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Effects of CO_2 and N fertilization on decomposition and N immobilization in ponderosa pine litter

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Abstract

Naturally senesced needles from ponderosa pine (*Pinus ponderosa* Dougl.), grown from seed in open-top chambers under three levels of CO_2 (350, 525 and 700 μl l^{-1}) and three levels of N fertilization (0, 10 and 20 g N m^{-2} yr^{-1}), were used in a field litterbag decomposition study and in a laboratory study on potential microbial and nonmicrobial N immobilization. The litterbag studies revealed no statistically significant effects of either CO_2 or N treatment on mass loss, N concentration, or N content over a 26-month period. The laboratory study of potential ^{15}N immobilization revealed no statistically significant effects of CO_2 or N treatment on either total or microbial immobilization. Elevated (CO_2) did have a significant negative effect on nonmicrobial immobilization, however. Natural abundance of ^{15}N was significantly greater with elevated (CO_2) in both live and naturally senesced needles under all N treatments. This pattern combined with ^{15}N natural abundance in soils suggests that saplings grown under elevated (CO_2) were either taking up more N from surface horizons or from a more recalcitrant soil N pool in either horizon.

Introduction

The long-term effects of elevated carbon dioxide (CO_2) on growth and carbon (C) sequestration are highly dependent on interactions with nitrogen (N) cycling. The interactions between N and elevated (CO_2) which have been studied thus far include 1. reduced tissue N concentration (increased growth per unit N uptake), 2. changes in litter quality and decomposition rate, and 3. changes in soil N mineralization rate (Diaz et al., 1993; Körner and Arnone, 1992; Norby et al., 1999; Zak et al., 1993). These effects could be very important in closed-canopy forests where more than 80% of N taken up by trees every year is recycled (Cole and Rapp, 1981). Free Air CO_2 Experiments (FACE) now in progress will provide information about ecosystem-level effects of elevated (CO_2) on N cycling. Meanwhile, we are forced to approach the problem through process-level studies based on greenhouse or open-top chamber experiments. The results of these process-level studies have been mixed and generally inconclusive, especially in regard to the litter quality-decomposition feedback (O'Neill, 1994; Randlett et al., 1996). Norby and Cotrufo (1998) reflected the consensus of the workshop on the effects of elevated (CO_2) on litter quality summarized in this volume by stating that the reduced decomposition – slowed N cycling hypothesis 'has been laid to rest'. Norby and Cotrufo (1998) go on to note that 'Rejecting the litter-quality hypothesis does not diminish the importance of other potential feedbacks on nitrogen availability ... or the need to characterize the processes that control decomposition in CO_2-enriched ecosystems'.

In this paper, we report the results of field and laboratory studies on the effects of elevated (CO_2) on decomposition and N immobilization in naturally-senesced needles from ponderosa pine (*Pinus pon-*

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derosa Dougl.) subjected to elevated (CO_2) and N fertilization in an open top chamber study. We also explore the implications of ^{15}N natural abundance in needles and soil for sources of N, including uptake under elevated (CO_2). These studies were conducted in conjunction with larger-scale field studies on the effects of elevated (CO_2) and N on plant physiological processes, soils, and C sequestration in ponderosa pine (Johnson et al., 1997, 1998).

Materials and methods

Site and treatment

The site is located at the Institute of Forest Genetics near the town of Placerville, Calfornia (840 m elevation; 38° 44′ N; 120° 45′ W). The soil is Aiken clay loam, a Xeric Haplohumult derived from andesite. During February through April 1991, 24 hexagonal open-top chambers (3.6 m in diameter) were established on the site. The original experimental design consisted of three levels of N (0, 10 and 20 g m^{-2} yr^{-1} of N as ammonium sulfate, applied in early spring), and four CO_2 treatments (ambient, no chamber; ambient, chambered; 525 μL L^{-1} CO_2; and 700 μL L^{-1} CO_2). The 10 g m^{-2} yr^{-1} N, 525 μL L^{-1} CO_2 treatment was excluded because of cost limitations. Seedlings were grown from seed (21 planting locations per chamber) and seedlings (21 per chamber), the latter being a backup in the event of excessive mortality. Treatments began in May, 1991. Seed-grown seedling survival was greater than 95%, and the seedling-grown stock was removed in October 1991. Water was delivered to each plot via a timed stand pipe to a looped 2.54 cm diameter manifold and low pressure spray heads. Each of the chambered treatments was replicated three times, and each of the unchambered treatments was replicated twice. Only the results from the chambered measurements will be reported here.

Methods

In August 1995 (fourth growing season), naturally-senesced needles were manually removed from the trees. This was accomplished by brushing senesced needles still attached to the trees by hand and collecting those that fell off. Subsamples of these needles were then weighed (3–5 g needles per bag), placed into 5 mm mesh litterbags, and set on the surface of the litter layer in a nearby ponderosa pine plantation (three replicates per chamber, for a total of nine replicates). Subsamples were taken for percent moisture and N analyses at that time. One of the replicates from each chamber was retrieved in October 1996 and all remaining samples were retrieved in October 1997. The samples were dried, weighed and analyzed for N at the Desert Research Institute on a Perkin-Elmer 2400 CHN Analyzer. Nitrogen contents (g bag^{-1}) were calculated from measured weights and N concentrations for each collection. Change in mass and N content were expressed as percentages of the initial values.

Samples of the senesced needles taken in August 1995 were subjected to tests for microbial and nonmicrobial N immobilization according to the following protocols. Triplicate samples of senesced needles (2 g per replicate) were leached over a 12 h period with 25 ml solution containing 8×10^{-5} *M* $(NH_4)_2SO_4$ containing 73.6% ^{15}N in a mechanical vacuum extractor (Centurion Corp.). At the end of this period, the samples were leached with 2 *M* KCl over a period of 4 h to remove readily exchangeable NH_4^+. Another set of samples (in triplicate) were treated with the same solution containing 5% $HgCl_2$ to preclude any biological N uptake. The extracts were discarded, and the solid samples were recovered for ^{15}N analyses. The ^{15}N analyses were conducted at the University of California, Davis using a Europa Scientific 'Integra' analyzer (Crewe, UK). This machine has the combined functions of an elemental analyzer and isotope ratio mass spectrometer. The solid sample materials (finely ground) are packaged in small tin capsules which are automatically introduced to a combustion tube. In the tube, the samples are flash combusted to H_2O, CO_2 and N_2+NO_x. The resulting gases are swept by a He carrier through further reactors where water is removed and NO_x is reduced to N_2. The N_2 and CO_2 are separated by chromatography before introduction to the mass spectrometer via an open split and inlet capillary which continuously samples the carrier gas stream. The mass spectrometer simultaneously monitors the ion currents from three adjacent mass species of the ion beam spectrum (m/z 28, 29 and 30 for N_2 and 44, 45 and 46 for CO_2). Thus, each sample gas appears as three simultaneous chromatography peaks which are integrated to calculate element totals and isotope ratios. Standard samples introduced at intervals in the sample batch are used to calibrate the measurements through comparison to known isotope standards.

Total and nonmicrobial ^{15}N immobilization were calculated from measured ^{15}N in treated samples minus ^{15}N natural abundance values for each sample. Microbial ^{15}N immobilization was calculated as the difference between ^{15}N in samples treated with $(^{15}NH_4)_2SO_4$ only and those treated with $(^{15}NH_4)_2SO_4$ plus $HgCl_2$. Live, current year needles sampled during August 1996 (as part of the final harvest of the study) were analyzed for total N and ^{15}N for comparison with the senesced needles.

Statistical analyses

Statistical analyses for treatment effects were performed using PROC GLM in Statistical Analysis System software. Two-way analysis of variance was performed on the data derived from this 3×3 (three CO_2×3 nitrogen) incomplete factorial experiment, with type IV sum of squares used to accommodate the excluded intermediate soil N and 525 μL L^{-1} CO_2 treatment combination and with n=3 for each included treatment combination. For comparison of total N and 15N on live and senesced foliage, a three-way analysis of variance was performed with three CO_2×three nitrogen×two status (live or senesced) treatments. The CO_2, N and status main treatment and ×N interaction effects were considered significant only when $P \leq 0.10$ according to the F test.

Results

Needle mass decreased by 8 to 15% over the 26-month litterbag experiment, but there were no treatment effects of either CO_2 or N (Figure 1 and Table 1). Nitrogen concentrations were lower in the initial samples with elevated (CO_2) and low N treatment levels, but this was not statistically significant and disappeared in the 14- and 26-month collections (Figure 2). No statistically significant differences in N concentration or content due to treatment were found at any time (Figure 2 and Table 1).

There were no statistically significant treatment effects on total ^{15}N immobilization or microbial ^{15}N immobilization in the laboratory studies on senesced needles. There was a consistent and statistically significant negative CO_2treament effect on nonmicrobial ^{15}N immobilization in all N treatments, but no statistically significant effects of N or CO_2×N treatments (Figure 3 and Table 1). We also found a statistically significant positive CO_2 treatment effect on ^{15}N natural abundance in senesced needles all N treatments.

Table 1. Results of unbalanced analysis of variance tests for effects of CO_2 and N treatments on live and senesced needles and on decomposition of senesced needles

Parameter	Treatment Effects	
	CO_2	N
Live needle N concentration	N.S.	N.S.
Senesced needle N concentration	N.S.	N.S.
Litterbag weight loss		
14 Months	N.S.	N.S.
26 Months	N.S.	N.S.
Litterbag N concentration		
14 Months	N.S.	N.S.
26 Months	N.S.	N.S.
Live needle ^{15}N	***	N.S.
Senesced needle ^{15}N	***	N.S.
^{15}N immobilization in senesced needles		
Microbial	N.S.	N.S.
Non-microbial	**	N.S.
Total	N.S.	N.S.

N.S.=not significant, *, ** and *** refer to 0.1, 0.05, and 0.01 significance levels, respectively. The CO_2×N interaction effects were not significant in any case and were therefore removed from the statistical analysis.

There were no effects of N or CO_2×N interaction treatments on either ^{15}N immobilization or ^{15}N natural abundance (Figure 3 and Table 1).

There were no statistically significant treatment effects on total N in live needles. There were statistically significant, negative CO_2 treatment effects on ^{15}N natural abundance in live needles, but no significant effects of either N or CO_2×N treatments (Figure 4). Similar to the senesced needles, ^{15}N natural abundance in live needles was significantly lower than in senesced needles and total N in live needles was significantly greater than in the senesced needles (Figures 3 and 4).

Discussion

Effects of elevated (CO_2) on litter decomposition and N immobilization

As in many other studies (see Introduction), elevated (CO_2) had no statistically significant effect on decomposition or N immobilization in the field litterbag study. Variability was quite high, however, and may have masked real effects. Elevated (CO_2) had no statistically significant effect on microbial N immobilization in senesced needles in the laboratory

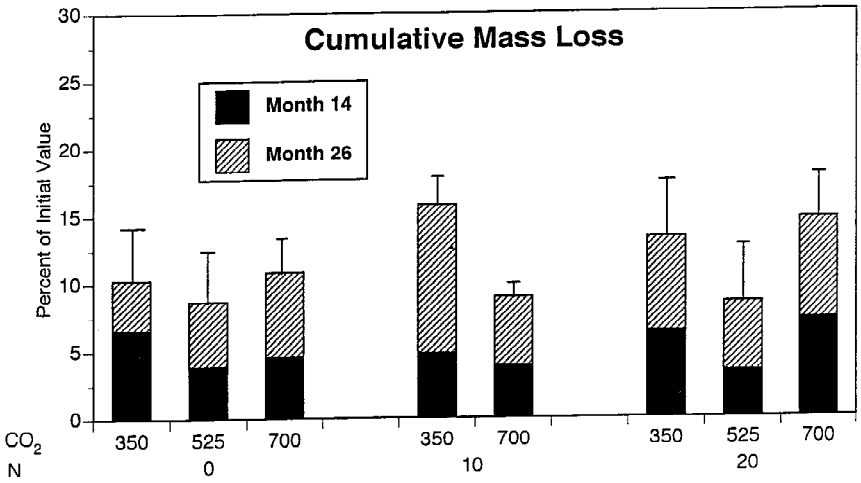

Figure 1. Percent mass loss in litterbag study of naturally senesced ponderosa pine needles treated with three levels of CO_2 (350, 525 and 700 μL L^{-1}) and three levels of N fertilization (0, 10 and 20 g N m^{-2} yr^{-1} as ammonium sulfate). Standard deviations are shown.

study but did have a highly significant, negative effect on nonmicrobial N immobilization. Nonmicrobial N immobilization can occur through chemical condensation reactions of phenols (originating from partially degraded lignin and some fungal pigments) with either amino acids or ammonia, resulting in the formation of 'brown, nitrogenous humates' (Mortland and Wolcott, 1965; Nömmik, 1965; Nömmik and Vahtras, 1982; Paul and Clark, 1989). The well-documented inhibitory effect of lignin on decomposition and N mineralization (Berg and McClaugherty, 1987; Berg et al., 1984; Cromack, 1973; Melillo et al., 1982) is due in part to the formation of stable nitrogenous compounds from lignin by-products through nonmicrobial processes, reducing N availability to decomposer organisms (Berg et al., 1984). Thus, lower nonmicrobial N immobilization with elevated (CO_2) could result in increased rates of litter decomposition, lower rates of N incorporation into stable humus forms, greater N availability to microorganisms over the short term, and possibly greater N availability to plants over the long term.

Nonmicrobial immobilization of ammonia in soils is enhanced by high pH because NH_3 is the reactive form (Nömmik and Vahtras, 1982); thus, nonmicrobial N immobilization is generally thought to be of minimal importance in most acid forest soils (Nömmik, 1970; Schimel and Firestone, 1989). However, Axelsson and Berg (1988) found nonmicrobial NH_4^+ immobilization in decomposing Scots pine (*Pinus sylvestris*) litter at pH 4. They also noted that nonmicrobial N immobilization decreased with increasing stages of litter decomposition. They attributed this to an inverse relationship between N concentration and NH_4^+ immobilization; thus, litter in later stages of decomposition had lower nonmicrobial N immobilization because it had higher N concentrations. We found no significant correlation between nonmicrobial N immobilization and N concentration in this study (r^2=0.003, P=0.79).

Effects of elevated (CO_2) on ^{15}N natural abundance

Elevated (CO_2) had a clear and highly significant positive effect on ^{15}N natural abundance in all N treatments. It appears that elevated (CO_2) caused the trees to take up a source of N more enriched in ^{15}N than was the case under ambient (CO_2). This is the opposite of what would be expected if free-living N_2-fixation were contributing to tree N uptake under elevated (CO_2); rather, the observed effect suggests that older sources of soil N are being tapped. Nadelhoffer and Fry (1988) found that isotopic fractionation causes discrimination against ^{15}N during decomposition such that older, more recalcitrant soil N tends to become enriched in ^{15}N. Their results suggest that there are two pools of naturally-labelled N in the soil: a surface pool which is low in ^{15}N and turns over rapidly and a subsoil pool which is relatively enriched in ^{15}N and is more recalcitrant. Högberg (1997) noted that soils with high rates of nitrification can have the reverse pattern (higher ^{15}N in surface horizons than in deeper horizons) because of discrimination against ^{15}N during nitrification, the latter of which is usually most active in surface horizons. In order to test this hypothesis, we analyzed

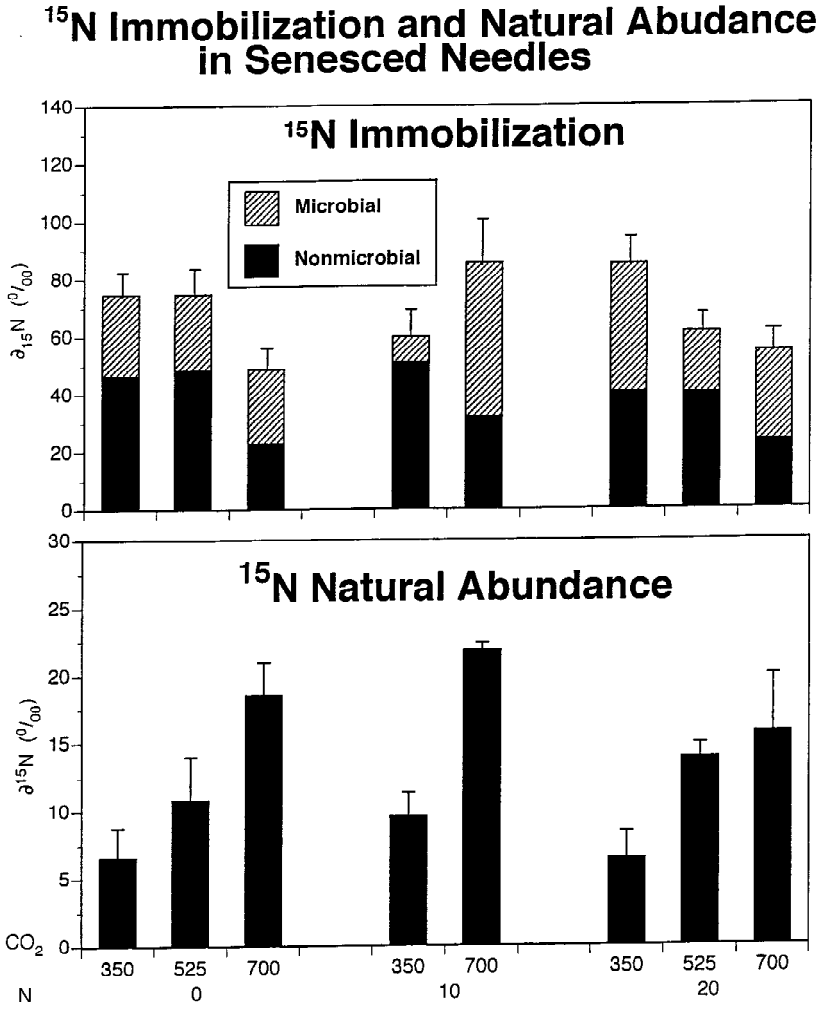

Figure 2. Nitrogen concentrations (top panel) and percent change in N content (lower panel) in a litterbag study of naturally senesced ponderosa pine needles treated with three levels of CO_2 (350, 525 and 700 μL L^{-1}) and three levels of N fertilization (0, 10 and 20 g N m^{-2} yr^{-1} as ammonium sulfate). Standard deviations are shown.

the soils from this site (taken before N fertilization or CO_2 treatments began) for ^{15}N. The results show that subsoils were consistently lower in ^{15}N than surface soils (Figure 5); thus, these soils appear to follow the pattern of high nitrification described by Högberg (1997). The soil ^{15}N results also suggest that, if rooting depth has any bearing on the observed patterns in foliar ^{15}N, trees under elevated (CO_2) were obtaining a greater proportion of their N from surface than from subsurface horizons. It is equally possible, of course, that trees under elevated (CO_2) were obtaining a more recalcitrant (and therefore ^{15}N-enriched) fraction of soil N from either the upper or lower soil horizons.

Previous studies at this site have shown that trees are taking up considerably more N per unit ground area under elevated (CO_2) (especially under the 700 μL L^{-1} treatment; Johnson et al., 1997). In the case of the low N (unfertilized) treatment, the additional N must come from either greater exploration of the soil or from increased 'mining' of soil N. Previous studies at this site have also established that root systems were better developed under elevated (CO_2) (Tingey et al., 1997; Walker et al., 1997), indicating that the soil was indeed being more extensively explored under elevated (CO_2). Other studies at this site have shown either a negative effect or no effect of elevated (CO_2) on soil N mineralization (Johnson et al., 1996, 1997),

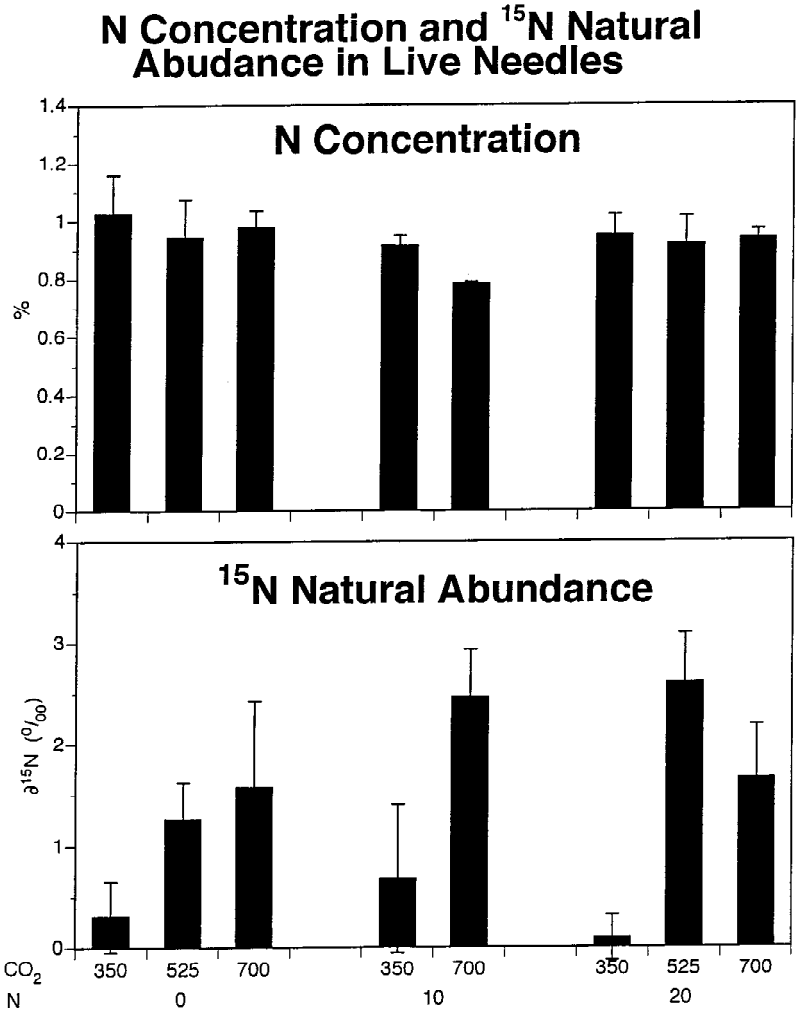

Figure 3. Microbial and nonmicrobial ^{15}N immobilization (top panel) during a laboratory study and ^{15}N natural abundance (lower panel) in naturally senesced ponderosa pine needles treated with three levels of CO_2 (350, 525 and 700 μL L^{-1}) and three levels of N fertilization (0, 10 and 20 g N m^{-2} yr^{-1} as ammonium sulfate). Standard deviations are shown.

suggesting that increased 'mining' was not occurring. However, we have found lower soil total N and increased C/N ratio with elevated (CO_2) after six years in this study (Johnson et al., in press), suggesting that soil 'mining' had occurred even if it was not detected by mineralization studies. In this context, it is very interesting to note the complete lack of N treatment effect on ^{15}N in either live or senesced needles. The lack of fertilizer effect in itself is not surprising, given that the fertilizer N was not labelled, but the lack of fertilizer effect on CO_2 response suggests that 'mining' for soil N occurred with elevated (CO_2) even with large additions of N.

Conclusions

Although field litterbag studies revealed no statistically significant effects of either CO_2 or N treatments, the clear and highly significant effect of elevated (CO_2) on nonmicrobial ^{15}N immobilization in senesced needles suggests that the reduced decomposition – slowed N cycling hypothesis has not been completely laid to rest in this case. Reduced nonmicrobial N immobilization in senesced needles under elevated (CO_2), if it holds true under normal forest stand conditions, could have many complex effects on ecosystem N cycling, including changing N availability to microbes and plants and long-term soil N accumulation

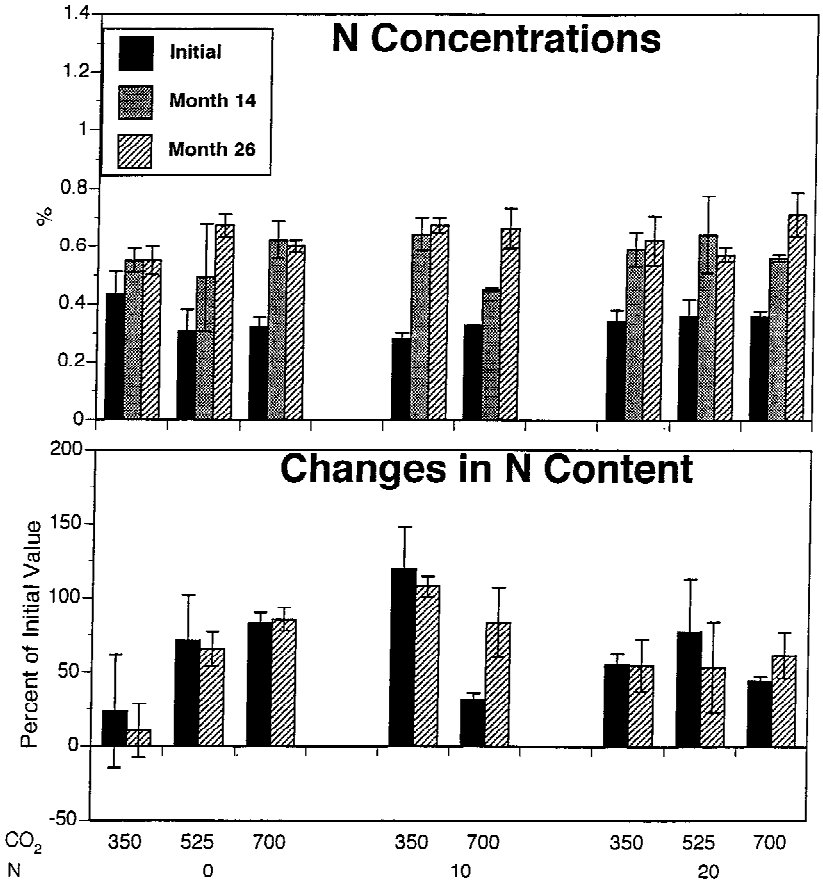

Figure 4. Nitrogen concentration (top panel) and ^{15}N natural abundance (lower panel) in live needles from ponderosa pine treated with three levels of CO_2 (350, 525 and 700 μL L^{-1}) and three levels of N fertilization (0, 10 and 20 g N m^{-2} yr^{-1} as ammonium sulfate). Standard deviations are shown.

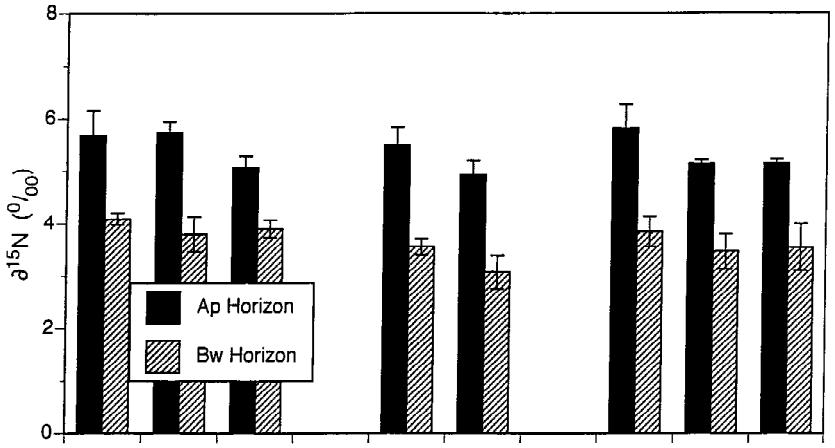

Figure 5. ^{15}N natural abundance in soils from the study site prior to treatment. Treatments included three levels of CO_2 (350, 525 and 700 μL L^{-1}) and three levels of N fertilization (0, 10 and 20 g N m^{-2} yr^{-1} as ammonium sulfate). Standard deviations are shown.

in humus. Further research is needed to determine how general this effect is.

The clearest treatment effect in this study was that of elevated (CO_2) on ^{15}N natural abundance in live and naturally senesced needles. This suggests that trees growing under elevated (CO_2) are able to tap older, more recalcitrant pools of soil N. This pattern is consistent with other results (Johnson et al., 1997 and in review) showing increased N uptake and reduced soil total N under elevated (CO_2).

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