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Effects of Long-term Exposure to Black Carbon Particles on Growth and Gas Exchange Rates of *Fagus crenata*, *Castanopsis sieboldii*, *Larix kaempferi* and *Cryptomeria japonica* Seedlings

Masahiro Yamaguchi, Yoko Otani[1)], Kenta Takeda[1)], I. Wuled Lenggoro[2)], Atsushi Ishida[3)], Kenichi Yazaki[4)], Kyotaro Noguchi[5)], Hiroyuki Sase[6)], Naoto Murao[7)], Satoshi Nakaba[8)], Kenichi Yamane[8)], Katsushi Kuroda[4)], Yuzou Sano[9)], Ryo Funada[8)] and Takeshi Izuta[8),*]

Faculty of Agriculture, Tokyo University of Agriculture and Technology, 3-5-8 Saiwaicho, Fuchu, Tokyo 183-8509, Japan
[1)]Graduate School of Agriculture, Tokyo University of Agriculture and Technology, 3-5-8 Saiwaicho, Fuchu, Tokyo 183-8509, Japan
[2)]Institute of Engineering, Tokyo University of Agriculture and Technology, 2-24-16 Nakacho, Koganei, Tokyo 184-8588, Japan
[3)]Center for Ecological Research, Kyoto University, 509-3, 2-chome, Hirano, Otsu, Shiga 520-2113, Japan
[4)]Forestry and Forest Products Research Institute, 1 Matsunosato, Tsukuba, Ibaraki 305-8687, Japan
[5)]Shikoku Research Center, Forestry and Forest Products Research Institute, Kochi, Kochi 780-8077, Japan
[6)]Asia Center for Air Pollution Research, 1182 Sowa Nishi-ku, Niigata-shi, Niigata 950-2144, Japan
[7)]Graduate School of Engineering, Hokkaido University, Kita 13, Nishi 8, Kita-ku, Sapporo, Hokkaido 060-8628, Japan
[8)]Institute of Agriculture, Tokyo University of Agriculture and Technology, 3-5-8 Saiwaicho, Fuchu, Tokyo 183-8509, Japan
[9)]Graduate School of Agriculture, Hokkaido University, Kita 9, Nishi 9, Kita-ku, Sapporo, Hokkaido 060-8589, Japan

*Corresponding author. Tel: +81-42-367-5728, E-mail: izuta@cc.tuat.ac.jp

ABSTRACT

To clarify the effects of black carbon (BC) particles on growth and gas exchange rates of Asian forest tree species, the seedlings of *Fagus crenata, Castanopsis sieboldii, Larix kaempferi* and *Cryptomeria japonica* were exposed to BC particles with sub-micron size for two growing seasons from 1 June 2009 to 11 November 2010. The BC particles deposited after the exposure to BC were observed on the foliar surface of the 4 tree species. At the end of the experiment, the amount of BC accumulated on the foliar surface after the exposure to BC aerosols were 0.13, 0.69, 0.32 and 0.58 mg C m^{-2} total leaf area in *F. crenata, C. sieboldii, L. kaempferi* and *C. japonica* seedlings, respectively. In August 2010, the exposure to BC particles did not significantly affect net photosynthetic rate under any light intensity, stomatal diffusive conductance to water vapour (g_s), stomatal limitation of photosynthesis, response of g_s to increase in vapour pressure deficit and leaf temperature under light saturated condition in the leaves or needles of the seedlings. These results suggest that the BC particles deposited on the foliar surface did not reduce net photosynthesis by shading, did not increase leaf temperature by absorption of irradiation light, and did not induce plugging of stomata in the leaves or needles of the seedlings. There were no significant effects of BC particles on the increments of plant height and stem base diameter during the experimental period and the whole-plant dry mass at the end of the experiment. These results indicate that the exposure to BC particles with sub-micron size for two growing seasons did not significantly affect the growth and leaf or needle gas exchange rates of *F. crenata, C. sieboldii, L. kaempferi* and *C. japonica* seedlings.

Key words: Forest tree species, Black carbon particles, Exposure, Growth, Gas exchange rates, Stomatal diffusive conductance

1. INTRODUCTION

Recently, East Asian countries face the problem of transboundary air pollution including aerosol particles and its effects on vegetation (Izuta and Funada, 2010). The aerosol particles present in the atmosphere vary in size from a few nm to a few hundred μm in diameter (Chin *et al*., 2007). The sub-micron sized particles are originated mainly from the anthropogenic sources (e.g. combustion) (Kasahara, 2004; Colvile, 2002). Because the particles with sub-micron size deposit quite slowly to terrestrial surfaces, sub-micron sized particles have relatively long atmospheric lifetimes in the absence of precipitation and are transboundary air pollutant (Fowler, 2002; Colvile, 2002). Furthermore, the deposition rates onto forests are substantially larger than those

onto short vegetation (Matsuda *et al*., 2010; Petroff *et al*., 2008; Fowler, 2002; Ruijgrok *et al*., 1997). Therefore, it is necessary to clarify the effects of sub-micron sized aerosol particles on growth and physiological functions of East Asian forest tree species.

Limited information on the effects of sub-micron sized aerosol particles on plants is available at the present time. Burkhardt *et al*. (2001) reported that the exposure to $NaNO_3$ particles (0.5-1.0 μm in diameter) for 3 to 4 hours increased transpiration rate in the leaves of *Sambucus nigra*. Hirano *et al*. (1995, 1991) reported that the exposure of *Cucumis sativus* and *Phaseolus vulguris* to black carbon (BC) particles within 3 min. reduced net photosynthetic rate by shading, and increased leaf temperature by absorption of irradiation light. These authors used BC with primary particle sizes of 0.03-0.2 μm (SEM-based sizes), and based on the fluidized bed-type aerosol generator they used, their system dispersed particles over a size range from sub-μm to few ten μm. However, there is no information on the long-term effects of sub-micron sized aerosol particles on growth and physiological functions such as photosynthesis of forest tree species.

The BC is a component of fine particulate matter. The main sources of BC are combustion of fossil fuels and biomass burning (WHO, 2012). The major source regions of BC are developing nations in the tropics and East Asia (Ramanathan and Carmichael, 2008). In Asian region, furthermore, it is predicted that BC emissions will increase (Ohara *et al*., 2007). To evaluate the effects of anthropogenic aerosol particles such as BC particles on forest ecosystems in East Asia, in the present study, we investigated the effects of BC particles with sub-micron size on growth and physiological functions of forest tree species native to East Asia.

2. MATERIALS AND METHODS

2.1 Plant Materials

On 8 May 2009, 3-year-old seedlings of *Fagus crenata* (deciduous broad-leaved tree), 2-year-old seedlings of *Castanopsis sieboldii* (evergreen broad-leaved tree), 1-year-old seedlings of *Larix kaempferi* (deciduous conifer) and 1-year-old seedlings of *Cryptomeria japonica* (evergreen conifer) were individually planted in 2 L pots filled with Kanuma pumice soil. During the experimental period from 1 June 2009 to 11 November 2010, the seedlings were grown in 6 phytotron chambers (around 2 × 2 × 2 m glasshouse with an air-conditioner system) located at Tokyo University of Agriculture and Technology (Fuchu, Tokyo, Japan). In the chambers, the seedlings received natural day length, and washing out of any particles deposited on the foliar surface was avoided. For each tree species, 10 seedlings were assigned to each chamber, for a total of 60 seedlings per species. The whole-plant dry mass, plant height and stem base diameter of the seedlings at the beginning of the experiment were 11.7 ± 3.3 g, 31.0 ± 3.7 cm and 6.6 ± 0.8 mm for *F. crenata*, 13.0 ± 2.9 g, 35.0 ± 3.4 cm and 4.7 ± 0.5 mm for *C. sieboldii*, 2.9 ± 0.8 g, 19.9 ± 2.9 cm and 4.1 ± 0.5 mm for *L. kaempferi*, and 2.2 ± 0.9 g, 19.2 ± 3.1 cm and 3.3 ± 0.6 mm for *C. japonica*, respectively.

In the chambers, air temperature and relative air humidity were maintained at 25.0 ± 1.0/18.0 ± 1.0°C (6:00-18:00/18:00-6:00) and 70 ± 5%, respectively, from 1 June to 7 December 2009 and from 17 April to 11 November 2010. From 7 January to 19 March 2010, the seedlings were grown under field condition to induce winter dormancy. During the winter dormancy period, to avoid washing out of any particles deposited on the leaf or needle surface, all the seedlings were protected from natural precipitation and snowfall by a transparent polyvinylchloride roof on rainy and snowy days. For approximately one month before and after the winter dormancy period, to acclimatize the seedlings to field condition, air temperature and relative air humidity in the chambers were maintained at 17.0 ± 1.0/12.0 ± 1.0°C (6:00-18:00/18:00-6:00) and 60 ± 5%, respectively. During the growth period, all the seedlings were irrigated as necessary with tap water and fertilized at the two-week intervals with 200 mL of liquid fertilizer (HYPONeX, N : P : K=6% : 10% : 5%, Hyponex Japan Co. Ltd., Japan) diluted 2,000 times.

2.2 Black Carbon Exposure

The seedlings were exposed to black carbon (BC) particles with sub-micron size generated by an aerosol generator system newly developed for this study. The system is based on an electrostatic-spray (Lenggoro *et al*., 2002) running for 10 min. and an ultrasonic nebulizer (Wang *et al*., 2008) running for 5 min. using suspension of BC particles. The source of BC is a combustion-derived nanopowders (suspension) with primary particle size around 30 nm, and mainly consisting of elemental carbon. The size distributions of generated and suspended aerosols in the dry condition (i.e. the solid BC particles) were measured by a real-time technique based on a differential mobility analyzer (DMA) method (Wang *et al*., 2008; Lenggoro *et al*., 2002). The measured mean size of BC particles was between 100-300 nm. This result indicates that the generated (dry) aerosols are formed by the aggregation of BC particles having primary particle size of around 30 nm. From 13 June 2009 to 6 January 2010 and from 21 March 2010 to 12 June 2010, the half of the seedlings were exposed to BC particles every two days with-

in 6:00-9:00 (BC treatment), and the other half of the seedlings were not exposed to the particles (control treatment). Because the deposition efficiency of submicro-meter sized aerosols is low, the BC particles were driven against the seedlings under windless condition in the present study. The windless condition was achieved by stopping the air circulation and air-conditioner system. To avoid the adverse effect of high air temperature on the seedlings during the stopping the system, it is necessary to conduct the BC exposure during the time when ambient air temperature and sunlight intensity were low. On the other hand, it was reported that aerosol particles has plugging effect on stomata (Hirano *et al*., 1995; Flückiger *et al*., 1979; Ricks and Williams, 1974). Because light stimulates stomatal opening (Roelfsema and Hedrich, 2005), exposure to BC was conducted within 6:00-9:00 to clarify the plugging effect of sub-micron sized BC particles on stomata. From 7 January to 20 March 2010, BC exposure could not be conducted, because the seedlings were not enclosed during winter dormancy period as mentioned above. At the end of the first growing season, the amount of BC deposited on foliar surface (M_{BC}) of the seedlings were lower than that observed in the field which may include particles larger than few micrometers (e.g. Matsuda *et al*., 2012). To achieve the greater M_{BC}, therefore, the exposure time was extended to 5 times and the seedlings grown in the BC treatment were exposed to BC particles every day within 6:00-9:00 from 13 June to 31 October 2010. In each treatment, 3 chamber replications for a total of 6 chambers were used.

2. 3 Measurements of Plant Growth

On 1 June 2009 and 1 November 2010, plant height and stem base diameter of all the seedlings were measured. Increments of plant height and stem base diameter during the experimental period were differences between plant height and stem base diameter on 1 June 2009 and those on 1 November 2010. On 12-17 November 2010, all the seedlings were harvested. The harvested seedlings were separated into the plant organs. The separated plant organs of the harvested seedlings were dried at 80°C in an oven for 1 week and weighed.

2. 4 Observation of BC Particles Deposited on the Foliar Surface

On 12-17 November 2010, the leaves or needles of *F. crenata, C. sieboldii, L. kaempferi* and *C. japonica* seedlings, which were exposed to BC in the growth chambers, were harvested to observe the BC particles deposited on the surface by field-emission scanning electron microscopy (FE-SEM). The harvested leaves and needles were air-dried in desiccators, fixed onto specimen stubs for FE-SEM and coated with platinum-palladium using a sputter coater or with platinum by vacuum evaporation. The foliar surface was observed under field-emission scanning electron microscopes (S-4800, HITACHI) at 0.5 to 2.5 kV.

2. 5 Quantification of BC Particles Deposited on Foliar Surface

On 12-17 November 2010, the leaves or needles of the harvested seedlings were washed with distilled water followed by the washing with chloroform for 20 seconds. To quantify M_{BC}, the particles suspended in the distilled water and chloroform were then collected on a quartz fiber filter (QR-100, Advantec MFS, Inc., Japan) by filtration. Filtration efficiency of BC particles suspended in water was considered to be more than 80% (Matsuda *et al*., 2012) and that in chloroform was confirmed as more than 90% according to the method of Matsuda *et al*. (2012) (data not shown). To determine the amounts of BC particles collected on the quartz fiber filter, the absorbance at 580 nm of the quartz fiber filters (A_{580}) were measured by a spectrophotometer with an integrating sphere (U-4100, Hitachi High Technologies Corp., Japan). In the quantification of BC, a calibration line was made between A_{580} of the filter and amount of BC collected on the filters measured by the thermal optical reflectance method using a DRI OC/EC carbon analyzer (Model 2001A). The M_{BC} was expressed on the basis of total leaf surface area. Leaf area of *F. crenata* and *C. sieboldii* seedlings was measured with an area meter (Hayashi Denko Co. Ltd., Japan). Leaf area of *L. kaempferi* seedlings was determined by image analysis software (LIA32, http://www.agr.nagoya-u.ac.jp/~shinkan/LIA32/index.html). Needle surface area of *C. japonica* was determined by the method of Sase *et al*. (1998).

2. 6 Leaf Gas Exchange Rates

To determine leaf or needle gas exchange rates, 3 seedlings per treatment-chamber combination were randomly selected for the analyses (9 determinations per treatment). Gas exchange rates of the leaves or needles of *F. crenata, C. sieboldii* (current-year leaves), *L. kaempferi* and *C. japonica* (current-year needles) were measured from 25 August to 4 September 2010. The measurements were performed using an infrared gas analyzer system (LI-6400, Li-Cor Inc., NE, U.S.A). Light-saturated net photosynthetic rate (A_{sat}), stomatal diffusive conductance to H_2O (g_s) and leaf temperature (T_{leaf}) were determined at air temperature of 25.0 ± 0.1 °C, 380 μmol CO_2 mol^{-1}, relative air humidity of 70 ± 5% and photosynthetic photon flux density (*PPFD*) of 1500 μmol m^{-2} s^{-1}. To obtain photosynthetic light-

response curve, net photosynthetic rates (A) were measured under different *PPFD* at the foliar surface ranging from 1500 to 0 μmol m^{-2} s^{-1}. On 16-24 August 2010, stomatal limitation of photosynthesis (L_s) and response of g_s to increase in vapour pressure deficit (*VPD*) were measured in the leaves or needles of *F. crenata, C. sieboldii, L. kaempferi* and *C. japonica*. To examine the relative limitation of net photosynthetic rate by stomatal diffusive resistance, the response of A to change in the intercellular CO_2 concentration (C_i) was measured by varying inlet CO_2 concentration from 0 to 500 μmol CO_2 mol^{-1}. Carboxylation efficiency (*CE*) was calculated from the linear relationship between A and C_i ranging from 0 to 400 μmol CO_2 mol^{-1}. The L_s was calculated by the following function:

$$L_s(\%)=[(A_o-A_{380})/A_o]\times 100$$

where A_o is the A estimated from *CE* and the assumption that C_i was equal to 380 μmol CO_2 mol^{-1}, and A_{380} is the A measured at inlet CO_2 concentration of 380 μmol CO_2 mol^{-1}. To examine the stomatal response to increase in *VPD* ($dg_s/dVPD$), the reduction rate of g_s with increase in leaf-to-air *VPD* from 1.5 to 3.5 kPa was measured by varying inlet H_2O concentration. The $dg_s/dVPD$ was calculated as slope of the regression line between vapour pressure deficit and relative value of g_s (g_s determined at 1.5 kPa *VPD* was defined as 1.0).

2.7 Statistical Analyses

Statistical analyses were performed with the IBM® SPSS® Advanced Statistics 19. To identify significant difference between the mean of 3 chamber replications in the control treatment and that in the black carbon treatment, paired t-test was performed ($p<0.05$).

3. RESULTS AND DISCUSSION

The FE-SEM images of foliar surface of *F. crenata, C. sieboldii, L. kaempferi* and *C. japonica* seedlings grown in black carbon (BC) treatment were shown in Fig. 1. The BC particles deposited after the BC exposure were observed in all the tree species. The amount of BC particles deposited on the foliar surface (M_{BC}) of *F. crenata, C. sieboldii, L. kaempferi* and *C. japonica* seedlings was indicated in Fig. 2. In all the tree species, the M_{BC} of the seedlings grown in the BC treatment was higher than that of the seedlings grown in the control treatment. These results indicate that the aerosol exposure chamber and generation system of BC particles with sub-micron size adopted in the present study is useful for relatively long-term experimental study on the effects of aerosol particles on the seedlings of forest tree species.

In the present study, the degree of increase in M_{BC} by the exposure to BC particles was little as compared with M_{BC} in the control treatment (Fig. 2). Because the

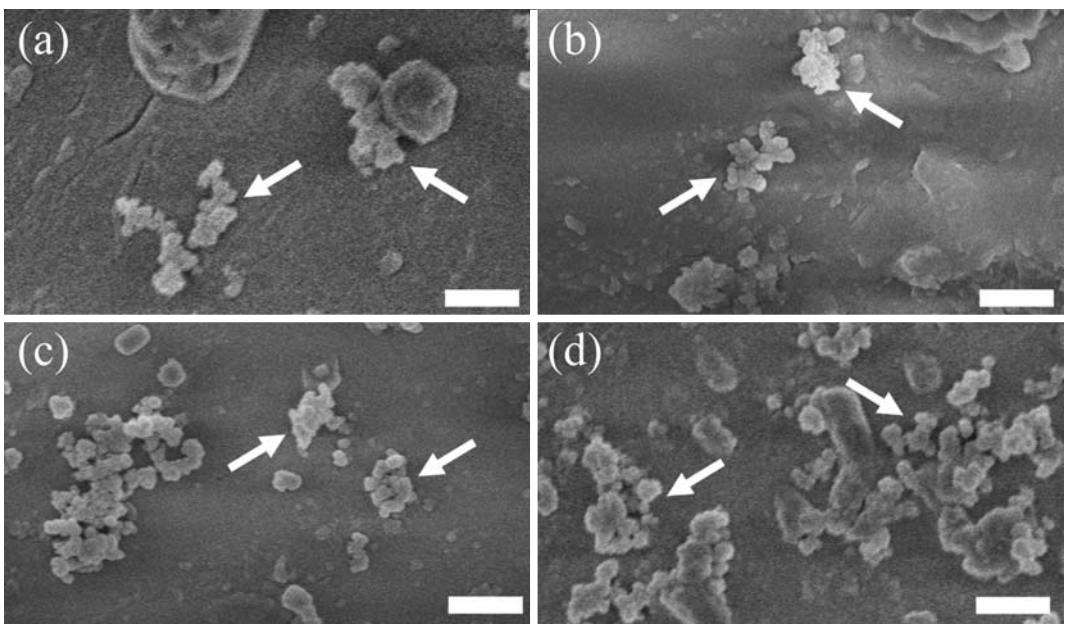

Fig. 1. Field-emission scanning electron micrographs showing black carbon (BC) particles deposited on the leaves or needles of (a) *F. crenata*, (b) *C. sieboldii*, (c) *L. kaempferi* and (d) *C. japonica* seedlings grown in the BC treatment. Arrows indicate the BC particles originated from the BC exposure. Bars=500 nm.

seedlings were grown in the glasshouse chambers, washing out of any particles deposited on the leaf or needle surface was avoided. Furthermore, the upper direction wind was always blowing (at most 0.5 m s^{-1}) in the chamber except for the exposure time, resulting in constant deposition of ambient BC, including both fine (below 1 μm) and coarse (above 1 μm) particles, onto the foliar surface of the seedlings. As a result, weight-based M_{BC} of the seedlings grown in the control treatment was relatively high (Fig. 2). On the other hand, difference in M_{BC} between the control treatment and the BC treatment can be considered as the M_{BC} accumulated by the exposure to BC particles. The M_{BC} accumulated by the exposure to BC particles consisted of mainly fine particles. As compared with M_{BC} in the control treatment, therefore, the weight-based M_{BC} accumulated by the exposure to BC particles was relatively low.

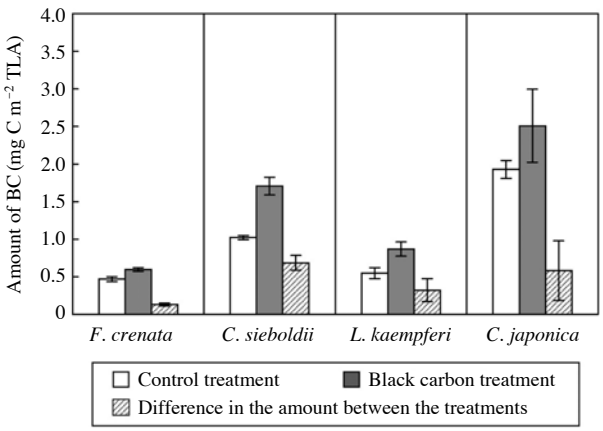

Fig. 2. Amount of black carbon (BC) particles deposited on the surface of the leaves or needles of *F. crenata, C. sieboldii, L. kaempferi* and *C. japonica* seedlings in November 2010. The amount of BC particles was expressed on the basis of total leaf area (TLA). Each value shows the mean of 3 chamber replications, and the standard deviation is given by vertical bar.

It is important for development of the generic dry deposition model to understand the relationship between generated amount of BC and M_{BC} of the seedlings. Because a "direct" deposition technique was used in the chamber, it is difficult to compare between the number concentrations of BC (solid) particles generated from the nozzle with those of suspended (non-deposited) in the chamber and deposited particles on the plants, even a real-time apparatus (such as a laser-based submicron-particle counter) was introduced in the chamber. Therefore, it is difficult to consider the relationship between generated amount of BC and M_{BC} to discuss the deposition process of BC in the chamber.

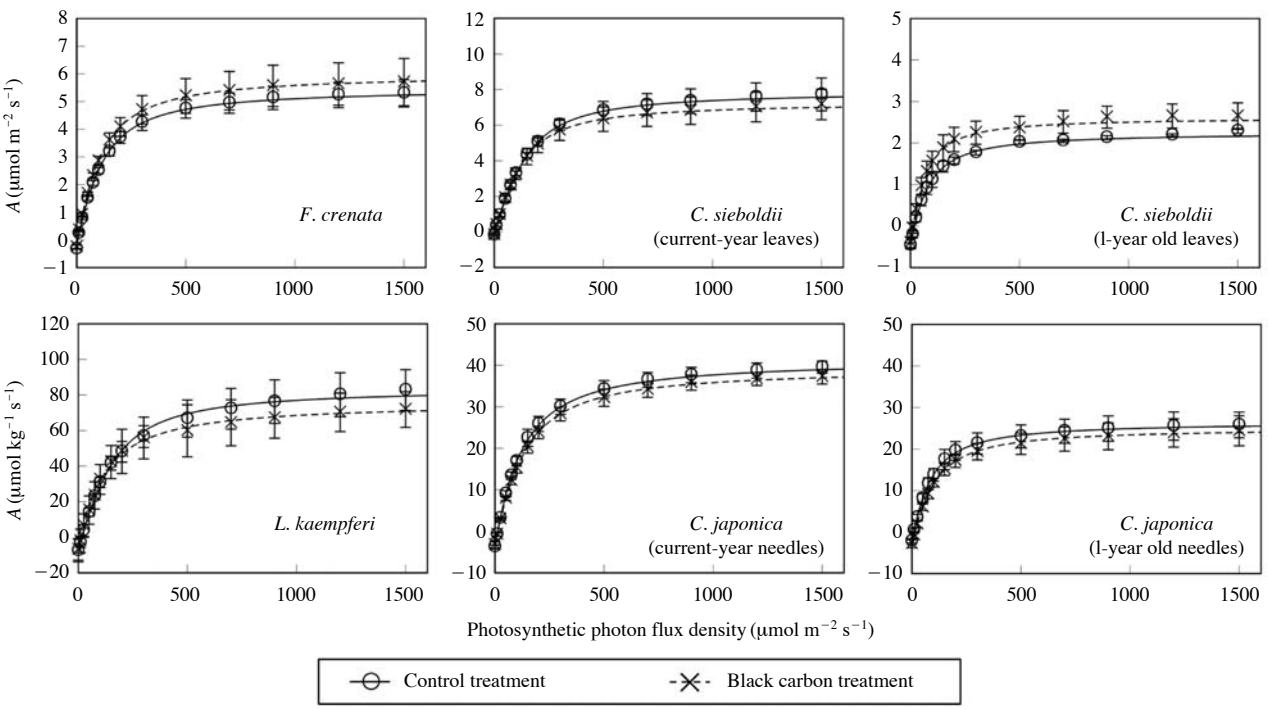

Fig. 3. Effects of black carbon particles on the response of net photosynthetic rates (*A*) to photosynthetic photon flux density in the leaves or needles of *F. crenata, C. sieboldii, L. kaempferi* and *C. japonica* seedlings in August 2010. Each value shows the mean of 3 chamber replications, and the standard deviation is given by vertical bar. The regression analysis was performed using non-rectangular hyperbolic function.

However, the species difference in M_{BC} was obvious especially between deciduous and evergreen tree species. The higher M_{BC} in evergreen tree species (*C. sieboldii* and *C. japonica*) as compared with that in deciduous tree species (*F. crenata* and *L. kaempferi*) (Fig. 2) can be attributed to longer leaf longevity in evergreen tree species. On the other hand, within the both evergreen and deciduous species, the M_{BC} in coniferous tree species was higher than that in the broad-leaved tree species (Fig. 2). This result was consistent with those reported by Beckett *et al*. (2000), Freer-Smith *et al*. (2005) and Hwang *et al*. (2011). Such greater capture efficiency of particles by shoots in coniferous tree species as compared with that in broad-leaved tree species can be explained by complex structure of shoot and smaller leaves (Beckett *et al*., 2000).

The effects of BC particles on the response of net photosynthetic rates to photosynthetic photon flux density (*PPFD*) (*A*-light curve) in the leaves or needles of *F. crenata, C. sieboldii, L. kaempferi* and *C. japonica* seedlings in August 2010 were indicated in Fig. 3. The exposure to BC particles did not significantly affect net photosynthetic rates in the leaves or needles of *F. crenata, C. sieboldii, L. kaempferi* and *C. japonica* seedlings under any *PPFD*. This result suggests that the BC particles deposited on the foliar surface did not significantly reduce net photosynthesis of the seedlings by shading. Table 1 indicates the effects of BC particles on net photosynthetic rate (A_{sat}) and stomatal diffusive conductance to water vapour (g_s) under light-saturated condition, stomatal limitation of photosynthesis (L_s), response of g_s to increase in vapour pressure deficit ($dg_s/dVPD$) and difference in temperature between leaf and air ($T_{leaf} - T_{air}$) in the leaves or needles of *F. crenata, C. sieboldii, L. kaempferi* and *C. japonica* seedlings in August 2010. There was no significant effect of BC particles on the $T_{leaf} - T_{air}$ in the leaves or needles under light-saturated condition. This result suggests that the BC particles deposited on the foliar surface did not increase leaf temperature by absorption of irradiation light. There were no significant effects of BC particles on the g_s, L_s and $dg_s/dVPD$ in the leaves or needles of *F. crenata, C. sieboldii, L. kaempferi* and *C. japonica* seedlings. These results suggest that the BC particles deposited on the leaves or needles did not induce plugging of stomata in *F. crenata, C. sieboldii, L. kaempferi* and *C. japonica* seedlings.

The effects of BC particles on the increments of plant height and stem base diameter of *F. crenata, C. sieboldii, L. kaempferi* and *C. japonica* seedlings during the experimental period were indicated in Fig. 4. There were no significant effects of BC particles on the increments of plant height and stem base diameter of the seedlings. The effects of BC particles on the whole-plant dry mass of *F. crenata, C. sieboldii, L. kaempferi* and *C. japonica* seedlings in November 2010 were

Table 1. Effects of black carbon particles on net photosynthetic rate (A_{sat}), stomatal diffusive conductance to water vapour (g_s) under light-saturated condition, stomatal limitaion of photosynthesis (L_s), response of g_s to increase in vapour pressure deficit ($dg_s/dVPD$) and difference in temperature between leaf and air (T_{leaf}-T_{air}) in the leaves or needles of *F. crenata*, *C. sieboldii*, *L. kaempferi* and *C. japonica* seedlings in August 2010.

Tree species	Treatment	A_{sat} ($\mu mol\ m^{-2}\ s^{-1}$)	g_s ($mol\ m^{-2}\ s^{-1}$)	L_s (%)	$dg_s/dVPD$** (kPa^{-1})	T_{leaf}-T_{air} (°C)
F. crenata	Control	5.30 (0.49)	0.143 (0.038)	23.7 (7.8)	−0.135 (0.037)	−0.13 (0.12)
	BC treatment	5.70 (0.85)	0.152 (0.043)	26.3 (2.6)	−0.160 (0.027)	0.00 (0.14)
	t-test	n.s.	n.s.	n.s.	n.s.	n.s.
*C. sieboldii**	Control	7.72 (0.92)	0.240 (0.070)	12.0 (2.4)	−0.294 (0.017)	0.71 (0.11)
	BC treatment	7.16 (0.85)	0.231 (0.009)	11.5 (0.7)	−0.322 (0.077)	0.33 (0.12)
	t-test	n.s.	n.s.	n.s.	n.s.	n.s.
		A_{sat} ($\mu mol\ kg^{-1}\ s^{-1}$)	g_s ($mol\ kg^{-1}\ s^{-1}$)	L_s (%)	$dg_s/dVPD$** (kPa^{-1})	T_{leaf}-T_{air} (°C)
L. kaempferi	Control	82.7 (11.5)	1.897 (0.223)	19.5 (1.6)	−0.240 (0.005)	1.36 (0.24)
	BC treatment	71.8 (10.0)	1.850 (0.149)	15.3 (3.4)	−0.221 (0.022)	1.59 (0.21)
	t-test	n.s.	n.s.	n.s.	n.s.	n.s.
*C. japonica**	Control	39.5 (1.5)	0.627 (0.035)	21.9 (5.8)	−0.302 (0.013)	1.62 (0.29)
	BC treatment	37.2 (1.7)	0.604 (0.018)	26.2 (2.2)	−0.283 (0.012)	1.46 (0.25)
	t-test	n.s.	n.s.	n.s.	n.s.	n.s.

Each value is the mean of three chamber replicates, and the standard deviation is shown in parentheses. n.s.=not significant (paired *t*-test, $p > 0.05$). Light-saturated condition: photosynthetic photon flux density (*PPFD*)=1500 $\mu mol\ m^{-2}\ s^{-1}$. *: measurements were conducted in current-year leaves or needles, **: slope of the regression line between vapour pressure deficit (*VPD*) and relative value of g_s (g_s determined at 1.5 kPa *VPD* was defined as 1).

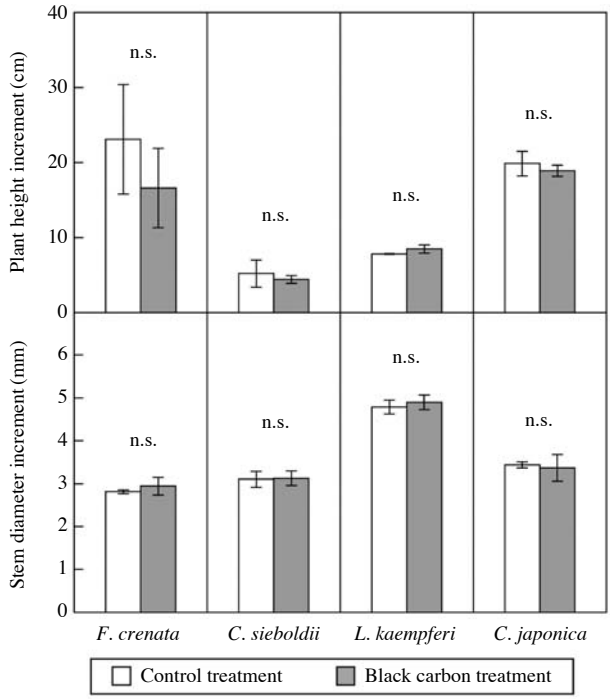

Fig. 4. Effects of black carbon particles on the increments of plant height (top) and stem base diameter (bottom) of *F. crenata, C. sieboldii, L. kaempferi* and *C. japonica* seedlings during the experimental period. Each value shows the mean of 3 chamber replications, and the standard deviation is given by vertical bar. n.s.=not significant (paired *t*-test).

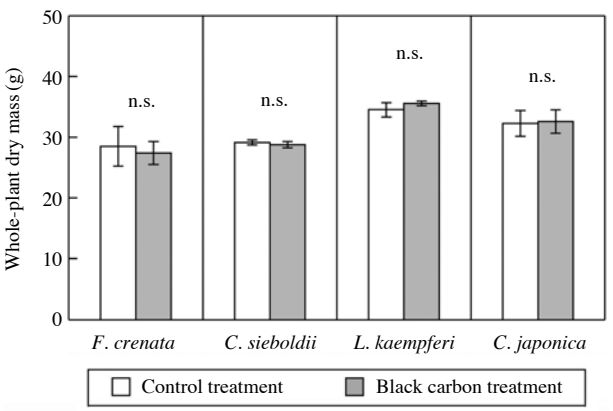

Fig. 5. Effects of black carbon particles on the whole-plant dry mass of *F. crenata, C. sieboldii, L. kaempferi* and *C. japonica* seedlings in November 2010. Each value shows the mean of 3 chamber replications, and the standard deviation is given by vertical bar. n.s.=not significant (paired *t*-test).

shown in Fig. 5. The exposure to BC particles did not significantly affect the whole-plant dry mass of the seedlings at the end of the experiment. These results indicate that the exposure to BC particles for two growing seasons did not significantly affect the growth of *F. crenata, C. sieboldii, L. kaempferi* and *C. japonica* seedlings. The M_{BC} accumulated by the exposure to BC particles were 0.13, 0.69, 0.32 and 0.58 mg C m^{-2} total leaf area (TLA) in *F. crenata, C. sieboldii, L. kaempferi* and *C. japonica* seedlings, respectively (Fig. 2). In *Cucumis sativus* and *Phaseolus vulguris*, net photosynthetic rate was reduced by shading effect of M_{BC} at >320 mg C m^{-2} projected leaf area (PLA) (i.e. 160 mg C m^{-2} TLA) (Hirano *et al.*, 1991). Using BC generated by the fluidized bed-type generator, with size range from sub-μm to few ten μm, Hirano *et al.* (1995) reported that leaf temperature of *C. sativus* and *P. vulguris* was increased due to the absorption of irradiation light by M_{BC} at >400 mg C m^{-2} PLA (i.e. 200 mg C m^{-2} TLA). With the existence of particle at few ten μm range, these weight-based M_{BC} (Hirano *et al.*, 1995) were quite high as compared with those observed in the present study (Fig. 2). As a consequence, it is possible that the reduction in net photosynthetic rate of the tree species was not observed in the present study, and hence the growth of the seedlings was not reduced by the exposure to BC particles. To evaluate the effects of BC particles on growth and leaf or needle gas exchange rates of forest tree species in the field, it is necessary to determine whether or not the amount of BC deposited on the foliar surface of forest trees grown in the field is higher than the amount observed in the present study.

Recently, Matsuda *et al.* (2012) reported that the amount of deposition of elemental carbon from the atmosphere to tropical forest in Thailand during the leafy season (i.e. growing season) in 2010 was estimated to be 0.34 mg C m^{-2} day^{-1}. This value corresponded to M_{BC} of 8.9 mg C m^{-2} TLA during the season, assuming that the leaf area index of the forest and the number of days during the season were 3.5 and 183, respectively (Matsuda *et al.*, 2012). The M_{BC} reported by Hirano *et al.* (1995, 1991) was very high as compared with that observed in the field and, thus, not realistic. To clarify the effects of BC particles on the growth and physiological function of forest tree species in the field, it is necessary to clarify the effects of BC particles deposited on the foliar surface at realistic level. In the present study, therefore, we tried to make the M_{BC} at ambient level, resulting in lower M_{BC} than that reported by Hirano *et al.* (1995, 1991). However, the M_{BC} in the present study (start in 2009) was also lower than that observed in the forest as reported in a recent study (Matsuda *et al.*, 2012). Because the objective of the present study is to clarify the effect of sub-micron sized (long-range transport) aerosol particles, the seedlings were exposed to sub-micron sized BC particles. Therefore, the M_{BC} accumulated by the exposure to BC

particles consisted of mostly sub-micron range particles with light mass per particle. On the other hand, in the field, the M_{BC} was not sorted out by particle size and consisted of not only fine particle (i.e. sub-micron size) but also coarse particle with relatively heavy mass per particle. As a result, the weight-basis M_{BC} in the present study was lower than that in the field. Therefore, it is necessary to sort out the weight of particles versus their sizes, and to clarify the relationship between field-observed amount and size range of BC deposited on the foliar surface and growth and physiological functions of forest tree species.

4. CONCLUSIONS

The exposure to BC particles with sub-micron size for two growing seasons dose not significantly affect the growth and leaf or needle gas exchange rates of *F. crenata, C. sieboldii, L. kaempferi* and *C. japonica* seedlings. To evaluate the effects of BC particles on forest tree species grown under field conditions, further study is needed to clarify the relationship between the amount of BC deposited on the foliar surface and the degree of the negative effects of BC on forest tree species.

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