

Microbial diversity and substrate properties drive the decomposition of spent mushroom substrate under nitrogen management

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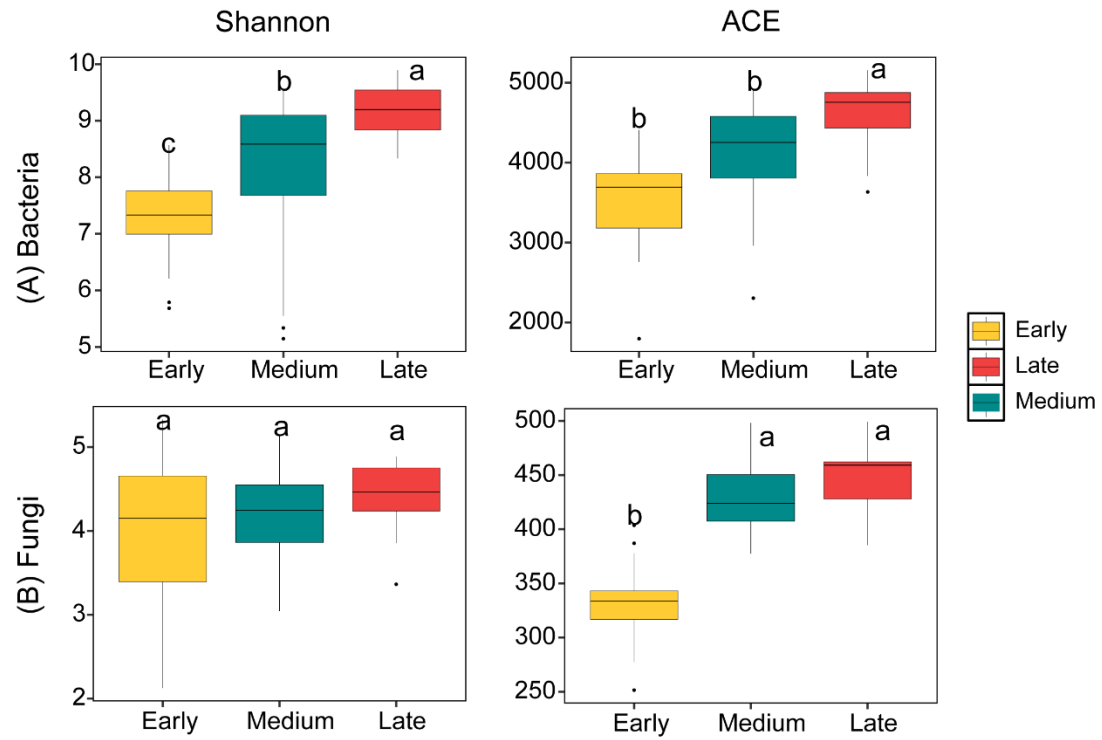


Fig. S1. Alpha diversity of bacterial and fungal communities across decomposition stages. Early (day 15), Medium (day 50), Late (day 110). Values are means $\pm$ SD ($n = 3$). Different letters indicate significant differences among stages ($P < 0.05$, one-way ANOVA with Tukey's HSD).

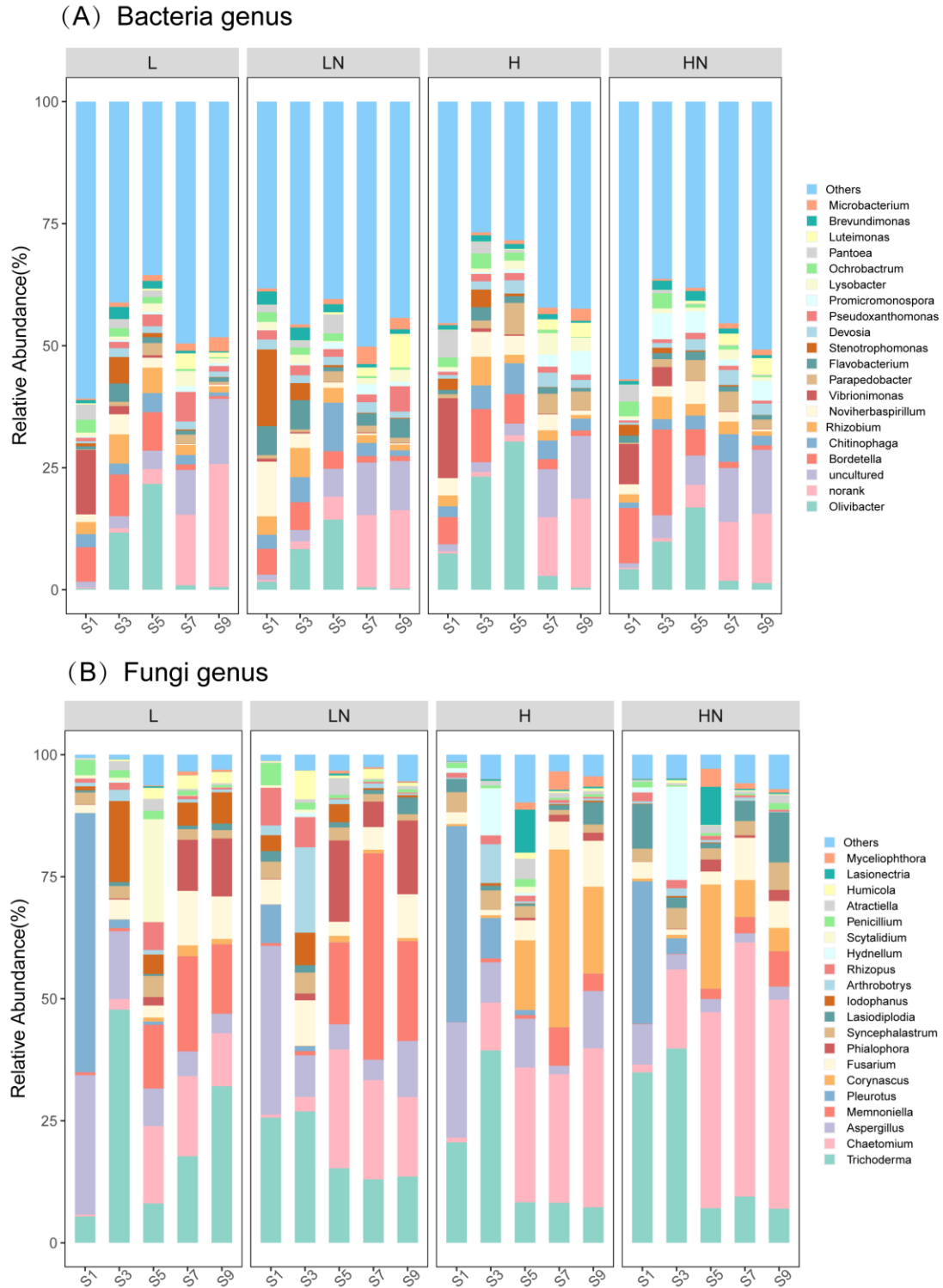


Fig. S2. Horizontal species composition of bacteria and fungi during decomposition of SMS. H: high SMS; HN: high SMS + N; L: low SMS; LN: low SMS + N. Sampling times: S1–S9 (days 5, 10, 15, 20, 35, 50, 70, 90, and 110). The top 20 genus are color-coded as shown in the legend. Relative abundance is based on 16S rRNA and ITS amplicon sequencing (n = 3).

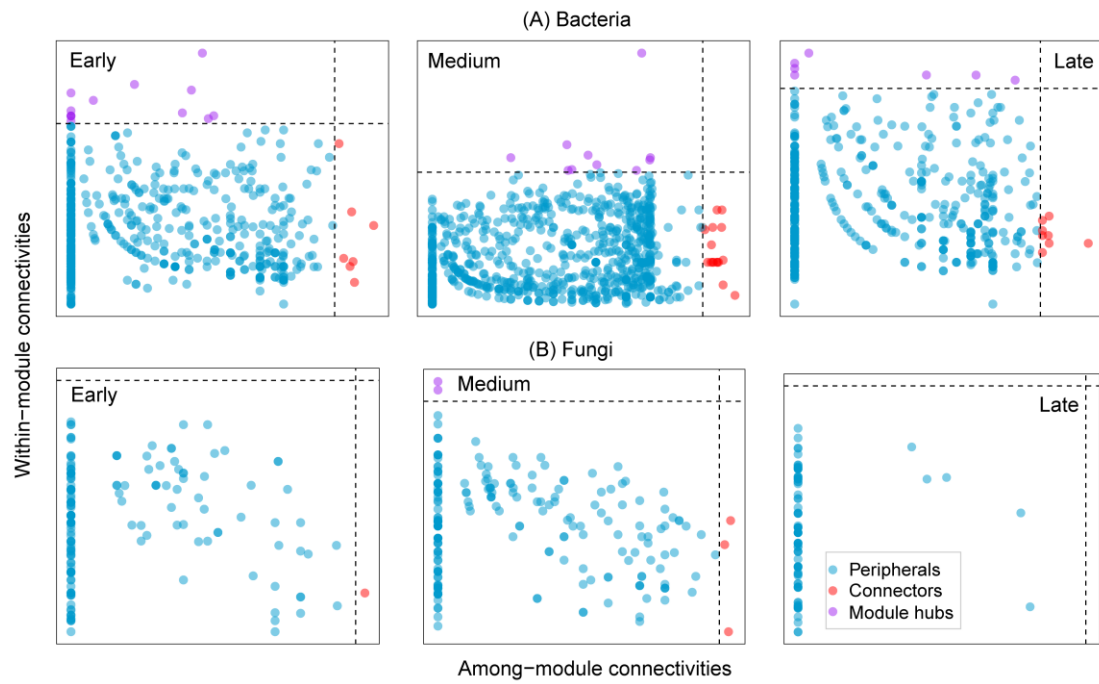


Fig. S3. Key species in the process of SMS decomposition. Stages: Early (day 15), Medium (day 50), Late (day 110). Zi: within-module connectivity; Pi: among-module connectivity. The dashed lines at $Z_i = 2.5$ and $P_i = 0.62$ define four node categories: peripherals (lower left), connectors (lower right), module hubs (upper left), and network hubs (upper right). Each symbol represents an OTU ($n = 3$ replicates per stage).

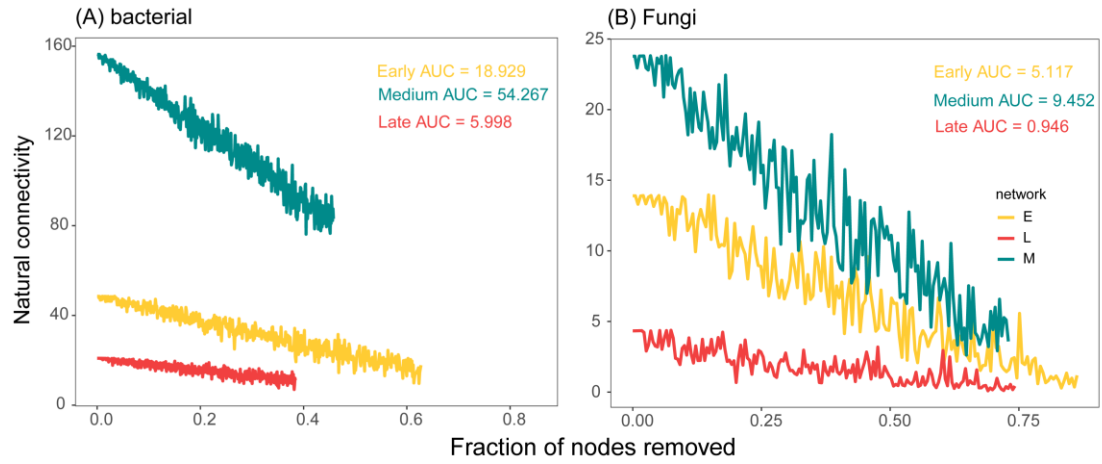


Fig. S4. Robustness of bacterial and fungal co-occurrence networks to random node removal across SMS decomposition stages. Stages: Early (day 15), Medium (day 50), Late (day 110). AUC values were calculated from natural connectivity decay curves; higher AUC = greater robustness. Data are based on networks constructed at each stage (Spearman, $|r| > 0.7$, $P < 0.01$).

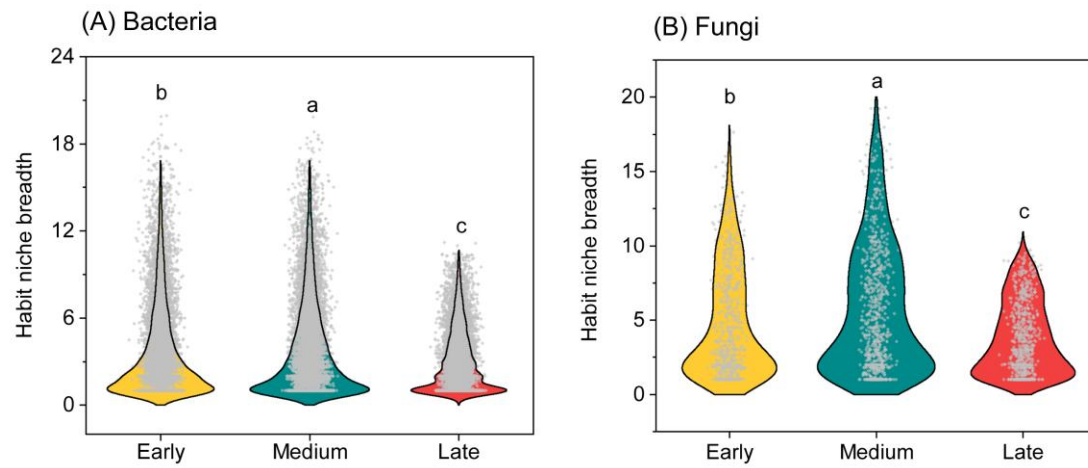


Fig. S5. Niche breadth of microbial community during the decomposition of SMS. Stages: Early (day 15), Medium (day 50), Late (day 110). Values are means $\pm$ SD ($n = 3$). Different lowercase letters indicate significant differences among stages ($P < 0.05$; one-way ANOVA with Tukey's HSD).

Table S1. Statistical analysis of significant differences in biomass loss and lignocellulose during various degradation stages of SMS by One-way ANOVA.

	Group	Early	Middle	Late	F-value	p-value
DR	H	5.06 ± 0.76a	3.69 ± 0.86b	2.04 ± 0.22c	25.893	0.001**
	HN	4.78 ± 0.22a	2.71 ± 0.72b	1.67 ± 0.13c	48.326	<0.001***
	L	6.19 ± 0.49a	3.87 ± 1.28b	1.94 ± 0.27c	22.674	0.002**
	LN	5.53 ± 0.34a	3.36 ± 1.09b	1.76 ± 0.28c	26.893	0.001**
BL	H	14.14 ± 7.65a	33.12 ± 8.04b	49.44 ± 6.26c	38.924	<0.001***
	HN	13.25 ± 7.56a	24.09 ± 4.83b	41.45 ± 7.73c	25.673	0.001**
	L	16.91 ± 8.07a	33.71 ± 4.85b	46.93 ± 3.69c	41.256	<0.001***
	LN	14.85 ± 6.84a	29.29 ± 4.25b	42.50 ± 2.53c	52.189	<0.001***
Hemicellulose	H	11.26 ± 1.87a	7.68 ± 2.52b	5.53 ± 0.60c	15.237	0.004**
	HN	11.41 ± 2.30a	10.47 ± 2.36ab	6.12 ± 0.94b	12.456	0.007**
	L	10.11 ± 1.42a	6.57 ± 1.25b	6.08 ± 0.40b	18.923	0.002**
	LN	10.01 ± 1.71a	9.07 ± 1.56ab	5.75 ± 0.85b	14.672	0.005**
Cellulose	H	27.99 ± 5.16	25.50 ± 0.70	22.33 ± 3.62	4.128	0.073
	HN	28.53 ± 5.76	26.30 ± 1.01	26.66 ± 1.00	1.845	0.239
	L	30.13 ± 1.31a	26.96 ± 1.15b	24.39 ± 1.07c	27.346	0.001**
	LN	31.74 ± 5.44a	25.31 ± 0.38b	27.87 ± 0.87ab	7.892	0.021*
Lignin	H	42.45 ± 9.40	45.48 ± 6.24	48.46 ± 4.07	1.245	0.352
	HN	40.81 ± 10.59	41.70 ± 4.07	44.48 ± 1.87	0.567	0.594
	L	38.46 ± 6.89	41.64 ± 0.25	42.59 ± 1.83	1.784	0.248
	LN	33.70 ± 7.69a	43.81 ± 2.14b	41.18 ± 1.43b	7.234	0.025

Data are presented as mean values ± standard deviation (n = 3). Lowercase letters indicate statistically significant differences among decomposition stages ($P < 0.05$). DR: decomposition rate; BL: biomass loss; H: High biomass; HN: High biomass+N; L: Low biomass; LN: Low biomass+N. * indicates a significant difference: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table S2. Statistical Analysis of Significant Differences in Biomass Loss and Lignocellulose among Treatments Using One-Way ANOVA.

	Period	H	HN	L	LN	F-value	p-value
DR	Early	5.06 ± 0.67a	4.78 ± 0.22b	6.19 ± 0.49a	5.53 ± 0.34ab	4.237	0.045*
	Middle	3.69 ± 0.86a	2.71 ± 0.72b	3.87 ± 1.28a	3.36 ± 1.09ab	5.892	0.018*
	Late	2.04 ± 0.22	1.67 ± 0.13	1.94 ± 0.27	1.76 ± 0.28	1.987	0.198
BL	Early	14.14 ± 7.65	13.25 ± 7.56	16.91 ± 8.07	14.85 ± 6.84	0.423	0.742
	Middle	33.12 ± 8.04a	24.09 ± 4.83b	33.71 ± 4.85a	29.29 ± 4.25ab	4.256	0.046*
	Late	49.44 ± 6.26	41.45 ± 7.73	46.93 ± 4.04	42.50 ± 2.95	3.892	0.056
Hemicellulose	Early	27.99 ± 5.16	28.53 ± 5.76	30.13 ± 1.31	31.73 ± 5.44	1.234	0.358
	Middle	25.50 ± 0.70	26.30 ± 1.01	26.96 ± 1.15	25.31 ± 0.38	2.845	0.106
	Late	22.33 ± 3.62a	26.66 ± 0.97b	24.39 ± 1.07ab	27.87 ± 0.87b	9.423	0.005**
Cellulose	Early	11.26 ± 1.87	11.41 ± 2.30	10.11 ± 1.42	10.01 ± 1.71	0.845	0.508
	Middle	7.68 ± 2.52a	10.47 ± 2.36b	6.57 ± 1.25a	9.07 ± 1.56ab	4.236	0.045*
	Late	5.53 ± 0.60	6.12 ± 0.94	6.08 ± 0.40	5.75 ± 0.85	1.234	0.358
Lignin	Early	42.45 ± 9.40	40.81 ± 9.59	38.46 ± 6.89	34.03 ± 7.69	1.645	0.256
	Middle	45.48 ± 6.24	41.70 ± 4.07	41.64 ± 0.26	43.81 ± 2.14	1.023	0.435
	Late	48.46 ± 4.07a	44.48 ± 1.73ab	42.59 ± 1.83b	41.18 ± 1.69b	4.892	0.033*

Data are presented as mean values ± standard deviation (n = 3). Lowercase letters indicate significant differences among treatments ($P < 0.05$). DR: decomposition rate; BL: biomass loss; H: High biomass; HN: High biomass+N; L: Low biomass; LN: Low biomass+N. * indicates a significant difference: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table S3. Standardized direct effects of predictor variables on endogenous variables in the SEM

Response Variable	Predictor Variable	Df	Std.Estimate	P Value	Significance
Enzyme	Community structure	53	0.920	< 0.001	***
Enzyme	Community diversity	53	-0.048	0.697	ns
Community structure	Community diversity	54	0.204	0.018	*
Residue property	Community Structure	53	-0.356	0.036	*
Residue property	Enzyme	53	0.146	0.186	ns
Decomposition Rate	Community diversity	51	0.444	< 0.001	***
Decomposition Rate	Enzyme	51	-0.050	0.392	ns
Decomposition Rate	Residue property	51	0.515	< 0.001	***

*Significance codes: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, ns = not significant.