

Table S1: Selected physicochemical characteristics of the soil and the biochars used in this study. WBC = wood-derived biochar; MBC = manure-derived biochar. All analyses were performed on a dry matter basis except for moisture, ash, fixed carbon, and volatile matter.

Parameter (unit)	Soil	WBC	MBC
pH	7.4	8.8	10.2
EC (µmho/cm)	245	477	13260
C	-	0.857	0.613
H	-	0.013	0.007
O	-	0.066	0.055
N (%)	0.003	0.003	0.010
P (%)	0.001	0.000	0.014
K (%)	0.014	0.003	0.017
Ca (%)	0.12	0.004	0.046
Mg (%)	0.009	0.001	0.019
S (ppm)	6.000	29.910	854.725
Na (%)	2.000	0.001	0.004
Fe (ppm)	1.67	904.310	3424.145
Zn (ppm)	0.20	31.940	119.500
Mn (ppm)	3.44	127.775	517.130
Cu (ppm)	0.10	13.210	46.375
B (ppm)	-	5.330	41.960
Moisture	-	0.056	0.533
Ash	-	0.058	0.148
Fixed Carbon	-	0.607	0.111
Volatile Matter	-	0.278	0.208
S BET (m ² /g)	-	419.040	7.050
O/C	-	0.058	0.069
H/C	-	0.188	0.138

Supplementary Table S2: Primers used in this study.

Gene target	Primer	Oligonucleotide sequence (5' – 3')	Reference
16S rRNA	515F-Y	GTGYCAGCMGCCGCGGTAA	Parada et al., 2016
	806RB	GGACTACNVGGGTWTCTAAT	Apprill et al., 2015

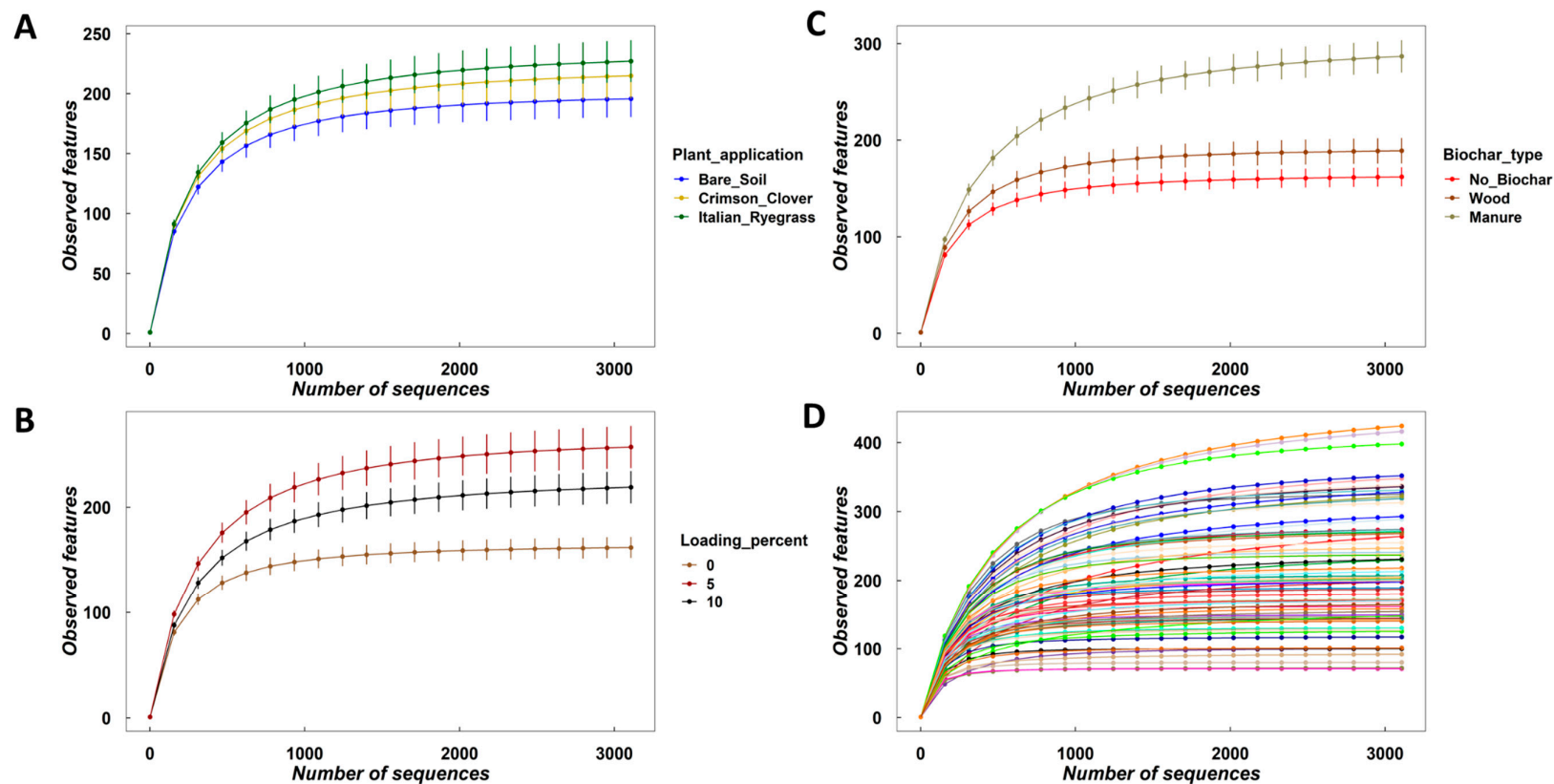
Supplementary Table S3: Alpha diversity metrics of prokaryote communities in soil. Values are mean ± SE. Different letters represent significant differences at $p < 0.05$ between treatments using Dunn’s pairwise comparison test.

Plant application	Biochar type	Loading percent	N	Richness		Diversity	
				Observed	Chao1	Shannon	Simpson
Soil	No Biochar	0	8	160.62 ± 17.74 de	163.53 ± 18.07 de	4.54 ± 0.18 a	0.98 ± 0.01 ab
	Wood	5	4	156.5 ± 4.29 def	156.58 ± 4.3 def	4.71 ± 0.07 ab	0.99 ± 0 ab
		10	4	205.5 ± 41.19 abdefg	208.23 ± 42.03 abdefg	4.92 ± 0.22 abc	0.99 ± 0 ab
	Manure	5	4	211.75 ± 54.36 abdefg	214.21 ± 55.8 abdefg	4.86 ± 0.31 abc	0.99 ± 0 ab
		10	4	279.5 ± 28.56 abc	290.66 ± 29.87 abc	4.91 ± 0.28 abc	0.98 ± 0.01 a
Crimson	No Biochar	0	8	138.75 ± 14.71 d	139.31 ± 14.74 d	4.58 ± 0.14 a	0.98 ± 0 ab
Clover	Wood	5	4	244.5 ± 44.79 abcefg	249.81 ± 45.98 abcefg	5.17 ± 0.21 bc	0.99 ± 0 b
		10	4	160.5 ± 21.27 defg	162.1 ± 22.04 defg	4.64 ± 0.17 ab	0.98 ± 0 a
	Manure	5	4	340.5 ± 31.05 ac	355.18 ± 34.97 ac	5.45 ± 0.08 c	0.99 ± 0 b
		10	4	266.75 ± 32.22 abcg	278.02 ± 35.48 abcg	4.94 ± 0.12 abc	0.98 ± 0 ab
Italian	No Biochar	0	8	186.62 ± 16.55 bdefg	188.98 ± 16.78 bdefg	4.72 ± 0.19 ab	0.98 ± 0.01 ab
Ryegrass	Wood	5	4	216 ± 19.98 abcdefg	217.95 ± 20.11 abcdefg	5 ± 0.15 abc	0.99 ± 0 ab
		10	4	151 ± 30.47 def	152.06 ± 31.1 def	4.58 ± 0.27 ab	0.98 ± 0 a
	Manure	5	4	372 ± 21.43 c	388.67 ± 24.26 c	5.51 ± 0.1 c	0.99 ± 0 b
		10	4	250.25 ± 27.42 abcfg	261.46 ± 29.96 abcfg	4.86 ± 0.19 ab	0.98 ± 0.01 ab

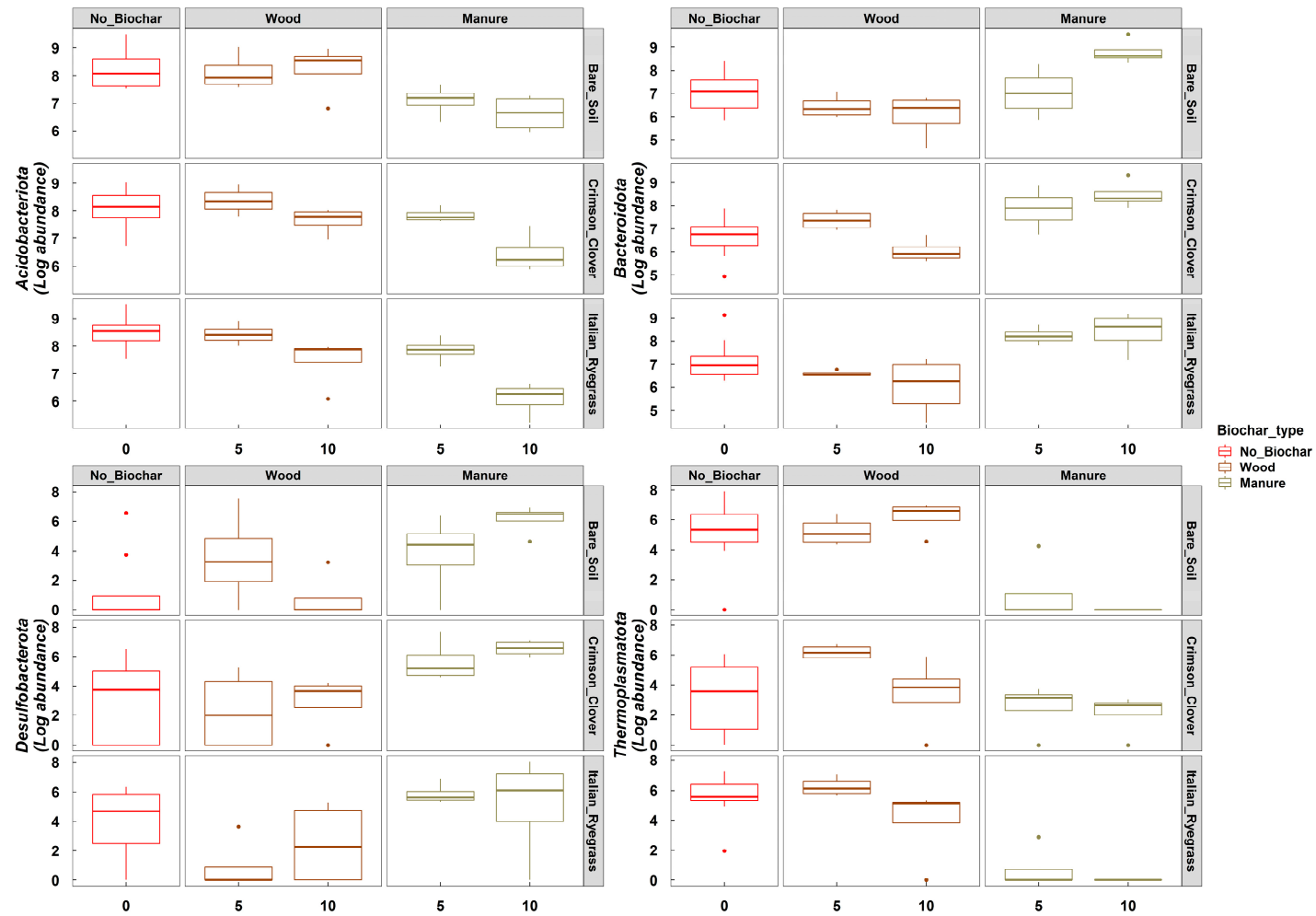
Supplementary Table S4: Physicochemical properties of the soil samples used in this study. Different letters represent significant differences at $p < 0.05$ between treatments using Dunn's pairwise comparison test. EC = Electrical conductivity; Mg = magnesium; K = potassium concentration; Na = sodium concentration; Ca = Calcium; NO₃ = Nitrate.

Plant	Biochar	Loading	N	pH	EC	NO ₃	P	K	Ca	Mg	Na
application	type	percent			(μmho/cm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)
Bare	No	0	8	7.88 ± 0.06 de	168 ± 26.7 c	4.62 ± 0.75 ab	27.5 ± 7.59 bcd	200.75 ± 36.99 def	1158.62 ± 52.24 bcd	103.5 ± 11.1 bc	24.88 ± 6.67 ef
Soil	Biochar										
		Wood	5	4	8.07 ± 0.05 def	111.25 ± 3.01 c	1.25 ± 0.95 cd	16 ± 2.27 bcd	184.5 ± 13.16 def	1066 ± 74.32 bcd	84.75 ± 5.63 c
		10	4	8.3 ± 0.04 abcd f	130 ± 2.8 bcd	1.75 ± 0.85 acd	12.75 ± 0.48 c	218.5 ± 8.26 abcd	967.75 ± 32.28 de	78.25 ± 2.1 c	33.25 ± 4.89 cdef
	Manure	5	4	8.95 ± 0.03 abc	1110.75 ± 202.79 ab	7 ± 1.08 ab	327.25 ± 2.78 ab	1619 ± 27.13 abc	2150 ± 77 abc	566 ± 11.16 ab	243.5 ± 18.18 abcd
		10	4	9.45 ± 0.05 a	1553.75 ± 256.02 a	3.25 ± 1.38 abc	617.75 ± 39.34 a	2896.25 ± 110.66 a	2756.75 ± 112.56 a	999 ± 48.55 a	456.25 ± 24.01 ab
	Crimson	No	0	8	7.69 ± 0.05 e	139 ± 7.51 cd	1.12 ± 0.55 cd	15.5 ± 1.99 c	142.62 ± 9.35 ef	1105.75 ± 28.8 bcd	92.62 ± 4.5 bc
Clover	Biochar										
		Wood	5	4	8.18 ± 0.05 cdef	122.5 ± 3.8 c	0.5 ± 0.29 cd	15.5 ± 2.63 cd	185.25 ± 13.35 def	1034.5 ± 43.13 cde	84.25 ± 5.44 c
		10	4	8.25 ± 0.03 bcd f	121.75 ± 2.17 c	0.5 ± 0.29 cd	12.5 ± 0.96 c	206 ± 11.25 bcd e	943 ± 39.91 de	76 ± 3.56 c	34 ± 3.27 cdef
	Manure	5	4	8.82 ± 0.09 ab cf	1162.25 ± 201.55 ab	9 ± 1.41 b	330.25 ± 11.78 ab	1605.25 ± 49.84 abc	2185 ± 62.32 ab	565.5 ± 15.61 ab	243.5 ± 20.02 abcd

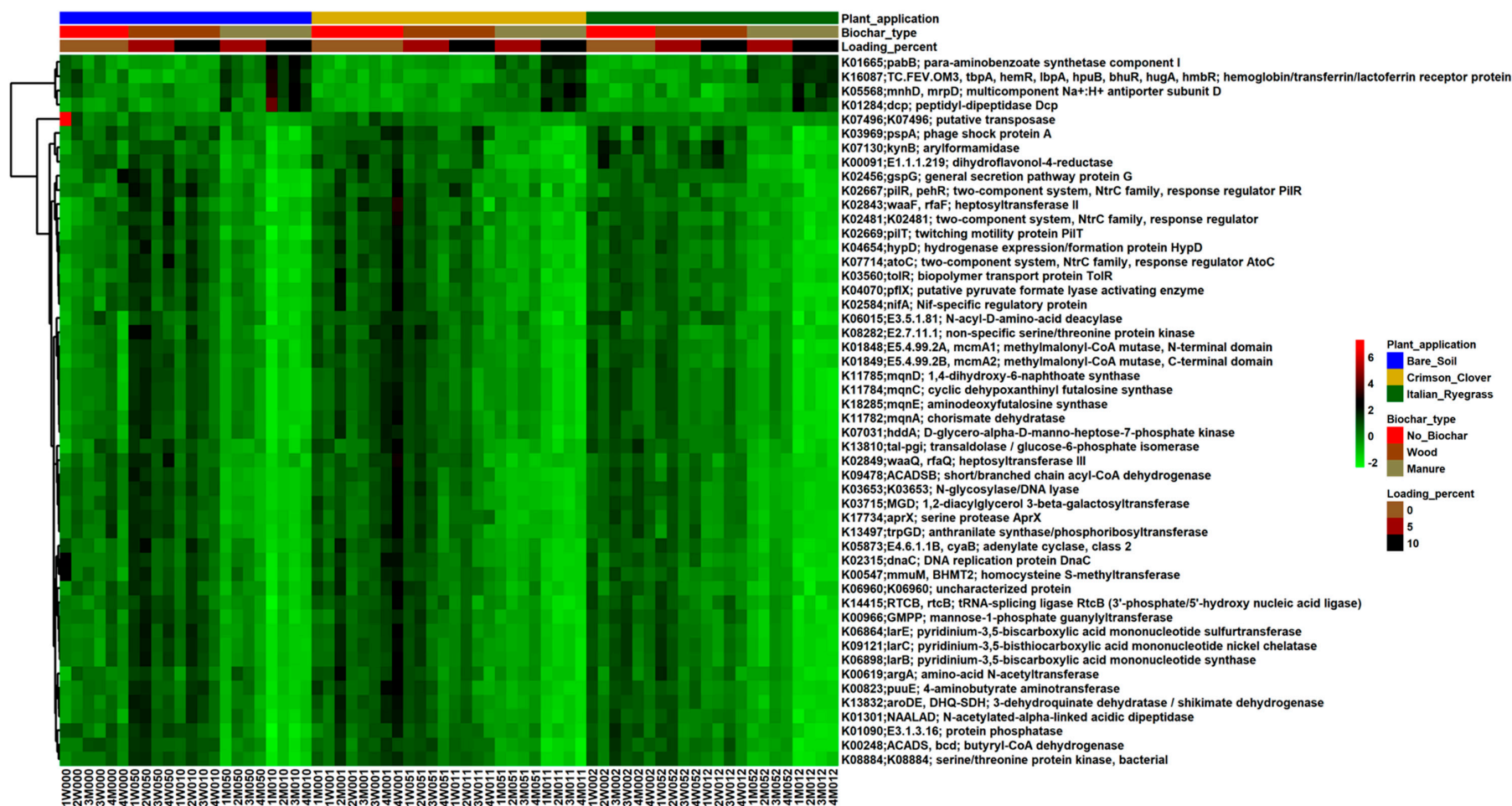
		10	4	9.38 ± 0.1 ab	1375 ± 325.37 a	3 ± 1.47 abcd	618.75 ± 20.75 a	2795.25 ± 55 ac	2768.25 ± 89.16 a	967.5 ± 24.87 a	415.5 ± 10.17 abc
Italian	No	0	8	7.74 ± 0.07 e	129.75 ± 6.62 c	0.25 ± 0.16 d	14.88 ± 1.95 c	134.5 ± 7.33 f	1048.75 ± 30.25 de	88.62 ± 3.44 c	16.62 ± 2.15 e
Ryegrass	Biochar										
		Wood	5	4	8.2 ± 0.04 cdef	116.25 ± 5.41 c	0.25 ± 0.25 cd	12 ± 0.71 c	160.25 ± 8.13 def	946 ± 45.84 de	78.25 ± 3.71 c
		10	4	8.3 ± 0.04 abcdf	125.25 ± 4.99 c	0.5 ± 0.29 cd	14.25 ± 1.31 cd	223.25 ± 15.76 abcd	896.5 ± 11.91 e	78.25 ± 3.61 c	35.25 ± 4.29 bcdf
	Manure	5	4	8.85 ± 0.06 abcf	843 ± 83.09 abd	0.5 ± 0.29 cd	324.5 ± 12.9 abd	1555.75 ± 37.39 abc	2172.25 ± 62.69 abc	564.25 ± 15.69 ab	230.25 ± 9.9 abcd
		10	4	9.4 ± 0.04 ab	1574.75 ± 230.12 a	0.75 ± 0.25 cd	626.25 ± 32.33 a	2957.75 ± 45.81 a	2807.5 ± 108.49 a	1011.25 ± 43.27 a	481.5 ± 19.96 a



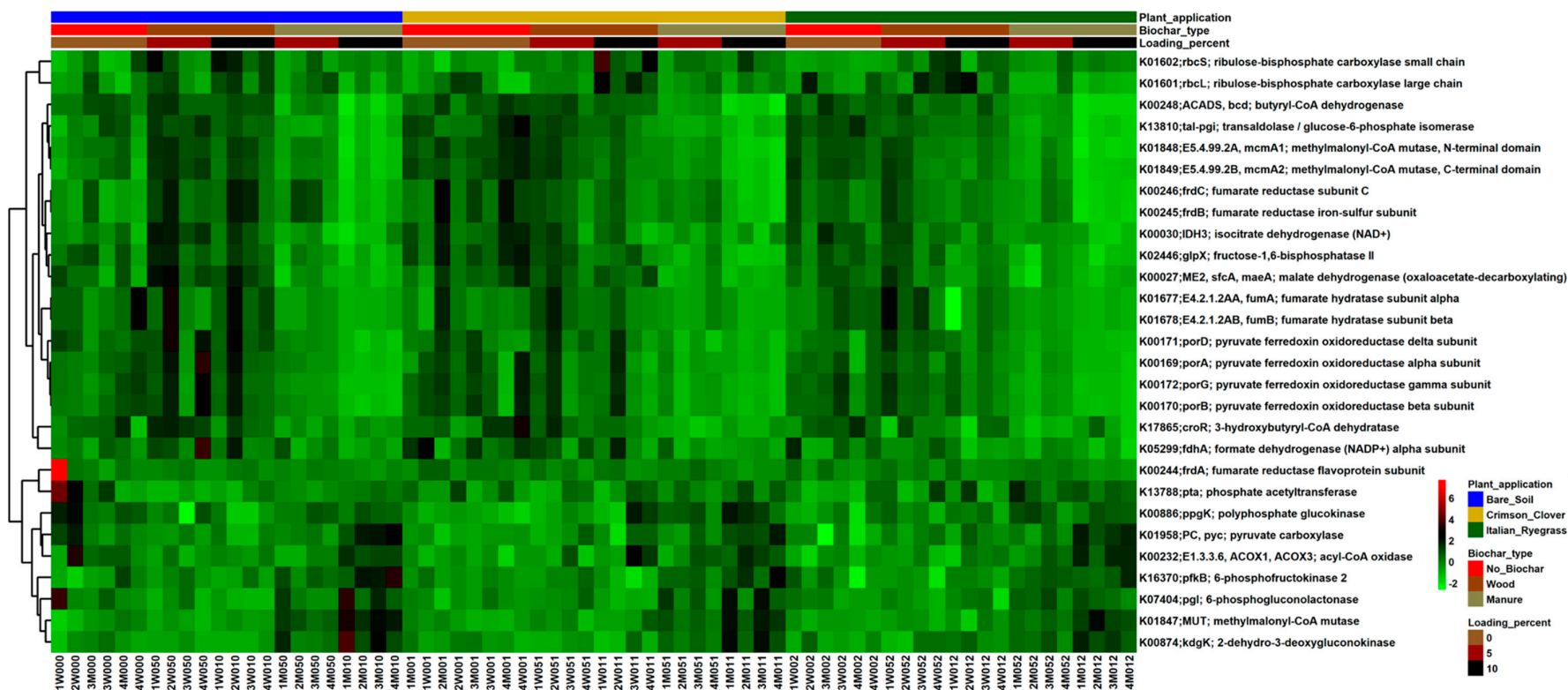
Supplementary Figure S1: Sample rarefaction curves depicting the richness (y-axis, as the number of unique ASVs recovered) determined at an equal sampling effort (x-axis, the number of individual sequences recovered in each sample) of prokaryotes in the soil. A. Comparison of bare soil (no crop), crimson clover, and Italian ryegrass. B. Comparison of different biochar loading percentages. C. Comparison of different biochar types. D. Comparison of individual samples. The rarefaction curves show that the rarefaction depth (3107) chosen in this study was enough to capture the prokaryote diversity of our soil samples.



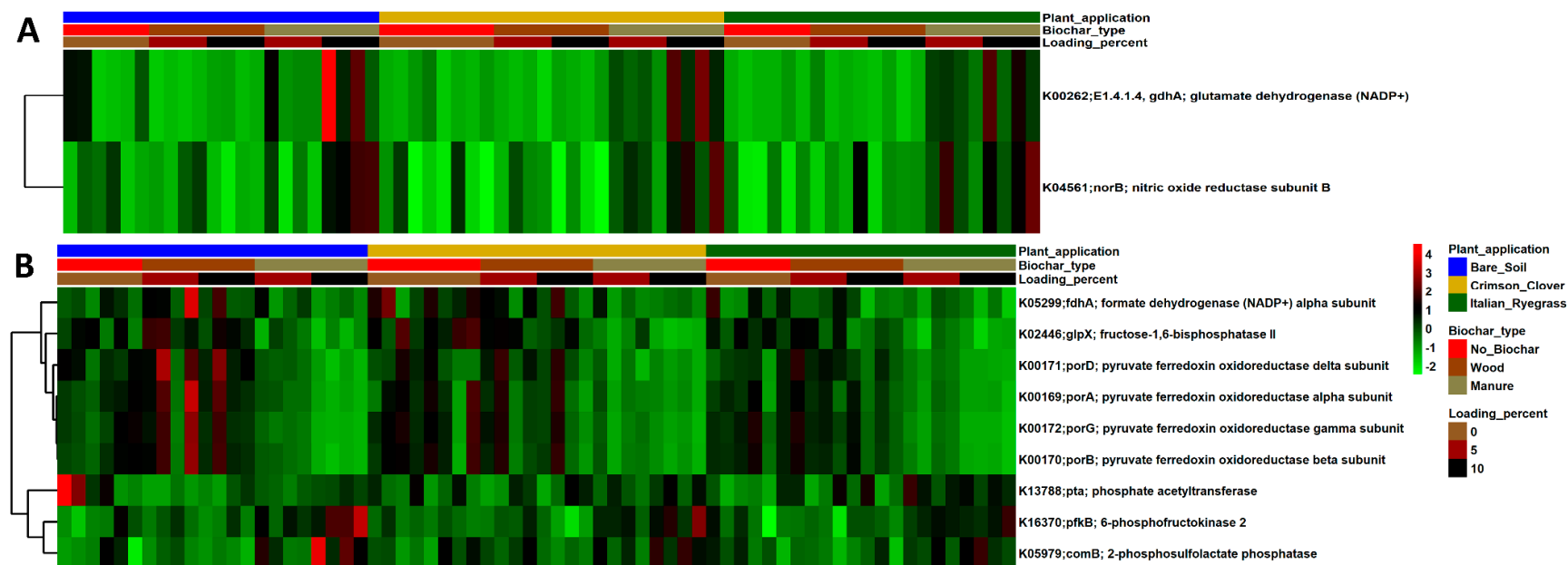
Supplementary Figure S2: Differentially abundant soil prokaryote phyla detected using ANCOM. On the x-axis is the biochar loading rate and the Y-axis the natural log abundance of the differentially abundant prokaryote phylum; n=4. Boxes represent 25-75% of the data, solid lines the median, the tips represent the minimum and maximum values excluding the outliers (1.5 times lesser or greater than the lower or upper quantiles) represented by dots outside of the boxes.



Supplementary Figure S3: Heatmap showing the difference in gene patterns observed between treatments for the fifty most significant genes (fifty lowest adjusted p-values). On the x-axis are samples and on the y-axis are hierarchically clustered significant genes. Gene abundances were transformed, scaled, and correlated. The heatmap shows that the gene patterns were different between biochar types and loading percent within biochar types.



Supplementary Figure S4: Heatmap showing the difference in carbon metabolism gene patterns observed between treatments. On the x-axis are samples and on the y-axis are hierarchically clustered significant genes. Gene abundances were transformed, scaled, and correlated. The heatmap shows that the gene patterns were different between biochar types and loading percent within biochar types regardless of crop type.



Supplementary Figure S5: Heatmap showing the pattern of significantly different (A) nitrogen and (B) methane metabolism genes observed between treatments. On the x-axis are samples and on the y-axis are hierarchically clustered significant genes. Gene abundances were transformed, scaled, and correlated. The heatmap shows that the gene patterns were different between biochar types and loading percent within biochar types irrespective of crop type.

REFERENCES

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