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Elevated atmospheric temperature and CO_2 altered the growth, carbon, and nitrogen distribution and the rhizosphere properties of *Platanus occidentalis* L. seedlings

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Abstract: The effects of elevated atmospheric temperature and CO_2 on the growth and plant carbon and nitrogen allocation of *Platanus occidentalis* L. and their influence on rhizosphere and soil microbial properties were evaluated through a rhizobag experiment in an environmentally controlled chamber. Seedlings of *P. occidentalis* L. were established in the central compartment of a rhizobag filled with forest soil and exposed to four treatment combinations (control (25 °C, 400 µmol mol^{-1} CO_2), T2 (30 °C, 400 µmol mol^{-1} CO_2), T3 (25 °C, 800 µmol mol^{-1} CO_2), and T4 (30 °C, 800 µmol mol^{-1} CO_2)) for 20 weeks. Elevated temperature and CO_2 enhanced the growth and dry matter yield and altered the C and N distribution of *P. occidentalis*. The pH, electrical conductivity, and concentration of dissolved sugar and organic carbon in soil solution were also altered, as well as the microbial activity and carbon utilization potential of soil microorganisms in the rhizosphere of *P. occidentalis*. Overall, the results showed that the changes in plant growth and physiology of *P. occidentalis* due to exposure to elevated temperature and carbon dioxide extended to the soil biological properties via plant-induced changes in substrate quality and quantity in the rhizosphere.

Key words: Carbon acquisition, carbon and nitrogen allocation, dissolved sugars, rhizosphere, soil microorganisms, soil solution

1. Introduction

The simultaneous increase of atmospheric temperature and carbon dioxide concentration are prominent features of global-scale environmental change. With the current trend of CO_2 and greenhouse gas emissions, it is predicted that in the next 50 years, CO_2 concentration may reach 790 µmol mol^{-1} while the temperature will increase by 0.2 °C per decade for the next two decades (IPCC, 2007), which will affect the health and productivity of forest ecosystems.

The interaction of climate change and forest ecosystems can be seen from different viewpoints. Forest trees and their communities are considered one of the key agents that could mitigate global climate change through carbon sequestration (Drake et al., 2011). On the other hand, plants' adaptation ability to climate change could influence the overall productivity of a particular ecosystem as well as its ability to mitigate climate change. Similarly, the extent of plants' response to climate change will influence the carbon and nutrient cycling and sequestration and is dependent on the prevailing environmental conditions (Drake et al., 2011). Several studies have shown that elevated atmospheric CO_2 and temperature, either singly or interactively, trigger unique physiological processes of plant species and modify plant diversity in an ecosystem in the long term (Ceulemans and Mousseau, 1994; Myster and Moe, 1995; Ceulemans et al., 1999; Finzi et al., 2001; Dawes et al., 2011). For example, the interactive effect of temperature and carbon dioxide on plant physiology and on carbon and nitrogen partitioning in plants could occur and alter the litter quality on forest soils, thereby affecting the long-term nitrogen and carbon cycling of the ecosystem (Hyvönen et al., 2007; Hungate et al., 2009). In turn, these changes in N and C cycling affect the overall ecosystem functioning, including the diversity of belowground communities of other organisms that will affect the plant community (Klironomos, 2002; Bever, 2003; Bartelt-Ryser et al., 2005; Dieleman et al., 2012).

Soil's overall productivity is significantly influenced by soil microbial communities through their involvement in primary soil processes such as organic cycling and nutrient transformation and dynamics (van Veen et al., 1991; Lin et al., 2004; Drigo, 2008; Wei et al., 2008; Phillips et al., 2012). These soil processes are also greatly influenced by rhizosphere processes. In fact, the rhizosphere has been identified as one of the key fine-scale components of the global carbon cycle (Coleman et al., 1992; Gobran et al.,

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1998), which has the ability to mediate soil interactions including plant nutrient acquisition, root colonization by rhizosphere microorganisms (Baker, 1989; Miller, 1990; Wood, 2012; Carrillo, 2013), and soil organic matter decomposition (Cheng and Coleman, 1990; Cheng and Johnson, 1998). Therefore, understanding biochemical processes in the rhizosphere in relation to increasing atmospheric CO_2 concentrations and temperatures is important in predicting the response of forest ecosystems to environmental changes. Cheng (1999) and Drigo et al. (2008) reported that plants grown in elevated CO_2 substantially increased C input to the rhizosphere as a priming effect, and they proposed several mechanisms of plant–rhizosphere interaction in response to elevated CO_2, depending on specific plant and soil conditions (Zak et al., 2003; Drigo et al., 2008). Changes in carbon and nutrient composition will induce changes in microbial community composition and structures in soil (Klamer et al., 2002; Janus et al., 2005; Lipson et al., 2005). Most of the studies conducted, however, are confined to grasses and other annuals; only a few have dealt with forest trees. In particular, few studies have dealt with the interactive effect of atmospheric temperature and carbon dioxide on rhizosphere processes, particularly on its impact on carbon and nutrient dynamics and microbial activity (Korner et al., 1996; Chen and Stark, 2000; Hernesma et al., 2005; Drigo et al., 2008; Teklay et al., 2010). Because of genetic diversity and other growth factors, it is therefore critical to evaluate these climatic parameters on the growth and development of important tree species, including its impact on rhizosphere processes (Ainsworth and Long, 2005; Aranjuelo et al., 2005a, 2005b; Drake et al., 2011).

Platanus occidentalis, a hardwood, is considered an important tree species for biomass production for renewable energy supply. Ideally, plant responses to environmental alterations would be best evaluated in mature trees on a long-term basis. We conducted this experiment on seedlings in order to determine their ability to respond to this particular environment, which may be necessary for identifying suitable species for forest rehabilitation programs in recent times. It is hypothesized that *Platanus occidentalis* has specific responses to environmental changes that would be extended to the rhizosphere and the plant's ability to adapt to its current environment. Hence, the objective of this study was to determine the growth response, carbon and nitrogen partitioning, and rhizosphere-induced changes to the rhizosphere properties of *P. occidentalis* exposed to elevated temperature and CO_2.

2. Materials and methods

2.1. Soil preparation and rhizobag assembly

The soil used in the experiments was collected from the subsurface layer (20–30 cm) of a forest plantation at the Korea Forest Research Institute (KFRI), Suwon City, Gyeonggi-do, South Korea. After air-drying, the soil samples for cultivation and chemical analysis were passed through 5- and 2-mm mesh sieves, respectively. The properties of the soil were described by Ultra et al. (2012). The soil is clayey and very strongly acidic (pH 3.8), belonging to the podzolic red-yellow forest soils (lithosols) developed from granitic parent material (Um, 1985).

A rhizobag system composed of two compartments was used for the experiment. The system had one central compartment (rhizosphere) in which the plants were grown, and an outer compartment serving as the bulk soil (Ultra et al., 2007). The central compartment was separated using a 25-µm nylon mesh mounted on a cylindrical plastic frame (70 mm in diameter, 200 mm in height). One kilogram of soil was placed in the central compartment and 9 kg in the outer compartment, totaling 10 kg of soil in a 6-L pot.

2.2. Plant establishment and treatment composition

The seedlings used in the experiment were generated from seeds of open-pollinated *P. occidentalis* of the KFRI plantations, Suwon City, South Korea. Seeds were germinated in seedling trays with vermiculite and maintained under screen-house conditions for 3 weeks. Seedlings with uniform growth were selected and planted in the central compartment. Pots were maintained at a moisture content of approximately 60% of field capacity in the growth chamber. Lost moisture was replenished every 2 days by adding the desired amount of water based on weight loss in each pot.

The experiment used four walk-in chambers (3 × 3 × 1.8 m) with controllable conditions corresponding to each treatment. The conditions in each chamber varied in temperature and carbon dioxide concentration depending on the treatment: T1 (25 °C, 400 µmol mol^{-1} CO_2), T2 (30 °C, 400 µmol mol^{-1} CO_2), T3 (25 °C, 800 µmol mol^{-1} CO_2), and T4 (30 °C, 800 µmol mol^{-1} CO_2). The carbon dioxide concentrations employed in the experiment was based on the projected CO_2 level in the next 50 years. The air was circulated through charcoal filters and the carbon dioxide was mixed into the air stream. Humidity was maintained at 60 ± 5%; light was provided at a photon flux density of 450 µmol m^{-2} s^{-1} during a 12-h photoperiod. Each treatment was composed of four plants established in a rhizobag as replications. The plants were grown for 20 weeks inside the chamber and the pots were constantly rearranged to avoid positional bias on plant growth and development.

2.3. Growth responses

Plant height and root collar diameter were monitored every 2 weeks for the first 8 weeks and every 4 weeks thereafter. The relative growth rate (RGR), as indicated by height, was calculated as $RGR = [\ln(X_2) - \ln(X_1)] / (t_2 - t_1)$, where X_1 was the height at time t_1 (start of experiment or

last measurement) and X_2 was the height at time t_2 (latest measurement). For biomass measurements, the leaves, stems, and roots were carefully removed and thoroughly rinsed twice with distilled water. The dry weights were recorded after drying the tissues at 70 °C for 3 consecutive days.

2.4. Pigment analysis

The leaves of *P. occidentalis* L. were excised and soaked in dimethyl sulfoxide in a glass vial. The vial was tightly capped and incubated at 70 °C for 2 h in the dark. The concentration of the extracted pigments (total chlorophyll, chlorophyll a, chlorophyll b, and carotenoid) was calculated based on their absorbance values at 664, 645, and 470 nm according to Lichtenthaler (1987).

2.5. Carbon and nitrogen analyses

Dried leaves, stems, and roots were ground (<0.1 mm). They were used to determine C and N concentration using a CHNS-elemental analyzer. Samples were analyzed in replicates of four. The C and N uptake in plant tissues was calculated based on the dry matter yield multiplied by the concentration.

2.6. Soil solution sampling and analysis

A soil moisture sampler (RHIZON FLEX, Rhizosphere Research Products, the Netherlands) was inserted into each compartment for periodic soil solution sampling. The soil solution was withdrawn with a 10-mL plastic syringe. The soil solution was stored at 4 °C and analyzed within 2 days of sampling.

The soil solution pH and electrical conductivity were determined using an electrode multiparameter analyzer (CONSORT C535). The total sugar content was analyzed based on the work of Ultra et al. (2012). The soil solution (125 µL) was mixed with 25 µL of an invertase solution and 100 µL of Na acetate buffer, pH 4.8. It was incubated for 2 h at 30 °C, mixed with 1.25 mL of color reagent, and boiled for 4 min in a water bath. After cooling, the mixture was centrifuged for 5 min at 12,000 rpm and then analyzed at 415 nm absorbance using spectrophotometer. The color reagent was prepared by dissolving 0.5 g of hydroxybenzoic acid hydrazide in 100 mL of solution containing 1.47 g of trisodium citrate × $2H_2O$, 0.15 g of $CaCl_2$ × $2H_2O$, and 2.0 g of NaOH. The amount of sugar was quantified based on the standard, which was made of a glucose solution.

The total oxidizable carbon in soil solution was determined based on the methods of Bartlett and Ross (1988). Briefly, 1.0 mL of soil solution was mixed with 0.5 mL of 10 mM Mn(III)-pyrophosphate solution and 0.5 mL of concentrated H_2SO_4. The mixture was vortexed and allowed to stand for at least 18 h. The optical density of the solution was determined at 495 nm absorbance on a spectrophotometer (SPECTRA MAX, Molecular Devices, USA). The concentration was evaluated against the standard, which was prepared from oxalic acid.

2.7. Soil sampling and chemical analysis

After sampling the roots of *P. occidentalis* L. from the central compartment, the soils in the central compartment designated as rhizosphere soil and outer compartments as bulk soils were sifted through a 2-mm sieve. Portions of 100 g were stored at 4 °C for the analysis of soil microbial properties; the remaining soil was air-dried and stored at 4 °C for chemical analysis. Soil pH and EC were measured in a 1:5 (soil:water) suspension using an electrode multiparameter analyzer (CONSORT C535). The carbon and nitrogen contents were determined using a CHNS-elemental analyzer (FlashEA 1112 Series, Thermo Electron Corp., Italy). Samples were analyzed in five replicates.

2.8. Soil microbial community level physiologic profile

Soil microbial activity was evaluated based on substrate (sole-carbon-source) utilization profiles that were established using a Biolog EcoPlate (Biolog, USA). The preparation and inoculation of the soil samples took place within 2 days of sampling. The plates contained 96 wells with three sets of 31 substrates plus one control (no substrate). A soil sample (10 g) was suspended in 0.1 M NaH_2PO_4 (pH 6) solution at a ratio of 1:9 (w/v). The soil suspension was diluted 1000-fold with 0.15 M NaCl, which enabled meaningful absorbance values to be measured during a 7-day incubation period, and 100 µL of the diluted suspension was inoculated into each well of a microplate. Thus, a single microplate contained three replicates of one sample. The microplates were incubated at 28 °C and their absorbance was measured at a wavelength of 590 nm using a microplate reader (SPECTRA MAX, Molecular Devices, USA) every 24 h for 7 days. The absorbance of the 31 substrates was used to calculate the average well color development (AWCD; Garland and Mills, 1991). AWCD values were plotted against the incubation period of the plate (Garland, 1997).

The Shannon–Weaver index (H, richness and evenness of response) and richness (R, the number of positive wells on the EcoPlate) of bacterial communities were calculated based on the EcoPlate readings after 72 h of incubation (Gomez et al., 2006).

2.9. Statistical analysis

The data were statistically analyzed using the SAS System for Windows, Version 8.01 (SAS Institute, USA) by two-way analysis of variance. For soil property data, 3-way analysis of variance was performed including the soil compartments. When significant interaction differences ($P < 0.05$) were indicated, Tukey's highly significant difference (HSD) tests were performed. The optical density (OD) data from the Biolog EcoPlate were transformed based on Weber et al. (2007) and subjected to factor analysis by principal component analysis using SPSS (PASW Statistics 17.0.3) based on Gomez et al. (2006). The OD of an individual substrate with high correlation to the extracted

principal components was also subjected to ANOVA and multiple mean comparisons by Tukey's HSD test.

3. Results

3.1. Growth response

Elevated atmospheric temperature and CO_2 had a significant interaction effect on plant height (P ≤ 0.002), relative height growth rate (RHGR; P ≤ 0.004), root collar diameter (P ≤ 0.000), shoot dry yield (P ≤ 0.001), root dry yield (P ≤ 0.003), total dry matter yield (P ≤ 0.001), and shoot:root dry yield ratio (P ≤ 0.004; Table 1). Plants exposed to 30 °C, 400 µmol mol^{-1} CO_2 and 30 °C, 800 µmol mol^{-1} CO_2 treatments were significantly taller and had higher RHGRs compared to those grown in 25 °C, 800 µmol mol^{-1} CO_2 treatment, but not those grown in 25 °C, 400 µmol mol^{-1} CO_2 treatment. The root collar diameter was the greatest in the 25 °C, 800 µmol mol^{-1} CO_2 treatment, but lowest in the 30 °C, 400 µmol mol^{-1} CO_2 treatment. The shoot dry yield was highest in the 30 °C, 800 µmol mol^{-1} CO_2 treatment, while the root dry yield was lowest at 25 °C, 400 µmol mol^{-1} CO_2. The total dry matter yield of plants exposed to 30 °C, 800 µmol mol^{-1} CO_2 was significantly higher than those of the other treatments, while the shoot/root dry yield ratio was generally higher when plants were exposed to 30 °C.

Elevated temperature and CO_2 had a significant interaction effect on chlorophyll a (P ≤ 0.004), chlorophyll b (P ≤ 0.014), chlorophyll a + b (P ≤ 0.024), carotene (P ≤ 0.001), ratio of chlorophyll a/chlorophyll b (P ≤ 0.028), and ratio of chlorophyll a + b/carotene (P ≤ 0.003) of *P. occidentalis* (Table 2). The chlorophyll a, b, and a + b and carotene contents were lower in plants exposed to 30 °C,

Table 1. Plant height, relative height growth rate (RHGR), root collar diameter, and dry matter yield of *Platanus occidentalis* exposed to elevated temperature and carbon dioxide levels[1].

Treatment	Plant height (cm)	RHGR (cm cm^{-1} $week^{-1}$)	Root collar diameter (mm)	Dry matter yield (g)			Shoot:root dry yields
				Shoots	Roots	Total	
25 °C, 400 µmol mol^{-1} CO_2	32.0 ± 6.0 b	0.129 ± 0.01 b	6.663 ± 0.46 b	4.112 ± 0.55 b	2.886 ± 0.16 a	6.998 ± 0.70 b	1.421 ± 0.11 c
30 °C, 400 µmol mol^{-1} CO_2	56.6 ±12.1 a	0.165 ± 0.14 a	5.127 ± 0.22 c	5.148 ± 0.91 b	1.769 ± 0.07 b	6.917 ± 0.88 b	2.920 ± 0.59 a
25 °C, 800 µmol mol^{-1} CO_2	44.0 ± 2.0 ab	0.150 ± 0.00 ab	7.820 ± 0.67 a	5.426 ± 0.10 b	2.940 ± 0.28 a	8.367 ± 0.38 b	1.854 ± 0.14 b
30 °C, 800 µmol mol^{-1} CO_2	61.3 ± 7.7 a	0.170 ± 0.01 a	7.537 ± 0.06 ab	7.652 ± 0.68 a	3.315 ± 0.62 a	10.968 ± 1.16 a	2.344 ± 0.33 ab
Temp.	0.000	0.001	0.006	0.002	ns	0.03	0.001
CO_2	0.001	0.004	0.000	0.001	0.004	0.000	ns
Temp. × CO_2	0.002	0.004	0.000	0.001	0.003	0.001	0.004

[1]Means followed by the same letter(s) within a column are not significantly different from each other based on Tukey's HSD test at 5% level.

Table 2. Photosynthetic pigments of *Platanus occidentalis* exposed to elevated temperature and carbon dioxide levels[1].

Treatment	Chl a	Chl b	Chl a + b	Car	Chl a/b	Chl/Car
	mg cm^{-2}					
25 °C, 400 µmol mol^{-1} CO_2	25.28 ± 2.26 b	10.68 ± 0.69 a	35.94 ± 2.88 a	5.56 ± 0.57 a	2.37 ± 0.11 a	6.48 ± 0.22 b
30 °C, 400 µmol mol^{-1} CO_2	18.68 ± 1.12 a	8.98 ± 0.26 b	27.66 ± 1.37 b	4.04 ± 0.37 b	2.08 ± 0.07 b	6.87 ± 0.30 a
25 °C, 800 µmol mol^{-1} CO_2	26.54 ± 3.36 b	11.09 ± 1.03 a	37.61 ± 4.66 a	5.67 ± 0.66 a	2.39 ± 0.13 a	6.64 ± 0.12 a
30 °C, 800 µmol mol^{-1} CO_2	24.53 ± 3.26 b	10.42 ± 0.99 a	34.94 ± 4.23 a	5.44 ± 0.70 a	2.35 ± 0.11 a	6.43 ± 0.14 b
Temp.	ns	ns	ns	ns	ns	ns
CO_2	ns	ns	ns	ns	ns	ns
Temp. × CO_2	0.004	0.014	0.024	0.001	0.028	0.003

[1]Means followed by the same letter(s) within a column are not significantly different from each other based on Tukey's HSD test at 5% level.

400 μmol mol^{-1} CO_2 compared to other treatments. The chlorophyll a/b ratio was also lower in the 30 °C, 400 μmol mol^{-1} CO_2 treatment compared to other treatments, while the chlorophyll/carotene ratio was higher in plants grown at 30 °C, 400 μmol mol^{-1} CO_2 and 25 °C, 800 μmol mol^{-1} CO_2 compared to other treatments.

The carbon concentration in leaf tissues increased when plants were exposed to elevated temperature and CO_2, while the leaf nitrogen concentrations decreased (Table 3). On the other hand, the C concentrations in the stem and roots did not vary significantly, while the N concentrations decreased with increased temperature and CO_2. Consequently, the C/N ratio in plant tissues increased when these plants were grown at high CO_2 concentrations and elevated temperature. The shoot, root, and total C uptakes of *P. occidentalis* increased due to the exposure of plants to elevated CO_2 and were synergistic with temperature (Table 4). Shoot and root uptake were not significantly affected by elevated temperature and CO_2. However, the total N uptake of *P. occidentalis* exposed to 30 °C, 400 μmol mol^{-1} CO_2 was significantly lower than the other treatments (Table 4).

3.2. Soil solution properties

The soil solution pH and electrical conductivity (EC) during the growing period varied significantly among treatments ($P \leq 0.001$), between samplings ($P \leq 0.003$), and between compartments ($P \leq 0.001$; Figure 1). The soil solution pH in the rhizosphere of plants grown in elevated temperature and CO_2 were generally lower compared to the control and the bulk soil (Figure 1a). Specifically, statistical differences were observed after 8 weeks of growth. The soil solution EC of these plants exposed to

Table 3. Carbon and nitrogen concentration on tissues of *Platanus occidentalis* exposed to elevated temperature and carbon dioxide levels[1].

Treatment	Leaf			Stem			Roots		
	C (%)	N (%)	C/N ratio	C (%)	N (%)	C/N ratio	C (%)	N (%)	C/N ratio
25 °C, 400 μmol mol^{-1} CO_2	43.3 ± 4.4 b	4.3 ± 0.4 a	10.0 ± 0.6 c	44.9 ± 4.3 a	0.99 ± 0.21 a	46.5 ± 8.6 c	42.7 ± 3.8 a	2.1 ± 0.2 a	20.3 ± 1.3 b
30 °C, 400 μmol mol^{-1} CO_2	44.2 ± 2.7 ab	3.8 ± 0.2 b	11.6 ± 0.2 b	44.4 ± 3.1 a	0.83 ± 0.15 a	54.9 ± 7.7 bc	39.7 ± 2.9 a	1.8 ± 0.2 b	21.1 ± 1.0 b
25 °C, 800 μmol mol^{-1} CO_2	46.3 ± 1.4 a	3.8 ± 0.1 b	12.1 ± 0.5 b	46.9 ± 3.9 a	0.81 ± 0.14 a	59.2 ± 11.1 ab	44.0 ± 4.4 a	1.5 ± 0.3 b	29.7 ± 5.4 ab
30 °C, 800 μmol mol^{-1} CO_2	46.8 ± 1.2 a	3.6 ± 0.4 b	13.2 ± 0.6 a	46.8 ± 4.3 a	0.68 ± 0.07 b	69.0 ± 6.3 a	43.6 ± 2.4 a	1.6 ± 0.2 b	26.9 ± 1.9 ab
Temp	ns	0.001	0.000	ns	0.005	0.003	ns	ns	ns
CO_2	0.002	0.005	0.000	ns	0.003	0.000	ns	0.000	0.000
Temp × CO_2	0.042	0.033	0.000	ns	0.001	0.000	ns	0.000	0.000

[1]Means followed by the same letter(s) within a column are not significantly different from each other based on Tukey's HSD test at 5% level.

Table 4. Carbon and nitrogen uptake of *Platanus occidentalis* exposed to elevated temperature and carbon dioxide levels.[1]

Treatment	Carbon (g plant^{-1})			Nitrogen (g plant^{-1})		
	Shoots	Roots	Total	Shoots	Roots	Total
25 °C, 400 μmol mol^{-1} CO_2	1.81 ± 0.12 c	1.23 ± 0.12 b	3.04 ± 0.12 c	0.12 ± 0.03 a	0.06 ± 0.02 a	0.18 ± 0.02 a
30 °C, 400 μmol mol^{-1} CO_2	2.28 ± 0.14 b	0.70 ± 0.04 c	2.98 ± 0.08 c	0.07 ± 0.04 a	0.03 ± 0.02 a	0.10 ± 0.01 b
25 °C, 800 μmol mol^{-1} CO_2	2.52 ± 0.08 b	1.29 ± 0.14 b	3.81 ± 0.12 b	0.11 ± 0.02 a	0.04 ± 0.02 a	0.15 ± 0.02 a
30 °C, 800 μmol mol^{-1} CO_2	3.58 ± 0.13 a	1.4 5± 0.16 a	5.03 ± 0.15 a	0.12 ± 0.02 a	0.05 ± 0.01 a	0.17 ± 0.02 a
Temp.	0.041	ns	ns	ns	ns	ns
CO_2	0.000	0.042	0.044	ns	ns	ns
Temp. × CO_2	0.032	0.048	0.026	ns	ns	0.044

[1]Means followed by the same letter(s) within a column are not significantly different from each other based on Tukey's HSD test at 5% level.

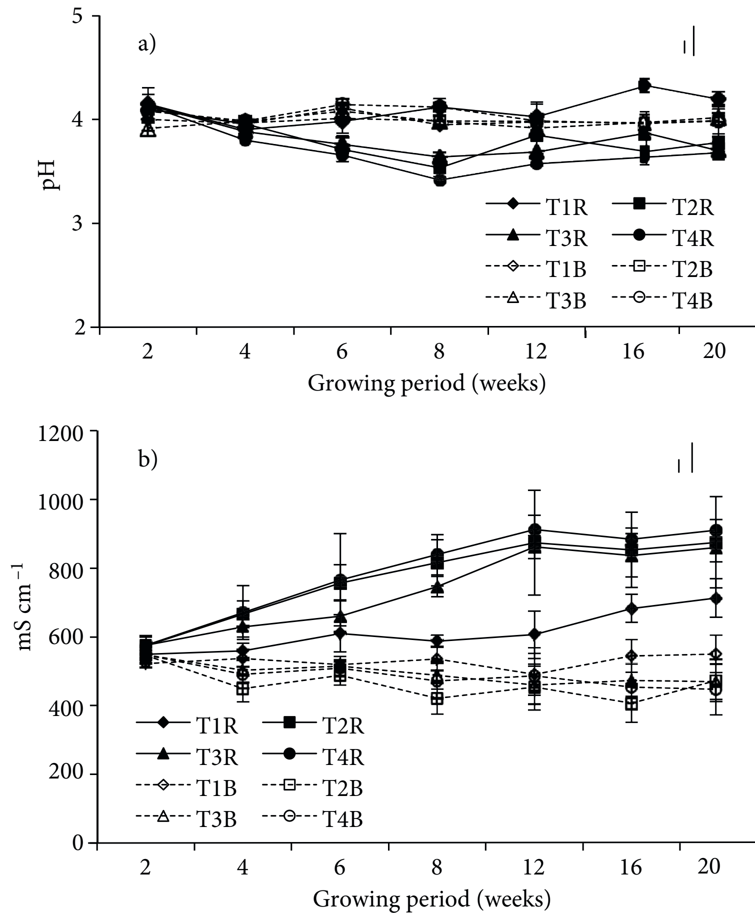

Figure 1. Soil solution pH (a) and soil solution electrical conductivity (b) from the rhizosphere and bulk soil of *P. occidentalis* grown in elevated temperature and CO_2 (T1: 25 °C, 400 µmol mol^{-1} CO_2; T2: 30 °C, 400 µmol mol^{-1} CO_2; T3: 25 °C, 800 µmol mol^{-1} CO_2; T4: 30 °C, 800 µmol mol^{-1} CO_2; R = rhizosphere, B = bulk). Bars in the right corner of the graphs represent the least significant difference (LSD) when comparing data points within the same sampling period (left) and between any data points (right). LSD value for pH: 0.125 and 0.215; EC: 86 and 128.

elevated temperature and CO_2 were significantly higher compared to the control (T1) and the bulk soil (Figure 1b).

The total sugar and dissolved oxidizable carbon (DOC) in the soil solution collected from the rhizosphere were relatively higher than those from the bulk soil; exposure of plants to elevated temperature and atmospheric carbon dioxide resulted in higher concentrations of sugars in soil solution, especially after 8 weeks of plant growth (Figure 2). The highest sugar and DOC concentrations were obtained in the rhizosphere of plants exposed to 30 °C, 400 µmol mol^{-1} CO_2, followed by samples obtained from the 30 °C, 800 µmol mol^{-1} CO_2 treatment.

3.3. Soil microbial activity and carbon utilization potential

To assess soil microbial activity and microbial community carbon utilization potential, Biolog EcoPlate data at 72 h of incubation were used. The Shannon–Weaver biodiversity index and the carbon utilization richness in the rhizosphere were significantly higher compared to the bulk soil ($P \leq 0.003$ and $P \leq 0.004$, respectively) and there was no interaction effect between temperature and CO_2. In the rhizosphere soils, the Shannon–Weaver index and the richness of substrate utilization of the soil microorganisms were significantly higher in the 800 µmol mol^{-1} CO_2 and 30 °C treatments compared to their counterparts (Table 5). On the other hand, the richness of substrate utilization of the soil microorganisms in rhizosphere exposed to high CO_2 treatments was significantly higher compared to their counterparts, regardless of the temperature levels (Table 5). AWCD from the rhizosphere was higher compared to those from the bulk soil ($P \leq 0.002$; data not shown) and in 72-h readings, soils exposed to 30 °C had higher AWCD

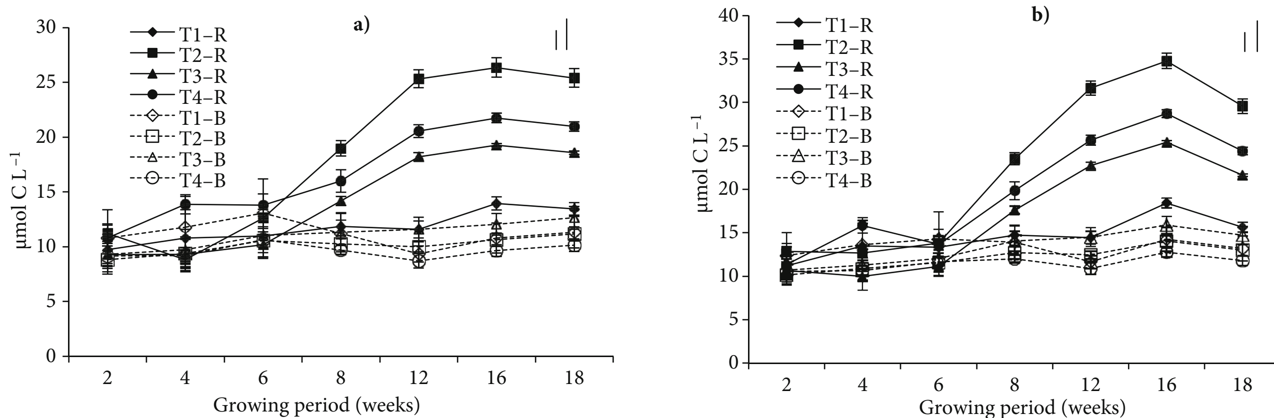

Figure 2. Concentration of total sugar (a) and total oxidizable organic carbon (b) in soil solution collected from the rhizosphere and bulk soil of *P. occidentalis* grown in elevated temperature and CO_2 (T1: 25 °C, 400 μmol mol^{-1} CO_2; T2: 30 °C, 400 μmol mol^{-1} CO_2; T3: 25 °C, 800 μmol mol^{-1} CO_2; T4: 30 °C, 800 μmol mol^{-1} CO_2; R = rhizosphere, B = bulk). Bars in the right corner of the graphs represent the least significant difference (LSD) when comparing data points within the same sampling period (left) and between any data points (right). LSD value for total sugar: 2.35 and 1.86; total oxidizable organic carbon: 2.45 and 2.10.

Table 5. Shannon–Weaver index, carbon utilization index, and AWCD based on optical density from Biolog EcoPlate analysis of rhizosphere soil of *P. occidentalis* grown in elevated temperature and CO_2 levels[1].

Treatments	Shannon			Richness			AWCD		
	Rhizo.	Bulk	R/B ratio	Rhizo.	Bulk	R/B ratio	Rhizo.	Bulk	R/B ratio
25 °C, 400 μmol mol^{-1} CO_2	3.25 ± 0.03 b	3.07 ± 0.03 a	1.06 ± 0.03 b	17.78 ± 0.18 c	16.78 ± 0.24 a	1.06 ± 0.02 b	1.06 ± 0.03 b	1.18 ± 0.04 a	0.90 ± 0.03 b
30 °C, 400 μmol mol^{-1} CO_2	3.24 ± 0.02 b	3.07 ± 0.02 a	1.09 ± 0.02 ab	18.56 ± 0.13 b	16.78 ± 0.28 a	1.11 ± 0.02 a	1.21 ± 0.04 a	0.98 ± 0.02 a	1.23 ± 0.03 a
25 °C, 800 μmol mol^{-1} CO_2	3.45 ± 0.02 a	3.07 ± 0.04 a	1.12 ± 0.03 a	17.67 ± 0.20 c	16.77 ± 0.33 a	1.05 ± 0.01 b	1.04 ± 0.04 b	1.07 ± 0.03 a	0.98 ± 0.03 b
30 °C, 800 μmol mol^{-1} CO_2	3.45 ± 0.03 a	3.04 ± 0.03 a	1.14 ± 0.03 a	20.22 ± 0.18 a	17.56 ± 0.25 a	1.15 ± 0.02 a	1.34 ± 0.02 a	0.99 ± 0.03 ab	1.35 ± 0.02 a
Temp.	ns	ns	0.002	0.000	ns	0.002	0.000	0.000	0.000
CO_2	0.03	ns	0.001	0.002	ns	0.000	0.000	0.000	0.000
Temp. × CO_2	0.02	ns	0.030	0.000	ns	ns	ns	0.020	ns

[1]Means followed by the same letter(s) within a column are not significantly different from each other based on Tukey's HSD test at 5% level.

readings regardless of the CO_2 levels (Table 4). The ratio of the Shannon–Weaver index of the rhizosphere to the bulk soil showed higher values in the high CO_2 treatment while the rhizosphere:bulk soil ratios, in terms of richness and AWCD, were significantly higher in 30 °C treatments, regardless of CO_2 levels.

Principal component analysis of logarithmic-transformed Biolog EcoPlate readings at 72 h resulted in a clustering of samples based on substrate utilization potential of rhizosphere organisms as affected by elevated temperature and CO_2. The first and the second principal components (PC1 and PC2) explained 42.17% and 17.87% of the data variance, respectively (Figure 3). Samples from the 800 μmol mol^{-1} CO_2 treatments were clustered toward the positive side of PC2, while those from 400 μmol mol^{-1} CO_2 treatments were clustered toward the negative side of PC2. PC1 separated the samples due to temperature effects on soils collected from 800 μmol mol^{-1} CO_2 treatments. The carbon substrates that contributed largely to extracted principal components based on the loading factor were D-galactonic acid γ-lactone, 4-hydroxybenzoic acid, D-mannitol, L-asparagine, and L-phenylalanine for PC1. For PC2, the carbon substrates that contributed to the extracted PC were D-galactonic-γ-lactone acid, Tween 40, 4-hydroxybenzoic acid, D-glucosaminic acid, and L-asparagine.

3.4. Correlations of plant and soil properties with soil microbial properties

Correlations of plant and soil parameters with soil microbial properties as determined by Biolog EcoPlate are

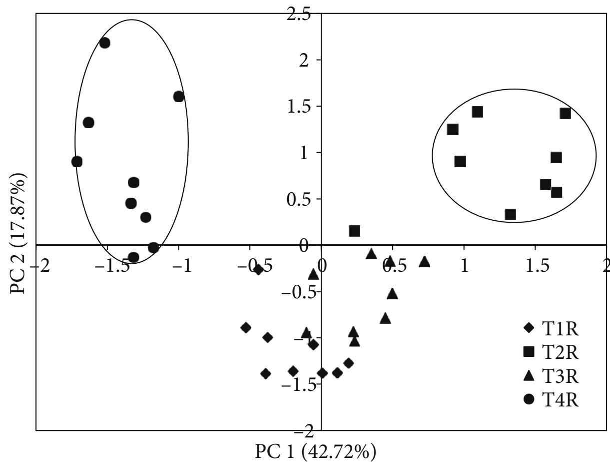

Figure 3. The biplot of PCA of optical density from Biolog EcoPlate analysis of rhizosphere soil of *P. occidentalis* grown in elevated temperature and CO_2. For legend description, please see Figure 1.

presented in Table 6. The Shannon–Weaver index positively correlated with stem and root C concentrations and the C/N ratio in stems and roots, but negatively correlated with soil solution pH. The substrate utilization richness had a negative correlation with the root dry weight, total dry yield, root C concentration, and root and total C uptake, but was positively correlated with soil solution EC. The AWCD and the extracted PC1 from the principal component analysis of rhizosphere soils negatively correlated with root, shoot, and total dry weights and root collar diameter, root C concentration, root N and C uptake, shoot C uptake, and total N and C uptake, but positively correlated with soil solution EC, total dissolved sugar, and total dissolved oxidizable carbon. PC2 had positive correlations with RGR, shoot dry weight, shoot/root dry yield, leaf and stem C/N ratio, total dissolved sugar, and total dissolved organic carbon, and soil EC. It showed negative correlations with leaf and stem N concentration and soil and soil solution pH.

4. Discussion

The growth response of trees to elevated temperature and atmospheric CO_2 depends on many factors, such as water and nutrient availability, the timing of the warming, the ability of the species to acclimate to new growing conditions (including the concentration of CO_2 and other atmospheric conditions), and how close trees already are to their thermal optimum for growth (Way and Oren, 2010, Dieleman et al., 2012). *Platanus occidentalis* responded positively to elevated temperature and CO_2 in terms of enhanced growth. However, for this fast-growing broadleaf tree, the interaction effects of elevated temperature and CO_2 were observed as manifested by variable plant heights and chlorophyll content. Elevated CO_2, or the combination of elevated CO_2 and temperature, increased growth rate and high pigment content, while high temperature alone increased plant height and lowered the chlorophyll content. Similarly, the dry matter yield of *P. occidentalis* showed an overall positive response to elevated CO_2 and temperature, while the shoot and total dry matter yield were increased synergistically by high CO_2 concentration and by elevated temperature. However, the root dry yield was reduced by elevated temperature alone, which resulted in the highest shoot/root dry yield ratio. This growth response of *P. occidentalis* is common for deciduous species, where the increase in shoot height growth is greater than increases in stem mass, implying that stem elongation, rather than general stem growth, is most affected at higher temperatures (Veteli et al., 2002; Wang et al., 2012; Borjigidai and Yu, 2013). This observation is supported by the much weaker response of stem diameter to temperature; stem diameter growth could only be doubled at high growth temperatures, while stem height growth increased up to eight-fold in the most extreme case of high temperature, leading to less tapered stems (Way and Oren, 2010). Myster and Moe (1995) observed similar responses in some horticultural species to warmer days, in which increases in day temperatures increased cell elongation but not cell width in the stems of many flowering plants. In addition, elevated temperature alone resulted in lower root biomass and high shoot/root dry matter ratio. Combined elevated temperature and CO_2 increased both the shoot and root dry matter. Plants exposed to both elevated temperature and CO_2 also had relatively high shoot/root ratios compared to either the control or those plants only exposed to elevated CO_2. This result demonstrated how the interaction of temperature and CO_2 impacted the plant growth of *P. occidentalis* by altering allocation between above- and below-ground components. Increase in temperature would favor shoot development more than root growth, but the combined effect of elevated temperature and CO_2 would counteract these shifts in growth trends (i.e. a lower root-to-shoot ratio). Previous studies on different plant species revealed no significant temperature effect on root dry matter yield, but nonetheless resulted in high shoot/root dry yield ratios (Veteli et al., 2002; Bronson et al., 2008; Burton et al., 2008; Wang et al., 2012). This observation was attributed to increased leaf mass and leaf surface area with increasing growth temperature, especially at growth temperatures above the current growing temperatures (Sperry et al., 1998; Ewers et al.. 2000; Hacke et al., 2000; Addington et al., 2006; Wang et al., 2012).

The carbon concentration and uptake increased at higher CO_2 concentrations, especially at elevated

Table 6. Correlations between plant and soil properties with the extracted principal components (PCs) at the end of the study from treatments exposed to elevated temperature and atmospheric carbon dioxide in combination. PC1 and PC2 are derived from principal component analysis of the microbial community substrate utilization potential of rhizosphere soil of *P. occidentalis* based on Biolog EcoPlate absorbance data after 72 h of incubation. (n = 12)

Parameters	PC1	PC2	Shannon–Weaver diversity index	Substrate utilization richness	AWCD
Plant parameters					
RGR	–0.123 ns	0.760**	–0.077 ns	–0.203 ns	–0.087 ns
Root dry weight	–0.821**	–0.174 ns	–0.088 ns	–0.694*	–0.867**
Shoot dry weight	–0.625*	0.601*	0.020 ns	–0.467 ns	–0.590*
Total dry yield	–0.789**	0.408 ns	–0.016 ns	–0.618*	–0.778**
Shoot:root dry yields	0.269 ns	0.706*	–0.052 ns	0.179 ns	0.321 ns
Root collar	–0.664*	–0.286 ns	0.090 ns	–0.555 ns	–0.731**
Nitrogen and carbon concentration					
Leaf-N	0.233 ns	–0.703*	0.109 ns	0.250 ns	0.223 ns
Leaf-C	–0.365 ns	0.347 ns	0.166 ns	–0.223 ns	–0.333 ns
Leaf C/N ratio	–0.392 ns	0.687*	–0.049 ns	–0.341 ns	–0.374 ns
Stem N	0.336 ns	–0.660*	–0.239 ns	–0.016 ns	0.228 ns
Stem C	–0.217 ns	–0.147 ns	0.640*	0.061 ns	–0.218 ns
Stem C/N ratio	–0.477 ns	0.621*	0.613*	0.035 ns	–0.360 ns
Root N	0.128 ns	–0.422 ns	–0.059ns	0.189 ns	0.144 ns
Root C	–0.633*	–0.380 ns	0.624*	–0.641*	–0.711**
Root C/N ratio	–0.285 ns	0.180 ns	0.702*	–0.375 ns	–0.337 ns
Nitrogen and carbon uptake					
Root N	–0.704*	–0.453 ns	–0.098 ns	–0.524 ns	–0.736**
Root C	–0.815**	–0.195 ns	–0.106 ns	–0.714**	–0.867**
Shoots N	–0.454 ns	0.570 ns	0.019 ns	–0.345 ns	–0.406 ns
Shoots C	–0.640*	0.574 ns	0.082 ns	–0.445 ns	–0.603*
Total N	–0.737**	0.341 ns	–0.025 ns	–0.555 ns	–0.706*
Total C	–0.792**	0.379 ns	0.026 ns	–0.603*	–0.782**
Soil solution					
Total dissolved sugar	0.396 ns	0.840**	–0.013 ns	0.637*	0.660*
Total dissolved organic C	0.404 ns	0.840**	–0.012 ns	0.265*	0.670*
pH	0.341 ns	–0.798**	–0.654*	0.245 ns	–0.348 ns
EC	0.587*	0.324 ns	0.065 ns	0.683*	0.687*
Soil pH	–0.253 ns	–0.628*	0.316 ns	0.156 ns	–0.218 ns
Soil EC	0.412 ns	0.676*	0.034 ns	0.404 ns	0.519 ns

*P < 0.05, **P < 0.01, ns = not significant.

temperatures, while the nitrogen concentration and uptake were significantly reduced in plant tissues. Consequently, the C/N ratios were increased due to elevated temperature and CO_2. Such a decrease in N concentration in tissues under elevated CO_2 is a consequence of dilution by carbohydrate accumulation, leaf structural material, and increases in plant internal demands for N (Ellsworth et al., 2004). Furthermore, N dilution may cause the immobilization of C in source tissues, leading to carbohydrate accumulation. Such changes of the C and N allocation in plant tissues could have a profound effect on the capability of plants to sustain the positive effects of elevated temperature and CO_2 in plant growth (Dieleman et al., 2012). Similarly, such changes would impact the below-ground environment by altering the C, N, and nutrient dynamics in the rhizosphere through increased flux of carbon into the soil and increased nutrient uptake (Drake et al., 2011). Such changes are important in identifying strategies in mitigating the rising atmospheric carbon dioxide and climate change as they bear significance in carbon and nutrient dynamics in forest ecosystems.

Although soil properties could be altered by changes in environmental conditions in the absence of growing plants, our results showed that exposure of *P. occidentalis* to elevated temperatures and CO_2 altered the soil properties, especially in the rhizosphere, similar to our previous results involving *P. densiflora* (Ultra et al., 2012). In this study, acidification in the rhizosphere of *P. occidentalis* exposed to elevated temperature could be attributed to enhanced production of organic acids and CO_2 by microbial activity as a result of enhanced root exudation as indicated by high dissolved sugar and DOC content in the rhizosphere. In addition, enhanced N uptake caused by elevated temperature and CO_2 could lead to rhizosphere acidification, especially because the main source of N applied in this experiment was urea, which hydrolyzes to ammonium (Marschner, 2012). The reduction of electrical conductivity of the soil solution could be attributed in part to the increase in solute concentration as a consequence of high mineralization rate and the priming effect of root exudates on OM mineralization in the rhizosphere, as triggered by elevated temperature and CO_2 (Phillips et al., 2012). Several studies have indicated that increased organic matter mineralization is associated with an increase in solute concentration from mineralized organic substrates and ion displacement from exchange sites due to organic acids released during decomposition (Stevenson, 1994; Cao et al., 1995; Drever and Stillings, 1997; Gobran et al., 1998; Turpault et al., 2007; Wood et al., 2012).

Dissolved organic matter in soil solution was principally produced by microbial activity, root exudates, and organic matter leachate, while the dissolved sugar is produced by plants as root exudates and secondarily from degradation of fresh organic materials by microbial activity (Grayston et al., 1996; Zhu and Cheng, 2011). Exposure of *P. occidentalis* to an elevated temperature resulted in the highest concentration of dissolved organic matter and total sugar in the rhizosphere's soil solution, followed by combined elevated temperature and CO_2, especially after 8 weeks of plant growth. This is in contrast to our previous results with *P. densiflora*, where the highest dissolved organic matter and sugar concentrations in soil solution were observed in plants exposed to combined elevated temperatures and CO_2 (Ultra et al., 2012). It appears that elevated temperature had promoted greater root exudation of *P. occidentalis* than when these plants were exposed to a combination of elevated temperature and CO_2 or CO_2 alone. This result is in agreement with Haase et al. (2007). In addition, it also showed that elevated temperature enhanced the decomposition and mineralization of organic matter, as evidenced by high DOC concentration and EC of soil solution.

Based on the short-term response of *P. occidentalis* seedlings to elevated temperature and CO_2, it is apparent that there were changes in microbial activity and carbon utilization potential of the microbial community in the rhizosphere. These changes could be attributed in part to the response of plants to environmental conditions and their subsequent rhizosphere inducement. Our data showed that temperature effects were more profound than elevated CO_2 in terms of the microbial activity, as indicated by AWCD and the richness of metabolized carbon, and such effects were compounded by increased CO_2 concentration. This could be due to enhanced root exudation and OM mineralization in soil, as indicated by positive correlations of AWCD and substrate utilization richness with total dissolved sugar and total dissolved oxidizable carbon. However, negative correlations of AWCD and substrate utilization richness with biomass yield, root carbon concentration, and N and C concentrations and uptake would indicate that the microbial activity is also negatively affected by plant growth, probably due to nutrient competition caused by enhanced nutrient removal via plant uptake (Castro et al., 2012). In contrast, elevated CO_2 had more influence on the Shannon–Weaver index than temperature, as estimated from the substrate utilization potential. Carbon utilization richness and AWCD, on the other hand, were influenced more by temperature than the increased CO_2. This shift in activity and substrate utilization potential of soil microorganisms is related to changes in soil chemical conditions and quantity and quality of root exudates as reflected by significant correlations with soil solution pH and the C and C/N ratios of roots (Table 5).

The biplot of PC1 and PC2 of the Biolog EcoPlate data shows the discrepancies of the soil microbial community

in terms of the microbial carbon utilization potential in the rhizosphere soil as affected by the exposure of *P. occidentalis* to elevated temperature and CO_2. The separation of soil samples between T2 and T4 indicates temperature's effect on substrate utilization at high CO_2 concentrations and could be the induced rhizosphere effect of temperature on the extent of plants' nutrient removal modifying the overall biochemical properties of the soil. This is indicated by the significant negative correlations of PC1 with dry matter yield and N and C concentration and uptake of *P. occidentalis*. On the other hand, PC2 was positively correlated with growth rate (0.760), C/N ratios of tissues (0.687), and the total dissolved sugar and oxidizable carbon in soil solution (0.84). PC2 seems to be related with the induced change in substrate quantity and quality deposited by root activity on the rhizosphere.

Overall, the results showed that the changes in plant growth and physiology of *P. occidentalis* seedlings due to exposure to elevated temperature and carbon dioxide extended to the soil biological properties via plant-induced changes in soil nutrients as a consequence of altered growth pattern and alteration of C and N partitioning in plants that results in changes in quality and quantity of root exudates and root turnover in the rhizosphere. Such changes are dependent on plant species and the prevailing growth conditions. This study also demonstrated that elevated temperature and carbon dioxide can affect microbial properties that may offer feedback to other processes in an ecosystem, as indicated by differences in the potential functional diversity of microbial communities estimated by community-level physiological profile using the Biolog EcoPlate.

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