Ecological interpretations of nitrogen isotope ratios of terrestrial plants and soils

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Abstract

Background Knowledge of biological and climatic controls in terrestrial nitrogen (N) cycling within and across ecosystems is central to understanding global patterns of key ecosystem processes. The ratios of ^{15}N:^{14}N in plants and soils have been used as indirect indices of N cycling parameters, yet our understanding of controls over N isotope ratios in plants and soils is still developing.

Scope In this review, we provide background on the main processes that affect plant and soil N isotope ratios. In a similar manner to partitioning the roles of state factors and interactive controls in determining ecosystem traits, we review N isotopes patterns in plants and soils across a number of proximal factors that influence ecosystem properties as well as mechanisms that affect these patterns. Lastly, some remaining questions that would improve our understanding of N isotopes in terrestrial ecosystems are highlighted.

Conclusion Compared to a decade ago, the global patterns of plant and soil N isotope ratios are more resolved. Additionally, we better understand how plant and soil N isotope ratios are affected by such factors as mycorrhizal fungi, climate, and microbial processing. A comprehensive understanding of the N cycle that ascribes different degrees of isotopic fractionation for each step under different conditions is closer to being realized, but a number of process-level questions still remain.

Keywords

Nitrogen, nitrogen isotopes, soil organic matter, plants, nutrient supplies, denitrification, decomposition

Introduction

Nitrogen (N) is a key limiting resource in many terrestrial ecosystems and its cycling affects almost all aspects of ecosystem function (Vitousek et al. 1997). The N cycle is complex, with multiple transformations, feedbacks, and interactions with other important biogeochemical elements. N supplies to plants limit primary productivity across a wide variety of ecosystems (LeBauer and Treseder 2008; Thomas et al. 2013). Because N concentrations in plants are also often limiting to herbivores, N supplies to plants can constrain the productivity of herbivores by limiting both the quantity and nutritional quality of plants (Augustine et al. 2003; Craine et al. 2010; Zavala et al. 2013). N supplies can also influence detritus-based food webs, leading to both positive and negative feedbacks on process rates of N cycling. For example, increased N availability can accelerate initial decomposition rates of plant litter, but can also decelerate the decomposition of biochemically recalcitrant organic matter in soils (Carreiro et al. 2000; Craine et al. 2007; Janssens et al. 2010; Melillo et al. 1982; Waldrop et al. 2004). Adding to these complexities, ecosystem N cycling rates can govern N losses. Trace N gas losses to the atmosphere are a strong forcing factor for global climate and increase with increasing soil N availability (Barnard et al. 2005; Hall and Matson 2003). N also plays a key role in limiting productivity in many aquatic ecosystems. Large losses of reactive N, such as NO_3^-, from soils can pollute groundwater and streams and ultimately reduce oxygen levels in river and estuarine environments (Howarth et al. 1996; Rabalais et al. 2002).

Understanding how patterns in terrestrial N cycling emerge within and across ecosystems is central to predicting patterns of plant productivity, ecosystem carbon sequestration,

nutrient fluxes to aquatic systems, and trace gas losses to the atmosphere (Galloway et al. 2008; Goll et al. 2012; Hudman et al. 2012; Pinder et al. 2012). Many specific N cycling processes can be difficult to measure, constraining the ability to generalize about the N cycle. Consequently, controls on N cycling are uncertain in many cases. No less uncertain is how N cycling responds to forcing factors such as changes in climate, increases in atmospheric CO_2, or greater N deposition. Such uncertainty in the mechanisms underlying how N is cycled across organism to landscape scales hampers parameterization of Earth system models and thus our ability to develop prognostic understanding of how ecosystems will respond and feedback to changes in climate (Thomas et al. 2015).

The ratios of ^{15}N:^{14}N in plants and soil have been used to infer N cycling process that are difficult to measure directly and challenging to scale (Amundson et al. 2003; Craine et al. 2009; Handley et al. 1999b; Hobbie and Högberg 2012; Högberg 1997; Martinelli et al. 1999). Although mechanistic understanding of controls over N isotope abundance was already well-developed over 25 years ago (Högberg 1997), during the past decade, a number of advances have been made in quantifying N isotope patterns of plants and soils at local to global scales as well as the mechanisms that underlie these patterns. As N isotopes of plants and soil are relatively straightforward to measure, a better mechanistic understanding of the patterns of natural abundance ^{15}N and their underlying causes is needed to infer spatial and temporal patterns of N cycling as well as their interpretation. The ratios of N isotopes in plants are more likely to reflect short-term variation in N cycling, e.g. annual time scales. Soil N isotopes integrate over longer time scales, e.g. centurial, and can include different processes than what control plant N isotope

composition (Bustamante et al. 2004). N isotopes are also a key to reconstructing past N availability, which helps us understand the current state and trajectory of N availability of ecosystems (Gerhart and McLauchlan 2014; McLauchlan et al. 2013).

This review has three main sections. First, we provide background on the main processes that affect plant and soil N isotope ratios. Second, we review the mechanisms that affect plant and soil N isotope patterns and the general ecological patterns of N isotopes in plants. Lastly, we identify some of the remaining questions that need to be answered in order to advance our understanding of N isotopes in terrestrial ecosystems and consequently the N cycle.

Background on the N cycle and N isotopes

The soil N pool accounts for less than 1% of global N reservoirs and plant N pools account for even less (Galloway et al. 2004). Both are essential for the functioning of ecosystems and the biosphere. N cycling rates and the predominant forms of bio-available N to plants varies among ecosystems. In cold ecosystems, dissolved organic N (DON) can be a dominant pool of N in soil solution (Schimel and Bennett 2004). In warmer ecosystems, either NH_4^+ or NO_3^- may dominate the inorganic N pool of an ecosystem (Kronzucker et al. 1997). Although plants benefit energetically from taking up the most reduced form of N, excessive uptake of NH_4^+ can be toxic (Miller and Cramer 2005) and plant N uptake preferences track the availability of different forms of N across different environmental conditions (Wang and Macko 2011). Losses of bioavailable N can be indicative of the N limitation status of plants and microbes and tend to increase with increasing external inputs and availability (e.g., Brookshire et al. 2012a; Vitousek et

al. 1989; Wang et al. 2007). Pathways of N loss from ecosystems are diverse. They include gaseous losses (e.g., denitrification), particulate losses through erosion (aeolian or hydrologic pathways), and leaching of organic and inorganic N.

Although the N cycle is composed of many processes that can be difficult to measure, the ratios of ^{15}N:^{14}N in plants or soils could shed light on patterns of key aspects of the N cycle. These aspects include N supply rates to ecosystems and plants, the availability of N to plants, the pathways by which N is lost from ecosystems, and the amounts of N lost. For the purposes of this review, we define *ecosystem N supply* as the total amount of N entering the ecosystem from the atmosphere by pathways such as biological fixation and deposition, and from bedrock weathering in some situations. *Soil N supply* is defined as the rates at which organic or inorganic N enters soil solution from organic matter decomposition and from different direct inputs to the ecosystem. Soil N supplies to plants can be measured as either net mineralization rates or a fraction of gross mineralization rates, but it is uncertain which of these better predict plant N uptake. In most ecosystems, inorganic N (NH_4^+ and NO_3^-) is the major N form for plant uptake, though some plants (directly and through mycorrhizal fungi) can take up DON, which precedes mineralization (Näsholm et al. 1998). The *availability* of N in soils to plants can be defined as the soil N supply relative to plant demand for N. Because the availability of N represents the balance of supply and demand, N availability can be even harder to measure directly than supply rates alone since plant N demand must also be assessed. Lastly, it is important to quantify the pathways by which N is lost from the ecosystem.

For some purposes, the absolute magnitude is sufficient, while in others the relative loss rates among pathways or relative to mineralization may be preferred.

As a natural component of the total N pool, approximately ~0.366 % of N is in the form of ^{15}N. The ratio of ^{15}N to ^{14}N present in a given pool can shed light on processes that are difficult to measure. Molecules containing ^{15}N are discriminated against in a number of processes associated with equilibrium and kinetic fractionations. N stable isotopic compositions are typically reported in δ notation, and expressed in per mil (‰) (Coplen 2011):

$$\delta^{15}N = (((^{15}N/^{14}N)_{sample}/(^{15}N/^{14}N)_{std}) - 1 \qquad \text{eq 1.}$$

where $(^{15}N/^{14}N)_{sample}$ is the N isotopic composition of a sample, and $(^{15}N/^{14}N)_{std}$ is the N isotopic composition of the standard material. The material used as a standard for the ratio of stable N isotopes is atmospheric molecular N, which by convention is set to 0 ‰. The fractionation between two substances A and B, which applies to all mass fractionation such as thermodynamic isotope effect and diffusion fractionations, can be expressed using the isotope fractionation factor (α):

$$\alpha = R_A/R_B, \qquad \text{eq. 2}$$

where R = the ratio of the heavy isotope to the lighter isotope in compounds A and B. Fractionation factors can also be expressed as discrimination (Δ), or fractionation (ε; also

referred to as the "isotope effect"), which is also normally expressed in ‰. These are defined (Coplen 2011) as

$$\Delta = \delta_A - \delta_B = (\alpha - 1) \approx \ln \alpha. \qquad \text{eq. 3}$$

The approximation in equation (3) is valid when α is low or under natural abundance. In general, patterns of natural abundance ^{15}N have proven difficult to explain with simple mixing models. A key reason for this is that many biochemical and abiotic reactions involving N have large fractionation factors, which may vary in their level of expression depending on the degree to which the reactions go to completion. There are few N sources that are sufficiently enriched or depleted relative to other pools of N in an ecosystem to serve as a distinct tracer (Robinson 2001). Using natural abundance $\delta^{15}N$ of plants or soils to infer N cycling processes is thus difficult because there is a single response variable with multiple drivers. Interpretations, however, can be refined by having multiple-responses, such as pairing N with O isotopes when studying NO_3^- (Högberg 1997) or with C isotopes when studying organic biomolecules (Baisden et al. 2002a). Yet, in most cases, natural abundance N isotopes can only be used to narrow down the mechanisms that might underlie plant or soil $\delta^{15}N$ patterns. Coupling measurements of $\delta^{15}N$ with direct measurements of N cycle processes is generally required to further narrow interpretations of patterns.

Plant N isotopes

Although N in plant biomass represents a small fraction of the total ecosystem N pool, the isotopic composition of plants can index short-term dynamics of N cycling, as

opposed to soil $\delta^{15}N$ which might represent long-term dynamics. Typically, plant leaves are used as an index of whole-plant $\delta^{15}N$. Although differences often exist among leaves, roots, and stems (Kolb and Evans 2002), the N isotope ratios generally correlate among plant fractions and any average differences are generally relatively minor. For example, across 90 grass species collected from 67 sites in four grassland regions of the world, the $\delta^{15}N$ of leaves averaged just 0.3 ‰ less than those of roots compared to a range of 18 ‰ for leaves and 14 ‰ for roots (Craine et al. 2005). Similarly, Dijkstra et al. (2003) reported differences in $\delta^{15}N$ of <1 ‰ between leaves and roots of natural meadows and forests in North America, with direction and magnitude of the differences depending on the functional type (forbs, legumes or grasses). In two North American hardwood tree species, $\delta^{15}N$ of leaves and wood were within 0.3 ‰ on average (Pardo et al. 2012). In that study, the greatest average difference in $\delta^{15}N$ occurred between roots and leaves for sugar maple (*Acer saccharum*) (2.1 ‰). Offsets between leaves and roots appear to be greatest for plants with ectomycorrhizal symbioses, e.g. ~4 ‰ enrichment in roots relative to leaves. That offset is dependent on the mycorrhizal status of the plants and on how much ectomycorrhizal mass is included with the roots (Hobbie and Colpaert 2003). In agricultural crops supplied with N-fertilizers, differences between leaves and roots can be larger (Robinson et al. 1998). For example, leaves of *Brassica campestris* grown with 12 mM NO_3^- had leaf and root $\delta^{15}N$ values of 0.2 ‰ and -6.7 ‰, respectively (Yoneyama et al. 2003).

At the global scale, foliar $\delta^{15}N$ ranges over 35 ‰ (Craine et al. 2009; Craine et al. 2012). The highest foliar $\delta^{15}N$ from a natural environment was 21.4 ‰, acquired from a prairie wildflower (*Callirhoe involucrata*) adjacent to a bison wallow in a tallgrass prairie in

Kansas, USA (Craine et al. 2012). The lowest foliar $\delta^{15}N$ recorded was -14.4 ‰, acquired from a fir tree (*Abies lasiocarpa*) near Lyman Glacier in Washington, USA (Hobbie et al. 2005). Across over 12,000 leaves collected globally (Craine et al. 2009; Craine et al. 2012), the mean $\delta^{15}N$ was 0.9 ‰ with 95% of the samples falling within a range of 15.5 ‰ (-7.8 ‰ to 8.7 ‰)

Locally, individual plants can vary in $\delta^{15}N$ by over 25 ‰ (Craine et al. 2012). The amount of variation in plant $\delta^{15}N$ observed at a particular site depends in part on the sampling intensity. Using data from Craine et al. (2012), the range of foliar $\delta^{15}N$ observed at a site increases logarithmically with the number of plants sampled, which includes additional species and replicates of the same species. When 10 plants are sampled at a site, the mean range averages 5.5 ‰. When 100 plants are sampled, the mean range averages 10.1 ‰. (Figure 1). In contrast to other studies (Nadelhoffer et al. 1996), ecosystems with low mean annual temperature do not necessarily show a greater range in foliar $\delta^{15}N$ than ecosystems with high mean annual temperature. There is no relationship between mean annual temperature or precipitation and the range of foliar $\delta^{15}N$ at a site, once the number of plants sampled is taken into account ($P > 0.2$).

Proximal causes of plant $\delta^{15}N$ variability

The variation observed within and among sites in foliar $\delta^{15}N$ is dependent on a large number of proximal factors. In the following sections, we discuss a number of these factors: the signature of deposited N, whether any N has been acquired from geologic sources, the amount of N acquired from symbiotic fixation by the plant, the form of N acquired, mycorrhizal symbioses, and the signature of the N lost from ecosystems.

Questions about the role of variation in the signature of soil organic matter (SOM) in determining plant $\delta^{15}N$ are addressed in a later section.

Deposition

Deposition of N can alter plant $\delta^{15}N$ when plants directly acquire N on leaf surfaces or by altering the signature of available N in the soil. NO_3^- in bulk precipitation tends to have an isotopic signature ranging from -3 ‰ to + 1 ‰ (Houlton and Bai 2009), likely reflecting anthropogenic NO_x originated from fossil fuel combustion and reduction (Felix et al. 2012). For systems receiving substantial amounts of rain from marine sources, the contribution of continental anthropogenic N sources can be traced using the dual (^{15}N, ^{18}O) isotopes of NO_3^-. For example, rain in Bermuda can be derived from cold-season continental USA sources (with NO_3^- that have low $\delta^{15}N$ and high $\delta^{18}O$ likely reflecting the contribution of fossil fuels) or warm-season marine sources (with high $\delta^{15}N$ and low $\delta^{18}O$ derived from natural atmospheric reactions). Consequently, the source of NO_3^- varies temporally resulting in a negative relationship between $\delta^{18}O$ and $\delta^{15}N$ with the lowest $\delta^{15}N$ and highest $\delta^{18}O$ derived from the continental USA (Altieri et al. 2013). In contrast, NO_3^- deposited onto ecosystems can have quite low $\delta^{15}N$ when it is generated from snow surfaces. Morin et al. (2009) showed that the photolysis of NO_3^- on snow surfaces can lead to deposition of highly ^{15}N-depleted NO_x downwind (as low as -40 ‰).

NH_4^+ in bulk precipitation not derived from marine sources tends to have lower $\delta^{15}N$ values than NO_3^- (Garten 1992; Koba et al. 2012; Xiao and Liu 2004; Zhang et al. 2008), possibly reflecting the agricultural sources of NH_4^+. The isotopic signature of atmospheric NH_4^+ is likely affected by marine sources. For example, a wide range in

$\delta^{15}N$ values of NH_4^+ in the bulk precipitation was collected near a bay in the eastern US (-8.3 to + 8.6 ‰) likely due to differences in whether NH_4^+ was derived from terrestrial or marine sources (Russell et al. 1998). However, the ranges of $\delta^{15}N$ for NH_4^+ and NO_3^- occasionally overlap (Russell et al. 1998) or NH_4^+ can have higher $\delta^{15}N$ than NO_3^- (Nadelhoffer et al. 1999).

For DON in precipitation, there are a limited number of measurements of its isotopic values. Cornell et al. (1995) first reported $\delta^{15}N$ of DON in precipitation on the ocean. DON values ranged from -7.3 to +7.3 ‰, with a trend towards ^{15}N-depletion in sites further from the ocean. Russell et al. (1998) also reported a wide range in $\delta^{15}N$ of DON (-0.5 to +14.7 ‰). Knapp et al. (2010) estimated $\delta^{15}N$ of total reduced N (DON + NH_4^+) in precipitation collected in Bermuda to be -0.6 ‰ compared to $\delta^{15}N$ of NO_3^- of -4.5 ‰ in the same samples.

The isotopic signatures of different compounds differ between wet and dry deposition (Heaton et al. 1997). For instance, Elliott et al. (2009) measured $\delta^{15}N$ of HNO_3 (gas; mean value = -3.2 ‰) and NO_3^- (particulate; +6.8 ‰). They found that $\delta^{15}N$ values of HNO_3 (gas) are an average of 3.4 ‰ higher than corresponding $\delta^{15}N$ of NO_3^- in wet deposition. For NO_3^-, a trend in higher $\delta^{15}N$ values in dry deposition than wet deposition has been reported elsewhere (Garten 1996), although one study (Mara et al. 2009) reported similar $\delta^{15}N$ values for NO_3^- in dry and wet depositions in a coastal region. Kawashima and Kurahashi (2011) reported quite high $\delta^{15}N$ of particulate NH_4^+ in some rural sites in Japan, which had much higher values than $\delta^{15}N$ of particulate NO_3^- (16.1‰ vs. -1‰). In contrast, $\delta^{15}N$ was higher in NO_3^- than in NH_4^+ on aerosol samples collected in a coastal sampling site (Yeatman et al. 2001).

Geologic N

Rocks contain approximately 99.9% of the fixed N on Earth (Capone et al. 2006). Such "geologic N" represents the accumulated products of physical and biological N_2 fixation after gaseous N losses and losses from weathering and erosion. The geological N pool is large and turns over at the scale of millions of years as high pressures and temperatures volatilize N in rock. Past work has indicated that N concentrations are greatest in sedimentary and low-grade meta-sedimentary rocks such as slate (~500 ppm on average) (Holloway and Dahlgren 2002), whereas high-grade metamorphic rocks such as gneiss and igneous rocks contain smaller quantities of fixed N. Rock-bound N can occur as organic N, NH_4^+, and, to a lesser extent in desert caliche deposits, as NO_3^-.

Substantial rock N contributions to sediments, ground- and surface-waters, and soil-systems are well known (Dahlgren 1994; Holloway et al. 1998; Holloway and Dahlgren 2002; Strathouse et al. 1980). Weathering of parent material contributes significant quantities of N to temperate coniferous forest ecosystems (Morford et al. 2011). Using natural N isotope composition, Morford et al. (2011) showed that the $\delta^{15}N$ of rock N was distinct from other N input sources in California, e.g. approximately 16‰ higher than symbiotically fixed N. Application of an N-isotope mixing model revealed a doubling of the forest N budget via weathering of geologic N. The potential for N isotope composition to reveal rock N sources in terrestrial and aquatic ecosystems is an area for open inquiry; however, current evidence indicates that rock mineral $\delta^{15}N$ is highly variable (from ~-11 to 24 ‰) (Holloway and Dahlgren 2002), making global-scale N isotope calculations of rock N inputs uncertain (Vitousek et al. 2013).

N_2 Fixation

There is virtually no variation in the $\delta^{15}N$ of N_2 in the global bulk atmosphere (Mariotti 1983) and only slight ^{15}N depletion in the soil atmosphere (*ca*. -0.2 ‰) imposed by diffusive flux of water vapor out of soil combined with gravitational settling of heavier isotopes and thermal diffusion of heavier isotopes to sites with lower temperatures (Severinghaus et al. 1996). The concentration of N_2 in the soil atmosphere is high enough that N_2 production by denitrification and diazotrophic N_2 consumption have limited potential to influence the $\delta^{15}N$ of soil N_2 (Barford et al. 1999).

During enzymatic fixation of N_2, the substrate N_2 binds reversibly to nitrogenase, facilitating potential discrimination against ^{15}N (Sra et al. 2004). The NH_3 produced is highly soluble and rapidly converts to NH_4^+ with the equilibrium in favor of ^{15}N-enriched NH_4^+ formation (Shearer and Kohl 1986). It is widely accepted, however, that nitrogenase in nature does not fractionate (Handley 2002). Although free-living diazotrophs fractionate slightly (~ -2.5 ‰), there is little fractionation by symbiotic fixation in legumes. Yet, nitrogenase *in vitro* has been reported to fractionate strongly (-17 ‰) (Sra et al. 2004). This raises the question as to how inherent fractionation by nitrogenase may be suppressed, particularly in symbiotic fixation (Handley 2002). Contrary to earlier perspectives (Handley and Raven 1992), Unkovich (2013) suggested that these differences in fractionation were due to reduced N_2 concentrations in nodules as a consequence of the O_2 barriers that also exclude N_2. Steps subsequent to the N_2 reduction may also contribute to compensating for nitrogenase fractionation, including gaseous N loss (e.g. as NH_3), export of ^{15}N-depleted ureides and the import of ^{15}N enriched amino acids (Unkovich 2013).

The $\delta^{15}N$ values of plants that rely exclusively on N_2 fixation are usually ~ 0 ‰, reflecting atmospheric isotopic N values (Handley 2002). Many N_2-fixing plants show significant departures from 0 ‰ due to differences in reliance on fixed N (Craine et al. 2009; Menge et al. 2009). Unkovich (2013) argued that variations in $\delta^{15}N$ of symbiotic N_2 fixation were not the product of N_2-fixation per se, but rather a combination of measurement errors, intra-plant fractionation events resulting in tissue differences and possible preferential losses of ^{15}N-depleted NH_3 (O'Deen 1989).

Nodules are commonly highly enriched in ^{15}N (e.g. $\delta^{15}N$ 2.5 – 6.3 ‰) (Shearer and Kohl 1986). A number of explanations for this enrichment have been provided, including losses of ^{15}N-depleted NH_3, export of ^{15}N-depleted ureides and the import of ^{15}N-enriched amino acids (Unkovich 2013). Nodules generally form a small proportion of the biomass of legumes and also represent a small proportion of the plant N (< 10%) even in plants exclusively dependent on N_2 fixation. As a consequence, enrichment of nodules has little effect on overall plant $\delta^{15}N$ values.

Transformations of plant-available nitrogen in soil

Feigin et al. (1974) first illustrated that the $\delta^{15}N$ of different types of soil inorganic N can be altered by transformations such as mineralization and nitrification. Although the subsequent number of studies on $\delta^{15}N$ of soil inorganic N remains small compared with the studies on bulk soils, many have revealed reduced $\delta^{15}N$ values for soil inorganic N (NO_3^- plus NH_4^+) than bulk soils in most cases (Binkley et al. 1985; Garten 1992; Koba et al. 1998).

The lower $\delta^{15}N$ of soil inorganic N relative to bulk soil N has been attributed to the isotopic fractionation during mineralization of larger molecules in the soil, although there is very little evidence for fractionation during this step (Högberg 1997). Consistent with previous research, Koba *et al*. (2010) demonstrated that soil inorganic N produced by organic matter mineralization and nitrification had more negative $\delta^{15}N$ values than bulk soil. Yet, the authors also reported that extractable organic N (EON) fraction had the highest $\delta^{15}N$ values among different N pools in the soil (Figure 2). This finding is somewhat unexpected; investigators typically assume that DON—comparable with EON in this case—has the same $\delta^{15}N$ of bulk soil N, because solubilization of organic N from bulk soil into the soil solution does not appear to induce isotopic fractionation (Amundson et al. 2003). This finding cannot be interpreted with the conventional view of N mineralization with negligible or small isotopic fractionation between SOM and EON. Koba et al. (2010) reported that the difference between the $\delta^{15}N$ of bulk soil N and EON was positively correlated with bulk soil C:N.

Soil microbial functioning is a likely driver of differences between $\delta^{15}N$ of bulk SOM and EON. Macko and Estep (1984) demonstrated ^{15}N-enrichment of a marine bacterium after their uptake of amino acids. An analysis of ten different soils from a range of ecosystem and climate types showed that soil microbial biomass was consistently ^{15}N-enriched relative to the total N pool (by approximately 3.2 ‰) and the extractable N pool (~3.7 ‰) (Dijkstra et al. 2006). Along these lines, soil microbial biomass can be ^{15}N-enriched compared with the substrate due to the excretion of ^{15}N-depleted N compounds (e.g. NH_3) (Collins et al. 2008). Dijkstra et al. (2008) hypothesized that soil microbial

biomass would excrete N with low $\delta^{15}N$ during deamination and associated transaminations of the incorporated organic N when C availability is low, resulting in the increase in $\delta^{15}N$ compared with the $\delta^{15}N$ of substrate. Expanding on these ideas, Coyle et al. (2009) suggested that greater ^{15}N-enrichment of the soil microbial biomass in lower C:N soils may result from relatively lower C availability, a feature realized in some grassland soils (Tiemann and Billings 2011).

The ^{15}N-enrichment in soil microbial biomass illuminates two points. The first is the discrepancy between the ^{15}N-depletion of soil inorganic N and the lack of reports of large isotopic fractionation during mineralization. Mineralization does not break $–NH_2$ bonds at the edges of organic matter molecules in the soil. Instead, mineralization is the consequence of incorporation and excretion of N by soil microbial biomass (Myrold and Bottomley 2008). Therefore, it is reasonable that soil inorganic N excreted from soil microbial biomass can be ^{15}N-depleted. Second, $\delta^{15}N$ of EON tends to be relatively elevated. The growing recognition that microbial necromass dominates inputs into SOM pools with longer turnover times (Berg and McClaugherty 2008; Gleixner 2013; Hobara et al. 2013; Liang and Balser 2010) is supported by the high $\delta^{15}N$ of these pools in the soil.

Nitrogen uptake

The isotopic fractionation that occurs during the uptake of soil N into plant tissue varies among plants and depends on the concentrations of N at the root surface. If the

concentration of N in soil solution is extremely low, roots essentially eliminate the possibility of N-isotope fractionation since net flow of N is from soils to roots. Concentrations of the N at root surfaces are difficult to measure but minimum soil solution N concentrations required for uptake of NO_3^- are extremely low (~0.3 – 9 μM) (Edwards and Barber 1976; Olsen 1950; Teo et al. 1992), and those for NH_4^+ are in the same range (~1.5 - 5 μM) (Abbes et al. 1995; Marschner et al. 1991). Soil amino acid concentrations are also commonly low, relative to [NO_3^-] and [NH_4^+], resulting in limited access of plant roots to these N-forms (Jones et al., 2005) and limited potential for fractionation. Nutrient uptake mechanisms allow roots to take up N at low concentrations and consequently deplete soil N to low concentrations (Miller and Cramer, 2004), which is consistent with the fact that observed fractionation is especially small when soil [N] are low (Evans 2001; Evans et al. 1996; McKee et al. 2002; Montoya and McCarthy 1995). It is only when soil [N] is high that significant fractionation may be common.

Discrimination against ^{15}N during uptake of NO_3^- by 38 plant species altered plant $\delta^{15}N$ by only 0.25 ‰ on average, although the extent of fractionation increased with increasing NO_3^- concentration (Mariotti et al. 1980). Evans (2001) used the fact that discrimination between NO_3^- and tissue can be ~0 ‰ across a range of NO_3^- concentrations to argue that there is no inherent fractionation during NO_3^- uptake processes. Furthermore, in aquatic systems, the cellular NO_3^- of phytoplankton has never been observed to be depleted in ^{15}N relative to the supply (Needoba et al. 2004) indicating that fractionation during uptake across cell membranes is unlikely. Fractionation with NH_4^+ may, however, be greater than with NO_3^- supply (Pennock et al. 1996; Yoneyama et al. 1991). Possible steps during NH_4^+ acquisition from soil that could result in isotopic fractionation are

NH_4^+ diffusion across the root boundary layer, active transport of NH_4^+ across the plasmalemma and assimilation into amino acids. Fractionation during uptake has been reported to result in increased $\delta^{15}N$ values of tissue NH_4^+ and decreased $\delta^{15}N$ values of organic N in rice (Yoneyama et al. 1991). This observation is consistent with fractionation during NH_4^+ assimilation into organic N.

The foregoing suggests that evidence for extensive fractionation of N during influx into cells, per se, is rather weak. Despite this, cytoplasmic pools of both NO_3^- and NH_4^+ are commonly enriched with ^{15}N, largely due to fractionation during reduction of NO_3^- to NO_2^- by nitrate reductase, the reduction of NO_2^- to NH_4^+ by nitrite reductase, and the subsequent assimilation into amino acids glutamine synthetase–glutamate synthase (Neerdoba et al. 2004). Nitrate reductase and glutamine synthetase both fractionate strongly against ^{15}N by *ca*. 15‰ and 17‰, respectively (Robinson 2001). In contrast, the reduction of NO_2^- to NH_4^+ is unlikely to fractionate *in situ* since cellular $[NO_2^-]$ is normally very low (Tcherkez 2011). Cumulatively, these fractionations cause the cytoplasmic inorganic N to become enriched in ^{15}N compared with soil N, whereas the organic N product is depleted in ^{15}N. The importance of these fractionations for plant $\delta^{15}N$ values varies with the locations of N reduction/assimilation, which can be leaves, roots, or both, depending on the species, environmental conditions and N source (Robinson et al. 1998). When reduction/assimilation occurs in the roots there is potential for the efflux of ^{15}N-enriched inorganic N from roots, resulting in both depletion of plant-^{15}N and enrichment of soil ^{15}N. Efflux of NO_3^- is commonly observed (Kronzucker et al. 1999) and a Nitrate Excretion Transporter (NAXT1) has been associated with NO_3^- efflux in *Arabidopsis* (Segonzac et al. 2007). Efflux of NH_4^+ has also been widely reported

(Britto et al. 2001). Both NO_3^- and NH_4^+ efflux are thought to function for regulation of cytoplasmic N concentrations. This might be especially important for NH_4^+ (Britto and Kronzucker 2002), which can be toxic when supplied at high concentrations (Miller and Cramer 2005). Apart from efflux of inorganic N, root exudation of amino acids also occurs (Farrar et al. 2003), resulting in the loss of ^{15}N-depleted organic N from the roots. Dissolved organic N losses are likely to be greatest when substrate [N] is high or when plant growth is relatively impaired.

Other factors that may also contribute to variations in plant tissue $\delta^{15}N$ are intra-plant fractionation between shoot and root combined with whether there is net influx or efflux of NH_3 from the shoot (O'Deen 1989). Although net efflux of NH_3 by tissue volatilization can increase tissue $\delta^{15}N$ due to the large isotope fractionation (Högberg 1997), when atmospheric concentrations of NH_3 are above a compensation point within leaves, net influx of ^{15}N-depleted atmospheric NH_3 can also decrease tissue $\delta^{15}N$ (Johnson and Berry 2013).

If the major N source for plants in soils is inorganic N, the $\delta^{15}N$ of plants should more closely correlate with the $\delta^{15}N$ of that source than total N (Cheng et al. 2010; Virginia 1982). Although a more comprehensive survey and broader sampling are required, published values of foliar $\delta^{15}N$ largely reflect the signatures of inorganic N available in soil (Figure 3). The vicinity of most plots to the identity line indicates that, in most non-boreal sites, plants mainly acquire NH_4^+ and NO_3^- from soil and this uptake occurs without any large isotopic fractionation.

Mycorrhizal influence on plant $\delta^{15}N$

Mycorrhizal symbioses are ubiquitous features of nearly all plant communities and many plants rely on mycorrhizal fungi to supply them with N (Smith and Read 2008). Mycorrhizal hyphae are narrower in diameter than roots and hence are more efficient in exploring soil for nutrients. Some mycorrhizal fungi are capable of producing enzymes to access organic forms of N. As a result of supplying a significant amount of N to plants and the known fractionation that occurs during N transfers to host plants, some of these fungi can greatly influence the N isotopic patterns in plants and other ecosystem pools (Hobbie and Högberg 2012).

Mycorrhizal fungi can be separated into three major types, arbuscular mycorrhizal (AM), ectomycorrhizal (EM), and ericoid fungi (Hobbie and Hobbie 2008). These fungal types differ considerably in the distance from the root that they can explore (Coleman et al. 2004) and enzymatic capabilities to access different forms of N (Read and Perez-Moreno 2003). These differences can influence foliar $\delta^{15}N$ of host plants. At the global scale, Craine et al. (2009) showed that the type of mycorrhizal fungi associated with plants can account for roughly one third of the variation in foliar $\delta^{15}N$ values of the more than 9,000 plants sampled. Moreover, the type of mycorrhizal association can significantly influence foliar $\delta^{15}N$ values, with ericoid and EM plants being more depleted in foliar $\delta^{15}N$ (3.2 ‰ and 5.9 ‰, respectively) than non-mycorrhizal plants. AM plants are intermediate in their isotopic values, being depleted on average by 2 ‰ relative to non-mycorrhizal plants.

The greater difference in foliar $\delta^{15}N$ between EM and ericoid plants and non-mycorrhizal plants arises because of the preferential retention of the ^{15}N by fungal biomass and the preferential transfer of ^{14}N to host plants (Hobbie and Colpaert 2003; Hobbie et al. 2000;

Hogberg et al. 1996; Taylor et al. 2003). Although AM plants are slightly depleted in ^{15}N relative to non-mycorrhizal plants, there is no clear indication that AM fungi retain a ^{15}N-enriched pool or transfer ^{15}N-depleted N to host plants (Azcon-G-Aguilar et al. 1998; Wheeler et al. 2000). However, it is difficult to quantify N retention by AM fungi and thus it is uncertain to what degree AM fungi contribute to variation in foliar $\delta^{15}N$ (Handley et al. 1999b). Some of the differences in foliar $\delta^{15}N$ between AM and non-mycorrhizal plants might be due to differences in the form of N directly acquired by the plants or the environments they tend to occupy.

Plant N isotopes have also been used to determine the role mycorrhizal fungi play in host plant N acquisition. Using a mass-balance approach based on the natural abundance values of ^{15}N in plant foliage, EM sporocarps, and soil, Hobbie and Hobbie (2006) devised an analytical model to quantify the amount of N transferred from EM fungi to host plants in N-limited environments. Their model showed that 61-86% of the N in arctic plants was supplied by mycorrhizal fungi. However, it is important to point out that estimates of the proportion of N in the host plant derived from mycorrhizal fungi are sensitive to the ^{15}N values of N sources. Hobbie and Hobbie (2006) used the $\delta^{15}N$ signature of bulk soil N as their N source. A later study divided the bulk soil N into inorganic and organic fractions that differ in their isotopic values. Hydrolysable amino acids were as much as 10 ‰ less than other fractions in bulk soil (Yano et al. 2010). Using the signature of the labile N instead of bulk soil, Yano et al. estimated that only 30-60% of the plant's N was supplied by mycorrhizal plants. It is therefore crucial to determine the $\delta^{15}N$ signature of available N sources to accurately understand the role EM fungi play in host plant N acquisition.

Ecosystem N losses

In ecosystems where the isotopic signature of N inputs does not differ significantly from that of the atmosphere, loss pathways are the primary factors that ultimately enrich ecosystem N, but they also influence the signature of N available to plants. Comprehensive synthesis of isotope systematics in gaseous and hydrologic (particulate and dissolved) N losses from ecosystems has been limited primarily by the inability to measure natural abundance losses of N_2 from denitrification against the large background of atmospheric N_2 (Houlton and Bai, 2009). Gaseous losses are expected to have large fractionation factors, but the observable expression of isotope effects depends strongly on the degree to which the reaction goes to completion (Bai and Houlton 2009; Craine et al. 2009).

Hydrologic losses (leaching and erosion) do not seem to be accompanied by fractionation, as the exported nitrate, DON and particulate N have similar $\delta^{15}N$ of ecosystem N. Therefore, gaseous losses appear primarily responsible for imprinting large scale patterns on the natural abundance $\delta^{15}N$ of plants and ecosystems (Houlton and Bai 2009). Systematic understanding of isotope effects associated with soil N loss pathways can best be organized by following the dominant soil N transformations from the mineralization of SOM into each loss pathway. The mineralization process itself will introduce variability into the $\delta^{15}N$ of NH_4^+ primarily reflecting the $\delta^{15}N$ of definable SOM pools or fractions, which varies with factors such as depth.

Once NH_4^+ has been produced in soil solution, it is subject to volatilization as NH_3 under alkaline conditions, which is most likely to occur in hotspots or hot moments. Significant NH_3 volatilization can follow animal excreta deposition or fertilizer application. The rate

limiting process, diffusion into the atmosphere, has a high fractionation factor (17.9 ‰) that can be calculated in the same manner as for other gases emitted from soil (Stern et al. 1999). Empirical measurements of $\delta^{15}N$ for NH_3 relative to residual soil or plant NH_4^+ have indicated ^{15}N depletion by up to 40 ‰ (Högberg 1997), but may also incorporate ^{15}N enrichment of the NH_4^+ pool due to co-occurring nitrification or a second diffusional fractionation during collection. Under typical situations with high rates of volatilization, not all of the NH_4^+ pool is lost, so strong expression of the fractionation is expected. Given the large size of ammonia volatilization losses in some ecosystems (Billen et al. 2013), further systematic studies would be beneficial.

Other gaseous losses, including NO_x, N_2O, and N_2, occur mainly during nitrification and denitrification. The well-known loss pathways correspond to a 'hole-in-the-pipe' model (Firestone and Davidson 1989), and are also often associated with hot spots and hot moments, suggesting that the reactions responsible for gaseous losses seldom consume the entire reactant pool. Significant ^{15}N enrichment of residual soil N pools can therefore be expected whenever processes that fractionate strongly against ^{15}N are the rate-limiting steps in gaseous loss pathways.

Few N isotope measurements of NO_x, N_2O, and N_2 are available at plot or ecosystem levels, so the potential for isotope effects is commonly assessed through biochemical fractionation factors (Högberg 1997; Mariotti et al. 1982). Reported soil and soil-emitted N_2O $\delta^{15}N$ values typically range between 0 and -40 ‰ (Pérez et al. 2001; Pérez et al. 2000; Pörtl et al. 2007; Van Groenigen et al. 2005; Xiong et al. 2009), and therefore suggest varying but often strong ^{15}N depletion in the gaseous loss pathway. The $\delta^{15}N$ values of denitrified N_2 emitted from soil to the atmosphere have not been successfully

measured. Processes are likely to follow those in groundwater systems, which are closed to atmospheric N_2. Under these conditions, a batch reaction model implies that ^{15}N-depletion can be expected as denitrification proceeds, and measurements are believed to demonstrate that the $\delta^{15}N$ in excess N_2 matches the $\delta^{15}N$ of the NO_3^- source after nearly complete denitrification (Böhlke and Denver 1995; Böhlke et al. 2002). Gaseous loss pathways including NO_x and the HONO pathway (Oswald et al. 2013) also appear likely to have significant biochemical fractionations.

The $\delta^{15}N$ signature of NO_3^- has been critical to interpreting patterns of denitrification in oceans (Sigman et al. 2000; Sigman et al. 2009) as well as terrestrial ecosystems (Bai and Houlton 2009; Brookshire et al. 2012b; Fang et al. 2015; Houlton et al. 2006; Houlton and Bai 2009). Both nitrification and denitrification fractionate ^{15}N strongly with similar fully-expressed organism-level isotope effects of 20-30 ‰ (Högberg 1997; Mariotti et al. 1982). This isotope effect decreases with increasing external NO_3^- concentrations and C quality, which affects NO_3^- uptake rate, likely resulting in a system-level isotope effect of just 10-15 ‰ (Kritee et al. 2012). A similarly low expression of an isotope effect has also been shown for natural soils (Houlton et al. 2006). Such ecosystem-level underexpression can result from heterogeneity in rate-limiting conditions in the soil environment. Houlton et al. (2006) found that at the wet sites in a Hawaiian forest rainfall gradient, saturating conditions likely drive denitrification to near-completion thus resulting in no net expression of fractionation, a pattern expected from closed-system microsite conditions (Mariotti et al. 1982; Sigman et al. 2001). Plants in these ecosystems are not strongly ^{15}N-enriched, despite high rates of denitrification.

Inorganic N lost from the ecosystem through leaching is either derived from the decomposition of organic matter or direct losses of depositional N. Although the $\delta^{15}N$ of NO_3^- may not be diagnostic of depositional N, the ^{18}O of NO_3^- differs globally by an average of 40 ‰ (range = 20- 60 ‰) between atmospheric and biospheric waters. As such, the dual natural abundance isotope distributions of NO_3^- ($\delta^{15}N$ and $\delta^{18}O$) have been used to partition NO_3^- in groundwater or streams into NO_3^- that derives from internal microbial nitrification and NO_3^- that passes directly from atmospheric sources (Durka et al. 1994). Most studies have found a uniformly low direct contribution of atmospheric NO_3^-. However, studies in some temperate regions exposed to chronic atmospheric N pollution show periods of NO_3^- loss, particularly during high flow and snow-melt, when up to 20% of NO_3^- derives directly from atmospheric sources. Brookshire et al. (2012b) showed that many tropical forests naturally export high levels of NO_3^- similar to that of polluted temperate forests but that an average of >98% of the NO_3^- derives from nitrification in the plant-soil system. An even more direct way to way to separate atmospheric from microbial effects on NO_3^- is through analysis of $\Delta^{17}O$ owing to the fact that ^{17}O is enriched in atmospheric NO_3^- due to mass-independent photochemical reactions while mass-dependent processes (e.g., nitrification and denitrification) do not affect $\Delta^{17}O$ (Fang et al. 2015; Michalski et al. 2004).

Interpreting patterns of plant $\delta^{15}N$ within and among ecosystems

Inferring sources of N to plants from plant $\delta^{15}N$

Variation in $\delta^{15}N$ among plants within an ecosystem has been interpreted as representing differences in fixation, mycorrhizal dependence, depth of acquisition within the soil profile, utilization of depositional N and the form of N that plants predominantly acquire (Vallano and Sparks 2013). Among ecosystems, variation in plant $\delta^{15}N$ can be affected by these same factors, but the form of N is unlikely to drive variation in stand-level signatures when the majority of available N is acquired by plants. For example, if all of the NH_4^+ and NO_3^- available to plants is acquired, differences in signatures between the two caused by fractionation during nitrification will not affect the mean signature of the inorganic N. Among ecosystems, soil and plant $\delta^{15}N$ can also be affected by variation in the ^{15}N value of atmospheric N deposition. When distal sources of N have similar values, as described earlier, plants or soils with higher $\delta^{15}N$ are often assumed to experience (or have) higher N availability.

As a result of the multiple potential influences on plant or soil $\delta^{15}N$, interpretations are not necessarily straightforward. Given the wide variation in signatures of atmospheric sources of N to plants and multiple factors in the soil that can affect plant $\delta^{15}N$, variation in plant $\delta^{15}N$ across spatial gradients or over time cannot only be interpreted as a signal of depositional N. Vallano and Sparks (2013) examined the foliar $\delta^{15}N$ of mature trees of four species along an urban-rural gradient that included variation in NO_2 concentrations

in the atmosphere. They found that after accounting for variation in soil $\delta^{15}N$, there was no relationship between NO_2 concentrations and foliar $\delta^{15}N$ for two species, a positive relationship for one, and a negative relationship for the fourth. The authors hypothesized that one species utilized enriched N in the atmosphere and the other depleted N. Yet, the signatures of N the plants were differentially accessing would have to differ by 20‰ to generate observed differences in $\delta^{15}N$ between the two species (Vallano and Sparks 2008). Even bryophytes that presumably rely on atmospheric N entirely (Binkley and Graham 1981) can vary by 8 ‰ within a narrow geographic region (Delgado et al. 2013).

Interpreting variation among species within a site is complicated due to the multiple processes influencing isotopic values. Although nitrification is a fractionating process, the assumption that variation among plants within a site reflects differences in uptake of NO_3^- vs. NH_4^+ might not be valid (Kahmen et al. 2008). Across a number of European grasslands, species that preferred NO_3^- relative to NH_4^+ under controlled conditions would be predicted to have lower foliar $\delta^{15}N$ than plants that preferred NH_4^+. Yet, plants that preferred NO_3^- were more enriched in ^{15}N, not less enriched. Among potential explanations for this pattern, NO_3^- may have been more enriched than NH_4^+ in the soils due to gaseous N loss subsequent to nitrification. Other differences among plants within a site could be due to differences in dependence on mycorrhizal fungi, or the depth in the soil profile from which N is acquired.

Because of the difference in ^{15}N signatures between N_2-fixing plants and non-N_2-fixing plants, natural abundance ^{15}N signatures have the potential to shed light on the dependence of different plants on recently fixed N. It is commonly assumed that plants relying exclusively on N_2 fixation have $\delta^{15}N$ of 0 ‰ (Robinson 2001), although this

assumption should be checked against cultivation of the plants in an N-free medium (Shearer and Kohl 1986). The fact that N_2-fixing plant $\delta^{15}N$ is approximately 0 ‰ has been used in mixing models to calculate the quantitative dependence of plants on N_2 fixation. The fraction of N derived from atmospheric N_2 is given by the following mixing model:

$$fN_{atm} = \frac{\delta^{15}N_{ref} - \delta^{15}N_{target}}{\delta^{15}N_{ref} - \delta^{15}N_{fix}},$$

where $\delta^{15}N_{ref}$ is the $\delta^{15}N$ for a reference plant that does not depend on N_2 fixation, $\delta^{15}N_{fix}$ is the $\delta^{15}N$ of a plant relying only on N_2 fixation (often assumed to be 0 ‰) and $\delta^{15}N_{target}$ is the $\delta^{15}N$ of the species for which dependence is being calculated (Shearer and Kohl 1986). Although this measurement has been widely applied, it can at best be considered an estimate. Finding a reference plant that is using the same soil N pool as the target species may be challenging. Uncertainty in the signature of N_2-fixing plants and determination of the signatures of non-N_2 fixing plants greatly reduces the utility of this approach. In addition, estimates of the signatures of plants need to include more than just the signature of foliar N when it is unrepresentative of the whole plant isotopic signature (Bouillet et al. 2008). Given the sensitivity of the two-pool mixing model to the signatures of either end member, the difference of even just 1 ‰ could have large effects on estimates.

Interpreting plant $\delta^{15}N$ as an indicator of N availability

N availability drives a significant amount of variation in plant $\delta^{15}N$ at local to regional scales. When N supplies are high relative to demand by plants and microbes, N accumulates in inorganic pools. Larger pools of NH_4^+ increase the likelihood of NH_3

volatilization and/or nitrification, which both increase the $\delta^{15}N$ of remaining inorganic pools. When NO_3^- pools are high, denitrification may also be more likely, which again can enrich the remaining inorganic pools in ^{15}N. Plants that experience greater N availability may reduce their dependence on mycorrhizal fungi. This reduced dependence on mycorrhizal fungi can enrich plants by reducing the depletion associated with N transfers from mycorrhizal fungi (Högberg et al. 2011). As N availability increases relative to C, soil microbial biomass is likely to become more enriched in ^{15}N, given greater N dissimilation compared to N assimilation, and the discrimination against ^{15}N associated with dissimilation (Dijkstra et al. 2008). Yet, the subsequent enrichment from gaseous N losses likely overrides this depleting factor.

N fertilization studies demonstrate that plants become enriched in ^{15}N as N availability increases. For example, an understory grass species in fertilized forest plots was enriched in ^{15}N by more than 11 ‰ relative to control plots (Johannisson and Högberg 1994). In a separate study, loblolly pine needles became enriched by as much as 5 ‰ with N fertilization (Choi et al. 2005).

Plant $\delta^{15}N$ also increases with increasing N availability across natural N supply or N availability gradients. In the Smoky Mountains, Tennessee, forests with high potential N mineralization had leaves that were enriched in ^{15}N by approximately 3 ‰ relative to stands with low potential N mineralization rates (Garten and Van Miegroet 1994). Craine et al. (2009) examined relationships between metrics of N supply or availability and foliar $\delta^{15}N$ across 15 studies. Consistently, when N supply or availability was measured *in situ*, $\delta^{15}N$ increased with N availability (Figure 4). There was less of a consistent relationship when N mineralization was measured as potential rates under standardized

conditions in the laboratory—positive correlations with $\delta^{15}N$ were only reported in 3 of the 5 studies (Craine et al. 2009).

Because sites with higher N availability are more likely to have plants with higher N concentration, plant N concentration tends to correlate positively with plant $\delta^{15}N$. At a global scale, foliar $\delta^{15}N$ increased logarithmically with increasing leaf N concentrations. On average, plants with foliar N concentrations of 40 mg N g^{-1} were enriched in ^{15}N by 4 ‰ more than plants with just 10 mg g^{-1} N (Craine et al. 2009). Stronger patterns can be present at local scales. For example, across 371 non-leguminous species in a tallgrass prairie, plants with foliar N concentrations of 40 mg N g^{-1} were 6.1 ‰ higher than plants with just 10 mg N g^{-1} (Craine et al. 2012).

Soil organic matter N isotopes

The processes that lead to variation in the isotopic ratio of SOM largely overlap with those for plants. Losses of depleted N from available pools enrich the remaining available N pool, which would enrich plants and microbes as well as the organic matter they produce. Yet, as soil organic matter turns over on slower time scales than plant organic matter, SOM $\delta^{15}N$ is likely to reflect longer term processes than plant $\delta^{15}N$. In this section, we focus on the patterns of $\delta^{15}N$ in SOM within and across soils as well as the likely mechanisms that generate these patterns.

Local and global range of soil organic matter $\delta^{15}N$

In the first broad survey of the $\delta^{15}N$ of SOM, Shearer and Kohl (1978) analyzed SOM $\delta^{15}N$ from over 100 soils from 20 US states. They examined the relationships between

SOM $\delta^{15}N$ and climate, depth, soil pH and land use. They reported that the average $\delta^{15}N$ of SOM was 9.2 ‰ with 90% of the samples ranging from 5 to 12 ‰, but could detect few geographic patterns.

Since the initial surveys of Shearer and Kohl (1978), our understanding of the patterns of SOM $\delta^{15}N$ and the mechanisms that underlie them has progressed substantially. Whereas Shearer and Kohl (1978) observed just 7 ‰ variation in the $\delta^{15}N$ of SOM, the global range of non-fertilized surface SOM $\delta^{15}N$ has now been quantified at ~30 ‰. The highest surface soil $\delta^{15}N$ recorded was 22.0 ‰ collected in South African fynbos on the Cape Peninsula (M. Cramer, unpublished). The highest surface SOM $\delta^{15}N$ not adjacent to marine ecosystems was 17.7 ‰, which was in the arid lowlands of Ethiopia (Terwilliger et al. 2008). The lowest surface SOM $\delta^{15}N$ was -7.8 ‰, collected from organic soils on moist acidic tundra (Bret-Harte et al. 2008). Among non-marine surface soils, 99% of the surface soil $\delta^{15}N$ samples fell within 17.6 ‰ (-5.0 ‰ – 12.6 ‰) and 95% of the samples fell within 14 ‰ (-3.5 ‰ – 10.5 ‰).

Local variation in SOM $\delta^{15}N$ has not been quantified as well as it has been for plants. Nevertheless, the $\delta^{15}N$ of surface SOM varied by as much as 16 ‰ along a 300-m transect in Zambian woodland savanna (Wang et al. 2013). When aggregated to the 0.1° latitude/longitude scale, the range of surface soil $\delta^{15}N$ increases logarithmically with increasing sampling density, but is independent of climate (Figure 1; $P > 0.05$ for MAT, MAP) (Craine et al. 2015). With 10 samples, the range is 4.1 ‰. With 100 samples, the range is 7.6 ‰.

^{15}N patterns related to litter and soil organic matter decomposition

Multiple studies of litter decomposition have demonstrated that litter $\delta^{15}N$ increases as decay proceeds. In a field decomposition study of grass and hardwood tree roots, the $\delta^{15}N$ of root litter increased by 1-3 ‰ over 5 years (Connin et al. 2001). Changes in isotopic composition during decomposition and microbial processing of leaf litter and SOM can vary with duration of incubation, differences in the mechanisms and controls on rates of decay, sequence of degradation of chemical compounds, and degree of incorporation of microbial biomass and residues.

Although loss of depleted N enriches organic matter throughout the continuum from litter to SOM, the early stages of decomposition can be associated with reductions in $\delta^{15}N$ as ^{15}N-depleted N is imported into microbial biomass. In one of the first studies of chemical changes during litter decomposition, the litter of pine needles decreased in $\delta^{15}N$ by 2 ‰ as relatively ^{15}N-depleted N was immobilized into litter over the first 22 months of decomposition (Melillo et al. 1989). Once net N mineralization began, N content of the litter began to decline and the $\delta^{15}N$ of the litter began to increase.

During the initial stages of decomposition, the $\delta^{15}N$ of organic matter can increase or decrease. The direction of change in N isotopic composition during litter decay result from differences in the degree of decomposition and nutrient availability. During the first year of a 2-year field incubation study of *Sphagnum* litter in a peatland, samples incubated in the oxic zone showed greater ^{15}N enrichment than litter in the anoxic zone (Asada et al. 2005). During a 3-year decay study in an alpine bog, *Sphagnum* showed enrichment in $\delta^{15}N$ while two vascular plant species showed declines in $\delta^{15}N$ (Bragazza

et al. 2010). *Spartina* biomass decaying in salt-marsh sediments also has exhibited declines in $\delta^{15}N$ over 18 months (Benner 1991).

The enrichment in $\delta^{15}N$ during organic matter decomposition is often attributed to incorporation of microbial biomass and residues into decaying litter and SOM. Over the course of a 6-month laboratory incubation of a cultivated soil, soil microbial biomass became significantly ^{15}N-enriched relative to bulk soil, while water-soluble N became ^{15}N-depleted (Lerch et al. 2011). The relationship between the ratio of microbial biomass enrichment relative to the water-soluble fraction and the C:N ratio of the water-soluble fraction followed an exponential decay model, indicating that the enrichment factor stabilized over the length of the incubation. The degree to which organic matter becomes enriched in ^{15}N during decay is likely influenced by the C- vs. N-limited status of the microbes performing the decomposition (Dijkstra et al. 2008), with enhanced ^{15}N enrichment of microbial biomass reflecting an increasing degree of N dissimilation linked to relative C limitation. This effect is driven in large part by discrimination against ^{15}N during transformations of organic N to NH_4^+, equilibrium isotope effects as NH_4^+ and NH_3 experience state changes, and discrimination against ^{15}N during subsequent loss of NH_3 from the cell.

Patterns among soil organic matter fractions

Trends in isotopic composition of SOM pools are consistent with enrichment in $\delta^{15}N$ with progressive decay and microbial alteration. Increasingly, conceptual models of SOM assume that most SOM in mineral soils is composed of microbially-processed OM (Gleixner 2013; Liang and Balser 2010; Schmidt et al. 2011). Hence, SOM with longer residence times in soil are expected to reflect isotopic signature of decomposers more

than of initial plant litter inputs. Consistent with this idea, SOM fractions generally show increasing values of $\delta^{15}N$ with decreasing particle size and increasing density or increasing mineral association (Baisden et al. 2002b; Billings 2006; Liao et al. 2006; Marin-Spiotta et al. 2009). For example, in a clay-rich tropical Oxisol, the $\delta^{15}N$ of SOM fractions increases with increasing microbial processing as evidenced by greater $\delta^{15}N$ of low C:N fractions (Figure 5).

Liao et al. (2006) quantified the C and N isotopic ratios of soils from sites where C_3 trees and shrubs replaced C_4 grasslands. Different physically-separated fractions of the soils varied by 6 ‰. The silt and clay fractions were most enriched in $\delta^{15}N$ and also had the longest radiocarbon-based mean residence times, suggesting stabilization of highly-processed organic matter in the fine-sized physical fractions. Similar patterns were observed in a highly-weathered wet tropical forest soil (Marin-Spiotta et al. 2009; Marín-Spiotta et al. 2008). The decline in C:N ratios typically seen with increasing SOM fraction $\delta^{15}N$ was associated with plant litter decay and incorporation of microbial biomass and products, as well as an increase in C mean residence time. In a study describing SOM chemistry in four soils representing a range of mineralogy and climate, Sollins et al. (2009) reported increases in $\delta^{15}N$ and decreases in C:N ratios with increasing density across a series of physical fractions that isolated organic matter of increasing radiocarbon mean residence time associated with different mineral types. Along a soil chronosequence in California annual grasslands, declining C:N and increasing $\delta^{15}N$ by up to 3 ‰ were observed with increasing mineral association in sequential density fractions (Baisden et al. 2002b). By using time-series to quantify multiple pool sizes and residence times, rather than a mean residence time, this study

found that changes in C:N and $\delta^{15}N$ were associated with pool size and might therefore reflect the degree of microbial transformation during mineral stabilization processes. Further supporting the idea of N isotopic enrichment with SOM transformations, Kramer et al. (2003) demonstrated a strong positive relationship between a common index of organic matter decomposition and microbial alteration, the alkyl-to-O-alkyl C ratio, and $\delta^{15}N$ in bulk soils and physical density SOM fractions.

The overall $\delta^{15}N$ signature of soils will depend on the signatures and the relative abundance of different fractions. For example, in a forest soil profile, most soil N (75-86%) was located in aggregates (Huygens et al. 2008). Consequently, values of $\delta^{15}N$ bulk soil were closely related to values of aggregates and displayed an increasing trend with increasing soil depth.

Patterns of soil organic matter $\delta^{15}N$ with depth

Variation in $\delta^{15}N$ with depth was shown early on to be substantial. Along an elevational gradient, Mariotti et al. (1980) showed that soil at just 50 cm deep can be enriched by up to 9 ‰ relative to surface soils. Wang et al. (2009) showed that soil at 90-cm depth can be enriched by up to 17.2 ‰ relative to surface soil in an African savanna. In a review of a global distribution of 88 soil profiles, Hobbie and Ouimette (2009) showed that $\delta^{15}N$ of SOM at 50 cm depth was enriched relative to surface litter by 9.6 ‰ for soils under ectomycorrhizal plant species and by 4.6 ‰ for plants under arbuscular mycorrhizal species. In contrast, in arid and semi-arid systems where soil pH is high, surface $\delta^{15}N$ values can be elevated by as much as 7 ‰ relative to deeper soils (Pataki et al. 2008).

The depth distribution of the $\delta^{15}N$ of SOM in a given soil profile is largely considered a function of the signature of inputs and losses that occur during the decomposition processes. Surface SOM $\delta^{15}N$ values typically are dominated by the $\delta^{15}N$ of incoming litterfall and root inputs. These vegetative components, in turn, exhibit $\delta^{15}N$ signatures indicative of their N source and internal allocation and re-allocation of N supplies (Robinson 2001). Assuming transport is generally downward, decomposition processes become a more dominant influence on soil $\delta^{15}N$ deeper in the soil profile – an effect that has been modeled consistently using C and N isotopes and abundances (Baisden et al. 2002a). As SOM age tends to increase with depth (Trumbore 2000; 2009), many studies assume that the degree of microbial processing of SOM generally increases with depth. Consistent with this, soil C:N tends to decline with depth (Marín-Spiotta et al. 2014) and SOM $\delta^{15}N$ often increases (Billings and Richter 2006; Compton et al. 2007; Piccolo et al. 1996). As discussed above, this is typically assumed to result from the fractionation associated with decay and microbial assimilation or dissimilation of N, with resulting ^{15}N-depletion or enrichment of microbial biomass, respectively (Dijkstra et al. 2006).

Although large contributions of microbial necromass to SOM are likely ubiquitous, their effect on soil profile $\delta^{15}N$ may be outweighed by the effect of gaseous losses dominating soil N cycling in surface soils. For example, Pataki et al. (2008) attributed ^{15}N enrichment of an alkaline soil in an arid ecosystems to ammonia volatilization and its large enrichment factor. It remains unclear whether this feature is ubiquitous in alkaline soils where NH_3 volatilization is a dominant process, or why other fractionating losses of nitrogenous gases (e.g. N_2O) do not appear to result in similar profiles.

In soils supporting aggrading forests with high vegetation nutrient demand, SOM decomposition can outweigh SOM formation (Richter et al. 1999). In these soils, increases in soil $\delta^{15}N$ with SOM decay can become evident within years, and during forest development, agriculturally well-mixed soil profiles can attain the vertical ^{15}N distribution typically seen in less disturbed profiles over decades (Billings and Richter 2006). This rapid shift in soil $\delta^{15}N$ with forest development is attributed to the accumulation of ^{15}N-enriched microbial necromass and, to a lesser extent, fractionation effects during SOM decay.

The mycorrhizal association of the dominant plant species is another important factor in explaining variation in vertical patterns of $\delta^{15}N$ in soils. Hobbie and Ouimette (2009) showed that almost all soil under ectomycorrhizal species had monotonically increasing soil $\delta^{15}N$, while 40% of the AM sites had the highest $\delta^{15}N$ at intermediate depth. There were no strong relationships between climate and the pattern of soil $\delta^{15}N$ with depth. Also, soils with higher nitrification rates did not appear to have greater vertical distributions of soil $\delta^{15}N$.

Vertical patterns in soil $\delta^{15}N$ also have the potential to be influenced by hydrologic movement of N. In a Hawaiian tropical rain forest, Marin-Spiotta et al. (2011) reported a soil ^{15}N profile with maximum $\delta^{15}N$ at intermediate depths. They attributed this pattern to differences in drainage and microbial processing in the upper and lower soil profile due to the presence of cemented or placic layers forming along hydrologic flow paths. Differences in the $\delta^{15}N$ above and below these layers were consistent with patterns in soil C:N ratios and the accumulation of organic matter at depth with isotopic and chemical

signatures more similar to the surface organic horizons. Thus, differences in decomposition (and the losses that occur therein) and the transport in preferential flowpaths of recent, surface organic matter to deeper mineral soil layers in very wet sites, with poor drainage, or with high shrink-swell capacity soils can also lead to vertical soil $\delta^{15}N$ profiles that differ from the more commonly observed enrichment with depth.

Interpreting differences between plant and soil $\delta^{15}N$

One of the most important steps in moving forward is an assessment of the relative merits of soil $\delta^{15}N$, plant $\delta^{15}N$, or the difference between the two for interpreting patterns of $\delta^{15}N$. The difference between plant $\delta^{15}N$ and soil $\delta^{15}N$ is generally referred to as the enrichment factor (Mariotti et al. 1981). It is called an "enrichment" factor based on the assumption that plant N is the end product of a series of enriching reactions that begin with soil organic matter. It is thought that standardizing patterns of plant $\delta^{15}N$ for underlying variation in $\delta^{15}N$ of SOM will remove variation in the signature of the source of $\delta^{15}N$ and better reveal N cycling patterns.

There are cases where enrichment factors appear to be better indicators than soil ^{15}N of N cycling rates among ecosystems. Emmett et al. (1998) compared enrichment factors among coniferous forests in order to normalize initial differences in soil $\delta^{15}N$ values for effect of land management practices, soil age, and climate. Among sites, surface soil $\delta^{15}N$ varied by ~6 ‰. Calculating the enrichment factor for these sites led to better relationships with N availability metrics than foliar $\delta^{15}N$.

When examined globally, plants are almost always more depleted in ^{15}N than soils. When comparing site-averaged foliar $\delta^{15}N$ and $\delta^{15}N$ of SOM (typically 0-20 cm) with data from Craine et al. (2009), in 92% of the sites, foliar $\delta^{15}N$ was less than that of soils (average difference of 3.3 ‰). Ericoid plants were the most depleted relative to SOM on average (-4.9 ± 0.2 ‰) with ectomycorrhizal plants (-4.0 ± 0.1 ‰) and arbuscular plants (-3.4 ± 0.1 ‰) showing similar levels of relative depletion. Even non-mycorrhizal plants were still depleted on average (-0.6 ± 0.2 ‰). Craine et al. (2009) reported that sites with high foliar $\delta^{15}N$ also had a large absolute difference between the $\delta^{15}N$ of leaf and SOM.

At the global scale, leaves are more depleted in ^{15}N than soils across the global climate spectrum even when factoring out mycorrhizal influences. To compare the global relationships between leaves and SOM, average SOM $\delta^{15}N$ was determined for 901 locations at the global scale assuming a depth of 30 cm using the data from Craine et al. (2015). In order to predict foliar $\delta^{15}N$ at each of these locations, we used the data from Craine et al. (2009) to establish relationships between foliar $\delta^{15}N$ and climate parameters (MAT, MAP) for plants of different mycorrhizal types assuming each plant had the global mean foliar N concentration in the dataset of 16.2 mg N g^{-1} (Craine et al. 2009). This allows one to calculate the $\delta^{15}N$ of a plant of a given mycorrhizal type anywhere in the global climate space. Given the soil $\delta^{15}N$ and predicted foliar $\delta^{15}N$ at each of the 901 locations, soils were more enriched than plants in 74% of the sites for the typical non-mycorrhizal plant and 99.9% of the sites for ericoid plants (Figure 6).

Three hypotheses have been offered to explain the consistent depletion of leaves relative to soils. First, solubilization of N leads to greater isotopic fractionation than previously thought. As discussed earlier, work on the signatures of microbial biomass suggest that

there are additional fractionation factors associated with mineralization that had not previously been considered when comparing plants and soils. Still, more research and modeling is needed to determine the potential influence of microbial enrichment on the net ^{15}N depletion of plants. Second, mycorrhizal transfers of N and fractionation during these transfers is stronger than previously thought. Although average differences among mycorrhizal types have been assessed, there are still uncertainties regarding the magnitude of fractionation under different conditions and the degree to which utilization of different forms of N contributes to the variation in $\delta^{15}N$ among plant species with different mycorrhizal symbioses. Third, the $\delta^{15}N$ of bulk SOM is not a good indicator of the signature of the pool that serves as a source of N to plants. Associated with differences in turnover, inorganic N is more likely to come from the relatively depleted non-mineral-associated pools rather than the enriched, mineral-associated pools. Critical knowledge gaps remain if we want to quantify the signatures of available N. More research is needed to link the signature of different SOM fractions and the source of N for plant and microbial uptake.

In order to link enrichment factors to N status or N availability, a number of conditions would have to be met or accounted for. Regarding sources of N, deposition would have to be a small source of N or have a similar signature as SOM. The greater ^{15}N enrichment of high-clay soils may make it seem like there is a lower enrichment in plants relative to SOM on high-clay soils than low-clay soils.

When comparing the signature of plants, both the depth of N acquisition and mycorrhizal type would have to be standardized. The presence of N fixers can also skew enrichment factors between soils and plant $\delta^{15}N$. N_2-fixing plants are typically excluded from

calculations of average plant $\delta^{15}N$, but rely on soil-derived N, too. Any difference in the signatures of N acquired by non-N_2-fixing and N_2-fixing plants would alter the calculated enrichment factor.

Although enrichment factors have been useful in determining N availability in some ecosystems, their application can be limited by the aforementioned processes that lead to variability in source N. Broad, cross-site studies that measure N cycling parameters as well as SOM and plant $\delta^{15}N$ are relatively rare. Given the difficulty in comparing N supplies or availability across broad contrasts where N is cycled fundamentally differently, e.g. organically vs. inorganically, the utility of enrichment factors to assess differences in N availability is unlikely to be tested soon. Instead, this technique is more likely to be useful across narrow contrasts with little variation in other factors. Specific interpretations of plant $\delta^{15}N$, soil $\delta^{15}N$, or enrichment factors will still need to occur on a case by case basis.

Interpreting N isotope patterns across climate gradients

As plant and soil $\delta^{15}N$ data have accumulated, $\delta^{15}N$ patterns and their interpretations have changed over time generating some confusion on how N cycling parameters might be changing along climate gradients. The first attempt to broadly synthesize relationships between climate and plant $\delta^{15}N$ was by Handley et al. (1999a) who found that foliar $\delta^{15}N$ declined linearly with increasing rainfall across 97 sites. Their working hypothesis to explain this pattern followed Austin and Vitousek (1998): dry sites have a more "open" N cycle with a greater importance of inputs and outputs compared to within-system cycling. Although Handley et al. found no influence of latitude on foliar $\delta^{15}N$, Martinelli et al.

(1999) reported that tropical leaves averaged 6.5 ‰ higher $\delta^{15}N$ than temperate leaves (3.7 vs. -2.8 ‰). These authors proposed a similar explanation, that tropical forests typically have a more "open" N cycle, with large inputs and outputs of N relative to internal N cycling.

Amundson et al. (2003) synthesized foliar $\delta^{15}N$ from 106 sites and demonstrated that foliar $\delta^{15}N$ increased with increasing mean annual temperature (MAT) and decreasing mean annual precipitation (MAP). They interpreted these patterns as indicating that hot, dry sites have both a greater proportion of N being lost through fractionating pathways and a more open N cycle. The authors suggested that because most undisturbed soils are near N steady state, an increasing fraction of ecosystem N losses with decreasing MAP and increasing MAT were ^{15}N-depleted forms (NO_3, N_2O, etc.).They concluded that wetter and colder ecosystems appeared to be more efficient in conserving and recycling mineral N.

A subsequent study of over 11,000 non-N_2 fixing plants at the global scale found that foliar $\delta^{15}N$ increased logarithmically with decreasing MAP (Craine et al. 2009). Foliar $\delta^{15}N$ increased linearly with increasing MAT, but only for those ecosystems with MAT > -0.5°C. Due to linkages observed at local scales between N availability and foliar $\delta^{15}N$, these global relationships between climate and foliar $\delta^{15}N$ were interpreted to suggest higher N availability in warm, dry ecosystems.

As was the case for plants, observations of the patterns of soil $\delta^{15}N$ with climate and their explanations have shifted over time. After the Shearer and Kohl (1978) synthesis of North American soils, there was a 20-year gap in synthesizing soil $\delta^{15}N$. In 1999, two

papers were published that began to frame global patterns of soil ^{15}N with respect to climate (Handley et al. 1999a; Martinelli et al. 1999). Handley et al. demonstrated that across 47 soils, low-latitude sites had lower $\delta^{15}N$ in SOM from surface mineral soils. With a different set of soils, Martinelli et al. showed the opposite pattern—tropical soils were more enriched in $\delta^{15}N$ than temperate soils. Combining the data of previous studies, Amundson et al. (2003) reported that average soil $\delta^{15}N$ followed similar patterns as foliar $\delta^{15}N$. Across 47 soils, average soil $\delta^{15}N$ to 50 cm increased with increasing MAT and decreased with increasing MAP ($P < 0.1$). This further supported their conclusion of greater proportions of fractionating losses in hot, dry ecosystems compared to the "more efficient" cold, wet ecosystems.

On the other hand, an extensive dataset of soil $\delta^{15}N$ that included key covariates (soil C and clay concentrations) suggests different mechanisms at work (Craine et al. 2015). Across 6,000 soil samples that were aggregated to 910 locations (0.1° latitude and longitude), the $\delta^{15}N$ of surface mineral soils was greater for sites with high MAT and low MAP, but there was no relationship between SOM $\delta^{15}N$ and MAT across ecosystems with MAT < 9.8°C. Soil $\delta^{15}N$ increased with decreasing C and N concentrations as well as decreasing C:N, similar to what is observed with increasing decomposition of organic matter and soil depth. Organic soils with a [C] of 450 mg C g^{-1} soil on average had a $\delta^{15}N$ of -0.2 ‰. Mineral soils with a [C] of 200 mg C g^{-1} soil on average had a $\delta^{15}N$ of 3.1 ‰. Mineral soils with a [C] of 20 mg C g^{-1} soil on average had a $\delta^{15}N$ of 5.0 ‰. In addition to soils with lower [C] being more enriched in $\delta^{15}N$, soils with higher clay concentrations were also more enriched in ^{15}N in a parallel manner to differences among soil fractions

that differ in clay content. Across soils, increasing clay concentrations by an order of magnitude increases soil $\delta^{15}N$ by 2.0 ‰.

When viewed independent of covariates, the relationships between climate and SOM $\delta^{15}N$ would suggest that a greater proportion of N was lost via fractionating processes in warm, dry ecosystems than cold or wet ecosystems. Yet, SOM C and N concentrations declined with increasing MAT and decreasing MAP, suggesting that the SOM of warm, dry ecosystems had been processed more on average than cold, wet ecosystems. In addition, SOM $\delta^{15}N$ was greater in ecosystems with higher clay concentrations and warm ecosystems tended to have higher clay concentrations than colder ecosystems. Amazon ecosystems (including white-sand forests) averaged 37% clay, while soil samples from all other ecosystems averaged just 11%. Many Amazonian forests had clay concentrations in excess of 60% (Quesada et al. 2010). After standardizing for variation in C concentrations (an index for the degree of microbial processing) and clay concentrations, SOM $\delta^{15}N$ did not vary with increasing MAT or MAP (Craine et al. 2015).

Hot ecosystems likely lose a similar proportion of N via gaseous pathways as cold ecosystems, as do wet and dry ecosystems. Compared to high-latitude ecosystems, tropical forests tend to lose a greater amount of N via fractionating pathways, but also non-fractionating pathways (Brookshire et al. 2012b). Tundra ecosystems are typically considered dominated by organic N cycling and net N mineralization is rare (Schimel and Bennett 2004). Yet, N_2O fluxes can be a high proportion of losses with relatively high gaseous N loss rates outside of the summer when mineralization and plant uptake are decoupled (Buckeridge et al. 2009; Filippa et al. 2009; Harms and Jones 2012).

In all, although the patterns of plant and soil $\delta^{15}N$ are clearer today than they were a decade ago, there is still uncertainty in how to interpret these patterns. On the one hand, plant and soil $\delta^{15}N$ might indicate greater N availability and greater relative importance of fractionating losses in hot, dry ecosystems compared to cold, wet ecosystems. On the other hand, taking into account covariates with soil $\delta^{15}N$ associated with the degree of processing of SOM and/or the proportion of mineral-associated organic matter, there may be little consistent difference in the relative proportion of fractionating losses across climate gradients. If so, there may be a need to reinterpret why foliar $\delta^{15}N$ is greater in hot, dry ecosystems than cold, wet ecosystems.

Summary and future research

Over the past decades, scientists from a broad array of disciplines have made significant strides in better understanding patterns of N isotopes in plants and soils and the processes that underlie variation in the patterns. Compared to a decade ago, we better understand such factors as the role of mycorrhizal fungi in influencing plant $\delta^{15}N$, the role of climate in determining both plant and soil $\delta^{15}N$, the changes in $\delta^{15}N$ that occur with microbial processing, the differences in $\delta^{15}N$ among soil fractions, and the signatures of $\delta^{15}N$ of different forms of N in the soil. Also, an integrated N cycle that ascribes different degrees of fractionation for each step under different conditions is close at hand. Progress in these areas sets the stage for further advances on a number of fronts.

First,.although the ^{15}N-enrichment of microbial biomass has been observed, there are still too few measurements of the ^{15}N signatures of microbial biomass to generalize their contribution to the isotopic values of plants under different environmental conditions. We

still cannot determine how much of the general ^{15}N-depletion of plants relative to soils is due to enrichment of microbial biomass during mineralization, or how differences in microbial communities affect isotopic values.

Second, the isotopic values of different forms of N in the soil need to be measured across a wider range of environmental conditions. We still cannot generalize patterns in values of dissolved organic N or inorganic N under a given set of conditions. Hence, we are limited in interpreting variation in plant $\delta^{15}N$ in terms of the form of N that plants acquire. A better understanding of the signatures of different forms of N is also essential for constraining the relative importance of different loss pathways in different ecosystems, and will assist in narrowing the range of fractionation factors associated with a particular process such as denitrification, which are still too broad to reliably constrain process-based modeling. Although we know that more-processed organic matter is more enriched in ^{15}N, the pairing of $\delta^{15}N$ signatures with measurements of soil microbial biomass and different organic matter pools, as well as other biochemical measures of microbial alteration and synthesis, will be critical to understanding the relative importance of fractionation associated with internal processes versus fractionation associated with loss pathways.

Third, more measurements of gaseous loss rates and the signature of gaseous loss products are necessary to test the degree to which plant or soil $\delta^{15}N$ reflect the relative importance of fractionating loss pathways. Along these lines, the signatures of deposited N are still too sparse to reliably incorporate into interpretations of $\delta^{15}N$ patterns. Too frequently, any changes in $\delta^{15}N$ over time are interpreted as resulting from changing

deposition patterns with little verification of the signatures of deposition and the potential relative importance of other pathways in affecting the ultimate sample signature.

Fourth, comprehensive modeling of whole-ecosystem N cycling with respect to isotopic fractionation is still in its early stages. Simple models of ecosystem-level $\delta^{15}N$ have assumed that at steady state, the signature of the N lost must be opposite in sign and of equal magnitude to the ^{15}N-values of the N in an ecosystem. Yet, pools of N differ in both their ^{15}N-values and turnover time, potentially decoupling the signatures of whole-ecosystem N and N exports. Better direct quantification of the isotopic composition of gaseous losses will help to test this. At the same time, multi-pool ecosystem models need to be deployed to further assess the sensitivity of whole-ecosystem $\delta^{15}N$ to turnover rates of different pools and pathways of N loss, and to test the processes that generate variation in SOM $\delta^{15}N$ with soil depth.

Lastly, this review demonstrates how much of the development of our understanding of N isotopes has come from integrating research across a broad range of disciplines. Fields as diverse as atmospheric chemistry, terrestrial ecosystem science, soil science, plant community ecology, plant ecophysiology, microbiology, molecular ecology, and mycorrhizal ecology all make substantial contributions to our emerging understanding of $\delta^{15}N$ signatures within ecosystems. By integrating approaches from these disciplines, we can make greater advances towards using N isotopes as a means of developing a predictive framework of ecosystem function.

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Figures

Figure 1. Range in $\delta^{15}N$ observed among a) sites for foliar $\delta^{15}N$ and b) 0.1° latitude and longitude for soils as a function of the number of samples measured per site or grid cell. Data from Craine et al. 2012 and Craine et al. 2015.

Figure 2. Relationship between bulk soil $\delta^{15}N$ and the $\delta^{15}N$ of organic N (closed squares), NH_4^+ (closed circles), and NO_3^- (open circles) across a range of soil depths from a subtropical forest in China (Koba et al. 2010). Each point represents a value derived from a particular soil depth (O horizon-100 cm) from three different locations within the forest.

Figure 3. Relationship between $\delta^{15}N$ of soil inorganic nitrogen and foliage $\delta^{15}N$. Data derived from multiple sources (Boddey et al. 2000; Cheng et al. 2010; Garten and Van Miegroet 1994; Pate et al. 1993; Takebayashi et al. 2010). When $\delta^{15}N$ of soil inorganic nitrogen was not provided in the reference, it was calculated as the mean value of $\delta^{15}N$-NH_4^+ and $\delta^{15}N$-NO_3^-, weighed by the size of these two N pools. Shown are the orthogonal fit (solid line; $y = 1.35 + 1.05x$; 95% CI for slope = 0.69 – 1.62, $r = 0.74$, $P < 0.001$). Identity line is shown dashed.

Figure 4. Effect leverage plots of standardized N supply and foliar $\delta^{15}N$ from nine studies after accounting for differences in mean foliar $\delta^{15}N$ among sites (Craine et al. 2009). N supply was measured either as in situ N mineralization or with resin bags and standardized between 0 and 1 for each study. $y = -3.09 + 3.59x$; $r^2 = 0.25$, $P < 0.001$.

Figure 5. Patterns in $\delta^{15}N$ and C:N concentrations of leaf litter and SOM physical density fractions across forests and pastures on highly-weathered Oxisols (0-10 cm) in the wet subtropical forest life zone of Puerto Rico. The degree of microbial decomposition generally increases from plant litter, Free LF (FLF; light fraction or particulate organic matter); occluded or intra-aggregate light fraction (OLF); and heavy fraction (HF; > 1.85 g/ml density. Data from Marín-Spiotta (2008).

Figure 6. Relationship between mean annual temperature (MAT) and the difference between the $\delta^{15}N$ of leaves and soils (0-30 cm). Predicted leaf $\delta^{15}N$ calculated for non-mycorrhizal plant with a foliar [N] of 16.2 mg g^{-1} at the same mean annual temperature and precipitation of soil. Soil data represent average soil $\delta^{15}N$ averaged for 901 0.1° latitude and longitude grid cells (Craine et al. 2015). Solid line represents same signature of leaves of non-mycorrhizal plant and soils. All points below line would have lower $\delta^{15}N$ in leaves than soils. To compare with other mycorrhizal types, dashed line shows expected difference between leaves of ericoid mycorrhizal plants, which are most depleted in ^{15}N relative to non-mycorrhizal plants, and soils.

Figure 1

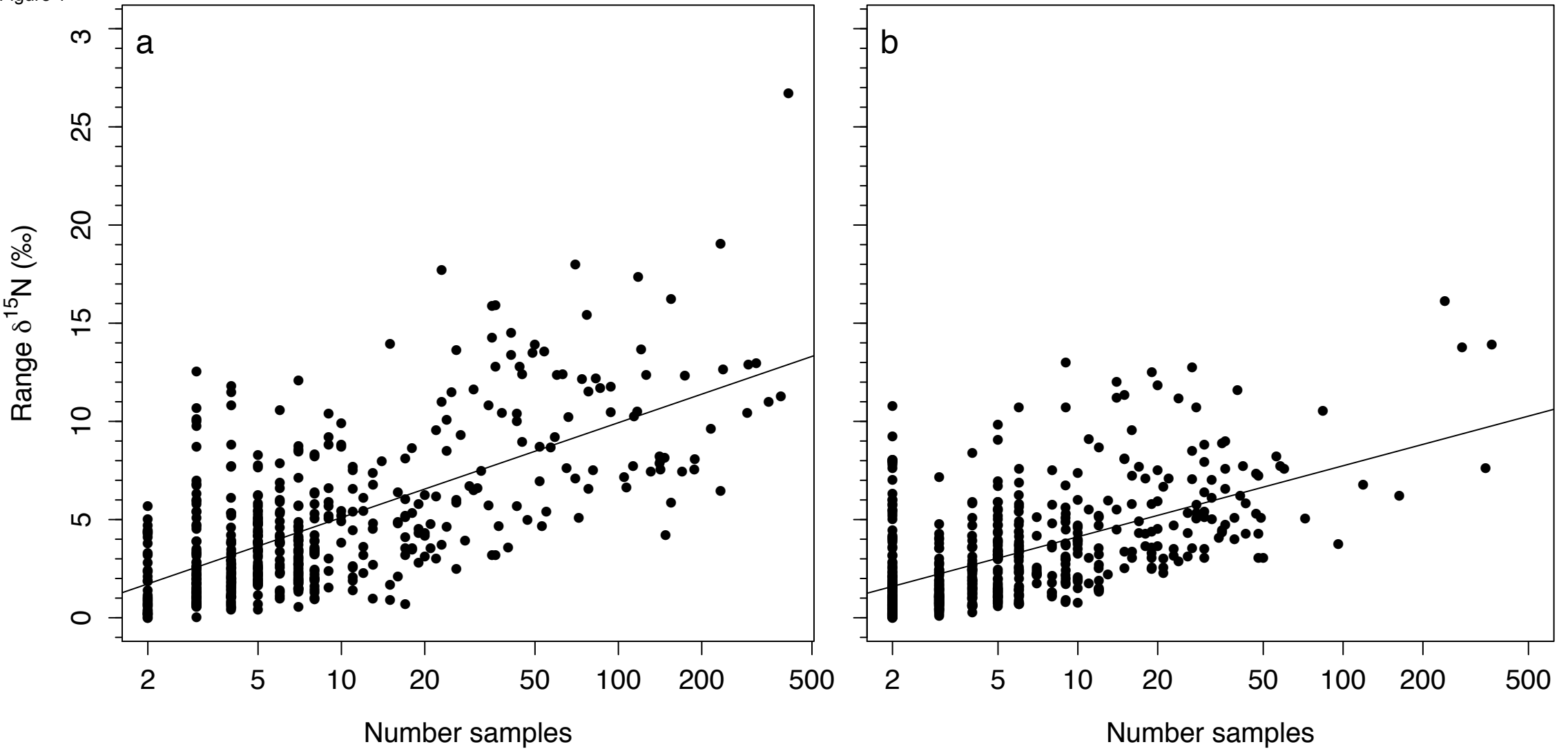
a
b
Range δ^{15}N (‰)
0
5
10
15
20
25
3
2
5
10
20
50
100
200
500
Number samples
Number samples

Figure 2

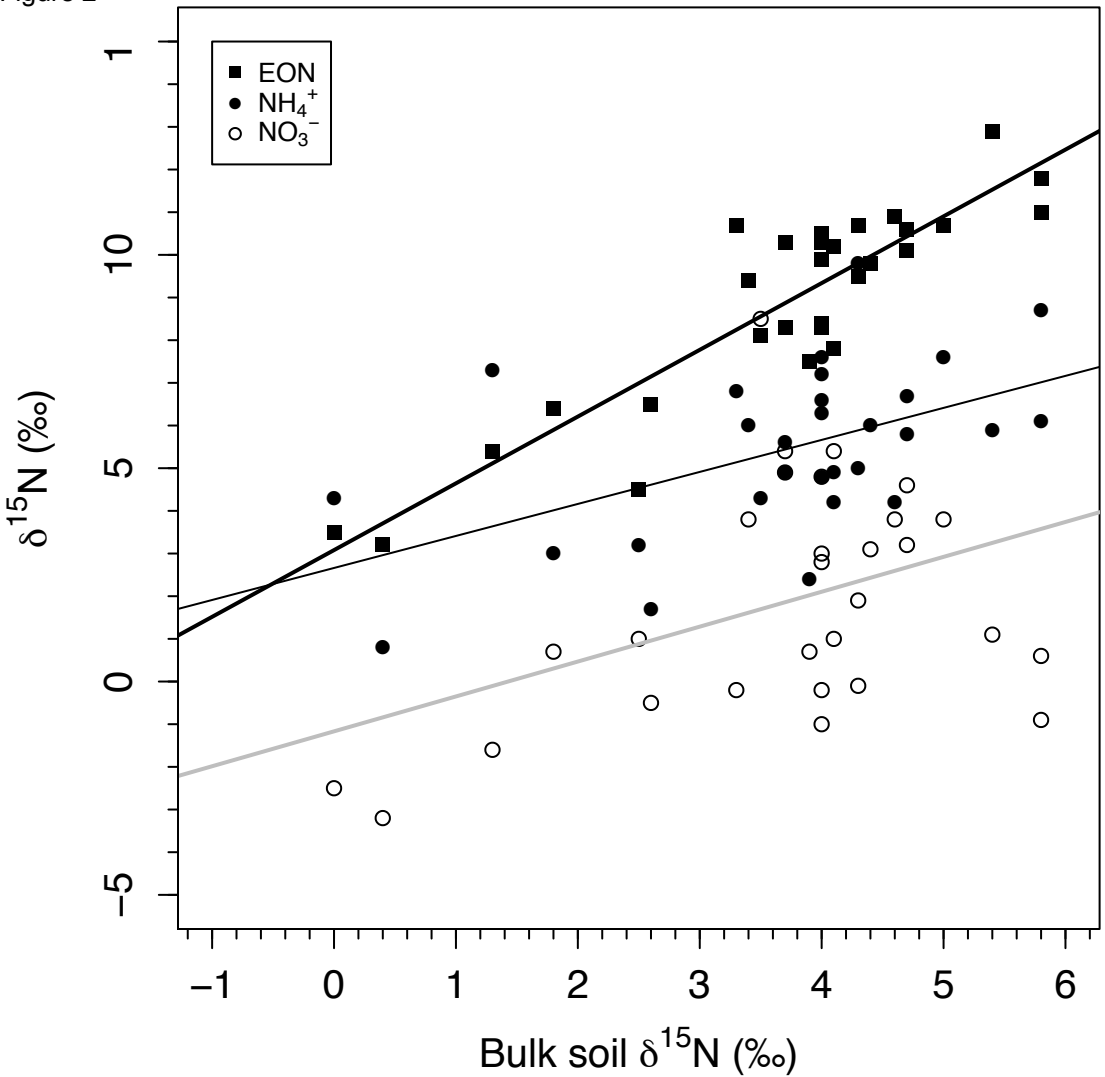

Figure 4

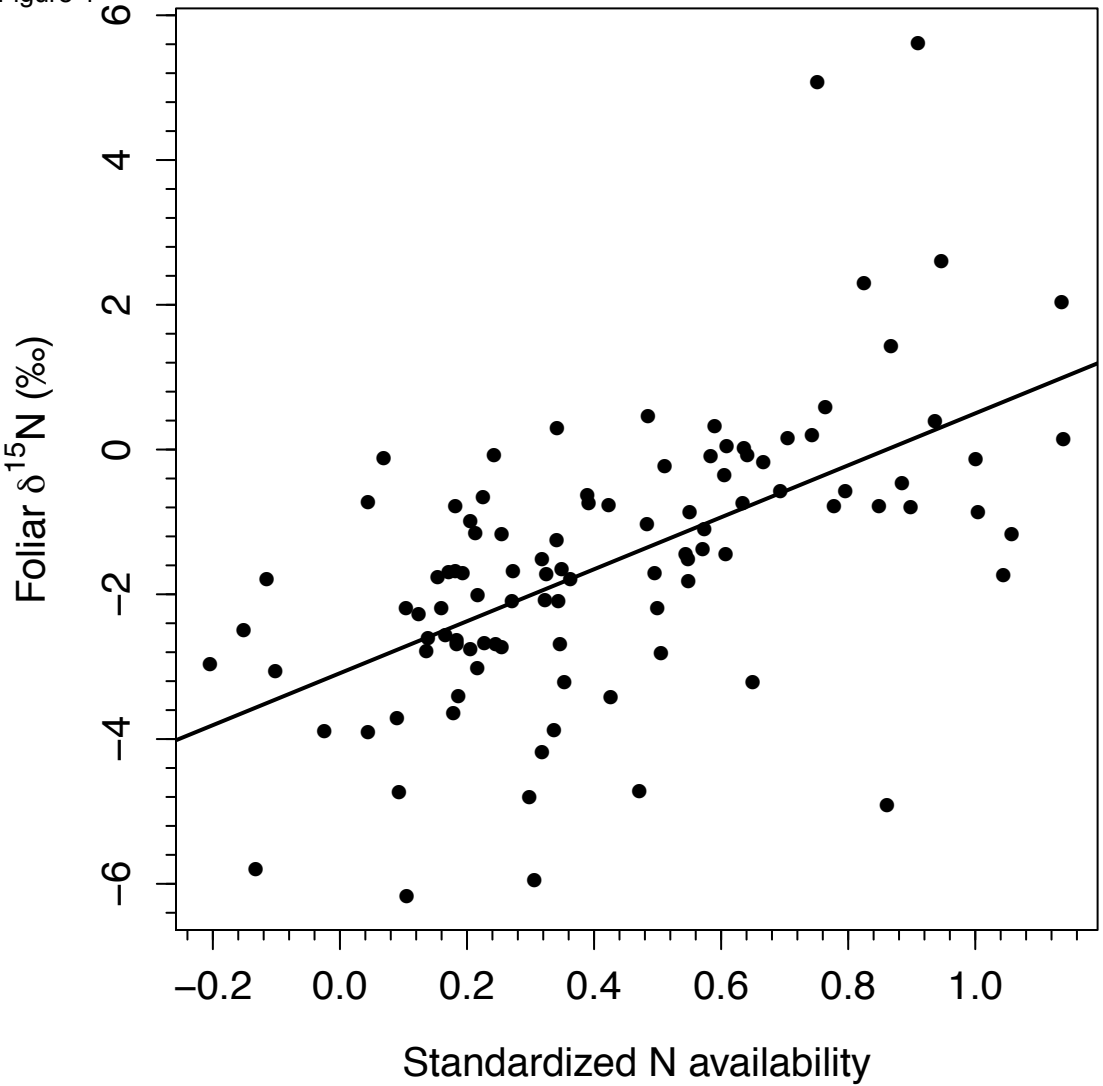
Foliar $\delta^{15}N$ (‰)
6
4
2
0
−2
−4
−6
−0.2
0.0
0.2
0.4
0.6
0.8
1.0
Standardized N availability

Figure 5

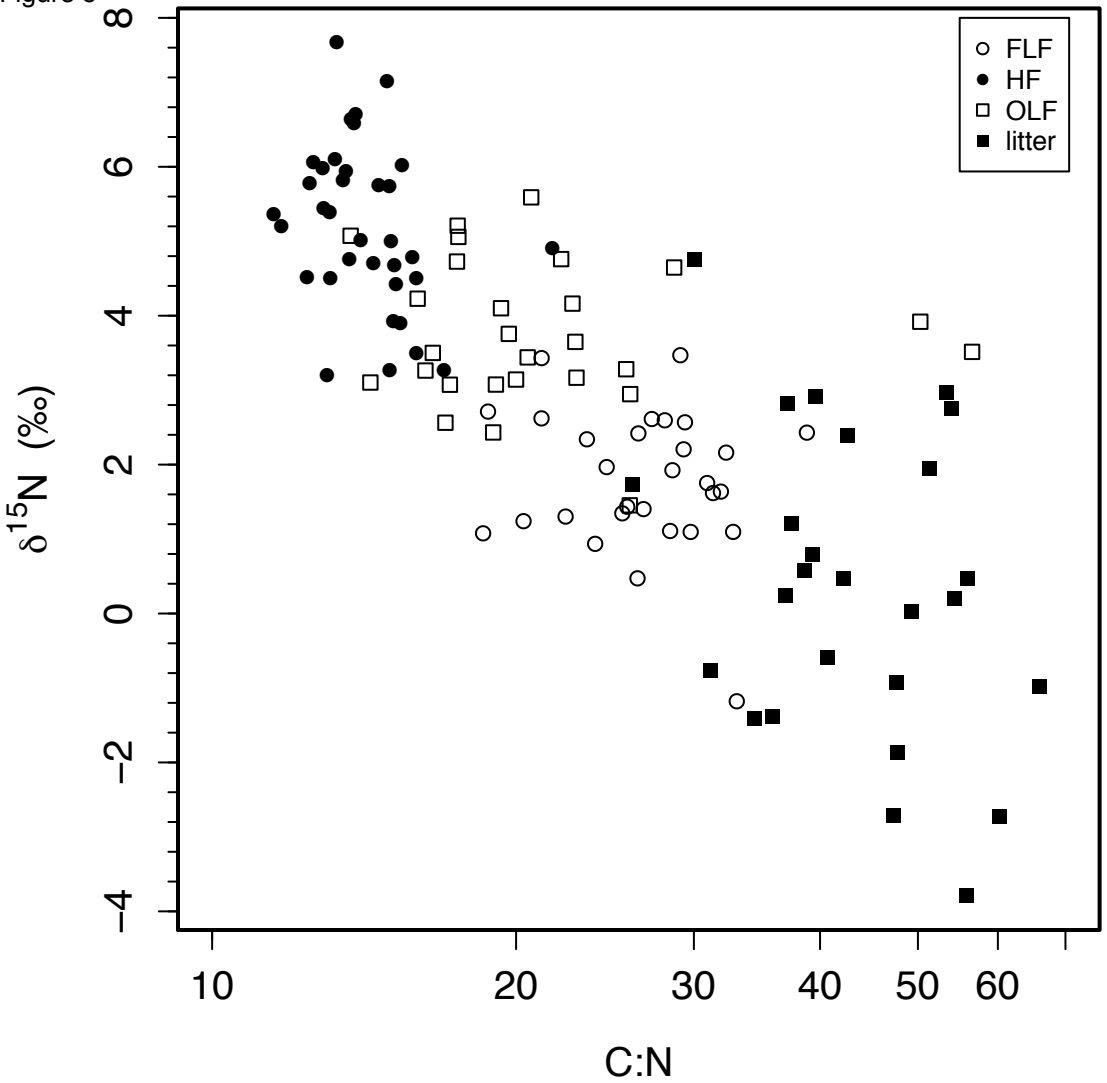

Figure 6

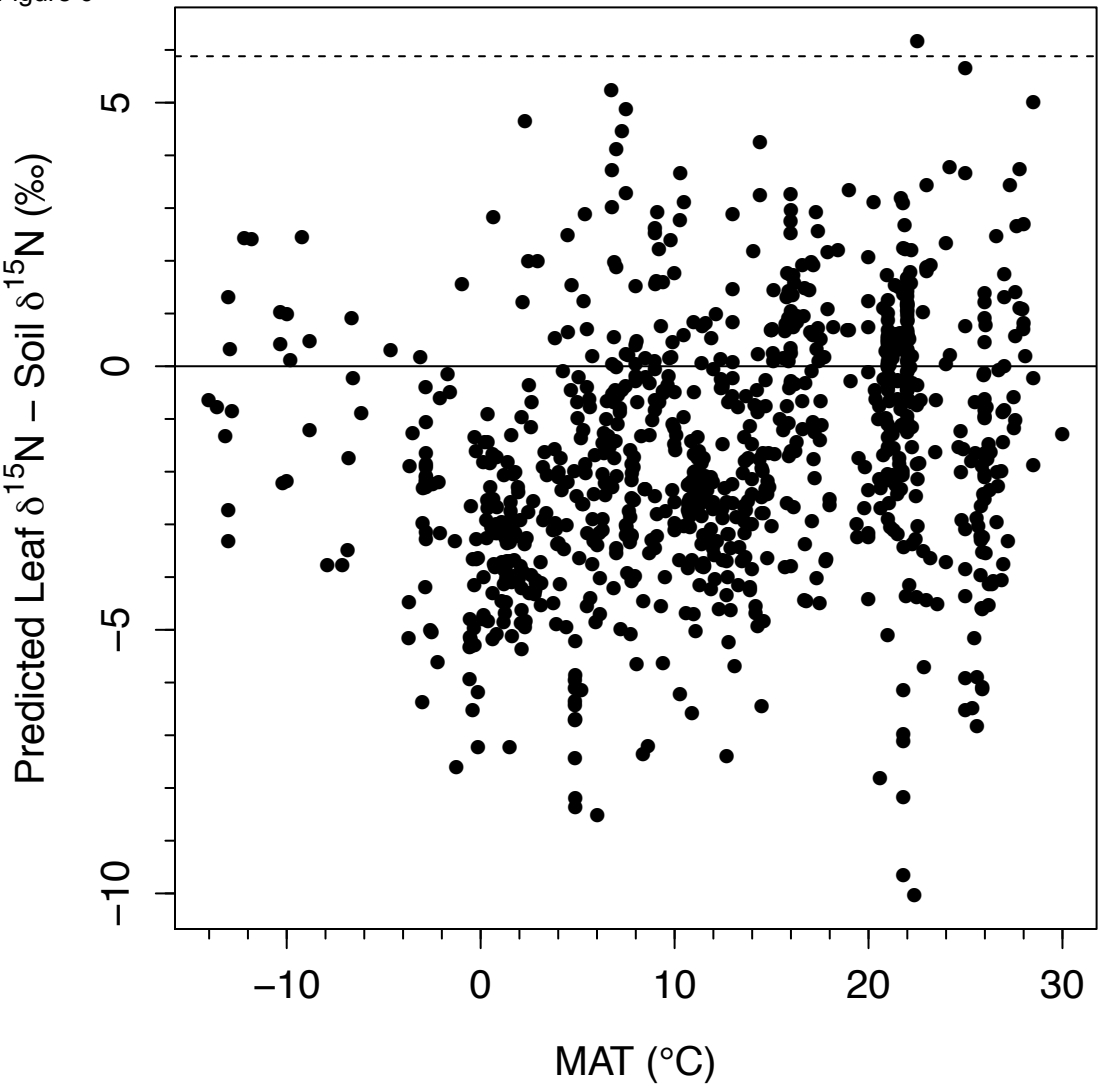

Figure 3