Text S1 Supplemental Materials and Methods

16S rRNA gene analysis

Genomic DNA was used as a template for PCR amplification targeting the V4 hypervariable region of the 16S rRNA gene to identify bacterial diversity. Barcoded primers 515F and 806R were employed for amplification. The PCR reaction mixture (20 μL) contained 10 μL Premix Taq, 1 μL each of forward and reverse primers (10 μmol/L), 1 μL template DNA (approximately 10–50 ng), and ddH_2O to a final volume (20 μL). The thermal cycling program consisted of initial denaturation at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s, with a final extension at 72 °C for 7 min.

PCR products were analyzed by 1% agarose gel electrophoresis and visualized using a gel imaging system to assess fragment length and concentration. Samples with clear target bands, free of non-specific amplification, were selected for downstream processing. Qualified PCR products were normalized using Gene Tools Analysis Software, and equal masses of each sample were pooled according to calculated volumes. The pooled amplicons were subsequently purified by gel extraction to obtain target DNA fragments.

Sequencing libraries were constructed according to the standard protocol of the NEBNext® Ultra™ II DNA Library Prep Kit for Illumina® (New England Biolabs, USA). The library preparation workflow included DNA fragmentation, end repair, A-tailing, adapter ligation, and PCR enrichment. DNA input quantities were strictly controlled (1–100 ng) to ensure library concentrations met sequencing requirements (≥2 nM). Library quality and concentration were assessed using an Agilent 2100 Bioanalyzer or a Qubit 3.0 fluorometer to confirm an appropriate fragment size distribution, with main peaks within the 300–500 bp range.

Paired-end sequencing (PE250) was performed on the Illumina NovaSeq 6000 platform (Guangdong Magigene Biotechnology Co., Ltd., Guangzhou, China). Sequencing depth was set to generate a minimum of 50,000 high-quality sequences per sample to ensure adequate coverage and data quality. Real-time monitoring of sequencing quality metrics was conducted, maintaining Q30 scores ≥85% and error rates ≤ 0.1%.

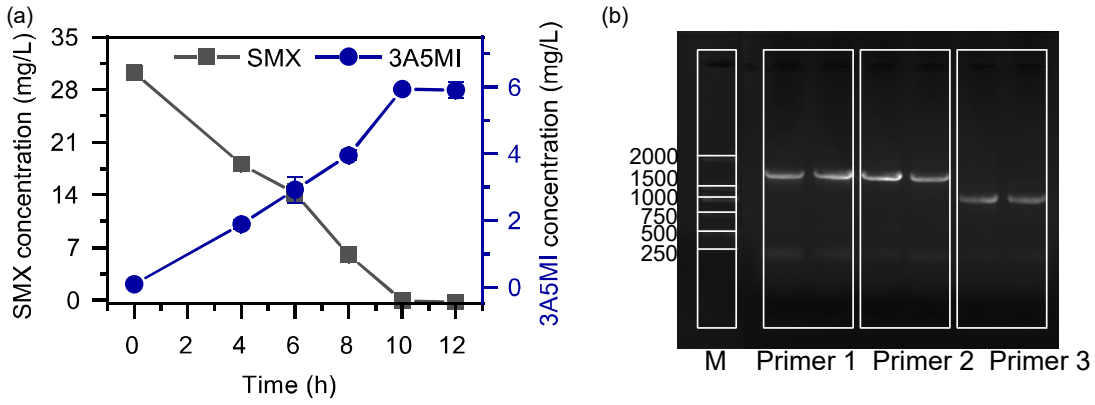

Fig. S1 (a) SMX biodegradation characteristics by *Paenarthrobacter* sp. strain M5 and (b) gel electrophoresis of SMX degradation gene *sadA* from strain M5

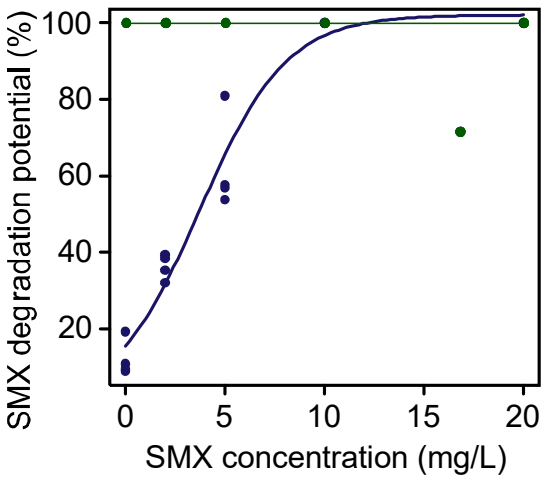

Fig. S2 *Ex situ* SMX degradation potential of naturally adapted (blue) and pre-adapted communities (green)

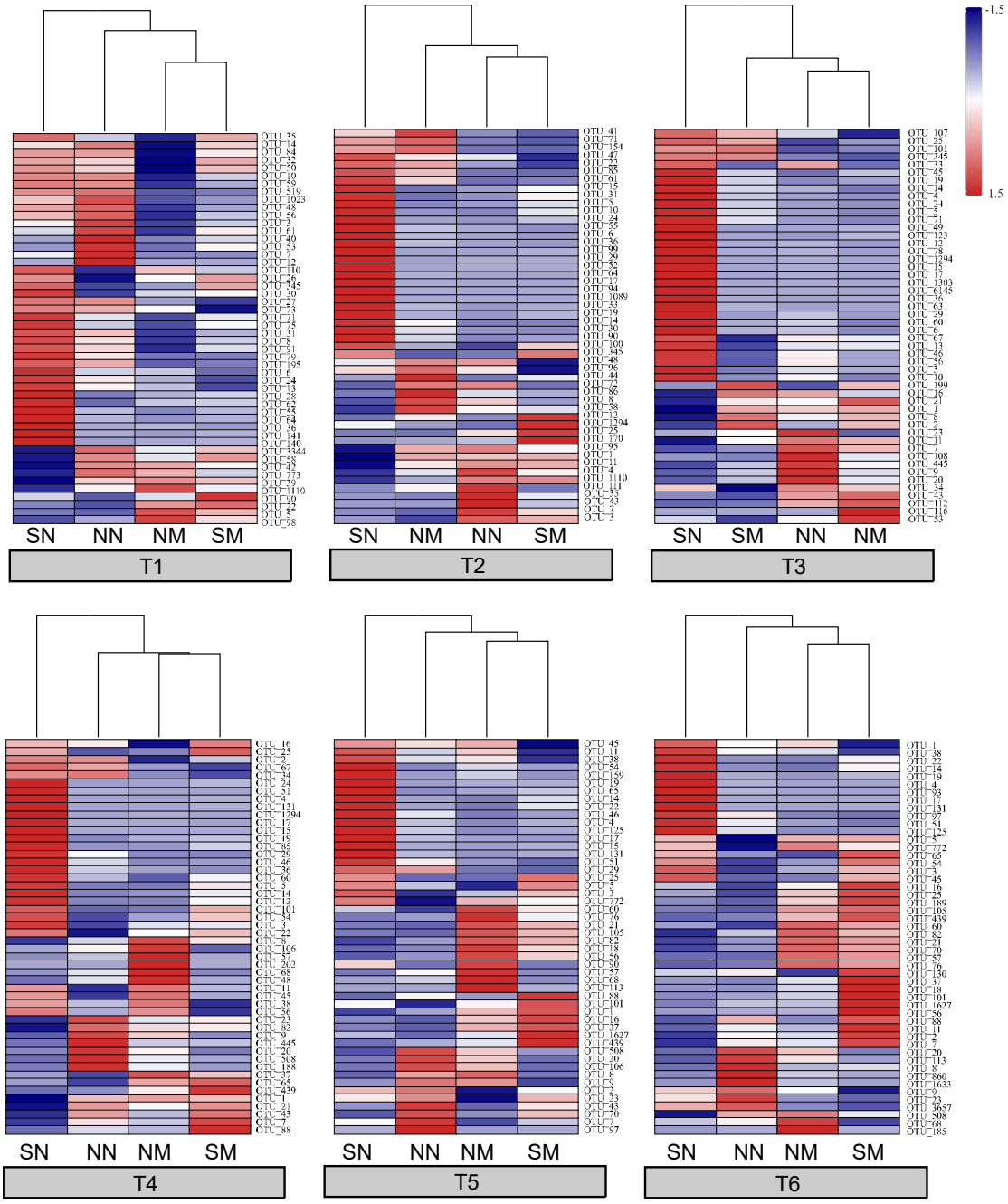

Fig. S3 Heatmap and hierarchical clustering of OTU abundance in different treatments at different time points

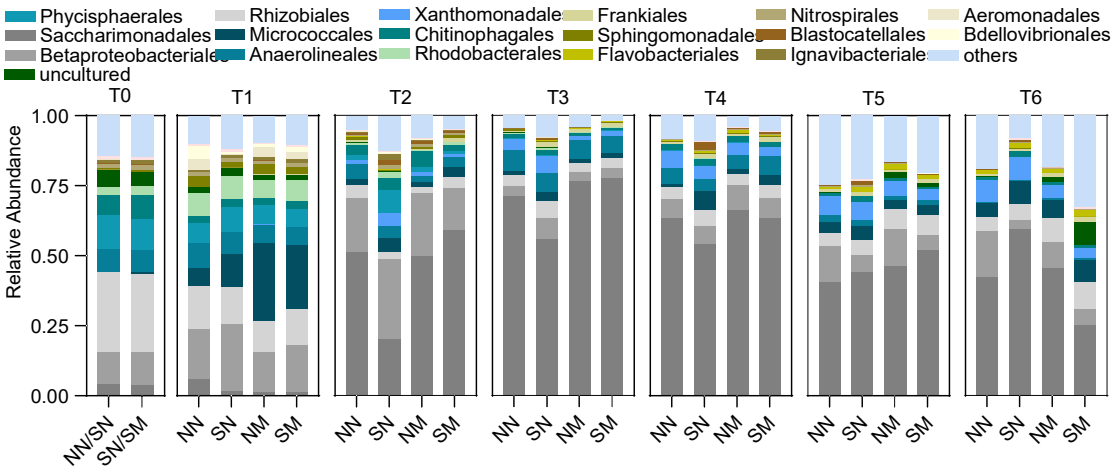

Fig. S4 Temporal dynamics of relative abundance at the order level in microbial communities of different treatment groups

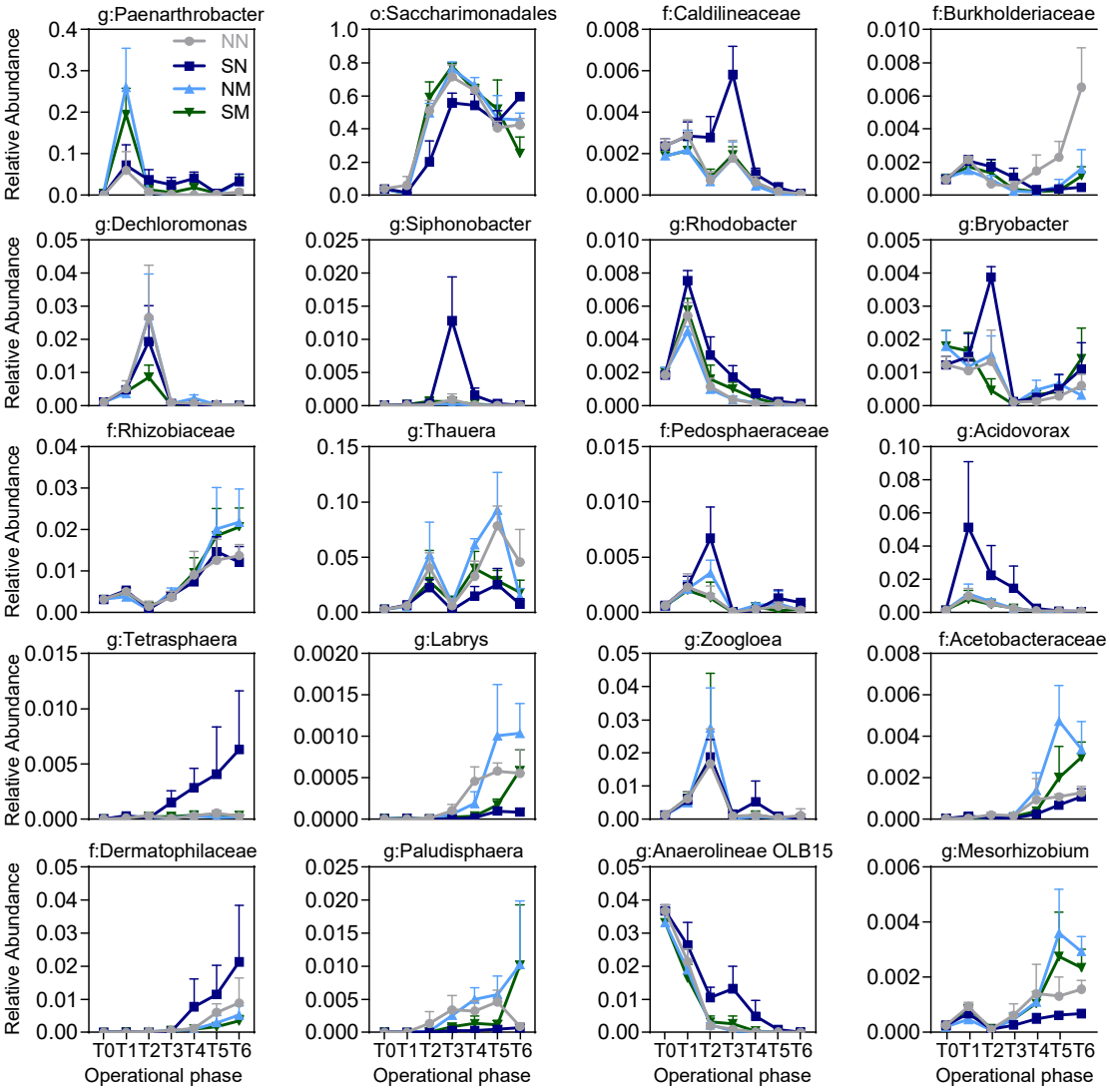

Fig. S5 Temporal dynamics of relative abundance of key genera based on the random forest algorithm in different treatment groups

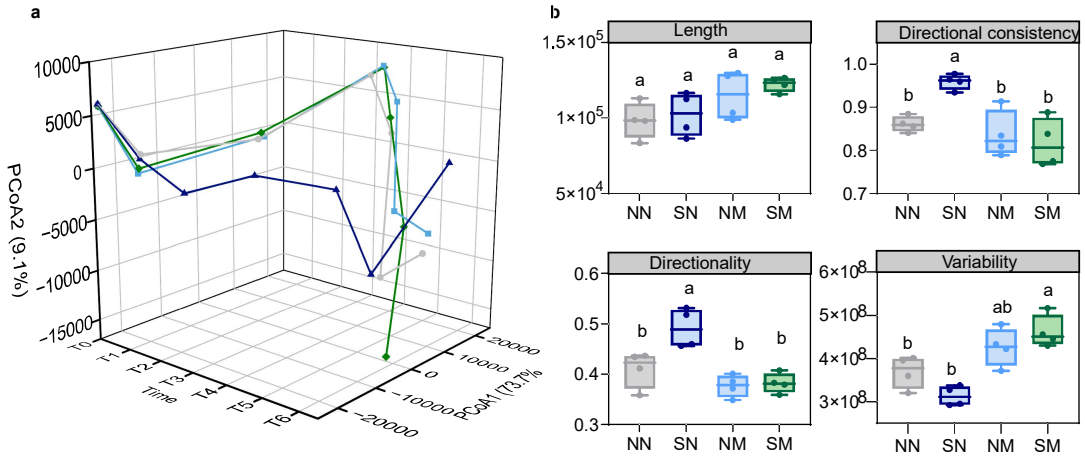

Fig. S6 (a) Community evolutionary trajectories and (b) statistical parameters based on Euclidean distance

Table S1 Adonis, Anosim, and MRPP analysis of microbial community structure differences across experimental phases

Inter-group difference	Stage	NN vs SN						NM vs SM						SN vs SM					
		Bray curtis		Euclidean		Jaccard		Braycurtis		Euclidean		Jaccard		Braycurtis		Euclidean		Jaccard	
		R^2	*p*	R^2	*p*	R^2	*p*	R^2	*p*	R^2	*p*	R^2	*p*	R^2	*p*	R^2	*p*	R^2	*p*
Adonis	T1	0.438	0.032	0.333	0.039	0.39	0.03	0.122	0.571	0.175	0.327	0.123	0.593	0.437	0.024	0.551	0.019	0.382	0.029
	T2	0.592	0.03	0.623	0.032	0.467	0.028	0.193	0.207	0.102	0.465	0.195	0.196	0.584	0.03	0.616	0.033	0.456	0.032
	T3	0.622	0.029	0.764	0.032	0.531	0.026	0.223	0.108	0.083	0.626	0.219	0.125	0.66	0.031	0.817	0.021	0.582	0.028
	T4	0.607	0.03	0.757	0.034	0.531	0.022	0.339	0.066	0.248	0.151	0.315	0.074	0.557	0.03	0.75	0.035	0.495	0.027
	T5	0.358	0.023	0.306	0.03	0.33	0.025	0.214	0.207	0.092	0.531	0.19	0.227	0.301	0.113	0.262	0.189	0.263	0.06
	T6	0.582	0.029	0.631	0.031	0.521	0.042	0.546	0.035	0.664	0.023	0.468	0.027	0.754	0.03	0.837	0.029	0.655	0.038
Anosim		R	*p*	R	*p*	R	*p*	R	*p*	R	*p*	R	*p*	R	*p*	R	*p*	R	*p*
	T1	0.906	0.026	0.562	0.042	0.906	0.036	-0.083	0.648	-0.104	0.883	-0.083	0.664	0.708	0.028	0.708	0.025	0.708	0.029
	T2	1	0.036	0.01	0.313	1	0.036	0.073	0.207	0.042	0.386	0.073	0.201	0.146	0.292	0.979	0.033	0.146	0.26
	T3	0.854	0.033	0.938	0.028	0.854	0.029	0.26	0.104	-0.01	0.528	0.26	0.088	0.448	0.026	0.25	0.101	0.448	0.026
	T4	0.979	0.034	1	0.024	0.979	0.028	0.573	0.062	0.25	0.116	0.573	0.069	0.542	0.03	0.083	0.405	0.542	0.027
	T5	0.542	0.029	0.417	0.029	0.542	0.031	0.125	0.247	-0.073	0.585	0.125	0.253	0.375	0.036	0.177	0.103	0.375	0.03
	T6	0.948	0.031	0.823	0.032	0.948	0.026	0.865	0.026	0.823	0.031	0.865	0.024	0.688	0.027	0.562	0.023	0.688	0.028
MRPP		δ	*p*	δ	*p*	δ	*p*	δ	*p*	δ	*p*	δ	*p*	δ	*p*	δ	*p*	δ	*p*
	T1	0.249	0.039	6853	0.027	0.395	0.025	0.206	0.521	7334	0.318	0.339	0.525	0.235	0.033	8119	0.035	0.377	0.037
	T2	0.566	0.027	20495	0.024	0.709	0.028	0.34	0.213	12797	0.294	0.531	0.036	0.583	0.025	21533	0.025	0.722	0.036
	T3	0.522	0.037	27435	0.026	0.654	0.037	0.101	0.11	3724	0.662	0.183	0.127	0.516	0.029	28832	0.026	0.636	0.033
	T4	0.351	0.026	15172	0.028	0.507	0.032	0.153	0.053	5295	0.118	0.264	0.046	0.328	0.029	14981	0.026	0.483	0.024
	T5	0.242	0.029	8320	0.021	0.384	0.03	0.301	0.265	15343	0.582	0.455	0.294	0.306	0.026	14883	0.218	0.461	0.035
	T6	0.275	0.029	10936	0.046	0.424	0.037	0.296	0.033	13735	0.022	0.448	0.028	0.368	0.029	18011	0.021	0.518	0.031