

Improvement of soil ecosystem multifunctionality by dissipating manure-induced antibiotics and resistance genes

Running title: Improvement of soil EMF by dissipating of antibiotics

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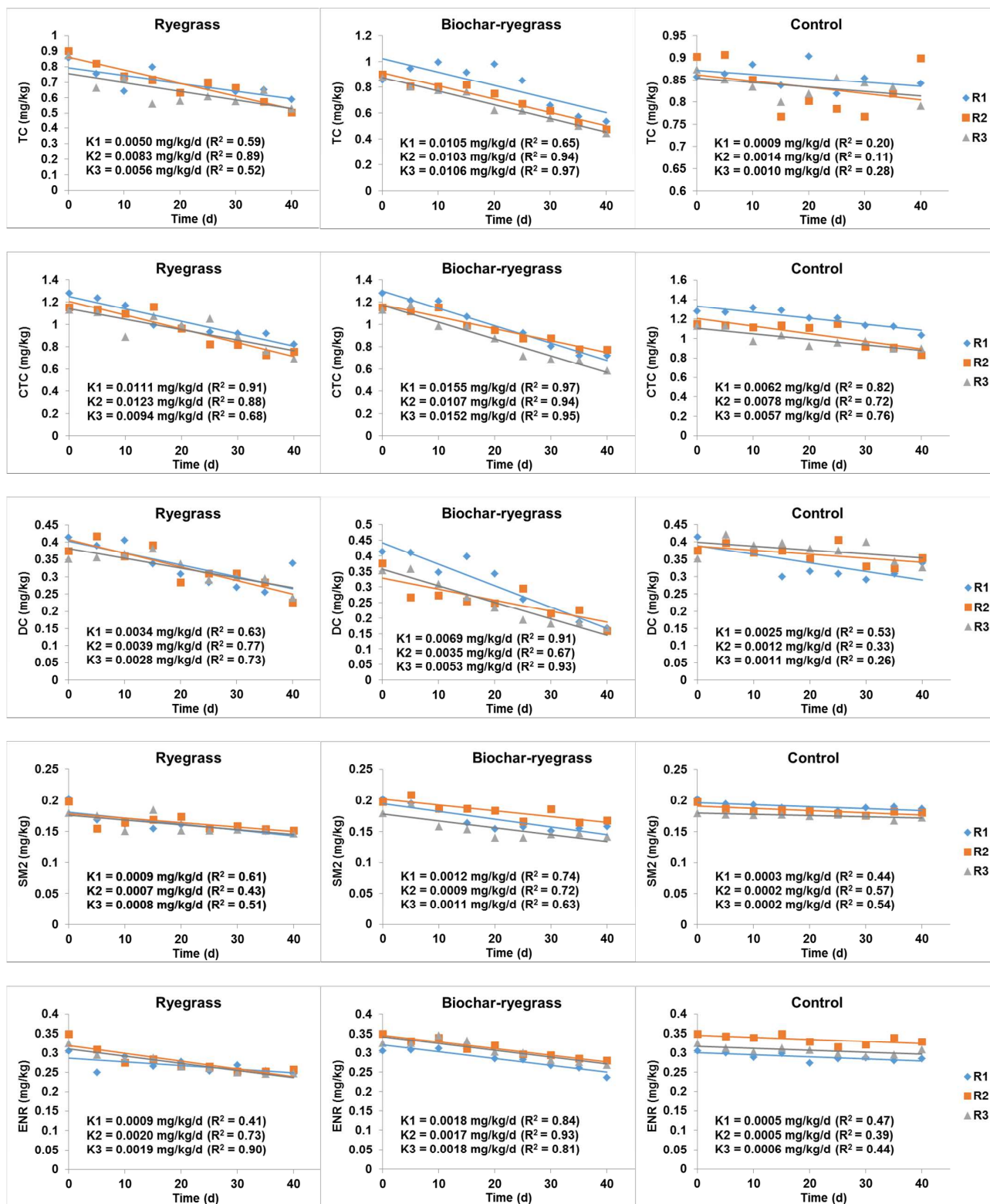
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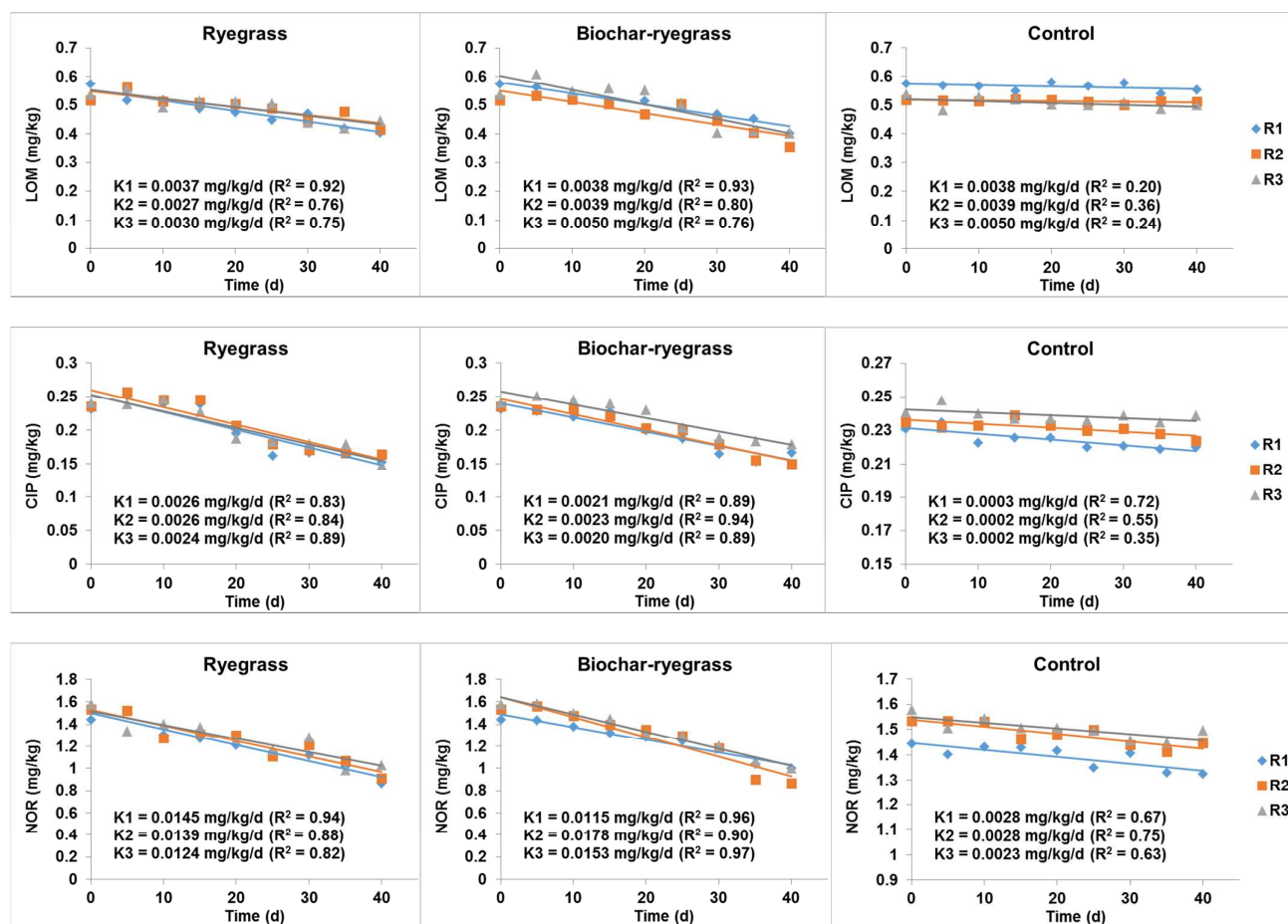


Figure S1 Concentrations of antibiotics in different treatments, control, ryegrass treatment and biochar-ryegrass treatment. TC, tetracycline; CTC, chlortetracycline; DC, doxycycline; SM2, sulfonamides; ENR, enrofloxacin; LOM, lomefloxacin; CIP, ciprofloxacin; NOR, norfloxacin. The antibiotics dissipation fitted zero-order reaction kinetics. The dissipation rates of various antibiotics were obtained through calculating the slope of antibiotic residues per day.

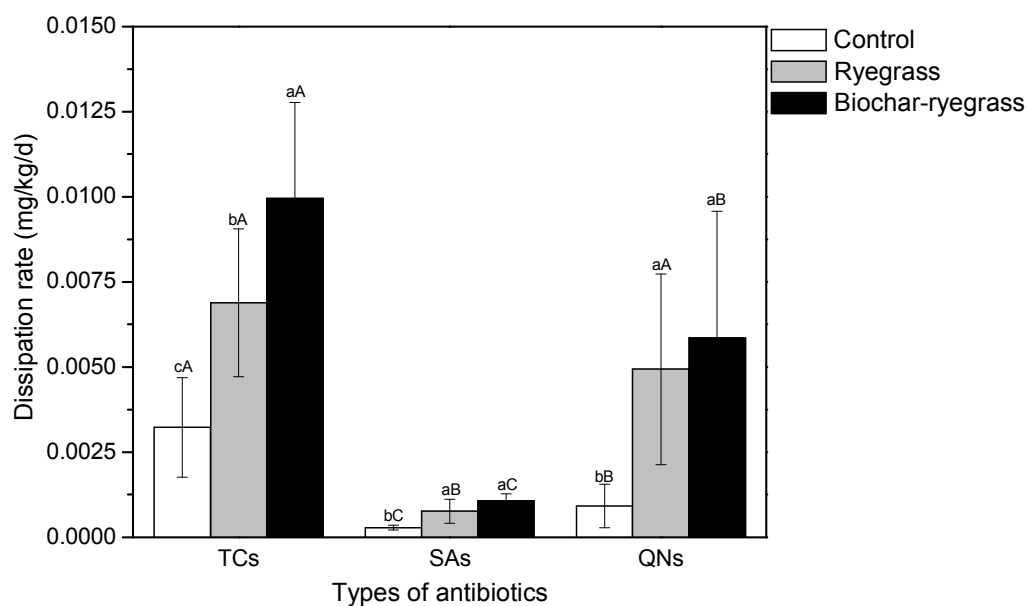


Figure S2 Dissipation rate of antibiotics in different treatments. Different lowercase letters indicated significant differences of same antibiotics in different treatments ($p < 0.05$); different uppercase letters indicated significant differences among different antibiotics in same treatments ($p < 0.05$). TCs included tetracycline antibiotics (TC, CTC, DC); SAs contain sulfa antibiotics (SM2); QNs contain quinolone antibiotics (ENR, NOR, CIP, LOM).

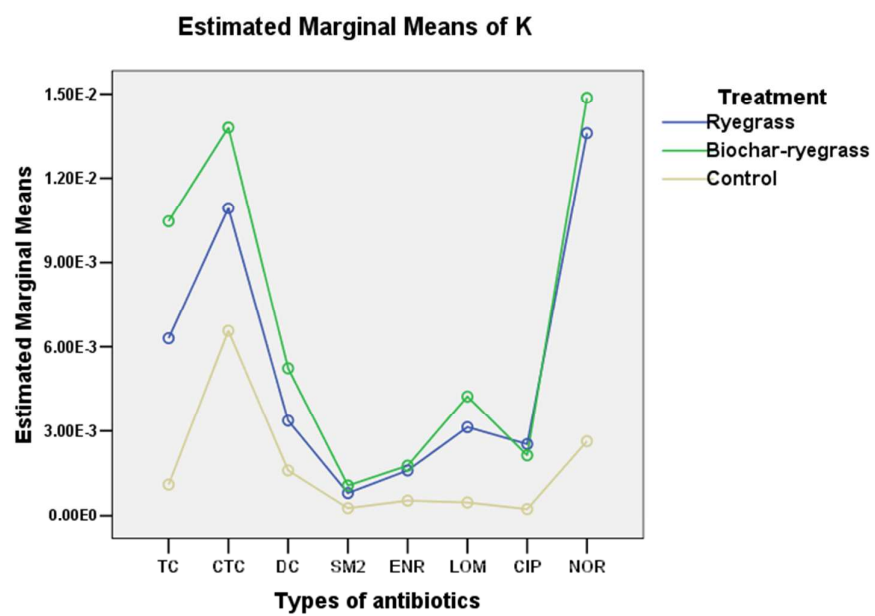


Figure S3 Two-way ANOVA was performed to estimate the effects of antibiotic types, treatment and their interactions on the dissipation of antibiotics.

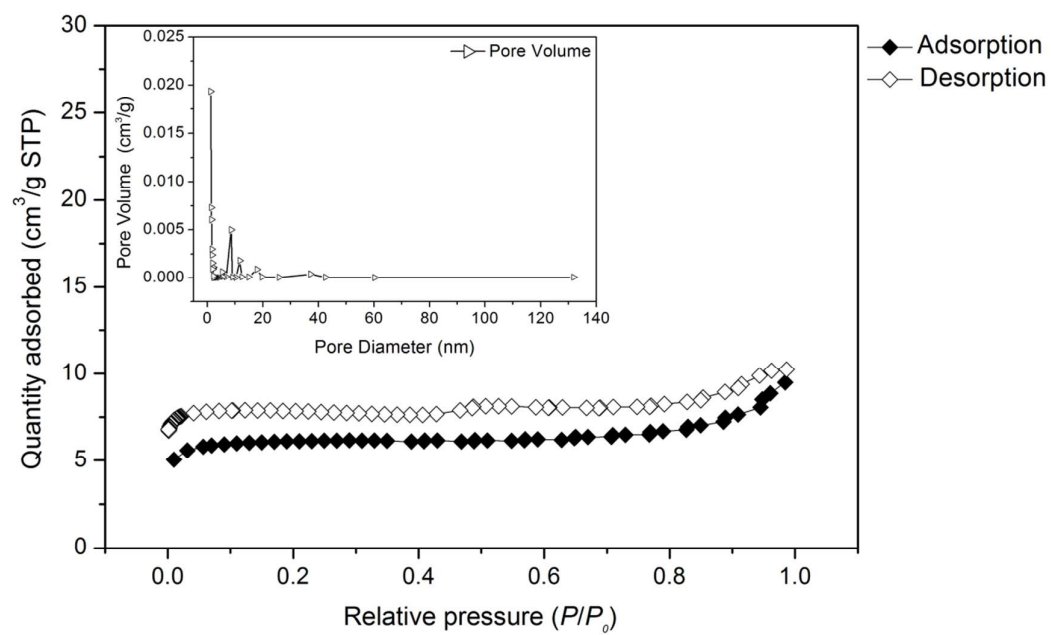


Figure S4 Adsorption-desorption N₂ curve and pore-size distribution curves of biochar.

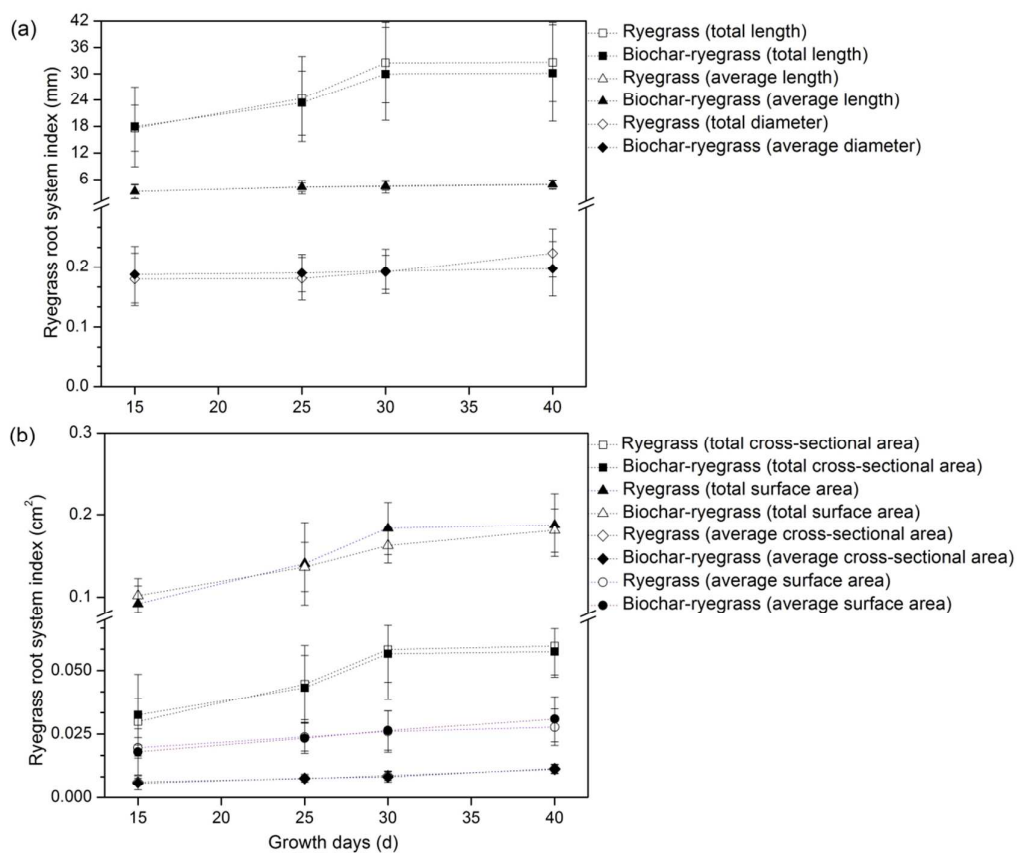


Figure S5 Root index of plant roots in different treatments. (a) Root length index; (b) Root area index.

Table S1 Primer information of antibiotic resistance genes (ARGs)

ARGs	Primer sequence (5'→3')	Length (bp)	Annealing temperature(°C)
<i>tet(W)</i>	FW_GAGAGCCTGCTATATGCCAGC	168	55
	RV_GGGCGTATCCACAATGTTAAC		
<i>tet(M)</i>	FW_ACAGAAAGCTTATTATATAAC	171	55
	RV_TGGCGTGTCTATGATGTTCAC		
<i>tet(O)</i>	FW_ACGGARAGTTTATTGTATACC	171	55
	RV_TGGCGTATCTATAATGTTGAC		
<i>tet(Q)</i>	FW_AGAATCTGCTGTTTGCCAGTG	169	55
	RV_CGGAGTGTCAATGATATTGCA		
<i>tet(H)</i>	FW_CAACCCATTACGGTGTGCTA	164	55
	RV_AAGTGTGGTTGAGAATGCCA		
<i>sul1</i>	FW_CGCACCGGAAACATCGCTGCAC	163	60
	RV_TGAAGTTCCGCCGCAAGGCTCG		
<i>sul2</i>	FW_TCCGGTGGAGGCCGGTATCTGG	191	60
	RV_CGGAATGCCATCTGCCTTGAG		
<i>gyrA</i>	FW_ACGTACTAGGCAATGACTGG	192	55
	RV_AGAAGTCGCCGTCGATAGAAC		
<i>qnrA</i>	FW_AGGATTTCTCACGCCAGGATT	124	55
	RV_CGCTTTCAATGAAACTGCA		
<i>ermF</i>	FW_CGA CAC AGC TTT GGT TGA AC	309	60
	RV_GGA CCT ACC TCA TAG ACA AG		
16S rRNA	515FW_GTGCCAGCMGCCGCGG	292	60
	806RV_GGACTACHVGGGTWTCTA-AT		

Table S2 Significant test of antibiotic resistance genes among samples

Genes	Treatments	0(d)	5(d)	10(d)	15(d)	20(d)	25(d)	30(d)	35(d)	40(d)
<i>tetM</i>	Control	aA	bA	aA	aA	aA	aA	aA	aA	aA
	Ryegrass	aA	aA	aA	aAB	aAB	aB	bB	bBC	bC
	Biochar-ryegrass	aA	bAB	bAB	bAB	bAB	bB	cB	cC	cD
<i>tetW</i>	Control	aB	aB	aAB	aAB	aAB	aAB	aAB	aAB	aA
	Ryegrass	aB	bB	abA	bB	bB	bC	bC	bC	bD
	Biochar-ryegrass	aA	bB	abA	bB	bB	bC	bC	cD	cE
<i>tetO</i>	Control	aA	aA	aA	aA	aA	aA	aA	aA	aA
	Ryegrass	aA	bB	cD	cD	cC	cD	cE	cE	cE
	Biochar-ryegrass	aA	bB	bB	bB	bB	bBC	bBC	bBC	bD
<i>tetQ</i>	Control	aC	aC	aB	aA	aA	aB	bC	bC	bC
	Ryegrass	aAB	aB	aAB	bAB	aA	aA	aA	aA	aA
	Biochar-ryegrass	aA	aAB	bAB	cAB	bB	bBC	cBC	cB	cC
<i>tetH</i>	Control	aB	aC	aC	aC	aBC	aAB	aA	aAB	aAB
	Ryegrass	aA	bB	aA	aA	aA	bAB	bAB	bB	bB
	Biochar-ryegrass	aA	aAB	bB	bC	bC	cCD	cCD	cCD	cD
<i>sul1</i>	Control	aAB	aB	aB	aAB	aAB	aAB	aA	aAB	aB
	Ryegrass	aA	aAB	abAB	bB	bB	bC	bD	bE	bE
	Biochar-ryegrass	aA	aB	abB	bB	cC	cD	cE	cF	cG
<i>sul2</i>	Control	aB	aA	aA	aB	aB	aC	aC	aC	aC
	Ryegrass	aA	bA	aA	bB	bB	bB	bBC	bC	bC
	Biochar-ryegrass	aA	cB	bB	cC	cD	cD	cE	cF	cG
<i>qnrA</i>	Control	aA	bB	bCD	bCD	cCD	cCD	cCD	cD	cC
	Ryegrass	aB	aB	aC	aC	aA	aA	aB	aC	aC
	Biochar-ryegrass	aA	bB	aC	aD	bC	bE	bF	bF	bF
<i>gyrA</i>	Control	aB	aC	aA	aA	aB	aB	aAB	aA	aAB
	Ryegrass	aA	aA	aA	aA	aAB	aAB	bAB	bC	aC
	Biochar-ryegrass	aA	bB	bB	bC	bC	bD	cD	cD	bE
<i>ermF</i>	Control	aA	aA	aAB	aAB	aA	aA	aB	aAB	aB
	Ryegrass	aA	aA	bD	bC	bC	bB	bD	bD	bD
	Biochar-ryegrass	aA	aA	aA	aAB	aA	bAB	bAB	bB	bC

Different lowercase letters indicate significant differences of sample among different treatments in same time period ($p < 0.05$); different uppercase letters indicate significant differences of samples among different time periods in same treatments ($p < 0.05$).

Table S3 Dissipation rate of antibiotic resistance genes

	<i>tetM/16S</i>	<i>tetW/16S</i>	<i>tetO/16S</i>	<i>tetQ/16S</i>	<i>tetH/16S</i>	<i>sul1/16S</i>	<i>sul2/16S</i>	<i>qnrA/16S</i>	<i>gyrA/16S</i>	<i>ermF/16S</i>
Control	-0.0013 ± 0.0002bE	-0.0012 ± 0.0002cE	0.0007 ± 0.0001cC	0.00005 ± 0.00001aD	0.0004 ± 0.0001aC	-0.0057 ± 0.0009bE	0.0163 ± 0.004aA	0.00005 ± 0.00001aD	-0.0021 ± 0.0004cE	0.0083 ± 0.001bB
Ryegrass	0.018 ± 0.001aA	0.0015 ± 0.0002bB	0.008 ± 0.003aB	0.00007 ± 0.00001aD	0.0005 ± 0.00001aC	0.0896 ± 0.009aA	0.0389 ± 0.007aA	0.00009 ± 0.00001aD	0.003 ± 0.0009bB	0.0248 ± 0.0005aA
Biochar-ryegrass	0.0662 ± 0.009aA	0.0083 ± 0.0008aB	0.0025 ± 0.0008bC	0.0001 ± 0.00002aD	0.0003 ± 0.0001aD	0.05186 ± 0.003aA	0.0706 ± 0.031aA	0.00003 ± 0.00001aE	0.0125 ± 0.006aB	0.0112 ± 0.004aB

Different lowercase letters indicate significant differences of same gene in different treatments ($p < 0.05$); different uppercase letters indicate significant differences among different genes in same treatments ($p < 0.05$). K = -Copy of ARGs/copy of 16S rRNA genes/day.

Table S4 Structure parameters of samples calculated from adsorption-desorption N₂

Stage	Samples	Surface area S_{BET} (m ² /g)	Micropore area S_{mi} (m ² /g)	External Surface Area S_{ex} (m ² /g)	Micropore volume V_{mi} (m ³ /g)
Early stage (1 st day)	Control	28.73	4.57	24.17	0.001921
	Ryegrass	29.23	5.15	24.08	0.002199
	Biochar-ryegrass	28.50	4.35	24.14	0.001781
Middle stage (25 th day)	Control	28.78	4.96	24.01	0.00208
	Ryegrass	28.75	5.51	23.19	0.002611
	Biochar-ryegrass	28.93	7.03	21.90	0.003351
Later stage (40 th day)	Control	28.99	5.34	23.63	0.002408
	Ryegrass	29.44	3.83	25.60	0.001775
	Biochar-ryegrass	55.20	5.99	49.21	0.002798

Table S5 Trace elements in the sample

Stage	Sample	Si (%)	Al (%)	Fe (%)	K (%)	Ca (%)	Ti (%)	S (%)	Zr (%)
Early stage	Control	61.894±0.713	14.864±0.588	11.963±0.025	5.839±0.056	2.873±0.030	1.707±0.017	0.636±0.055	0.110±0.002
	Ryegrass	60.965±0.713	15.314±0.588	11.673±0.025	5.909±0.056	2.846±0.019	1.726±0.017	0.696±0.059	0.116±0.003
	Biochar-ryegrass	61.145 ±0.731	15.002 ±0.601	12.014 ±0.022	5.726 ±0.057	3.202 ±0.033	1.723 ±0.018	0.598 ±0.047	0.109 ±0.002
Middle stage	Control	60.994 ±0.718	15.335 ±0.616	12.002 ±0.025	5.828 ±0.057	2.972 ±0.030	1.730 ±0.018	0.754 ±0.056	0.114 ±0.002
	Ryegrass	62.270±0.696	15.662 ± 0.590	10.976 ± 0.024	5.718 ±0.054	2.618 ±0.028	1.686 ± 0.017	0.605 ± 0.055	0.100 ±0.002
	Biochar-ryegrass	61.380±0.701	15.363 ±0.603	11.482 ± 0.025	5.960 ±0.056	2.819 ±0.029	1.734 ±0.018	0.707 ±0.056	0.115 ±0.002
later stage	Control	61.115 ±0.725	15.186 ±0.622	12.054 ±0.025	5.757 ±0.057	3.202 ±0.033	1.764 ±0.018	0.313 ±0.055	0.116 ±0.002
	Ryegrass	62.244 ±0.690	15.524 ±0.586	11.052 ±0.023	5.702 ±0.054	2.628 ±0.028	1.746 ±0.017	0.625 ±0.051	0.099 ±0.001
	Biochar-ryegrass	61.318 ±0.685	15.301 ±0.589	11.407 ± 0.024	5.787 ±0.054	3.172±0.031	1.646±0.017	0.650 ±0.053	0.103 ±0.002
Stage	Sample	Mn (%)	V (%)	Sr (%)	Cr (%)	Rb (%)	Y (%)	Ni (%)	Nb (%)
Early stage	Control	0.097±0.004	0.074±0.007	0.048±0.001	0.028±0.004	0.026±0.001	0.018±0.001	0.015±0.002	0.009±0.001
	Ryegrass	0.099±0.004	0.069±0.007	0.045±0.001	0.028±0.003	0.024±0.002	0.019±0.002	0.016±0.002	0.008±0.001
	Biochar-ryegrass	0.092±0.004	0.071 ±0.007	0.049 ±0.001	0.034 ±0.003	0.026 ±0.002	0.021 ±0.001	0.016 ±0.002	0.008 ±0.001
Middle stage	Control	0.091 ±0.004	0.065 ±0.007	0.045 ±0.001	0.031 ±0.004	0.022 ±0.002	0.021 ±0.001	0.013 ±0.002	0.007 ±0.001
	Ryegrass	0.079 ±0.004	0.065 ±0.007	0.043 ±0.001	0.025 ±0.004	0.027 ±0.001	0.016 ±0.001	0.012 ±0.002	0.007 ±0.001
	Biochar-ryegrass	0.114 ±0.004	0.067 ±0.007	0.045 ±0.001	0.031 ±0.004	0.029 ±0.001	0.017 ±0.001	0.014 ±0.002	0.007 ±0.001
later stage	Control	0.114 ±0.004	0.071 ±0.007	0.049 ±0.001	0.037 ±0.004	0.029 ±0.002	0.018 ±0.001	0.016 ±0.002	0.008 ±0.001
	Ryegrass	0.100 ±0.004	0.061 ±0.007	0.043 ±0.001	0.032 ±0.004	0.023 ±0.001	0.019 ±0.001	0.018 ±0.002	0.006 ±0.001
	Biochar-ryegrass	0.124 ±0.005	0.064 ±0.007	0.043 ±0.001	0.233 ±0.004	0.023 ±0.001	0.017 ±0.001	0.040 ±0.002	0.006 ±0.001

Table S6 Correlation coefficient between root index and total residue of antibiotics

Values Pearson coefficient *Correlation at the 0.05 (2-tailed).	Total residue of antibiotics	Pearson correlation	Total length	Average length	Total diameter	Total cross-sectional area	Total surface area	Average cross-sectional area	Average surface area	indicated the correlation (r). is significant level
	Ryegrass	<i>r</i>	-0.123	-0.091	-0.026	-0.145	-0.465*	-0.109	-0.206	
	Biochar-ryegrass	<i>r</i>	-0.113	-0.082	-0.019	-0.129	-0.427*	-0.099	-0.197	

**Correlation is significant at the 0.01 level (2-tailed).

Table S7 Correlation coefficient between ecosystem multifunctionality and antibiotics

	Pearson correlation	TC	CTC	DC	SM2	ENR	LOM	CIP	NOR
Soil respiration	<i>r</i>	-0.023	0.021	0.026	0.075	0.031	-0.109	0.026	-0.052
Soil ammonification	<i>r</i>	-0.219	-0.171	-0.087	-0.127	0.023	-0.167	-0.032	-0.385*
Soil nitrification	<i>r</i>	-0.124	-0.022	-0.089	0.043	-0.028	-0.081	-0.007	-0.528*

Values indicated the Pearson correlation coefficient (*r*). The significant ones were bolded.

*Correlation is significant at the 0.05 level (2-tailed).

Table S8 pH values of samples

Stage	Samples	pH
Early stage (1 st day)	/	10.34±0.25
	Biochar	
	Control	7.65±0.16
	Ryegrass	7.62±0.28
Middle stage (25 th day)	Biochar-ryegrass	7.64±0.25
	Control	7.55±0.36
	Ryegrass	7.09±0.19
	Biochar-ryegrass	7.55±0.15
Later stage (40 th day)	Control	7.48±0.31
	Ryegrass	6.92±0.16
	Biochar-ryegrass	7.5±0.19