

Combined Use of Biochar and Microbial Agents Can Promote Lignocellulosic Degradation Microbial Community Optimization during Composting of Submerged Plants

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3.1. Changes of temperature during composting

Temperature can be used to assess the compost maturity, which is also an important inducing factor for microbial changes at different composting stages. No significant difference between temperature change trend was found for all treatments (Fig. S1). Temperature had risen to the maximum rapidly at the beginning 3 to 9 days, due to the suitable carbon-nitrogen ratio and porosity fermentation conditions, thermophilic microorganisms decomposed organic matter and multiplied rapidly, as well as producing a large amount of heat and gas at this period. The highest temperature of the reactors was 53.1 to 54.8 °C, subsequently, the temperature fluctuated down and gradually stabilized. The possible reason could be that the content of organic acid and inorganic acid increased rapidly in the early stage of high temperature composting period, and the activity of some thermophilic microorganisms was blocked and the number reduced [1]. The addition of homemade microbial agent (H groups) increased the average temperature but did not shorten the thermophilic stage. The microbial activity might be stimulated by the addition of microbial agents, and the temperature was higher than that of the other treatment groups. Significantly higher temperature was observed with biochar and wetland sediment addition than the control treatment, indicating that the additives provided long lasting microbial activity, which was conducive to the degradation of compost substrate. The temperature rose again within 21–24 days, indicating that the turning pile and microbial agent supplement provided long lasting microbial activity, which was conducive to the degradation of lignocellulose macromolecules and secondary fermentation of undecomposed material in the pile.

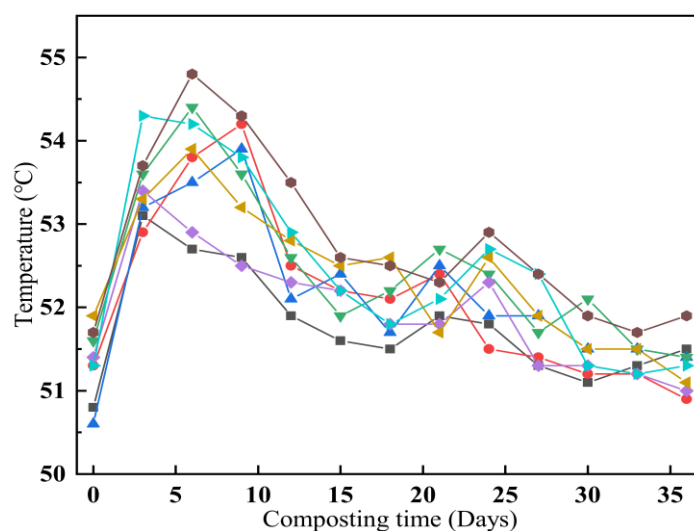


Figure S1 Variation in temperature during co-composting

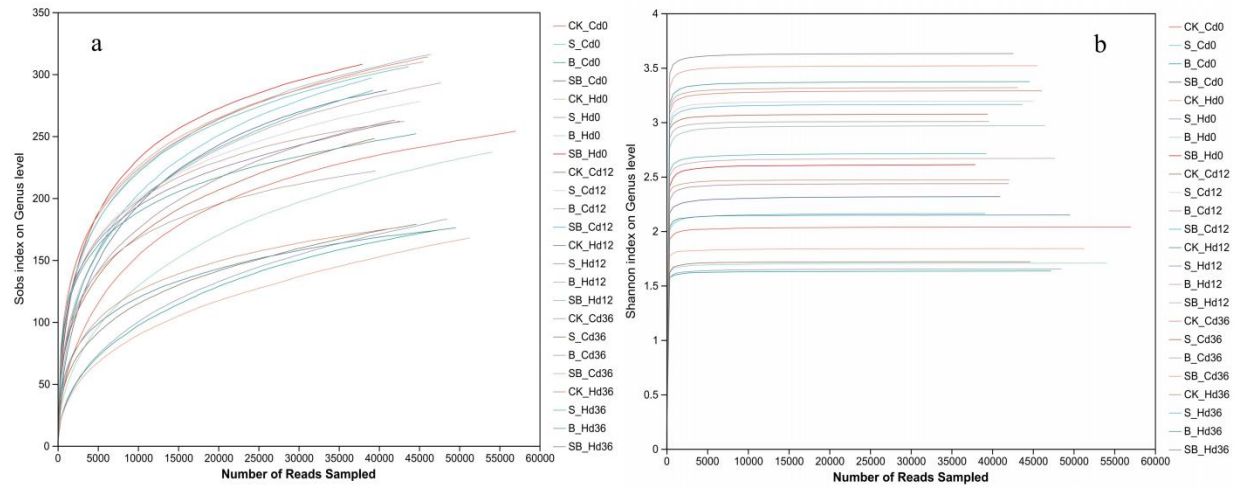


Figure S2 Sobs (a) and Shannon (b) rarefaction curves

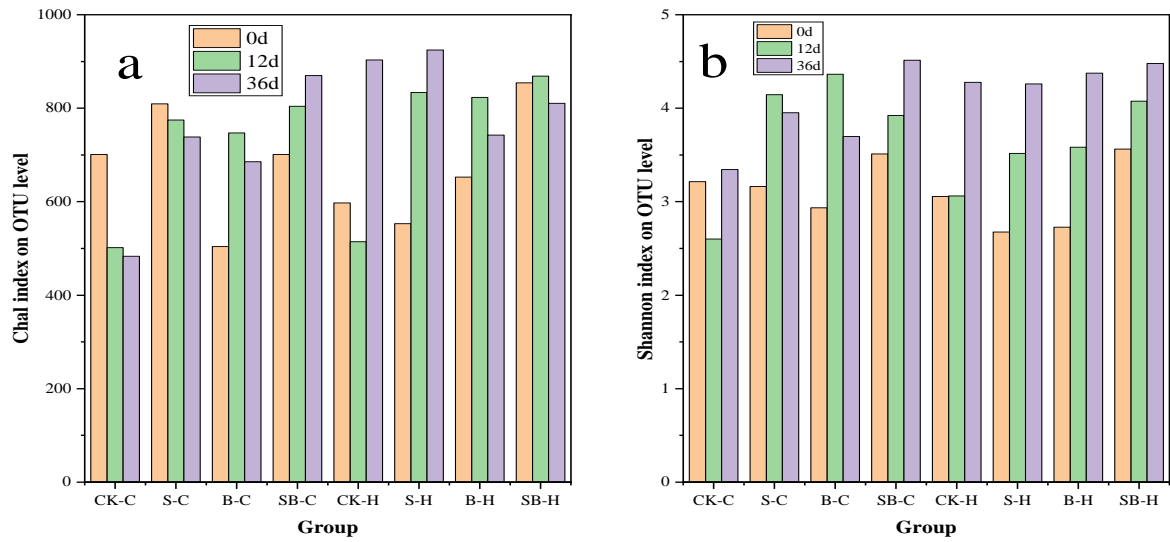


Figure S3 The a diversity of different treatments or additives (a:Chao index; b:Shannon index)

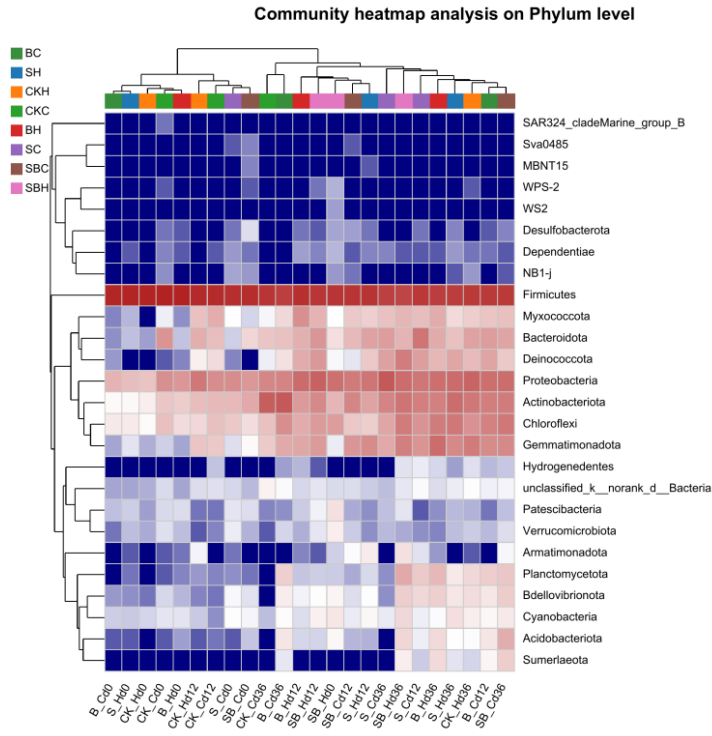


Figure S4 Community heatmap analysis on **phyla level**

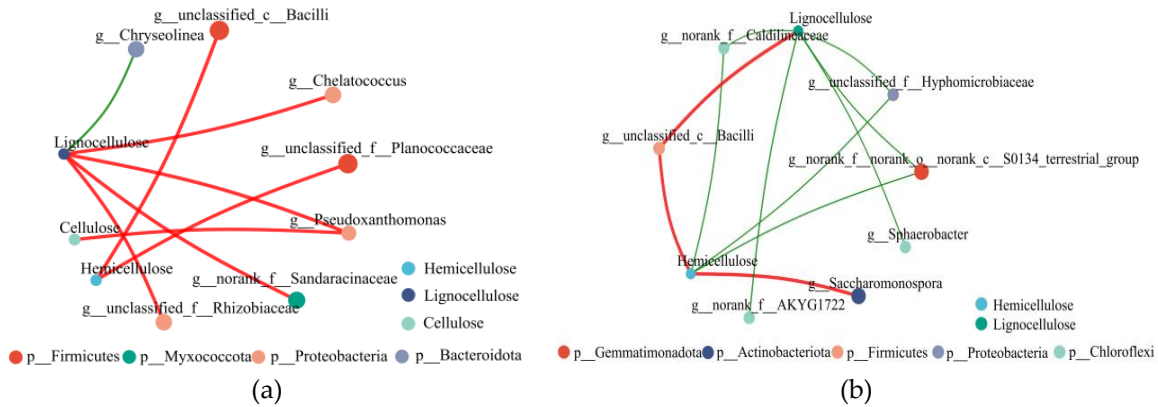


Figure S5 Co-occurrence network between lignocellulosic content and major genus in the top 20 in abundance during the thermophilic (12 d) **(a)** and cooling stages (36 d) **(b)** (Spearman's $r > 0.5$, FDR-adjusted $p < 0.05$) (red represents positive effects; green represents negative effects). LIG, lignocellulose; CELL, cellulose; HEMI, hemicellulose

Table S1 Changes in physical-chemical parameters for 8 treatments during composting process

Treatment/sample	pH	EC(ms cm ⁻¹)	TOC(%)	TN(%)	cellulose(%)	hemicellulose(%)	lignin(%)	FA(g kg ⁻¹)	HA(g kg ⁻¹)	GI(%)
CK-C0	6.5(0.1)abc	3.33(0.10)ab	76.80(1.54)	2.74(0.07)	15.6(0.31)ab	7.65(0.15)ab	4.89(0.11)ab	4.75(0.17)	3.06(0.14)c	58.94(1.18)ab
S-C0	6.7(0.1)a	3.42(0.11)ab	77.93(1.56)	2.69(0.08)	15.7(0.30)ab	7.91(0.16)a	4.93(0.12)ab	4.31(0.19)	3.17(0.11)bc	62.11(1.24)a
B-C0	6.3(0.1)c	3.37(0.10)ab	78.33(1.57)	2.68(0.09)	15.5(0.36)b	7.56(0.20)ab	4.96(0.10)a	4.53(0.16)	3.16(0.10)bc	60.38(1.21)a
SB-C0	6.5(0.1)abc	3.48(0.09)ab	77.03(1.54)	2.62(0.09)	16.3(0.29)ab	7.77(0.14)ab	4.90(0.09)ab	4.62(0.15)	3.27(0.11)abc	61.77(1.24)a
CK-H0	6.4(0.1)bc	3.59(0.11)a	77.70(1.55)	2.67(0.08)	15.9(0.32)ab	7.45(0.15)b	4.88(0.10)ab	4.58(0.15)	3.03(0.12)c	52.56(1.05)c
S-H0	6.6(0.1)ab	3.56(0.11)a	77.63(1.55)	2.69(0.10)	15.6(0.33)ab	7.38(0.15)b	4.95(0.09)a	4.35(0.16)	3.02(0.14)c	56.02(1.12)b
B-H0	6.7(0.1)a	3.47(0.12)ab	77.73(1.55)	2.66(0.08)	15.8(0.31)ab	7.56(0.19)ab	4.77(0.11)ab	4.71(0.13)	3.49(0.12)ab	55.59(1.11)bc
SB-H0	6.3(0.1)c	3.22(0.10)b	78.13(1.56)	2.64(0.07)	16.4(0.33)a	7.51(0.18)ab	4.64(0.10)b	4.52(0.17)	3.56(0.11)a	56.63(1.13)b
CK-C12	8.0(0.1)d	4.76(0.12)b	69.23(1.38)ab	3.12(0.09)c	13.8(0.28)ab	5.25(0.11)ab	5.16(0.12)	3.62(0.11)a	4.06(0.15)de	82.31(1.65)cd
S-C12	8.1(0.1)cd	4.82(0.14)ab	67.93(1.36)abc	3.37(0.11)bc	14.2(0.28)a	5.12(0.09)ab	5.11(0.09)	3.64(0.11)a	4.13(0.14)cde	79.74(1.59)d
B-C12	8.3(0.1)bc	5.02(0.15)a	67.33(1.35)abcd	3.45(0.10)ab	13.3(0.27)bcd	4.87(0.10)abc	5.15(0.11)	3.08(0.12)bc	4.26(0.12)cde	86.27(1.73)abc
SB-C12	8.2(0.1)bcd	4.77(0.11)b	63.87(1.28)d	3.56(0.12)ab	12.5(0.25)e	5.36(0.12)cd	5.08(0.11)	2.99(0.14)bc	4.97(0.13)a	88.33(1.77)ab
CK-H12	8.6(0.1)a	4.79(0.14)ab	70.53(1.41)a	3.28(0.10)bc	12.9(0.26)cde	5.36(0.21)a	5.17(0.10)	3.07(0.11)bc	4.43(0.11)bcd	83.66(1.67)bcd
S-H12	8.4(0.1)ab	5.13(0.15)ab	67.13(1.34)abcd	3.33(0.10)bc	13.4(0.27)bcd	4.77(0.10)de	5.13(0.08)	3.23(0.10)b	4.02(0.10)e	86.77(1.74)abc
B-H12	8.3(0.1)bc	5.11(0.16)ab	65.57(1.31)bcd	3.51(0.11)ab	12.8(0.26)de	4.97(0.11)bcd	5.20(0.09)	3.68(0.12)a	4.49(0.15)bc	89.64(1.79)a
SB-H12	8.1(0.1)cd	4.55(0.13)b	64.50(1.29)cd	3.69(0.11)a	13.6(0.27)abc	4.56(0.10)e	5.33(0.11)	2.87(0.11)c	4.76(0.14)ab	90.60(1.81)a
CK-C36	7.9(0.1)a	3.36(0.10)a	65.33(1.10)a	3.37(0.10)b	11.4(0.23)d	5.55(0.14)a	4.33(0.09)a	2.58(0.12)	4.82(0.16)de	104.00(2.08)b
S-C36	7.6(0.1)bcd	2.82(0.08)cd	62.27(1.29)ab	3.63(0.11)ab	12.5(0.27)bc	5.11(0.24)bc	4.27(0.07)ab	2.41(0.13)	4.66(0.17)d	105.16(3.09)b
B-C36	7.6(0.1)bcd	2.96(0.09)bcd	62.03(1.41)abc	3.69(0.13)ab	12.5(0.25)bc	4.66(0.08)de	4.33(0.08)a	2.43(0.11)	5.05(0.14)cde	105.78(2.12)b
SB-C36	7.5(0.1)cd	2.99(0.10)bc	60.33(1.46)bc	3.81(0.11)a	13.2(0.26)ab	4.51(0.19)eg	4.22(0.09)ab	2.51(0.12)	5.73(0.15)a	115.24(2.30)a
CK-H36	7.8(0.1)ab	2.82(0.08)cd	64.43(1.38)a	3.60(0.12)ab	11.8(0.24)cd	5.37(0.11)ab	4.35(0.10)a	2.66(0.11)	5.21(0.12)bcd	104.38(2.09)b
S-H36	7.7(0.1)abc	2.74(0.07)d	61.47(1.41)abc	3.80(0.11)a	12.5(0.25)bc	4.88(0.10)cd	4.28(0.09)ab	2.47(0.09)	5.45(0.17)abc	105.65(2.11)b
B-H36	7.5(0.1)cd	3.14(0.09)ab	58.43(1.44)bc	3.77(0.11)a	12.9(0.26)ab	4.36(0.13)fg	4.19(0.08)ab	2.42(0.10)	5.62(0.16)ab	106.35(2.13)b
SB-H36	7.4(0.1)d	2.88(0.08)cd	58.20(1.61)c	3.86(0.12)a	13.6(0.27)a	4.21(0.09)g	4.04(0.08)b	2.45(0.11)	5.86(0.13)a	116.57(2.33)a

Notes: Data are reported as Mean (SD) with n = 3. Different letters following the mean values indicate a significant difference ($p < 0.05$) between different treatments

for each composting phase (i.e. day 2, 12,36).

Table S2: Richness and diversity index of composting in different treatments at 97% level

Group	Chao			Shannon			Coverage		
	0d	12d	36d	0d	12d	36d	0d	12d	36d
CK-C	701.1	501.6	483.1	3.213	2.600	3.343	0.997	0.997	0.997
S-C	809.3	774.8	738.6	3.162	4.144	3.952	0.995	0.996	0.995
B-C	504.3	747.2	685.5	2.934	4.364	3.697	0.997	0.996	0.996
SB-C	700.8	804.3	869.9	3.512	3.922	4.513	0.996	0.995	0.996
CK-H	597.2	514.6	903.0	3.056	3.063	4.277	0.997	0.998	0.996
S-H	553.1	833.3	924.6	2.676	3.518	4.261	0.997	0.995	0.995
B-H	652.4	823.1	742.7	2.728	3.582	4.375	0.997	0.996	0.996
SB-H	854.4	868.6	810.4	3.562	4.076	4.480	0.995	0.996	0.996

Table S3: The twenty most abundant OTUs identified to genus and sorted by total bacteria sequences

<i>OTU ID</i>	<i>Phylum</i>	<i>Class</i>	<i>Order</i>	<i>Family</i>	<i>Genus</i>	<i>% Total</i>
OTU903	<i>Firmicutes</i>	<i>Bacilli</i>	<i>Bacillales</i>	<i>Bacillaceae</i>	<i>Bacillus</i>	22.89
OTU648	<i>Firmicutes</i>	<i>Bacilli</i>	<i>Bacillales</i>	<i>Planococcaceae</i>	<i>Lysinibacillus</i>	10.31
OTU64	<i>Firmicutes</i>	<i>Bacilli</i>	<i>Bacillales</i>	<i>Bacillaceae</i>	<i>Bacillus</i>	4.64
OTU873	<i>Firmicutes</i>	<i>Bacilli</i>	<i>Brevibacillales</i>	<i>Brevibacillaceae</i>	<i>Brevibacillus</i>	4.41
OTU177	<i>Firmicutes</i>	<i>Bacilli</i>	<i>Bacillales</i>	<i>Bacillaceae</i>	<i>Bacillus</i>	3.25
OTU341	<i>Firmicutes</i>	<i>Bacilli</i>	<i>Bacillales</i>	<i>Planococcaceae</i>	<i>Lysinibacillus</i>	2.88
OTU1019	<i>Firmicutes</i>	<i>Bacilli</i>	<i>Bacillales</i>	<i>Bacillaceae</i>	<i>Bacillus</i>	2.60
OTU622	<i>Firmicutes</i>	<i>Bacilli</i>	<i>Bacillales</i>	<i>Bacillaceae</i>	<i>Bacillus</i>	2.59
OTU141	<i>Firmicutes</i>	<i>Bacilli</i>	<i>Bacillales</i>	<i>Planococcaceae</i>		2.06
OTU892	<i>Firmicutes</i>	<i>Bacilli</i>	<i>Bacillales</i>	<i>Bacillaceae</i>	<i>Bacillus</i>	2.04
OTU518	<i>Actinobacteriota</i>	<i>Actinobacteria</i>	<i>Pseudonocardiales</i>	<i>Pseudonocardiaceae</i>	<i>Saccharomonospora</i>	1.91
OTU135	<i>Firmicutes</i>	<i>Bacilli</i>	<i>Brevibacillales</i>	<i>Brevibacillaceae</i>	<i>Brevibacillus</i>	1.59
OTU953	<i>Deinococcota</i>	<i>Deinococci</i>	<i>Deinococcales</i>	<i>Trueperaceae</i>	<i>Truepera</i>	1.18
OTU181	<i>Firmicutes</i>	<i>Bacilli</i>				1.01
OTU111	<i>Firmicutes</i>	<i>Bacilli</i>	<i>Brevibacillales</i>	<i>Brevibacillaceae</i>	<i>Brevibacillus</i>	0.87
OTU1322	<i>Firmicutes</i>	<i>Bacilli</i>	<i>Bacillales</i>	<i>Bacillaceae</i>	<i>Bacillus</i>	0.86
OTU723	<i>Gemmatimonadota</i>	<i>S0134_terrestrial_group</i>				0.86
OTU590	<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Hyphomicrobiaceae</i>		0.79
OTU1069	<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Granulosicoccales</i>			0.79
OTU654	<i>Gemmatimonadota</i>	<i>S0134_terrestrial_group</i>				0.79

Table S4: The network nodes and edges number in co-occurrence

Group	nodes number	edges number
CK-C	319	11299
S-C	410	16652
B-C	315	10149
SB-C	413	17521
CK-H	357	14003
S-H	385	14265
B-H	374	13948
SB-H	429	17939

References:

1. Zhao, Y.; Zhao, Y.; Zhang, Z.; Wei, Y.; Wang, H.; Lu, Q.; Li, Y.; Wei, Z. Effect of thermo-tolerant actinomycetes inoculation on cellulose degradation and the formation of humic substances during composting. *Waste Manage.* **2017**, *68*, 64-73. <http://doi.org/10.1016/j.wasman.2017.06.022>