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Forest Ecology and Management 196 (2004) 173–186
Forest Ecology and Management
www.elsevier.com/locate/foreco

Effects of chronic N additions on tissue chemistry, photosynthetic capacity, and carbon sequestration potential of a red pine (*Pinus resinosa* Ait.) stand in the NE United States

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Abstract

Temperate forests are predicted to play a key role as important sinks for atmospheric carbon dioxide, which could be enhanced by nitrogen (N) deposition. However, experimental evidence suggests that the impact of N deposition on temperate forest productivity may not be as great as originally assumed. We investigated how chronic N addition affects needle morphology, nitrogen metabolism (or partitioning) photosynthetic capacity and foliage productivity. The investigation is based at the Harvard Forest (MA) as part of a now 15-year long N amendment study. Foliar N content in red pine (*Pinus resinosa*) of the high N treatment has significantly increased, but this increase was accompanied by a de-coupling of the photosynthesis–N relationship. In addition needle longevity in the high N trees was significantly lower compared to the control trees. Conifers of the high N treatment did not use the surplus of N to optimize the amount of photosynthetically active metabolites. Instead N accumulated as soluble protein (other than Rubisco), amino acids and chlorophyll. Photosynthetic capacity in the control trees was about 50% higher than in the fertilized trees. These results indicate that the increase in leaf N is not accompanied by a greater capacity for carbon assimilation in the high N treatment. Using a simple model (PnET-Day) of canopy photosynthesis and carbon allocation, we assessed the long-term effect of these physiological changes on ecosystem carbon balance. The model results emphasize and reinforce the large difference between rates of carbon accumulation predicted to occur if net photosynthesis remained linearly related to foliar N concentration, and rates measured in the field.

Keywords: Photosynthesis; Needle longevity; Nitrogen; Chlorophyll; NPP; *Pinus resinosa*

1. Introduction

Atmospheric nitrogen (N) deposition has been identified as the probable cause of both forest decline (McNulty et al., 1996; Schulze, 1989) and enhanced productivity in temperate forests (Schindler and Bayley, 1993; Townsend et al., 1996). A significant portion of the large carbon (C) sink presumed to be in the northern hemisphere (Ciais et al., 1995; Fan et al., 1998) has been ascribed to fertilization by N deposition. This mechanism is especially important as N deposition rates will remain unchanged or increase

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doi:10.1016/j.foreco.2004.03.032

over much of the developed and developing world, even as sulfur deposition has dropped considerably (Galloway, 1995; Holland et al., 1999). Whether N deposition has positive or negative effects on an ecosystem depends on the N status of the system and the rate and duration of N deposition (Aber, 1992; Aber and Driscoll, 1997). Most added N enters the soil and is rapidly immobilized (Boyce et al., 1996; Magill et al., 2000; Nadelhoffer et al., 1999). If the remaining available N does not exceed the capacity for N uptake by vegetation net primary productivity (NPP) and C sequestration may be enhanced. If deposition rates exceed the capacity for N uptake, nutrient imbalances can lead to forest decline due to N saturation (Aber et al., 1989; Agren and Bosatta, 1988; Nihlgard, 1985).

One of the most consistent responses of forests to N inputs in the high concentration range is higher foliar N concentrations (Ericsson et al., 1993; Magill et al., 2000; Naesholm et al., 1994; van Dijk and Roelofs, 1988). However, over the low to medium range of N deposition, large surveys in Europe (Cape et al. 1990, Bauer et al., 2000) and in the US (Aber et al., 2003) showed that N concentration is relatively insensitive. Along a transcontinental transect through Europe foliar N remained conservative for spruce and beech ecosystems even though N deposition reached 20 kg N ha^{-1} per year while sulphur deposition peaked at over 40 kg S ha^{-1} per year (Bauer et al., 2000). Changes in ecosystem N cycling will also have a strong impact on carbon cycling. The relationship between foliar N and maximum photosynthetic capacity (Field and Mooney, 1986; Evans, 1989; Reich et al., 1997) predicts that increased foliar N content should lead to a proportional C gain per unit mass of foliage. However, the few studies that have examined leaf physiological responses to long-term atmospheric pollution, have found that photosynthetic capacity may or may not be enhanced in trees (Lange et al., 1987; Weidner and Kraus, 1987). In fact, due to other nutrient imbalances, these forest canopies can exhibit higher leaf turnover rates and reduced leaf area (Lange et al., 1989; Oren and Zimmermann, 1989). As a consequence, persistent or even chronic nutrient shortage has been shown to reduce forest productivity and long-term carbon sequestration potential (Bauer et al., 2000).

At the Harvard Forest chronic N amendment study (Magill et al., 1997 and this issue), foliar N content of red pine (*Pinus resinosa* Ait.) has more than doubled in response to chronic N additions, but recently wood production rates have decreased significantly (Magill et al., 2000 and this issue). The purpose of this study is to explore the effect of long-term N additions on nitrogen metabolism, photosynthetic capacity and carbon sequestration potential of a temperate forest ecosystem following 15 years of chronic N additions (Magill et al., 1997 and this issue). Three primary questions will be addressed. How does excess supply of N change the biochemistry of N partitioning in red pine needles? Does the additional N in the foliage support a higher photosynthetic capacity? What is the predicted effect of the change in foliar physiology on sequestration of C at the stand level?

2. Material and methods

2.1. Study site and experimental treatments

This investigation was part of the chronic N amendment study at the Harvard Forest in central Massachusetts (42°30′N, 72°10′W). Chronic additions of nitrogen have been carried out in adjacent red pine (*Pinus resinosa*) and mixed deciduous stands since 1988 on land that was formerly cultivated or used as a pasture (Magill et al., 1997 and this issue). The red pine stand is an even-aged stand that was planted in 1926 on a soil classified as a Typic Dystrochrept. Monthly temperatures range from 19 °C in July to −12 °C in January, with an average annual precipitation of 112 cm. Total N deposition to the site averages at about 8 kg N ha^{-1} per year (Ollinger et al., 1993). Of the 30 m × 30 m plots, which are used for the different treatments, we chose the control and the high N application for this study. N is applied as a concentrated solution of NH_4NO_3 divided into six equal monthly doses (May to September) totaling 50 (low N) and 150 (high N) kg N ha^{-1} per year. Within the first year of N application significant nitrate losses were recorded in the pine stand, which continues to increase (Magill et al. this issue). N retention since the start of the fertilizer application has averaged 85% of deposition plus additions for the 10-year period from 1989 to 1999. However, most of the N

was retained in the soil organic matter pool (84%) and woody biomass increment decreased in the high N pine plot relative to the control (Magill et al., 2000 and this issue).

2.2. Sample collection and chemical analysis

Needle sampling was carried out in July 2000 and was restricted to the control (ambient N deposition) and high N plot of the red pine stand, due to limited canopy access. Two 25 m high walk-up towers were erected using standard construction scaffolding, which allowed sampling of two trees in both plots. From each tree, needles were collected from up to three individual branches. Needle samples were equally divided for subsequent C and N metabolite analysis, as well as leaf area and dry weight determination. Total C and N were measured with a Fison CHN analyzer (Milan, Italy) from needle material, which was dried at 75 °C and ground with a micro-dismembrator (Braun Bio-Tek). Needle material for protein analysis was filled in test tubes, shock-frozen in liquid nitrogen and stored on dry ice. The frozen leaf material was ground with a pre-chilled mortar and pestle to a fine powder and transferred immediately into a pre-weighed test tube. The frozen leaf powder (50–100 mg FW) was mixed with 1.5 ml of extraction buffer consisting of 100 mM Tris–HCl, 20 mM $MgCl_2$, 10 mM $NaHCO_3$, 1 mM NaEDTA, 5 mM DTT and 10% (v/v) glycerol (pH 8.0 with NaOH). The test tube was stirred vigorously and immediately centrifuged at $15{,}000 \times g$ for 10 min. The supernatant was transferred into a new vial and used for analysis of soluble protein with a microplate reader (Bio-Tek EL-309, Vermont, USA) based on the method of Bradford (1976) using bovine serum albumine (BSA) as the standard (Jones et al., 1989). Chlorophyll was extracted from fresh leaf material with dimethylformamide (DMF) as the solvent (Porra et al., 1989), and chlorophyll concentrations were calculated according to the equations given by (Wellburn, 1994). Carbohydrates (soluble sugars and starch) were measured following the procedures in Bauer et al. (2001). Both soluble sugars and starch were analyzed colorimetrically as glucose equivalents with the anthrone reaction (Allen, 1989) using a microplate reader. Structural carbon was calculated as the difference between total C concentration and the sum of soluble sugars and starch concentration.

Needles for amino acid analysis were chopped finely and placed in 1 ml of 5% ice cold perchloric acid. The tubes were kept on ice during transport to the laboratory and stored frozen at −20 °C until further analyses. The frozen samples were thawed and refrozen (−20 °C) three times (Minocha et al., 1994), centrifuged at $13{,}500 \times g$ for 10 min and 100 μl of the supernatant was used for dansylation according to the procedure of Minocha et al. (1990) with the following modifications. After a 60 min incubation period, 50 μl of L-alanine (100 mg ml^{-1} in water) in place of proline was added to the reaction mixture to react with the excess dansylchloride. One half of the dansylated mixture (135 μl) was placed in a new microfuge tube, to which 730 μl of methanol and 135 μl of 2.9 N acetic acid were added, in order to remove excess sodium carbonate in the form of CO_2. The suspension was mixed and filtered with a 0.45 μm nylon syringe filter. The dansylated free amino acids were detected using a modified HPLC method (Warner and Dorsey, 1997). A 40% gradient of a mobile phase A (100% acetonitrile) was run for 37.5 min at 1 ml min^{-1} using 25 mM sodium acetate buffer (pH 5.94) containing 1-propanol (3%) and acetonitrile (10%) as mobile phase B. The amino acids were quantified based on an external standard. The liquid chromatographic system consisted of a Perkin-Elmer series 410 pump, a Hitachi AS-4000 auto-sampler (Hitachi Limited, Tokyo, Japan) fitted with a 100 μl loop (10 μl actual injection volume), a Phenomenex LUNA C18 (3 μm particle size, 100 mm × 4.6 mm, i.d. cartridge column: #00D-4251-EO; Phenomenex, Torrance, CA) and a Shimadzu RF-10A_{XL} fluorescence detector (Shimadzu Scientific Instruments Inc., Columbia, MD). The excitation and emission wavelengths were set at 340 and 515 nm, respectively. A Gilson 712 HPLC System Controller (version 1.2) was used to integrate the data (Gilson Inc., Middleton, WI, USA). Along with the 19 common amino acids in plants, two other uncommon amino acids, ornithine and γ-amino butyric acid (GABA) can be detected, while threonine, asparagines and tyrosine could not be quantified by this method. The free amino acid (FAA) pool of the red pine needles is dominated by the major amino acids glutamic acid, arginine, proline, leucine, lysine and γ-amino butyric acid. Therefore N allocated to the FAA was calculated as the sum of N in these six amino acids only.

2.3. Photosynthesis measurements

For a subset of needles CO_2 response curves at saturating light (A/C_i-curves) were carried out in the canopy with a Li-Cor 6400 (Li-Cor Inc., Lincoln, Nebraska) photosynthesis system. The cuvette CO_2 concentration was controlled with a CO_2 mixer attached to the system, allowing a stepwise change of CO_2 in the inflowing air stream. Light levels were maintained at a photosynthetic photon flux density of 1500 μmol m^{-2} s^{-1} by a red–blue light source. In addition, steady state light response was measured for all needle age classes on the sample branches to determine maximum photosynthetic capacity at saturating light (P_{max}) and ambient CO_2 concentration. The A/C_i-curves were analyzed with the PhotosynAssistant software (Dundee Scientific, UK) in order to determine A_{max} and maximum carboxylation capacity V_{cmax} following the calculation procedure as described in Bauer et al. (2001). The program provides initial parameter estimation for V_{cmax}, J_{max} (the maximum electron transport capacity) and TPU (triose phosphate utilization) based on the algorithms for the biochemical model of leaf photosynthesis by Farquhar et al. (1980) and von Caemmerer and Farquhar (1981). All input parameters were set according to Harley and Sharkey (1991). After calculating the initial parameter estimates, the program uses an iterative procedure to calculate the rate for V_{cmax} from the gas exchange data.

2.4. Estimated canopy carbon balance

A simple model of canopy photosynthesis and carbon allocation was used to assess the potential effect of changes in foliar physiology on ecosystem carbon balances. The model (PnET-Day) has been tested extensively against data collected at the Harvard Forest including whole-canopy gross photosynthesis data acquired by eddy covariance methods and wood and foliar NPP data (Aber et al., 1995, 1996). In PnET-Day, photosynthetic capacity is predicted from foliar N content. For all runs, we used the same canopy and stand parameters as in the previous work (Aber et al., 1995) with the exception of foliar N concentration and needle longevity data reported for both control and high N stands below. For the high N site, we also used foliar data from this study, but then ran the model again constraining A_{max} to the values reported here, rather than the value estimated from foliar N concentration. The constrained model was run both with and without a proportional reduction in foliar dark respiration rates. The model was driven with measured time-series climate data from the Harvard Forest for the period 1991–1998. Results represent both the immediate effect on carbon gain and the feedback with foliar production over this 8-year period.

3. Results

The N application resulted in significant changes in the morphology of control and high N needles (Table 1). Needles of the high N red pine trees are both longer and heavier with changes ranging from 14 to about 42% as compared to the control trees. Because of the parallel increase in needle dry weight and area, specific leaf area (SLA (cm^2 g^{-1})) of the red pine needles showed no significant differences between control and high trees. Despite the large changes in needle morphology, the differences between control and high N needles remained proportional with high N trees having greater needle area and length for any given dry weight (Fig. 1A and B). Needle retention also changed significantly in response to N application (Fig. 2). Three-year old needles were rare to absent in the high N stand, while in the control stand an average of 40% of all needles were still present in the third year. On an average, needles in the control stand had a half-life of 2.5 years, while those in the high N stand averaged 1.5 years.

The N concentrations in the needles of high N trees were significantly higher than those in the control trees (Table 2). Mass and area based N concentrations increased between 55 and 110% in the high N treatment. In both treatments 1-year-old needles generally had the highest N concentration. The C/N ratio in the high N needles was significantly lower in the control trees. In absolute terms the C/N ratio decreased between 36 and 50%. The change in the C/N ratio was entirely due to the large changes in total N, because total carbon does not change significantly due to treatment or needle age (Table 2). Concentrations of soluble sugars and starch, however, decreased in the high N pine needles and also changed significantly with needle age.

Table 1
Morphological characteristics (average ± S.E.) for red pine trees from the control and the high N treatment

	Age	Control	High N	Change (%)	PT	PNa
Specific leaf area ($cm^2\ g^{-1}$)	0	34.68 ± 2.39 a	37.92 ± 2.18 a	9.34	0.707	<0.001
	1	29.15 ± 1.87 ab	28.74 ± 0.66 b	−1.41		
	2	28.81 ± 1.95 ab	27.59 ± 0.98 c	−4.22		
	3	22.01 ± 0.41 b				
Needle dry weigth (mg $needle^{-1}$)	0	35.02 ± 7.52 a	39.98 ± 2.69 a	14.15	<0.001	<0.001
	1	50.83 ± 3.21 ac	72.22 ± 2.05 b	42.08		
	2	54.33 ± 5.34 bc	74.88 ± 2.77 b	37.82		
	3	73.74 ± 3.66 b				
Needle area (cm^2 $needle^{-1}$)	0	116.30 ± 17.88 a	151.17 ± 13.02 a	29.99	<0.001	0.002
	1	147.48 ± 11.80 a	207.27 ± 5.69 b	40.54		
	2	153.68 ± 10.35 a	205.54 ± 10.46 b	33.75		
	3	162.28 ± 11.04 a				
Needle length (cm $needle^{-1}$)	0	11.23 ± 0.80 a	12.94 ± 0.64 a	15.19	<0.001	<0.001
	1	13.39 ± 0.55 b	16.93 ± 0.25 b	26.46		
	2	13.45 ± 0.36 b	16.48 ± 0.54 b	22.55		
	3	14.18 ± 0.18 b				

Needles ($n = 3$–6, except for 3-year-old control needles with $n = 2$) were collected in the upper third of the crown from three to four branches of two trees per treatment. Different letters in the same column indicate significant differences between needle age classes within each treatment (LSD, $P = 0.05$). Change (%) indicates the increase or decrease of each morphological parameter of high N needles relative to the control. The last two columns contain the significance levels from the multivariate analysis of variance (GLM) with treatment (P_T) and needle age (P_{NA}) as the main factors.

In needles of high N trees protein increased by 65–80%, while chlorophyll increased by 25–120% (Fig. 3a and b). Nitrogen partitioned to the FAA increased by several orders of magnitude (Fig. 3c). The composition of the free amino acid pool in the control needles was almost equally dominated by glutamate, gaba and leucine (Table 3). Individually all amino acids increased in the high N pine needles due to the N

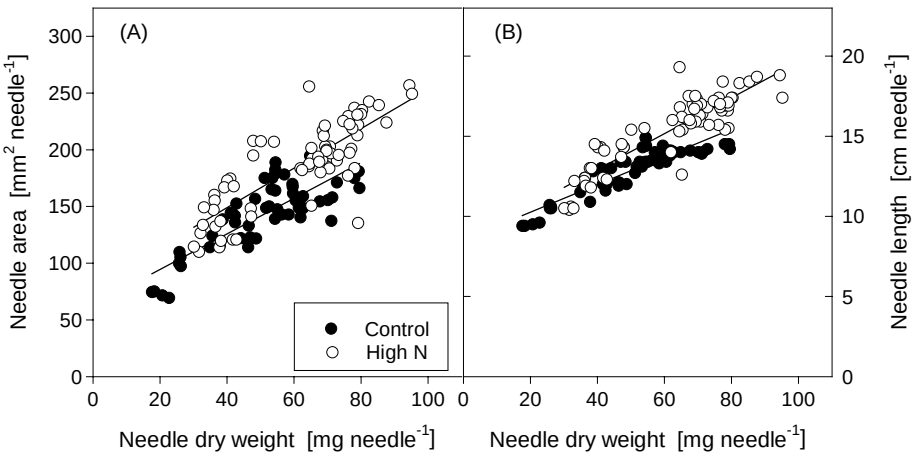

Fig. 1. Relationship between needle dry weight and needle area (A), and between needle dry weight and needle length (B) for red pine trees of the control ($n = 55$, black circles) and the high N treatment ($n = 67$, white circles). Each data point represents the value for individual needles across all needle age classes (0–3 years).

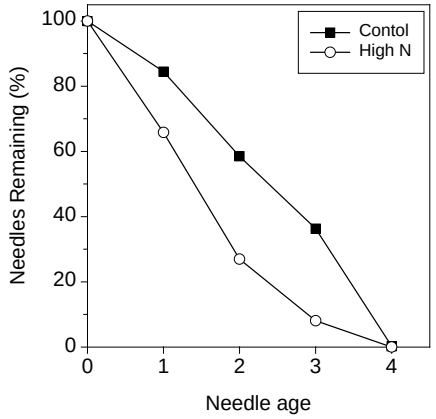

Fig. 2. Needle retention (as percentage remaining live needles) on branches of control (black circles) and high N (white circles) red pine trees. Each data point represents the average ± S.E. ($n = 3$) per needle age class. Needle retention was calculated based on a census in the previous year of this study.

application, but the large increase in the FAA was entirely dominated by the change in arginine. The increase in arginine and consequently in FAA resulted in a lower contribution of the remaining amino acids to the composition of the FAA in high N needles. Not only did the change in arginine concentration dominate the composition of the FAA (Fig. 4a), it also correlated significantly with the change in total N concentration of the high N pine needles (Fig. 4b).

The contribution of N partitioned into protein, chlorophyll and amino acids increased significantly with increasing needle nitrogen concentration (Fig. 5a–c). However, protein nitrogen tended to reach a limit at high N concentration (Fig. 5a), while chlorophyll and amino acids continued to increase linearly with foliar N. In relative terms, protein and chlorophyll contributed equally to total N in control and high N needles, whereas amino acid bound nitrogen increased linearly with increasing needle nitrogen concentration (Fig. 5d–f). Above a concentration of

Table 2
Total nitrogen on mass and area basis, C/N ratio, total carbon and carbohydrates of red pine needles from the control and the high N treatment (average ± S.E.)

Age	Control	High N	Change (%)	P_T	P_{NA}
Specific leaf area ($cm^2\ g^{-1}$)					
0	34.68 ± 2.39 a	37.92 ± 2.18 a	9.34	0.707	<0.001
1	29.15 ± 1.87 ab	28.74 ± 0.66 b	−1.41		
2	28.81 ± 1.95 ab	27.59 ± 0.98 c	−4.22		
3	22.01 ± 0.41 b				
Needle dry weigth (mg per needle)					
0	35.02 ± 7.52 a	39.98 ± 2.69 a	14.15	<0.001	<0.001
1	50.83 ± 3.21 ac	72.22 ± 2.05 b	42.08		
2	54.33 ± 5.34 bc	74.88 ± 2.77 b	37.82		
3	73.74 ± 3.66 b				
Needle area (cm^2 per needle)					
0	116.30 ± 17.88 a	151.17 ± 13.02 a	29.99	<0.001	0.002
1	147.48 ± 11.80 a	207.27 ± 5.69 b	40.54		
2	153.68 ± 10.35 a	205.54 ± 10.46 b	33.75		
3	162.28 ± 11.04 a				
Needle length (cm per needle)					
0	11.23 ± 0.80 a	12.94 ± 0.64 a	15.19	<0.001	<0.001
1	13.39 ± 0.55 b	16.93 ± 0.25 b	26.46		
2	13.45 ± 0.36 b	16.48 ± 0.54 b	22.55		
3	14.18 ± 0.18 b				

Different letters in the same column indicate significant differences between needle age classes within each treatment (LSD, $P = 0.05$). The last two columns contain the significance levels from the multivariate analysis of variance (GLM) with treatment (P_T) and needle age (P_{NA}) as the main factors. The corrected ANOVA model for total carbon concentration was not significant ($P = 0.626$).

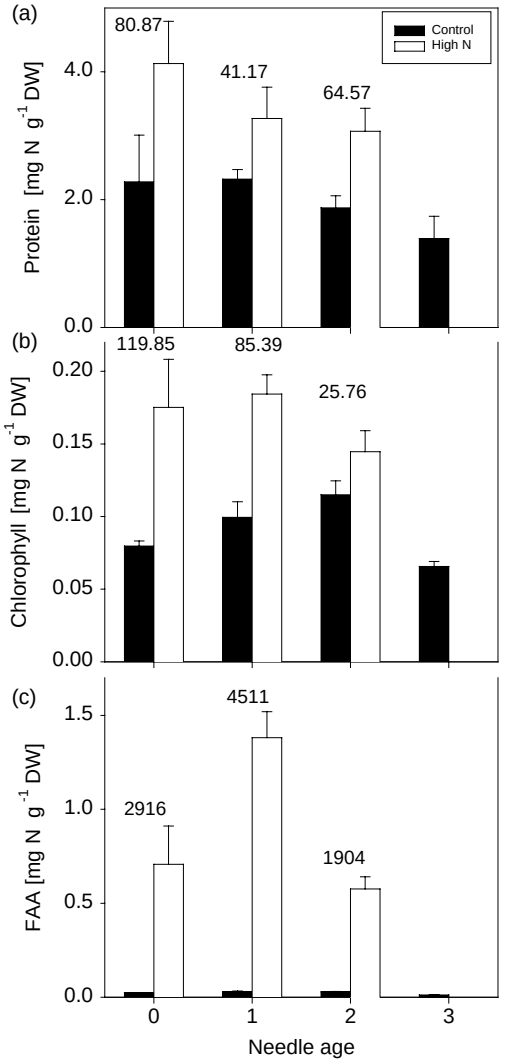

Fig. 3. Nitrogen partitioning (average ± S.E.) to soluble protein, chlorophyll and to the free amino acid pool (FAA) in red pine needles from control (black bars) and high N (white bars) trees. Needles ($n = 3$–6, except for 3-year-old control needles with $n = 2$) were collected in the upper third of the crown from three to four branches of two trees per treatment. Numbers on top of the data bars indicate the percentage increase or decrease of a particular metabolite in high N needles relative to the control. Differences between treatments (P_T) and within needle age classes (P_{NA}) were calculated with a multivariate analysis of variance (GLM).

about 14 mg N g^{-1} DW the amino acid contribution to the total needle N pool more than doubled.

Photosynthetic capacity of red pine needles from the high N treatment was significantly lower than that of control trees (Table 4). Light saturated photosynthetic rates of the high N pine needles were about 30–60% lower (mass- and area-related) than rates of control needles. Photosynthetic capacity changed significantly between different needle age classes following the changes in needle mass. Also, CO_2 saturated photosynthetic response and the capacity for carboxylation (V_{cmax}) and electron transport (J_{max}) declined in high N pine needles (Table 4, Fig. 6).

Predictions from the PnET-Day model emphasize the stand-level impact of the alterations in photosynthetic capacity reported here. Without implementing lower photosynthetic capacity, total annual gross photosynthesis, total foliar production, and total wood production would be expected to increase by 25–50% (Fig. 7). Constraining the model with a 30% (mass basis) reduction in net photosynthesis corresponding to the reduction in photosynthetic capacity of 1-year-old needles and no change in dark respiration rates results in a nearly 90% reduction in net photosynthesis and NPP over the 8-year period of simulation. Even with lower respiration and A_{max}, both carbon gain and NPP are reduced by about 80% after 8 years. It is significant that wood production has fallen dramatically in the high N pine plot from 1988 to the present and tree mortality has become acute (Magill et al., this issue). In addition further reductions in productivity were observed in the three-year period of measurements (1996–1999) surrounding the physiological studies presented here (Magill et al., unpublished data). Annual wood production in the high N pine essentially has approached zero.

4. Discussion

The Harvard Forest chronic N amendment study represents an extreme situation of a temperate forest under simulated inputs of high amounts of inorganic nitrogen beyond the levels commonly experienced in the region (Ollinger et al., 1993). Results from this experiment to date suggest that identical changes in forest structure and biogeochemistry are encountered here as in European forest ecosystems (Bredemeier et al., 1998). However, there is still a lack of information on how atmospheric N deposition will affect canopy photosynthesis and in turn carbon sequestration potential. There is growing evidence to suggest

Table 3
Amino acid concentrations (μmol AA g^{-1} DW) in red pine needles from the control and the high N treatment (average ± S.E.). Needles ($n = 3$–6, except for 3-year-old control needles with $n = 2$) were collected in the upper third of the crown from three to four branches of two trees per treatment

Age	Control	High N	Change (%)	P_T	P_{NA}	FAA (%)
Glutamate						
0	0.49 ± 0.04	2.06 ± 0.17	316.8	<0.001	0.001	−50.2
1	0.63 ± 0.13	1.27 ± 0.13	102.3			−85.0
2	0.61 ± 0.03	0.87 ± 0.06	43.5			−80.2
3	0.33 ± 0.06					
Arginine						
0	n.d.	11.01 ± 3.50	n/a			n/a
1	0.01 ± 0.00	23.26 ± 2.39	182,313	<0.001	0.014	12,487
2	0.02 ± 0.01	8.64 ± 1.05	35,896			4,839
3	n.d.					
Proline						
0	0.22 ± 0.01	0.46 ± 0.12	105.4	0.042	0.209	−74.9
1	0.38 ± 0.11	0.72 ± 0.12	91.0			−86.2
2	0.28 ± 0.01	2.97 ± 1.23	971.8			20.3
3	0.25 ± 0.04					
Gaba						
0	0.30 ± 0.02	2.02 ± 0.29	561.8	<0.001	0.887	−29.8
1	0.54 ± 0.03	1.88 ± 0.12	247.0			−74.7
2	0.54 ± 0.08	1.71 ± 0.28	216.4			−58.0
3	0.22 ± 0.03					
Leucine						
0	0.53 ± 0.02	0.88 ± 0.06	65.6	<0.001	<0.001	−80.5
1	0.40 ± 0.03	0.61 ± 0.04	50.1			−89.0
2	0.41 ± 0.02	0.47 ± 0.02	14.3			−84.2
3	0.02 ± 0.00					
Lysine						
0	0.06 ± 0.00	0.54 ± 0.12	791.8	<0.001	0.169	−13.7
1	0.07 ± 0.01	0.57 ± 0.06	732.0			−40.1
2	0.06 ± 0.01	0.29 ± 0.01	383.2			−33.3
3	0.03 ± 0.01					

Also given are the change (%) in amino acid concentration, the significant levels from the multivariate analysis of variance (GLM) with treatment (P_T) and needle age (P_{NA}) as the main factors, and the change (%) in contribution to the free amino acid pool (FAA) due to the N treatment.

that increases in temperate forest productivity due to N deposition may not be as great as several studies have suggested. Most N deposited in forest ecosystems is immobilized within the soil (Buchmann et al., 1996; Magill et al., 1997; Tietema et al., 1998). Many studies have found a significant, positive relationship between the rate of N deposition and N losses due to leaching (mostly of nitrate) for a given forest/soil type (Bredemeier et al., 1998; Gundersen, 1995; Peterjohn et al., 1996). The fraction of N deposited on forests that is acquired by trees is significantly less (on average only 20%) than has been previously assumed (Nadelhoffer et al., 1999 and this issue). In addition, loss of mobile cations due to soil acidification and nitrate leaching can lead to nutrient limitations (especially for Mg and Ca), which has been implicated as a primary cause for forest decline (Schulze, 1989).

Long-term additions of inorganic N in the red pine stand have led to an increase in foliar N (Magill et al., 1997 and this issue), leaching of nitrate and cations,

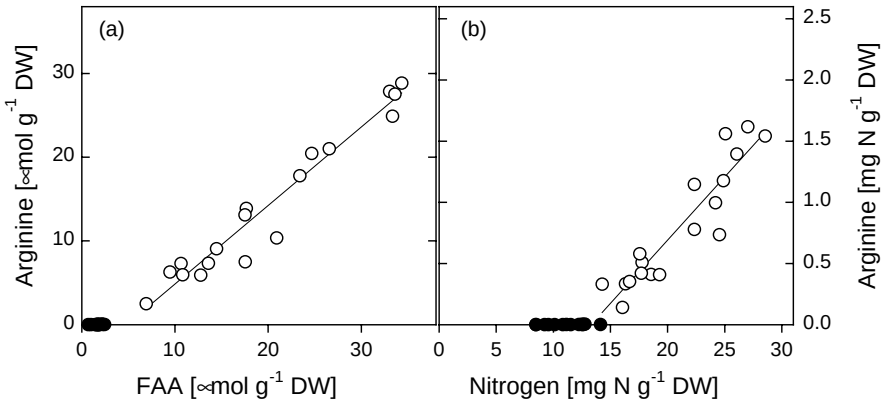

Fig. 4. Relationship between arginine concentration and free amino acid concentration (a) and between nitrogen allocated to arginine and total nitrogen concentration (b) in red pine needles from control (black circles) and high N (white circles) trees. Needles ($n = 3$–6, except for 3-year-old control needles with $n = 2$) were collected in the upper third of the crown from three to four branches of two trees per treatment. In both figures a linear regression was calculated for the needles of the high N treatment with $P < 0.001$ for both regression lines.

reductions in element N ratios in foliage and increased production of secondary metabolites (Magill et al., 2000; Minocha et al., 2000). Similar significant changes were observed in terms of foliar N partitioning. Nitrogen allocated to protein, chlorophyll, and amino acids increased substantially, but only the increase in amino acid bound nitrogen had a significant effect on needle nitrogen concentration. The changes in key metabolites indicate that the pattern of photosynthetic N partitioning under conditions of chronic N additions has changed and that nitrogen is diverted away from photosynthesis causing a decoupling in the functional allocation of photosynthetically used nitrogen. As a result photosynthetic capacity is down regulated because most of the excess N is not invested into the primary processes of carboxylation.

Plants are known to respond to various forms of abiotic and biotic environmental stress by changes in metabolite levels. High leaf N concentrations can result from a disproportionately high fraction of non-protein or non-photosynthetic protein N. Increases in leaf N concentration in trees resulting from high N deposition are typically accompanied by increases in individual amino acids and the FAA (Bauer et al., 2000; Ericsson et al., 1993; Ericsson et al., 1995; van Dijk and Roelofs, 1988), polyamines (Minocha et al., 2000), and under certain circumstances nitrate (Gebauer and Stadler, 1990; van Dijk and Roelofs, 1988). Leaf level changes in the concentration of individual amino acids and in the free amino acid pool occur even at very low levels of N deposition without any significant change in total N (Bauer et al., 2000; Naesholm et al., 1994; Schneider et al., 1996). Under conditions of high N supply, trees store N in the form of nitrate, amino acids and protein. While nitrate storage can represent a significant trade off because of high maintenance costs, storage of organic N, either as amino acid or protein, is much more favorable (Pate and Layzell, 1990), because the organic N form is readily available for plant growth when external supply decreases.

The most important amino acid for the high N red pine trees is arginine, which is also the most frequently observed N storage compound because of the favorable N/C ratio. In addition, arginine, together with other key amino acids such as glutamate or gaba, are believed to signal N saturation from the shoot to the root (Rennenberg et al., 1998) and therefore have the potential to inhibit further root N uptake (Imsande and Touraine, 1994; Marschner et al., 1996). The implication at the stand level would be to what extent a metabolic root–shoot signal can influence the whole ecosystem N cycling, because as a result, more unused soil N is likely lost to deeper soil layers and into aquifers. The changes in the composition of individual amino acids found in red pine are in agreement with other studies. The significant increase in glutamate and in particular in arginine concentration in the high N needles is a

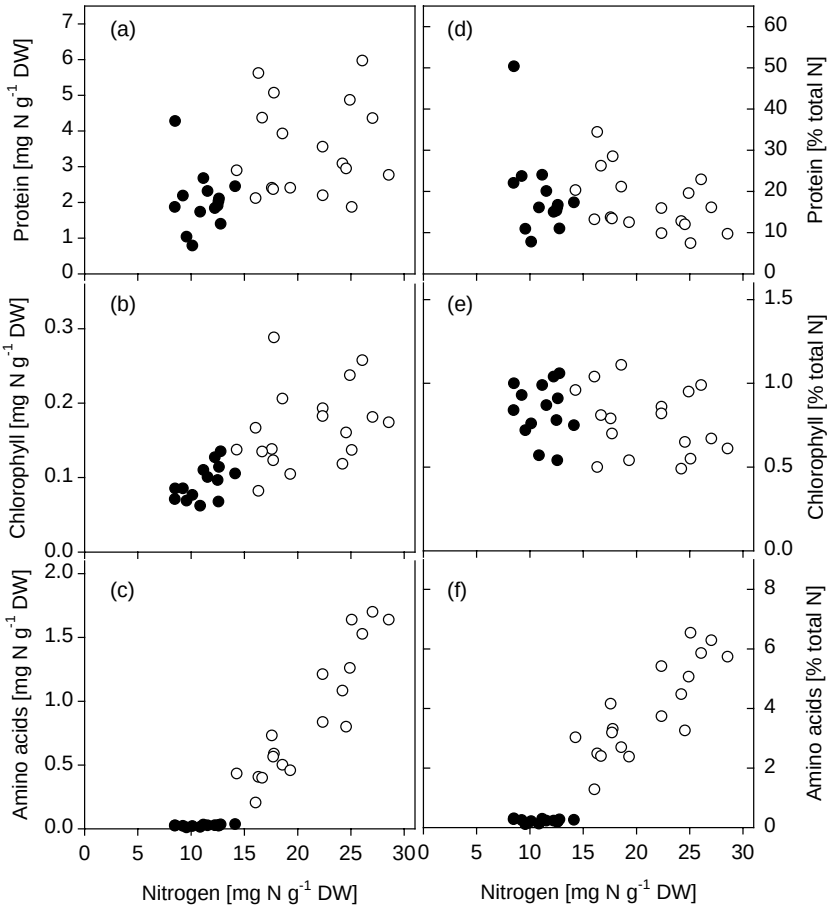

Fig. 5. Relationship between total N concentration and N allocated to protein, chlorophyll and amino acids (a, b, c) and percent N allocated to protein, chlorophyll and amino acids (d, e, f) in red pine needles from control (black circles) and high N (white circles) trees. Needles ($n = 3$–6, except for 3-year-old control needles with $n = 2$) were collected in the upper third of the crown from three to four branches of two trees per treatment. Linear regressions in panels a, b and in d, e were calculated for the entire data set of control and high N trees, whereas linear regressions in panels c and f were restricted to the values for the high N trees.

characteristic response of a formerly N limited forest to chronic additions of N (Rennenberg et al., 1998). There is however no clear evidence as to what extent increased arginine concentrations inhibit root N uptake.

Due to significantly shorter needle life span, the larger needles in the high N treatment do not compensate for lower photosynthetic rates and in turn do not lead to higher canopy photosynthesis. Modeling stand productivity using data on leaf N and photosynthetic capacity, as reported here, demonstrated that the disruption of the functional N partitioning might lead to significant reductions in carbon sequestration potential (Bazzaz, 1997). In the long term, surplus consumption of N paired with limitations of other nutrients could constrain the capacity of the high N trees to further increase total foliar mass (Aber et al., 1998; Asner et al., 1997). Severe nutrient shortages can cause a limitation in sink strength/size for recent photosynthates, which is likely to negatively feedback on photosynthetic N partitioning (Stitt and Krapp, 1999). Carbon assimilation of the high N red pine trees is limited because of significant reductions in needle retention and the low amount of carbohydrates to supply substrate for the carboxylation processes.

Table 4
Photosynthetic capacity of red pine needles from control and high N trees (average ± S.E.) expressed on leaf mass- and leaf area-basis

Age	Control	High N	Change (%)	P_T	P_{NA}
P_{max} (mass) (nmol g^{-1} s^{-1})					
0	74.23 ± 7.16 ac	34.88 ± 3.99 a	−53.00	<0.001	0.001
1	45.08 ± 6.08 b	31.17 ± 3.82 a	−30.86		
2	43.54 ± 3.56 b	21.01 ± 2.40 b	−51.75		
3	52.87 ± 1.79 a				
P_{max} (area) (μmol m^{-2} s^{-1})					
0	2.23 ± 0.22 a	0.88 ± 0.09 a	−60.40	<0.001	0.008
1	1.66 ± 0.25 b	1.11 ± 0.15 b	−33.01		
2	1.61 ± 0.16 b	0.78 ± 0.09 a	−51.56		
3	2.41 ± 0.01 a				
V_{cmax}					
	12.18 ± 1.66	9.45 ± 1.10	−22.41	0.280	
J_{max}					
	26.70 ± 2.69	16.50 ± 1.76	−38.20	0.027	

The upper part of the table contains the age-dependent rates for light saturated photosynthesis rates (P_{max}). The last two columns contain the significance levels from the multivariate analysis of variance (GLM) with treatment (P_T) and needle age (P_{NA}) as the main factors. The lower part of the table contains the grand means for the biochemical limitations of photosynthesis (μmol m^{-2} s^{-1}) calculated according the biochemical model of photosynthesis von Caemmerer and Farquahr (1981) with the parameters according Harley et al. (1991). Statistical differences between treatments for V_{cmax} and J_{max} were calculated with a *t*-test for independent samples.

The connection between the foliar N partitioning and photosynthetic capacity emphasizes the importance of key physiological processes at the leaf level for modeling of stand level dynamics. Reductions in photosynthetic capacity due to excess N supply feeds back on the ecosystem carbon balance. Results from the PnET-Day simulation suggest that feedback between reduced net carbon gain and allocation to future foliar production will lead to significant declines in both foliar and wood production—trends which have been observed in the high N stand.

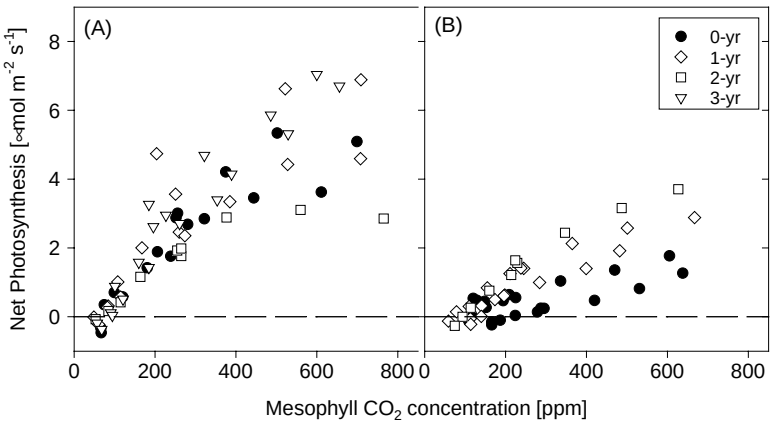

Fig. 6. Relationship between net photosynthetic rate and mesophyll internal CO_2 concentration (A/C_i-curve) for different needle age classes of control (A) and N fertilized red pine trees (B). Needles ($n = 3$–6, except for 3-year-old control needles with $n = 2$) were collected in the upper third of the crown from three to four branches of two trees per treatment.

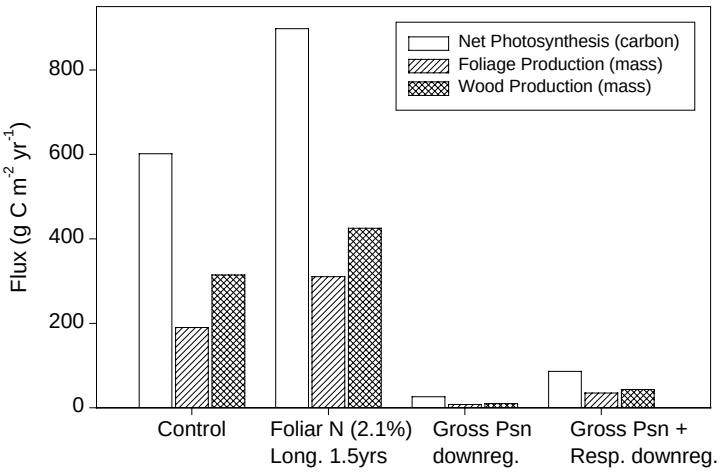

Fig. 7. Gross photosynthesis, foliar production and wood production as predicted by the PnET-Day model after 8 years under four different growth scenarios: (1) control with recent conditions for needle N (1.1%) and needle longevity (2.5 years), (2) high N (2.1%N and 1.5 years of needle longevity), (3) high N with down-regulation of photosynthesis (30% less than control, mass basis), and (4) high N with down-regulation of both photosynthesis and foliar dark respiration.

Acknowledgements

The authors would like to thank Mr. Benjamin Mayer for analysis of free amino acids at the USDA Forest Service, Durham, NH.

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