

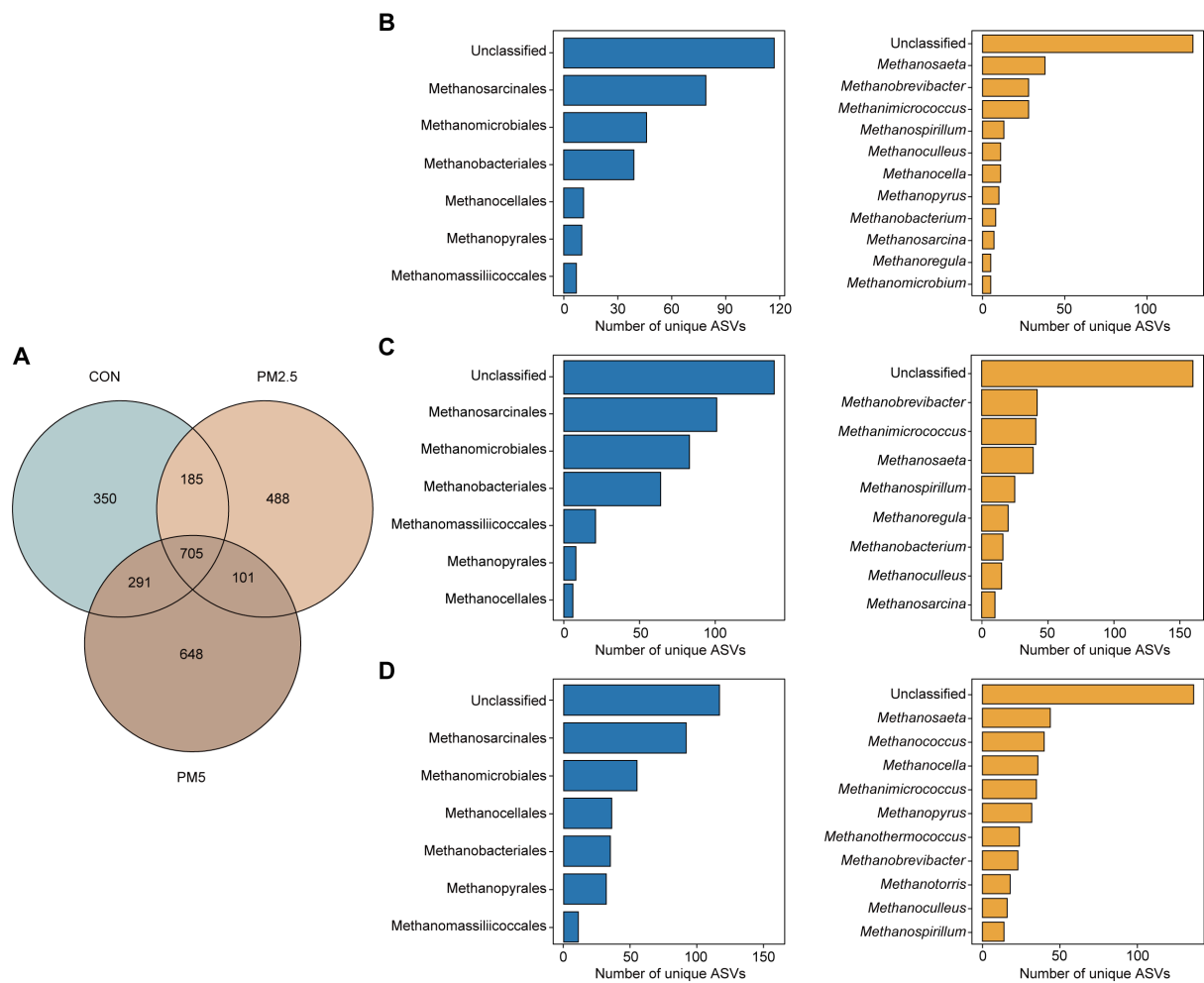


Fig. S1. Venn diagram of archaeal ASVs and taxonomic composition of group-specific taxa. **A**, Venn diagram showing the shared and unique archaeal ASVs among the CON, PM2.5, and PM5 groups. **B-D**, Bar plots illustrating the taxonomic distribution of specific ASVs unique to the CON (**B**), PM2.5 (**C**), and PM5 (**D**) groups. Blue bars represent the order level, and yellow bars represent the genus level.

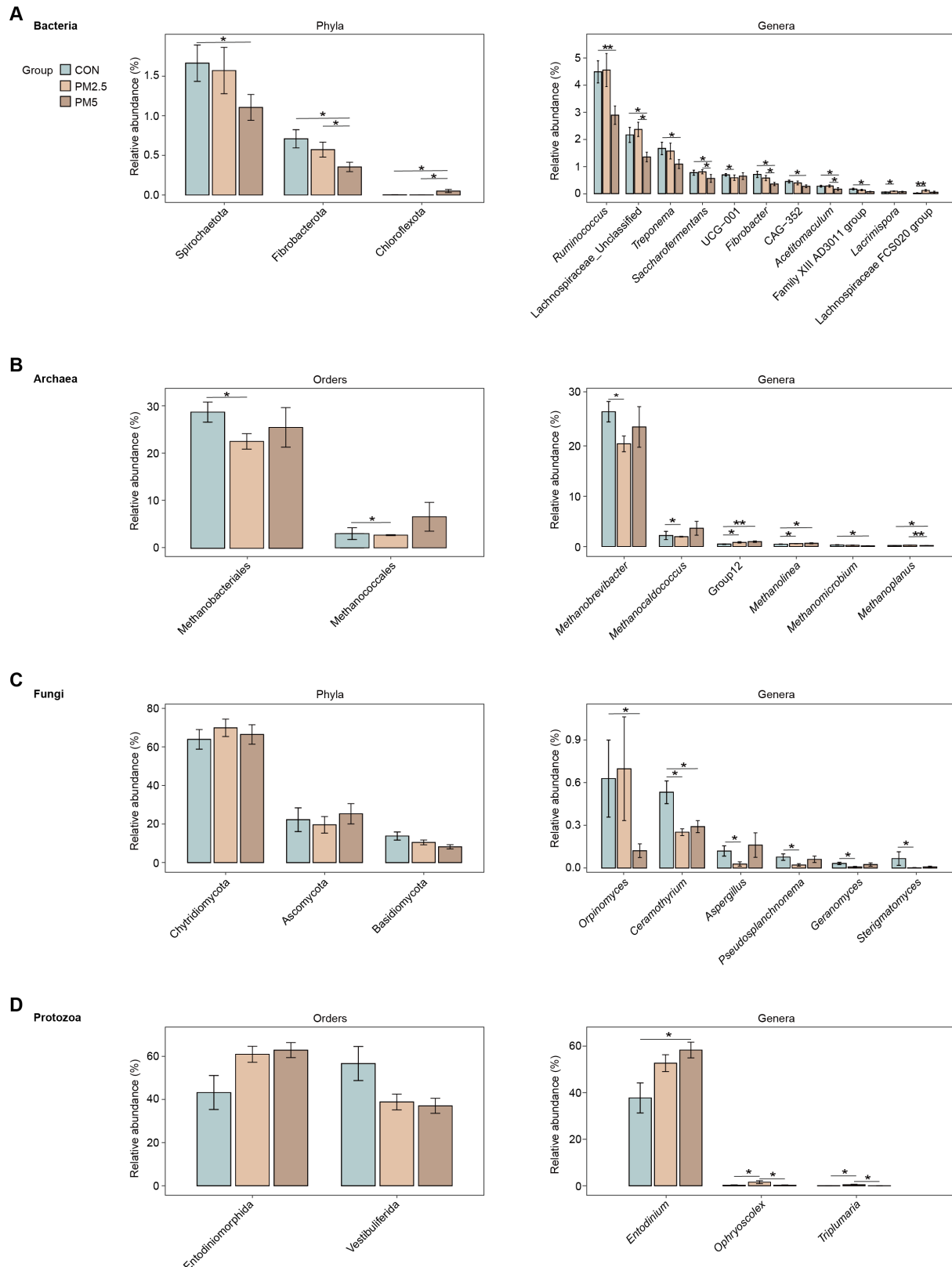


Fig. S2. Differential relative abundances of microbial communities across treatments. **A**, Bacterial differential analysis at the phylum and genus levels. **B**, Archaeal differential analysis at the order and genus levels. **C**, Fungal differential analysis at the phylum and genus levels. **D**, Protozoal differential analysis at the order and genus levels. Significant differences are indicated as $*P < 0.05$, $**P < 0.01$ (Wilcoxon rank-sum test). Data are presented as means with SEM. SEM, standard error of the mean.

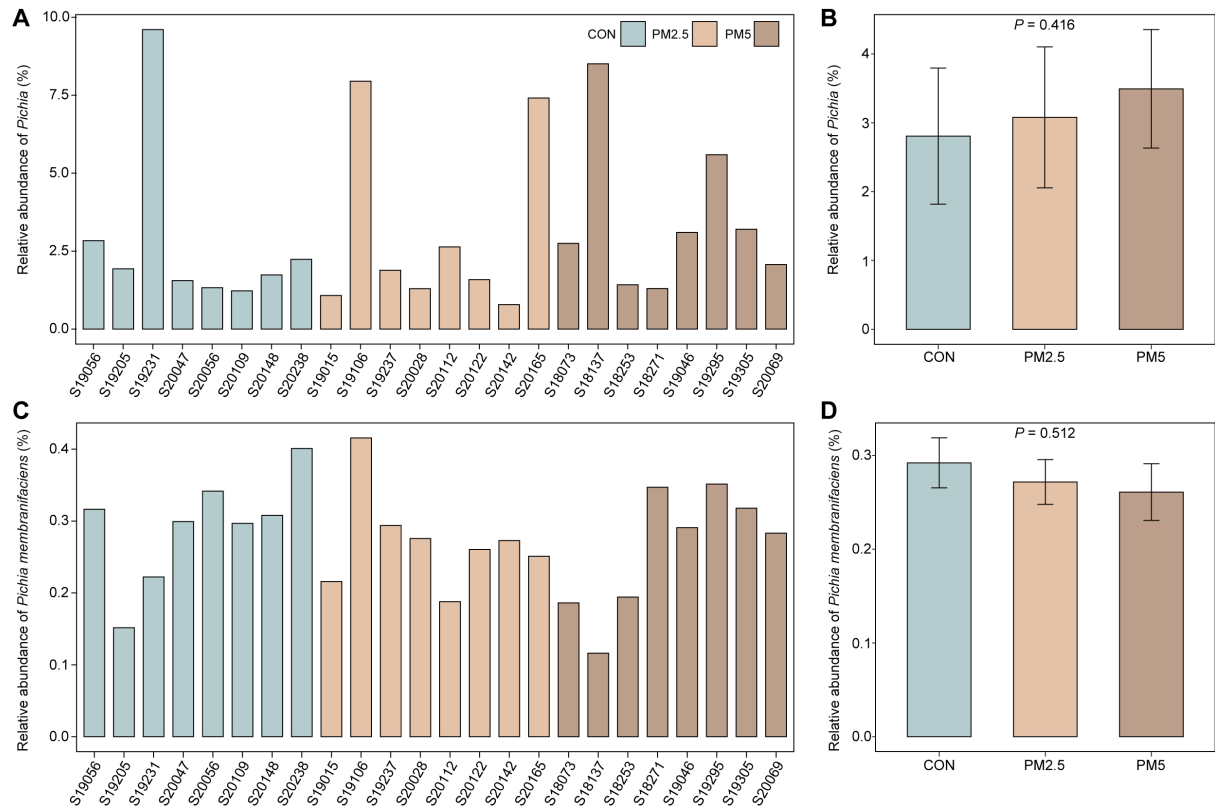


Fig. S3. Relative abundance of the genus *Pichia* and the species *Pichia membranifaciens* across experimental groups. **A**, The relative abundance of the genus *Pichia* in individual samples, as determined by 18S rRNA amplicon sequencing. **B**, Group-level comparison of the relative abundance of the genus *Pichia* among the CON, PM2.5, and PM5 groups. **C**, The relative abundance of *Pichia membranifaciens* in individual samples, as determined by metagenomic sequencing. **D**, Group-level comparison of the relative abundance of *Pichia membranifaciens* among the experimental groups. For panels B and D, data are presented as means $\pm$ SEM. SEM, standard error of the mean. The *P*-values indicate statistical significance among the groups, determined using the Wilcoxon rank-sum test.

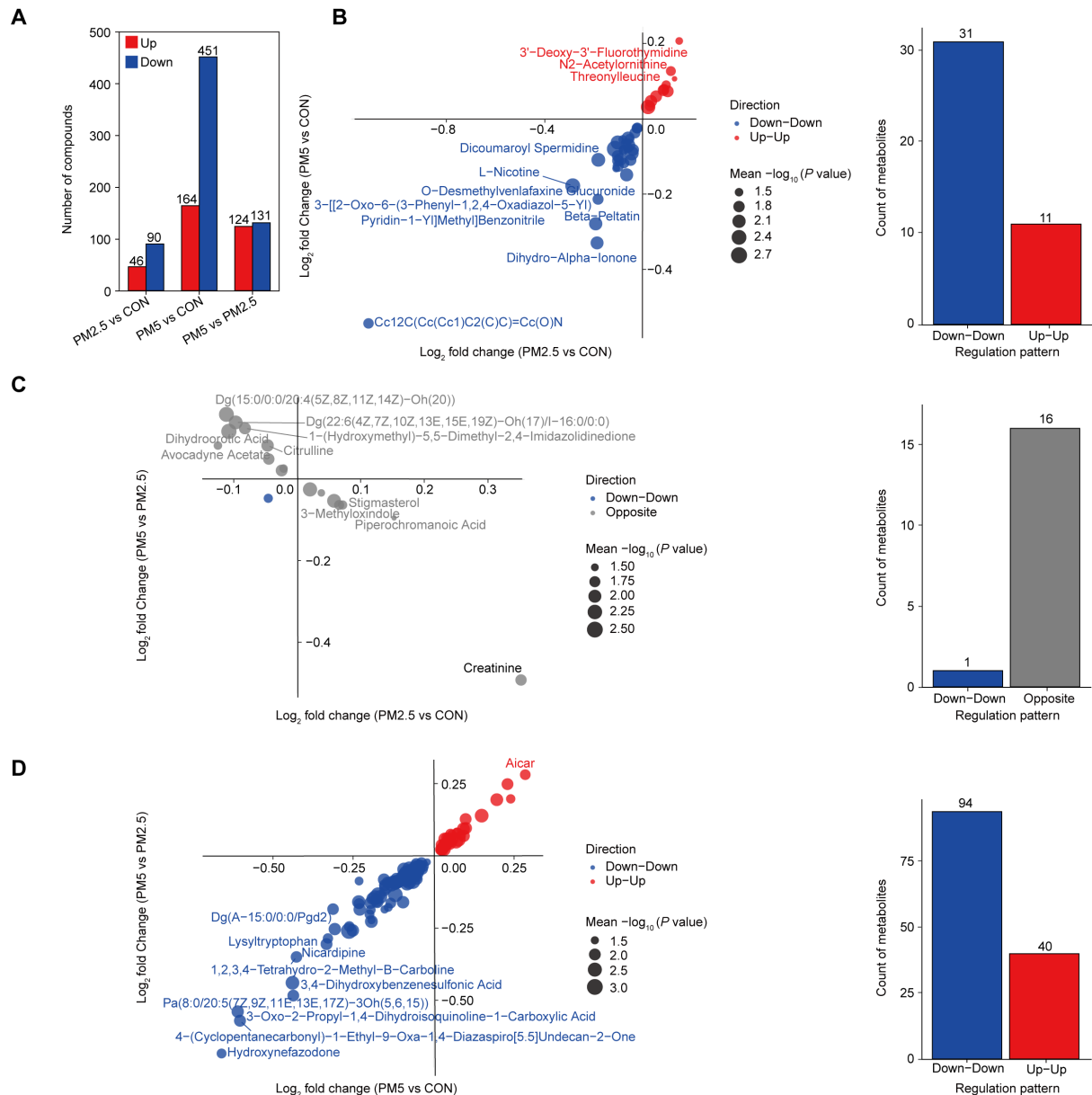


Fig. S4. Differential metabolites and regulation patterns across treatments. **A**, Numbers of significantly upregulated and downregulated metabolites for each pairwise comparison. **B-D**, Two-dimensional $\log_2$ fold change scatter plots combined with bar charts summarizing regulation patterns. **B**, common differential metabolites between PM2.5 vs CON and PM5 vs CON; **C**, PM2.5 vs CON and PM2.5 vs PM5; **D**, shows PM5 vs CON and PM2.5 vs PM5. Red dots indicate metabolites upregulated in both comparisons (Up-Up), blue dots downregulated in both (Down-Down), and gray dots opposite regulation trends (Opposite). Bubble size represents average $-\log_{10}(P \text{ value})$. Bar charts summarize numbers of metabolites in each regulatory pattern.

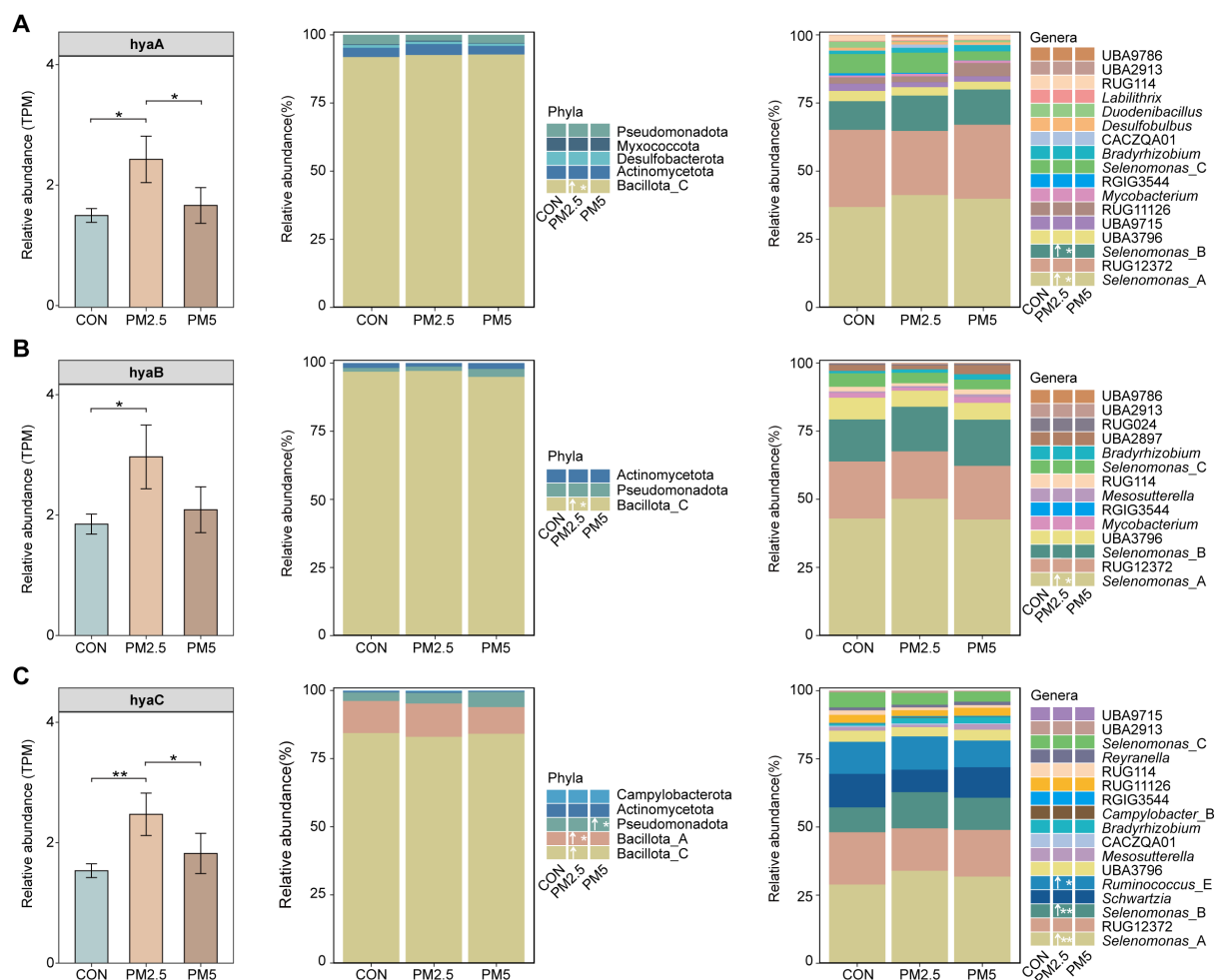


Fig. S5. Relative abundance and taxonomic composition of hydrogenase genes. A-C, Relative abundance of *hyaA* (A), *hyaB* (B), and *hyaC* (C) genes (left), with phylum- and genus-level contributions (middle and right) across CON, PM2.5, and PM5 groups. Upward arrows indicate significant increases, while downward arrows indicate significant decreases in the treatment groups compared with the CON group. Significant differences are indicated as $*P < 0.05$ and $**P < 0.01$ (Wilcoxon rank-sum test). Data in the bar charts are presented as mean $\pm$ SEM. SEM, standard error of the mean.

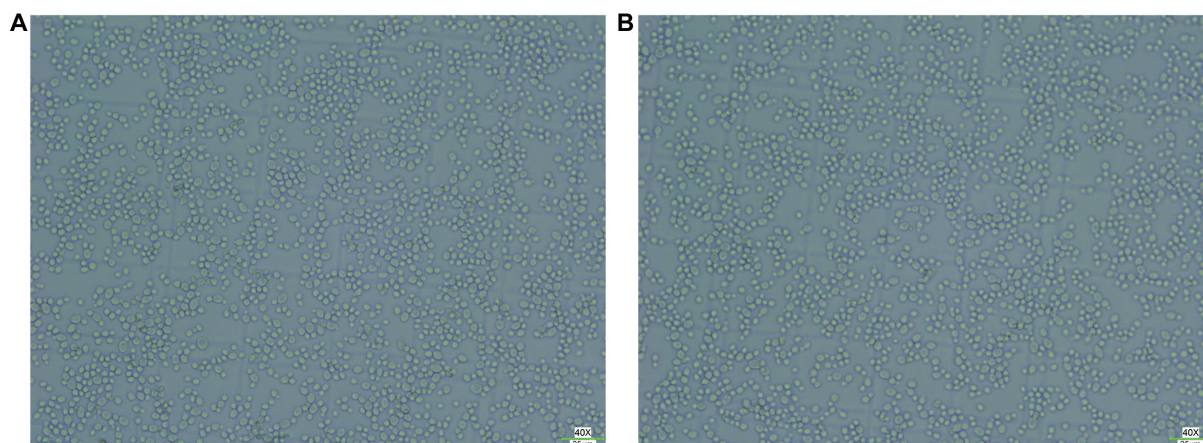


Fig. S6. Viability assessment of *Pichia membranifaciens* M12 prior to experimental administration. **A**, Prior to *in vitro* inoculation, the viability of the yeast suspension was assessed using 1% methylene blue staining. **B**, Prior to daily *in vivo* administration, the viability of the prepared yeast culture was verified using 1% methylene blue staining.

Table S1. List of the 73 yeast strains isolated from rumen samples.

Strains ID	Species	Genus	Subsequent experiments ¹
A1	<i>Candida catenulata</i>	<i>Candida</i>	–
A7	<i>Candida catenulata</i>	<i>Candida</i>	–
A15	<i>Candida catenulata</i>	<i>Candida</i>	–
B1	<i>Saccharomyces cerevisiae</i>	<i>Saccharomyces</i>	–
B2	<i>Trichosporon asahii</i>	<i>Trichosporon</i>	+
B3	<i>Pichia kudriavzevii</i>	<i>Pichia</i>	–
B5	<i>Pichia membranifaciens</i>	<i>Pichia</i>	–
B6	<i>Pichia kudriavzevii</i>	<i>Pichia</i>	–
B8	<i>Candida catenulata</i>	<i>Candida</i>	–
B12	<i>Pichia membranifaciens</i>	<i>Pichia</i>	–
B13	<i>Candida tropicalis</i>	<i>Candida</i>	+
B14	<i>Candida catenulata</i>	<i>Candida</i>	–
B15	<i>Candida catenulata</i>	<i>Candida</i>	–
B17	<i>Geotrichum candidum</i>	<i>Geotrichum</i>	+
B21	<i>Candida catenulata</i>	<i>Candida</i>	–
B22	<i>Candida catenulata</i>	<i>Candida</i>	–
B24	<i>Saccharomyces mikatae</i>	<i>Saccharomyces</i>	+
B26	<i>Saccharomyces cerevisiae</i>	<i>Saccharomyces</i>	+
B28	<i>Pichia membranifaciens</i>	<i>Pichia</i>	–
B29	<i>Candida catenulata</i>	<i>Candida</i>	–
B30	<i>Candida catenulata</i>	<i>Candida</i>	–
B32	<i>Candida catenulata</i>	<i>Candida</i>	–
B33	<i>Candida catenulata</i>	<i>Candida</i>	–
B34	<i>Candida catenulata</i>	<i>Candida</i>	–
B35	<i>Candida catenulata</i>	<i>Candida</i>	–
B36	<i>Candida catenulata</i>	<i>Candida</i>	–
B37	<i>Pichia kudriavzevii</i>	<i>Pichia</i>	–
B38	<i>Pichia kudriavzevii</i>	<i>Pichia</i>	–
B39	<i>Pichia membranifaciens</i>	<i>Pichia</i>	–
B41	<i>Candida catenulata</i>	<i>Candida</i>	–
B42	<i>Candida catenulata</i>	<i>Candida</i>	–
B43	<i>Candida catenulata</i>	<i>Candida</i>	–
B44	<i>Candida catenulata</i>	<i>Candida</i>	–
B45	<i>Candida catenulata</i>	<i>Candida</i>	–
B46	<i>Pichia membranifaciens</i>	<i>Pichia</i>	–
B49	<i>Cyberlindnera fabianii</i>	<i>Cyberlindnera</i>	+
B50	<i>Candida catenulata</i>	<i>Candida</i>	–
B51	<i>Pichia membranifaciens</i>	<i>Pichia</i>	–
B53	<i>Pichia membranifaciens</i>	<i>Pichia</i>	–
B54	<i>Pichia membranifaciens</i>	<i>Pichia</i>	–
B55	<i>Pichia kudriavzevii</i>	<i>Pichia</i>	–
B56	<i>Saccharomyces cerevisiae</i>	<i>Saccharomyces</i>	–
B59	<i>Candida catenulata</i>	<i>Candida</i>	–
B61	<i>Candida catenulata</i>	<i>Candida</i>	–
M1	<i>Pichia membranifaciens</i>	<i>Pichia</i>	–
M2	<i>Pichia kudriavzevii</i>	<i>Pichia</i>	–
M3	<i>Pichia kudriavzevii</i>	<i>Pichia</i>	–

M4	<i>Candida catenulata</i>	<i>Candida</i>	–
M5	<i>Pichia kudriavzevii</i>	<i>Pichia</i>	–
M6	<i>Pichia kudriavzevii</i>	<i>Pichia</i>	–
M7	<i>Cyberlindnera jadinii</i>	<i>Cyberlindnera</i>	+
M8	<i>Pichia kudriavzevii</i>	<i>Pichia</i>	–
M9	<i>Candida catenulata</i>	<i>Candida</i>	–
M10	<i>Candida catenulata</i>	<i>Candida</i>	–
M11	<i>Candida catenulata</i>	<i>Candida</i>	–
M12	<i>Pichia membranifaciens</i>	<i>Pichia</i>	+
M13	<i>Candida catenulata</i>	<i>Candida</i>	–
M14	<i>Candida catenulata</i>	<i>Candida</i>	–
M17	<i>Candida catenulata</i>	<i>Candida</i>	–
M18	<i>Pichia kudriavzevii</i>	<i>Pichia</i>	–
M19	<i>Saccharomyces cerevisiae</i>	<i>Saccharomyces</i>	–
Y1	<i>Pichia kudriavzevii</i>	<i>Pichia</i>	–
Y2	<i>Candida catenulata</i>	<i>Candida</i>	–
Y3	<i>Wickerhamomyces anomalus</i>	<i>Wickerhamomyces</i>	+
Y4	<i>Pichia kudriavzevii</i>	<i>Pichia</i>	+
Y6	<i>Pichia kudriavzevii</i>	<i>Pichia</i>	–
Y7	<i>Pichia kudriavzevii</i>	<i>Pichia</i>	–
Y9	<i>Candida catenulata</i>	<i>Candida</i>	+
Y10	<i>Pichia kudriavzevii</i>	<i>Pichia</i>	–
Y11	<i>Pichia kudriavzevii</i>	<i>Pichia</i>	–
Y12	<i>Pichia kudriavzevii</i>	<i>Pichia</i>	–
Y13	<i>Candida catenulata</i>	<i>Candida</i>	–
Y14	<i>Candida catenulata</i>	<i>Candida</i>	–

¹ “+” indicates strains selected for downstream experiments.

Table S2. Effects of different rumen-derived yeasts on rumen fermentation parameters *in vitro*.

Items	CON	B2	Y3	Y4	M7	Y9	M12	B13	B17	B24	B26	B49	SEM	P value ¹
pH	6.12 ^e	6.24 ^{bcd}	6.23 ^{cd}	6.24 ^{bcd}	6.24 ^{bcd}	6.30 ^{ab}	6.29 ^{abc}	6.32 ^a	6.20 ^d	6.23 ^{cd}	6.20 ^d	6.22 ^d	0.026	<0.001
Concentration of VFAs, mmol/L														
Acetate	67.59 ^{bc}	66.95 ^{bc}	66.57 ^{bc}	67.78 ^{bc}	66.74 ^{bc}	71.36 ^a	65.22 ^c	69.27 ^{ab}	67.78 ^{bc}	67.44 ^{bc}	69.86 ^{ab}	69.67 ^{ab}	1.409	0.007
Propionate	25.67 ^e	26.22 ^{de}	27.56 ^{bc}	29.39 ^a	27.07 ^{cd}	27.79 ^{bc}	29.38 ^a	29.37 ^a	29.69 ^a	28.42 ^{ab}	28.78 ^{ab}	28.76 ^{ab}	0.569	<0.001
Isobutyrate	1.48 ^{ab}	1.24 ^{bc}	0.78 ^d	1.15 ^{bc}	1.31 ^{abc}	1.24 ^{bc}	1.64 ^a	1.11 ^c	0.43 ^e	1.46 ^{abc}	1.29 ^{bc}	1.48 ^{ab}	0.150	<0.001
Butyrate	16.87 ^{ab}	17.37 ^a	15.06 ^c	15.55 ^{bc}	15.95 ^{bc}	15.64 ^{bc}	13.79 ^d	15.86 ^{bc}	15.47 ^c	15.58 ^{bc}	16.14 ^{bc}	16.06 ^{bc}	0.579	<0.001
Isovalerate	3.20	3.38	3.07	3.12	3.19	3.04	3.12	3.21	3.19	3.31	3.27	3.31	0.207	0.889
Valerate	2.79 ^{ab}	2.94 ^a	2.63 ^{bc}	2.32 ^d	2.81 ^{ab}	2.68 ^{abc}	1.78 ^e	1.65 ^e	1.67 ^e	1.82 ^e	2.45 ^{cd}	2.78 ^{ab}	0.132	<0.001
Total VFAs	117.60 ^{abc}	118.09 ^{abc}	115.66 ^{bc}	119.30 ^{abc}	117.07 ^{abc}	121.74 ^a	114.93 ^c	120.47 ^{ab}	118.22 ^{abc}	118.02 ^{abc}	121.78 ^a	122.06 ^a	2.118	0.019
A : P	2.63 ^a	2.55 ^{ab}	2.42 ^{cd}	2.31 ^{ef}	2.47 ^{bc}	2.57 ^a	2.22 ^f	2.35 ^{de}	2.28 ^{ef}	2.37 ^{cde}	2.43 ^{cd}	2.42 ^{cd}	0.046	<0.001
Molar proportion of VFAs, %														
Acetate	57.47	56.69	57.54	56.81	57.01	58.62	56.77	57.50	57.33	57.14	57.36	57.07	0.528	0.070
Propionate	21.83 ^h	22.21 ^{gh}	23.82 ^{cde}	24.67 ^{cd}	23.12 ^{ef}	22.83 ^{fg}	25.57 ^a	24.38 ^{bcd}	25.11 ^{ab}	24.09 ^{cd}	23.63 ^{def}	23.57 ^{def}	0.424	<0.001
Isobutyrate	1.26 ^{ab}	1.05 ^{bcd}	0.68 ^e	0.96 ^{cd}	1.12 ^{bcd}	1.02 ^{bcd}	1.43 ^a	0.93 ^{de}	0.36 ^f	1.23 ^{abc}	1.07 ^{bcd}	1.21 ^{abcd}	0.128	<0.001
Butyrate	14.34 ^{ab}	14.70 ^a	13.03 ^c	13.02 ^c	13.63 ^{bc}	12.85 ^c	11.98 ^d	13.16 ^c	13.08 ^{bc}	13.20 ^c	13.26 ^c	13.16 ^c	0.404	<0.001
Isovalerate	2.73	2.86	2.66	2.62	2.73	2.50	2.71	2.66	2.70	2.81	2.68	2.71	0.159	0.753
Valerate	2.37 ^{ab}	2.49 ^a	2.27 ^{ab}	1.94 ^d	2.40 ^{ab}	2.20 ^{bc}	1.54 ^e	1.37 ^e	1.41 ^e	1.54 ^e	2.01 ^{cd}	2.28 ^{ab}	0.097	<0.001

¹ Different superscript letters within a row indicate significant differences among the groups ($P < 0.05$). Significance was tested using one-way ANOVA with yeast treatment as a fixed effect, and post hoc pairwise comparisons were performed with Duncan's multiple range test.

SEM = standard error of the mean; VFA = volatile fatty acids; A : P = acetate to propionate ratio.

Table S3. Effects of different rumen-derived yeasts on gas production *in vitro*.

Items	CON	B2	Y3	Y4	M7	Y9	M12	B13	B17	B24	B26	B49	SEM	<i>P</i> value ¹
Gas production, mL														
Total gas production	119.13 ^{ab}	121.13 ^a	111.50 ^{cd}	120.75 ^a	111.00 ^{cd}	108.88 ^{de}	103.00 ^e	111.70 ^{cd}	111.75 ^{cd}	112.88 ^{bcd}	117.63 ^{abc}	114.38 ^{abcd}	3.150	<0.001
Hydrogen	2.98 ^a	2.98 ^a	2.78 ^{abc}	2.55 ^c	2.61 ^{bc}	2.87 ^{abc}	2.57 ^{bc}	2.75 ^{abc}	2.70 ^{abc}	2.71 ^{abc}	2.92 ^{ab}	2.89 ^{abc}	0.148	0.044
Methane	24.32 ^b	27.95 ^a	22.86 ^{bcd}	20.55 ^{cd}	21.34 ^{bcd}	22.88 ^{bcd}	20.15 ^d	22.18 ^{bcd}	20.68 ^{cd}	23.46 ^{bc}	23.26 ^{bcd}	23.61 ^{bc}	1.330	<0.001
Methane/total VFAs	0.207 ^b	0.236 ^a	0.198 ^{bcd}	0.173 ^d	0.182 ^{bcd}	0.188 ^{bcd}	0.175 ^{cd}	0.184 ^{bcd}	0.175 ^{cd}	0.199 ^{bc}	0.191 ^{bcd}	0.194 ^{bcd}	0.011	<0.001

¹ Different superscript letters within a row indicate significant differences among the groups ($P < 0.05$). Significance was tested using one-way ANOVA with yeast treatment as a fixed effect, and post hoc pairwise comparisons were performed with Duncan's multiple range test.

SEM = standard error of the mean; VFA = volatile fatty acids.

Table S4. Effects of *Pichia membranifaciens* M12 supplementation on concentration of dissolved gas, methane, and carbon dioxide production in dairy cows.

Items	CON	PM2.5	PM5	SEM	<i>P</i> value ¹
Dissolved gas					
Dissolved methane, mmol/L	1.36 ^a	0.78 ^b	0.97 ^b	0.065	<0.001
Dissolved hydrogen, μ mol/L	15.43 ^a	11.52 ^b	15.04 ^a	0.959	0.017
Gas production					
Carbon dioxide production, kg/day	12.44	12.57	11.49	0.696	0.510
Methane production, g/day	495.00 ^a	460.89 ^{ab}	418.91 ^b	12.825	0.048
Methane yield, g/kg of DMI	20.27 ^a	19.32 ^a	16.49 ^b	0.542	0.009
Methane intensity, g/kg of ECM	13.63 ^a	12.54 ^{ab}	11.22 ^b	0.397	0.044

¹ Different superscript letters within a row indicate significant differences among the groups ($P < 0.05$). Significance of differences among the three groups was tested using one-way ANOVA with Tukey's post hoc test.

SEM = standard error of the mean; DMI, dry matter intake; ECM, energy-corrected milk.

Table S5. Effects of *Pichia membranifaciens* M12 supplementation on feed intake and apparent nutrient digestibility in dairy cows.

Items	CON	PM2.5	PM5	SEM	<i>P</i> value ¹
Dry matter intake, kg/day	24.50 ^{ab}	23.97 ^b	25.38 ^a	0.381	0.042
Apparent nutrient digestibility, %					
Dry matter	68.30	69.00	68.54	0.144	0.126
Crude protein	74.99 ^{ab}	76.14 ^a	74.05 ^b	0.342	0.039
Ether extract	60.69 ^b	62.20 ^b	70.30 ^a	1.518	0.016
Neutral detergent fiber	63.13 ^a	63.90 ^a	60.54 ^b	0.528	0.020
Acid detergent fiber	59.10	59.07	57.94	0.624	0.698

¹ Different superscript letters within a row indicate significant differences among the groups ($P < 0.05$). Significance of differences in dry matter intake among the three groups was tested using a linear mixed-effects model with Satterthwaite's method, and pairwise comparisons were adjusted with Tukey's method. Significance of differences in apparent nutrient digestibility among the three groups was tested using one-way ANOVA with Tukey's post hoc test.

SEM = standard error of the mean.

Table S6. Effects of *Pichia membranifaciens* M12 supplementation on milk production and milk composition in dairy cows.

Items	CON	PM2.5	PM5	SEM	<i>P</i> value ¹
Milk production, kg/day	34.96	35.70	35.58	1.299	0.910
4% fat-corrected milk (FCM), kg/day	36.03	36.21	36.27	1.047	0.987
Energy-corrected milk (ECM) ² , kg/day	36.68	36.99	37.59	1.070	0.836
Milk composition					
Milk fat, %	4.15	4.09	4.14	0.106	0.914
Milk protein, %	3.57 ^a	3.42 ^b	3.67 ^a	0.067	0.045
Milk lactose, %	5.11	5.20	5.01	0.077	0.236
Milk urea nitrogen, %	15.40	15.94	15.52	0.451	0.681
Total solids, %	13.39	13.27	13.43	0.156	0.773
Milk fat yield, kg/day	1.44	1.46	1.51	0.065	0.758
Milk protein yield, kg/day	1.25	1.22	1.30	0.046	0.385
Milk lactose yield, kg/day	1.80	1.86	1.84	0.090	0.892
Milk solids yield, kg/day	4.72	4.70	4.89	0.144	0.574

¹ Different superscript letters within a row indicate significant differences among the groups ($P < 0.05$). Significance of differences among the three groups was tested using a linear mixed-effects model with Satterthwaite's method, and pairwise comparisons were adjusted with Tukey's method.

² Energy-corrected milk ECM (kg/d) = $[0.39 \times \text{milk fat (\%)} + 0.24 \times \text{milk protein (\%)} + 0.17 \times \text{milk lactose (\%)}] \times \text{milk production (kg/d)}/3.14$.

SEM = standard error of the mean.

Table S7. Effects of *Pichia membranifaciens* M12 supplementation on serum parameters related to protein metabolism, energy substrates, and liver function in dairy cows.

Items	CON	PM2.5	PM5	SEM	<i>P</i> value ¹
Protein metabolism					
Total protein, g/L	74.77	73.08	72.90	1.604	0.685
Albumin, g/L	34.25	35.53	34.96	0.786	0.522
Globulin, g/L	40.52	37.54	37.94	2.195	0.605
Albumin to globulin ratio	0.90	0.97	0.96	0.059	0.648
Urea, mmol/L	5.07	5.22	4.91	0.176	0.484
Creatinine, μ mol/L	62.92	70.00	64.00	2.437	0.104
Energy substrates					
Glucose, mmol/L	3.44	3.33	3.28	0.069	0.282
Free fatty acids, mmol/L	0.16	0.14	0.14	0.020	0.747
Triglycerides, mmol/L	0.14	0.14	0.14	0.007	0.966
HDL cholesterol, mmol/L	3.75	3.83	3.80	0.166	0.947
LDL cholesterol, mmol/L	3.27	3.30	3.39	0.229	0.938
Total cholesterol, mmol/L	6.63	6.67	6.78	0.414	0.966
Liver function					
Alanine aminotransferase, U/L	27.00	29.83	29.83	1.032	0.104
Aspartate aminotransferase, U/L	79.33	78.83	80.25	5.412	0.983
Alkaline phosphatase, U/L	39.25	39.00	50.75	4.719	0.255
Total bilirubin, μ mol/L	3.66	3.55	3.64	0.114	0.767

¹ Significance of differences among the three groups was tested using one-way ANOVA with Tukey's post hoc test.

SEM = standard error of the mean; HDL = High-density lipoprotein; LDL = Low-density lipoprotein.

Table S8. Effects of *Pichia membranifaciens* M12 supplementation on rumen fermentation parameters in dairy cows.

Items	CON	PM2.5	PM5	SEM	<i>P</i> value ¹
pH	6.45	6.42	6.45	0.066	0.939
Redox potential, mv	-307.17	-301.25	-294.42	6.478	0.392
Ammonia nitrogen, mg/dL	11.34	12.56	11.74	1.128	0.745
Lactate, mmol/L	0.44	0.47	0.46	0.021	0.537
Concentration of VFAs, mmol/L					
Acetate	70.54 ^a	62.51 ^b	65.86 ^{ab}	2.144	0.043
Propionate	23.59	22.36	21.40	1.119	0.403
Isobutyrate	0.63	0.58	0.69	0.090	0.762
Butyrate	10.54	10.22	11.16	0.375	0.240
Isovalerate	1.03	1.08	1.07	0.070	0.070
Valerate	1.07	1.07	0.99	0.051	0.482
Total VFAs	107.40	97.81	101.17	3.332	0.140
Acetate to propionate ratio	3.03	2.85	3.11	0.104	0.198
Molar proportion of VFAs, %					
Acetate	65.69	64.01	65.11	0.562	0.135
Propionate	21.94	22.72	21.09	0.626	0.198
Isobutyrate	0.60	0.60	0.68	0.089	0.785
Butyrate	9.81 ^b	10.47 ^{ab}	11.08 ^a	0.294	0.016
Isovalerate	0.96	1.10	1.06	0.055	0.278
Valerate	1.00	1.10	0.98	0.040	0.109

¹ Different superscript letters within a row indicate significant differences among the groups ($P < 0.05$). Significance of differences among the three groups was tested using one-way ANOVA with Tukey's post hoc test.

SEM = standard error of the mean; VFA = volatile fatty acids.

Table S9. Effects of *Pichia membranifaciens* M12 supplementation on microbial crude protein supply to the small intestine in dairy cows.

Items	CON	PM2.5	PM5	SEM	<i>P</i> value ¹
Urine volume, L/day	36.32	36.54	30.75	2.221	0.191
Urinary PD, mmol/day					
Allantoin	694.72	743.34	750.92	53.521	0.730
Uric acid	49.65	46.59	57.53	6.007	0.365
Endogenous PD	56.56	58.01	56.64	1.082	0.585
PD absorption, mmol/day	795.93	847.95	865.09	54.570	0.658
Microbial crude protein, g/day	3102.70	3325.90	3416.25	243.93	0.656

¹ Significance of differences among the three groups was tested using one-way ANOVA with Tukey's post hoc test.

SEM = standard error of the mean; PD = purine derivatives.

Table S10. Effects of *Pichia membranifaciens* M12 supplementation on microbe numbers.

Items	CON	PM2.5	PM5	SEM	<i>P</i> value ¹
Bacteria (log ₁₀ / L)	14.02	14.40	14.65	0.263	0.365
Archaea (log ₁₀ / L)	11.35	11.07	11.29	0.107	0.400
Fungus (log ₁₀ / L)	7.37	7.48	7.45	0.140	0.636
Protozoa (log ₁₀ / L)	11.55	12.40	12.24	0.270	0.158

¹ Significance of differences among the three groups was tested using the Kruskal-Wallis method, and pairwise comparisons were conducted with the Wilcoxon rank-sum test.

SEM = standard error of the mean.

Table S11. Summary of sequencing output and ASV richness across domains.

Domain	Total sequences	Average per sample	Number of ASVs
Bacteria	860,457	35,852	5,865
Archaea	850,110	35,272	3,523
Eukaryota	830,990	34,625	73,686

ASV = amplicon sequence variant.

Table S12. Effects of *Pichia membranifaciens* M12 supplementation on alpha diversity of rumen microbial communities.

Items	CON	PM2.5	PM5	SEM	<i>P</i> value ¹
Bacteria					
Observed ASVs	709	733	694	20	0.612
ACE ²	711	735	696	20	0.605
Chao1	711	735	695	20	0.564
Shannon	5.83	5.95	5.84	0.05	0.379
Simpson	0.99	0.99	0.99	<0.01	0.379
Archaea					
Observed ASVs	372 ^b	408 ^a	371 ^b	13	0.039
ACE ²	372 ^b	408 ^a	371 ^b	13	0.043
Chao1	372 ^b	408 ^a	371 ^b	13	0.039
Shannon	4.93	5.12	4.93	0.08	0.102
Simpson	0.98	0.99	0.98	<0.01	0.113
Fungi					
Observed ASVs	2312	2867	2315	258	0.226
ACE ²	5300	5783	4995	322	0.102
Chao1	4477	5046	4193	277	0.120
Shannon	6.3	6.23	6.2	0.12	0.675
Simpson	0.98	0.97	0.98	0.01	0.228
Protozoa					
Observed ASVs	4141	3886	4431	253	0.406
ACE ²	8787	8210	8650	704	0.617
Chao1	7569	7103	7578	603	0.651
Shannon	6.24	6.29	6.35	0.07	0.793
Simpson	0.98	0.98	0.98	<0.01	0.728

¹ Different superscript letters within a row indicate significant differences among groups ($P < 0.05$). Significance of differences among the three groups was tested using the Kruskal-Wallis method, and pairwise comparisons were conducted with the Wilcoxon rank-sum test.

² SEM = standard error of the mean; ASV = amplicon sequence variant; ACE = abundance-based coverage estimator. Good's coverages were identified more than 99%.

Table S13. The composition and nutrient levels of the basal diet.

Items	Proportion
Diet composition, % DM	
Corn meal	5.59
Steam-flaked corn	3.82
Barley	0.79
Soybean meal	5.29
Extruded soybean	0.23
Distillers dried grains with solubles	2.66
Soybean hulls	1.42
Brewers' grains	7.50
Cottonseed	3.86
Beet pulp	10.20
Double-low rapeseed meal	3.81
Corn silage	36.45
Alfalfa hay	10.47
Wheat straw	6.00
Palmitic acid fat powder	0.17
Uridan	0.10
Sodium bicarbonate	0.38
Montmorillonite	0.07
Expanded urea	0.12
Premix ¹	1.07
Total	100.00
Nutrient composition, % DM	
Dry matter	48.83
Organic matter	92.19
Crude protein	16.06
Ether extract	3.74
Neutral detergent fiber	41.16
Acid detergent fiber	23.63
Crude ash	7.81
Calcium	2.34
Phosphorus	0.78
Net energy for lactation (Mcal/kg) ²	1.60

¹ The premix provided the following for per kg of diets: vitamin A 210,000 IU, vitamin D 70,000 IU, vitamin E 1,165 mg; biotin 2 mg, β - carotene 3.3 mg, Mn 1,580 mg, manganese methionine 300 mg, Cu 600 mg, copper methionine 120 mg, Zn 3,250 mg, zinc methionine 660 mg, I 55 mg, Se 18 mg, selenium yeast 3.3 mg, Co 11 mg, rumen protected methionine 2.7%, salt 18.3%, Mg 5.5%, potassium carbonate 7.4%.

² Net energy for lactation (Mcal/kg) was calculated according to NRC (2001).

Table S14 The real-time PCR primers used in this study

Microbes	Primers	Sequences	Annealing temperature (°C)
Bacteria	F	CCTACGGGAGGCAGCAG	60
	R	ATTACCGCGGCTGCTGG	
Archaea	F	GTGCTCCCCCGCCAATTCCT	59
	R	GCGGTGTGTGCAAGGAGC	
Protozoa	F	GCTTTCGWTGGTAGTGTATT	55
	R	CTTGCCCTCYAATCGTWCT	
Fungi	F	GAGGAAGTAAAAGTCGTAACAAGGTTTC	60
	R	CAAATTCACAAAGGGTAGGATGATT	