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Composition of Organic Carbon-Based Compounds in the Stem Wood of *Quercus mongolica* Seedlings Grown Under Elevated CO_2 and/or O_3 Concentrations

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In this study, we examined the composition of organic constituents of stem woody tissue together with tree growth in *Quercus mongolica* var. *grosseserrata* Blume seedlings raised under controlled CO_2 and/or O_3 concentrations in a Free-Air Concentration Enrichment system. After exposure to ambient air (control), elevated CO_2 concentration (550 μmol mol^{-1} CO_2), elevated O_3 concentration (double that of the control), and a combination of elevated CO_2 and O_3 concentrations during a growing season, we measured the diameter and length of stem, and biomass of sampled seedlings and quantified the lignin, extractive, and holocellulose contents of the woody tissue of current-year stems. We confirmed that the growth of seedlings was enhanced under an elevated CO_2 concentration condition. In line with this, the extractive content was lower in woody tissue formed under an elevated CO_2 concentration than that formed under ambient air, whereas holocellulose content showed an inverse pattern. Elevated O_3 concentration itself did not change the organic constituents of the woody tissue, but it reduced the influence of an elevated CO_2 concentration. We thus assume that *Q. mongolica* formed woody tissue with a low extractive content under the high CO_2 concentration condition, although this response was possibly mitigated by an elevated O_3 concentration. Extractives contains antimicrobial components such as tannins, flavonoids, quinones, and terpenoids. The decrease in extractives within the widely distributed *Q. mongolica* in East Asia may have a non-negligible impact on C cycling in the future earth with high atmospheric CO_2 concentration.

Keywords: carbon dioxide, ozone, holocellulose, lignin, solvent extractive, stem wood, organic constituent

INTRODUCTION

Emissions of atmospheric carbon dioxide (CO_2) and tropospheric ozone (O_3) have been increasing because of anthropogenic activities (e.g., IPCC, 2013; Ainsworth et al., 2020; Takahashi et al., 2020). Elevated atmospheric CO_2 and O_3 concentrations can influence forest productivity (Agathokleous et al., 2020; Ainsworth et al., 2020), and further impacts on carbon (C) cycling in forest ecosystems

have been investigated. Many studies have demonstrated that elevated CO_2 concentrations enhance the rate of photosynthesis and the biomass growth of trees (e.g., Zak et al., 2011; Walker et al., 2019), although photosynthetic downregulation has also been observed with long-term exposure to elevated CO_2 concentrations (e.g., Norby et al., 2010; Sigurdsson et al., 2013). As for elevated O_3 concentrations, previous research has reported that it leads to a reduction of the rate of photosynthesis, acceleration of leaf senescence, increases in dark respiration, and changes in C allocation for seedlings and/or adult trees (e.g., Matyssek et al., 2010; Watanabe et al., 2013, 2018; Kitao et al., 2015, 2021; Yamaguchi et al., 2019).

Elevated CO_2 and/or O_3 concentrations may influence C cycling *via* the decomposition of organic matter. Organic matter is decomposed *via* a process of leaching, fragmentation, and chemical alteration. The chemical alteration is regulated by not only decomposer and environmental factors but also organic matter quality, which is partly determined by the composition of organic carbon-based compounds (Chapin et al., 2011). Elevated CO_2 and O_3 concentrations change the composition of the organic constituents of tree leaves (e.g., Oksanen et al., 2005; Chen et al., 2015) and leaf litter (e.g., Kasurinen et al., 2006; Parsons et al., 2008). Additional research has reported that leaf and leaf litter changes influence decomposition rates (e.g., Kasurinen et al., 2006, 2017; Parsons et al., 2008). This effect may also be present in other tissues. Organic composition changes under elevated CO_2 and/or O_3 concentrations have also been detected in woody tissue (Kaakinen et al., 2004; Mattson et al., 2005; Luo et al., 2008; Luo and Polle, 2009; Richet et al., 2012), which constitutes a large C pool in the form of woody debris and dead wood in forest ecosystems (Aalde et al., 2006). Thus, studying the organic composition of woody tissues formed under elevated CO_2 and/or O_3 concentrations provides important insight into understanding their impact on C cycling in forest ecosystems.

In terms of decomposition, the organic constituents of woody tissue can be divided into lignin, extractives, and holocellulose; among these constituents, lignin and extractives are strongly related to organic decomposition (Taylor et al., 2002; Oliveira et al., 2010) as determinants of organic matter quality (Chapin et al., 2011). Lignin is a structural component, constituting 18–35% of woody tissue (Pettersen, 1984). The lignin content of woody tissue formed under elevated CO_2 concentrations increases in hybrid poplars, *Populus* × *euramericana* and *Populus tremula* × *alba* (Luo and Polle, 2009; Richet et al., 2012), but does not change in other tree species (Luo et al., 2008). Similarly, elevated O_3 concentrations increase the lignin content in some tree species (Kaakinen et al., 2004; Richet et al., 2011, 2012). Extractives are non-structural components consisting of various compounds, such as tannins, flavonoids, quinones, and terpenoids, which are related to the durability of wood against fungi and termites (Taylor et al., 2006; Oliveira et al., 2010; Santana et al., 2010). Under elevated CO_2 concentrations, extractive contents have been shown to either not change (Kaakinen et al., 2004) or increase in *Betula pendula* (Kostiainen et al., 2006, 2008); however, elevated O_3 concentrations have been shown to decrease the extractive content of *Populus tremuloides* (Kostiainen et al., 2008), have no effect on *Acer saccharum* (Kaakinen et al., 2004), and increase the extractive content of *Betula papyrifera* (Kostiainen et al., 2008). Holocellulose consists of structural components (i.e., cellulose and hemicellulose) and accounts for 65–75% of woody tissue (Pettersen, 1984). Elevated CO_2 concentrations decrease the cellulose and hemicellulose contents in *Betula* species (Kaakinen et al., 2004; Kostiainen et al., 2006), although smaller changes have been observed in other tree species (Kaakinen et al., 2004; Kostiainen et al., 2008). Elevated O_3 concentrations do not affect the contents of cellulose and hemicellulose (Kaakinen et al., 2004; Kostiainen et al., 2006, 2008). Thus, for some species, recalcitrant lignin, antimicrobial extractive such as tannins, flavonoids, quinones, and terpenoids (Valette et al., 2017; Broda, 2020), and holocellulose contents may change in response to elevated CO_2 and/or O_3 concentrations.

Quercus mongolica var. *crispula* is a representative deciduous broadleaf tree species in the temperate zone of East Asia (Menitsky, 2005). Thus, the response of *Q. mongolica* to elevated CO_2 and/or O_3 concentrations may have an impact on a wide range of the temperate forest in this area. In response to elevated CO_2 and/or O_3 concentrations, this species is tolerant to high O_3 concentrations (Yamaguchi et al., 2011) and changes the mineral composition of its leaves under elevated CO_2 and O_3 concentrations (Shi et al., 2016). However, the influence of elevated CO_2 and/or O_3 concentrations on the organic composition of its woody tissue remains unknown.

In this study, we aimed to clarify whether elevated CO_2 and/or O_3 concentrations induce a change in the composition of organic carbon-based compounds of *Q. mongolica* woody tissue. To achieve this objective, we evaluated the growth of seedlings and analyzed the organic constituents of the stem woody tissue of *Q. mongolica* seedlings grown over 180 days under controlled CO_2 and/or O_3 concentrations in Free-Air Concentration Enrichment (FACE) systems. In the FACE system of seedling scale, there is a threat that seedlings raised under elevated CO_2 concentration grow rapidly and exceed the height of the FACE system. On the other hand, the stem growth of seedlings was changed under elevated O_3 concentrations during one growing season for deciduous broadleaf tree species (Matsumura et al., 1998). Thus, we adopted one growing season as the experimental period of this study and used the current-year stem formed under treatments for analyzing the organic constituents.

MATERIALS AND METHODS

Plant Materials

This study used 2-year-old *Q. mongolica* seedlings. The ecological characteristics of *Q. mongolica* are well known: frequent sprout formation (Sakai and Sakai, 1998), moderate shade tolerance (Higo, 1994), occurrence in mid-successional stage forests (Higo, 1994), and flush and succeeding-type leaf emergence (Kikuzawa, 1983). On March 4, 2011, *Q. mongolica* seedlings were planted in the nursery (soil pH ≈5.5) of the Forestry and Forest Products Research Institute in Tsukuba, Japan (36°00′ N, 140°08′ E, 20 m a.s.l.). Seedlings were planted 50 cm from each other. The

contents of carbon, nitrogen, exchangeable calcium, magnesium, and potassium in soil of the nursery were 41.0 g kg^{-1}, 3.40 g kg^{-1}, 6.16 $cmol_c$ kg^{-1}, 0.80 $cmol_c$ kg^{-1}, and 0.39 $cmol_c$ kg^{-1}, respectively (Yamada et al., 2021). The annual mean air temperature at the nearest automated meteorological data acquisition site (36°03′ N, 140°08′ E, elevation 25 m) was 14.3°C and the annual precipitation was 1395 mm in 2011 (Japan Meteorological Agency, 2021). The daily mean air temperature and precipitation in the nursery during a growing season in 2011 was shown in **Figure 1**.

Experimental Design

We used the same experimental design and facility as those of Tobita et al. (2019), although the experimental period (1 growing season) was shorter than that of Tobita et al. (2019), who used much smaller seedlings (1-year-old) than those in the present study (2-year-old). *Q. mongolica* seedlings were grown in FACE systems. These seedlings were exposed to ambient air ([Control]), elevated CO_2 ([CO_2]; 550 μmol mol^{-1} CO_2), elevated O_3 ([O_3]; twice ambient O_3), or a combination of elevated CO_2 and O_3 ([CO_2 + O_3]; 550 μmol mol^{-1} CO_2 and twice ambient O_3), based on a simulation for 2060 projections (IPCC, 2007; **Figure 2**). Ambient O_3 concentration was monitored using an O_3 analyzer (Model EG-3000F, Ebara Jitsugyo Co., Ltd., Kanagawa, Japan), and elevated O_3 concentration was continuously regulated to 2× ambient O_3 concentration during daytime. We set 12 frames of the FACE system as three replicates of four treatments (i.e., [Control], [CO_2], [O_3], and [CO_2 + O_3]) and planted six seedlings in each frame, for a total of 72 seedlings.

Each frame of the FACE system was 2-m wide × 2.5-m high. Two transparent windscreens (Sky Coat 5 polyolefin film, C.I. Takiron Corp., Osaka, Japan) of 50-cm in length were installed into each frame at heights of 15 and 75 cm to facilitate the mixing of CO_2 and O_3. The windscreen is drip-proof and fog-proof film, but has no function of UV protection. CO_2 and O_3 gasses were injected with ambient air *via* polyethylene tubes connected to the frame at a height of 2 m at 20-cm intervals. Gaseous CO_2 and O_3 were generated from liquid CO_2 (Air Water Inc., Osaka, Japan) and an ozone generator (Model PZ2A, Kofloc, Kyoto, Japan). CO_2 and O_3 were supplied during the daytime from May 20 to November 16, 2011. The CO_2 and O_3 supplies were controlled using a proportional integral derivative control system with digital controllers (Model SDC35, Azbil Corp., Tokyo, Japan).

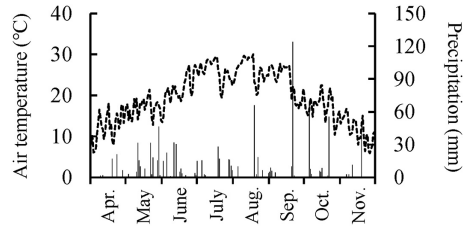

FIGURE 1 | Daily mean air temperature and precipitation in the experimental nursery from April 1st to November 30th, 2011.

On the basis of monitoring using an infrared CO_2 analyzer (Model LI-820, LI-COR, Inc., Lincoln, NE, United States), over the course of the treatments, the mean (± standard error) CO_2 concentrations during the daytime (7:00–17:00) were 378 ± 2.5 and 562 ± 16.9 ppm for the ambient and elevated CO_2 concentration conditions, respectively. According to measurements obtained using an O_3 analyzer (Model EG-3000F, Ebara Jitsugyo Co., Ltd., Kanagawa, Japan) and an O_3 monitor (Model 205, 2B Technologies, Boulder, CO, United States), during treatments, the mean (± standard error) O_3 concentrations during the daytime (7:00–17:00) were 36.1 ± 1.0 and 65.3 ± 2.0 ppb for the ambient and elevated O_3 concentration conditions, respectively.

Measurement of Aboveground Biomass

We measured the stem length and diameter at the base of the seedlings on April 14 (start of the experiment) and November 15 to 16 (end of the experiment) for all 72 seedlings. At the end of the experiment, we harvested the aboveground portion of these seedlings and divided them into leaves, stems, and other parts (i.e., branches and twigs). Furthermore, from September 5, 2011, to the end of the experiment, we collected fallen leaves every day. These samples were oven-dried at 70°C for >72 h using a hot air circulating oven (GT-120P, ALP Co., Ltd., Tokyo, Japan), and then weighed. The dry weights of leaves and fallen leaves were considered to be the leaf biomass; the dry weight of stems was considered to be the stem biomass; and the aboveground biomass was the sum of the dry weight of leaves, stems, and other parts.

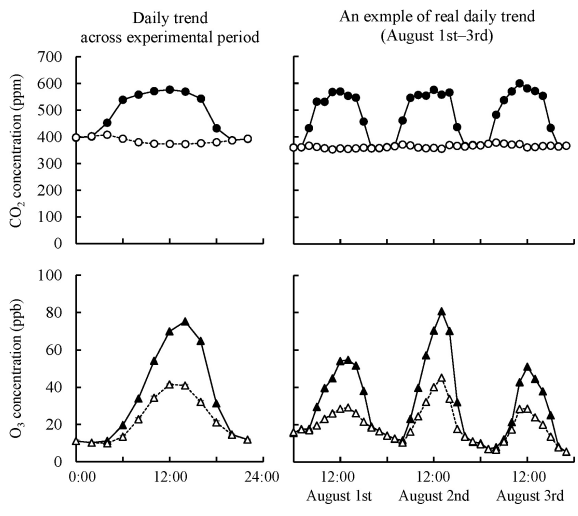

FIGURE 2 | Daily trend of CO_2 and O_3 concentrations in the chamber of the FACE system across experimental period and an example of real daily trend on August 1st–3rd, 2011. Open circle, ambient CO_2 as mean value of [Control] and [O_3] treatments; solid circle, elevated CO_2 as mean value of [CO_2] and [CO_2 + O_3] treatments; open triangle, ambient O_3 as mean value of [Control] and [CO_2] treatments; solid triangle, elevated O_3 as mean value of [O_3] and [CO_2 + O_3] treatments.

Measurement of Organic Constituents of Woody Tissue

We randomly selected three seedlings from each of the 12 frames (36 seedlings total) and used their dried stems to quantify the organic constituents of their woody tissues. We separated the current-year stem, formed under treatments within the FACE system, from the other parts. The bark of the current-year stem was removed using a chisel to eliminate the influence of bark chemicals, which may be altered under elevated CO_2 concentrations (Eberhardt et al., 2015). The remaining portion of the stem (i.e., woody tissue) was ground into fine particles that could pass through a 4-mm-mesh screen.

Fine particle samples (0.5 g) were dried at 105°C for 24 h, weighed, and used to determine water content ($WC_{fineparticle}$). Next, we performed solvent extraction according to JIS P 8010-1976, with some modifications. We added 150 mL of ethanol/benzene solution (1/2, v/v) into 1.0–1.5 g of fine particle sample and conducted Soxhlet extraction for 6 h. The ethanol/benzene extractives solution was evaporated and then dried under reduced pressure in a desiccator. We weighed the dried extractives to determine the percentage of dry weight of extractives to that of fine particle sample (extractive content), which was calculated using the water content value, $WC_{fineparticle}$. The ethanol/benzene extraction residue was air-dried and used to quantify the lignin content, according to JIS P 8008-1976, but with some modifications (described as follows).

A 0.5-g sample of the ethanol/benzene extraction air-dried residue was dried at 105°C for 24 h and weighed and its water content was determined ($WC_{residue}$). Next, we added 15 mL of 72% H_2SO_4 solution into the residue, stirred the solution, and let it stand at 20°C for 4 h. We then added 560 mL of distilled water into the residue solution and refluxed it for 4 h. After cooling, the residue solution was suction filtered through a glass filter (1 GP 16). The filtrate was adjusted to 2 L by adding distilled water, and the absorbance of acid-soluble lignin (ASL) at 210 nm (As) was measured using ultraviolet-visible spectrometer (UV-2400PC, Shimadzu, Kyoto, Japan), whereas the absorbance of distilled water (Ab) was measured as a blank sample. The filtered residue was dried at 105°C for 24 h and weighed as dry weight of Klason lignin (KL). The percentage of ASL (ASL content) and KL (KL content) to dry weight of the fine particle sample was calculated using Formulas 1 and 2, respectively. We further calculated the total lignin content as the sum of ALS and KL.

$$[\text{Formula1}]\text{ASL}(\%) = \{V \times (As - Ab)/AC\}/[SW/\{(100 - PE)/100\}] \times 100$$

$$[\text{Formula2}]\text{KL}(\%) = KLW/[SW/\{(100 - PE)/100\}] \times 100$$

where V, AC, and KLW are the volume of residue solution diluted filtrate (i.e., 2 L), absorption coefficient of lignin (i.e., 110), and dry weight of KL, respectively. SW is the dry weight of the residue, which was calculated using the water content value, $WC_{residue}$, and PE is the percentage of the dry weight of ethanol/benzene extraction to that of the fine particle sample.

In this study, the percentage of holocellulose (holocellulose content) to dry weight of the fine particle sample was calculated by subtracting the lignin and extractive contents from 100%.

Analysis of ethanol/benzene extractives, ASL, KL, and holocellulose was conducted twice for each fine particle sample, as replicate measurements; we used the mean value of the two measurements for statistical analysis. However, the dry weight of current-year stem was so low for two seedlings grown in the [Control] treatment that small amount of fine particle sample obtained; this amount of fine particle was not enough for twice measurement. We thus used only a single value for the two samples for statistical analysis.

Statistical Analysis

Values for the base diameter and stem length at the start and the end of the experiment, stem and aboveground biomass, and the extractive content of woody tissue were transformed using a Box-Cox transformation to meet data normality. We then analyzed the effects of elevated CO_2 and/or O_3 concentrations on seedling growth, i.e., differences in diameter at base and stem length between the start and the end of the experiment, using a generalized linear mixed model with a Gaussian error distribution (Formula 3). In Formula 3, measurement time means a categorical variable, which shows the start and the end of experiment. In this analysis, we considered individual seedling number, which is nested by each chamber in the FACE system, to be a random effect.

$$\begin{aligned}[\text{Formula3}]&\text{Base diameter or stem length}\\ &= CO_2\text{concentration} + O_3\text{concentration}\\ &+ \text{Measurement time} + CO_2\text{concentration}\\ &\times O_3\text{concentration} + CO_2\text{concentration}\\ &\times \text{Measurement time} + O_3\text{concentration}\\ &\times \text{Measurement time} + CO_2\text{concentration}\\ &\times O_3\text{concentration} \times \text{Measurement time}\\ &+ (\text{FACEchamber}/\text{IndividualNo})\end{aligned}$$

We further analyzed the effects of elevated CO_2 and/or O_3 concentrations on seedling biomass (i.e., aboveground, leaf, and stem biomass) and the organic composition of woody tissue (i.e., extractives, total lignin, ASL, KL, and holocellulose contents) using a generalized linear mixed model with a Gaussian error distribution (Formula 4). In this analysis, we considered each chamber in the FACE system to be a random effect.

$$\begin{aligned}[\text{Formula4}]&\text{Each biomass or organic constituent variable}\\ &= CO_2\text{concentration} + O_3\text{concentration}\\ &+ CO_2\text{concentration} \times O_3\text{concentration} + (\text{FACEchamber})\end{aligned}$$

We determined the significance of each fixed effect (i.e., CO_2 concentration, O_3 concentration, Measurement time, and their interaction) using an analysis of deviance (type II test; Fox and Weisberg, 2019). Furthermore, differences in the growth and

TABLE 1 | Size and biomass (mean ± standard deviation) of *Q. mongolica* seedlings at the start and end of the experiment, after treatments of atmospheric CO_2 and O_3 concentration ([Control]), elevated CO_2 concentration ([CO_2]), elevated O_3 concentration ([O_3]), and a combination of elevated CO_2 and O_3 concentrations ([$CO_2 + O_3$]).

Size or biomass of seedling	Treatment			
	[Control]	[CO_2]	[O_3]	[$CO_2 + O_3$]
Diameter at base (mm)				
at start of experiment	4.61 ± 0.08^{a}	4.23 ± 0.32^{a}	4.16 ± 0.16^{a}	4.03 ± 0.16^{a}
at end of experiment	10.15 ± 0.74^{b}	14.05 ± 1.27^{c}	10.46 ± 0.65^{b}	12.39 ± 0.81^{bc}
Stem length (cm)				
at start of experiment	15.17 ± 0.39^{a}	14.35 ± 1.10^{a}	15.81 ± 0.67^{a}	15.66 ± 1.58^{a}
at end of experiment	63.20 ± 12.18^{b}	91.43 ± 11.35^{c}	72.73 ± 5.38^{bc}	97.09 ± 6.57^{c}
Aboveground biomass (g)	30.92 ± 7.61^{b}	64.48 ± 12.88^{a}	37.29 ± 5.21^{ab}	52.44 ± 7.56^{ab}
Leaf biomass (g)	12.55 ± 2.13^{b}	24.49 ± 4.49^{a}	15.88 ± 2.25^{ab}	21.61 ± 2.79^{ab}
Stem biomass (g)	7.84 ± 1.58^{b}	15.01 ± 2.68^{a}	9.72 ± 1.23^{ab}	12.26 ± 1.77^{ab}

Different letters indicate significant differences between treatments and between the start and the end of experiment (Tukey test: $p < 0.050$).

organic constituent variables across the four treatments were assessed by the Tukey method using GLHT function based on general linear hypotheses (Hothorn et al., 2008). All statistical analyses were conducted in R3.6.1 (R Core Team, 2019).

RESULTS

Q. mongolica Seedling Growth

Seedling base diameter was influence by CO_2 concentration, measurement time, and their interaction ($p < 0.001$; **Tables 1**, **2**). Seedling base diameters were similar across treatments at the start of the experiment. At the end of the experiment, the diameter at the base was higher in the [CO_2] treatment than in the [O_3] and [Control] treatments ($p < 0.001$). Stem length was also influenced by CO_2 concentration, measurement time, and their interaction ($p < 0.010$; **Tables 1**, **2**). At the end of the experiment, stem length was higher in the [CO_2] and [$CO_2 + O_3$] treatments than in the [Control] treatment ($p < 0.016$).

In terms of biomass growth, CO_2 concentration affected aboveground, leaf, and stem biomass at the end of the experiment (**Tables 1**, **2**; $p = 0.001$, $p = 0.001$, $p = 0.005$, respectively), all of which were higher in the [CO_2] treatment than in the [Control] treatment ($p < 0.018$).

Organic Carbon Component of *Q. mongolica* Seedling Stem Wood

With respect to total lignin, we detected an interaction between CO_2 and O_3 concentrations (**Table 3**; $p = 0.020$); however, there was no difference across treatments (**Figure 3**; $p > 0.078$). The KL content showed a similar result as that for total lignin: there was an interaction between elevated CO_2 and O_3 concentrations ($p = 0.021$), but no difference across treatments ($p > 0.150$). Neither CO_2 nor O_3 concentration influenced ASL content ($p > 0.067$).

CO_2 concentration affected the stem woody tissue extractive content (**Table 3**; $p = 0.029$). The mean (± standard error) percentage of extractive content in the

TABLE 2 | Results of the analysis of deviance (type II test) for stem size and aboveground, leaf, and stem biomass at the start and end of the experiment.

Explained variable	Fixed effect	df	Residual deviance	*p* value
Stem diameter at base (mm)	CO_2 concentration	1	10.95	***<0.001***
	O_3 concentration	1	1.21	0.271
	Measurement time	1	146.11	***<0.001***
	CO_2 concentration × O_3 concentration	1	1.28	0.258
	CO_2 concentration × Measurement time	1	14.70	***<0.001***
	O_3 concentration × Measurement time	1	1.11	0.292
	CO_2 concentration × O_3 concentration × Measurement time	1	1.72	0.189
Stem length (cm)	CO_2 concentration	1	6.63	***0.010***
	O_3 concentration	1	1.58	0.208
	Measurement time	1	331.75	***< 0.001***
	CO_2 concentration × O_3 concentration	1	0.07	0.795
	CO_2 concentration × Measurement time	1	12.98	***<0.001***
	O_3 concentration × Measurement time	1	1.12	0.290
	CO_2 concentration × O_3 concentration × Measurement time	1	0.33	0.566
Aboveground biomass (g)	CO_2 concentration	1	10.09	***0.001***
	O_3 concentration	1	0.09	0.770
	Interaction of CO_2 and O_3 concentrations	1	1.15	0.283
Leaf biomass (g)	CO_2 concentration	1	10.34	***0.001***
	O_3 concentration	1	0.01	0.935
	Interaction of CO_2 and O_3 concentrations	1	1.28	0.258
Stem biomass (g)	CO_2 concentration	1	7.73	***0.005***
	O_3 concentration	1	0.00	0.994
	Interaction of CO_2 and O_3 concentrations	1	1.89	0.170

Aboveground, leaf, and stem biomass were analyzed for the end of experiment. A bold value shows a significance of the fixed effect.

TABLE 3 | Results of the analysis of deviance (type II test) for the organic constituents of woody tissue.

Explained variable	Fixed effect	df	Residual deviance	*p* value
Total lignin (%)	CO_2 concentration	1	0.13	0.714
	O_3 concentration	1	1.13	0.287
	Interaction of CO_2 and O_3 concentrations	1	5.38	***0.020***
Acid soluble lignin (%)	CO_2 concentration	1	1.95	0.162
	O_3 concentration	1	3.35	0.067
	Interaction of CO_2 and O_3 concentrations	1	0.12	0.729
Klason lignin (%)	CO_2 concentration	1	0.00	0.955
	O_3 concentration	1	0.45	0.503
	Interaction of CO_2 and O_3 concentrations	1	5.35	***0.021***
Extractives (%)	CO_2 concentration	1	4.76	***0.029***
	O_3 concentration	1	0.00	0.984
	Interaction of CO_2 and O_3 concentrations	1	3.03	0.082
Holocellulose (%)	CO_2 concentration	1	1.91	0.167
	O_3 concentration	1	0.72	0.397
	Interaction of CO_2 and O_3 concentrations	1	8.30	***0.004***

A bold value shows a significance of the fixed effect.

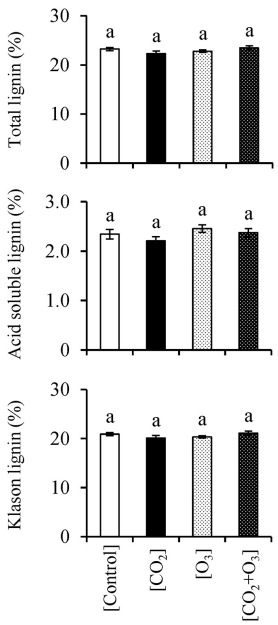

FIGURE 3 | Total lignin, acid-soluble lignin, and Klason lignin contents in *Q. mongolica* seedlings subjected to conditions of atmospheric CO_2 and O_3 concentration ([Control]), elevated CO_2 concentration ([CO_2]), elevated O_3 concentration ([O_3]), and a combination of elevated CO_2 and O_3 concentrations ([CO_2 + O_3]). Bars indicate standard errors. Different letters indicate significant differences among treatments (Tukey method: $p < 0.050$).

[CO_2] treatment was 2.88 ± 0.09%, which was lower than that in the [Control] treatment (3.63 ± 0.32%; $p = 0.028$; **Figure 4**).

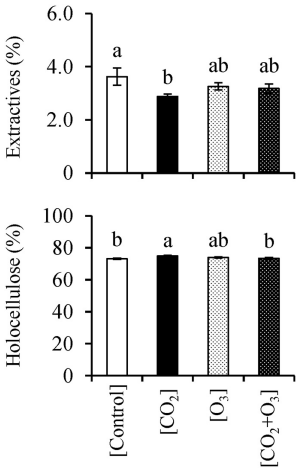

FIGURE 4 | Ethanol/benzene extractives and holocellulose contents in *Q. mongolica* seedlings subjected to conditions of atmospheric CO_2 and O_3 concentration ([Control]), elevated CO_2 concentration ([CO_2]), elevated O_3 concentration ([O_3]), and a combination of elevated CO_2 and O_3 concentrations ([CO_2 + O_3]). Bars indicate standard errors. Different letters indicate significant differences among treatments (Tukey method: $p < 0.050$).

There was also a significant interaction between CO_2 and O_3 concentrations for holocellulose content (**Table 3**; $p = 0.004$). The holocellulose content (mean ± standard error) for the [CO_2] treatment was 74.8 ± 0.47%, which was higher than that for the [Control] and [CO_2 + O_3] treatments ($p < 0.042$; **Figure 4**), which corresponded to 73.2 ± 0.36% and 73.4 ± 0.52%, respectively.

DISCUSSION

Effect of Elevated CO_2 and/or O_3 Concentrations on *Q. mongolica* Growth

Elevated CO_2 concentrations enhanced stem growth, resulting in increased leaf, stem, and aboveground biomass (**Table 1**). This suggests that the growth of *Q. mongolica* seedlings was accelerated by an elevated CO_2 concentration, as reported for many other tree species (e.g., Richet et al., 2012; Tobita et al., 2019). Conversely, we observed minor changes in biomass growth under the elevated O_3 concentration condition (**Table 1**), unlike in other species (e.g., Watanabe et al., 2007; Richet et al., 2012), whose growth and biomass have been shown to decrease under such conditions. However, our results are similar to those of Kitao et al. (2015), who investigated the response of the same species to elevated CO_2 and O_3 concentrations over 2 years. Yamaguchi et al. (2011) also suggested the sensitivity to O_3 is low for *Q. mongolica* by summarizing the critical level of O_3 concentrations for biomass growth of various tree species. Thus, we hypothesize that *Q. mongolica* is tolerant to elevated O_3 concentrations. This species showed the reduction of net photosynthesis rate under high O_3 concentration (Kitao et al., 2015), similar to other tree species (Yamaguchi et al., 2011). On the other hand, Kitao et al. (2015) suggested that C

allocation is increased into the shoot and leaves under elevated O_3 concentrations for *Q. mongolica* while similar tendency was not observed for other species (Watanabe et al., 2008). Additionally, *Q. mongolica* often shows partly determinant growth, i.e., additional shoot elongation, as a response to the change in environment (Kitao et al., 2006), and *Quercus* species carried C synthesized in a given year over to the additional growth in the next year (e.g., Vernay et al., 2018). The key to high O_3 concentration tolerance may be a change in the C allocation pattern, which is induced by partly determinant growth type and carry-over effect, that may be specific to this tree species.

In this study, the interactive effect of the combination of elevated CO_2 and O_3 concentrations on biomass growth was not clear (**Table 1**). Kitao et al. (2015) reported an increase in *Q. mongolica* seedling biomass growth over their 2-year experiment, with a significant increase in C allocation into the shoots (cf., Kitao et al., 2021). A longer experimental period may provide more evidence of the species-specific responses of shoot–root allocation to elevated CO_2 and O_3 concentrations. Our results demonstrate that stem length growth is enhanced under a combination of elevated CO_2 and O_3 concentrations. Elevated O_3 concentration enhances stem length growth in *Cryptomeria japonica* (Hiraoka et al., 2017), and a slight increase in stem length was observed under the elevated O_3 concentration condition in our study (**Table 1**). Rapid biomass growth under elevated CO_2 concentrations may promote an increase in stem length under elevated O_3 concentrations.

Effect of Elevated CO_2 and/or O_3 Concentrations on the Organic Carbon Components of *Q. mongolica* Woody Tissue

The experimental period was only 1 growing season in this study. Thus, our results for organic carbon component may be different from a long-term experiment. However, our experiment gives an insight at least for newly formed wood tissue and supplies basic information for long-term experiment.

We did not detect a change in the total lignin, ASL, and KL contents (**Figure 3**), irrespective of the increase in biomass growth noted under an elevated CO_2 concentration (**Table 1**). The lignin content of the woody tissue may be stable in *Q. mongolica*, similar to that of other species (e.g., *Populus nigra*; Luo et al., 2008). The deposition of lignin on cell walls enhances the physical strength of stem wood tissue (e.g., Gril et al., 2017), which is necessary to keep the shape of tree, i.e., standing stem against gravity. We thus think that the increase in biomass growth is accompanied by additional lignin production to keep above a certain level of lignin content. Conversely, the stability of lignin content under elevated O_3 concentrations differs from the results noted in previous reports, which found an increase in lignin content in *Populus tremuloides*, *Betula papyrifera*, and a hybrid poplar (Kaakinen et al., 2004; Richet et al., 2011, 2012). Richet et al. (2012) reported a decrease in biomass growth under elevated O_3 concentrations with an increase in lignin content. In this study, a reduction of biomass growth under elevated O_3 concentrations was not observed (**Table 1**). Therefore, we speculate that less change in lignin content in *Q. mongolica* is due to the lack of a change in biomass growth under elevated O_3 concentrations.

We found that the woody tissue extractive contents were reduced under elevated CO_2 concentrations (**Figure 4**). This finding differs from that for other tree species (Kaakinen et al., 2004; Kostiainen et al., 2006, 2008), whose extractive contents have been reported to remain unchanged or increase under similar conditions. Conversely, Luo et al. (2008) noted a reduction of soluble phenolics, components of extractives, under elevated CO_2 concentrations. Thus, we suggest that the elevated CO_2 concentration reduced the extractive content of woody tissue in *Q. mongolica*. Luo et al. (2008) hypothesized that the biosynthesis for biomass growth precedes that for carbon-based secondary compounds such as phenolics and tannins. The synthesis of extractive may not keep up with the production of structural components, i.e., lignin and holocellulose. The extractives have the antimicrobial function, i.e., inhibition of degradative capabilities of rot fungi and disruption of parts of fungal cell (Valette et al., 2017). In this study, the difference in extractive content was very small (i.e., 0.75%) between the [Control] (3.63%) and [CO_2] (2.88%) treatments (**Figure 4**); however, this equated to a 21% decrease (=0.75% / 3.63% × 100) in the extractive content. Kirker et al. (2013) reported that the initial decomposition rate of extractive-free wood block exposed to brown-rot fungi was 5–10 fold higher than unextracted wood block for 3 hardwood species. Thus, we predicted that the reduction of extractive contents under elevated CO_2 concentration causes measurable increase in the decomposition rate of dead wood and woody debris.

On the other hand, the decrease in extractive content under the elevated CO_2 concentration treatment became unclear upon the addition of O_3, i.e., the combination of elevated CO_2 and O_3 concentrations (**Figure 4**). Similar differences among treatments were observed for stem biomass (**Table 1**). Elevated O_3 concentration itself did not change the extractive content, but it may have an effect to mitigate the impact of high CO_2 concentration on extractive content.

Elevated CO_2 concentration enhanced the holocellulose content (**Figure 4**), although it was only indirectly determined (i.e., woody tissue excluding lignin and extractives). This result is different from those of previous reports for other species (Kaakinen et al., 2004; Kostiainen et al., 2006, 2008), which demonstrated no change or a decrease in cellulose and hemicellulose contents under similar conditions. However, Luo and Polle (2009) reported that the cell wall fraction increases under elevated CO_2 concentrations in a hybrid poplar. For *Q. mongolica*, the size of the vessels and the number of cambial cells increase under elevated CO_2 concentrations (Watanabe et al., 2010). In *Q. mongolica*, the holocellulose content may increase *via* a change in structure of the woody tissue under elevated CO_2 concentrations. On the other hand, there was a very small increase (i.e., 1.6%) in holocellulose content from the [Control] (73.2%) to [CO_2] (74.8%) treatments, indicating only a 2% increase (=1.6% / 73.2% × 100) in the holocellulose content.

The small increase may not influence the decomposition rate drastically. Furthermore, an elevated O_3 concentration did not change the holocellulose content, but it reduced the impact of high CO_2 concentrations (**Figure 4**); a similar result was obtained for the cellulose content in *Betula pendula* seedlings (Kostiainen et al., 2006). Therefore, an increase in holocellulose content may be mitigated by elevated O_3 concentrations in *Q. mongolica*.

CONCLUSION

In this study, we noted a decrease in the extractive content of *Q. mongolica* under elevated CO_2 concentrations, whereas the holocellulose content increased. As part of the rapid biomass growth response of *Q. mongolica* seedlings (**Table 1**), the production of structural components (i.e., lignin and holocellulose) may precede that of non-structural components (i.e., extractives). Extractives inhibit biodegradation, irrespective of the small amount in woody tissue (Oliveira et al., 2010; Kirker et al., 2013) *via* their antimicrobial function such as inhibition of degradative capabilities of rot fungi and disruption of parts of fungal cell (Valette et al., 2017). The decrease in extractive content suggests that dead wood and woody debris may be rapidly decomposed under high atmospheric CO_2 concentration in *Q. mongolica*. *Q. mongolica* is distributed widely in the temperate forests of Russian Far East, Mongolia, the northeastern part of China, the Korean Peninsula, and Japan. This decrease in extractive content may have a non-negligible impact on C cycling on the global scale. Furthermore, our results showed a possibility that elevated O_3 concentrations mitigate the impact of elevated CO_2 concentrations; this must be tested. Additionally, we only evaluated the organic constituents of woody tissue in one of the representative species in this area. More information for the response of various species is necessary to enhance the prediction accuracy for the impact of elevated CO_2 and/or O_3 concentrations on C cycling in the future forests.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

SU analyzed the data of the experiment and prepared the manuscript. SH and KH measured the amount of each organic constituents of wood stem. HT got the research grants and measured the growth of seedlings. MK got the research funds and built the experiment system. All authors contributed to the article and approved the submitted version.

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