



Foliar $\delta^{15}\text{N}$ patterns along successional gradients at plant community and species levels

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[1] Relationships to ^{15}N abundances have been found in aridity, rainfall, soil age and latitudinal gradients across large spatial and temporal scales and patterns at intermediate spatial and temporal scale remain unclear. An investigation on ^{15}N abundances at intermediate spatial and temporal scale was conducted in a series of successional fields (Blandy Experimental Farm) in northern Virginia. Foliar and soil samples were collected from these fields in mid-summer 2004 and 2005. The results showed that foliar $\delta^{15}\text{N}$ signatures decreased as successional age increased at both the plant community and species levels. There were also significant decreases in soil $\delta^{15}\text{N}$ as succession proceeded. These results provide a clear example that $\delta^{15}\text{N}$ signatures in certain ecosystems, like an early successional field, could change significantly at time scales within human lifespans and such dynamics should be considered when modeling $\delta^{15}\text{N}$ spatial patterns. **Citation:** Wang, L., P.-J. L. Shaner, and S. Macko (2007), Foliar $\delta^{15}\text{N}$ patterns along successional gradients at plant community and species levels, *Geophys. Res. Lett.*, 34, L16403, doi:10.1029/2007GL030722.

1. Introduction

[2] Nitrogen (N) is a unique nutrient in that it is highly controlled by biological processes. The natural abundance of ^{15}N is an integrator of N cycling and reflects numerous processes occurring in the soil, plant and atmosphere [Robinson, 2001]. The $\delta^{15}\text{N}$ signature of any ecosystem component (e.g. leaves, roots or soil organic matter) is a result of a combination of processes that affect the ^{15}N composition, such as different N sources and effects of isotopic fractionation. Natural abundance stable isotope compositions along natural gradients have been of great interest in ecological studies with correlations to ^{15}N abundance being found with aridity [Aranibar *et al.*, 2004; Swap *et al.*, 2004], rainfall [Austin and Vitousek, 1998], soil age [Brenner *et al.*, 2001] and latitudinal gradients [Sah *et al.*, 2006]. Stable isotope compositions have also been used to indicate agricultural history [Koerner *et al.*, 1999]. However, most of these studies have been accomplished at large geographical scales (>1000 km) and long time scales (>1000 years), patterns of stable isotope abundance along natural gradients at intermediate spatial and temporal scale remain unclear. The present study attempts to assess whether there are foliar $\delta^{15}\text{N}$ patterns at much shorter time scales (less than 100 years) and much

smaller spatial scales (sites that are within 5 km) as secondary succession develops from early to late succession ages.

[3] It was hypothesized that there is a relationship between foliar $\delta^{15}\text{N}$ and succession age, based on two pieces of existing knowledge. First, it is known that mycorrhizal fungi (especially ectomycorrhizal (EM) fungi) discriminate against ^{15}N when transferring soil N to their host plants; consequently, the foliar $\delta^{15}\text{N}$ signatures decrease with an increase in mycorrhizal association [Hobbie and Colpaert, 2004]. This has been described in theoretical work [Hobbie *et al.*, 2005] and has been demonstrated through field observations and laboratory experiments [Hogberg *et al.*, 1996; Hobbie and Colpaert, 2004]. For vesicular-arbuscular mycorrhizal (VAM) fungi, the impact of isotope discrimination is inconclusive but is presumably much smaller than for EM fungi [Hogberg, 1997; Handley *et al.*, 1999a]. Second, it is also known that during secondary succession from abandoned agriculture fields to forests, the density of VAM fungi first increases with time and then decreases in the late successional forest sites, and there is a shift in the dominant vegetation from herbaceous VAM fungal hosts to woody EM fungal hosts [Johnson *et al.*, 1991]. Because the VAM association intensity increases and the type of mycorrhizal associations change (VAM to EM) as succession develops, both of which influencing a decrease in plant ^{15}N content, there should be a negative correlation between successional age and plant $\delta^{15}\text{N}$ signatures. However, such a correlation has not been reported, and only few attempts have been made to explore such a relationship in primary succession [Hobbie *et al.*, 1998, 2005].

[4] This study was conducted in a series of successional fields at the Blandy Experimental Farm (BEF) of the University of Virginia. The BEF offers an excellent opportunity to study foliar isotopic changes during secondary succession because the land-use and fertilizer-use history of each successional field is well-documented.

2. Materials and Methods

2.1. Study Site

[5] The BEF study site is located in Clarke County, Virginia (39°09'N, 78°06'W). The BEF can be described as an ecosystem consisting of agricultural fields, successional fields, deciduous woodlands and ephemeral wetlands. The woodlots are mature, second-growth, oak-hickory-elm forests, aged at around 85 to 120 years. The mid-successional fields have been free of agricultural activities for 15–20 years, and the early-successional fields have been free from any mowing and/or agricultural activities for 5 years. The successional ages are calculated as from abandonment to 2005 [Riedel and Epstein, 2005; Shaner *et al.*, 2007;

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Wang et al., 2007]. The annual average temperature during 2003–2004 was 14°C and the average precipitation was 127 cm. Compared to the long-term average (1971–2000), 15°C and 100 cm, 2003–2004 was slightly wetter and cooler (data obtained from Martinsburg, VA weather station, 40 km from BEF). The soil in all of the successional fields is silt loam and the soil type is ultisols according to US Soil Taxonomy. Topography at all successional fields is gently sloping (<10%) [Riedel and Epstein, 2005].

[6] Three sites including a 5-year-old field, a 19-year-old field and an 85-year-old woodlot, which are adjacent to each other, were chosen for this study. An additional site, presumed to be 18-year old (located approximately 2 km away), was analyzed and not included in the evaluation since the age and land use history were found to be not as well known and subject to modern fertilizer influence (see online datasets). The 5-year-old field supported corn and barley before abandonment in 2000; the 19-year-old field has been managed for agriculture and grazing since at least 1958 until abandonment in 1986. From 1958 through 1968 this field was managed under a 5 year crop rotation of 3 years pasture (orchard grass and clover) and two years for crop (corn and barley). From 1969 to 1986, the field was mowed annually for hay and grazed by cattle the remainder of the growing season [Riedel and Epstein, 2005]. The 5-year-old field is relatively homogeneous and dominated by *Solidago spp.* (Goldenrod), there are other dicots such as *Asclepias syriaca* (Common milkweed), *Phytolacca America* (Pokeweed) and *Carduus spp.* (Thistle), and some shrub seedlings in the understory such as *Rhamnus cathartica* (Buckthorn) and *Celastrus orbiculatus* (Oriental bittersweet). The 19-year-old field is a mixture of woody and shrub species such as *R. cathartica*, *C. orbiculatus* and *Ailanthus altissima* (Tree of Heaven), and dicots such as *Solidago spp.*, *Daucus carota* (Wild carrot), *A. syriaca*, *P. America* and *Carduus spp.* The common species in the woodlot (85-year-old field) are woody plants such as *Quercus alba* (White oak), *Q. rubra* (Red oak), *Carya spp.* (Hickory), *Asimina triloba* (Pawpaw), *Juglans nigra* (Black walnut), *Celtis occidentalis* (Hackberry); shrubs such as *C. orbiculatus* and *Lonicera spp.* (Honeysuckle); dicots such as *Polygonum spp.* (Lady's thumb) and *P. America*.

2.2. Field Sampling

[7] In September 2004 and October 2005, based on the vegetation composition at each successional age, a systematic foliage sampling was conducted in the three successional fields (Table 1). The sampling period was between 1 and 2 days each year to minimize effects of potential foliar $\delta^{15}\text{N}$ changes along the growing season. The new leaves from the upper crown were collected from all the individuals (the tree leaves for the late successional stage were collected from the middle canopy by climbing). Upon collection, foliar samples were identified to genus or species. Plant foliage samples from between 5 and 8 species were taken from each of the successional fields (16 different species in total). For most species in each successional field, five different individuals were sampled for foliage but the number varied based on availability (Table 1). In each successional age, the average value of each species was used to represent the community foliar $\delta^{15}\text{N}$ value. In October 2005, five soil

samples (0–10 cm, A horizon only in all sampled systems) were sampled from each of the three successional fields using soil corer with 5 cm diameter. The surface litter and organic layers, which only exist in 85-year-old field, were carefully removed and only mineral soils were collected for analysis. Plant roots and gravel were removed from soil samples by sieves in the laboratory. The average of five samples was used to indicate the soil $\delta^{15}\text{N}$, %C, %N and C/N ratio at each successional age.

2.3. Chemical Analyses

[8] Foliar and soil samples were dried at 60°C for 72 hours. After drying, they were ground and homogenized for isotope and elemental analysis. Stable N isotope analysis was performed using a Micromass Optima Isotope Ratio Mass Spectrometer (IRMS) connected to an elemental analyzer (EA) (GV/Micromass, Manchester, UK). The N stable isotope compositions are reported in the conventional form (‰):

$$\delta^{15}\text{N}(\text{‰}) = \left[\left(\frac{{}^{15}\text{N}/{}^{14}\text{N}_{\text{sample}}}{{}^{15}\text{N}/{}^{14}\text{N}_{\text{standard}}} \right) - 1 \right] \times 1000$$

where $({}^{15}\text{N}/{}^{14}\text{N})_{\text{sample}}$ is the N isotopic composition of a sample, and $({}^{15}\text{N}/{}^{14}\text{N})_{\text{standard}}$ is the N isotopic composition of the standard material. The standard material for stable N isotopes is atmospheric molecular N (AIR). Reproducibility of these measurements is approximately 0.2‰. To explore what factors determine foliar $\delta^{15}\text{N}$ patterns, foliar %C and %N, as well as soil %C and %N were measured using the elemental analyzer (EA).

2.4. Statistical Analyses

[9] Foliar and soil $\delta^{15}\text{N}$, %C and %N data are normally distributed (SAS v. 9.1, PROC UNIVARIATE) and were not transformed. At the plant community level, a two-way ANOVA with the sampling time (2004 vs. 2005) and the successional ages as two main factors (SAS v. 9.1, PROC GLM) was performed to test the foliar $\delta^{15}\text{N}$ differences between the three successional ages; the mean foliar $\delta^{15}\text{N}$ values of all individuals for each species at each successional age were used in this analysis. Because no significant effect with sampling time was found, the foliar $\delta^{15}\text{N}$ changes along succession were reported based on the pooled data of year 2004 and 2005. Mean separations were determined by a Tukey *post hoc* test at $\alpha = 0.05$ (SAS v. 9.1 PROC GLM). Soil %C, %N, soil C/N ratio and soil $\delta^{15}\text{N}$ values were compared between successional ages using one-way ANOVA and mean separations were calculated by a Tukey *post hoc* test at $\alpha = 0.05$. To illustrate the relationship between %N and $\delta^{15}\text{N}$ values in soils and plants, Pearson correlation analyses were performed between foliar $\delta^{15}\text{N}$ and foliar %N for both 2004 and 2005 data, and between foliar $\delta^{15}\text{N}$ (2004 and 2005 data) and soil $\delta^{15}\text{N}$ values in 2005 (PROC CORR).

[10] All species were used to test the foliar $\delta^{15}\text{N}$ patterns at the community level. At the species level, only those species that occurred in at least two successional ages were used to test for a species-specific response of foliar $\delta^{15}\text{N}$ during succession. Based on this criterion, six species were selected including *S. altissima*, *C. orbiculatus*, *R. cathartica*, *P. persicaria*, *P. americana* and *L. maackii* (Amur honeysuckle). For each species, a two-way ANOVA with the

Table 1. Species and Number of Samples That Were Collected in 2004 and 2005 as Well as the Potential Mycorrhizal Infection Type for Each Species^a

Successional Age (yrs)	2004	2005	Mycorrhizae Type
5	<i>Celastrus orbiculatus</i> (5)	<i>Celastrus orbiculatus</i> (5)	VAM
	<i>Rhamnus cathartica</i> (5)	<i>Rhamnus cathartica</i> (5)	VAM
	<i>Polygonum persicaria</i> (3)	<i>Polygonum persicaria</i> (5)	VAM
	<i>Phytolacca americana</i> (3)	<i>Phytolacca americana</i> (5)	NM
	<i>Solidago altissima</i> (5)	<i>Solidago altissima</i> (3)	VAM
		<i>Rubus spp.</i> (1)	VAM
		<i>Triodia flava</i> (?) (1)	NA
19	<i>Celastrus orbiculatus</i> (5)	<i>Celastrus orbiculatus</i> (5)	VAM
	<i>Rhamnus cathartica</i> (5)	<i>Rhamnus cathartica</i> (5)	VAM
	<i>Lonicera maackii</i> (3)	<i>Lonicera maackii</i> (3)	VAM
	<i>Polygonum persicaria</i> (3)	<i>Polygonum persicaria</i> (3)	VAM
	<i>Phytolacca americana</i> (3)	<i>Phytolacca americana</i> (5)	NM
	<i>Solidago altissima</i> (5)	<i>Solidago altissima</i> (5)	VAM
		<i>Toxicodendron radicans</i> (1)	NA
85	<i>Celastrus orbiculatus</i> (5)	<i>Celastrus orbiculatus</i> (3)	VAM or NM
	<i>Carya spp.</i> (5)	<i>Carya spp.</i> (5)	VAM
	<i>Lonicera maackii</i> (5)	<i>Lonicera maackii</i> (5)	VAM
	<i>Polygonum persicaria</i> (4)	<i>Polygonum persicaria</i> (5)	VAM
	<i>Asimina triloba</i> (5)	<i>Asimina triloba</i> (4)	NA
	<i>Phytolacca americana</i> (3)	<i>Phytolacca americana</i> (5)	NM
	<i>Lindera benzoin</i> (5)	<i>Lindera benzoin</i> (5)	VAM
	<i>Quercus spp.</i> (3)	<i>Quercus spp.</i> (5)	VAM and EM

^aNumber of samples is given in parentheses. Species are vesicular-arbuscular mycorrhizal (VAM), ectomycorrhizal (EM), and non-mycorrhizal (NM); NA means data not available.

sampling time (2004 vs. 2005) and the successional ages as the two main factors (SAS v. 9.1, PROC GLM) were used to test the foliar $\delta^{15}\text{N}$ differences. For most species no differences in foliar $\delta^{15}\text{N}$ were found between the sampling times; therefore, the foliar $\delta^{15}\text{N}$ changes along succession were reported based on pooled data for the years 2004 and 2005. For *C. orbiculatus*, foliar $\delta^{15}\text{N}$ changes in the two sampling time were reported separately since there was a significant sampling time effect. Mean separations were determined by Tukey *post hoc* test at $\alpha = 0.05$ (SAS v. 9.1 PROC GLM).

3. Results and Discussion

[11] There is a clear and consistent pattern that foliar $\delta^{15}\text{N}$ decreases as secondary succession develops from early succession to late succession at both the community level and species level (Figure 1). At the community level, successional age explained 55% of the total variance of foliar $\delta^{15}\text{N}$ (Figure 1a). Similar trends existed at the species level, with five of the six species (*C. orbiculatus*, *R. cathartica*, *P. persicaria*, *P. americana*, and *S. altissima*) showing decreasing trends in foliar $\delta^{15}\text{N}$ as successional ages increase (Figure 1b–1g). The only species that did not show a significant relationship between foliar $\delta^{15}\text{N}$ and successional age was *L. maackii* (Figure 1h). These results agree with *Hobbie et al.* [1998] following a re-analysis of the data from that report. In that study, carried out on a primary succession series at Glacier Bay, Alaska, *Hobbie et al.* [1998] reported the foliar $\delta^{15}\text{N}$ signature changes along the successional sequence for the different species, and no consistent relationship between foliar $\delta^{15}\text{N}$ and successional age was found. It is believed that the lack of a relationship between foliar $\delta^{15}\text{N}$ and successional age in that study was the result of the N-fixing activity interaction with mycor-

rhizal associations in the early successional ages. In that study, the effect of N-fixation ($\delta^{15}\text{N}$ signature for N fixation is approximately 0‰ or slightly negative) increased the foliar $\delta^{15}\text{N}$ signature for the early succession ages (because most foliar $\delta^{15}\text{N}$ signature are negative in that system, Figure 1i). A re-analysis of the *Hobbie et al.* [1998] data, removing the values of N-fixing species and those from earliest succession ages, reveals a strong negative relationship between foliar $\delta^{15}\text{N}$ signatures and succession age ($r = -0.97$, $p < 0.0001$, Figure 1i).

[12] It is further suggested that a major driver for the variation in foliar $\delta^{15}\text{N}$ along succession is the change in soil $\delta^{15}\text{N}$. This conclusion is based on these lines of evidence: 1) there was a significant decrease in soil $\delta^{15}\text{N}$ as succession developed (Table 2), 2) there were strong correlations between foliar and soil $\delta^{15}\text{N}$ in both 2004 and 2005 ($r = 0.95$ and 0.97 , respectively, $p < 0.001$ for both cases); 3) the foliar $\delta^{15}\text{N}$ pattern of most individual species (five of the six observed) that occurred at multiple successional ages mirrored the community foliar $\delta^{15}\text{N}$ pattern in both 2004 and 2005 (Figures 1a–1h), which exactly track the soil $\delta^{15}\text{N}$ patterns (Table 2). Because of the relatively small spatial scale (all study sites are within 5 km) and short time scale (~100 years for the whole series), it is unlikely that any across site variations in soil clay content or N deposition, which are known to affect plant and soil $\delta^{15}\text{N}$ signatures, influenced the $\delta^{15}\text{N}$ significantly. As well, N-fixing species are rare in all of the succession fields (Table 1), posing little effect on the foliar $\delta^{15}\text{N}$ patterns.

[13] Another potential factor that may contribute to the variation in individual foliar $\delta^{15}\text{N}$ is rooting depth [*McKane et al.*, 1990]. However, the species selected included both woody species and herbaceous species, and almost all of them (five out of six) showed significant $\delta^{15}\text{N}$ decline with succession. For the herbaceous species, *S. altissima* and

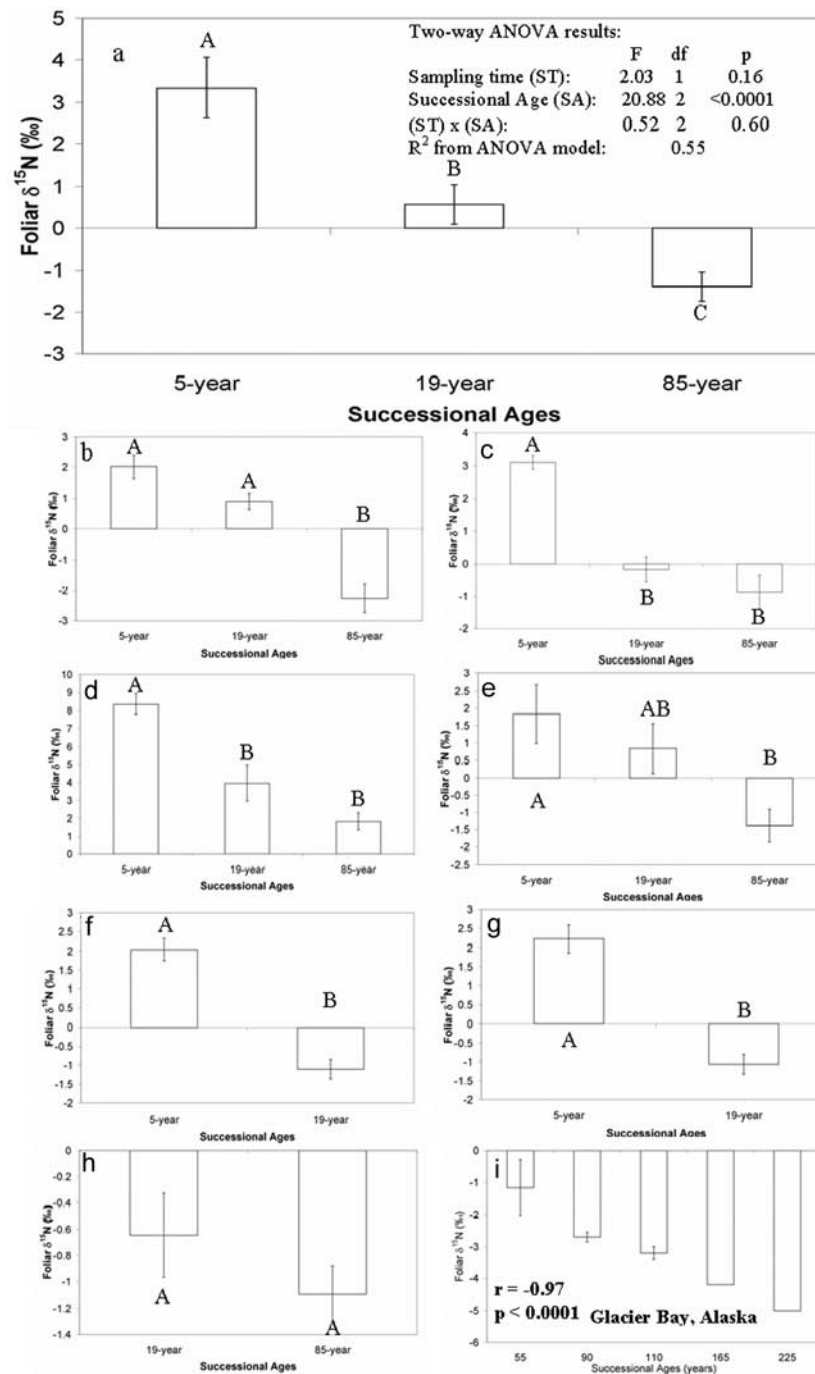


Figure 1. (a) Plant community level foliar $\delta^{15}\text{N}$ variations at different successional ages. Each bar (error bar is the standard error) represents an average of 2004 and 2005 data. (b–h) The species level foliar $\delta^{15}\text{N}$ variations at different successional ages. The species are: (b) *Celastrus orbiculatus* (2004), (c) *Celastrus orbiculatus* (2005), (d) *Phytolacca America*, (e) *Polygonum persicaria*, (f) *Rhamnus cathartica*, (g) *Solidago altissima*, and (h) *Lonicera maackii*. Each bar (error bar is the standard error) represents an average of 2004 and 2005 data except Figures 1b and 1c. (i) Re-analysis of the Hobbie *et al.* [1998] data by removing the values of nitrogen-fixing species and the values from earliest succession age (Each bar represents average across all species at each successional age).

P. persicaria, the root distribution was observed by removal of several individuals in the field. No observable differences in root depth were found among successional ages. In addition, as succession develops, there is a vegetation composition shift from herbaceous species to woody species, and the rooting depth of woody species is generally

greater than herbaceous species [Jackson *et al.*, 1996; Burch *et al.*, 1997]. If rooting depth is the influential for the observed foliar $\delta^{15}\text{N}$ pattern, and would increase as succession developed, since soil $\delta^{15}\text{N}$ increases with soil depth and deeper rooting depths would therefore generate higher foliar $\delta^{15}\text{N}$. This is opposite to that which was observed.

Table 2. Foliar and Soil %C, %N, C/N Ratio, Soil $\delta^{15}\text{N}$, and Soil-Plant $\delta^{15}\text{N}$ Difference Changes Along Succession in 2005^a

	5 Years	19 Years	85 Years
Soil C, %	2.00 ± 0.12 (A)	1.51 ± 0.21 (A)	4.12 ± 0.74 (B)
Soil N, %	0.19 ± 0.01 (A)	0.15 ± 0.02 (A)	0.32 ± 0.05 (B)
Soil C/N ratio	10.6 ± 0.1 (A)	9.8 ± 0.4 (A)	12.8 ± 0.3 (B)
Foliar C, %	45.82 ± 0.55 (A)	48.19 ± 0.62 (B)	48.63 ± 0.43 (B)
Foliar N, %	2.68 ± 0.18 (A)	2.60 ± 0.18 (A)	2.82 ± 0.13 (A)
Foliar C/N ratio	19.1 ± 1.3 (AB)	20.8 ± 1.3 (A)	18.1 ± 0.7 (B)
Soil $\delta^{15}\text{N}$	5.6 ± 0.3 (A)	3.8 ± 0.4 (B)	2.6 ± 0.3 (B)
$\delta^{15}\text{N}$ difference	1.9	3.7	4.4

^aShown are mean ± 1 standard error. The different letters indicate different means at $\alpha = 0.05$ using Tukey *post hoc* test.

Therefore it is unlikely that rooting depth contributed to the foliar $\delta^{15}\text{N}$ patterns observed here.

[14] Clearly, these limited data do not allow for a complete explanation for the changes in $\delta^{15}\text{N}$ of the soils along the successional gradients. Studies from other systems suggest the possibility that when N availability is high, the entire system is more open to N transformations and modification; for example, ^{14}N can be preferentially removed from the system through the processes such as ammonia volatilization [Swap *et al.*, 2004] and nitrate leaching [Vitousek *et al.*, 1989; Austin and Vitousek, 1998], leading to an enrichment of soil $\delta^{15}\text{N}$. When N availability is low, the entire system becomes more closed, leading to less loss of N and diminished fractionation in soil ^{15}N . The N cycling openness has also been suggested to explain the soil $\delta^{15}\text{N}$ difference between tropical and temperate forests [Martinelli *et al.*, 1999]. In the present successional system, it could be that higher soil $\delta^{15}\text{N}$ in early successional ages is affected by the greater openness in N cycling although the factors that influence this are not well-understood. Soil C/N in the late successional age was higher than those in early and middle successional ages along this successional gradient (Table 2). Since higher soil C/N generally indicates more N limitation for soil microbes [Chapin *et al.*, 2002], the later successional stands may have had less openness for N cycling owing to lower N availability for soil microbes. Another potential mechanism for the observed $\delta^{15}\text{N}$ decreasing trend is the increase of losses of dissolved organic N (DON) along succession. The DON might have high $\delta^{15}\text{N}$ values and the higher losses will lead the whole-ecosystem $\delta^{15}\text{N}$ depleted as proposed by Handley *et al.* [1999b].

[15] Although the foliar $\delta^{15}\text{N}$ compositions along succession are clearly influenced by soil $\delta^{15}\text{N}$ changes, mycorrhizal associations may also play an important role. The differences in $\delta^{15}\text{N}$ between soil and plant increase as succession develops (Table 2). The larger difference between plants and soils as succession develops fits well with the change of mycorrhizal association intensity and association type along succession. In addition, there is a positive correlation between foliar $\delta^{15}\text{N}$ and %N in both 2004 and 2005 ($r = 0.50$, $p = 0.01$ and $r = 0.45$, $p = 0.008$ respectively), which is a potential indicator of plant-mycorrhizal interactions [Hobbie *et al.*, 2000].

[16] In summary, this study shows that there is a consistent foliar $\delta^{15}\text{N}$ decrease in an entire successional series (e.g. from early to middle and late succession) at both

community and species levels. This study shows that foliar and soil $\delta^{15}\text{N}$ patterns can appear over relatively short time and small spatial scales, which suggests the need for considering $\delta^{15}\text{N}$ temporal dynamics in certain ecosystems when modeling $\delta^{15}\text{N}$ spatial patterns. Several lines of evidence show that the major driver of foliar $\delta^{15}\text{N}$ change along the succession is soil $\delta^{15}\text{N}$ change although mycorrhizal associations may also play an important role. The same direction in $\delta^{15}\text{N}$ changes in plants and soils indicate N cycling during secondary succession is an open-system process and the system becomes less open toward late successional stage.

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