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Multi-Walled Carbon Nanotube Application Alters Stomatal Behavior in Boreal Shrubs Under Drought Conditions

Sahari Inoue [1,2,*,†], Binyam Tedla [1,2,‡], Jean-Marie Sobze [1] and Raymond Thomas [3]

[1] Centre for Boreal Research, Northern Alberta Institute of Technology, Peace River, AB T8S 1R2, Canada; btedla@lakeheadu.ca (B.T.)
[2] Faculty of Natural Resources Management, Lakehead University, Thunder Bay, ON P7B 5E1, Canada
[3] Department of Biology, Biotron Experimental Climate Change Research Centre, Faculty of Science, Western University, London, ON N6A 5B7, Canada; rthoma2@uwo.ca
* Correspondence: sinoue@lakeheadu.ca
† Current address: Research Faculty of Agriculture, Hokkaido University, Sapporo 060-8589, Japan.
‡ Current address: Natural Resources and Forestry Program, College of New Caledonia, Prince George, BC V2N 1P8, Canada.

Abstract

Seedling establishment on reclaimed boreal sites is frequently constrained by drought and other abiotic stresses. Carbon nanomaterials have been shown to influence stress physiology in crops, but their effects on native boreal species are poorly understood. We tested whether carboxylic acid-functionalized multi-walled carbon nanotubes (MWCNTs) alter drought responses in three shrubs widely used in reclamation: *Shepherdia canadensis* (L.) Nutt, *Cornus sericea* L., and *Viburnum edule*. Seedlings received two irrigations with MWCNTs suspensions (0 (control), 10, or 30 mg L^{-1}) before exposure to well-watered or drought conditions in a greenhouse. Drought reduced photosynthesis, stomatal conductance, and transpiration and increased *Ci*/*Ca* across species, consistent with declining leaf water potential. MWCNTs did not broadly modify these responses, but the highest concentration (30 mg L^{-1}) further suppressed stomatal conductance in *C. sericea* and *V. edule* during mid- to late drought. *S. canadensis* showed little responsiveness. These effects suggest that MWCNT-associated stomatal closure may limit water loss under stress but also constrain CO_2 uptake, offering no clear photosynthetic benefit. MWCNT impacts were subtle, species- and dose-dependent, and centered on stomatal regulation. Application in reclamation should therefore be approached cautiously, balancing potential water-saving benefits against possible reductions in carbon assimilation and growth.

Keywords: *Shepherdia canadensis* (L.) Nutt.; *Cornus sericea* L.; *Viburnum edule*; nanoparticle; reclamation; gas exchange; morpho-physiological response; nursery practice

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1. Introduction

Reclamation processes following boreal resource development, such as oil sands mining, typically involve the reconstruction of soils using a substrate layer (e.g., tailings sand or overburden) and a cover soil layer (salvaged peat, mineral soils, or upland surface and mineral soils), which serves as the medium for vegetation growth [1,2]. However, planted seedlings face significant challenges in reclaimed soil due to abiotic stressors, including low nutrient availability, high pH, salinity, and drought conditions [3], which contribute to slow growth and transplant shock [3,4]. Thus, enhancing seedling morpho-physiological traits during nursery production is critical for improving drought resilience and increasing survival in these challenging environments.

Reclaimed sites in boreal regions are highly prone to water deficits due to the physical limitations of reconstructed soils and harsh microclimatic conditions. Substrate materials, such as tailings sand, typically have a low water-holding capacity and high drainage, whereas overburden may retain water but often lacks sufficient aeration [5]. In addition, poor soil–root contact and limited root permeability following transplanting further restrict water uptake [6]. These limitations are compounded by surface exposure to high solar radiation, wind, and temperature extremes, and by the lack of ground cover in early stages of reclamation [7,8]. Together, these factors lead to frequent drought stress, impaired seedling establishment, and low survival rates. Therefore, improving drought tolerance at the seedling stage through nursery-based interventions is critical for successful reclamation outcomes.

Nanotechnology offers emerging opportunities in agriculture and environmental applications [9–11]. Nanoparticles (1–100 nm), including carbon-based nanomaterials such as multi-walled carbon nanotubes (MWCNTs), exhibit unique properties that enable rapid cellular uptake and may enhance water uptake and drought tolerance in plants [12]. However, their effects on plant growth and physiology are inconsistent, with studies reporting positive [13], negative, or negligible effects depending on species, concentration, and application method [14–16]. For instance, MWCNTs can enhance the drought tolerance of plants by altering gene expression in crop species, activating water stress-related genes [17,18], and affect plant growth and development [19–21]. They can stimulate the production of antioxidants and osmoprotectants in seedlings, which protect them from oxidative stress caused by drought exposure [22]. MWCNTs can also enhance the photosynthetic capacity, improving their carbon assimilation rate, thereby increasing their water-use efficiency [23].

The effects of MWCNTs on plants vary depending on the type, concentration, and species of the plant used. For instance, Zhang et al. [23] reported that low concentrations (5 mg/L) had no effect on rice seedlings, whereas 20 mg/L promoted growth and increased chlorophyll content. Tiwari et al. [24] found that higher concentrations (>20 mg/L) inhibited growth in maize, whereas exposure to 50 mg/L of carboxyl-functionalized MWCNT increased dry biomass. While some studies have explored the use of MWCNTs to promote seed germination in boreal shrub species [25–27], their effects on drought-related physiological traits during the seedling stage remain largely unstudied. Conversely, studies on drought mitigation using MWCNTs have focused primarily on agricultural crops or model herbaceous species. To our knowledge, no prior studies have evaluated the effects of MWCNTs on drought physiology in native boreal shrubs used for reclamation.

In this study, we explored the potential of carboxyl-functionalized MWCNT, delivered via irrigation during seedling production, to alleviate drought-induced physiological stress in native boreal shrub species. We evaluated the combined effects of drought and MWCNT application on three native shrubs commonly used in boreal reclamation: *Shepherdia canadensis* (L.) Nutt., *Cornus sericea* L., and *Viburnum edule*. *S. canadensis* is a nitrogen-fixing, perennial deciduous shrub widely distributed across North America, including Alberta [28–30]. *C. sericea* is a fast-growing deciduous woody shrub native to much of North America and a vital source of food for many wild animals, including ruminants and birds [31]. *V. edule* is a cold-adapted shrub common to northern Canada and Alaska. Because MWCNT effects are often species- and concentration-dependent, we also examined whether responses varied among these shrubs across a moderate concentration range, with the aim of identifying patterns relevant to their potential use in reclamation. This study aimed to assess whether carboxyl-functionalized MWCNTs influence physiological drought responses in these native species during nursery production, providing insights into their potential relevance for early-stage reclamation practices.

2. Materials and Methods

2.1. Plant Material, Propagation, and Greenhouse Conditions

The study was conducted over a five-month period in the greenhouse at the Centre for Boreal Research (Northern Alberta Institute of Technology, Peace River, Alberta, Canada). One-year-old seedlings of three native boreal shrub species, Canadian buffaloberry (*Shepherdia canadensis* (L.) Nutt.), red-osier dogwood (*Cornus sericea* L.), and low-bush cranberry (*Viburnum edule*), were propagated from seeds collected near Peace River, Alberta.

Seeds of *S. canadensis* and *C. sericea* were cold-stratified at 4 °C for 12 and 8 weeks, respectively. Seeds of *V. edule* underwent a sequential stratification regime consisting of cold (4 °C), warm (20 °C), and cold (4 °C) treatments for 2, 3, and 3 months, respectively. Germination was carried out in trays containing a 1:1 (*v*/*v*) mixture of peat and vermiculite, maintained at approximately 24 °C during the day and 14 °C at night, under an 18 h photoperiod.

After completing one growing season, seedlings were overwintered in cold storage and utilized the following spring for the experiment. They were transplanted into plastic pots (18 cm height; 16 cm top diameter, 14 cm bottom diameter) containing a standard peat-based substrate.

Throughout both the propagation and experimental phases, the greenhouse environment was maintained at approximately 24 ± 3 °C (day) and 14 ± 3 °C (night), with ~60% relative humidity and an 18 h photoperiod supplemented by high-pressure sodium lighting.

2.2. Experiment Design

The experiment was a split-plot with three replicate blocks per species. Soil moisture was assigned at the main-plot level (well-watered, drought). Carboxyl-functionalized multi-walled carbon nanotube (MWCNT) concentration (0 (control), 10, 30 mg L^{-1}) was applied at the subplot level within each moisture main plot. Within subplots, seedlings were randomized completely.

2.3. Application of MWCNT and Soil Moisture Treatments

Carboxylic acid-functionalized MWCNT (outer diameter, 10–20 nm; length, 10–30 μm; purity, >95 wt%) (Table 1) was purchased from commercial suppliers Cheap Tubes Inc. (Grafton, VT, USA). MWCNT was weighed and mixed with deionized water and ultrasonicated by a probe sonicator (Fisher Scientific, FB-505, Hampton, NH, USA) at 40% amplitude for 40 min to maximize dispersion [32–34]. The solution was delivered to the growing medium twice during the experiment (1 and 2 months after transplantation). Each plant was watered with 15 mL of MWCNT solution. To minimize cross-treatment mixing and maintain consistent water retention, each pot was placed in an individual plastic tray to capture any immediate drainage. The dispersion stability and persistence of MWCNTs in the substrate (e.g., via dynamic light scattering, zeta potential analysis, or microscopy) were not assessed, as the objective of this study was to evaluate seedling physiological responses to MWCNT application rather than characterize nanomaterial behavior.

Table 1. Properties of carboxyl-functionalized multi-walled carbon nanotubes (COOH-MWCNTs) used in the experiment.

MWCNT Type	Outer Diameter (nm)	Inside Diameter (nm)	Length (μm)	Purity (wt%)	Functional Content (wt%)	Ash (wt%)	Specific Surface Area (m^2/g^{-1})	Bulk Density (g/cm^{-3})	True Density (g/cm^{-3})
COOH-MWCNTs	<8	2–5	10–30	>95	5.58	<1.5	500	0.27	~2.1

All seedlings were watered every other day until two weeks after the second application of MWCNT. Then, drought stress was induced by withholding water for 14 days. The drought stress continues until the seedling reaches the wilting point. Soil volumetric water content (SVWC) was monitored using an SVWC sensor (5TE soil moisture and temperature, Decagon Devices Inc., Pullman, WA, USA) (Figure 1). Seedlings in the well-watered treatment were watered every other day to maintain moisture at ~0.3 to 0.35 m^3/m^3 (SVWC).

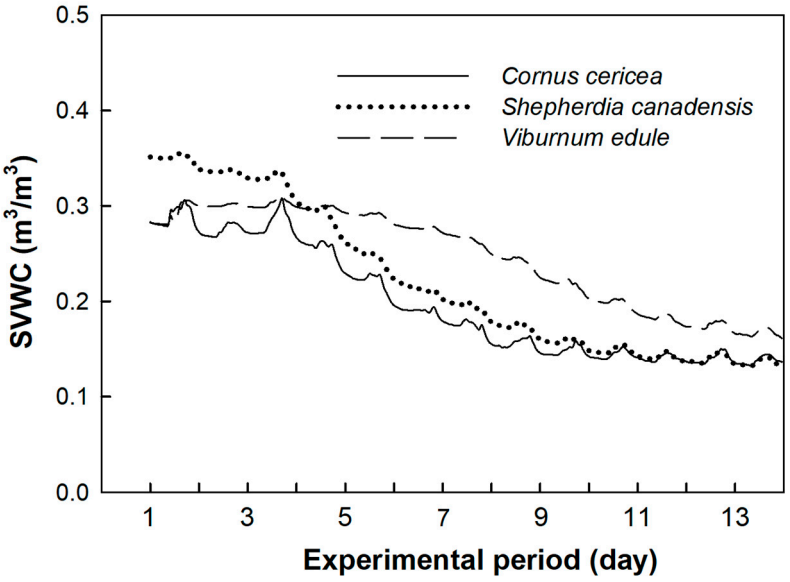

Figure 1. Soil volumetric water content (SVWC) over the experimental period in the drought treatment for *Shepherdia canadensis*, *Cornus sericea*, and *Viburnum edule*. The dotted line represents *S. canadensis*, the solid line *C. sericea*, and the dashed line *V. edule*.

2.4. Foliar Gas Exchange Measurement

Measurements were conducted every two days from the initiation of the drought treatment. Gas exchange was measured non-destructively on the same seedlings across time to allow repeated measurements. For each species and treatment combination, three seedlings were measured within each of two replicate blocks (n = 3 per block), totaling six seedlings per species per treatment.

Foliar gas exchange was measured using a PP CIRAS-3 open gas exchange system, and measurements were made from samples placed in a PLC Universal Leaf Cuvette (PP Systems, Amesbury, MA, USA). A healthy and fully expanded leaf (4th or 5th from the top) was measured from three randomly selected seedlings from each treatment replicate grown under the following conditions: 22 °C air temperature, 50% relative humidity, 800 µmol m^{-2} s^{-1} photosynthetically active radiation, and 400 µmol mol^{-1} CO_2. The measurements were carried out between 9:00 a.m. and 2:00 p.m. The net photosynthetic rate (P_n), stomatal conductance (g_s), transpiration rate (E), and instantaneous water-use efficiency (*iWUE*) were obtained from these measurements.

2.5. Leaf Water Potential Measurement

Midday leaf water potential (Ψ_L) was measured to determine the whole plant water status. Two seedlings were randomly chosen from different plant species in each treatment combination. A fifth of a fully expanded and healthy leaf was cut and placed in a pressure chamber (Model 1000, PMS Instrument Co., Albany, OR, USA), which was pressurized using a nitrogen tank. The pressure (Ψ) was recorded when the initial xylem sap was

observed from the cut end of the petiole. The measurement was conducted from 12:00 to 14:00 h.

2.6. Data Analysis

Gas exchange data were analyzed using a split-split plot ANOVA design. Soil moisture treatment (well-watered vs. drought) was assigned at the main plot level, MWCNT concentration (0, 10, 30 mg L^{-1}) at the subplot level, and measurement day as a repeated sub-subplot factor. Measurements were taken repeatedly from the same seedlings over time. Each species was analyzed separately.

Prior to analysis, data were evaluated for normality and homogeneity of variance using probability plots and residual scatterplots. Following assumption checks, a three-way ANOVA was performed using R software (Version 3.6.0, R Development Core Team 2019). Effects were considered significant at $p \leq 0.05$. When a significant main effect or interaction involving MWCNT concentration was detected, Fisher's Least Significant Difference (LSD) post hoc test was used to compare treatment means.

3. Results

Drought-stressed seedlings exhibit a progressive reduction in midday leaf water potential (Ψ_L) during the experimental period, becoming significantly lower than the well-watered seedlings from day 7 (Figure 2). In contrast, well-watered seedlings maintained relatively stable Ψ_L throughout the experiment, with average values of −1.33 MPa for *S. canadensis*, −0.91 MPa for *C. cericea*, and −0.89 MPa for *V. edule*.

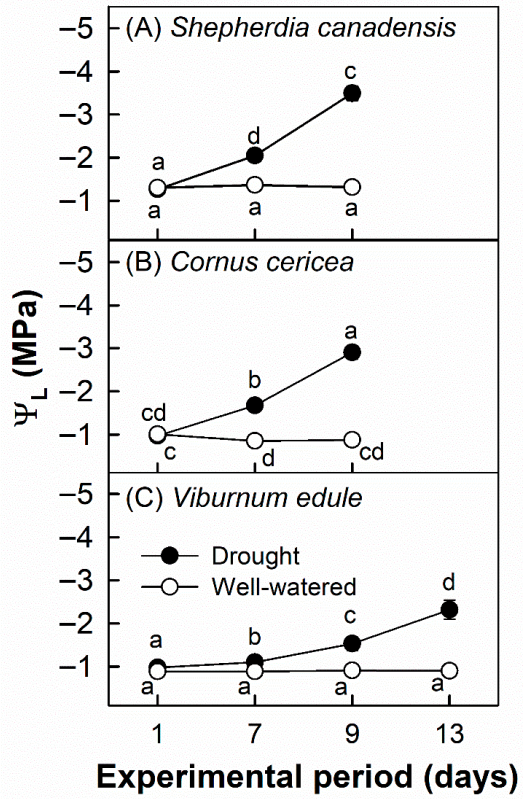

Figure 2. Leaf water potential (Ψ_L) in *S. canadensis*, *C. sericea*, and *V. edule* under well-watered and drought conditions. Each bar represents the mean ± SE (n = 4) for each treatment. Values were pooled across MWCNT concentrations due to the absence of a significant MWCNT effect. Different letters indicate significant differences between treatments (Fisher's LSD, $p \leq 0.05$).

As shown in Table 2, the response of the physiological parameters measured was not affected by the effect of MWCTN concentration alone. However, species-specific differences were observed in the gas exchange responses. The response pattern of P_n differed between species (Figure 3). Water-withholding treatments led to a significant change in P_n on day 7 and day 11 for *S. canadensis* and *V. edule*, respectively (Figure 3A,C). In contrast, there was an initial increase in photosynthesis for *C. cericea* followed by a decrease (Figure 3B), indicating an optimal water potential threshold for maximizing photosynthetic potential (~−1.5 MPa). Accompanying the reduction in gas exchange, the decline in g_s was by 31.4% and 25% for *S. canadensis* and *C. cericea*, respectively (Table 2; Figure 3D).

Table 2. Summary of F- and *p*-values from split-split plot ANOVA for physiological responses in *S. canadensis*, *C. sericea*, and *V. edule* under two soil moisture regimes (M), three MWCNT concentrations (C), and repeated measurements across time (D). Measurement day was treated as a repeated sub-subplot factor with six time points for *S. canadensis* and *C. sericea*, and seven for *V. edule*. Significant effects ($p \leq 0.05$) are shown in bold. Degrees of freedom (DF) for each main effect and interaction are provided below the corresponding columns. Variables include net photosynthetic rate (P_n), stomatal conductance (g_s), transpiration rate (E), intrinsic water use efficiency (*iWUE*), and the ratio of intercellular to ambient CO_2 concentration (Ci/Ca).

Variable	M		C		M × C		D		M × D		C × D		M × C × D	
	F	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***
S. canadensis														
P_n	10.26	0.085	0.44	0.673	0.09	0.913	18.38	**<0.01**	11.83	**<0.01**	0.76	0.669	0.29	0.978
g_s	13.76	0.066	1.91	0.262	0.02	0.982	19.49	**<0.01**	9.68	**<0.01**	1.64	0.143	0.71	0.707
IWUE	5.36	0.147	0.03	0.971	0.02	0.979	12.66	**<0.01**	23.68	**<0.01**	0.22	0.993	0.22	0.992
E	33.42	**0.029**	1.48	0.330	0.05	0.955	31.60	**<0.01**	9.84	**<0.01**	1.40	0.226	0.73	0.688
Ci/Ca	4.50	0.168	0.24	0.794	0.34	0.728	21.76	**<0.01**	17.56	**<0.01**	0.22	0.992	0.18	0.997
	(DF = 1)		(DF = 2)		(DF = 2)		(DF = 5)		(DF = 5)		(DF = 10)		(DF = 10)	
C. cericea														
P_n	11.97	**0.041**	0.88	0.501	0.24	0.803	51.29	**<0.01**	81.31	**<0.01**	0.57	0.824	1.05	0.427
g_s	10.48	**0.048**	0.99	0.468	0.13	0.883	34.45	**<0.01**	58.67	**<0.01**	0.92	0.527	2.18	**0.049**
iWUE	14.14	**0.033**	1.51	0.352	1.18	0.419	30.88	**<0.01**	58.97	**<0.01**	0.42	0.928	0.31	0.974
E	10.02	0.051	0.54	0.630	0.10	0.904	38.73	**<0.01**	51.01	**<0.01**	0.60	0.803	2.07	0.060
Ci/Ca	20.19	**0.021**	0.90	0.494	0.98	0.470	44.67	**<0.01**	47.72	**<0.01**	0.59	0.809	0.35	0.957
	(DF = 1)		(DF = 2)		(DF = 2)		(DF = 5)		(DF = 5)		(DF = 10)		(DF = 10)	
V. edule														
P_n	0.15	0.736	0.02	0.983	1.03	0.435	18.60	**<0.01**	7.543	**<0.01**	1.55	0.152	0.72	0.727
g_s	11.65	0.076	0.73	0.535	0.25	0.789	19.86	**<0.01**	10.72	**<0.01**	2.21	**0.033**	1.88	0.072
IWUE	0.25	0.666	0.12	0.893	0.96	0.458	11.89	**<0.01**	4.424	**<0.01**	1.24	0.298	0.41	0.950
E	546.7	**<0.01**	0.33	0.739	0.92	0.471	24.09	**<0.01**	10.47	**<0.01**	2.40	**0.021**	2.70	**0.011**
Ci/Ca	0.001	0.975	0.83	0.500	1.73	0.288	9.99	**<0.01**	3.668	**<0.01**	1.77	0.093	0.42	0.948
	(DF = 1)		(DF = 2)		(DF = 2)		(DF = 6)		(DF = 6)		(DF = 12)		(DF = 12)	

The effects of MWCNT concentration on g_s were species- and time-dependent. In *C. cericea*, a significant decline in g_s was observed mid-experiment period (day 7) in seedlings treated with the highest MWCNT concentration (30 mg L^{-1}) under drought conditions (Table 2; Figure 4A). The difference within the MWCNT treatments disappeared with the progress of drought severity. In *V. edule*, g_s remained unaffected by MWCNT treatment during the first week of water-withholding, but declined significantly in the second week under 30 mg L^{-1} treatment, showing 19% and 31% decline on day 9 and day 11, respectively, compared to MWCNT 10 mg/L. Relative to control seedlings (0 mg L^{-1}), g_s under 30 mg L^{-1} declined by 29% on day 7 and 15% on day 11 (Figure 4B). A similar trend was observed in the transpiration rate (E) of *V. edule*, where drought-exposed seedlings treated with 30 mg L^{-1} MWCNT exhibited significantly lower E compared to other concentrations

on day 9. By day 11, the differences among MWCNT treatments diminished, with all treatments showing reduced *E* under drought. However, on day 13, *E* remained relatively stable under the 30 mg L^{-1} treatment, while seedlings under the 0 mg L^{-1} (control) and 10 mg L^{-1} treatments showed a further significant decline.

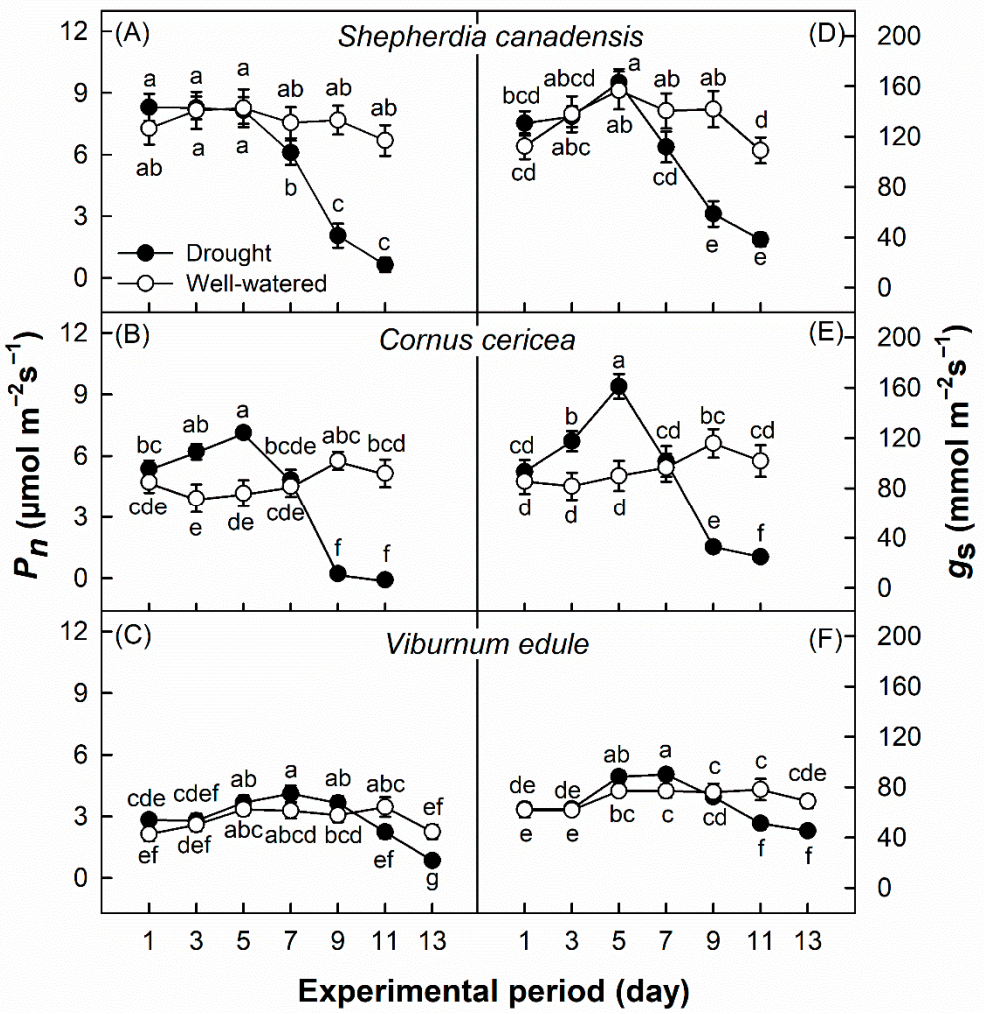

Figure 3. Net photosynthetic rate (P_n; panels (**A**–**C**)) and stomatal conductance (g_s; panels (**D**–**F**)) measured in *S. canadensis*, *C. sericea*, and *V. edule* under well-watered (○) and drought (●) conditions over the experimental period. Each point represents mean ± SE (n = 18). Data were pooled across MWCNT concentrations (0 (control), 10, and 30 mg L^{-1}) due to the absence of a significant MWCNT effect or interaction (see Table 2). Statistically significant effects were detected for soil moisture, measurement day, and their interaction. Different letters denote significant differences between treatments (Fisher's LSD, $p \leq 0.05$).

In the first half of the water-withholding treatment, the increase in *IWUE* was observed only for *C. cericea*; however, *IWUE* was found to fall rapidly in the second week of the experiment for drought seedlings (Figure 5D–F). The transpiration response pattern was similar to the photosynthesis and stomatal conductance (Figure 5A–C). Up to the beginning of the second week of water-withholding, there was no difference in *Ci*/*Ca* between well-watered seedlings and those exposed to drought treatments in both *S. canadensis* and *C. cericea*. However, *Ci*/*Ca* was significantly higher for both species in the second week of the experiment period. This observation was only seen at the last measurement for *V. edule*. Accompanying the rapid increase in *Ci*/*Ca* was a steep simultaneous decline in g_s Figures (3D–F and 5G–I).

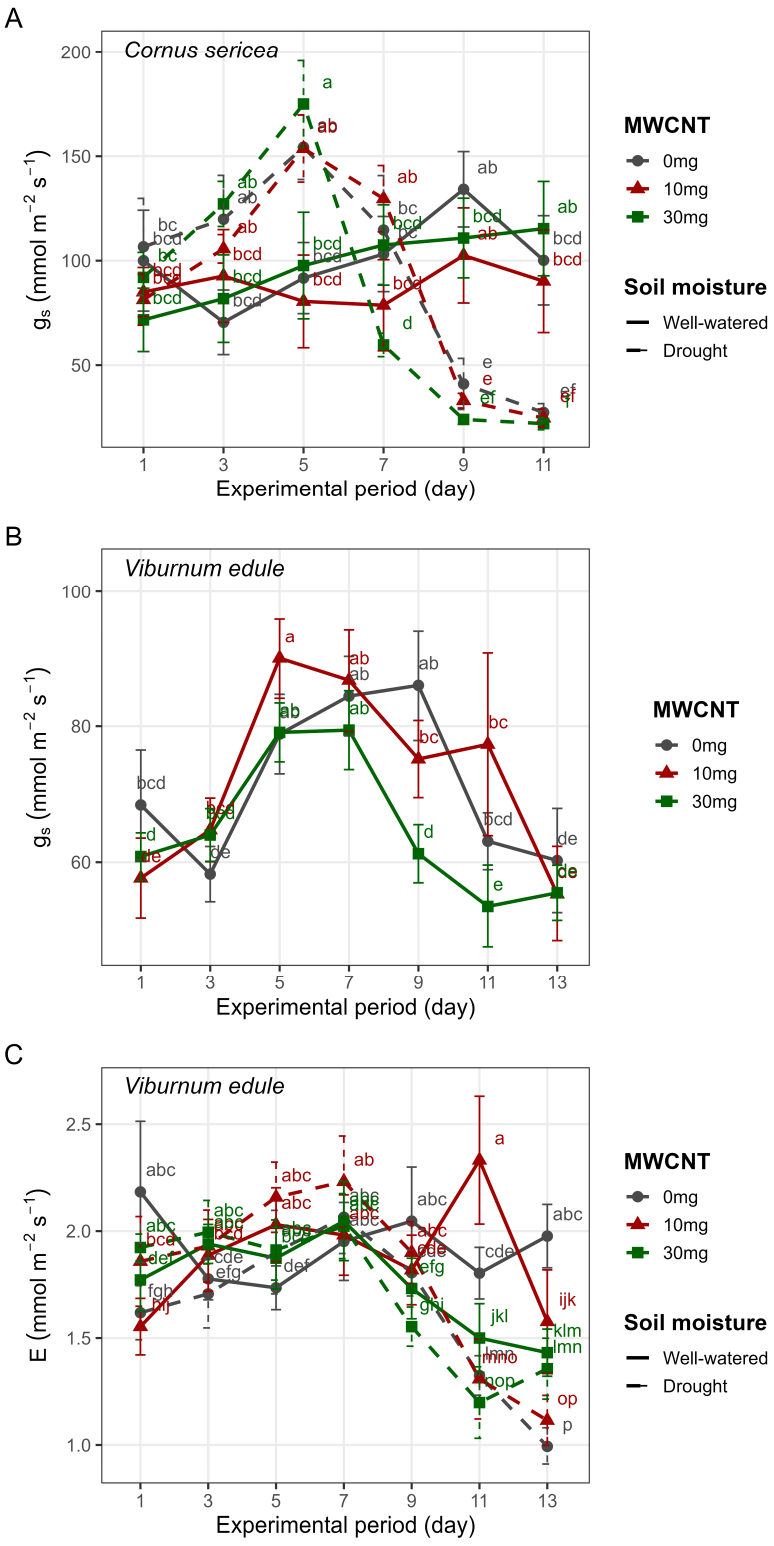

Figure 4. Stomatal conductance (g_s) measured in *C. sericea* (**A**) and *V. edule* (**B**), and transpiration rate (*E*) in *V. edule* (**C**), during the experimental period under different MWCNT concentrations (0 (control), 10, and 30 mg L^{-1}) and two soil moisture treatments (well-watered and drought). In panels (**A**,**C**), data are shown for all combinations of MWCNT concentration and soil moisture (significant MWCNT × moisture × day interaction); in panel (**B**), data are pooled across soil moisture levels (no significant interaction). Solid and dashed lines represent well-watered and drought treatments, respectively; colors represent MWCNT concentrations. Data are presented as mean ± SE of six seedlings (n = 6 per species × treatment). Different letters indicate statistically significant differences among treatments ($p \leq 0.05$; Fisher's LSD test).

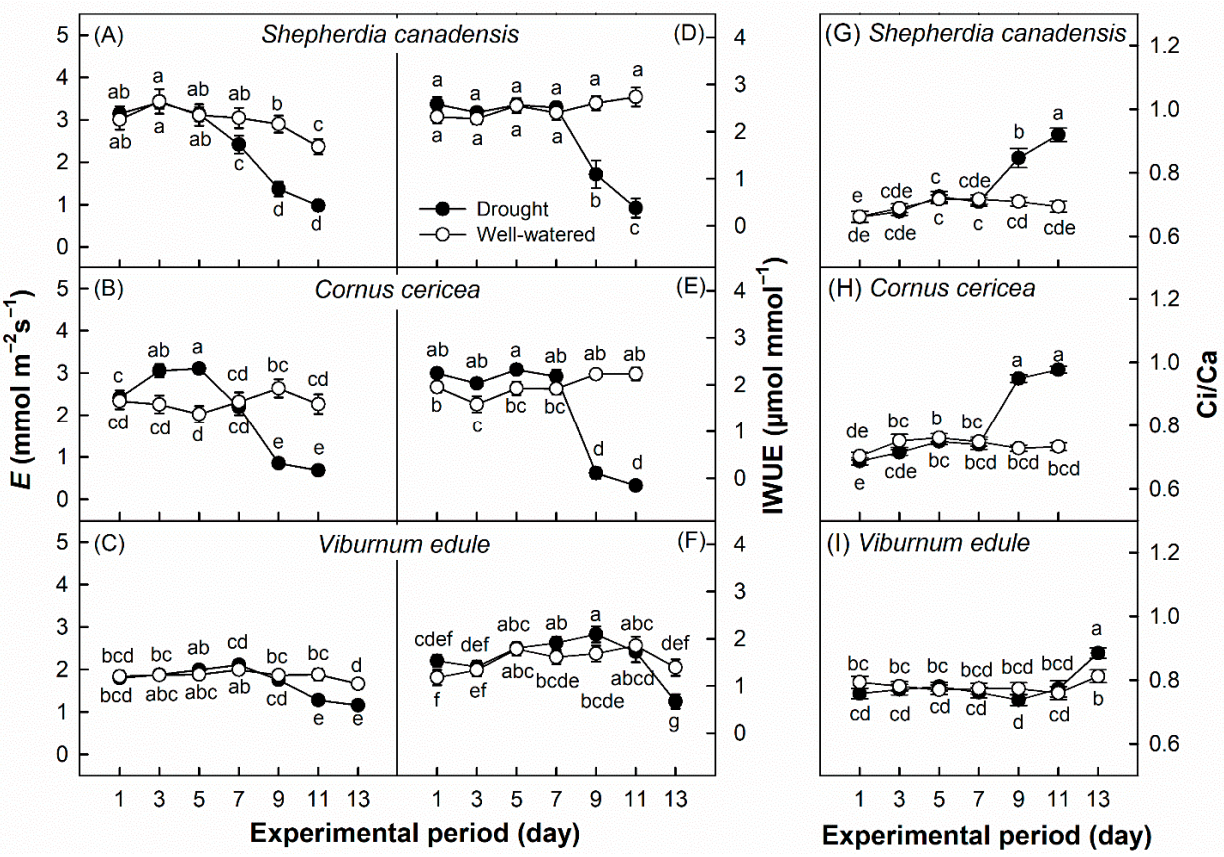

Figure 5. Transpiration rate (*E*), instantaneous water use efficiency (*IWUE*), and the ratio of intercellular to ambient CO_2 concentration (Ci/Ca) measured in *S. canadensis* (**A**,**D**,**G**), *C. sericea* (**B**,**E**,**H**), and *V. edule* (**C**,**F**,**I**) during the experimental period under well-watered (○) and drought (●) conditions. Data were pooled across MWCNT concentrations due to the absence of significant MWCNT effects (see Table 2). Values are presented as mean ± SE (n = 18). Significant effects of soil moisture, measurement day, and their interaction were detected. Different letters denote statistically significant differences among treatments (Fisher's LSD, $p \leq 0.05$).

4. Discussion

This study evaluated whether carboxyl-functionalized multi-walled carbon nanotubes (MWCNTs), delivered via irrigation, can mitigate drought-induced physiological stress in native boreal shrub species commonly used in land reclamation. Many previous studies, especially in agricultural crops and model herbaceous plants, such as *Vigna radiata*, *Spinacia oleracea*, and *Arabidopsis thaliana*, have reported that carbon nanotubes enhance photosynthesis (P_n) and chloroplast activity. These effects are often linked to stimulation of Rubisco, increased chlorophyll content, or improved electron transport [35–38]. However, such responses were not observed in our study. Instead, the physiological effects of MWCNTs were subtle, species-specific, and primarily evident in stomatal conductance (g_s) under drought conditions. While some prior studies [39] have reported reductions in g_s with MWCNT exposure, our findings provide new evidence from native woody perennials. This includes species-specific g_s suppression under drought, a response not widely documented in the existing literature. These results highlight the importance of evaluating MWCNT effects in ecologically relevant species and stress conditions when considering potential applications in forest restoration or reclamation.

The present study revealed that reductions in g_s were modulated by MWCNT concentration in both *C. sericea* and *V. edule*, but with different response patterns. In *C. sericea*, a significant interaction indicated that seedlings treated with 30 mg L^{-1} MWCNT exhibited

earlier g_s suppression under drought conditions compared to other concentrations, with effects most evident by day 7. In *V. edule*, g_s suppression at 30 mg L^{-1} was detected as a main effect of concentration, emerging by days 9 and 11 and independent of soil moisture treatment, suggesting a more general sensitivity to MWCNT exposure. In contrast, *S. canadensis* did not exhibit clear physiological responses to the application of MWCNTs. Previous studies have reported that MWCNTs can alter membrane function by modifying lipid composition, increasing membrane rigidity and permeability, and enhancing aquaporin expression or activity, which together may influence water transport and indirectly affect stomatal regulation [40]. Similar concentration-dependent declines in g_s have been reported by Izad et al. [39] in *Orthosiphon stamineus*, but only at much higher MWCNT levels (700–2100 mg L^{-1}), where reductions in g_s, P_n, and transpiration rate of up to ~50% relative to controls were accompanied by increased water use efficiency. Taken together, these findings suggest that native boreal shrubs can exhibit stomatal sensitivity to MWCNTs at substantially lower concentrations than some herbaceous species, but with species-specific differences in whether effects are drought-dependent or constitutive.

In addition, recent evidence suggests that MWCNTs can upregulate stress-responsive genes related to ABA signaling, reactive oxygen species detoxification, and drought acclimation (e.g., [41,42]). For example, Zhuzhukin et al. [41] reported that low concentrations of MWCNTs enhanced the expression of drought- and stress-related genes (e.g., DREB2, PR-1, PR-10, LEA8) in birch seedlings, whereas higher concentrations downregulated several of these genes. Although we did not measure gene expression in our study, such molecular responses may help explain the significant g_s suppression observed under 30 mg L^{-1} MWCNT, which could reflect an amplification of endogenous drought-response pathways and a shift toward protective, though potentially growth-limiting, physiological adjustment.

The species-specific responses observed here are notable. *V. edule* exhibited delayed but significant g_s suppression under 30 mg L^{-1} MWCNT, while *C. sericea* responded earlier in the drought cycle. This may reflect inherent differences in stomatal regulation or MWCNT sensitivity. In contrast, *S. canadensis* did not exhibit clear physiological responses to MWCNTs, suggesting either lower responsiveness or higher tolerance to MWCNTs exposure in this species. This raises the possibility that higher concentrations than those tested here may be required to elicit measurable responses in *S. canadensis*. Such interspecific differences highlight the importance of species screening when evaluating novel soil amendments for ecological applications. Previous work also shows that MWCNTs can affect gas exchange in contrasting ways, depending on the species and stress type; for example, Alp et al. [42] reported that MWCNT application alleviated the decline in g_s under copper stress.

The MWCNT concentrations tested (0, 10, 30 mg L^{-1}) provided preliminary insights into physiological responses, but they may not fully capture potential threshold or hormetic effects that have been reported elsewhere (e.g., [19,41]). The chosen range was selected to reflect concentrations previously used in both agricultural and forestry contexts, where the range of 10–30 mg L^{-1} MWCNTs has been shown to improve stress tolerance without apparent toxicity in plants (e.g., [41,43]). In contrast, higher concentrations (>50 mg L^{-1}) have been associated with negative effects on plant physiology and morphology in several species [44], suggesting that 30 mg L^{-1} may already approach the upper threshold of physiological safety. Thus, while the aim of the present study was not to establish toxicity thresholds, the selected concentrations capture a range likely to be operationally effective for drought mitigation while avoiding confounding toxic effects. Future work should incorporate broader concentration gradients (e.g., 1–100 mg L^{-1}) to better characterize

dose–response relationships, identify thresholds or hormetic patterns, and assess potential toxicity or environmental persistence under field conditions.

Additional insights into species-specific drought physiology were provided by analysis of intercellular to ambient CO_2 concentration (Ci/Ca). In *S. canadensis* and *C. sericea*, no significant differences were observed between droughted and control plants until the second week of drought, when Ci/Ca values increased significantly, indicating the onset of photosynthetic stress. In contrast, *V. edule* maintained stable Ci/Ca levels until the final measurement, suggesting a delayed physiological response to water deficit compared to the other species. An increase in the Ci/Ca ratio typically reflects non-stomatal limitations, where internal biochemical constraints, rather than reductions in stomatal conductance, restrict CO_2 assimilation. This interpretation is consistent with Brodribb and Hill [45], who reported sharp increases in Ci/Ca and declines in PSII efficiency (Fv/Fm) in *Podocarpus lawrencii* under extreme drought, reflecting photoinhibition and metabolic suppression, not just CO_2 diffusion limitation. Such reductions in photochemistry and Calvin cycle activity may represent irreversible photosynthetic damage under prolonged stress.

While our findings provide preliminary insights into the physiological effects of MWCNTs during early seedling development, their implications for reclamation must be considered cautiously. Our assessment was limited to gas exchange and leaf water potential, and did not include other informative drought tolerance indicators such as chlorophyll fluorescence, osmolyte accumulation, or root morphology [46–48], which could help distinguish between protective adjustments and stress-induced damage. In addition, long-term growth, survival, and ecological performance under natural field conditions remain unknown, as do potential trade-offs between short-term drought protection and sustained carbon assimilation or biomass accumulation. The environmental fate and persistence of carbon-based nanomaterials in boreal soils are also poorly understood, raising important questions about potential ecotoxicological risks and soil–microbe interactions [49,50]. Addressing these uncertainties will require extended trials that integrate broader physiological traits, follow seedling performance beyond the nursery stage, and evaluate environmental safety under operational reclamation scenarios.

In conclusion, this study demonstrates that carboxyl-functionalized MWCNTs can modulate stomatal behavior in native boreal shrubs, but in a species-specific and drought-intensity-dependent manner. *C. sericea* exhibited earlier reductions in g_s under drought at the highest MWCNT concentration, whereas *V. edule* showed g_s suppression as a main effect of MWCNT concentration, independent of water status. *S. canadensis* displayed little responsiveness within the tested range. These findings suggest that carboxyl-functionalized MWCNTs may help mitigate transpiration losses during water limitation, but at the potential cost of reduced CO_2 diffusion and photosynthetic capacity. Such dual effects underscore the importance of optimizing MWCNT application to balance protective water-saving mechanisms with growth sustainability. Further research should extend the concentration gradients, incorporate molecular and biochemical analyses, and evaluate long-term performance under operational field conditions to fully assess the potential of MWCNTs as a drought mitigation strategy in forest restoration and reclamation.

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