Elevated CO_2 Exacerbates Flooding-Induced Mortality in Black Spruce While Enhancing Adventitious Root Formation in Tamarack

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Abstract

Anthropogenic increases in atmospheric CO_2 concentrations, coupled with climate change-induced alterations in hydrological regimes, are increasing flood frequency across many boreal regions. Prolonged flooding creates oxygen-deprived soils that force roots to shift from aerobic respiration to anaerobic fermentation, an inefficient metabolic pathway that compromises carbohydrate utilization and leads to root dieback. We examined the interactive effects of flooding and CO_2 levels on two ecologically important boreal species: black spruce (Picea mariana [Mill.] Britton, Sterns & Poggenb.) and tamarack (Larix laricina [Du Roi] K. Koch). Seedlings were exposed to a 28-day flooding event under ambient (AC, $400 \mu mol\ mol^{-1}$) and elevated (EC, $800 \mu mol\ mol^{-1}$) CO_2 concentrations.

Flooding significantly reduced root respiration (-16% during treatment; -36% 35 days post-recovery), root biomass (-66%), root volume (-58%), and overall growth (-70%). While Photosystem II quantum efficiency declined under flooding, photosynthetic rates remained stable. Species responses diverged markedly: flooded black spruce exhibited 2.1× higher mortality under EC (59%) versus AC (28%), whereas tamarack developed adventitious roots 2.9× more frequently under EC (53%) than AC (18%) with no mortality. Contrary to our hypothesis, EC failed to mitigate flood stress or enhance recovery through carbohydrate supplementation.

These findings demonstrate that elevated CO_2 exacerbates flood vulnerability in black spruce while promoting tamarack's adaptive capacity through morphological plasticity. Such differential responses suggest future climate conditions may drive compositional shifts in boreal forests, favoring tamarack in increasingly flood-prone landscapes.

Keywords: Black spruce; Tamarack; Climate change

Introduction

Atmospheric CO_2 concentrations have risen significantly over the past century due to anthropogenic activities [1]. Elevated CO_2 generally enhances plant photosynthetic CO_2 assimilation [2-5], water-use efficiency [6], and biomass accumulation. Additionally, it alters carbon allocation among plant organs, stimulating biomass investment in both coarse and fine roots while increasing total root length [7]. These changes are accompanied by shifts in root distribution within the soil profile, including deeper rooting depth and higher root turnover rates [7]. Elevated CO_2 also enhances root respiration due to increased carbohydrate availability [7]. While these effects are observed in both controlled environments and Free-Air CO_2 Enrichment (FACE) experiments, the magnitude of response tends to be lower in field conditions [8].

Elevated CO_2 is a major driver of global temperature increases [9] and the heightened frequency and severity of hydrological extremes such as flooding [10,11]. Flooding raises water tables, reducing soil oxygen availability and forcing tree roots to shift from aerobic respiration to anaerobic fermentation (Taiz & Zeiger, 2010) [12]. Fermentation generates only 1/18th the ATP per unit of carbohydrate compared to aerobic respiration, leading to inefficient energy production and impaired root function [12]. This inefficiency can hinder water and nutrient uptake [12] and reduce rooting depth [13]. Since roots are critical for nutrient and water absorption (Grant et al. 2012) [7], their dysfunction can suppress aboveground physiological processes and productivity [13-16]. Prolonged hypoxia or anoxia may further exacerbate these effects

by inducing root mortality (Parad et al. 2013) [12]. However, elevated CO_2 may partially mitigate flooding stress by increasing photosynthetic carbon supply to roots [7], potentially compensating for inefficient carbohydrate utilization under hypoxia. Conversely, deeper rooting under elevated CO_2 could increase root exposure to flooding, complicating these interactions. Despite these possibilities, little is known about how elevated CO_2 influences tree physiological responses to flooding or alters flood resistance.

The Canadian boreal forest encompasses diverse topographies, with peatlands covering nearly 12% of the land area [17]. Two dominant peatland tree species, black spruce (*Picea mariana* (Mill.) Britton, Sterns & Poggenb.) and tamarack (*Larix laricina* (Du Roi) K. Koch) [18,19], are widely distributed across Canada. Black spruce alone constitutes ~37% of Ontario's growing forest stock [20]. Both species endure periodic flooding, which, while detrimental to tree health, plays a vital ecological role by transporting nutrients (Benke, 2001; Mosepele et al. 2009; Junk et al. 1989; Sparks, 1995; Tockner et al. 2000). Notably, tamarack exhibits flood tolerance through adventitious root formation, facilitating oxygen transport to submerged roots [21], whereas black spruce lacks this trait. This difference raises intriguing questions about species-specific responses to elevated CO_2 under flooding.

Black spruce and tamarack thrive across diverse boreal landscapes, including nutrient-poor, waterlogged peatlands [13]. Both species have evolved flood-resistant adaptations [13,22], but it remains unclear whether elevated CO_2 further enhances their resilience or elicits divergent responses. To investigate this, we subjected two-year-old seedlings of both species to a 28-day flooding treatment under ambient and elevated CO_2, assessing ecophysiological traits and recovery dynamics. We hypothesized that elevated CO_2 would bolster flood resistance, thereby mitigating flooding-induced stress.

Materials and Methods

Seedling material and preparation

Plant material

We used black spruce (*Picea mariana* (Mill.) BSP) and tamarack (*Larix laricina* (Du Roi) K. Koch) seedlings for this study. Black spruce seed cones were collected from a wetland stand at the Lakehead University Jack Haggerty Forest (48°29′2″ N, 89°29′23″ W), approximately 37km north of Thunder Bay, Ontario. Seed extraction followed protocols from The Woody Plant Seed Manual [23]. After collection, cones were rinsed, soaked in cold water for 4 hours, drained, and air-dried on metal trays for 20 hours. They were then oven-dried at 54.44°C (130°F) (4-hour ramp-up, 11-hour hold) before cooling to room temperature. Seeds were extracted by shaking the dried cones. Tamarack seeds (Seed Zone 19, north of Lake Nipigon, Ontario) were obtained from the Ontario Tree Seed Centre (Angus, Ontario).

Seed stratification and germination

Seeds of both species were soaked in cold water for 24 hours, drained, and placed in moist paper towel-lined containers for cold stratification (4°C, 3 weeks). Stratified seeds were sown in 60mL Styrofoam cells (Beaver Plastics Ltd., Acheson, Alberta) filled with a peat moss: vermiculite mix (3:2 v:v), with 3-4 seeds per cell. The blocks were placed in polyethylene misting tents for 34 days under controlled greenhouse conditions (Argus Control Systems Ltd., Surrey, British Columbia): 23°C Day temperature, 17°C night temperature, 16h photoperiod (supplemented with high-pressure sodium lamps to maintain above 400µmol m^{-2} s^{-1} PAR). The blocks were misted daily during germination and subsequently watered when substrate moisture dropped to 35% (Delta-T ML2x probe & HH2 moisture meter).

Seedling establishment and fertilization

After germination, seedlings were irrigated twice weekly with starter fertilizer 11-41-8 (100.1mg/L N, 373.1mg/L P, 72.8mg/L K) for the first 2 weeks. Forestry seedling standard fertilizer (20-8-20 N-P-K) was applied thereafter. Nutrient concentrations were started at 50mg/L N, 20mg/L P, and 50mg/L K50 and gradually increased to 200mg/L N, 80mg/L P, and 200mg/L K over 2 months (Forestry Notes, 2009).

The environmental conditions were maintained at 22°C/17°C (day/night), 16-h photoperiod, and 60-70% RH.

Cold hardening

When seedlings reached ~15cm height, cold hardening was initiated according to Landis (1999): temperature was reduced by 3°C/week until reaching 7°C (day) and 4°C (night) over 5 weeks, photoperiod gradually shortened from 16h to 8h, fertilization switched to conifer finisher fertilizer (50mg/L N, 312.5mg/L P, 437.5mg/L K), and watering reduced to once weekly.

A blackout curtain ensured darkness during the night phase. Cold hardening was confirmed after 30 days by root color change (white → golden brown) and completion of terminal bud settings, and tamarack needle yellowing/shedding. Two seedlings per block were destructively sampled (roots washed and inspected) to verify hardening.

Seedling storage

Hardened seedlings were removed from containers, wrapped in plastic film, and stored in plastic-lined boxes at -3°C.

Experiment design

The study employed a split-split plot design with CO_2 treatment (two levels: ambient [400 µmol mol^{-1}] and elevated [800µmol mol^{-1}]) as the whole plot (each was replicated in two separate greenhouses) and flooding treatment (flooded vs. non-flooded) as the split plot, and species (black spruce vs. tamarack) as the split-split plot. The experiment was conducted in four controlled-environment greenhouses at the Lakehead University Forest Ecology Research Complex (Thunder Bay Campus). Each CO_2 level was duplicated in two independent greenhouses.

Seedlings were removed from cold storage, thawed for 24 h at 17°C in darkness, and potted in 5×5×6-inch pots filled with a peat moss: vermiculite mix (3:2 v/v). Seedlings of each species were randomly allocated to greenhouses, then randomly assigned to flooding treatments (5 seedlings per species per treatment and per greenhouse). The seedlings (5 per species per bin) were placed in clear plastic bins (n=2 bins per greenhouse) for water inundation. Seedlings in the control treatment were placed in identical bins with drainage holes (6 × 0.5cm) to prevent water-logging. Bins were randomly positioned on growth benches to minimize spatial bias.

Environmental conditions

The day and night temperatures in all the greenhouses were at 10°C and 7°C, respectively, for the initial 10 days and were then increased by 1.5°C per week for 7 weeks. The photoperiod started at 8h and gradually increased to 16h at a rate of 2 hours a week. High-pressure sodium lamps were used to extend the natural photoperiod and to supplement the natural light when it fell below 400μmol m^{-2} s^{-1} PAR (on cloudy days, early morning and evening). The CO_2 enrichment as achieved using CO_2 generators (model GEN-2E; Custom Automated Products Inc., Riverside, California, USA).

During the seven weeks leading up to the start of the flooding treatment, the seedlings were watered when the volumetric water content of the growing medium reached about 35% as described previously. The seedlings were fertilized once a week at a concentration of 50mg/L N, 20mg/L P, and 50mg/L K in the initial two weeks of the experiment and then increased to 200mg/L N, 80mg/L P, and 200mg/L K for the remainder of the experiment.

Flooding treatment

The flooding treatment was implemented by filling the plastic bin with water up to the seedlings' root collar position. Seedlings in the control group were watered as described previously. 0.5 cm holes were drilled in the bottom of control bins to avoid water accumulation. The flooded bins were drained completely after 28 days and the recovery process of seedling physiological processes was monitored for 36 days.

Chlorophyll fluorescence measurement

The maximum quantum yield of Photosystem II (Fv/Fm) was measured at 4-day intervals during the flooding treatment and weekly during the 35 days period after the termination of the treatment using the built-in FMS-2 portable pulse-modulated fluorometer (Hansatech Instruments Ltd. Norfolk, UK) in the PP-Systems CIRAS-3 gas exchange system (PP System Inc., Amesbury, MA, USA). The measurement was taken after at least 60 minutes dark adaptation on four randomly selected seedlings from each treatment combination. Black spruce measurements were taken on current-year foliage. Three readings were recorded on each sample and averaged for subsequent data analysis.

Foliage gas exchange measurement

We assessed the responses of foliar and root physiological traits by measuring foliar gas exchange and root respiration using a PP-Systems CIRAS-3 open gas exchange system equipped with a Parkinson conifer leaf chamber with automatic environment control (PP System Inc., Amesbury, MA, USA) and an FMS-2 portable pulse-modulated fluorometer (Hansatech Instruments Ltd. Norfolk, UK). Foliar gas exchanges were measured 17 days after initiating the flooding treatment, and 6 days and 35 days after the treatment ended. Five stable readings were recorded per sample and averaged for analysis. All measurements were performed on the same foliage of the same seedlings under identical chamber conditions. Seedlings in the ambient CO_2 treatment were measured at 400μmol mol^{-1}, while those in the elevated CO_2 treatment were measured at 800μmol mol^{-1}. All measurements were taken at 400μmol m^{2}s^1 PAR and 25°C temperature. The sample foliage was removed from the tree after the final measurement for leaf area determination Regent WinSEEDLE (Regent Instruments, Quebec, Canada).

Root respiration measurement

Due to the destructive nature of sampling and limited seedling availability, root respiration was measured only twice: 17 days after flooding initiation and 35 days after the end of the flooding treatment. To measure respiration, roots were carefully extracted, washed, and surface-dried in the dark. Four seedlings per treatment combination were measured. The total area and length of the sample roots were measured using Regent WinRhizo system (Regent Instruments, Quebec, Canada).

Morphological and biomass measurement

Total project leaf area was determined using Regent WinSeedle system (Regent Instruments, Quebec, Canada). Seedling height and root collar diameter (RCD) were measured at the beginning and end of the experiment to determine increments and relative growth rates. The biomass of foliage, stem, and roots was measured after 16 hours of oven drying at 70°C. The root (leaf) mass ratio was calculated by dividing root (leaf) biomass by seedling biomass. Specific leaf area was calculated by dividing leaf area by leaf biomass. Specific root length was calculated by dividing total root length by total root biomass.

To assess root characteristics, the roots of four randomly selected seedlings per treatment combination (per greenhouse) were carefully extracted, washed, and analyzed using the Regent WinRhrizo system (Regent Instruments, Quebec, Canada) to quantify total root length (cm), total root surface area (cm^2), and total root volume (cm^3).

Adventitious roots and mortality

Adventitious root formation was determined by the emergence of white roots on the surface of the growing media after

the flooding treatment. Seedling mortality was determined by the loss of foliage, branch brittleness, and absence of photosynthetic activity.

Data analysis

The data was analyzed using a split-plot ANOVA, with CO_2 treatment at the whole plot, flooding treatment as the split plot, and species as the split-split plot. The assumptions of normality and homogeneity were assessed using the Shapiro tests and examining the residual plots, respectively. Since both assumptions were met for all the variables, no data transformation was necessary. When ANOVA showed a significant interaction, a Tukey's post-hoc test was used to compare the means. All the analyses were conducted using the "R" statistics software ("R", Geneva Switzerland).

To analyze the number of adventitious roots and percent mortality, two different models were used due to the fact that these factors were measured as a count. A logistic regression with an identity link was used for mortality analysis and a generalized linear model with a Poisson distribution and an identity link were used to analyze adventitious roots.

Results

Physiological responses

Photosynthetic rate did not differ significantly in response to CO_2 or flooding treatments in either species after 17 days of flooding or 35 days post-flooding. However, six days after flooding ceased, the photosynthetic rate was significantly lower in flooded seedlings ($6 \mu mol \ m^{-2} \ s^{-1}$) compared to controls ($18 \mu mol \ m^{-2} \ s^{-1}$) (Table 1). Elevated CO_2 significantly enhanced photosynthesis in both species, though the increase was markedly greater in *L. laricina* than in *P. mariana* (Figure 1, Figures 2A-2B, Table 1).

Table 1: Probabilities for the effects of CO_2, flooding, and species on photosynthesis (A) and stomatal conductance (g_s). Values of 0.05 or less are printed in bold.

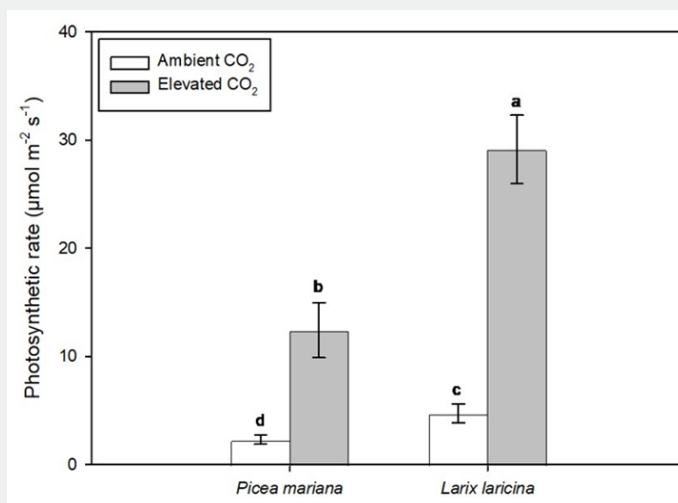| Terms	17 Days of Flooding		6 Days of Recovery		35 Days of Recovery	
	A	gs	A	gs	A	gs
CO_2	0.846	**0.029**	**0.01**	**0.025**	0.885	0.649
flooding	0.2	0.174	**0.027**	**0.048**	0.105	0.107
CO_2 × flooding	0.393	0.845	0.185	0.853	0.787	0.47
species	0.798	0.543	**0.009**	**0.025**	0.889	0.275
CO_2 × species	0.838	0.523	**0.028**	0.44	0.228	0.164
flooding ×species	0.397	0.775	0.087	0.266	0.16	0.841
CO_2 × flooding × species	0.452	0.83	0.151	0.845	0.422	0.693

Figure 1: Interactive effects of CO_2 (AC 400µmol mol⁻¹ vs. EC 800µmol mol⁻¹) and species on photosynthesis (A) 6 days following the termination of a 28-day flood treatment.
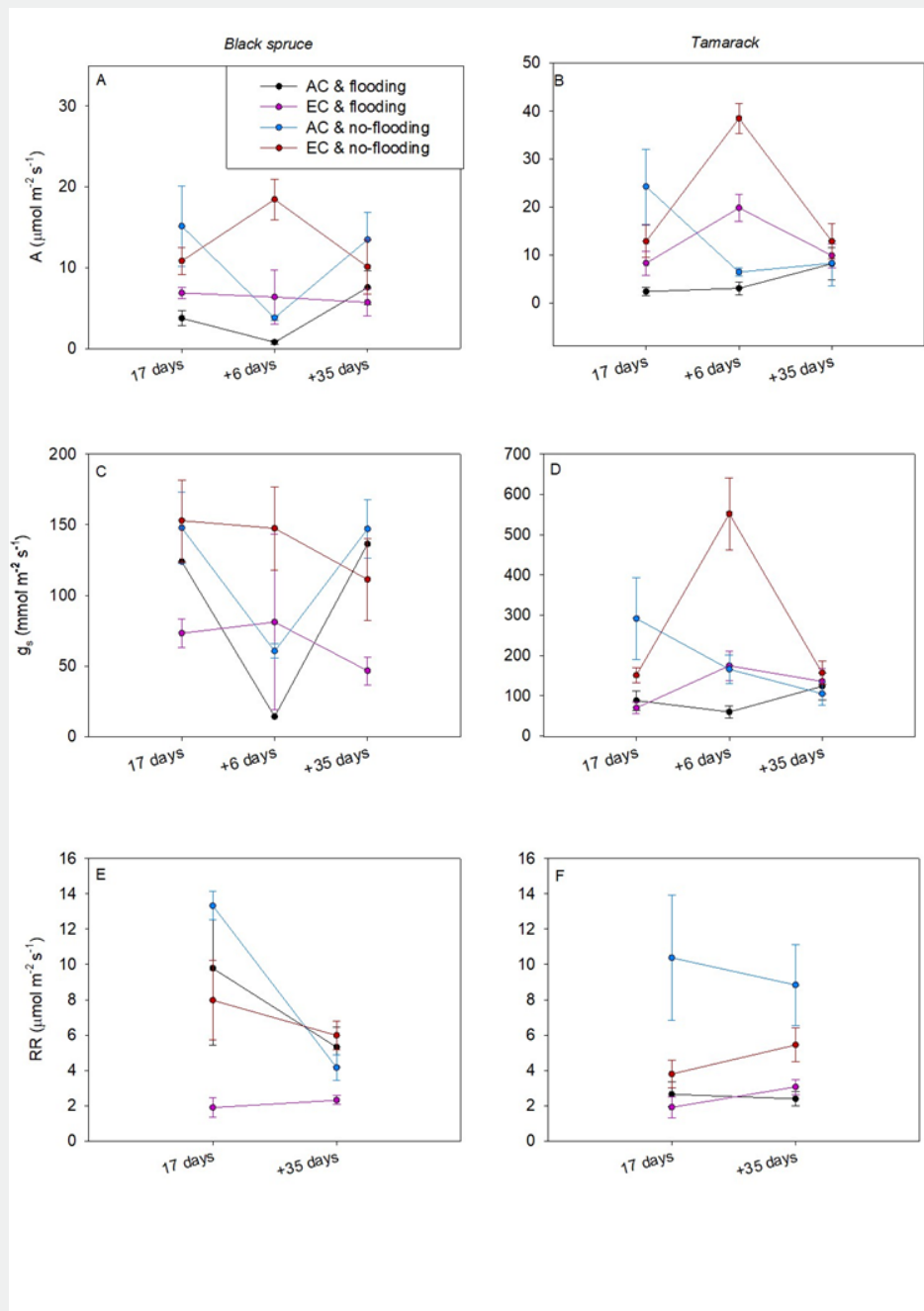
Figure 2: The effects of CO_2 (AC 400µmol mol^{-1} vs. EC 800µmol mol^{-1}) and flooding on photosynthesis (*A*), stomatal conductance (g_s), and root respiration (RR) in black spruce and tamarack. The measurements were taken after 17 days of flooding treatment, and +6 and +35 days after the termination of a 28-day flooding treatment.

Stomatal conductance was significantly reduced under elevated CO_2 after 17 days of flooding (AC: 655mmol m^{-2} s^{-1}, EC: 112mmol m^{-2} s^{-1}) but increased six days post-flooding (AC: 75mmol m^{-2} s^{-1}, EC: 239mmol m^{-2} s^{-1}). No significant CO_2 effect was observed 35 days after flooding ended (Table 1). Flooded seedlings exhibited significantly lower stomatal conductance six days post-flooding (Flood: 83mmol m^{-2} s^{-1}, Control: 231mmol m^{-2} s^{-1}) (Table 1, Figures 1C-1D). Flooding had no significant effect at other measurement points. Additionally, *L. laricina* showed higher stomatal conductance (238 mmol m^{-2} s^{-1}) than *P. mariana* (76mmol m^{-2} s^{-1}) six days post-flooding (Figures 2C-2D, Table 1).

Flooding significantly suppressed root respiration during the treatment (Day 17: Flood = 7.43 vs Control = 8.87µmol m^{-2} s^{-1}) and persisted 35 days post-recovery (Flood = 3.28 vs Control = 5.11µmol m^{-2} s^{-1}; Figure 2E-2F, Table 2). In contrast, elevated CO_2 stimulated root respiration at 35 days post-flooding (AC = 4.18 vs EC = 4.20µmol m^{-2} s^{-1}; Table 2, Figure 2E-2F).

Table 2: P-values for the effects of CO_2, flooding, species, and their interactions on root respiration (RR) after 17 days of flooding and 35 days after the termination of a 28-day flooding treatment. Significant probabilities (≤ 0.05) are printed in bold.

Terms	RR 17 days of Flooding	RR 35 of Recovery
CO_2	0.157	0.003
flooding	0.003	0.007
CO_2 × flooding	0.06	0.1
species	0.127	0.956
CO_2 × species	0.495	0.643
flooding ×species	0.789	0.273
CO_2 × flooding × species	0.394	0.11

The maximum quantum yield (Fv/Fm) showed significant depression under flooding but fully recovered by 35 days post-treatment (Figure 3, Table 4). Notably, no intermediate measurements were taken between days 17-34 to characterize this recovery trajectory. Treatment interactions and other factors exhibited minimal effects, with only isolated exceptions observed (Table 4, Figure 3).

Table 3: P-values for the effects of CO_2, flooding, species, and their interactions on the maximum quantum yield of Photosystem II (Fv/Fm) after 6, 10, 18, 22, and 26 days of flooding treatment, and +4, +8, +12, 16, and +35 days after the termination of a 28-day flooding treatment. Significant probabilities (≤ 0.05) are bolded.

Terms	6 Days	10 Days	18 Days	22 Days	26 Days	+4 Days	+8 Days	+12 Days	+16 Days	+35 Days
CO_2	0.143	0.501	0.955	0.595	0.392	0.24	0.007	0.11	0.042	0.874
flooding	0.163	0.254	0.311	**0.016**	**0.005**	**0.013**	**0.007**	**0.008**	**0.002**	**0.006**
CO_2 × flooding	**0.0141**	0.087	0.679	0.517	0.193	0.818	0.127	0.104	0.073	0.806
species	0.867	0.878	0.997	0.319	0.133	0.337	0.192	0.09	0.108	0.076
CO_2 × species	0.122	0.181	0.464	0.837	0.623	0.313	0.072	0.215	0.102	0.311
flooding × species	0.481	0.368	0.376	0.049	0.238	0.168	0.266	0.06	0.128	0.098
CO_2 × flooding × species	0.904	0.815	0.163	0.21	0.947	0.355	0.119	0.095	0.118	0.366

Table 4: P-values for the effects of CO_2, flooding, species, and their interactions on the maximum quantum yield of Photosystem II (Fv/Fm) after 6, 10, 18, 22, and 26 days of flooding treatment, and +4, +8, +12, 16, and +35 days after the termination of a 28-day flooding treatment. Significant probabilities (≤ 0.05) are bolded.

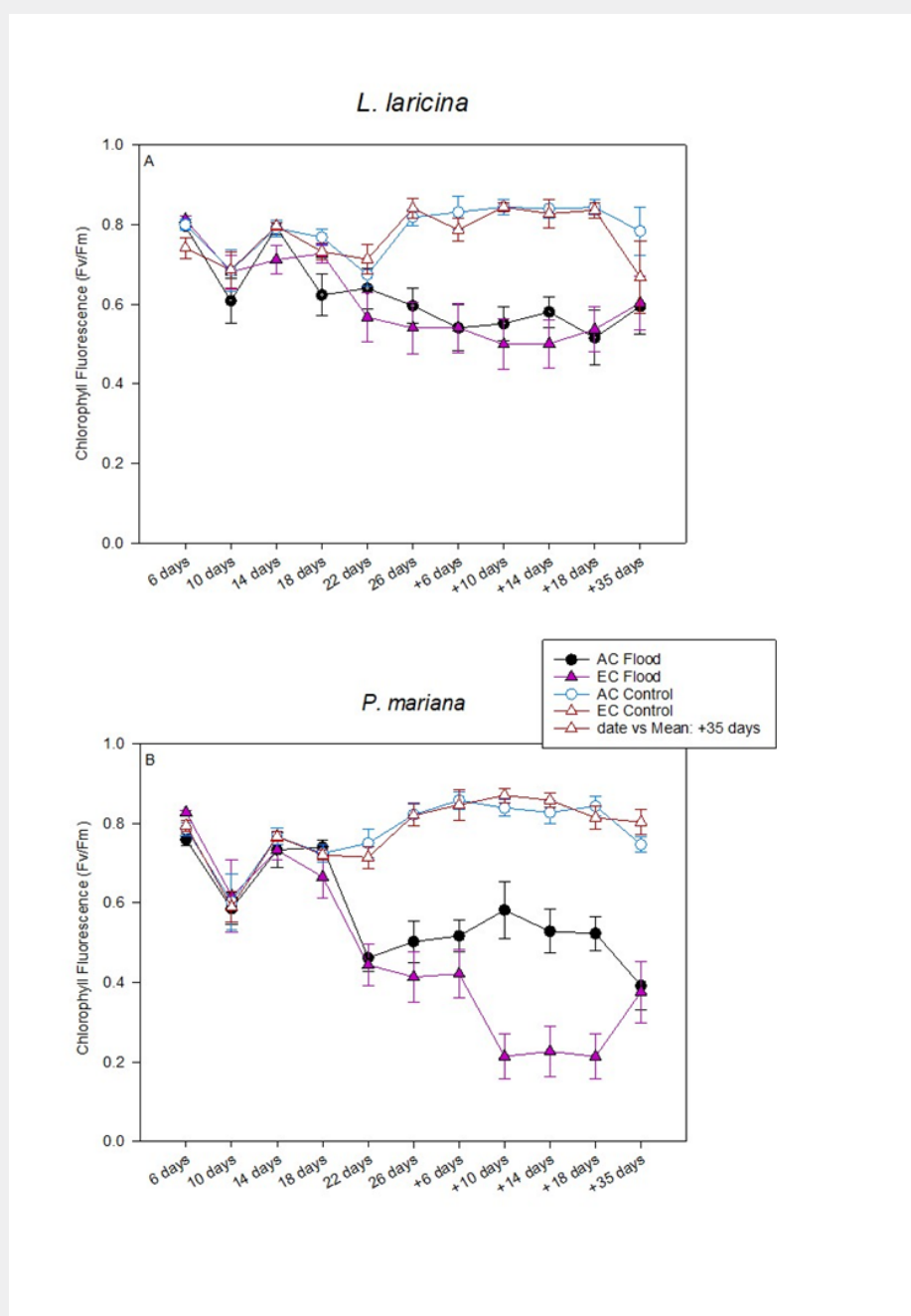| Terms	RGR-H	SLA	RGR-RCD	SRL	A-root	Vol-root
CO_2	**0.004**	0.277	0.288	0.383	0.358	0.426
flooding	0.129	**0.009**	0.164	0.103	**0.006**	**0.003**
CO_2 × flooding	0.444	0.788	0.207	0.866	0.291	0.419
species	**0.044**	0.201	**0.027**	**0.003**	0.4	0.109
CO_2 × species	0.458	0.714	0.401	0.144	0.809	0.896
flooding × species	0.413	0.403	**0.056**	0.715	0.381	0.351
CO_2 × flooding × species	0.59	0.488	0.355	0.692	0.8	0.872

Figure 3: Fv/Fm of black spruce and tamarack measured after 6, 10, 18, 22, and 26 days of flooding treatment, and +4, +8, +12, 16, and +35 days after the termination of a 28-day flooding treatment.
Note: AC = 400μmol mol^{-1} CO_2; EC = 800μmol mol^{-1} CO_2.

Growth and morphological responses

Elevated CO_2 significantly enhanced height relative growth rate (RGR) in both species (AC: 0.16cm cm^{-1} vs. EC: 0.46cm cm^{-1}) but had no significant impact on specific leaf area (SLA), diameter growth, or root traits (Table 4). Neither flooding nor treatment interactions significantly affected height RGR (Table 2).

Flooding markedly increased SLA (Control: 105cm^2 g^{-1} vs. Flooded: 410cm^2 g^{-1}) while reducing root surface area (Control: 370cm^2 vs. Flooded: 155cm^2) and root volume (Control: 6.4cm^3 vs. Flooded: 2.7cm^3; Table 4). Seedling biomass was severely impacted by flooding, with significant reductions in total biomass (Flooded: 4.32g vs. Control: 14.49g), stem biomass (3.22g vs. 11.31g), root biomass (1.03g vs. 3.07g), and needle biomass (2.09g vs. 5.37g). However, root mass ratio increased under flooding (0.24 vs. 0.21; Table 5).

Table 5: P-values for the effects of CO_2, flooding, species, and their interactions on the foliage biomass, root biomass, total seedling biomass, leaf/root ratio, and root mass ratio of black spruce and tamarack seedlings.

Terms	Foliage	Root	Seedling	Leaf/Root Ratio	Root Mass Ratio
CO_2	0.242	0.093	0.252	0.634	0.618
flooding	**0.002**	**0.001**	**0.006**	0.149	**0.049**
CO_2 × flooding	0.71	0.808	0.168	0.969	0.559
species	0.652	0.09	0.731	**0.009**	0.161
CO_2 × species	0.345	0.452	0.523	0.728	0.613
flooding × species	0.62	0.28	0.455	0.157	0.243
CO_2 × flooding × species	0.903	0.696	0.851	0.462	0.25

Species-specific responses

Tamarack (*Larix laricina*) exhibited superior growth performance compared to black spruce (*Picea mariana*), with higher height RGR (0.47 vs. 0.27cm cm^{-1}), faster diameter growth (0.32 vs. 0.14cm cm^{-1}), but lower specific root length (705 vs. 1109cm g^{-1}; Table 4).

Adventitious root formation and mortality

Flooding triggered adventitious root development in tamarack but not in black spruce. Elevated CO_2 further promoted this response, with significantly more tamarack seedlings developing adventitious roots under high CO_2 (17/32) compared to ambient CO_2 (6/32; χ^2 = 8.47, *p* < 0.01 for CO_2 × flooding interaction). Flooding also induced mortality in black spruce (59% under elevated CO_2 vs. 28% under ambient CO_2; χ^2 = 7.58, *p* < 0.01), while all tamarack seedlings survived regardless of treatment.

Discussion

CO_2 elevation fails to mitigate flooding effects

Contrary to our hypothesis, elevated CO_2 did not alleviate flooding-induced stress or enhance post-flooding recovery in either species. The underlying assumption that CO_2-induced increases in photosynthetic carbon gain would compensate for root carbohydrate inefficiency under flooding was not supported. While short-term CO_2 enrichment typically stimulates photosynthesis, prolonged exposure often triggers photosynthetic downregulation through reduced leaf biomass allocation and diminished photosynthetic capacity [12]. Additionally, elevated CO_2 may have exacerbated root hypoxia by increasing dissolved CO_2 in the rhizosphere, lowering pH and amplifying flooding damage- a mechanism consistent with the disproportionately higher mortality observed in flooded *Picea mariana* under elevated CO_2 (59% vs. 28% under ambient CO_2). This negative effect likely offsets any potential physiological benefits of CO_2 enrichment. Our findings align with the theory that flooding primarily disrupts root carbohydrate utilization rather than directly impairing photosynthesis, as evidenced by sustained photosynthetic rates despite significant biomass reductions.

Persistent flooding impacts on root function

Flooding induced long-term suppression of root respiration, with depressed rates persisting 35 days post-recovery in both species, a pattern well-documented in prior studies [13-16]. This suggests that while shoots tolerated 28-day flooding, root systems sustained severe, lasting damage. The mechanistic basis for reduced respiration likely shifted over time: during active flooding, hypoxia suppressed cytochrome respiration while inducing fermentative pathways [12], whereas post-recovery declines probably reflected preferential mortality of high-activity fine roots. This interpretation is supported by our observations of reduced root mass and altered allocation patterns following flooding.

Differential photosystem II sensitivity

Photosystem II (PSII) exhibited greater flooding sensitivity than gas-exchange parameters. While photosynthetic CO_2 assimilation and stomatal conductance remained stable, PSII quantum efficiency (Fv/Fm) declined significantly from day 22 of flooding through 35 days post-recovery. As Fv/Fm reflects the efficiency of electron transport in oxygen-evolving PSII complexes [12], its sustained depression indicates chronic photochemical impairment despite maintained carbon fixation. The delayed onset of Fv/Fm reduction suggests both species tolerate short-term flooding without photochemical damage, consistent with their natural occurrence in flood-prone habitats [13,17,24].

Species-specific adaptive strategies

The species diverged markedly in flooding responses, with CO_2 enrichment further amplifying these differences. *Larix laricina's* capacity to form adventitious roots—enhanced under elevated CO_2 (17/32 vs. 6/32 seedlings; *p* < 0.01) mirrors observations by Calvo-Polanco et al. [21] and likely explains its survival across treatments. In contrast, *P. mariana* suffered high mortality (59% under elevated CO_2), lacking such morphological adaptations. These results suggest future atmospheric CO_2 levels may favor *L. laricina* dominance in floodplains while disadvantaging *P. mariana*, potentially altering boreal wetland species composition [25].

Author Contribution

K Richard: developed the research plan, carried out the experiment, conducted data analysis, wrote an MScF thesis

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