

N/P/K Ratios and CO₂ Concentration Change Nitrogen-Photosynthesis Relationships in Black Spruce

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Abstract

The relationship between photosynthesis and leaf nitrogen concentration is often used to model forest carbon fixation and ratios of different nutrient elements can modify this relationship. However, the effects of nutrient ratios on this important relationship are generally not well understood. To investigate whether N/P/K ratios and CO₂ concentration ([CO₂]) influence relationships between photosynthesis and nitrogen, we exposed one-year-old black spruce seedlings to two [CO₂] (370 and 720 μmol·mol⁻¹), two N/P/K ratio regimes (constant (CNR) and variable (VNR) nutrient ratio) at 6 N supply levels (10 to 360 μmol·mol⁻¹). It was found that photosynthesis (P_n) was more sensitive to nitrogen supply and N/P/K ratios under the elevated [CO₂] than under ambient [CO₂]; under the elevated [CO₂], P_n declined with increases in N supplies above 150 μmol·mol⁻¹ in the CNR treatment but was relatively insensitive to N supplies of the same range in the VNR treatment. Further, our data suggest that the nutrient ratio and the CO₂ elevation effects on photosynthesis were via their effects on the maximum rate of carboxylation (V_{cmax}) but not electron transport (J_{max}) or triose phosphate utilization (TPU). The results suggest that the CO₂ elevation increased the demand for all three nutrient elements but the increase was greater for N than for P and K. The CO₂ elevation resulted in greater photosynthetic use efficiencies of N, P and K, but the increases varied with the nutrient ratio treatments. The results suggest that under elevated [CO₂], higher net photosynthetic rates demand different optimal N-P-K ratios than under the current [CO₂].

Keywords

Maximum Rate of Carboxylation, Photosynthetic Electron Transport, Triose Phosphate Utilization, Nutrient Use Efficiency

1. Introduction

The close relationship between photosynthesis and nitrogen in plants ([1]-[8]) is often used to predict photosynthesis and growth [9] [10] [11] [12] [13] as well as in ecosystem carbon flux models [9]-[14]. The relationship can explain much of the variation in plant performance even without considering the effects of other nutrient elements, such as phosphorus (P) and potassium (K) [15] [16]. Plants are generally more sensitive to N than to other nutrient elements [17] because N is required in the greatest quantity and is more closely related to the amount and functioning of photosynthetic machines [18] [19]. However, other elements can interact with N in affecting plant physiological processes and modify the N-photosynthesis relationship as well as affect N uptake [20] [21] [22]. For instance, high N supply induces K deficiency [22]; high N/K ratios reduce plant growth [20] [21] [22] [23]; and high K supply negatively affects N and P uptake [22] [24]. The N/P ratio influences the synthesis of photosynthetic enzymes and the shape of N-photosynthesis response curves [22] [25]. The nature and consequence of interactions among different nutrient elements depend on the specific concentration of each element along the concentration ranges defined by the critical deficiency concentration (CDC) and the critical toxic concentration (CTC) [2] [18] [20] [21] [22]. Too low a concentration will expose plants to the risk of growth suppression from CDC restraint while at too high concentration plants will face the risk of growth suppression from CTC [2] [18] [21]. Increasing nutrient supply between the CDC and CTC generally results in greater plant growth, but the pattern of the response varies with nutrient element [18] [21]. For example, responses to P and K are generally steeper than to N, particularly in the lower portion of the range [3] [18] [20] [22] [26] [27] [28]. However, N-photosynthesis relationships are generally investigated with P and K concentrations maintained constant [29] [30] [31] [32] [33] with few exceptions where N/K and N/P ratios are kept constant [29] [34] [35] [36].

Elevations in atmospheric CO₂ concentration can also modify the N-photosynthesis relationship [37] [38] [39] [40] [41]. Global climate change models predict that the atmospheric CO₂ concentration will double from the level of 2000 by the end of this century [42] [43]. While CO₂ elevations generally stimulate photosynthesis and enhance its nitrogen use efficiency, the stimulation may not sustain in the long term [44] [45] [46] [47] because of photosynthetic acclimation or down-regulation [48] [49] [50] [51] [52]. The degree of photosynthetic down-regulation is closely correlated to N supply [53] [54] [55]. Most studies suggest that photosynthetic down regulation is a result of nutrient limitation [51] [56] [57] [58] [59]. Consequently, CO₂ elevations may be less beneficial to trees growing on nutrient-poor sites [30]. Conversely, fertilization can increase CO₂ stimulation of photosynthesis and growth [49] [56] [58] [59]. CO₂ elevations generally increase nutrient demand [60] and the increase is generally greater for N than for P and K [18] [26] [27] [28] because plants require a greater amount of N than P and K [18] [26] [27] [28]. Elevated [CO₂] and increased N supply can have synergistic effects on photosynthesis and biomass production

[30] [31] [39] [50] [61]. However, other nutrient elements can also interact with [CO₂]. For example, a low P supply can suppress the CO₂ stimulation of photosynthesis in some species [25] [62]. Therefore, a good understanding of how CO₂ elevation, nutrient supply and nutrient ratios affect the relationship between N supply and the photosynthesis and growth of plants is critical for an accurate and reliable prediction of plant growth trends under future climate conditions [8] [14] [63] [64].

Black spruce grows on sites with a wide range of N levels [65]. However, its physiological responses to CO₂ elevation are generally examined under optimal nutrient regimes [29] [66] [67] [68] [69]. The objective of this study was to investigate how elevated CO₂, nitrogen supply and their interactions with P, K and N/P/K ratios affect the relationship between N and photosynthesis in black spruce. Since the increases in nutrient demand induced by CO₂ elevations are proportionally greater for N than for P and K, and CTC is reached faster for P and K than for N under elevated CO₂ when N/P/K ratios are maintained constant, we hypothesize that N/P/K ratios and CO₂ will interactively affect the N-photosynthetic relationship.

2. Materials and Methods

2.1. Plant Materials

One-year-old black spruce seedlings (*Piceamariana* [Mill.] B.S.P.) were obtained from the Hill's Greenhouses Ltd. in Murillo (west of Thunder Bay). The seedlings were relatively uniform in size at the beginning of the experiment (H = 22.8 ± 0.16 cm, RCD = 2.05 ± 0.02 cm). The seedlings were transplanted into containers of 13 cm height and 12 cm diameter with a mixture of peat moss and vermiculite (1:1; v/v).

2.2. Experiment Design

There were two CO₂ treatments (AC 370, EC 720 μmol·mol⁻¹), two nutrient ratio treatments within each CO₂ treatment (constant N/P/K ratio (CNR) vs. variable N/P/K ratio (VNR)), and six levels of N supply within each nutrient ratio treatment (10, 80, 150, 220, 290 and 360 μmol N mol⁻¹ solution). The CO₂ treatments were implemented in four greenhouses with identical dimensions and design (two replicates for each CO₂) in the Forest Ecology Complex of Lakehead University Thunder Bay campus. In the CNR treatment, the N/P/K ratios were 5/2/5 in all 6 N treatments; in the VNR treatment, P and K concentrations were the same in all 6 N treatments (60 μmol·mol⁻¹ P, 150 μmol·mol⁻¹ K). There were 4 seedlings per treatment combination (2 × 6 × 4 = 48 seedlings in each greenhouse).

The day/night air temperatures in the greenhouses were controlled at 25°C-26°C/16°C-17°C and the photoperiod at 16 hours in all the greenhouses. The natural light was supplemented using high-pressure sodium lamps on shorter days. All the experiment conditions (temperature, [CO₂] and light) were moni-

tored and controlled using a computerized Argus environment control system (Argus Control Systems Ltd, Vancouver, BC, Canada). Seedlings were watered as needed (generally every two days) to maintain the volumetric water content of the growing medium above 30%, as determined using an HH2 Moisture Meter and ML2X ThetaProbe (Delta-T Devices, Cambridge, U.K.). The experiment lasted 3.5 months.

2.3. Gas Exchange Measurement

Photosynthetic responses to $[\text{CO}_2]$ were measured at 50, 150, 250, 370, 550, 720, 1000 and 1400 $\mu\text{mol}\cdot\text{mol}^{-1}$ $[\text{CO}_2]$ using a PP CIRAS open gas exchange system with a conifer leaf cuvette (PP System Inc. Amesbury, MA, USA). Other environment conditions in the leaf chamber were 25°C air temperature, 800 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ photosynthetically active radiation (saturated) and 50% RH. The measurements were taken on the current year foliage on the terminal shoot. All measurements were made between 0730-1130 h *in situ*. Following the measurement, the foliage used for the gas exchange measurement was harvested and scanned for projected leaf area using WinSEEDLE (Regent Instruments Inc., Quebec City, Canada) and subsequently dried at 75°C for 48 hours for calculating specific leaf area and nutrient analyses. The A/Ci data were analysed using the Plantecophys package of R 4.0.2 to determine the maximum rate of Rubisco carboxylation (V_{cmax}) and light saturated rate of photosynthetic electron transport (J_{max}).

Leaf concentrations of nitrogen, Phosphorus and potassium were determined as described in [70]. Photosynthetic N-use efficiency (PNUE), P-use efficiency (PPUE) and K-use efficiency (PKUE) were calculated by dividing the net photosynthetic rate measured at the corresponding growth $[\text{CO}_2]$ by the corresponding nutrient concentration.

2.4. Statistical Analysis

The data were examined graphically for the normality of distribution (probability plots for residuals) and homogeneity of variance (scatter plots). Since both assumptions for the Analysis of variance (ANOVA) were met, no data transformation was necessary. When ANOVA showed a significant effect ($p \leq 0.05$) for N supply or an interaction, Fisher's Least Significant Difference (LSD) post hoc test was conducted. The analyses were conducted using the R software package.

3. Results

3.1. Net Photosynthetic Rate (P_n)

The response pattern of photosynthesis (P_n) to N supply was affected by both $[\text{CO}_2]$ and nutrient ratio regime (significant 2- and 3-way interactions in **Table 1**). At the ambient $[\text{CO}_2]$, the general response patterns were similar in the two nutrient ratio regimes: P_n increased with N increases from 10 to 150 $\mu\text{mol}\cdot\text{mol}^{-1}$ and then decreased with further increases in N supply (**Figure 1**). Under the elevated $[\text{CO}_2]$, however, the response patterns diverged between the two nu-

trient ratio regimes at N supplies above 150 $\mu\text{mol}\cdot\text{mol}^{-1}$: P_n in the CNR treatment decreased with further increases in N supply as in the ambient CO_2 treatment, but no such decreases occurred in the VNR treatment (**Figure 1**).

3.2. Biochemical Parameters

Both $[\text{CO}_2]$ and nutrient ratio regime influenced the response of maximum rate of carboxylation (V_{cmax}) to N supply but neither affected the response of light saturated rate of electron transport (J_{max}) (**Table 1**). While V_{cmax} increased with increasing N supply from 10 to 150 $\mu\text{mol}\cdot\text{mol}^{-1}$ in both $[\text{CO}_2]$ treatments, the response differed between the two CO_2 treatments at higher N levels: V_{cmax} declined with further increases in N supply under the ambient $[\text{CO}_2]$ but it plateaued under the elevated $[\text{CO}_2]$ (**Figure 2(a)**). The response pattern of V_{cmax} to N supply in the CNR nutrient ratio regime was similar to that in the ambient $[\text{CO}_2]$ treatment while the response in the VNR was similar to that under the elevated $[\text{CO}_2]$ (**Figure 2(a)** & **Figure 2(b)**). J_{max} generally increased with increasing N supply from 10 to 150 $\mu\text{mol}\cdot\text{mol}^{-1}$ and declined with further increases in N supply, but the difference between two adjacent N levels was not always statistically significant (**Figure 2(c)**). The CO_2 elevation significantly increased J_{max}

Table 1. P values for the effects of CO_2 concentration (C), nutrient ratio (NR), nitrogen supply (N) and their interactions on physiological variables in black spruce seedlings.

Variable	Treatment effects						
	C	NR	N	C × NR	C × N	NR × N	C × NR × N
P_n	0.022	0.001	<0.001	<0.001	0.003	<0.001	0.013
V_{cmax}	0.021	0.084	<0.001	0.132	<0.001	0.027	0.566
J_{max}	0.050	0.626	<0.001	0.513	0.109	0.383	0.357
TPU	0.039	0.886	<0.001	0.930	0.103	0.341	0.293
g_s	0.100	0.274	0.314	0.746	0.250	0.229	0.427
NUE	0.024	0.299	<0.001	0.028	0.476	0.159	0.324
PUE	0.052	0.364	0.012	0.012	0.103	<0.001	0.003
KUE	0.014	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Notes: P_n = net photosynthetic rate, V_{cmax} = maximum rate of carboxylation, J_{max} = light saturated rate of electron transport, g_s = stomatal conductance, NUE = photosynthetic nitrogen-use efficiency, PUE = phosphorus-use efficiency, KUE = potassium-use efficiency, N_a , P_a and K_a are leaf area based foliar N, P and K concentrations. 1-year old seedlings were grown under two $[\text{CO}_2]$ (ambient $[\text{CO}_2]$ = 370 $\mu\text{mol}\cdot\text{mol}^{-1}$, elevated $[\text{CO}_2]$ = 720 $\mu\text{mol}\cdot\text{mol}^{-1}$), 6 N concentrations (10, 80, 150, 220, 290 and 360 $\mu\text{mol}\cdot\text{mol}^{-1}$), and two nutrient ratio regimes (CNR—constant N/P/K ratios at 5/2/5 and VNR where the concentration was 60 $\mu\text{mol}\cdot\text{mol}^{-1}$ for P and 150 $\mu\text{mol}\cdot\text{mol}^{-1}$ K at all six N concentrations) for 3.5 months. Significant effects ($p \leq 0.05$) were bold-faced.

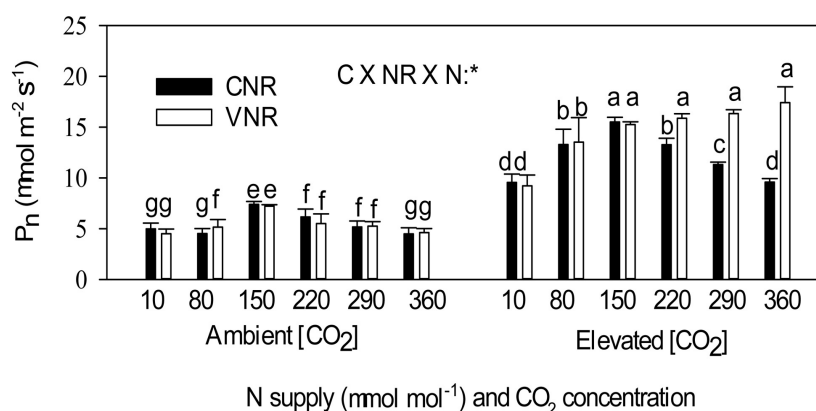


Figure 1. Net photosynthetic rate (mean \pm SEM) in black spruce seedlings grown under two [CO₂] (ambient [CO₂] 370 $\mu\text{mol}\cdot\text{mol}^{-1}$; elevated [CO₂] 720 $\mu\text{mol}\cdot\text{mol}^{-1}$), 6 N concentrations (10, 80, 150, 220, 290 and 360 $\mu\text{mol}\cdot\text{mol}^{-1}$), and two nutrient ratio regimes (CNR—constant N/P/K ratios at 5/2/5 and VNR where the same concentrations of 60 $\mu\text{mol}\cdot\text{mol}^{-1}$ P and 150 $\mu\text{mol}\cdot\text{mol}^{-1}$ K were used at all six N levels). The photosynthesis was measured at the corresponding growth [CO₂]. The significant 3-way interactions ($P < 0.05$) were labelled with lower case letters and means with different letters were significantly different from each other ($P \leq 0.05$).

(from 72.8 to 101.5 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$). However, neither nutrient ratio regime nor [CO₂] had a significant effect on the response of J_{max} to N supply (**Table 1**).

3.3. Foliar Nutrient Use Efficiency

The CO₂ elevation significantly increased photosynthetic nitrogen-use efficiency (PNUE) and the increase was greater in the VNR than CNR treatment (**Table 1**, **Figure 3(a)**). Nutrient ratio regime had opposite effects on PNUE under different CO₂ treatments: under the ambient [CO₂], PNUE was significantly lower in the VNR than in CNR; under the elevated [CO₂], however, PNUE was significantly greater in the VNR than CNR (**Table 1** and **Figure 3(a)**). PNUE generally decreased with the increasing N supply (**Table 1** and **Figure 3(b)**).

The photosynthetic phosphorus-use efficiency (PPUE) was affected by both nutrient ratio and [CO₂] (**Table 1**): under the elevated [CO₂] and VNR, PPUE increased with increasing N supply from 10 to 80 $\mu\text{mol}\cdot\text{mol}^{-1}$ and then became relatively stable with further increases in N supply; under the elevated [CO₂] and CNR, there was not much change in PPUE as N supply increased from 10 to 150 $\mu\text{mol mol}^{-1}$ N but it decreased with further increases in N supply; there was generally very little variation in PPUE under the ambient [CO₂] either with nutrient ratio or N supply (**Figure 3(c)**).

The responses of photosynthetic potassium-use efficiency (PKUE) to [CO₂], nutrient ratio and N supply were similar to those of PPUE with the exception that PKUE increased with increasing N up to 150 $\mu\text{mol mol}^{-1}$ under the elevated [CO₂] and VNR as compared to 80 $\mu\text{mol mol}^{-1}$ N for PPUE (**Table 1**, **Figure 3(c)** and **Figure 3(d)**).

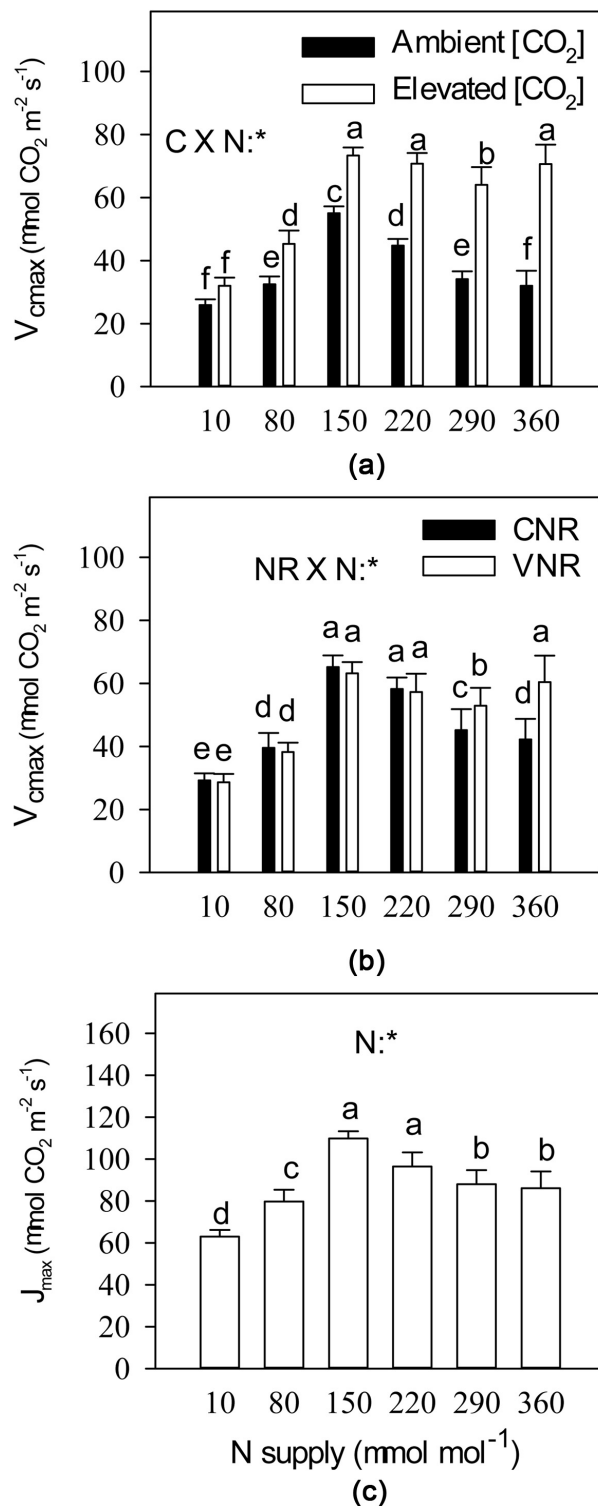


Figure 2. Effects of CO₂ concentration (C), nitrogen supply (N) and Nutrient ratios (NR) on maximum rate of carboxylation (*V_{cmax}*) and light saturated rate of electron transport (*J_{max}*) in black spruce seedlings. Significant effects ($P \leq 0.05$) were indicated with “*” and means (+SEM) different letters were significantly different from each other. Other explanations are as in **Figure 1**.

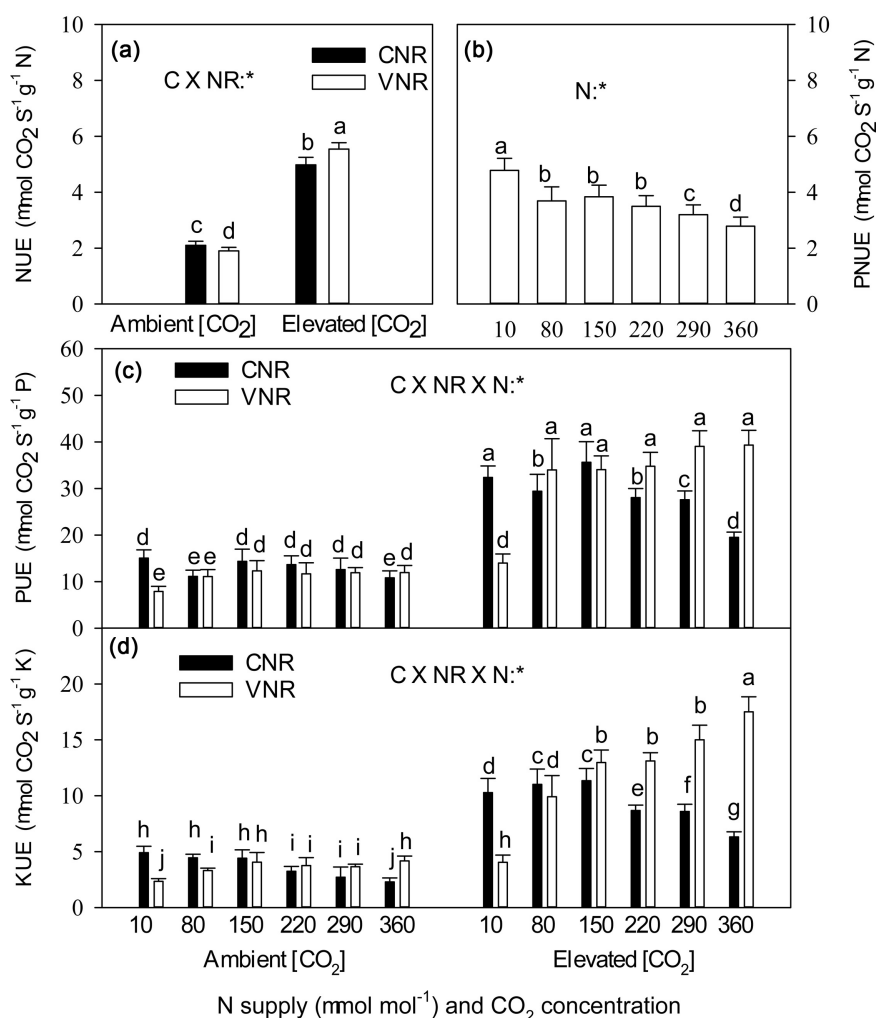


Figure 3. Effects of CO₂ concentration (C), nitrogen supply (N) and Nutrient ratios (NR) on photosynthetic nitrogen-use efficiency (NUE), photosynthetic phosphorus-use efficiency (PUE), and photosynthetic potassium-use efficiency (KUE) in black spruce seedlings. Other explanations are as in **Figure 1** & **Figure 2**.

4. Discussions

The results of this study support the hypotheses that constant N/P/K ratios (CNR) would lead to reductions in photosynthetic rate at high N supplies (>150 $\mu\text{mol mol}^{-1}$ N) under elevated [CO₂] and that CO₂ elevations would modify nitrogen-photosynthesis relationships. The fact that photosynthesis in the variable N/P/K ratios (VNR) (or constant P & K concentrations) did not show any sign of declines at higher N supply levels under the elevated CO₂ suggests that the decline in photosynthesis in the CNR treatment was attributed to toxic levels of P or K or both. Further, under the ambient [CO₂], photosynthesis at high N (>150 $\mu\text{mol mol}^{-1}$ N) declined in both CNR and VNR, suggesting that the decline in photosynthesis under the ambient CO₂ was probably attributed to nitrogen toxicity. The above results also suggest that the CO₂ elevation increased the demand for nitrogen to greater extents than for phosphorus and potassium. However, the

CO₂ elevation modified the nitrogen-photosynthesis relationship only in the VNR treatment. Thus, it can be concluded that the highest nitrogen supply used in this study was not toxic to black spruce under the elevated CO₂ and that the phosphorus and/or potassium levels in the CNR were too high at nitrogen supplies greater than 150 μmol mol⁻¹ while those in the VNR was too low to maximize photosynthesis. This study suggests that neither P and K concentrations nor N/P/K ratios should be kept constant at the current optimums when increasing fertilization are used to increase plant productivity in the future. Instead the concentration combination of the three key nutrient elements should be considered as a single integrated factor. Further research is warranted to determine optimal combinations of these elements under the future doubled CO₂ environment. The results of this study are consistent with the concepts of critical deficiency concentration and critical toxic concentration as used by some other researchers [18] [28] [29]. Our data suggest that the nutrient ratios and the CO₂ elevation affected photosynthesis via their effects on V_{cmax} but not J_{max}. These results are in agreement with the findings of [14], but contradicted the results of [71]. The effects probably reflected specific performance of black spruce seedlings growing in our wide range of N supplies and various nutrient ratios.

The nutrient ratios modified the responses of photosynthetic use-efficiency of N, P and K (PNUE, PPUE and PKUE, respectively) to [CO₂] and N supply. The CNR treatment suppressed PNUE under the elevated [CO₂]. PPUE and PKUE, however, were suppressed by the CNR only at higher N supplies under elevated [CO₂]. As discussed previously on the suppressions of CDC and CTC, the decreases in PPUE and PKUE were probably attributable to the CTC effect of K. The PKUE under the elevated [CO₂] increased with increases of N supply in the VNR treatment (K availability remained equal across all N supply levels), indicating the enhanced effect of increasing N supply on PKUE; the reversed trend occurred to the PKUE in the CNR treatment (K supply increased with increases of N supply), indicating the high K availability decreased the PKUE, and this high K availability caused the seedlings passively absorbing K, which led [K] passing over the level of CTC of K. In addition, the CO₂ elevation in this study generally resulted in greater PNUE, PPUE and PKUE. The enhancing effect of CO₂ elevation on PNUE agreed with the findings of [72], and the negative relationship between PNUE and leaf [N] was consistent with the results of [35]. The changes of the PNUE provided evidence that the relationship between photosynthesis and nitrogen was modified by nutrient ratios.

The nutrient ratios changed the relationship between photosynthesis and leaf [N] in this study. [73] has pointed out that the effects of elevated [CO₂] on V_{cmax} are largely through the changes in leaf [N] [73]. The results in this study indicated that the relationship between V_{cmax} and leaf [N] was also affected by N/P/K ratios. The high leaf N concentration at the high N supply levels resulted in greater V_{cmax} with the VNR treatment, but not in the CNR treatment. The effect was probably due to the toxic effects of K. Elevated [CO₂] decreases leaf [N] [49]

[74] and reduces net photosynthetic rates measured at a common [CO₂]. Cao *et al.* ([3]) reported that the magnitude of photosynthetic acclimation in white birch (*Betula papyrifera* Marsh.) in response to CO₂ elevation decreases with greater leaf N concentrations. Zhang and Dang [70] found that no photosynthetic down-regulation occurs in white birch seedlings in response to CO₂ elevation at various levels of N supply when N/P/K ratios are maintained constant. However, in this current study, the N_a increased with the increasing N supply, but this ascending trend of leaf [N] did not show consistent corresponding increases of net photosynthetic rate at the high N supply levels under the elevated [CO₂], rather the trend was modified by the nutrient ratios, that the CNR decreased P_n and the VNR increased it. [3] reports that CO₂ elevation increases P_n with no significant effect on N_a. The changes in leaf [K] due to the effect of nutrient ratio treatments also showed a positive effect on P_n, indicating that photosynthetic responses were not only correlated to leaf [N], but also to leaf [K], supporting the conclusion of [3] that the relationship between photosynthesis and leaf [N] is influenced by nutrient ratio.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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