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Carbon balance of a winter wheat-root microbiota system under elevated CO_2

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Abstract

We examined the carbon budget of young winter wheat plants and their associated microorganisms as affected by a doubling of the atmospheric CO_2 concentration (700 μmol mol^{-1}). Plants were grown hydroponically in pre-sterilised sand at a controlled irradiance and temperature regime. Net photosynthesis (P_N) and respiration (R_D) rates of roots and shoots were measured continuously, plant growth and carbon distribution in the plant-root medium-associated microorganism system were determined destructively in interval-based analyses. P_N in elevated CO_2 grown plants (EC) was 123 % of that in the control (AC) plants when averaged over the whole life span (39-d-old plants, 34 d in EC), but the percentage varied with the developmental stage being 115, 88, and 167 % in the pretillering, tillering, and posttillering phase, respectively. There was a transient depression of P_N, higher amplitude of day/night fluctuations of the chloroplast starch content, and depression of carbon content in rhizosphere of EC plants during the period of tillering. After 34 d in EC, carbon content in shoots, roots, and in rhizodepositions was enhanced by the factors 1.05, 1.28, and 1.96, respectively. Carbon partitioning between above and belowground biomass was not affected by EC, however, proportionally more C in the belowground partitioning was allocated into the root biomass. Carbon flow from roots to rhizodepositions and rhizosphere microflora was proportional to P_N; its fraction in daily assimilated carbon decreased from young (17 %) to older (3-4 %) plants.

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Additional key words: carbon partitioning; photosynthesis; plant growth; microbial biomass; respiration rate; *Triticum aestivum* L.

Introduction

The response of plant growth to an environmental factor cannot be simply estimated from the response of photosynthesis. While P_N determines the amount of carbon available for plant metabolism, C partitioning coordinates the photosynthetic potential of the whole plant with absorption capacity for nutrients and water, and codetermines soil microbiota vital to the overall health of the plant. Moreover, the proportion of C lost when converting fixed C to structural biomass must be included into the growth calculations (see Lloyd and Farquhar 1996). The necessary values can be obtained from simultaneous measurements of photosynthesis, respiration, and biomass production. Numerous studies addressed the effect of EC on photosynthesis, saccharide metabolism, and shoot carbon allocation (*e.g.*, Farrar and Williams 1991, Stitt 1991, Sage 1994, Roumet *et al.* 1996). However, studies linking photosynthesis and respiration of shoots and roots with detailed analysis of plant growth and C accumulation and utilisation in the root medium (soil) are rare (*e.g.*, Whipps 1985) but essential for understanding of the whole physiology and carbon balance of plants (see Rogers *et al.* 1997 for review).

The aim of our investigation was to determine (*1*) carbon distribution in winter wheat plants and in the root medium during the early stages of plant ontogeny, and (*2*) the effect of doubling the atmospheric CO_2 concentration on C balance in a plant-root medium system. Photosynthesis was the only significant source of C in our system. The main parameters investigated were P_N, R_D of roots and shoots, plant growth rate, total C content, and microbial C content in the root medium. We combined continuous measurements of gas exchange and interval-based measurements of C allocation. Carbon balance in the system was estimated from diurnal courses of P_N and R_D of shoots, R_D of roots and microorganisms, and from C accumulation in plant organs, root medium, and microbial biomass.

Materials and methods

Plants: We used winter wheat (*Triticum aestivum* L. cv. Ritmo) inoculated with a suspension of active bacteria isolated from the rhizosphere of soil-grown wheat of the same cultivar.

Inoculation and germination of seeds: Inoculum of rhizosphere bacteria was prepared from roots of plants in the 3-5 leaf stage which were grown in soil (cambisol, C 1.3 %, C/N 9.1, pH 6.4) in the same irradiance/temperature conditions as the experimental plants. The procedure is described in detail by Elhottová *et al.* (1997). Seeds were sterilised for 20 min with 0.05 % $HgCl_2$, placed into an excess of thawed inoculum, and gently shaken at 25 °C for 20 min. Inoculated seeds were then incubated on sterile moistened filter paper at 20 °C for four to five days. At this stage all seedlings had 3 seminal roots about 10 mm long.

Plant culture: The seedlings were aseptically transferred to a sterile whole plant growth chamber consisting of two glass cylinders (60×400 mm for the top cylinder and 60×200 mm for the bottom cylinder) separated by a perforated plexiglas disk. One day after seedling transfer, the perforations were hermetically sealed with vulcanisable silicon rubber (*Stomaflex* impression material, *Spofa Dental*, Czech Republic). The root cylinder was filled with coarse silica sand (grain size 1-2 mm) free of organic carbon (combusted for 5 h at 550 °C and washed in distilled water). The middle disk, top, and bottom plexiglass covers were equipped with gas and/or nutrient solution inlet and outlet ports and thermocouple connectors. There was a DC microfan (*Micronel V300*, Switzerland) on the inner side of the top cover. The whole growth chamber assembly was sealed. The root cylinder was connected to a darkened 500 cm^3 infuse flask with sterile, carbon-free, full-strength nutrient solution (IBP solution, 12 mM NO_3^-), and the level of solution in sand was adjusted to reach the seminal roots. In this way, the sand was saturated with nutrient solution during the whole experiment. The shoot and root compartments were supplied with air pumped through a bacterial filter (*Whatman*).

Two series with four growth chambers each were placed in a temperature- and irradiance-controlled growth cabinet (*Sherer*, USA). The chambers which were used for analysis of plants in the 3-5 leaf stage (12-13 d after planting, DAP) contained 7 plants, those used for analyses in the stages of early (18-21 DAP) and late (24-34 DAP) tillering contained 4 and 2 plants, respectively. Both series of chambers were supplied with air pumped from the atmosphere outside the laboratory building. The air entering one of two chamber series was enriched with CO_2 from a tank with 5 % CO_2 in nitrogen, using a mass flow meter and controller (*Tesla VK175*, Czech Republic). Day/night CO_2 concentrations in the AC and EC series of growth chambers were 300/360 and 690/730 μmol mol^{-1}, respectively. Humidity in both input air streams was controlled by a two-way Peltier dew point generator (*MGK-4, Walz*, Germany). The day/night air temperature was 20/17 °C. Air humidity in the shoot cylinder varied from 60 to 90 %. A photon flux rate (PAR) of 350 μmol m^{-2} s^{-1} (at the upper leaf level) was supplied by fluorescence tubes and incandescent lamps for 15 h per day.

Experimental design: Four identical experimental runs with the two growth chambers series for AC and EC (4 chambers per series) were organised (32 chambers altogether). Gas exchange was measured in two runs. Destructive analyses were done in each run. Water and CO_2 exchange were measured for 24 h, alternatively for one AC a one EC chamber. The growth chambers with the same number of plants were always selected for comparison. Measurements began when the seedlings were 5 d in the growth chamber and were continued for 5-6 d with the same two chambers. Leaf area was measured on intact plants each second day after the 24 h period of gas exchange measurement was completed. Before the end of the 5-6 d gas exchange period, we measured the CO_2 response of photosynthesis for both CO_2 treatments. Then, plants and the sand medium were used for destructive analyses (see below). Monitoring of gas exchange continued immediately in two other chambers with older plants.

Gas exchange measurements: An open gas system was used for monitoring the water vapour and CO_2 exchange rates. The shoot part of the growth chamber was ventilated separately from the root compartment with flow of air increasing from 8.3 to 41.7 $cm^3\ s^{-1}$ as the leaf area cumulated (0.011-0.004 $m^3\ s^{-1}\ m^{-2}$ of leaf area). The root part was bubbled with air of the same inlet composition but flow rate was about 10 times lower. The CO_2 content in both outlet air streams passing the roots or leaves was analysed alternatively by an infrared gas analyser (*LI-Cor 6250*, USA or *INFRALYT IV*, Germany) every three minutes. The humidity of air entering the chamber was calculated from dew point settings, of air leaving the chambers from measurements done by a capacitance sensor (*Vaisala*, Finland) and by a thermistor. Leaf temperature was measured by three thermocouples attached to the leaf surface.

Destructive analyses: Following the period of gas exchange analysis, we measured leaf and root area, root length (*DELTA T* Image Analysis System, England), fresh and dry masses of leaves, roots, and seed residues. Total carbon and nitrogen contents were analysed for leaves and roots (*Carlo Erba CHN* analyser). Before the root length and area were measured, roots with adhering sand were washed in 0.1 % hexametaphosphate. The suspension obtained was used for the determination of rhizosphere carbon. 10 cm^3 of the suspension was collected for the analysis of total C, and the rest was divided into the fractions with particles smaller than 2.7 and 0.3 μm^3, using filtration through glass and membrane filters (*Whatman*). The filters were boiled 6 times repeatedly in redistilled water to remove carbon residue. Carbon in microbial biomass was calculated as the difference between the C content in the filtrates. The unattached sand and nutrient solution from the root part of the chamber were mixed and analysed for total C content and microbial biomass carbon. Microbial biomass C was measured using a fumigation incubation method (Jenkinson and Powlson 1976). Carbon content in the suspension, filtrates, and sand was analysed using the dichromate semi-micro method (Hejzlar and Kopáček 1990). All destructive analyses were done in 4-5 replications.

Samples of leaves were taken two times (26 and 34 d after planting), in the ends of light and dark periods for transmission electron microscopy (TEM) images of chloroplast ultrastructure. Number and area of starch grains were calculated from TEM micrographs.

Calculation of carbon balance parameters: We calculated the total amount of carbon assimilated during the light period and lost by respiration during the subsequent dark period from measurements of CO_2 fluxes recorded at 0.5-2.0 h intervals. Carbon lost by respiration of roots and microorganisms, and water transpired from leaves were calculated similarly. For the days when the alternative CO_2-growth treatment was measured, we used interpolation for assessment of the amount of C fixed and water lost. The C release into the root medium was assumed to be equal to the amount of C utilised by microbial populations living in the rhizosphere and in the sand. The growth rate of the microbial population was estimated from the fluctuations of microbial biomass in the rhizosphere and unattached sand using the model of Chapman and Gray (1986). The following assumptions were included: (*1*) the yield factor for microflora is 0.4 (Newman and Watson 1977); (*2*) the specific maintenance

rate of microorganisms in rhizosphere is 0.02 h^{-1} (Newman and Watson 1977); (*3*) the specific maintenance rate of microorganisms in surrounding sand is 0.0008 h^{-1} (Chapman and Gray 1986). As the only source of carbon in sand was C penetrating from rhizosphere, a limitation of microbial growth by substrate deficiency can be expected here. (*4*) The growth of microbial populations is balanced in a given period of time.

For the estimation of C allocation into shoots and roots, and C remaining in the seed residue, we used C contents in dry matter. Carbon allocated into shoots for the given period of gas exchange measurements was calculated from: (*1*) the leaf area of measured plants at the beginning of the period, (*2*) areal dry mass and (*3*) C content estimated in the previous sampling period for plants of the same age and CO_2 treatment, and (*4*) from dry mass and (*5*) C content of investigated plants determined in the period of gas exchange measurements. The values (*1*) to (*3*) were used for estimation of C content at the beginning of the period of gas exchange measurement, values (*4*) and (*5*) for calculation of C content at the end of that period. A similar principle was used for the interval-based calculation of C allocated into the roots.

Results

Carbon accumulated significantly faster in shoots and in root medium (rhizosphere + unattached sand) of EC than AC plants after 28 d of plant growth (Fig. 1). Mean shoot/root C ratio was 3.8 ± 1.7 (mean ± SD, n = 32). Almost all tillers appeared between 22 and 26 DAP.

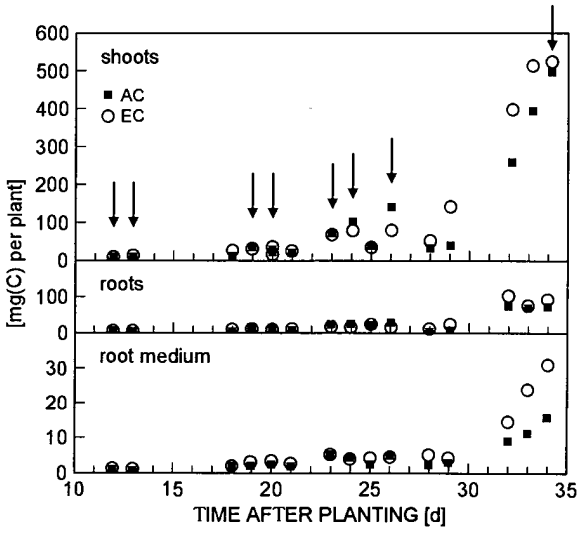

Fig. 1. Carbon accumulation in shoots, roots, and root medium of winter wheat during 34 d of plant growth in growth chambers. Each symbol represents the value for one growth chamber with plants grown in CO_2 concentration of 350 (AC, ■) and 700 (EC, ○) μmol mol^{-1}. The arrows indicate days in which gas exchange was also measured.

When averaging over the whole experimental life span (arithmetical means of plants analysed during 5-34 DAP), about 60 % of daily assimilated C was allocated into new biomass of leaves, 10 % was spent in respiration of leaves at night, and

Table 1. Carbon partitioning in a plant-microbe system expressed as % of assimilation rate [mg(C) plant^{-1} d^{-1} ± standard deviation). Means from 7 samplings at different plant age (12, 13, 19, 20, 24, 26, and 35 d) are given, numbers in parentheses represent coefficients of variation in % of the related mean value. *The difference between the C flow from shoots into the below-ground part and the C flow from seed into the root represents an average contribution of seed to daily C income.

C partitioning in plant-microbe system	Growth CO_2 concentration [μmol mol^{-1}]	
	350	700
Assimilation rate [mg C per plant and day] = 100 %	14.2 ± 17.4	17.5 ± 30.9
Shoot: % C allocated to biomass	56 (17)	61 (28)
% C lost by respiration in darkness	9 (12)	12 (25)
% C flow to the belowground parts	34 (28)	27 (55)
Seed: % C flow into roots*	40 (24)	37 (42)
Root: % C allocated to biomass	10 (40)	10 (30)
% C lost by respiration	21 (38)	17 (30)
% C flow to rhizodepositions	10 (76)	10 (94)
Rhizodepositions: % C allocated to rhizodepositions	4 (76)	4 (94)
% C lost by microbial respiration	6 (76)	6 (94)

30 % was translocated into the roots (Table 1). About one half of the latter was lost by root respiration and the remaining half was used in equal proportions for root biomass and release into the rhizosphere. Three fifths of the released C were respired by soil microorganisms. About 30 % of the daily assimilated C was respired in shoots (shoot mitochondrial respiration on light is not included in the figure), roots, and microbiota in both CO_2 treatments. Average C assimilation rate per plant in EC plants was 123 % of that in AC plants. Five percent more of daily assimilated C was allocated into biomass of leaves and 7 % less into the root-root medium system in the EC treatment than in the AC one. The variability of C assimilation per plant was high here due to growth of leaf area. The variability was remarkably higher in the EC than in the AC plants. Thus, the effect of EC appeared non-significant. The rise in variability at EC was caused by a transient inhibition of photosynthesis between 23 and 26 DAP and is analysed later. The variability of C flow from roots into rhizodepositions was caused by a decrease in C release from roots as the plants developed: 17 % of the assimilated C was released from roots of 18-d-old plants but only 3-4 % from roots of 39-d-old plants.

Total C content in the rhizosphere of plants in EC was higher than that in the AC treatment, except for 24 and 26 DAP. The transiently lower C content in the rhizosphere of the EC treatment was reflected by a lower microbial biomass at 26 DAP (Fig. 2*A,B*). Carbon content in unattached (non-rhizosphere) sand in the EC plants was generally higher than in the AC ones, before 21 and after 27 DAP, but no differences were found in the period of 23-26 DAP (Fig. 2*C*). Microbial biomass carbon in unattached sand showed no systematic difference between the CO_2 treatments.

The period of plant growth between 23 and 26 DAP was characterised by a lower C assimilation in the EC treatment (Fig. 3). P_N was down-regulated in EC plants in this period (Fig. 4). This phenomenon was not observed in earlier and later periods

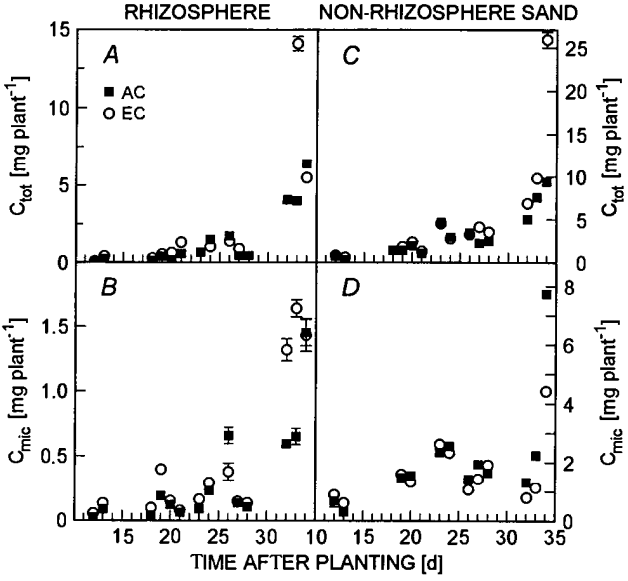

Fig. 2. Total carbon content (C_{tot}) and microbial biomass (C_{mic}) in the rhizosphere of winter wheat plants (*A,B*) and in the non-rhizosphere sand (*C,D*). Symbols (the same as in Fig. 1) and bars show means ± SD ($n = 4$).

of plant growth (values not shown). We assume that a feedback inhibition of P_N occurred as a result of excessive accumulation of saccharides in mesophyll cells. We checked this possibility by measurement of the area of starch grains in transverse

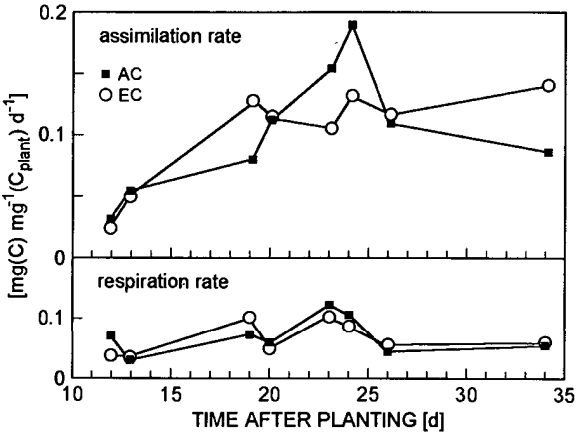

Fig. 3. Assimilation and respiration rates of winter wheat plant during 34 d of plant growth in growth chambers. Respiration rate was calculated as a sum of leaf respiration in the dark and root respiration. Each symbol represents a mean of 3-4 daily integrals measured over 6-8 d for growth chambers with 350 (AC, ■) and 700 (EC, ○) μmol(CO_2) mol^{-1}.

section of chloroplasts. The day/night change in the area of starch grains per chloroplast section was higher in plants grown under EC at 26 DAP as compared to the AC plants. We did not find this difference at the 34 DAP (Table 2). The

Table 2. Area of starch grains per chloroplast of plants grown in control and elevated CO_2 treatments for 26 and 34 d. Values for samples taken at the end of light and dark periods are given. The projected area of starch grains is expressed as the % of the total area of a chloroplast cross section. Mean values and standard deviations for *n* chloroplasts are given.

Time after planting [d]	End of	Growth CO_2 concentration [μmol mol^{-1}]	
		350	700
26	day	11.3 ± 6.2 (*n*=21)	17.14 ± 13.6 (*n*=14)
	night	2.6 ± 3.9 (*n*=10)	0 (*n*=10)
34	day	10.5 ± 3.6 (*n*=10)	8.6 ± 5.4 (*n*=13)
	night	1.2 ± 1.6 (*n*=13)	1.6 ± 3.3 (*n*=27)

inhibition of P_N (Figs. 3 and 4) and decreased C content in the rhizosphere (Fig. 2*A,B*) in the EC plants at about 24 DAP suggested a division of the whole period of plant growth into three periods after relative assimilation rate: (*1*) an initial period, when assimilation rate of EC plants was higher than that of the AC plants, (*2*) a transient period, during which assimilation rate of the EC plants was lower than in the AC plants, and (*3*) a period when the inhibition of assimilation rate disappeared. Diagrams of the diurnal C budgets calculated separately for these periods are given in Fig. 5.

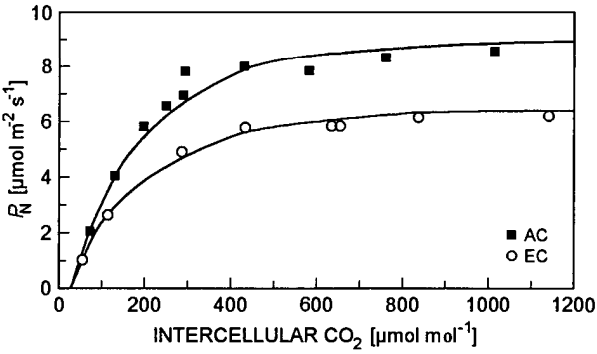

Fig. 4. CO_2 response of net photosynthetic rate (P_N) recorded for both CO_2 growth treatments on the 24th and 25th days after planting when an inhibition of P_N was observed. For symbols see Fig. 1.

The C budget of plants in the initial period (from 5 to 20 DAP) was characterised by an extensive flow of C from seed to roots (Fig. 5*A,B*). P_N was slightly higher in the EC treatment (~114 % of AC). About 10 (AC) and 14 (EC) % of the daily assimilated C was respired in shoots during the dark period, 27 (19) % was respired in roots, 31 (31) % of assimilated C was transported into the roots, 15 (17) % was released from roots into the rooting zone. We did not find any differences in the proportion of photosynthates accumulated in shoots, roots, and root medium between the CO_2 treatments.

In the transient period, the C budget differed noticeably between the CO_2 treatments (Fig. 5*C,D*). Daily C assimilation in EC plants was reduced to 65 % of that in AC plants due to a decrease in P_N (to 88 %) and in leaf area per plant. The

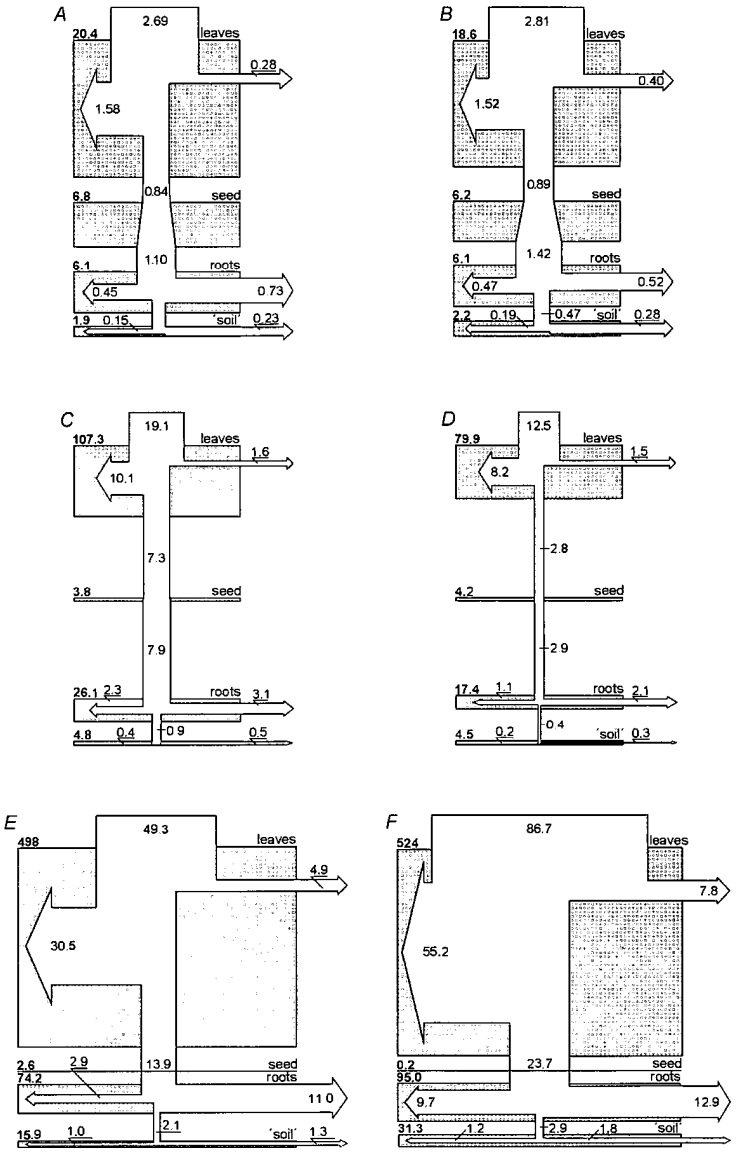

Fig. 5. Diurnal carbon budget of plant-microbe systems exposed to 350 (*A, C, E*) and 700 (*B, D, F*) μmol(CO_2) mol^{-1} in the initial period (5-20 DAP; *A, B*), transient period of tillering (21-26 DAP; *C, D*), and final period of elongation growth (27-34 DAP; *E, F*). The filled area is proportional to the carbon accumulation in the plant organs or in the root medium including 'soil' microorganisms. Width of the arrows indicates daily flow of carbon into (allocation) or out of (respiration) the sinks. The figures are in mg of carbon (*bold*, accumulation) or in mg C d^{-1} $plant^{-1}$ (*regular*, flows) and represent means from 2-4 independent measurements. Respiration of soil microorganisms was calculated from the model described in Materials and methods.

loss of C by root respiration per unit C incorporated into the roots was 45 % higher in the EC than AC treatment. Reduced P_N in the EC plants was accompanied by a overproportional depression of C flow into the roots and into root medium. Phenologically, this period was characterised by the appearance of tillers.

In the last period (Fig. 5*E,F*), total assimilation rate in EC plants was 75 % higher than in the AC plants, largely because of higher P_N. Enhanced leaf area played a minor role. The amount of C translocated from shoots to roots in the EC treatment increased in similar way (by 71 %) as did C assimilation. The surplus of C translocated into roots in the EC plants was used predominantly for root growth. The loss of C by root respiration per unit C translocated to roots was by 30 % lower in the EC plants than in the AC treatment.

Discussion

The results of this experiment with wheat plants grown hydroponically (in sand) under unlimited nutrient supply did not show a time-consistent effect of a doubled CO_2 concentration on P_N and C balance. In very young plants (10 to 25 d after seed watering), the CO_2 enrichment had a small positive effect on C fixation and distribution. In the period of tillering (26-31 d) of EC plants we found a decrease in P_N followed by a decrease in C translocation into roots, with a reduction of C content in rhizosphere and with enhanced respiration costs of root construction. Consequently, a reduced growth rate of plants and changes in the C balance of the plant-root medium system were observed. After tillering (32- to 39-d-old plants), EC stimulated assimilation rate and C allocation to shoots, roots, and rhizodepositions. The proportions of daily assimilated C distributed into shoots and belowground part of the system remained unchanged for both CO_2 treatments. However, relative C investment into the roots construction was doubled in EC (11 % of daily assimilated C) compared to the AC (6 %). This resulted in slightly increased root/shoot ratio of C content under EC in 39-d-old plants (0.18 compared to 0.15 in AC).

The increased root C accumulation and root/shoot ratio at EC coincide with 87 and 41 % of those reviewed by Rogers *et al.* (1994). On the other hand, it contradicts the suggestion that an increase in C allocation to the roots is not the direct effect of a doubling CO_2 concentration, but the indirect effect of more rapid nutrient depletion in the root medium (Christ and Körner 1995, Lambers *et al.* 1995). We believe that there was no nutrient deprivation in our plants. The absence of an outstanding positive effect of EC on growth in early stage of plant development contradicts earlier findings (Billes *et al.* 1993, Paterson *et al.* 1996, Soussana *et al.* 1996). Nevertheless, it can be explained on the basis of growth analysis. Literature clearly supports the view that enhanced tillering is the key factor of the increase in above-ground biomass of young cereal plants (Lawlor and Mitchell 1991, Weigel *et al.* 1994, Barnes *et al.* 1995, Christ and Körner 1995). Thus, the major effect of EC on shoot biomass can be expected in the posttillering phase. However in our experimental system, the number of tillers (2-6 per plant) was not affected by CO_2 treatment. We checked and excluded the possibility that the rubber sealant limited

production of tillers by a physical constriction of plant bases. Nevertheless, we still observed marked growth stimulation in the posttillering phase resulting from increased P_N per unit of leaf area.

The observed growth rate of plants and root/shoot ratio were low when compared with winter wheat grown in soil (Masle *et al.* 1990). Our results for plant growth are, however, in the same range as those of Christ and Körner (1995) who measured growth of wheat plants in hydroponics. Reduced growth and root/shoot ratio in "artificial" conditions may have resulted from the specific root environment; coarse silica sand, and full strength nutrient solution. We used sand of 1-2 mm particle size to avoid errors with the measurements of C distribution caused by the adhesion of microorganisms to fine particles (Marshall 1971). The adverse effect of coarse texture on root growth is known, similar to the effects of "luxury" content of mineral nutrients and water (Evans 1972, Merckx *et al.* 1985, Zagal *et al.* 1993).

The assimilation rate was higher in the initial stages of growth, dropped in the period of tillering, and increased again after tillers had appeared. The transient decrease of P_N in the EC treatment was accompanied by the accumulation of starch grains in chloroplasts. It indicates a change in the rates of production and utilisation of saccharides (Ehret and Jolliffe 1985, Farrar and Williams 1991, Stitt 1991) and an imbalance in C sink-source relations (Radin *et al.* 1986). Significant role of sink-source relationships in crop response to CO_2 was pointed out by Lawlor and Mitchell (1991). Woodrow (1992) explained species-specific responses to EC by the genetically fixed variable potential to form sinks for assimilates. Similar mechanisms could transiently inhibit photosynthesis during onset of tillering. This is later overcome by the elongation growth. Sharma-Natu *et al.* (1997) recently reported similar transient down-regulation of photosynthesis in the post-anthesis period in two cultivars of wheat.

The release of C from roots into the root medium ranged from 3 to 17 % of daily assimilated C, the loss of C by root respiration ranged from 15 to 22 %. These values fall into the range found by other authors (Barber and Martin 1976, Merckx *et al.* 1985, Vančura 1988, Liljeroth *et al.* 1990, Whipps 1990). Respiration cost in roots calculated from our values was 770-1830 g(C) kg^{-1}(C) incorporated into the roots. These values are higher by about one order than the minimum respiration cost of root construction estimated by Lambers (1987), suggesting that more C has been spent for maintenance and transport events than for the synthesis of new tissue over the whole period of plant growth.

Total C content in the rhizosphere of the plants was enhanced in the EC treatment in the initial and final periods of growth when P_N was increased as well. This enhancement was not due to the modified activity of the roots, but resulted from an increased size of the root system. Similar results were published by Billes *et al.* (1993). In the transient period of tillering, C release into the rhizosphere decreased for both CO_2 treatments; smaller amounts of C were found under EC, which was caused by both a smaller root system and reduced C release rates from roots into the rhizosphere. It can be explained by an inhibition of P_N and C translocation into roots as well as by the high respiration cost of roots. In the same period, we observed an accumulated storage of PHB (poly-β-hydroxybutyrate), endogenous compounds in

the rhizosphere bacteria (Elhottová *et al.* 1997). As these compounds are produced by bacteria in conditions of unbalanced growth, *e.g.*, after a change of nutrient status (Tunlid and White 1992), we suggest that not only the amount but also the composition of exudates might be affected in the tillering period.

In conclusion, first, the effect of EC on plant growth and C partitioning in young winter wheat plants (10-39 d from seed watering) varied with plant development: the generally stimulative effect on both P_N and growth turned temporary to the adverse one during the phase of tillering. Excess accumulation of starch in chloroplasts suggests that the activation of the meristems in newly forming but not yet rapidly growing tillers coincides with a transient imbalance between sinks and sources of assimilates. Second, the developmental fluctuations in P_N and shoot growth are closely linked to the growth and C balance of the root-root microbiota system. The components of the C budget, including the release of organic material from roots into the surrounding medium, were in proportion to C assimilation rate. Third, EC, with its stimulative effect on growth, did not change significantly carbon partitioning between the aboveground and belowground biomass. However, redistribution of the belowground C in favour of the root biomass resulted in an increase of root/shoot C ratio under EC. The enhanced accumulation of C in the root medium in the later period of plant growth (after tillering) support an assumption that increased allocation of C into roots under EC promotes C release from roots into the soil (van de Geijn and van Veen 1993).

References

Barber, D.A., Martin, J.K.: The release of organic substances by cereal roots in soil. - New Phytol. **76**: 69-80, 1976.

Barnes, J.D., Ollerenshaw, J.H., Whitfield, C.P.: Effects of elevated CO_2 and/or O_3 on growth, development and physiology of wheat (*Triticum aestivum* L.). - Global Change Biol. **1**: 129-142, 1995.

Billes, G., Rouhier, H., Bottner, P.: Modifications of the carbon and nitrogen allocations in the plant (*Triticum aestivum* L.) soil system in response to increased atmospheric CO_2 concentration. - Plant Soil **157**: 215-225, 1993.

Chapman, S.J., Gray, T.R.G.: Importance of cryptic growth, yield factors and maintenance energy in models of microbial growth in soil. - Soil Biol. Biochem. **18**: 1-4, 1986.

Christ, R.A., Körner, C.: Responses of shoot and root gas exchange, leaf blade expansion and biomass production to pulses of elevated CO_2 in hydroponic wheat. - J. exp. Bot. **46**: 1661-1667, 1995.

Ehret, D.L., Jolliffe, P.A.: Leaf injury to bean plants grown in carbon dioxide enriched atmospheres. - Can. J. Bot. **63**: 2015-2020, 1985.

Elhottová, D., Tříska, J., Šantrůčková, H., Květoň, J., Šantrůček, J., Šimková, M.: Rhizosphere microflora of winter wheat plants cultivated under elevated CO_2. - Plant Soil **197**: 251-259, 1997.

Evans, G.C. (ed.): The Quantitative Analysis of Plant Growth. - Blackwell, Oxford 1972.

Farrar, J.F., Williams, M.L.: The effect of increased atmospheric carbon dioxide and temperature on carbon partitioning, source-sink relations and respiration. - Plant Cell Environ. **14**: 819-830, 1991.

Hejzlar, J., Kopáček, J.: Determination of low chemical oxygen demand values in water by the dichromate semi-micro method. - Analyst **115**: 1463-1465, 1990.

Jenkinson, D.S., Powlson, D.S.: The effect of biocidal treatments on metabolism in soil. V. A method for measuring soil biomass. - Soil Biol. Biochem. **8**: 209-213, 1976.

Lambers, H.: Growth, respiration, exudation and symbiotic associations: the fate of carbon translocated to the roots. - In: Gregory, P.J., Lake, J.V., Rose, D.A. (ed.): Root Development and Function. Pp. 125-146. Cambridge University Press, Cambridge - London - New York - New Rochelle - Melbourne - Sydney 1987.

Lambers, H., Vandenboogaard, R., Veneklaas, E.J., Villar, R.: Effect of global environmental change on carbon partitioning in vegetative plants of *Triticum aestivum* and closely related *Aegilops* species. - Global Change Biol. **1**: 397-406, 1995.

Lawlor, D.W., Mitchell, R.A.C.: The effects of increasing CO_2 on crop photosynthesis and productivity: a review of field studies. - Plant Cell Environ. **14**: 807-818, 1991.

Liljeroth, E., van Veen, J.A., Miller, H.J.: Assimilate translocation to the rhizosphere of two wheat lines and subsequent utilization by rhizosphere microorganisms at two soil nitrogen concentrations. - Soil Biol. Biochem. **22**: 1015-1021, 1990.

Lloyd, J., Farquhar, G.D.: The CO_2 dependence of photosynthesis, plant growth responses to elevated atmospheric CO_2 concentrations and their interaction with soil nutrient status. I. General principles and forest ecosystems. - Funct. Ecol. **10**: 4-32, 1996.

Marshall, K.C.: Sorptive interactions between soil particles and microorganisms. - In: McLaren, A.D., Skujins, J. (ed.): Soil Biochemistry. Vol. II . Pp. 409-445. Marcel Dekker, New York 1971.

Masle, J., Farquhar, G.D., Gifford, R.M.: Growth and carbon economy of wheat seedlings as affected by soil resistance to penetration and ambient partial pressure of CO_2. - Aust. J. Plant Physiol. **17**: 465-487, 1990.

Merckx, R., den Hartog, A., van Veen, J.A.: Turnover of root derived material and related microbial biomass formation in soils of different texture. - Soil Biol. Biochem. **17**: 565-569, 1985.

Newman, E.I., Watson, A.: Microbial abundance in the rhizosphere: a computer model. - Plant Soil **48**: 17-56, 1977.

Paterson, E., Rattray, E.A., Killham, K.: Effect of atmospheric CO_2 concentration on C-partitioning and rhizosphere C-flow for three plant species. - Soil Biol. Biochem. **28**: 195-201, 1996.

Radin, J.W., Mauney, J.R., Guinn, G.: Diurnal osmotic cycling in cotton leaves as an indicator of source-sink balance. - Plant Cell Environ. **9**: 49-352, 1986.

Rogers, H.H., Runion, G.B., Krupa, S.V.: Plant responses to atmospheric CO_2 enrichment with emphasis on roots and rhizosphere. - Environ. Pollut. **83**: 155-189, 1994.

Rogers, H.H., Runion, G.B., Krupa, S.V., Prior, S.A.: Plant responses to atmospheric carbon dioxide enrichment: Implications in root-soil-microbe interactions. - In: Allen, L.H., Jr., Kirkham, M.B., Olszyk, D.M., Whitman, C.E. (ed.): Advances in Carbon Dioxide Effects Research. Pp. 1-34. American Sociey of Agronomy, Madison 1997.

Roumet, C., Bel, M.P., Sonie, L., Jardon, F., Roy, J.: Growth response of grasses to elevated CO_2: a physiological plurispecific analysis. - New Phytol. **133**: 595-603, 1996.

Sage, R.F.: Acclimation of photosynthesis to increasing atmospheric CO_2: The gas exchange perspective. - Photosynth. Res. **39**: 351-368, 1994.

Sharma-Natu, P., Khan, F.A., Ghildiyal, M.C.: Photosynthetic acclimation to elevated CO_2 in wheat cultivars. - Photosynthetica **34**: 537-543, 1997.

Soussana, J.F., Casella, E., Loiseau, P.: Long-term effects of CO_2 enrichment and temperature increase on a temperate grass sward. II. Plant nitrogen budgets and root fraction. - Plant Soil **182**: 101-114, 1996.

Stitt, M.: Rising CO_2 levels and their potential significance for carbon flow in photosynthetic cells. - Plant Cell Environ. **14**: 741-762, 1991.

Tunlid, A., White, D.C.: Biochemical analysis of biomass, community structure, nutritional status, and metabolic activity of microbial communities in soil. - In: Stotzky, G., Bollag, J.M. (ed.): Soil Biochemistry. Vol. 7. Pp. 229-262. Marcel Dekker, New York 1992.

Vančura, V.: Plant metabolites in soil. - In: Vančura, V., Kunc, F. (ed.): Soil Microbial Associations. Pp. 57-191. Academia, Praha 1988.

van de Geijn, S.C., van Veen, J.A.: Implications of increased carbon dioxide levels for carbon input and turnover in soil. - Vegetatio **104/105**: 283-292, 1993.

Weigel, H.J., Manderscheid, R., Jager, H.J., Mejer, G.J.: Effects of season-long CO_2 enrichment on cereals. 1. Growth performance and yield. - Agr. Ecosystem Environ. **48**: 231-240, 1994.

Whipps, J.M.: Effect of CO_2 concentration on growth, carbon distribution and loss of carbon from the roots of maize. - J. exp. Bot. **36**: 644-651, 1985.

Whipps, J.M.: Carbon economy. - In: Lynch, J.M. (ed.): The Rhizosphere. Pp. 59-97. Wiley & Sons, New York 1990.

Woodrow, I.: Modelling photosynthesis and transpiration in plants growing in an atmosphere enriched in CO_2. - In: Murata, N. (ed.): Research in Photosynthesis. Vol. IV. Pp. 795-802. Kluwer Academic Publ., Dordrecht - Boston - London 1992.

Zagal, E., Bjarnason, S., Olsson, U.: Carbon and nitrogen in root zone of barley (*Hordeum vulgare* L.) supplied with nitrogen fertilizer at two rates. - Plant Soil **157**: 51-63, 1993.