Phyton (Austria) Special issue: "APGC 2004"	**Vol. 45**	Fasc. 4	(117)-(124)	1.10.2005

Effects of Free-Air CO_2 Enrichment on Leaf and Panicle Temperatures of Rice at Heading and Flowering Stage

By

H. OUE[1], M. YOSHIMOTO[2] & K. KOBAYASHI[3]

Key words: Free-air CO_2 enrichment, heading and flowering stage of rice, panicle temperature, panicle transpiration conductance, heat balance on a panicle.

Summary

OUE H., YOSHIMOTO M. & KOBAYASHI K. 2005. Effects of free-air CO_2 enrichment on leaf and panicle temperatures of rice at heading and flowering stage. – Phyton (Horn, Austria) 45 (4): (117)-(124).

The elevated CO_2 concentration in the atmosphere could induce global warming and physiological changes in plants, e.g. stomatal closure. Through FACE (Free Air CO_2 Enrichment) experiments in a paddy field, it has been confirmed that CO_2–induced stomatal closure decreases transpiration, which causes a rise of leaf temperature (T_l). Thus, with elevated CO_2, the probability of heat-induced spikelet sterility (HISS) of rice could increase. The objectives of this study were as follows: (1) Measure differences in stomatal conductance (g_s), panicle transpiration conductance (g_p), T_l, and panicle temperature (T_p) of rice plants between FACE plots (E-CO_2) and ambient plots (A-CO_2) at Wuxi, China (31° 37' N, 120° 28' E) at heading and flowering stages. (2) Model the heat balance on a panicle surface. (3) Simulate T_p and clarify the impacts of FACE on T_p using the model. Panicles emerged and flowered about 4 days earlier in E-CO_2 than in A-CO_2. The measured T_p was 1 - 2 °C higher in E-CO_2 than in A-CO_2, which was equivalent to or even higher than the difference in T_l between in the two plots. The g_p decreased with panicle age after heading, while g_s increased asymptotically with the increase of photosynthetically active radiation. But, under both CO_2 conditions, g_p was not significantly different for the same days after heading. By modelling heat balance on a panicle surface and by simulating T_p, influences of elevated CO_2 on T_p were quantified. First, lower g_p due to older age after flowering. Second, higher air temperature (T_a) followed by higher T_l. Third, higher long wave radiation emitted from leaves (L_l). The effects of T_a and g_p on T_p were significant but the effect of L_l on T_p was not significant. On the same number of days after heading, the net effect of g_p on T_p would be almost zero. Therefore, raised T_a would be a critical factor for the increase in T_p.

---

[1] Faculty of Agriculture, Ehime University, 3-5-7 Tarumi, Matsuyama 790-8566, Japan, e-mail: oue@agr.ehime-u.ac.jp

[2] National Institute for Agro-Environmental Sciences, 3-1-3 Kannondai, Tsukuba, Ibaraki 305-8604, Japan.

[3] Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan.

Introduction

The elevated CO_2 concentration in the atmosphere may induce global warming, which could increase the probability of heat-induced spikelet sterility (HISS) in rice during anthesis (MATSUI & al. 1997a). Elevated CO_2 concentration may also induce stomatal closure on leaves (KIMBALL & IDSO 1983). In the FACE (Free Air CO_2 Enrichment) experiment in a paddy field, it has been confirmed that stomata close under elevated CO_2, resulting in decrease of transpiration, which causes a rise of leaf temperature T_l and air temperature T_a within the rice canopy (OUE & al. 2003). Increased temperatures could exacerbate the HISS of rice (MATSUI & al. 1997b). The objectives of this study were as follows: (1) Measure differences in stomatal conductance (g_s), panicle transpiration conductance (g_p), T_l, and panicle temperature (T_p) of rice plants between FACE plots (E-CO_2) and ambient plots (A-CO_2) at heading and flowering stage. (2) Model the heat balance on a panicle surface. (3) Simulate T_p and clarify the impacts of FACE on T_p.

Material and Methods

Observations

Observations were conducted in farmers' rice paddy fields in Wuxi, China (31° 37' N, 120° 28' E), from 2 to 4 September 2002 and from 25 August to 4 September, 2003 at the heading, flowering and ripening stages. For the elevated CO_2 treatment, a 'FACE ring' was used (OKADA & al. 2001), consisting of eight CO_2-emitting tubes in an octagonal arrangement, from which pure CO_2 was released into the air above the plant canopy towards the centre of the ring without the use of blowers. The FACE rings were 12 m in diameter, with nominal usable areas were about 80m^2 leaving a 1-m buffer zone from the emission tubes. Detailed characteristics and performance were described by OKADA & al. 2001. The target CO_2 concentration in the FACE rings was 200 μmol mol^{-1} above ambient CO_2 concentration 24 hours per day from transplanting through harvest.

Rice (*Oryza sativa* L. cv. Wuxiangjing 9) was transplanted with a density of 24 stubs per m^2 in all fields. Heading was observed on 25 August in E-CO_2 and 29 August in A-CO_2 in 2002 and 25 August in E-CO_2 and 30 August in A-CO_2 in 2003. Thus, heading in E-CO_2 was 4 - 5 days earlier than in A-CO_2.

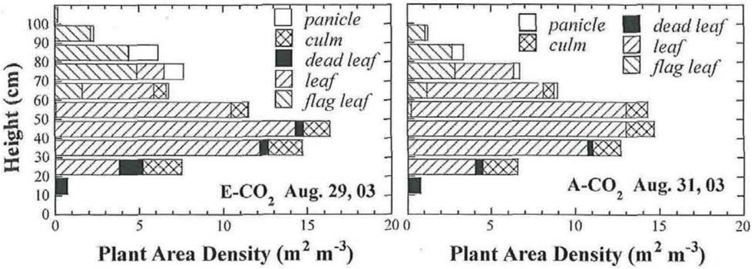

Fig. 1. Vertical profiles of plant area density in E-CO_2 and in A-CO_2 after heading, August 29 and 31 2003.

Vertical profiles of leaf stomatal conductance (g_s) and panicle transpiration conductance (g_p) were measured using a portable photosynthesis system (LI-6400, Li-COR, USA) from around

0900 to 1800 LST on two continuous days in E-CO_2 and in A-CO_2. The g_s was measured for 3 to 5 leaves in a canopy layer every 10 cm with height. The g_p was also measured by the same cuvette as used for leaves. The area of a panicle for g_p measurement was calculated by multiplying average area of a grain by a number of grains of the panicle. All the measurements were made at prevailing light and climatic conditions, and the CO_2 concentration in the chamber of the LI-6400 was controlled to 350 μmol mol^{-1} in A-CO_2 and 550 μmol mol^{-1} in E-CO_2 using the CO_2 injector. Vertical profiles of leaf temperature (T_l) and panicle temperature (T_p) were measured using a portable infrared thermometer (THI-500, TASCO, Japan) in each plot every hour during the daytime. The T_l and T_p were measured manually by pointing the thermometer at a leaf or a panicle horizontally in a canopy layer every 10 cm with height. Vertical profiles of plant area density in each plot were measured by sampling two stubs of rice in both plots, by cutting them at intervals of 10 cm, by dividing them into components of leaves, panicles and culms and by scanning them, examples of which are shown in Fig. 1. Though the measurement was made two days earlier in E-CO_2 than in A-CO_2, panicle area was larger in E-CO_2 because heading was five days earlier. Leaf area index (LAI; not accounting for dead leaves) was 6.30 in E-CO_2 and 6.05 in A-CO_2.

Radiation and heat balance on a panicle

Radiation and heat balance on a panicle of sunlit and shaded sides at the top of the canopy are as follows:

Shortwave radiation absorbed by a panicle (S_p) is written as

$$S_p = (1-r_p)\, F_p\, [\sec(\theta)\, S_d + 2\, d_f\, S_f], \tag{1}$$

where S_d and S_f are downward direct and diffused shortwave radiations, respectively, the ratio of which was set to 0.5. Parameters r_p and F_p are the albedo of a panicle and contribution factor to cutting off the radiation by a panicle (OUE 2001), which were set to 0.35 and 0.35 after OUE 2003. The θ is the solar zenith angle, and d_f is the diffusivity factor for radiation, which was 1.66 (= sec 53°).

Heat balance on a panicle is written as

$$S_p + F_p\, d_f\, (L_l + L_a) = H_p + lE_p + 2\, F_p\, d_f\, \sigma T_p^{\,4}, \tag{2}$$

where L_l and L_a are longwave radiations from a leaf surface adjacent to the panicle and from the atmosphere, respectively. T_p is panicle temperature. The H_p and lE_p are sensible and latent heat fluxes on a panicle, which are written as

$$H_p = c_p \rho\, c_h\, u\, (T_p - T_a), \tag{3}$$

$$lE_p = c_p \rho\, c_e\, u\, [e_{sat}(T_p) - e_a] / \gamma, \tag{4}$$

where T_p and $e_{sat}(T_p)$ are panicle temperature and saturated vapour pressure at T_p. The T_a and e_a are air temperature and vapour pressure adjacent to the panicle. The u is wind speed at the top of the canopy. The c_p is the specific heat of air at constant pressure, ρ is the density of air and γ is the psychrometric constant. Parameters c_h and c_e are transfer coefficient of a panicle for sensible heat and latent heat, respectively. In this study, c_h was set to 0.04, which is as the same level as that of a leaf (OUE 2003). The parameter c_e is written as

$$c_e = \frac{1}{u\left(\dfrac{1}{c_h\, u} + \dfrac{1}{g_p}\right)}. \tag{5}$$

These equations were solved to calculate T_p with measured climatic conditions and g_p in A-CO_2 and in E-CO_2.

Results and Discussion

Effects of elevated CO_2 on stomatal conductance (g_s) and on panicle transpiration conductance (g_p)

Fig. 2 (a)-(b) shows relationships between photosynthetic photon flux density (PPFD) and stomatal conductance g_s in A-CO_2 and in E-CO_2. Measured g_s was classified by the height of canopy layer in order to quantify effects of leaves' aging. Non-rectangular hyperbolic functions were applied for regressions. The g_s was higher in A-CO_2 than in E-CO_2 for the same canopy layers because of stomatal closure under higher CO_2 concentration as observed earlier (e.g. KIMBALL & IDSO 1983). Stomatal closure under higher CO_2 concentration raised leaf temperature as shown in Fig. 5. The g_s was about 1.5 cm s^{-1} in A-CO_2 and 1.2 cm s^{-1} in E-CO_2 under light saturation at the top canopy layer, and was reduced 20-70 % by elevated CO_2 in this growing stage. In addition, the g_s was lower in lower canopy layer because of senescence. This effect was more apparent in E-CO_2 than in A-CO_2, which suggests that leaves age faster in E-CO_2 than in A-CO_2.

Fig. 2 (c)-(d) shows relationships between PPFD and panicle transpiration conductance g_p measured more than 6 days after the first heading day in each plot. The g_p had no relation to PPFD in both plots. This result reveals that transpiration from panicles hardly took place through the stomata (e.g. ISHIHARA &

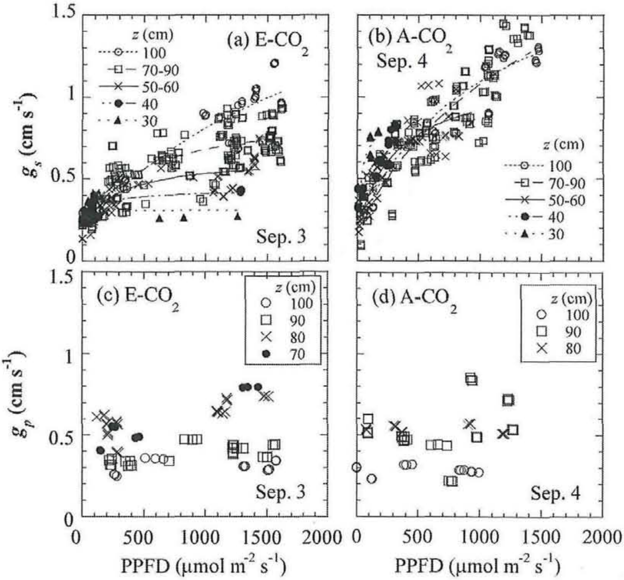

Fig. 2. (a)-(b) Relationships between PPFD and g_s and (c)-(d) relationships between PPFD and g_p classified by the height of canopy layer in E-CO_2 (September 3 2002) and in A-CO_2 (September 4 2002) after heading.

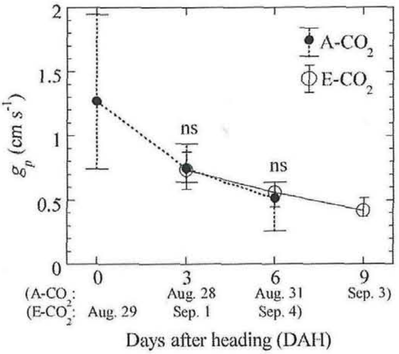

Fig. 3. Changes of g_p against the days-after-heading (DAH) in E-CO$_2$ and in A-CO$_2$. The solid and open circles represent averages of g_p in A-CO$_2$ and in E-CO$_2$ respectively measured from 9: 00 to 16:00. The FACE effect for g_p was not significant (ns) on DAH = 3 and 6 at $p < 0.05$.

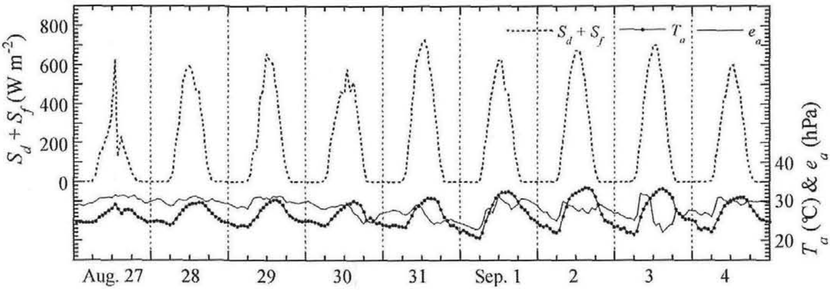

Fig. 4. Daily variations of climatic conditions; global solar radiation ($S_d + S_f$), air temperature (T_a) and vapour pressure (e_a) at the height of 200 cm above the ground in the paddy field during the observation of g_p.

al. 1990) but mainly through the cuticle. The g_p was higher in lower canopy layer as a whole because the panicle was younger and contained more moisture.

Changes of g_p against the days-after-heading (DAH) in each plot are shown in Fig. 3. In A-CO$_2$, heading began on August 29 2002, which was 4 days later than in E-CO$_2$. The g_p was very high just after heading, which reached about 170 % of g_s in A-CO$_2$ and 200 % of g_s in E-CO$_2$ under light saturation. The reason for the high g_p could be the high moisture content of panicle. The g_p declined asymptotically as the increase of DAH. The difference of g_p between the two plots was statistically insignificant on DAH = 3 and 6 at $p < 0.05$, while the difference of g_p between DAH were significant at $p < 0.05$ in both plots. Thus, the FACE effect for g_p was not significant but the DAH effect was significant. Because climatic conditions did not differ so much during the observation (Fig. 4), the reduction of g_p as the increase of DAH could be regulated mainly by the change of moisture content of panicles. These results reveal that the difference of g_p between the two plots was not directly due to elevated CO_2 but due to the time lag of heading, i.e. the accelerated phenology by elevated CO_2.

These results suggest that panicle temperature (T_p) could not be directly influenced by elevated CO_2 but could be 'indirectly' influenced by increased long wave radiation from leaves, whose temperature would be raised by stomatal closure, and by raised air temperature within the rice canopy (OUE & al. 2003). Additionally, T_p could be influenced by reduced g_p compared between the two plots on the same day.

Observed effects of elevated CO_2 on leaf and panicle temperature

Fig. 5 shows vertical profiles of leaf temperature (T_l) and panicle temperature (T_p) in A-CO_2 and in E-CO_2 on September 2 2002. Temperature at the height of 0 cm denotes water surface temperature.

Both T_l and T_p were higher in E-CO_2 than in A-CO_2 in most canopy layers except for T_l at the lowest canopy layer and water surface temperature. The difference of T_l between the two plots reached 1.6 °C. The difference of T_p between the two plots reached 2.4 °C and T_p reached 34.1 °C. Higher T_l in E-CO_2 was mainly attributed to lower g_s as shown in Fig. 2 (a)-(b). Higher T_p in E-CO_2 would have mainly been attributed to lower g_p as shown in Fig. 3 because of time lag of heading and to higher air temperature in the canopy and higher infrared radiation from leaves, which will be discussed later.

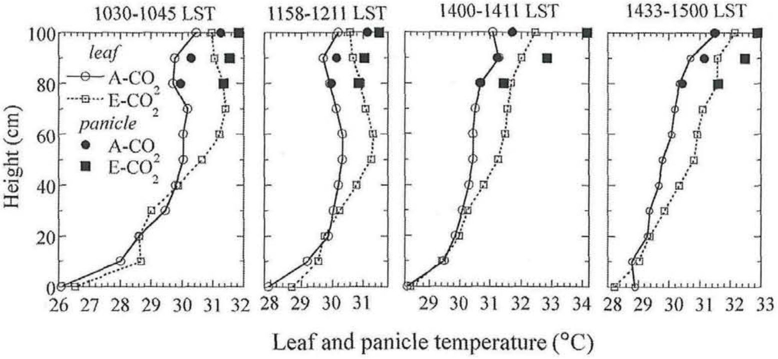

Fig. 5. Vertical profiles of leaf temperature and panicle temperature on September 2, 2002. Panicle temperature was measured over 80 cm because little panicles are at 70 cm and nothing below 60 cm.

In both plots, T_p was higher than T_l in the upper two canopy layers, i.e. at the height of 90 - 100 cm. This is because of lower g_p than g_s. However, T_p at the height of 80 cm was almost the same as T_l. Thus, the difference between T_p and T_l was larger in an upper layer. Panicles in an upper layer, in general, are older than those in a lower layer. In this observation, panicles in E-CO_2 at the top of the canopy, which was at ripening stage, were older than those in A-CO_2, and panicles at the height of 80 cm were just after heading in both plots. Thereby, g_p of a panicle just after heading in a lower layer is higher than that in a higher layer and g_p at 80 cm is even higher than g_s at the same height in E-CO_2 as shown in Figs. 2 and 3. This caused smaller difference between T_p and T_l in a lower canopy layer. These differences in g_p and g_s are also the reason why the differences between T_p and T_l were nearly the same for both plots or larger in E-CO_2 than in A-CO_2.

Simulation of effects of elevated CO_2 on T_p with the heat balance model

Effects of elevated CO_2 were simulated with the heat balance model on a panicle surface (Eqs. 1-5). Effects included lower g_p because of time lag of heading, higher air temperature in the canopy, and higher infrared radiation from leaves on T_p for the four instances in Fig. 5.

Comparisons between calculated T_p based on the model and measured T_p in both plots are shown in Fig. 6. The model calculation reproduced measured T_p quite successfully with statistical insignificance at $p < 0.01$ in A-CO_2 and at $p < 0.05$ in E-CO_2.

To assess effects of elevated CO_2 on T_p, contributions of changes of g_p, T_a and L_l based on the model were shown in Table 1. The effects of T_a and g_p on T_p were statistically significant but the effect of L_l on T_p was not significant at $p<0.05$.

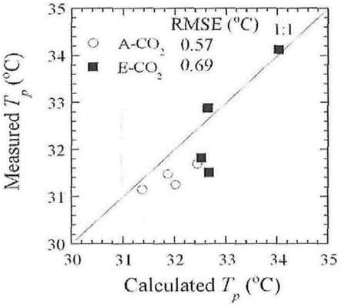

Fig. 6. Comparisons between calculated T_p based on the model and measured T_p in A-CO_2 and in E-CO_2 for the four instances in Fig. 5. Differences between calculated and measured T_p were not significant at $p < 0.01$ in A-CO_2 and at $p < 0.05$ in E-CO_2.

The effects of raised T_a and increased L_l are attributed to stomatal closure by elevated CO_2 and the effect of decreased g_p is attributed to the time lag of heading. So, on the same number of days after heading, the net effect of g_p on T_p would be almost zero. On the other hand, raised T_a would be a critical factor because T_p is strongly depended on it. The model simulations, which are not shown in the figure, reveal that T_p would reach up to 33.5 °C and 35.5 °C if T_a were 35.0 °C just after heading, and 6 days after heading at ripening stage, respectively.

Simulation with the heat balance model on a panicle surface revealed that increase in T_a within the canopy due to stomatal closure of leaves under elevated CO_2 and due to the global warming would exacerbate heat-induced spikelet sterility in rice. The increase in T_p due to elevated CO_2 could also affect the quality of rice seed because the increase in T_p was larger at ripening stage than at heading and flowering stage.

Table 1. Simulated increases in T_p by the changes of g_p, T_a and L_l under elevated CO_2 for the four instances in Fig. 5. The g_p used for this simulation were measurements in A-CO_2 on Sep. 1 and in E-CO_2 on Sep. 3.

Time	Parameters in A-CO_2 Changes in E-CO_2 are in the brackets			Increases in T_p (°C)		
	g_p (cm s^{-1})	T_a (°C)	L_l (W m^{-2})	due to g_p	due to T_a	due to L_l
10:30	0.69 (-0.17)	31.27 (+0.43)	467.29 (+3.15)	0.17	0.33	0.01
12:00	0.82 (-0.42)	31.04 (+1.00)	465.76 (+2.30)	0.53	0.76	0.01
14:00	0.82 (-0.45)	32.07 (+0.74)	471.15 (+8.54)	1.08	0.44	0.07
15:00	0.64 (-0.27)	32.01 (+0.23)	473.98 (+3.81)	0.61	0.15	0.03

Acknowledgements

We would like to thank Prof. J. G. ZHU, Institute of Soil Science, Chinese Academy of Science, China for his cooperation in the FACE experiment. We also thank Drs. M. OKADA and H. Y. KIM and Mr. H. NAKAMURA for their supports for setting the FACE apparatus. This research was financially supported by the research project named 'Elucidation of Global Warming Impacts in Agriculture, Forestry and Fishery and Development of Mitigation Techniques' funded by the Ministry of Agriculture, Forestry and Fisheries of Japan.

References

ISHIHARA K., KIYOTA E. & IMAIZUMI N. 1990. Transpiration and photosynthesis characteristics of the panicle in comparison with the flag leaf in the rice plant. - Jpn. J. Crop Sci. 59 (2): 321-326. (in Japanese with English abstract)

KIMBALL B. A. & IDSO S. B. 1983. Increasing atmospheric CO_2: effects on crop yield, water use, and climate. - Agric. Water Manag. 7: 55-72.

MATSUI T., OMASA K. & HORIE T. 1997a. High temperature-induced spikelet sterility of Japonica rice at flowering in relation to air temperature, humidity and wind velocity conditions. - Jpn. J. Crop Sci. 66 (3): 449-455.

— , NAMUCO O. S., ZISKA L. H. & HORIE T. 1997b. Effects of high temperature and CO_2 concentration on spikelet sterility in Indica rice. - Field Crops Res. 51: 213-219.

OKADA M., LIEFFERING M., NAKAMURA H., YOSHIMOTO M., KIM H. Y. & KOBAYASHI K. 2001. Free-air enrichment (FACE) using pure CO_2 injection: system description. - New Phytologist 150: 251-260.

OUE H. 2001. Effects of vertical profiles of plant area density and stomatal resistance on the energy exchange processes within a rice canopy. - Journal of the Meteorological Society of Japan. 79 (4): 925-938.

— 2003. Evapotranspiration, photosynthesis and water use efficiency in a paddy field (II) – prediction of energy balance and water use efficiency by numerical simulations based on a multilayer model. - Journal of Japan Society of Hydrol. and Water Resources 16 (4): 389-407. (in Japanese with English abstract)

— , YOSHIMOTO M., KUWAGATA T. & KOBAYASHI K. 2003. Influences of elevated CO_2 (FACE) on transpiration, photosynthesis and micrometeorology in a rice paddy field. – IUGG2003 Abtsracts B: 397-398.

Phyton (Austria) Special issue: "APGC 2004"	**Vol. 45**	Fasc. 4	(125)-(131)	1.10.2005

Effects of Elevated CO_2 and Nitrogen Availability on Nodulation of *Alnus hirsuta* Turcz.

By

H. TOBITA 1), M. KITAO 1), T. KOIKE 2) & Y. MARUYAMA 1)

Key words: Nitrogen fixation, nitrogen resorption efficiency, leaf nitrogen, C:N ratio, leaf litter.

Summary

TOBITA H., KITAO M., KOIKE T. & MARUYAMA Y. 2005. Effects of elevated CO_2 and nitrogen availability on nodulation of *Alnus hirsuta* Turcz. - Phyton (Horn, Austria) 45 (4): (125)-(131).

The effects of elevated CO_2 and soil nitrogen (N) availability on nodulation and N_2 fixation of alder (*Alnus hirsuta* Turcz.) were investigated. Our objective was to determine if elevated CO_2 concentrations enhance nodule mass and alter the inhibitory effect of soil N on nodulation. Potted seedlings of alder were grown at either ambient or elevated CO_2 concentrations (36 Pa and 72 Pa CO_2), with different levels of N supplied as liquid fertilizer (52.5, 5.25 and 0 mgN pot^{-1} week^{-1} for High-N, Low-N and N-free, respectively) in a natural daylight phytotron.

Elevated CO_2 increased both whole-plant mass and nodule mass per plant, but the ratio of nodule mass to whole-plant mass (NMR) was not affected. At High-N, NMR declined under both CO_2 treatments, indicating an inhibitory effect of soil N availability on nodulation regardless of CO_2 concentration. The total amount of N_2 fixation in plants without added N was enhanced under elevated CO_2 conditions, with an increase of nodule mass. At elevated CO_2, both whole-plant N concentration and area-based leaf N decreased.

Leaf litter C:N ratio increased (+14%) under elevated CO_2, suggesting that leaf litter decomposition rates are decreased by elevated CO_2. However, area-based leaf litter N did not decrease under elevated CO_2 due to decreased retranslocation of leaf N per area during leaf senescence. Furthermore, leaf area per plant increased under elevated CO_2 in each of the N-treatments. These results suggest that total leaf litter N input to soil should increase under elevated concentrations of atmospheric CO_2, and that alder may increase soil N availability under elevated CO_2.

---

1) Hokkaido Research Center, Forestry and Forest Products Research Institute, 7 Hitsujigaoka, Toyohira, Sapporo, Hokkaido 062-8516, Japan. Fax: +81-11-851-4167, e-mail: tobi@ffpri.affrc.go.jp

2) Boreal Forest Conservation Research, Hokkaido University Forests, Sapporo, Hokkaido 060-0809, Japan.

Introduction

Many plant species grown under elevated CO_2 ($[CO_2]^e$) exhibit an enhanced CO_2 assimilation rate and growth when other environmental resources do not limit productivity (NORBY & al. 1999). Forest ecosystems are usually nitrogen (N) -limited (VITOUSEK & HOWARTH 1991), and plants growing under low N conditions usually respond much less to $[CO_2]^e$ (HARTWIG & al. 1996). As trees with symbiotic N_2-fixing capability are largely independent of soil N, they could be more responsive to $[CO_2]^e$ than non-N_2-fixers. In fact, increased growth, N_2 fixation, and nitrogenase activity of nodules or the nodule mass have been documented (NORBY 1987, ARNONE & GORDON 1990, SCHORTEMEYER & al. 1999, THOMAS & al. 2000). However, added fertilisers usually reduce nodule formation or N_2 fixation (EKBLAD & HUSS-DANELL 1995, KOIKE & al. 1997, VOGEL & al. 1997). Because the supply of photosynthate to nodules may increase by enhanced photosynthesis under $[CO_2]^e$ (NORBY 1987), these inhibitions may be reduced as concentrations of atmospheric CO_2 rise (THOMAS & al. 2000).

Nitrogen derived from N_2 fixation cycles into the soil mainly through leaf litter. Many tree species were reported to have decreased leaf N and whole-plant N under $[CO_2]^e$ (COTRUFO & al. 1998), which may result in decreased leaf litter N (O'NEILL & NORBY 1996). Since the decline in leaf N under $[CO_2]^e$ conditions was less in N_2-fixers than in non-N_2-fixers (COTRUFO & al. 1998), the decrease in the leaf litter N of N_2-fixers may also be small. An increase in N biomass through N_2 fixation by litter N under $[CO_2]^e$ conditions may promote the productivity of associated non-N_2-fixers (NORBY 1987, HARTWIG & al. 1996).

Actinorhizal N_2-fixing species, including *Alnus hirsuta* Turcz., can contribute significant amounts of fixed N to temperate forest ecosystems (DAWSON 1983, KOIKE & al. 1997). We examined the effects of $[CO_2]^e$ and soil N availability on nodulation and growth of *A. hirsuta* to determine if $[CO_2]^e$ enhances nodule mass, alters nodulation, or increases total N_2 fixation and leaf litter N input of *A. hirsuta* seedlings.

Material and Methods

One-year-old seedlings of *Alnus hirsuta* Turcz. obtained from a commercial nursery (Oji Forestry & Landscaping, Sapporo, Japan), were transplanted into five liter pots filled with 1:1 (v/v) Kanuma pumice and clay loam and grown in a natural daylight phytotron. The CO_2 partial pressure was regulated with a CO_2 controller (DAIWA Air, Sapporo, Japan) at either 36 Pa (ambient) or 72 Pa (elevated) CO_2 from mid-May. Each treatment was replicated twice. Seedlings were supplied with nitrogen (N) at 52.5 mgN pot^{-1} $week^{-1}$ (High-N), 5.25 mgN pot^{-1} $week^{-1}$ (Low-N) or no N ('N-free') in 0.5x Hoagland solution. Other nutrient concentrations were the same. Air temperature was maintained at 26/16 °C (day/night) from May to September. Pots were set in trays with water in order to avoid desiccation. After 100 days (late-August), six seedlings of each treatment were harvested, and dry mass and N content of each organ were determined by combustion using an NC analyzer (NC-800; Sumica Chem., Osaka, Japan). Twenty-five seedlings were harvested before treatment to provide a baseline for the initial mass and N content of plants.

Leaf gas exchange measurements were made on six mature leaves of each treatment on day 59 (mid-July). Light-saturated net photosynthetic rates per leaf area (P_{max}) were determined using an open gas exchange system (LI-6400; Li-Cor Inc., Lincoln, NE, USA) at each CO_2 condition (36 Pa or 72 Pa CO_2).

Total N_2 fixation (TNF) was calculated only in the N-free treatments. Because symbiotic N_2 fixation was considered to be the only source of N at N-free, TNF was considered to be equal to the total plant N (TPN) increment during two harvests. The average specific nitrogenase activity (SNA) was calculated as: $SNA = TPN * NW^{-1} * [day]^{-1}$, where NW is the average nodule mass per plant during two harvests and [day] is the period of the treatment when it was harvested (TISSUE & al. 1997).

Leaf litter from each individual was collected daily as leaves abscised after gradually decreasing the ambient temperature from 20/10 °C to 14/10 °C (day/night) from September to November. Area-based N resorption efficiency (RE) before senescence was calculated as: $RE = 100 * (1 - [N]_{fallen} * [N]_{green}^{-1})$, where $[N]_{fallen}$ was average area based leaf litter N within each treatment and $[N]_{green}$ was average area-based leaf N of each plant harvested in August (VAN HEERWAARDEN & al. 2003).

Analysis of variance (ANOVA) was used to evaluate the effects of CO_2 and N-treatments on biomass, leaf area, P_{max} and N content at the probability level $p<0.05$ using StatView Version 5.0 (Abacus Concepts, Inc., Berkeley, CA). The probability level $p<0.1$ was considered to indicate a trend. Analysis of co-variance (ANCOVA) was used to compare the relationship between whole-plant mass and nodule mass per plant. Statistically significant differences of SNA of the seedlings in the N-free treatment between CO_2 treatments were tested using Student's t-test ($p<0.05$).

Results and Discussion

Elevated CO_2 ($[CO_2]^e$) had a significant positive effect on whole-plant mass, leaf mass and leaf area per plant as well as on the P_{max} of mature leaves despite of N treatments (Table 1). Trees growing under $[CO_2]^e$ conditions tended to have a higher nodule mass per plant than control plants at ambient CO_2 concentrations (Fig. 1). Elevated CO_2, however, had no effect on the relationship between whole-plant mass and nodule mass (Fig. 2, ANCOVA, $p>0.1$) as reported for *Alnus rubra* (HIBBS & al. 1995), suggesting that $[CO_2]^e$ increased the nodule mass of *A. hirsuta* as a function of the increase in whole-plant mass rather than by an increase in the ratio of nodule mass to whole-plant mass (NMR).

Table 1. Whole-plant mass (g $plant^{-1}$), leaf mass (g $plant^{-1}$) and area (m^2 $plant^{-1}$) per plant, and light-saturated photosynthesis (P_{max}) (μmol m^{-2} s^{-1}) of *Alnus hirsuta*. * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$, n.s. = not significant at $p<0.1$.

	High-N		Low-N		N-free		Source of variance		
	ambient	elevated	ambient	elevated	ambient	elevated	CO_2	N	CO_2xN
whole-plant mass	34.5	43.5	34.3	40.6	39.2	51.6	**	n.s.	n.s.
leaf mass	13.1	16.3	12.6	14.8	14.3	17.7	*	n.s.	n.s.
leaf area	0.37	0.42	0.34	0.40	0.43	0.50	*	*	n.s.
P_{max}	13.8	16.7	12.8	14.3	11.4	14.2	***	**	n.s.

Increased N availability had a negative effect on both nodule mass (Fig. 1) and NMR (Fig. 2) regardless of CO_2 concentration, indicating that the inhibitory effect of high soil N availability on nodulation of *A. hirsuta* was retained under $[CO_2]^e$. Some *Alnus* species had a similar inhibitory effect on nodulation under

$[CO_2]^e$ (NORBY 1987, KOIKE & al. 1997, TEMPERTON & al. 2003), though some legume species showed no limitation (THOMAS & al. 2000).

The whole-plant N content after 100 days tended to be higher under $[CO_2]^e$ (Table 2). The enhanced whole-plant N uptake under $[CO_2]^e$ with N-free soil amendment means that N_2 fixation increased under $[CO_2]^e$. On the other hand, the average specific nitrogenase activity (SNA) tended to decrease under $[CO_2]^e$ (2.5 mmolN g nodule^{-1} day^{-1} under ambient conditions and 2.2 mmolN g nodule^{-1} day^{-1} under $[CO_2]^e$; t-test, $p=0.06$). Some studies of *Alnus* species have shown that $[CO_2]^e$ increased the total amount of N_2 fixation per plant by having greater nodule nitrogenase activity (TEMPERTON & al. 2003), by greater nodule mass (HIBBS & al. 1995) or by both (NORBY 1987, ARNONE & GORDON 1990, VOGEL & al. 1997). This current work suggests that the increase in N_2 fixation under $[CO_2]^e$ occurred mainly because of an increase in nodule mass proportionate with whole-plant mass, and not by increased SNA.

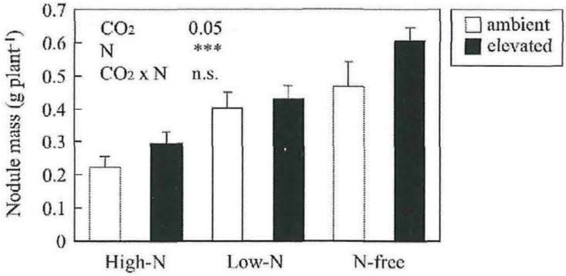

Fig. 1. Nodule mass per plant of *Alnus hirsuta* after 100 days. Values shown are means + SE (n = 6). *** = p<0.001, n.s. = not significant at p<0.1.

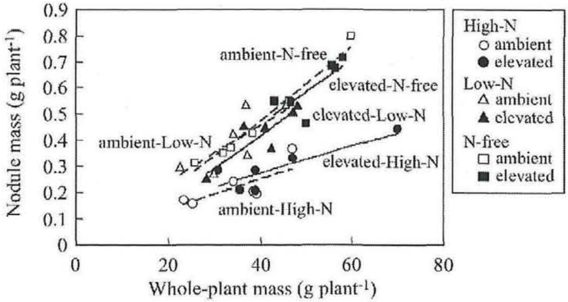

Fig. 2. Relationships between whole-plant mass and nodule mass per plant. Lines represent statistically significant (p<0.05) power functional regression at each treatment [(nodule mass) = a * (whole-plant mass)^b]. Dotted lines; ambient CO_2, solid lines; elevated CO_2.

Since the nitrogenase activity of nodules was not measured, we could not evaluate N_2 fixation at High-N and Low-N. In an earlier study, it was reported that $[CO_2]^e$ had no effect on the proportion of nitrogen derived from symbiotic fixation (VOGEL & al. 1997, SCHORTEMEYER & al. 1999). As whole-plant N accretion was larger under $[CO_2]^e$ in each of the N-treatments (Table 2), it is possible that N_2 fixation at High-N and Low-N are greater under $[CO_2]^e$ conditions than under ambient concentrations.

Elevated CO_2 had a negative effect on whole-plant N concentration and mass-based and area-based leaf N (Table 2), but no effect on area-based leaf litter N (Fig. 3a). These results suggest that the resorption efficiency (RE) of leaf N was affected by CO_2 concentrations, and indeed RE tended to decrease under $[CO_2]^e$ at low N and N-free (Fig. 3b). Thus, N resorption from leaves to seedlings before abscision, seems to be lower in $[CO_2]^e$ than in ambient CO_2. Because $[CO_2]^e$ increased total leaf area (Table 1) and had no effect on area-based leaf litter N in all N-treatments (Fig. 3a), total per plant leaf litter N input into soil may increase under $[CO_2]^e$. VOGEL & al. 1997 also reported that total leaf litter N of *Alnus glutinosa* increased with $[CO_2]^e$, though autumnal leaf N resorption of *A. glutinosa* was minimally affected by $[CO_2]^e$.

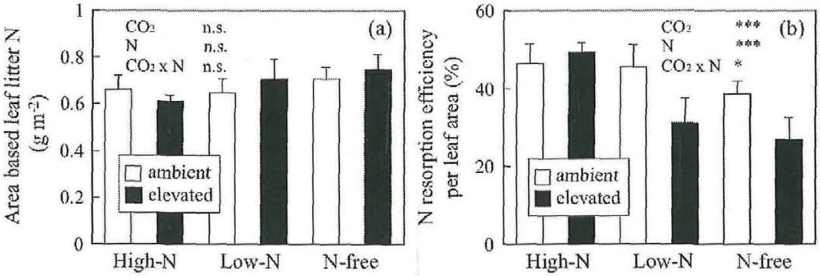

Fig. 3. Area based leaf litter N (a) and N resorption efficiency (b) of *Alnus hirsuta*. Values shown are means + SE (n = 6). * = $p<0.05$, *** = $p<0.001$, n.s. = not significant at $p<0.1$.

Table 2. Whole-plant N increment (g plant^{-1}), whole-plant N concentration (mg g^{-1}), leaf N (mass and area based, mg g^{-1}, g m^{-2}), and leaf litter N (mass based, mg g^{-1}) and C:N ratio of *Alnus hirsuta*. * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$, n.s. = not significant at $p<0.1$.

	High-N		Low-N		N-free		Source of variance		
	ambient	elevated	ambient	elevated	ambient	elevated	CO_2	N	CO_2xN
plant N increment	0.72	0.81	0.61	0.72	0.80	0.90	0.07	*	n.s.
whole-plant N	21.4	19.2	18.5	18.0	20.9	17.7	***	*	n.s.
mass based leaf N	34.8	31.0	31.1	29.9	36.1	28.7	***	0.07	*
area based leaf N	1.23	1.20	1.13	1.11	1.18	1.02	*	**	0.08
leaf litter N	18.1	15.8	17.7	15.4	19.2	16.3	***	n.s.	n.s.
leaf litter C:N	29.0	33.2	29.0	33.9	26.8	32.3	***	n.s.	n.s.

Elevated CO_2 increased the C:N ratio of leaf litter of *A. hirsuta* (14 ± 0.35 %, mean of three N-treatments ± SE) compared to ambient CO_2 (Table 2). The initial C:N ratio of leaf litter is one of the main factors that affects decomposition rates (O'NEILL & NORBY 1996). Though *Alnus glutinosa* exhibited a modest increase of 8 % in litter C:N ratio (25.9 ± 0.35) under $[CO_2]^e$, VOGEL & al. 1997 proposed that there would be little effect of CO_2 concentration on litter decomposition rates. Further study will be needed to evaluate the effect of $[CO_2]^e$ on leaf litter decomposition rates.

In conclusion, it was suggested that *A. hirsuta* would accumulate a greater biomass N through increased N_2 fixation by increased nodule mass, and may increase soil N availability by increased leaf litter N under $[CO_2]^e$.

Acknowledgements

We would like to thank Mrs. H. TAOKA and Mr. K. MIMA for assistance.

References

ARNONE III J. A. & GORDON J. C. 1990. Effect of nodulation, nitrogen fixation and CO_2 enrichment on the physiology, growth and dry mass allocation of seedlings of *Alnus rubra* Bong. - New Phytol. 116: 55-66.

COTRUFO M. F., INESON P. & SCOTT A. 1998. Elevated CO_2 reduces the nitrogen concentration of plant tissues. - Global Change Biol. 4: 43-54.

DAWSON J. O. 1983. Dinitrogen fixation in forest ecosystems. - Can. J. Microbiol. 29: 979-992.

EKBLAD A. & HUSS-DANELL K. 1995. Nitrogen fixation by *Alnus incana* and nitrogen transfer from *A. incana* to *Pinus sylvestris* influenced by macronutrients and ectomycorrhiza. - New Phytol. 131: 453-459.

HARTWIG U. A., ZANETTI S., HEBEISEN T., LÜSCHER A., FREHNER M., FISCHER B., VAN KESSEL C., HENDREY G. R., BLUM H. & NÖSBERGER J. 1996. Symbiotic nitrogen fixation: One key to understand the response of temperate grassland ecosystems to elevated CO_2? - In: KÖRNER C. & BAZZAZ F. A. (Eds.), Carbon dioxide, populations, and communities, pp. 253-264. - Academic Press, San Diego.

HIBBS D. E., CHAN S. S., CASTELLANO M. & NIU C.-H. 1995. Response of red alder seedlings to CO_2 enrichment and water stress. - New Phytol. 129: 569-577.

KOIKE T., IZUTA T., LEI T. T., KITAO M. & ASANUMA S. 1997. Effects of high CO_2 on nodule formation in roots of Japanese mountain alder seedlings grown under two nutrient levels. - In: ANDO T., FUJITA K., MAE T, MATSUMOTO H., MORI S. & SEKIYA J. (Eds.), Plant nutrition - for sustainable food production and environment, pp. 887-888. - Kluwer Academic Publishers, Japan.

NORBY R. J. 1987. Nodulation and nitrogenase activity in nitrogen-fixing woody plants stimulated by CO_2 enrichment of the atmosphere. - Physiol. Plant. 71: 77-82.

—, WULLSCHLEGER S. D., GUNDERSON C. A., JOHNSON D. W. & CEULEMANS R. 1999. Tree responses to rising CO_2 in field experiments: implications for the future forest. - Plant Cell Environ. 22: 683-714.

O'NEILL E. G. & NORBY R. J. 1996. Litter quality and decomposition rates of foliar litter produced under CO_2 enrichment. - In: KOCH G. W. & MOONEY H. A. (Eds.), Carbon dioxide and terrestrial ecosystems, pp. 87-103. - Academic Press, San Diego.

SCHORTEMEYER M., ATKIN O. K., MCFARLANE N. & EVANS J. R. 1999. The impact of elevated atmospheric CO_2 and nitrate supply on growth, biomass allocation, nitrogen partitioning and N_2 fixation of *Acacia melanoxylon*. - Aust. J. Plant Physiol. 26: 737-747.

TEMPERTON V. M., GRAYSTON S. J., JACKSON G., BARTON C. V. M., MILLARD P. & JARVIS P. G. 2003. Effects of elevated carbon dioxide concentration on growth and nitrogen fixation in *Alnus glutinosa* in a long-term field experiment. - Tree Physiol. 23: 1051-1059.

THOMAS R. B., BASHKIN M. A. & RICHTER D. D. 2000. Nitrogen inhibition of nodulation and N_2 fixation of a tropical N_2-fixing tree (*Gliricidia sepium*) grown in elevated atmospheric CO_2. - New Phytol. 145: 233-243.

TISSUE D. T., MEGONIGAL J. P. & THOMAS R. B. 1997. Nitrogenase activity and N_2 fixation are stimulated by elevated CO_2 in a tropical N_2-fixing tree. - Oecologia 109: 28-33.

VAN HEERWAARDEN L. M., TOET S. & AERTS R. 2003. Nitrogen and phosphorus resorption efficiency and proficiency in six sub-arctic bog species after 4 years of nitrogen fertilization. - J. Ecol. 91: 1060-1070.

VITOUSEK P. M. & HOWARTH R. W. 1991. Nitrogen limitation on land and in the sea: How can it occur? - Biogeochem. 13: 87-115.

VOGEL C. S., CURTIS P. S. & THOMAS R. B. 1997. Growth and nitrogen accretion of dinitrogen-fixing *Alnus glutinosa* (L.) Gaertn. under elevated carbon dioxide. - Plant Ecol. 130: 63-70.

Zeitschrift/Journal: Phyton, Annales Rei Botanicae, Horn

Jahr/Year: 2005

Band/Volume: 45_4

Autor(en)/Author(s): Oue H., Yoshimoto M., Kobayashi K.

Artikel/Article: Effects of Free-Air CO2 Enrichmnet on Leaf and Panicle Temperatures of Rice at Heading and Flowering Stage. 117-124