Supplementary Information

for

Formation of Char-like, Fused-ring Aromatic Structures from a Nonpyrogenic Pathway During Decomposition of Wheat Straw

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Figure S1. Plot of mass remaining in visible straw residues at selected sampling times under the aerobic (black squares) and anaerobic (gray squares) conditions. Fitted lines: wheat straw decomposition under aerobic (black dashed line) and anaerobic (gray dashed line) conditions.



Figure S2. ¹³C NMR with spectral editing for identification of functional groups in fresh wheat straw, wheat straw residues after 360day decomposition under (Ae) aerobic and (An) anaerobic conditions: (a-c) unselective cross polarization/total side band suppression (CP/TOSS) spectra for reference, with a contact time of 1 ms at a spinning speed of 5 kHz; (d-f) corresponding dipolar dephased CP/TOSS spectra showing nonprotonated carbons and carbons of mobile segments such as CH₃ acquired after 40 µs of decoupling gated off; (g-i) selection of sp³-hybridized carbon signals by a ¹³C chemical shift anisotropy (CSA) filter, which in particular identifies O-C-O carbons near 105 ppm, which are typical of sugar rings, with CSA filter time of 35 µs, other parameters as in CP/TOSS; (j-l) selection of nonprotonated or mobile sp³ – hybridized C signals with a 35-µs CSA filter and 40-µs dipolar dephasing, which in particular identifies O-C_q-O carbons near 105 ppm; (m-o) corresponding selection of protonated sp³-hybridized C signals with a 35-µs CSA filter and short 50-µs cross polarization (CP), which identifies O-CH-O carbons near 105 ppm; and (p-r) selection of relatively immobile CH and CH₂ signals with small residual CH₃, which is achieved by the difference between a short-CP spectrum and a spectrum of short CP combined with dipolar dephasing. All the spectra were normalized based on the highest peak. Recycle delays for all the spectra were 1 s.



Figure S3. Comparison of DP/MAS ¹³C NMR spectra after 68-µs dipolar dephasing (DD) of fresh wheat straw (dashed red line) and wheat straw following (An) anaerobic incubation for 360 days (solid line), scaled vertically to match.



Figure S4. Exchange with protonated and nonprotonated spectral editing (EXPANSE) NMR spectra (a, d) and dipolar-dephased doublequantum/single-quantum (DQ/SQ) NMR spectra (b, e) of ¹³C-labeled fresh wheat straw (a, b) and degraded wheat straw after 360-day decomposition under aerobic conditions (d, e). (c) Proposed aromatic structures in the fresh wheat straw. (f) Proposed aromatic structure in the degraded wheat straw. Numerals were used to indicate the C-C bond.



Figure S5. Dipolar-dephased DQ/SQ spectra of degraded wheat straw after 360-day incubation under anaerobic conditions.

Table S1. Basic soil properties at the beginning of the experiment.

рН	Organic C	Total N	Extractable (Olsen) P	Extractable K	Sand (2-0.05 mm)	Silt (0.05-0.002 mm)	Clay (<0.002 mm)
6.15	7.4 (g kg ⁻¹)	0.53 (g kg ⁻¹)	3.3 (mg kg ⁻¹)	152 (mg kg ⁻¹)	150 (g kg ⁻¹)	590 (g kg ⁻¹)	260 (g kg ⁻¹)

Note: Soil pH was determined in 1:2.5 (w/v) soil-to-water ratio extracts;

Soil organic carbon (SOC) was determined by the Walkley-Black method;¹

Total N (TN) was determined by the Kjeldahl method;²

Olsen P was extracted by 0.5 M sodium bicarbonate (pH 8.5) and determined using the Mo-Sb colorimetry method;³

The exchangeable K was extracted by 1.0 M ammonium acetate (pH 7.0) and determined by flame photometry;⁴

The particle size distribution was determined by the hydrometer method following dispersion of the particles with a chemical dispersant.⁵

	220-188	188-161	-	161-140	140)-93	120-93	-	93-66			66-45	5	45-0	
	Ketone/	COO/		Arc	omatic C	ıtic C			O-alkyl C		OCH ₂ /OCH ₃				
Sample	aldehyde	N-C=O	Total	Arom C-O	Arom C _{non}	Arom C-H	Anomeric C	Total	OC _q	OCH	Total	OCH ₂	OCH ₃	Alkyl C	Aromaticity
Fresh straw	0.5	3.5	17.4	6.3	5.5	5.6	14.0	45.9	2.7	43.2	15.3	4.1	11.2	3.4	17.4
Ae-30d	0.7	3.7	20.3	7.0	6.7	6.6	12.4	40.3	1.8	38.5	17.6	4.3	13.3	5.0	20.4
Ae-60d	0.6	3.7	21.2	7.2	7.1	6.9	12.3	39.9	1.9	38.0	17.5	4.3	13.2	4.8	21.2
Ae-90d	0.6	3.8	22.3	7.7	7.3	7.3	12.7	41.1	1.9	39.2	15.3	4.4	10.9	4.2	22.3
Ae-180d	1.0	4.6	23.2	8.1	8.1	7.0	11.9	37.2	2.3	34.9	16.4	4.4	12.0	5.7	23.2
Ae-360d	1.2	7.5	31.1	10.1	11.3	9.7	8.6	28.5	2.9	25.6	14.8	4.9	9.9	8.3	31.1
An-30d	0.7	3.1	17.2	6.2	5.2	5.8	13.7	43.8	1.5	42.3	17.5	3.9	13.6	4.0	17.2
An-60d	0.7	2.6	16.5	6.2	5.0	5.3	14.7	44.6	1.8	42.8	17.7	3.7	14.0	3.1	16.6
An-90d	0.4	2.8	17.7	6.3	5.3	6.1	14.1	44.8	2.1	42.7	16.7	3.9	12.8	3.4	17.7
An-180d	0.7	2.9	15.6	6.0	4.7	4.9	14.6	44.4	1.6	42.8	17.8	3.7	14.1	4.0	15.6
An-360d	0.8	3.0	18.9	6.6	5.9	6.4	13.1	43.0	2.4	40.6	16.1	3.9	12.2	5.1	18.9

Table S2. Relative proportions (%) of C functional groups in fresh wheat straw and wheat straw residues derived from the integration of ¹³C DP/MAS and ¹³C DP/MAS with DD NMR spectra. The ppm ranges for each carbon form are shown.

Сгор	Grain Production Mg year (10 ⁶ tons) (FAO, 2016 ⁶)	Straw-to-grain ratio (Lal, 2005 ⁷ ; Kim & Dale, 2004 ⁸ Gelaw et al., 2014 ⁹)	Shoot-to-root ratio (Bolinder et al., 1997 ¹⁰ ; Dubey et al., 2009 ¹¹ ; Gelaw et al., 2014 ⁹)	Straw residue production (10 ⁶ tons)	Root residue production (10 ⁶ tons)			
		Cereal						
Barley (Hondeum vulgare L.)	143.1	1.5	7.1	214.6	30.2			
Corn (Zea may L.)	1291.9	1	6.3	1291.9	205.1			
Millet	30.3	3	2.8	90.9	32.5			
Oat	23.4	1	2.5	23.4	9.4			
Rice (Oryza sativa L.)	952.1	1.5	5.7	1428.2	250.6			
Rye (Secale cereale L.)	13.4	1.5	5.0	20.1	4.0			
Sorghum (Sorghum bicolor L.)	66.3	1.5	5.9	99.4	16.9			
Wheat (Triticum aestivum)	881.1	1.5 7.4		1321.7	178.6			
Others (e.g. Triticale)	25.6	1.3-1.7	4.4-5.4	33.3	6.2			
		Legumes						
Bean	28.0	1.5	5.2	42.0	8.1			
Board bean	6.1	1.5	5.2	9.2	1.8			
Chink pea	12.1	0.2	2.3	2.4	1.1			
Lentil	6.4	1.0	1.5	6.4	4.3			
Pea	15.6	1.0	3.0	15.6	5.2			
Pulses	4.1	1.0	2.5	4.1	1.6			
Soybean	346.8	1.0	5.2	346.8	66.7			
Others (e.g. cow peas)	7.8	1.0	2.1-3.3	7.8	2.4			
		Oil crops						
Rapeseed	84.1	1.5	4.1	126.2	30.8			
Seed cotton	81.4	1.5	3.0	122.1	40.7			
Sunflower	49.9	2.6	5.7	129.7	22.8			
Others	6.8	2.5	3.8	17.0	4.5			
		Sugar crop						
Sugar beet	285.3	0.25	5.6	71.3	12.7			
Sugarcane	2013.7	0.25	2.8	503.4	179.8			
Others	1.0	0.25	2.8	0.3	0.1			
Fiber								
Fibre crops	1.1	1.9	2.1	2.1	1.0			
		Tuber						
Cassava	281.9	0.25	-	70.5	-			
Sweet potato	176.0	0.25	-	44.0	-			

Table S3. The world crop straw and root productions in 2016.

Potato	475.9	0.25	-	119.0	-		
Others (e.g. fibre crops nes)	76.4	0.25	-	19.1	-		
Total crop production	6182.4	1116.7					
Upland crop production	4754 3	866 2					
(subtract rice production from the total production)							

"-" means no date.

Table S4. Lignin contents of shoot and root tissues for different crops.

Сгор	Lignin of shoots (%)	Lignin of roots (%)	References
Wheat	3.5-8.6	9.5-12.6	El Hawary et al., 2015 ¹² ; Rasse et al., 2005 ¹³
Corn	3.5-6.6	8.3-9.5	Youssef et al., 2018 ¹⁴ ; Rasse et al., 2005 ¹³
Cotton	4	8.3	El Hawary et al., 2015^{12}
Soybean	9.1	24.7	Youssef et al., 2018 ¹⁴
Legume	7.4-10.2;8.8	17.3-24.0	Tian and Kang, 1998 ¹⁵
Ryegrass	4-6	9-15	Lorenz and Rattan, 2005 ¹⁶ ; de Neergaard et al., 2001 ¹⁷
Mean	6.3	14.3	

Supplementary Methods

S.1 ¹³C cross-polarization/total sideband suppression (CP/TOSS) with or without dipolar dephasing (DD)

Because ¹³C CP/TOSS spectra can be easily and quickly acquired, the first step for our advanced NMR characterization of wheat straw samples was to acquire ¹³C CP/TOSS spectra of all the samples as the first approximations of their chemical structures (Supplementary Figure 3). All the samples were run in a double resonance probe head using 4-mm sample rotors at a spinning speed of 5 kHz, with a CP time of 1 ms, 90° pulse length of 4 µs for ¹H, and a recycle delay of 1 s. Four-pulse total suppression of sidebands was applied prior to detection, and two-pulse phase-modulated decoupling was used for optimum resolution. In addition, CP/TOSS with 40-µs dipolar dephasing (DD) was used to generate a sub-spectrum with nonprotonated C and mobile groups [e.g., OCH₃, CCH₃, and -(CH₂)_n-].

S.2 High-speed quantitative ¹³C direct polarization / magic angle spinning NMR (DP/MAS) and DP/MAS with dipolar dephasing (DP/MAS with DD)

Quantitative ¹³C DP/MAS spectra and DP/MAS spectra after recoupled 68- μ s dipolar dephasing were recorded at a spinning speed of 14 kHz, with a 90° ¹³C pulse length of 4 μ s. Recycle delays of 100 s were used, after tests by the cross polarization/spin lattice relaxation time/total side band suppression (CP/T₁ -TOSS) technique, which shows that all C sites were relaxed by >95%. The details of these techniques have been described elsewhere.¹⁸

S.3 ¹³C chemical-shift-anisotropy (CSA) with or without dipolar dephasing

To separate the signals of anomeric C from those of aromatic C, both of which may resonate between 120 and 90 ppm, the aromatic C signals were selectively suppressed by a five-pulse ¹³C CSA filter with a CSA filter time of 35 µs. To further select the signals of nonprotonated anomeric C, this CSA filter was combined with a dipolar dephasing time of 40 µs. In a complementary experiment, selected spectra of protonated anomerics (O-CH-O, acetals) were obtained by CSA filtering after short CP.

S.4 Spectral editing of immobile CH₂ and CH.

The combined spectrum of these chemical groups was obtained with good sensitivity in a simple spectral-editing experiment. First, a CP/ TOSS spectrum was recorded using a short CP of 40 μ s. It showed predominantly protonated C in immobile segments, but residual peaks of quaternary C resulted from two bond magnetization transfer. Second, a CP/TOSS spectrum was acquired using a short CP of 40- μ s dipolar dephasing. It contained only the residual signals of quaternary C or mobile segments (including CH₃ groups with >50% efficiency). This residual spectrum was then subtracted from the first CP/TOSS spectrum. The resulting difference spectrum represented immobile CH₂ and CH, with a small CH₃ contribution.

S.5 The estimate of char-like carbon derived from wheat-straw decomposition.

S.5.1 The amount of nonpyrogenic BC derived from wheat straw after one-year decomposition was calculated by Eq. 1.

[1]

$$C_{wheat} = \frac{M_r \times C_s \times \chi_{arom} \times \chi_{fused}}{M_0 \times T} \times 1000$$

C_{wheat} (g/kg): the amount of nonpyrogenic fused-ring aromatic C derived from wheat straw decomposition

Mr (g): final mass of wheat straw residue after 360 days decomposition

 $M_0(g)$: mass of fresh wheat straw

- Cs: the fraction of carbon in wheat straw residue (%)
- χ_{arom}: aromatic C (93-161 ppm) / total C (0-220 ppm)

 χ_{fused} : fused ring aromatic C [(integral of 107 and 142 ppm signals in DP/TOSS spectra with t_{deph} =0.86 ms) / (integral of 107 and 142 ppm signals in DP/TOSS spectra with t_{deph} =0) – 3%]. Note: 3% was obtained from the NMR result of fresh wheat straw.

T: correction coefficient for regular T_2 relaxation (0.59)

S.5.2 The amount of nonpyrogenic BC derived from crop shoot and root decomposition worldwide was estimated by Eq. 2.

 $C_{nonpyrogenic} = (\sum M_{shoot} \times C_1 \times P) + (\sum M_{root} \times C_2)$

Cnonpyrogenic: the amount of nonpyrogenic fused-ring aromatic C derived from decomposition of crop residues

 $\sum M_{shoot}$: upland crop straw residues (4.8×10¹² kg or 4.8 Pg) of crop residues (aboveground biomass), such as wheat straw, maize stover, rape stalks, cotton stalks and so on, annually produced worldwide (based on estimates of FAO, 2016; Supplementary Table S3).

[2]

- C₁: the amount of fused-ring aromatic C derived from one kg of crop residues (aboveground biomass) after one year of aerobic degradation, $C_1 \approx C_{wheat}$. As mentioned in Eq.1, C_1 was positively related to χ_{arom} (or content of lignin) of crop straw. Normally, the concentrations of lignin in different crops straw are as follow: lignin _{rice} < lignin _{rape} < lignin _{wheat}, and lignin _{maize} < lignin _{cotton} < lignin _{soybean} (ref^{14,19}), and hence we estimated that the χ_{arom} of all crop straw was approximately equal to the χ_{arom} of wheat straw. Similarly, we assumed C₁ was approximately equal to C_{wheat}.
- ∑ M_{root}: total amount (0.9×10¹² kg or 0.9 billion tons) of upland crop straw roots (belowground biomass), such as wheat straw, maize stover, rape stalks, cotton stalks and so on, annually produced worldwide (based on estimates of FAO, 2016; Supplementary Table S3).
- C₂: the amount of fused-ring aromatic C derived from one kilogram of crop roots after one year of aerobic degradation, $C_2 \approx C_1 \times (14.3 \div 6.3)$. (Lignin content of roots for different crops obtained by the previous studies was on average more than double that of shoots (see Supplementary Table S4), and hence we estimated $C_2 \approx C_1 \times (14.3 \div 6.3)$.)
- P: the assumed percentage of crop residues incorporated into the soil (70%).^{20,21}

Supplementary References

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