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Variability in C_3-Plant Cell-Wall Biosynthesis in a High-CO_2 Atmosphere by Solid-State NMR Spectroscopy

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Abstract: We have used a frequency-selective rotational-echo double-resonance (REDOR) solid-state NMR experiment to measure the concentrations of glycine−glycine pairs in proteins (and protein precursors) of intact leaves of plants exposed to both high- and low-CO_2 atomospheres. The results are interpreted in terms of differences in cell-wall biosynthesis between plant species. We illustrate this variability by comparing the assimilation of label in cheatgrass and soybean leaves labeled using ^{15}N-fertilizer and $^{13}CO_2$ atmospheres. Cheatgrass and soybean are both C_3 plants but differ in their response to a high-CO_2 environment. Based on REDOR results, we determined that cheatgrass (a plant that seems likely to flourish in future low-water, high-CO_2 environments) routes 2% of the assimilated carbon label that remains in the leaf after 1 h in a 600-ppm $^{13}CO_2$ atmosphere to glycine-rich protein (or its precursors), a structural component of cell walls cross-linked to lignins. In contrast, soybean under the same conditions routes none of its assimilated carbon to glycine-rich protein.

Introduction

Cheatgrass (*Bromus tectorum*) is an invasive weed that is displacing native vegetation throughout the Great Basin area of Utah, Idaho, Oregon, and California.[1] Cheatgrass outcompetes the seedlings of native and desirable species like sage for soil moisture and seems likely to flourish in future high-CO_2 conditions.[2] Cheatgrass matures early and dries quickly into dense mats rich in lignin and aromatics that are easily ignited.[1] Parts of the Great Basin that used to burn once every 20 or 30 years are now having annual fires. A cheatgrass fire spread across 350,000 acres of Utah in 2007 destroying valuable cattle.[3] Many of the fires threatening Los Angeles and San Diego in 2008 were attributed to cheatgrass invasions.[3]

Cheatgrass is a C_3 plant[4] just like soybean[5] (*Glycine max*). This means that the first step in carbon assimilation is the production of triose phosphates by ribulose bisphospate carboxylase-oxygenase (Rubisco) in the Calvin cycle.[5] We are interested in how cheatgrass achieves high efficiency in transporting water and assimilating carbon. The notion is that some of these traits might be transferred to help offset anticipated water shortages for C_3 food crops grown in future arid high-CO_2 environments.[6,7]

In the summer of 2008, we grew cheatgrass outdoors in pots containing $^{15}N_2$-labeled ammonium nitrate. We then ^{13}C-labeled a cluster of about 12 leaves for 1 h. Each leaf was approximately 10 cm long and 0.5 cm wide. We used 200-ppm $^{13}CO_2$ for one pot and 600-ppm $^{13}CO_2$ for another. The higher concentration is the expected atmospheric CO_2 level in 50 years or so.[8] We examined lyophilized leaves using $^{13}C\{^{15}N\}$ rotational-echo double resonance[9,10] (REDOR) solid-state NMR (with and without frequency selection) and compared the results to similar labeling experiments performed on soybeans in 2006.

The results were dramatically different. First, soybean leaves made and stored starch, whereas cheatgrass made no labeled starch. The only major product of photosynthesis in cheatgrass was sucrose, which is immediately available for transport out of the leaf. Second, soybean leaves made glycine-rich protein (GRP), or its precursors possibly stored in vescicles, at low CO_2 but not at high CO_2. GRPs are structural proteins[11] that are sometimes cross-linked to lignin to strengthen leaf cell walls,[12] particularly those of water-transporting xylem cells.[13] We have found that the glycine that goes into GRP is largely the product

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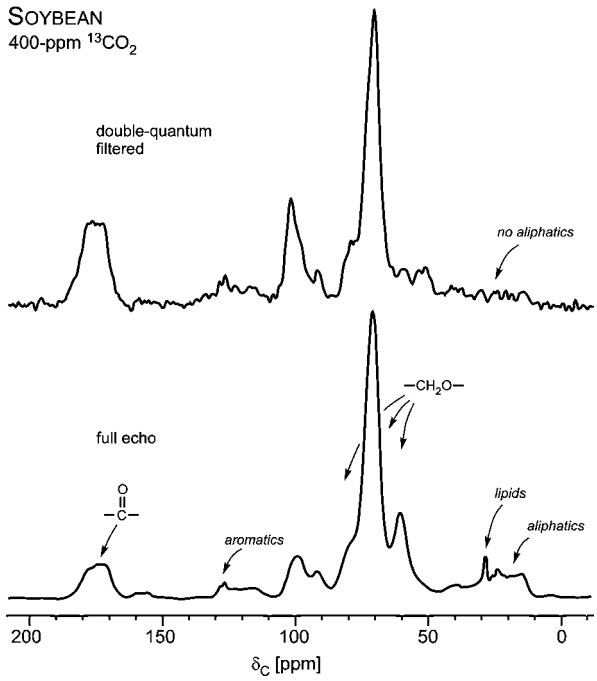

Figure 1. 125-MHz ^{13}C full-echo spectra of a soybean leaf labeled for 1 h by 400-ppm $^{13}CO_2$ with (top) and without (bottom) scalar-J-based double-quantum filtering (DQF). The DQF-REDOR echo (283712 scans) formed after 56 rotor periods and the standard REDOR full echo (51008 scans) after 12 rotor periods. Magic-angle spinning was at 7143 Hz.

of the Rubisco photorespiratory oxygenase pathway. Cheatgrass made as much GRP as soybean at low CO_2 but continued to make GRP at high CO_2.

Our interpretation of these comparisons is that soybean utilizes a control system in the biosynthesis of GRP (possibly involving a fast-acting glycine-dependent riboswitch[14,15]) that is activated under an indicator of short-term water stress. This indicator is a low internal-CO_2 concentration within the leaf,[16] which in the field results from increased stomatal resistance to gas exchange. Under high external-CO_2 conditions, either the riboswitch, or perhaps a less pronounced CO_2-dependent stomatal response for soybean, results in a high internal-CO_2 concentration which turns off the GRP pathway. Cheatgrass has apparently evolved under consistent water-stress conditions to run the GRP biosynthetic pathway full out, which suggests no riboswitch (or the equivalent) and no control.

Materials and Experimental Methods

Growth of Plants. Cheatgrass and soybeans were grown outdoors (June−August, 2006−8) on the roof of the Washington University McMillen Laboratories building. The plants were grown in 30-cm-diameter pots filled with a mixture of one-third perlite and two-thirds top soil. Approximately 3 weeks after planting, the pots were fertilized each day with 100 mL of a 1 g/L $^{15}NH_4{}^{15}NO_3$ solution. The ^{15}N label (99 atom % ^{15}N, Isotec, Miamisburg, OH) was used to distinguish ^{13}C label in amino acids and proteins from that in organic acids. This high level of fertilizer suppressed symbiotic nitrogen fixation, as evidenced later by the scarcity of soybean root nodules. The ^{15}N enrichment of the leaves was approximately 50%, as determined by solid-state ^{15}N NMR,[16] less than that of the ammoniun nitrate because of unlabeled nitrogen sources in the top soil. The plants were watered by hand, typically every 2−4 h during the day.

Labeling with $^{13}CO_2$. Labeling with $^{13}CO_2$ (99 atom % ^{13}C) was performed approximately 8 weeks after planting. The labeling was performed between 10 am and 2 pm on sunny, cloud-free days with temperatures between 80−90 °F at noon in St. Louis. The windy conditions on the roof of the McMillen Laboratories building resulted in soybean plants that were short (60 cm after 8 weeks) with thick stems and large leaves. The target central leaf of a soybean trifoliolate (fifth or sixth node, uniform green coloration, typical surface area of 50 cm^2) was enclosed within the equivalent of a compact-disk jewel case,[17] which loosely fit around the stem and allowed the labeling gas (200, 400, and 600 ppm by volume, Praxair, Inc., Cahokia, IL) to enter one end of the case, sweep over both surfaces of the leaf, and exit from the other end.

A cluster of about a dozen leaves from several cheatgrass plants in a single pot was also labeled. Each leaf was about 10-cm long and 0.5-cm wide. The labeling was begun after confirmation of active photosynthesis by accumulation of moisture on the inner surface of the case. Gas mixtures in pressurized 6-L cylinders containing 21% O_2, 79% N_2, and the desired $^{13}CO_2$ concentration (200 and 600 ppm by volume) flowed through the labeling chamber for 1 h. The gas flow was constant and was the equivalent of a turnover of 100 volumes of the labeling chamber per minute. This allowed a rapid complete exchange of $^{13}CO_2$ for $^{12}CO_2$ so that short labeling times were practical. An electric fan blew exiting gas away from the plants. Humidification of the labeling gas was achieved by bubbling through 50 mL of water so that the relative humidity inside the labeling chamber was maintained above 80%, as confirmed by an HMT333 humidity and temperature sensor (Vaisala, Helsinki, Finland). At the end of the labeling period, the leaves were cut from their stems and immersed in liquid nitrogen, a procedure that required less than 10 s. The frozen leaves were lyophilized, after which 175 mg (of typically 250 mg) were chopped into approximately 1-mm fragments by hand with a razor blade, packed into a magic-angle spinning rotor, and examined by solid-state NMR.

Solid-State NMR. Spectra were obtained using a 6-frequency transmission-line probe,[18] having a 12-mm long, 6-mm inner-diameter analytical coil and a Chemagnetics/Varian magic-angle spinning ceramic stator. Lyophilized samples were contained in thin-wall Chemagnetics/Varian 5-mm outer-diameter zirconia rotors. The rotors were spun at 6250 or 7143 Hz with the speed under active control to within ±2 Hz. The spectrometer was controlled by a Tecmag pulse programmer. Radiofrequency pulses for ^{13}C (125 MHz) and ^{15}N (50.3 MHz) were produced by 2-kW American Microwave Technology power amplifiers. Proton (500 MHz) radiofrequency pulses were generated by a 2-kW Amplifier Systems tube amplifier driven by a 50-W American Microwave Technology power amplifier. The π-pulse lengths were 8 μs for ^{13}C and 9 μs for ^{15}N. A 12-T static magnetic field was provided by an 89-mm bore Magnex superconducting solenoid. Proton−carbon cross-polarization magic-angle spinning transfers were made with radiofrequency fields of 62.5 kHz. Proton dipolar decoupling was 100 kHz during data acquisition.

Rotational-Echo Double Resonance. REDOR was used to restore the dipolar couplings between heteronuclear pairs of spins that are removed by magic-angle spinning.[19] REDOR experiments are always done in two parts, once with rotor-synchronized dephasing pulses (S) and once without (full echo, S_0). The dephasing pulses change the sign of the heteronuclear dipolar coupling, and this interferes with the spatial averaging resulting from the motion of the rotor. The difference in signal intensity (REDOR difference, $\Delta S = S_0 - S$) for the observed spin in the two parts of the REDOR experiment is directly related to the corresponding distance to the

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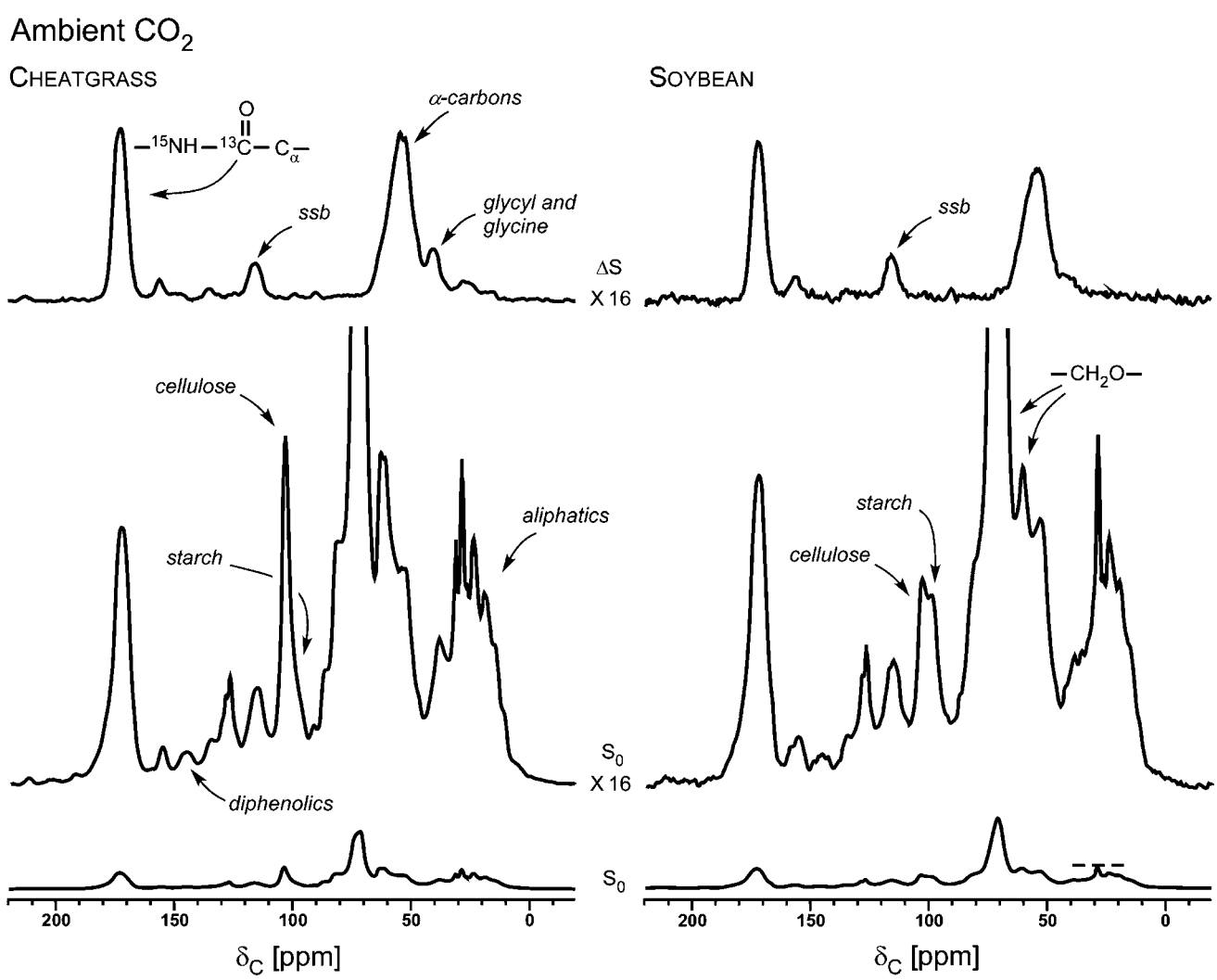

Figure 2. 125-MHz $^{13}C\{^{15}N\}$ REDOR spectra of natural-abundance-^{13}C, ^{15}N-fertilizer-labeled leaves of cheatgrass (left) and soybean (right). The full-echo spectra (S_0) are shown at the bottom of the figure and the REDOR differences (ΔS) at the top. The starch and cellulose assignments (arrows, S_0 spectra) are for the anomeric carbons near 100 ppm. Only directly bonded $^{13}C-^{15}N$ pairs were detected in ΔS by a short dipolar evolution period. The cheatgrass spectra were the result of the accumulation of 52176 scans and the soybean spectra of 14284 scans. Magic-angle spinning was at 7143 Hz.

dephasing spin.[19] All REDOR spectra were collected with standard xy-8 phase cycling[20] on both observed and dephasing channels. In the $^{13}C\{^{15}N\}$ REDOR experiments reported here, short dipolar evolution periods were used (12 or 16 rotor periods) so that only directly bonded $^{13}C-^{15}N$ pairs were detected.

Frequency-Selective REDOR. A frequency-selective REDOR experiment[10] combined a REDOR pulse sequence with DANTE inversion to reintroduce selectively the dipolar interaction between ^{13}C observed spins and directly bonded ^{15}N dephasing spins, the latter within a narrow frequency range. A full-echo signal was obtained without the DANTE pulses while the frequency-selective recoupled signal was obtained by applying two DANTE $\pi/2$ pulses.[21] The parameters of $^{13}C\{^{15}\underline{N}\}$ (the underline denotes frequency selection) for ^{13}C,^{15}N-labeled cheatgrass and soybean leaves were as follows: magic-angle spinning at 6250 Hz, a 1.5-ms 62.5-kHz $^1H-^{31}C$ Hartmann−Hahn CP match, 8-μs ^{13}C π pulses, 10-μs ^{15}N π pulses, frequency-selected ^{15}N $\pi/2$ pulses at δ_N 85, and proton decoupling at 100 kHz. Each DANTE $\pi/2$ pulse was composed of four 1.1-μs DANTE pulses with (nominal) rf amplitude of 50 kHz and a separation from one another of one rotor period (160 μs). The pulse width was adjusted for optimum signal inversion at 85 ppm.

Double-Quantum Filter. The high-efficiency *J*-based double-quantum-filter pulse sequence introduced for solids by Mueller et al.[22] was used to separate label from natural-abundance ^{13}C signals. REDOR ^{15}N pulses created dephasing during the two $\tau-\pi-\tau$ periods of the sequence.[23] A single ^{15}N $\pi/2$ pulse was inserted between the ^{13}C coherence-transfer pulses for both full-echo (without ^{15}N π pulses) and dephased-echo (with ^{15}N π pulses) REDOR acquisitions. The ^{15}N $\pi/2$ pulse ensured that bilinear coherence generated during the first $\tau-\pi-\tau$ dephasing period was not refocused as observable single-quantum coherence during the second period.[23] As a result, double-quantum filtering and REDOR dephasing could be done simultaneously rather than sequentially.

Results and Discussion

Natural-Abundance Background. The full-echo ^{13}C NMR spectrum of a lyophilized soybean leaf labeled for 1 h with 400-ppm $^{13}CO_2$ is shown in Figure 1 (bottom). About half of the total integrated spectral intensity is due to label and half to ^{13}C at natural-abundance.[16,17] Because the Calvin cycle in the leaf chloroplasts is isotopically saturated in about 5 min,[17] subsequent biosynthesis results in uniformly ^{13}C-labeled products.

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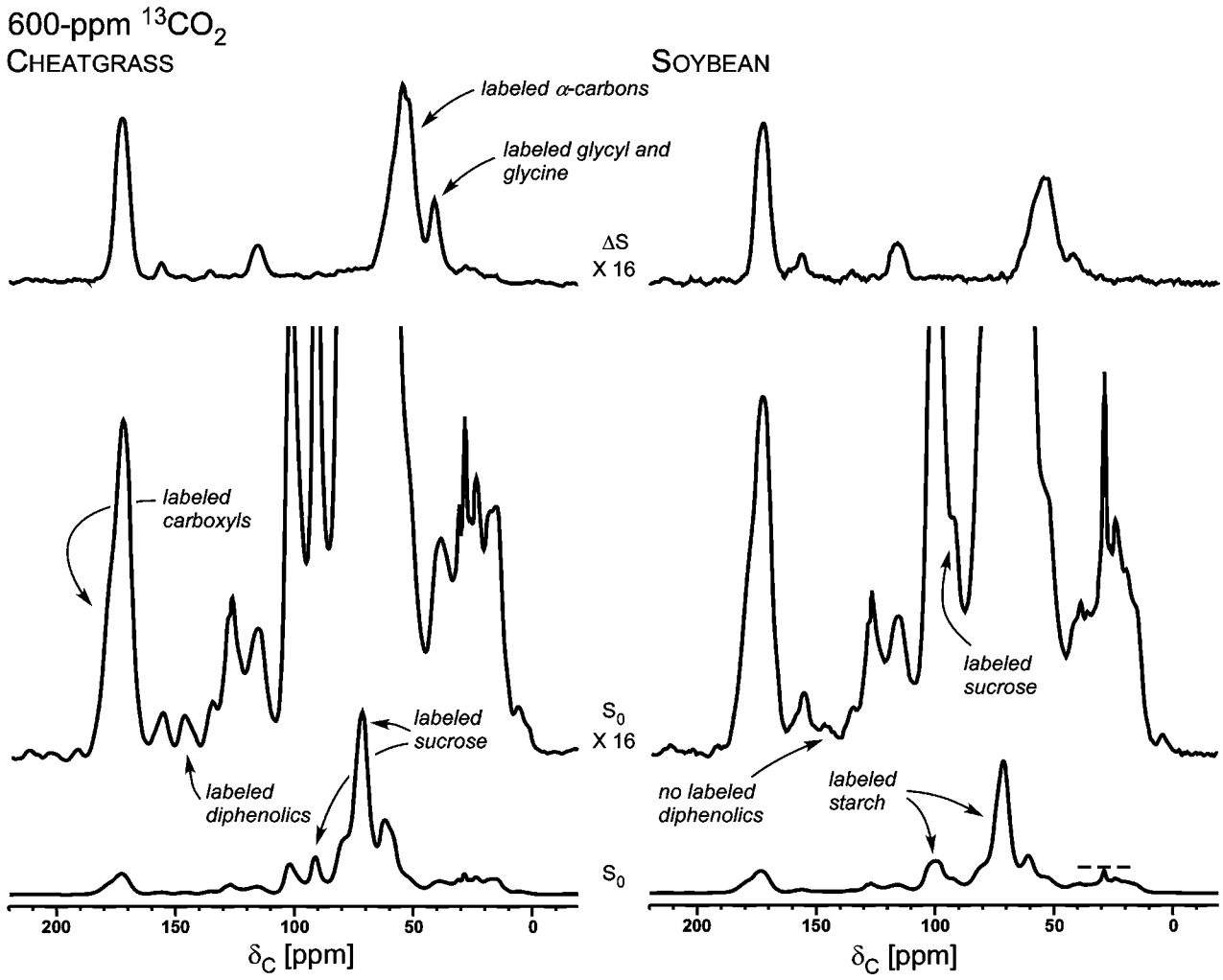

Figure 3. 125-MHz $^{13}C\{^{15}N\}$ REDOR spectra of ^{15}N-fertilizer-labeled leaves of cheatgrass (left) and soybean (right) exposed to 600-ppm $^{13}CO_2$ for 1 h. The full-echo spectra (S_0) are shown at the bottom of the figure and the REDOR differences (ΔS) at the top. Only directly bonded $^{13}C-^{15}N$ pairs were detected in ΔS by a short dipolar evolution period. Identification of incorporated label was made qualitatively by comparison to Figure 2 using the lipid peaks at 29 ppm as an indicator of the natural-abundance ^{13}C concentration (dotted line, bottom right). The cheatgrass spectra were the result of the accumulation of 42260 scans and the soybean spectra of 17444 scans. Magic-angle spinning was at 7143 Hz.

The $^{13}C-^{13}C$ scalar or dipolar couplings can then be used to suppress the natural-abundance contribution through a double-quantum filter[23] (Figure 1, top). The problems with this approach for removing the background are a loss of sensitivity due to homogeneous decay and the pulse imperfections of the filter, as well as some distortions in relative signal intensities because of variations in scalar couplings. Nevertheless, the filter is valuable because we can establish with its use that after 1 h of exposure to $^{13}CO_2$ sugars, and some protein carbons are massively ^{13}C labeled but lipids and most aliphatic side chain carbons are not (Figure 1). We will take advantage of this result in our analysis of labeled-carbon assimilation in cheatgrass and soybean leaves by subtracting the corresponding natural-abundance ^{13}C spectra of unlabeled leaves (normalized by weight and scans) from the spectra of leaves labeled by $^{13}CO_2$ with scaling of the natural-abundance spectra for minor leaf-to-leaf compositional variations so that ^{13}C label in the aliphatic region (including the lipid peak at δ_C 29) of the difference is minimized.

Line Assignments. The natural-abundance $^{13}C\{^{15}N\}$ REDOR full-echo spectra of cheatgrass and soybean leaves are qualitatively similar (Figure 2, bottom) but with some significant differences. The cheatgrass is lower in starch content than soybean but higher in structural cellulose[24] (Figure 2, middle expansion) and higher also in diphenolics like the isodityrosines of plant cell-wall cross-links[25−29] with their unique 145-ppm oxygenated aromatic-carbon chemical shift.[30] The REDOR differences show approximately the same protein content for both kinds of leaves, but with higher concentrations of glycyl and glycine methylene carbons (42 ppm) and polyamine species (around 30 ppm) in cheatgrass (Figure 2, top).

Using the lipid-peak intensity at 29 ppm as a guide (cf. above), we see qualitatively that both cheatgrass and soybean leaves assimilate about the same amount of ^{13}C label after exposure to 600-ppm $^{13}CO_2$ for 1 h (compare the bottom spectra of Figure 2 with the corresponding spectra of Figure 3 with the dotted lines as a guide). The label in cheatgrass is primarily in sucrose (characteristic anomeric-carbon peak at 92 ppm) but

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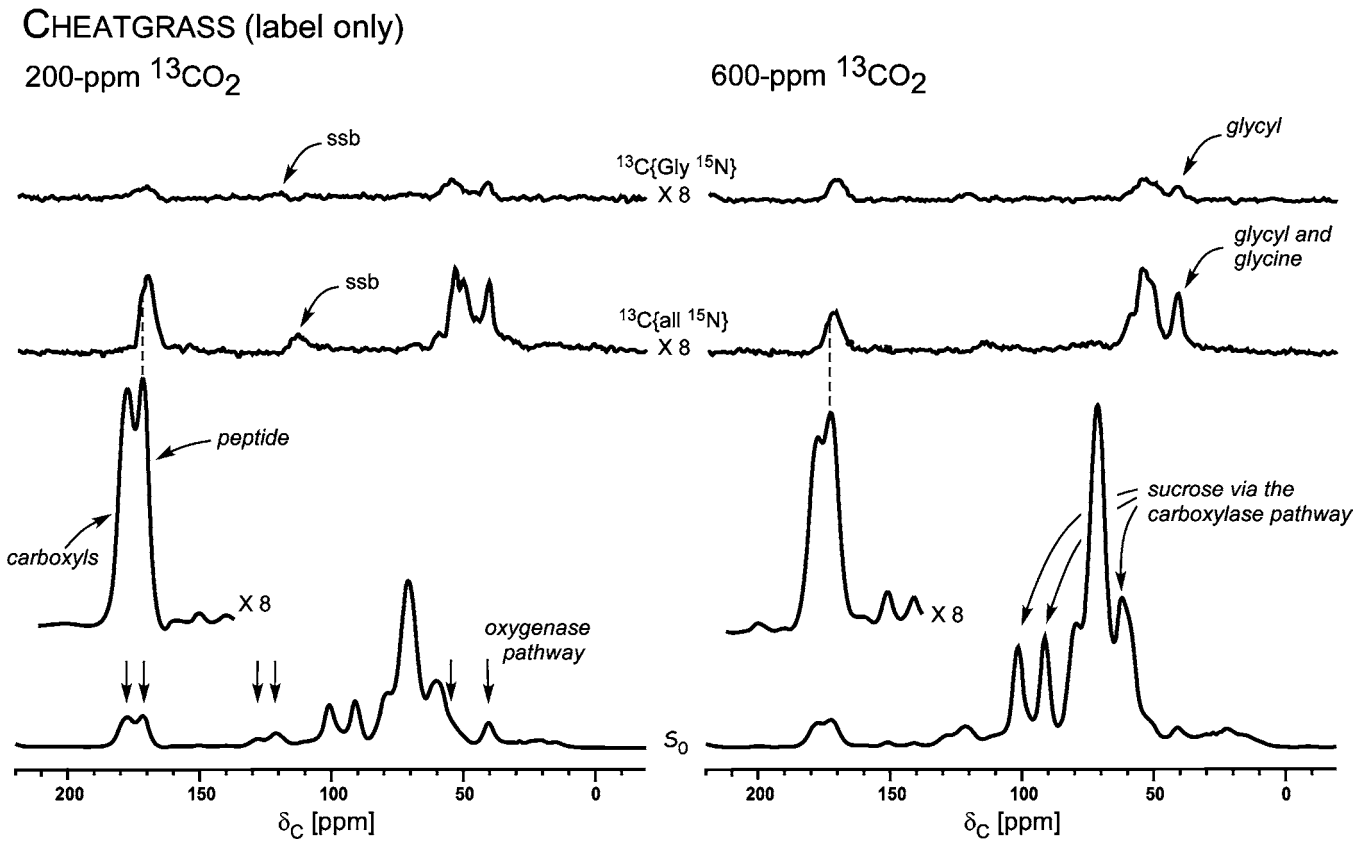

Figure 4. 125-MHz $^{13}C\{^{15}N\}$ and frequency-selected $^{13}C\{^{15}\underline{N}\}$ REDOR spectra of ^{15}N-fertilizer-labeled leaves of cheatgrass exposed for 1 h to 200-ppm $^{13}CO_2$ (left) and 600-ppm $^{13}CO_2$ (right). The selected ^{15}N dephasing frequency was at δ_N 85, an amide-nitrogen frequency which is primarily but not exclusively due to glycyl residues in proteins. The spectra have been scaled as described in the caption to Figure 3. Contributions from the natural-abundance background have been removed by subtraction. The full-echo spectra (S_0) are shown at the bottom of the figure and the REDOR differences (ΔS) at the middle and top. Only directly bonded $^{13}C-^{15}N$ pairs were detected in ΔS by a short dipolar evolution period. The cheatgrass spectra on the left (bottom and middle) were the result of the accumulation of 53428 scans and on the left (top) 106832 scans. The cheatgrass spectra on the right (bottom and middle) were the result of the accumulation of 42260 scans and on the right (top) 227416 scans. Magic-angle spinning was at 7143 Hz for the REDOR spectra and 6250 Hz for the frequency-selected REDOR spectra.

with significant amounts of label also in diphenolics, organic acids, protein, and protein precursors including glycyl and other α-carbons (Figure 3, left). The label in soybean is mostly in starch, sucrose, and protein (Figure 3, right). Comparisons of the current results with earlier labeling experiments on soybeans for 6 min[17] indicate that with 200-ppm $^{13}CO_2$ labeling conditions approximately 10 times as much label accumulates in the leaf after 60 min. That is, most of the ^{13}C label assimilated by photosynthesis is retained in (not exported from) the soybean leaf after 1 h.

Cheatgrass under Low and High $^{13}CO_2$ Concentrations. At 200-ppm $^{13}CO_2$, the photorespiratory oxygenase pathway of Rubisco accounts for about 30% of the total carbon assimilation of a C_3 leaf.[17] The oxygenase pathway involves O_2 as a cosubstrate with ribulose bisphosphate for Rubisco.[5] This pathway produces glyoxylate in chloroplasts which is converted to glycolate and then glycine in peroxisomes. Two glycines either condense in mitochondria to a single serine (which is subsequently converted to phosphoglycerate and returned to the Calvin cycle) with the release of photorespiratory CO_2[5] or the glycines enter leaf biosynthesis intact.[16,17] The latter pathway in cheatgrass leaves labeled by 200-ppm $^{13}CO_2$ leads to the labeled glycine carboxyl and glycyl peptide carbonyl-carbon peaks (170−176 ppm, and their spinning sidebands, 120−130 ppm), and labeled glycyl and glycine methylene-carbon peaks (42 ppm) observed in Figure 4 (parallel arrows, bottom left). The total integrated intensity of these peaks is about 30% of that of the sucrose from the carboxylase pathway (60−100 ppm), as expected for spectra in which natural-abundance ^{13}C signals have been subtracted and only signals from ^{13}C labels appear. Most of the α-carbon intensity (50 ppm), as well as some of the carbonyl-carbon intensity (172−180 ppm), may arise from the carboxylase pathway for both 200- and 600-ppm $^{13}CO_2$ labeling conditions.

At 600-ppm $^{13}CO_2$, the products in the leaf in the form of glycine and glycyl compounds are reduced by approximately 10%, while that of the carboxylase pathway increases by more than a factor of 2 (Figure 4, right). These variations are due to the change in relative concentrations of the competitive CO_2 and O_2 substrates at the Rubisco active site.[31] The REDOR differences (Figure 4, middle and top) show a minor dependence on the $^{13}CO_2$ concentration. For example, the C_α ΔS (55 ppm) is larger than the carbonyl-carbon ΔS (172−175 ppm) for 600-ppm but not 200-ppm $^{13}CO_2$ labeling (Figure 4, middle). This result indicates an accumulation of labeled free amino acids (including glycine) at the higher labeling concentration.

The top REDOR difference is from a frequency-selective $^{13}C\{^{15}\underline{N}\}$ experiment. The dephasing ^{15}N frequency was δ_N 85, which is at the high-field side of the peptide amide-nitrogen peak.[16] This shift position is primarily but not exclusively due

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to glycyl peptide nitrogens.[32] (The ^{15}N chemical shift scale we are using is relative to solid ammonium sulfate. To convert to the more conventional liquid-ammonia scale used in solution-state NMR, add 20 ppm.) About one-third to one-half of the high-field methylene-carbon intensity at 42 ppm in Figure 4 (bottom) is due to glycyl peptide with the remainder due to free glycine. These estimates are based on differences in the two types of REDOR ΔS's in Figure 4 (middle and top) for the spectra of both 200-ppm and 600-ppm $^{13}CO_2$ labeling conditions. The nitrogen of free glycine has δ_N 12 and so is excluded from contributing to $^{13}C\{^{15}\underline{N}\}$ dephasing by the DANTE frequency selection.

The low-field carbonyl-carbon REDOR difference arises exclusively from peptide linkages (the $^{13}C{=}O$ and ^{15}N of free glycine are two bonds apart and do not recouple significantly for short REDOR evolution periods).[19] From the sizable $^{13}C\{^{15}\underline{N}\}$ ΔS's (Figure 4, top), we see that much of the carbonyl ^{13}C label in protein is adjacent to ^{15}N in glycyl residues, particularly for the 600-ppm $^{13}CO_2$ labeling. In addition, most of this labeled peptide carbonyl intensity has a chemical shift of less than 172 ppm; that is, to the right of the dotted lines in Figure 4, a carbon chemical-shift region primarily due to glycyl residues in α-helices and β-strands.[33] This carbonyl-carbon label is therefore primarily in a glycyl residue and is linked to an ^{15}N peptide nitrogen also in a glycyl residue, $-NHCH_2{}^{13}C(=O)$ $^{15}NHCH_2C(=O)-$, Gly-Gly.

We conclude that cheatgrass is routing much of the product of its oxygenase pathway to proteins (or protein precursors) rich in Gly-Gly sequences. Such sequences occur mainly in glycine-rich proteins (GRPs) having up to 70% glycyl residues that are structural components of plant cell walls.[11] By contrast, for example, the Gly-Gly peptide-bond concentration in Rubisco is less than 1%.[34] Detection of GRP in situ by conventional methods is difficult, but antibody stains have been used to show that GRP is cross-linked to lignins, particularly in the xylem.[13] At 600-ppm $^{13}CO_2$, the label in the GRP represents approximately 2% of the total assimilated carbon label retained in the cheatgrass leaf (Figure 4, right, comparison of two times the 42-ppm glycyl methylene-carbon peak, top, and the 72-ppm main carbohydrate peak, bottom). This estimate assumes an average ^{15}N isotopic enrichment of 50% (cf. above). Production of high concentrations of cell-wall cross-linked GRP by cheatgrass is consistent with the observation of ^{13}C label in other cell-wall components including diphenolics (Figure 3, left) and may be a property of a still developing young leaf.

Differences in Cheatgrass and Soybean Carbon Assimilation. We highlight the difference in carbon assimilation between cheatgrass and soybean as a function of external-CO_2 concentration using REDOR ΔS's with and without ^{15}N frequency selection (Figure 5). The dotted lines are near δ_C 172, just as in Figure 4. The routing of label for cheatgrass and soybean is roughly similar at 200-ppm $^{13}CO_2$, although there is significantly more signal intensity to the right of the dotted line for cheatgrass (more Gly-Gly sequences). However, the routing of label is totally different at 600-ppm $^{13}CO_2$. Cheatgrass continues to make GRP in a high-CO_2 environment whereas soybean does not.

Soybean routes carbon from the oxygenase pathway of Rubisco along three pathways:[16,17] (i) to phosphoglycerate via glycine and serine in support of photosynthesis: (ii) directly to protein; and (iii) to single-carbon insertions and CO_2 via total decarboxylation of glycine.[35] The selection in the leaf between these three options depends on environmental conditions and is made on a time scale of less than 2 min.[17] This means that at least partial control must be as fast-acting as, for example, a glycine-dependent riboswitch[14,15] and not some slower gene-activation process.[36,37] Consistent with this hypothesis, the deposition of the major product of photosynthesis as starch would facilitate comparison within soybean leaves of rapidly changing levels of glycine and glycerate to signal internal-CO_2 concentration and hence water status.

In the field, low internal-CO_2 conditions within the leaf result from water stress and increased stomatal resistance to gas exchange. The plants in our experiments were not water stressed and had open stomata, as evidenced by active transpiration and fogging of the labeling-chamber windows when the gas flow was reduced. A low internal-CO_2 concentration was achieved by low external $^{13}CO_2$-labeling conditions. In this situation, soybean routes some glycine from the oxygenase pathway directly to protein production including Gly-Gly sequences (Figure 5, top right, black). However, under high-CO_2 conditions, no Gly-Gly sequences are produced (Figure 5, top right, red) as soybean routes most of the glycine from the oxygenase pathway to phosphoglycerate and the Calvin cycle[15,16] (Figure 3, right). We suspect that the soybean control mechanism that selects between pathway options i−iii is not optimized for 600-ppm CO_2 conditions but rather for the approximately 275-ppm

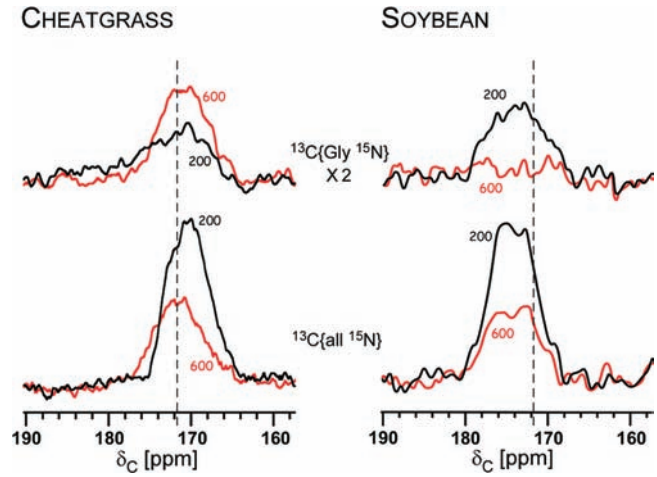

Figure 5. 125-MHz $^{13}C\{^{15}N\}$ REDOR (bottom) and frequency-selective $^{13}C\{^{15}\underline{N}\}$ REDOR (top) carbonyl-carbon difference spectra (ΔS) of ^{15}N-fertilizer labeled leaves of cheatgrass (left) and soybean (right) exposed for one hour to 200-ppm $^{13}CO_2$ (black) and 600-ppm $^{13}CO_2$ (red). The selected ^{15}N dephasing frequency (top spectra) was at δ_N 85 which is primarily but not exclusively due to glycyl residues in proteins. The spectra have been scaled as described in the caption to Figure 3. Contributions from the natural-abundance background have been removed by subtraction. Only directly bonded $^{13}C-^{15}N$ pairs were detected by a short dipolar evolution period. The four cheatgrass spectra are from Figure 4. The soybean difference spectra at the bottom of the figure were the result of the accumulation of 50188 scans (black) and 17444 scans (red) and at the top of the figure 113920 scans (black) and 131072 scans (red). Magic-angle spinning was at 7143 Hz for the REDOR spectra and 6250 Hz for the frequency-selected REDOR spectra.

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CO_2 and high-water environment that was common for soybean growth over the last half-million years.[38]

Carbon assimilation in cheatgrass bypasses a CO_2-concentration-dependent control involving either a riboswitch or a stomatal response (or the equivalent). The oxygenase pathway maintains full production of GRP under both high- and low-CO_2 environments (Figures 4 and 5). Presumably, the evolution of the ability of cheatgrass to thrive in arid climates has dictated GRP-stregthened cell walls for optimal water conservation and transport. We believe that the high-CO_2 environments expected in the near future, together with periodic episodes of water stress, will combine to result in a decided growth advantage for C_3 plants like cheatgrass over C_3 crop plants like soybean.[2]

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