Supplemental Materials for: '**Physicochemical changes in pyrogenic organic matter (biochar) after 15 months field-aging'**

by Mukherjee et al.

Supplemental Table S1. Characteristics of soils used in this study.

Soil	latitude	longitude	Soil Series	% sand	% silt	% clay
BY soil[a]	34.4836	-84.3227	Edneyville loam	63	27	10
PR soil[b]	29.6833	-82.0333	Tavares or Candler series	95	3	2

[b] Plant Science Research and Education Unit, Citra, FL. This site had not been fertilized or grazed for the past 10 yr. Soils were deep and extremely well-drained fine sands of the Tavares or Candler series (sandy hyperthermic, uncoated Typic Quartzipsamments)

Supplemental Table S2. Elemental composition of fresh and aged biochars and soil/biochar mixtures.

Biochar or Soil/Biochar	C[a]	N[a]	H[a]	O[b]	P[c]	K[c]	Mg[c]	Ca[c]	S[c]	B[c]	Zn[c]	Mn[c]	Fe[c]	Cu[c]	O/C
	(mg g^{-1})														
Fresh Oak-250	626 ± 32	1.9 ± 0.3	31 ± 0.4	342 ± 32	0.4	3.4	0.6	7.1	0.2	0.00	0.0	0.1	0.2	0.00	0.8
Fresh Oak-400	679 ± 57	3.7 ± 0.7	42 ± 0.7	276 ± 57	1.3	6.4	1.1	11.8	0.2	0.01	0.0	0.2	0.0	0.00	0.4
Fresh Oak-650	754 ± 14	4.6 ± 0.4	28 ± 1.2	213 ± 15	0.9	6.3	0.5	10.3	0.1	0.00	0.0	0.1	0.0	0.00	0.2
Fresh Pine-250	624 ± 4	0.0 ± 0.0	26 ± 0.7	350 ± 5	0.1	0.6	0.5	2.4	0.1	0.00	0.0	0.1	0.0	0.01	0.7
Fresh Pine-400	758 ± 7	0.7 ± 0.9	37 ± 0.4	204 ± 7	0.1	1.0	0.7	4.7	0.2	0.00	0.0	0.1	0.0	0.00	0.4
Fresh Pine-650	552 ± 0	0.0 ± 0.0	33 ± 0.4	416 ± 8	0.1	0.5	0.2	2.7	0.1	0.00	0.0	0.1	0.0	0.00	0.2
Fresh Grass-250	494 ± 31	12 ± 2	36 ± 1.0	458 ± 31	1.4	5.0	3.0	8.2	0.6	0.00	0.1	0.1	0.1	0.01	0.8
Fresh Grass-400	523 ± 4	14 ± 0.2	46 ± 0.1	417 ± 4	4.2	15.3	3.7	9.9	0.6	0.01	0.2	0.2	0.1	0.01	0.6
Fresh Grass-650	557 ± 5	5.7 ± 0.4	30 ± 1.0	408 ± 6	3.3	7.9	5.8	16.9	0.6	0.01	0.2	0.3	0.2	0.01	0.5
Aged Oak-250	594 ± 22	2.5 ± 0.3	47 ± 6.2	343 ± 20	0.1	1.8	0.7	8.2	0.2	0.00	0.0	0.1	0.0	0.01	0.6
Aged Oak-400	710 ± 19	3.0 ± 0.7	50 ± 0.6	222 ± 3	nm	nm	nm	nm	nm	nm	nm	nm	nm	nm	0.3
Aged Oak-650	813 ± 2	2.8 ± 1.2	76 ± 7.8	304 ± 32	0.8	0.9	0.5	11.6	0.1	0.00	0.0	0.1	0.0	0.01	0.4
Aged Pine-250	560 ± 53	1.2 ± 0.6	56 ± 4.2	429 ± 5	nm	nm	nm	nm	nm	nm	nm	nm	nm	nm	0.8
Aged Pine-400	634 ± 72	1.4 ± 0.4	46 ± 38	379 ± 3	nm	nm	nm	nm	nm	nm	nm	nm	nm	nm	0.6
Aged Pine-650	780 ± 101	0.8 ± 0.8	90 ± 21	539 ± 18	nm	nm	nm	nm	nm	nm	nm	nm	nm	nm	0.7
Aged Grass-250	575 ± 20	9.8 ± 1.2	43 ± 0.8	379 ± 16	0.2	1.4	2.9	12.5	0.3	0.00	0.2	0.2	0.2	0.02	0.7
Aged Grass-400	635 ± 8	15 ± 2.1	27 ± 34	319 ± 46	nm	nm	nm	nm	nm	nm	nm	nm	nm	nm	0.5
Aged Grass-650	704 ± 24	15 ± 1.8	35 ± 1.6	238 ± 14	1.9	1.3	3.0	15.9	0.5	0.00	0.3	0.3	0.5	0.02	0.3

Supplemental Table S2. Continued.

Biochar or Soil/Biochar Mix	C[a]	N[a]	H[a]	O[b]	P[c]	K[c]	Mg[c]	Ca[c]	S[c]	B[c]	Zn[c]	Mn[c]	Fe[c]	Cu[c]	O/C
	(mg g^{-1})														
Aged BY	27 ± 4	2.2 ± 0.3	4.7 ± 0.7	966 ± 5	1.1	0.2	0.4	5.7	0.2	0.0	0.0	0.2	1.7	0.0	-
BY/Aged Oak-250	49 ± 6	3.1 ± 0.3	7.1 ± 0.1	947 ± 0	nm	nm	nm	nm	nm	nm	nm	nm	nm	nm	-
BY/Aged Oak-650	54 ± 4	3.4 ± 0.1	7.9 ± 0.3	935 ± 4	nm	nm	nm	nm	nm	nm	nm	nm	nm	nm	-
Aged PR	3.3 ± 3.8	0.2 ± 0.2	0.7 ± 0.1	991 ± 1	0.4	0.2	0.2	2.2	0.1	0.0	0.0	0.0	0.6	0.0	-
PR/Aged Oak-250	10 ± 2	0.4 ± 0.1	0.8 ± 0.1	987 ± 0	nm	nm	nm	nm	nm	nm	nm	nm	nm	nm	-
PR/Aged Oak-650	11 ± 0.0	0.5 ± 0.0	1.1 ± 0.1	993 ± 1	nm	nm	nm	nm	nm	nm	nm	nm	nm	nm	-

Notes/Abbreviations:

nm: not measured

BY: agricultural Entisol, Gainesville, Florida, USA

PR: forest Spodosol, Marion County, Florida, USA

a: measured via high temperature catalyzed combustion (CHN analyzer)

b:calculated from the following equation: % O = 100 - % (C+N+H)

c: analyzed on ICP after total digested acid extraction procedure (AOAC 985.01)

Note: The O concentration was calculated by weight difference, assuming biochars were consisted of C, H, N, and O

Supplemental Table S3. Physicochemical characteristics of fresh and aged biochars (average, n =3).

Biochar or Soil/Biochar Mix	pH	VM	AC	SA ($m^2 g^{-1}$)		CA	PA	TA	CEC	AEC
		(%)		CO_2	N_2	(mmol g^{-1})			($cmol_c$ kg^{-1})	
Fresh Oak-250	3.5 ± 0.0	66.0 ± 4.4	1.4 ± 0.1	275 ± 89	1.2 ± 0.8	5.0 ± 0.1	3.2 ± 0.7	8.1 ± 2.4	35.6 ± 6.1	0.0
Fresh Oak-400	6.7 ± 0.2	51.9 ± 5.2	2.6 ± 0.2	250 ± 74	2.0 ± 1.2	3.9 ± 0.3	2.4 ± 0.3	6.0 ± 0.2	10.7 ± 2.3	0.0
Fresh Oak-650	9.1 ± 0.0	36.4 ± 1.1	3.7 ± 0.2	533 ± 42	226 ± 7.0	3.9 ± 0.1	1.4 ± 0.1	4.7 ± 0.1	8.9 ± 1.8	0.0
Fresh Pine-250	3.1 ± 0.1	61.1 ± 1.6	0.3 ± 0.1	335 ± 112	0.3 ± 0	5.7 ± 0.1	0.4 ± 0.4	7.2 ± 0.1	45.2 ± 2.1	0.0
Fresh Pine-400	5.1 ± 0.1	58.6 ± 1.0	0.5 ± 0.2	329 ± 122	3.1 ± 1.5	4.9 ± 0.1	1.2 ± 0.2	6.1 ± 0.1	12.4 ± 1.1	0.0
Fresh Pine-650	6.8 ± 0.1	25.2 ± 4.7	1.1 ± 0.1	604 ± 65	254 ± 104	4.4 ± 0.3	0.1± 0.0	4.4 ± 0.0	10.4 ± 2.2	0.0
Fresh Grass-250	4.5 ± 0.0	62.5 ± 2.9	6.8 ± 0.2	222 ± 75	3.4 ± 1.6	6.2 ± 0.0	1.9 ± 0.2	8.0 ± 0.1	58.2 ± 15.5	0.0
Fresh Grass-400	7.8 ± 0.0	51.4 ± 6.4	13.2 ± 0.2	178 ± 42	6.0 ± 6.2	3.9 ± 0.3	1.9 ± 0.0	5.9 ± 0.4	18.2 ± 6.9	0.0
Fresh Grass-650	10.0 ± 0.0	33.0 ± 1.2	15.9 ± 0.5	433 ± 82	73 ± 39	3.9 ± 0.1	1.1 ± 0.1	4.7 ± 0.0	35.9 ± 6.9	0.0
Aged Oak-250	4.00 ± 0.0	66.1 ± 0.9	2.0 ± 0.3	208 ± 17	0.6 ± 0.0	1.9 ± 0.3	3.8 ± 0.3	4.7 ± 0.3	135.6 ± 3.7	94.3 ± 72.5
Aged Oak-400	5.60 ± 0.0	50.2 ± 1.5	2.2 ± 0.2	283 ± 20	0.7 ± 0.0	nm	nm	nm	nm	nm
Aged Oak-650	6.7 ± 0.3	29.4 ± 4.3	2.9 ± 0.8	556 ± 10	35 ± 19	1.1 ± 0.5	0.8 ± 0.0	0.8 ± 0.3	110.6 ± 25.3	51.2 ± 13.3
Aged Pine-250	3.70 ± 0.0	63.7 ± 2.3	1.1 ± 0.5	249 ± 2	0.5 ± 0.1	nm	nm	nm	nm	nm
Aged Pine-400	5.00 ± 0.0	63.2 ± 2.5	1.1 ± 0.5	222 ± 14	1.7 ± 0.9	nm	nm	nm	nm	nm
Aged Pine-650	5.40 ± 0.0	24.7 ± 1.4	0.3 ± 0.2	577 ± 10	0.5 ± 0.1	nm	nm	nm	nm	nm
Aged Grass-250	4.20 ± 0.0	65.2 ± 1.4	7.7 ± 0.1	238 ± 17	4.6 ± 2.2	2.2 ± 0.0	2.5 ± 0.7	4.2 ± 0.3	205.8 ± 84.3	75.3 ± 33.8
Aged Grass-400	6.00 ± 0.0	53.1 ± 2.4	8.2 ± 0.5	237 ± 2	1.8 ± 0.1	nm	nm	nm	nm	nm
Aged Grass-650	7.00 ± 0.0	41.5 ± 3.1	10.0 ± 0.3	517 ± 9	39 ± 8	0.8 ± 0.5	1.2 ± 0.5	1.2 ± 0.3	238.2 ± 22.7	106.1 ± 14.8

Notes/Abbreviations: VM = volatile matter, AC = ash content, SA = surface area as measured by CO_2 and N_2 sorptometry, respectively. CA = carboxylic acidity, PA = phenolic acidity, and TA = total acidity are surface acid functional groups as measured by Boehm titration (Boehm, 1964; Goertzen et al., 2010), CEC and AEC = cation and anion exchange capacity, respectively, measured at pH 6-7, nm = not measured

Supplemental Table S4. ANOVA test (*p* values) for differences between means of biochar parameters for each treatment type; parent biomass (oak, pine and grass), condition (fresh and aged), and highest treatment temperature (HTT: 250, 400 and 650 ^{o}C) and their interactive effects.

Sources	DF	Analyses								
		C%	O/C	VM	Ash	N_2-SA	CO_2-SA	pH	CEC[1]	AEC[2]
Biomass Type	2	0.095	0.213	0.682	0.001	0.676	0.024	0.001	0.018	0.227
Condition	1	0.198	0.548	0.216	0.130	0.050	0.297	0.001	na	na
Biomass. x Cond.	2	0.429	0.070	0.089	0.129	0.452	0.072	0.009	na	na
HTT	2	0.092	0.011	<0.001	0.069	0.020	<0.001	<0.001	0.004	0.500
Biomass x HTT	4	0.713	0.844	0.014	0.172	0.707	0.165	0.028	na	na
Cond. x HTT	2	0.214	0.269	0.803	0.181	0.045	0.369	0.001	na	na

Abbreviations: DF = Degree of Freedom, SA = Surface Area, VM = Volatile Matter, CEC = Cation Exchange Capacity at pH 7, AEC = Anion Exchange Capacity at pH 7, HTT = Highest Treatment Temperature

na: Not Applicable - Not enough data points to run ANOVA because those properties were only analyzed on four instead of nine aged biochar samples

1. n = 18
2. n = 4

Supplemental Table S5. Correlation coefficients (R^2) for linear relationships between various properties of aged biochars only. Underlined numbers are inverse correlations and bold numbers are significant at $p < 0.05$.

	R^2							
	N_2 SA[a]	CO_2 SA[a]	VM[a]	Ash [a]	pH [a]	CEC [b]	AEC [b]	O/C [a]
CO_2 SA[a]	0.44							
VM[a]	-0.26	-0.90						
AC[a]	0.21	0.00	0.00					
pH[a]	0.56	0.46	-0.52	0.19				
CEC[b]	0.07	0.01	0.01	0.72	0.08			
AEC[b]	0.18	0.07	-0.01	0.55	0.19	0.93		
O/C[a]	-0.37	-0.10	0.14	-0.15	-0.66	-0.14	-0.33	
C%[a]	0.28	0.76	-0.90	-0.02	0.59	-0.05	-0.00	-0.28

Notes/Abbreviations:
SA = surface area, VM = volatile matter, AC = ash content, CEC and AEC = cation and anion exchange capacity measured at pH 6-7.
a: n = 9
b: n = 4

Supplemental Table S6. Correlation coefficients (R^2) between various properties of fresh biochars only (n = 9), negative signs indicate negative correlations. The underlined values are significant at $p < 0.05$.

	R^2						
	N_2-SA [a]	CO_2-SA [a]	VM[a]	Ash [a]	pH [a]	CEC [a]	O/C [a]
CO_2-SA[a]	0.84						
VM[a]	-0.73	-0.67					
AC[a]	-0.01	-0.04	-0.08				
pH[a]	0.27	0.17	-0.63	0.43			
CEC[a]	-0.23	-0.18	0.24	0.06	-0.19		
O/C[a]	-0.61	-0.56	0.63	0.02	-0.38	0.71	
C%[a]	0.01	0.05	0.00	-0.29	0.00	-0.37	-0.22

Notes/Abbreviations:

SA = surface area, VM = volatile matter, AC = ash content, CEC = cation exchange capacity at pH 7

a: n = 9

Supplemental Table S7. Correlation coefficients (R^2) between various properties of biochars (pooled fresh and aged together, n = 18). Negative values indicate inverse correlations. The underlined values are significant at $p < 0.05$.

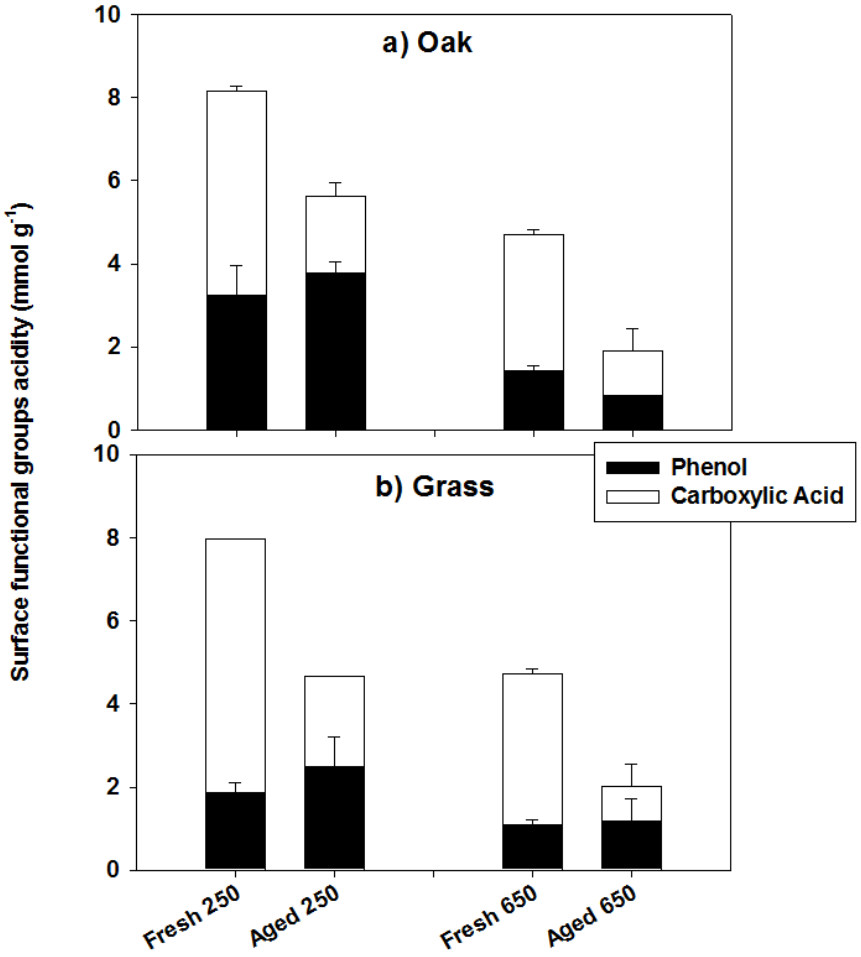
	R^2							
	N_2-SA[a]	CO_2-SA[a]	VM[a]	Ash [a]	pH [a]	CEC [a]	AEC [b]	O/C[a]
CO_2-SA[a]	0.92							
VM[a]	-0.83	-0.84						
AC[a]	-0.01	-0.00	-0.02					
pH[a]	0.34	0.33	-0.59	0.34				
CEC[a]	-0.00	0.00	0.01	0.32	0.02			
AEC[b]	0.02	0.03	-0.01	0.32	0.10	0.93		
O/C[a]	-0.36	-0.37	0.57	-0.01	-0.71	0.04	-0.00	
C%[a]	0.36	0.34	-0.30	-0.15	0.23	-0.12	-0.01	-0.68

Notes/Abbreviations:

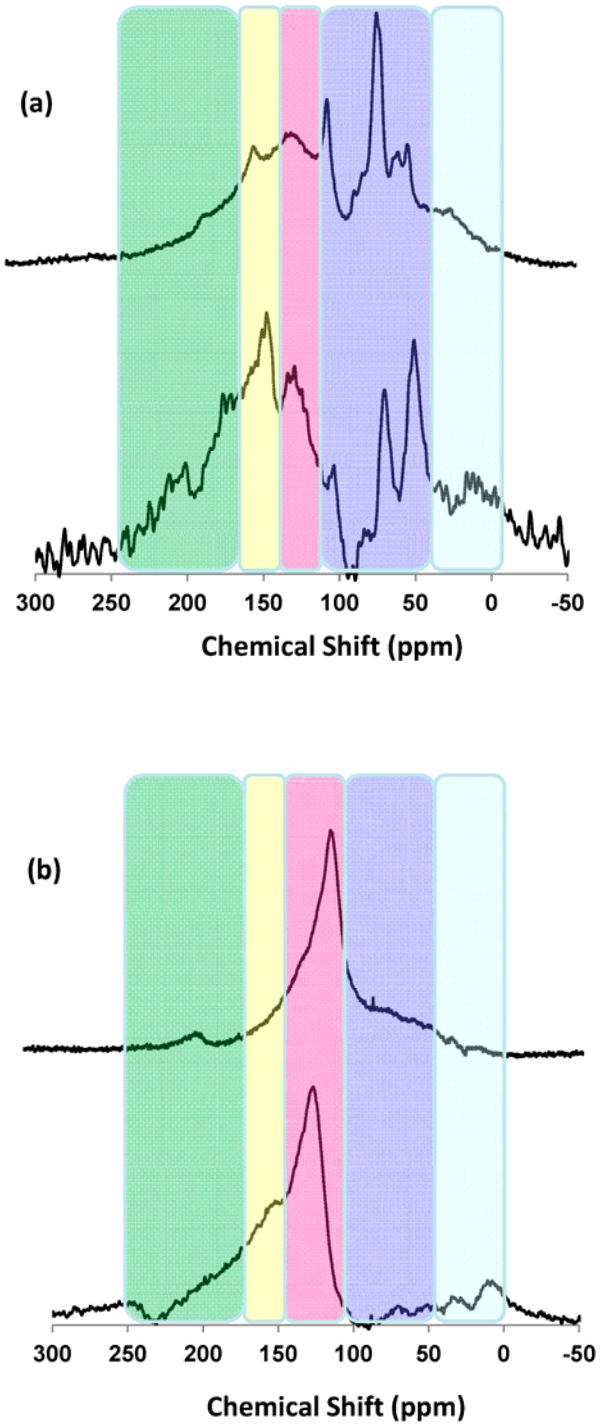
SA = surface area, VM = volatile matter, AC = ash content, CEC = cation exchange capacity at pH 7, AEC = anion exchange capacity at pH 7

a: n = 18

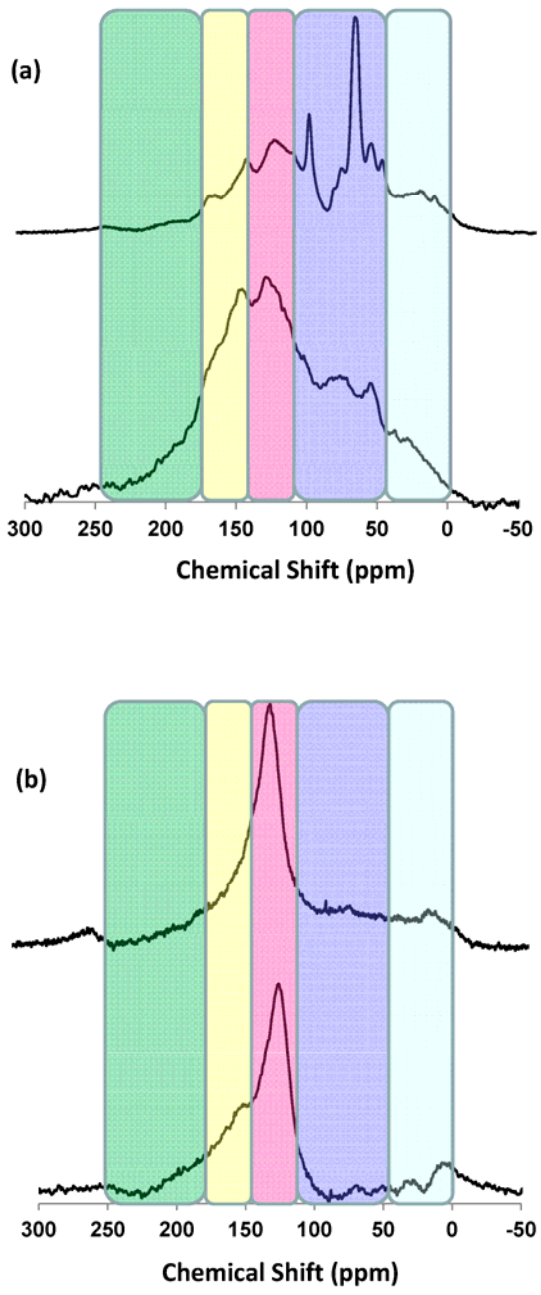
b: n = 4



Supplemental Figure S1. Comparison between the acidic surface functional group content of fresh and aged a) oak and b) grass biochars produced at 250 and 650 ^{o}C determined using the Boehm titration method (Boehm, 1964; Goertzen et al., 2010) as detailed in Mukherjee et al. (2011). Error bar shows the standard deviation of three replicate analyses. This method was recently called into question for use with biochar by a study that showed that the presence of inorganic basic and acidic components in biochar (e.g. carbonates, oxides, silica) that can interfere with the titration results. (Tsechansky and Graber, 2014) . Thus, the acidic surface functional group quantities may be invalid and are not presented in the main manuscript.



Supplemental Figure S2. ^{13}C CP-MAS NMR spectra of fresh (top) and aged (bottom) oak biochars prepared at (a) 250^{o} and (b) 650 ^{o}C. Shaded regions correspond to spectral regions representing functional groups as described in text.



Supplemental Figure S3. ^{13}C CP-MAS NMR spectra of fresh (top) and aged (bottom) grass biochars prepared at (a) 250^{o} and (b) 650 ^{o}C. Shaded regions correspond to spectral regions representing functional groups as described in text.

References for Supplemental Information Section

Boehm, H.P., Diehl, E., Heck, W., and Sappok, R., 1964. Surface oxides of carbon. Angewandte Chemie International Edition 3, 669-677.

Goertzen, S.L., Thériault, K.D., Oickle, A.M., Tarasuk, A.C., Andreas, H.A., 2010. Standardization of the Boehm titration. Part I. CO_2 expulsion and endpoint determination. Carbon 48, 1252-1261.

Mukherjee, A., Zimmerman, A.R., Harris, W.G., 2011. Surface chemistry variations among a series of laboratory-produced biochars. Geoderma, 163, 247-255.

Tsechansky, L., Graber, E.R., 2014. Methodological limitations to determining acidic groups at biochar surfaces via the Boehm titration. Carbon 66, 730-733.