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Global Measurement of Nitrous Oxide Isotopomers using Cavity Ring-down Spectroscopy

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GLOBAL MEASUREMENT OF NITROUS OXIDE ISOTOPOMERS USING CAVITY RING-DOWN SPECTROSCOPY

by

AMY ELIZABETH STEIKER

B.A., University of Colorado, 2008

A thesis submitted to the Faculty of the Graduate School of the University of Colorado in partial fulfillment of the requirement for the degree of Master of Arts

Department of Ecology and Evolutionary Biology

2014
This thesis entitled:
Global Measurement of Nitrous Oxide Isotopomers using Cavity Ring-down Spectroscopy
written by Amy Elizabeth Steiker
has been approved for the Department of Ecology and Evolutionary Biology

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The final copy of this thesis has been examined by the signatories, and we
Find that both the content and the form meet acceptable presentation standards
Of scholarly work in the above mentioned discipline.
Nitrous oxide continues to increase in the atmosphere mainly due to heightened microbial production from fertilized agricultural systems. Our ability to constrain the anthropogenic influence on \( N_2O \) production at a global scale is mainly hampered by both the spatiotemporally heterogeneous emission of \( N_2O \) from soil, as well as uncertainties regarding the transport dynamics of \( N_2O \) between the stratosphere and troposphere. Monitoring the stable isotopic composition of \( N_2O \) in air can help to better constrain \( N_2O \) emission sources, since many pools of \( N_2O \) exhibit distinctly different isotopic signatures. The intramolecular position of \( ^{15}N \) (\( \beta \) position \( ^{15}N^{14}N^{16}O \) versus \( \alpha \) position \( ^{14}N^{15}N^{16}O \)) can be used in conjunction with the total \( ^{15}N \) isotopic composition of \( N_2O \) (\( \delta ^{15}N^{\text{bulk}}_{N_2O} \)) to further elucidate source fluxes, as \( N_2O \) produced from biological sources exhibits unique site-specific isotopic signatures that are independent of the substrate value. A subset of 19 sites from the NOAA/ESRL Cooperative Sampling Network was measured in order to describe the global distribution and seasonality of \( N_2O \) isotopomers. Simultaneous and continuous measurement of \( N_2O \) mole fraction, \( \delta ^{15}N^{\text{bulk}}_{N_2O}, \delta ^{15}N_{N_2O}, \delta ^{15}N_{N_2O-N_2O}, \) and \( \delta ^{15}N_{N_2O-N_2O} \) was conducted using the Picarro G5101-i wavelength-scanned cavity ring-down spectrometer (CRDS) coupled with a quantum cascade laser capable of the mid-infrared wavelength detection needed for \( N_2O \). A significant (\( p < 0.05 \)) negative correlation between mixing ratio and \( \delta ^{15}N^{\text{bulk}}_{N_2O} \) is exhibited across 13 annual (spring 2013-2014) site means, which is consistent with a stronger \( N_2O \) source signal in the northern mid-latitudes. While
significant isotopic differences within and between sites are observed at most sites, long-term measurement uncertainties greater than 0.5‰, as well as the use of discrete versus continuous sampling, may limit our ability to detect tropospheric trends. Suggested quality assurance protocols including water removal and cavity temperature monitoring may help to reduce these uncertainties with future CRDS users.
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CHAPTER 1

INTRODUCTION

The advent of inexpensive and abundant synthetic fertilizer produced by the Haber-Bosch process in the mid-20th century was a pillar of the “Green Revolution,” or the widespread expansion of agriculture on a global level. While the resultant increase in food availability has obvious benefits, it has come at a cost to both ecological and human health, in part due to excessive fertilizer inputs. Such inputs increase the levels of reactive nitrogen (i.e. inorganic reduced and oxidized forms of nitrogen and organic N-containing molecules; Galloway et al., 2008). Between 1860 and the early 1990’s, this reactive nitrogen increased by a factor of ~10, yet the human population only grew by factor of ~4.5 (Galloway et al., 2004). Humans now produce roughly twice the amount of nitrogen as what is contributed naturally to the environment (Leach et al., 2012) and five times the natural amount in the United States alone (United States Environmental Protection Agency, 2011).

Nitrous oxide (N$_2$O), a greenhouse gas with roughly 300 times the global warming potential of CO$_2$, is one form of reactive nitrogen that has increased over 20% since pre-industrial times from 270 parts per billion (ppb) (Machida et al., 1995) to over 325 ppb today. N$_2$O is currently increasing at a rate of 0.8 ppb yr$^{-1}$, mainly due to increased
microbial production from fertilized agricultural systems (Denman et al., 2007). As the third most powerful greenhouse gas (Denman et al., 2007) and the primary species responsible for stratospheric ozone destruction (Ravishankara et al. 2009), N₂O is directly threatening ecosystem and human health. With agricultural soils accounting for roughly 25% of the N₂O emitted into the atmosphere (Denman et al., 2007), it is important to understand the controls over the production of N₂O in order to implement agricultural policies aimed at reducing emissions.

While we have a reasonable understanding of the point-level controls over microbial N₂O production (Robertson and Tiedje, 1987; Stein and Yung, 2003), those controls cause substantial spatiotemporal variation in N₂O production, leaving our ability to measure and model emissions at larger scales poorly constrained (Baggs, 2008). Additionally, the influence of abiotic controls over N₂O intra-annual variability remains largely uncertain, which exacerbates the difficulty in constraining N₂O sources. Top-down inversion estimates (e.g. Hirsch et al., 2006; Miller et al., 2012) are larger than bottom-up inventories of N₂O, particularly in northern tropical latitudes, which further exemplifies the need to better constrain these sources given the rapidly expanding agricultural and industrial activities of those regions.

In order to resolve these discrepancies and uncertainties, stable isotopic approaches can be used to better differentiate the relative contributions of different N₂O sources to the atmosphere. Both biological and physical processes discriminate, or fractionate, against molecules containing heavy isotopes, creating distinctly unique isotopic signatures of N₂O from a stratospheric versus industrial source, for example. Additionally, N₂O produced from nitrification can exhibit a distinctly different isotopic signature than N₂O from
denitrification, which can increase our ability to understand the effects of agricultural practices on microbial processes without alteration of the natural system. The position specific heavy nitrogen isotope (\(^{15}\text{N}\)) of \(\text{N}_2\text{O}\) can also be used to further elucidate the enzymatic processes responsible for the formation of \(\text{N}_2\text{O}\). While these site-specific, or isotopomer, signatures have been shown to be independent of their precursors (Popp et al., 2002; Toyoda et al., 2002; Sutka et al., 2006), overlap across microbial taxa can confound the final \(\text{N}_2\text{O}\) product value.

On a global scale, previous research concerning the behavior of \(\text{N}_2\text{O}\) isotopes is limited. Observations regarding both the temporal change and seasonal patterns of the tropospheric \(\text{N}_2\text{O}\) isotopic signature remain inconsistent (Toyoda et al. 2013), and there has yet to be published research comparing \(\text{N}_2\text{O}\) isotopes across latitudinal gradients. This is due in part to the labor- and time-intensive analytical method using traditional isotope ratio mass spectrometry. However, new technology utilizing cavity ring-down spectroscopy (CRDS) involves the continuous and simultaneous measurement of both \(\text{N}_2\text{O}\) mixing ratio and isotopomers without the need for pre-concentration, thereby enabling the large throughput of samples required for atmospheric monitoring.

1.1 Objectives

Given the remaining uncertainties of global \(\text{N}_2\text{O}\) dynamics and the information that can be gained from site-specific isotopes, my main objective of this project is to measure the \(\text{N}_2\text{O}\) mixing ratio and isotopic species from sites within NOAA/ESRL Cooperative Sampling Network across a latitudinal gradient. Discrete flask samples are collected in pairs on a weekly basis at most sites, and analyzing at least one year of samples at sites
capturing a variety of source signals (e.g. marine, industrial, or arctic environments) will provide answers to the following questions:


2. How do sites with different source signals differ in terms of N₂O isotopic seasonal cycle and interannual trends?

3. Does the CRDS analyzer have the precision needed to resolve global N₂O isotopic signals?

I hypothesize that long term monitoring of N₂O isotopes can lead to a better understanding of global changes to the nitrogen cycle, and one year of data collection will provide a proof of concept regarding analytical precision and seasonal and latitudinal variability. Although there are known changes in δ¹⁵N_{bulk}-N₂O since pre-industrial times (Bernard et al., 2006; Park et al., 2012; Sowers et al. 2002), changes in the isotopomer signal remain uncertain (Toyoda et al. 2013), and therefore more frequent and spatially varied measurements should help to resolve this uncertainty. Since an isotopic seasonal cycle was observed at Cape Grim, Tasmania (Park et al., 2012), yet no significant isotopic trends were observed at Hateruma Island, Japan by Toyoda et al. (2013), I hypothesize that significant differences will be observed at different NOAA sites both in terms of intra-annual variability and annual means. Additionally, prototype testing of the new Picarro CRDS analyzer revealed analytical uncertainty equal to reported isotopomer precision using IRMS yet greater than that of IRMS for δ¹⁵N_{bulk}. I therefore hypothesize that the CRDS analyzer is adequate for
isotopomers and $\delta^{15}\text{N}_{\text{bulk}}$ on decadal time scales, but may not meet the precision needed to detect $\delta^{15}\text{N}_{\text{bulk}}$ intra-annual variability.
CHAPTER 2

BACKGROUND AND LITERATURE REVIEW

2.1 The Global Nitrogen Cycle

2.1.1 Nitrogen in Terrestrial Systems

The two major pathways of nitrogen inputs to soil are via nitrogen fixation and nitrogen deposition. Biological nitrogen fixation is the energy intensive reduction of atmospheric N$_2$ to NH$_3$. This process utilizes the nitrogenase enzyme and is mainly carried out by symbiotic prokaryotes associated with legumes, cyanobacteria, and heterotrophic bacteria (Sprent and Sprent, 1990). Ammonia is a major contributor to both wet and dry nitrogen deposition (Asman et al., 1998; Flechard and Fowler, 1998) and can lead to increased soil and aquatic acidification due to H$^+$ release from post-depositional reactions (ApSimon et al., 1987). Beyond the other prominent reactive forms of N deposition, namely NO$_x$ produced from fossil fuel combustion and nitrate, dissolved organic nitrogen in precipitation is now suggested to contribute to roughly 30% of global N deposition (Cornell et al., 2003; Jickells, 2006).

Organic soil N is also converted to NH$_3$ through the process of mineralization. Additional plant- and microbe- available N can be derived from polymers which, once
depolymerized to monomers, is directly available to plants. This additional mechanism challenges the classical paradigm of plant-microbe competition through immobilization (Schimel and Bennet 2004). While mineralized N in the form of NH$_4^+$ can be immobilized, nitrification can also occur, which is an oxidative process whereby NH$_4^+$ is first oxidized to NH$_2$OH (hydroxylamine), which is then converted to NO$_2^-$ (nitrite) and further oxidized to NO$_3^-$ (nitrate). Gross mineralization rates are dependent on the stoichiometry of soil microorganisms: a greater soil organic matter C:N compared to the energy needs of microorganisms will lead to greater competition with plants and higher immobilization rates, whereas lower C:N increases soil N availability, leading to greater rates of mineralization and nitrification.

Nitrogen is lost from the soil though several mechanisms including denitrification, leaching, ammonia volatilization, and abiotic removal. Denitrification is a series of anaerobic reactions in which NO$_3^-$ is reduced to N$_2$ with the intermediate products of NO$_2^-$, NO, and N$_2$O. Nitrate leach rates are ultimately controlled by soil moisture, though biomass and soil characteristics such as texture determine moisture levels throughout the soil profile (Singh and Sekhon, 1979). Ammonia volatilization occurs more frequently in soils with high pH and is also correlated with soil temperature, soil moisture, and soil drying rate (Ernst and Massey, 1960). Fertilizers containing urea and ammonium based fertilizers also lead to large ammonia losses from agricultural fields (Bussink and Oenema, 1998).

2.1.2 Nitrogen in Marine Systems

Similar to terrestrial systems, nitrogen takes many oxidized and reduced forms in the ocean and is controlled by redox conditions and microbial energy needs. In shallow
depths of the open ocean, a loop occurs in which NH$_4^+$ produced from N$_2$ fixation and NO$_3^-$ assimilation is concurrently immobilized by phytoplankton and mineralized back to NH$_4^+$ via viruses and grazers (Zehr and Kudela, 2011). In deeper depths, particulate nitrogen is also mineralized to NH$_4^+$, which is then converted to NO$_3^-$ through nitrification in chemolithotrophs (Zehr and Kudela 2011). NO$_3^-$ is circulated to higher depths through upwelling and advection and can be converted to NH$_4^+$ via assimilation, whereby the nitrate reductase enzyme reduces NO$_3^-$ to NH$_4^+$ in order to create amino acids and build biomass in a variety of microorganisms (Zehr and Kudela 2011). While N$_2$ fixation and the NH$_4^+$ immobilization and mineralization loop also occur in the oxygen minimum zones of the ocean, denitrification, anammox, and dissimilatory nitrate reduction to ammonia (DNRA) are additional anaerobic processes that mainly occur in anoxic or suboxic conditions. Denitrification, for example, mainly occurs in benthic sediments and in parts of the oxygen minimum zone water column (Naqvi et al., 2008). Anammox, or anaerobic ammonia oxidation, is a more recently discovered process that is also found to occur in suboxic conditions and plays a major role in the removal of oceanic nitrogen through N$_2$O and N$_2$ (Kuypers et al. 2003, 2005; Thamdrup & Dalsgaard, 2002).

2.1.3 Anthropogenic Changes to the Nitrogen Cycle

While agriculture is the primary cause of the increase in reactive nitrogen since pre-industrial times, fossil fuel emissions are the second largest contributor of anthropogenic N and produce NO$_x$ mainly from combustion in vehicles and electricity generating units (Galloway et al., 2004). This has led to numerous environmental concerns, including water and air pollution, losses in biodiversity, and global climate change (Vitousek et al., 1997), as
well as human health concerns including methemoglobinemia (Townsend et al., 2003, Townsend and Howarth, 2010). Increased nitrogen deposition, for example, can lead to eutrophication (Vitousek et al., 2007) and soil acidification (Högberg et al., 2006). NO\textsubscript{x} in combination with VOCs forms tropospheric ozone and particulate matter, both of which adversely affect the environment and human respiratory health (Galloway et al., 2004).

The natural oceanic nitrogen cycle has also been altered considerably by human activity. Duce et al. (2008) estimate that in 2000, 80% of the total \( \sim 67 \) Tg N yr\(^{-1}\) of N\textsubscript{r} deposited in the ocean was anthropogenic and is projected to increase substantially in the coming decades. The obvious primary implication of this added source of oceanic N\textsubscript{r} is increased net primary productivity, which Duce et al. (2008) calculate to be an order of magnitude greater than the amount prior to 1860. While this increase in productivity may lead to increased oceanic CO\textsubscript{2} uptake, confounding variables such as reduced carbon immobilization due to stoichiometric constraints (Riebesell et al., 2007) and reduced productivity due to ENSO-driven stratification increase (Behrenfeld et al., 2006) complicate predictions of the ocean’s response to anthropogenic N\textsubscript{r} inputs.

### 2.2 Production Pathways of Nitrous Oxide

As one major form of reactive nitrogen, N\textsubscript{2}O is contributing to the increase in environmental problems caused by the anthropogenic augmentation of the nitrogen cycle, most notably climate change and stratospheric ozone loss. Nitrous oxide concentration has increased from roughly 270 ppb in pre-industrial times (Machida et al., 1995) to over 325 ppb today due to anthropogenic activities including agriculture, fossil fuel combustion,
industry, cattle and feedlots, and biomass burning (Flückiger et al., 1999; Forster et al., 2007; Thorne et al., 2009). Agricultural activity contributes 215.9 Mt CO₂ equivalent to the atmosphere each year, which is approximately 68% of all N₂O emissions in the United States (U.S. EPA 2010). Clearly there is a need to respond to and mitigate this continuing increase in N₂O from both an ecological and societal perspective. Gaining a better understanding of the main sources responsible for N₂O production, and the ways in which agricultural practices can reduce this production, will aid in this remediation.

2.2.1 Soil Microbial Processes

The two main pathways involved in soil production of N₂O include nitrification and denitrification. Nitrifiers produce N₂O as a byproduct during NH₂OH oxidation as well as through the reduction of nitrite, which is referred to as nitrifier denitrification. N₂O is also formed as an intermediate product during the reduction of NO₃⁻ to N₂ during denitrification. Although bacterial nitrification and denitrification are still considered to be the dominant N₂O producing processes in soil, many other mechanisms are now known to contribute to N₂O formation, although scaling the relative contributions of each process to regional or global scales remains a major challenge given its spatiotemporal variability. Additional sources beyond nitrification and denitrification include abiotic processes such as chemical decomposition of NH₂OH, chemodenitrification, and ammonium nitrate decomposition; anaerobic processes including dissimilatory nitrate reduction to ammonium (DNRA) and co-denitrification of organic N compounds with NO and N₂O; and coupled nitrification-denitrification in which nitrate produced by nitrite oxidizers is immediately denitrified in situ by denitrifiers (Butterbach-Bahl et al., 2013).
Ammonia oxidizing bacteria are mainly comprised of the γ and β Proteobacteria, with Nitrosomonas and Nitrosospira being the main genera within the β class (Purkhold et al., 2000). Until recently, bacteria were thought to be the main domain responsible for nitrification; however, there is now evidence that archaea are also an important contribution to this process (Prosser & Nicol 2008). Through the recovery of amo (ammonia monooxygenase) genes, mesophillic crenarchaea have been found to be ubiquitous among both marine and terrestrial environments (e.g., Wuchter et al., 2006, Leininger et al., 2006, Nicol et al., 2008). In terms of the relative abundance of nitrifying archaea to bacteria, Leninger et al. (2006) found that archaeal amoA genes were more abundant than bacterial amoA in the majority of sampled soils. While this may suggest a greater role of archaeal nitrification, these results do not take into account functionality; in other words, it is unknown whether or not this gene is operating or if it is as multifunctional as bacterial amoA.

Denitrifying microbes are far more phylogenetically diverse than nitrifiers, consisting of various groups of bacteria, archaea, and fungi (Kizawa et al., 1991; Zumft, 1993). In general, denitrification is a property of gram-negative, motile bacteria; however they have been found to belong in all major physiological groups with the exceptions of Enterobacteriaceae, obligate anaerobes, and gram-positive bacteria (excluding Bacillus spp.; Zumft, 1993). The most well-studied denitrifiers include Pseudomonas denitrificans, Pseudomonas perfectomarinus, and Paracoccus denitrificans (Stein and Yung, 2003). The key enzyme in the dissimilatory denitrification process is nitrite reductase, which is used during the reduction of nitrite to nitric oxide. The two genes, nirK and nirS both encode for this enzyme, producing cytochrome cd1- and copper-containing nitrite reductase,
respectively. nirK is only found among 30% of denitrifiers, yet is found to be more physiologically diverse and more present in soil systems (Coyne et al., 1989). nirS is more widely distributed, yet is more predominant in marine sediments (Braker et al., 2000).

2.2.2 Environmental Controls

Adding to the complexity of understanding N₂O production at larger scales, numerous environmental conditions affect these processes simultaneously. As with any microbially-mediated biogeochemical process, such factors include climate, substrate availability, and soil chemical and physical conditions. For example, soil moisture content is a major control over nitrification and denitrification, generally decreasing and increasing these processes with greater soil moisture, respectively (Davidson, 1991; Davidson, 1993). In general, soils with a water filled pore space (WFPS) of 40-60% usually produce N₂O via nitrification (Linn and Doran, 1984), while wetter soils with a WPFS of over 80% usually emit N₂O from denitrification (Smith and Arah, 1990). However, broad generalizations about moisture controls over both pathways can be misleading, as shown by multiple studies (e.g. Bollmann and Conrad, 1998; De Klein and Van Longtestijn, 1996). The relative dominance of nitrification versus denitrification for N₂O production can also vary with time. For example, Peréz et al. (2001) found that denitrification remains the dominant producer of N₂O up to three days following irrigation, while nitrification prevails after the fifth day. Such temporal variation in the predominant pathway (as well as overall rates) can combine with spatial variation in control agents such as soil texture, temperature, soil carbon, pH, substrate C:N, and overall substrate availability (Bollmann and Conrad, 1998; Kuenen and Robertson, 1994; Mosier, 1998).
All of the above is true in unmanaged systems, but fertilizer additions and other management practices in agricultural landscapes create even greater spatiotemporal variation, as well as the largest soil effluxes of N$_2$O on earth (Johnson et al., 2005; Matson et al., 1998; Venterea and Rolston, 2000). Most of those large fluxes, however, tend to occur in small fractions of the landscape, and at distinct (but short) time periods. In other words, soil N$_2$O emissions are notorious for displaying a substantial non-normal distribution in time and space, greatly challenging our ability to quantify and model emissions at scales of relevance to farmers.

Although the correlation between increased agriculture and soil-emitted N$_2$O remains fairly obvious, other indirect influences on the N$_2$O budget including climate warming, land use change, and changes in the hydrologic cycle are more complex. An increase in temperature should generally increase rates of soil microbial activity, which should effectively increase N$_2$O emissions regardless of substrate availability. A decrease of 0.5 ppb yr$^{-1}$ in the N$_2$O growth rate between 1991 and 1993 can be partly contributed to a decrease in Northern Hemispheric temperature, as well as decreased fertilizer use and changes in stratospheric circulation from the Mt. Pinatubo eruption (Thompson et al., 1994). Land use change might also play a major role in the N$_2$O emission rate, although this effect has yet to be quantified on a global scale (Folland et al., 2001). Since tropical ecosystems are the largest natural source of N$_2$O, the continued rates of rapid conversion to agriculture and pastureland could potentially decrease emission rates (Peréz et al., 2000).

While changes in soil moisture affect overall N$_2$O emissions, it is difficult to determine what effects, if any, global warming-induced changes in the hydrologic cycle have had on the N$_2$O budget. Peréz et al. (2000) found that less N$_2$O was emitted from dry
season soil in Brazil compared with the wet season, although a maximum difference of ~7 ppm was observed between two different wet season years. Although global precipitation has increased by 2% since the beginning of the 20th century, major spatial and temporal variations exist, including a statistically insignificant increase in wet season rainfall in northern Amazonia and parts of Brazil (Hulme et al., 1998). Due to this variability, it is unlikely that changes in precipitation patterns have influenced N2O mixing ratios or isotopic signatures due to climate warming, and instead increased irrigation should be considered as having a greater impact on the N2O budget.

2.2.3 Microbial Community Controls

In addition to environmental controls on soil processes, biological factors such as microbial community structure need to be considered in attempting to quantify N2O emissions. In particular, the effects of fertilizer addition on microbial communities can also play a role in the processes responsible for N2O production. Although there are numerous studies describing fertilizer effects on plant communities (e.g., Gilliam, 2006; Gough et al., 2000; Stevens and Carson, 2002; Suding et al., 2005), this interaction is not as well understood for soil microbes. Studies on diversity have shown that ammonia oxidizer populations are more diverse in non-fertilized systems versus improved soils (Webster et al., 2002). This is thought to be due to the negative effects associated with decreased pH levels. However, Ramirez et al. (2010) did not observe this correlation consistently, with only one of the two study sites exhibiting this relationship. They postulate that the controls over diversity may differ from those over community structure, with pH only affecting diversity at or below 6.5.
These community shifts could have the potential to influence N₂O emissions depending on whether or not nitrifying and denitrifying communities are responding to excess nitrogen inputs. Avrahami et al. (2002) looked at both the shifts in bacterial communities and N₂O emitted from soils with varying ammonium additions. Neither diversity nor structure changed significantly with ammonium inputs; however, these samples were incubated at only 4°C for 4 weeks, which is much colder than an average growing season soil temperature in the environment. In contrast to the lack of response with ammonia oxidizers, denitrifying communities did exhibit a significant shift in structure. Although a shift did not occur among ammonia oxidizers, it is possible that the incubation time of only 4 weeks was not long enough to allow for a change in this slow-growing group. Denitrifiers, on the other hand, are relatively fast-growing organisms, and therefore a shift in structure should occur within this short time period.

In terms of N₂O emission, Avrahami et al. (2002) produced similar results to previous studies (e.g., Müller et al., 1998; Schuster and Conrad, 1992; Skiba and Smith, 2000), showing an overall increase in N₂O released from more fertilized soils, and a shift toward nitrification-dominated N₂O emission. This is a result of increased nitrification levels due to increased ammonium, which thereby increases the nitrate substrate needed for denitrification. The lack of a community shift in ammonia oxidizers from increased fertilizer suggests that N₂O emissions are controlled mainly by physiology. However, more research needs to be done concerning the possibility of longer-term shifts in slow-growing ammonia oxidizers.
2.3 Nitrous Oxide in the Troposphere

2.3.1 Seasonal and Interannual Variability

The atmosphere currently contains over 325 ppb of nitrous oxide, which has been increasing in the atmosphere at a steady rate of 0.80 ppb yr\(^{-1}\) since the 1970's (Forster et al., 2007). The mixing ratio of N\(_2\)O is greater in northern versus southern latitudes and seasonal cycle amplitudes range from ± 0.3 to 0.9 ppb depending on location (Liao et al., 2004; Neison et al., 2011; Fig. 2.0). These amplitudes are all less than 1% of the total mixing ratio, which is consistent with the relatively long lifetime of N\(_2\)O in the atmosphere at roughly 114 years (Montzka et al., 2003). The late summer decline in N\(_2\)O in the northern hemisphere is attributed mainly to stratospheric exchange of N\(_2\)O-poor air (Morgan et al., 2004; Neison et al., 2004; Neison et al., 2011) and is in direct contrast to the increased biological emission of N\(_2\)O that is expected in the summer. This exchange is due to the Brewer-Dobson circulation in the winter, which leads to the introduction of air from the middle and upper stratosphere into the lower troposphere (Holton et al., 1995; Neison et al., 2004; Liang et al., 2008, 2009). While Neison et al. (2011) found strong correlations at most northern sites between N\(_2\)O mole fraction and stratospheric temperature, Trinidad Head, California exhibited a stronger correlation with Pacific Northwest upwelling. In the southern hemisphere, N\(_2\)O is more seasonally variable and a weaker Brewer-Dobson circulation occurs. While Liao et al. (2004) were not able to detect a significant seasonal amplitude in the southern hemisphere using NOAA CCGG discrete flask data, Neison et al. (2011) concluded that the stratosphere does contribute significantly to the seasonality of southern hemispheric N\(_2\)O using AGAGE data, with N\(_2\)O minima occurring in the fall.
Figure 2.0: (a) N₂O mixing ratio values measured by NOAA/ESRL GMD since 1977, separated into latitudinal bins, from: [http://www.esrl.noaa.gov/gmd/hats/combined/N2O.html] (b) Mace Head, Ireland N₂O seasonal cycle using monthly means from NOAA CCGG and AGAGE networks (from Nevison et al., 2011)

2.3.2 Global Sources and Sinks of N₂O

Of the estimated 18.3 Tg N-N₂O emitted to the atmosphere in 2000, natural sources including soil, ocean, and atmosphere accounted for 57% of total emissions, while anthropogenic sources including direct and indirect agriculture, energy, industry, biomass burning, and anthropogenic ocean inputs were responsible for 43% of total sources (Syakila and Kroeze, 2011; Table 2.0). Prather and Ehhalt et al. (2001) estimated that the pre-industrial source was only 10.7 Tg N-N₂O yr⁻¹, implying that anthropogenic activity...
largely contributed to this increase if natural sources remained unchanged. According to the IPCC Fourth Assessment Report, anthropogenic contributions including fossil fuel combustion, agriculture, biomass and biofuel burning, human excreta, and estuaries make up 61% of the total natural sources (Denman et al., 2007), whereas the more recent aforementioned estimates by Syakila and Kroeze (2011), which take into account indirect agricultural emissions in both soils and oceans, make up 74% of natural sources. Agricultural sources account for 63% of all anthropogenic N₂O sources, and 27% of total sources (Syakila and Kroeze 2011). IPCC guidelines underestimate N₂O emissions, and it is possible that deep ocean anthropogenic source that Syakila and Kroeze (2011) account for may help to refine current inventory estimates.
<table>
<thead>
<tr>
<th>Source</th>
<th>Estimate (Tg N₂O yr⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Natural</strong></td>
<td></td>
</tr>
<tr>
<td>Soils</td>
<td>6.6</td>
</tr>
<tr>
<td>Ocean</td>
<td>3.8</td>
</tr>
<tr>
<td>Atmospheric chemistry</td>
<td>0.6</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>11</strong></td>
</tr>
<tr>
<td><strong>Anthropogenic</strong></td>
<td></td>
</tr>
<tr>
<td>Direct agriculture</td>
<td>4.1</td>
</tr>
<tr>
<td>Land deposition</td>
<td>0.4</td>
</tr>
<tr>
<td>Ocean deposition</td>
<td>0.2</td>
</tr>
<tr>
<td>Rivers, estuaries, coastal zones</td>
<td>0.6</td>
</tr>
<tr>
<td>Fossil fuel combustion/industry</td>
<td>0.7</td>
</tr>
<tr>
<td>Biomass/biofuel burning</td>
<td>0.7</td>
</tr>
<tr>
<td>Human excreta</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>6.9</strong></td>
</tr>
<tr>
<td><strong>Source Total</strong></td>
<td><strong>17.9</strong></td>
</tr>
<tr>
<td><strong>Pre-industrial source total</strong></td>
<td><strong>10.7</strong></td>
</tr>
</tbody>
</table>

Table 2.0: N₂O source estimates for the year 2006, from Ciais et al. 2013

Once emitted to the troposphere, N₂O is transported to the stratosphere and eventually destroyed either by photodissociation or the reaction with electronically excited oxygen atoms (O(¹D)), accounting for 90% and 10% of its destruction, respectively (Crutzen and Oppenheimer, 2008; Minschwaner et al., 1993). A small soil surface sink was identified by Syakila and Kroeze (2011), which occurred in severely nitrate-limited soils. Soil denitrifiers can use N₂O as an electron acceptor in these conditions. While this sink is considered negligible in the global N₂O budget, it is possible that this sink could influence a
smaller national or regional scale, as this sink occurs mostly at temperate to high latitudes (Syakila and Kroeze, 2011).

2.3.3 Human and Environmental Effects of Increased N₂O

N₂O can lead to an increase in tropospheric ozone, as nitric oxides formed in the stratosphere from the reaction with O(^1D) and N₂O can be transported to the troposphere. The following series of reactions with NOₓ lead to ozone formation (Revell et al. 2012):

\[
\begin{align*}
\text{OH} + \text{CO} + \text{O}_2 & \rightarrow \text{HO}_2 + \text{CO}_2 \\
\text{HO}_2 + \text{NO} & \rightarrow \text{NO}_2 + \text{OH} \\
\text{NO}_2 + h\nu & \rightarrow \text{NO} + \text{O} \\
\text{O} + \text{O}_2 + \text{M} & \rightarrow \text{O}_3 + \text{M}
\end{align*}
\]

Surface level ozone can lead to crop damage (Chameides et al., 1994) and human respiratory problems (von Mutius, 2000). Additionally, N₂O can lead to stratospheric ozone destruction catalytically through NOₓ, which is formed from the reaction between O(^1D) and N₂O (Crutzen 1970):

\[
\begin{align*}
\text{NO} + \text{O}_3 & \rightarrow \text{NO}_2 + \text{O}_2\text{NO} + \text{O}_3 \rightarrow \text{NO}_2 + \text{O}_2 \\
\text{NO}_2 + \text{O} & \rightarrow \text{NO} + \text{O}_2\text{NO}_2 + \text{O}_3 \rightarrow \text{NO}_3 + \text{O}_2 \\
\text{O}_3 + \text{O} & \rightarrow 2\text{O}_2\text{NO}_3 + h\nu \rightarrow \text{NO} + \text{O}_2 \\
2\text{O}_3 & \rightarrow 3\text{O}_2
\end{align*}
\]

The decrease in stratospheric ozone can cause several detrimental human health effects due to the increase in the UV-B component of solar ultraviolet radiation (UVR). The prevalence of melanoma continues to increase due to excessive UVR exposure and is projected to double within the next ten years (Norval et al., 2007). Additionally UV-B
radiation can cause both acute and chronic effects on eye health such as photoconjunctivitis and cataract, respectively, as well as overall immunosuppression (Norval et al., 2007). Although N₂O has become the leading ozone depleting substance due to the drastic decrease in CFC production after the Montreal Protocol, confounding factors such stratospheric cooling leading to an increased N sink (Rosenfield and Douglass, 1998) and a decrease in NOₓ caused by the projected strengthening of the Brewer-Dobson circulation (Fomichev et al., 2007) may reduce the effectiveness of N₂O in stratospheric ozone destruction (Revell et al., 2012).

2.4 Nitrous Oxide Stable Isotopes

Given the spatiotemporal heterogeneity of N₂O emission from soil, determining the relative contributions of nitrification-derived N₂O versus denitrification-derived N₂O can help elucidate the biological controls over emission. Traditional methods of partitioning these aforementioned sources usually involve incubation studies using acetylene inhibition, which is used to block N₂O emitted from nitrification. Not only are these methods invasive, but they can also be inaccurate due to the disruption of the natural system. Stable isotopic approaches, however, are non-invasive and can be useful tools for partitioning sources of N₂O. This technique stems from the fact that various processes exhibit unique isotopic signatures due to biological and/or physical isotopic discrimination, i.e. fractionation. Stable isotopic measurements are reported relative to a known standard as a delta value in units of per mil (‰):

\[ \delta = ((R_{sample}/R_{standard}) - 1) \times 1000 \]
where R = heavy/light isotope. Stable isotopes of both nitrogen and oxygen in N₂O are measured relative to atmospheric N₂ and O₂, both of which are reported as 0‰.

Measuring the intramolecular, or site-specific, position of ¹⁵N (¹⁵N¹⁴N¹⁶O versus ¹⁴N¹⁵N¹⁶O) is the most recent advancement in N₂O stable isotope research, and holds promise for providing greater constraints over N₂O dynamics. The terminal and central N atoms are referred to as the β and α positions, respectively, of this asymmetric molecule (N-N-O). The site-specific position is measured as site preference (SP):

\[
SP = \delta^{15}N^\alpha - \delta^{15}N^\beta
\]

The combined position-independent ¹⁵N-N₂O signal is referred to δ¹⁵Nbulk:

\[
\delta^{15}N_{\text{bulk}} = (\delta^{15}N^\alpha + \delta^{15}N^\beta) / 2
\]

While ¹⁵N¹⁴N¹⁶O and ¹⁴N¹⁵N¹⁶O are referred to as isotopomers, and ¹⁴N¹⁴N¹⁶O can be referred to as an isotopologue of ¹⁴N¹⁴N¹⁸O, neither term is accurate when used to compare SP and δ¹⁸O values. Toyoda et al. (2013) recently proposed using the term ‘isotopocule’ to describe all isotopically substituted species of a molecule. However, the term ‘isotopomer’ can be used accurately to describe both SP and δ¹⁵Nbulk values, and will be used throughout this discussion.

2.4.1. δ¹⁵N_{bulk} and δ¹⁸O

The delta values of bulk ¹⁵N-N₂O are distinctly different between production via nitrification versus denitrification, ranging from -45‰ to -66‰, and -13‰ to -28‰, respectively (Barford et al., 1999; Pérez et al., 2001; Webster and Hopkins, 1996; Yoshida, 1988). The reasons for this difference lie mainly in the substrate values, consumption of N₂O in denitrification, and the diffusivity of N₂O out of the soil. The latter two processes
leave behind heavier molecules, enriching the leftover N\textsubscript{2}O, which is therefore why denitrification-derived N\textsubscript{2}O is far more enriched in \textsuperscript{15}N than from nitrification. $\delta^{15}\text{N}_{\text{bulk-N}_2\text{O}}$ from soil is most depleted in agricultural systems (Opdyke et al., 2009; Park et al., 2011; Pérez et al., 2001) and most enriched in natural temperate climates (Bol et al., 2003; Yamulki et al., 2001; Table 2.1). Pérez et al. (2001) found that N-fertilized soils emit N\textsubscript{2}O that is more depleted on average in \textsuperscript{15}N than from non-fertilized soils. This $^{15}\text{N}$ depletion is mainly due to the ability of bacteria to discriminate against heavier isotopes more easily with increased substrate availability.

In the ocean, N\textsubscript{2}O production was once thought to be mainly attributed to the activity of ammonia-oxidizing bacteria (AOB) either through $\text{NO}_3^-$ reduction to $\text{NO}_2^-$ during nitrification or during nitrifier-denitrification (Ostrom et al., 2000), and to some degree denitrifier activity in suboxic or anoxic regions of the ocean. However, the isotopic signature of N\textsubscript{2}O derived from AOB is far more depleted in $\delta^{15}\text{N}$ (-68‰ to -10‰; Frame and Casciotti, 2010; Yoshida, 1988) than the overall marine signal of -6‰ to 21‰ (Toyoda et al., 2013), suggesting that other sources producing a more enriched signal need to be accounted for. Santoro et al. (2011) found that the ammonia oxidizing archaea (AOA) enrichment culture CN25 produces N\textsubscript{2}O with bulk $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values similar to the overall marine signal, emphasizing the need quantify archaeal N\textsubscript{2}O emissions and incorporate them into N\textsubscript{2}O budget estimates.
<table>
<thead>
<tr>
<th>Pool</th>
<th>$\delta^{15}N_{\text{bulk}}$-$N_2O$ Signature</th>
<th>Process</th>
<th>$\delta^{15}N_{\text{bulk}}$ Enrichment Factor ($\varepsilon = \delta_{\text{product}} - \delta_{\text{substrate}}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil</td>
<td>Tropical forest: -18.0‰ to -6.6‰ (Park et al., 2011; Perez et al., 2000)</td>
<td>Nitrification</td>
<td>-66‰ to -45‰ (Stein and Yung, 2003)</td>
</tr>
<tr>
<td></td>
<td>Agricultural field: -41.5‰ to -5.3‰ (Opdyke et al., 2009; Park et al., 2011; Perez et al., 2001)</td>
<td>Nitrifier Denitrification</td>
<td>-35.1‰ ± 2.7‰ (Sutka et al., 2003; 2004)</td>
</tr>
<tr>
<td></td>
<td>Grassland: -6.1‰ to -3.4‰ (Bol et al., 2003; Yamulki et al., 2001)</td>
<td>Denitrification</td>
<td>NO$_3^-$ $\rightarrow$ N$_2$O: -10‰ to -37‰</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>N$_2$O $\rightarrow$ N$_2$: -7‰ to -30‰ (Park et al., 2011)</td>
</tr>
<tr>
<td>Ocean</td>
<td>-6‰ to 21‰ (Toyoda et al., 2013)</td>
<td>Nitrification</td>
<td>Same as soil</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Denitrification</td>
<td>Same as soil</td>
</tr>
<tr>
<td>Fossil fuels/Industry</td>
<td>-19.7‰ ± 27.7‰ (Toyoda et al., 2013) Coal: 1.8‰ (Ogawa and Yoshida, 2005b)</td>
<td>Thermal decomposition</td>
<td>-3.6‰ (Ogawa and Yoshida, 2005b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hydrogen reduction: N$_2$O + H $\rightarrow$ N$_2$ + OH</td>
<td>1.2‰ (Ogawa and Yoshida, 2005b)</td>
</tr>
<tr>
<td>Biomass Burning</td>
<td>-8‰ (Ogawa and Yoshida, 2005a)</td>
<td>Thermal decomposition: N$_2$O+M $\rightarrow$ N$_2$+O+M</td>
<td>-3.6‰ (Ogawa and Yoshida, 2005a)</td>
</tr>
<tr>
<td>Troposphere</td>
<td>6.69‰ (Toyoda et al., 2013)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stratosphere</td>
<td>10.0‰ (Toyoda et al., 2013)</td>
<td>N$_2$O photolysis</td>
<td>-43.0‰ to -16.0‰ (Toyoda et al., 2004)</td>
</tr>
</tbody>
</table>

Table 2.1: Reported $\delta^{15}N_{\text{bulk}}$-$N_2O$ values from the major pools of $N_2O$, as well as enrichment factors of the processes within pools.

The $^{18}O$ signature of $N_2O$ can be used along with bulk $\delta^{15}N$ values to differentiate between $N_2O$ produced from nitrification versus denitrification, as $N_2O$ reduction to NO removes lighter $^{18}O$ from the $N_2O$ pool, producing $\delta^{18}O$-$N_2O$ values that are more enriched than that of nitrifier-produced $N_2O$ (Snider et al. 2012). $\delta^{18}O$-$N_2O$ produced via nitrification in soil ranges from 13‰ to 35‰ (Snider et al., 2012), yet $\delta^{18}O$-$N_2O$ from denitrification is
much less constrained given the reported fractionation range of over 30‰ during N₂O reduction (Wahlen and Yoshinari, 1985; Wada and Ueda, 1996). δ¹⁸O values are additionally challenging to interpret since both soil air and soil water are used as oxygen sources during nitrification, which can have distinctly different ¹⁸O values (Schmidt and Voerkelius, 1989).

Beyond characterizing the isotopic signature of natural sources of N₂O, Toyoda et al. (2008) estimated the δ¹⁵Nbulk and δ¹⁸O values of automobile emissions to be -4.9 ± 8.2‰ and 43.5 ± 13.9‰, respectively. The new car used in the study produced a more enriched signal in δ¹⁵Nbulk, which is an indication of N₂O reduction caused by new three-way catalysts. This range of δ¹⁵Nbulk values is lower than the average δ¹⁵Nbulk of the troposphere, and may aid in quantifying the automobile contribution to the global N₂O budget. Additionally, this range is significantly lower than the δ¹⁵Nbulk value range of coal combustion (9.2 ± 5.3‰; Ogawa and Yoshida, 2005b), and therefore the relative contribution of these industrial sources of N₂O can be better elucidated using δ¹⁵N.

While most terrestrial sources of N₂O are more isotopically depleted than the average tropospheric value of 6.69‰ (Toyoda et al., 2013), photochemical reactions in the stratosphere favor the destruction of lighter N₂O molecules, producing a remaining enriched N₂O pool (δ¹⁵Nbulk = 10.0‰; Toyoda et al., 2013) that mixes back into the troposphere through to stratosphere-troposphere exchange (STE). The enrichment factor for stratospheric photolysis of N₂O ranges from -43.0 ± 3.7‰ to -16 ± 0.6‰, with lighter values observed in the higher tropopause (15.8 km) and heavier values at lower altitude (15.0 km; Toyoda et al., 2004).
Firn air and ice core bulk $\delta^{15}$N measurements can be used to confirm bottom-up approaches to the isotopic fractionation factors in the biosphere. These measurements are useful mainly for determining the temporal changes in $N_2O$, which capture anthropogenic alterations since pre-industrial times. Bernard et al. (2006) sampled firn air and ice cores from Greenland and Antarctica, measuring the isotopic values of total $^{15}$N and $^{18}$O, as well as site-specific $^{15}$N since 1700 AD. All isotopes exhibited depletion through time, ranging from $-1.6\%$ for $\delta^{18}$O to $-2.8\%$ for $\delta^{15}$N. Sowers et al. (2002) also found $\delta^{15}$N and $\delta^{18}$O to be more enriched in the past than the present tropospheric averages. Air aged between 1785 and 1819 AD trapped in Greenland ice cores was determined to be $1.9\%$ and $2.9\%$ heavier in $\delta^{15}$N and $\delta^{18}$O, respectively, than current values. This confirms the findings of Pérez et al (2001), supporting the hypothesis that an increase in agriculture and fertilizer use continues to deplete the $\delta^{15}$N signature of the troposphere. Toyoda et al. (2013) also confirmed this depletion in $\delta^{15}$N$_{\text{bulk}}$ since 1999, but found the rate of depletion to be smaller than the aforementioned previous estimates. This enrichment could be due to the recent increase in manure versus synthetic fertilizer (Toyoda et al., 2011b), which is projected to continue throughout this century (Bouwman et al., 2011). Therefore, monitoring the change in tropospheric $\delta^{15}$N$_{\text{bulk}}$-$N_2O$ on decadal timescales is essential to confirm these potential shifts in microbial $N_2O$ production.

2.4.2 Intramolecular position of $^{15}$N- $N_2O$

Although bulk $\delta^{15}$N and $\delta^{18}$O values can aid in better distinguishing source and sink processes of $N_2O$, several limitations of this approach exist. Most notably, it is difficult to constrain the reduction rate of $N_2O$ to $N_2$ during denitrification, producing a wide range of
Additional confounding variables include the variability of substrate isotopic values (Tilsner et al., 2003) and N₂O reduction reaction rate constants (Vieten et al., 2007). Unlike δ¹⁵Nbulk-N₂O, it has been shown that SP values are independent of the substrate's isotopic composition in microbial processes (Popp et al., 2002; Toyoda et al., 2002; Sutka et al., 2006).

NO₂⁻ reduction by *Nitrosomonas multiformis* and other denitrifiers produce N₂O with a site preference near 0‰ (Sutka et al., 2003, 2004, 2006; Toyoda et al., 2005). A site preference near 30‰ has been observed in NH₄⁺ fertilized soil (Well et al., 2008) and from pure culture studies of the soil nitrifiers *Nitrosomonas europaea, Nitrosospira multiformis,* and *Methylosinus trichosporium* during hydroxylamine oxidation and ammonia oxidation (Sutka et al., 2006), as well as from the archaeal enrichment culture CN25 (Santoro et al., 2011). Frame and Casciotti (2010) also found the SP of marine ammonia oxidizing bacteria to vary substantially from 36.3‰ during hydroxylamine oxidation to -10.7‰ during nitrifier-denitrification depending on O₂ concentration (Fig. 2.1).
The difference in SP is thought to arise from discriminatory enzymatic processes during the formation of \( \text{N}_2\text{O} \) in nitrification. Nitric oxide reductase is the enzyme responsible for creating \( \text{N}_2\text{O} \) by forming \( \text{N} = \text{N} \) from two NO molecules. Stein and Yung (2003) hypothesized that a sequential binding of each NO molecule to the NOR enzyme will lead to a preference for \( ^{14}\text{N}^{b} \) compared with simultaneous binding. An NOR sequential binding pathway in nitrifying bacteria versus simultaneous binding for denitrifiers would support the previous results; however, limited evidence exists on these enzymatic
processes. Toyoda et al. (2002) also support the N-O break of hyponitrite (ONNO⁻) as the cause of δ¹⁵Nα enrichment. Schmidt et al. (2004) emphasize that the enrichment in δ¹⁵Nα-N₂O and δ¹⁸O-N₂O produced from nitrification processes is not actually a product of nitrification per se, but rather the form of nitric oxide reductase present. The cNOR enzyme systems from denitrifiers (e.g. Paracoccus denitrificans) utilize the simultaneous, or parallel NO reduction process, whereas the P450nor NORs characteristic of fungal denitrifiers sequentially reduce the two NO molecules, leading to a site preference. A major limitation to this approach is that nitrifier denitrification shares a similar preference with the reduction of NO₃⁻ and NO₂⁻ during denitrification (-0.5‰) (Sutka et al. 2006). In this case, the site preference approach needs to be used in conjunction with total ¹⁵N so that the total δ¹⁵N signature of denitrification can first be determined before partitioning the two nitrifier processes.

Since the various microbial reactions involved in both nitrification and denitrification involve the formation and cleavage of the Nα-O bond, the δ¹⁵Nα preference is more pronounced compared with non-microbial sources of N₂O, and is reported as smaller than the tropospheric SP average across different soils and fertilizer regimes (Table 2.2). Perez et al. (2001) were the first to report site preference values from soil emissions, finding a 9.3‰ enrichment of Nα compared with Nβ between the day of irrigation to four days afterwards. Since nitrification generally increases with decreased soil moisture content, this implies that the microbial processes responsible for N₂O production during nitrification may exhibit a distinct fractionation factor relative to the central N atom. Bol et al. (2003) also found temporal differences in SP after fertilization application on grassland soil, suggesting that nitrification is the dominant N₂O emitting process directly after
fertilization but denitrification dominates after the initial application phase. Additionally, Park et al. (2011) determined denitrifiers to be the dominant producer of ‘background’ tropical forest N₂O emissions, yet the SP values were not as useful for differentiating between agricultural and natural systems versus the use of δ¹⁵N_{bulk}. 
<table>
<thead>
<tr>
<th>Pool</th>
<th>Site Preference-N₂O signature</th>
<th>Process</th>
<th>SP Enrichment Factor ((\varepsilon = \delta_{\text{product}} - \delta_{\text{substrate}}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil</td>
<td>Tropical forest: 9.7‰ ± 7.9‰ (Park et al., 2011)</td>
<td>Nitrification</td>
<td>(\delta_{\text{product}} = 31.4%) to 35.6‰ (Sutka et al., 2006) <strong>SP values independent of substrate</strong></td>
</tr>
<tr>
<td></td>
<td>Agricultural field: 1.5‰ to 9.3‰ (Opdyke et al., 2009; Park et al., 2011; Perez et al., 2001)</td>
<td>Nitrifier Denitrification</td>
<td><strong>SP values independent of substrate</strong> (\delta_{\text{product}} = 0.1%) (Sutka et al., 2006) (\delta_{\text{product}} = -0.5%) (Sutka et al., 2006) <strong>SP values independent of substrate</strong></td>
</tr>
<tr>
<td></td>
<td>Grassland: 2.2‰ to 6.7‰ (Bol et al., 2003; Yamulki et al., 2001)</td>
<td>Denitrification</td>
<td></td>
</tr>
<tr>
<td>Ocean, rivers, estuaries</td>
<td>Sub-trop NP: 0 to 8‰ (Popp et al., 2002) ETNP: 11.0 ± 1.2‰ (Yamagishi et al., 2007) Western NP: 35.7‰ (Toyoda et al., 2002) RE: 23.3‰ (Toyoda et al., 2013)</td>
<td>Nitrification</td>
<td>Same as soil</td>
</tr>
<tr>
<td>Fossil fuels/Industry</td>
<td>17.8‰ ± 5.6‰ (Toyoda et al., 2008)</td>
<td>Thermal decomposition</td>
<td>(\varepsilon N\alpha = 14.3%) (\varepsilon N\beta = -21.6%) (Ogawa and Yoshida, 2005b)</td>
</tr>
<tr>
<td></td>
<td>Hydrogen reduction: N₂O + H (\rightarrow) N₂ + OH</td>
<td>Thermal decomposition: N₂O+M(\rightarrow)N₂+O+M</td>
<td>(\varepsilon N\alpha = 12.5%) (\varepsilon N\beta = -10.1%) (Ogawa and Yoshida, 2005b)</td>
</tr>
<tr>
<td>Biomass Burning</td>
<td>-2.7‰ ± 1.9‰ (Toyoda et al., unpublished)</td>
<td>Thermal decomposition: N₂O+M(\rightarrow)N₂+O+M</td>
<td>(\varepsilon N\alpha = 14.3%) (\varepsilon N\beta = -21.6%) (Ogawa and Yoshida, 2005b)</td>
</tr>
<tr>
<td>Troposphere</td>
<td>18.45‰ (Toyoda et al., 2013)</td>
<td>N₂O photolysis</td>
<td>(\varepsilon^{(15)N\alpha} = -59.1%) to -22.2‰ (\varepsilon^{(15)N\beta} = -33.1%) to -7.1‰ (Toyoda et al., 2004)</td>
</tr>
<tr>
<td>Stratosphere</td>
<td>21‰ (Toyoda et al., 2013)</td>
<td>N₂O photolysis</td>
<td></td>
</tr>
</tbody>
</table>

Table 2.2: Reported SP values from the major pools of N₂O, as well as enrichment factors of the processes within pools.
Limited studies have been conducted assessing the temporal variability of SP in the troposphere, both on a seasonal and interannual level. Röckmann and Levin (2005) found a depletion in $\delta^{15}\text{N}_{\text{bulk}}, \delta^{15}\text{N},$ and $\delta^{15}\text{N}-\text{N}_2\text{O}$ from 1990-2002 (-0.04‰ ± 0.003‰, -0.014‰ ± 0.016‰, and -0.063‰ ± 0.014‰, respectively, indicating an increase in SP) using archived Antarctic air samples but did not assess seasonal variability due to the sparse dataset. Röckmann et al. (2003), however, did not detect any significant change in N$_2$O SP from Antarctic firn air, but this may have been due to firn modeling and/or instrument uncertainty. Park et al. (2012) detected both a seasonal and interannual trend in $\delta^{15}\text{N}_{\text{bulk}}$ and SP using both Antarctic firn air and archived air samples from Cape Grim, Tasmania. SP showed a positive yet statistically insignificant temporal trend. While Park et al. (2012) attribute this increase to a possible overall increase in fertilizer-induced nitrification, the smaller recent depletion in $\delta^{15}\text{N}_{\text{bulk}}$ measured by Toyoda et al. (2013) was instead speculated to be due to an increase in denitrification from manure use. In addition to this discrepancy, Toyoda et al. (2013) did not detect any clear temporal trend in SP from their sampling site at Hateruma Island (HAT), Japan.

Park et al. (2012) and Toyoda et al. (2013) also produced conflicting results concerning the potential seasonal cycle of N$_2$O isotopomers. Park et al. (2012) found a significant season cycle at Cape Grim for $\delta^{15}\text{N}_{\text{bulk}}, \delta^{18}\text{O},$ and $\delta^{15}\text{N}$ (peak-to-peak amplitudes of ~0.8‰, ~0.14‰, and ~0.75‰, respectively), which are all negatively correlated with N$_2$O mixing ratio. The authors assign this relationship to the stratospheric influence of N$_2$O -poor and -enriched air in the local fall, and the increase in N$_2$O -rich and -depleted emission from ocean ventilation in the local summer. They also emphasize that the large seasonal amplitude of $\delta^{15}\text{N}$ could not be due solely to stratosphere-troposphere exchange,
but rather is a result of these two processes, as well as seasonal changes in ocean temperature and N\textsubscript{2}O solubility. Toyoda et al. (2013), however, were not able to detect a seasonal cycle at HAT for any isotopomer or isotopologue, as these values were neither in phase with N\textsubscript{2}O mixing ratio nor were the amplitudes larger than their instrumental precision. This site may have been more influenced by terrestrial and/or anthropogenic sources, causing greater variability from the annual mean. This lack of a coherent intra-annual trend could also simply mean that the fluxes influencing the change in mixing ratio may not be large enough to significantly influence the isotopomers. Therefore it is clear that uncertainties remain regarding temporal and spatial variation in N\textsubscript{2}O isotopomers, which may be resolved with increased sampling frequency and location.

2.4.3 Isotope Ratio Mass Spectrometry

Given the identical masses of the major N\textsubscript{2}O isotopologues (mass-to-charge ratios (m/z) of 44, 45, and 46) compared to CO\textsubscript{2}, $\delta^{15}$N\textsubscript{bulk} can easily measured using isotope ratio mass spectrometry (IRMS). On the other hand, position-specific $\delta^{15}$N is not as straightforward due to the identical masses of these isotopomers. Due to electron ionization of N\textsubscript{2}O in the ion source, NO\textsuperscript{+} fragments are produced and can be quantified by tuning to a m/z of 30 ($^{14}$N\textsubscript{16}O\textsuperscript{+}) and 31 ($^{15}$N\textsubscript{16}O\textsuperscript{+} and $^{14}$N\textsubscript{17}O\textsuperscript{+}). This technique was originally employed in 1953 using enriched N\textsubscript{2}O from ammonium nitrate thermal decomposition (Friedman and Bigeleisen, 1953), yet it was not until 1999 that isotopomers were measured using IRMS at natural abundance (Toyoda and Yoshida, 1999; Brenninkmeijer and Röckmann, 1999). Assuming that the NO\textsuperscript{+} fragments always contain the alpha position N, $\delta^{15}$N\textsubscript{a} can be measured directly by correcting for the $^{14}$N\textsubscript{17}O
isotopologue detected at m/z 31. Santrock et al. (1985) determined that $^{17}$O naturally varies with $^{18}$O in a linear fashion using the equation:

$$
\delta^{17}O = k(\delta^{18}O)
$$

where $k \approx 0.5$. Therefore, the $\delta^{18}$O-$N_2O$ can be determined using this equation and both the m/z 45 ($^{14}$N$^{14}$N$^{17}$O, $^{14}$N$^{15}$N$^{16}$O, and $^{15}$N$^{14}$N$^{16}$O) and 46 measurement ($^{14}$N$^{14}$N$^{18}$O, $^{14}$N$^{15}$N$^{17}$O, $^{15}$N$^{14}$N$^{17}$O, and $^{15}$N$^{15}$N$^{16}$O), which can then be used to calculate the $^{15}$N$^{16}$O$^+$ contribution of m/z 31 (Brenninkmeijer and Röckmann, 1999).

While these calculations are relatively straightforward, there are several uncertainties involved, including mass independent fractionation of $^{17}$O. $^{17}$O excess anomalies from the expected aforementioned relationship have been observed in tropospheric N$_2$O, presumably from heavy O$_3$ in gaseous ammonia degradation (Röckmann et al., 2001). Given that the abundance of $^{17}$O is $\sim$one tenth of that of $^{15}$N, and because the $^{17}$O excess is known to be roughly equal to 1‰ in atmospheric N$_2$O, this interference is not known to be a major source of error. Of much greater concern is the known scrambling effect of ions within the IRMS ion source. The reaction of O($^{1}$D) + N$_2$O can yield NO$^+$ containing $\beta$ position N, and it is possible for O($^{1}$D) to form from the ion source filament (Brenninkmeijer and Röckmann, 1999). This contribution of ion source-derived NO$^+$ needs to be quantified using pure enriched isotopomers, which have been shown to be consistent under normal operating conditions at 8-9% of measured NO$^+$ (Toyoda and Yoshida, 1999; Brenninkmeijer and Röckmann, 1999).

2.4.4 Cavity Ring-down Spectroscopy
Cavity Ring-down spectroscopy (CRDS) is a method of analyzing both trace gas mixing ratios and isotopomers using optical absorption. The theoretical foundation behind this method includes Beer-Lambert’s law:

\[ A = 1 - \frac{I}{I_o} \]

where \( A \) = light absorbance through a gas and \( I \) = light intensity, such that:

\[ I(\lambda) = I_o(\lambda) e^{(-\alpha L)} \]

where \( \lambda \) = wavelength of the light, \( \alpha(\lambda) \) = absorption coefficient (usually measured in cm\(^{-1}\)), and \( L \) = distance through the gas. The absorption coefficient is dependent on the specific gas composition, pressure, and temperature of the optical system, and \( \alpha \) (ppm/cm) changes linearly with different gas mixture compositions such that:

\[ \alpha(\lambda) = \sum \alpha_i(\lambda) \]

The absorption of a particular gas species is dependent on its vibrational and rotational energy states, and these states are determined by weight, configuration, and binding energies of the atoms within a molecule (Rothman et al., 2009). When transmission resonance occurs, the laser light builds up in the cavity, and the laser is then shut off once the intensity reaches a threshold as determined by the cavity electronics. Light then begins to decay exponentially, which occurs at a faster rate with more gas absorbance, and visa versa. This absorption can be quantified using the following equation:

\[ \tau = \frac{1}{(c \alpha_c)} \]

where \( \tau \) = cavity ring down time, \( c \) = speed of light, and \( \alpha_c \) = absorption coefficient. In general, longer ring down times are desirable in order to optimize the number of sample points taken as the laser is tuned across the desired spectral frequencies. Each decay event corresponds to an absorbance and wavelength value, as determined by the wavelength
monitor. Ultimately, the concentration of the gas species of interest is derived from the integrated area under the absorption line peak. While the height of the peak correlates linearly with gas concentration, the width and shape is dependent on temperature, pressure, and gas matrix and therefore it is important to hold these conditions constant. This technique allows for direct measurement of isotopomers since each one presents a unique absorption line.

The Picarro G5101i-i Analyzer utilizes a mid-infrared quantum cascade laser capable of wavelength emission from 3.44 to 84 μm. Nitrous oxide absorbs in the mid-infrared wavelength range (3.5-5 μm). The laser housing includes a thermoelectric cooling device (TEC), heat spreader, and optimal lens used to collimate and direct the laser light to the optical detector. The TEC is based on the Peltier effect, where electric current through the junction of two different metals produces a change in temperature (Day and Arnone, 2011). The laser is focused through the optical detector into the optical cavity, where three highly reflective (> 99.9%) mirrors are positioned such that the beam travels around the cavity in a closed path. Power transmission is optimized when the incoming light oscillations are in phase with previously circulating light. In order to obtain wavelength transmission resonance, the laser is tuned either by changing the laser wavelength or the cavity length via mirror positioning. The analyzer operates at a flow rate of ca. 30 sccm and can handle an N₂O mixing ratio of 0.3 to 1 ppm with sampling pressure between 300 and 1000 Torr. A ring down is performed at a rate of 25 MHz (Balslev-Clausen, 2011), which corresponds to an effective path length of 8 kilometers. Sampling points from the ring down events are used to create a fitting curve, which corresponds to one sample point (Fig. 2.2). These points are created approximately every 4 seconds.
2.4.5 $\delta^{15}$N-$N_2O$ Calibration

Currently no international standard exists for $N_2O$ isotopologues. While it is possible to directly calibrate $\delta^{15}$N$_{\text{bulk}}$-$N_2O$ to a primary standard, previous methods used to calibrate position-specific $\delta^{15}$N have resulted in large discrepancies between laboratory groups, even when performing similar calibration methodologies (Westley et al., 2007). The primary calibration of $\delta^{15}$N$_{\text{bulk}}$ involves the decomposition of $N_2O$ to $N_2$, which can then be analyzed on an IRMS versus the international $N_2$ air standard. Kaiser et al. (2003) performed the complete conversion of $N_2O$ to $N_2$ and $O_2$ using a pure gold tube as a catalyst heated to over $930^\circ$C, which is then frozen on silica gel and separated using a GC before being introduced into a mass spectrometer. $\delta^{18}$O was measured against an $O_2$ working
standard calibrated versus vSMOW. A negligible amount of $\delta^{18}O$ converted to CO$_2$ in this process, however Kaiser et al. (2003) did not detect any significant fractionation.

Westley et al. (2007) compared the two major calibration methodologies for site-specific $\delta^{15}N$: thermal decomposition of ammonium nitrate versus the trace addition of position specific labeled N$_2$O. While both methods were found to be problematic in terms of characterizing the ion source scrambling effect, Westley et al. (2007) were able to successfully replicate and subsequently recommend the ammonium nitrate approach. The ammonium nitrate thermal decomposition calibration approach of Toyoda and Yoshida (1999) involves the heating of NH$_4$NO$_3$ to 225-230°C in order for the following reaction to occur:

$$5\text{NH}_4\text{NO}_3 \rightarrow \text{NNO} + 2\text{H}_2\text{O}$$

This method of N$_2$O production is advantageous because the $^{15}N$ at the alpha position originates entirely from nitrate, while ammonium is responsible for the beta position (Friedman and Bigeleisen, 1950). Sutka et al. (2003) first performed the labeled calibration approach using N$^{15}$NO as a tracer, yet Kaiser et al. (2004) found various errors in their methods, including issues in the volumetric measurement of their labeled standard gases and their slope correction. Kaiser et al. (2004) eliminated the need for volumetric addition of the isotopic tracer by first introducing their reference gas into both the reference and sample bellows of a dual-inlet IRMS followed by the addition of doubly labeled N$_2$O ($^{15}N^{15}$NO) to the sample bellows only.

Theoretically, the use of doubly labeled N$_2$O eliminates the problems associated with the ion source scrambling effect since mass 31 will form from regardless of whether the $^{15}N$ originated from the alpha or beta position of the original N$_2$O molecule.
Additionally, they quantified and corrected for the purity of their labeled tracers unlike Sutka et al. (2003) by solving for both $N_c(^{15}N^{14}N^{16}O)/N_c(^{15}N^{2^{15}}O)$ $N_c(^{14}N^{15}N^{16}O)/N_c(^{15}N^{2^{16}}O)$, where $N_c$ equals the number of molecules from the double labeled tracer, by assuming that these ratios are equal to one another. A mixing series of working standards and labeled tracer was measured against the original working standard, and a linear least squares regression slope of both and $45d$ versus $46d$ and $31d$ versus $46d$ was then applied to the theoretical calculations of the site specific $^{15}N$. The final absolute calibration of the working standards was achieved by first adjusting the addition of the tracer impurity ratios mentioned above in order to fit the $45d$ versus $46d$ slope, and then by adjusting the beta position ratio to fit the measured $31d$ versus $46d$ slope. This method requires the accurate calibration of both $\delta^{18}O-N_2O$ and $\delta^{15}N^{bulk}-N_2O$ of the working standard. The delta values Kaiser et al. (2004) obtained for free tropospheric $N_2O$ using this technique differ greatly compared to Yoshida and Toyoda (2000), which the authors mainly attributed to potential errors in their working calibration to the primary isotopic reference materials air-$N_2$ and VSMOW. Westley et al. (2007) point out that both Kaiser et al. (2004) and Toyoda and Yoshida (1999) fail to take into account not only the different rates at which each isotopomer of $N_2O$ forms the fragment NO+ ion in the ion source, but also how ion source conditions including pressure and accelerating voltage play a role in fragmentation rates.
CHAPTER 3

SAMPLING AND ANALYSIS METHODOLOGY

3.1 NOAA Flask Network

The NOAA/ESRL Global Monitoring Division (GMD) Cooperative Sampling Network is a global air sampling network that consists of over 70 sites sampled weekly. These discrete samples are collected at the land surface as pairs of 2.5 L glass flasks from NOAA baseline observatories, cooperative fixed sites, and commercial ships. The sample vessels are two-port 2.5 L Pyrex flasks equipped with glass-piston stopcocks sealed with Teflon PTFE O-rings (Maserie, 2001). Using a portable battery-powered pump, flasks are filled in series as pairs by flushing and pressurizing to approximately 20 kPa above ambient pressure (Maserie, 2001). Four different water vapor removal methodologies are used during the filling procedure (Table 3.0).
These samples are then sent back to NOAA where they are measured for mixing ratios of several gas species including CO$_2$, CH$_4$, CO, H$_2$, N$_2$O, and SF$_6$. In addition, stable isotopes of CO$_2$ and CH$_4$ are also measured at CU-Boulder’s Institute of Arctic and Alpine Research (INSTAAR) Stable Isotope Laboratory. Nitrous oxide concentration is measured at the Greenhouse Gas Measurement Lab at NOAA using a gas chromatograph. As a World
Meteorological Organization, the NOAA GMD is considered a Central Calibration Laboratory for N$_2$O, and therefore all measurements are tied to standard gases that have been calibrated for N$_2$O concentration. A subset of 44 sites were originally used for this project, with 19 of those sites containing complete annual datasets (Fig. 3.1).

Figure 3.1: Subset of the NOAA CCGG Cooperative Sampling Network sites used to measure N$_2$O isotopomers. Sites highlighted in red denote complete annual datasets (n = 19).

3.2 Flask Measurement Methodology

Three instruments were utilized in this project: The initial instrument (Instrument A) was in operation from the spring to mid-December of 2013 until the TEC failed. Instrument B operated from January through May of 2014. Over this time period, the long term uncertainty was determined to be larger than Instrument A and it was determined
that the laser cavity temperature was not stable, also due to the coolbox TEC malfunctioning. The final repair of Instrument B is referred to as Instrument C due to significant differences in performance.

Each sample run includes an internal standard (MULD-001), a ‘known-unknown’ quality control standard (hereby referred to as the ‘trap’ standard; SCUL-001), and the paired flask samples. The runs are structured such that the internal and trap standards run before and after batches of no more than 8 samples (4 pairs). This ensures that the internal standard runs roughly every 2 hours in order to account for cavity drift and instability. Eight sample flasks are attached to a manifold using Swagelok Ultra-Torr vacuum fittings. The sample extraction system consists of the flask manifold, a beaded glass water trap submerged in a -62°C ethanol bath, and a rotary vane vacuum pump. Swagelok ¼ turn plug valves and couplings connect the pump, manifold, standard gases, and Ultra Zero Air (AirGas, Inc.) to the inlet of the Picarro G5101-i via ⅛” stainless steel tubing. The beaded glass water trap is connected to this tubing by Swagelok Ultra-Torr vacuum fittings. Each gas cylinder is fitted with stainless steel, high-purity single stage regulator (AirGas Inc. model Y11-C444A CGA) equipped with a Kel-F seat and attached to the extraction line with a Swagelok VCO fitting. A Swagelok ¼ turn plug valve is connected to the inlet of the Picarro G5101-i in order to toggle between the extraction system and the Ultra Zero Air (Fig. 3.2).
While the extraction system is evacuated to < 0.02 mbar, the inlet valve is open to Ultra Zero Air at 5 PSIG. Once the evacuation is complete, the extraction line opens to MULD-001, and standard air flows at 5 PSIG through the beaded glass water trap up through the inlet turn valve in order to pressurize the line. Standard gases flow through the same system as the samples in order to follow the Identical Treatment principle of standard and reference materials (Werner and Brand, 2001). The inlet valve is then opened to the extraction system, and the gas-mixing ratio is monitored manually as it enters the laser cavity and stabilizes. The sampling time begins roughly 5 minutes after the sample first enters the inlet, although this time can vary for flask samples due to low pressure. Once the N₂O mixing ratio value has stabilized, the sample runs for a total of 5 minutes. A spreadsheet is used during the run, along with the online NOAA/ESRL/GMD Session Builder program created by Ken Maserie, to record each sample ID, fill date, and time of analysis.
3.3 Instrument Calibration

3.3.1 Laboratory Intercomparison

Eleven laboratories including INSTAAR’s Stable Isotope Lab (SIL) participated in an intercomparison of N\textsubscript{2}O isotopic calibration in order to assess the current agreement of N\textsubscript{2}O isotopomer values between laboratories, led by laboratories at Empa (Dübendorf, Switzerland) and the Tokyo Institute of Technology (Mohn et al., 2014). Three standard gases (S1, S2, and Target) calibrated by Tokyo Tech were analyzed without knowing the calibrated isotopic or mixing ratio values. First, due to the limited mole fraction range of the Picarro G5101-i and in order to minimize linearity effects, standards S1 and S2 were first diluted to ambient concentration using two 1 L Mylar bags fitted with a Swagelok ¼ turn plug valve and a rubber septum. The following procedure was done for both S1 and S2. One evacuated Mylar bag was filled directly with standard, while the other bag was filled with a blend of Ultra Zero Air (AirGas, Inc.) and standard. This was done by connecting the Mylar bag to an extraction line, vacuum pump, and Ultra Zero Air, as well as an inline septum and Swagelok ¼ turn plug valve placed directly upstream of the Mylar bag. The Mylar bag and extraction line were first flushed with Ultra Zero Air and evacuated. Using an airtight syringe, 5 ccs were removed from the first Mylar bag filled with standard. With the evacuated Mylar valve open and the valve closed upstream of the extraction line septum, the standard was injected. Once the Ultra Zero Air was introduced into the extraction line, the valve upstream of the extraction line septum was opened, allowing the air and standard to fill the Mylar bag. The Ultra Zero Air flowed at 5 psig for 46 seconds in order to obtain ambient N\textsubscript{2}O concentration. The Target gas was diluted slightly by 35.7 ppb on average by
first evacuating a Mylar bag, then filling it with Ultra Zero Air for 5 seconds at 5 psig, and then filling the bag with the Target gas.

The Mylar bags containing each standard were connected to the sampling line using Swagelok fittings. The sampling line includes a beaded glass water trap submerged in a -62°C ethanol bath. Samples were analyzed by a Picarro G5101-i, capable of simultaneous N,O concentration, bulk δ^{15}N-N,O, δ^{15}Nα-N,O, and δ^{15}Nβ-N,O measurement. Samples were introduced for 3 minutes followed by 5 minutes of analysis at a sampling interval of ~4.3 seconds and flowing at ~30 sccm. The sampling line was evacuated to < 0.02 mbar between each sample. Standards S1, S2, and Target were run against a pressurized whole-air internal reference from Niwot Ridge, Colorado (30 km west of Boulder, 3.5 km above sea level). Additionally, a quality control reference also filled at Niwot Ridge was run as a “known unknown.” These internal references have only been roughly calibrated through the Biogeochemistry and Paleoproteomics Laboratory at Michigan State University. For this preliminary calibration, one aliquot of each reference gas was added to a 500 mL glass flask, first flushed with 10 times the flask volume of the reference gas and measured by isotope ratio mass spectrometry using the methodology of Ostrom and Ostrom (2012).

One hundred second running means were taken for each 5-minute sample, then averaged to produce the raw sample value. The internal reference was measured at least 3 times throughout each run, as the first, last, and middle samples. A drift correction was first applied to the raw reference values. Samples were then corrected to the preliminary calibrated reference values. Absolute differences between each measured value and the calibrated value of the “known unknown” internal reference greater than 0.3 ppb, 1.1‰, 1.6‰, and 1.4‰ for N,O concentration, bulk δ^{15}N-N_{2}O, δ^{15}Nα-N_{2}O, and δ^{15}Nβ-N_{2}O,
respectively lead to a sampling flag. Sampling flag limits were determined by taking the standard deviation of the raw internal reference values for the prior 12 runs, which were then averaged and multiplied by 3. Standards S1, S2, and Target were measured over 3 runs with no sampling flags.

3.3.2 Calibration Methodology for CRDS

The absolute calibration of the G5101-i poses unique challenges given the lack of international isotopomer standards and the need for standard analysis at ambient mixing ratios. The latter challenge can be additionally problematic if the calibration is to be confirmed using an IRMS, since either pure N₂O or a high volume of ambient standard is likely needed. Therefore, a serial dilution method is warranted in order to achieve ambient concentration within a synthetic air matrix. Five hundred mL each of 98%+ δ¹⁵Nα and δ¹⁵Nβ standards were obtained from Cambridge Isotope Laboratories (Tewksbury, MA) in 75 cc stainless steel flasks fitted with a ¼” NPT Whitey valve. The following procedure is conducted for each isotopomer standard. The standard flask is attached to a Swagelok fitting with a rubber septum. This small volume is first evacuated and the standard flask Whitey valve is then opened to fill this volume. A gas tight syringe is used to remove 1 cc of this standard gas. A 300 mL stainless steel lecture flask is used as the first dilution vessel. This flask is attached to a stainless steel manifold with a septum connected to a vacuum pump and a cylinder of Ultra Zero Grade air (AirGas Inc.; 76.5 - 80.5% Nitrogen, 19.5 - 23.5% Oxygen). The manifold is first evacuated and the 1 cc of standard gas is injected into a small volume directly upstream of the evacuated lecture flask. The manifold and lecture flask then opens to the Ultra Zero Grade air at 100 PSIG such that the standard aliquot is
flushed along with the zero air into the lecture flask. The mole fraction of N₂O within this first dilution at 100 PSI of synthetic air is calculated to be 5.131e⁻⁴ mol/mol air. A second dilution is then performed in a similar manner: The 300 mL dilution vessel is attached to an evacuated small volume with a septum. 1.25 cc of diluted N₂O (2.86e⁻⁸ moles) is extracted using an air tight syringe. This aliquot is then introduced to the flushed and evacuated dilution vessel as mentioned in the first dilution method. This second dilution yields an N₂O mole fraction of 328.92 nmol/mol. This method can be performed with varying ratios of δ¹⁵Nα and δ¹⁵Nβ in order to create a range of isotopic standards.

3.4 Sample Value Corrections

The data points obtained from the Picarro G5101-i are considered ‘raw’ in that while a rough calibration was conducted during the manufacturing of the instrument, samples need to be corrected to a calibrated standard during each run in order to account for instrument drift. An original Microsoft Visual Basic Macro, as well as an original R script was created to process all data from this project. Raw DAT files from the instrument first needed to be converted to CSV format in order to be compatible with R, which was used for all run corrections. A Microsoft VB Macro was created to automate this process, since a standard full run produces ~8000 rows of data separated into 8 or 9 data files. The Macro script accepts the run number as the input, which corresponds to the folder name where the raw DAT files exist. The script then loops through each DAT file, delimiting and reformatting the columns, which are then saved as CSV files (Appendix, Table A1).

Similar to the Macro script, the only input needed in the run correction R script is also the run number. First each run CSV file is bound together into one data frame and the
dates are converted from Epoch time into easily interpretable date and time entries. The run spreadsheet created during analysis is then read into R and the times corresponding to each sample are matched with the corresponding data frame row numbers. A data frame is then created from the mean values and standard deviation of raw mixing ratio, $\delta^{15}N_{\text{bulk}}$, $\delta^{15}N_{\alpha}$, $\delta^{15}N_{\beta}$, water concentration, outlet valve value (a representation of flask pressure), Data Acquisition System (DAS) temperature, and cavity temperature. Individual sample precision of mixing ratio and isotopologue is also calculated by taking the standard deviation of each 100-second rolling mean. The final raw value of each sample is determined by taking the mean of each 100-second rolling mean value. The raw data correction is performed using the calibrated values of internal standard MULD-001 to create “virtual” raw standard values at each sample with the assumption that a linear drift occurs between standard measurements. The difference between calibrated MULD-001 values and the virtual values is then applied to the raw values of each sample. This script allows for automatic bulk reprocessing, as the entire script exists within a loop. Specific run numbers can be entered as needed and standard values can be easily changed and reprocessed.
CHAPTER 4

RESULTS AND DISCUSSION

4.1 Instrument Performance

4.1.1 Allan Variance

Allan variance is a commonly used metric in optical and laser applications for frequency stability. The Allan variance values provide information regarding the measurement noise over various averaging times and is expressed as:

\[
\sigma^2_A(\tau) = \frac{1}{2(m-1)} \sum_{i=1}^{m-1} [A_i(p) - A_{i+1}(p)]^2
\]

Where \( A_i(p) \) represents consecutive sections of data at an interval of \( p \) and a total observation time of \( \tau \). The Allan variance results are best represented by plotting the square root of variance, or Allan deviation, on a logarithmic scale. The smallest Allan deviation value corresponds to the time interval yielding the greatest instrument stability. Allan variance values representative of each instrument are shown in Table 4.0. Time intervals under 100 seconds were chosen, as 100 second running means are used to calculate sample values.
Among all three instruments, the smallest variance for N₂O mixing ratio and isotopic species occurred after 500 seconds, making this the ideal averaging time in terms of instrument noise reduction (Fig. 4.0). However, this is an unrealistic time interval to use for NOAA CCGG flask samples given their finite volume and the needs of other flask users. Instead, an averaging time of 100 seconds was determined to be a reasonable interval, yielding enough sample points for 150 mL of air and an Allan variance of less than 0.5‰ for each isotopomer and < 0.01 ppb for N₂O mixing ratio.

Table 4.0: Allan variance values calculated from ~24 hour analysis of standard cylinder SCUL-001 using the *allanvar* R package.

<table>
<thead>
<tr>
<th></th>
<th>Instrument A</th>
<th>Instrument B</th>
<th>Instrument C</th>
</tr>
</thead>
<tbody>
<tr>
<td>N₂O (ppb)</td>
<td>0.008</td>
<td>0.003</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>[67 sec.]</td>
<td>[59.5 sec.]</td>
<td>[64 sec.]</td>
</tr>
<tr>
<td>δ¹⁵Nα</td>
<td>0.43‰</td>
<td>0.53‰</td>
<td>0.43‰</td>
</tr>
<tr>
<td></td>
<td>[67 sec.]</td>
<td>[59.5 sec.]</td>
<td>[64 sec.]</td>
</tr>
<tr>
<td>δ¹⁵Nβ</td>
<td>0.48‰</td>
<td>0.43‰</td>
<td>0.30‰</td>
</tr>
<tr>
<td></td>
<td>[67 sec.]</td>
<td>[59.5 sec.]</td>
<td>[64 sec.]</td>
</tr>
</tbody>
</table>
4.1.2 Cavity Temperature Stability

Due to the peak shape dependence on cavity temperature and pressure, cavity temperature stability is necessary in order to produce reliable values using CRDS. Each of the three instruments produced significant differences in the mean cavity temperature of each sample (both flagged and unflagged flasks and cylinders were used in this dataset) (Fig. 4.1). The largest standard deviation of 0.023 °C across all runs occurred during the use of Instrument B, with a mean standard deviation of .0043 °C for each run. This is over three times greater than the mean standard deviation of each Instrument C run of 0.0012 °C. While Instrument A shared a similarly high mean standard deviation of 0.0030 °C, the
outliers towards the end of Instrument A’s lifetime skew this value, and therefore the median standard deviation of 0.0011 °C is more representative of the variation in cavity temperature. The larger instrument uncertainty of Instrument B is a clear result of its poor cavity temperature stability, and it is therefore essential to use cavity temperature as diagnostic parameter for instrument performance monitoring.

![Cavity temperature throughout the analysis of Instruments A, B, and C, represented by colors blue, red, and purple, respectively.

Figure 4.1: Cavity temperature throughout the analysis of Instruments A, B, and C, represented by colors blue, red, and purple, respectively.

4.1.3 Water Interference

Due to the presence of a water absorption line in the G5101-\textit{i} wavenumber spectrum (see section 2.4.4), water concentration causes a major interference in all raw isotopomer values. Eight pairs of NOAA flasks were analyzed with and without the ethanol water trap (hereby referred to as ‘dry’ and ‘wet’ samples, respectively) and with an equal amount of flasks analyzed with the trap first, and visa versa, in order to control for potential sample pressure bias. This experiment was also conducted for tank standards...
MULD-001 and SCUL-001 as a control, and the difference between groups was less than the instrument uncertainty in all N₂O species. Additionally, data were retained only for flasks without pair difference or analysis flags.

The mean water concentration during analysis of the wet flasks ranged from 0.32% from LLN (Method G; dried during sampling) to 2.60% from NAT (Method N; not dried), with sites HUN, KEY, NAT, BMW, and UTA also analyzed. A significant linear relationship exists for all isotopomers between wet water concentration and the value of wet minus dry corrected values (Fig. 4.2). Mixing ratio values are smaller for wet flasks compared to their dry values, and this difference increases as the water concentration increases. The opposite is true for δ¹⁵N⁰bulk-N₂O, δ¹⁵Nα-N₂O, and δ¹⁵Nβ-N₂O: all wet flask isotopomer values increase compared to their dry values, and this difference also increases as initial water concentration increases.
Figure 4.2: Linear relationship of flask water concentration and the difference between wet and dry corrected values of (a) N₂O mixing ratio (R² = 0.96; y = -4.53x + 3.16); (b) δ¹⁵Nbulk (R² = 0.95; y = 4.41x + 1.07); (c) δ¹⁵N (R² = 0.91; y = 4.82x + 2.0); and (d) δ¹⁵N (R² = 0.95; y = 3.99x + 0.15)

While the y intercept should be close to 0 for each species, this is only the case for δ¹⁵N, which is perhaps consistent with the fact that the water absorption line is closest to that of ¹⁵N¹⁴N¹⁶O. However, it is unclear why flasks analyzed without the water trap would have higher delta and mixing ratio values even with 0% water, especially given the
consistent values of the standard gases both with and without the trap. Regardless of this discrepancy, it is clear that the additional water trap used on this system is essential for drying flask samples completely, as even sites with sampling method codes indicative of condenser use (namely KEY, LLN, and BMW) exhibited high water values. The median water concentration of all unflagged flasks used in this project is 0.01%, yet even the wet BMW samples, which were supposedly dried during sampling, averaged 1.06% water. Although a water trap was used on all NOAA flask samples during this project, water and/or flask pressure (see Section 4.2.3) was a predictor of high pair differences and is a parameter that should be considered along with other instrument quality assurance indicators including drift and precision.

4.2 Data Quality Control

All samples within a run are subject to three quality control measures for N₂O mixing ratio and isotopomer: An analysis (‘A’) flag, a trap (‘T’) flag, and a pair (‘P’) flag. The ‘A’ flag is an indication of instrument drift between MULD-001 measurements within a sample run. The instrument’s long-term uncertainty is used to determine the drift limit, which is calculated as the overall mean of the standard deviation of each daily run’s MULD-001 raw values. For each run set, or the samples between each MULD-001 measurement, an ‘A’ flag is applied if the absolute value of the difference between MULD-001 values is greater than twice the instrument uncertainty. The ‘T’ flag is used to evaluate the accuracy of the analysis through the Trap performance. All samples in a run will get a ‘T’ flag if the difference between the mean of each corrected N₂O species Trap value and the calibrated value is greater than twice the long-term instrument uncertainty. A ‘N’ flag can also occur if
a Trap aliquot was not present in a run. Finally the ‘P’ flag ensures consistency within each NOAA flask pair. Three times the instrument long-term uncertainty is used as a threshold for pair difference. Pair differences may be an indication of issues with sampling or analysis error.

4.2.1 Analysis Flag Results

The mean standard deviations of MULD-001 per sample run is highest for all N₂O species during Instrument B analysis, and is especially larger than Instrument A and C in δ¹⁵Nβ uncertainty. Instrument A and C produced comparable uncertainties, with δ¹⁵Nβ performance slightly better during Instrument C analysis (Table 4.1). Although δ¹⁵Nα uncertainty was consistent across all three instruments, this was the largest out of each isotopic species for Instruments A and C and lowest for B.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>N₂O Mixing Ratio (ppbv)</td>
<td>0.08</td>
<td>0.18</td>
<td>0.08</td>
<td>0.12</td>
</tr>
<tr>
<td>δ¹⁵Nbulk (‰)</td>
<td>0.35</td>
<td>1.13</td>
<td>0.45</td>
<td>0.66</td>
</tr>
<tr>
<td>δ¹⁵Nα (‰)</td>
<td>0.78</td>
<td>0.86</td>
<td>0.76</td>
<td>0.81</td>
</tr>
<tr>
<td>δ¹⁵Nβ (‰)</td>
<td>0.57</td>
<td>2.27</td>
<td>0.43</td>
<td>1.18</td>
</tr>
<tr>
<td>SP (‰)</td>
<td>1.15</td>
<td>2.62</td>
<td>0.83</td>
<td>1.64</td>
</tr>
</tbody>
</table>

Table 4.1: Mean raw standard deviation of MULD-001 N₂O species throughout the lifetime of instruments A, B, and C, and overall mean standard deviation.

Instrument A maintained a standard deviation below 1.5‰ for each isotopomer until the problems arose with temperature instability towards the end of its lifetime (Fig. 4.3b).
Instrument B produced a significantly higher uncertainty in $\delta^{15}N^B$ and $\delta^{15}N^{\text{bulk}}$ compared to the other instruments steadily throughout its duration. Instrument C performed worse in $\delta^{15}N^C$ uncertainty compared to Instrument A prior to its cavity temperature malfunction, but otherwise maintained comparable uncertainties in the other isotopic species.
Fig. 4.3: Internal standard MULD-001 raw standard deviation of N$_2$O mixing ratio (a) and isotopomer (b) during each sample run.
4.2.2 Trap Flag Results

The performance of the Trap cylinder, SCUL-001, is an indication of instrument accuracy and drift over time (Fig. 4.4). In terms of accuracy, the Trap isotopomer values were only determined through a rough calibration and therefore the values corrected using internal standard MULD-001 may not be a reliable index. Both internal standards, however, were calibrated for N₂O mixing ratio through NOAA GMD/ESRL, and should therefore shed light on mixing ratio accuracy.

Across all instruments, 58% of Trap mixing ratio values fell within the range of its calibrated value ± instrument uncertainty, with Instrument C performing best at 74% of trap values retained within this range. Instrument A trap values were systematically higher than the calibrated value with 45% of values falling within the uncertainty range and 80% of values greater than calibrated. No drift during Instrument A is evident (simple linear model 1 regression, p = 0.83; R² = 6.8e⁻⁴). Instrument B also produced higher calculated values versus the calibrated value 69% of the time, and exhibited a slight drift towards higher mixing ratio values (p = 0.05, R² = 0.03). 84% of Trap values analyzed by Instrument C are lower than calibrated but no significant drift exists (p = 0.73, R² = 2.6e⁻³). Overall, significant drift towards a lower mixing ratio value did occur, yet a small R² indicates that there was a large amount of variation through all runs (p = 0.04, R² = 0.02).

Trap δ¹⁵Nbulk values exhibited a significant drift towards heavier values during Instrument A (simple linear model 1 regression; p = 9.2e⁻⁴, R² = 0.14). This is perhaps a result of the instrument temperature malfunction that occurred towards the end of its
lifetime. No significant drift in Δ15N\textsubscript{bulk} occurred during either Instrument B (p = 0.89, R² = 1.7e-4) or C (p = 0.17, R² = 0.04). Similarly, no significant slope occurred during all three instruments in Δ15N\textsuperscript{a} (simple linear model 1 regression of Instrument A; B; and C: p = 0.55, R² = 5.1e-3; p = 0.75, R² = 9.9e-4; p = 0.62, R² = 5.4e-3, respectively). Finally, Δ15N\textsuperscript{b} drift was evident in instrument A (p = 2.5e-5, R² = 0.22) and C (p = 0.04, R² = 0.09) but no drift occurred during analysis with instrument B (p = 0.99, R² = 6.7e-7). Since Δ15N\textsubscript{bulk} values are a product of both Δ15N\textsuperscript{a} and Δ15N\textsuperscript{b}, the drift in Δ15N\textsubscript{bulk} is most likely due to the instability of Δ15N\textsuperscript{b}.

![Figure 4.4: Trap standard SCUL-001 performance across each instrument. N₂O mixing ratio and isotopomer values corrected to internal standard MULD-001. Black lines represent calibrated value of each N₂O species; shaded area equals calibrated value ± instrument-specific uncertainty.](image)
4.2.3 Pair Flag Results

NOAA flask pair retention is much higher for isotopomer values than for N₂O mixing ratio. For each isotopomer, over 95% of the flask pairs were retained on the sole basis of pair difference, yet just over 75% of pairs analyzed on all three instruments were within the pair difference limits for N₂O mixing ratio (Table 4.2). The highest rate of mixing ratio value retention occurred during using Instrument B and was lowest during Instrument A. Less than half of flask pairs were retained after taking Analysis flagging into account during the use of Instrument A, with almost 60% ‘A’ and ‘P’ flag retention overall. Conversely, the highest isotopomer retention also occurred during Instrument A, although the isotopomer retention rates across instruments do not vary greatly compared to mixing ratio. Slightly more pairs were retained for isotopomers after both ‘A’ and ‘P’ flags at 64% compared to mixing ratio values.

<table>
<thead>
<tr>
<th>Inst.</th>
<th>Total Pairs</th>
<th>Unflagged Pair dif N₂O (%)</th>
<th>All Unflagged N₂O (Retained for A and P; %)</th>
<th>Retained δ¹⁵Nbulk (%)</th>
<th>Retained δ¹⁵Nα (%)</th>
<th>Retained δ¹⁵Nβ (%)</th>
<th>Retained SP (%)</th>
<th>All Unflagged Isotopomers (Retained for A and P; %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inst. A</td>
<td>452</td>
<td>66.6</td>
<td>47.8</td>
<td>95.8</td>
<td>97.6</td>
<td>99.1</td>
<td>97.8</td>
<td>64.4</td>
</tr>
<tr>
<td>Inst. B</td>
<td>387</td>
<td>86.3</td>
<td>75.7</td>
<td>98.5</td>
<td>98.7</td>
<td>99.7</td>
<td>99.4</td>
<td>63.6</td>
</tr>
<tr>
<td>Inst. C</td>
<td>165</td>
<td>76.4</td>
<td>52.1</td>
<td>100</td>
<td>100</td>
<td>99.4</td>
<td>99.4</td>
<td>62.4</td>
</tr>
<tr>
<td>Total</td>
<td>1004</td>
<td>75.8</td>
<td>59.3</td>
<td>97.5</td>
<td>98.4</td>
<td>99.4</td>
<td>98.8</td>
<td>63.8</td>
</tr>
</tbody>
</table>

Table 4.2: Retention rates of N₂O mixing ratio and isotopomer of NOAA flask pairs for each instrument. Only pairs without ‘A’ and ‘P’ flags in all isotopic species are retained for isotopomers.

Temporal flagging patterns throughout the duration of this project are evident in each N₂O species (Fig. 4.5). During the first ~10 runs of Instrument A, ‘A’ and ‘P’ flagging occurred frequently and there are clear deviations from average isotopic pair values. Pair
flags without additional Analysis flags are most common in $\text{N}_2\text{O}$ mixing ratio values and are rare in each isotopomer, especially after the initial problematic runs. Most isotopic pair removals are a result of only Analysis flags, which is concurrent with the high retention rates from pair differences.
Figure 4.5: NOAA flask performance for (a) N₂O mixing ratio; (b) δ¹⁵N
bulk; (c) δ¹⁵Nα; and (d) δ¹⁵Nβ as indicated by Pair flag, Analysis flag (± instrument uncertainty), and both Pair and Analysis flag. Left, center, and right shaded regions signify analysis by instruments A, B, and C, respectively.

In order to investigate the factors influencing the high number of N₂O mixing ratio pair flags, a two tailed two-sample t test assuming unequal variances was conducted on all flasks with and without a ‘P’ flag for both H₂O concentration and pressure. The mean H₂O concentration for unflagged and flagged flasks was 0.018% and 0.034%, respectively. With
a p-value of $= 2.1 \times 10^{-6}$ we can reject the null hypothesis that the two H$_2$O means are equal and therefore flagged flasks have a statistically greater H$_2$O concentration. However, both groups are not normally distributed and several high H$_2$O outliers exist. Therefore this may not be the sole predictor of pair differences. A statistical difference is also present for mean flask pressure during analysis ($p = 2.62 \times 10^{-16}$, with significantly greater values in the unflagged group (mean = 5.46 mbar) versus the flagged group (mean = 3.89 mbar). Flask pressure in both groups are more normally distributed than H$_2$O values, so therefore pressure may be a more reliable predictor of a mixing ratio pair difference.

Beyond looking at H$_2$O and pressure means between flagged and unflagged flasks, the mean H$_2$O and pressure differences between each pair can also shed light on the potential variables responsible for N$_2$O mixing ratio pair differences. In the flagged group, both the mean H$_2$O difference (0.015%) and pressure difference (2.49e$^{-17}$ mbar) were significantly greater ($p = 4.3 \times 10^{-4}$ and $p = 8.0 \times 10^{-7}$ for H$_2$O and pressure, respectively) than the H$_2$O and pressure differences in the unflagged group (0.003% and 6.99e$^{-22}$ mbar, respectively).

4.2.4 NOAA Mixing Ratio Comparison

Because the NOAA/ESRL CCGG group measures N$_2$O mixing ratio before analysis at INSTAAR, the comparison between the two laboratories and methodologies can provide additional quality assurance information. Across all CRDS instruments, 90.1% of flask values were greater than those measured by NOAA/ESRL CCGG (Fig. 4.6). The mean difference in values (INSTAAR – NOAA CCGG) is 0.46 ppb. Since the Trap values analyzed by Instrument A and B were also greater than calibrated, it is possible that the internal
standard MULD-001 was calibrated inaccurately. However, the majority flask values remained higher than NOAA CCGG values during analysis by Instrument C (Fig 4.6b), while 84% of Trap values were lower than calibrated.
Figure 4.6: Comparison of NOAA CCGG and INSTAAR Stable Isotope Lab N$_2$O mixing ratio analysis. (a) Mixing ratio values analyzed by both labs including NOAA CCGG outliers and sampling flags; (b) Mixing ratio value difference between labs with a line at difference = 0

While internal standard calibration may be a cause of this offset, the flask pressure may be an additional variable causing inaccurate mixing ratio values. The offset between NOAA CCGG and INSTAAR increases substantially below 5 PSI (Fig. 4.7a), and this offset is significantly greater (mean = 0.76 ppb) compared with flasks greater than 5 PSI (mean = 0.28 ppb, p << 0.05; Fig 4.7b). A simple linear model 1 regression reveals that a drift towards a smaller offset does exist for both low and high pressure flasks throughout the project (p = 2.4e-6, R$^2$ = 0.07; p = 6.0e-5, R$^2$ = 0.03, respectively). However, higher offsets occurring during analysis by Instrument A possibly due to temperature instability may influence this relationship given the low R$^2$ values in both groups.

The cause of this increase in calculated mixing ratio values in low pressure flasks could be due to the inability of the raw measurements to properly stabilize given the 300 Torr lower limit provided by the Picarro specifications. The offset could also be due to the inability of the ethanol trap to effectively remove water under lower pressure, thereby causing spectral interference in N$_2$O isotopomer absorption at the wavelengths optimized in the Picarro G5101-I analyzer.
Figure 4.7: Flask pressure relationship with NOAA CCGG offset: (a) Mixing ratio value difference as a function of mean flask pressure during INSTAAR analysis; (b) NOAA CCGG offset by INSTAAR analysis run number separated by mean flask pressure. Left, center, and right shaded regions signify analysis by instruments A, B, and C, respectively.
4.3 Latitudinal Trends

Out of the 19 retained sites, 14 were used to describe N₂O mole fraction and isotopomers across latitudinal and longitudinal gradients due to inconsistent start and end dates. The retained annual means of each N₂O species were calculated from a start date of 2013.3-2013.5 to end dates from 2014.3-2014.5. Flask values that differed more than two times the instrument uncertainty of the previous and subsequent sample were also manually removed as outliers. N₂O mole fraction exhibits statistically significantly different values between sites for both INSTAAR and NOAA/CCGG data (One-way Model 1 ANOVA; p < 0.05). The highest values occur in the northern mid-latitudes and the lowest values in the southern tropics, with the exception of Bukit Kototabang, Indonesia (Fig. 4.8a). The difference between the lowest annual mean at SMO (INSTAAR = 326.48 ppb ± 0.33; NOAA/CCGG = 326.22 ppb ± 0.46) and the highest at HUN (328.15 ppb ± 0.92; NOAA/CCGG = 327.72 ppb ± 0.85) is 1.67 ppb and 1.5 ppb as calculated by INSTAAR and NOAA/CCGG, respectively. This variation is roughly only 0.5% of the global mean mixing ratio. Across longitudes, the highest N₂O mixing ratios occur over the United States and Europe land masses with the exception of the CIB site, which is roughly 200 km northwest of Madrid, Spain (Fig. 4.12a). While most coastal sites have lower N₂O values, BKT, LLN, and KEY have mixing ratios above the global mean, which are all in closer proximity to combustion sources than the other coastal sites.
Figure 4.8: NOAA annual site means from start dates between 2013.3-2013.5 to end dates from 2014.3-2014.5 by latitude of (a) N₂O mixing ratio measured by NOAA CCGG and INSTAAR; (b) δ¹⁵N<sub>bulk</sub>; (c) δ¹⁵N<sub>α</sub>; (d) δ¹⁵N<sub>β</sub>, and (e) Site Preference.

To compare these rather sparse data to a more complete dataset, all terrestrial surface flask N₂O mole fraction data in 2010 was compiled, which excluded ocean sites and sites with the potential for a large industrial signal (including HPB, HUN, and OXK). A polynomial regression analysis was used to determine whether or not latitude predicts N₂O mixing ratio (Fig. 4.9). The R² value of 0.896 confirms that latitude can be used to predict
the vast majority of N\textsubscript{2}O values. The largest absolute residuals occur at higher latitudes beginning at 30°N, which may be explained by the influence of industry, such as LLN in Lulin, Taiwan, or by an agricultural influence, like at site SGP in South Great Plains, Oklahoma.

The reason that this trend is not linear, but rather sinusoidal, is due to several reasons. A small source due to the small amount of land mass and low temperatures explain the low N\textsubscript{2}O levels in the south. The concentration increases in the tropics due to heightened microbial activity and increased land mass. The N\textsubscript{2}O concentration is greatest in mid-northern latitudes due to agricultural and industrial activities, and then subsides at higher latitudes due to decreased temperature and anthropogenic activity, but remains higher than the concentration at low latitudes because of heightened stratospheric mixing and influence from the mid northern latitudes.

Hirsch et al. (2006) used inverse modeling to partition the relative amount of N\textsubscript{2}O emitted from different latitudinal regions, with 0 to 4%, 16 to 32%, 50 to 64%, and 16 to 23% emitted from -90° to -30°, -30° to 0°, 0° to 30°, and 30° to 90°, respectively. These relative contributions are consistent with both the 2010 and 2013 data, where the lowest concentrations are found in the most southern region of the world, which then increases and peaks at ~35°N, and then decreases again in the most northern latitudes. The anomalously high mixing ratio at Bukit Kototabang, Indonesia additionally confirms the conclusion by Hirsch et al. (2006) that the N\textsubscript{2}O signal from the tropics is continuing to increase above bottom-up inventory estimates.
Figure 4.9: $\text{N}_2\text{O}$ mole fraction (ppb) and latitude with overlay of line of best fit, determined by the third degree polynomial regression model: $\hat{Y} = 323.3 + 0.0195X - 9e^{-5}X^2 - 2.05e^{-6}X^3$

The isotopic variation across latitudes is also statistically significant (One-way Model 1 ANOVA; $p < 0.05$) for $\delta^{15}\text{N}^\text{bulk}$, $\delta^{15}\text{N}_\alpha$, $\delta^{15}\text{N}_\beta$, and SP. Lower SP values may be attributed to a biomass burning or soil signal, which is consistent with the lower than average values at LLN, SGP, and HUN. The SP mean at ICE is the lowest of all 14 sites, yet a higher value would be expected with a stronger stratospheric signal and lack of depleted anthropogenic sources in the arctic. Three anomalously high $\delta^{15}\text{N}_\beta$ flask pair values bring this mean 0.44‰ lower than it would be with these outliers removed (17.70‰ versus 18.14‰), but the mean is still lower than the overall mean across all sites (18.42‰).

While the uncertainty and pair difference flagging protocols removed most outliers, site IZO contains large variances in $\delta^{15}\text{N}_\alpha$ and SP (4.76‰ and 7.79‰, respectively), which is far greater than the medians of both species across sites (0.51‰ and 0.91‰,
respectively). With IZO completely removed as one of the 14 annual mean sites, the isotopic variation across latitudes remains significant, and additionally a significant negative correlation between $\delta^{15}\text{N}_{\text{bulk}}$ and N$_2$O mixing ratio is observed using both mixing ratio data from this project and from NOAA/ESRL CCGG (Pearson Product Moment correlation: $p = 0.03$ and 0.001, respectively; Fig. 4.10). This correlation is consistent with what we would expect across sites with varying terrestrial and marine inputs. The coastal sites in the Southern Hemisphere, SMO and NAT, are the most enriched in $\delta^{15}\text{N}_{\text{bulk}}$ with the smallest mixing ratio, which may be due to the enriched signal of marine N$_2$O (Popp et al. 2002, Toyoda et al. 2002) and the smaller mixing ratio signal in the Southern Hemisphere as modeled by Hirsch et al. (2006) and Huang et al. (2008). Conversely, the most depleted $\delta^{15}\text{N}_{\text{bulk}}$ annual mean occurs at HUN. The high carbon monoxide mixing ratio observed at HUN confirms the presence of combustion sources, which should also lead to greater emission of depleted N$_2$O.

The biggest outlier in this $\delta^{15}\text{N}_{\text{bulk}}$ – mixing ratio relationship using data from this project is at UTA, with both a high mixing ratio and $\delta^{15}\text{N}_{\text{bulk}}$, yet the mixing ratio means at UTA, SGP, and KEY from NOAA/ESRL CCGG are more consistent with an agricultural signal in the Midwestern United States. A higher $R^2$ and steeper regression slope using NOAA ESRL/CCGG mixing ratios is due to a smaller variance in mixing ratio across all annual means and suggests poorer precision using the Picarro G5101-i. Both $\delta^{15}\text{N}_1$ and $\delta^{15}\text{N}_2$ are also significantly negatively correlated with NOAA/ESRL CCGG mixing ratios ($p = 7.8\times10^{-5}$ and $p = 0.02$, respectively) but only $\delta^{15}\text{N}_1$ is significantly correlated with INSTAAR mixing ratio values and SP values show no significant correlation with either mixing ratio dataset.
Figure 4.10: Correlation between N₂O mixing ratio and δ¹⁵N_{bulk} of annual means of 13 retained sites. (a) Mixing ratio data from this project. Least squares linear regression (R² = 0.35): y = -0.48x + 161.39; (b) Mixing ratio data from NOAA/ESRL CCGG. Least squares linear regression (R² = 0.63): y = -0.77x + 256.03

Across all unflagged flask data, however, significant correlations are observed between mixing ratio and δ¹⁵N_{bulk}, SP and δ¹⁵N, SP and δ¹⁵N, and δ¹⁵N and δ¹⁵N, (Fig. 4.11). The negative correlation between SP and δ¹⁵N is consistent with the tropospheric values from Yoshida and Toyoda (2000). Although each of these correlations are significant, low regression R² values for all relationships except for SP and δ¹⁵N suggest that either analytical uncertainty or a non background signal may be influencing these values. The high SP – δ¹⁵N R² value could also simply suggest that SP is more influenced by δ¹⁵N than by δ¹⁵N, which may again be a cause of poor analytical precision.
Figure 4.11: Correlations across all unflagged flask data between (a) Mixing ratio measured by NOAA/ESRL CCGG and $\delta^{15}N_{\text{bulk}}$ ($R^2 = 0.23; y = -0.47 + 159.42$), (b) Site Preference and $\delta^{15}N_c$ ($R^2 = 0.28; y=0.72x +7.38$), (c) Site Preference and $\delta^{15}N_c$ ($R^2 = 0.50, y = -0.81x + 15.80$), and (d) $\delta^{15}N_c$ and $\delta^{15}N_c$ ($R^2 = 0.054; y = 0.28x -7.38$)

The trends across land masses are also consistent with expected anthropogenic emissions. The NOAA/ESRL CCGG $N_2O$ mixing ratio data reveal increasing values across Europe, but no significant difference across the United States (Fig. 4.12a). Likewise, no difference is seen in SP across the US, and although $\delta^{15}N_{\text{bulk}}$ decreases from Utah to Florida,
the mean at UTA may not be reliable as discussed previously. Although substantial agricultural $\text{N}_2\text{O}$ emissions occur over the Cornbelt (Miller et al., 2012), the KEY site may not be capturing this signal and could instead be influenced more by local industrial or marine sources. Significant differences in both mixing ratio and $\delta^{15}\text{N}_{\text{bulk}}$ occur between MID and SMO, which share a similar longitude but reside in opposite hemispheres. These differences again reflect the higher and more depleted mixing ratio and delta value, respectively, in the Northern Hemisphere. Another significant difference in $\delta^{15}\text{N}_{\text{bulk}}$ and SP occurs between BKT and LLN, yet no significant difference is seen in mixing ratio values.
Figure 4.12: NOAA annual site means from start dates between 2013.3-2013.5 to end dates from 2014.3-2014.5 by longitude of (a) N₂O mixing ratio measured by NOAA CCGG and INSTAAR, (b) δ¹⁵Nbulk, (c) δ¹⁵Nα, (d) δ¹⁵Nβ, and (e) Site Preference.

4.4 Seasonal Trends

The mixing ratio and isotopomer values across the full year of analysis for each of the 19 sites is shown in Figure 4.13. While visible outliers were removed for the site comparison discussed in the previous section, outliers that passed the analysis and pair difference flagging routines were retained below, with the instrument-specific uncertainties and low flask pressure indicated. Additionally, the N₂O mixing ratio data in Figure 4.13 are only from flasks that were retained for isotopomer values in order to accurately analyze the two datasets. Since many flasks that were retained for isotopomers were flagged for mixing ratio, NOAA CCGG values are also shown in order to fill these gaps and compare with INSTAAR values.

The variability in N₂O mixing ratio is not consistent across sites. Interestingly, the sites exhibiting the greatest difference in highest versus lowest values tend to have the greatest mean and visa versa. Using INSTAAR mixing ratio data, the greatest difference in
highest versus lowest values occurs at site UTA (Wendover, Utah), however the low flask pair mean value of 324.04 ppb seems to be a clear outlier, as the rest of the values are greater than 327 ppb. The MID N₂O variation shares a similar outlier issue, with a flask pair ∼3 ppb less than the rest of the flask values during that year. These sites aside, the greatest variation occurs at site HUN (Hegyhatsal, Hungary) with an annual difference of 3.51 ppb, which may be a result of a variable regional industrial influence. With the exception of BKT, all sites with a variation greater than 2 ppb are located in the Northern Hemisphere, while southern hemispheric and coastal sites vary by less than 2 ppb. The variability of this dataset is similar to the 3 ppb magnitude of detrended data collected by Liao et al. (2004) and the 0.1% season amplitude compared to mean mixing ratio at Mace Head, Ireland reported by Hirsch et al. (2006).

The minimum N₂O values in all mid- to upper- northern hemispheric sites occur in the summer months between June and August of 2013. The minimums at IZO, KEY, and LLN occurred earlier, between April and May. This is consistent with the seasonal cycles described by Nevison et al. (2011), where the change in N₂O concentration is strongly anti-correlated with stratospheric temperature at the MHD (Mace Head, Ireland) site. In the southern hemispheric sites of BKT, SMO, NAT, PSA, and SPO, the minimum values all occur in March and April of 2013. Again, these data are in agreement with the autumn southern hemispheric minima shown by Nevison et al. (2011). This observed difference between hemispheres is caused by a longer duration of the polar vortex in the Southern Hemisphere, leading to a delay in the N₂O minimum (Nevison et al., 2011). However, tropospheric transport processes in the Northern Hemisphere maintain a steady N₂O
minimum, whereas a larger interannual variability is observed in the Southern Hemisphere (Liang et al., 2008; Ishijima et al., 2010).

Although these data are in line with the timing of mixing ratio seasonal trends observed in previous research (Hirsch et al., 2006; Jiang et al., 2007; Liao et al., 2004; Nevison et al., 2004; Nevison et al. 2011), most sites do not exhibit clear seasonal trends with the exception of ICE and SPO, perhaps due to their remote locations and proximity to the poles. As Nevison et al. (2011) pointed out, detecting the relatively small seasonal variability in N₂O using discrete sampling is less than ideal, and therefore this lack of coherent seasonal patterns at most sites with only ~one year of data is expected.

The poor δ¹⁵N· precision of Instrument B limits the ability to observe isotopic changes across seasons within each site, and therefore the seasonal changes in δ¹⁵N· are perhaps the most accurate and reliable isotopomer due to the dependence of both the δ¹⁵Nbulk and SP values on δ¹⁵N·. According to Park et al. (2012), a negative correlation between δ¹⁵N· and mixing ratio is expected, at least at sites influenced by a marine signal, due to the opposing effects of stratospheric mixing and ocean ventilation. Coastal sites with a potential seasonal isotopic signal in δ¹⁵N· include LLN and BMW (Fig. 4.13), but no coherent patterns exist at other remote marine sites such as SMO or GMI. However one of the most intelligible displays of δ¹⁵N· seasonality and mixing ratio anti-correlation is exhibited at HUN (Fig. 4.13), with a clear δ¹⁵N· maximum and minimum in the summer and winter, respectively. Although some seasonal patterns are evident in N₂O isotopomers, particularly in δ¹⁵N·-N₂O, these results should be interpreted cautiously, as most sites do not exhibit clear patterns and only a limited amount of unflagged flask pairs within the year of analysis exist.
IZO $\delta^{15}$N$_{\text{小幅}}$ N$_2$O

IZO $\delta^{15}$N$_{-}$ N$_2$O

IZO $\delta^{15}$N$_{0}$ N$_2$O

IZO Site Preference

IZO N$_2$O Mixing Ratio

KEY $\delta^{15}$N$_{\text{小幅}}$ N$_2$O

KEY $\delta^{15}$N$_{-}$ N$_2$O

KEY $\delta^{15}$N$_{0}$ N$_2$O

KEY Site Preference

KEY N$_2$O Mixing Ratio
Figure 4.13: Annual mixing ratio and isotopomer values by NOAA site in alphabetical order, retained after Analysis and Pair flag removal. Values in blue, red, and purple correspond to analysis by Instrument A, B, and C, respectively. Mixing ratio values measured by NOAA CCGG in black diamonds. High and low pressure flasks are represented by triangle and square symbols, respectively.

While filtering for Analysis and Pair flags creates the largest dataset among all sites, more comprehensive filtering schemes present the most conservative and accurate dataset. Due to the high uncertainty values of Instrument B, the flasks measured by this instrument were flagged using the mean of Instrument A and C uncertainties (Appendix, Fig. A1). Furthermore, given the problems with low flask pressure (see 4.2.4), flasks with less than 5 PSI were removed as an additional filtering scheme (Fig. 4.14). The additional pressure filter highlights the air volume problems with several sites including ICE, IZO, KEY, MID, NAT, and UTA, as the majority of unflagged values were removed due to low pressure.
KEY $\delta^{15}$N bulk N$_2$O

KEY $\delta^{15}$N ice N$_2$O

KEY $\delta^{15}$N silicate N$_2$O

KEY Site Preference

KEY N$_2$O Mixing Ratio

LLN $\delta^{15}$N bulk N$_2$O

LLN $\delta^{15}$N ice N$_2$O

LLN $\delta^{15}$N silicate N$_2$O

LLN Site Preference

LLN N$_2$O Mixing Ratio
Figure 4.14: Annual mixing ratio and isotopomer values by NOAA site in alphabetical order. Data filtered by analysis and pair flags, as well as flask pressure greater than 5 PSI. In addition, samples measured by instrument B were filtered using the mean uncertainties of instruments A and C.
CHAPTER 5

CONCLUSIONS

With current uncertainties regarding the global N₂O budget including source fluxes, stratospheric influence, and seasonal variation, there is a clear need to increase both large and small scale monitoring efforts. Additionally, published research on the spatial and temporal variability of N₂O isotopocules on a global scale is limited and inconsistent. This is mainly due to the difficulty in detecting isotopic trends, which is a result of both the relatively long lifetime of N₂O in the troposphere, leading to a low signal:noise in isotopic source signals, and also the inability to resolve these small signals given current analytical uncertainties. A major goal of this project was to therefore assess whether or not the new Picarro G5101-i CRDS instrument was capable of resolving these signals and therefore provide the ability to monitor N₂O isotopomers in discrete NOAA CCGG flask samples as is currently taking place for CO₂ and CH₄ isotopologues.

The poor precision of Instrument B hampers the ability to detect seasonal variability within sites and across a spatial gradient. Instrument B aside, the average uncertainty of Instrument A and C for δ¹⁵Nbulk, δ¹⁵Nα, and δ¹⁵Nβ is 0.40‰, 0.77‰, and 0.50‰, respectively, with a smaller repeated measurement standard deviation from the
intercomparison project of 0.23‰, 0.27‰, and 0.74‰, respectively, on Instrument A. Both of these measures of precision for δ\textsuperscript{15}N\textsubscript{bulk} are larger than recent reported precisions using IRMS (e.g., Park et al. 2012: 0.15‰; Toyoda et al., 2013: 0.1‰) but comparable to IRMS for site preference measurements (e.g., Park et al. 2012: δ\textsuperscript{15}N\textsubscript{α} = 0.8‰, δ\textsuperscript{15}N\textsubscript{β} = 0.9‰; Toyoda et al., 2013: δ\textsuperscript{15}N\textsubscript{α} and δ\textsuperscript{15}N\textsubscript{β} = 0.3‰). Given the ability of Park et al. (2012) to detect a seasonal cycle at Cape Grim with larger uncertainty in both δ\textsuperscript{15}N\textsubscript{α} and δ\textsuperscript{15}N\textsubscript{β} than those from this study, it is conceivable to observe intra-annual trends with the current CRDS specifications. However, the data from this study are highly variable within each site due to several factors including the large overall uncertainty across all instruments, and therefore the data from this pilot year of sampling do not provide clear results regarding the instrument’s capabilities.

Another hindrance to detecting seasonal trends is the use of discrete samples as opposed to in situ monitoring. Nevison et al. (2011), for example, had more difficulty discerning N\textsubscript{2}O mixing ratio seasonality with NOAA/ESRL CCGG discrete samples as opposed to the frequent (∼40 minute) sampling at Advanced Global Atmospheric Gases Experiment (AGAGE) baseline sites. With poorer mixing ratio precision using this CRDS system than with the gas chromatograph used at NOAA, these trends may be even harder to detect. This was evident from the data collected from this project, which had much higher variability in mixing ratio within each site than the unflagged values measured by NOAA/ESRL CCGG. Given the CRDS feature of continuous sampling, a reasonable option beyond discrete sampling would be the implementation of permanent Picarro G5101-i analyzers at these baseline monitoring sites.
The major factor influencing measurement drift is cavity temperature stability. The instability of Instrument B may have been identified sooner by including it as an additional flagging parameter after processing each run. A standard deviation of 0.002°C of the mean temperature for each sample is a recommended flagging threshold on this CRDS analyzer, which may be indicative of a critical malfunction of the TEC or laser itself. Additionally, drift can be improved throughout a sample run by ensuring that the internal standard is analyzed at an interval of no greater than two hours.

In terms of improving sample performance, flask pair disagreement was mainly dependent on low flask pressure, which resulted in delayed measurement stability. While the minimum pressure specification is publicized as 400 mbar, the results of this study suggest that 500 mbar is a more appropriate threshold, as flasks below 500 mbar began to exhibit much greater differences in mixing ratio compared to NOAA/ESRL CCGG measurements than flasks with a higher pressure. Although most flasks are filled to slightly above ambient pressure, most sites underwent analysis by at least three different laboratories or instruments before adding this additional N₂O isotope measurement path, which caused logistical problems with the network to begin with. If the analysis of discrete samples were to continue using this Picarro CRDS analyzer, it is recommended that the flasks are either measured first at INSTAAR before other isotopic or VOC measurements, or flasks measured for VOCs be excluded all together, leaving only sites measured for CO₂ isotopes. Again, establishing a continuous monitoring program at a NOAA baseline site would eliminate the issue of low sample pressure all together.

Water interference is another major problem associated with the NOAA flask samples. Several types of water traps were tested, and the cold ethanol trap was
determined to be the most effective method compared to chemical desiccants such as Drierite. Although many NOAA sites utilize drying methods during sampling, water must be trapped during extraction in addition to this, as even flasks with only 0.5% water produced delta values several per mil different than the same sample dried through the ethanol trap. A linear trend between water concentration and the difference between wet and dry values was observed, however no water correction was applied due to the intercept being greater than 0‰ in $\delta^{15}$N$_{bulk}$ and $\delta^{15}$N$\text{\textsubscript{N}}$. Samples with higher water concentration were more likely to be flagged for pair difference, and those that remained unflagged can be removed through outlier filtering within each site.

All samples retained for pair difference and analytical uncertainty flags exhibited a strong correlation between $\delta^{15}$N$_{bulk}$ and mixing ratio, with delta values increasing as mixing ratio decreased. This relationship is consistent with both the expected low mole fraction and isotopically depleted stratospheric influence of tropospheric N$_2$O as well as a terrestrial or industrial emission source of depleted N$_2$O. The mean annual differences in $\delta^{15}$N$_{bulk}$ across sites were also consistent with the latitudinal gradient of N$_2$O mixing ratio, and this relationship has yet to be shown on a global scale until now. While significant differences in SP between sites exist, these differences should be interpreted cautiously given the high uncertainty and standard deviation within each site. One site in particular that exhibits unique isotopic signatures and potential seasonal patterns without any indication of sampling or analytical errors is HUN (Hegyhatsal, Hungary), which is notably influenced by industry as confirmed by a high average carbon monoxide mixing ratio compared to baseline sites. Due to uncertain estimates of industrial and agricultural sources of N$_2$O, increased isotopic monitoring of areas with heightened levels of these
activities including the US Cornbelt and Southeast Asia, in addition to continuous baseline monitoring, may be an appropriate development in future research. Furthermore, while the polar sites that were investigated (SPO, PSA, ICE, and TIK) did not produce clear isotopic seasonal patterns, these sites are heavily influenced by stratospheric back-flux. The initiation of N₂O isotopic monitoring at these additional sites may help to quantify the stratospheric influence of seasonal N₂O variability, which is essential in order to refine the global N₂O budget.

The SP uncertainty produced from the higher performing instruments is comparable to the uncertainties associated with IRMS, and analytical issues present in this study including cavity temperature and water interference can easily be resolved with additional quality assurance flagging regimes. Although it is still unclear whether or not tropospheric δ¹⁵N_{bulk} or SP exhibits a season cycle, monitoring these isotopomers over a longer decadal time scale can provide answers to questions concerning large-scale shifts in source emissions, including changes in both the type and quantity of fertilizer application.
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GMI $\delta^{15}$N$_{bulk}$ - N$_2$O

GMI $\delta^{15}$N$^-$ - N$_2$O

GMI Site Preference

GMI N$_2$O Mixing Ratio

HPB $\delta^{15}$N$_{bulk}$ - N$_2$O

HPB $\delta^{15}$N$^-$ - N$_2$O

HPB Site Preference
Figure A1: Annual mixing ratio and isotopomer values by NOAA site in alphabetical order. Data retained after analysis and pair flag removal. In addition, samples measured by instrument B were filtered using the mean uncertainties of instruments A and C.
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Note: This is a sample table and the numbers are randomly generated. The actual data should be sourced from financial reports or official financial statements.
Table A1: Raw flask data in order of analysis run number. The following parameters were measured and recorded: Run number, NOAA site, NOAA flask ID number, NOAA flask event number, flask fill date, corrected mixing ratio and isotopomer values, single measurement precisions (calculated by taking the standard deviation of each 100-second running mean), mixing ratio and isotopomer flags, Run date, mixing ratio and isotopomer raw values and standard deviations, outlet valve value and standard deviation, Das temperature and standard deviation, and cavity temperature and standard deviation.