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Growth reduction and alteration of nonstructural carbohydrate (NSC) allocation in a sympodial bamboo (*Indocalamus decorus*) under atmospheric O₃ enrichment



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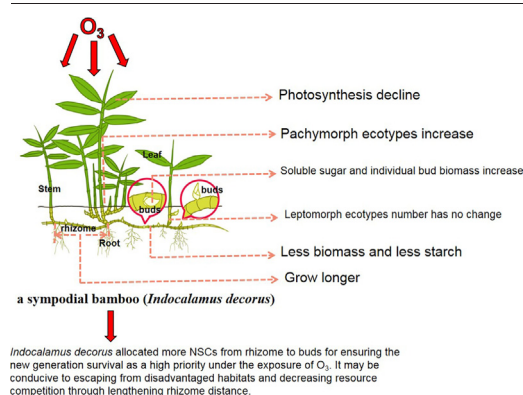
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HIGHLIGHTS

- The most biomass reductions induced by elevated O₃ happened in rhizome.
- More starch in rhizome transferred to soluble sugar in buds.
- Elevated O₃ increased individual rhizome bud biomass and rhizome bud to rhizome biomass ratio.
- Elevated O₃ did not affect scattered ecostyle number but induced monopodial (clumped) ecotypes number decreased.
- The mean distance between two ramets lengthened possible for escaping and decreasing resource competition.

GRAPHICAL ABSTRACT



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ABSTRACT

Regional increases in atmospheric O₃, mainly produced photochemically from anthropogenic precursor gases, have phytotoxicity due to its strong oxidizing properties. To determine the response of bamboo physiology to elevated O₃ levels, three-year-old dwarf bamboo (*Indocalamus decorus*) clones were exposed to three O₃ concentrations (Ambient-AA, 21.3 to 80.9 ppb in the daytime; -AA + 70, 70 ppb O₃ above ambient; -AA + 140, 140 ppb O₃ above ambient) in open-top chambers for one growing season in Beijing, China. Gas exchange, biomass, growth, soluble sugar, and starch contents were examined at the end of the experiment. Our findings indicated that: (1) elevated O₃ treatments decreased the photosynthesis rate, total biomass, and bud numbers but increased individual bud biomass and rhizome bud to rhizome biomass ratio. The most severe reduction was observed in new rhizome biomass (35.9% reduction in AA + 70 and 57.2% reduction in AA + 140), whereas individual bud biomass increased by 50% and 75% in the AA + 70 and AA + 140 groups compared with AA, respectively; (2) the starch contents in the rhizome decreased by 28.4%, whereas soluble sugar increased by 38.1% in the AA + 140 rhizome buds compared to AA; (3) only the culm numbers of pachymorph rhizomes (clumped) decreased, whereas no changes in leptomorph rhizomes were observed. However, the mean distance between two ramets was lengthened by 49.4% and 86.5% in AA + 70 and AA + 140, respectively. In conclusion, *Indocalamus decorus* allocated more nonstructural carbohydrates (NSCs) from the rhizome to the buds to

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form stronger buds and ensure the survival of newer generations as a high priority in response to O₃ exposure. *Indocalamus decorus* may be conducive to escaping from disadvantaged habitats and decreasing resource competition by lengthening the distance between two ramets.

1. Introduction

Atmospheric O₃ is an important air pollutant and oxidant that threatens both human and environmental health, including the biological processes of animals and plants (Matyssek et al., 2014; Li et al., 2015). Given the rapid economic development and urbanization over the past two decades, atmospheric O₃ pollution caused by the emission of precursor gases (NO_x and VOCs) is becoming a serious problem in China. This is particularly true in rapidly developing urban clusters such as Beijing, Tianjin, Shanghai, and the Pearl River Delta regions (Pei et al., 2020; Zhang et al., 2019; Zhao et al., 2021). The Beijing Municipal Environmental Protection Bureau recently reported that the daily (8 h) maximum O₃ average concentration in Beijing has increased from 183.4 ppb in 2013 to 202.6 ppb in 2015 (BMEPB, 2013; BMEPB, 2015; Li et al., 2020). Furthermore, the mean O₃ concentrations in 2020 (108.6 ppb) are higher than those in 2019 (96.8 ppb) (Li et al., 2021). This increasing trend highlights the challenge. Therefore, additional research is necessary to characterize the species-specific effects of O₃ pollution.

Carbon reserves serve as important indicators of carbon limitation in plants and carbohydrates are the main product of photosynthesis (Hoch, 2015). Plants store these carbohydrates primarily in the form of sugars and starch, which constitute nonstructural carbohydrates (NSCs) (Bansal and Germino, 2009). NSC pools in different plant organs can serve as buffers to counteract decreases in C assimilation in response to environmental stressors such as O₃ exposure (Meier et al., 1990), heat (Marias et al., 2017), and drought (Francisco et al., 2018; Schnbeck et al., 2018). O₃ could induce not only visible foliar injury (Wan et al., 2014), photosynthesis inhibition (Li et al., 2015), and biomass reduction (Wang et al., 2007; Leung et al., 2020), but also alter photosynthetic partitioning. The photosynthetic partitioning especially concerning carbohydrate allocation between the above- and below-ground structures of many species, thereby impairing the balance between structural and nonstructural carbohydrates (Meier et al., 1990; Chen et al., 2018; Grantz and Gunn, 2010). NSCs could also buffer the imbalance between the supply and demand of C across plant tissues on diurnal and seasonal scales (Martínez-Vilalta et al., 2016; Hartmann and Trumbore, 2016). Therefore, the concentrations and proportions of NSCs in different organs might reflect the status of carbon supply in the entire plant, which provides insights into the strategy and ability of plants to grow and survive when confronted with environmental disturbances.

The responses of trees (deciduous or evergreens), crops, and grass to O₃ exposure are species-specific and reflect differences in the NSCs of different organs. Some studies have reported that NSC components such as starch decrease under elevated O₃ levels in the roots of spruce seedlings (*Picea abies*; Braun et al., 2004), Scots pine (*Pinus sylvestris*; Anttonen and Kärenlampi, 1995), loblolly pine (*Pinus taeda*; Meier et al., 1990), ponderosa pine (*Pinus ponderosa*; Andersen et al., 1997), and an aspen clones (*Populus tremuloides*; Coleman et al., 1995). However, Chen et al. (2015) demonstrated that under exposure to the same O₃ concentrations, *Phoebe bournei* allocated more sucrose, glucose, fructose, and starch from its leaves to fine roots, whereas *Pinus massoniana* exhibited no observable changes in nonstructural carbohydrates. Lux et al. (1997) also found that the roots and stems of *Fagus sylvatica* stored larger amounts of starch in response to ambient O₃, whereas *Picea abies* accumulated high amounts of soluble carbohydrates in needles, particularly glucose. Nevertheless, current studies have largely focused on leaf or root allocation (Landhäusser and Lieffers, 2003; Myers and Kitajima, 2007; Poorter and Kitajima, 2007; Merchant et al., 2010; Chen et al., 2018), whereas the whole-plant distribution (including the buds) of NSCs has remained largely uncharacterized.

Bamboo, a member of the grass family Poaceae, belongs to the subfamily Bambusoideae, which consists of 1642 species (Vorontsova et al., 2016) and is widely distributed in tropical, subtropical, and warm temperate regions in the Asia Pacific region, as well as in the Americas and Africa (Lin et al., 2020). China has the most bamboo species (almost 46%) and the largest bamboo distribution area (around 1/3 of the total bamboo forest) in the world (Vorontsova et al., 2016). Bamboo is a unique fast-growing evergreen perennial clonal grass species with lignification culms, which mainly relies on asexual reproduction and possesses a well-developed below-ground rhizome-root system. Bamboo biomass increases on average at a rate of 10%–30% annually, which far exceeds the 2%–5% growth rates of other timber species (Atanda, 2015). In addition to their ecological relevance, some bamboo species could also help alleviate food security, energy security, and climate change (Ramakrishnan et al., 2020). Several studies have characterized the responses of bamboo to environmental stressors such as CO₂ (Li et al., 2013; Zhuang et al., 2018), temperature (Li et al., 2013), nitrogen addition (Chen et al., 2017), and O₃ (Zhuang et al., 2011, 2018). Zhuang et al. (2011) reported that the O₃ tolerance of *Oligostachyum lubricum* was stronger than that of *Phyllostachys edulis*. Luo et al. (2010) found that O₃ exposure affected lignification and related enzyme activity in bamboo (*Phyllostachys pubescens* Mazel) shoots. Zhuang et al. (2018) reported that elevated O₃ significantly decreased leaf biomass and nutrient uptake of both *Phyllostachys edulis* and *Oligostachyum*. However, although the concentration of NSCs in tree tissues is considered a very effective indicator of carbon supply and demand (Körner, 2003; Martínez-Vilalta et al., 2016; Hartmann and Trumbore, 2016), little is known regarding the impacts of O₃ on NSC allocation and compensation among different bamboo organs including the buds.

The rhizome is the clonal organ of bamboo and possesses considerable storage capability. Storage organs such as the rhizome can offer quick compensation to local damage in other organs (de Kroon and van Groenendael, 1997; Dong et al., 2010), especially when the storage in other plant parts is insufficient (Dong et al., 2010). There are two basic growth forms of rhizome in bamboo species. Leptomorph rhizomes, which occur as spreading ramets, are long and slender, with indefinite subterranean development. Based on their diageotropic characteristics, the growth of these ramets is classified as monopodial and indeterminate (Mclure, 1967).

In contrast, pachymorph rhizomes occur as short and thick clumping ramets. The growth of this type of rhizome is classified as sympodial and determinate (Mclure, 1967). Leptomorph rhizomes enable plants to escape from less favorable patches where resource levels are low or competitive stress is high, whereas pachymorph rhizomes enable plants to consolidate or maintain favorable patches (de Kroon and Hutchings, 1995; Chen et al., 2011). The growth patterns of a given plant species are genetically determined but can be modified by its environmental conditions (Amiaud et al., 2008; Dong et al., 2010). To increase the odds of survival in adverse environments, some rhizomatous clonal plants were reported to increase asexual reproduction (increase ramet buds number) or performed physiological integration when ramets were growing under drought (Wang et al., 2008), overgraze (Gao et al., 2013), severe sand burial (Chen et al., 2010), or lack of nutrients (Abrahamson et al., 1991; Hutchings, 2010; Gao et al., 2013). Some clonal plants were reported to possess longer rhizomes and more ramets to increase their ability to quickly escape unfavorable habitats and reach a better habitat patch through exploring (Wang et al., 2008). Chen et al. (2011) reported that the mean spacer length between original plants and ramets increased with burial depth to overcome sedimentation stress. Similar responses have also been reported among clonal plants under grazing pressure (Amiaud et al., 2008) and low nutrient conditions (Ye et al., 2006). However, few studies have examined the

plasticity of bamboo growth forms in response to abiotic stressors such as high O₃ concentrations, a common atmospheric pollutant in urban areas.

Indocalamus decorus, a sympodial rhizome bamboo species including both typical leptomorph (short) rhizomes and pachymorph (long) rhizomes (McClure, 1966), can adapt to a wide range of environmental conditions, low temperatures, drought, and barren soil. Therefore, this species is widely used in soil stabilization and landscaping (hedging, stratified planting, etc.). Specifically, our study sought to characterize whether and how elevated O₃ levels affected the growth and NSC allocation among different bamboo organs, including the buds.

We also investigated the plasticity of clonal growth forms in the sympodial rhizome bamboo *Indocalamus decorus* as an initial step toward understanding bamboo growth strategies in response to abiotic stress. The proportion and changes in rhizome ecotypes (leptomorph and pachymorph) and the spacer length of bamboo could provide insights into the plasticity of the responses of these species to different environmental stressors such as O₃ exposure. Therefore, our study aimed to: (1) investigate the growth response of *I. decorus* to O₃ stress, including aboveground and underground growth, and (2) examine the response of gas exchange/ photosynthesis and NSC allocation in different bamboo organs to elevated O₃ levels. In this context, we hypothesized that: (1) *I. decorus* might store and transfer carbohydrates from the well-developed underground root system or mature leaves prior to new organs such as buds under elevated O₃ conditions; (2) bamboo produce more buds and longer rhizomes under elevated O₃ conditions to increase the odds of effectively escaping from adverse environments and reaching more favorable conditions.

2. Materials and methods

2.1. Experimental site and plant material

The experimental site was located at Chang Ping district, a suburb of Beijing, China (40°12'N, 116°80'E). The annual mean precipitation of the experimental site was 550.3 mm and the mean air temperature was 11.8 °C. Beijing has a typical temperate and monsoonal climate with four clearly distinct seasons. The soil in the experimental site is generally characterized as moist with moderate fertility, and therefore provided the required conditions to promote the growth of *I. decorus*.

Rhizome cuttings (8 to 10 cm) of *I. decorus* with uniform color, bud numbers, and size were obtained from current-year new rhizomes, all of which grew from similar five-year-old mother culms in March 2017 from a nursery in Nanjing. Afterward, new cuttings were re-transplanted in the original soil for 2 years. Next, 100 uniform bamboo clones with abundant original soil were selected and sent to Beijing in January 2019. All two-year-old bamboo clones were planted in 100 pots (top diameter: 27.5 cm, base diameter: 18.5 cm, height: 21 cm) with 5 kg of soil, then randomly distributed on the ground of 9 open-top chambers (OTCs) after acclimating the environment within the OTCs from January 15th to May 29th. The soil used in the pots was the local soil, which was generally characterized as moist with moderate fertility with an organic content of 16.4 g kg⁻¹, a total nitrogen content of 0.9 g kg⁻¹, an available phosphorus content of 102.1 mg kg⁻¹, and a pH of 8.3. All bamboo clones were watered as needed and fertilized once with 4 g of specialized carbamide and potassium sulfate fertilizer (N14:P14:K14, Wuhan Greenovo Biotechnology Co., Ltd., China) prior to the experiment.

2.2. OTC system

The OTCs were made of an aluminum alloy frame covered with transparent plastic film with a 10-mm thickness (90% transparency). The OTCs were 2.5 m in height and 2 m in diameter, with a ground area of 3.14 m², and were 2 m apart from each other. To prevent rain from intruding into the chamber while still allowing the ambient air to circulate freely, a rain cap was constructed and placed 25 cm above the frustum yet. The average air temperature inside and outside the chamber was no more than 2 °C from 8:30 to 17:30. More details of OTC design, operation, O₃

concentration control, and distribution control can be found in Li et al. (2015). O₃ concentrations were evenly distributed, both vertically and horizontally, within the OTCs. The Model 49i O₃ analyzer (Thermo Scientific, MA, USA) was calibrated every month with a Model 49i-PS O₃ analyzer (Thermo Scientific, MA, USA).

2.3. Experimental design and sampling

Three O₃ treatments were randomly assigned to nine OTCs. The O₃ concentrations evaluated herein were based on a preliminary study with lower O₃ concentrations (the same species but not the same plants we used in this experiment). AA + 40 and AA + 80 were monitored for one whole growing season but we identified no foliar injury or any changes in net photosynthesis rates and other indexes such as MDA or pigments. Due to the increasing O₃ concentrations in Beijing (Wang et al., 2012; BMEPB, 2015; Li et al., 2020; Li et al., 2021) and the O₃ tolerance of the bamboo employed in this study, the O₃ concentrations in the present study were selected to match the levels in ambient air (AA), in addition to ambient air + 70 ppb O₃ (AA + 70) and ambient air + 140 ppb O₃ (AA + 140). O₃ exposure started on June 1st and ended on October 12nd. O₃ was applied through fumigation from 8:30 to 17:30 (9 h) each day.

The average ambient air (AA) O₃ concentration in the experimental sites was 47.7 ppb, ranging from 21.3 to 80.9 ppb throughout the day during the experimental periods (Fig. 1). The average O₃ concentrations of AA + 70 and AA + 140 were 123.5 and 176.6 ppb (Fig. 1), respectively, meaning that the O₃ control could meet the target requirements. O₃ was applied daily except for rainy days, for a total of 87 days of O₃ exposure during the experimental period (Fig. 1). AOT40 was calculated as the accumulated hourly mean O₃ concentrations over 40 ppb during the O₃ fumigation period (Karenlampi and Skarby, 1996). At the end of the experiment, the AOT40 values of AA, AA + 70, and AA + 140 were 6.87, 50.36, and 82.34 ppm.h, respectively (Supplemental Fig. 2).

Approximately 10 *I. decorus* bamboo clones were placed into each chamber. The bamboo clones with the same treatments were interchanged to different positions of each chamber or different chambers with the same treatments every other week. Four uniform *I. decorus* bamboo clones within the same chamber were marked as sampling bamboo for biomass and NSC measurements. NSC and biomass samples were obtained at the end of the experiment. Growth measurements were conducted using the remaining bamboo clones.

2.4. Gas exchange measurement

Leaf gas exchange was measured using a portable Li-Cor 6400 photosynthesis system with a standard leaf chamber (LCF) (Li-Cor Inc., Lincoln NE, USA). Three pots of uniform bamboo clones were selected randomly from 10 pots per chamber. On each selected clone, two fully expanded, current-year new (expanded around June) and old leaves (expanded

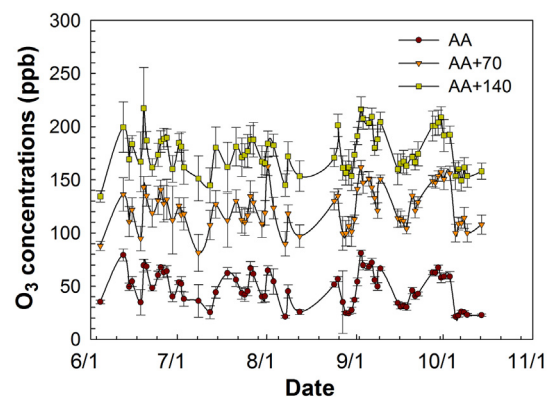


Fig. 1. Mean O₃ concentrations of three treatments during the experiment. Data are shown as mean ± SE (N = 3).

around March) undamaged by pests or insects were marked to conduct gas exchange measurements. The gas exchange measurement began at approximately 9:00–11:00 am on September 10th of 2019. The mean air temperature on the day of measurement was 27.43 °C.

2.5. Growth and biomass

The numbers of culms from leptomorph (monopodial) rhizomes, numbers of culms from pachymorph (sympodial) rhizomes, leaf number, rhizome number, total rhizome length, rhizome bud number, and the mean distance between ramets were assessed after harvesting at the end of the experiment (10th October 2019). All harvested samples were divided into two groups. One group was used for determining the growth index mentioned above, and the other group was used for biomass and NSC measurements after oven-drying. Roots were extracted and carefully collected after high-pressure water flushing in a basin with a filter. Biomass (separated as new rhizomes, stems, roots, buds, and leaves) was determined by drying up at 70 °C to a constant weight prior to analysis.

2.6. NSC contents

NSC contents (soluble sugar content + starch content) were determined as described by Zhou et al. (2009) and Wang et al. (1993) with slight modifications. First, 0.05 g of the dried plant tissue samples were accurately weighed and ground in a mortar with liquid nitrogen to determine the soluble sugar contents. The sample was then washed with 5 ml of distilled water, then transferred to a 10 ml centrifuge tube and soaked in boiling water for 30 min. Next, the sample was centrifuged at 4000 rpm for 10 min after it cooled down, after which the supernatants were collected. This procedure was repeated twice and the supernatants were combined to obtain a total volume of 50 ml. Afterward, 1 ml of this extract was thoroughly mixed with 1 ml distilled water and 5 ml of anthrone reagent (0.1 g C₁₄H₁₀O dissolved in 100 ml of 72% sulfuric acid). The mixture was shaken, soaked in boiling water for 10 min, and placed in a water bath to cool to room temperature. Finally, a UV–visible spectrophotometer was used to determine the sample absorbance at 620 nm. The same steps were repeated to create a glucose standard curve, after which the soluble sugar content (mg·g⁻¹) in the plant tissues was calculated according to the standard curve. For starch content determination, 7.5 ml of 30% HClO₄ was added to the residue that was left over after the extraction of soluble sugar. The mixture was allowed to sit overnight, placed in a water bath at 80 °C for 10 min to extract the starch fraction, and centrifuged at 4000 rpm for 10 min after cooling down. The supernatant was then transferred to a 50 ml volumetric flask to ensure that the volume was constant. Starch content (mg·g⁻¹) was determined as described by Grantz and Yang (2000).

2.7. Statistical analyses

The effects of O₃ on all parameters were examined by one-way ANOVA. Post-hoc comparisons were conducted using the least significant difference (LSD) test. Prior to analysis, all data were checked for normality (Kolmogorov-Smirnov test) and homogeneity of variance (Levene's test). If the data were not normally distributed and/or their variance was not homogeneous, non-parametric tests were used. Results were considered significant at $p \leq .05$. All analyses were performed using the SPSS statistics software (Version 17.0, SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Gas exchange

Our findings demonstrated that elevated O₃ levels significantly decreased the gas exchange capacity of *I. decorus* in both old and new leaves. Old and new leaves exhibited significant differences in net photosynthetic rate (Pn) and stomatal conductance (Gs) among the three tested treatments.

The Pn values of the new leaves were 2.1, 1.7, and 2.1 times higher than those of old leaves, whereas the Gs values of the new leaves were 1.6, 1.7, and 1.9 times higher than those of the old leaves. The Pn and Gs values of the old leaves under the AA + 140 treatment were 34.9% and 32.4% lower than the control, respectively (Fig. 2). In contrast, the AA + 70 and AA treatments had no significant differences in Pn and Gs after almost 5 months of fumigation (Fig. 2). Furthermore, the Pn values of the new leaves in the AA + 70 and AA + 140 groups were 17.3% and 34.5% lower than those of the control (AA), respectively (Fig. 2). The Gs values of the new leaves were not affected by the elevated O₃ levels.

3.2. Growth

Our findings indicated that the leaf number, the number of culms from pachymorph (sympodial) rhizomes (NSPR), total rhizome number, total bud number, and total rhizome length decreased significantly under elevated O₃ conditions. The leaf number decreased by 21.6% and 25.7% in AA + 70 and AA + 140 compared with AA, respectively (Fig. 3). The bamboo species evaluated herein exhibits both leptomorph and pachymorph rhizome ecotypes. However, our results indicated that only the culm numbers of pachymorph (sympodial) rhizomes (NSPR) decreased by 46.1% and 43.1% in AA + 70 and AA + 140, respectively, whereas the number of culms from leptomorph (monopodial) rhizomes (NSLR) remained largely unaffected in response to O₃ exposure. The total rhizome number, total bud number, and total rhizome length decreased by 43.4%, 34.1%, and 21.2% under AA + 70, and by 59.9%, 41.5%, and 41.4% under AA + 140, respectively. However, elevated O₃ levels increased the mean distance between ramets by 49.4% and 86.5% in AA + 70 and AA + 140, respectively

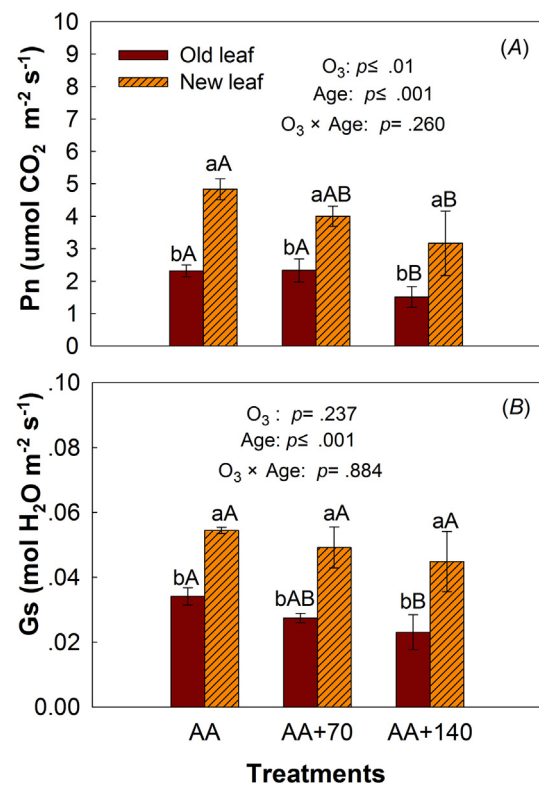


Fig. 2. Effects of elevated O₃ and leaf age on the (A) net photosynthesis rate (Pn) and (B) stomatal conductance (Gs) of *Indocalamus decorus*. O₃: O₃ effects; Age: Age effects; O₃ × Age: interaction of O₃ and age. Different lowercase letters above the bars indicate significant differences between leaf ages in the same O₃ treatments when $p \leq .05$. Different uppercase letters above the bars indicate significant multiple comparisons results of the same age leaf among the three treatments when $p \leq .05$. Data are shown as mean ± SE (N = 3).

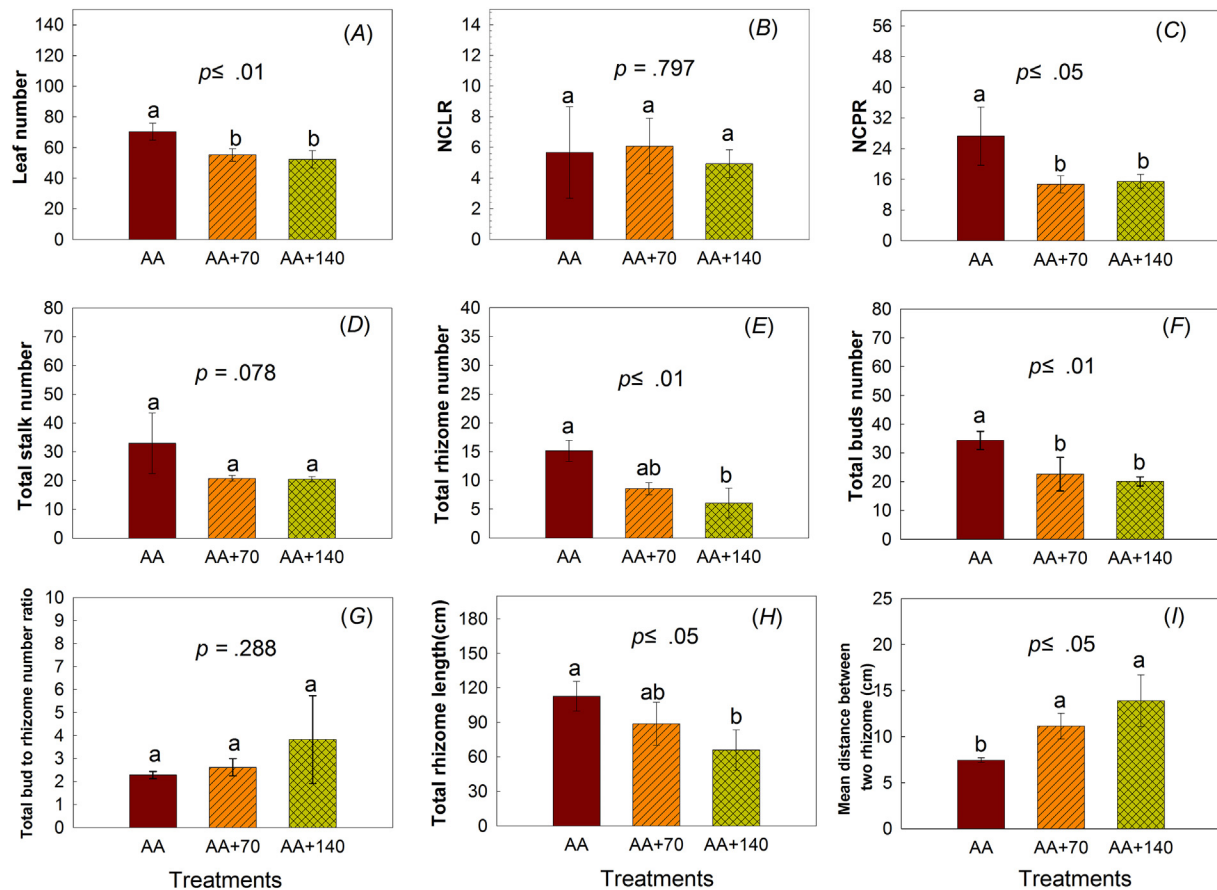


Fig. 3. Effects of elevated O_3 concentrations on the growth characteristics of *Indocalamus decorus*. Different letters after the numbers indicate significant multiple comparison results among the three O_3 treatments ($p \leq .05$). (A): leaf number; (B) NCLR: numbers of culms from leptomorph (monopodial) rhizome; (C): NCPR: number of culms from pachymorph (sympodial) rhizome; (D): total culm number; (E): total rhizome number; (F): total bud number; (G): total bud to total rhizome number ratio; (H): total rhizome length; (I): mean distance between two ramets. Data are shown as mean \pm SE ($N = 3$).

(Fig. 3). AA + 70 and AA + 140 exhibited no significant differences in any of the aforementioned parameters.

3.3. Biomass

The elevated O_3 treatments significantly reduced the total biomass (Fig. 4F). The reduction percentages of the total biomass were 27.1% in AA + 70 and 43.7% in AA + 140, which was mainly attributed to a reduction in the biomass of new rhizomes (Fig. 4A), rhizome roots (Fig. 4C), and leaves (Fig. 4E). New rhizomes exhibited the most severe reduction in biomass, reaching 35.9% in AA + 70 and 57.2% in AA + 140 (Fig. 4A). However, elevated O_3 had no significant effects on stem biomass (Fig. 4B), rhizome bud biomass (Fig. 4D), and underground to aboveground biomass ratio (Fig. 4H). Moreover, no significant differences in biomass were observed in the AA + 70 and AA + 140 treatment groups (Fig. 4). In contrast, elevated O_3 levels significantly increased the individual bud biomass and rhizome bud to rhizome biomass ratio. Specifically, the individual bud biomass increased by 50% and 75% in the AA + 70 and AA + 140 treatments compared with AA, respectively (Fig. 4G), whereas the rhizome bud to rhizome biomass ratio increased 0.86 and 1.92 times in AA + 70 and AA + 140, respectively (Fig. 4I).

3.4. NSC contents, amounts, and allocation proportions in all organs

3.4.1. NSC contents

O_3 fumigation had no effects on the soluble sugar contents of new rhizomes, stems, rhizome roots, and leaves. O_3 fumigation significantly increased the soluble sugar contents in rhizome buds by 40.3% in AA + 70

and 38.1% in AA + 140, and no significant differences were identified between these treatments (Fig. 5A). Moreover, O_3 fumigation significantly reduced the starch contents in the new rhizomes by 28.4% in AA + 140 (Fig. 5B).

Regarding NSC contents, the rhizome buds exhibited higher NSC contents, whereas new rhizomes and leaves had lower contents. The NSC contents of the rhizome buds increased by 36.8% in AA + 70 and 45.0% in AA + 140. However, new rhizomes in the AA + 140 group exhibited a significant reduction of 21.3%. The NSCs in leaves were significantly decreased by 14.1% in AA + 70 and 15.4% in AA + 140 (Fig. 5C). No differences in soluble sugar, starch, or NSC contents were found between AA + 70 and AA + 140.

3.4.2. NSC amounts

Our findings indicated that soluble sugar and starch were most accumulated in the leaves and NR. Elevated O_3 concentrations decreased the amounts of soluble sugar in the leaf by 14.3% in AA + 70 and 28.0% in AA + 140 (Fig. 6). The starch and total NSC amounts exhibited consistent decline trends in new rhizomes, leaves, and rhizome roots under the O_3 treatments. New rhizomes exhibited the most severe reduction (31.2% in starch and 24.7% in NSCs in AA + 140) (Fig. 6). The total amounts of soluble sugar, starch, and NSCs of plants decreased by 23.1%, 32.6%, and 31.5% in AA + 70, and 47.1%, 54.6%, and 52.5% in AA + 140.

3.4.3. NSC allocation proportions

Elevated O_3 concentrations also induced opposite NSC allocation proportion trends in rhizome and rhizome buds. Specifically, new rhizomes exhibited a significant decrease, whereas rhizome buds exhibited a significant

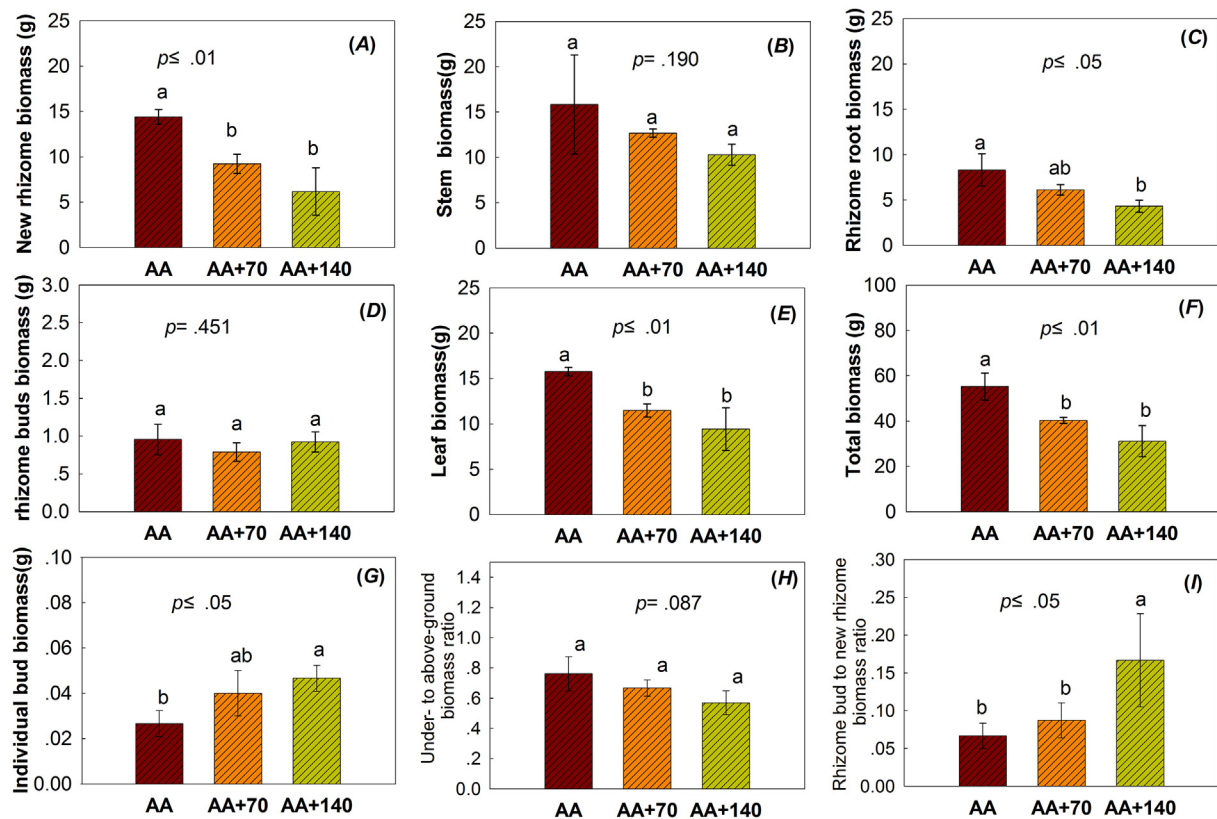


Fig. 4. Biomass and ratios of different structures of *Indocalamus decorus* under three O₃ treatments. (A): New rhizome biomass; (B): stem biomass; (C): rhizome root biomass; (D) rhizome bud biomass. (E): leaf biomass; (F): total biomass; (G): individual bud biomass; (H): underground to aboveground biomass ratio; (I): rhizome bud to new rhizome biomass ratio. Different letters after the numbers indicate significant multiple comparison results among the three O₃ concentration treatments ($p \leq .05$). Data are shown as mean \pm SE ($N = 3$).

adverse increase in soluble sugar, starch, and NSC proportions (Fig. 7). The soluble sugar proportion of rhizome buds was 1.2% in AA, and then increased to 1.9% in AA + 70, and 3.3% in AA + 140 (Fig. 7A). The starch amount proportion of NR was 48.2% in AA, then decreased to 41.5% in AA + 70, followed by 31.2% in AA + 140. The starch amount proportion of rhizome buds increased by 62.3% in AA + 70 and 232.2% in AA + 140 (Fig. 7B). The percentages of NSCs exhibited a similar trend to that of starch (Fig. 7C).

4. Discussion

O₃-induced reductions in Pn and biomass have been widely reported in trees (Li et al., 2015), crops (Wang et al., 2007; Zhang et al., 2020), and grasses (Dolker and Agrawal, 2019). However, very few studies have characterized the effects of O₃ on bamboo. Our study identified Pn reductions of 34.9% in AA + 140 (around 190 ppb) and 27% in AA + 70 (about 120 ppb) (Fig. 1), which were lower than most species from a meta-analysis of Zhang et al. (2021) (−31.6% at 101 ppb O₃ on average) considering the higher O₃ dose in our experiment. According to another meta-analysis published by Li et al. (2017), the tolerance of *Indocalamus decorus* to O₃ was comparable with that of most evergreen broad-leaved tree species (30% reductions at 158 ppb) but higher than most deciduous broad and needle leaf tree species (27% reductions at 79 ppb and 36% for deciduous broad-leaves at 94 ppb). This relatively high O₃ tolerance could be partly attributed to the low stomatal conductance of *I. decorus*, which limits O₃ entry (Fig. 2). Furthermore, *I. decorus* also has a highly developed mixed type underground rhizome system, which confers notable advantages to clonal plants (McClure, 1966). The rhizome serves as both an asexual reproductive organ and a storage pool, buffer, and water/nutrient pipeline, thus allowing plants to resist adverse environmental conditions (Fig. 4; Fig. 5).

In our experiment, the most severe O₃-induced biomass reduction was observed in the rhizome (Fig. 4). The decline in the rhizome biomass was largely due to a decrease in carbohydrate assimilation associated with a lower photosynthetic rate. Moreover, the rhizome may transfer NSCs to the aboveground structures for compensation, as well as to the buds to meet the nutrient demands of the next generation. The U/A ratio (underground to aboveground biomass ratio) (0.57) of *I. decorus* was higher than the root to shoot ratio of sub-tropical and temperate vegetation, which is approximately 0.23–0.56 (IPCC, 2019; Mokany et al., 2006).

Upon exclusively comparing the above-ground biomass reduction of *I. decorus* with that of other plant species such as Shantung maple (Li et al., 2015), we concluded that O₃ had relatively slight impacts on *I. decorus* without considering the severe reduction in the underground part. Therefore, the underground structures of *I. decorus* must also be considered to avoid underestimating the reductions induced by elevated O₃ levels. Starch storage in the root system is a sensitive indicator of carbohydrate reserves in a tree (Ziegler, 1991a, 1991b; Lehtila, 2000; Liu et al., 2007). Our results indicated that the starch contents in the rhizome decreased significantly under elevated O₃ conditions (Figs. 4, 6), which was consistent with previous studies in spruce (Braun et al., 2004) and aspen (Coleman et al., 1995). Moreover, although the rhizome roots in this study were extracted and collected very carefully after high-pressure water flushing in a basin with a filter, there were still small inevitable losses of the finest roots, which could result in an underestimation of the NSCs in the roots.

Clonal organs (rhizomes) can store carbohydrates and produce meristems (buds). Bud number and bud biomass are among the key traits of clonal plants, indicating the potential to produce offspring ramets (Gao et al., 2008). We hypothesized that more buds might be produced after O₃ fumigation, as reported in several studies that evaluated the responses

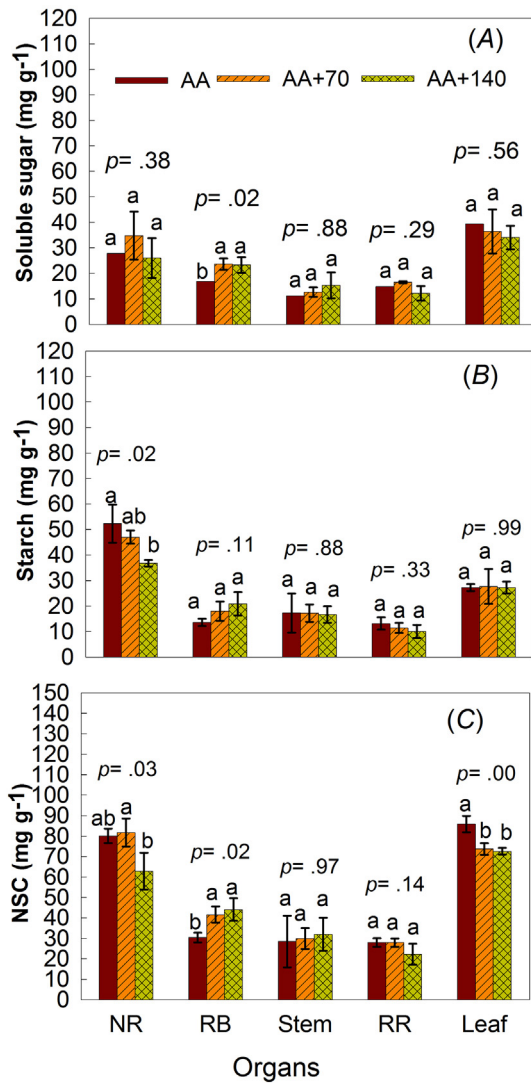


Fig. 5. Effects of elevated O₃ levels on the soluble sugar (A), starch (B), and nonstructural carbohydrate (NSC, C) contents of all organs of *Indocalamus decorus*. The uppercase letters above the bars indicate the multiple comparison results of the same treatment among different plant parts. The lowercase letters above the bars indicate the multiple comparison results of the same plant organ among the three O₃ treatments when the O₃ effects were significant (p ≤ .05). NR: New rhizome; RB: Rhizome bud; RR: Rhizome root; TB: Total biomass. Data are shown as mean ± SE (N = 3).

of rhizomatous clonal plants to soil water status (Wang et al., 2008), herbivory (Gao et al., 2013), sand burial (Chen et al., 2010), and nutrient patches (Gao et al., 2013) to increase asexual reproduction. However, our findings indicated that although the total bud number was decreased due to low photosynthesis (Fig. 2) and severe biomass reduction (Fig. 4), the soluble sugar and NSC contents in buds, as well as the bud biomass to total rhizome biomass ratio and the individual bud biomass increased significantly in response to elevated O₃ levels (Fig. 3, Fig. 4, Fig. 5). These findings demonstrate that more energy was still allocated to the buds through the rhizome when the whole bamboo plant was fumigated with high O₃ concentrations. This increased energy allocation to bud development is likely an adaptation to increase renewal rates, enhance survival rates and growth potential, or escape from adverse environments in the next growing season (Wang et al., 2008; Hutchings, 2010; Gao et al., 2013; Agathokleous et al., 2021). Moreover, our findings suggested that the bamboo plants responded to O₃ fumigation by forming relatively larger and stronger buds with higher survival rates instead of increasing their numbers. This result was demonstrated by an increase in the individual bud biomass and rhizome bud to new rhizome biomass ratio, coupled with a largely unchanged total bud to rhizome number ratio and a decrease in the total bud number (Fig. 4G, I, 3G, F). Agathokleous et al. (2021) also demonstrated that moderate O₃ concentrations (approximately 60 ppb) increased the number of buds per unit of biomass of hybrid larch (*Larix gmelinii* var. *japonica* × *L. kaempferi*) saplings over three growing seasons. This indicated that the physiological mechanisms of the plastic responses of bud formation might be conserved among plants with different functional characteristics.

C storage mainly occurs in the form of sugars and starch, which make up the NSC fraction of the plant. Starch is stable and better suited for energy storage, whereas soluble sugar acts as a direct energy supply (Dietze et al., 2014). Given that the starch contents in the rhizome decreased significantly in the elevated O₃ treatments but both the starch contents and soluble sugar in rhizome buds increased, the high contents of soluble sugar inside the buds should have resulted from rhizome starch hydrolysis, as previously observed in the needles of Wengernalp trees (Magell et al., 1994). Very few reports have focused on the effect of O₃ on NSC allocation in bamboo buds. However, related studies have been conducted on other species. Lux et al. (1997) found that the fructose and pinitol (the main constituents of soluble sugar) levels in the buds of spruce trees (*Picea abies*) grown in ambient O₃ were significantly higher than those of their counterparts grown in filtered air. Similar results were also found in American pondweed (*Potamogeton nodosus* Poiret) winter buds, which exhibited soluble sugar concentrations that have been associated with tuber and winter bud sprouting (Spencer et al., 2001). Therefore, preserving the bud biomass (Fig. 4) while increasing the NSC contents in response to O₃ fumigation appears to be a good trade-off to ensure the survival of the next generation, as an inhibition in photosynthesis results in whole biomass reduction. The rhizome is the clonal organ of bamboo, which has considerable storage capability. The increased NSC contents in the buds indicate that the storage

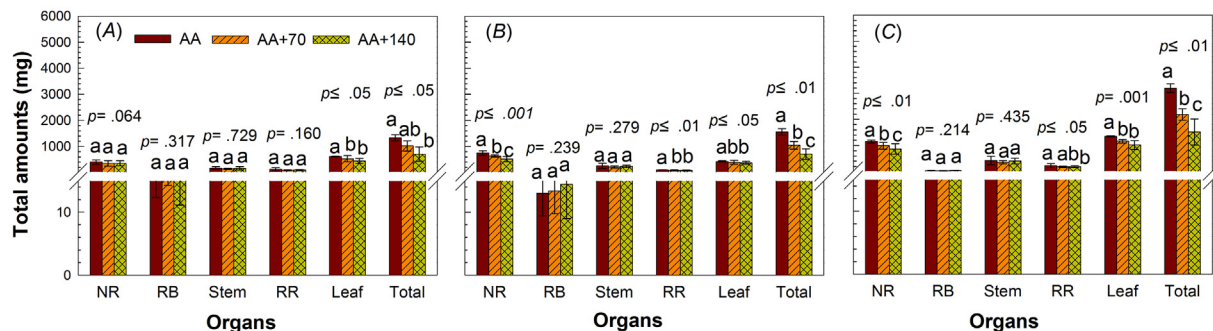


Fig. 6. Total amount of soluble sugar (A), starch (B), and NSCs (C) in all organs of *Indocalamus decorus*. The lowercase letters in the graph indicate the multiple comparison results of the same plant organ among three O₃ treatments when the O₃ effect was significant (p ≤ .05). NR: New rhizome; RB: Rhizome bud; RR: Rhizome root; Total: Total biomass. Data are shown as mean ± SE (N = 3).

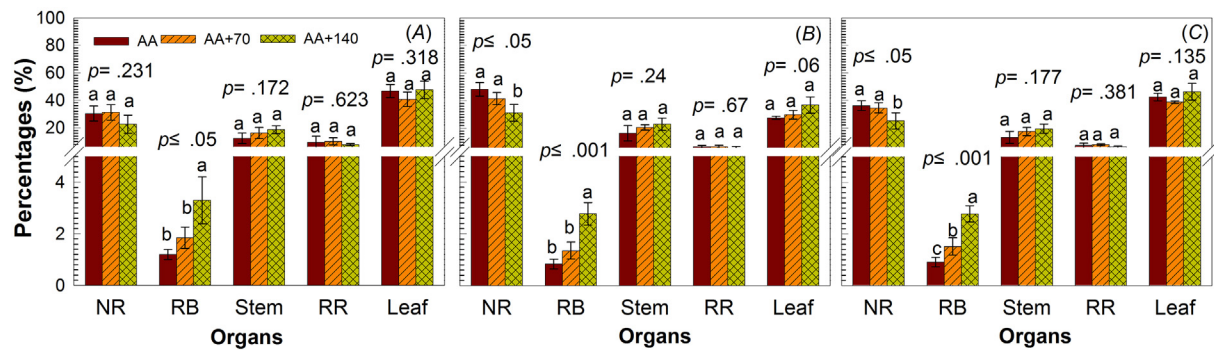


Fig. 7. Percentages of soluble sugar (A), starch (B), and nonstructural carbohydrates (NSCs, C) in five organs of *Indocalamus decorus* under three O₃ treatments. The lowercase letters indicate the multiple comparison results among three O₃ treatments when the O₃ effect was significant ($p \leq .05$). NR: New rhizome; RB: Rhizome buds; RR: Rhizome root. Data are shown as mean \pm SE (N = 3).

organ offers quick compensation to buds as a priority when under local damage (de Kroon and van Groenendael, 1997; Dong et al., 2010). Other studies have also reported that the high amounts of soluble sugars could have other functions in addition to energy storage and transport, such as radical scavenging (Malhotra and Khan, 1984). Therefore O₃ tolerance might be improved in new shoots of *I. decorus*, which will grow from new buds. In general, the optimal allocation theory found in many ecological models involving plant biomass allocation, in which biomass is preferentially allocated to the plant part obtaining the resource that is essential but limiting for growth (Poorter and Nagel, 2000; Shipley and Meziane, 2002), appears to be a reasonable explanation of the biomass allocation strategies of clonal plants suggested by our results.

Additionally, our study identified no significant decline in starch or soluble sugar contents in the leaves of *I. decorus* (Fig. 5), which was consistent with previous findings (*Fagus sylvatica* in Lux et al., 1997; *Gossypium barbadense* in Grantz and Yang, 2000; *Taxus wallichiana* in Cao et al., 2017; *Phoebe zhennan* in Chen et al., 2018). In contrast, Neufeld et al. (2012) reported an increase in starch amounts in tolerant cutleaf coneflower (*Rudbeckia laciniata* L.), whereas Chen et al. (2018) found that elevated O₃ levels increased the NSC contents in low O₃ treatments in *Phoebe bournei* but no change in high O₃ treatment. These observations suggest that the response of leaf nonstructural carbohydrates to O₃ was very species-specific and depended on the O₃ concentrations, fumigation duration, and developmental stage. We hypothesized that the fact that elevated O₃ levels did not affect the starch and soluble sugar contents of leaves might be a specific strategy of *Indocalamus decorus* to maintain leaf survival similar to other evergreen species under stress. Based on the findings of Chen et al. (2018), we assumed that the O₃ dose accumulation (AOT40) in our experiment might have exceeded a certain threshold (Supplemental Fig. 2), which constrained C acquisition and resulted in a decrease in energy reserves for growth or repair as a protection mechanism in leaves. Therefore, the leaves lost their role as a functional energy reserve to increase soluble sugar for injury repair. Our results suggested that *I. decorus* transferred carbohydrates from its underground structures to other organs, either under stress or directly damaged, to ensure survival.

The bamboo culm is a one-time structure formed by the current-year bamboo shoot during the springtime. The stem biomass had no response to elevated O₃ because when the O₃ fumigation started, the new bamboo shoot had already sprouted, and the bamboo stem was almost formed by then. Interestingly, different from our second hypothesis, we observed an increase in the mean rhizome distance between two ramets and not in the total length of the rhizome, which may also be a strategy for decreasing resource competition in adverse O₃ environments and increasing the opportunity to escape from adverse environments and reach more favorable conditions (Wang et al., 2008). Increased rhizome spacing may also provide the opportunity for other more O₃-tolerant competitors to grow interspersed with the bamboo, as more light would penetrate between the culms. However, the greater spacing would allow O₃ to penetrate more

thoroughly through the canopy, which may have exacerbated the adverse effects of O₃ to *I. decorus* in our experiments.

The total length of the rhizome was decreased due to: 1) a significant decline in biomass. The O₃ accumulated dose may exceed the threshold for a significant biomass reduction in the rhizome, thus limiting the elongation growth; 2) the pot limitation. Although the pots used in our experiments were as large as possible, the underground system may also be restricted to some extent. Moreover, the culm numbers from pachymorph (sympodial) rhizomes decreased (Fig. 3) under elevated O₃, which may also be a strategy to integrate environmental resources for survival.

The above-described NSC allocation (carbon metabolism) patterns (i.e., prioritizing the growth of the subsequent generation) are mainly the result of the physiological integration and foraging behavior of clonal plants. However, the plasticity of the clonal integration response to air pollution in a natural environment is likely more complex than the results from the OTCs experiments. Moreover, the physiological responses to multiple stressors, especially the interaction with other environmental factors and the carry-over effects of the next generation, also need to be evaluated in field conditions in the future.

5. Conclusions

Bamboo is a uniquely lignified evergreen perennial grass species. However, very few studies have evaluated the adverse effects of O₃ pollution on these plants. Our study provides quantitative insights into the effects of O₃ on gas exchange, growth, biomass, and nonstructural carbohydrate allocation in all organs of the native dwarf bamboo *I. decorus*. Our results indicated that *I. decorus* has a relatively high O₃ tolerance capability. Bamboo has a well-developed below-ground rhizome-root system, and therefore the biomass reduction induced by O₃ would be seriously underestimated if not considering the underground part. The rhizomes work as a buffer and nutrient pool to ensure that the current plant components obtain essential and limited resources for growth and reproduction. The rhizomes also allow plants to escape adverse conditions and avoid resource competition by changing the mean rhizome distance between ramets.

CRedit authorship contribution statement

LL and WXK jointly designed the experiment. LL and WXK provided financial support. LJJ and LL ran the experiment, collected and analyzed data. LL drafted the manuscript. WXK, WWF, FL, LX and WC assisted in manuscript revision.

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Declaration of competing interest

I declare on behalf of my co-authors that the work described was original research that has not been published previously, and not under consideration for publication elsewhere, in whole or in part. No conflict of interest exists in the submission of this manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2022.154096>.

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