

Free-Air CO₂ Enrichment of Sorghum: Soil Carbon and Nitrogen Dynamics

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The positive impact of elevated atmospheric CO₂ concentration on crop biomass production suggests more carbon inputs to soil. Further study on the effect of elevated CO₂ on soil carbon and nitrogen dynamics is key to understanding the potential for long-term carbon storage in soil. Soil samples (0- to 5-, 5- to 10-, and 10- to 20-cm depths) were collected after 2 yr of grain sorghum [*Sorghum bicolor* (L.) Moench.] production under two atmospheric CO₂ levels: 370 [ambient] and 550 μL L⁻¹ [free-air CO₂ enrichment; FACE] and two water treatments (ample water and limited water) on a Trix clay loam (fine, loamy mixed [calcareous], hyperthermic Typic Torrifluvents) at Maricopa, AZ. In addition to assessing treatment effects on soil organic C and total N, potential C and N mineralization and C turnover were determined in a 60-d laboratory incubation study. After 2 yr of FACE, soil C and N were significantly increased at all soil depths. Water regime had no effect on these measures. Increased total N in the soil was associated with reduced N mineralization under FACE. Results indicated that potential C turnover was reduced under water deficit conditions at the top soil depth. Carbon turnover was not affected under FACE, implying that the observed increase in soil C with elevated CO₂ may be stable relative to ambient CO₂ conditions. Results suggest that, over the short-term, a small increase in soil C storage could occur under elevated atmospheric CO₂ conditions in sorghum production systems with differing water regimes.

The historic rise in atmospheric carbon dioxide (CO₂) concentration has been caused primarily by fossil fuel burning and land use change associated with industrial and/or population expansion over the last century (Houghton et al., 1990; Keeling and Whorf, 1994). This steady rise is significant because CO₂ is the major mobile form of carbon (C) in the atmosphere, which can influence the biosphere, geosphere, and hydrosphere. Of special note is the continuing debate about potential changes to the global climate being induced by increased atmospheric CO₂. Furthermore, because CO₂ is a primary input for crop growth, questions concerning the potential of highly managed agricultural soils to store surplus atmospheric C as an amelioration measure have arisen. The dynamics of C in terrestrial ecosystems has become an important topic because the global C budget is not in balance (Schlesinger, 1991).

Over the last few decades, numerous studies have shown that elevated atmospheric CO₂ often enhances aboveground crop biomass production (Kimball, 1983; Strain and Cure, 1985; Amthor, 1995). However, future economic and environmental concerns dictate a further understanding of how varying cropping systems will be altered by a future CO₂-enriched world. The amount of crop biomass produced in the field may depend on the differences in CO₂ effects on the crop species used in agroecosystems. Ultimately, production levels will affect future food security issues, residue management, and belowground processes inclusive of soil C storage potential. Increased C uptake and assimilation generally results in increased crop growth under CO₂-enriched conditions. It is known that C₃ and C₄ photosynthetic types respond differently to elevated CO₂ with regard to carbon metabolism and water use (Rogers et al., 1983; Amthor, 1995). Crops with a C₃ photosynthetic pathway often exhibit greater growth response compared with crops with a C₄ pathway (Amthor, 1995; Amthor and Loomis, 1996; Rogers et al., 1997). The CO₂-concentrating mechanism used by C₄ species limits the response to CO₂ enrichment (Amthor and Loomis, 1996). However, like C₃ species, plants with the C₄ pathway can exhibit growth stimulation due to lowered stomatal conductance and increased water use efficiency (Rogers et al., 1983).

In addition to assessment of aboveground biomass, more recent efforts have concentrated on the potential effects of CO₂ on belowground responses. Reviews by Rogers et al. (1994; 1996) indicate that a significant proportion of the increase in biomass found

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Abbreviation: FACE, free-air CO₂ enrichment.

under CO₂-enriched conditions is allocated belowground. Positive shifts in residue inputs, above- and belowground, to the soil system are important because they may influence soil C accumulation. However, in addition to the quantity of inputs affected by the rise in CO₂, factors such as residue composition and exogenous nutrient supplies will also affect the rate and extent of organic C turnover, thereby controlling soil C storage patterns. Elevated atmospheric CO₂ has been shown to alter the chemical composition of plant tissue. Reviews of the literature suggest that tissue nutrient concentration is often reduced for CO₂-enriched plants (Conroy, 1992; Rogers et al., 1999).

Reduction in tissue N concentration is of particular interest because this often results in an increased residue C/N ratio, which may influence rates of residue decomposition, soil C dynamics, and plant N availability in agroecosystems. Studies on decomposition of cotton [*Gossypium hirsutum* (L.)] residue produced under free-air CO₂ enrichment (FACE) showed little difference in C mineralization, but N mineralization was decreased for FACE residue (Torbert et al., 1995). Evaluation of soybean [*Glycine max* (L.) Merr.] and grain sorghum [*Sorghum bicolor* (L.) Moench.] residues from an open-top chamber study indicated that N mineralization was reduced by high CO₂; however, differences in quantity and quality of residue affected C mineralization (Torbert et al., 1998). Initially, C mineralization was only reduced with high CO₂ in sorghum, but in the long term, a reduction in CO₂-C mineralized per gram of residue added was observed for both crops due to elevated CO₂. These findings were complemented by open top chamber field results. Measurement of soil solution below the rooting zone indicated more nitrate leaching under ambient CO₂, suggesting a slower release of N from decomposing residue under elevated CO₂ (Torbert et al., 1996). The cumulative effect of elevated CO₂ treatment (5 yr) indicated that soil C storage was greater in the soybean system (Prior et al., 2003). This was attributed to differences in soil C storage mechanisms between the two crops based on δ¹³C results reported by Torbert et al. (1997). In FACE experiments conducted with irrigated cotton (3 yr) and wheat (2 yr), FACE resulted in soil C accumulation in both cases (Wood et al., 1994; Prior et al., 1997). For cotton, available N limited C cycling early on, but over the long term, FACE soil had greater C mineralization in both water regimes. Carbon turnover increased in the water stress treatment and decreased under non-water stress conditions. A differential effect of CO₂ and irrigation treatment on residue structure and/or composition may have altered soil C cycling, suggesting that increased soil C storage is more likely when soil water is not limiting in a high CO₂ environment. For FACE wheat, potential N mineralization was unaffected by CO₂, indicating that factors other than N may have limited C mineralization and turnover. Overall, these findings suggested that more soil C storage occurs in CO₂-enriched wheat systems regardless of water treatment. The diverse findings from previous work highlight the importance of assessing management systems that are reflective of various crop, soil, and environmental conditions to better predict the impact of increasing atmospheric CO₂ on terrestrial soil C storage patterns.

More field work investigating elevated CO₂ and its interaction with other environmental factors is needed to determine how

different cropping systems will influence soil C storage patterns. Evaluating the effects of changing CO₂ level on a C₄ grain sorghum crop is important because sorghum represents one of the five major cereal crops (Doggett, 1988; Bennett et al., 1990). Major sorghum production regions are located in the USA, Mexico, Asia (e.g., China and India), and throughout Africa (FAO, 1996). Because sorghum is a major food staple for many developing countries (FAO, 1996), especially in semiarid regions (Doggett, 1988; Bennett et al., 1990), it is essential to evaluate how changes in the global environment may affect sustainability of these systems. Our objective was to investigate the cumulative effect of 2 yr of FACE and soil water stress in a grain sorghum production system on soil N and C mineralization and C turnover.

Materials and Methods

A FACE application system (Hendrey et al., 1993) was used to achieve CO₂ enrichment (200 μL L⁻¹ above ambient) in the field under two soil water regimes (ample or limited water) for two growing seasons (1998 and 1999). Control plots were maintained under ambient CO₂ conditions (370 μL L⁻¹). A large-diameter (22 m) PVC torus, fitted with a uniformly spaced series of 32 individually valved vertical vent pipes (2 m height), was used for controlled release of CO₂. Exposure within these circular arrays was regulated by a computer program based on an algorithm keyed to wind velocity, wind direction, and CO₂ concentration. Carbon dioxide was added upwind from open sectors of vent pipes in amounts equivalent to wind speed such that the circular plot within the array was uniformly fumigated. Carbon dioxide exposure commenced after planting each year and was terminated at harvest. Dummy arrays encompassed sorghum grown under ambient CO₂. Four FACE and four ambient plots were established.

The soil series at the study site located at the Maricopa Agriculture Center for Resources and Extension of the University of Arizona at Maricopa, AZ (33°10'N, 112°0'W) is a Trix clay loam (fine, loamy, mixed [calcareous], hyperthermic Typic Torrifluvents). Grain sorghum [*Sorghum bicolor* (L.) Moench. cv. Dekalb DK54] was grown; details of cultural practices used have been reported by Ottman et al. (2001). Sowing occurred on 15 and 16 July 1998 and on 14 and 15 June 1999 on a 0.76-m row spacing. Each of the main circular FACE and control plots was split into semicircular halves, with each half receiving an ample (Wet) or limited (Dry) water irrigation regime. The water was applied using flood irrigation. Only two irrigations were applied to the Dry treatment each season, compared with seven (1998) or six (1999) to the Wet treatments. Wet plots were irrigated when 30% of the available water in the rooting zone was depleted. The plots were then irrigated with an amount calculated to replace 100% of the potential evapotranspiration since the last irrigation adjusted for rainfall (Fox et al., 1992). Amounts were further adjusted to meet the 100-mm minimum irrigation amount. The Dry plots were planned to receive one third the number of irrigations and irrigation amounts as the wet plots; this was achieved by applying water only twice, once at the start and again at midseason. The total amounts of irrigation plus rain during 1998 were 1218 and 474 mm to the Wet and Dry plots, respectively. In 1999 they were 1047 and 491 mm, respec-

tively. Fertilizer was applied at 279 kg N ha⁻¹ and 41 kg P ha⁻¹ in 1998 and 265 kg N ha⁻¹ and 41 kg P ha⁻¹ in 1999.

Soil samples were taken to investigate the cumulative effect of 2 yr of FACE and water treatments on soil microbial activity. Composite soil samples were collected (25 random cores per plot) on 15 Nov. 1999, before harvest, at 0- to 5-, 5- to 10-, and 10- to 20-cm depth increments, stored at 5°C, transported by plane to Auburn, AL, and processed for the incubation study within 2 d. Methods used by Torbert et al. (1998) were used for determination of potential C and N mineralization. Pre-incubation soil samples were dried (60°C), ground to pass a 0.15-mm sieve, and analyzed for total N (Fison NA1500 CN Analyzer; Fison Instruments Incorp., Beverly, MA). Soil organic C was determined with a LECO CR12 Carbon Determinator (LECO Corp., Augusta, GA) (Chichester and Chaison, 1992). Soil inorganic N (NO₂-N + NO₃-N and NH₄-N) was extracted with 2 mol L⁻¹ KCl and measured before and after incubation by standard colorimetric procedures using a Technicon Autoanalyzer (Technicon Industrial Systems, 1973a, 1973b). Sieved soil samples (2-mm sieve) were weighed (25 g dry weight basis) and placed in plastic containers. Deionized water was added to adjust soil water content to -20 kPa at a bulk density of 1.3 Mg m⁻³. Containers were placed in sealed glass jars with 10 mL of water (humidity control) and a 10-mL vial of 1 mol L⁻¹ NaOH (CO₂ trap). Jars were incubated in the dark at 25°C and removed after 30 and 60 d. Carbon dioxide in NaOH traps was determined by titrating excess base with 0.25 mol L⁻¹ HCl in the presence of BaCl₂. Potential C mineralization was the difference between blanks and CO₂-C captured in sample traps. Potential N mineralization was the difference between final and initial inorganic N contents for the incubation. Potential C mineralization divided by total organic C was used to calculate C turnover.

The experimental design was a split-plot with a randomized complete block arrangement of the main-plot factor (two CO₂ levels: ambient and FACE) for which there were four blocks. The second factor (water regime: wet and dry) was assigned to subplots (each half of the study plot within main plots). Statistical analysis of data was performed using the Mixed procedure of the Statistical Analysis System (Littell et al., 1996). A significance level of *P* < 0.05 was established a priori.

Results

Soil Characteristics

The impacts of 2 yr of atmospheric CO₂ and water regime treatments on soil C, N, and C/N ratio for this sorghum cropping system are shown in Table 1. No significant treatment interactions were noted for these soil variables at the depth intervals evaluated. For soil C, the main effect of CO₂ was significant at all depth increments; soil C was higher under FACE. The main effect of water regime was significant for soil C only at the bottom depth increment (10–20 cm), with this value being higher under the dry water regime. Water regime had no impact on soil N, but, similar to soil C, the significant main effects of CO₂ indicated that N was increased by FACE. For soil, the C/N ratio at the 0- to 5-cm and 10- to 20-cm depths, the main effect of CO₂ was significant, but water regime was not; the ratio was lower under FACE at these depths.

Table 1. Soil C, N, and C/N ratio as affected by 2 yr of exposure to atmospheric CO₂ level and water treatment; these were the soil conditions existing at the start of the incubation study.

Depth	Ambient CO ₂		FACE†		Pr > F‡		
	Dry	Wet	Dry	Wet	CO ₂	H ₂ O	CO ₂ × H ₂ O
g kg ⁻¹							
Soil C							
0–5 cm	8.31§	8.56	9.40	9.17	0.041	0.961	0.152
5–10 cm	8.40	8.58	9.39	9.28	0.029	0.813	0.300
10–20 cm	8.64	8.32	9.62	8.98	0.035	0.022	0.343
Soil N							
0–5 cm	0.64	0.66	0.81	0.81	0.001	0.649	0.546
5–10 cm	0.64	0.65	0.81	0.82	<0.001	0.567	1.000
10–20 cm	0.66	0.64	0.78	0.79	0.010	0.825	0.392
g g ⁻¹							
Soil C/N ratio							
0–5 cm	13.05	13.08	11.60	11.36	<0.001	0.352	0.234
5–10 cm	13.10	13.21	11.63	11.38	0.002	0.383	0.035
10–20 cm	13.10	13.10	12.54	11.37	0.036	0.241	0.236

† Free-air CO₂ enrichment.

‡ Probability of a greater F by chance between the CO₂ or water treatments and for the CO₂ × H₂O interaction.

§ Values represent means of four replications.

A significant CO₂ × H₂O regime interaction at the 5- to 10-cm depth indicated that the soil C/N ratio for both water regimes under ambient CO₂ was higher than FACE and that the FACE-dry treatment was slightly higher than its wet counterpart.

Soil Nitrogen Mineralization

In general, net N immobilization occurred during the 0- to 30-d incubation period of the incubation study, suggesting that initially large proportions of available C relative to N led to net N immobilization (Table 2). Significant interactive effects of CO₂ with water treatment were observed at the top two depth increments (0–5 and 5–10 cm). At the 0- to 5-cm depth, N was less limiting under the ambient CO₂-wet regime because values were less negative compared with the other treatments, which were similar to each other. At the 5- to 10-cm depth, the wet regimes under both CO₂ treatments showed more mineralization relative to their dry counterparts. However, under dry conditions, ambient CO₂ exhibited more N immobilization compared with FACE. At the 10- to 20-cm depth, the significant main effect of water regime suggested that N was less limiting under wet conditions regardless of CO₂ level.

Patterns of N immobilization were still noticeable at the 30- to 60-d incubation period, especially under FACE. During this incubation period, there were no significant treatment interactions or main effects of water treatment at the various depth intervals; however, significant main effects of CO₂ were noted at all soil depths. Ambient CO₂ soil exhibited more N mineralization than FACE soils, suggesting that N content had become less of a limiting factor in residue decomposition under ambient CO₂ conditions.

For the total incubation period (0–60 d), patterns of N immobilization at the top depth (0–5 cm) were similar to those observed at the 0- to 30-d incubation period. At the next two depths (5–10 and 10–20 cm), main effects of CO₂ and water treatment were significant. Free-air CO₂ enrichment resulted in more N immobi-

Table 2. Soil N mineralization as affected by atmospheric CO₂ level and water treatment.

Depth	Ambient CO ₂		FACE†		Pr > F‡		
	Dry	Wet	Dry	Wet	CO ₂	H ₂ O	CO ₂ × H ₂ O
mg kg ⁻¹							
0–30 d							
0–5 cm	-48.20§	-15.35	-49.63	-48.55	0.002	0.003	0.004
5–10 cm	-32.74	-3.02	-21.68	-7.45	0.200	<0.001	0.010
10–20 cm	-31.76	-8.22	-31.81	-20.47	0.145	0.001	0.148
30–60 d							
0–5 cm	4.93	-0.39	-5.36	-5.54	0.056	0.467	0.494
5–10 cm	10.66	7.04	-3.79	-3.44	0.010	0.580	0.505
10–20 cm	8.36	9.77	-8.63	-6.51	0.001	0.638	0.925
0–60 d							
0–5 cm	-43.26	-15.74	-55.00	-54.08	0.024	0.035	0.044
5–10 cm	-22.07	4.01	-25.47	-10.89	0.032	<0.001	0.145
10–20 cm	-23.41	1.55	-40.44	-26.98	0.002	0.001	0.124

† Free-air CO₂ enrichment.

‡ Probability of a greater F by chance between the CO₂ or water treatments and for the CO₂ × H₂O interaction.

§ Values represent means of four replications.

lization at these soil depths. Furthermore, the dry regime exhibited more N immobilization compared with wet conditions.

Soil Carbon Mineralization

For the 0- to 30-d period, the main effects of CO₂ and water regime and their interaction were significant at the 0- to 5-cm depth increment (Table 3). The noted interaction indicated that the ambient-dry treatment exhibited lower C mineralization vs. other treatment combinations, which were similar to each other. At the 5- to 10-cm depth, the main effect of water regime was significant; soil respiration was lower under Dry compared with Wet conditions. A trend ($p = 0.07$) for a main effect of CO₂ was noted at the last depth increment, with C mineralization being lower under the ambient CO₂ treatment. At the 30- to 60-d incubation period, no significant treatment effects were noted at any of the soil depths evaluated. For the total incubation period

Table 3. Soil C mineralization as affected by atmospheric CO₂ level and water treatment.

Depth	Ambient CO ₂		FACE†		Pr > F‡		
	Dry	Wet	Dry	Wet	CO ₂	H ₂ O	CO ₂ × H ₂ O
mg kg ⁻¹							
0–30 d							
0–5 cm	99.58§	171.69	168.32	174.34	0.092	0.009	0.019
5–10 cm	79.60	123.52	105.13	113.41	0.520	0.045	0.152
10–20 cm	71.46	75.77	99.60	91.51	0.073	0.865	0.580
30–60 d							
0–5 cm	122.30	103.89	103.01	108.66	0.574	0.572	0.302
5–10 cm	94.86	88.20	95.51	111.51	0.331	0.581	0.206
10–20 cm	104.79	87.28	92.29	79.74	0.275	0.115	0.781
0–60 d							
0–5 cm	221.88	275.57	271.32	282.99	0.094	0.004	0.025
5–10 cm	174.47	211.72	200.64	224.92	0.290	0.113	0.720
10–20 cm	176.25	163.05	191.90	171.25	0.244	0.108	0.709

† Free-air CO₂ enrichment.

‡ Probability of a greater F by chance between the CO₂ or water treatments and for the CO₂ × H₂O interaction.

§ Values represent means of four replications.

(0–60 d), results were somewhat similar to those observed at the 0- to 30-d incubation period. A significant CO₂ × H₂O interaction for this period was noted at the 0- to 5-cm depth increment; as seen at 0 to 30 d, the ambient-dry treatment exhibited lower C mineralization vs. other treatment combinations. No significant treatment effects were noted at the 5- to 10-cm or 10- to 20-cm depths for the total incubation period.

Soil Carbon Turnover

At the 0- to 30-d incubation period, a significant CO₂ × H₂O interaction was seen at the 0- to 5-cm depth increment (Table 4). In this case, the ambient-dry treatment exhibited the lowest soil C turnover relative to the other treatment combinations. At the 5- to 10-cm depth increment, the main effect of water regime was significant, with soil C turnover being lower under dry conditions. Experimental treatments had no impact on soil C turnover at the last depth increment for the 0- to 30-d incubation period. This was also true for all depth increments at the 30- to 60-d incubation period. For the total incubation period (0–60 d), the main effect of water regime was the only significant treatment effect observed. This was true for the 0- to 5-cm depth where soil C turnover was lowest under dry conditions.

Discussion

Soil C is an indicator of overall soil health and influences terrestrial ecosystem sustainability. To understand global C cycling, it is essential to evaluate the potential impacts of the rising level of atmospheric CO₂ on soil C dynamics in agroecosystems. In the current study, soil C, along with soil N, was found to increase at all evaluated soil depth increments (i.e., between 0 and 20 cm) following two growing seasons under FACE (Table 1). Previous reports from this study indicated that FACE increased total biomass production (Ottman et al., 2001). Findings included an increased stover production, which represented greater residue inputs to the soil; this helps explain the increase in soil C observed in our study. Likewise, stover production was increased by the wet moisture regime, but this was not reflected in the soil C (or N) levels. Others have reported that elevated CO₂ can cause significant increases in sorghum root production (Prior et al., 2003), which, in conjunction with aboveground stover production, constitutes the total input to the soil system. It is possible that increased rooting might explain the CO₂ × H₂O interaction noted for soil C/N ratio at the 5- to 10-cm depth. However, the impact of FACE on sorghum roots for this study has not been reported, so this contention cannot be supported at this point. Although the impact of moisture regime was reflected in soil C and N, differences were observed in the potential C and N mineralization assessment (Tables 2 and 3).

Evaluation of potential N mineralization of soil collected after two growing seasons indicated that N was highly immobilized (Table 2). This finding is consistent with inputs of sorghum residue having a high C/N ratio. We observed that there was more N immobilization under FACE conditions. This is likely due to a higher C/N ratio for plant material produced

under high CO₂ conditions as reported by Torbert et al. (2000). This increased N immobilization under FACE also resulted in the soil C/N ratio being lower under these conditions because more N remained in the decomposing sorghum residue. The increase in soil N under FACE vs. the ambient CO₂ treatment was relatively larger than the increase observed for soil C (Table 1). This finding was not only observed in the surface layer but was also noted at the lower depth increments where root residue inputs most likely had a more dominant effect.

The impact of the wet regime resulted in reduced N immobilization at all depths (Table 2). This was likely due to the increased inputs of residue (and therefore N) under these conditions. Over the long term, the N mineralized from wet conditions would have been more vulnerable to losses. Therefore, increased N mineralization under wet conditions may have contributed to the lack of differences observed for total N between the moisture regimes after two growing seasons.

Treatment effects had a significant effect on C mineralization at the top soil depth (Table 3) that was directly proportional to the treatment effects for stover production (Ottman et al., 2001). The increased biomass with the moisture regime resulted in an increase in C mineralization at the top depth. Likewise, the lowest stover inputs occurred under ambient-dry conditions. This resulted in the lowest C mineralization compared with the other treatment combinations. No other significant treatment effects were observed at the other depth increments.

With soil C turnover, the wet regime resulted in significantly higher turnover compared with the dry regime, but no significant CO₂ effect or interaction was observed (Table 4). This indicates that factors other than higher residue inputs found under FACE limited soil C turnover compared with ambient conditions. A possible explanation may be related to a differential effect of CO₂ and irrigation treatment on residue structure/composition, thereby altering decomposition patterns. This is consistent with the fact that we found more soil C under FACE, with no impact of moisture regime (Table 1).

These results are somewhat consistent with previous elevated CO₂ studies with other cropping systems (Wood et al., 1994; Prior et al., 1997). In a 3-yr FACE cotton study, it was suggested that soil C storage is more likely under non-limiting soil water conditions when CO₂ concentration is raised (Wood et al., 1994); results indicated that factors other than total biomass input may affect soil C cycling. In another FACE study, an evaluation of soils after 2 yr of wheat residue inputs indicated that more C storage may occur under elevated CO₂ for irrigated and non-irrigated systems (Prior et al., 1997). Soil C and N were increased after 2 yr of FACE. However, water regime had no effect on these measures.

Results from this study indicate that changes over the short term will occur with C and N. The increased CO₂ resulted in reduced N mineralization, resulting in increased total N in the soil. The changes in C mineralization and C turnover suggest that a small increase in soil C storage could occur under elevated atmospheric CO₂ conditions in sorghum production systems regardless of the moisture regime.

Table 4. Soil C turnover as affected by atmospheric CO₂ level and water treatment.

Depth	Ambient CO ₂		FACE†		Pr > F‡		
	Dry	Wet	Dry	Wet	CO ₂	H ₂ O	CO ₂ × H ₂ O
	—————mg kg ⁻¹ —————						
0–30 d							
0–5 cm	12.05	20.02	17.82	19.00	0.239	0.003	0.012
5–10 cm	9.48	14.52	11.12	12.20	0.801	0.037	0.154
10–20 cm	8.22	9.00	10.38	10.15	0.176	0.812	0.666
30–60 d							
0–5 cm	14.70	12.20	11.02	11.88	0.332	0.571	0.269
5–10 cm	11.32	10.35	10.20	12.02	0.866	0.659	0.177
10–20 cm	12.15	10.62	9.55	8.88	0.146	0.326	0.694
0–60 d							
0–5 cm	26.75	32.22	28.85	30.88	0.692	0.003	0.093
5–10 cm	20.80	24.88	21.32	24.22	0.980	0.130	0.777
10–20 cm	20.38	19.62	19.92	19.02	0.570	0.377	0.935

† Free-air CO₂ enrichment.

‡ Probability of a greater F by chance between the CO₂ or water treatments and for the CO₂ by H₂O interaction.

§ Values represent means of four replications.

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