Alteration of belowground carbon dynamics by nitrogen addition in southern California mixed conifer forests

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[1] Nitrogen deposition rates in southern California are the highest in North America and have had substantial effects on ecosystem functioning. We document changes in the belowground C cycle near ponderosa pine trees experiencing experimental nitrogen (N) addition (50 and 150 kg N ha^{-1} a^{-1} as slow release urea since 1997) at two end-member sites along a pollution gradient in the San Bernardino Mountains, California. Despite considerable differences in N deposition between the two sites, we observed parallel changes in microbial substrate use and soil enzyme activity with N addition. $\Delta^{14}C$ measurements indicate that the mean age of C respired by the Oa horizon declined 10–15 years with N addition at both sites. N addition caused an increase in cellulolytic enzyme activity at the polluted site and a decrease in ligninolytic enzyme activity at the unpolluted site. Given the likely differences in lignin and cellulose ages, this could explain the difference in the age of microbial respiration with N addition. Measurements of fractionated soil organic matter did not show the same magnitude of changes in response to N addition as were observed for respired C. This lesser response was likely because the soils are mostly composed of C having turnover times of decades to centuries, and 9 years of N amendment were not enough to affect this material. Consequently, $\Delta^{14}C$ of respired CO_2 provided a more sensitive indicator of the effects of N addition than other methods. Results suggest that enhanced N deposition alone may not result in increased soil C storage in xeric ecosystems.

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1. Introduction

[2] Nitrogen (N) is the element limiting primary production in most terrestrial ecosystems [*Vitousek and Howarth*, 1991]. Fertilizer use and fossil fuel combustion have more than doubled the input of biologically available N into ecosystems, mainly in the northern hemisphere [*Vitousek et al.*, 1997]. This generally leads to an enhancement of aboveground plant productivity; however, at very high levels, N deposition can lead to N saturation, characterized by the alleviation of N limitations on primary productivity, soil acidification, and increased N losses from the ecosystem [*Aber et al.*, 1989]. Enhanced N deposition is widespread [*Galloway et al.*, 1995], and N saturation has been observed in numerous temperate forest ecosystems in North America (summarized by *Fenn et al.* [1998]).

[3] N deposition has also been demonstrated to affect belowground carbon cycling, although the net result on soil carbon (C) storage is unclear. Changes in litter quality and quantity, as well as in microbial community composition and activity can all affect decomposition and soil respiration rates. The degree to which each of these changes is expressed will vary with climate. Most results to date explore changes in mesic, temperate systems, but in xeric or cryic ecosystems, where decomposition rates are limited by lack of water or freezing temperatures, the rate of response to N deposition may be dramatic, as evidenced by rapid loss of centuries old C in tundra soils exposed to N + P addition [*Mack et al.*, 2004; *Nowinski et al.*, 2008].

[4] As aboveground primary production generally increases with N deposition, leaf litter inputs to the soil surface are higher [*Vitousek and Howarth*, 1991]. Litter quality (as measured by C:N, lignin:N or N content of litter) also increases with N deposition. Higher quality litter is associated with higher rates of decomposition [*Swift et al.*, 1979; *Aber and Melillo*, 1982; *Melillo et al.*, 1982] and decomposition increases in response to N addition [*Knorr et al.*, 2005], at least in the short term. Most sequential coring studies suggest that fine root production does not change or decreases with N addition, although C and N budget-based methods infer increased root N inputs [*Nadelhoffer*, 2000]. Plants may allocate a smaller proportion of C to the rhizosphere due to decreasing nutrient needs, but with increased N concentrations in roots, the overall effect of

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changing belowground litter quality and quantity is unclear [*Nadelhoffer*, 2000].

[5] The makeup of the microbial community is also altered by N addition or deposition. Numerous enzyme assay studies have found that cellulolytic enzymes are more active with greater N, while lignin-oxidizing enzymes are less active with N enrichment [*Carreiro et al.*, 2000; *Frey et al.*, 2004; *Saiya-Cork et al.*, 2002; *Sinsabaugh et al.*, 2002]. The decrease in lignin degradation may reflect reduced activity of microorganisms capable of degrading lignin, particularly white-rot fungi, and/or the N-mediated enhancement of recalcitrance, while cellulose degradation may be enhanced because of reduced N limitation of some soil organisms that degrade cellulose [*Fog*, 1988].

[6] In short-term experiments, the enhancement of cellulose decomposition, which occurs early in the decomposition process, generally dominates the response to N additions, while in long-term experiments, the depression of lignin degradation becomes more important as cellulose has largely been lost [*Fog*, 1988]. These factors can contribute to differing responses to N amendment across ecosystems and soil fractions depending on the amount of labile C. For example, *Neff et al.* [2002] found that in alpine tundra soils where decomposition rates are low most of the year due to cold temperatures, N addition accelerated decomposition in the light fraction where largely labile C resides, while having little effect on the heavy fraction where more recalcitrant C resides. Consequently, long-term N addition in xeric or cryic ecosystems might not result in lower decomposition, as frequently seen in mesic systems, because undecomposed litter and consequently, cellulose stores, are likely to be more abundant.

[7] This study explores the effects of N amendment on two xeric sites in the San Bernardino Mountains of southern California that vary in background N deposition rate and pollution. Owing to high rates of fossil fuel combustion, nitrogen deposition rates in southern California are the highest in the United States, with up to 70 kg N ha^{-1} being deposited annually. Nitrogen saturation symptoms have been observed at the polluted end-member site and over the western end of the San Bernardino Mountains [*Fenn et al.*, 1996].

[8] Previous studies at these sites have shown a wide variety of changes associated with the pollution gradient. The high pollution site is highly N enriched exhibiting severe symptoms of N saturation, as demonstrated by high nitrate concentrations in soil and in stream water runoff as well as N enrichment of vegetation. In contrast, N cycling remains conservative at the low pollution site [*Fenn and Poth*, 1999b; *Fenn et al.*, 1996, 2008]. In addition to these N eutrophication effects, the high pollution site has experienced severe soil acidification and decreasing base saturation as a result of N deposition over the past half century [*Wood et al.*, 2007]. These forests are less likely to decline from soil acidification effects due to relatively high base saturation, even in high deposition sites. However, forest health is affected by air pollution in the San Bernardino Mountains because the combined effects of ozone and N deposition increase susceptibility to bark beetles, reduce root biomass, and increase drought stress and risk of severe fire occurrence [*Jones et al.*, 2004; *Grulke et al.*, 2008].

Table 1. Site Information for Camp Osceola and Camp Paivika[a]

	Camp Osceola	Camp Paivika
Mean annual temperature (°C)	10.6	12.9
Mean annual precipitation (cm)	90	98
Elevation (m)	2135	1580
Ozone concentration (ppb h^{-1})	62	80
N deposition (kg ha a^{-1})	7.5	71.1
pH (A horizon)	5.77	3.98

[a]Temperature from *Fenn and Poth* [1999a, 1999b], elevation from *Fenn et al.* [2005], precipitation and ozone from *Grulke et al.* [2002], N deposition from *Fenn et al.* [2008], and pH from *Grulke et al.* [1998].

[9] Ozone is also present at high levels in the polluted site (Table 1) and has several effects on ecosystem functioning [*Fenn and Bytnerowicz*, 1993]. Ozone damage to ponderosa pine needles at the high pollution site causes them to be replaced more frequently, resulting in increased allocation to aboveground plant tissues [*Grulke and Balduman*, 1999]. The variation in $\delta^{13}C$ signatures and N contents of ponderosa pine is consistent with depressed photosynthesis and higher N availability in the more polluted end of the gradient. Consequently, litter inputs of C and N are much higher on the polluted end of the gradient, which ultimately results in extremely thick litter layers [*Grulke et al.*, 1998; *Grulke and Balduman*, 1999].

[10] Changes in decomposition and respiration observed along the gradient appear to differ from those resulting from N amendment in more mesic environments. *Fenn and Dunn* [1989] found that heterotrophic respiration rates and fungal diversity in recently fallen litter increased with increasing pollution inputs. CO_2 evolution rates were positively correlated with litter N concentration and negatively correlated with litter Ca concentration, both of which would be associated with younger needles and consequently may result from ozone damage more than increased soil N availability [*Fenn and Dunn*, 1989]. It is difficult to separate the effects of N from the effects of ozone and litter inputs in a gradient study. Therefore, N addition treatments were begun at two of the end-member sites in 1997, and we sampled the different levels of N amendment at both sites in 2006. We combined radiocarbon measurements of organic matter and microbially respired CO_2 with measures of C stocks and fluxes to quantify differences in C cycling with 9 years of N amendment in sites representing high and low ends of the pollution gradient.

2. Methods

2.1. Research Sites

[11] Two sites were chosen along a gradient of pollution in the San Bernardino Mountains east of Los Angeles, which have been receiving elevated N inputs since at least 1950 [*Miller and McBride*, 1999]. Dominant species at the sites include ponderosa pine (*Pinus ponderosa*), Jeffrey pine (*Pinus jeffreyi*), and to a lesser extent California black oak (*Quercus kelloggi*), incense cedar (*Calocedrus decurrens*), white fir (*Abies concolor*), and sugar pine (*Pinus lambertiana*) [*Fenn and Dunn*, 1989]; our study considered N amendment of ponderosa and Jeffrey pine only. The soils are derived from partially weathered granitic rock. Soils at Camp Paivika (CP) are coarse-loamy, mixed mesic, Ultic Haploxerolls of the

Shaver series, while soils at Camp Osceola (CAO), near Barton Flats, are coarse-loamy, frigid, Xerumbrepts [*Arkley*, 1981]. Other site characteristics are reported in Table 1. The plots selected have not burned in the last 100 years, but there has been recent thinning of trees at both sites by the National Forest Service.

[12] Nitrogen addition treatments were performed at both sites in order to separate effects of N from other pollutants. N has been added annually each autumn since 1997 in the form of granular urea formaldehyde, a formulation in which N is released slowly by microbial activity [*Corke and Robinson*, 1966], resulting in the buildup of soil N stores over the long-term [*Martikainen et al.*, 1989]. Consequently, urea formaldehyde generally has less impact on the microbial community than other types of fertilizer [*Aarnio and Martikainen*, 1995; *Holopainen and Heinonentanski*, 1993; *Martikainen et al.*, 1989]. N was added at levels of 50 and 150 kg N ha^{-1} a^{-1}, to the drip line around ponderosa and Jeffery pine trees at least 5 m apart. These N additions brought total estimated N deposition rates to ~70, 120 and 220 kg N ha^{-1} a^{-1} (control, +50 and +150) at CP, and ~8, 58 and 158 kg N ha^{-1} a^{-1} at CAO.

2.2. Soil Sampling

[13] Samples of Oi (organic horizon of plant and animal parts, only slightly decomposed) and Oa (organic horizon of highly decomposed, amorphous residues) and uppermost A (mineral) horizons were taken in the (N/NE) quadrant ~0.5 m from the bole of ponderosa pine trees at the long-term N addition experiment sites. Three trees were selected randomly for each treatment (control, +50 and +150) and treated as replicates. Soils for incubations were collected in midwinter 2006 for SOM and incubation analysis and mid-summer for enzymatic analysis by cutting a 15 × 15 cm square of litter (and Oa at CAO) then taking a 5 cm diameter core of top 10 cm of the A horizon (and Oa at CP). In order to minimize disturbance on the treatment plots, only one sample was taken per plot. Soils were brought back to the lab, weighed, and homogenized, and subsamples were dried for gravimetric moisture analysis. Bulk density was calculated using the wet weights and gravimetric moisture contents. C contents from combustion yields and bulk densities were used to calculate the C inventories, although because the A horizon integrates different depths at the two sites due to differences in the upper part of the soil profile, the total C inventories are not comparable for this horizon.

2.3. Organic Matter Fractionation

[14] Organic matter (OM) samples used in incubation studies (see below) were physically fractionated based on particle size and density. Oa and A horizon materials were first sieved using a 1 mm sieve and the >1 mm size fraction (coarse) was stored. Oi soils consisted entirely of the coarse fraction. The <1 mm size fraction was separated into high and low density fractions by centrifugation using a sodium polytungstate ($Na_6[H_2W_{12}O_{40}]$) solution with a density of 2 g/mL [*Gaudinski et al.*, 2000], called the "heavy" (>2 g/mL) and "light" (<2 g/mL) fractions. Following fractionation, soils were dried, ground, and combusted for radiocarbon and $\delta^{13}C$ analysis. C content values were obtained from manometric determination of CO_2 yield on combustion and used to calculate soil C stocks.

[15] In order to ascertain the relationship of cellulose to respiration and the other soil fractions, holocellulose was extracted from the Oa horizon soils (<1 mm size fraction) from CP using a 2:1 toluene:ethanol extraction, followed by an ethanol-only extraction, then finally by bleaching with a sodium chorite/acetic acid solution [*Leavitt and Danzer*, 1993]. After extraction, samples were rinsed with deionized water and dried, and $\Delta^{14}C$ and $\delta^{13}C$ analyses were performed.

2.4. $\Delta^{14}C$ and ^{13}C Analysis

[16] Soil and gas samples were analyzed for radiocarbon content at the Keck Carbon Cycle AMS Facility at UC Irvine. Gas samples were taken directly from the evacuated canisters in which they were collected. Soil organic matter samples were deposited in quartz tubes, evacuated, sealed, and placed in a 900°C combustion oven for two hours, following which the CO_2 was obtained by cracking the tube. The rest of the purified CO_2 was converted to graphite using the sealed zinc tube reduction method and a Fe catalyst [*Xu et al.*, 2007]. $\Delta^{14}C$ units are used to report these data because we are modeling changes in the absolute number of ^{14}C molecules over time, and are defined as:

$$\Delta^{14}C = \left[\frac{\left(\frac{^{14}C}{^{12}C}\right)_{\text{sample},-25}}{0.95\left(\frac{^{14}C}{^{12}C}\right)_{\text{OX1},-19} \exp^{\left((2006-1950)/8267\right)}} - 1 \right] * 1000$$

. . .or the deviation from unity, in parts per thousand, of the $^{14}C/^{12}C$ ratio of the sample from that of an internationally accepted standard (Oxalic Acid I, corrected for radioactive decay of $\Delta^{14}C$ between the year of sampling and 1950) [*Stuiver and Polach*, 1977]. Samples were normalized to a $\delta^{13}C$ of −25‰, assuming $\Delta^{14}C$ fractionation is twice that of $\delta^{13}C$, so that $\Delta^{14}C$ measurements do not reflect mass dependent fractionation. $\delta^{13}C$ was measured concurrently with $\Delta^{14}C$ on the Keck Carbon Cycle AMS and used for fractionation correction [*Southon et al.*, 2004]. Higher precision $\delta^{13}C$ measurements were made on an aliquot of purified CO_2 with a Gasbench II interface to a continuous flow stable isotope ratio mass spectrometer (Delta-Plus CFIRMS).

2.5. Modeling Turnover Time

[17] The turnover time of soil C was estimated from $\Delta^{14}C$ values. The $\Delta^{14}C$ of newly fixed plant C reflects the $\Delta^{14}C$ signature of the atmosphere. Since the 1950s, the $\Delta^{14}C$ of atmospheric CO_2 has varied [*Levin and Kromer*, 2004], mainly because of atmospheric weapons testing. The $\Delta^{14}C$ signature of the atmosphere increased dramatically during the late 1950s and early 1960s (Figure 1). Since then, $\Delta^{14}C$ values have declined as 'bomb' $\Delta^{14}C$ is fixed into ocean and terrestrial C reservoirs, and CO_2 from the burning of fossil fuels, which are devoid of radiocarbon, is added to the atmosphere (−1000‰; the Suess Effect). This allows us to use radiocarbon to determine mean ages and turnover times on annual to decadal time scales [*Broeker and Peng*, 1982] in addition to the centennial to millennial times scales addressed with traditional radiocarbon dating.

[18] Because most of the C in the Oi and Oa horizons was likely accumulated over time following fire, and thus not at

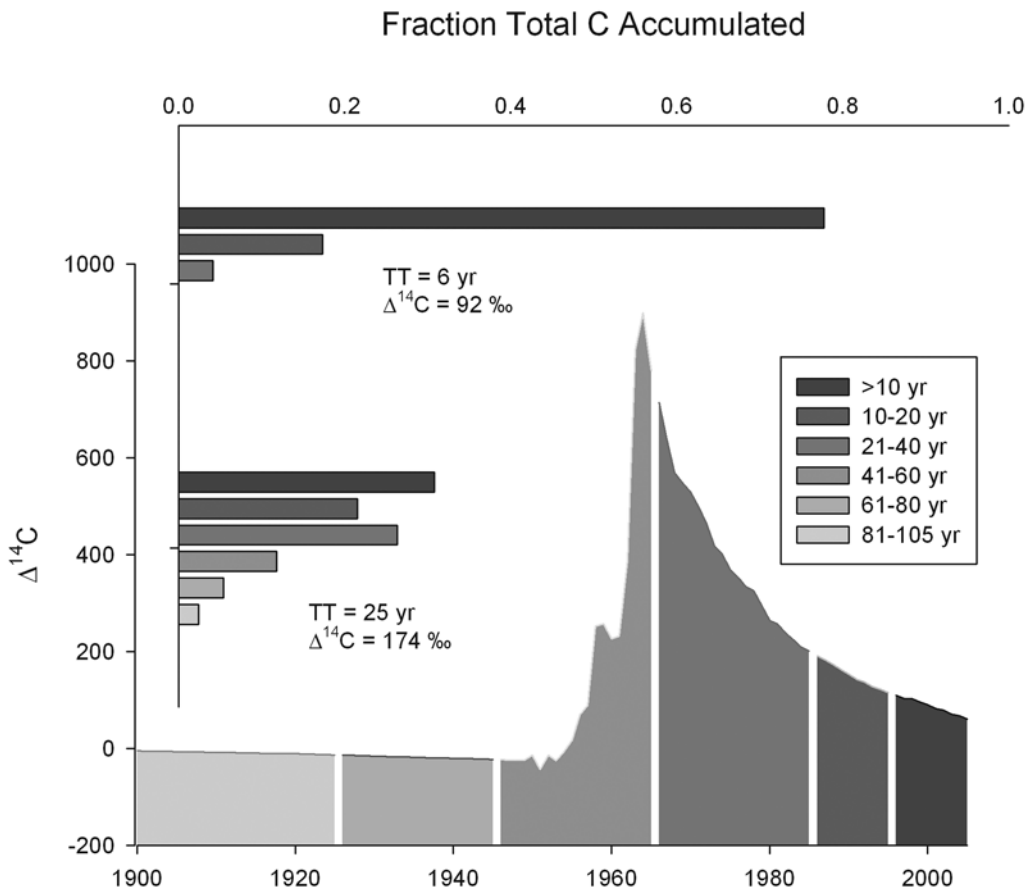

Figure 1. Fraction of total C accumulated from different age groups. $\Delta^{14}C$ signature of the atmosphere since 1900 from *Levin and Kromer* [2004] and Trumbore laboratory.

steady state, we used a vertical accumulation model to estimate input and decomposition rates from the amount and $\Delta^{14}C$ values of accumulated O horizon material [*Trumbore and Harden*, 1997; *Hahn and Buchmann*, 2004]. This approach is illustrated in Figure 1, which shows the relative fraction of C of different ages accumulated over the last century, assuming different turnover rates. Pools with relatively long turnover times (~100 years) would have approximately equal parts prebomb (~0‰) and postbomb carbon (≫0‰), while C pools cycling on decadal (e.g., 25 years) timescales would be comprised almost entirely of postbomb C (≫0‰). Extremely fast cycling C pools would be comprised of C from only the last few years, which may have a radiocarbon signature close to that of a mixture of prebomb and postbomb C. The two can be differentiated by examining estimates of the total amount of C that should have accumulated; otherwise, rates of C input (litterfall) or loss (heterotrophic respiration) can be used to determine which age or turnover time is more reasonable [*Gaudinski et al.*, 2000].

2.5.1. Accumulation Model

[19] The turnover time of C within each fraction was estimated by matching modeled and measured $\Delta^{14}C_{OM}$ and C inventory values, within one standard deviation of the mean, given specified input values which were deemed reasonable given prior measurements at the sites. For each fraction, we assumed annual C inputs are constant over time (I), and layers build up vertically. After its addition to soil (in year y), we assume the amount of carbon declines with time according to a first order decay constant (k, in a^{-1}), such that the amount in 2006 is given by:

$$C_y = I \bullet e^{-k(2006-y)}$$

[20] We assumed C accumulation began in 1900, since it appears that neither site has burned in the twentieth century (R. Minnich, personal communication, 2007), although the model was not particularly sensitive after 75 years. Hence the total C is:

$$C_{total} = \Sigma\, C_y \text{ (from 1900 to y)}$$

The $\Delta^{14}C$ signature of the C added to the accumulating organic layer each year depends on two factors: the radiocarbon signature of local CO_2 in a given year ($\Delta^{14}C_{atm(t)}$) and any time lag that occurs between when C is fixed from the atmosphere and added to the soil ($\Delta^{14}C_{atm(t-lag)}$). The atmospheric $\Delta^{14}C$ signature at both sites is lower than the northern hemisphere average because of the influence of local combustion of fossil fuel CO_2. We corrected for this by measuring the $\Delta^{14}C$ signature of annual grasses in 2006 to determine the contribution of fossil fuel CO_2 [*Hsueh et al.*, 2007] and determining the ratio of the percent fossil fuel in the sample to the total fossil fuel emissions of the Los

Angeles metro area. We then used historical data on the Los Angeles metro area population (U.S. census) and U.S. per capita emissions [*Marland et al.*, 2007] to back calculate the historical $\Delta^{14}C$ record at each site. Time lags between fixation of C and addition to a given soil pool were calculated as follows: the age of the material added to the Oi layer reflected the amount of time spent on the tree (mean needle age of 2 years at CP and 4 years at CAO) [*Grulke and Balduman*, 1999]. For the Oa horizons, we added the time spent in the Oi horizon (derived from turnover time modeling), assuming that was the source of new Oa material. Over time, the radiocarbon signature of C added in year y will decline slightly due to radioactive decay at rate λCy, where λ is the decay constant of $\Delta^{14}C$ ($\ln(2)/5730 = 0.00012097\ a^{-1}$).

[21] The ratio of $\Delta^{14}C/^{12}C$ in litter inputs each year (R_{lag}) is:

$$R_{lag} = \left(\Delta^{14}C_{atm(t-lag)}/1000\right) + 1$$

The radiocarbon signature of the remaining C fixed in year y is:

$$RCy = R_{lag} * \bullet\ e^{-\lambda(2006-y)}$$

Total $\Delta^{14}C$ values for soil organic matter were calculated as follows:

$$\begin{aligned}\Delta^{14}Ctotal &= \left(\Sigma\ R_{Cy} - 1\right) \bullet 1000 \\ &= \left[\left(\Sigma R_{Cy} \bullet C_y\right)/\Sigma\ C_y - 1\right] \bullet 1000\end{aligned}$$

Each fraction was modeled as one pool, although it is clear that some fractions represent a mixture of faster and slower cycling material.

2.5.2. Steady State Model

[22] The A horizon and Oa heavy fraction are not necessarily lost during fire and are less likely to be accumulating C; therefore, a steady state model was used to calculate turnover times for these pools [*Gaudinski et al.*, 2000]. The steady state model, which is the same as the accumulation model assuming an accumulation timescale $\gg$ turnover time of the material, calculates the change in C inventory as a function of the inputs and the decay of C present.

$$dC/dt = I - kC$$

Thus, the C inventory at time (t) is:

$$C_t = I - kC_{t-1} + C_{t-1}$$

The $\Delta^{14}C_{OM}$ at time (t) is as:

$$\Delta^{14}C_{OM(t)} = \left(R_{OM(t)} - 1\right) \bullet 1000$$

where

$$R_{OM(t)} = \left[I \bullet R_{atm(t)} + C_{(t-1)} \bullet R_{OM(t-1)} \bullet (1 - k - \lambda)\right]/C_t.$$

Inputs, I, are calculated as k*C, and λ is the radioactive decay constant for $\Delta^{14}C$ [*Stuiver and Polach*, 1977]. As time lags of C inputs into these fractions were not well constrained, they were not used, and the turnover times reported reflect a combination of time spent in that soil fraction, as well as time spent elsewhere. Again, each fraction was modeled as one pool, though it is clear that mineral-associated phases in particular integrate faster and slower cycling pools.

2.6. Incubations

[23] Soils were stored at 7°C for 3–4 weeks in order to allow roots to die. Three subsamples of the homogenized soil were taken, weighed, put into aluminum foil containers, and placed in humidified Mason jars, which were then scrubbed with soda lime to remove atmospheric CO_2. Additional soil was kept aside for gravimetric moisture analysis. Samples from CAO were substantially drier than samples from CP, therefore, water was added to the CAO samples to make moisture levels equivalent. CO_2 concentrations were measured daily using a Licor 6252 Infrared Gas Analyzer [*Davidson and Trumbore*, 1995]. In order to keep conditions constant, jars were scrubbed whenever the CO_2 concentrations exceeded 4%. At the end of the incubation period, the air inside the jar was collected using evacuated steel canisters and saved for $\delta^{13}C$ and $\Delta^{14}C$ analysis. After incubations were complete jar volumes were determined and the C flux was calculated and expressed as CO_2 increase per gram of C.

2.7. Enzyme Assays

[24] Activities of β-glucosidase (BG) and polyphenol oxidase (PPO) were measured according to *Allison et al.* [2008], adapted from *Sinsabaugh* [1994]. Three soil samples from each treatment were collected for enzyme analysis in summer 2006. Samples were taken from the litter layer by cutting a 10 × 10 cm square, while the Oa horizon was sampled with a 2.5 cm diameter corer. Samples were returned to the lab and homogenized after 5–7 days of storage at 7°C. Three 2 g samples were taken from each treatment for enzyme activity analysis, while some of the remaining soil was analyzed for gravimetric moisture. Each sample was added to 60mL of a 50 mM sodium acetate buffer (pH = 5) and blended for 2 min. to make a homogenate. 50 μL of the homogenate was combined with 150 μL of substrate. The substrate for the polyphenol oxidase assay was 50 mM pyrogallol/50 mM EDTA and the substrate for β-glucosidase was 5 mM pNP- β-glucopyranoside. Each assay and control was replicated 8 times. Plates were placed on a shaker and incubated for 1–2 h. Following incubation, 1 M NaOH was added to the β-glucosidase samples to terminate the reaction and develop the color. Absorbance was measured at 405 nm for both assays. Activities are expressed as μmol h^{-1} $gDOM^{-1}$, where DOM indicates dissolved organic matter.

2.8. Statistics

[25] Statistical analyses were performed in SPSS. ANOVAs were used with N addition level and study site as the independent variables.

3. Results

3.1. Soil C Inventory

[26] Treatments have not had a dramatic effect on C storage in the last 9 years (Table 2). The amount of C in the Oi, Oa, and top 10 cm of the A horizons at CP (the more polluted site), was 1.9 times higher than at CAO (the less polluted site), although this was not statistically significant

Table 2. C Contents, and $\Delta^{14}C$ and ^{13}C Signatures of Fractionated Organic Matter[a]

	C Contents (kg m^{-2})			$\Delta^{14}C$ (‰)			$\delta^{13}C$ (‰)		
	+0	+50	+150	+0	+50	+150	+0	+50	+150
Camp Osceola									
Oi total	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	130 ± 25	74 ± 10	116 ± 41	−25.2 ± 0.1	−24.9 ± 0.3	−25.3 ± 0.2
Oa total	2.5 ± 0.9	2.9 ± 2.8	1.8 ± 1.1						
Oa coarse	2.1 ± 0.8	2.4 ± 2.3	1.5 ± 0.9	210 ± 19	150 ± 32	168 ± 12	−25.1 ± 0.2	−24.7 ± 0.4	−25.0 ± 0.5
Oa f. light	0.4 ± 0.1	0.5 ± 0.5	0.3 ± 0.2	206 ± 8	103 ± 32	92 ± 57	−25.4 ± 0.1	−25.2 ± 1.1	−26.3 ± 1.2
Oa f. heavy	0.02 ± 0.01	0.01 ± 0.01	0.00 ± 0.00	125 ± 6	40 ± 27	62 ± 22	−24.7 ± 0.1	−24.3 ± 0.1	−24.4 ± 0.4
A total	2.6 ± 0.1	3.1 ± 0.3	3.6 ± 0.8						
A light	1.9 ± 0.1	2.2 ± 0.5	2.9 ± 0.2	48 ± 13	83 ± 63	78 ± 56	−24.0 ± 0.4	−24.6 ± 0.7	−24.8 ± 0.3
A heavy	0.8 ± 0.0	0.8 ± 0.2	0.7 ± 0.1	18 ± 21	33 ± 1	33 ± 12	−23.8 ± 0.1	−23.7 ± 0.0	$-23.4_{n=1}$
Camp Paivika									
Oi total	4.0 ± 0.3	1.5 ± 1.2	4.5 ± 2.2	88 ± 17	96 ± 9	91 ± 24	−26.7 ± 0.0	−27.0 ± 0.5	−27.0 ± 0.3
Oa total	1.1 ± 0.7	2.3 ± 0.4	1.9 ± 0.4						
Oa coarse	0.4 ± 0.3	1.8 ± 0.4	1.1 ± 0.2	209 ± 9	153 ± 28	150 ± 31	−26.0 ± 0.3	−26.7 ± 0.3	−25.0 ± 2.8
Oa f. light	0.6 ± 0.4	0.4 ± 0.1	0.8 ± 0.6	226 ± 29	186 ± 21	188 ± 22	−23.4 ± 4.9	−26.5 ± 0.3	−26.4 ± 0.3
Oa f. heavy	0.06 ± 0.04	0.13 ± 0.02	0.08 ± 0.02	76 ± 7	86 ± 9	60 ± 8	−25.7 ± 0.4	−25.8 ± 0.1	−25.0 ± 0.7
A total	5.3 ± 2.0	5.4 ± 0.6	6.3 ± 0.8						
A light	4.3 ± 1.8	4.4 ± 0.1	5.1 ± 0.7	73 ± 19	151 ± 17	91 ± 35	−25.8 ± 0.2	−25.8 ± 0.5	−26.3 ± 0.2
A heavy	1.0 ± 0.4	1.0 ± 0.0	1.2 ± 0.2	7 ± 9	27 ± 11	−19 ± 9	−25.2 ± 0.5	−25.3 ± 0.3	−25.6 ± 0.2

[a]Refer to the Methods section for description of fractionation procedures.

(p = 0.06, control, *t* test). The location of C storage also differed between the sites: at CAO, 45% of the total C was stored in the Oa horizon, compared to 10% at CP. There was 10 times more C in the Oi horizon at CP than at CAO ($p < 0.001$, control, *t* test). The overall C inventories are not necessarily comparable between sites, however, because the A horizons integrate different profile depths, and we did not sample the entirety of that horizon. The only major treatment difference at CP was that the 50 kg N ha^{-1} a^{-1} treatment had less C than the control (*t* test, p = 0.03). At CAO, the A horizon control treatment had significantly less C than the 150 kg N ha^{-1} a^{-1} treatment (*t* test, p = 0.01). However, spatial variability in C stocks was quite large, and our sampling is insufficient to determine differences in C stocks among treatments.

3.2. Organic Matter $\Delta^{14}C$ and ^{13}C

[27] Turnover times were estimated by matching the C inventory and $\Delta^{14}C$ for different isolated fractions using means (and 1 standard deviation) obtained for the control sites (Table 3). After accounting for the differences in age of needle inputs, turnover times in the Oi horizon (6–10 years) were similar between sites. The Oa coarse and light fraction turnover times were also comparable at CAO and CP (coarse: CAO: 11–50 years., CP: 17–45 years.; light: CAO: 11–53 years., CP: 16–34 years). However, C in the A light fraction had longer turnover times at CAO than at CP (CAO: 140–200, CP: 90–160 years). In contrast, the Oa heavy fraction had faster turnover times at CAO compared to CP (CAO:65–74, CP:100–120 years). There were no differences between the A heavy fractions, with both sites having mean turnover times of 200–300 years.

[28] N addition treatments resulted in the depression of $\Delta^{14}C$ signatures of some soil fractions (Table 2). At CP, $\Delta^{14}C$ values of OM in the Oi layer did not change with N addition. However, radiocarbon signatures of the Oa coarse and fine-heavy fractions and fractions isolated from the A horizons were significantly affected by N addition (p = 0.047, Oa coarse; p = 0.003, Oa fine light; p = 0.018, Oa fine heavy; p = 0.003, A light; p = 0.019, A heavy; ANOVA) (Table 2 and Figure 2). Cellulose extracted from the Oa fine fraction at CP (which did not differ with treatment) had mean $\Delta^{14}C$ signatures nearly identical to those of the Oa coarse fraction (which differed with N addition; Figure 3).

[29] At CAO, effects of N addition on the Oi and A horizons were variable and showed no consistent effect (Figure 2). The $\Delta^{14}C$ signatures of the Oa coarse and fine-light fractions were similar in the control treatments, but both decreased with N addition, the fine-light fraction more

Table 3. Modeled Mean Ages of Soil Fractions in the Controls[a]

	Camp Osceola				Camp Paivika			
Soil Horizon	Mean Age (years)	Inputs (g m^{-2} a^{-1})	Error (%) ($\Delta^{14}C$, C)	Lag (years)	Mean Age (years)	Inputs (g m^{-2} a^{-1})	Error (%) ($\Delta^{14}C$, C)	Lag (years)
Oi total	6–10	50–80	10,25	4	6–8	450–500	20,25	2
Oa coarse	10–48	20–100	10,50	12	16–43	10–20	10,25	10
Oa fine light	10–51	5–20	10,50	12	15–31	20–30	10,25	10
Oa fine heavy	65–74	0.2–0.3	1 SD $\Delta^{14}C$	0	100–120	0.5–0.6	1 SD $\Delta^{14}C$	0
A light	140–200	9–14	1 SD $\Delta^{14}C$	0	90–150	29–47	1 SD $\Delta^{14}C$	0
A heavy	190–340	2–4	1 SD $\Delta^{14}C$	0	255–330	3–4	1 SD $\Delta^{14}C$	0

[a]Modeling procedures are defined in the Methods section. The first error listed is for the modeled $\Delta^{14}C$ signature, and the second is for the C inventory. This is calculated as the percent difference from the mean, and the error allowed generally corresponded to the range of values measured. In Oa heavy and A horizons, all solutions where modeled $\Delta^{14}C$ was within 1 standard deviation of the mean measured value were given. Lag is the number of years elapsed between the time the C was last in the atmosphere and the time it entered the pool of interest.

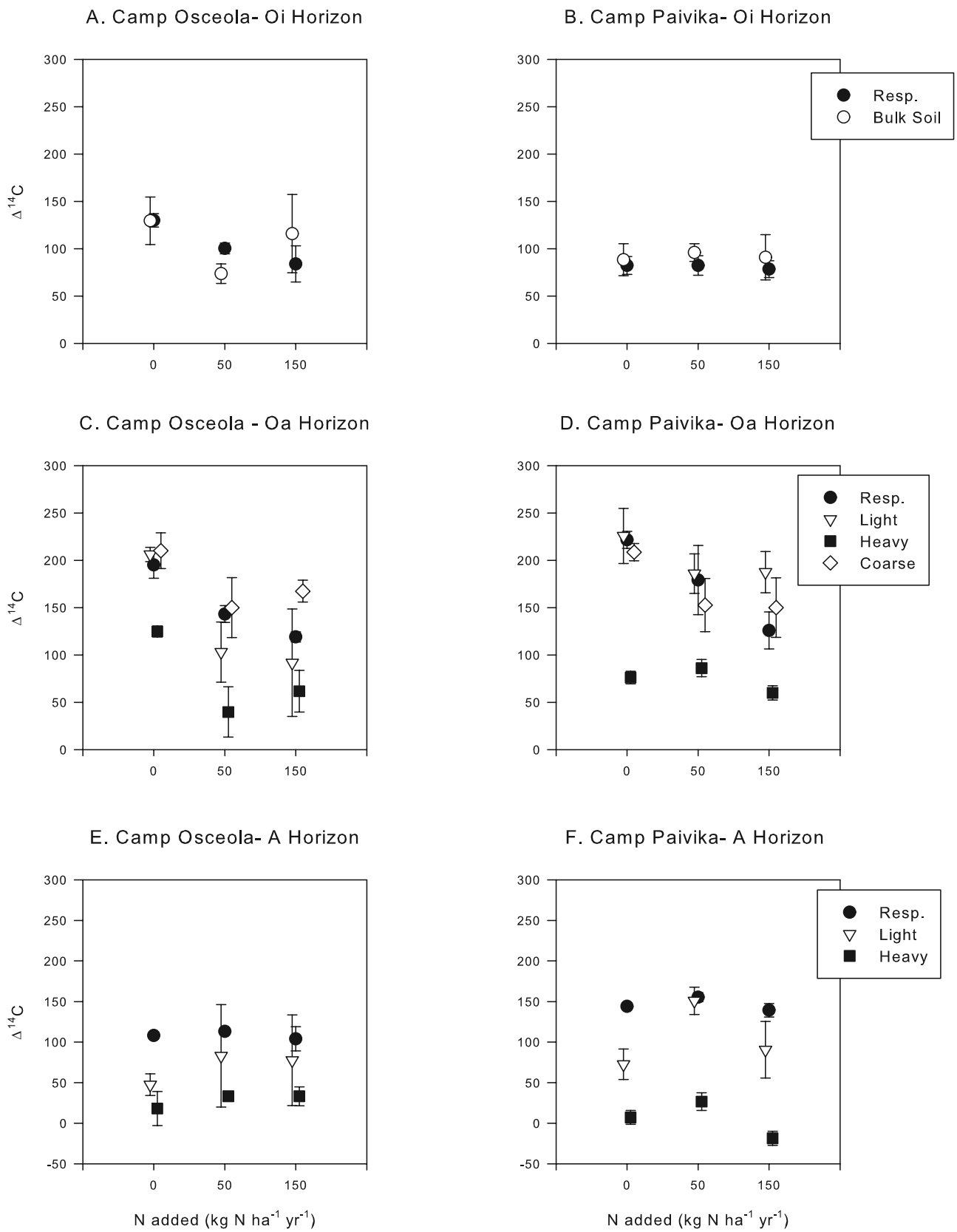

Figure 2. Comparison of SOM and heterotrophic respiration $\Delta^{14}C$. Black circles represent respiration, open circles represent bulk SOM, open triangles represent the light fraction (<1 mm), black squares represent the heavy fraction (<1 mm), and open diamonds represent the coarse fraction (>1 mm). (a, c, and e) Camp Osceola is the clean site, and (b, d, and f) Camp Paivika is the polluted site. Error bars represent the standard deviation.

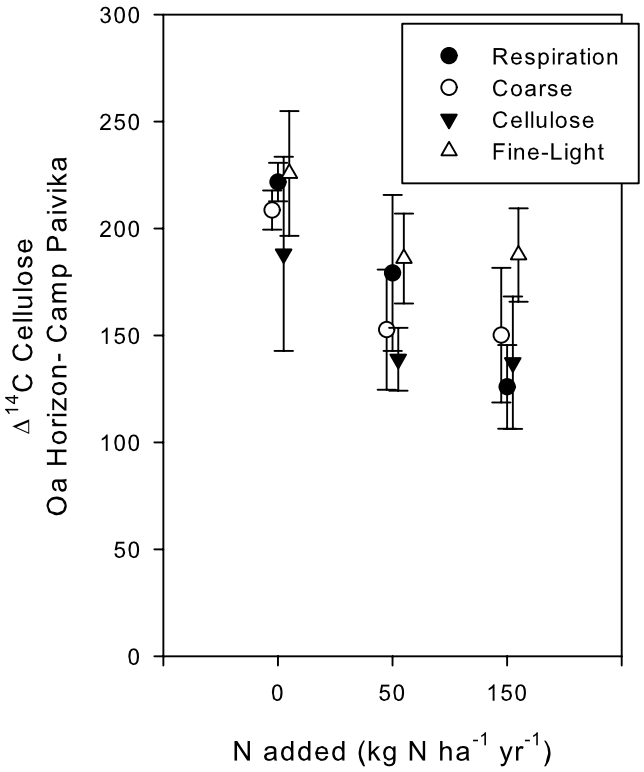

Figure 3. Comparison of cellulose, coarse fraction (>1 mm), light-fine fraction, and heterotrophic respiration $\Delta^{14}C$ in the Oa horizon at Camp Paivika, the polluted site. Error bars represent the standard deviation.

dramatically (Table 2 and Figure 2). The $\Delta^{14}C$ signatures of the Oa fine-heavy fraction were much lower than the other Oa fractions, and declined with N addition ($p = 0.005$, ANOVA). While significant treatment differences were identified in fractions isolated from the A horizons at CAO, we are not confident that they reflect more than spatial or sampling variability, and the amount of C represented in the A horizon is not large compared to the Oa and Oi horizons at this site.

3.3. Heterotrophic Respiration

[30] Specific respiration rates at both sites showed a rapid decrease with depth (Figure 4). Specific respiration rates at CAO exceeded those at CP in the Oa and A horizons ($p < 0.001$, control, paired *t* test) (Figure 4). Owing to lower amounts of C in the soil at CAO, the potential for C loss was similar between sites (Figure 5).

[31] The $\Delta^{14}C$ signatures of respired CO_2 were significantly greater than the 2006 atmospheric $\Delta^{14}C$-CO_2 signature, and the highest $\Delta^{14}C$ values were respired from the Oa horizon (Figure 6). The $\Delta^{14}C$ signature of CO_2 respired from the Oi horizon was higher at CAO, consistent with the longer time lag (greater age) of C inputs to the Oi horizon at that site compared to CP.

[32] Respiration rates did not exhibit a consistent response to N addition. N addition at moderate levels (50 kg ha^{-1}) resulted in increased specific respiration rates in both the Oi and Oa horizons at CP, but only in the Oi horizon at CAO. However, with greater N additions, respiration rates returned to control levels or below, except in the case of the CAO Oi horizon, where respiration rates were between control and 150 kg ha^{-1} values. The A horizons at both sites showed inconsistent trends.

[33] In contrast, respiration $\Delta^{14}C$ values shifted dramatically with N addition in the Oa horizon; the $\Delta^{14}C$ of respiration dropped ~100‰ between the control and +150 treatments at both sites. This lowered $\Delta^{14}C$ signature corresponds to a 10–15 year decrease in the mean age of respired C with N addition (Figure 1). At CAO, there was a decline in the Oi horizon's $\Delta^{14}C$ signature as well, corresponding to an 8 year decrease in the mean age of respired C. There was no clear trend in the A horizon at either site.

[34] Comparing the $\Delta^{14}C$ signatures of respired CO_2 from the Oa horizon with the $\Delta^{14}C$ of OM fractions that comprise potential microbal C sources (substrates) suggests that N addition may shift the relative importance of each substrate (Figure 2). At both CAO and CP, the Oa horizon's respiration $\Delta^{14}C$ signature showed a stronger reduction with N addition than did the OM fractions' $\Delta^{14}C$ signatures. For control treatments, the $\Delta^{14}C$ signatures of respired CO_2 and the coarse and fine-light fractions were similar, while in the +150 treatment, the $\Delta^{14}C$ of respired CO_2 falls between coarse, light and heavy fractions (see Figure 2).

[35] A consistent change in the relationship between OM and respired C with N addition is not apparent in the other horizons (Figure 2). In the Oi horizon, there was little difference between the $\Delta^{14}C$ signatures of respiration and bulk OM between or within treatments at either site (Figure 2), except the +50 treatment at CAO had lower OM $\Delta^{14}C$ signatures than the other treatments (Figure 2). In the A horizon, the respired C had higher $\Delta^{14}C$ signatures than either the light or heavy fraction, except in the +50 treatment at CP (Figure 2).

3.4. Enzymes

[36] Enzyme activities of β-glucosidase (BG) and polyphenol oxidase (PPO) varied between sites and with N addition. At CP, N addition increased BG activity in both the Oi and Oa horizons (Figure 7). N addition had no significant effect on PPO activity, although activity was higher in the Oi horizon's +50 treatment. Low enzyme activity in the control treatments may reflect the anomalously low field moisture levels in the collected soils more than treatment effects.

[37] At CAO, BG activity in the Oi horizon was significantly higher in the +50 treatment than in the other two treatments (Figure 7). However, the soils collected from the +150 treatment were also anomalously dry on the date sampled, which again may have artificially depressed enzyme activity. The Oa horizon showed no significant differences in BG activity, but PPO activity decreased significantly in response to N in both horizons.

[38] Comparing between sites, both BG and PPO activities were substantially higher in the Oi horizon at CAO ($p = 0.005$, BG control, $p = 0.005$, PPO control, *t* test), in agreement with the overall higher specific respiration rates for that site's Oi horizon. No pattern was evident in the Oa horizon (Figures 4 and 7).

4. Discussion

4.1. Response to Short-Term N Addition Treatments

[39] We observed changes in respiration rates, $\Delta^{14}C$ signatures of OM and respiration, and enzyme activities in

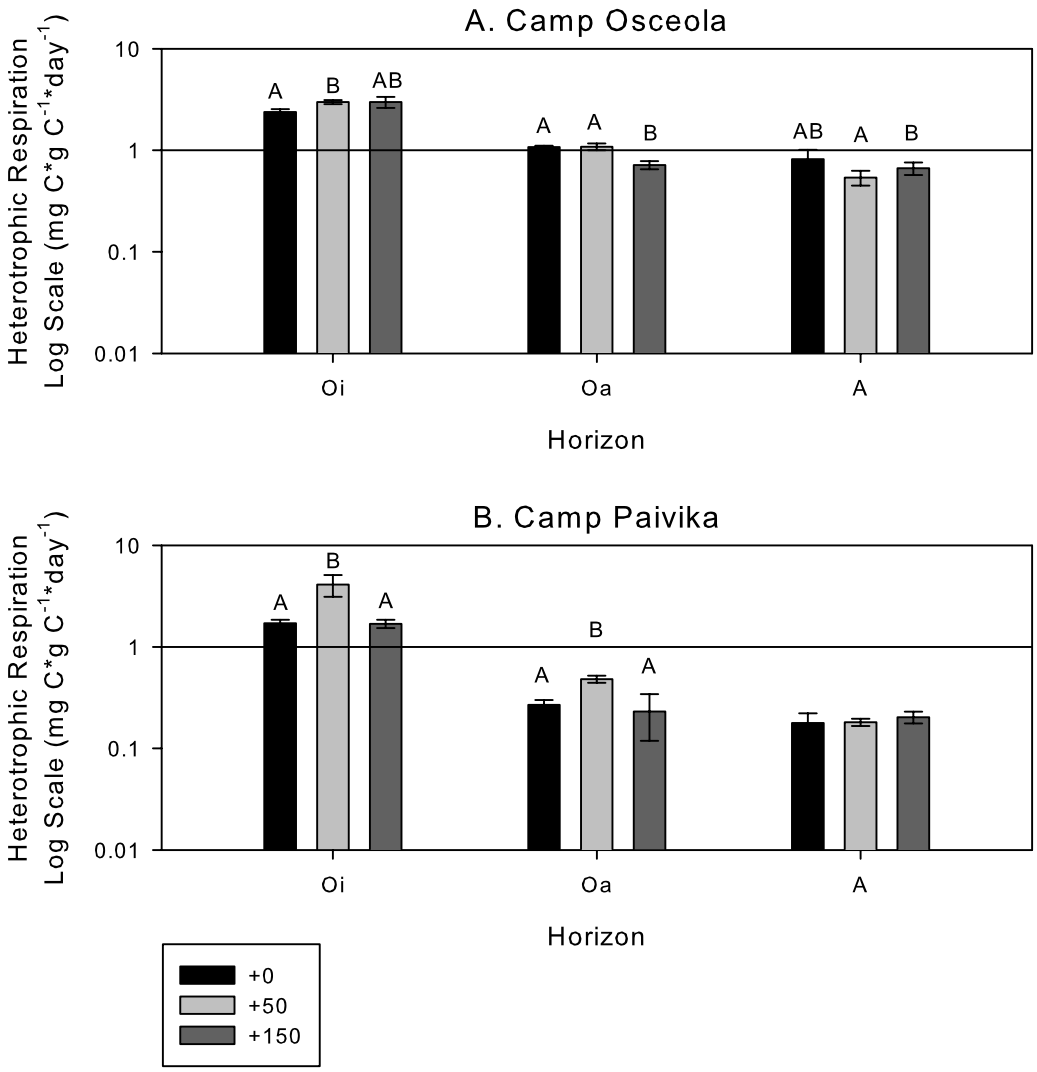

Figure 4. Heterotrophic respiration rates in mg g C^{-1} d^{-1}. (a) Camp Osceola, the clean site, and (b) Camp Paivika, the polluted site. The black bars are the control, the light gray bars are the 50 kg N ha^{-1} treatment, and the dark gray bars are the 150 kg N ha^{-1} treatment. Different letters represent significant differences between the treatments (*t* test, $p < 0.05$). Error bars represent the standard deviation.

response to N addition treatments. Short-term N addition at intermediate levels (50 kg ha^{-1} a^{-1}) appeared to stimulate decomposition at both sites. Oi respiration rates increased with N additions of 50 kg ha^{-1}, but did not increase further with additions of 150 kg ha^{-1}. At CP, rates actually declined to control levels in the 150 kg ha^{-1} plots (Figure 4). Effects of N addition were more dramatic in the $\Delta^{14}C$ signature of respiration than in the rates.

[40] One of the most notable results of this study is the 100‰ drop in the $\Delta^{14}C$ signature of Oa horizon respiration in response to N addition. This change in respiration $\Delta^{14}C$ integrates both shifts in microbial activity and in the substrates available for decomposition. The $\Delta^{14}C$ signature of respiration from the Oa control soils reflects the signature of its coarse and light fraction substrates, which declined with N addition (Figures 2 and 5) (CAO Oa coarse, $p = 0.040$; CAO Oa light, $p = 0.018$, CP Oa coarse = 0.047; CP Oa light = 0.155, ANOVA). Therefore, the decrease in the $\Delta^{14}C$ signature with N addition could result either from a shift in the organic matter available for decomposition or a shift in the relative contributions of different soil fractions to the total respiratory flux (Figure 2). Because the relationship between the $\Delta^{14}C$ signature of respiration and the signatures of the substrates was not constant with N addition (Figure 2), both shifts likely contribute in this case. The $\Delta^{14}C$ signature of decomposing substrates is indeed changing with N amendment, but the relative contributions of the coarse and light fractions to the respiratory flux also change at both sites, suggesting a change in microbial substrate use as well.

[41] The conclusion that shifts in $\Delta^{14}C$ signatures with N addition are a result of changes in microbial substrate use is also supported by the results of the enzyme assays, which showed decomposition at CAO shifts away from lignin, which is decomposes slowly and thus higher, older $\Delta^{14}C$ signatures, and, at CP, toward the decomposition of cellulose, which cycles faster and would thus have lower, more recent $\Delta^{14}C$ signatures (Figure 7). This change in enzyme activity with N addition is well supported by the literature [*Carreiro*

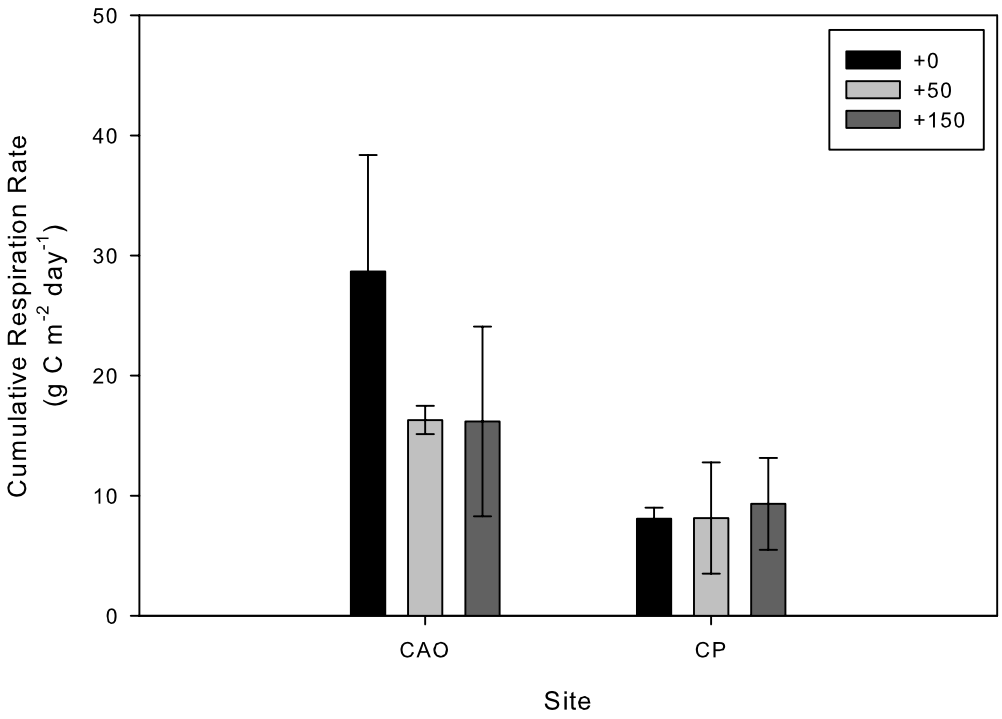

Figure 5. Idealized cumulative respiration rates by site and treatment. Respiration rates represent the maximum possible respiration as they were measured in laboratory incubations where water was not limiting as it is in the field. The black bars represent the control, the light gray bars are the 50 kg N ha^{-1} treatment, and the dark gray bars are the 150 kg N ha^{-1} treatment. CAO is the clean site, and CP is the polluted site. Error bars correspond to 1 standard deviation of the mean.

et al., 2000; *Frey et al.*, 2004; *Saiya-Cork et al.*, 2002; *Sinsabaugh et al.*, 2002], although a recent study of a xeric grassland found that only glucosidase activity was enhanced by added N [*Stursova et al.*, 2006].

[42] The alteration of enzyme activities with N addition may result in changes in the preservation of OM components. Lower OM $\Delta^{14}C$ signatures are a result of younger (lower $\Delta^{14}C$) inputs or faster substrate turnover times. At CP, cellulose had significantly lower $\Delta^{14}C$ signatures than the <1 mm fraction from which it was derived (+50: $p = 0.033$, *t* test; +150: $p = 0.084$, *t* test), which is comparable to the light fraction, as the heavy fraction contains minimal amounts of C. Lignin comprised most (54.5%, ± 0.7%) of the light fraction and polysaccharides (i.e., cellulose) comprised a much smaller portion (8.6% ± 1.6%) (J. Neff and D. Fernandez, unpublished data, 2007). Consequently, to satisfy the mass balance requirements, lignin must have a higher $\Delta^{14}C$ signature than cellulose. Further, since lignin typically has longer turnover times than cellulose in the same soil horizon [*McClaugherty and Berg*, 1987], it would be generally expected to have higher $\Delta^{14}C$ values. Therefore, a decrease in the $\Delta^{14}C$ signature of respiration with N addition would suggest that cellulose is younger, is contributing more to heterotrophic respiration than lignin, or both.

[43] *Fog* [1988] suggests that decomposition is suppressed with high lignin litters and in long-term N addition experiments, and a meta-analysis by *Knorr et al.* [2005] found that high levels of N addition inhibited litter decay. *Berg et al.* [1982] and *Berg and Matzner* [1997] proposed that N suppresses late-stage decomposition where the degradation of lignin and humus should dominate. The depression of respiration rates after long-term N addition has been observed in mesic temperate forests [e.g., *Nohrstedt et al.*, 1989; *Bowden et al.*, 2004; *Burton et al.*, 2004], and suggests that the decline in lignin decomposition becomes more important than the increase in the cellulose decomposition under long-term or high levels of N addition. The suppression of lignin decomposition by N may result from the incorporation of N into lignin compounds, which makes these compounds more difficult to decompose and increases the humus component [*Bollag et al.*, 1983; *Liu et al.*, 1985; *Gallo et al.*, 2004]. In addition, increased N concentrations have been observed to cause the downregulation of ligninolytic enzymes in white-rot fungi [*Keyser et al.*, 1978].

[44] The apparent decrease in residence time of OM with N addition could result from (a) faster decomposition rates or (b) shorter lag times for inputs. Faster decomposition rates are supported by *Fenn and Dunn* [1989] at these same study sites, which found microbial respiration rates were ~30% higher in litter from high pollution sites compared to controls. Shorter needle lag times may be a factor in site differences, as needles are shed more rapidly at the high end of the pollution gradient [*Grulke et al.*, 1998], consequently needles entering the Oi horizon have lower $\Delta^{14}C$ signatures. However, this would not cause the differences between treatments. N has been shown to affect root production [*Nadelhoffer*, 2000], but roots are generally confined to the A horizon due to the dryness of the sites [*Grulke et al.*, 1998], therefore, changes in root inputs would not cause the changes seen in the Oi and Oa horizons. Consequently, changes in

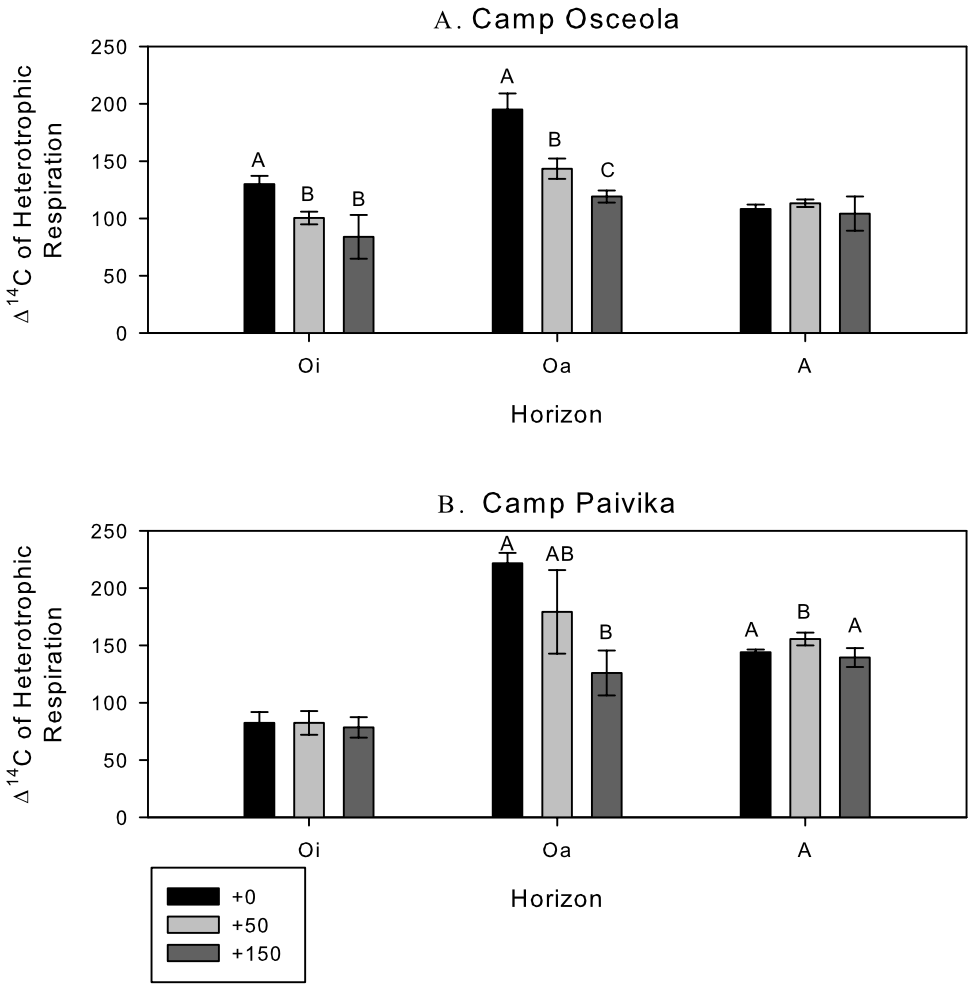

Figure 6. $\Delta^{14}C$ of heterotrophic respiration. The black bars represent the control, the light gray bars are the 50 kg N ha^{-1} treatment, and the dark gray bars are the 150 kg N ha^{-1} treatment. Different letters represent significant differences between the treatments (*t* test, $p < 0.05$). (a) Camp Osceola is the clean site, and (b) Camp Paivika is the polluted site. Error bars represent the standard deviation.

decomposition rates are likely the main cause of differences between treatments. These, in turn, will eventually affect the rate and $\Delta^{14}C$ signature of inputs into subsequent pools.

[45] The change in the decomposition rates of different substrates also has consequences for what C is left to accumulate in the soil. C left in a soil fraction is a balance between what enters and leaves the fraction. An increase in the decomposition rate of cellulose in a particular fraction would result in a relative increase in the proportion of lignin and an increase in apparent age. If less than 100% of the C decomposed, the apparent age in the "next" soil fraction would decrease due to enhanced inputs of younger C. Alternatively, overall faster decomposition rates of a pool would result in decreased turnover times and apparent age. The largest effects of N addition occurred in the Oa rather than Oi and A horizons (Figure 2). In the Oa horizon, the signatures of the coarse and light-fine fraction tended to be similar in the control treatment and declined with N addition, indicating that the mean age of C in those fractions was decreasing (i.e., residence time was decreasing) relative to the control, which could be caused by either increased low $\Delta^{14}C$ inputs or faster turnover (Figure 2). This decline was steeper in the coarse fraction with N amendment at CP, while the light fraction showed larger decreases in $\Delta^{14}C$ signature at CAO (Figure 2).

[46] The coarse fraction represents organic matter in the early stages of decomposition, whereas the fine fraction represents material that has been partially broken down by physical or biological means. The coarse fraction contained more woody debris, and therefore more cellulose than the fine fraction (J. Neff and D. Fernandez, unpublished data, 2007); consequently, the acceleration of cellulose degradation, as indicated by the increase in β-glucosidase activity, would result in faster turnover times in this fraction. Cellulose extracted from the <1 mm fraction at CP had lower $\Delta^{14}C$ values than the fine-light fraction in the N addition treatments (+50: $p = 0.033$, *t* test; +150: $p = 0.084$, *t* test), with values more similar to those of the coarse fraction (Figure 3). This supports the evidence of faster cellulose decomposition (e.g., lower turnover time) with N amendment as suggested by the enzyme assays. The light fraction at CAO may have experienced larger changes than the coarse fraction because the coarse fraction is several times larger and any changes in cycling would have an obvious effect on the light

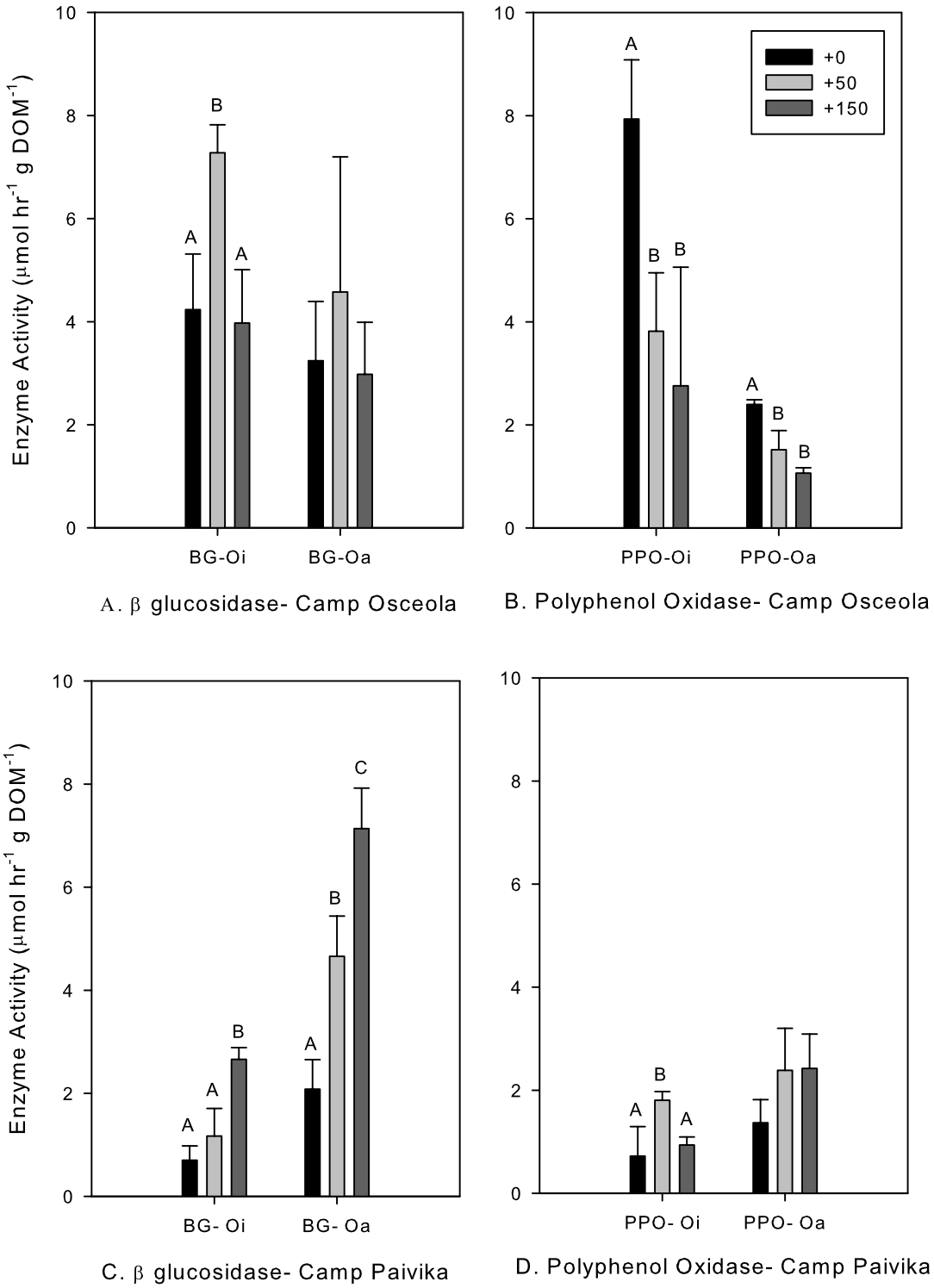

Figure 7. Enzyme activities of (left) β glucosidase and (right) polyphenol oxidase. (a and b) Camp Osceola is the clean site, and (c and d) Camp Paivika is the polluted site. The black bars represent the control, the light gray bars are the 50 kg N ha^{-1} treatment, and the dark gray bars are the 150 kg N ha^{-1} treatment. Both the Oi and Oa horizons are shown in the same plot. Different letters represent significant differences between the treatments (t test, $p < 0.05$). Error bars represent the standard deviation.

fraction, but would contribute proportionately less to the coarse fraction.

[47] The dramatic changes in the $\Delta^{14}C$ signature are probably less apparent in the OM than in the respiration because it takes much longer for a change in C loss to affect such a large pool. Additionally, these sites experience conditions unfavorable to decomposition for most of the year due to the lack of moisture. Therefore, any alteration in

decomposition would take many years to affect the bulk OM, and only 9 years of N amendment had occurred at the time of sampling.

4.2. Responses to Long-Term Pollution Inputs

[48] Climatic factors, including cold winters and dry hot summers, combine to slow decomposition and build up organic matter in surface O horizons over time in these sites. The turnover times of Oi horizon fresh litter, the most rapidly cycling pool, ranged from 6 to 10 years, while the Oa horizon had turnover times on the order of decades, and the A horizon on the order of a century or more. The radiocarbon-based estimates of Oi turnover are in accord with litter bag estimates [*Jenny et al.*, 1949], while the Oa estimates are similar to other radiocarbon estimates from similar forests, which indicate decadal turnover times [*Trumbore et al.*, 1996; *Rasmussen et al.*, 2005]. These soils are developed on granitic parent material and do not have large amounts of clay minerals to stabilize organic matter for millennial timescales [*Trumbore et al.*, 1996; *Rasmussen et al.*, 2005].

[49] CP, the more polluted site, differs significantly from CAO in belowground C cycling, reflecting altered patterns of both C inputs and decomposition. The combination of ozone damage and abundant N has shortened needle lifetimes at CP and increased surface litter inputs to the Oi horizon while turnover times remained the same (6–10 years in the Oi), resulting in high Oi carbon stocks at CP. [*Grulke et al.*, 1998; *Grulke and Balduman*, 1999; *Fenn and Poth*, 1999a]. The mean age of Oi material at CAO is greater, however (reflected in higher $\Delta^{14}C$ values), because of preaging of litter material due to longer leaf lifetimes.

[50] Responses of decomposition rates to N addition may be expected to vary with timescale and degree of decomposition. For many decades it was assumed that N limited microbial decomposition above a threshold C:N ratio according to Liebig's law of the minimum. This was further supported by the observations that materials with lower C:N ratios decayed more quickly than their counterparts [*Swift et al.*, 1979; *Aber and Melillo*, 1982; *Melillo et al.*, 1982]. While this may be the case for cellulose and other labile materials, this theory does not explain the findings of many long-term N addition experiments or the response of recalcitrant substrates to N addition [*Fog*, 1988]. In general, studies in mesic temperate forests have found that increased N levels in soil often result in decreased decomposition and soil respiration rates [*Bowden et al.*, 2004; *Burton et al.*, 2004; *Magill and Aber*, 1998]. However, little change or enhanced respiration and C loss is observed in xeric or cryic ecosystems, where more C is in early stages of decomposition due to temperature or moisture limitation of microbial activity most of the year [*Fog*, 1988]. This is analogous to observed differences between short and long-term N additions, with respiration or mass loss rates being higher initially, but eventually lowering in response to N addition [*Berg and Matzner*, 1997].

[51] A meta-analysis by *Knorr et al.* [2005] found that decomposition of high quality (low lignin) litter was enhanced by N addition, but decomposition of low quality (high lignin) litter was reduced. If some substrates are more susceptible to enhanced decomposition than others, the character and age structure of residual OM would be expected to change over the long-term. For example, the difference in age between respired CO_2 (which reflects more rapidly decomposing materials) and the OM-CO_2 (which reflects the residues that do not decompose rapidly) might be expected to increase over time. However, in a xeric environment, where decomposition is limited by climatic conditions, it is unclear on what timescale we might expect chronic N addition to manifest itself. Radiocarbon-based turnover times in low density material in Oa and A horizons tended to be similar between sites, unlike the respiration results which found depressed CO_2 evolution at high pollution sites (Table 3 and Figure 4). Specific respiration rates based on incubations where water was added demonstrate potential decomposition rates and were higher at CAO. Enzyme activities, another reflection of potential decomposition rates, were also higher at CAO. Both results suggest that short-term decomposition rates are higher at CAO: that during the months when decomposition is not limited by moisture supply, decomposition proceeds more rapidly. Over the long-term, N deposition has been observed to lead to an overall slowing of decomposition rates [e.g., *Magill and Aber*, 1998; *Burton et al.*, 2004]. If residual material not decomposed in the Oi horizon is more resistant to decomposition, the long-term effect would be slower decomposition in the Oa horizon with increased N levels, as we observed here. Early in the deposition period or at lower deposition levels, N appears to have the potential to increase overall decomposition rates, but eventually this effect becomes overwhelmed by the decrease in lignin decomposition associated with long-term increases in N availability.

5. Conclusions

[52] $\Delta^{14}C$ in incubations and enzyme activities both provide sensitive indicators of belowground responses to N addition. Results at two sites along a pollution gradient broadly support an evolving picture of short- and long-term responses in the literature, which involves shifts in plant allocation and microbial communities. Short-term N addition treatments caused a shift toward decomposition of younger, more labile material (cellulose) as observed in changes in $\Delta^{14}C$ of respired CO_2. Low levels of N addition increased specific respiration rates at both sites, indicating that the more polluted CP still had the capacity to respond positively to N addition, despite its long history of N deposition. However, the higher N addition treatment did not result in further increases in respiration rate at either site, and in many cases, resulted in a decline in respiration rate. This suggests that the point at which the enhanced degradation of labile compounds becomes less influential than the decreased degradation of refractory compounds has been reached between +50 and +150 kg N ha^{-1} a^{-1}. On the longer term, chronic pollution seems to cause some decline in decomposition rates, as evidenced by the lower respiration rates at CP, again supporting the *Berg and Matzner* [1997] hypothesis. It is difficult to draw conclusions about the effects of long-term N deposition from a comparison of the two sites because their responses are complicated by other factors, particularly climatic differences and other covarying pollutants. Consequently, changes in C storage and allocation may or may not be a direct result of N deposition. Whether a site shows changes in C storage will depend on the history of N deposition and the type of C

available. Small or medium increases in available N may result in enhanced cellulose degradation without much effect on lignin decomposition, but at some point the depression of lignin decomposition begins to outweigh the enhancement of cellulose decomposition.

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