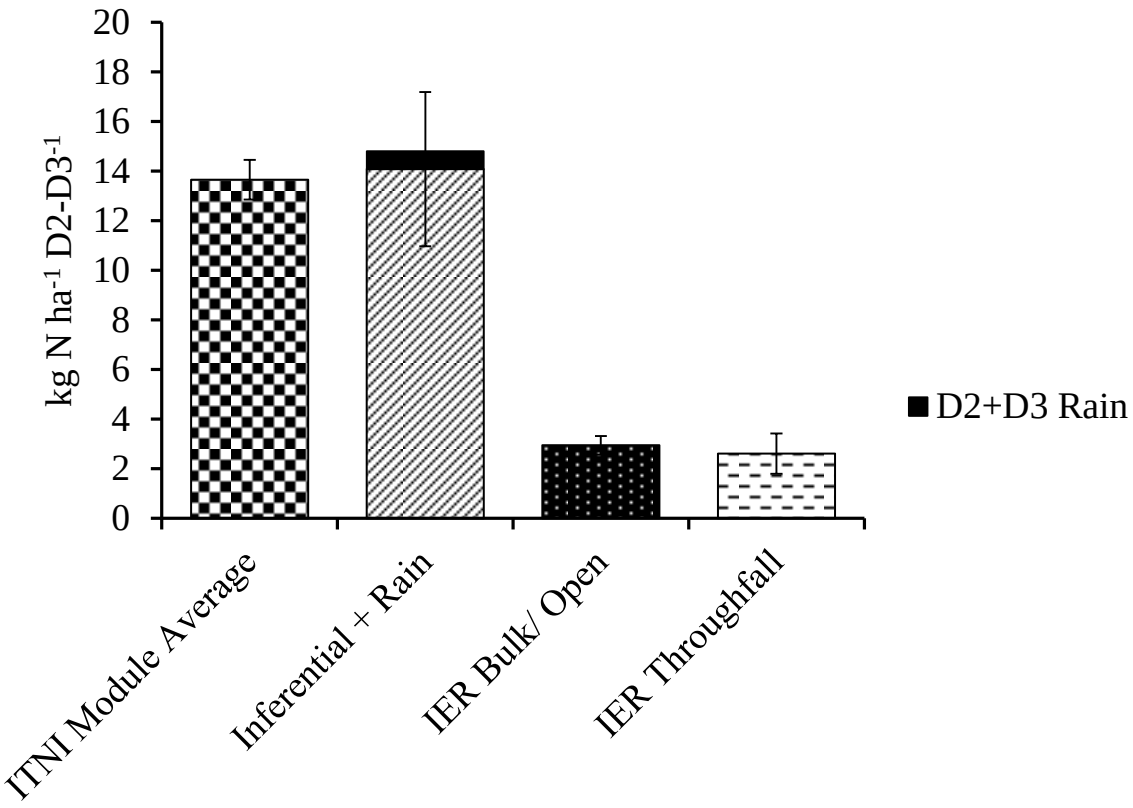


Figure 2.10. Comparison among the deposition methods for Deployment 2 plus Deployment 3.



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