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Plant species, atmospheric CO_2 and soil N interactively or additively control C allocation within plant-soil systems

FU Shenglei[1,2] & Howard Ferris[2]

1. Institute of Ecology, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China;
2. Department of Nematology, University of California, Davis, CA 95616, USA
Correspondence should be addressed to Fu Shenglei (email: sfu@scbg.ac.cn)

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Abstract Two plant species, *Medicago truncatula* (legume) and *Avena sativa* (non-legume), were grown in low- or high-N soils under two CO_2 concentrations to test the hypothesis whether C allocation within plant-soil system is interactively or additively controlled by soil N and atmospheric CO_2 is dependent upon plant species. The results showed the interaction between plant species and soil N had a significant impact on microbial activity and plant growth. The interaction between CO_2 and soil N had a significant impact on soil soluble C and soil microbial biomass C under *Madicago* but not under *Avena*. Although both CO_2 and soil N affected plant growth significantly, there was no interaction between CO_2 and soil N on plant growth. In other words, the effects of CO_2 and soil N on plant growth were additive. We considered that the interaction between N_2 fixation trait of legume plant and elevated CO_2 might have obscured the interaction between soil N and elevated CO_2 on the growth of legume plant. In low-N soil, the shoot-to-root ratio of *Avena* dropped from 2.63±0.20 in the early growth stage to 1.47±0.03 in the late growth stage, indicating that *Avena* plant allocated more energy to roots to optimize nutrient uptake (i.e. N) when soil N was limiting. In high-N soil, the shoot-to-root ratio of *Medicago* increased significantly over time (from 2.45±0.30 to 5.43±0.10), suggesting that *Medicago* plants allocated more energy to shoots to optimize photosynthesis when N was not limiting. The shoot-to-root ratios were not significantly different between two CO_2 levels.

Keywords: elevated CO_2, legume species, microbial biomass, shoot-to-root ratio.

Primarily due to diverse uses of fossil fuel and changes in the pattern and intensity of land use, atmospheric CO_2 has risen from ca. 280 μL/L before 1750 to a present mean value of 370 μL/L[1]. It was predicted that atmospheric CO_2 concentration could exceed 500 μL/L during the first half of this century under current emission rate[2]. Various studies have shown that plant growth response to elevated CO_2 differs strongly with plant species and some of the studies showed that plant growth response to elevated CO_2 was interactively controlled by soil N availability[3−9]. However, Van Kessel *et al.*[10] found that total the above-ground biomass of *Trifolium repens* (a legume species) accumulated doubled under elevated CO_2 but was independent of N fertilizer. In contrast, *Lolium perenne* (a non-legume species) did not respond to elevated CO_2 but increased significantly in high N soil. In other words, no interactions between CO_2 and soil N effects on plant growth were found in Van Kessel *et al.*'s study[10]. Soussana and Hartwig (1996)[11] found a

significant interaction between N_2 fixation trait of legume and elevated CO_2. They found that legume species usually showed stronger responses to elevated CO_2 than non-legume plant species. The N_2 fixation trait was likely a key factor causing the strong responses of the growth of legume species to elevated CO_2. Therefore, how soil N interacts with legume species regarding their growth response to elevated CO_2 warrants investigation.

Due to a much higher CO_2 concentration (i.e. 2000－6000 μL/L) in soil than in atmosphere (i.e.380 μL/L), the responses of soil processes to elevated CO_2 are basically indirect owing to improved plant carbohydrate status[6]. It was reported that elevated CO_2 resulted in an increase in soil microbial biomass C or rhizosphere bacterial biomass[12−14]. C input to soil such as rhizodeposition and root exudates was enhanced in response to elevated CO_2[14,15]. A few studies showed that the responses of soil processes to elevated CO_2 were interactively controlled by soil N availability[4,6,8,9]. Hu *et al.*[6] found that the effect of elevated CO_2 on soil microbes was highly dependent upon the competition for soil N between plants and soil microorganisms. However, other studies reported no interaction between elevated CO_2 and soil N on soil processes[10,16]. Cotrufo and Gorissen[16] found that soil microbial biomass increased under elevated CO_2 at both soil N levels but no interaction was observed between elevated CO_2 and soil N treatment. Since the effects of elevated CO_2 on soil processes are indirect through the response of plant to elevated CO_2, whether there is an interactive effect between CO_2 and soil N on soil processes is highly dependent upon plant species.

It was reported that the allocation of C among plant shoots and roots was an important component of ecosystem C budget and a good indicator of potential C sequestration. Therefore, the response of shoot-to-root ratio to elevated CO_2 has been widely investigated in recent years[17−19]. Rogers *et al.*[20] reviewed 264 studies on changes of root-to-shoot ratio (reverse of shoot-to-root ratio) as influenced by CO_2 level and found that 59.5% of the studies reported to increase, 37.5% reported to decrease and 3.0% reported no response. They concluded that crop type, plant age, nutrient availability, water, light, and temperature were responsible for the wide range response of shoot-to-root ratios to elevated CO_2 and pointed out that the driving force of shoot-to-root ratio change was the capability of plant to integrate all resources in order to optimize its functions to survive if resources were limiting. Based on this, we hypothesize that shoot-to-root ratio will increase under legume species because soil N might not be limiting to the growth of legume plant with an N_2 fixation trait but the shoot-to-root ratio will decrease under non-legume plant if soil N is limiting, and these changes will be enhanced under elevated CO_2.

In the present study, we selected a legume species (*Medicago truncatula*) and a non-legume species (*Avena sativa*) and grew them in growth chambers under both ambient and elevated CO_2 levels with low and high soil N. Plant growth, the shoot-to-root ratio, soil microbial biomass C, N and soil soluble C were measured. Our objectives were to test the hypothesis that whether C allocation within plant-soil system was interactively or additively controlled by atmospheric CO_2 and soil N was dependent upon plant species.

1 Methods and materials

1.1 Soil and seedling

The soil used in the present experiment was a local Yolo loam (53% sand, 29% silt and 18% clay). Sand (1∶1, v/v) was added to the soil in order to provide a "low-N" soil mixture. The soil mixture was sieved through a 6.35-mm sieve and homogenized using a cement mixer. The total C and N of the soil mixture were 300 mg/kg soil and 40 mg/kg soil, respectively. The mineral N of the soil mixture was 18 mg/kg soil with a pH of 7.6. The experimental microcosms were containers of 9 cm in diameter and 12 cm in height. Each microcosm was filled with 600 g soil mixture and the moisture was initially adjusted to 90% of field water holding capacity.

Medicago seeds were germinated on 1% water agar plates after acid scarification with conc. H_2SO_4 (for 8 min.) and cold treatment (4℃ for 36 h.); the seedlings were transplanted into experimental microcosms. *Avena* seeds were germinated directly in microcosms.

1.2 Experimental design

This experiment was conducted at the Controlled Environmental Facility (CEF) at the University of California, Davis using growth chambers (Controlled Environments Ltd., Winnipeg, Manitoba, Canada). At CEF, all environmental variables (i.e. CO_2 concentration, temperature, humidity, light intensity) of the growth chambers were controlled by a central computer system and all settings were checked and calibrated regularly. However, due to the high occupancy and demand of the growth chambers, only two chambers were assigned and used for this experiment. In order to check the potential chamber effect, *Medicago truncatula* was grown in these two chambers under the same environmental conditions (i.e. ambient CO_2 concentration, 22℃, 70% and 300 μmol·m^{-2}·s^{-1} for temperature, humidity and light intensity) and harvested when plants were mature. Results showed that the plant growth (shoot, root) was not significantly different between two chambers, indicating there was no "chamber effect" for this experiment (Table 1). In the two growth chambers, CO_2 concentration was set at the “ambient” level for one chamber and at “elevated” (ambient + 150 μL/L) level for the other. Temperature, humidity and light intensity were controlled at 22℃, 70% and 300 μmol·m^{-2}·s^{-1} for both growth chambers, respectively.

In each growth chamber, two plant species (*Medicago truncatula* or *Avena sativa*) were grown in separate microcosms. For each plant species, half were randomly treated as “low-N” and the half as “high-N”. The background mineral N (total of NH_4^+-N and NO_3^--N) was 18.3±0.7 mg/kg soil. In the “high-N” soil treatment, 0.33 mg/kg soil of NH_4NO_3-N was added with water to each microcosm daily. In the “low-N” soil treatment, only water was added daily to all microcosms to keep soil moisture at the same level as those microcosms in “high-N” treatment. Four (1st, 2nd, 3rd, 4th) destructive sequential samplings were conducted when plants were 5, 7, 9 and 11 weeks old, respectively. There were 8 replicates for each treatment at each sampling time. Therefore, the total microcosms used in this study were 256 (2 chambers ×2 plant species ×2 soil N levels ×4 sampling times ×8 replicates).

1.3 Measurements

Plant materials were harvested and separated into shoots and roots. The weights of shoots and roots were recorded after oven-drying at 70℃. The shoot-to-root ratios were then calculated. Total C and total N of plant roots were determined using an Infrared Mass Spectrometer (IRMS, Europa Hydra 20/20) at the UC Davis Isotope Facility.

Soil microbial biomass-C was measured using the fumigation-extraction method[21]. Briefly, 20 g of each soil sample was fumigated with $CHCl_3$ vapor for 48 h and was then extracted with 60 mL of 0.5 mol/L K_2SO_4 solution after removal of $CHCl_3$. Control samples were extracted the same way as fumigated samples except without fumigation process. Total C of the fumigated and control samples were measured using TOC analyzer (Shimadzu 5050A). The difference of total C between fumigated and control sample was the mineralized microbial biomass C, and 0.38 was used as K_c for microbial biomass calculation[22]. Soil C extracted with 0.5 mol/L K_2SO_4 from the unfumigated control samples was considered to be soil soluble C. Soil microbial biomass-N was measured using the persulfate-oxidation method[23].

500 g of soil samples was dried and ground to fine powder before it was submitted for analysis. 10 subsamples of the soils were used for measurements of soil total C and N content using the Carlo-Erba combustion method and a dynamic flash combustion system coupled with gas chromatographic (GC) separation and thermal conductivity detection (TCD). The

Table 1 *T*-test for potential “chamber effect” when *Medicago truncatula* was grown in two growth chambers (A, B) under the same environmental conditions

Variables	A[a)]	B[a)]	*F* value	*P* value
Shoot (S)	0.35±0.01[b)]	0.34±0.03	0.02	0.8914[c)]
Root (R)	0.14±0.01	0.13±0.01	0.27	0.6099
Total	0.49±0.02	0.47±0.04	0.07	0.7924
S/R	2.47±0.08	2.60±0.11	0.62	0.4368

a) A, B were the codes for two growth chambers; b) value and standard errors (n = 15); c) significant level was set at $P < 0.05$.

method has a detection limit of 0.01% for carbon and 0.04% for nitrogen and is generally reproducible within 5% (relative).

1.4 Statistics

Statistical analyses for all data were performed with GLM procedures using SAS software[24].Three-way ANOVA was performed to check the main effects and their interactions of atmospheric CO_2, soil N concentration and plant species on the plant growth, soil microbial activities and C allocation between shoots and roots. Significance levels were set at $P < 0.05$.

2 Results

2.1 Plant biomass

In most cases, plant growth responded significantly to the enrichment of atmospheric CO_2 as well as to the enrichment of soil N, however, there was no interaction between atmospheric CO_2 and soil N on plant growth. Soil N had a large impact on plant growth but this impact was dependent upon plant species (Table 2). In other words, there was a significant interaction between plant species and soil N ($P < 0.05$). The shoot-to-root ratio of *Avena* decreased significantly in low-N soil, and the shoot-to-root ratio of *Medicago* increased significantly over time in high-N soil. Nevertheless, the shoot-to-root ratios of the plants were not significantly different between the two CO_2 levels at both soil N levels.

In low-N soil, shoot weights of *Medicago* and root weights of both plant species were significantly greater under elevated CO_2 than under ambient CO_2 by the 4th sampling date when plants were 11 weeks old. There were no differences in these weights at earlier sampling dates. Total plant biomass (sum of shoots and roots) of *Avena* was significantly higher than that of *Medicago* at all times (Figs. 1(a) and 2(a)). The shoot-to-root ratio of *Medicago* maintained a mean value of 3.04±0.12 and did not change over time. However, the shoot-to-root ratio of *Avena* decreased significantly after the 1st sampling when plants were 5 weeks old, and it dropped from 2.63±0.20 to 1.47±0.03 (Fig. 3(a)).

In high-N soil, shoots and roots of both *Medicago* and *Avena* increased significantly under elevated CO_2 after the 1st sampling date when plants were 5 weeks old. Total plant biomass of *Avena* was significantly higher than that of *Medicago* (Figs. 1(b) and 2(b)). The shoot-to-root ratio of *Medicago* increased significantly over time (from 2.45±0.30 to 5.43±0.10) but the shoot-to-root ratio of *Avena* was relatively stable with a mean value of 2.59±0.14 (Fig. 3(b)).

2.2 Soil microbial C and N

The responses of soil microbial biomass C and microbial N to elevated CO_2 or to enrichment of soil N were different under different plant species. There was a significant interaction ($P < 0.005$) between CO_2 and soil N on soil microbial C under *Medicag*, but there was no interaction between CO_2 and soil N on soil microbial C under *Avena*. There was a significant interaction ($P < 0.05$) between plant species and soil N effects on soil microbial C (Table 3).

In low-N soil, microbial biomass C (C_{mic}) was significantly higher at the 3rd and 4th than at the 1st and 2nd sampling dates under both plant species; and this was more pronounced under *Avena*. No significant difference of microbial C was found between two CO_2 levels (Fig. 4(a)). The specific microbial biomass (normalized by plant roots) under *Medicago* decreased through time and the decrease was more pronounced

Table 2 Three-way ANOVA for plant biomass

Source	Three-way ANOVA							
	wk5[a)]		wk 7		wk 9		wk 11	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
CO_2	NS[b)]	***[c)]	***	***	***	NS	***	***
Species	***	***	***	***	***	***	***	***
CO_2×species	NS	NS	NS	NS	NS	NS	NS	NS
N		***	***	***	NS	***	***	***
CO_2×N	NS	NS	NS	NS	NS	***	NS	NS
SpeciesN	***	NS	NS	***	NS	NS	***	***
CO_2×species×N	NS	NS	NS	NS	NS	NS	NS	NS

a) Sampling dates; b) NS indicates “not significant”; c)**, *** = significant at $P < 0.05$, and 0.01 level, respectively.

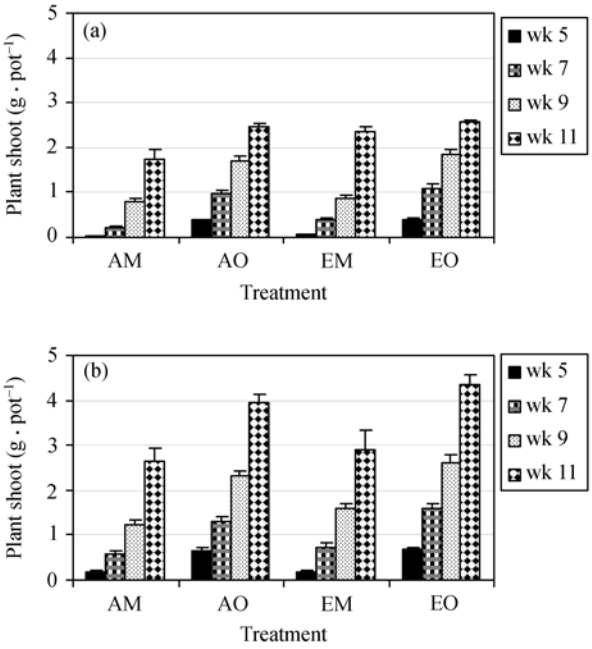

Fig. 1. (a) Dry weight of plant shoots ($g \cdot pot^{-1}$) in low-N soil; (b) dry weight of plant shoots in high-N soil. A, E, M and O refer to "ambient CO_2, elevated CO_2, *Medicago truncatula* and *Avena sativa*", respectively. wk 5, wk7, wk 9 and wk 11 refer to sequential sampling times when plants are 5, 7, 9 and 11 weeks old. Bars are expressed with means and standard errors from 8 observations.

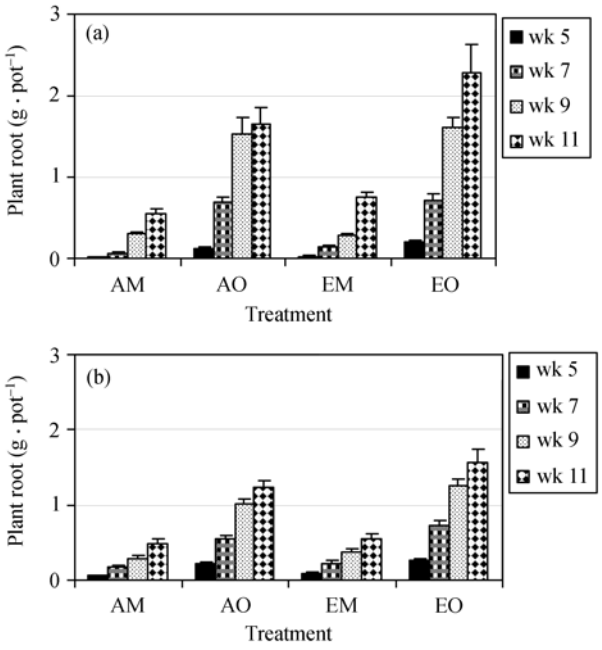

Fig. 2. (a) Dry weight of plant roots ($g \cdot pot^{-1}$) in low-N soil; (b) dry weight of plant roots in high-N soil. Footnotes are the same as in Fig. 1.

under elevated CO_2. The specific microbial biomass under *Avena* stabilized after it dropped significantly from 1st sampling date. In general, the specific microbial biomass associated with *Medicago* (107.27±27.08 mg $C_{mic} \cdot g^{-1}$ root) was higher than that associated with *Avena* (35.02±5.15 mg $C_{mic} \cdot g^{-1}$ root) (Fig. 5(a)). There was no difference in microbial N (N_{mic}) between the

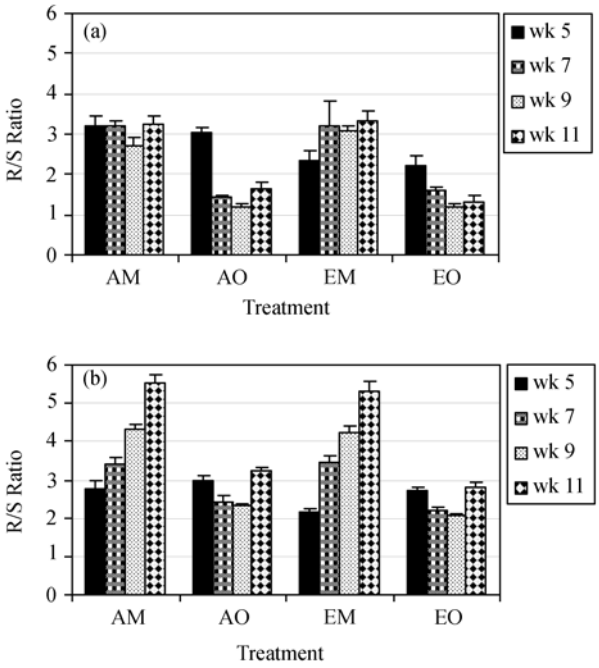

Fig. 3. (a) Shoot-to-root ratios in low-N soil; (b) shoot-to-root ratios in high-N soil. Footnotes are the same as in Fig. 1.

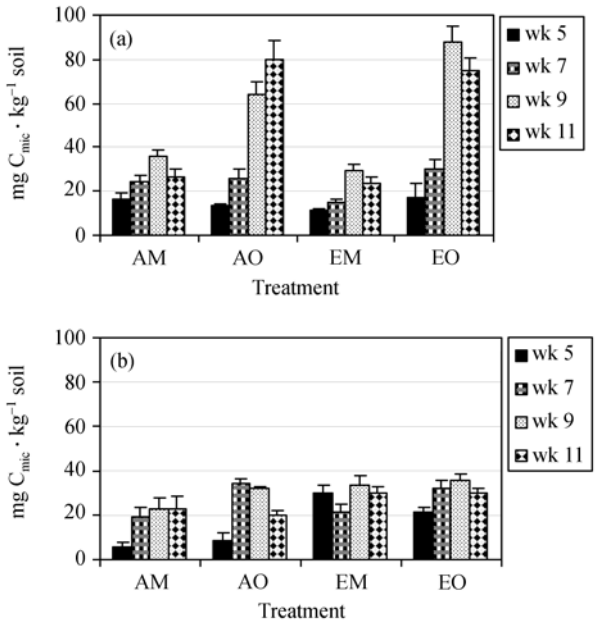

Fig. 4. (a) Soil microbial biomass (mg $C_{mic} \cdot kg^{-1}$ soil) in low-N soil; (b) soil microbial biomass in high-N soil. Footnotes are the same as in Fig. 1.

Table 3 Three-way ANOVA for soil microbial C (C_{mic}) and microbial N (N_{mic})

Source	Three-way ANOVA							
	wk5		wk7		wk9		wk11	
	C_{mic}	N_{mic}	C_{mic}	N_{mic}	C_{mic}	N_{mic}	C_{mic}	N_{mic}
CO_2	***	NS	NS	NS	**	NS	NS	***
Species	NS	NS	***	NS	***	NS	***	***
CO_2 x species	NS	NS	NS	NS	NS	NS	NS	**
N	NS	***	NS	**	***	NS	***	**
CO_2 x N	***	NS	NS	NS	NS	NS	NS	NS
Species x N	NS	NS	NS	NS	***	NS	***	NS
CO_2 x species x N	NS	NS	NS	NS	***	NS	NS	NS

** = significant at $P < 0.05$ level; *** = significant at 0.01 level; NS indicates "not significant".

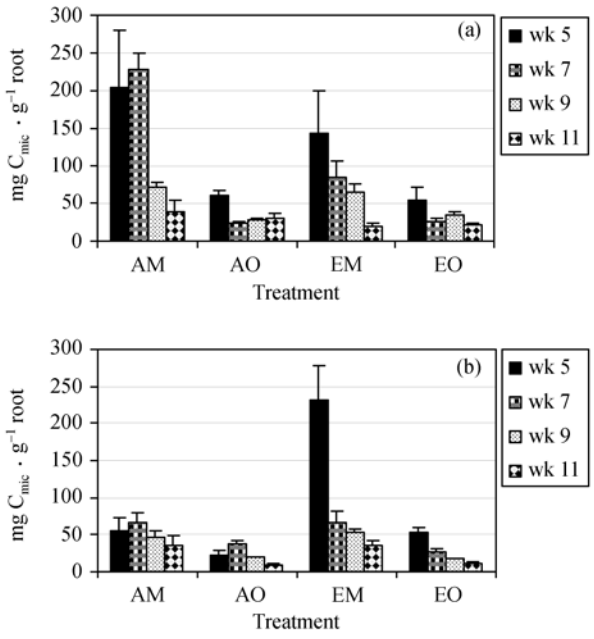

Fig. 5. (a) Specific microbial biomass (mg C_{mi}·g^{-1} root, soil microbial biomass normalized by plant root biomass) in low-N soil; (b) specific microbial biomass in high-N soil. Footnotes are the same as in Fig. 1.

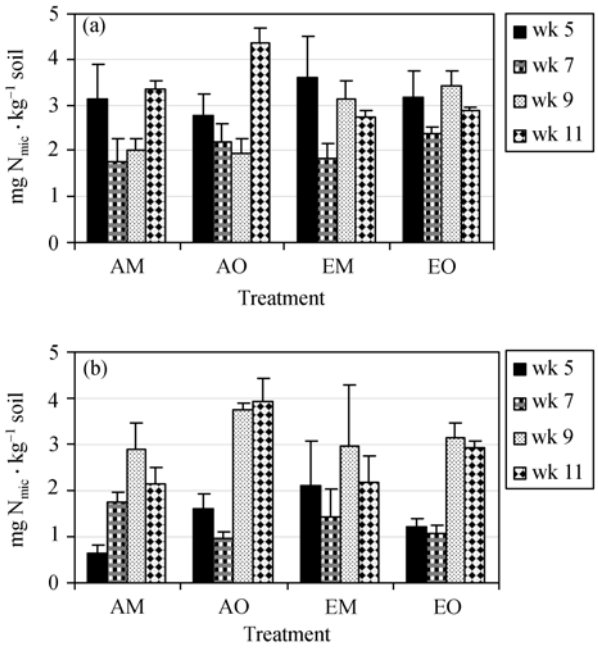

Fig. 6. (a) Soil microbial N (mg N_{mic}·kg^{-1} soil) in low-N soil; (b) soil microbial N in high-N soil. Footnotes are the same as in Fig. 1.

two plant species and between the two CO_2 concentrations; and microbial N did not change through time (Fig. 6(a)).

In high-N soil, microbial biomass C under *Medicago* was significantly higher under elevated CO_2 than under ambient CO_2 level at the 1st, 3nd and 4th sampling dates, with the greatest effect at the 1st sampling date. Microbial biomass C under *Avena* increased significantly on the 1st and 4th sampling dates (Fig. 4(b)). The specific microbial biomass had a decreasing trend over time under both *Medicago* and *Avena*. In general, the specific microbial biomass under *Medicago* (73.29±22.96 mg C_{mic}·g^{-1} root) was higher than that under *Avena* (26.75±5.11 mg C_{mic}·g^{-1} root), and it was significantly higher under elevated CO_2 than under ambient CO_2 for both plant species on the 1st sampling date (Fig. 5(b)). No significant difference in microbial N (N_{mic}) was found between the two plant species or between the two CO_2 levels; but microbial N increased significantly at late growth stages than at early stages (Fig. 6(b)).

2.3 C-to-N ratio of plant roots

In general, N content of plant roots increased with the addition of soil N but C content of plant roots did not change much for both plant species. As a result, C-to-N ratio of plant roots decreased with the addition

Table 4 C and N content of plant roots at different growth stages

Species	CO_2	N	Early growth stage			Late growth stage		
			C (%)	N (%)	C-to-N ratio	C (%)	N (%)	C-to-N ratio
Medicago	A[a)]	low	33.1±1.4[b)]	3.0±0.2	11.1±0.6	35.1±1.2	2.4±0.1	14.9±0.3
		high	36.0±1.5	2.7±0.1	13.7±0.5	39.1±0.7	3.3±0.1	11.9±0.4
	E	low	34.7±1.3	2.9±0.2	12.0±0.4	36.9±0.5	2.4±0.1	15.1±0.2
		high	36.5±1.1	3.2±0.2	11.9±1.0	36.2±0.7	3.2±0.1	11.3±0.2
Avena	A	low	36.8±1.5	0.8±0.0	49.0±1.7	33.0±1.5	0.5±0.0	64.8±1.2
		high	35.0±1.2	1.7±0.2	21.9±2.0	39.0±0.7	0.9±0.0	42.9±1.4
	E	low	32.6±1.1	0.6±0.0	53.9±2.2	34.4±1.3	0.5±0.0	64.3±1.5
		high	34.1±2.0	1.4±0.1	25.1±1.9	35.0±1.7	0.8±0.0	47.3±2.4

a) A, E refer to "ambient CO_2, elevated CO_2", respectively; b) data are means with standard errors (n = 8).

of soil N for both plant species, and this was more pronounced for *Avena*. N content of plant roots was inversely related to their C-to-N ratios. In addition, the C-to-N ratio of plant roots of *Medicago* was significantly lower than that of *Avena* at both soil N levels. In low-N soil, the C-to-N ratio of plant roots ranged from 11.1 to 15.1 for *Medicago* and ranged from 49.0 to 64.8 for *Avena*. In high-N soil, the C-to-N ratio of plant roots ranged from 11.3 to 13.7 for *Medicago* and ranged from 21.9 to 47.3 for *Avena*. Neither C content nor N content of plant roots responded to elevated CO_2, consequently, C-to-N ratio of plant roots did not respond to elevated CO_2 (Table 4).

2.4 Soil soluble C and soil mineral N

Soil soluble C under *Medicago* responded significantly to CO_2 elevation and soil N enrichment, and a significant interaction ($P < 0.005$) was found between elevated CO_2 and soil N on soil soluble C under *Medicago*. Soil soluble C under *Avena* did not respond to elevated CO_2, but increased significantly in high-N soil, no interaction was found between elevated CO_2 and soil N on soil soluble C under *Avena*. Although there was no significant difference in soil soluble C between the two plant species, the concentration of soil soluble C in high-N soil was significantly higher than that in low-N soil under both plant species (Figs. 7(a) and (b)).

In general, no significant difference of soil mineral N concentration was found between ambient CO_2 and elevated CO_2 treatments at both soil N levels, nevertheless, the changing pattern of soil mineral N concentration under *Medicago* was different from that under *Avena*. In low-N soil, soil mineral N level decreased significantly over time under *Avena*, however, it

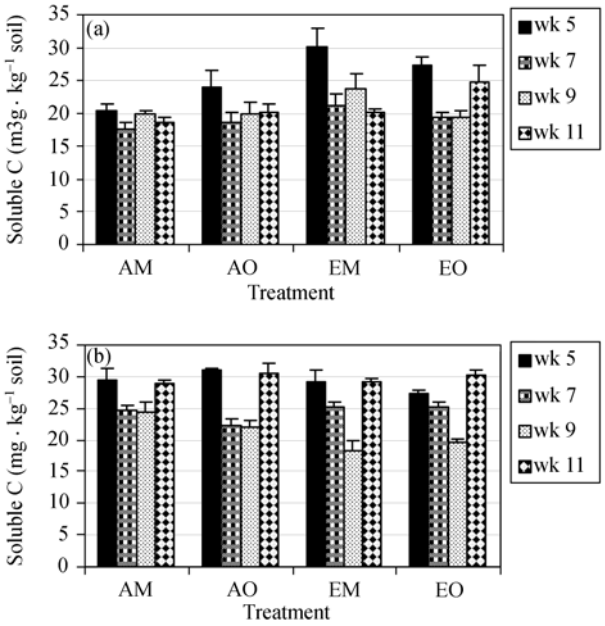

Fig. 7. (a) Soil soluble C (mg·kg^{-1} soil) in low-N soil; (b) soil soluble C in high-N soil. Footnotes are the same as in Fig. 1.

showed a large increase of soil mineral N (compared to background soil N level) at the 1st sampling time before a rapid and continuous decline under *Medicago* (Fig. 8(a)). In high-N soil, soil mineral N level under *Avena*, showed a similar pattern to that in low-N soil. An increase of soil mineral N (compared to background soil N level) at the 1st sampling time was also observed under *Medicago* but it stayed relatively stable after an initial decline (Fig. 8(b)).

3 Discussion

3.1 Plant biomass

In the present study, we found that both legume (*Medicago*) and non-legume (*Avena*) species respond-

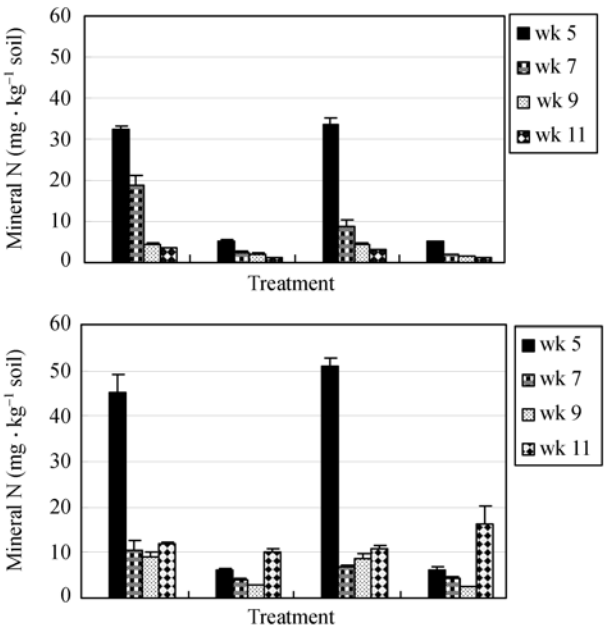

Fig. 8. (a) Soil mineral N (mg·kg^{-1} soil) in low-N soil; (b) soil mineral N in high-N soil. Footnotes are the same as in Fig. 1.

ed to soil N and atmospheric CO_2, which was different from the findings in Van Kessel *et al.*'s study[10]. Van Kessel *et al.*[10] found that legume species only responded to CO_2 but not to soil N and non-legume species only responded to soil N but not to CO_2. We considered that the N-fixation trait of legume species might have the complicated effect of soil N on plant growth in these two studies because the N-fixing capability of legume species was more likely to be optimized in field conditions (i.e. Van Kessel *et al.*'s study) than in pot studies (i.e. our study). In fact, Suter *et al.*[19] demonstrated that the response of plant growth to elevated CO_2 was much stronger in field condition than in controlled environment. In the field study like Van Kessel et al.' s[10], soil N might not be a limiting factor to the growth of legume species because of sufficient N_2 fixation; therefore, the growth of legume species did not respond to soil N enrichment. Niklaus *et al.*[25] reported that about 90% of N for legume plant was from symbiotic N_2 fixation, and thus legume growth was not limited by soil N. We postulated that the statement made by Niklaus *et al.* might be true in the field conditions but not in pot study. Based on our observation, root growth and nodule formation were constrained so that N-fixing capability could not be optimized and soil N was still a limiting factor to the growth of legume species, this limitation was more pronounced in low-N soil. In the present study, N limitation to the growth of legume plant in low-N soil was indicated in the following two aspects: 1) In general, N content of plant roots was lower in low-N soil (with one exception) than in high-N soil. As a result, C-to-N ratio of plant roots was higher in low-N soil than in high-N soil; 2) N content of plant roots was lower at the late plant growth stage than at the early growth stage, consequently, C-to-N ratio of plant roots was higher at the late plant growth stage than at the early growth stage (Table 4). As to *Avena*, N limitation was even more pronounced because the responses of N content and C-to-N ratio were even larger to soil N change and plant age. In the present study, no interaction was found between elevated CO_2 and soil N effects on the growth of either *Medicago* or *Avena*. However, there was a significant interaction between elevated CO_2 and soil N effects on soil microbial biomass C and soluble C under *Medicago* though not under *Avena*.

In low-N soil, the shoot-to-root ratio of *Medicago* did not change much over time; however, the shoot-to-root ratio of *Avena* decreased (resulting from increase of root growth) significantly during late growth stages. In high-N soil, the shoot-to-root ratio of *Medicago* increased (resulting from the increase of shoot growth) significantly over time but the shoot-to-root ratio of *Avena* was relatively stable. Similarly, Gedroc *et al.*[26] found that plants grown at low-nutrient levels had lower shoot-to-root ratio than those grown at high-nutrient levels. This illustrated two different survival strategies of plants regarding C allocation. When soil nutrient (i.e. N) is limiting to plant growth, the plant allocates more energy (C) to roots[27,28] to optimize the uptake of nutrients from soil. When soil nutrient is not limiting to plant growth, the plant allocates more energy to shoots[29] to optimize the leaf area for photosynthesis. This was consistent with the former part of our hypothesis that shoot-to-root ratio increases under legume species when soil N is not limiting to its growth but decreases under non-legume species when soil N is limiting. Nevertheless, the shoot-to-root ratios of the plants were not significantly different between two CO_2 levels.

3.2 Soil microbial C and N

Although many studies reported that soil microbial biomass was enhanced under elevated CO_2[30−32], Zak *et al*.[33] found that soil microbial biomass, microbial immobilization, and nitrification were equivalent at ambient and elevated CO_2 in either low- or high-N soil. In the present study, we found that the response of soil microbial C to elevated CO_2 was dependent on both plant species and soil N level. Overall, no significant difference in soil microbial N and soil soluble C was observed between elevated CO_2 and ambient CO_2 in either low- or high-N soil. In addition, no significant difference in microbial C was found between two CO_2 levels in low-N soil. However, microbial C under *Medicago* increased significantly under elevated CO_2 than ambient CO_2 in high-N soil. Significant interactions were found between elevated CO_2 and soil N effects on soil microbial biomass C and soil soluble C under *Medicago*.

Microbial biomass C increased over time under both plant species (more pronounced under *Avena*) in low-N soil, but it was found only closely related to root biomass. The specific microbial biomass (normalized by plant root biomass) decreased through time, indicating the root vitality and/or activity decreased with plant age. In general, the specific microbial biomass under *Medicago* was greater than that under *Avena*; it was most likely due to higher quality (i.e. N content) of root exudates of *Medicago* (legume species). Lower C-to-N ratio of *Medicago* roots provided indirect evidence for that. Microbial N was not significantly different between two CO_2 levels in either low- or high-N soil, indicating that elevated CO_2 did not alter the retention of microbial N. In other words, plants grew more under elevated CO_2 and demanded more soil mineral N; they might win the competition for mineral N in soil against microorganisms but might not have a mechanism to get the N out of microbial biomass.

4 Conclusion

Both atmospheric CO_2 and soil N had significant impact on plant growth, but these impacts varied with plant species. There was no interaction between atmospheric CO_2 and soil N on plant growth, but there was an interaction between atmospheric CO_2 and soil N on soil soluble C and microbial biomass under legume plant (*Medicago*). We considered that the interaction between N_2 fixation trait and elevated CO_2 might have obscured the interaction between soil N and elevated CO_2 effects on the growth of legume plant (*Medicago*). In low-N soil, the plant growth of non-legume plant (*Avena*) was seriously limited by soil N so that more energy was allocated to roots for N uptake; therefore, the shoot-to-root ratio of *Avena* decreased significantly. In high-N soil, the growth of legume plant (*Medicago*) was not limited by soil N so that more energy was allocated to shoots for photosynthesis; therefore, the shoot-to-root ratio of *Medicago* increased significantly. This was consistent with part of our hypothesis that the shoot-to-root ratio of plant increases when soil nutrient is not limiting to plant growth and the shoot-to-root ratio of plant decreases when soil nutrient is limiting. Nevertheless, the changes of shoot-to-root ratio were not enhanced under elevated CO_2. Overall, the findings of the present study are consistent with our hypothesis that whether C allocation within plant-soil system is interactively or additively controlled by atmospheric CO_2 and soil N is dependent upon plant species.

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