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Natural abundance ^{15}N in soil and litter across a nitrate-output gradient in New Hampshire

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Abstract

Stable isotopes of nitrogen are potentially a valuable tool for regional assessments of nitrogen saturation because they provide an integrated measure of the past nitrogen cycling history of a site. We measured $\delta^{15}N$ of soil and litter, as well as net nitrification potential, at three sites across a nitrate-loss gradient in the White Mountains, New Hampshire to test the hypotheses: (1) that $\delta^{15}N$ in soil and litter increase across a spatial gradient of nitrate loss; and (2) that $\delta^{15}N$ in soil and litter is elevated when nitrification is elevated. $\delta^{15}N$ was found not to vary significantly among the three sites. Patterns of leaf litter and forest floor $\delta^{15}N$, however, were strongly influenced by species composition in individual plots. Beech litter had significantly higher $\delta^{15}N$ than yellow birch, sugar maple, and red maple. The conifer-dominated plots had significantly lower $\delta^{15}N$ in both the organic soil horizons and in litter than did the hardwood-dominated plots. When we adjusted for spatial heterogeneity in mineral soil $\delta^{15}N$ values by using an enrichment factor, $\delta^{15}N_{foliar} - \delta^{15}N_{Bs}$, in place of absolute soil $\delta^{15}N$ values, a positive relationship was found with net nitrification for hardwoods. $\delta^{15}N$ may also be a useful tool for evaluating species differences in nitrogen cycling and nitrogen uptake. The distinct pattern we observed of decreasing $\delta^{15}N$ across the continuum from hardwood-dominated to conifer-dominated sites may suggest that local drivers (for example, nitrification rate) regulate the absolute value of foliar $\delta^{15}N$, while species-driven factors (e.g., timing and type of uptake) control the foliar $\delta^{15}N$ value of one species relative to another in the same plot.

Keywords: Nitrogen cycling; N saturation; Species patterns

1. Introduction

Stable isotopes of nitrogen can be used to evaluate patterns of nitrogen cycling in forest ecosystems (Gebauer and Schulze, 1991; Nadelhoffer and Fry, 1994; Emmett et al., 1998; Austin and Vitousek, 1998; Hobbie et al., 1999). In several studies, natural abundance ^{15}N of soil and foliage has been used to identify areas with higher rates of nitrification (Garten, 1993; Garten and Van Miegroet, 1994; Emmett et al., 1998). Elevated rates of nitrification may occur in an early stage of nitrogen saturation, the condition that occurs when available nitrogen within an ecosystem exceeds plant and microbial demand (Aber et al., 1989). As an ecosystem nears nitrogen saturation, nitrate loss in surface water increases and may lead to harmful ecological effects. These may include: (1) soil and surface water acidification (Reuss and Johnson, 1986); (2) deterioration of both water quality in groundwater used for drinking (Skeffington and Wilson, 1988; Hauhs et al., 1989) and of coastal waters via eutrophication (Nilsson and Grennfelt, 1988; Fisher et al., 1988); and (3) plant nutrient imbalances that have been implicated in forest decline (Friedland et al., 1984; Waring, 1987; Schulze, 1989; Aber et al., 1995).

In contrast to co-ordinated regional studies such as the NITREX study in Europe (Wright and Van Breemen, 1995; Wright and Rasmussen, 1998), experimental research on nitrogen saturation in the U.S. has not generated a regional-scale evaluation of ecosystem susceptibility to nitrogen saturation (Aber et al., 1998). In particular, some complex questions remain unanswered, such as what is the time course to nitrogen saturation for forested catchments receiving the

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moderate levels of nitrogen deposition in the northeastern U.S. (in contrast to higher levels of nitrogen deposition observed in Europe). Most evaluations of the nitrogen cycling and nitrogen status of an ecosystem rely on long-term monitoring of streamwater and precipitation chemistry to develop input-output budgets of nitrogen for the ecosystem. This approach provides much useful information about individual sites, including the seasonal and annual variability of nitrogen fluxes. However, the intensity and long-term nature of such measurements limits their use for large-scale regional assessments of nitrogen cycling or nitrogen saturation.

Stable nitrogen isotope ratios, in contrast, are a potentially valuable property for regional assessments of nitrogen saturation. Stable isotopes have the potential advantages both of being feasible for investigating large areas and of providing, not simply instantaneous information, but rather, an integrated measure of the nitrogen cycling history of a site with a single sampling (Robinson, 2001). When used to record the effect of large disturbances in the nitrogen cycle, stable isotope measurements showed a clear relationship between elevated nitrification and nitrate loss induced by clear-cutting and increases in litter and organic soil horizon δ^{15}N (Pardo et al., 2002). Increases in foliar δ^{15}N were observed after N saturation was induced via N additions to a red spruce stand in Vermont (Pardo et al., 1998; McNulty et al., 2005). Näsholm et al. (1997) observed elevated foliar δ^{15}N in spruce stands in southern Sweden with elevated nitrate leaching. Another study (Pardo et al., 2001), also suggests that smaller disruptions of the nitrogen cycle (e.g. a period of elevated nitrification and nitrate loss) caused detectable increases in δ^{15}N values in organic soil horizons. These studies suggest that δ^{15}N could be used to compare sites with respect to nitrogen saturation. This approach was successfully applied in a European study where measurements of plant and soil δ^{15}N increased along a nitrogen deposition gradient (Emmett et al., 1998).

In this study, we measured net nitrification and δ^{15}N of soil and litter from three sites across a nitrate-loss gradient to test the hypotheses: (1) that δ^{15}N in soil and litter increases across a spatial gradient of nitrate loss; and (2) that δ^{15}N in soil and litter is elevated when nitrification is elevated.

1.1. Background on stable isotopes in forest ecosystems

Isotope data are reported as δ^{15}N values, which represent the per mil (‰) difference between the isotopic composition of the sample and that of atmospheric dinitrogen:

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

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

Stable isotopes of nitrogen are useful in ecological research because natural abundance of ^{15}N records the net effect of nitrogen transformations on the soil and plants (Handley and Raven, 1992; Högberg, 1997). Microbes discriminate against the heavier ^{15}N during microbial transformations of nitrogen, creating products that are depleted in ^{15}N (lower ^{15}N:^{14}N ratio) and leaving the source pool enriched in ^{15}N (higher ^{15}N:^{14}N ratio; Mariotti et al., 1981; Shearer and Kohl, 1986). Nitrogen mineralization produces ^{15}N-depleted ammonium and causes a direct enrichment of the ^{15}N in soil organic nitrogen (Létolle, 1980). Nitrification produces ^{15}N-depleted nitrate and ^{15}N-enriched ammonium (Handley and Raven, 1992). When ^{15}N-enriched ammonium is retained in the soil and ^{15}N-depleted nitrate is leached from the ecosystem, the net effect of nitrification is to enrich the soil in ^{15}N (Shearer and Kohl, 1986; Nadelhoffer and Fry, 1994; Högberg, 1997).

The δ^{15}N of plants is a function of the δ^{15}N of the source nitrogen (Handley and Raven, 1992). Plants generally have a lower δ^{15}N value than soils (Fry, 1991; Nadelhoffer and Fry, 1994; Högberg, 1997), because they assimilate inorganic nitrogen which, as a result of microbial transformations, is depleted in ^{15}N relative to soil (Ledgard et al., 1984; Nadelhoffer and Fry, 1988) and they typically reflect the δ^{15}N of the inorganic N they take up (Nadelhoffer and Fry, 1994). If the inorganic source of nitrogen for plants became enriched in ^{15}N, the plants would also become enriched in ^{15}N (Emmett et al., 1998; Pardo et al., 2002).

Thus, if ecosystem nitrification and consequent nitrate loss rates are high, soil and, therefore, plants should become enriched in δ^{15}N (Handley and Raven, 1992; Nadelhoffer and Fry, 1994). In contrast, in an ecosystem where nitrate loss is low, and nitrogen is cycled very tightly, there is less opportunity for fractionation to be expressed in the product pool. In the limit, when all of a source pool of nitrogen (e.g., for nitrification, the source pool is NH_4^+ and the product pool is NO_3^-) is transformed by microbes, mass balance requires that the ratio of ^{15}N to ^{14}N will not change (Mariotti et al., 1981). Thus, in an ecosystem with a tight nitrogen cycle and low nitrate losses, one expects that soil and plant δ^{15}N should not become enriched. Among ecosystems with varying nitrogen cycling regimes and nitrate loss patterns, the δ^{15}N of soil and plant material would be expected to be lower at the sites with low nitrate losses and higher at sites with higher nitrate losses. Thus, we expected that δ^{15}N in soil and litter would increase across a gradient of increasing streamwater nitrate loss at our study sites.

2. Materials and methods

2.1. Description of study sites

The study described here was conducted on three watersheds in the White Mountains of New Hampshire across a gradient of increasing nitrate output: Cone Pond Watershed (CPW); the Hubbard Brook Experimental Forest Watershed 3 (HBEF W3); and the Bowl Research Natural Area (Bowl; Fig. 1). These sites span the range of nitrate concentration observed in streamwater from 96 streams in northern New England (Hornbeck et al., 1997). Research has been conducted at CPW since 1980 (continuously since 1989), at HBEF W3 continuously since 1963, and at the Bowl for 18 months during 1973–1974 and continuously since 1994. The sites are similar in soils, hardwood species present, nitrogen deposition, and climate

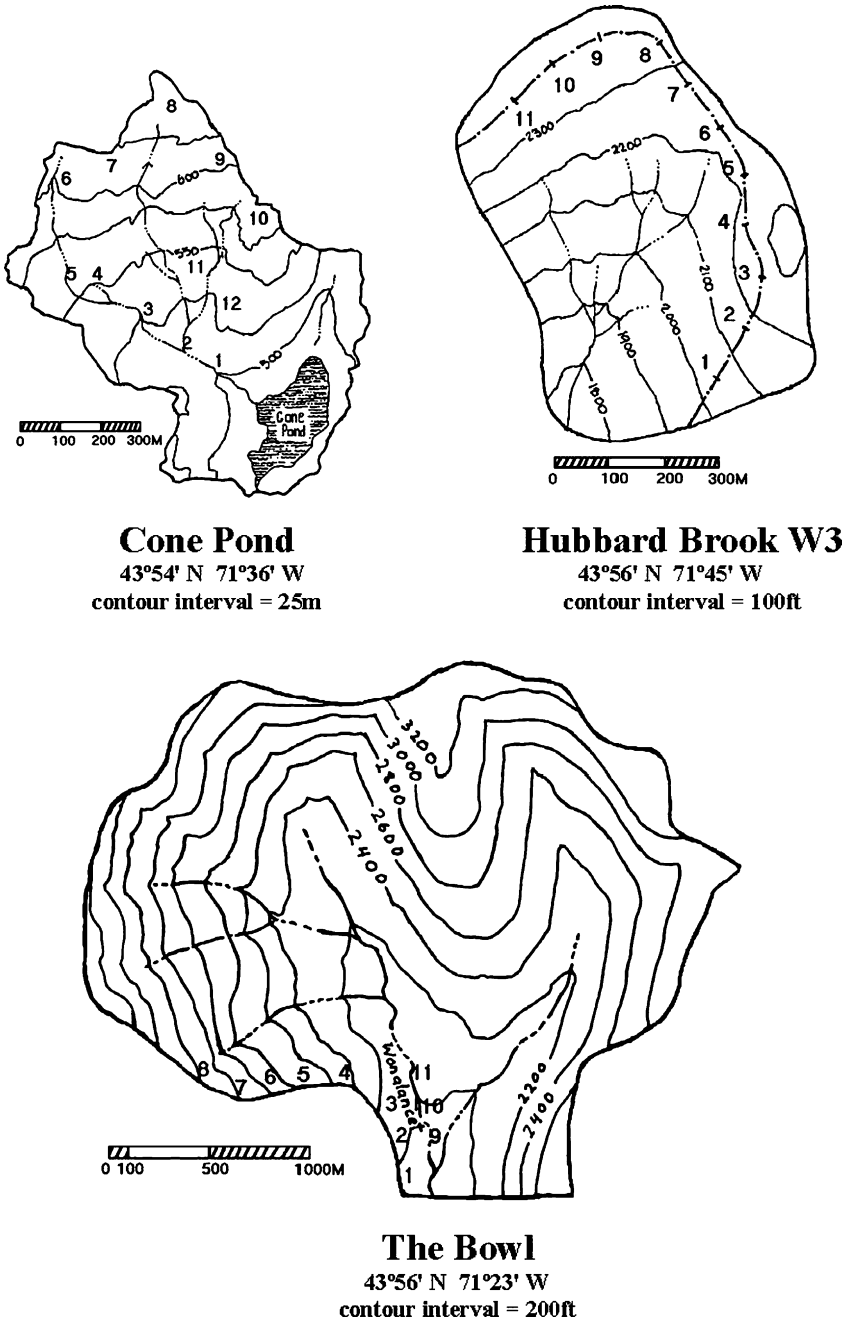

Fig. 1. Sampling locations in Cone Pond Watershed, Watershed 3 at the Hubbard Brook Experimental Forest, and the Bowl Research Natural Area, New Hampshire. Soil pit locations are marked.

(Table 1). They differ in streamwater nitrate loss, species composition, size, slope, and land-use history (Table 1). The Bowl is a much larger watershed which is dominated by hardwoods in the lower, flat portion of the watershed, and by conifers in the steeper upper elevations, above 900 m (Martin and Bailey, 1999). The CPW is dominated by conifers with patches of hardwoods. The HBEF W3 is dominated by hardwoods with patches of conifers stands at upper elevations. The CPW and the Bowl have been subject to natural disturbances only and are considered old-growth forests; the HBEF W3 has been logged (most recently about 1910–1917; Likens et al., 1977). The sites are described in detail elsewhere (Bailey et al., 1995; Likens and Bormann, 1995; Martin, 1979).

2.2. Soil sample collection

Soil samples were collected in July and August 1995 using a 22 cm × 22 cm template for the Oie and Oa horizon, and a 4 cm slide-hammer soil corer for the upper mineral horizons, followed by a soil auger to sample the deeper mineral soil. At HBEF W3, sampling locations were situated at 100 m intervals along the W3 trail. At the Bowl, eight sampling locations were situated at 200 m intervals along the Wiggins trail and three sampling locations were situated at 200 m intervals along the West Branch stream. At both sites, soil pits were randomly located between 20 and 120 m from the trail (Fig. 1). At the HBEF W3 and the Bowl, 11 soil pits were sampled. At CPW,

Table 1
Characteristics of study sites

Characteristic	Cone Pond Watershed[a]	Hubbard Brook Experimental Forest W3[b]	Bowl Research Natural Area[c]
Location	43°54′N, 71°36′W	43°56′N, 71°45′W	43°56′N, 71°23′W
Size	33 ha	43 ha	206 ha
Soil type	Coarse-loamy mixed frigid Typic, Lithic and Aquic Haplorthods, some Histosols, little clay, sandy loam texture	Coarse-loamy, mixed, frigid Aquic, Lithic, and Typic Haplorthods little clay, sandy loam texture	Coarse-loamy, mixed, frigid Aquic, Lithic, and Typic Haplorthods, little clay, sandy loam texture
Bedrock	Interbedded quartzite and mica schist[d]	Schists, quartzites and granulites	Syenite and granite
Forest type	Mixed conifer with some hardwoods	Northern hardwood	Mixed conifer and hardwood
Species composition	*Picea rubens* 35%[e] *Abies balsamea* 12% *Tsuga canadensis* 16% *Acer saccharum* 7% *Fagus grandifolia* 8% *Betula alleghaniensis* 11%	*Acer saccharum* 31%[f] *Fagus grandifolia* 36% *Betula alleghaniensis* 18%	*Acer saccharum* 15%[g] *Betula* 33% *Fagus grandifolia* 17% *Picea rubens* 14% *Abies balsamea* 8%
Land-use history	Severe forest fire in 1820[f]	Last logged 1910–1917	Wind disturbance only
Stand age	Old growth some trees up to 260 years[h]	~80 years	Old growth 35–200 years
Mean annual nitrogen deposition	490 mol ha^{-1} y^{-1}[i]	492 mol ha^{-1} y^{-1}[j]	~492 mol ha^{-1} y^{-1}[k]
Streamwater nitrogen outflux	14 mol ha^{-1} y^{-1}[i]	201 mol ha^{-1} y^{-1}[j]	285 mol ha^{-1} y^{-1}[l]
Streamwater nitrate concentration	0 μmol L^{-1}[i]	21 μmol L^{-1}[j]	28 μmol L^{-1}[l]

[a] Data for Cone Pond Watershed are from Bailey et al. (1995), except where otherwise noted.
[b] Data for Hubbard Brook Experimental Forest are from Likens and Bormann (1995), except where otherwise noted.
[c] Data for the Bowl Research Natural Area are from Martin (1979), except where otherwise noted.
[d] Hornbeck et al. (1997).
[e] J. Hornbeck (personal communication).
[f] T. Siccama (personal communication).
[g] Martin and Bailey (1999).
[h] Buso et al. (1984)
[i] Data from Hornbeck et al. (1997) means for the period October 1991–September 1994.
[j] Data from G.E. Likens (www.hubbardbrook.org) for the period 1965–1992.
[k] Deposition was assumed to be similar to that at Hubbard Brook based on previous comparisons (Martin, 1979).
[l] Data from Martin et al. (2000) and Martin (1979), averaged for the two periods: May 1973–October 1974 and June 1994–October 1995.

samples were collected near twelve previously-established, randomly located soil pits (Hyman et al., 1998). Oie and Oa horizons were separated in the field; mineral horizons were separated in the laboratory. Samples were air dried immediately upon returning to the laboratory. Oie horizons were sieved through a 6 mm mesh, all other horizons were sieved through a 1 mm mesh (rather than 2 mm), which was necessary to remove the fine gravel at the Bowl, where gravel content was high.

2.3. Litter collection and preparation

Newly fallen litter samples were collected from CPW and the HBEF W3 in one sampling in October 1995, and from all three sites in September and October 1996; in 1996, there were two collections at each site in order to span the periods that litter of different species falls. Samples were collected at the same 11–12 sites where soils were sampled (Fig. 1). Fresh litter was collected over a 24 h rain-free period on three nylon mesh litter traps with a total area of 1.5 m^2 at each pit. The traps were staked into place. Leaves were sorted by species and oven dried at 65 °C for 48 h. In 1996, samples from the two collection dates were combined after sorting by species.

For each watershed, in order to calculate mean litter $\delta^{15}N$ by plot (over all species), a mass-weighted mean was calculated based on the mass collected by species at each site.

2.4. Sample preparation and $\delta^{15}N$ analysis

Dried soil and litter samples were pulverized in a shatterbox (SPEX Chemical and Sample Prep, model 8500, Metuchen, NJ), oven dried at 65 °C and loaded into tin capsules for isotope analysis. Isotopic analyses were performed using a Dumas combustion system in continuous flow mode (Carlo Erba) followed by a VG Prism mass spectrometer (Laboratory 1: Harvard University) or using a Finnigan Delta-S mass spectrometer (Laboratory 2: Boston University Stable Isotope Laboratory).

Samples were analyzed on two different instruments, therefore, it was necessary to make extensive comparisons in order to ensure that the measurements were equivalent. To verify the precision of the isotopic analysis, 10% of the samples were analyzed in triplicate. The standard deviation of the replicates was 0.138‰ at Laboratory 1, and 0.11‰ at Laboratory 2. The precision of the analysis for peptone standards in the nitrogen mass range of most of the samples was approximately ±0.15‰ (S.D.) at Laboratory 1 and ±0.21‰ at

Laboratory 2. Apple leaves from the National Institute of Standards and Technology (Standard Reference Material #1515) were run at Laboratory 2 only, and had a standard deviation of 0.20‰ and a mean of 0.35‰.

To verify the accuracy of the two laboratories, we compared the means of peptones and of 25 samples that were run in both laboratories. The difference between Laboratory 1 and Laboratory 2 (Laboratory 1 value minus Laboratory 2 value) for the peptones was −0.12‰ and for the 25 test samples was −0.01‰. These differences were considered negligible since they were not greater than the precision of analysis.

2.5. Nitrification sample collection and analysis

For measurement of net nitrification, soil samples were collected from three conifer and three hardwood stands at each of the 3 watersheds from the Oe + Oa horizon and at 10–20 cm (from the surface of the mineral soil) in November 1996 using a slide-hammer soil corer (4 cm in diameter × 10 cm long). We sampled the 10–20 cm depth because that enabled us to have a more similar horizon (generally the Bs horizon) at each site; thus we were able to better examine differences between the watershed rather than differences caused by small scale spatial heterogeneity in the depth of the A, E or Bh horizons. In order to make the samples more representative, each sample was composited from three sub-samples which were collected from randomly selected locations near the sampling site. For the Oea samples, 2–3 cores were taken side-by-side at each of the three sub-sites to ensure enough soil mass for analysis. At CPW, the conifer sites were adjacent to soil pits 7, 10, and 12, and hardwood sites were adjacent to soil pits 2, 4, and 11. Samples at the HBEF were collected from the Bear Brook Watershed (BBW) which is adjacent to the reference watershed 6, and is assumed to be indistinguishable from the other undisturbed watersheds at the HBEF. The soil samples from conifer sites at the HBEF were collected from a single, 1 ha spruce-fir stand along the ridge top of this hardwood-dominated watershed. The hardwood soil samples at the HBEF were from three sites along a low-elevation transect used for long-term litter collection (Hughes and Fahey, 1994; www.hubbardbrook.org). At the Bowl, conifer soil samples were collected adjacent to soil pits 5, 6, and 7; hardwood soil samples were collected adjacent to soil pit 9, and from two sites near soil pit 10.

Sub-samples were pooled in the field and stored at 4 °C until the following day when they were sieved at 6 mm for the Oea horizon and 2 mm for the 10–20 cm mineral soil. One set of samples was extracted immediately. A second set of samples was incubated aerobically at constant field moisture content for 28 days in a dark incubator at 25 °C, and then extracted as described below. Net nitrification extractions were performed by adding 10 g of wet soil to 100 mL of 2N KCl, mechanically shaking for 1 h and allowed to settle overnight at 4 °C. Samples were extracted using glass fiber filters (Whatman #42) under suction. Wet/dry soil mass ratio was measured for each sample by drying a 10 g sub-sample of wet soil at 65 °C for 48 h. Extracted solution was frozen until analysis. Extracted solution samples were analyzed for ammonium (Technicon Instruments Co., 1973) and nitrate (Willis, 1980) on a Technicon Auto-Analyzer at the USDA Forest Service Laboratory (Durham, NH).

In 1997, soil samples were collected from hardwood and conifer sites at Cone Pond and the Bowl and only from hardwood sites at HBEF west of W6. Samples were collected three times in 1997, in May, July, and October, using a pin block method. In the pin block method, long (13.2 cm) nails are driven through holes along the edge of a 15 cm × 15 cm square frame that has been placed on the forest floor to enclose a "box" of soil for sampling. Oe and Oa were collected as a single horizon, and mineral soil was discarded. The same laboratory procedures detailed above were followed except samples were incubated for 10 days in 1997.

2.6. Statistical analysis

For soils, the effects of watershed, horizon, and stand type were tested using ANOVA and compared using the Student–Newman–Keuls statistic at the $\alpha < 0.05$ level (Montgomery, 1991). For litter, the effects of watershed, year, species and stand type were tested using ANOVA and compared using the Student–Newman–Keuls statistic at the $\alpha < 0.05$ level. All statistical analyses were conducted using SAS (SAS Institute Inc., 1988).

We examined the potential relationships between enrichment factor ($\delta^{15}N_{foliar} - \delta^{15}N_{Bs}$) and nitrification using Spearman's Rank Correlation Analysis ($\alpha = 0.05$).

3. Results

The soils data showed a pattern of increasing $\delta^{15}N$ with depth, a pattern that has been reported at many other sites (Riga et al., 1971; Ledgard et al., 1984; Nadelhoffer and Fry, 1988; Högberg et al., 1996). The mean $\delta^{15}N$ for the Oie horizon ranged from −0.77 to −0.05‰. The mean $\delta^{15}N$ for the Oa horizon ranged from 3.31 to 3.75‰. The mean $\delta^{15}N$ for the Bs horizon ranged from 7.11 to 7.61‰. There were, however, no significant differences in soil $\delta^{15}N$ among the sites for a given horizon (Fig. 2).

At all three sites there were distinct patterns of leaf litter $\delta^{15}N$ by species (Fig. 3). For example, beech litter had significantly higher $\delta^{15}N$ than yellow birch, sugar maple, and red maple at all three sites for both years ($p < 0.0025$). Beech litter $\delta^{15}N$ was also significantly higher than spruce and hemlock at CPW ($p = 0.0001$) and than spruce and fir at the Bowl ($p = 0.0025$). Beech litter $\delta^{15}N$ was consistently higher than sugar maple litter $\delta^{15}N$ within a plot (Fig. 4). Yellow birch, red maple, and sugar maple had similar $\delta^{15}N$ values. Spruce and hemlock had similar $\delta^{15}N$ values. The less abundant species (black cherry, ash, mountain maple and balsam fir) had varying $\delta^{15}N$ values at the different sites and for different years.

There were no significant differences in litter $\delta^{15}N$ between 1995 and 1996 at CPW; at HBEF W3, beech and yellow birch $\delta^{15}N$ were higher in 1995 than in 1996 ($p = 0.006$; Fig. 3). Litter $\delta^{15}N$ was lower at Cone Pond than at HBEF W3 for most of the dominant species ($p = 0.0001$; Fig. 3). There were no

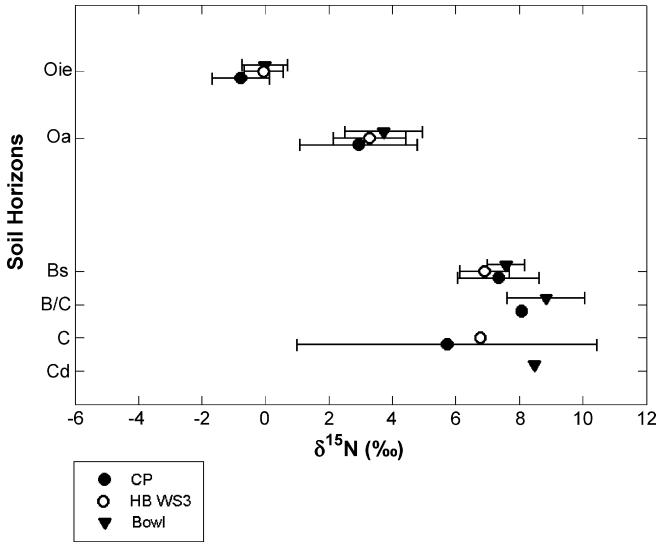

Fig. 2. δ^{15}N in soil at Cone Pond Watershed, Watershed 3 at the Hubbard Brook Experimental Forest, and the Bowl Research Natural Area Watershed means of δ^{15}N in soil by horizon for Oie, Oa, Bs, B/C, C, and Cd horizons with 95% CI. Cone Pond Watershed (CP; filled circles), Hubbard Brook Experimental Forest W3 (HBEF W3; open circles), and Bowl Research Natural Area (Bowl; filled triangles).

significant differences in mean litter δ^{15}N between the other sites.

In order to evaluate within-site variability, we compared soil and litter δ^{15}N by plot (Fig. 5). The conifer-dominated plots at the Bowl (Fig. 5) had significantly lower δ^{15}N in the organic horizons ($p = 0.0001$) and in litter ($p = 0.0002$) than the hardwood-dominated plots. The conifer-dominated plots at CPW (Fig. 5) had significantly lower δ^{15}N in the Oie horizon ($p = 0.0023$) and in litter ($p = 0.03$) than the hardwood-dominated plots. To further examine the relationship between species composition and δ^{15}N in litter, we separated the data from CPW into four categories according to stand type: pure hardwood; mixed hardwood; mixed conifer; and pure conifer (Fig. 6). Because the separation between hardwoods and conifers is distinct at the Bowl (i.e. there are no mixed hardwood and conifer sites), we compared only the pure hardwood to pure conifer stands (Fig. 6). Because there are few conifers at HBEF W3, we could not compare the litter δ^{15}N across stand types within that site. At CPW, litter δ^{15}N was highest for the pure hardwoods (−0.6‰), and declined across the categories, with the lowest δ^{15}N observed at the pure conifer sites (−4.1‰; Fig. 6). These differences were significant ($p < 0.0001$), except between mixed hardwood and mixed conifer. At the Bowl, litter δ^{15}N was significantly higher ($p = 0.002$) for the pure hardwoods (−1.2‰) than for pure conifer sites (−3.2‰; Fig. 6).

In general, net nitrification increased across the nitrate-loss gradient, however, there was considerable variability (Fig. 7). Net nitrification increased with increasing nitrate loss across the spatial nitrate-loss gradient for the Oea horizon for hardwood-dominated sites. N mineralization rates were lower for the mineral soil samples than for the Oea horizon samples at the HBEF ($p = 0.0006$). For conifer dominated sites at CPW and the Bowl, a similar pattern was observed in 1997: the Bowl had higher values than CPW for Oea horizon samples ($p = 0.0001$; Fig. 7). Net nitrification at CPW was lower for conifer-dominated sites than for hardwood-dominated sites; at the Bowl, this pattern was observed in 1996, but not 1997. The conifer site at HBEF W3 had higher net nitrification than the Bowl conifer-dominated sites for both horizons in 1996 (Fig. 7). The conifer-dominated site at HBEF W3 also had higher net nitrification than hardwood-dominated sites at HBEF W3 for both horizons in 1996.

The enrichment factor, defined as $\delta^{15}N_{foliar} - \delta^{15}N_{Bs}$, is a method of comparing δ^{15}N values from different sites by normalizing for the spatial heterogeneity in mineral soil δ^{15}N values (Fig. 8; Garten, 1993; Garten and Van Miegroet, 1994). In order to evaluate whether the enrichment factor reflects ecosystem nitrogen cycling rates, we compared the enrichment factor with an independent measure of nitrogen cycling, net nitrification (Fig. 9). At the highest levels of net nitrification, the enrichment factor was higher; for lower values of net nitrification, there was considerable scatter in the data (Fig. 9). For hardwoods in 1996, there was a positive correlation between net nitrification and enrichment factor (Spearman rank correlation coefficient = 0.56; $p = 0.05$).

4. Discussion

4.1. Soil $\delta^{15}N$

Soil δ^{15}N did not increase along the gradient of increasing nitrate loss. In fact, there were no differences among the sites for any horizon (Fig. 2). This finding is particularly surprising for the two organic horizons, which, because of their relatively short turnover time of approximately 12–15 years (Gosz et al., 1976), are expected to be most responsive to alterations in the nitrogen cycle (Pardo et al., 2002). The turnover time in the mineral soil is on the scale of centuries, and, therefore, should be less reflective of disturbances in recent decades (Gaudinski et al., 2000). These results can lead to two alternative conclusions: (1) that there are, in fact, no differences in soil δ^{15}N among the sites at the watershed scale; or (2) that in order to detect differences in soil δ^{15}N it would be necessary to examine processes at a smaller scale, a scale closer to that on which nitrogen transformations occur. Indeed, another study (Pardo et al., 2006) found that local drivers of N cycling (C:N, nitrification:mineralization) were better predictors of foliar δ^{15}N (at the plot scale) than was N deposition, a regional driver of N cycling. When N deposition is considerably higher, as occurs in Europe, increases in δ^{15}N values of sites across a nitrate loss gradient have been reported (Nohrstedt et al., 1996).

4.2. Year-to-year variation in leaf litter $\delta^{15}N$

The low variability in litter δ^{15}N (Fig. 5) is significant in two respects. First, this result is in contrast to litter nitrogen concentration, which can vary more than 25% from year to year (Hughes and Fahey, 1994). Second, the constancy of litter δ^{15}N from year to year demonstrates that litter δ^{15}N is a stable and

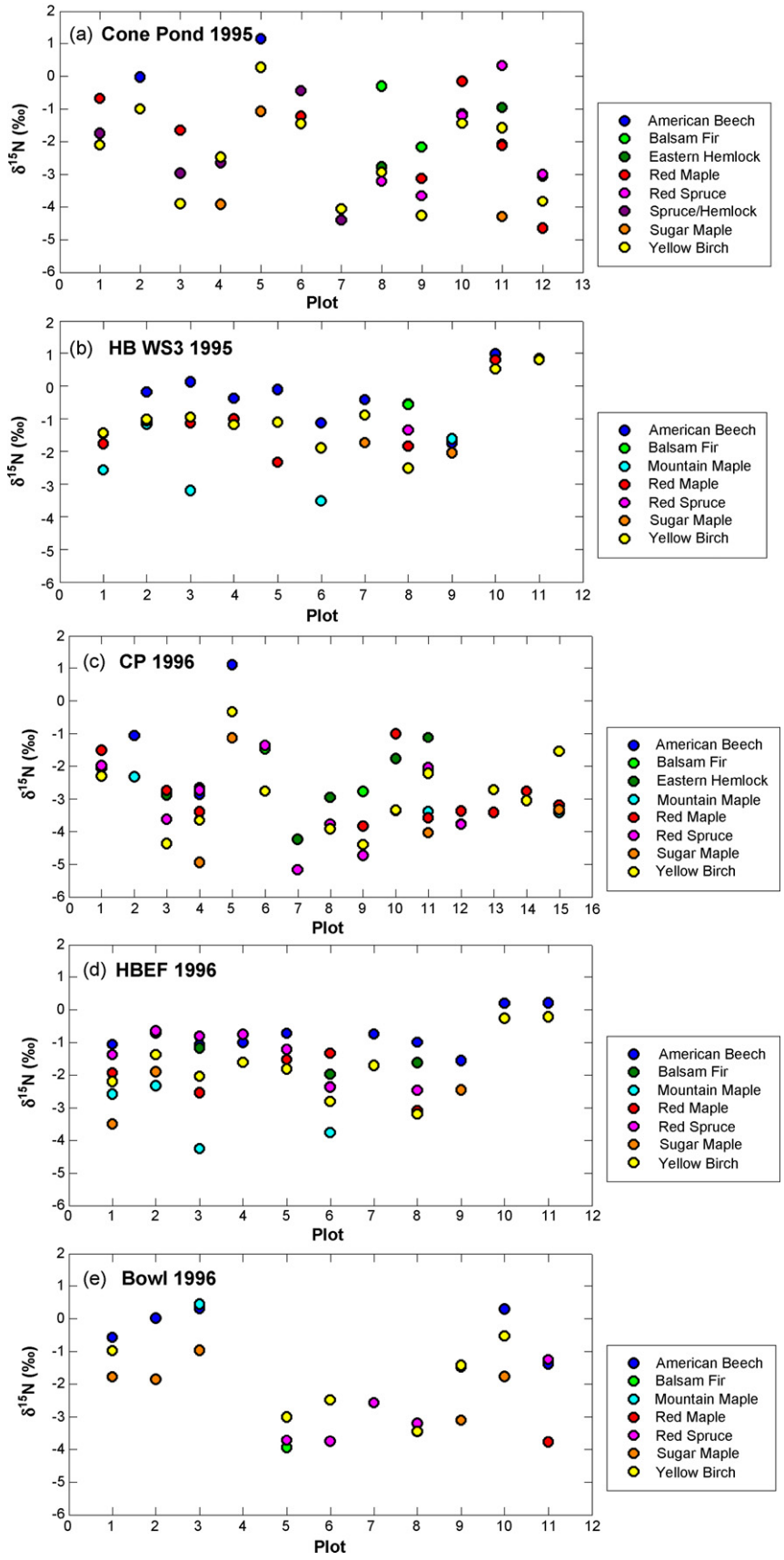

Fig. 3. $\delta^{15}N$ in litter by species at Cone Pond Watershed, Watershed 3 at the Hubbard Brook Experimental Forest, and the Bowl Research Natural Area: (a) Cone Pond 1995, (b) Hubbard Brook Watershed 3 1995, (c) Cone Pond 1996, (d) Hubbard Brook Watershed 3 1996, (e) Bowl 1996.

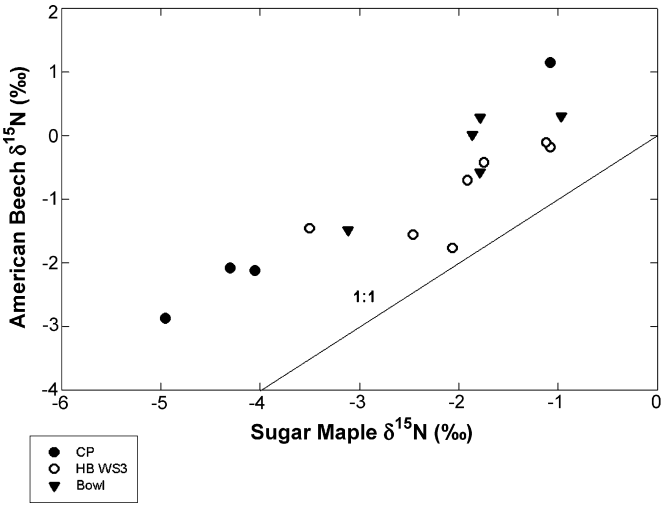

Fig. 4. δ^{15}N in litter for American beech and sugar maple at Cone Pond Watershed, Watershed 3 at the Hubbard Brook Experimental Forest, and the Bowl Research Natural Area. Paired litter samples from the same plot are shown. In all cases, beech litter δ^{15}N was higher than sugar maple litter δ^{15}N. Cone Pond Watershed (CP; filled circles), Hubbard Brook Experimental Forest W3 (HBEF W3; open circles), and Bowl Research Natural Area (Bowl; filled triangles).

good indicator of the δ^{15}N available to plants. In a study of other watersheds at the HBEF, there was no significant change in foliar δ^{15}N over an 11-year period (Pardo et al., 2002).

4.3. Species differences in soil and litter δ^{15}N

We observed distinct differences in litter δ^{15}N by tree species (Fig. 3); indeed, the patterns of litter and forest floor δ^{15}N as a function of species composition (Fig. 6) may facilitate interpretation of the factors regulating foliar δ^{15}N. The strongest pattern that we observed was that foliar δ^{15}N of beech was higher than δ^{15}N of sugar maple (Fig. 4), with yellow birch δ^{15}N generally in between where these species occur in the same plot. The absolute value of the foliar δ^{15}N for both species varies over about 4‰ across all plots and sites (Fig. 4). This pattern has been observed at other sites in northeastern North America (Nadelhoffer et al., 1999; Pardo et al., 2002, 2006, 2007). There are several possible explanations for the differences in litter δ^{15}N by species: (1) differences in rooting depth; (2) local differences in nitrogen cycling and nitrification rate; (3) ammonium versus nitrate uptake preference; and (4) effects of mycorrhizal association.

Rooting depth could be significant because, just as total soil δ^{15}N increases with depth, typically the δ^{15}N of exchangeable inorganic nitrogen also increases with depth in the soil (Nadelhoffer and Fry, 1994; Schulze et al., 1994). Therefore shallow-rooted trees, such as spruce trees, might be expected to have litter that was more depleted in ^{15}N than trees with deeper roots, such as beech. We evaluated the relationship between litter δ^{15}N by species and soil δ^{15}N for each horizon in order to determine whether there were positive correlations. A positive correlation between litter δ^{15}N and soil δ^{15}N from a particular horizon could suggest that a given species derived most of its nitrogen from that horizon. We did not find any consistent patterns relating litter δ^{15}N to total soil δ^{15}N by horizon for any of the species. Future research to evaluate the importance of rooting depth in controlling plant δ^{15}N might include measuring the δ^{15}N of ammonium and nitrate by horizon at each site, and comparing these values to the litter δ^{15}N by species.

Nitrogen cycling rate and the rate of nitrate loss from an ecosystem (or a portion of an ecosystem) can also affect the δ^{15}N of the inorganic nitrogen. Because plant uptake is assumed to be a non-fractionating process, the δ^{15}N of foliar tissue should be similar to that of the inorganic nitrogen that the plants take up (Högberg, 1997). Therefore, factors that regulate the availability and δ^{15}N of plant-available inorganic nitrogen also will affect the δ^{15}N of foliar tissue. Several factors may control nitrogen cycling by affecting the rate at which nitrogen becomes available to microbes. These include litter quality (e.g., C, N, lignin:N) and other abiotic factors. Soil thickness affects the size of the total available nitrogen pool. Drainage class influences water run-off and therefore will affect soil moisture (Gilliam et al., 2001). Temperature can affect the composition of the microbial community and the rate of microbial activity (Balser et al., 2002). All these factors regulate the microbially mediated processes which influence inorganic nitrogen availability and δ^{15}N in forest soils. Species composition can affect N cycling rate (Zak et al., 1986; Finzi et al., 1998; Templer et al., 2003; Lovett et al., 2004). The fact that species composition seems to affect the absolute value of litter δ^{15}N of all tree species within a plot (Fig. 6) without shifting the relative ranking of species by foliar δ^{15}N value (Figs. 3 and 4) suggests species-driven differences in nitrification rate may affect the foliar δ^{15}N.

In an ecosystem where nitrification occurs (without substantial denitrification), the ammonium pool will be more ^{15}N-enriched than the nitrate pool (Garten, 1993; Schulze et al., 1994). In ecosystems with high rates of nitrification and nitrate loss, the ammonium pool may be considerably enriched in ^{15}N relative to the nitrate pool (as long as denitrification is not high, as is the case at these sites; Bohlen et al., 2001; Groffman and Pardo, unpublished data, www.hubbardbrook.org). In either situation, the more a plant exhibits preferential uptake of ammonium, the more enriched in ^{15}N the plant litter will be (Emmett et al., 1998). In order to determine whether such species differences in uptake preference would be measurable in the field, one would need to know the δ^{15}N and the amount of all the nitrogen taken up by plants.

Finally, recent work has suggested that mycorrhizal associations may play a significant role in plant nutrition, especially in nitrogen-limited ecosystems (Chapin et al., 1993; Aber et al., 1998; Hobbie et al., 1999). Most research evaluating the effect of mycorrhizal association on foliar δ^{15}N has been conducted in severely nitrogen-limited arctic and sub-arctic ecosystems (Michelsen et al., 1996; Nadelhoffer et al., 1996; Hobbie et al., 1999). It is difficult to evaluate the possible importance of mycorrhizal association in these temperate forests which may be approaching N saturation.

The processes that affect the δ^{15}N of plant material are interrelated: (1) several factors may lead to an increase in litter δ^{15}N

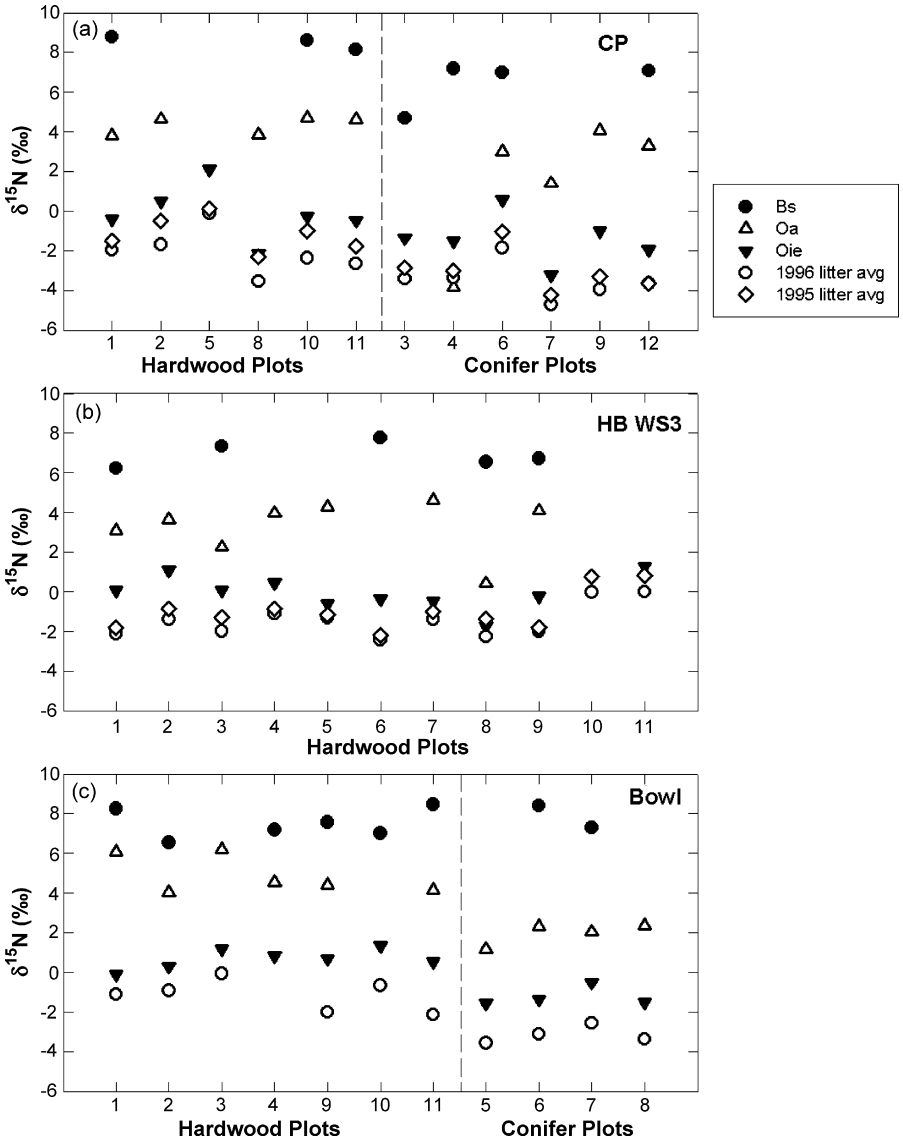

Fig. 5. δ^{15}N in soil and litter by sampling location at Cone Pond Watershed, Watershed 3 at the Hubbard Brook Experimental Forest, and the Bowl Research Natural Area Sampling locations correspond to plots shown in Fig. 1. Litter values are mass-weighted means by plot. (a) Cone, (b) W3, (c) Bowl.

(e.g. a deep-rooted species with an ammonium preference at a site with high nitrification and nitrate losses); and (2) litter inputs are a strong determinant of the δ^{15}N of organic horizon soil. ^{15}N-depleted litter will lower the δ^{15}N of the organic soil horizons. Conversely, ^{15}N-enriched litter will lead to ^{15}N-enriched organic horizons.

4.4. Patterns in net nitrification

There are several factors that affect nitrification rate in forest ecosystems, including nitrogen and carbon availability, temperature, soil drainage, and litter/organic matter quality (Paul and Clark, 1989). We expected higher rates of nitrification in the Oea horizon compared to the mineral soil based on more favorable conditions for nitrification (higher organic matter content, carbon and nitrogen availability, soil temperature and moisture). Similarly, we expected higher net nitrification in the hardwood-dominated sites than in conifer-dominated sites at the Bowl and CPW based on the higher quality of hardwood litter. The pattern of higher net nitrification in Oea horizon of the conifer-dominated sites at the HBEF was unexpected (Fig. 7). Because the site is on a ridge top and is less steep than much of the watershed, higher soil moisture may have stimulated microbial activity. Subsequent measurements in other conifer-dominated areas at the HBEF, however, suggest that the higher rate of nitrification we measured in the conifer stand is anomalous (Bohlen et al., 2001; www.hubbardbrook.org). Conifer-dominated sites represent a very small area of HBEF W3, so this result has little quantitative bearing on the interpretation of nitrogen cycling for the entire watershed.

Two dominant factors that regulate streamwater nitrate loss are nitrate availability and hydrologic flow. An increase in nitrification along the nitrate-loss gradient would be expected: the greater the nitrate produced, the greater the quantity available to be lost from the ecosystem. Recent work suggests that most nitrate lost in streamwater is microbially produced

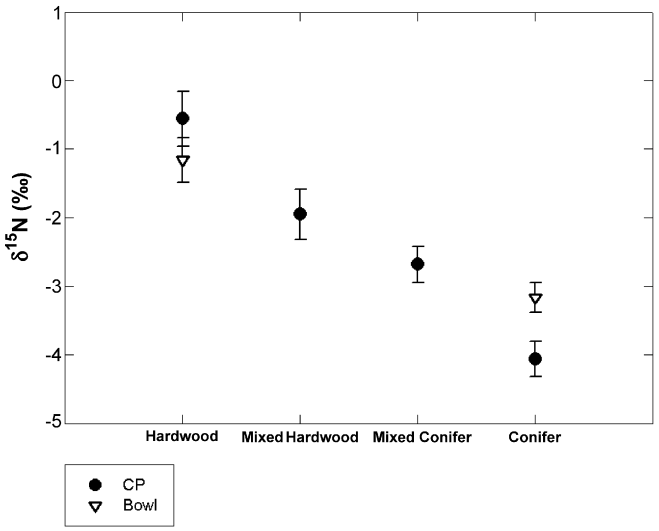

Fig. 6. δ^{15}N in litter by species composition at Cone Pond Watershed and the Bowl. Means of litter δ^{15}N by stand type; bars represent standard error.

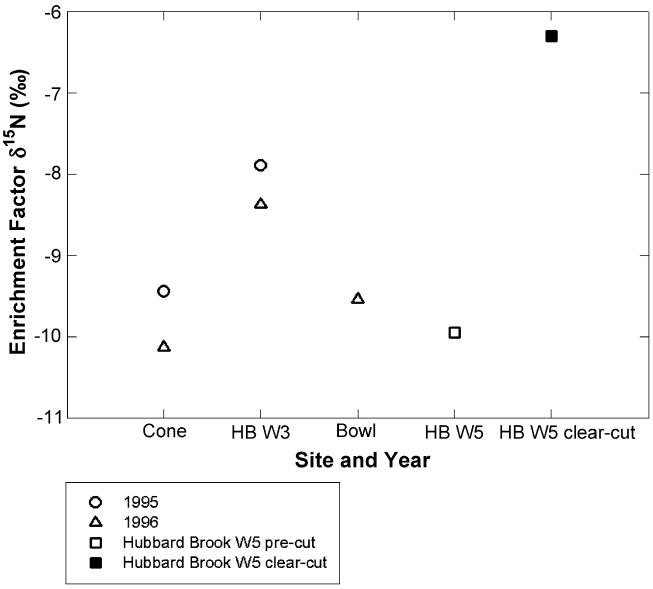

Fig. 8. Enrichment factor. The enrichment factor, defined as $\delta^{15}N_{foliar} - \delta^{15}N_{Bs}$ is a method of comparing δ^{15}N values from different sites by normalizing for the spatial heterogeneity in mineral soil δ^{15}N values. Values from another watershed at the HBEF, W5, are shown to contrast the enrichment factor before and immediately after clearcutting.

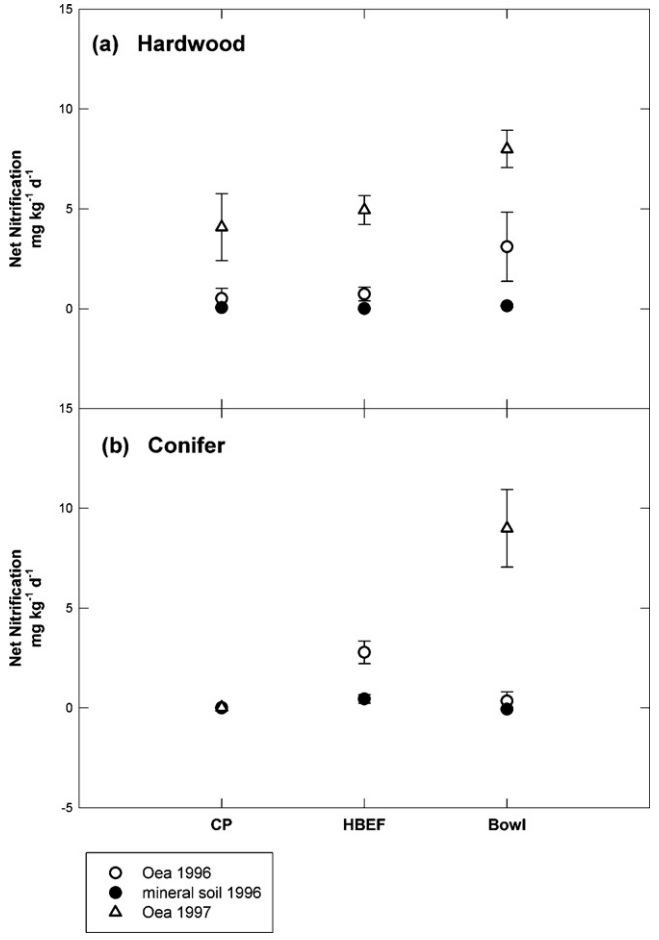

Fig. 7. Net nitrification at Cone Pond Watershed, Watershed 3 at the Hubbard Brook Experimental Forest, and the Bowl Research Natural Area in 1996 and 1997: (a) hardwood, (b) conifer. The sites are arranged on the x axis in order of increasing streamwater nitrate output.

within the ecosystem (Kendall et al., 1996; Burns and Kendall, 2002). We observed this pattern at the HBEF and the Bowl (Pardo et al., 2004). Thus, one might expect higher nitrification at sites with higher nitrate loss.

Other studies have suggested that HBEF and the Bowl are hydrologically distinct and that the differences in nitrate loss are less a function of differences in N saturation than in hydrology (Pardo et al., 2004; Houlton et al., 2003). The Bowl is steep-sided with shallow soils at upper elevations and flat bottomed with deep, gravelly soils at the base of the watershed (Martin et al., 2000), which may facilitate rapid drainage and subsequent storage of N outside the rooting zone (Pardo et al., 2004).

Another important issue in comparing nitrogen cycling at these three sites is the difference in land-use history which may have altered the available nitrogen pool (Goodale and Aber, 2001). The CPW experienced a severe forest fire several years after a hurricane (ca. 1820; Buso et al., 1984). This fire appears to have depleted the nitrogen pool in soil to such an extent that differences in nitrogen cycling rate (net nitrification) are still detectable between burned and unburned areas of the watershed (Hornbeck and Lawrence, 1996). The legacy of the fire appears also to have affected the foliar δ^{15}N values, most likely because of the continuing low nitrification rates. Severe fire often initially causes foliar and surface soil δ^{15}N to increase because of the removal of ^{15}N-depleted organic matter and subsequent disturbance-induced high nitrification and nitrate loss (Mack personal communication; Stephan and Kavanaugh, 2005; Smithwick et al., 2005). HBEF W3 likely lost a significant amount of nitrogen following logging in the early part of the century, while the Bowl has been subject only to natural disturbances, and therefore would be expected to be the most nitrogen-replete of the three ecosystems. The pattern of net nitrification in all sites generally follows the pattern that was

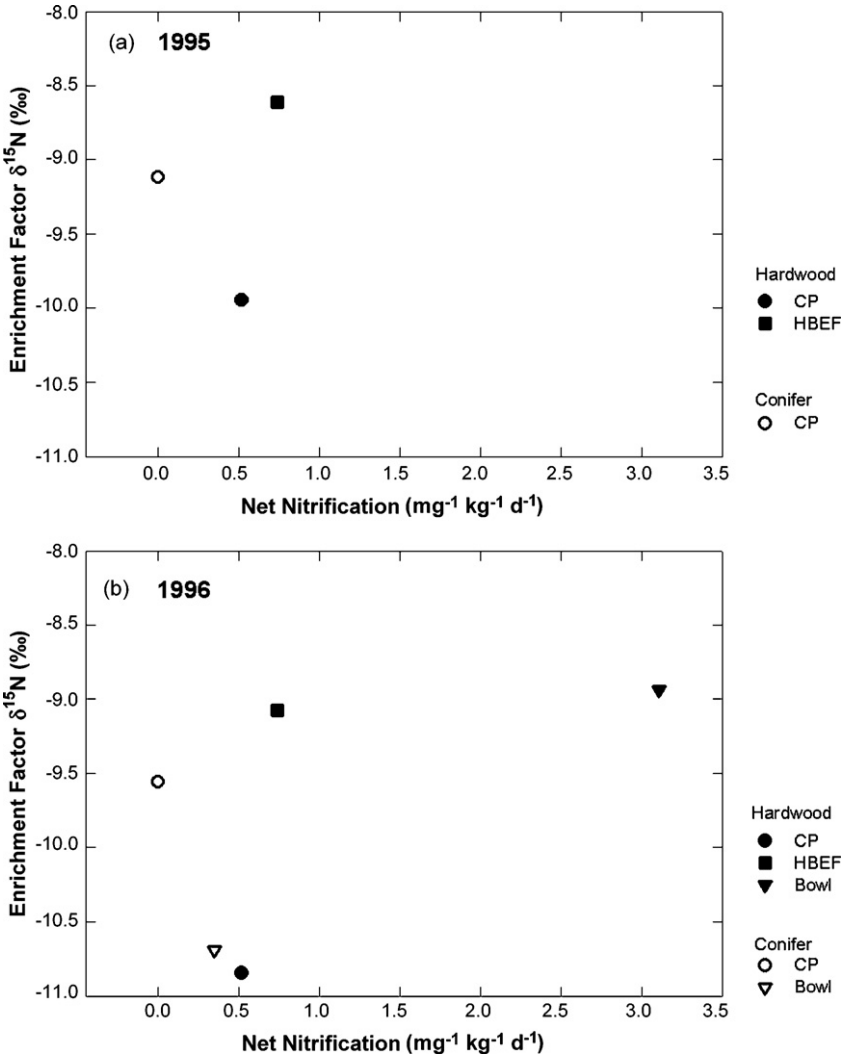

Fig. 9. Enrichment factor as a function of net nitrification. (a) Enrichment factor calculated using litter collected in 1995. (b) Enrichment factor calculated using litter collected in 1996. The enrichment factor, defined as $\delta^{15}N_{foliar} - \delta^{15}N_{Bs}$ is a method of comparing δ^{15}N values from different sites by normalizing for the spatial heterogeneity in mineral soil δ^{15}N values. The enrichment factor is plotted vs. 1996 net nitrification potential.

expected based on the respective land-use histories at the three sites, except for the conifer-dominated site at the HBEF (Fig. 7).

4.5. Enrichment factors

Previous studies have demonstrated positive correlations between the enrichment factor and nitrification rate for areas with differences in nitrogen cycling rate within a watershed (Garten, 1993; Garten and Van Miegroet, 1994). In a study comparing sites across a deposition gradient in Europe, a strong correlation was found between the enrichment factor versus nitrification and other measures of nitrogen cycling rate (Emmett et al., 1998). Other studies have calculated a different enrichment factor using the difference between roots and soil in a given horizon (Högberg et al., 1996) or the difference between the organic horizon and mineral horizons (Vervaet et al., 2002). The enrichment factor in this study shows different patterns for hardwood and conifer-dominated sites. The pattern for the hardwood-dominated sites (Fig. 9) may suggest a threshold–above a certain nitrification rate (perhaps $\sim$1 mg kg^{-1} d^{-1} at these sites), the enrichment factor will be high, below a certain rate it will be low (Fig. 9). There appears to be no pattern in the enrichment factors in the conifer sites, although if indeed there is a threshold above which the enrichment factor is high, the nitrification rate in the conifer-dominated sites at both the Bowl and CPW may simply be too low to yield a higher enrichment factor. Other studies report enrichment factors ranging from -8‰ to -4‰ for Walker Branch, Tennessee (Garten, 1993), from -10‰ to -4‰ for the Great Smoky Mountain National Park (Garten and Van Miegroet, 1994) and from -8‰ to -1‰ for sites across Europe (Emmett et al., 1998). The enrichment factors measured in this study are similar to those from the Great Smoky Mountain National Park (Garten and Van Miegroet, 1994).

5. Conclusions

In summary, although mean δ^{15}N did not vary significantly between soil horizons among the three sites across this nitrate-loss gradient in New Hampshire, differences in litter δ^{15}N

amongst species were strong and consistent. Leaf litter and organic soil $\delta^{15}N$ were both highly responsive to species composition in individual plots. These differences between species may provide a valuable key for understanding how different species influence and are influenced by nitrogen cycling. Most notably, the influence of species composition on the absolute value of litter $\delta^{15}N$ of all tree species within a plot did not shift the relative ranking of species by foliar $\delta^{15}N$ value which suggests that species-driven differences in nitrification rate may affect the foliar $\delta^{15}N$. Further studies of the factors controlling species differences in foliar $\delta^{15}N$ could be useful for understanding the pattern and timing of N uptake by species and ultimately how species composition affects that ability of a stand to retain N.

The enrichment factor may also provide a tool for comparing sites with different nitrogen cycling patterns. At sites with high nitrification and nitrate loss, the enrichment factor should be higher; at sites with low nitrification and nitrate loss, the enrichment factor should be lower. The positive correlation between the enrichment factor and net nitrification we observed at the hardwood sites could suggest a threshold level of nitrification above which the enrichment factor will be high. However, comparison of watersheds is complicated by the broad range of factors that control nitrogen availability and the $\delta^{15}N$ of plant-available nitrogen, so that the enrichment factor may be best suited for evaluating within-site differences or very large differences between watersheds.

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