Biogeochemistry (2013) 112:275–291
DOI 10.1007/s10533-012-9724-1

Patterns in δ^{15}N in roots, stems, and leaves of sugar maple and American beech seedlings, saplings, and mature trees

L. H. Pardo · P. Semaoune · P. G. Schaberg · C. Eagar · M. Sebilo

Received: 24 December 2010 / Accepted: 1 March 2012 / Published online: 1 April 2012

Abstract Stable isotopes of nitrogen (N) in plants are increasingly used to evaluate ecosystem N cycling patterns. A basic assumption in this research is that plant δ^{15}N reflects the δ^{15}N of the N source. Recent evidence suggests that plants may fractionate on uptake, transport, or transformation of N. If the dominant source of plant N is via roots, a difference in δ^{15}N by tissue type would suggest fractionation on transport and assimilation of N. In order to evaluate differences between species and plant parts, we measured δ^{15}N in root, stem, and leaf tissues of individual sugar maple (*Acer saccharum*; SM) and American beech (*Fagus grandifolia*; BE) plants ranging in age from germinants to mature trees at the Hubbard Brook Experimental Forest, New Hampshire (USA). For SM, root δ^{15}N > stem δ^{15}N > leaf δ^{15}N; for BE seedlings, root δ^{15}N > stem δ^{15}N and root δ^{15}N > leaf δ^{15}N. These differences suggest that fractionation occurs during plant transport and assimilation of N. Beech δ^{15}N (root, stem, and leaf) was consistently higher than SM δ^{15}N for 1–7 year-old seedlings. At one site, we found no differences with age in foliar δ^{15}N (range: 4.1–4.8 ‰) for seedlings, saplings, and trees which suggests that it may be possible to compare foliar δ^{15}N of plants of different ages at some sites. However, at another site, foliar and root δ^{15}N were higher for trees than 1–2 year-old seedlings. This study suggests that physiological differences in N assimilation and transport processes that differ by species likely control plant δ^{15}N.

Keywords Stable isotopes · Isotopic fractionation · Nitrogen · Species patterns · Northern hardwood forest

L. H. Pardo (✉) · P. G. Schaberg
USDA Forest Service, Northern Research Station,
705 Spear St., South Burlington, VT 05403, USA
e-mail: lpardo@fs.fed.us

P. Semaoune · M. Sebilo
UPMC Univ Paris 06, UMR 7618 Bioemco, Paris, France

C. Eagar
USDA Forest Service, NRS, Durham, NH, USA

Introduction

Nitrogen (N) plays a key role in biogeochemical cycling within forests. Among the many factors that regulate N cycling in temperate forest ecosystems, species composition has been shown to be an important determinant of ecosystem N cycling dynamics (Finzi et al. 1998; Lovett et al. 2004; Templer et al. 2007). In the northeastern US, for example, species composition has been shown to influence soil C:N, rates of nitrification, and NO_3^- loss in northern hardwood forests (Lovett and Rueth 1999; Lovett et al. 2004). However, many questions remain about the factors that influence and are influenced by species patterns in N content and cycling. Interpreting observed species patterns could help elucidate some of these controls on N cycling dynamics.

Over the past decade, stable N isotopes have been increasingly utilized to assess ecosystem N cycling and status, including responses to disturbance (Evans 2007; Pardo et al. 2006). They have also been used to explore species differences. Variation in tissue $\delta^{15}N$ by tree species has been reported for northeastern North America (Pardo et al. 2006; Templer et al. 2007). For example, at over 50 plots from Ontario, Canada to Maine, USA where both sugar maple (*Acer saccharum*; SM) and American beech (*Fagus grandifolia*; BE) were growing, foliar $\delta^{15}N$ was consistently higher for BE than for SM (Pardo et al. 2006). In boreal and tundra ecosystems, functional differences in nutritional strategy (Chapin et al. 1993; McKane et al. 2002) appear to explain species differences in $\delta^{15}N$ (Nadelhoffer et al. 1996).

Patterns in N isotopes are ultimately the result of isotopic fractionations that lead to the creation of pools within an ecosystem with distinct isotopic signatures (Nadelhoffer and Fry 1994; Pardo and Nadelhoffer 2009). Isotopic fractionation occurs during physical, enzymatic, and other biological processes that discriminate against the heavier ^{15}N and in favor of the lighter ^{14}N when chemical bonds are broken (Mariotti et al. 1982; Nadelhoffer and Fry 1994). Therefore, ^{15}N:^{14}N ratios of reaction products are typically lower than ratios of substrates, leading to higher ^{15}N:^{14}N, or *^{15}N enrichment,* of residual substrates in relation to *^{15}N depletion* of products (Mariotti et al. 1981; Robinson 2001; Shearer and Kohl 1986). When the depleted product is removed from the system (via leaching, gaseous losses etc.), the residual N pools (soil, vegetation and inorganic N pools—NH_4^+ and NO_3^-) become enriched in ^{15}N. For example, following nitrification, if ^{15}N-enriched NH_4^+ is retained in the soil and ^{15}N-depleted NO_3^- is leached from the ecosystem, the net effect of nitrification is to enrich the soil in ^{15}N (Högberg 1997; Nadelhoffer and Fry 1994; Shearer and Kohl 1986). These processes typically lead to a pattern of increasing soil $\delta^{15}N$ with depth (Evans 2007; Mariotti et al. 1980; Nadelhoffer and Fry 1988; Pardo et al. 2002). The $\delta^{15}N$ of plant-available N, thus, may vary as a function of the form of N and these transformations and soil processes.

Stable N isotopes represent a potentially powerful tool for unraveling the complexities of the N cycle in forest ecosystems. They have been shown to record elevated nitrification and denitrification (Piccolo et al. 1994; 1996), N saturation (Emmett et al. 1998; Pardo et al. 2006), precipitation volume (Austin and Vitousek 1998; Handley et al. 1999), climate and precipitation driven changes in source $\delta^{15}N$ (Houlton et al. 2007), and disturbance from clear-cutting (Pardo et al. 2002). Yet, within the sometimes striking patterns observed in plant tissue $\delta^{15}N$, there is often tremendous variability which can make interpretation difficult (Pardo and Nadelhoffer 2009). This variability may be caused by measurable controlling factors. For example, variation in soil moisture in Scottish sites was associated with variation in foliar $\delta^{15}N$ across the growing season (Handley et al. 1999). In contrast, prior research at the Hubbard Brook Experimental Forest (HBEF) suggests little variation over the growing season (Pardo et al. 2002). In order to correctly interpret plant ^{15}N data, it is essential to better understand the extent of variation in plant tissue $\delta^{15}N$ and, where possible, to identify causes of systematic variation.

A number of other factors may influence the $\delta^{15}N$ of plant tissue: $\delta^{15}N$, form, and concentration of N source; site characteristics including rate of N cycling; species; mycorrhizal association; and prior land use history (Evans 2001; Evans et al. 1996; Pardo and Nadelhoffer 2009; Pritchard and Guy 2005; Yoneyama and Kaneko 1989; Yoneyama et al. 1991). Different N forms (ON, NH_4^+, or NO_3^-) may have different isotopic signatures because of the isotopic fractionations that occur during N transformations (Mariotti et al. 1982; Nadelhoffer and Fry 1994). During nitrification, for example, the NO_3^- produced is depleted in ^{15}N relative to the NH_4^+ substrate (Handley and Raven 1992), similarly, during denitrification, $\delta^{15}N$ of the residual NO_3^- pool (the substrate) will increase. Thus, in an ecosystem with high nitrification and negligible denitrification, it would be expected that NH_4^+ in soil solution would have a higher $\delta^{15}N$ than NO_3^- in solution [although few studies have gathered the data necessary to compare $\delta^{15}N$ of NO_3^- and NH_4^+ of plant-available N in forest soils (Garten 1993; Koopmans et al. 1997; Koba et al. 1998)]. Consequently, for such an ecosystem, plants that preferentially take up NO_3^- should be depleted relative to plants that preferentially take up NH_4^+. In a broad analysis of over 30 sites across the northeastern US, Pardo et al. (2006) proposed that a likely cause for species differences in foliar $\delta^{15}N$ (higher $\delta^{15}N$ in BE compared to SM) was differences in rooting depth. Since soil $\delta^{15}N$ increases with depth at the HBEF

(Pardo et al. 2001, 2002), it is expected that the $\delta^{15}N$ of available soil N would also increase with depth and cause plants that get their N from deeper horizons, such as BE to have higher tissue $\delta^{15}N$ than SM, which is more shallow rooted (Yanai et al. 2008). Little is known about how $\delta^{15}N$ varies with plant age from germination to maturity. It is possible that as seedlings mature to trees and rooting depth increases, that foliar $\delta^{15}N$ will also increase.

A fundamental assumption often made is that plant $\delta^{15}N$ reflects the $\delta^{15}N$ of the N source (Mariotti et al. 1982; Nadelhoffer and Fry 1994). Inherent in this assumption is that there is no fractionation on uptake, storage, transport, and retranslocation of N (Nadelhoffer and Fry 1994). If the assumption is correct, then, for a plant with a single N source, the different plant tissues would all have the same $\delta^{15}N$. A simple way to test whether there is fractionation on within-plant transport of N is to measure the $\delta^{15}N$ of the different plant parts. The most robust way to do this is to measure the $\delta^{15}N$ in different parts within a single plant (preferably using the whole plant), rather than comparing the mean root $\delta^{15}N$ of several plants to the mean leaf $\delta^{15}N$, because there is considerable variability in the $\delta^{15}N$ values for a given species within a given site (Pardo et al. 2006; Templer et al. 2007). Recent work (Emmerton et al. 2001a, b; Kolb and Evans 2003; Pritchard and Guy 2005; Yoneyama et al. 2001, 2003) has called into question the previously held view that plants do not fractionate on uptake/assimilation of N (Evans et al. 1996; Högberg 1997; Mariotti et al. 1982; Nadelhoffer and Fry 1994), by showing that in some cases, fractionation on uptake or assimilation may occur. The question then becomes how specific factors (species, site conditions, etc.) influence the occurrence and extent of fractionation. As ^{15}N is used more routinely in terrestrial ecosystems to evaluate N cycling dynamics, it is critical to refine our understanding of what determines foliar or plant $\delta^{15}N$ so that misinterpretation of ecosystem N cycling is avoided. A better understanding of the factors that influence the isotopic signature of plant tissue could potentially facilitate prediction of the form or timing of N uptake which could be useful in evaluating stand level N dynamics.

The main objectives of this study were to evaluate whether there is fractionation on within-plant N transport and to assess the extent of species differences and variation in $\delta^{15}N$ among tissues of the dominant trees in a northern hardwood forest. We used a novel approach: we measured $\delta^{15}N$ in root, stem, and leaf tissues of individual SM and BE plants ranging in age from germinants to mature trees in order to evaluate differences between species and plant parts. Because we measured the $\delta^{15}N$ within individual plants, analyzed many samples, evaluated plants of different ages and for the young plants, measured the whole plant, we were better able to observe patterns. Prior work has typically only compared means of roots to means of shoots. We hypothesized that:

(1) there would be no differences in $\delta^{15}N$ among tissues within a plant,
(2) root and stem $\delta^{15}N$ in BE would be greater than root and stem $\delta^{15}N$ in SM (as is leaf $\delta^{15}N$),
(3) $\delta^{15}N$ in plant tissue would increase with age (as rooting depth increased), and
(4) plant $\delta^{15}N$ would not change across the growing season.

Materials and methods

Site description

The study was conducted at the HBEF, in the White Mountains of central New Hampshire (43°56′N, 71°45′W). The HBEF is a northern hardwood forest which extends over 3,160 ha; the south-facing watersheds, where most prior research has been conducted, range in elevation from 500–800 m (Likens et al. 1977). The climate is predominantly humid continental; mean annual precipitation is approximately 1,400 mm. The dominant tree species in the south-facing watersheds are SM (~35 % of basal area), BE (~35 %), and yellow birch (*Betula allegheniensis*; ~20 %) (Siccama "unpublished data", http://www.hubbardbrook.org/data/dataset_search.php). Soils are well-drained spodosols, Typic, Aquic, and Lithic Haplorthods with little clay and a sandy loam texture (Lawrence et al. 1986). Soils are acidic with mineral soil pH values about 4.5 or less, and are approximately 60 cm deep (Johnson 1995) with a 3–15 cm forest floor (Huntington et al. 1988; Likens et al. 1977). The bedrock is medium to coarse-grained Sillimanite schist of the Rangely formation.

The study was conducted at two sites at the HBEF: the TDE plot (43°56′N, 71°45′W) with an elevation of

486 m, is 20 m in diameter and dominated by SM and BE. The NuPert site, situated 300–900 m west of watershed 6 (the reference watershed), ranges in elevation from 700 to 760 m; SM represent 70–85 % of stems (Berger et al. 2001). The NuPert site can be wetter than the TDE site (Berger et al. 2001). For this study, we sampled only trees from four (45 × 45 m^2) control plots. The NuPert site was included in the study because it provided the opportunity to collect root and foliar samples from the same tree (a highly destructive process not possible in most research sites). We expected that the differences in elevation would not affect the foliar $\delta^{15}N$, because we have not observed differences with elevation across a gradient from 540 to 770 m in our long-term monitoring of foliar $\delta^{15}N$ at this site (Pardo et al. 2002; Pardo "unpublished data").

The region was settled by Europeans in the late 1800s and selectively logged from about 1900 to 1917 (Whittaker et al. 1974). After an ice-storm on January 1998, some trees were damaged, resulting in broken branches or the collapse of tree trunks due to heavy ice loads (Jones and Mulherin 1998; Rhoads et al. 2002). At the HBEF, for the first year after the ice storm, nitrate leaching increased in damaged areas (Houlton et al. 2003). At the NuPert site, a damage survey of 340 trees showed that 66 % of trees were damaged (Huggett et al. 2007). The area around the TDE plot was not damaged.

Sample collection

Samples of SM and BE of different ages were collected randomly at the sites on different dates in June–August 2008 (Table 1). About 10 × 10 cm^2 of soil around the

Table 1 Number of samples collected by age, date, and site

Species	Site	Date	Age group	Plant tissue				Number of plants
				Leaf	Root	Shoot	Stem	
BE	NuPert	10 June 08	3–7$^+$ years	2	2		2	2
			Tree	1				1
	TDE	29 July 08	1–2 years	10	10		10	10
			3–7$^+$ years	14	13		14	15
			Sapling	5				5
		20 August 08	Tree	4				4
SM	NuPert	9 June 08	1–2 years	1				1
		10 June 08	1–2 years	5	6		6	6
		22 July 08	Tree		20			20
		21 August 08	Tree	20				20
	TDE	19 June 08	Germinant		2	3		3
			1–2 years	17	17		15	17
			3–7$^+$ years	2	2			2
		26 June 08	Germinant		9	9		9
			1–2 years	16	16		17	17
			3–7$^+$ years	4	7		6	7
			Sapling	3	1		3	3
			Tree	4				4
		29 July 08	Germinant		9	7		9
			1–2 years	17	18		17	18
			3–7$^+$ years	12	3		11	12
			Sapling	5			1	5
		20 August 08	Tree	4				4

Species sampled were *Fagus grandifolia* (BE) and *Acer saccharum* (SM)

seedlings was excavated by hand to ensure that the entire root system was removed. Germinants were separated into shoot and root. Seedlings were separated into three parts (leaf, stem, and root) in the laboratory. We aged each seedling by counting terminal bud scars. From mature trees, fine roots (diameter $\leq$ 2 mm) were collected from five randomly chosen dominant SM trees per NuPert plot on 22 July 2008 by following a root from the trunk until we reached fine roots. Leaves from the same trees were collected on 21 August 2008. Leaves were also collected from mature BE and SM trees at the TDE site on 21 August 2008. For mature trees and saplings, we collected a sub-sample of foliage and fine roots. Based on our previous work at this site, and the very low overall variability in foliar δ^{15}N over the 17 years that it has been monitored (Pardo et al. 2002; Pardo "unpublished data"), it appears that the sub-samples we collect represent the patterns observed at the site. Additionally, over the monitoring period at this site, we have not observed changes in foliar δ^{15}N across the growing season or between green leaves and fresh litter. In this study, we also did not observe patterns in foliar δ^{15}N across the growing season. Thus, we do not expect the separation of foliar and root collection to alter the interpretation of δ^{15}N data in this study. In order to further investigate patterns with age, we collected 10 plants each of seedlings (age class 2), saplings, and trees in July and August 2009 from both NuPert and TDE plots. All leaf samples from mature trees were collected from the upper, sunlit canopy using a shotgun.

Sample analysis

Tissue samples were oven-dried at 65 °C, pulverized in a shatterbox (SPEX Chemical and Sample Prep, model 8500, Metuchen, NJ, USA), oven-dried at 65 °C, and loaded into a capsule for isotope analysis. Samples were analyzed for N content and N stable isotope ratios at the Center for Stable Isotope Biogeochemistry at University of California, Berkeley (CSIB) via a CE Instruments 1500 elemental analyzer (Wigan, UK) coupled with a Finnigan MAT Delta^{Plus} XL isotope ratio mass spectrometer in continuous flow (Thermo Scientific, Bremen, Germany). We report all isotope data as δ^{15}N values, which represent the per mil (‰) difference between the isotopic composition of the sample and that of atmospheric dinitrogen (which is defined as 0 ‰):

$$\delta^{15}\mathrm{N} = \left[\left(R_{\mathrm{sample}}/R_{\mathrm{standard}}\right) - 1\right] \times 1000 \quad (1)$$

where R_{sample} represents the sample isotope ratio (^{15}N/^{14}N), and R_{standard} is ^{15}N/^{14}N for atmospheric N_2, or 0.0036765.

The standard deviation of the 8.3 % of samples analyzed in triplicate was 0.10 ‰; the precision of apple leaf standard, NIST 1515, was 0.09 ‰ (mean δ^{15}N = 0.69 ‰).

Statistical analysis

We analyzed the patterns in individual plants in order to increase the statistical power of our analysis. We separated seedlings into three age classes: age class 1 = 1–2 years; age class 2 = 3–7 years; age class 3 > 7 years. We grouped plants into age classes because of the uneven age distribution of sugar maple seedlings that results from uneven mast events (every 3–5 years: Godman et al. 1990). Because the ages and distribution of seedlings were not uniform, it was therefore difficult to collect equal numbers of samples which resulted in unequal samples sizes. To assess differences among plant parts, we used restricted maximum likelihood via PROC MIXED on the difference in δ^{15}N between the parts in individual plants. Analogous to conducting paired t tests, this approach provided greater statistical power for testing the consistency of differences in δ^{15}N among plant parts and helped to account for tree-to-tree differences associated with the heterogeneity of soils within a site. Plant parts were fixed effects in the model. The Kenward–Rodgers denominator degree of freedom method was employed. Differences in plant parts were assessed via least squares means using the Tukey–Kramer adjustment. For δ^{15}N, the effects of site, species, and plant part were tested using restricted maximum likelihood or pseudo-maximum likelihood, depending on the distribution of the response variable. We used the Shapiro–Wilk W test for normality. In cases where the response variable was normally distributed we used PROC MIXED. In cases where the response variable was not normally distributed we used PROC GLIMMIX with a gamma distribution and a log link function to account for the right skewed data. The Kenward–Rogers denominator degrees of freedom method was used with both procedures. Site, species, and plant part and were fixed effects in the

models. Differences in $\delta^{15}N$ were assessed via least squares means using the Tukey–Kramer adjustment. Within each site for each species for $\delta^{15}N$, the effects of age and plant part were tested using methods described above for $\delta^{15}N$. Site, species, and plant part were fixed effects in the models. Differences between plant parts in the same plant were defined as $\Delta\delta^{15}N$, for example $\Delta\delta^{15}N_{root-leaf} = \delta^{15}N_{root} - \delta^{15}N_{leaf}$. For $\Delta\delta^{15}N$, the effects of site, species and age were tested using methods described above for $\delta^{15}N$. Site, species and age were fixed effects in the models. All statistical analyses were performed using SAS software (Version 11.0) and an alpha level of 0.05.

Results

Differences among plant parts

In general, in an individual plant, root $\delta^{15}N$ was significantly higher than shoot $\delta^{15}N$ for both SM and BE (Fig. 1). For SM germinants, root $\delta^{15}N >$ shoot $\delta^{15}N$; for SM seedlings, root $\delta^{15}N >$ stem $\delta^{15}N >$ leaf $\delta^{15}N$; and for SM trees, root $\delta^{15}N >$ leaf $\delta^{15}N$ (Fig. 2; Table 2). All differences were significant. For BE seedlings, root $\delta^{15}N >$ stem $\delta^{15}N$ and root $\delta^{15}N >$ leaf $\delta^{15}N$ (Fig. 2); there was no significant difference between stem $\delta^{15}N$ and leaf $\delta^{15}N$ (Table 2).

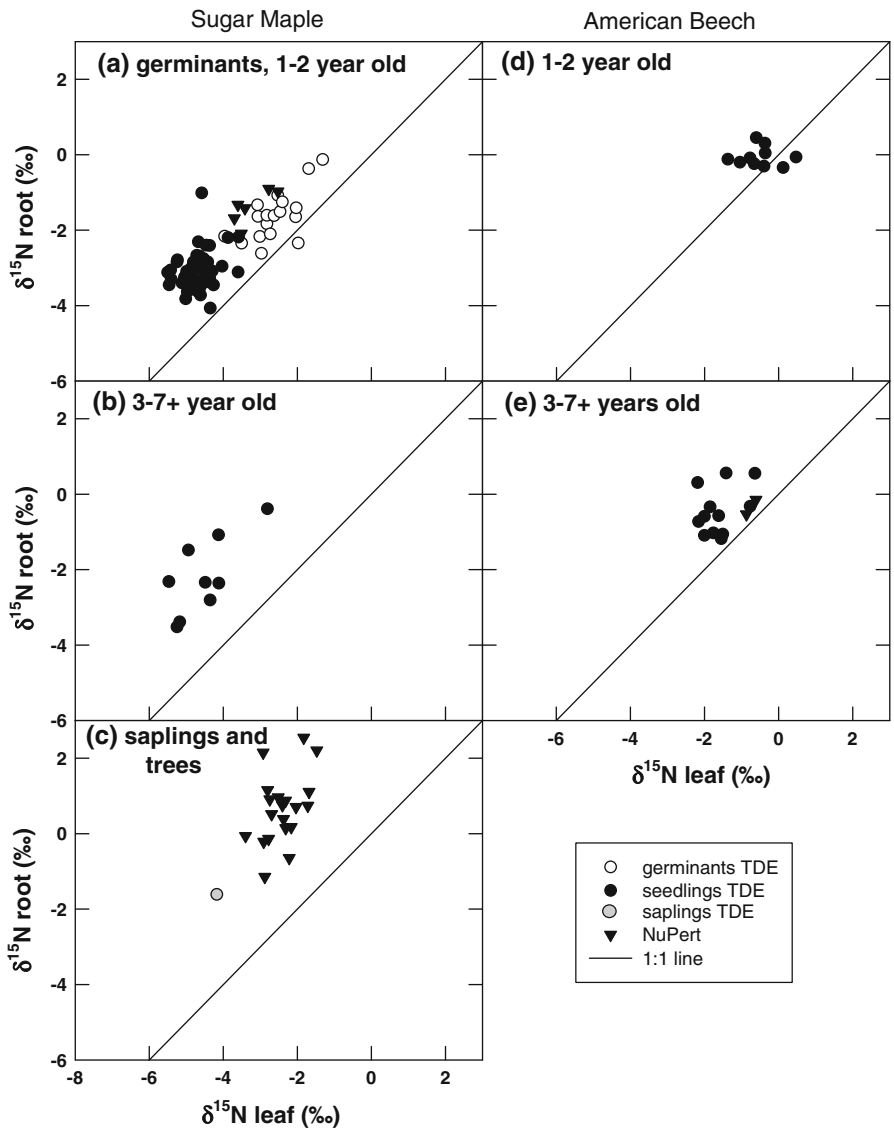

Fig. 1 Root versus leaf $\delta^{15}N$ for **a** sugar maple (SM) germinants and 1–2 years old seedlings, **b** SM 3–7+ years old seedlings, **c** SM saplings and SM trees, **d** American beech (BE) 1–2 years old seedlings, **e** BE 3–7+ years old seedlings

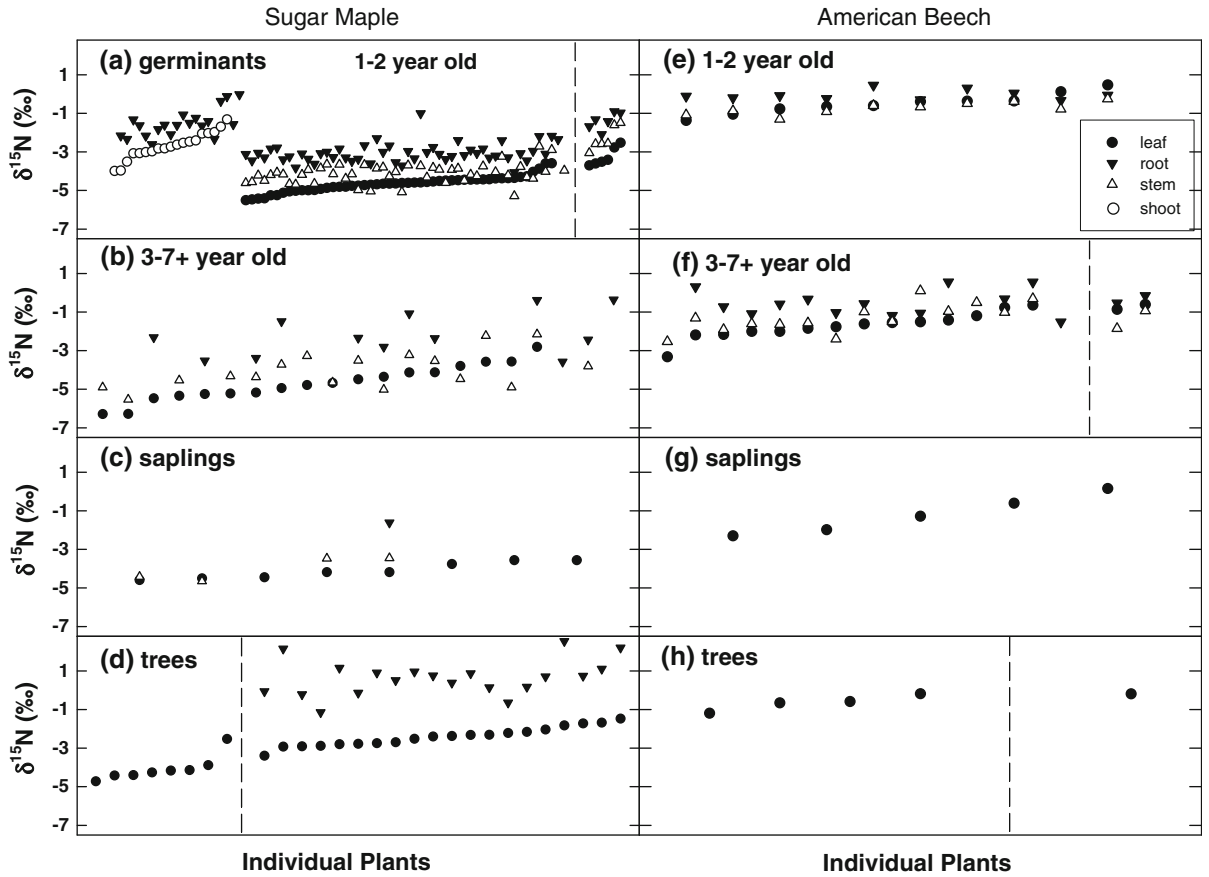

Fig. 2 Root, stem, leaf and shoot δ^{15}N for individual plants of **a** sugar maple (SM) paired shoot and root of germinants and 1–2 years old seedlings, **b** SM 3–7+ years old seedlings, **c** SM saplings, **d** SM trees, **e** BE 1–2 years old seedlings, **f** BE 3–7+ years old seedlings, **g** American beech (BE) saplings, **h** BE trees. *Points* to the *right* of the *dashed line*are from the NuPert plot; *all other points* are from the TDE plot

Table 2 Statistical results: differences between plant part δ^{15}N

	Mean Δ δ^{15}N	*n*	*P*
SM			
Root-leaf	2.1	87	<.0001
Root-shoot	1.0	18	<.0001
Root-stem	1.1	63	<.0001
Stem-leaf	0.58	76	<.0001
BE			
Root-leaf	0.8	24	<.0001
Root-stem	0.77	24	<.0001
Stem-leaf	0.08	26	0.47

Comparison of $\Delta\delta^{15}$N between species

We evaluated the differences in δ^{15}N between plant parts (Fig. 1). The largest differences ($\Delta\delta^{15}$N) reported were between root δ^{15}N and leaf δ^{15}N. All $\Delta\delta^{15}$Ns were consistently greater in SM than BE (Table 3).

Differences by site

Generally, SM foliage and root δ^{15}N was higher at the NuPert site than at the TDE site. For SM, mean foliar δ^{15}N for age class 1 (1–2 years) and for trees was higher at the NuPert site than at the TDE site

Table 3 Statistical results: comparison of plant part $\delta^{15}N$ by species

	BE	SM	*n*	*P*
TDE				
$\delta^{15}N$				
Root	−.20	−2.90	82	<.0001
Stem	−1.06	−4.10	95	<.0001
Leaf	−0.92	−4.70	82	<.0001
$\Delta\delta^{15}N$				
Root-leaf	.84	1.70	81	<.0001
Root-stem	.75	1.15	78	.0017
Stem-leaf	.15	0.60	85	0.0008

(Fig. 2a, d; Table 4). Similarly, for age class 1, root and stem $\delta^{15}N$ were higher at the NuPert site than at the TDE site (Fig. 2a, d; Table 4). For BE, we were able to compare only age class 2 (3–7 years) at the NuPert and TDE sites and found no significant differences between the sites for root, stem, or leaf $\delta^{15}N$ (Table 4).

Patterns with age

We evaluated patterns with age for each species and site. At the TDE plot, SM foliar $\delta^{15}N$ was higher for germinants than for age classes 1–3, saplings or trees, which were not different from each other (Fig. 2; Table 5). At the TDE plot, SM root $\delta^{15}N$ was higher for germinants than for age class 2, which was higher than age class 1; all differences were significant. There were no significant differences in SM stem $\delta^{15}N$ between age classes 1 and 2 at the TDE plot. In contrast, at the NuPert plot, $\delta^{15}N$ of SM foliage and roots for trees was significantly higher than for age class 1 (Table 5).

For BE at the TDE plot, foliar $\delta^{15}N$ for age class 1 (−0.5 ‰) was significantly greater than for age classes 2 and 3, and age class 3 was significantly lower than saplings and trees; there were no other significant differences among age classes (Table 5). There were no significant differences in root $\delta^{15}N$, which ranged from −0.8 to 0 ‰ among age classes 1–3. Stem $\delta^{15}N$ for age class 1 was significantly greater than age class 3, whereas age class 2 was not significantly different from the other age classes.

Patterns in $\Delta\delta^{15}N$ with age

For SM, $\Delta\delta^{15}N_{root-leaf}$ was higher for older plants. $\Delta\delta^{15}N_{root-leaf}$ was higher for trees than age class 1 seedlings in NuPert, and higher for age class 2 than age class 1 seedlings than germinants at the TDE plot (Table 5). Similarly, at the TDE plot, $\Delta\delta^{15}N_{root-stem}$ was higher for age 2 than age 1. Differences by age for $\Delta\delta^{15}N_{root-leaf}$ and $\Delta\delta^{15}N_{root-stem}$ for BE and for $\Delta\delta^{15}N_{stem-leaf}$ for SM were not significant. For BE, in the TDE plot, $\Delta\delta^{15}N_{stem-leaf}$ for age 2 was greater than for age 1; other differences among age classes 1–3 were not significant. There were no differences in $\Delta\delta^{15}N$ values between sites for age class 1 (Table 5).

Table 4 Statistical results: comparison of plant part $\delta^{15}N$ by site

	Age class	NuPert	TDE	*n*	*P*
$\delta^{15}N$					
SM					
Root	1	−1.4	−3.07	58	<.0001
Stem	1	−2.3	−4.1	56	<.0001
Leaf	1	−3.3	−4.7	56	<.0001
Leaf	Tree	−2.41	−4.07	28	<.0001
BE					
Root	2	−.34	−.39	10	.92
Stem	2	−1.4	−.96	9	.43
Leaf	2	−0.74	−1.51	9	.13
$\Delta\delta^{15}N$					
SM					
Root-leaf	1	1.86	1.6	56	.26
Root-stem	1	.91	1.07	54	.36
Stem-leaf	1	.94	.53	54	.06

Table 5 Statistical results: comparison of plant part $\delta^{15}N$ by age

	Germinant	Age1	Age2	Age3	Sapling	Tree	*n*	*P*
TDE								
$\delta^{15}N$								
SM								
Root	−1.54	−3.07	−2.17				84	<.0001
Stem		−4.13	−3.87				64	.17
Leaf/shoot	−2.69	−4.66	−4.82	−4.01	−4.10	−4.07	103	<.0001
BE								
Root		−.05	−.39	−.78			23	.06
Stem		−.73	−.96	−1.63			24	.013
Leaf		−.50	−1.51	−1.92	−1.20	−.66	33	0.0015
$\Delta\delta^{15}N$								
SM								
Root-leaf	1.00	1.59	2.34				59	.0008
Root-stem		1.07	1.64				56	.0006
Stem-leaf		0.53	0.86				61	0.05

	Age1	Tree	*n*	*P*
NuPert				
SM				
$\delta^{15}N$				
Root	−1.40	0.66	26	<.0001
Leaf	−3.26	−2.41	26	0.0012
$\Delta\delta^{15}N$				
Root-leaf	1.86	3.07	26	.0023

Differences by species and patterns with sampling date

Beech $\delta^{15}N$ was consistently higher than SM $\delta^{15}N$ for age classes 1 and 2 within the TDE plot for leaf, stem, and root tissues (Fig. 3; Table 3). There were no differences by date among germinant, 1–3 year-old SM seedlings for root, stem, or leaf $\delta^{15}N$ for the three sampling dates (Table 1).

Discussion

Patterns with plant part

Our approach had several advantages over prior mean-based $\delta^{15}N$ studies: (1) by measuring differences within individual plants, we reduced variation caused by soil and plant heterogeneity and we had more power to detect differences, and (2) for seedlings, we reduced biases that could occur from sub-sampling by measuring entire plants.

When roots and leaves have the same N source (e.g. soil solution), a difference in their N isotopic composition would reflect a fractionation during transformation and transport within the plant. Our results (root $\delta^{15}N >$ leaf $\delta^{15}N$) suggest a pattern of fractionation during N transformation and transport which leads to assimilation of ^{15}N-enriched N in roots and of ^{15}N-depleted N in leaves.

Our data, which showed a difference of 2.1 ‰ (0.09 ‰ std. dev.) between root and leaf $\delta^{15}N$ for SM and of 0.8 ‰ (0.14 ‰ std. dev.) between root and leaf for BE, were similar to previous studies in the northeastern US which suggested that root $\delta^{15}N$ is greater than leaf $\delta^{15}N$ in trees (Table 6). In single species plots of SM, BE, red oak (*Quercus rubra)*, and Eastern hemlock (*Tsuga canadensis*) in the Catskill

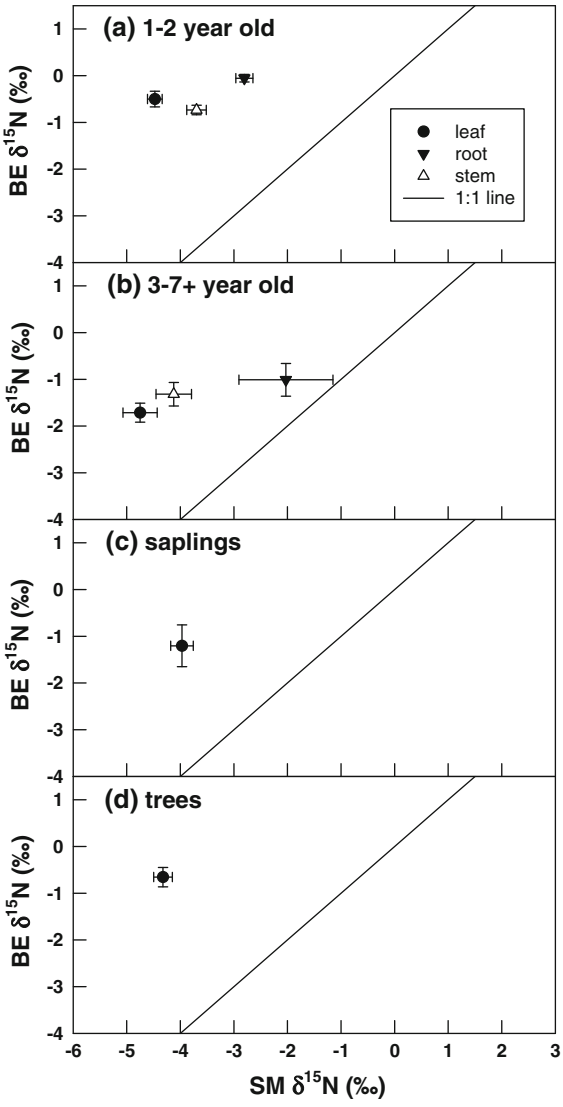

Fig. 3 Comparison of American beech (BE) versus sugar maple (SM) root, stem, leaf and shoot δ^{15}N for **a** 1–2 years old seedlings, **b** 3–7+ years old seedlings, **c** saplings, **d** trees

Mountains, New York, plot mean root δ^{15}N was found to be higher than mean leaf δ^{15}N (Templer et al. 2007; Table 6). At Harvard Forest, Massachusetts, in an oak-dominated hardwood plot, fine root δ^{15}N of all species combined was −1.6 ‰ while foliar δ^{15}N was lower for the individual species (red oak, birch (*Betula)*, red maple (*A. rubrum)*, and BE) and ranged from −3.9 to −2.3 ‰; in a red pine plot, fine root δ^{15}N in the forest floor was −1.2 ‰ and foliage was −1.8 ‰ (Nadelhoffer et al. 1999; Table 6), again supporting the pattern of greater root δ^{15}N than leaf δ^{15}N we observed in this study. Similarly, at the Bear Brook Watershed, Maine, fine root δ^{15}N of BE and SM combined was 0.18 ‰ while mean foliar δ^{15}N was −2.1 ‰ (Nadelhoffer et al. 1995; Nadelhoffer "unpublished data"). Furthermore, in a seedling experiment, root δ^{15}N was greater than leaf δ^{15}N, with differences of 1.5 ‰ for *Q. rubra* and 2.3 ‰ for *Q. alba* observed (Kolb and Evans 2003). Similar patterns are reported for other tree species (Table 6).

In some other regions, the pattern of greater root δ^{15}N than leaf δ^{15}N has been observed less consistently. In Sweden, for *Picea abies* at Gårdsjön (Emmett et al. 1998) and *Pinus sylvestris* at Norrliden (Högberg et al. 1996), root δ^{15}N > leaf δ^{15}N. However, for other European forests, the opposite pattern was observed; for *P. abies* at Klosterhede in Denmark and *P. sitchensis* at Aber in the UK, (Emmett et al. 1998) root δ^{15}N < leaf δ^{15}N. When root δ^{15}N < leaf δ^{15}N for trees, in some cases the differences reported were small, while in other cases the magnitude of the difference was not reported (see Table 6).

One important factor influencing the δ^{15}N of plants is mycorrhizal association. Hobbie and Colpaert (2003) found that when tree species were infected in the lab, root δ^{15}N was greater than leaf δ^{15}N, while plants without mycorrhizal infection have the opposite pattern. For ectomycorrhizal (ECM) species, strong fractionation during fungal uptake and transfer of ^{15}N-depleted N to the plant has been shown to cause this pattern (Hobbie and Hobbie 2006). However, we note that, although SM is associated with arbuscular mycorrhizal (AM) fungi, we observed a larger difference between root and leaf than for BE. Similarly, foliar δ^{15}N of BE was higher than that for sugar maple, in contrast to the expected pattern the foliar δ^{15}N AM > ECM (Nadelhoffer et al. 1996).

One possible explanation for differences in root and leaf δ^{15}N within an individual plant is different sources of N for the root compared to the leaf. If roots took up their N from the soil solution, whereas leaves took up their N directly from deposition, then these tissues could have different δ^{15}N values without any fractionation occurring within the plant. Germinants get a good deal of their N from the seed and most of the rest via roots. Thus, the fact that root δ^{15}N > shoot δ^{15}N in germinants, as was observed for older plants, suggests that foliar uptake alone is not what drives differences in δ^{15}N between plant parts. It seems unlikely that canopy uptake at the HBEF could

Table 6 Comparison of root and leaf $\delta^{15}N$ for tree species

Species	Common name	Field or lab study	Mycorrhizal status[a]	$\delta^{15}N_{root}$	$\delta^{15}N_{leaf}$	$\delta^{15}N_{root} - \delta^{15}N_{leaf}$	Reference
$\delta^{15}N_{root} > \delta^{15}N_{leaf}$							
Acer saccharum	Sugar maple	F		2.7 ± 0.2	−1 ± 0.4		Templer et al. (2007)
Acer saccharum, Betula alleghaniensis, Fagus grandifolia	Sugar maple, yellow birch, American beech	F		0.18	−2.10		Nadelhoffer et al. (1995)
Fagus grandifolia	American beech	F		1 ± 0.3	−0.5 ± 0.2		Templer et al. (2007)
Inga laurina, Prestoea Montana, Syzygium jambos, Tabebuia heterophylla	Sacky sac bean, sierra palm, malabar plum, roble blanco	F		3.0 ± 0.5	0.4 ± 0.4		Marin-Spiotta et al. (2009)
Nothofagus betuloides	Guindo	F		−5.4	−10.7	5.3	Boeckx et al. (2005)
Picea abies	Norway spruce	F					Emmett et al. (1998)
Picea rubens	Red spruce	F		−1.23	−1.46		Nadelhoffer et al. (1995)
Pinus resinosa	Red pine	F		−1.2	−1.8		Nadelhoffer et al. (1999)
Pinus sylvestris	Scots pine	F	ECM			1–5	Högberg et al. (1996)
Pinus sylvestris	Scots pine	L	M	2.18 ± 0.14–2.89 ± 0.12	1.27 ± 0.22–1.62 ± 0.22	0.91	Hobbie and Colpaert (2003)
Pinus sylvestris	Scots pine	L	M	3.09 ± 0.13–2.28 ± 0.08	0.07 ± 0.13 to −0.38 ± 0.31	3.02	Hobbie and Colpaert (2003)
Quercus, Acer rubrum, Betula, Fagus grandifolia	Red and black oak, red maple, black and paper birch, American beech	F		−1.63	−3.93 to −2.33		Nadelhoffer et al. (1999)
Quercus alba	White oak	L	NM			2.3	Kolb and Evans (2002)
Quercus rubra	Red oak	F		0.6 ± 0.2	−1.3 ± 0.2		Templer et al. (2007)
Quercus rubra	Red oak	L	NM			1.5	Kolb and Evans (2002)
Tsuga canadensis	Eastern hemlock	F		0.1 ± 0.42	−0.6 ± 0.02		Templer et al. (2007)
$\delta^{15}N_{root} < \delta^{15}N_{leaf}$							
Nothofagus betuloides	Guindo	F		−8.2	−7.5	−0.7	Boeckx et al. (2005)
Picea abies	Norway spruce	F					Emmett et al. (1998)
Picea sitchensis	Sitka spruce	F					Emmett et al. (1998)
Pinus sylvestris	Scots pine	L	NM	1.95 ± 0.12	2.15 ± 0.10	−0.2	Hobbie and Colpaert (2003)

Table 6 continued

Species	Common name	Field or lab study	Mycorrhizal status[a]	$\delta^{15}N_{root}$	$\delta^{15}N_{leaf}$	$\delta^{15}N_{root} - \delta^{15}N_{leaf}$	Reference
Pinus sylvestris	Scots pine	L	NM	1.35 ± 0.12	1.97 ± 0.10	−0.62	Hobbie and Colpaert (2003)
Pinus sylvestris	Scots pine	F	ECM				Högberg et al. (1996)

F field experiments, *L* lab experiments

[a] Mycorrhizal status is noted when reported: *M* unspecified mycorrhizal association, *ECM* ectomycorrhizal, *NM*-non-mycorrhizal. Entries without $\delta^{15}N$ data were included when only root $\delta^{15}N$ to leaf $\delta^{15}N$ pattern was reported

provide enough input at a different enough $\delta^{15}N$ value to account for the difference we observed in root and leaf $\delta^{15}N$, given that the amount of N mineralized in the soil is one to two orders of magnitude greater (Venterea et al. 2003) than that available during the growing season via deposition ($\sim$1 kg N ha^{-1} year^{-1}; Likens et al. 2005). There have been few studies that directly assessed the fraction of N taken up via the canopy in forests in the northeastern US, particularly in northern hardwood forests. In a spruce-fir stand in Maine that received about 6 kg N ha^{-1} year^{-1} ambient deposition, more than 70 % of N applied aerially (18–20 kg N ha^{-1} year^{-1} as NH_4NO_3) was retained by the forest canopy (Gaige et al. 2007). However, because this application of relatively high N input compared to ambient deposition occurred over the growing season, canopy uptake may have been maximized. Furthermore, spruce-fir stands intercept and retain considerably more deposition N in the canopy (Gaige et al. 2007; Lovett and Lindberg 1993) than hardwood stands. Thus, we expect at the HBEF that the dominant source of N nutrition for BE and SM is the soil solution.

Another possible explanation for differences in root and leaf $\delta^{15}N$ within an individual plant is that different forms of N may be assimilated in roots compared to leaves. For example, NO_3^- may be reduced and assimilated in the roots and the ^{15}N-enriched residual NO_3^- assimilated in the leaves (Evans 2001; Evans et al. 1996). Finally, the form of the N taken up may itself influence the extent of fractionation (Gessler et al. 1998; Kreuzwieser et al. 1997; Marschner et al. 1991; Serna et al. 1992). For example, a recent study by Ariz et al. (2011) found consistently higher root and shoot $\delta^{15}N$ values for seven herbaceous and two tree species that were fed with a single N source of NO_3^- than those fed solely with NH_4^+. However, root $\delta^{15}N$ was lower than shoot $\delta^{15}N$ for carob (*Ceratonia siliqua* sp.) when fed with NH_4^+ (Ariz et al. 2011, supplemental files).

Because we did not measure the $\delta^{15}N$ of the N plants took up, we are not able to assess whether fractionation occurred on uptake. Based on 10 SM seedlings collected in 2009 at both the TDE and NuPert plots, we found variation of 1–2 ‰ in whole plant $\delta^{15}N$ values (i.e., a weighted mean based on plant part $\delta^{15}N$ and mass). If there were no fractionation on uptake and source $\delta^{15}N$ did not vary spatially or

temporally, one would expect a constant whole plant $\delta^{15}N$ value.

In summary, because of the assumption that there is no fractionation on uptake/assimilation, it has often been asserted that foliar $\delta^{15}N$ reflects the ^{15}N of the inorganic N taken up by plants (Falkengren-Grerup et al. 2004; Boeckx et al. 2006). If there is fractionation on uptake or within-plant transport or transformation, the relationship between the $\delta^{15}N$ of plant-available N and that of the plant may be more complex. Although dramatic shifts in the $\delta^{15}N$ of the source N should still be reflected by shifts in plant $\delta^{15}N$, our study shows that caution needs to be exercised in assuming that plant $\delta^{15}N$ is equivalent to that of its source.

Patterns with age

We expected plant $\delta^{15}N$ to increase with age as root depth increased, because soil $\delta^{15}N$ increases with depth at this site (Pardo et al. 2002) and root $\delta^{15}N$ in the mineral soil (at 50 cm depth) was higher than that in the forest floor (Pardo et al. 2006). Thus, we assumed that as rooting depth increased from seedlings to trees, the deeper roots would have access to inorganic N that would be more enriched in ^{15}N. At the NuPert plots in 2008, we observed higher $\delta^{15}N$ in SM foliage and roots for trees compared to seedlings in age class 1; at the TDE plot, we observed higher $\delta^{15}N$ in SM roots for age class 2 compared to age class 1. However, we found no difference in $\delta^{15}N$ between seedling and tree foliage in the NuPert plots in samples collected from 10 plants each of seedlings (age class 2), saplings, and trees in July and August 2009. Sapling foliar $\delta^{15}N$ in 2009 in the NuPert plots was significantly greater than seedling or tree foliar $\delta^{15}N$. At the TDE plot, SM foliar $\delta^{15}N$ was very consistent across all age classes from 1 to trees (ranging from 4.1 to 4.8 ‰), which suggests that, at some sites, it may be appropriate to compare foliar $\delta^{15}N$ of plants of different ages. It is possible that at the NuPert site, if more age classes had been represented and sample sizes had been larger, that the same pattern would have been observed. It is also plausible that site differences between NuPert and the TDE plot may have contributed to the pattern of increased $\delta^{15}N$ with age. The increase in $\Delta\delta^{15}N_{root-leaf}$ and $\Delta\delta^{15}N_{root-stem}$ for SM with age was observed both at the TDE and NuPert plots. This pattern suggests that whatever the mechanism controlling the difference in $\delta^{15}N$ between roots and leaves, this difference increases with age—whether this is a function of transport length or serial fractionation and transport/assimilation cycles is not known. The site characteristics that differ between the two sites—the NuPert site is higher in elevation, significantly wetter at times due to springs/seeps (Berger et al. 2001), and may have differences in nitrification rate or % nitrification—might affect the $\delta^{15}N$ values at the site. Such factors have been reported to affect $\delta^{15}N$ (Garten 1993; Pardo et al. 2006). In order to evaluate whether wetness increased foliar $\delta^{15}N$, in 2009, we collected leaves from 10 seedlings in the TDE plot in dry areas and 10 seedlings in a wet, near-stream area. We found no significant difference in foliar $\delta^{15}N$ ($\alpha = 0.05$). We assume that the 1998 ice storm did not alter $\delta^{15}N$ in tissue samples collected in 2008; in fact, samples collected in the growing season after ice storm damage elsewhere at the HBEF did not show any response in foliar $\delta^{15}N$ following this disturbance (Pardo et al. 2002), which does not appear to have altered nitrification rates (Houlton et al. 2003).

The elevated $\delta^{15}N$ value of germinant roots and shoots in SM at the TDE site may be attributable to the ^{15}N in the seed, rather than soil sources of N. We did not measure germinants at the NuPert site, so we cannot evaluate whether the pattern of germinants $\delta^{15}N$ being higher than seedlings would exist there as well.

Patterns with date

In contrast to studies that have reported variation in foliar $\delta^{15}N$ across the growing season (Handley et al. 1999), we did not observe variation over time in any plant part. This is not surprising, given the low variation in mean $\delta^{15}N$ from litter and leaf samples combined, which were collected at slightly different points during the growing season each year at this site over 10 years (SE = 0.08 ‰; Pardo et al. 2002, 2006). It has been suggested that variation in $\delta^{15}N$ across the growing season may be most pronounced when water availability varies (Handley et al. 1999), although others have not observed this pattern (Jung et al. 1997). Precipitation at the HBEF is spread evenly across the growing season (Likens et al. 1977). There were no differences between fresh litter and leaf $\delta^{15}N$

at this site (Pardo et al. 2002), which suggests that there is no significant fractionation on resorption.

Patterns with species

In an earlier study, Pardo et al. (2006) ruled out the following explanations for the higher $\delta^{15}N$ in BE than SM: (1) NH_4^+ vs. NO_3^- preference would not explain the pattern that we observed, given the prior observation that NH_4^+ $\delta^{15}N > NO_3^-$ $\delta^{15}N$ and that BE prefer NO_3^- while SM prefer NH_4^+ (Rothstein et al. 1996; Templer and Dawson 2004, Socci and Templer 2011. (2) Nitrification rate should lead to the opposite pattern (SM > BE) to that observed, because soil around SM should have higher nitrification rates, which, when NO_3^- leaching occurs should cause the plant-available inorganic N to be ^{15}N enriched (Lovett et al. 2004). (3) Mycorrhizal association should not lead to the pattern that we observed, as BE is ECM and SM is AM, and prior studies have shown that foliage in plants associated with ectomycorrhizal fungi (ECM) have a lower $\delta^{15}N$ than those associated with arbuscular fungi (AM) (Michelsen et al. 1998; Schmidt and Stewart 2003).

It was suggested that the factors that were more likely to have affected foliar $\delta^{15}N$ were rooting depth or physiological factors such as phenology (Pardo et al. 2006) (For example, an earlier leaf out date might give one species access to an N pool that was systematically different, perhaps more depleted, relative to a species that leafed out later). Based on our data for SM and BE in the TDE site, it does not appear that rooting depth drives foliar $\delta^{15}N$. If rooting depth were an important factor, one would expect that the difference between BE (deeper rooted) and SM would increase with age, because tree roots would have the potential to access deeper soil pools that were enriched in ^{15}N. However, the difference between BE and SM leaves did not vary much from seedlings (4.2 ‰) to trees (3.4 ‰; Table 4). There is an increase >2 ‰ between the Oie and Oa horizon at this site (Pardo et al. 2002), which may be the cause of the increase in SM root $\delta^{15}N$ between age classes 1 and 2 at the TDE plot. The difference between the Oie and Bs horizons ($\sim$8 ‰) is considerably greater than that observed, for example, in foliar $\delta^{15}N$ between trees and 1–2 years old seedlings in the NuPert plots.

One explanation for differences between SM and BE $\delta^{15}N$ may be that the assumptions in some of the above explanations do not hold. For example, if BE prefer NH_4^+ and SM prefer NO_3^- or if plant-available NH_4^+ is not consistently enriched relative to NO_3^-, we would expect that the $\delta^{15}N$ of the N source was regulating the plant $\delta^{15}N$. However, the most likely explanation for differences between SM and BE $\delta^{15}N$ is physiological differences in the uptake, transport, and assimilation of N between these species. The results of Ariz et al. (2011) suggest that the form of N taken up is the major control on plant $\delta^{15}N$, but that species, N concentration of source and transport mechanism affect plant $\delta^{15}N$ as well. Previous studies have led to the suggestion that internal processes were more important in controlling plant $\delta^{15}N$ than external factors including source $\delta^{15}N$ (Kolb and Evans 2003). Our within-plant analyses are consistent with this suggestion.

Conclusions

This paper presents, to our knowledge, the first extensive measurements of $\delta^{15}N$ in different plant parts of the same plant for tree species. By demonstrating systematic differences between roots and shoots in over 200 individual plants of sugar maple and American beech of different ages, we make a significant contribution to understanding the factors that regulate foliar $\delta^{15}N$. We observe that root $\delta^{15}N$ is consistently higher than shoot $\delta^{15}N$, with stem $\delta^{15}N$ typically falling between root and shoot. Rooting depth appears not to be a significant factor controlling foliar $\delta^{15}N$. We report that all plant parts of American beech have a higher $\delta^{15}N$ than sugar maple. We show that fractionation likely occurs on transport/assimilation, thus providing evidence that foliar $\delta^{15}N$ is not likely to directly reflect the $\delta^{15}N$ of the N source. This has significant implications for the increasing number of studies utilizing foliar $\delta^{15}N$ in order to interpret N cycling dynamics.

Further refining understanding of the mechanisms that control the differences in tissue $\delta^{15}N$ in SM and BE could advance understanding of their respective roles in stand level N cycling dynamics. If the response of these two species to the various factors that can influence plant $\delta^{15}N$ (form, concentration, relative abundance of NH_4^+ vs. NO_3^-) were known, it could be possible to make better predictions of stand level N retention or loss. For example, if the

isotopic values indicate higher nitrate use by BE than by SM, stand level shifts away from BE (e.g., as a result of beech bark disease) would be expected to lead to increased nitrate leaching. If the relative $\delta^{15}N$ values of SM and BE in the same stand are a function of differences in timing of uptake (phenological stage can influence the plant ^{15}N (Ariz et al. 2011)), these relative differences might be expected to shift under changing climatic conditions. Several lines of future research could help unravel the questions remaining about the factors controlling plant $\delta^{15}N$ values: (1) better quantification of $\delta^{15}N$ in sources of plant N (NH_4^+, NO_3^-, DON), (2) controlled experiments to identify whether there is fractionation on uptake, in particular evaluating whether form of N affects fractionation in these species, and (3) measurement of the fractionation involved in N transformations and transport within plants of different species.

Acknowledgments This research was supported, in part, by US National Science Foundation Grants DEB 98-10221 and DEB-0423259 (Hubbard Brook Long Term Ecological Research). This research was conducted at the HBEF, which is operated by the Northern Research Station, USDA Forest Service, Newtown Square, PA. This paper is a contribution to the Hubbard Brook Ecosystem Study. We appreciate the contributions of Bénédicte Bachelot to this project. We thank Annie Rendall for lab and field work, Stefania Mambelli and Paul Brooks for isotope analyses, John S. Stanovick for statistical analysis, Molly Robin-Abbott for data analysis and graphics, Natalie Cleavitt, Amey Bailey, and Ian Halm for field and general guidance, and Alyssa Kilanowski for field assistance. We appreciate the reviews of an earlier version of the manuscript by Tim Fahey, Kim Kolb, and Bob McKane. We thank Teresa Dias and Cristina Cruz for helpful discussions. We appreciate the comments of two anonymous reviewers.

References

Ariz I, Cruz C, Moran JF, González-Moro MB, García-Olaverri C, González-Murua C, Martins-Loução MA, Aparicio-Tejo PM (2011) Depletion of the heaviest stable N isotope is associated with NH_4^+/NH_3 toxicity in NH_4^+-fed plants. BMC Plant Biol 11:83. http://www.biomedcentral.com/1471-2229/11/83

Austin AT, Vitousek PM (1998) Nutrient dynamics on a precipitation gradient in Hawai'i. Oecologia 113:519–529

Berger TW, Eagar C, Likens GE, Stingeder G (2001) Effects of calcium and aluminum chloride additions on foliar and throughfall chemistry in sugar maples. For Ecol Manage 149:75–90

Boeckx P, Paulino L, Oyarz C, van Cleemput O, Godoy R (2005) Soil $\delta^{15}N$ patterns in old-growth forests of southern Chile as integrator for N-cycling. Isotopes Environ Health Stud 41(3):249–259. doi:10.1080/10256010500230171

Boeckx P, Van Meirvenne M, Raulo F, Van Cleemput O (2006) Spatial patterns of $\delta^{13}C$ and $\delta^{15}N$ in the urban topsoil of Gent, Belgium. Org Geochem 37:1383–1393

Chapin FS III, Moilanen L, Kielland K (1993) Preferential use of organic nitrogen for growth by a non-mycorrhizal arctic sedge. Nature 361:150–153

Emmerton KS, Callaghan TV, Jones HE, Leake JR, Michelsen A, Read DJ (2001a) Assimilation and isotopic fractionation of nitrogen by mycorrhizal fungi. New Phytol 151:503–511

Emmerton KS, Callaghan TV, Jones HE, Leake JR, Michelsen A, Read DJ (2001b) Assimilation and isotopic fractionation of nitrogen by mycorrhizal and nonmycorrhizal subarctic plants. New Phytol 151:513–524

Emmett BA, Kjønaas OJ, Gundersen P, Koopmans C, Tietema A, Sleep D (1998) Natural abundance of ^{15}N in forests across a nitrogen deposition gradient. For Ecol Manage 101: 9–18

Evans RD (2001) Physiological mechanisms influencing plant nitrogen isotope composition. Trends Plant Sci 6:121–126

Evans RD (2007) Soil nitrogen isotope composition. In: Lajtha K, Michener RH (eds) Stable isotopes in ecology and environmental science, 2nd edn. Blackwell, Oxford, pp 83–98

Evans RD, Bloom AJ, Sukrapanna SS, Ehleringer JR (1996) Nitrogen isotope composition of tomato (*Lycopersicon esculentum* Mill. cv. T-5) grown under ammonium or nitrate nutrition. Plant Cell Environ 19:1317–1323

Falkengren-Grerup U, Michelsen A, Olsson MO, Quarmby C, Sleep D (2004) Plant nitrate use in deciduous woodland: the relationship between leaf N, ^{15}N natural abundance of forbs and soil N mineralisation. Soil Biol Biochem 36:1885–1891

Finzi AC, Van Breemen N, Canham CD (1998) Canopy tree—soil interactions within temperate forests: species effects on soil carbon and nitrogen. Ecol Appl 8:440–446

Gaige E, Dail DB, Hollinger DY, Davidson EA, Lee JT, Fernandez IF, Sievering H, Rodrigues C, Hughes H, White A, Halteman W (2007) Changes in canopy processes following whole-forest canopy nitrogen fertilization of a mature spruce-hemlock forest. Ecosystems. doi:10.1007/s10021-007-9081-4

Garten CT (1993) Variation in foliar ^{15}N abundance and the availability of soil nitrogen on Walker Branch Watershed. Ecology 74:2098–2113

Gessler A, Schneider S, Von Sengbusch D, Weber P, Hanemann U, Huber C, Rothe A, Kreutzer K, Rennenberg H (1998) Field and laboratory experiments on net uptake of nitrate and ammonium by the roots of spruce *Picea abies* and beech *Fagus sylvatica* trees. New Phytol 138:275–285

Godman RM, Yawney HW, Tubbs CH (1990) *Acer saccharum* Marsh. Sugar maple. In: Burns RM, Honkala BH (eds) Silvics of North America, Vol 2 hardwoods, Agriculture Handbook 654. Forest Service, USDA, Washington, DC, p 78

Handley LL, Raven JA (1992) The use of natural abundance of nitrogen isotopes in plant physiology and ecology. Plant Cell Environ 15:965–985

Handley LL, Austin AT, Robinson D et al (1999) The ^{15}N natural abundance ($\delta^{15}N$) of ecosystem samples reflects measures of water availability. Aust J Plant Physiol 26:185–199

Hobbie EA, Colpaert JV (2003) Nitrogen availability and colonization by mycorrhizal fungi correlate with nitrogen isotope patterns in plants. New Phytol 157:115–126

Hobbie JE, Hobbie EA (2006) ^{15}N in symbiotic fungi and plants estimates nitrogen and carbon flux rates in arctic tundra. Ecology 87:816–822

Högberg P (1997) Tansley Review No. 95. ^{15}N natural abundance in soil-plant systems. New Phytol 137:179–203

Högberg P, Högbom L, Schinkel H, Högberg M, Johannisson C, Wallmark H (1996) ^{15}N abundance of surface soils, roots and mycorrhizas in profiles of European forest soils. Oecologia 108:207–214

Houlton BZ, Driscoll CT, Fahey TJ, Likens GE, Groffman PM, Bernhardt ES, Buso DC (2003) Nitrogen dynamics in ice-storm damaged forest ecosystems: implications for nitrogen limitation theory. Ecosystems 6:431–443. doi: 10.1007/s10021-002-0198-1

Houlton BZ, Sigman DM, Schuur EAG et al (2007) A climate-driven switch in plant nitrogen acquisition within tropical forest communities. Proc Natl Acad Sci USA 104:8902–8906

Huggett BA, Schaberg PG, Hawley GJ, Eagar C (2007) Long-term calcium addition increases growth release, wound closure, and health of sugar maple (*Acer saccharum*) trees at the Hubbard Brook Experimental Forest. Can J For Res 37:1692–1700

Huntington TG, Ryan DF, Hamburg SP (1988) Estimating soil nitrogen and carbon pools in a northern hardwood forest ecosystem. Soil Sci Soc Am J 52:1162–1167

Johnson CE (1995) Soil nitrogen status eight years after whole-tree clear-cutting. Can J For Res 25:1346–1355

Jones KF, Mulherin ND (1998) An evaluation of the severity of the January 1998 ice storm in northern New England. Report for FEMA Region 1 Cold Regions Research and Engineering Laboratory, Hanover, NH 33

Jung K, Gebauer G, Gehre M, Hofmann D, Weißflog L, Schüürmann G (1997) Anthropogenic impacts on natural nitrogen isotope variations in *Pinus sylvestris* stands in an industrially polluted area. Environ Pollut 97:175–181

Koba K, Tokuchi N, Yoshioka T, Hobbie EA, Iwatsubo G (1998) Natural abundance of nitrogen-15 in a forest soil. Soil Sci Soc Am J 62:778–781

Kolb KJ, Evans RD (2002) Implications of leaf nitrogen recycling on the nitrogen isotope composition of deciduous plant tissues. New Phytol 156:57–64

Kolb KJ, Evans RD (2003) Influence of nitrogen source and concentration on nitrogen isotopic discrimination in two barley genotypes (*Hordeum vulgare* L.). Plant Cell Environ 26:1431–1440

Koopmans CJ, Van Dam D, Tietema A, Verstraten JM (1997) Natural ^{15}N abundance in two nitrogen saturated forest ecosystems. Oecologia 111:470–480

Kreuzwieser J, Herschbach C, Stulen I, Wiersema P, Vaalburg W, Rennenberg H (1997) Interactions of NH_4^+ and L-glutamate with NO_3^- transport processes of non-mycorrhizal Fagus sylvatica roots. J Exp Bot 48:1431–1438

Lawrence GB, Fuller RD, Driscoll CT (1986) Spatial relationships of aluminum chemistry in the streams of the Hubbard Brook Experimental Forest, New Hampshire. Biogeochemistry 2:115–135

Likens GE, Bormann FH, Pierce RH, Eaton JS, Johnson NM (1977) Biogeochemistry of a Forested Ecosystem. Springer, New York, p 146

Likens GE, Buso DC, Butler TJ (2005) Long-term relationships between SO_2 and NO_x emissions and SO_4^{2-} and NO_3^- concentration in bulk deposition at the Hubbard Brook Experimental Forest, New Hampshire. J Environ Monit 7:964–968

Lovett GM, Lindberg SE (1993) Atmospheric deposition and canopy interactions of nitrogen in forests. Can J For Res 23:1603–1616

Lovett GM, Rueth H (1999) Soil nitrogen transformations in beech and maple stands along a nitrogen deposition gradient. Ecol Appl 9:1330–1344

Lovett GM, Weathers KC, Arthur MA, Schultz JC (2004) Nitrogen cycling in a northern hardwood forest: do species matter? Biogeochemistry 67:289–308

Marin-Spiotta E, Silver WL, Swanston CW, Ostertag R (2009) Soil organic matter dynamics during 80 years of reforestation of tropical pastures. Glob Change Biol 15: 1584–1597. doi:10.1111/j.1365-2486.2008.01805.x

Mariotti A, Pierre D, Vedy JC et al (1980) The abundance of natural nitrogen-15 in the organic matter of soils along an altitudinal gradient. Catena 7:293–300

Mariotti A, Germon JC, Hubert P et al (1981) Experimental determination of nitrogen kinetic isotope fractionations: some principles; illustration for denitrification and nitrification processes. Plant Soil 62:413–430

Mariotti A, Mariotti F, Champigny ML, Amarger N, Moyse A (1982) Nitrogen isotope fractionation associated with nitrate reductase activity and uptake of NO_3^- by Pearl Millet. Plant Physiol 69:880–884

Marschner H, Häussling M, George E (1991) Ammonium and nitrate uptake rates and rhizosphere pH in non-mycorrhizal roots of Norway spruce [*Picea abies* (L.) Karst.]. Trees 5:14–21

McKane RB, Johnson LC, Shaver GR et al (2002) Resource-based niches provide a basis for plant species diversity and dominance in arctic tundra. Nature 415(6867):68–71

Michelsen A, Quarmby C, Sleep D, Jonasson S (1998) Vascular plant ^{15}N natural abundance in heath and forest tundra ecosystems is closely correlated with presence and type of mycorrhizal fungi in roots. Oecologia 115:406–418

Nadelhoffer KJ, Fry B (1988) Controls on natural nitrogen-15 and carbon-13 abundances in forest soil organic matter. Soil Sci Soc Am J 52:1633–1640

Nadelhoffer KJ, Fry B (1994) Nitrogen isotope studies in forest ecosystems. Methods in Ecology. pp 22–44

Nadelhoffer KJ, Downs MR, Fry B, Aber JD, Magill AH, Melillo JM (1995) The fate of ^{15}N-labelled nitrate additions to a northern hardwood forest in eastern Maine, USA. Oecologia 103:292–301

Nadelhoffer K, Shaver G, Fry B, Giblin A, Johnson L, McKane R (1996) ^{15}N natural abundances and N use by tundra plants. Oecologia 107:386–394

Nadelhoffer KJ, Downs MR, Fry B (1999) Sinks for ^{15}N-enriched additions to an oak forest and a red pine plantation. Ecol Appl 9:72–86

Pardo LH, Nadelhoffer KJ (2009) Using nitrogen isotope ratios to assess terrestrial ecosystems at regional and global scales. In: West JB, Bowen G, Dawson T, Tu K (eds) Isoscapes: understanding movement, pattern, and process on earth through isotope mapping. Springer, New York

Pardo LH, Hemond HF, Montoya JP, Siccama TG (2001) Long-terms patterns in forest floor nitrogen-15 natural abundance at Hubbard Brook, N.H. Soil Sci Soc Am J 65:1279–1283

Pardo LH, Hemond HF, Montoya JP, Fahey TJ, Siccama TG (2002) Response of the natural abundance of ^{15}N in forest soils and foliage to high nitrate loss following clear-cutting. Can J For Res 32:1126–1136

Pardo LH, Templer PH, Goodale CL et al (2006) Regional assessment of N saturation using foliar and root $\delta^{15}N$. Biogeochemistry 80:143–171

Piccolo MC, Neill C, Cerri C (1994) Natural abundance of ^{15}N in soils along forest-to-pasture chronosequences in the western Brazilian Amazon Basin. Oecologia 99:112–117

Piccolo M, Neill C, Mellilo JM et al (1996) ^{15}N natural abundance in forest and pasture soils of the Brazilian Amazon Basin. Plant Soil 182:249–258

Pritchard ES, Guy RD (2005) Nitrogen isotope discrimination in white spruce fed with low concentrations of ammonium and nitrate. Trees 19:89–98. doi:10.1007/s00468-004-0367-2

Rhoads AG, Hamburg S, Fahey TJ, Siccama TG, Hane EN, Battles J, Cogbill C, Randall J, Wilson G (2002) Effects of an intense ice storm on the structure of a northern hardwood forest. Can J For Res 32:1763–1775

Robinson D (2001) $\delta^{15}N$ as an integrator of the nitrogen cycle. Trends Ecol Evol 16:153–162

Rothstein DE, Zak DR, Pregitzer KS (1996) Nitrate deposition in northern hardwood forests and the nitrogen metabolism of *Acer saccharum* Marsh. Oecologia 108:338–344

Schmidt S, Stewart GR (2003) $\delta^{15}N$ values of tropical savanna and monsoon forest species reflect root specialisations and soil nitrogen status. Oecologia 134:569–577

Serna M, Borras R, Legaz F, Primo-Millo E (1992) The influence of nitrogen concentration and ammonium/nitrate ratio on N-uptake, mineral composition and yield of citrus. Plant Soil 147:13–23

Shearer G, Kohl D (1986) N_2 fixation in field settings, estimations based on natural ^{15}N abundance. Aust J Plant Physiol 13:699–757

Socci AM, Templer PH (2011) Temporal patterns of inorganic nitrogen uptake by mature sugar maple (*Acer saccharum* Marsh.) and red spruce (*Picea rubens* Sarg.) trees using two common approaches. Plant Ecol Divers 4:141–152

Templer PH, Dawson TE (2004) Nitrogen uptake by four tree species of the Catskill Mountains, New York: implications for forest N dynamics. Plant Soil 262:251–261

Templer P, Arthur M, Lovett G, Weathers K (2007) Plant and soil natural abundance $\delta^{15}N$: indicators of relative rates of nitrogen cycling in temperate forest ecosystems. Oecologia 153:399–406

Venterea RT, Lovett GM, Groffman PM, Schwarz PA (2003) Landscape patterns of net nitrification in a northern hardwood-conifer forest. Soil Sci Soc Am J 67:527–539

Whittaker RH, Bormann FH, Likens GE, Siccama TG (1974) The Hubbard Brook Ecosystem Study: forest biomass and production. Ecol Monogr 44:233–254

Yanai RD, Fisk MC, Fahey TJ, Cleavitt NL, Park BB (2008) Identifying roots of northern hardwood species: patterns with diameter and depth. Can J For Res 38:2862–2869. doi:10.1139/X08-125

Yoneyama T, Kaneko A (1989) Variations in the natural abundance of ^{15}N in nitrogenous fractions of komatsuna plants supplied with nitrate. Plant Cell Physiol 30:957–962

Yoneyama T, Omata T, Nakata S, Yazaki J (1991) Fractionation of nitrogen isotopes during the uptake and assimilation of ammonia by plants. Plant Cell Physiol 32(8):1211–1217

Yoneyama T, Matsumaru T, Usui K, Engelaar WMHG (2001) Discrimination of nitrogen isotopes during absorption of ammonium and nitrate at different nitrogen concentrations by rice (*Oryza sativa* L.) plants. Plant Cell Environ 24:133–139

Yoneyama T, Ito O, Engelaar WMHG (2003) Uptake, metabolism and distribution of nitrogen in crop plants traced by enriched and natural ^{15}N: progress over the last 30 years. Phytochem Rev 2:121–132