

Free-Air Carbon Dioxide Enrichment of Soybean: Influence of Crop Variety on Residue Decomposition

S. A. Prior,* H. A. Torbert, G. B. Runion, H. H. Rogers, D. R. Ort, and R. L. Nelson

ABSTRACT

Elevated atmospheric CO₂ can result in larger plants returning greater amounts of residue to the soil. However, the effects of elevated CO₂ on carbon (C) and nitrogen (N) cycling for different soybean varieties have not been examined. Aboveground residue of eight soybean [*Glycine max* (L.) Merr.] varieties was collected from a field study where crops had been grown under two different atmospheric CO₂ levels [370 μmol mol⁻¹ (ambient) and 550 μmol mol⁻¹ (free-air carbon dioxide enrichment, FACE)]. Senesced residue material was used in a 60-d laboratory incubation study to evaluate potential C and N mineralization. In addition to assessing the overall effects of CO₂ level and variety, a few specific variety comparisons were also made. Across varieties, overall residue N concentration was increased by FACE, but residue C concentration was only slightly increased. Overall residue C to N ratio was lower under FACE and total mineralized N was increased by FACE, suggesting that increased N₂ fixation impacted residue decomposition; total mineralized C was also slightly increased by FACE. Across CO₂ levels, varietal differences were also observed with the oldest variety having the lowest residue N concentration and highest residue C to N ratio; mineralized N was lowest in the oldest variety, illustrating the influence of high residue C to N ratio. It appears (based on our few specific varietal comparisons) that the breeding selection process may have resulted in some varietal differences in residue quality which can result in increased N or C mineralization under elevated CO₂ conditions. This limited number of varietal comparisons indicated that more work investigating varietal influences on soil C and N cycling under elevated CO₂ conditions is required.

THE WELL DOCUMENTED anthropogenic-driven increase in atmospheric CO₂ concentration (Keeling and Whorf, 1994) has raised questions related to the ability of highly managed agricultural systems to store soil carbon (Rogers et al., 1994). Biomass production represents the primary means of carbon input to the soil system. However, the decomposition rate of these residue inputs is influenced by tissue chemistry and exogenous nutrient sources. An important topic related to the ability of soil systems to store carbon (C) is nitrogen (N) availability, since this element can limit the amount of C that can be fixed in terrestrial ecosystems. Although the availability of N could limit plant response to elevated CO₂ (Strain and Cure, 1985), it is well established that

elevated CO₂ can often enhance biomass production under ideal nutrient conditions (Rogers and Dahlman, 1993; Amthor, 1995; Kimball et al., 2002). In addition to these CO₂-induced increases in productivity, shifts in tissue quality or nutrient content have been observed in plants grown under elevated CO₂ (Conroy, 1992; Cotrufo et al., 1998; Prior et al., 1997a) which could influence soil C and nutrient cycling in cropping systems (Torbert et al., 2000; Prior et al., 2003).

Whether changes in the composition of plant residue in a CO₂-rich environment may limit decomposition rates and nutrient cycling is not entirely known (Van Veen et al., 1991; Norby and Cotrufo, 1998; Rogers et al., 1999; Torbert et al., 2000). In an incubation study using soil collected after a 3-yr FACE (free-air carbon dioxide enrichment) cotton experiment, alterations in soil C and N cycling patterns were observed for FACE under different irrigation regimes (Wood et al., 1994). In this study, increased soil C turnover corresponded weakly with soil organic C concentrations or biomass production, suggesting that changes in residue quality may have been a factor influencing C cycling. In a wheat FACE study (Prior et al., 1997b), different results were noted between the interaction of FACE and the soil water conditions compared to the FACE cotton system study. For cotton, decreased C turnover occurred when soil moisture was not limiting, whereas potential C turnover in the wheat system was similar regardless of soil moisture conditions. Others have examined residue decomposition as affected by elevated CO₂ independent of the cumulative impact to the soil (Torbert et al., 1995, 1998; Henning et al., 1996). For example, mature cotton residues (leaf, stem, and root) produced under FACE were examined separately using differing soil series (sandy loam, silt loam, and clay loam) having no elevated CO₂ exposure history (Torbert et al., 1995). Differences in soil series were found to exert an important control on decomposition of residues produced under elevated CO₂. Further, increased levels of easily decomposable components compensated for higher C to N ratios, resulting in similar decomposition rates among residues from different CO₂ treatments. While soil C mineralization showed little effect, the N mineralization rates of amended soils were impacted. The release of inorganic N into soil solution was slower with FACE, resulting in decreased N mineralization similar to findings reported in the aforementioned FACE studies (Wood et al., 1994; Prior et al., 1997b). The impacts of CO₂ treatment on mineralization patterns were also reported by Henning et al. (1996), examining individual plant parts of sorghum and soybean material taken at physiological maturity, and by Torbert et al. (1998),

S.A. Prior, H.A. Torbert, G.B. Runion, and H.H. Rogers, USDA-ARS National Soil Dynamics Laboratory, 411 South Donahue Drive, Auburn, AL 36832. D.R. Ort, USDA-ARS Photosynthesis Research Unit, 1201 West Gregory Drive, Urbana, IL 61801. R.L. Nelson, USDA-ARS, 232 National Soybean Research Center, 1101 West Peabody Drive, Urbana, IL 61801. Received 30 Apr. 2005. *Corresponding author (sprior@acesag.auburn.edu).

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677 S. Segoe Rd., Madison, WI 53711 USA

Abbreviations: FACE, free-air carbon dioxide enrichment.

Table 1. Characteristics of the eight soybean varieties evaluated in the incubation study.

Variety	General characteristics
NN5	non-nodulated; Williams mutant with <i>rj5</i> and <i>rj6</i> alleles; Maturity Group III
Williams	nodulated; Maturity Group III; seed protein 42%
LG00-15593	nodulated, Maturity Group III, seed protein 48%; [Williams (3) × Wisconsin Black]
Pana	nodulated; Maturity Group III
Lincoln	nodulated; Maturity Group III; released 1943; best public cultivar separated by >50 yr (vs. HS93-4118)
HS93-4118	nodulated; Maturity Group IV; released 2000; best public cultivar separated by >50 yr (vs. Lincoln)
Flyer	nodulated; Maturity Group IV; released 1988; CO ₂ non-responder
Spencer	nodulated; Maturity Group IV; released 1988; CO ₂ responder

evaluating senesced residues of these species collected at maturity, indicating some differences between crop species which were attributed to differences in residue quality. For example, Torbert et al. (1998) reported that low soil N availability limited microbial decomposition in both grain sorghum and soybean, with net N immobilization conditions persisting throughout most of the incubation period. Also, as was observed with the cotton plant decomposition, the release of inorganic N into the soil solution with soybean was slower under elevated CO₂ conditions. Collectively, these laboratory incubation studies indicate that the limitations on N availability were imposing an important controlling effect on the residue decomposition processes in soil, but some differences occurred among crop species examined.

More work is needed to accurately determine whether differences in crop residue, due to elevated CO₂, could alter soil N cycling and the ability of highly managed agricultural systems to store soil C. One aspect which has not been investigated is whether elevated CO₂ will alter decomposition patterns of residues from different varieties within a given crop species. Our objective was to investigate the effects of elevated CO₂ on C and N cycling for residues of eight soybean varieties in a 60-d laboratory incubation study.

MATERIALS AND METHODS

Plant material for this incubation study was collected from an elevated atmospheric CO₂ study conducted using FACE exposure technology. The details of the FACE exposure system used in this study have been previously described in detail by Miglietta et al. (2001). The CO₂ regimes were ambient (370 μmol mol⁻¹) and FACE (550 μmol mol⁻¹), and eight soybean varieties were evaluated (Table 1); the experiment was conducted using a randomized complete block design with four replications. The FACE rings were 20 m in diameter (octagonal shape plots) and within these the variety plots were 1.9 m² in size. Carbon dioxide exposure of the crop was from emergence to harvest, during daylight hours, and concurrent weather information was collected. An on-site weather station (MetData 1-type; Campbell Scientific, Logan, UT) measured air temperature and relative humidity at a height of 3 m. A quantum sensor (Model QSO; Apogee Instruments, Logan, UT) measured incident photosynthetic photon irradiance at a

height of 3 m. Data were averaged and logged at 10 min intervals throughout the growing season. Tipping bucket rain gauges (Model 52202; R.M. Young, Traverse City, MI) were distributed throughout the field and recorded rainfall (0.0001-m increments) throughout the season. Weather data is posted on the SoyFACE website (<http://www.soyface.uiuc.edu/weather.htm>, verified 23 Jan. 2006); the Illinois State Water Survey weather station (<http://www.sws.uiuc.edu/data/climatedb/>, verified 23 Jan. 2006) in Urbana, IL (40°05' N, 88°14' W) is situated 3 km north of the SoyFACE site and at the same altitude.

The soil series at the soybean FACE facility, located on 32 ha of farmland within the Crop Sciences Research and Education Center of the University of Illinois at Urbana, IL (40°2' N, 88°13' W, 228 m above sea level; <http://www.soyface.uiuc.edu>, verified 23 Jan. 2006), is a Flanagan–Drummer silty clay loam (fine-silty, mixed, mesic Typic Endoaquolls). The soil is a deep, organically rich “prairie soil” typical of northern and central Illinois. The field is tile-drained and has a history of continuous crop cultivation to arable crops for over 100 yr. Agronomic practices in use at the site are typical for this region of Illinois. No applications of additional phosphorus or potassium were required and, according to standard regional practices for soybean production, no nitrogen was added to the field. Basic information on the properties of the soil is shown in Table 2. The field was sown using a mechanical seed planter to a density of about 200 000 plants ha⁻¹ on row spacings of 0.38 m. The experimental plots were over-sown by hand on the day of planting and thinned after emergence to ensure uniform plant density. The soybean crop was grown under rainfed and conventional tillage conditions.

Final harvest was performed in October 2001 and seeds were separated from residue material (pod hulls, leaves, and stems) which would normally remain in the field at harvest. Residue material was dried at 65°C (until weight loss was complete) and ground in a Wiley mill to pass a 0.44-mm screen. A subsample of this ground residue material was collected for each variety for analysis in the incubation procedures. Total C and N contents of residue materials were determined using a Fisons NA1500 CN Analyzer (Fisons Instruments, Beverly, MA).

The Flanagan–Drummer silty clay loam soil used in this study was collected from the plow layer (0–15 cm) by taking multiple shovel samples from locations where a crop had not been planted; these were combined into a composite sample which was thoroughly mixed and screened (2-mm mesh size); any remaining organic debris was removed from the sample using tweezers. This processed composite sample was used as the source of soil for evaluation of potential soil C and N cycling using methods of Torbert et al. (2000). Soil water con-

Table 2. Characteristics of the Flanagan–Drummer silty clay loam soil used in the incubation study.

pH	EC [†]	C	N	P	K	Ca	Mg	Mn	Fe	Zn	B	Cu	Na
	S m ⁻¹	— g kg ⁻¹ —		mg kg ⁻¹									
6.8	0.025	19.4	1.8	10.5	91.5	1369.5	494.0	12.7	5.5	0.9	0.6	0.8	25.0

[†] Electrical conductivity.

Table 3. Initial residue N concentration, C concentration, and C to N ratio of eight soybean varieties as affected by CO₂ level (N = 4).

Variety	Residue C			Residue N			Residue C to N ratio		
	Ambient	FACE†	Overall variety mean‡	Ambient	FACE	Overall variety mean	Ambient	FACE	Overall variety mean
	g kg ⁻¹								
NN5	424.27	425.50	424.89b	11.07	10.50	10.78bcd	38.51	40.63	39.57bc
Williams	422.55	425.21	423.88bc	12.76	14.05	13.40a	33.34	30.36	31.85d
LG00-15593	427.07	428.01	427.54a	11.36	11.95	11.65bc	38.05	36.19	37.12c
Pana	421.24	421.66	421.45d	8.40	12.55	10.47cd	50.49	34.33	42.41b
Lincoln	427.99	427.40	427.69a	7.78	9.73	8.75e	57.05	45.48	51.27a
HS93-4118	419.13	419.48	419.30e	9.77	11.27	10.52cd	44.64	37.56	41.10bc
Flyer	422.45	427.26	424.86b	9.22	10.79	10.00d	46.28	40.01	43.14b
Spencer	421.38	423.88	422.63cd	11.09	12.59	11.84b	38.70	33.75	36.22cd
Overall CO ₂ mean	423.26	424.80		10.18	11.68		43.38	37.29	
CO ₂		0.0099§			0.0001			0.0002	
Variety		<0.0001			<0.0001			<0.0001	
CO ₂ × variety		0.3679			0.1201			0.1042	

† Free-air carbon dioxide enrichment.

‡ Variety main effect means with the same letters are not significantly different from each other.

§ Pr > F for main effects and interactions determined from ANOVA under Proc Mixed.

tent of the composite sample was measured after drying soil subsamples at 105°C for 72 h. Sieved soil samples were weighed (25 g dry weight basis) and placed in plastic 118.3-mL specimen cup containers (Fisher Scientific, Hampton, NH); these cups were sealed to ensure uniform soil water content until all soil and residue samples could be weighed. All pre-weighed samples were stored at 4°C before initiation of the incubation study. Pre-weighed residue samples were then incorporated with the pre-weighed soil samples at a rate of 0.1 g. Residue was incorporated with the soil since the major field experiment was managed under conventional tillage conditions and the long-term cultivation history of this area was conventional tillage. After residue had been mixed into the soil, the specimen cup containers were tamped to a uniform height to achieve a bulk density of 1.3 Mg m⁻³ (the typical range of bulk density for this soil type is 1.0 to 1.3 Mg m⁻³). Deionized water was then carefully added to adjust soil water content (soil water content equivalent to -20 kPa). Specimen cup containers were then placed in sealed 946-mL glass Ball Mason Jars (Alltrista Consumer Products Co., Muncie, IN) with 10 mL of water (placed into the bottom of the jar for humidity control) and a 10-mL vial of 1 M 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 1 M HCl in the presence of BaCl₂. Potential C mineralization was the difference between CO₂-C

captured in sample traps and in blanks. Soil inorganic N (NO₃-N and NH₄-N) was extracted with 2 M KCl and measured by standard colorimetric procedures using a Autoanalyzer III (Bran Luebbe, Buffalo Grove, IL). Potential N mineralization was the difference between inorganic N contents of samples compared to blanks (soil with no plant residue additions).

Data were analyzed as an ANOVA using the Proc Mixed procedure of the Statistical Analysis System (Littell et al., 1996) of SAS. A significance level of *P* < 0.10 was established a priori. As previously stated, the study was a randomized complete block design with four replications. Main effects of CO₂ treatment and soybean variety were often significant while their interactions were rarely significant; all data are presented with appropriate Pr > F values (Tables 3–6). However, pre-planned comparison among varieties were conducted using contrast statements (Tables 7 and 8); these specific varietal comparisons included the following: (i) Flyer vs. Spencer; (ii) Williams vs. LG00-15593; (iii) HS93-4118 vs. Lincoln; and (iv) NN5 vs. nodulating soybeans.

RESULTS

The initial residue characteristics in terms of C concentration, N concentration, and C to N ratio for the eight soybean varieties as affected by CO₂ level were eval-

Table 4. Nitrogen mineralization and C mineralization of incorporated residues for eight soybean varieties as affected by CO₂ level during the 0- to 30-d incubation period (N = 4).

Variety	N mineralization			C mineralization		
	Ambient	FACE†	Overall variety mean‡	Ambient	FACE	Overall variety mean
	mg kg ⁻¹					
NN5	-11.28	-12.56	-11.92b	512.97	533.28	523.12cd
Williams	-9.20	-9.21	-9.20e	552.51	543.74	548.13a
LG00-15593	-10.33	-9.96	-10.14de	539.57	546.50	543.04ab
Pana	-13.27	-9.77	-11.52bc	539.95	551.95	545.95a
Lincoln	-13.82	-12.96	-13.39a	534.46	506.96	520.71d
HS93-4118	-11.60	-10.97	-11.28bcd	549.50	529.58	539.54abc
Flyer	-12.13	-10.99	-11.56bc	530.26	543.74	537.00abcd
Spencer	-11.52	-9.29	-10.41cde	516.35	536.15	526.25bcd
Overall CO ₂ mean	-11.64	-10.71		534.45	536.49	
CO ₂		0.0148§			0.7064	
Variety		<0.0001			0.0799	
CO ₂ × variety		0.0879			0.2051	

† Free-air carbon dioxide enrichment.

‡ Variety main effect means with the same letters are not significantly different from each other.

§ Pr > F for main effects and interactions determined from ANOVA under Proc Mixed.

Table 5. Nitrogen mineralization and C mineralization of incorporated residues for eight soybean varieties as affected by CO₂ level during the 30- to 60-d incubation period (N = 4).

Variety	N mineralization			C mineralization		
	Ambient	FACE†	Overall variety mean	Ambient	FACE	Overall variety mean
	mg kg ⁻¹					
NN5	19.60	20.39	20.00	310.23	339.29	324.76
Williams	16.20	17.82	17.01	347.86	492.42	420.14
LG00-15593	16.58	20.30	18.44	358.99	359.99	359.49
Pana	16.62	18.00	17.31	372.08	342.63	357.35
Lincoln	15.20	15.15	15.18	346.15	338.48	342.32
HS93-4118	13.81	19.46	16.63	357.46	362.44	359.95
Flyer	15.21	14.90	15.06	307.28	423.81	365.55
Spencer	17.04	16.64	16.84	354.25	347.04	350.65
Overall CO ₂ mean	16.28	17.83		344.29	375.76	
CO ₂		0.1184‡			0.0693	
Variety		0.2356			0.2622	
CO ₂ × variety		0.7521			0.1166	

† Free-air carbon dioxide enrichment.

‡ Pr > F for main effects and interactions determined from ANOVA under Proc Mixed.

uated (Table 3). For initial residue C concentration, the main effect of CO₂ was significant and FACE increased this measure slightly (0.36%). The main effect of variety was also significant for residue C; the HS93-4118 variety had the lowest value while the two highest values were associated with the LG00-15593 and Lincoln varieties. There was no significant interaction for residue C; the same was true for initial residue N and residue C to N ratio. The main effect of CO₂ was significant for residue N; FACE increased the overall residue N by approximately 15%. The main effect of variety was also significant for residue N. In this case, the Lincoln variety had the lowest residue N while the highest value was noted with the Williams variety. The main effect of CO₂ was found to be significant for the initial residue C to N ratio; this measure was lowered by FACE (14%) compared to ambient CO₂. The main effect of variety was significant for residue C to N ratio, with the highest observed being Lincoln with 51.3 and the lowest being Williams at 31.9.

During the 0- to 30-d incubation period, N of incorporated residues for the eight soybean varieties was

clearly immobilized as reflected by the negative N mineralization values shown in Table 4. The main effect of CO₂ and variety was significant, as well as the interaction. In this case, the Pana and Spencer varieties had significantly less N immobilized under conditions of FACE compared to ambient CO₂. Carbon mineralization of incorporated residues for this period was fairly consistent for the eight soybean varieties across CO₂ levels. The main effect of CO₂ was not significant. Main effect of variety was significant; however, these differences were small. There was no significant interaction for C mineralization during the 0- to 30-d incubation interval.

During the 30- to 60-d incubation period, potential N mineralization of incorporated residues of the eight soybean varieties under the different CO₂ levels was clearly observed (Table 5) relative to the 0- to 30-d incubation interval (Table 4). However, there were no significant effects for CO₂, variety, or their interaction. For corresponding measures of C mineralization during this incubation period, the main effect of CO₂ was significant; FACE increased overall C mineralization by approximately 9%. Main effect of variety was not significant

Table 6. Nitrogen mineralization and C mineralization of incorporated residues for eight soybean varieties as affected by CO₂ level during the 0- to 60-d incubation period (N = 4).

Variety	N mineralization			C mineralization		
	Ambient	FACE†	Overall variety mean‡	Ambient	FACE	Overall variety mean
	mg kg ⁻¹					
NN5	8.32	7.83	8.07a	823.20	872.57	847.89b
Williams	7.00	8.62	7.81a	900.37	1036.16	968.26a
LG00-15593	6.26	10.35	8.30a	898.57	906.49	902.53b
Pana	3.35	8.23	5.79ab	912.03	894.57	903.30b
Lincoln	1.38	2.19	1.79c	880.61	845.44	863.02b
HS93-4118	2.21	8.49	5.35ab	906.96	892.03	899.49b
Flyer	3.09	3.91	3.50bc	837.54	967.55	902.55b
Spencer	5.51	7.35	6.43ab	870.60	883.19	876.90b
Overall CO ₂ mean	4.64	7.12		878.74	912.25	
CO ₂		0.0147§			0.0737	
Variety		0.0169			0.0839	
CO ₂ × variety		0.6605			0.1487	

† Free-air carbon dioxide enrichment.

‡ Variety main effect means with the same letters are not significantly different from each other.

§ Pr > F for main effects and interactions determined from ANOVA under Proc Mixed.

Table 7. Pre-planned varietal comparisons for residue C concentration, N concentration, and C to N ratio.

Variable	Varietal comparison	Ambient vs. ambient	Pr > t †	FACE‡ vs. FACE	Pr > t
C, g kg ⁻¹	Lincoln vs. HS93-4118	427.99 vs. 419.13	<0.0001	427.40 vs. 419.48	<0.0001
	Williams vs. LG00-15593	422.55 vs. 427.07	0.0076	425.21 vs. 428.01	0.0912
	Flyer vs. Spencer	422.45 vs. 421.38	0.5120	427.26 vs. 423.88	0.0426
	NN5 vs. Nodulated	424.27 vs. 423.12	0.3498	425.50 vs. 424.70	0.5182
	Lincoln vs. HS93-4118	7.78 vs. 9.77	0.0527	9.73 vs. 11.27	0.1318
N, g kg ⁻¹	Williams vs. LG00-15593	12.76 vs. 11.36	0.1682	14.05 vs. 11.95	0.0423
	Flyer vs. Spencer	9.22 vs. 11.09	0.0685	10.79 vs. 12.59	0.0793
	NN5 vs. Nodulated	11.07 vs. 10.05	0.1878	10.50 vs. 11.84	0.0823
	Lincoln vs. HS93-4118	57.05 vs. 44.64	0.0052	45.48 vs. 37.56	0.0671
	Williams vs. LG00-15593	33.34 vs. 38.05	0.2711	30.36 vs. 36.19	0.1743
C to N ratio	Flyer vs. Spencer	46.28 vs. 38.70	0.0792	40.01 vs. 33.75	0.1454
	NN5 vs. Nodulated	38.51 vs. 44.08	0.0880	40.63 vs. 36.81	0.2373

† Differences between varieties determined using contrast statement under Proc Mixed.

‡ Free-air carbon dioxide enrichment.

and no significant interaction was noted for C mineralization covering the 30- to 60-d incubation period.

Potential N mineralization of incorporated residues for eight soybean varieties as affected by CO₂ level for the total incubation period (i.e., 0–60 d) is shown in Table 6. The main effect of CO₂ was significant and total N mineralization was increased by FACE. The overall N mineralization mean was 4.64 for ambient CO₂ compared to 7.12 mg kg⁻¹ for FACE, which represented an increase of 53%. The main effect of variety was significant, with the Lincoln and Flyer varieties exhibiting the lowest N mineralization values. No significant interaction was noted for total N mineralization. For corresponding total C mineralization, the main effect of CO₂ was significant. The overall C mineralization mean was 878.7 mg kg⁻¹ for ambient compared to 912.3 mg kg⁻¹ for FACE, which represented an increase of only 3.8%. The main effect of variety was significant with the

Williams variety exhibiting the highest C mineralization value; overall, differences between varieties were not great. No significant interaction was noted for C mineralization for the total incubation period (0–60 d).

Pre-planned varietal comparisons were also performed based on some specific varietal characteristics listed in Table 1. Flyer and Spencer were compared due to their differential response to CO₂ enrichment. Williams and LG00-15593 were compared due to their differences in seed protein content. The best publicly released cultivars that are separated by >50 yr (HS93-4118 and Lincoln) were compared. A comparison between the only non-nodulating variety (NN5) and the mean of all other nodulating varieties was also done. The results of these comparisons for initial residue characteristics and mineralization patterns are exhibited in Tables 7 and 8, respectively. Significant differences were observed between some of these variety comparisons,

Table 8. Pre-planned varietal comparison for N and C mineralization.

Variable	Comparison	Ambient vs. ambient	Pr > t †	FACE‡ vs. FACE	Pr > t
		mg kg ⁻¹			
0–30 d					
N min	Lincoln vs. HS93-4118	-13.82 vs. -11.60	0.0384	-12.96 vs. -10.97	0.0612
	Williams vs. LG00-15593	-9.20 vs. -10.33	0.2842	-9.21 vs. -9.96	0.4760
	Flyer vs. Spencer	-12.13 vs. -11.52	0.5653	-10.99 vs. -9.29	0.1100
	NN5 vs. Nodulated	-11.28 vs. -11.70	0.6038	-12.56 vs. -10.45	0.0099
	Lincoln vs. HS93-4118	534.46 vs. 549.50	0.3286	506.96 vs. 529.58	0.1444
C min	Williams vs. LG00-15593	552.51 vs. 539.57	0.4001	543.74 vs. 546.50	0.8568
	Flyer vs. Spencer	530.26 vs. 516.35	0.3660	543.74 vs. 536.15	0.6205
	NN5 vs. Nodulated	512.97 vs. 537.51	0.0385	533.28 vs. 536.95	0.7515
	30–60 d				
N min	Lincoln vs. HS93-4118	15.20 vs. 13.81	0.6157	15.15 vs. 19.46	0.1255
	Williams vs. LG00-15593	16.20 vs. 16.58	0.8888	17.82 vs. 20.30	0.3731
	Flyer vs. Spencer	15.21 vs. 17.04	0.5118	14.90 vs. 16.64	0.5300
	NN5 vs. Nodulated	19.60 vs. 15.81	0.0755	20.34 vs. 17.47	0.1674
	Lincoln vs. HS93-4118	346.15 vs. 357.46	0.8142	338.48 vs. 362.44	0.6189
C min	Williams vs. LG00-15593	347.86 vs. 358.99	0.8171	442.42 vs. 359.99	0.0082
	Flyer vs. Spencer	307.28 vs. 354.25	0.3315	423.81 vs. 347.04	0.1156
	NN5 vs. Nodulated	310.23 vs. 349.51	0.2876	339.29 vs. 380.97	0.2553
	0–60 d				
N min	Lincoln vs. HS93-4118	1.38 vs. 2.21	0.7678	2.19 vs. 8.49	0.0277
	Williams vs. LG00-15593	7.00 vs. 6.26	0.7905	8.62 vs. 10.35	0.5346
	Flyer vs. Spencer	3.09 vs. 5.51	0.3856	3.91 vs. 7.35	0.2205
	NN5 vs. Nodulated	8.32 vs. 4.11	0.0506	7.83 vs. 7.02	0.7007
	Lincoln vs. HS93-4118	880.61 vs. 906.96	0.6132	845.44 vs. 892.03	0.3730
C min	Williams vs. LG00-15593	900.37 vs. 898.57	0.9723	1036.16 vs. 906.49	0.0160
	Flyer vs. Spencer	837.54 vs. 870.60	0.5264	967.55 vs. 883.19	0.1102
	NN5 vs. Nodulated	823.20 vs. 886.67	0.1119	872.57 vs. 917.92	0.2527

† Differences between varieties determined using contrast statement under Proc Mixed.

‡ Free-air carbon dioxide enrichment.

which differed between ambient and/or FACE conditions. The implications of these differences are discussed in detail below.

DISCUSSION

Differences in residue N were noted among varieties, with the oldest variety (Lincoln) having the lowest value (Table 3). Lincoln also had one of the highest residue C values. Likewise, these differences were reflected in the residue C to N ratio of the Lincoln variety, which was higher than the other varieties evaluated. It is possible that differences between the newer varieties and this old variety may be reflective of the breeding process impacting the selection of factors influencing residue quality characteristics.

Overall differences in these residue characteristics could also be attributable to changes in atmospheric CO₂ level (Table 3). Although FACE led to an increase in residue C, this change was relatively small. More importantly, residue N was increased by FACE and residue C to N ratio was lower under FACE (Table 3), which was likely due to increased symbiotic N₂ fixation capabilities involving *Bradyrhizobium*. Others have reported that CO₂ enrichment increases nodulation and biological N₂ fixation in leguminous crops (Zanetti et al., 1996; Kimball et al., 2002). Using isotope dilution methods, Torbert et al. (2004) reported that N₂ fixation in soybean was significantly higher under elevated CO₂ compared to ambient CO₂ in a 3-yr field study. This increase in the level of biologically fixed N₂ in soybean would account for the increased N levels observed in the FACE treatment compared to ambient CO₂ treatments in this study.

Some specific comparisons between varieties were made to determine if certain criteria or varietal characteristics (Table 1) influenced residue quality under different levels of atmospheric CO₂ (Table 7). For example, the Flyer and Spencer varieties were directly compared since Flyer is thought to represent a non-responder to CO₂ while Spencer represents a CO₂ responder (Ort and Nelson, personal communication). In this comparison, both varieties showed an increase in residue N under both ambient and elevated CO₂ conditions. Further, Flyer was found to exhibit a high C to N ratio compared to Spencer under ambient CO₂ conditions, with a similar trend being noted between these varieties under elevated CO₂ conditions.

Another comparison was made between varieties that exhibited specific differences in seed protein content; Williams with 42% seed protein and LG00-15593 with 48% seed protein (Table 7). It was thought that shifts in seed protein content may also be accompanied by shifts in N allocation to non-yield residue components of the soybean crop. There was some indication that the variety with the highest seed protein (LG00-15593) had the lowest residue N (due to more N allocation to the seed). However, in this comparison, both varieties exhibited similar C to N ratios under ambient and elevated CO₂ conditions; this finding could be attributable in part to corresponding increases in residue C.

The Lincoln and HS93-4118 varieties were compared since they represent the best publicly released cultivars that are separated by >50 yr (Table 7); Lincoln was released in 1943 and HS93-4118 in 2000. In this case, both varieties showed a reduction in residue C under both CO₂ levels. Further, residue N was higher for HS93-4118 under ambient CO₂ conditions, with a similar trend being noted between these varieties under elevated CO₂ conditions. These shifts resulted in the old release (Lincoln) having a higher C to N ratio under both ambient CO₂ and FACE conditions compared to the respective HS93-4118 counterparts. This suggests that breeding selection (often done for yield improvement) resulted in the HS93-4118 variety having a higher residue quality in terms of higher residue N, resulting in a lower residue C to N ratio.

The non-nodulating variety (NN5) was also compared to the average of the nodulating soybeans (Table 7). In this comparison, both showed no difference in residue C. Although no difference was observed for residue N in the ambient comparison, a significant difference in residue N occurred in the elevated CO₂ comparison. While this may reflect a stimulation of N₂ fixation for nodulated soybeans under elevated CO₂, it did not alter residue C to N ratio under high CO₂ conditions. In fact, NN5 was found to exhibit a higher C to N ratio only under ambient CO₂ conditions.

Differences in potential N mineralization were found to occur over time based on the length of the incubation period and the soybean varieties evaluated (Tables 4–6). Immobilization of residue N clearly occurred during the first 30 d for all varieties (Table 4); however, over the next interval (30–60 d; Table 5), the opposite occurred as more N was released into soil solution thereby reflecting an overall increase in potential N mineralization. Although it has been reported that the C to N ratio of soybean residue is generally lower than that of non-nodulating crop species (Parr and Papendick, 1978) resulting in comparatively faster N mineralization, microbial decomposition processes in our case were sufficient to cause immobilization of residue N during the first 30 d. Even though N immobilization occurred, the inherent N fertility of the soil resulted in no negative impact on residue decomposition as relatively high C mineralization occurred during this period (Table 4). However, during the following incubation period (30–60 d), continuing microbial decomposition processes resulted in a release of N from the soybean residue, with the overall impact for the entire incubation period being net N mineralization (Table 5). Likewise, the cumulative assessment of potential N mineralization (0–60 d) reflected that overall mineralization of residue N was increased by FACE (Table 6).

As in our earlier specific comparisons, we also examined specific mineralization patterns (Table 8). For the non-nodulating variety (NN5) vs. that of the nodulating soybeans comparison, overall N mineralization patterns were similar in the elevated CO₂ comparison except for the 0- to 30-d period when NN5 exhibited greater N immobilization. On the other hand, more N was mineralized for NN5 in the ambient CO₂ comparison

for the 30- to 60- and 0- to 60-d periods. These mineralization patterns generally follow the shifts noted with residue C to N ratio.

Differences in N mineralization patterns between the non-responder to CO₂ (Flyer) and the CO₂ responder (Spencer) were also examined. While the CO₂ responder clearly had higher total N mineralization (all incubation periods) compared to the non-responder, no significant increase in net N mineralization due to CO₂ enrichment was observed for the CO₂ responder (Table 8). A comparison of corresponding C mineralization patterns showed no significant differences. This indicates that yield response to CO₂ treatment may not always impact our ability to predict N and C mineralization patterns. Likewise, this was true for soybean varieties having different seed protein values (i.e., Williams with seed protein of 42% vs. LG00-15593 with seed protein of 48%) in terms of N mineralization comparisons (Table 8). However, this was not the case for C mineralization in the Williams vs. LG00-15593 comparison. In this case, there was a consistent pattern of lower C mineralization for the LG00-15593 variety (higher seed protein) for the comparison made under FACE conditions. This difference ranged from a reduction of approximately 19% (30–60 d) to 13% (0–60 d).

For the Lincoln vs. HS93-4118 comparison (i.e., best public release varieties separated by >50 yr), there was a clear response to elevated CO₂, with the newer variety (HS93-4118) having a greater cumulative N mineralization response (0–60 d; Table 8). As suggested earlier, the breeding selection process may have unknowingly impacted residue quality (e.g., lower residue C to N ratio), resulting in higher N mineralization for the HS93-4118 variety which was most exaggerated under elevated CO₂ conditions (Table 8).

CONCLUSIONS

During the last 50 yr, there has been a substantial release of CO₂ to the atmosphere and numerous studies have shown positive growth responses to CO₂ enrichment. In addition, although changing CO₂ level can also alter residue quality and decomposition patterns, these responses have been shown to be influenced by such factors as crop species and soil type (Torbert et al., 2000). However, it is not known whether variety breeding efforts for yield response (or other desired factors) were also selecting for differential CO₂ growth responses. This work clearly shows that elevated CO₂ can impact overall residue quality and that CO₂ had greater effect on increasing overall N mineralization more than C mineralization. Based on our few varietal comparison, it appears that in some cases, the breeding selection process may have resulted in varietal differences in residue quality which can result in increased N or C mineralization under elevated CO₂ conditions.

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