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A stable isotopic study to determine carbon and nitrogen cycling in a disturbed southern Californian forest ecosystem

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Abstract. This study utilized isotope analyses to contrast nitrogen and carbon dynamics at four sites located along an air pollution gradient in the San Bernardino National Forest in southern California. Natural ^{15}N and ^{13}C abundances along with nutritional and edaphic properties were determined in soil, litter, and vegetation samples. Mean bulk nitrogen $\delta^{15}\text{N}$ values of soil and vegetation at Camp Paivika (CP), the most polluted site, were at least 1.7‰ more enriched than the other, less polluted sites. Mean soil $\delta^{15}\text{NH}_4^+$ was also significantly enriched in ^{15}N at CP compared to Barton Flats (BF), the least polluted site, by 3.8‰. Soil $\delta^{15}\text{NO}_3^-$ signatures were not statistically different among sites. The litter $\delta^{15}\text{NH}_4^+$ values followed a trend similar to that of the soil. Furthermore, the litter $\delta^{15}\text{NO}_3^-$ at CP was significantly depleted in ^{15}N compared to the other sites. The isotopic discrimination for the eventual production of nitrate from organic nitrogen in soil and litter was maximized at CP and minimized at BF. A stable carbon isotopic gradient of decreasing soil, litter, and foliar $\delta^{13}\text{C}$ was also observed with increasing site pollution level. These results support the hypothesis that chronic atmospheric deposition has enhanced nitrogen cycling processes and has affected carbon metabolism at CP.

1. Introduction

Northern temperate forest ecosystems have traditionally been viewed as nitrogen limited [Aber *et al.*, 1989; Garner, 1994]. Deposition of nitrogenous pollutants on forest ecosystems in North America and Europe has been occurring at enhanced rates over the past 50 years. The potential impact of chronic atmospheric nitrogen on forest biogeochemistry, forest biodiversity, soil chemistry, water quality, and air quality has led to the concept of nitrogen saturation. This condition results when the availability of mineral nitrogen exceeds the total combined plant and microbial demand [Aber *et al.*, 1989].

A nitrogen-saturated ecosystem is unable to retain additional nitrogen from sources such as atmospheric deposition, soil mineralization, and nitrogen fixation. High nitrogen deposition causes soil acidification, which increases losses of calcium and potassium and mobilizes aluminum. Leaching of NO_3^- into groundwater and stream water impairs water quality and can cause coastal eutrophication [Shortle and Smith, 1988; Skeffington and Wilson, 1988; Fisher and Oppenheimer, 1991; Vitousek *et al.*, 1997].

From a climatic perspective, increased nitrogen deposition may temporarily stimulate carbon dioxide assimilation by vegetation, but over long timescales, the imbalance in the supply of nitrogen versus phosphate and acidification will eventually lead to reduced carbon sequestration in plants and soil [Asner *et al.*, 1997]. Furthermore, fluxes of the chemically and radiatively active trace

gases nitric oxide (NO) and nitrous oxide (N_2O) are likely to increase as a result of heightened nitrogen deposition to the ecosystem [Vitousek and Matson, 1985; Anderson *et al.*, 1988; Anderson and Poth, 1989].

Differences in the isotopic composition of nitrogen in various ecosystem components can provide information about potential sources of nitrogen and relative importance of various nitrogen cycling processes in the ecosystem [Owens, 1987; Nadelhoffer and Fry, 1988, 1994; Evans and Ehleringer, 1993]. Several fertilization experiments have demonstrated a connection between nitrogen availability and ^{15}N abundance in forest soils and foliage. Forests that are subjected to high nitrogen loads have been shown to export larger amounts of nitrogen (in both gaseous and liquid forms) from the system by nitrification and denitrification, resulting, through isotope fractionation, in a retention of ^{15}N [Shearer and Kohl, 1986; Högberg, 1990, 1991; Högberg and Johannison, 1993; Johannison and Högberg, 1994]. An enrichment in foliar $\delta^{15}\text{N}$ values was observed across a natural gradient of nitrogen-poor to nitrogen-rich sites in a deciduous forest in Tennessee [Garten, 1993; Garten and Van Miegroet, 1994].

Although the biogeochemical cycles of carbon and nitrogen are intimately coupled in terrestrial ecosystems, there have been few reports describing simultaneous determination of carbon and nitrogen isotopic variations in forests [Gebauer and Schulze, 1991; Hobbie, 1994]. Dual-isotopic measurements of different compartments of a healthy and a declining forest stand in Germany showed no difference in the carbon isotopic composition of the vegetation. However, the vegetation and litter at the declining site were more enriched in ^{15}N than at the healthy site [Gebauer and Schulze, 1991].

Fertilizer addition experiments have most often been performed to simulate the effects of excess atmospheric nitrogen

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San Bernardino National Forest

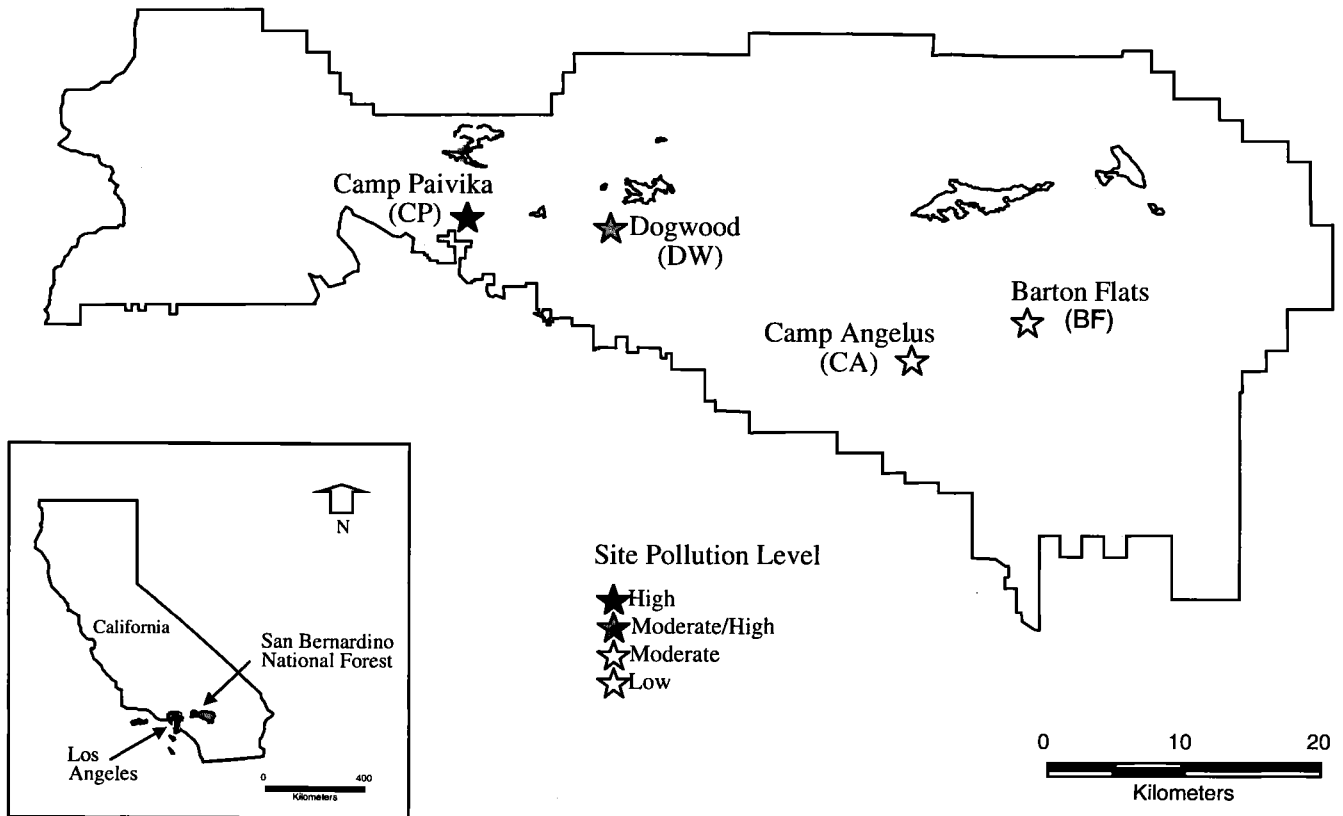


Figure 1. Location of the study sites across the west to east air pollution gradient.

deposition to forest ecosystems. However, this simulation is somewhat limited [Skeffington and Wilson, 1988]. Precise understanding of whether and how biogeochemical processes are altered by air pollution requires field studies of natural distributions. The San Bernardino National Forest (Figure 1) provides an ideal location for studying ecosystem responses to elevated nitrogen additions that occur normally rather than with fertilization. It is located 120 km east of Los Angeles and, like other natural ecosystems in southern California, has been subjected to anthropogenic nitrogen deposition from the Los Angeles air basin for at least the last 40 years. Visible foliar injury, decreased needle retention, cone production stem density, and increased susceptibility to insects have all been associated with elevated ozone concentrations in this forest [Miller *et al.*, 1989].

A pollution gradient exists across the coniferous forests in the San Bernardino Mountains (SBM), with concentrations decreasing from west to east. This air pollution gradient was originally established for ozone [Miller *et al.*, 1986, 1989] and later confirmed for nitrogen and sulfur as well [Fenn and Bytnerowicz, 1993]. The four sites used in this study lie across this nitrogen deposition gradient with Camp Paivika (CP) at the western end of the transect and Barton Flats (BF) at the eastern end (Figure 1). Total nitrogen deposition at CP is estimated to be 35–45 kg ha⁻¹ yr⁻¹ (Table 1), and the site is considered to be highly impacted by air pollution. BF is the least polluted site and receives ~5 times less nitrogen annually than CP [Fenn and Bytnerowicz, 1993]. Mineralization, nitrification, and litter decomposition rates are greatest at the western end of the transect [Fenn and Dunn, 1989; Fenn, 1991; Fenn *et al.*, 1996]. Results for a number of nutri-

tional and edaphic indicators and from nutrient cycling model simulations suggest that the forest at CP might be nitrogen saturated [Fenn *et al.*, 1996].

2. Methods

2.1. Description of Study Sites

The four study sites are located along a 37 km west to east air pollution gradient within a mixed conifer forest in the San Bernardino Mountains, east of Los Angeles, California (Figure 1). The sites are part of 18 permanent research plots that have been studied by the U.S. Department of Agriculture (USDA) Forest Service since 1972. These plots are relatively homogeneous with respect to tree cover, which comprises at least 50 Ponderosa (*Pinus ponderosa*) or Jeffrey pines (*P. jeffreyi*) with diameter at breast height (DBH) of at least 10 cm. Other overstory species found in the Ponderosa pine sites are incense cedar (*Caleocedrus decurrens*), California black oak (*Quercus kelloggii*), and sugar pine (*P. lambertiana*). Along the west to east transect the tree species composition shifts from the Ponderosa pine type to the closely related Ponderosa-Jeffrey pine type [McBride and Miller, 1977]. The selected sites have not burned in the past 40 years.

The sites have been classified as receiving high-, moderate-, or low-air pollution exposure on the basis of 24 hour averages of O₃ concentrations [Miller *et al.*, 1986]. Nitrogen and sulfur compounds concentrations follow the same trend along the west-east transect as ozone. Nitrogen deposition declines steeper than ozone across the horizontal pollution gradient, which is thought to

Table 1. Selected Site Characteristics Across the West to East Air Pollution Gradient

Site	Elevation ^a , m	Annual Mean Precipitation ^a , mm	N Deposition, kg N ha ⁻¹ yr ⁻¹	NO Flux (Dry / Wet) ^b , ng N m ⁻² s ⁻¹
CP	1600	580	(35-45) ^b	8.1 / 21.6
DW	1725	1070	N/A	N/A
CA	1830	750	N/A	N/A
BF	1946	540	6 ^c	1.5 / 10

N/A, not available; CP, Camp Paivika; DW, Dogwood; CA, Camp Angelus; BF, Barton Flats.

^a From *Arkley et al.* [1977].

^b From *Fenn et al.* [1996].

^c N deposition at BF is the estimated N deposition at Camp Osceola, located 2 km east of BF, from *Fenn and Bytnerowicz* [1993].

be a result of the high deposition velocity of nitric acid, the main component of the nitrogenous, dry deposition in the area [*Fenn and Bytnerowicz*, 1993]. Air pollution exposures are greatest on the western end of the gradient because of the proximity of major urban centers in the valley to the west. Camp Paivika is located on the crest overlooking the urban area to the west and is the site with the highest air pollution exposure. Dogwood (DW), located ~9 km east of CP, is also polluted but to a lesser extent than CP. Camp Angelus (CA) is located ~30 km east of CP and receives moderate to low levels of polluted air. Finally, Barton Flats is the easternmost and least polluted site. Site characteristics are summarized in Table 1.

Parent material of the soils at the sites consists of partially weathered or decomposed granitic rock, except for CA, which is mainly stony granitic colluvium. Soils in the San Bernardino Mountains are sandy with considerable fine gravel (2-5 mm), are well drained, and have a low water-holding capacity. Soils at the four sites have weakly developed B horizons and become increasingly coarse textured with depth. At CP and DW, soils are mainly coarse-loamy, mixed, mesic Ultic Haploxerolls of the Shaver series. The Shaver series is dark in color to a depth of >50 cm with no significant clay accumulation in the subsoil. At CA the soil is a stony, coarse-sandy loam. Soil at BF is mostly coarse-loamy, mixed, frigid Xerumbrept [*Arkley et al.*, 1977; *Arkley*, 1981].

2.2. Sample Collection

All samples were collected during July 1995 and included at each site.

1. The first type was vegetation samples. Ten *Pinus ponderosa* of two age classes (five current and five 1 year old needles) and five *Quercus kelloggii* (California black oak) trees were collected at approximately the same canopy heights at all sites. Each individual tree foliage sample was made up of needles or leaves from three individual branches from that tree. The foliage samples were rinsed with deionized water, freeze-dried, and pulverized prior to the isotopic analyses.

2. The second type was litter samples. Five per site, from the layer above the mineral soil and underneath the undecomposed litter, were collected at four locations within a 4 m circle within each plot and represented a mixture of decomposition states of leaves and needles. The litter samples were freeze-dried and pulverized.

3. The last type was soils. Mineral soils (0-5 cm, five per site) were collected at the same locations used for the litter collection. After freeze-drying, the soils were sieved through a 2 mm mesh screen. The soil samples were pulverized prior to bulk nitrogen and carbon isotopic analyses.

2.3. Sample Analyses

2.3.1. Nutrient determination of ammonium and nitrate. Inorganic nitrogen was extracted from the soil and litter samples

with a 2 M KCl solution in deionized water, with a soil to extractant ratio of 1:4 (weight to volume) and a litter to extractant ratio of 1:10 (weight to volume) [*Lindau and Spalding*, 1984]. The soil or litter slurry was manually shaken for ~3 min and automatically shaken for at least 1 hour. The extracts were centrifuged, filtered through precombusted (at 550°C for 1 hour) Whatman 42 filters, and frozen until analysis. Ammonium (NH₄⁺) concentrations were determined colorimetrically by an automated indophenol method with a Technicon Autoanalyzer II [*Parsons et al.*, 1984], and nitrate (NO₃⁻) was determined by copperized cadmium reduction in combination with diazotization with an Alpkem "Flow Solution" Autoanalyzer with a model 510 spectrophotometric detector (Pestorp Analytical Corporation, unpublished report, 1992). Concentrations were calculated on a soil/litter dry weight basis. The pH was determined on a 1:2 mixture of soil/litter and 0.01 M CaCl₂.

2.3.2. Stable isotopic analyses. The stable isotope analyses included determinations of the isotopic compositions of the organic carbon and bulk nitrogen in the soil, vegetation, and litter samples, as well as determinations of the δ¹⁵NH₄⁺ and δ¹⁵NO₃⁻ in the soil and litter samples.

2.3.2.1. Carbon and nitrogen: The litter and soil material was mixed with purified (850°C for 1 hour), coarsely ground CuO (BDH Chemical) and pure granular Cu (Alpha Resources Inc.). The mixed samples were placed into precombusted (550°C for 1 hour) quartz tubes, which were vacuum sealed and combusted at 850°C for 1 hour. The resulting CO₂ and N₂ gases were cryogenically separated and purified on a vacuum line and analyzed for their isotopic signatures [*Macko et al.*, 1987]. The stable isotopic measurements were conducted on a VG-PRISM stable isotope ratio mass spectrometer, except for the vegetation samples which were analyzed on a VG-OPTIMA stable isotope ratio mass spectrometer coupled to a Carbon-Nitrogen-Sulfur (CNS) elemental analyzer. The overall precision for determination of the carbon and nitrogen isotopic compositions was of the order of ± 0.2‰.

Natural abundances of ¹⁵N and ¹³C are typically expressed as per mil (‰) deviations from a primary standard by the equation:

$$\delta^{15}\text{N} \text{ or } \delta^{13}\text{C} = 1000 \left(\frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \right),$$

where R is the ratio of the heavy to the light isotope. By definition the isotopic composition of the standard reference material is 0‰ [*Hoefs*, 1987]. A substance containing more of the heavy isotope relative to another substance is referred to as "enriched" or "heavier", whereas one containing less of the heavy isotope is described as "depleted" or "lighter". A notation commonly used to report the isotopic fractionation during a reaction is "delta del (Δ)". This isotopic discrimination can be approximated by the instantaneous difference in δ¹⁵N between product and substrate: Δ_{p-s} ≈ δ¹⁵N_p - δ¹⁵N_s. So Δ_{p-s} represents the degree to which a

reaction favors the lighter isotope over the heavier. Depending on the environmental conditions, the discrimination factors for nitrification can range from -5 to -35%, whereas the discrimination factors for denitrification can range from -11 to -33% [Delwiche and Steyn, 1970; Mariotti et al., 1980, 1981, 1982].

Carbon concentrations of the samples were determined from manometer deflections during the cryogenic separation of the N_2 and CO_2 . Nitrogen concentrations were calculated from the ion gauge pressure intensity of the sample in a calibrated volume of the mass spectrometer [Korontzi, 1997]. Carbon and nitrogen contents of the plant samples were determined during the isotopic analysis on the VG-OPTIMA CNS elemental analyzer. The C:N values are reported as the molar C:N ratios for the samples.

2.3.2.2. Ammonium and nitrate: NH_4^+ and NO_3^- were distilled from soil and litter extracts using a Labconco Rapid Distillation system (RapidStill II) according to the procedures described by Velinsky et al. [1989] and Ostrom [1992]. Distillation proceeded until 1.5 times the original volume sample was collected in the recovery flask to ensure complete recovery of the NH_4^+ . Initially, the NH_4^+ in the sample was converted to NH_3 by raising the pH to >10 with the addition of 3 mL of 5 N NaOH. The NH_3 distilled out of the sample was converted back to NH_4^+ with 0.03 N HCl and then bound to a zeolite molecular sieve (Union Carbide Ionsiv W-85) by ion exchange. The zeolite was initially dried at 160°C for 1 hour. While stirring the solution containing the sieve for 1 hour, an optimal binding pH of 5.5-6.0 was maintained by addition of either 0.05 N Na_2CO_3 or 0.03 N HCl. The sieve was then vacuum filtered onto a precombusted (at 550°C for 1 hour) Whatman 42 glass fiber filter and oven-dried at 60°C overnight. Approximately 1.5 g of Devardas alloy (50% Cu, 45% Al, and 5% Zn) were added to the cooled distilled sample to reduce all the residual NO_3^- to NH_4^+ . The distillation was then repeated [Russell et al., 1998]. The Devardas alloy was ground to

a fine powder prior to addition to the sample to avoid fractionation [Kreitler, 1975]. Two zeolite bindings were performed for each NH_4^+ and NO_3^- sample to ensure full recovery. The two sieves were combined to form one sample, and the bound NH_4^+ was converted to N_2 gas for isotope ratio analysis as above. The overall precision for determining NH_4^+ and NO_3^- isotopic composition was better than 0.3‰ [Korontzi, 1997], which is in agreement with previously reported values [Kreitler, 1975; Velinsky et al., 1989; Russell et al., 1998].

2.3.3. Statistical analyses. Differences among sites were tested with one-way analysis of variance (ANOVA), followed by Student-Newman-Keuls multiple-comparison or Student *t* test procedures. Data were tested for normality using the Kolmogorov-Smirnov test (with Lilliefors' correction). When the data failed the normality test, a Kruskal-Wallis one-way ANOVA on ranks was used. A significance level of 95% ($P \leq 0.05$) was used for all hypotheses tested.

3. Results and Discussion

3.1. Nutritional and Edaphic Parameters

3.1.1. Molar C:N ratios, total nitrogen fractions, and pH. Soil and vegetation molar C:N ratios were significantly lower at CP than at BF (Figures 2a and 2b). Mean molar C:N ratios for soil ranged from 23 at CP to 33 at BF. The related range for the vegetation was from 24 at CP to 39 at BF. Litter molar C:N ratios were not significantly different (Figure 2c).

Nitrogen levels in foliage of oak and pine trees at CP were higher than at the other sites (Table 2). Current and 1 year old needles were significantly higher in percent N and had lower molar C:N ratios at CP than did pine samples at the other sites. Similarly, the higher percent N in oak foliage at CP was accompanied by lower molar C:N ratios, compared to the eastern, less polluted sites (Tables 2 and 3). Soil and litter were more acidic at CP than at BF (Table 2). Similar findings for the C:N ratios, the foliage nitrogen concentrations, and the soil and litter pH have been reported by Fenn et al. [1996] for the same sites in the San Bernardino National Forest.

It has been observed that as litter decomposition proceeds, its C:N ratio decreases [Haynes, 1986]. Litter decomposition has been found to be faster at CP compared with the other sites [Fenn and Dunn, 1989; Fenn, 1991] and is further supported from the higher mineral nitrogen concentrations at this site reported here (Figures 3a and 3b). However, no statistical differences were observed in the litter molar C:N ratios among sites (Figure 2c). This could be attributed to two facts: first, each litter sample included a mixture of materials at different states of decomposition, and second, each litter sample consisted of varied percentages of oak and pine foliage, with dissimilar molar C:N ratios (Table 3). Therefore the litter molar C:N ratio was a poor indicator of nitrogen availability.

Differences in nitrogen levels and C:N ratios between CP and the eastern sites (CA and BF) reflected patterns of nitrogen availability and uptake. The higher percent N in foliage and litter and the lower molar C:N ratios in soil and vegetation at CP are indicative of the nitrogen enrichment and increased nitrogen uptake at this site compared to the two eastern sites. These characteristics are thought to be among the primary indicators of nitrogen-saturated ecosystems [Aber et al., 1989; Stams and Schipholt, 1990].

Acidification of soil has also been identified as a symptom of nitrogen saturation [Skeffington and Wilson, 1988]. Possible reasons for soil acidification at the western, highly polluted sites include heightened nitrification rates, chronic acidic deposition, and root uptake of NH_4^+ . Low pH values at CP and DW have been found to be linearly correlated with low percent base saturation at those sites [Fenn et al., 1996].

In addition to site-specific differences, species-specific differences were also observed. For example, a significant age dependence was observed in the ponderosa pine needles. Current needles exhibited higher percent N and lower molar C:N ratios than the 1

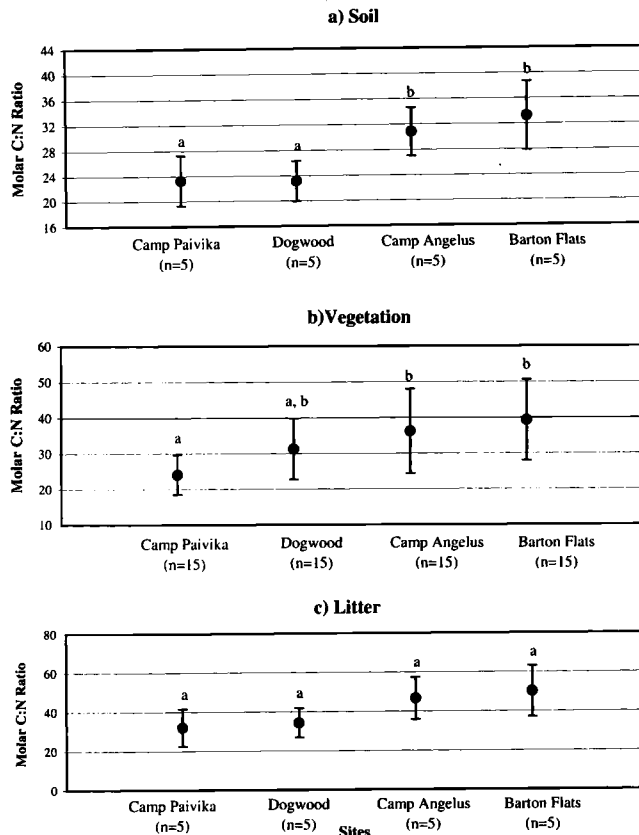


Figure 2. Molar C:N ratio variations across the west to east air pollution gradient. Means followed by the same letter are not statistically different (mean \pm σ).

Table 2. Vegetation Percent Nitrogen and pH in Soil and Litter

Site	Oak Foliage Percent N	Current Needles Percent N	1 Year Old Needles Percent N	Soil pH	Litter pH
CP	3.3 (0.2)	2.4 (0.3)	2.0 (0.2)	3.8 (0.5)	3.9 (0.4)
DW	2.6 (0.5)	1.7 (0.2)	1.4 (0.1)	4.7 (0.5)	5.0 (0.3)
CA	2.5 (0.3)	1.5 (0.4)	1.4 (0.3)	5.4 (0.4)	5.4 (0.5)
BF	2.2 (0.3)	1.8 (0.5)	1.1 (0.1)	5.6 (0.6)	5.4 (0.4)

Numbers in parentheses are standard deviations of the site mean.

year old needles at all sites except CA (Tables 2 and 3). This observed difference is common [Clancy *et al.*, 1995]. Oak foliage at each site contained significantly higher total nitrogen and had lower molar C:N ratios than the pine needles (Tables 2 and 3). These differences indicate species-specific variations in nitrogen use and suggest that oak trees are a greater sink for assimilated nitrogen compared to pine trees [Cole, 1981].

3.1.2. Extractable ammonium and nitrate concentrations.

The NO_3^- concentrations in soil and litter extracts were several times higher at CP than at the less polluted sites (Figures 3a and 3b). Across the west to east air pollution gradient the mean soil NO_3^- -N ranged from 11.9 mg (kg dry weight)⁻¹ at CP to 1.0 mg (kg dry weight)⁻¹ at BF. The mean litter NO_3^- -N concentrations ranged from 38.6 mg (kg dry weight)⁻¹ at CP to 6.2 mg (kg dry weight)⁻¹ at BF. The NH_4^+ concentrations in soil extracts were similar among sites (Figure 3a). The NH_4^+ levels in the litter, however, were at least 5 times higher at CP compared to the other sites (Figure 3b). The mean litter NH_4^+ -N concentrations ranged from 141.7 mg (kg dry weight)⁻¹ at CP to 15.6 mg (kg dry weight)⁻¹ at BF.

The soil and litter exchangeable nitrogen concentrations at the study sites were directly correlated with nitrogen deposition. Total inorganic nitrogen levels in soil and litter were highest at CP, as expected, because this site is exposed to the highest atmospheric nitrogen deposition. The lowest levels were observed at the eastern sites (CA and BF). The higher NO_3^- concentrations in soil and litter at CP indicates that nitrification was enhanced compared to the other sites. Other processes that could also have resulted in high mineral nitrogen concentrations, such as less leaching and less denitrification, were probably not as important because of the prolonged summer drought. Gaseous losses of nitrogen are higher at CP than BF anyway [Fenn *et al.*, 1996]. Nitrification at the eastern sites was low, which is characteristic of a nitrogen limited system [Haynes, 1986; Aber, 1992].

These results support previous findings. Fenn *et al.* [1996] found nitrification rates in soil and litter to be highest at CP relative to the other sites. Similarly, Fenn and Dunn [1989] determined faster litter decomposition rates at the western, more polluted sites than at the eastern, less polluted sites in the San Bernardino National Forest. Soil inorganic nitrogen levels at CP and

Table 3. Plant-Specific Molar C:N Ratios

Site	Oak Foliage	Current Needles	1 Year Old Needles
CP	18 (1)	24 (3)	30 (2)
DW	21 (4)	33 (3)	40 (2)
CA	23 (2)	41 (9)	44 (9)
BF	28 (3)	37 (3)	53 (5)

Numbers in parentheses are standard deviations of the site mean.

DW were comparable to other ecosystems in southern California [Fenn *et al.*, 1996].

Accumulation of NH_4^+ in litter was evident at CP, not only from its high concentration but also from the substantially higher NH_4^+ -N to NO_3^- -N ratio in litter than in soil (3.7 versus 0.7, respectively; Figures 3a and 3b). This was partially anticipated because nitrification and NH_4^+ removal processes, such as NH_4^+ uptake by plants, are more efficient in soil than in litter and because this site receives the highest amounts of atmospheric mineral nitrogen.

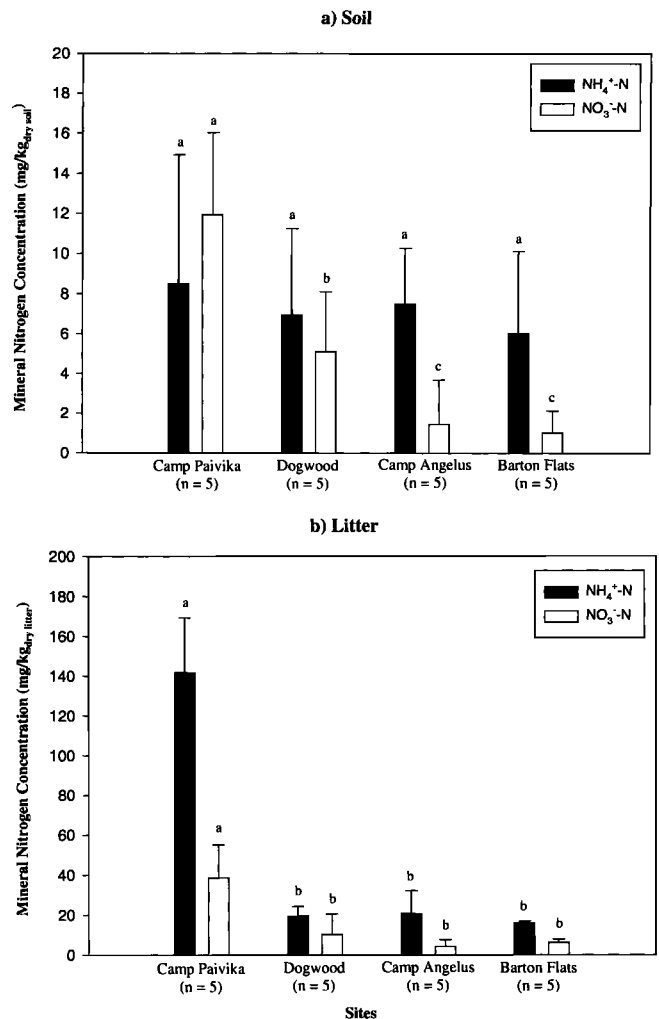


Figure 3. Mineral nitrogen concentrations across the west to east air pollution gradient. Means followed by the same letter are not statistically different (mean \pm σ).

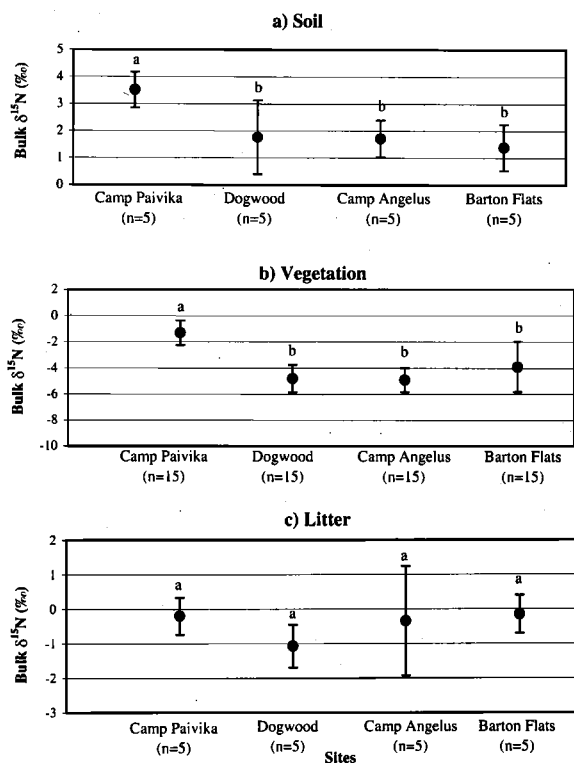


Figure 4. Bulk $\delta^{15}\text{N}$ values across the west to east air pollution gradient. Means followed by the same letter are not statistically different (mean \pm σ).

3.2. Stable Isotopic Data

3.2.1. Bulk nitrogen isotopic variations among sites. Bulk nitrogen isotopic analyses of soil and vegetation samples showed a distinct variation in isotopic signature across the nitrogen depositional gradient. Significantly heavier soil and vegetation $\delta^{15}\text{N}$ values were found at the westernmost site (CP) than at the easternmost site (BF) (Figures 4a and 4b). Likely, the isotopic variation between CP and the other sites resulted from isotope discrimination during the different nitrogen cycling processes and primarily reflected the forest response to increased atmospheric nitrogen loading. The isotopic composition of the soil, litter, and foliage samples at all sites were within the normal range of values reported for other forest ecosystems [Korontzi, 1997].

Soil bulk $\delta^{15}\text{N}$ values at CP ranged from +3.0 to +4.3‰ and were significantly enriched compared to the three other sites which ranged from +0.2 to +3.6‰ (Figure 4a). The enrichment in soil ^{15}N at CP can be attributed to extended periods of enhanced rates of mineralization and, more importantly, nitrification coupled with losses of nitrogenous gases. Ecosystems with high rates of microbial activity tend to have higher soil $\delta^{15}\text{N}$ values [Mariotti et al., 1981]. The turnover of the microbial biomass in the soil organic matter may have also led to the higher soil $\delta^{15}\text{N}$ values, especially if one considers that a microbial population may be larger in a nitrogen-rich than in a nitrogen-limited soil environment. Immobilization of the heavier NH_4^+ (Figure 5a) likely contributed to an enrichment of microbes in ^{15}N at this site. Furthermore, increased losses of nitrogenous gases, depleted in ^{15}N , from the soil during nitrification and denitrification may have also led to an enrichment of soil $\delta^{15}\text{N}$ values [Högberg, 1990; Handley and Raven, 1992; Nadelhoffer and Fry, 1994]. Fenn et al. [1996] found that emissions of NO and N_2O from dry soils were 5 and 10 times higher, respectively, at CP than at BF.

Vegetation was isotopically more enriched in ^{15}N at CP than at the other sites along the transect (Figure 4b), and the $\delta^{15}\text{N}$ values ranged from -2.1 to +0.4‰. Foliage $\delta^{15}\text{N}$ values at the other three

sites ranged from -7.1 to -1.6‰. No species-specific differences were observed in the $\delta^{15}\text{N}$ values at any site.

The vegetation at each site was more depleted in ^{15}N than the soil (Figures 4a and 4b), suggesting preferential uptake of a form of nitrogen which was depleted in ^{15}N from the soil by the plants. For most plant species a mixture of NH_4^+ and NO_3^- appears to produce optimum growth, but one form might be preferred over the other [Haynes, 1986]. The results from this study suggest a preferential assimilation of NO_3^- , which was similar isotopically to the vegetation, as opposed to the soil NH_4^+ , which was more enriched than the vegetation (Figures 6a, 6b, 5a, 5b, and 4b). Field studies have also suggested that deciduous trees and conifers preferentially assimilate NO_3^- rather than NH_4^+ [Nadelhoffer et al., 1984]. The $\delta^{15}\text{N}$ values of the oak foliage and the pine needles at each site were statistically indistinguishable from the soil $\delta^{15}\text{NO}_3^-$ (Figures 6a and 6b), confirming in vitro experiments [Mariotti et al., 1980; Yoneyama and Kaneko, 1989] which showed that there is no isotopic effect during NO_3^- uptake by plants. The observation that plant material exhibited lower $\delta^{15}\text{N}$ values than the soil in which the plants were growing is consistent with general observations of ^{15}N distribution in forests [Nadelhoffer and Fry, 1994].

It should be noted that the oak foliage and the pine needles were slightly enriched relative to the soil NO_3^- at CP but not at the other sites (Figures 6a and 6b). This could suggest that the ratio of NH_4^+ to NO_3^- in the mixture taken up by oak trees might be greater at CP. It has been observed that in acidic soils, forest trees prefer NH_4^+ [Krajina et al., 1973]. This might also explain the enrichment in ^{15}N in vegetation at this site because the soil $\delta^{15}\text{NH}_4^+$ was more enriched at CP than at the other sites (Figure 5a). Foliar ^{15}N enrichment at CP could also be attributed to higher losses of volatile nitrogen (e.g., NH_3 , amines, N_2O and NO_x) from the plants [Farquhar et al., 1980, 1983].

As mentioned above, heightened nitrification in the soil (Figure 3a) along with increased nitrogenous gaseous losses [Fenn et al., 1996] likely contributed to the enrichment of the soil $\delta^{15}\text{N}$ at CP. Furthermore, nitrification enriched the soil $\delta^{15}\text{NH}_4^+$, which

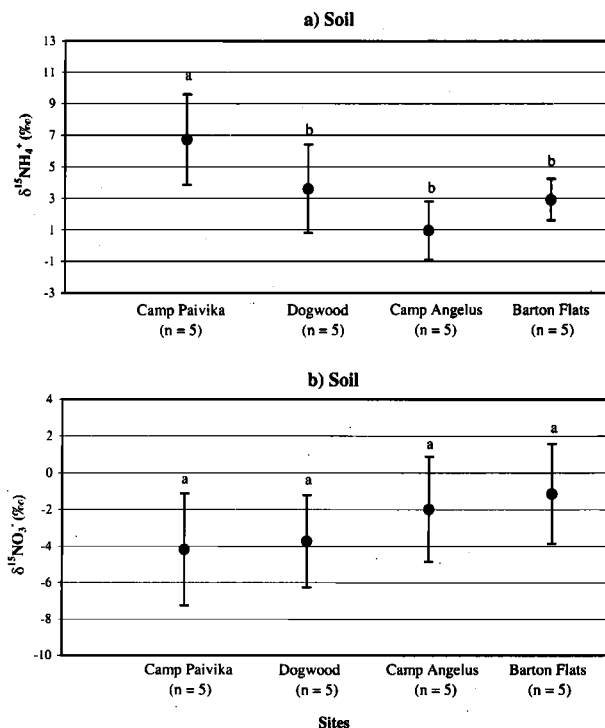


Figure 5. Soil $\delta^{15}\text{NH}_4^+$ and $\delta^{15}\text{NO}_3^-$ values across the west to east air pollution gradient. Means followed by the same letter are not statistically different (mean \pm σ).

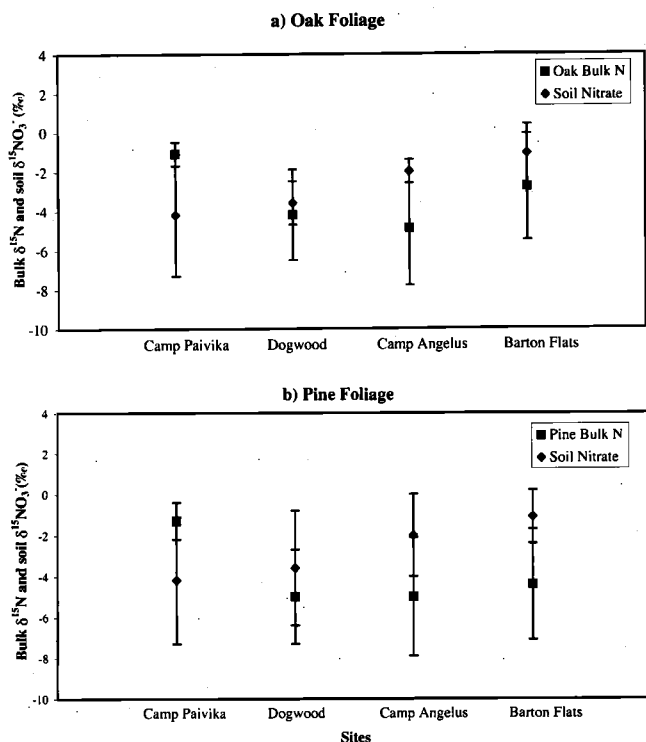


Figure 6. Vegetation bulk $\delta^{15}\text{N}$ and soil $\delta^{15}\text{NO}_3^-$.

in turn contributed to the higher plant $\delta^{15}\text{N}$ values at this site. Several previous studies support these speculations. The $\delta^{15}\text{N}$ values of soil and *Picea abies* needles were observed to increase with increasing nitrogen fertilization [Högberg *et al.*, 1992; Johannisson and Högberg, 1994]. A positive correlation between ^{15}N abundance of *Pinus sylvestris* foliage and nitrogen losses was found in nitrogen-fertilized stands [Högberg and Johannisson, 1993]. Increased losses of ^{15}N -depleted compounds were suggested to account for the enriched needle $\delta^{15}\text{N}$ values found at a declining *P. abies* forest in Germany [Gebauer and Schulze, 1991]. Furthermore, the same observed pattern of increasing ^{15}N natural abundance in foliage along a naturally occurring gradient of increasing soil nitrogen availability was also found in Walker Branch Watershed, Tennessee, and was positively correlated with net nitrification rates in the soil [Garten, 1993; Garten and Van Miegroet, 1994].

Litter bulk $\delta^{15}\text{N}$ values (Figure 4c) ranged from -1.7 to +2.3‰ with no noticeable pattern observed among sites. Because no species-specific differences were found in the foliage $\delta^{15}\text{N}$ values at any site, the litter bulk $\delta^{15}\text{N}$ values were not affected by species composition. Litter bulk $\delta^{15}\text{N}$ values were slightly more depleted than the soil bulk $\delta^{15}\text{N}$. This was expected because total nitrogen in plants was more depleted than that in soil (Figures 4a and 4b). In addition, the litter samples were more enriched in ^{15}N than their source plant nitrogen. This could be the result of translocation of compounds, depleted in ^{15}N , from senescent foliage, as woody tissues have been found to be depleted relative to leaves [Virginia *et al.*, 1989; Gebauer and Schulze, 1991].

Enrichment in the $\delta^{15}\text{N}$ during decomposition can be $<0.5\%$ even after significant loss of nitrogen has occurred [Mellilo *et al.*, 1989; Macko *et al.*, 1994]. The standard deviation of the litter $\delta^{15}\text{N}$ values at all sites was $>0.5\%$. This may explain the fact that no statistical differences were observed among sites even though litter decomposition was higher at CP. Furthermore, this suggests that litter bulk $\delta^{15}\text{N}$ values are poor indicators of nitrogen availability.

3.2.2. Ammonium and nitrate isotopic variations among sites. To further investigate nitrogen cycling processes at sites

across the atmospheric pollution gradient, the isotopic composition of NH_4^+ and NO_3^- were determined in soil and litter samples (Figures 5a, 5b, 7a and 7b). Soil $\delta^{15}\text{NH}_4^+$ ranged from +2.8 to +10.7‰ at CP and from -1.1 to +6.8‰ at the other sites. Soil $\delta^{15}\text{NO}_3^-$ were similar at all sites and ranged from -7.2 to +2.9‰. Litter $\delta^{15}\text{NH}_4^+$ values ranged from -2.1 to +0.4‰ at CP and from -6.1 to -1‰ at the other sites. The respective litter $\delta^{15}\text{NO}_3^-$ values ranged from -11.0 to -6.4‰ and from -9.0 to +1.1‰.

The NH_4^+ and NO_3^- isotope data provided further evidence for the existence of a spatial nitrogen isotope gradient. The nitrogen isotopic data combined with the nutrient data suggest that nitrogen cycling varied directly as a function of atmospheric nitrogen deposition at all of the four study sites. In both litter and soil the extent of the nitrogen isotope discrimination for the eventual production of NO_3^- from organic nitrogen (ΔNO_3^- : organic N) was diminished with decreasing levels of pollution (Figures 8a and 8b). A difference of approximately -8‰ was found in soil and litter between the mean $\delta^{15}\text{N}$ of the bulk nitrogen and the mean $\delta^{15}\text{NO}_3^-$ at CP. The respective difference at BF was only about -2‰.

Similarly, the isotopic discrimination for nitrification (ΔNO_3^- : NH_4^+) in soil and litter was highest at CP and lowest at CA and BF (Figures 9a and 9b). The difference between the mean $\delta^{15}\text{NH}_4^+$ and the mean $\delta^{15}\text{NO}_3^-$ values in the soil was about -11‰ at CP and about -4‰ at BF. The respective difference in the litter was about -8‰ at CP and about +2‰ at BF. At CA and BF the $\delta^{15}\text{NO}_3^-$ values in litter were more positive than their $\delta^{15}\text{NH}_4^+$ signatures. This is opposite to the observations in the litter at CP and DW and in the soil at all sites. This could have resulted from denitrification. The summer dry conditions that persisted at all sites were not particularly favorable for denitrification. However, its impact on litter $\delta^{15}\text{NO}_3^-$ values of the two less polluted sites (CA and BF) might have been greater because of the smaller litter NO_3^- pool at these sites compared to CP.

The strongly enriched soil $\delta^{15}\text{NH}_4^+$ values (Figure 5a), coupled with depleted $\delta^{15}\text{NO}_3^-$ values (Figure 5b) at CP, suggest rapid turnover of a large NH_4^+ pool due to nitrification with continuous replenishment by atmospheric deposition and mineralization. Soil

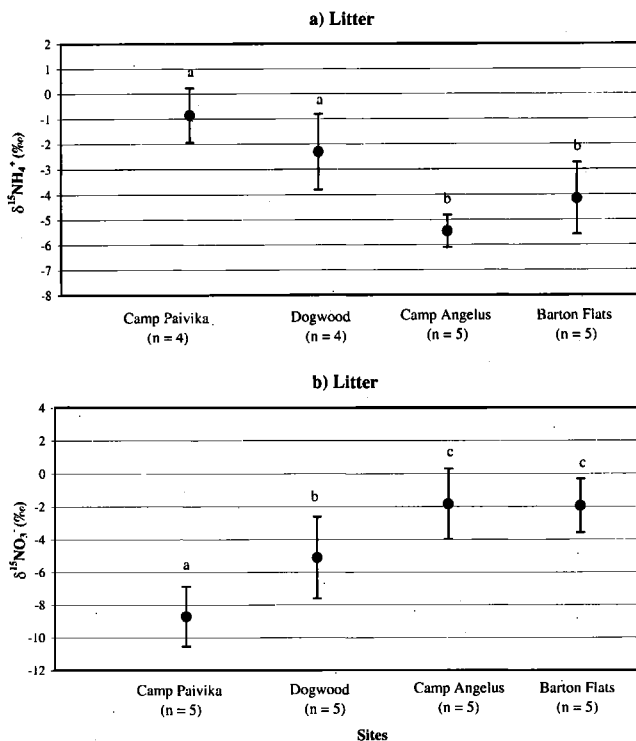


Figure 7. Litter $\delta^{15}\text{NH}_4^+$ and $\delta^{15}\text{NO}_3^-$ values across the west to east air pollution gradient. Means followed by the same letter are not statistically different (mean \pm σ).

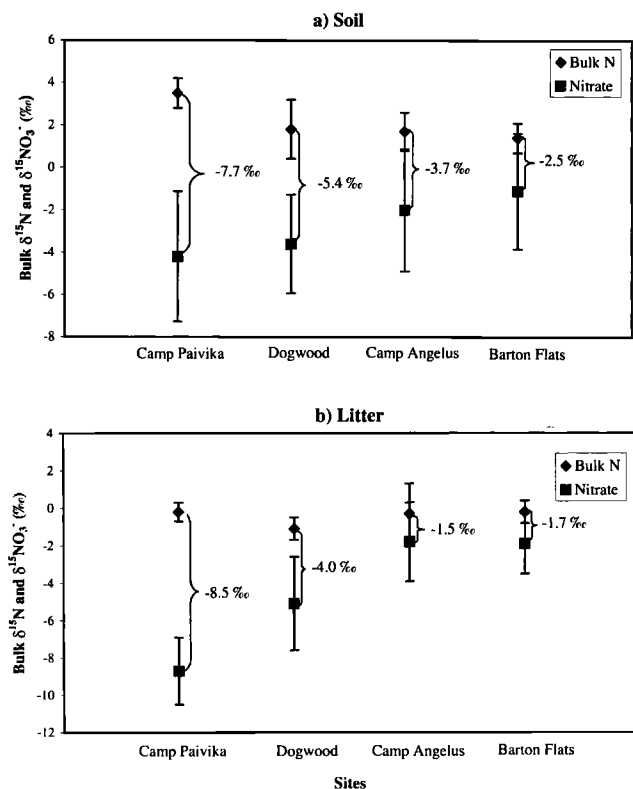


Figure 8. Isotopic discrimination, $\Delta_{\text{NO}_3^- - \text{organic N}}$, for the eventual conversion of organic nitrogen to nitrate across the west to east air pollution gradient.

$\delta^{15}\text{NH}_4^+$ was significantly more enriched at CP than at the less polluted sites. This was likely a result of nitrification, which favors utilization of the lighter isotope, and because NH_4^+ supply was not a limiting factor. The isotope data were further supported by the soil nutritional analysis data. Soil NO_3^- concentrations were highest at CP (Figure 3a), indicating also the predominance of nitrification and the availability of a large NH_4^+ pool. Isotopic values of soil NO_3^- were not statistically different among sites. Soil NO_3^- at the less polluted sites tended to be isotopically similar to the total soil $\delta^{15}\text{N}$ (Figure 8a), indicating the limited supply of NH_4^+ from mineralization and/or atmospheric deposition that would serve as a substrate for nitrification. This situation is characteristic of a nitrogen-limited ecosystem [Heaton, 1986].

The fact that the soil NO_3^- at CP was not isotopically different from the other sites could be the net effect of different counteracting processes. Although oxidation of a continuously replenished NH_4^+ pool would initially produce a very depleted NO_3^- , increased losses of nitrogenous gases during either nitrification or denitrification would enrich the remaining NO_3^- . Nitrogenous emissions have been found to be several times higher at CP than at BF [Fenn et al., 1996], although loss of nitrogen gases during nitrification usually accounts for only a small percentage of the NH_4^+ converted to NO_3^- .

A pattern similar to the soil of strongly enriched $\delta^{15}\text{NH}_4^+$ and depleted $\delta^{15}\text{NO}_3^-$ (Figures 7a and 7b) was also observed in the litter at CP (Figures 5a and 5b). Litter NH_4^+ was significantly more enriched at CP than at the less polluted sites. Litter NO_3^- at the two less polluted sites was isotopically enriched relative to NO_3^- at the highly polluted sites. These isotope trends in the litter suggested, once more, increased nitrogen cycling and the predominance of nitrification at CP. The litter isotope data were further supported by the nutrient analysis data (Figure 3b), which illustrated enhanced nitrification at CP, as in the case of the soil. Similar to what was observed in the soil, litter NO_3^- at the less

polluted sites had an isotopic composition closer to that of the total nitrogen (Figure 8b), again indicating that the less polluted sites were nitrogen limited.

3.2.3. Stable carbon isotopic variations among sites. The carbon isotopic composition of all the terrestrial samples analyzed were consistent with reported ranges of $\delta^{13}\text{C}$ values for C_3 plants [Rounick and Winterbourn, 1986] and organic matter in forest soils under C_3 vegetation [Balesdent et al., 1987]. A significant gradient of decreasing soil, litter, and foliar $\delta^{13}\text{C}$ was observed with increasing site pollution level (Figures 10a, 10b and 10c). Soil $\delta^{13}\text{C}$ values ranged from -26.1 to -25.3‰ at CP and from -24.7 to -24.2‰ at BF. Litter and vegetation $\delta^{13}\text{C}$ values were likewise more depleted at CP (ranging from -27.2 to -26.1‰ and -28.8 to -26.0‰, respectively) than at BF (ranging from -25.5 to -24.2‰ and -27.4 to -25.3‰, respectively). Garten and Taylor [1992] noted a >2‰ enrichment in the $\delta^{13}\text{C}$ of conifer needles over broadleaf deciduous trees, but no such differences were observed among species in the present study.

It has been suggested that such variations in the $\delta^{13}\text{C}$ of plants can arise from differences in available soil water. Plants from drier environments tend to have higher $\delta^{13}\text{C}$ values than plants from wetter environments [Farquhar et al., 1989; Garten and Taylor, 1992]. However, in this study, sampling was conducted during the summer, when dry conditions predominate at all sites. The assertion that the difference in $\delta^{13}\text{C}$ among sites did not result from variances in water use efficiency was further supported by two facts: first CP and BF receive similar amounts of precipitation annually (Table 1), and second, even though DW receives the highest amounts of precipitation annually, there was no depletion in ^{13}C of plants at DW relative to CP.

Another source of variation in vegetation $\delta^{13}\text{C}$ values among sites was eliminated by sampling at approximately the same canopy heights at all sites. Foliage from greater heights has been shown to be more enriched in ^{13}C than that at lower levels [Heaton and Crossley, 1995; Garten and Taylor, 1992]. Likewise, differences in radiation fluxes could introduce differences in the $\delta^{13}\text{C}$ values among sites. In general, foliar $\delta^{13}\text{C}$ values become more enriched with increasing irradiance [Farquhar et al., 1989]. However, radiation fluxes are similar between CP and BF (M. Poth, unpublished data, 1995).

Moreover, it has been observed that carbon isotope discrimination declines with increasing altitude, and this has been attrib-

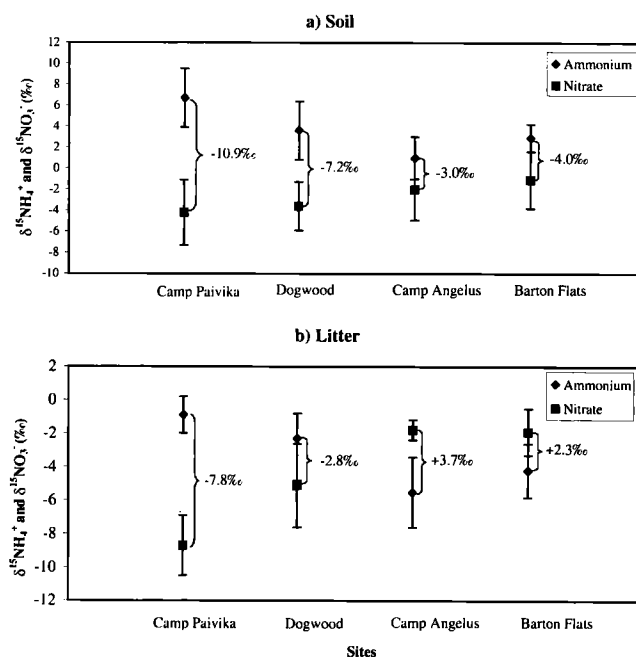


Figure 9. Isotopic discrimination, $\Delta_{\text{NO}_3^- - \text{NH}_4^+}$, for the process of nitrification across the west to east air pollution gradient.

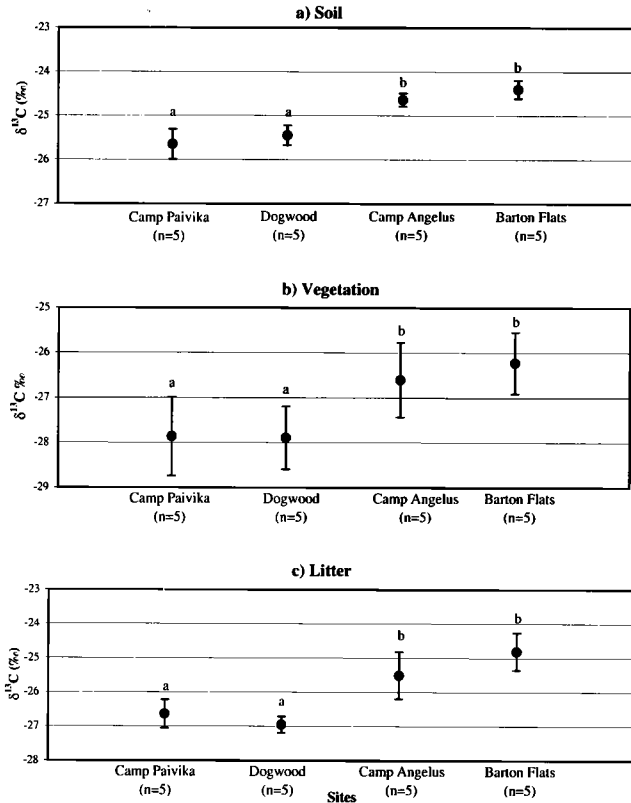


Figure 10. δ¹³C values across the west to east air pollution gradient. Means followed by the same letter are not statistically different (mean ± σ).

uted to increased carboxylation efficiencies with increasing altitude [Körner *et al.*, 1988, 1991]. It is believed that altitudinal differences alone could not have caused the enrichment in ¹³C at BF because the elevation difference between CP and BF is small (0.346 km). Körner *et al.* [1988] proposed a regression line that relates the carbon isotopic composition of plants with elevation. For trees, in particular, they determined the following equation: $Y = -29.38 + 0.880 X$ ($R^2 = 0.58$), where $Y = \delta^{13}C$ (‰) and X is the altitude difference between sites (km). The above equation was applied to predict whether the elevation difference between CP and BF could have produced the observed differences in the organic carbon stable isotopic composition. For the elevation difference between CP and BF ($X = 0.346$ km) the predicted δ¹³C difference was found to be 0.3‰. According to this relationship, the vegetation at BF should have had an average δ¹³C value of about -27.6‰. Nevertheless, foliage at BF was significantly more enriched (mean = -26.2‰).

Considering that environmental parameters, including site altitude and light, and sampling factors, such as canopy height and soil depth, did not account for the carbon isotopic differences among sites, the depletion in ¹³C at the highly polluted sites was most likely the consequence of the long-term exposure of the forest to air pollution. Changes in the isotopic composition of leaf tissues resulting from exposure to gaseous air pollutants have been documented previously. Both an enrichment [Farquhar *et al.*, 1989] and a depletion [Heaton and Crossley, 1995] of foliar ¹³C in plants subjected to air pollution compared with plants growing in clean air have been observed. The data in the present study are in agreement with the findings of Heaton and Crossley [1995].

The δ¹³C values suggest that the increased nitrogen content of the foliage did not actually enhance photosynthesis, as would have been expected [Field and Mooney, 1986]. One plausible explanation is that the depletion of δ¹³C at the highly polluted sites may

have resulted from depressed rates of carbon assimilation by physiologically stressed trees. Reduction in carbon fixation in the forests in the San Bernardino Mountains has been documented by Miller [1989] and has been linked to their exposure to increased ozone levels. Inhibition of photosynthesis could also have resulted from nitrogen saturation. Nitrogen saturation can lead to nutrient imbalances in roots and leaves through soil acidification, which in turn can reduce photosynthetic efficiency [Asner *et al.*, 1997; Vitousek *et al.*, 1997]. The combined effect of ozone exposure and increased nitrogen deposition may have resulted in decreased photosynthesis in the San Bernardino National Forest.

A different interpretation for the observed stable carbon isotope gradient is that the depleted δ¹³C values at the highly polluted sites may have resulted from fixation of ¹³C-depleted atmospheric CO₂ produced by fossil fuel combustion. Fossil fuel combustion has been found to produce isotopically light CO₂ (-25 to -35‰ [Friedman and Irsa, 1967]). Evans *et al.* [1986], using gas exchange methods, found that the net fractionation during photosynthesis was not affected by changes in the isotopic composition of the source CO₂. In such a case, assimilation of an isotopically depleted CO₂ at the more polluted sites could influence the stable carbon isotopic composition of the forest species and generate the observed isotopic gradient.

3.2.4. Link between carbon and nitrogen isotope ratios. Linking stable carbon with stable nitrogen isotopic measurements is a more powerful approach toward identifying ecosystem components and biogeochemical processes that are most sensitive to disturbances, since one of the factors controlling photosynthesis is nitrogen availability. Distributions of paired bulk δ¹⁵N and δ¹³C values of soil and vegetation illustrated that at CP the enrichment in ¹⁵N was coupled with a depletion in ¹³C (Figures 11a and 11b). Furthermore, litter was more depleted in ¹³C at CP and DW, but

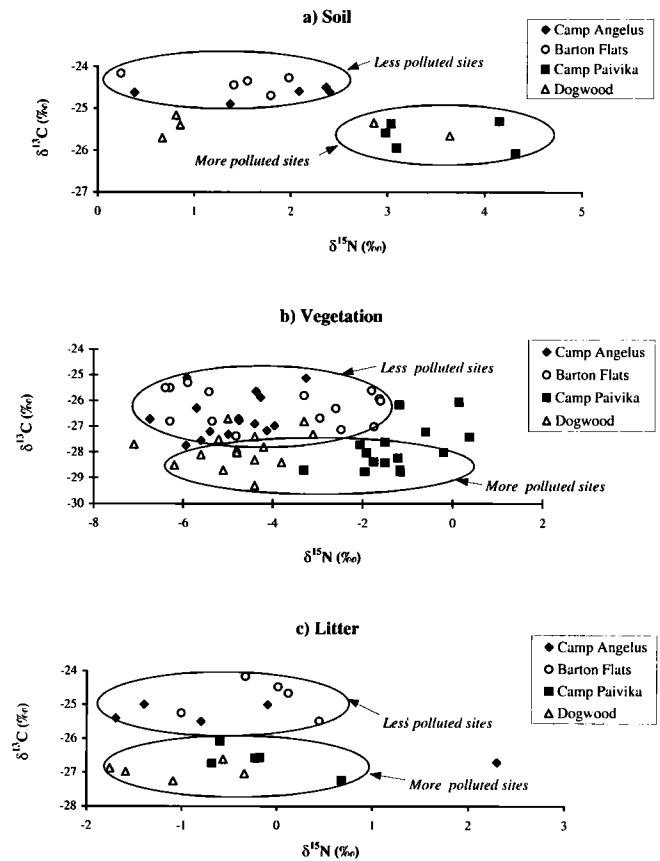


Figure 11. δ¹³C values versus δ¹⁵N values at all sites. Means followed by the same letter are not statistically different (mean ± σ).

this depletion was not accompanied by an enrichment in ^{15}N , as in the case of soil and vegetation (Figure 11c).

The correlation between stable carbon and stable nitrogen isotopic signatures determined in this study is believed to be indicative of anthropogenically induced changes in the San Bernardino National Forest. Both the carbon and the nitrogen cycles in this forest have been affected by air pollution. The $\delta^{13}\text{C}$ values provided insight into the process of photosynthesis, and the $\delta^{15}\text{N}$ values allowed insight into patterns of nitrogen soil transformations, uptake by plants, and return to the soil.

4. Conclusions and Summary

Heightened nitrogen deposition into forest ecosystems has received increasing attention in the last 10 years because it can contribute to forest decline. This study attempted to trace anthropogenically induced changes in the nitrogen cycling and the carbon metabolism of the San Bernardino National Forest by examining natural abundances of stable nitrogen and carbon isotopes across an air pollution gradient. The following conclusions can be drawn from this study.

1. In the San Bernardino National Forest, there was a spatial gradient in exchangeable nitrogen concentrations, directly correlated with nitrogen deposition. Higher total nitrogen levels were found in soil, litter, and foliage at CP compared with the other sites. The NH_4^+ and NO_3^- concentrations in soil and litter were comparable to those determined by Fenn *et al.* [1996] for the same sites.

2. A distinct spatial influence was also noted in the stable nitrogen isotopic composition of the different terrestrial compartments. Soil and foliar $\delta^{15}\text{N}$ values in the San Bernardino National Forest were positively correlated with sizes of available nitrogen pools at CP and BF. The ^{15}N enrichment at CP reflects the efficacy of excess nitrogen deposition to stimulate nutrient cycling processes at this site. These results are in agreement with previous studies that investigated the nitrogen isotopic composition of soil and foliage at locations with experimentally induced [Högberg *et al.*, 1992; Högberg and Johannison, 1993; Johannison and Högberg, 1994] or naturally occurring [Garten, 1993; Garten and Van Miegroet, 1994] increased soil nitrogen.

3. The soil and litter $\delta^{15}\text{NH}_4^+$ were significantly more enriched at CP than at the less polluted sites. Soil $\delta^{15}\text{NO}_3^-$ values were not different among sites, whereas litter $\delta^{15}\text{NO}_3^-$ values were significantly depleted at CP compared to the other sites. The more enriched $\delta^{15}\text{NH}_4^+$ values, combined with the depleted $\delta^{15}\text{NO}_3^-$ values and the higher NO_3^- concentrations in soil and litter at CP, suggest that a large NH_4^+ pool was maintained by atmospheric inputs and mineralization, while nitrification was proceeding at a faster rate at CP [Fenn *et al.*, 1996] than at the other sites.

4. Nitrogen isotope fractionation effects were directly related to the exposure of the site to pollution. Fractionations were maximized at the highly polluted sites and minimized at the less polluted sites. The isotopic discrimination for the overall conversion of organic nitrogen to NO_3^- ($\Delta_{\text{NO}_3^- \text{ organic N}}$) in the soil and litter was highest at CP and lowest at the less polluted sites. The greater convergence between $\delta^{15}\text{N}$ and $\delta^{15}\text{NO}_3^-$ values in soil and litter at CA and BF indicated nitrogen-limited systems, as opposed to CP.

5. A gradient of decreasing $\delta^{13}\text{C}$ values in soil, litter, and vegetation was found with increasing site pollution level. The depletion at the highly polluted sites could be attributed to either decreased photosynthetic rates caused by air pollution or assimilation of a more depleted CO_2 source. The first explanation is consistent with previous studies that investigated the effects of ozone on the San Bernardino National Forest [Miller, 1989] and with recent studies that suggest that nitrogen saturation can eventually lead to decreased carbon fixation [Asner *et al.*, 1997; Vitousek *et al.*, 1997].

6. Air pollution has induced changes in the nitrogen cycling of the forest at CP. An enrichment in ^{15}N coupled with a depletion in ^{13}C in soil and vegetation indicates anthropogenically induced changes in the San Bernardino National Forest.

Nitrogen saturation does not necessarily imply a disastrous, visible impact on forest health [Aber *et al.*, 1989; Skeffington, 1990]. Both the isotopic data and the nutritional parameters determined in this study support the hypothesis put forth by Fenn *et al.* [1996] that CP might be approaching a nitrogen-saturated state. This hypothesis is also supported by nitrogen fertilization experiments conducted at CP, which showed no foliar growth response in *P. Ponderosa*, as would occur in a nitrogen-limited system [Kiefer and Fenn, 1997].

The potential long-term effects of nitrogen saturation on the nutritional status of the San Bernardino National Forest are unknown. The results of this study do not imply that the forest at CP is approaching or will approach a declining state. Visible injury to vegetation in the San Bernardino National Forest has thus far been attributed to ozone [Miller *et al.*, 1982; Miller, 1989]. However, Aber *et al.* [1989] pointed out that because ozone has the potential to decrease photosynthetic rates, the combined stresses of excess nitrogen deposition and ozone can intensify the effects of nitrogen saturation and ultimately lead to forest decline.

A tangible concern with nitrogen saturation in this forest ecosystem is stream water quality. Increased NO_3^- concentrations have been reported in chaparral watersheds in the San Gabriel Mountains, north/northeast of Los Angeles [Riggan *et al.*, 1985, 1994]. Preliminary stream water analyses in the San Bernardino National Forest confirm the hypothesis that western watersheds, exposed to the highest atmospheric nitrogen loading, exhibit higher streamwater NO_3^- concentrations ($\sim 5.5 \text{ mg L}^{-1}$) than eastern watersheds ($\sim 0.18 \text{ mg L}^{-1}$) (M.E. Fenn, personal communication, 1998).

This study establishes the use of stable isotope analysis at natural abundance levels to investigate the effects of excess atmospheric nitrogen deposition to forest ecosystems. Stable isotopes provide a powerful tool to follow anthropogenically induced changes to the nitrogen and carbon biogeochemical cycles and to indicate which processes or components are most sensitive to human perturbation. Garten [1993] and Garten and Van Miegroet [1994] proposed that natural variations in foliar ^{15}N abundance may serve as an indicator of nitrogen availability. The results of this study are consistent with their hypothesis. This study further shows that natural abundances of ^{15}N , $^{15}\text{NH}_4^+$, and $^{15}\text{NO}_3^-$ in soil can serve as sensitive indicators of enhanced nitrogen transformations in forest soils.

Future work should include additional ^{15}N natural abundance measurements of dry deposition, precipitation, surface water, groundwater, and soil NO_x as well as investigation of seasonal isotopic variations. Total nitrogen, as well as inorganic and organic nitrogen stable isotopic measurements are needed to link all aspects of the nitrogen cycle, understand the effects of human perturbations to ecosystems, and develop predictive ecosystem models. Additional dual stable isotopic measurements of carbon and nitrogen can also be very useful in revealing the responses and interactions of the two biogeochemical cycles under increased anthropogenic activities.

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