

Supplementary Information

Deciphering the microbiome potential and metabolic profiling of animal waste co-composting reveals the co-occurrence network of non-microbial and microbial biostimulants to strengthen conservative practices in sustainable agriculture

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Table S1 The physico-chemical and microbiological properties of initial soils used in the pot-assay for the cultivation of red *Amaranthus*

Soil parameters	Estimated data
pH	7.550±0.008
EC (mS/cm)	0.132±0.013
Ammonical N (NH ₄ -N) (kg/ha)	221.61±8.36
Olsen P (P ₂ O ₅ -P) (kg/ha)	55.59±5.47
Available K (K ₂ O-K) (kg/ha)	239.46±1.99
Total culturable bacteria count (10 ⁸ CFU/g)	9.00±2.05
Total culturable actinomycetes count (10 ⁵ CFU/g)	27.33±2.84
Total culturable fungi count (10 ² CFU/g)	41.33±7.31

Table S2 The physico-chemical properties of KPJ over the decomposition period (Tabulated data of Fig. 1).

Formulations	Day(s)	pH	EC (mS/cm)	Organic C (%)	NH ₄ -N (%)	P ₂ O ₅ -P (%)	K ₂ O-K (%)
FKPJ	0	5.933± 0.009k	9.561± 0.062j	0.544± 0.021ef	0.047± 0.002e	0.013± 0.000g	0.104± 0.001j
	15	6.007± 0.003j	12.928± 0.102c	0.856± 0.009c	0.089± 0.002bc	0.024± 0.000b	0.115± 0.000i
	30	7.000± 0.006e	13.333± 0.034ab	0.776± 0.026cd	0.103± 0.001a	0.016± 0.000ef	0.124± 0.001gh
	45	7.067± 0.003d	12.105± 0.013e	0.694± 0.028de	0.069± 0.001d	0.014± 0.000g	0.126± 0.000g
	60	7.217± 0.003c	10.648± 0.026g	0.645± 0.039def	0.044± 0.001e	0.013± 0.000g	0.132± 0.000ef
	75	7.293± 0.003b	9.893± 0.044i	0.634± 0.035def	0.027± 0.001f	0.015± 0.000f	0.136± 0.001cd
	90	7.393± 0.003a	9.827± 0.015ij	0.513± 0.027f	0.019± 0.001f	0.021± 0.000d	0.142± 0.001b
LKPJ	0	5.907± 0.012l	9.799± 0.018ij	0.510± 0.015f	0.044± 0.001e	0.010± 0.001h	0.106± 0.000j
	15	5.433± 0.009m	13.261± 0.088b	1.378± 0.057b	0.096± 0.005ab	0.016± 0.000e	0.123± 0.001h
	30	6.167± 0.009i	13.563± 0.029a	1.753± 0.041a	0.086± 0.005bc	0.022± 0.001c	0.133± 0.002ef
	45	6.833± 0.003h	12.402± 0.192d	1.918± 0.047a	0.082± 0.005c	0.025± 0.001a	0.134± 0.001de
	60	6.937± 0.012g	11.704± 0.045f	1.918± 0.036a	0.086± 0.005bc	0.024± 0.000ab	0.131± 0.000f
	75	6.960± 0.006f	10.185± 0.028h	1.809± 0.149a	0.044± 0.005e	0.021± 0.001d	0.138± 0.000c
	90	7.020± 0.012e	9.554± 0.239j	1.836± 0.052a	0.030± 0.009f	0.020± 0.000d	0.145± 0.000a

DMRT was performed to analyze the data. The data is represented here as mean ± SEM (n=3). The different letters indicate statistical differences among the treatments (p<0.05), whereas a similar letter indicates no statistical differences among treatments (p<0.05).

Table S3 The non-microbial biostimulant potential of KPJ over the decomposition period (Tabulated data of Fig. 2)

Formulations	Day(s)	Indole content (µg/ml)	Gibberellin content (µg/ml)	Phenolics content (µg/ml)	Flavonoid content (µg/ml)	Germination index (%)
FKPJ	0	9.240± 0.397a	851.667± 4.410ef	233.958± 2.320e	97.333± 3.844b	81.875± 1.812c
	15	8.646± 0.370ab	1621.667± 29.202a	312.500± 6.683d	129.333± 6.642a	73.235± 1.057c
	30	8.635± 0.171ab	1245.000± 12.583b	235.208± 5.594e	87.667± 0.882bc	153.169± 10.834a
	45	8.417± 0.352bc	1101.667± 8.819bcd	160.417± 2.534f	89.667± 6.741bc	78.387± 8.923c
	60	8.333± 0.085bc	950.000± 20.817de	111.250± 1.301g	78.333± 1.764cd	82.329± 8.466c
	75	8.365± 0.063bc	696.667± 13.642fg	91.458± 0.908gh	67.000± 2.646def	104.684± 13.267bc
	90	7.719± 0.266cd	371.667± 18.782i	60.000± 0.000h	58.000± 1.155ef	90.040± 11.812c
LKPJ	0	7.688± 0.100cd	486.667± 38.442hi	235.000± 10.897e	73.333± 1.453cde	139.712± 10.550ab
	15	7.583± 0.052cd	653.333± 92.075gh	465.833± 35.047a	133.667± 7.126a	78.522± 11.082c
	30	8.010± 0.290bcd	1033.333± 174.005cd	415.833± 12.836b	52.333± 3.180f	103.351± 19.295bc
	45	7.302± 0.099d	1198.333± 26.194bc	371.667± 3.411c	130.333± 2.603a	87.846± 27.583c
	60	8.271± 0.253bc	973.333± 12.019de	303.333± 5.512d	63.000± 7.937def	72.988± 20.327c
	75	8.010± 0.260bcd	1043.333± 29.627cd	262.500± 6.166e	86.333± 6.360bc	111.111± 0.180abc
	90	8.281± 0.321bc	840.000± 49.329ef	225.000± 18.215e	58.333± 12.468ef	101.881± 18.508bc

DMRT was performed to analyze the data. The data is represented here as mean ± SEM (n=3). The different letters indicate statistical differences among the treatments (p<0.05), whereas a similar letter indicates no statistical differences among treatments (p<0.05).

Table S4 Culture-based microbial dynamics of KPJ over the decomposition period (Tabulated data of Fig. 3)

Formulations	Day(s)	TBC (10 ¹⁴ CFU/ml)	TAC (10 ⁸ CFU/ml)	TFC (10 ³ CFU/ml)	TFNF (10 ⁸ CFU/ml)	TPSB (10 ⁷ CFU/ml)	TKSB (10 ⁷ CFU/ml)	TRZ (10 ⁸ CFU/ml)
FKPJ	0	1.201± 0.069f	0.075± 0.003d	2.000± 1.000c	1.128± 0.127c	10.680± 0.869bcd	1.101± 0.040c	6.000± 2.309ef
	15	26.000± 2.966bc	5.853± 0.958bc	0.667± 0.120d	3.180± 0.081bc	9.133± 2.546bcd	13.413± 0.903b	200.000± 22.745b
	30	30.827± 0.762b	4.933± 0.312c	0.167± 0.067d	4.047± 0.285bc	20.167± 0.260b	29.453± 10.138a	119.333± 9.333c
	45	4.600± 0.361f	0.500± 0.058d	0.063± 0.026d	0.127± 0.007c	0.015± 0.003d	0.398± 0.020c	0.833± 0.273f
	60	20.800± 1.510cd	0.123± 0.007d	0.127± 0.055d	0.062± 0.005c	0.017± 0.007d	0.014± 0.002c	7.333± 1.202ef
	75	0.433± 0.067f	0.026± 0.002d	3.120± 0.545b	0.293± 0.081c	0.012± 0.002cd	0.527± 0.071c	3.667± 1.453ef
	90	13.913± 0.808de	0.239± 0.105d	6.153± 0.533a	0.045± 0.011c	0.016± 0.002d	1.447± 0.133c	248.000± 4.619a
LKPJ	0	0.250± 0.032f	1.572± 0.184d	2.000± 0.577b	0.631± 0.043c	4.390± 0.618cd	0.640± 0.134c	0.030± 0.006f
	15	32.987± 2.294b	7.467± 2.094b	0.400± 0.115d	56.800± 20.85a	14.500± 1.815bc	15.600± 3.816b	8.667± 1.764ef
	30	6.880± 1.617ef	16.880± 1.792a	0.200± 0.058d	19.000± 4.957b	64.533± 15.721a	3.097± 1.022c	44.667± 4.807d
	45	46.800± 10.193a	2.433± 0.722d	0.033± 0.009d	0.888± 0.053c	0.006± 0.002cd	1.373± 0.631c	0.333± 0.088f
	60	3.833± 0.623f	0.090± 0.004d	0.107± 0.020d	0.022± 0.012c	0.033± 0.009d	0.021± 0.003c	6.667± 1.453ef
	75	1.367± 0.219f	0.007± 0.001d	0.327± 0.012d	0.006± 0.001c	0.016± 0.004d	0.109± 0.016c	2.667± 0.882ef
	90	2.707± 0.154f	0.152± 0.007d	0.633± 0.078d	0.022± 0.003c	0.003± 0.001cd	0.507± 0.047c	26.000± 8.505de

DMRT was performed to analyze the data. The data is represented here as mean ± SEM (n=3). The different letters indicate statistical differences among the treatments (p<0.05), whereas a similar letter indicates no statistical differences among treatments (p<0.05).

Table S5 Bacterial taxa of KPJ in ecosystem services

Bacterial species	Relative abundance (%)					
	FKPJ0	FKPJ30	FKPJ60	LKPJ0	LKPJ30	LKPJ60
1. Carbohydrate decomposition:						
<i>Clostridium cellulosi</i>	0.000	0.104	0.057	0.000	0.000	0.000
<i>Butyrivibrio</i>	0.000	0.067	0.016	0.063	0.133	0.000
<i>Cellulosilyticum lentocellum</i>	0.000	0.000	0.000	0.000	0.074	0.000
<i>Chloroflexi</i> sp. ADurb.Bin325	0.000	0.000	0.000	0.000	0.055	0.000
<i>Chloroflexi</i> sp. HGW-Chloroflexi-4	0.000	0.000	0.000	0.000	0.059	0.000
<i>Clostridium homopropionicum</i>	0.000	0.000	0.000	0.000	0.000	0.036
<i>Selenomonas ruminantium</i>	0.000	0.000	0.000	0.036	0.048	0.000
<i>Ruminococcus torques</i>	0.000	0.052	0.000	0.000	0.000	0.000
2. Phytohormone synthesis and transporter:						
<i>Comamonas aquatica</i>	0.000	0.319	0.082	0.000	0.564	0.087
<i>Lactobacillus harbinensis</i>	0.000	0.000	0.000	0.817	0.000	0.000
<i>Lactobacillus mucosae</i>	0.000	0.067	0.000	0.000	0.000	0.000
<i>Pseudomonas syringae</i>	0.000	0.000	0.107	0.000	0.000	0.000
<i>Stenotrophomonas maltophilia</i>	0.000	0.037	0.123	0.000	0.000	0.000
<i>Azospirillum</i>	0.000	0.000	0.074	0.000	0.000	0.000
<i>Rhizobium</i>	0.000	0.000	0.115	0.000	0.000	0.000
<i>Arcobacter butzleri</i>	0.000	0.052	0.000	0.000	0.906	0.224
<i>Prevotella</i> sp. CAG:891	0.080	0.000	0.000	0.000	0.000	0.000
<i>Bacillus cereus</i>	0.044	0.000	0.000	0.000	0.000	0.000
<i>Ruminococcus</i> sp. CAG:177	0.000	0.000	0.041	0.000	0.136	0.000
3. Nitrogen fixation:						
<i>Bacillus cereus</i>	0.044	0.000	0.000	0.000	0.000	0.000
<i>Bacillus flexus</i>	0.000	0.052	0.000	0.000	0.000	0.000
<i>Bacillus pumilus</i>	0.000	0.000	0.000	0.000	0.000	0.039
<i>Klebsiella pneumoniae</i>	1.622	0.000	0.000	0.000	0.000	0.000
<i>Paraburkholderia</i>	0.000	0.000	0.041	0.000	0.000	0.005
<i>Pseudomonas stutzeri</i>	0.000	0.000	0.041	0.000	0.000	0.000
<i>Bacillus cereus</i>	0.044	0.000	0.000	0.000	0.000	0.000
<i>Streptomyces</i>	0.000	0.089	0.066	0.040	0.000	0.000

Bacterial species	Relative abundance (%)					
	FKPJ0	FKPJ30	FKPJ60	LKPJ0	LKPJ30	LKPJ60
4. Nutrient cycling:						
<i>Acidobacteria bacterium</i>	0.000	0.000	0.057	0.000	0.000	0.000
<i>Acidovorax caeni</i>	0.000	0.089	0.000	0.000	0.180	0.000
<i>Denitrobacterium detoxificans</i>	0.000	0.111	0.049	0.000	0.066	0.000
<i>Pseudoxanthomonas</i>	0.000	0.000	0.057	0.000	0.000	0.000
<i>Candidatus Accumulibacter</i>	0.000	0.000	0.008	0.000	0.000	0.000
<i>Desulfovibrio desulfuricans</i>	0.000	6.581	1.107	0.000	0.597	0.082
5. Soil bioremediation:						
<i>Dechloromonas sp.</i>	0.000	0.000	0.074	0.000	0.000	0.000
<i>Alicyclophilus denitrificans</i>	0.000	0.045	0.000	0.000	0.000	0.000
<i>Lysinibacillus alkalisoli</i>	0.000	0.201	0.000	0.000	0.000	10.989
<i>Desulfovibrio desulfuricans</i>	0.000	6.581	1.107	0.000	0.597	0.082

Table S6 Potential pathogenic microbial species abundance (relative abundance) in KPJ

Species	FKPJ0	FKPJ30	FKPJ60	LKPJ0	LKPJ30	LKPJ60
<i>Pseudomonas aeruginosa</i>	0.00	0.18	0.59	0.00	0.12	0.14
<i>Pseudomonas syringae</i>	0.00	0.00	0.11	0.00	0.00	0.00
<i>Stenotrophomonas maltophilia</i>	0.00	0.04	0.12	0.00	0.00	0.00
<i>Bacillus cereus</i>	0.04	0.00	0.00	0.00	0.00	0.00
<i>Klebsiella pneumoniae</i>	1.62	0.00	0.00	0.00	0.00	0.00
<i>Acinetobacter baumannii</i>	0.00	0.10	0.00	0.00	0.07	0.00
<i>Ascochyta rabiei</i>	0.00	0.04	0.00	0.00	0.00	0.00
<i>Beauveria bassiana</i>	0.07	0.08	0.04	0.00	0.09	0.00
<i>Bordetella bronchiseptica</i>	0.00	0.08	0.10	0.00	0.00	0.00
<i>Bordetella pertussis</i>	0.00	0.10	0.43	0.00	0.05	0.03
<i>Candida tropicalis</i>	0.00	0.13	0.00	0.00	0.00	0.00
<i>Clostridioides difficile</i>	0.07	0.00	0.05	0.07	0.11	0.04
<i>Clostridium perfringens</i>	0.00	0.00	0.00	0.02	0.07	0.00
<i>Enterococcus faecalis</i>	0.06	0.00	0.00	0.15	0.00	0.00
<i>Enterococcus faecium</i>	0.06	0.00	0.00	0.08	0.00	0.00
<i>Escherichia coli</i>	7.11	0.19	0.06	0.03	0.10	0.00
<i>Lactococcus lactis</i>	1.32	0.00	0.00	0.00	0.00	0.00
<i>Mycobacterium tuberculosis</i>	0.00	0.00	0.07	0.00	0.00	0.00
<i>Pseudomonas savastanoi</i>	0.00	0.00	0.04	0.00	0.00	0.00
<i>Rhizopus oryzae</i>	0.00	0.18	0.19	0.00	0.19	0.05
<i>Salmonella enterica</i>	0.17	0.00	0.00	0.00	0.00	0.00
<i>Shigella flexneri</i>	0.05	0.00	0.00	0.00	0.00	0.00
<i>Streptococcus pneumoniae</i>	0.04	0.00	0.00	0.05	0.05	0.03
<i>Streptococcus suis</i>	0.04	0.00	0.00	0.00	0.00	0.00
Cumulative relative abundance (%)	10.65	1.10	1.79	0.40	0.85	0.29

Table S7 The notable AMR gene families and AROs and their relative abundance in KPJ.

AMR gene family	FKPJ0	FKPJ30	FKPJ60	LKPJ0	LKPJ30	LKPJ60
ATP-binding cassette (ABC) antibiotic efflux pump	15.37	15.03	15.23	16.87	15.98	17.48
Major facilitator superfamily (MFS) antibiotic efflux pump	5.96	2.93	2.60	8.13	3.58	4.10
Elfamycin resistant EF-Tu	4.95	6.41	5.50	5.23	6.28	5.80
Fluoroquinolone resistant gyrA	3.67	4.66	4.56	6.77	4.83	5.02
Antibiotic resistant fusA	3.58	3.55	2.31	3.96	3.45	3.52
Resistance-nodulation-cell division (RND) antibiotic efflux pump	3.36	8.22	12.57	1.74	7.46	6.89
Rifamycin-resistant beta-subunit of RNA polymerase (rpoB)	2.78	4.00	3.43	3.83	4.05	3.41
ARO terms	FKPJ0	FKPJ30	FKPJ60	LKPJ0	LKPJ30	LKPJ60
ARO:3003735	3.57	3.55	2.31	3.95	3.45	3.52
ARO:3003438	2.82	1.54	1.54	1.90	1.92	1.86
ARO:3003291	2.73	0.99	0.67	1.79	1.20	1.14
ARO:3003287	2.73	0.41	0.22	1.76	0.68	0.59
ARO:3004681	2.25	2.99	1.95	1.94	2.74	2.87
ARO:3004049	1.01	2.45	2.62	0.74	1.94	2.34
ARO:3000535	1.94	2.20	2.42	2.65	2.60	2.66
ARO:3002522	0.62	1.98	1.77	0.83	1.80	2.04
ARO:3005008	0.71	1.75	2.44	0.36	1.63	1.88
ARO:3003950	1.90	1.76	2.32	1.84	1.63	2.52
ARO:3004721	0.75	1.46	1.28	2.92	1.14	1.27
ARO:3003737	1.41	1.25	0.95	2.61	1.45	1.34
ARO:3003974	0.00	0.04	0.07	2.61	0.02	0.03

Note: The relative abundance of AMR gene family or ARO was calculated using the formula: (Read abundance of specific AMR gene family or ARO in a sample) / (The total read abundance of AMR gene families or AROs in a sample) X 100.

Table S8 Properties of microbial co-occurrence network of KPJ

Network Properties	FKPJ	LKPJ
Nodes	428	305
Edges	16505	7005
Positive edges (%)	91.64	96.47
Negative edges (%)	8.36	3.53
Number of modules	11	14
Network diameter	37	34
Average degree	77.126	45.934
Average clustering coefficients	0.930	0.909
Average path length	14.626	15.519
Density	0.181	0.151