

Microbial dynamics, fermentation, and aerobic stability of conventional and BMR corn **hybrids** ensiled at **low moisture** with or without a **combo inoculant**

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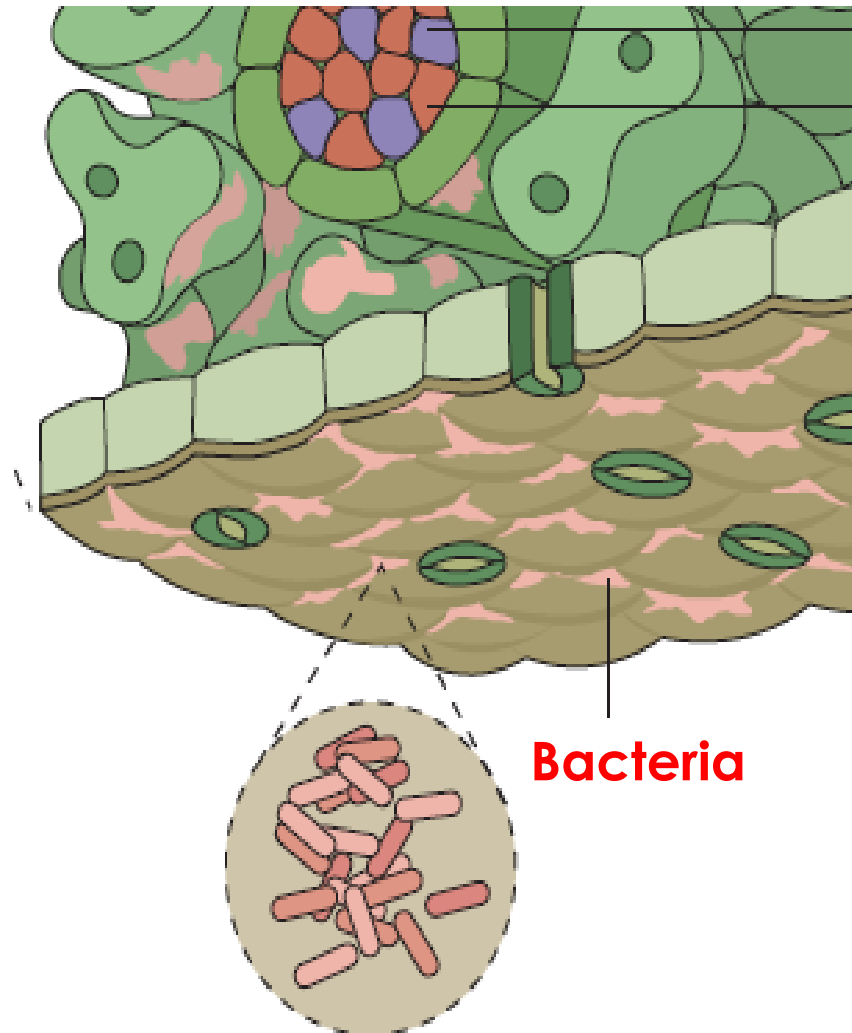
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Introduction

- Ensiling whole corn > 35% DM can result in poorly compacted, ↓ quality silos due to microbial aerobic spoilage.
- The microbial community of corn silages produced in **suboptimal conditions** have not been studied in as much detail compared with optimally stored silages (Muck, 2013).



Objectives

Evaluate the effects of inoculant and corn hybrid (high DM %) on:

- **Nutritional composition, fermentation, and aerobic stability**
- **Bacterial and fungal taxonomic profiles**
- **OTUs richness and shifts**

...before and after ensiling.



Materials and Methods

- **Corn field at Piedmont Research Station (NCDA-NCSU), Salisbury, North Carolina.**

Treatments

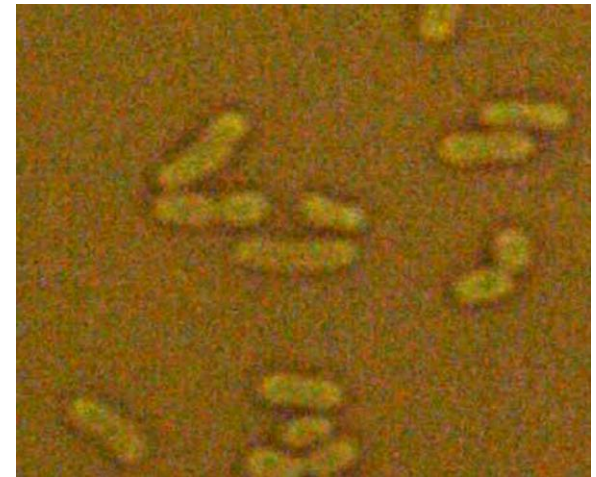
- Factorial combination (4 x 2) of:
 - ▣ Four corn hybrids (**HYB**):

Label	Hybrid	Company	Type	Relative Maturity
MCN	TMF2R737	Mycogen	Conventional	112
MBR	F2F817	Mycogen	BMR	116
PCN	P2089YHR	Pioneer	Conventional	120
PBR	P1449XR	Pioneer	BMR	114

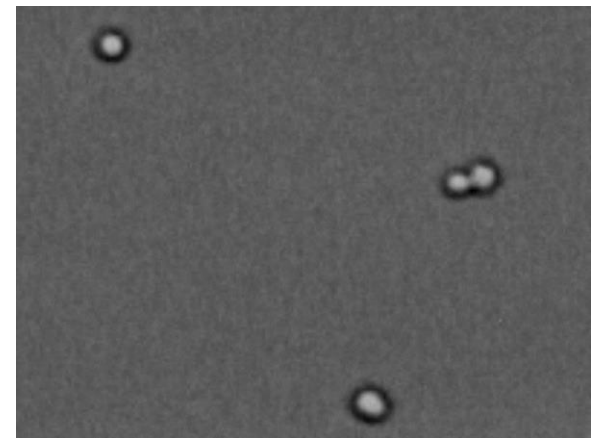
- ▣ Two additive levels (**ADV**)
 - Sterile dd water (**CON**) or inoculant (**INO**) were applied at 1 ml/kg of fresh corn.

Combo Inoculant

- *L. buchneri* 40788 (log 5.6 cfu/g of fresh corn)
- *P. pentosaceus* 12455 (log 5 cfu/g of fresh corn)
- Enzymes from *T. reesei*.



L. buchneri



P. pentosaceus

Corn Harvest

- 6 plots/hybrid were randomly located within field.
- At **~41% DM** and chopped to a TLC of 1.9 cm with kernel processing.
- 2 replicated piles (4.3 kg each, fresh basis) were obtained from each plot.

Ensiling

- CON or INO randomly assigned to a pile/plot/hybrid ($2 \times 6 \times 4 = 48$ piles).
- Forage was packed into 7.6L mini-silos and stored at 23°C ($\pm 1^{\circ}\text{C}$) for 100 d.
 - Density: ~ 192 kg of DM/ m^3

Experimental Design and Statistical Analysis

- **CRD with a 4 (HYB) × 2 (ADV) factorial treatment arrangement.**
 - 6 replicates per treatment.
- **Model contained effect of HYB, ADV, and their interaction.**
 - Data analyzed with Proc. GLM of SAS v9.4.
 - SLICE option used to analyze interactions.
 - Means separated by the LSD test.
 - Significance $P \leq 0.05$.



Results and Discussion

Day 0 of Ensiling

Bacteria (16S V4 region)

Fungi (ITS region)

Day 0 Results

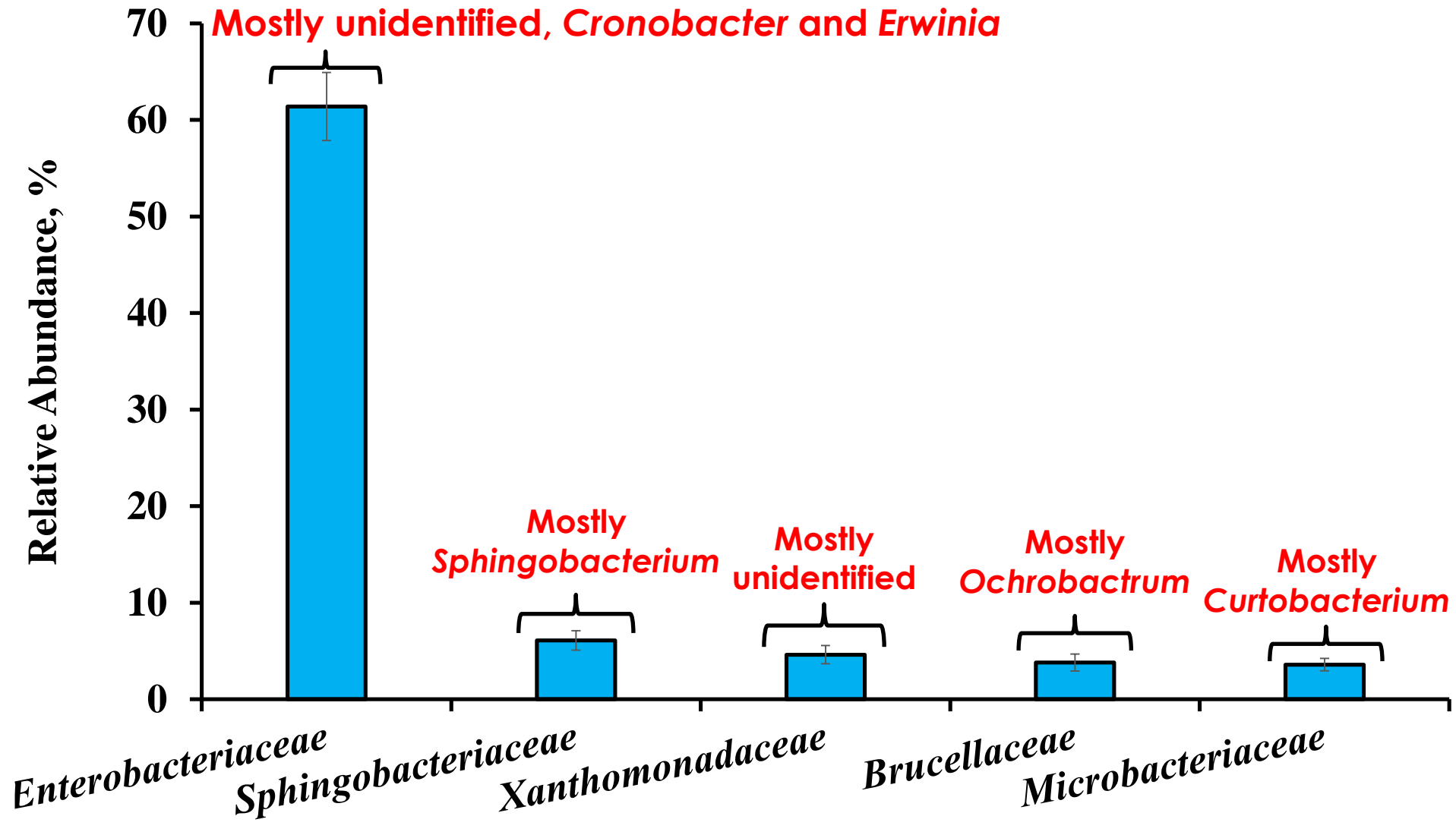
Item	MCN	PCN	MBR	PBR	SEM
DM (%)	44.0 ^a	42.0 ^b	38.1 ^c	41.3 ^b	0.665
% of DM					
CP	5.96 ^b	5.46 ^c	6.85 ^a	5.98 ^b	0.182
WSC	3.32 ^b	3.48 ^b	5.49 ^a	3.66 ^b	0.356
NDF	45.5 ^a	41.8 ^b	44.7 ^a	42.3 ^b	0.94
Log cfu/fresh g					
LAB	6.73 ^c	7.06 ^b	7.71 ^a	7.67 ^a	0.101
Yeasts	6.63	6.65	6.63	6.77	0.087
Molds	5.94	5.93	5.70	5.89	0.107

Bacteria (16S V4 region)

Alpha diversity

Taxonomic profile

Enterobacteriaceae Dominates

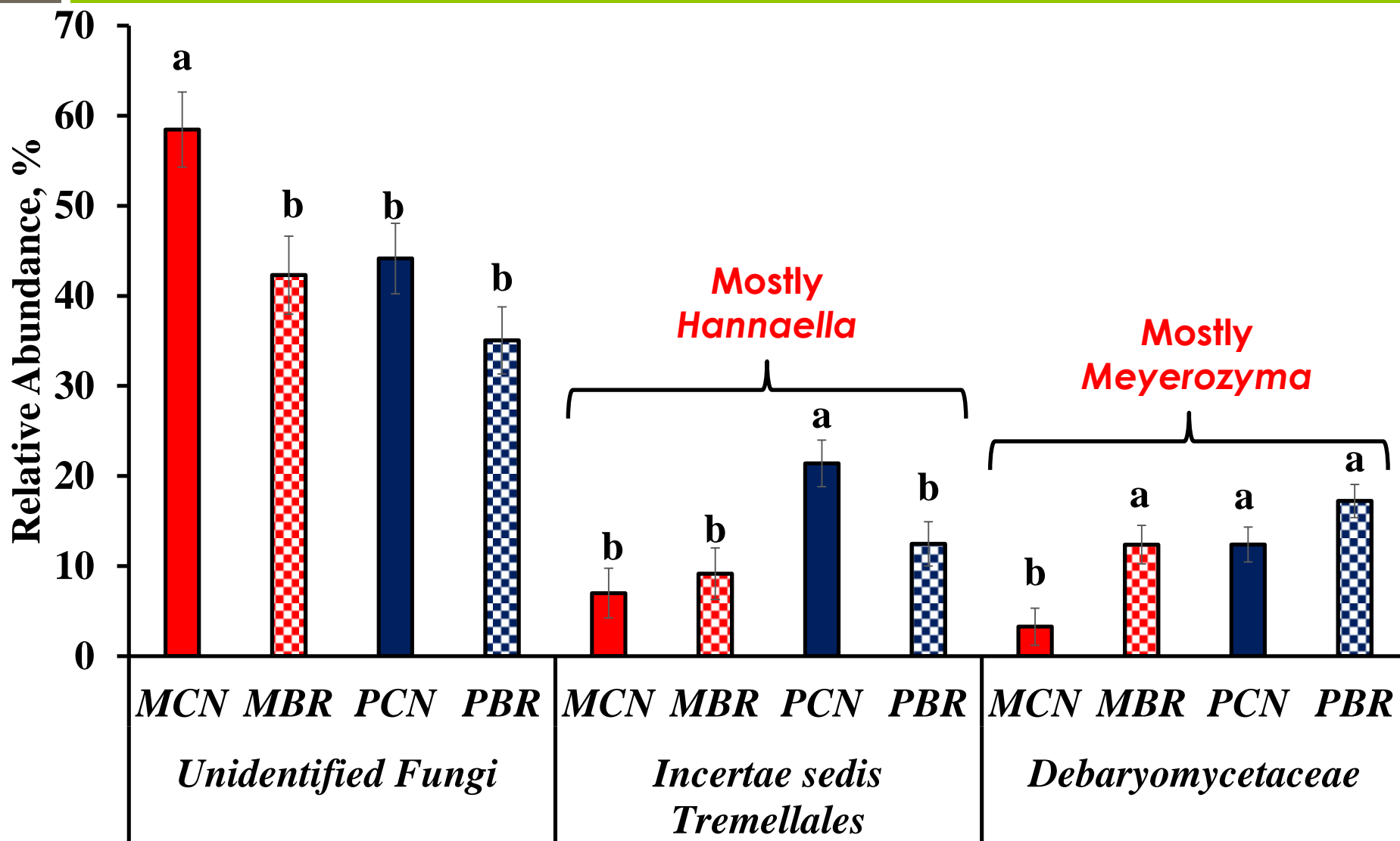


Fungi (ITS region)

Alpha diversity

Taxonomic profile

Unidentified Fungi Dominates



Day 100 of Ensiling

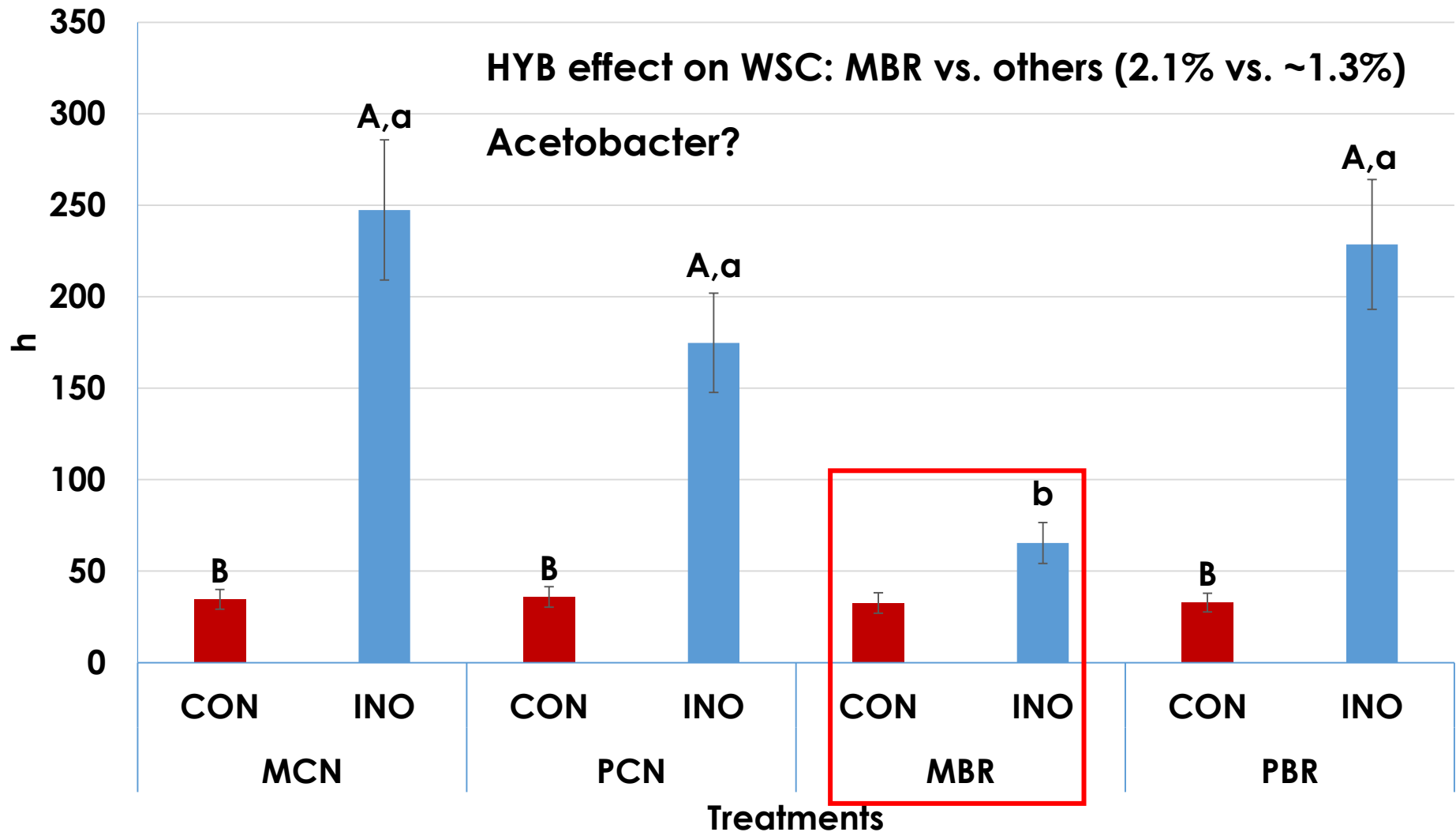
Bacteria (16S V4 region)

Fungi (ITS region)

INO Effects

Item	CON	INO	Change	SEM
DM recovery, %	94.6	94.0	=	1.07
NH ₃ -N, % of N	8.0	9.7	↑	0.39
pH	3.80 ^B	4.02 ^A	↑	0.041
Lactic acid, % of DM	4.82 ^A	2.65 ^B	↓	0.436
Acetic acid, % of DM	0.51 ^B	1.69 ^A	↑	0.132
Yeasts	5.13 ^A	3.78 ^B	↓	0.440

Aerobic Stability: ADV × HYB



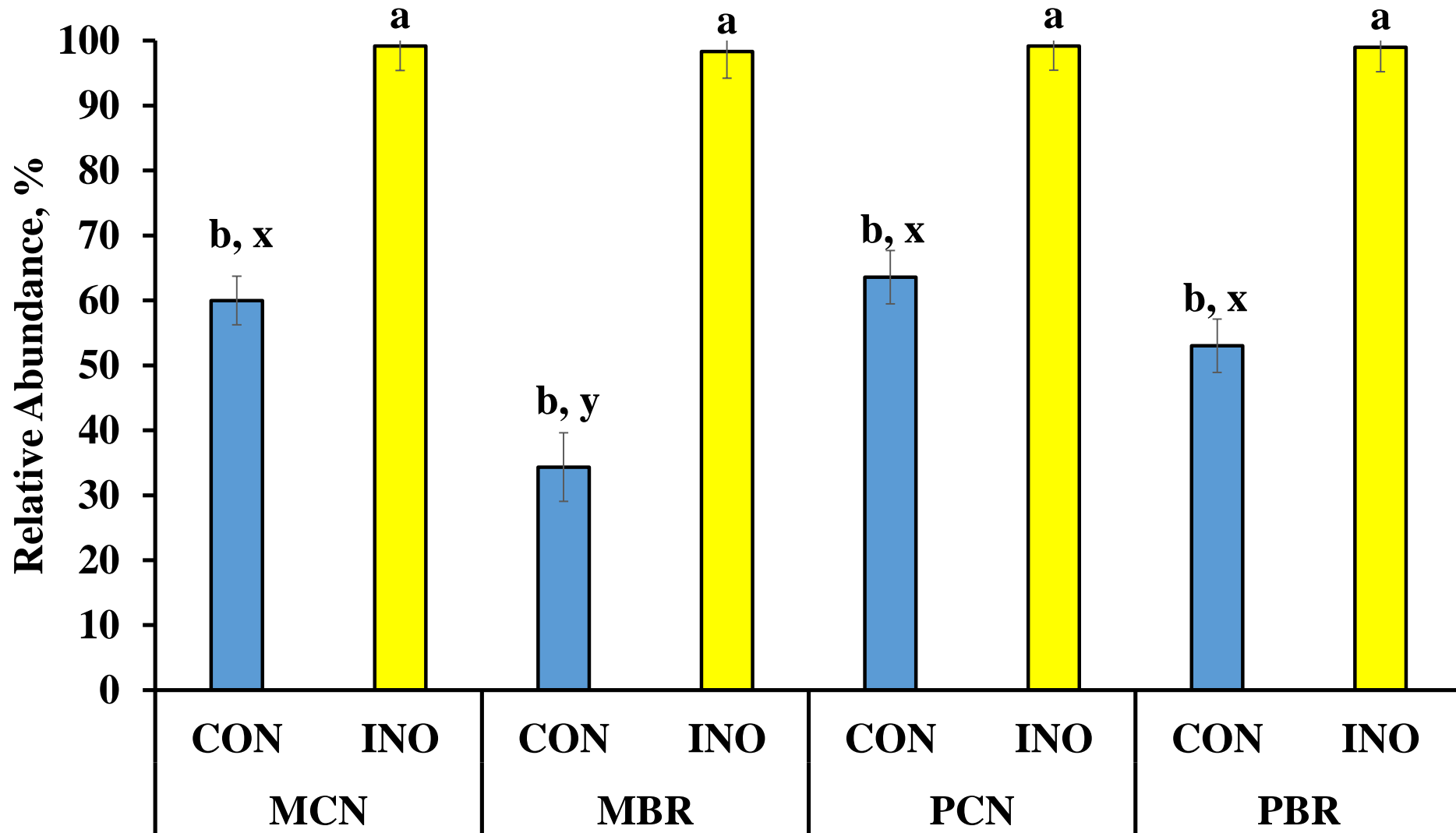
Bacteria (16S V4 region)

Alpha diversity

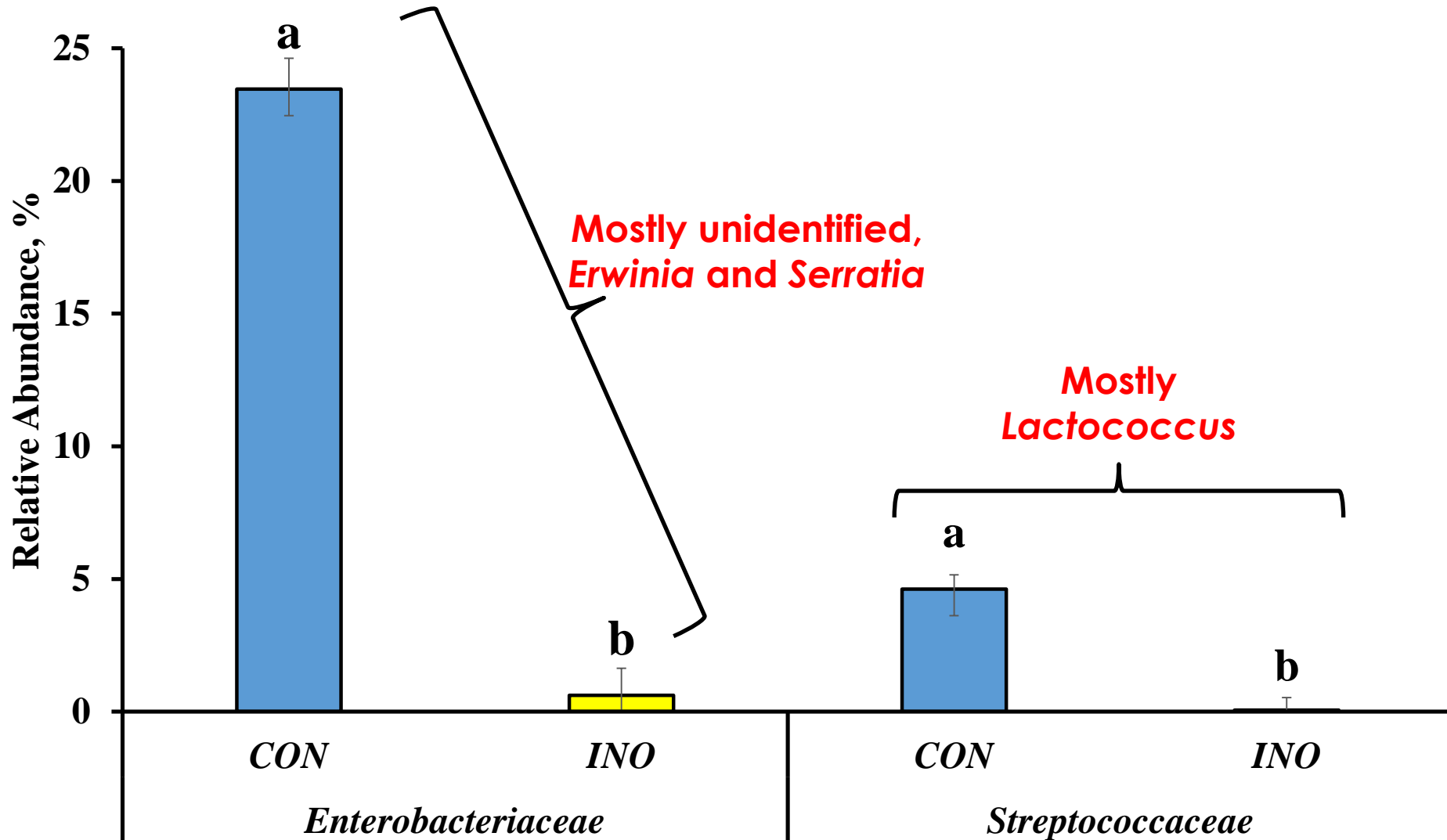
Taxonomic profile

Beta diversity

Lactobacillaceae Dominates

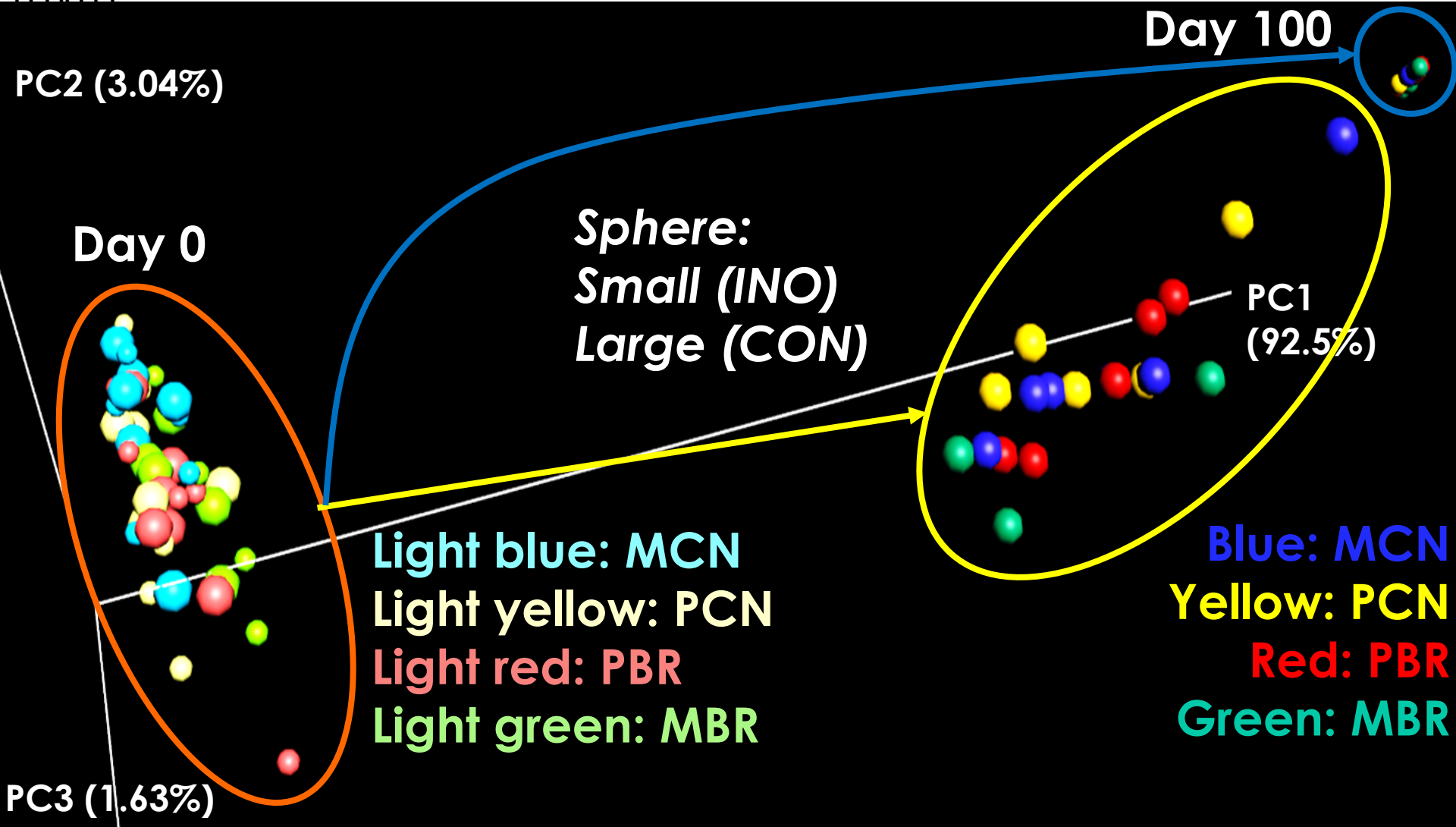


INO ↓ *Enterobacteriaceae*



Weighted UNIFRAC PCoA Plot

**ANOSIM: d 0 vs. 100 ($P = 0.001$) and within d 100 between the CON and INO ($P = 0.001$)



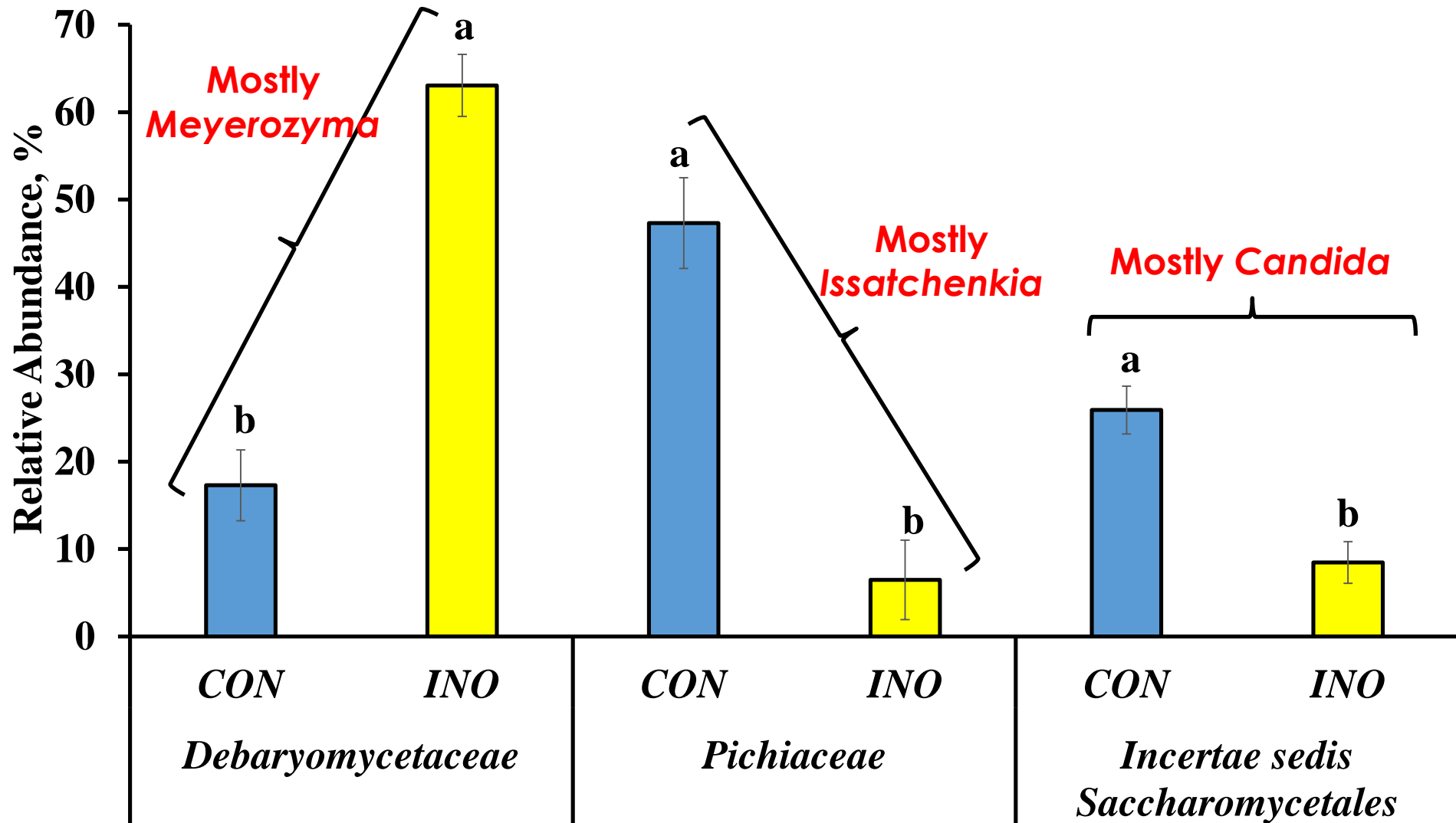
Fungi (ITS region)

Alpha diversity

Taxonomic profile

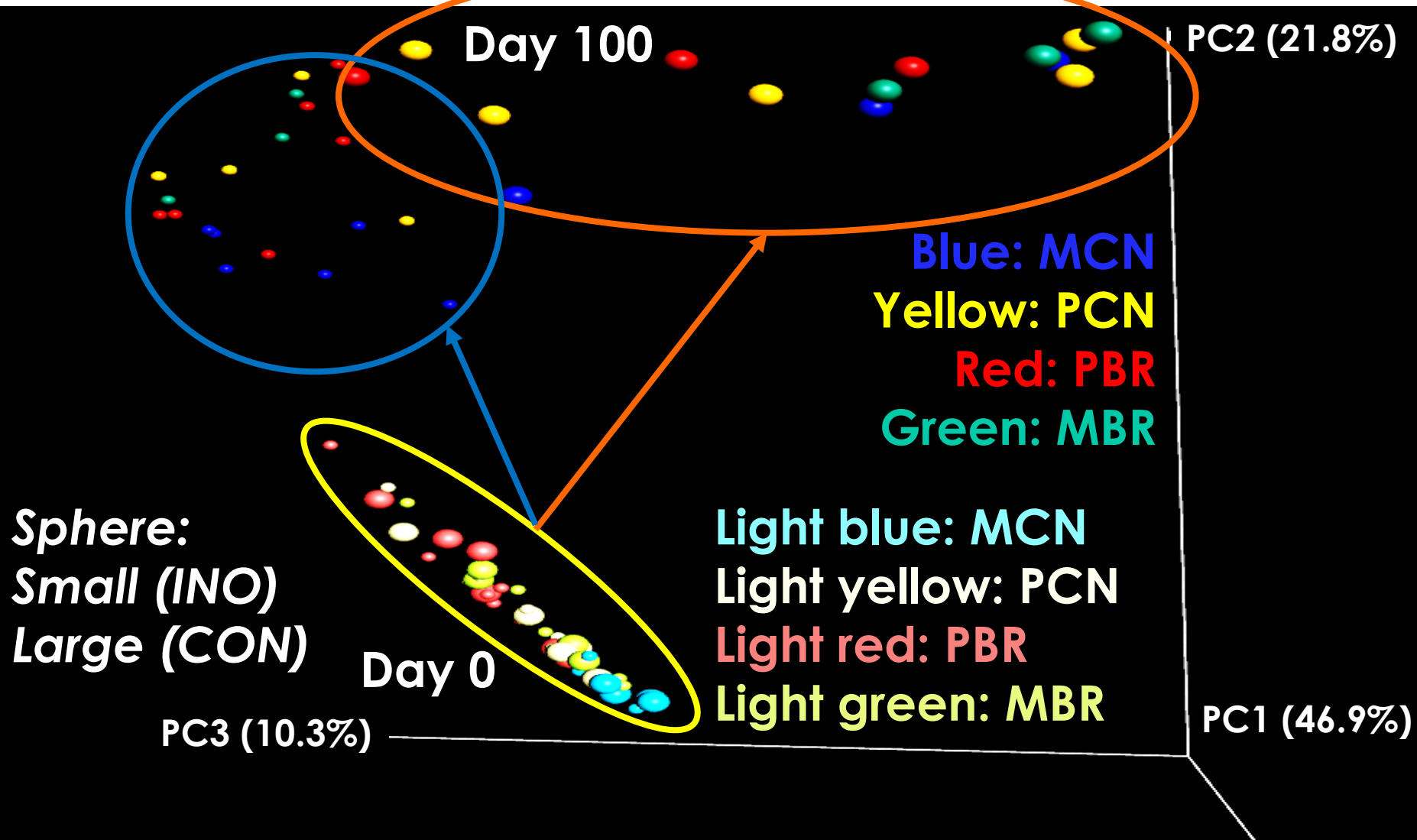
Beta diversity

↑ *Debaryomycetaceae* ↓ *Pichiaceae*



Bray-Curtis PCoA Plot

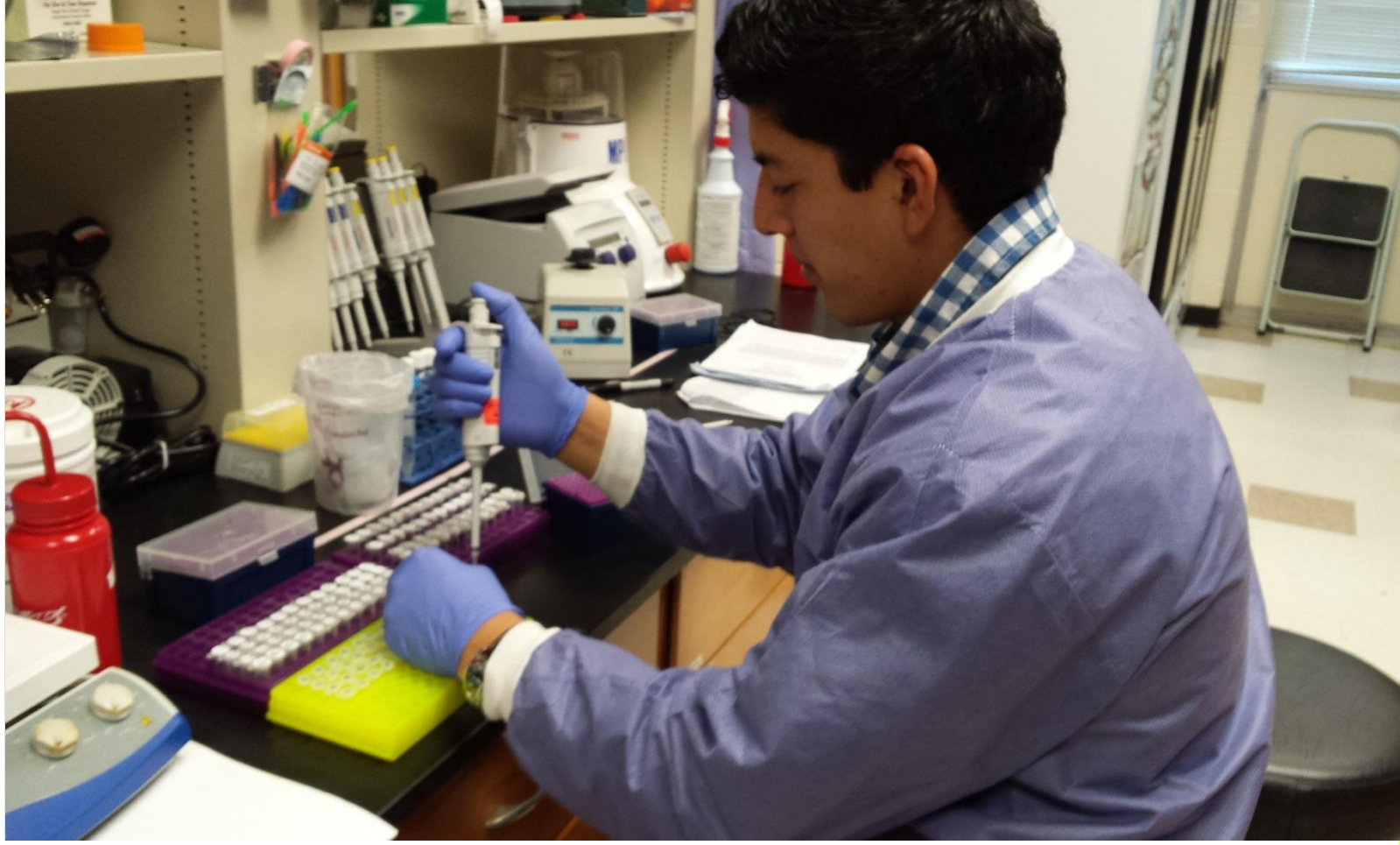
****ANOSIM: d 0 vs. 100 ($P = 0.001$) and within d 100 between the CON and INO ($P = 0.001$)**



Alpha Diversity

Alpha Diversity

Item	<u>Bacteria</u>		<u>Fungi</u>	
	OTUs	Evenness	OTUs	Evenness
Day 0				
MCN	434 ^c			0.022 ^c
PCN	444 ^c			0.032 ^{ab}
MBR	654 ^a	~0.025	~77.1	0.029 ^{bc}
PBR	534 ^b			0.038 ^a
SEM	16	0.0011	2.78	0.0033
Day 100				
CON	276		43.6 ^b	0.055 ^a
INO	61	~0.016	59.8 ^a	0.033 ^b
SEM	8.7	0.0011	2.95	0.0047



Conclusions

Conclusions

- **Ensiled corn (100 d):**
- **Aerobic stability was extended at least 5× for all INO-treated HYB except for MBR.**
 - **Similar DM recovery vs. CON.**
- **INO ↓ bacterial and ↑ fungal diversity across all HYB.**
- **Bacteria:**
 - **INO:** *Lactobacillaceae* (> 98%).
 - **CON:** *Lactobacillaceae* (~50%),
Enterobacteriaceae (~24%)
Leuconostocaceae (~14%)

Conclusions

- **Fungi: INO had**
 - ▣ **↑ *Debaryomycetaceae*, ↓ *Pichiaceae* and i.s. *Saccharomycetales*.**
- **Large differences exist between CON and INO bacterial and fungal community structures.**
 - ▣ **INO microbial communities were closer and more consistent.**

“INO improved dry corn silage quality by a consistent shift in the microbial community structure during ensiling across HYB, except for MBR”

Thanks!

Questions?

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- Lallemand Animal Nutrition (Milwaukee, WI) provided the inoculant.

