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Impact of CO_2 enrichment and variable nitrogen supplies on composition and partitioning of essential nutrients of wheat

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Abstract

This study was conducted to determine effects of nitrogen supply (75 and 150 kg(N) ha^{-1}) and CO_2 enrichment on partitioning of macro and micro nutrients in wheat (*Triticum aestivum* L. cv. HD-2285). Plants were grown from seedling emergence to maturity inside open top chambers under ambient CO_2 (CA, 350 ± 50 μmol mol^{-1}) and elevated CO_2 (CE, 600 ± 50 μmol mol^{-1}). Leaves, stems and roots of the same physiological age were analyzed for carbon, nitrogen, calcium, copper, iron, zinc and manganese content at 40, 60 and 90 d after germination. C, Cu, Mn and Zn content was higher in the stem, leaves and roots on dry mass basis under CE than CA. However, N and Fe contents decreased in CE grown plants. Ca content was unaffected due to CE and variable N supplies.

Additional key words: calcium, carbon, copper, iron, manganese, nitrogen, *Triticum aestivum*, zinc.

Introduction

The direct impact of rising atmospheric CO_2 concentration on plants includes a potential increase in photosynthetic rate (*e.g.* Ulman *et al.* 2000, Srivastava *et al.* 2002) and growth (*e.g.* Griffin and Luo 1999) that may significantly alter the structure and productivity of both natural and agricultural plant community (Drake *et al.* 1997).

Interaction between elevated CO_2 and variable nitrogen supplies on crops like soybean (Campbell *et al.* 1988), sugar beet (Demmers-Derks *et al.* 1998), tobacco (Geiger *et al.* 1999), rice (Aben *et al.* 1999) and cotton (Broker *et al.* 2000) showed that high CO_2 can increase the Rubisco efficiency and cause mobilization of nitrogen for their growth and development (Theobald *et al.* 1998). Thus the crop plants which show increased Rubisco efficiency under CE may require less amount of N for optimum biomass production (Sage *et al.* 1988). Therefore, the objective of the present experiment was to examine the effects of various doses of nitrogenous fertilizer on partitioning of various macro and micro nutrients in wheat and to determine whether the nutritional balance was modified in plants growing in a CO_2 enriched environment.

Materials and methods

Plants and treatments: Wheat (*Triticum aestivum* L. cv. HD-2285) was grown in pots filled with sandy loam soil (3:1). Nitrogen doses [recommended – 150 kg ha^{-1} (N_{150}) and low – 75 kg ha^{-1} (N_{75})] was given in the form of three split doses. Pots were sufficiently supplied with water. A modified naturally lit open top chamber (OTC), as described by Rogers *et al.* (1996) was developed to study crop responses to elevated CO_2. The height and diameter of the OTC were 1.8 m and 1.6 m, respectively. One set of plants was grown under ambient CO_2 (CA, 350 ± 50 μmol mol^{-1}) and other group of plants was grown under elevated CO_2 (CE, 600 ± 50 μmol mol^{-1}). A total of 16 pots were used in each chamber for the treatment. The concentration of CO_2 in the chamber was monitored

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Abbreviations: CA, CE - ambient and elevated CO_2 concentrations (350 ± 50 and 600 ± 50 μmol mol^{-1}, respectively); N_{75}, N_{150} - nitrogen doses 75 and 150 kg ha^{-1}, applied in three split doses.
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using infra-red gas analyser (Model *LI-6200*, *Licor*, Lincoln, USA).

Plant materials (leaves, stems and roots) were sampled at vegetative, flowering and anthesis stages (40, 60 and 90 d after germination). Roots of the plants were removed by washing the soil of the pots without disrupting the small little branches of the roots. Each plant was separated into leaves, stem and roots and dried at 80 °C for 4 h and then at 60 °C till constant mass. Dry mass was recorded by electronic balance (*Sartorius 1212 MP*, Gottingen, Germany).

Total nitrogen concentration: Nitrogen in the form of reduced nitrogen in 0.1 g samples of dried leaves, stems and roots subjected to digestion and distillation was determined by *N-Kjeltech Auto 1030* analyzer, following the procedure detailed in *Tecator* manual, 1987 (*Tecator Company*, Hoganas, Sweden).

Carbon estimation: Estimation of total carbon content was done by wet digestion following the modified Walkley-Black method (Walkley and Black 1934). The dried sample (0.05 g) was oxidized with a mixture of potassium dichromate and concentrated sulphuric acid using the heat of dilution of acid. The unused potassium dichromate was estimated by back titration with ferrous ammonium sulphate.

Estimation of macro and micronutrients: Samples were digested using mixture of HNO_3 and $HCLO_4$ (9:4) according to Bhargava and Raghupathi (1993). The digested material was used for the estimation of Ca, Mn, Zn, Cu and Fe using atomic absorption spectrophotometer (*Perkin Elmer Analyst*™ *700*, CT, USA).

Statistical analysis: The experiment was conducted in randomized block design. There were five replications for each treatment. For estimation of nutrients four plants from each replication were mixed together and then required amount was weighed in triplicate. Statistical analysis of the data was done by analysis of variance (ANOVA) given by Panse and Sukhatme (1967). The critical difference (CD) values were calculated at 5 % probability level.

Results

The carbon content of plants increased under CE. The C content of leaves was higher at N_{150} as compared to N_{75} under elevated CO_2. The maximum increase in the C content was recorded at 90 d after germination (11.5 % at N_{75} and 19.16 % at N_{150}) (Table 1). Similar results were obtained in stems and roots where maximum C content was recorded at both the N doses under CE at 90 d after sowing (Tables 2 and 3).

In contrast to carbon, nitrogen concentration in leaves, stems and roots of CE plants were lower than in CA plants. The decrease due to CO_2 enrichment was more pronounced under N_{75} as compared to N_{150}. The decrease was maximum at 40 d at both N doses (Tables 1,2). C:N ratio in leaves, stems and roots was higher at CE and N_{75} as compared to CA and N_{150}. However, C:N ratio in stem was less when compared to that of leaf and root.

Table 1. Effects of ambient (CA) and elevated (CE) CO_2 concentration and different nitrogen supply (N_{75}, N_{150}) on nutrients of wheat leaves at different growth stages. Values are mean ± SD of five replications, * - $P < 0.05$. Contents of C, N and Ca are expressed in [%(d.m.)] and those of Mn, Zn, Cu and Fe in [µg g^{-1}(d.m.)].

Age	Treatments		C	N	C/N	Ca	Mn	Zn	Cu	Fe
40 d	N_{75}	CA	38.05±0.08	2.94±0.24	12.94±1.09	0.44±0.03	12.50±2.67	24.00±2.83	8.80±0.25	18.44±1.26
		CE	40.80±0.57	2.09±0.04	19.52±0.60*	0.49±0.04	14.30±1.77	21.30±2.55	11.50±0.41	15.30±1.40
	N_{150}	CA	39.24±2.84	3.19±0.11	12.29±0.45	0.46±0.01	13.89±1.23	27.50±2.33	9.50±0.32	20.22±2.97
		CE	41.61±0.55	2.70±0.03*	15.41±0.04	0.55±0.01	16.00±1.10*	25.80±2.55	13.00±0.80*	16.84±1.41*
60 d	N_{75}	CA	38.50±2.30	2.46±0.01	15.65±0.98	0.52±0.05	14.50±3.11	32.50±3.30	10.72±0.23	23.05±1.88
		CE	41.56±0.47	1.78±0.11*	23.34±1.67*	0.58±0.03	15.40±3.25	36.40±1.50	13.03±0.50	20.13±1.30*
	N_{150}	CA	39.46±3.10	2.92±0.03	13.51±0.93	0.54±0.01	14.95±1.81	34.30±3.62	11.40±0.74	23.78±1.90
		CE	44.67±4.68	2.66±0.31	16.79±0.21	0.61±0.08	16.10±1.57	37.60±1.24	14.60±1.23*	22.66±1.57
90 d	N_{75}	CA	39.96±2.82	2.18±0.25	18.33±3.46	0.49±0.23	13.40±1.24	28.10±1.92	7.60±0.43	21.55±3.27
		CE	44.54±2.16	1.64±0.31*	27.15±6.59*	0.53±0.01	16.20±1.40*	31.60±1.95	8.20±0.85	18.12±1.24
	N_{150}	CA	42.58±3.85	2.65±0.14	16.05±0.59	0.52±0.23	14.70±1.70	30.30±5.08	9.30±1.22	23.26±1.44
		CE	50.72±4.47	2.45±0.32	20.70±4.57*	0.57±0.06	18.00±4.07*	34.20±3.80	9.70±0.36	22.84±2.60

N_{150} had more pronounced effect on Cu content in leaf when compared to N_{75} under CE than under CA and the maximum increase in Cu content in leaf was recorded at 40 d (Table 1). Increase in Cu content in stem and a root was also observed under CE. The maximum increase was obtained at 90 d under CE at both the N doses (Tables 2, 3).

Minor increase in Mn content was observed in leaves grown at CE under both N doses. In stem no significant difference in Mn content was observed at later growth stages but at 40 d, higher increase in Mn due to CO_2 enrichment was obtained under N_{150} when compared to N_{75}. In roots consistent increase in Mn was observed at both nitrogen doses due to CO_2 enrichment (Tables 1, 2, 3).

Iron content in wheat leaves was reduced due to CO_2 enrichment under both N applications and highest change in Fe content was observed at vegetative stage. No significant difference in Fe content was observed in stems. In roots N_{150} had more pronounced effect when compared to N_{75} under CE at 60 d (Table 3).

No significant variation in Ca content was observed in leaf, stem and roots under different CO_2 and N treatments.

Zn content in leaf was reduced in the initial stages of plant growth and then showed an increasing trend in later growth stages due to CE. The maximum increase in Zn content in leaf was recorded at 90 d under both N applications. However, in stem higher Zn content was obtained at initial stages but decreased later. Zn content in root showed similar pattern to stem and its accumulation slowly decreased with plant age.

Table 2. Effects of ambient (CA) and elevated (CE) CO_2 concentration and different nitrogen supply (N_{75}, N_{150}) on nutrients of wheat stems at different growth stages. Values are mean ± SD of five replications. * - $P < 0.05$. Contents of C, N and Ca are expressed in [%(d.m.)] and those of Mn, Zn, Cu and Fe in [μg g^{-1}(d.m.)].

Age	Treatments		C	N	C/N	Ca	Mn	Zn	Cu	Fe
40 d	N_{75}	CA	37.39±4.47	1.73±0.18	21.61±3.47	0.53±0.06	9.80±1.10	27.20±1.53	6.50±0.67	13.65±1.88
		CE	39.08±5.69	1.45±0.10*	26.95±2.09*	0.56±0.04	10.70±1.74	34.00±3.90*	7.40±0.77	12.46±2.86
	N_{150}	CA	37.61±2.28	2.02±0.23	18.62±1.04	0.53±0.04	10.60±1.75	31.70±1.57	7.20±0.82	14.98±2.98
		CE	40.33±4.13	1.89±0.16*	21.33±3.95	0.57±0.11	20.41±1.57*	39.70±2.05*	8.60±0.29*	14.07±2.67
60 d	N_{75}	CA	38.03±4.00	1.47±0.16	25.87±0.02	0.52±0.06	11.80±2.81	34.94±5.32	5.90±0.51	16.48±3.76
		CE	41.23±6.75	1.25±0.14	32.98±1.68*	0.56±0.04	12.90±5.09	37.20±2.21	7.20±0.75	14.73±2.69
	N_{150}	CA	39.33±4.51	1.87±0.17	21.01±0.51	0.54±0.04	12.80±2.97	38.10±3.34	6.50±0.58	17.02±2.66
		CE	43.65±5.49	1.72±0.17	25.37±0.69*	0.56±0.07	13.90±2.18	39.50±5.18	7.70±0.35*	12.94±1.57*
90 d	N_{75}	CA	39.66±3.93	1.22±0.13	32.51±6.89	0.48±0.01	12.00±2.25	24.70±2.66	5.50±0.39	12.38±2.55
		CE	45.30±4.54	1.09±0.12	41.55±0.45*	0.47±0.01	12.40±2.60	27.00±4.71*	7.60±0.69	10.74±0.82
	N_{150}	CA	41.24±5.71	1.91±0.15	21.59±4.70	0.51±0.12	12.60±3.46	27.40±1.37	5.90±0.61	14.51±2.97
		CE	47.26±3.95	1.65±0.16	28.64±0.31*	0.50±0.03	12.60±2.62	28.64±2.17	8.40±0.25*	11.92±1.12

Table 3. Effects of ambient (CA) and elevated (CE) CO_2 concentration and different nitrogen supply (N_{75}, N_{150}) on nutrients of wheat roots at different growth stages. Values are mean ± SD of five replications. * - $P < 0.05$. Contents of C, N and Ca are expressed in [%(d.m.)] and those of Mn, Zn, Cu and Fe in [μg g^{-1}(d.m.)].

Age	Treatments		C	N	C/N	Ca	Mn	Zn	Cu	Fe
40 d	N_{75}	CA	13.23±1.73	0.45±0.01	29.35± 2.91	0.72±0.05	18.00±1.98	37.10±2.21	14.17±0.84	22.27±2.09
		CE	14.24±3.17	0.32±0.10*	44.50±24.85*	0.74±0.03	23.60±1.57*	49.60±2.66*	17.50±1.20*	20.82±2.47
	N_{150}	CA	15.36±1.33	0.58±0.00	26.48± 2.29	0.80±0.01	22.00±3.32	42.20±2.05	15.80±1.11	27.42±3.30
		CE	16.62±2.23	0.46±0.03	36.13± 7.09*	0.82±0.00	25.00±3.62	51.40±3.61*	19.20±2.10*	26.60±4.67
60 d	N_{75}	CA	18.17±1.44	0.40±0.07	45.42±11.80	0.77±0.03	16.30±1.53	34.60±2.83	15.40±0.99	32.30±1.46
		CE	20.07±1.16	0.29±0.01*	69.20± 2.32*	0.84±0.06	22.70±1.34*	39.00±5.09	18.70±1.56*	26.51±1.26*
	N_{150}	CA	21.03±0.67	0.52±0.01	40.44± 0.75	0.82±0.22	19.50±2.04	38.70±3.11	17.50±0.66	34.25±1.53
		CE	24.32±2.83	0.41±0.01	59.31± 8.95*	0.88±0.11	39.36±3.30*	41.90±5.49	19.50±1.54	23.07±1.10*
90 d	N_{75}	CA	18.25±1.05	0.33±0.05	55.30±11.93	0.82±0.03	18.70±2.35	34.20±3.65	12.80±0.56	27.03±5.16
		CE	20.90±2.38	0.28±0.04	74.64± 2.86*	0.86±0.00	26.40±2.90*	37.30±7.92	14.60±2.20	24.36±3.30
	N_{150}	CA	23.24±1.22	0.62±0.23	37.48±13.32	0.84±0.06	20.20±2.98	38.20±2.98	14.50±1.66	34.40±1.70
		CE	27.54±1.48	0.39±0.08*	70.62±19.63	0.88±0.16	27.80±2.25*	40.50±3.75	16.51±1.88	29.00±1.26*

Discussion

The carbon content was higher in plants at CE than at CA. The maximum C content was observed in leaves and lowest in roots. Increase in N supply caused further increase in C content of both CA and CE plants. Similar increase in C content has been reported by van Ginkel *et al.* (1997) in root, stem and leaves of *Lolium perenne* in response to CE. However, Gorissen and Cotrufo (2000) have reported reduced C content of roots due to CE.

Low N concentration in leaves, stem and roots was recorded when the wheat plants were grown under CE. Maximum reduction of N in all plant parts occurred at N_{75}. Similarly lowering of N concentration under CE has been reported in wheat (Hocking and Meyer 1991, Manderscheid *et al* 1995) and soybean (Reeves *et al.* 1994). However, reduction in N concentration under CE was less when N supply to soil was increased. Rogers *et al.* (1996) have reported that high CO_2 induced reduction in leaf N concentration disappear when N supply was enhanced in cotton.

An increase in C content and reduction in N concentration of plant tissues increased the C/N ratio. Monje and Bugbee (1988) have reported similar increase in C/N ratio in wheat under CE. The highest increase in C/N ratio occurred in roots followed by leaves and stem. Srivastava *et al.* (2001) have reported that under CE partitioning of C is more towards roots compared to shoot in mungbean. However, increase in N supply to soil lowered the C/N ratio.

The elevated CO_2 also affect the content of other macro and micronutrients like Ca, Mn, Zn, Fe and Cu in all the parts of wheat plants. The Ca content showed marginal increase whereas Fe content were lower under CE in stem, leaves and roots at all the stages of growth. Mn, Zn and Cu content were higher in CE grown plant as compared to CA but the increase was not significant at any stage. Baxter *et al.* (1994) have also reported similar increase in P, Mg and K content in three grass species under CE. In this study, plants grown under CE and high N application (N_{150}) showed more gain in nutrient content compared to plants grown under CE and low N application (N_{75}). Therefore, CE and high N grown plants removed more nutrients from the soil. A reduction in plant nutrient concentration is often found at elevated CO_2 (Wong 1979, Conroy *et al.* 1992, Schaffer *et al.* 1997) and our findings are in contrast to them.

These results suggest that CO_2 enrichment may affect the uptake of micro and macronutrients as well as their allocation to different plant parts. However further studies are required to confirm the changes in their critical concentrations and process of nutrient uptake as affected by increase in atmospheric CO_2 in near future.

References

Aben, S.K., Ghannown, O., Conroy, J.P.: Nitrogen requirements for maximum growth and photosynthesis of rice *Oryza sativa* L. cv. Jarrah grown at 36 and 70 Pa CO_2. - Aust. J. Plant Physiol. **26**: 759-766, 1999.

Baxter, R., Gantley, M., Ashenden, T.W., Farrar, J.F.: Effects of elevated carbon dioxide on three grass species from montane pasture. II. Nutrient uptake, allocation and efficiency of use. - J. exp. Bot. **45**: 1267-1278, 1994.

Bhargava, B.S., Raghupathi, H.B.: Analysis of plant materials for macro and micronutrients. - In: Tandon, H.L.S. (ed.): Methods of Analysis of Soils, Plants, Water and Fertilizers. Pp. 49-82. Fertilizer Dev. Consul. Org., New Delhi 1993.

Broker, F.L., Shafer, S.R., Wei, C.M., Horton, S.J.: CO_2 enrichment and N fertilization effects on cotton plant residue chemistry and decomposition. - Plant Soil **220**: 89-98, 2000.

Campbell, W.J., Allen, L.H., Jr., Bower, G.: Effects of CO_2 concentration on Rubisco activity, amount and photosynthesis in soybean leaves. - Plant Cell Environ. **14**: 807-818, 1988.

Conroy, J.P., Milham, P.J., Barlow, E.W.R.: Effect of nitrogen and phosphorus availability on the growth response of *Eucalyptus grandis* to high CO_2. - Plant Cell Environ. **15**: 843-847, 1992.

Demmers-Derks, H., Mitchell, R.A.G., Mitchell, V.J., Lawlor, D.W.: Response of sugar beet (*Beta vulgaris* L.) yield and biochemical composition to elevated CO_2 and temperature at two nitrogen applications. - Plant Cell Environ. **21**: 829-836, 1998.

Drake, B.G., Gonzalez-Meler, M.A., Long, S.P.: More efficient plants: a consequence of elevated carbon dioxide? - Annu. Rev. Plant Physiol. Plant mol. Biol. **48**: 607-640, 1997.

Geiger, M., Haake, V., Ludewig, F., Sonnewald, U., Stitt, M.: The nitrate and ammonium nitrate supply have a major influence on the response of photosynthesis, carbon metabolism, nitrogen metabolism and growth to elevated carbon dioxide in tobacco. - Plant Cell Environ. **22**: 1177-1183, 1999.

Gorissen, A., Cotrufo, M.F.: Decomposition of leaf and root tissue of three perennial grass species grown at two levels of atmospheric CO_2 and N supply. - Plant Soil **224**:75-84, 2000.

Griffin, K.L., Luo, Y.: Sensitivity and acclimation of *Glycine max* (L.) Merr. Leaf gas exchange to CO_2 partial pressure. - Environ. exp. Bot. **42**: 141-153, 1999.

Hocking, P.J., Meyer, C.P.: Effects of CO_2 enrichment and nitrogen stress on growth, and partitioning of dry matter and nitrogen in wheat and maize. - Aust. J. Plant Physiol. **18**: 339-356, 1991.

Manderscheid, R., Bender, J., Jager, H.J., Weigel, H.J.: Effects of season long CO_2 enrichment on cereals. II. Nutrient concentrations and grain quality. - Agr. Ecosyst. Environ. **54**: 175-185, 1995.

Monje, O., Bugbee, B.: Adaptation to high CO_2 concentration in an optimal environment; radiation capture, canopy quantum yield and carbon use efficiency. - Plant Cell Environ. **21**: 315-324, 1998.

Panse, V.G., Sukhatme, P.T. (ed.): Statistical Methods for Agricultural Workers. - Indian Council of Agricultural Research, New Delhi 1967.

Reeves, D.W., Rogers, H.H., Prior, S.A., Wood, C.W., Runion, G.B.: Elevated atmospheric CO_2 effects on sorghum and soybean nutrient status. - J. Plant Nutr. **17**: 1939-1954, 1994.

Rogers, G.S., Milham, P.J., Thiband, M.C., Conroy, J.P.: Interaction between rising CO_2 concentration and nitrogen supply in cotton. I. Growth and leaf nitrogen concentration. - Aust. J. Plant Physiol. **23**: 119-125, 1996.

Sage, R.F., Sharkey, T.D., Seeman, J.R.: The *in vivo* response of the ribulose-1,5-bisphosphate carboxylase activation state and the pool sizes of photosynthetic metabolites to elevated CO_2 in *Phaseolus vulgaris* L. - Planta **174**: 407-416, 1988.

Schaffer, B., Whiley, A.W., Searle, C., Nissen, R.J.: Leaf gas exchange, dry matter partitioning, and mineral element concentrations in mango as influenced by elevated atmospheric carbon dioxide and root restriction. - J. amer. Soc. hort. Sci. **122**: 849-855, 1997.

Srivastava, A.C., Pal, M., Sengupta, U.K.: Changes in nitrogen metabolism of *Vigna radiata* in response to elevated CO_2. - Biol. Plant. **45**: 395-399, 2002.

Srivastava, A.C., Sengupta, U.K., Pal, M.: Growth, CO_2 exchange rate and dry matter partitioning in mungbean grown under elevated CO_2. - Indian J. exp. Biol. **39**: 572-577, 2001.

Theobald, J.C., Michell, R.A.C., Parry, M.A.J., Lawlor, D.W.: Estimating the excess investment in ribulose-1,5-bisphosphate carboxylase/oxygenase in leaves of spring wheat grown under elevated CO_2. - Plant Physiol. **118**: 945-955, 1998.

Ulman, P., Čatský, J., Pospíšilová, J.: Photosynthetic traits in wheat grown under decreased and increased CO_2 concentration, and after transfer to natural CO_2 concentration. - Biol. Plant. **43**: 227-237, 2000.

Van Ginkel, J.H., Gorissen, A., Van Veen, A.: Carbon and nitrogen allocation in lolium perenne in response to elevated atmospheric CO_2 with emphasis on soil carbon dynamics. - Plant Soil **188**: 299-308, 1997.

Walkley, A., Black, C.A.: An examination of Degjareff methods for determining soil organic matter and proposed modification of the chromic acid titration method. - Soil Sci. **37**: 29-38, 1934.

Wong, S.C.: Elevated atmospheric partial pressure of CO_2 and plant growth. II. Interaction of nitrogen nutrition and photosynthetic capacity in C_3 and C_4 plants. - Oecologia **44**: 68-74, 1979.