Natural ^{15}N Abundance of Plants and Soil N in a Temperate Coniferous Forest

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ABSTRACT

Measurement of nitrogen isotopic composition (δ^{15}N) of plants and soil nitrogen might allow the characteristics of N transformation in an ecosystem to be detected. We tested the measurement of δ^{15}N for its ability to provide a picture of N dynamics at the ecosystem level by doing a simple comparison of δ^{15}N between soil N pools and plants, and by using an existing model. δ^{15}N of plants and soil N was measured together with foliar nitrate reductase activity (NRA) and the foliar NO_3^- pool at two sites with different nitrification rates in a temperature forest in Japan. δ^{15}N of plants was similar to that of soil NO_3^- in the high-nitrification site. Because of high foliar NRA and the large foliar NO_3^- pool at this site, we concluded that plant δ^{15}N indicated a great reliance of plants on soil NO_3^- there. However, many δ^{15}N of soil N overlapped each other at the other site, and δ^{15}N could not provide definitive evidence of the N source. The existing model was verified by measured δ^{15}N of soil inorganic N and it explained the variations of plant δ^{15}N between the two sites in the context of relative importance of nitrification, but more information about isotopic fractionations during plant N uptake is required for quantitative discussions about the plant N source. The model applied here can provide a basis to compare δ^{15}N signatures from different ecosystems and to understand N dynamics.

Key words: nitrogen isotope ratio; nitrogen availability; nitrogen dynamics; nitrate reductase activity; foliar NO_3^-; modeling.

INTRODUCTION

The supply of nitrogen (N) often limits the growth of plants, the composition of communities, the productivity of ecosystems, and ecosystem processes (Vitousek and Howarth 1991). Thus, it is crucial for a better understanding of ecosystem processes to determine how N is transformed, used, and recycled in ecosystems.

Variations in ^{15}N natural abundance (expressed as δ^{15}N) of plant tissue and soil have been examined to gain insights into patterns of N cycling in terrestrial ecosystems (Nadelhoffer and Fry 1994; Handley and Scrimgeour 1997; Högberg 1997). Plant δ^{15}N is determined by (1) the source(s) of N (soil, precipitation, N_2 fixation, and so on), (2) the depth(s) in the soil from which N is taken up, (3) the form(s) of soil N used (organic N, NH_4^+, and

Table 1.   General Description of Ryuoh Mountain Slope

Variable	Position in Mountain Slope	
	Upper Site[a]	Lower Site[a]
Plant species composition	Cryptomeria japonica	Cryptomeria japonica
	Hydrangea hirta	Leucosceptrum stellipilum
	Lindera triloba	Hydrangea hirta
	Pieris japonica	Lindera triloba
DBH of Cryptomeria japonica (cm)	9.4–25.0	12.4–38.2
Fine root biomass (g m^{-2})	1068	726
Net mineralization rates (mg N kg soil^{-1} d^{-1})	0.18–1.46	0.00–1.69
	0.03–0.25	0.19–0.45
	1.82–2.65	1.95–4.48
	6.77–15.91	5.77–12.41
Net netrification rates (mg-N kg-soil^{-1} d^{-1})	0.00–0.02	0.00–2.46
	0.00–0.09	0.04–0.44
	0.16–0.94	1.85–4.36
	0.20–3.03	1.66–12.04
Gross mineralization rates (mg-N kg-soil^{-1} d^{-1})	2.39–8.50	7.31–47.53
Gross nitrification rates (mg-N kg-soil^{-1} d^{-1})	0.01–0.04	0.04–11.07
Soil C/N ratio	80.9–107.8	57.3–86.0
	20.5–21.6	17.5–21.2

[a]Upper Site (812–851-m a.s.l.) and Lower Site (765–812-m a.s.l.) are defined according to the results on different N transformation patterns observed by Hirobe and others (1998). Data are shown as ranges.

NO$_3$$^-$), (4) influences of mycorrhizal symbioses, (5) fractionations during and after N uptake by plants, and (6) plant phenology (Högberg 1997). Because of these potential factors that can affect plant δ^{15}N, a simple comparison of δ^{15}N between plant and soil N cannot always provide definitive evidence about N dynamics in plant–soil systems (Handley and Scrimgeour 1997; Evans 2001; Robinson 2001). The δ^{15}N values often show repeatable patterns in similar environments (Handley and Scrimgeour 1997; Michelsen and others 1998). Such patterns of plant δ^{15}N thus have great potential to provide integrated information on ecosystem N dynamics. A generally accepted theory that explains the δ^{15}N patterns can reveal new insights into the mechanical details of ecosystem N dynamics (Handley and others 1999). Modeling approaches (Koopmans and others 1997; Hobbie and others 1999; Brenner and others 2001) are likely to be useful for this purpose because δ^{15}N in ecosystems cannot be determined so simply (Handley and Scrimgeour 1997).

A theoretical model that explains the isotopic composition of inorganic soil N as a function of the relative rates of N transformation in soil, particularly the rate of N immobilization versus nitrification (Shearer and others 1974), indicated that plant δ^{15}N might correlate positively with N availability in forest soils. Garten and Van Miegroet (1994) successfully refined this model to apply to soil–plant systems, illustrating that the changes in plant δ^{15}N can be interpreted in the context of different N cycling mechanisms. Their model (Garten and Van Miegroet 1994) is more appropriate than simply matching δ^{15}N values between soil N pools and plants but it has not been tested extensively.

The aim of this study was to test the existing theory (Garten and Van Miegroet 1994) to determine if δ^{15}N can provide useful information on N dynamics, which should be consistent with other data on plants and soils. We first determined intraplant variation in δ^{15}N for some plant species to find whether foliar δ^{15}N can be representative of plant δ^{15}N. Second, we measured δ^{15}N of soil N in the forest floor to supplement the results of Koba and others (1998) to complete a description of δ^{15}N variations in a forest ecosystem. We have no good indicators for organic N and NH$_4$$^+$ uptake by plants in the field except tracer experiments. However, nitrate reductase activity (NRA) of plants is a good indicator for NO$_3$$^-$ uptake and use by plants (Lee and Stewart 1978) as well as the NO$_3$$^-$ pool in plant tissues. Therefore, finally we measured foliar NRA and the NO$_3$$^-$ pool as a check on our δ^{15}N interpretations.

MATERIALS AND METHODS

This study was carried out at Mt. Ryuoh, Shiga Prefecture, Japan (35°1'N, 136°20'E). Mean annual

Table 1. Continued

Comments	Data Source
	L. Koyama (unpublished)
Nov. 1997	L. Koyama (unpublished)
O layer + mineral soil 0–80 cm; May 1993	Kasuya and Shimada (1996) and their unpublished data
Mineral soil 0–50 cm; Oct. 1994	Koba and others (1998)
Mineral soil 0–5 cm; Aug. 1995	Hirobe and others (1998)
Mineral soil 0–5 cm; Nov. 1995	Tokuchi and others (2000)
Oe + a layer; Nov. 1996	Hobara and Tokuchi (1998)
Mineral soil 0–50 cm; Oct. 1994	Koba and others (1998)
Mineral soil 0–5 cm; Aug. 1995	Hirobe and others (1998)
Mineral soil 0–5 cm; Nov. 1995	Tokuchi and others (2000)
Oe + a layer; Nov. 1996	Hobara and Tokuchi (1998)
Mineral soil 0–5 cm; Nov. 1995	Tokuchi and others (2000)
Mineral soil 0–5 cm; Nov. 1995	Tokuchi and others (2000)
Oi layer; June 1996	S. Hobara (unpublished)
Mineral soil 0–5 cm; Nov. 1995	Tokuchi and others (2000)

precipitation and soil temperature (at 5-cm depth) from 1986 to 1991 were 2050 mm and 10°C, respectively. A forested area with a mean slope of 38.5° was chosen for the study site (Hirobe and others 1998; Tokuchi and others 1999). Dominant overstory vegetation on the slope was a 45-year-old *Cryptomeria japonica* D. Don plantation, which had reached canopy closure. This watershed had low annual N input (3.3 kg N ha^{-1} y^{-1}, by bulk precipitation) and low N drainage water loss (0.6 kg N ha^{-1} y^{-1}; Ohrui and Mitchell 1997).

A transect on a slope (5 m wide and 135 m long) was established at an elevation of 765–851-m above sea level (a.s.l.). There are distinct differences in N cycling patterns in soils between the upper and lower part of this slope (Hirobe and others 1998; Hobara and Tokuchi 1998; Koba and others 1998; Tokuchi and Iwatsubo 1999; Tokuchi and others 2000). The characteristics are summarized in Table 1. The most distinctive characteristic at this site is that soils from an upper part of the slope have both low net nitrification potentials and small pools of NO_3^-, while high net nitrification rates and large NO_3^- pools are observed in soils from the lower part of the slope (Table 1). Hirobe and others (1998) reported that surface mineral soils (at 0–5-cm depth) showed (i) low to zero net nitrification rates in the upper part (812–851-m a.s.l.), (ii) large net

nitrification rates in the lower part (765–802-m a.s.l.), and (iii) variable net nitrification rates in the transition zone (802–812-m a.s.l.). This dichotomy on the NO_3^- production along this slope was also observed in forest floors (Hobara and Tokuchi 1998) and in deeper mineral layers (Koba and others 1998). Accordingly, we divided the transect into two sites, the Upper Site (812–851-m a.s.l.) with a low NO_3^- supply rate, and the Lower Site (765–812-m a.s.l.) with a high NO_3^- supply rate.

The predominant understory species along this slope are *Leucosceptrum stellipilum* (Miq.) Kitam. Et Murata, *Hydrangea hirta* (Thunb.) Siebold, *Lindera triloba* (Sieb. Et Zucc.) Blume [*Parabensoin trilobum* (Sieb. Et Zucc.) Nakai], and *Pieris japonica* D. Don. *Pieris japonica* was observed almost exclusively at the Upper Site and *Leucosceptrum stellipilum* was observed only at the Lower Site, while *Hydrangea hirta* and *Lindera triloba* occurred at both the Upper Site and the Lower Site (L. Koyama unpublished). *Cryptomeria japonica, Leucosceptrum stellipilum, Hydrangea hirta*, and *Lindera triloba* are associated with arbuscular mycorrhiza (AM; Fujimaki and others 2001). Colonization percentage of AM of *Lindera triloba* was higher at the Upper Site (72–84%) than at the Lower Site (21%–57%), although no significant difference in colonization was found among the other species (Fujimaki and others 2001). *Pieris ja-*

ponica is highly infected by ericoid mycorrhiza (ERM), while none of the species appeared to be colonized by ectomycorrhiza (ECM) at these sites. The fine roots of *Cryptomeria japonica* were concentrated in the forest floor at the Upper Site and in shallow mineral soils (at 0–30-cm depth) at the Lower Site (Kasuya and Shimada 1996).

Whole plants were collected to determine the internal variations of $\delta^{15}N$ from both the Upper Site and the Lower Site, except for *Leucosceptrum stellipilum* which was present only at the Lower Site. Collections were made in June (for *Pieris japonica*, *Hydrangea hirta*, and *Leucosceptrum stellipilum*) and in September (for *Lindera triloba*) in 1995. The plants were divided into several organs according to each life form or morphological features. Whole plant $\delta^{15}N$ was calculated as the weighted average by using the data on N contents (L. Koyama unpublished):

$$\text{Whole plant } \delta^{15}N = \sum (\delta^{15}N \text{ organ}$$
$$\times \text{ mg N organ})/\text{mg N whole plant} \quad (1)$$

Collection of tree leaves for isotopic analysis began in 1994. The canopy of *Cryptomeria japonica* was too high to obtain leaves, especially at the Lower Site. In addition, *Cryptomeria japonica* was commercially planted and any damage, including intensive sampling activities, had to be avoided. Thus, we collected only a few samples of this species from the lower part of the canopy at the Lower Site (June 1994) and from several positions in the canopy at the Upper Site (June 1994 and 1995).

Leaves of four understory species were collected during three successive years (June 1995, July and October 1996, and August 1997) from the Upper Site and the Lower Site. Care was taken to collect the leaves from the entire canopy to eliminate intercanopy differences in foliar chemistries. Each sample was a composite of at least ten leaf samples from a single plant with the exception of *Leucosceptrum stellipilum*, which had large leaves (two to four leaves for one composite).

Besides seasonal measurement of foliar $\delta^{15}N$, we conducted intensive measurements on foliar N status in August 1997 to get an insight into the relationships between foliar $\delta^{15}N$ and other indicators (foliar NRA, NO_3^-, and N content). Collection of samples in August 1997 was finished strictly within 2 h of solar noon on a sunny day because light intensity is one of the controlling factors for NRA. Foliar NRA was measured as in Gebauer and others (1984) and Koyama and others (2001) to find the relative levels of NRA of the already endogenous enzyme under conditions of nonlimiting amounts of substrate. Foliar NO_3^- was also measured to confirm the NO_3^- use of plants. We did not collect samples from *Pieris japonica* at the Lower Site, or from *Leucosceptrum stellipilum* at the Upper Site. Detailed descriptions of the procedure for NRA and foliar NO_3^- analyses is reported in Koyama and others (2001). Foliar N content was analyzed using the NC analyzer (SUMIKA, NC-900, Osaka, Japan) with the ground sample as well as $\delta^{15}N$ analysis (see below).

Forest floor samples were collected in September 1995 and October 1997. From the Lower Site, only the Oi layer was sampled because the Oe + a layer was so thin (Hobara and Tokuchi 1998; Tokuchi and Iwatsubo 1999) and because root density in the forest floor at the Lower Site was quite small (3% of total root mass; Kasuya and Shimada 1996). Samples of leaves and forest floor were dried at 40°C and finely ground in a vibration mill and dried again at 105°C for $\delta^{15}N$ measurements.

The resin bag technique (Binkley and Matson 1983; Giblin and others 1991, 1994; Pate and others 1993; Hirobe and others 2001) was used to collect large enough quantities of NO_3^- in the soil at the Upper Site for $\delta^{15}N$ measurement. Ion-exchange resins (IER; cation and anion, 80 g each) were put into a cylinder bag (PVC ring with nylon mesh). The 80 g of resin has a multifold capacity for ion exchange over one year as calculated from soil solution chemistry (Tokuchi and Iwatsubo 1999), and thus isotope fractionation during absorbance onto IER is neglected by the absorption of all ions through IER bags. At 831- and 840-m a.s.l. at the Upper Site, the bags were buried 10 cm beneath the forest floor from October 1994 to October 1995. We think that isotopic data from these IER likely provide time-integrated information for isotopic composition. We used only NO_3^- data here because $\delta^{15}N$ of NH_4^+ in the soil solution must be different from that in the extractable pool of NH_4^+ in soil as a result of isotopic fractionation during ion exchange [$\delta^{15}N$ of IER-captured NH_4^+ was $-4.86 \pm 0.85‰$ ($n = 6$; mean $\pm$ SE) beneath the forest floor and $-1.52 \pm 0.61‰$ ($n = 6$) at 10-cm depth in mineral soil]. After mixing the collected resin, about 10 g of resins (captured more than 0.2 mg of NO_3^-–N) were extracted by 100 ml of 2 M KCl. This extraction was repeated three times to prevent potential isotopic fractionation during extraction.

In October 1997, soil extracts of the field-collected forest floor were prepared for $\delta^{15}N$ analysis of inorganic N (NH_4^+ and NO_3^-) by shaking the soil sample with 2 M KCl (100 g dry soil vs. 700 ml KCl) for 1 h. For an accurate $\delta^{15}N$ measurement, the
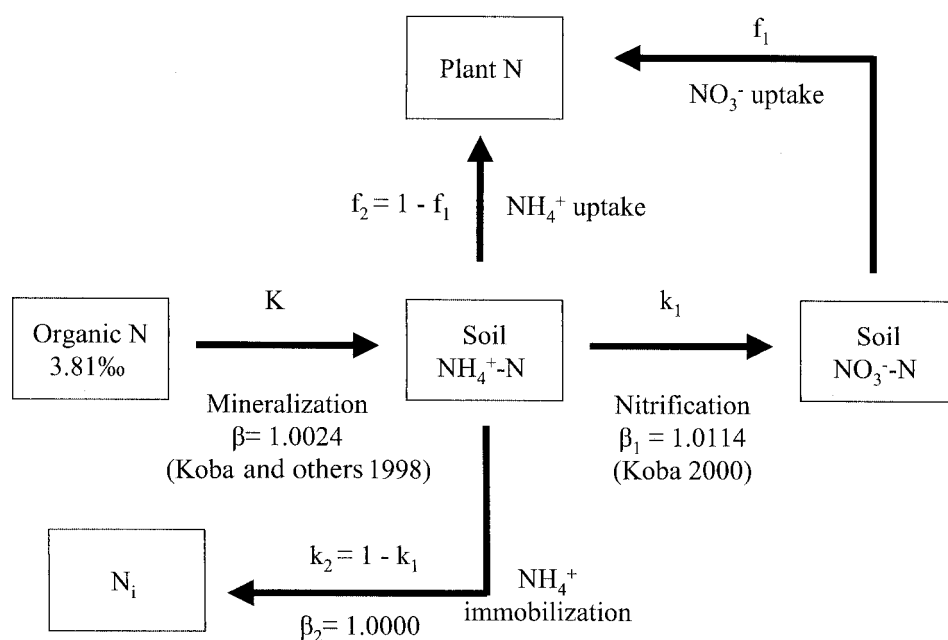
Figure 1. Model of soil N transformation and N uptake by plants (Garten and Van Miegroet 1994). The rate constants for mineralization, nitrification, and NH_4^+ immobilization are denoted by K, k_1, and k_2, respectively. The fractional uptake of NO_3^- and NH_4^+ is denoted by f_1 and f_2, respectively. Pool N_i is immobilized N prior to incorporation into organic soil N. Each fractionation constant (β) is the ratio of the rate of the process for ^{14}N relative to ^{15}N.

maximum volume for our Kjeldahl distillation glassware (1-l flask) was 700 ml of KCl but the amounts of NO_3^- in extracts from the Oe + a layer were too small to determine isotopic compositions.

From extracts of IER and the forest floor, NH_4^+ and NO_3^- were collected separately through semimicro Kjeldahl distillation with MgO and Devarda's alloy (Mulvaney 1993). Liberated NH_3 gas from an aliquot was trapped in diluted H_2SO_4 solution (0.025 N). Isotopic analysis was conducted on N_2 gas following combustion of the NH_4^+ absorbed onto cation-exchange resin (Garten 1992).

Nitrogen isotope compositions of plant tissues, soils, and NH_4^+ absorbed onto cation-exchange resin were measured using a Finnigan Mat Delta-S or 252 (Finnigan MAT, Bremen, Germany), followed by a manual cryopurification of combustion products in a vacuum system (Minagawa and others 1984) or coupled with an elemental analyzer (Carlo, Erba, Milan, Italy).

Results of ^{15}N natural abundance are expressed as

$$\delta^{15}N\ (‰) = (R_{sample}/R_{standard} - 1) \times 1000$$

(2)

where R = mass 29/mass 28 and the standard is the atmospheric N_2 ($\delta^{15}N = 0‰$). The precision based on multiple analysis of laboratory standards [KNO_3, $(NH_4)_2SO_4$, and DL-alanine] was better than $\pm$ 0.18‰ (as standard deviation for both inorganic and organic N).

Data were analyzed by STATISTICA (Statsoft Japan, Tokyo, Japan) for one-way analysis of variance (ANOVA) and t-test. The Mann–Whitney U-test, Kruskal–Wallis test, and Spearman's correlation coefficient were used for comparison of $\delta^{15}N$ data. Differences were considered significant at $P < 0.05$.

A model for this forest ecosystem (Figure 1) was constructed after Garten and Van Miegroet (1994) using STELLA® modeling software (High Performance Systems, Inc., Lyme, NH) with apparent isotopic fractionation factors [1.0024 and 1.0114 for mineralization (β) and nitrification (β_1), respectively] and $\delta^{15}N$ of soil N of 3.81‰ (the value for 0–5 cm depth at the Upper Site). These apparent fractionation factors were calculated with $\delta^{15}N$ data of 0–50 cm mineral soils (Koba and others 1998; Koba 2000). The isotopic fractionation factor for NH_4^+ immobilization (β_2) was set to 1.000 after Garten and Van Miegroet (1994), and f_1 and f_2 were the fractional contributions of NO_3^- uptake and NH_4^+ uptake to total N uptake ($f_1 + f_2$), respectively (Figure 1). The model was run for varying rates of nitrification (k_1) relative to NH_4^+ immobilization (k_2), with the constraint that $k_1 + k_2 = 1$, and for varying f_1 and f_2 to plant N (Garten and Van Miegroet 1994).

RESULTS

Plant ^{15}N natural abundances

The internal variations in $\delta^{15}N$ (expressed by $\delta^{15}N$ of current leaves $\delta^{15}N$ of each plant organ) were

Table 2. Internal Variation of $\delta^{15}N$ for Each Plant Species

		$\delta^{15}N$ Current Leaf $-\delta^{15}N$ Each Organ (‰)[a]					
Plant Species	N^b	Whole Plant[c]	Root	Current Shoot	Branch	Belowground Shoot	Old Leaf
Leucosceptrun stellipilum	4	0.47±0.27	1.54±0.55	0.12±0.23		0.14±0.53	
Hydrangea hirta	4	−0.35±0.21	−0.61±0.28	−0.27±0.11	0.26±0.11		
Lindera triloba	6	−0.27±0.27	−0.37±0.40	−0.04±0.08	−0.47±0.21		
Pieris japonica	6	0.43±0.23	0.25±0.31	0.53±0.3	0.91±0.19		0.43±0.21

[a]*The difference in $\delta^{15}N$ between current leaf and each organ or calculated whole plant body are expressed. Data are mean ± 1 SE.*
[b]*N = number of individuals.*
[c]*Whole-plant $\delta^{15}N$ was calculated by data of N content from unpublished data of L. Koyama.*

Table 3. Natural Abundance of ^{15}N of Different Plants

		Variation of $\delta^{15}N$ (‰) of Plants*				
Position	Plant Species	Jun 94	Jun 95	Jul 96	Oct 96	Aug 97
Upper Site	*Hydrangea hirta*	No data	0.36 ± 0.20 (4)[aX]	−1.38 (1)[aXY]	−0.11 ± 0.35 (3)[aX]	−0.85 ± 0.23 (10)[aX]
	Lindera triloba	No data	−0.38 ± 0.32 (11)[aX]	−1.08 ± 0.18 (20)[abX]	−1.71 ± 0.25 (15)[bX]	−1.48 ± 0.18(10)[abX]
	Pieris japonica	No data	−2.63 ± 0.31 (13)[aY]	−2.63 ± 0.29 (21)[aY]	−2.97 ± 0.22 (20)[aY]	−2.72 ± 0.24 (10)[aY]
	Cryptomeria japonica	−2.79 ± 0.28 (4)[a]	−2.48 ± 0.16 (3)[aXY]	No data	No data	No data
Lower Site	*Leucosceptrum stellipilum*	No data	−3.74 ± 0.29 (5)[bY]	−3.09 ± 0.14 (16)[abY]	−1.23 ± 0.21 (10)[aXY]	−1.94 ± 0.26(10)[abX]
	Hydrangea hirta	No data	0.23 ± 0.50 (9)[aX]	−0.47 ± 0.24 (21)[aX]	−0.63 ± 0.19 (14)[aX]	−1.16 ± 0.25 (10)[aX]
	Lindera triloba	No data	−1.26 ± 0.21 (8)[abXY]	−1.22 ± 0.14 (23)[aX]	−1.69 ± 0.20 (10)[abY]	−2.04 ± 0.15 (10)[bX]
	Pieris japonica	No data	0.18 ± 0.50 (5)[aX]	−2.21 ± 0.61 (6)[aXY]	−2.17 ± 0.58 (5)[aXY]	No data
	Cryptomeria japonica	−2.07 ± 0.30 (5)	No data	No data	No data	No data

*Data are mean ± SE. The number of samples is given in parentheses. Values within a species in each site with different lower-case letters are significantly different and values within a period with different upper-case letters are significantly different in each site (Kruskal–Wallis test, $P < 0.05$).

small in all understory species (less than 1‰), although there was a 1.5‰ difference between leaves and roots of *Leucosceptrum stellipilum* (Table 2). Variations in ^{15}N abundances among species were small, with average $\delta^{15}N$ values ranging from –2.97‰ to 0.36‰ at the Upper Site and from –3.74‰ to 0.23‰ at the Lower Site (Table 3). Seasonal $\delta^{15}N$ variations were found for *Lindera triloba* at both sites and for *Leucosceptrum stellipilum* at the Lower Site (Table 3). The largest difference of 2.5‰ was observed for *Leucosceptrum stellipilum* (between June 1995 and October 1996, Table 3).

At the Upper Site, *Pieris japonica* had the lowest $\delta^{15}N$ values over the entire sampling interval (Table 3), and the differences in average values between the most enriched and the most depleted in each period were small (2.99‰ at maximum in June 1995, Table 3). At the Lower Site, the ranking in $\delta^{15}N$ fluctuated over the sampling periods (Table 3) and the differences among species were less than 4‰ (3.97‰ at maximum in June 1995, Table 3).

^{15}N natural abundances of N sources

The $\delta^{15}N$ values of total N in the Oi layer [Table 4 for the data at the Upper Site and –2.31 ± 0.06‰ (n = 7) at the Lower Site] were similar to those for *Cryptomeria japonica* leaves at both sites (Table 3). At the Upper Site, the $\delta^{15}N$ value for the Oe + a layer was significantly higher than that for the Oi layer (Table 4).

Extractable NH_4^+ in the Oe + a layer had significantly lower $\delta^{15}N$ values than total N in the Oe + a layer, but they were not significantly different from the $\delta^{15}N$ values of total N in the Oi layer (Table 4). The $\delta^{15}N$ value of NO_3^- on IER of the forest floor was not significantly different from extractable NH_4^+ of the forest floor (Table 4).

Foliar NRA, NO_3^-, N content, and $\delta^{15}N$

All understory species had NRA at both the Upper Site and the Lower Site with the exception of *Pieris japonica* (Table 5), which was distributed almost exclusively at the Upper Site. *Hydrangea hirta* and *Lindera triloba* at the Lower Site had a significantly

Table 4. δ^{15}N of N Sources in Different Compartments for Soils at the Upper Site

N Forms	Sampling Point	δ^{15}N (‰)[a]
Total N	Oi layer	$-3.04\pm0.11(4)$
	Oe + a layer	$1.73\pm0.25(9)$
Exchangeable NH_4^+	Oe + a layer	$-3.55\pm1.52(3)$
NO_3^- absorbed onto IER	Beneath forest floor	$-2.66\pm0.94(6)$
	Beneath the surface mineral soil[b]	$-4.56\pm0.56(6)$

[a]Data are mean ± 1 SE and number of samples is given in parentheses.
[b]At 10-cm depth in mineral soil.

Table 5. NRA, NO_3^- Pool, and N Content of Plant Leaves

	NRA (μg N g^{-1} h^{-1})*		NO_3^- (mg N g^{-1})*		Leaf N (%)*	
Plant Species	Upper Site	Lower Site	Upper Site	Lower Site	Upper Site	Lower Site
Leucosceptrum stellipilum	No data	$30.5 \pm 3.4\ (10)^A$	No data	$3.13 \pm 0.24\ (10)^A$	No data	$3.55 \pm 0.05\ (10)^A$
Hydrangea hirta	$0.5 \pm 0.3\ (9)^{Ab}$	$0.9 \pm 0.1\ (9)^{Ba}$	$0.01 \pm 0.01\ (10)^{Aa}$	$0.02 \pm 0.01\ (10)^{Ba}$	$2.86 \pm 0.07\ (10)^{Ba}$	$2.73 \pm 0.10\ (10)^{Ba}$
Lindera triloba	$0.2 \pm 0.2\ (9)^{Bb}$	$1.1 \pm 0.1\ (10)^{Ba}$	$0.03 \pm 0.01\ (10)^{Ab}$	$0.29 \pm 0.07\ (10)^{Ba}$	$3.13 \pm 0.10\ (10)^{Ab}$	$3.89 \pm 0.15\ (10)^{Aa}$
Pieris japonica	Not detected	No data	$0.01 \pm 0.00\ (10)^A$	No data	$1.46 \pm 0.03\ (10)^c$	No data

*Data are mean ± 1 SE. Number of samples is given in parentheses. Values within a site with different upper-case letters are significantly different (ANOVA, P < 0.05). Values within a species with different lower-case letters are significantly different (t-test, P < 0.05).

higher NRA than at the Upper Site (Table 5). At the Upper Site, *Hydrangea hirta* had a higher NRA than *Lindera triloba*, and *Leucosceptrum stellipilum* had the highest NRA at the Lower Site (Table 5).

Plant leaves at the Upper Site had small NO_3^- pools, while *Lindera triloba* and *Leucosceptrum stellipilum* at the Lower Site had large NO_3^- pools (Table 5). *Leucosceptrum stellipilum* had the largest pool at the Lower Site, and *Lindera triloba* at the Lower Site had a larger pool than at the Upper Site (Table 5).

Pieris japonica had the lowest and *Lindera triloba* had the highest leaf N content at the Upper Site (Table 5). At the Lower Site, *Hydrangea hirta* had a lower N content than the other two species (Table 5). There was a significant difference in N content for *Lindera triloba* between the Upper Site and the Lower Site, whereas *Hydrangea hirta* had a similar N content at both sites (Table 5).

The relationships between foliar δ^{15}N and other measures of N status are shown in Figure 2. Significant correlation was found between δ^{15}N and foliar NRA for *Lindera triloba* at the Lower Site (Figure 2a). *Leucosceptrum stellipilum* showed a significant correlation between δ^{15}N and foliar NO_3^- (Figure 2b), whereas no correlation was found between δ^{15}N and leaf N content for any species (Figure 2c).

DISCUSSION

Isotopic composition of plants and N sources: simple comparison

In Figure 3 we summarize data on ^{15}N abundance of plants and soil N on the Ryuoh Mountain slope from 1994 to 1997 in a format similar to that of Garten (1993). Because internal variation in δ^{15}N among different plant parts was very small (Table 2), foliar δ^{15}N was used as a surrogate for whole-plant δ^{15}N. Although seasonal variation in δ^{15}N was found (Table 3), it was much smaller than the range of soil-N pools, and we used the pooled data of plant δ^{15}N in Figure 3 to show general trends in ^{15}N distribution in forest ecosystems. Details about the isotopic composition of total N and extractable inorganic N in mineral soils (at 0–50-cm depth) were presented elsewhere (Koba and others 1998). Briefly, δ^{15}N values of three types of N (total N, NH_4^+ and NO_3^-) in mineral soils generally increased with soil depth, and their order in δ^{15}N in soil was determined by net nitrification rates (Koba and others 1998). At the Lower Site, where net nitrification is high (Table 1), the order was NH_4^+ < total N < NO_3^-, while at the Upper Site, where net nitrification rates were quite low, the order was total N ≤ NH_4^+ < NO_3^- (Table 1). Significant differences were found between the two sites (Figure
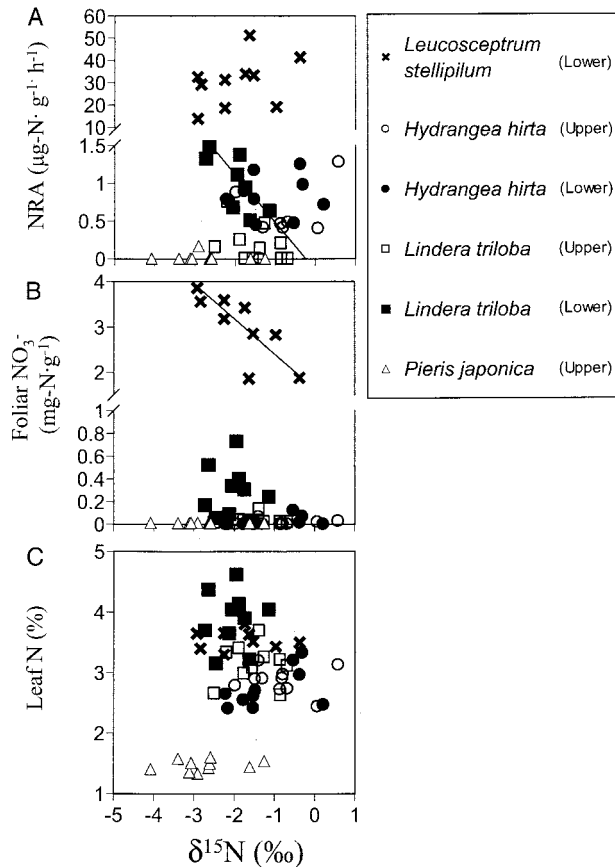
Figure 2. Relationships between $\delta^{15}N$ and other foliar N status. **A** $\delta^{15}N$ and foliar NRA, **B** $\delta^{15}N$ and foliar NO_3^-, **C** $\delta^{15}N$ and foliar N content. Foliar samples were collected in August 1997. Significant correlations were found between $\delta^{15}N$ and foliar NRA for *Lindera triloba* at the Lower Site ($P < 0.05$) and between $\delta^{15}N$ and foliar NO_3^- for *Leucosceptrum stellipilum* ($P < 0.001$).

3) for *Pieris japonica* ($P < 0.005$), total N in the Oi layer ($P < 0.01$), total N in mineral soils ($P < 0.05$), and extractable NH_4^+ in mineral soils ($P < 0.05$). It is noteworthy that only *Pieris japonica* had significantly different $\delta^{15}N$ between these two sites where soil N dynamics are quite different (Table 1).

Plant $\delta^{15}N$ values were lower than those of mineral soils at both sites (Figure 3). This pattern is consistent with many other studies (Gebauer and Schulze 1991; Garten 1993; Garten and Miegroet 1994; Michelsen and others 1996, 1998; Nadelhoffer and others 1996, 1999; Miller and Bowman 2002). However, the range of plant $\delta^{15}N$ values was as small (5.7‰ at the Upper Site and 6.6‰ at the Lower Site) as that observed in a North American temperate forest (Nadelhoffer and others 1999). This result is in contrast to large differences in foliar $\delta^{15}N$ reported for strongly N-limited ecosystems

(Schulze and others 1994; Michelsen and others 1996, 1998; Nadelhoffer and others 1996).

The isotopic similarity between plants and soil NO_3^- at the Lower Site signals the potentially great reliance of plants upon NO_3^- (Figure 3). This interpretation is corroborated by higher net nitrification in soils (Table 1), higher foliar NRA, and a larger foliar NO_3^- pool (Table 5) at the Lower Site than at the Upper Site. The relationship between $\delta^{15}N$ and foliar NRA or NO_3^- observed for some species (Figures 2a and b) strongly supports the idea that greater reliance on NO_3^- determined plant $\delta^{15}N$. The same inverse relationship between $\delta^{15}N$ and NRA that we found for *Lindera triloba* at the Lower Site (Figure 2a) was found by Miller and Bowman (2002) for some alpine species, suggesting that these alpine plants may rely on NO_3^- with low $\delta^{15}N$ for their N nutrition to a greater degree than NH_4^+ and organic N.

In addition, the only large differences in $\delta^{15}N$ among tissues occurred in *Leucosceptrum stellipilum*, which is believed to rely heavily on NO_3^- (Table 5). In this species, root $\delta^{15}N$ was 1.07‰ less than whole-plant $\delta^{15}N$ and was 1.54‰ less than leaf $\delta^{15}N$ (Table 2). Evans and others (1996) reported that plant roots had lower $\delta^{15}N$ than plant leaves when the N source was NO_3^- only and suggested that this difference resulted from partial reduction of NO_3^- in roots, thereby slightly enriching NO_3^- that is transported to and subsequently reduced in leaves. Yoneyama and Kaneko (1989) reported a similar pattern between leaves and roots when NO_3^- was the sole N source. This difference with regard to *Leucosceptrum stellipilum* also suggests that NO_3^- is an important N source for this species.

Although NO_3^- uptake can explain the isotopic similarity between plant and soil NO_3^- for most of the species studied, it is impossible to apply that explanation to *Pieris japonica*, which is an ERM species and has almost no ability to use NO_3^- (Table 5). This family is known to not use NO_3^- (Haynes and Goh 1978) but to rely more on complex organic N through ERM. Previous studies showed that plants with ERM have low $\delta^{15}N$ values (Schulze and others 1994; Michelsen and others 1996, 1998; Nadelhoffer and others 1996; Hobbie and others 2000), although these plants apparently cannot use NO_3^-, which is considered to be the most ^{15}N-depleted among N sources. Schulze and others (1994) and Nadelhoffer and others (1996) suggested that the shallow root system of ERM plants is responsible for the low $\delta^{15}N$ values, while Michelsen and others (1996) suggested that this is caused by their great reliance upon organic N in fresh litter (which closely reflects the low $\delta^{15}N$ values of foliage). $\delta^{15}N$

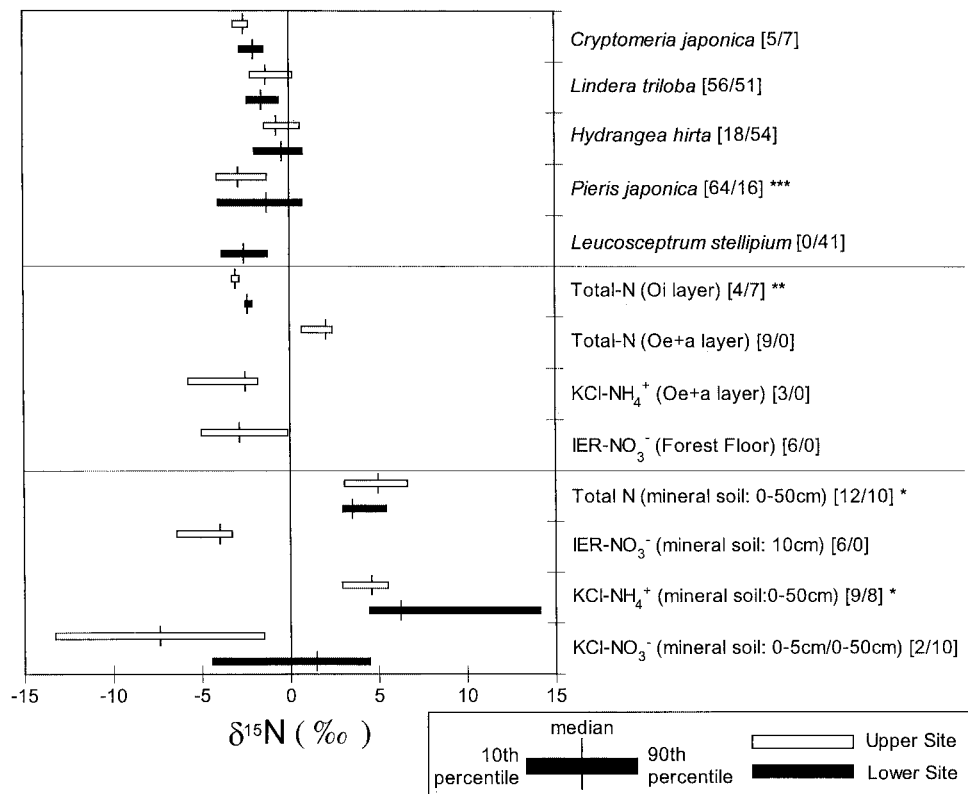**Figure 3.** Median values with 10th and 90th percentiles for measurements of natural ^{15}N abundance in plants, total soil N (Total N), dissolved inorganic N in soil solution captured by IER, and exchangeable inorganic N (KCl) in Ryuoh Mt., Japan. White bars show the data for the Upper Site and dark bars are for the Lower Site. Data on total soil N and exchangeable inorganic N in mineral soils are from Koba and others (1998). The variability in data for KCl–NO_3^- at the Upper Site is caused by only two data points because of the small pool size of NO_3^- (Koba and others 1998). The two numbers in brackets are the number of samples at the Upper Site and at the Lower Site, respectively. Significant difference between the two sites are *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.005$.

of the forest floor does not fully explain the low $\delta^{15}N$ of *Pieris japonica* at the Lower Site in this forest ecosystem (Figure 3), and more work is needed on ERM plants having low $\delta^{15}N$ in many ecosystems.

On the other hand, there are many N sources with similar $\delta^{15}N$ in soils at the Upper Site (Figure 3). With wide and overlapped spectra of isotopic signatures, simple comparisons of plant and soil $\delta^{15}N$ data could not provide any extensive elucidation for N sources of plants.

Interpretation of $\delta^{15}N$ by the model

The model assumes that plant $\delta^{15}N$ values are a function of (1) varying plant uptake of soil NO_3^- and NH_4^+, (2) the isotopic composition of soil N pools, and (3) relative rates of soil N transformations (Garten and Van Miegroet 1994). This model also suggests that there are several possible cases that can contribute to changing plant $\delta^{15}N$ values as a function of relative importance of nitrification (see Figure 6 in Garten and Van Miegroet 1994). To test this theory, the model in Ryuoh (Figure 1) after Garten and Van Miegroet (1994) was applied to the data shown in Table 6 where $\delta^{15}N$ data at the Lower Site was standardized by the addition of 1.05‰ (the

difference of soil N at 0–5 cm depth between the two sites) in order to compare the data from the two sites in the same context.

Simulated $\delta^{15}N$ of soil NH_4^+, NO_3^-, and plant N is presented in Figure 4a as a function of the ratio $k_2/(k_1 + k_2)$, that is, the ratio of immobilization to (immobilization + nitrification). Consistent with expectations based on prior simulations (Figure 3 in Shearer and others 1974; Figure 6 in Garten and Van Miegroet 1994), simulation results showed that soil inorganic N and plant N with constant f_1 become enriched in ^{15}N as the ratio $k_2/(k_1 + k_2)$ decreases, that is, NH_4^+ immobilization becomes less important (Figure 4a). $\delta^{15}N$ measured at both sites (Table 6) is plotted in Figure 4b. Note that the ratio decreases from the Upper Site (0.99) to the Lower Site (0.91) but the difference is quite small (0.08). Standardized $\delta^{15}N$ of soil inorganic N (Table 6) was more positive at the Lower Site than at the Upper Site, which is consistent with simulated results (Figure 4b), although the difference in $\delta^{15}N$ of soil inorganic N between the two sites was larger than simulated. Furthermore, standardized plant $\delta^{15}N$ values (Table 6) at the Lower Site were more positive where the relative importance of nitrifica-

Table 6. Data Used for the Model[a]

	$\delta^{15}N$ (‰)		
	Upper Site	Lower Site[b]	(Raw Data)
Soil total N (mineral soil: 0–5 cm)	3.86	3.86	(2.81)
Soil NH_4^+ (mineral soil: 0–5 cm)	2.86	10.09	(9.04)
Soil NO_3^- (mineral soil: 0–5 cm)	−7.39	−4.99	(−6.04)
Soil NH_4^+ (mineral soil: 0–50 cm)	4.86	9.27	(8.22)
C. japonica[c]	−2.66	−1.02	(−2.07)
L. triloba[c]	−1.18	−0.43	(−1.48)
H. hirta[c]	−0.49	0.53	(−0.52)
P. japonica[c]	−2.75	−0.40	(−1.45)
L. stellipilum[c]	No data	−1.38	(−2.43)

Ratio		
	Upper Site	Lower Site
$k_2/(k_1 + k_2)$[d]	0.99	0.91

[a]*For soil N only $\delta^{15}N$ data collected from both sites were selected for use (from Koba and others 1998).*
[b]*The data at the Lower Site were adjusted by the addition of 1.05‰ to compare $\delta^{15}N$ data in the single model output. The raw data for the Lower Site were shown in parenthesis.*
[c]*Averaged value for three years.*
[d]*Calculated by the data from Tokuchi and others (2000).*

tion is greater, which is consistent with simulation output when f_1 is constant between the two sites (Figure 4b). We conclude that the model is partially validated and that $\delta^{15}N$ of soil N and plants can be interpreted successfully in terms of relative importance of nitrification (compared with NH_4^+ immobilization). Moreover, Figure 4b shows that the reason that plant $\delta^{15}N$ did not differ between the two sites with clearly different N cycling patterns (Table 1) can be attributed to the relatively small difference in the ratio of $k_2/(k_1 + k_2)$ at these sites (Table 6). With a difference in this ratio of 0.08, one would expect to see a difference in plant $\delta^{15}N$ values on the order of only 0.9‰ between the Upper and Lower Sites from the model (Figure 4b).

Plant $\delta^{15}N$ data plotted among various curves for f_1 in Figure 4b further imply that plants' reliance on NO_3^- is relatively constant (0.2–0.4) between the two sites. However, it is almost impossible for this model in its present form to estimate their reliance quantitatively because isotopic fractionations during assimilation of soil N (for example, see Högberg and others 1999; Emmerton and others 2001a, 2001b; Kohzu and others 2000; Yoneyama and others 2001 but see Mariotti and others 1980; Shearer and Kohl 1989; Evans and others 1996) can alter plant $\delta^{15}N$. The magnitude of this effect cannot be estimated quantitatively for different plant species with different physiological traits, such as mycor-

rhizal infections, or preference of N forms (see Handley and Scrimgeour 1997; Evans 2001; Robinson 2001). For example, *Pieris japonica*, whose ability to use NO_3^- proved to be negligible (Table 5), had a $\delta^{15}N$ signature suggesting that this species relies on NO_3^- to some extent (Figure 4b; approximately 0.4 and 0.3 for f_1 at the Upper Site and the Lower Site, respectively). Instead, isotopic fractionations during N uptake via ERM (Emmerton and others 2001a, 2001b) may be responsible for its low $\delta^{15}N$. The lack of information about isotopic fractionation during plant N uptake should be overcome in order to apply the model to estimate f_1 quantitatively in the future.

In addition to isotopic fractionations during N uptake by plants, the model should include organic N. Our model simulated $\delta^{15}N$ of N_i (immobilized N; Figure 1), which was always very close to simulated $\delta^{15}N$ of NH_4^+ (data not shown) because isotopic fractionation during immobilization was set to 1.000, showing the same trend as NH_4^+ and NO_3^-: as $k_2/(k_1 + k_2)$ increases the $\delta^{15}N$ value decreases. This N_i pool can be considered one of the organic N pools in soils available for plants because microbial cells turn over rapidly and release immobilized N back into the soil (Hodge and others 2000). Thus, even considering uptake of organic N, we can interpret plant $\delta^{15}N$ in the same manner by using the model if N_i can be considered a good proxy of
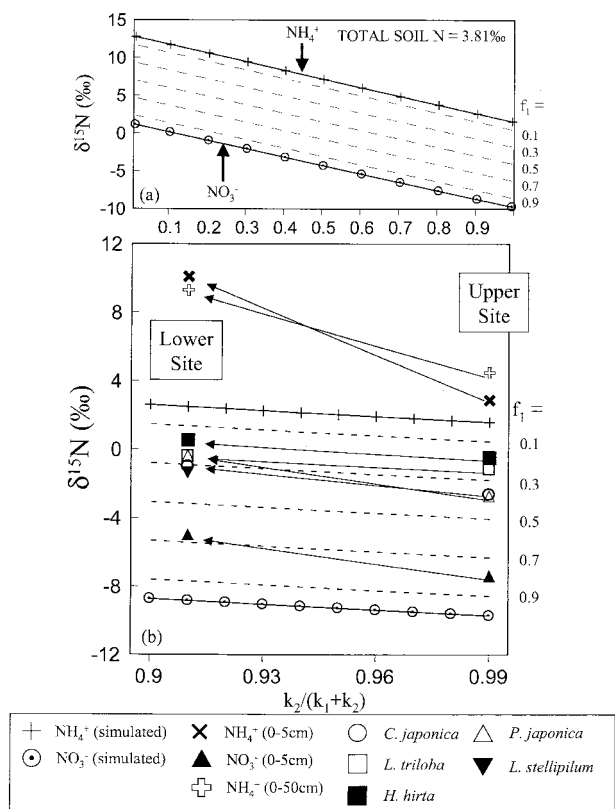
Figure 4. Predicted δ^{15}N values for (**a**) inorganic soil N pools and (**b**) foliar N as a function of the changing rate of N immobilization (k_2) to nitrification (k_1) in soil after Garten and Van Miegroet (1994). The parallel lines represent different outcomes when the fractionational uptake of soil NO_3^- by plant (f_1) is varied between 0.1 and 0.9.

preted as an example of how tightly N cycles in soil or of N openness (Austin and Vitousek 1998) because this ratio suggests how readily NH_4^+ is converted into NO_3^-, which has a higher capacity to be lost from an ecosystem than NH_4^+. Comparisons of δ^{15}N of different ecosystems have already been conducted in the context of water availability (Handley and others 1999) and N excess (Martinelli and others 1999). These comparisons include the analysis of δ^{15}N data with the model in such works that allow one to characterize N dynamics in a certain ecosystem more successfully with δ^{15}N by taking advantage of integration over seasonal and spatial variations in soil N transformations, minimal site disturbance, ease of sample collection, and potential for measurement on archived foliage samples (Garten and Van Miegroet 1994).

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available organic N. However, more information about the production, availability, and isotopic signature of organic N, as well as isotopic fractionation factors during the processes regarding organic N, is required to successfully include organic N in the model.

Although δ^{15}N signatures are not a simple tracer of N source, the model tested here provides a basis for interpreting δ^{15}N with respect to ecosystem N dynamics. This model, via relative importance of immobilization [$k_2/(k_1 + k_2)$] to soil N cycle and of NO_3^- to plant N demand (f_1), permits one to understand the N status in a soil–plant system established by multiple N transformation processes. Without elaborate experimental designs (for example, see Nordin and others 2001), it must be difficult to determine f_1 quantitatively, but NRA and foliar NO_3^- measurements can be effective in providing field evidence of NO_3^- uptake. The ratio $k_2/(k_1 + k_2)$, highlighted in the model, can also be inter-

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