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A new *in vitro* model of the healthy human ileum and its associated microbiota

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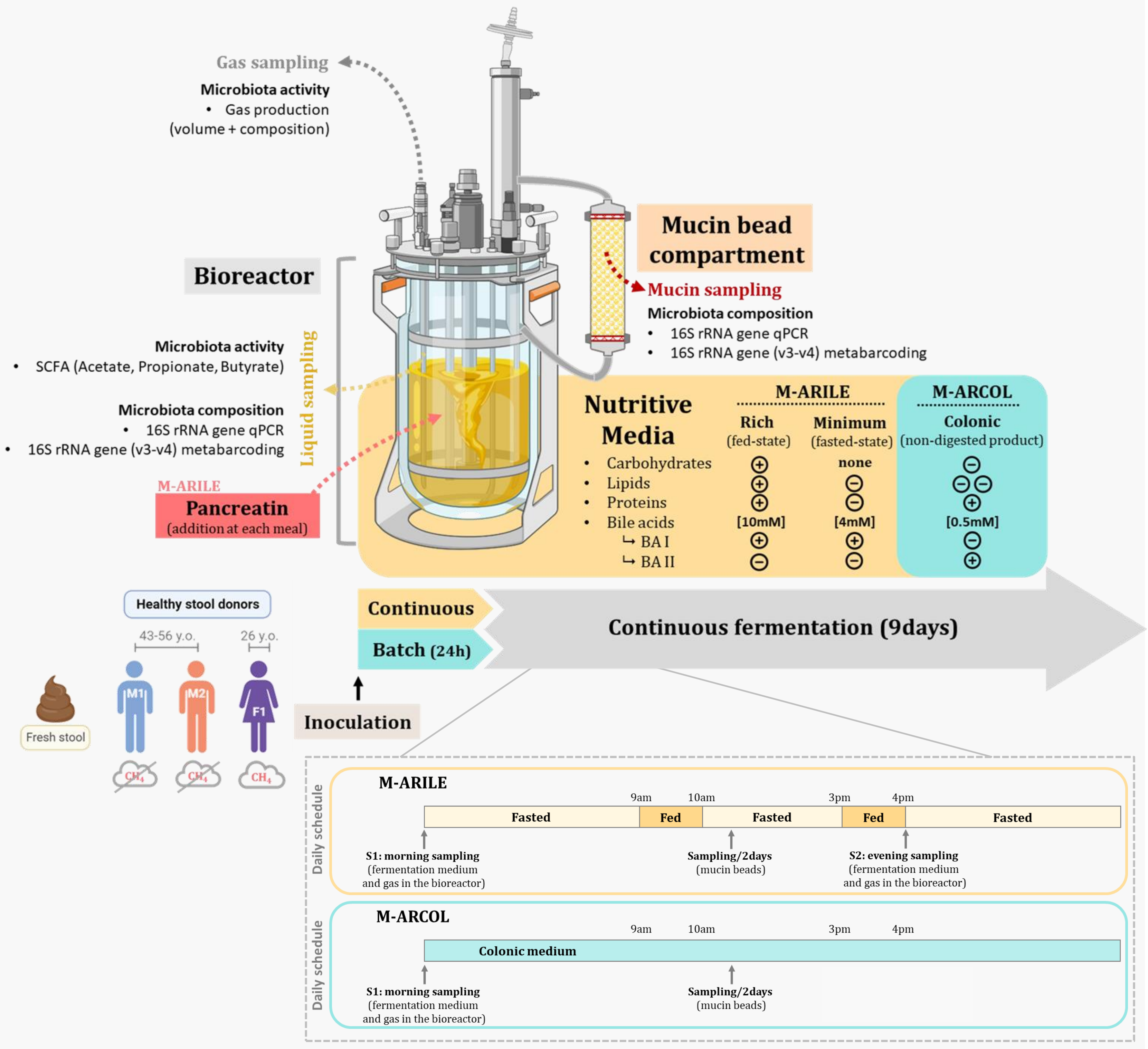
INTRODUCTION

The human small intestine is the main site of food digestion and nutrient absorption. Its microbiota certainly plays a key role in host health, but until now it was largely understudied due to sampling invasiveness, especially in healthy volunteers.

Up to now, there is no available *in vitro* system simulating the ileal compartment and its associated microbiota, that has been fully developed and validated based on *in vivo* data in humans.

Aim of this study: Development of an *in vitro* dynamic model of the healthy human ileum microbiota, reproducing both luminal and mucosal microenvironments.

MATERIAL & METHODS



Gas sampling
Microbiota activity
- Gas production (volume + composition)

Bioreactor

Liquid sampling

Microbiota activity
- SCFA (Acetate, Propionate, Butyrate)

Microbiota composition
- 16S rRNA gene qPCR
- 16S rRNA gene (v3-v4) metabarcoding

M-ARILE
Pancreatin (addition at each meal)

Mucin bead compartment

Mucin sampling
Microbiota composition
- 16S rRNA gene qPCR
- 16S rRNA gene (v3-v4) metabarcoding

Nutritive Media	**M-ARILE** Rich (fed-state)	**M-ARILE** Minimum (fasted-state)	**M-ARCOL** Colonic (non-digested product)
Carbohydrates	⊕	none	⊖
Lipids	⊕	⊖	⊖⊖
Proteins	⊕	⊖	⊕
Bile acids	[10mM]	[4mM]	[0.5mM]
↳ BA I	⊕	⊕	⊖
↳ BA II	⊖	⊖	⊕

Healthy stool donors
43-56 y.o. — M1, M2
26 y.o. — F1
Fresh stool
CH_4

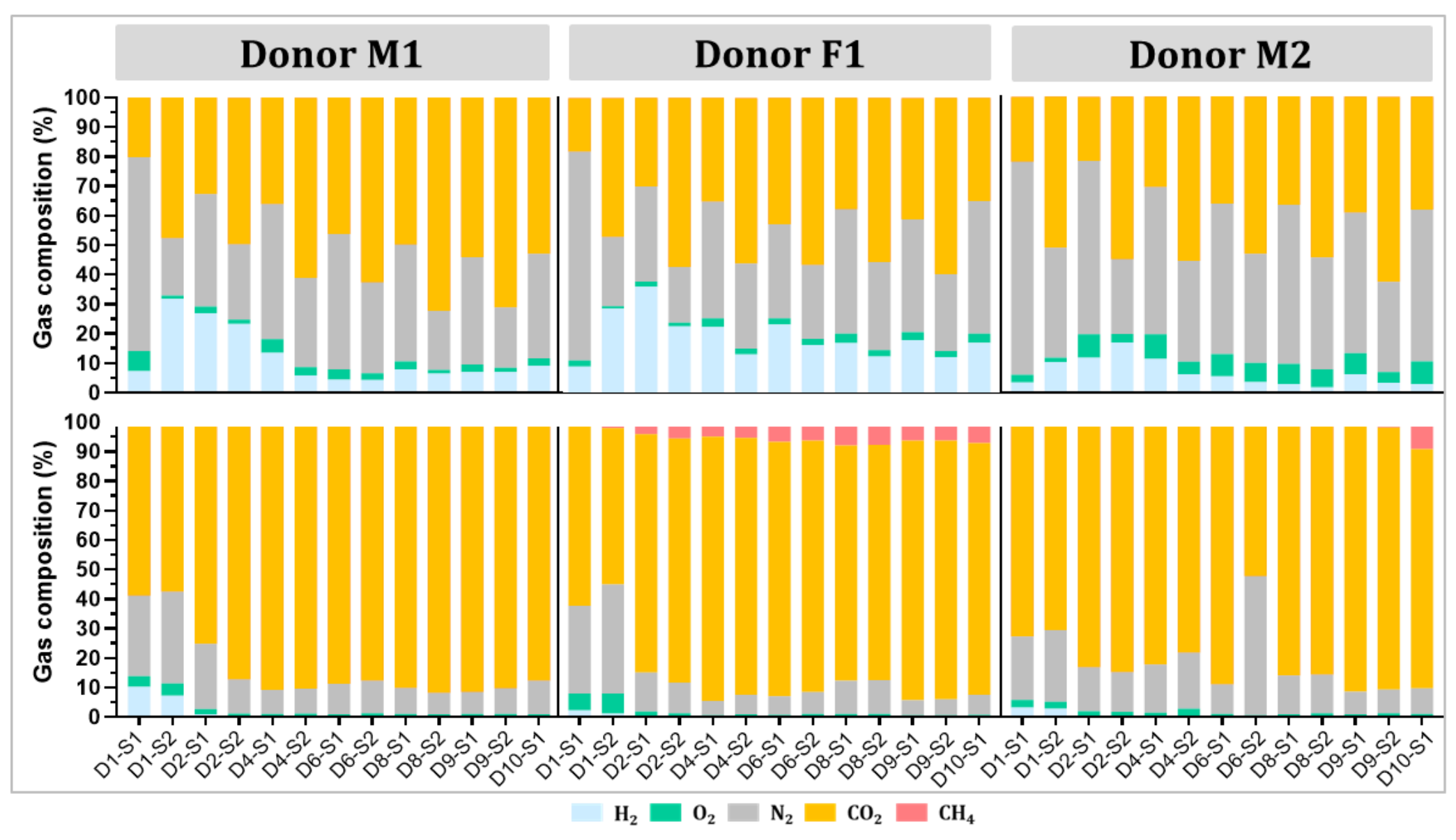
Inoculation → Batch (24h) → Continuous → Continuous fermentation (9days)

Daily schedule

M-ARILE: 9am, 10am, 3pm, 4pm — Fasted | Fed | Fasted | Fed | Fasted
S1: morning sampling (fermentation medium and gas in the bioreactor)
Sampling/2days (mucin beads)
S2: evening sampling (fermentation medium and gas in the bioreactor)

M-ARCOL: 9am, 10am, 3pm, 4pm — Colonic medium
S1: morning sampling (fermentation medium and gas in the bioreactor)
Sampling/2days (mucin beads)

RESULTS Microbiota activity



Gas composition

. Gas production stabilized at day 4 (D4) in M-ARILE and at day 2 (D2) in M-ARCOL.

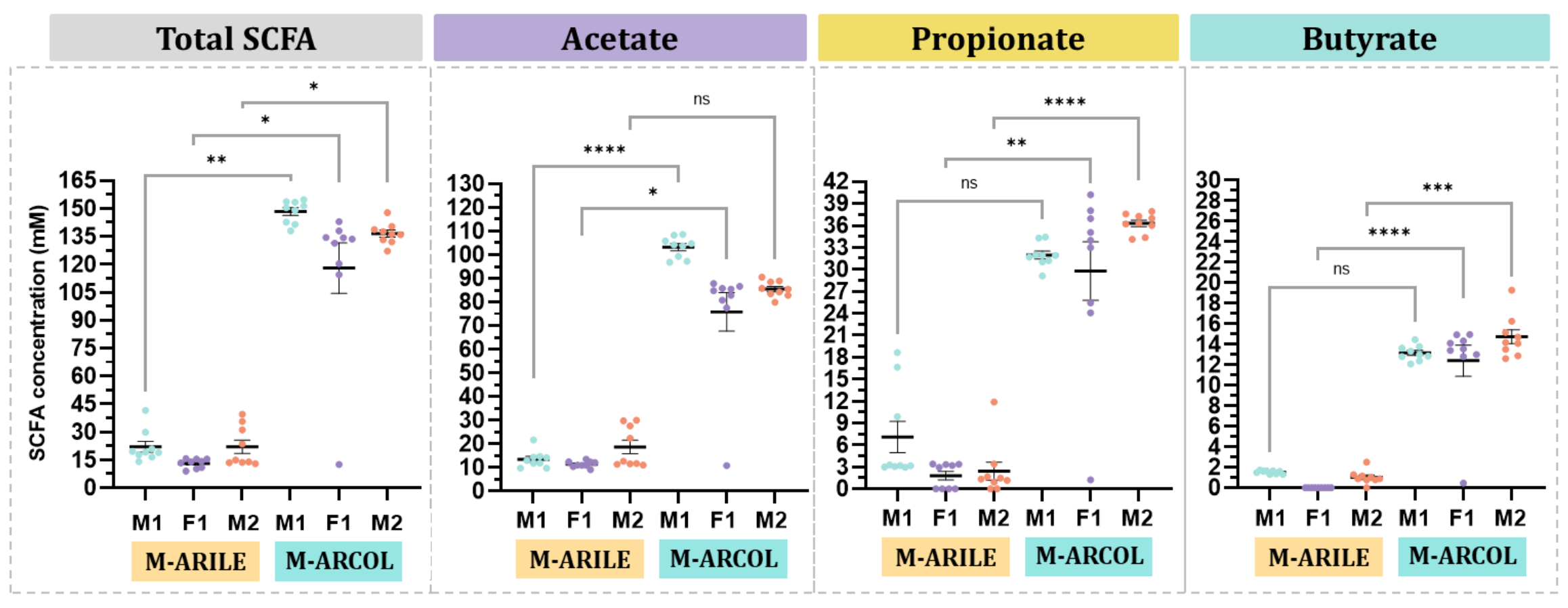
. Variations in CO_2 percentages between S1 & S2, only in M-ARILE and for all donors.

. No methane production in M-ARILE but CH_4 measured in M-ARCOL for donor F1.

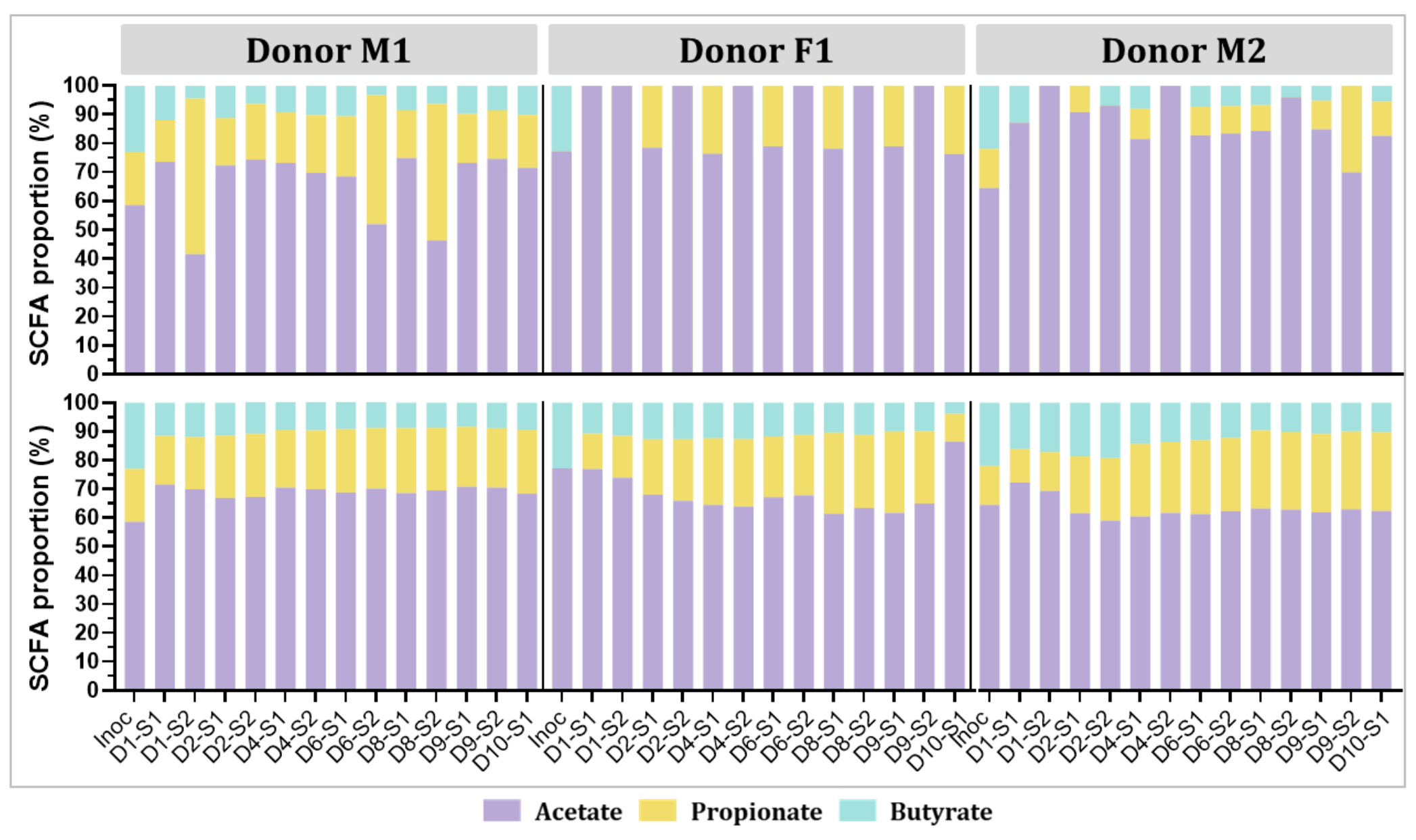
SCFA concentrations

. Lower total SCFA, acetate, propionate and butyrate concentrations in M-ARILE *versus* M-ARCOL for most of the donors.

. No butyrate production for donor F1 in M-ARILE.



Kruskal-Wallis test on mean stabilized SCFA production (D6 to D10) ±SEM with Dunn's multiple comparisons test (n=3). ns: not significant; *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001



SCFA composition

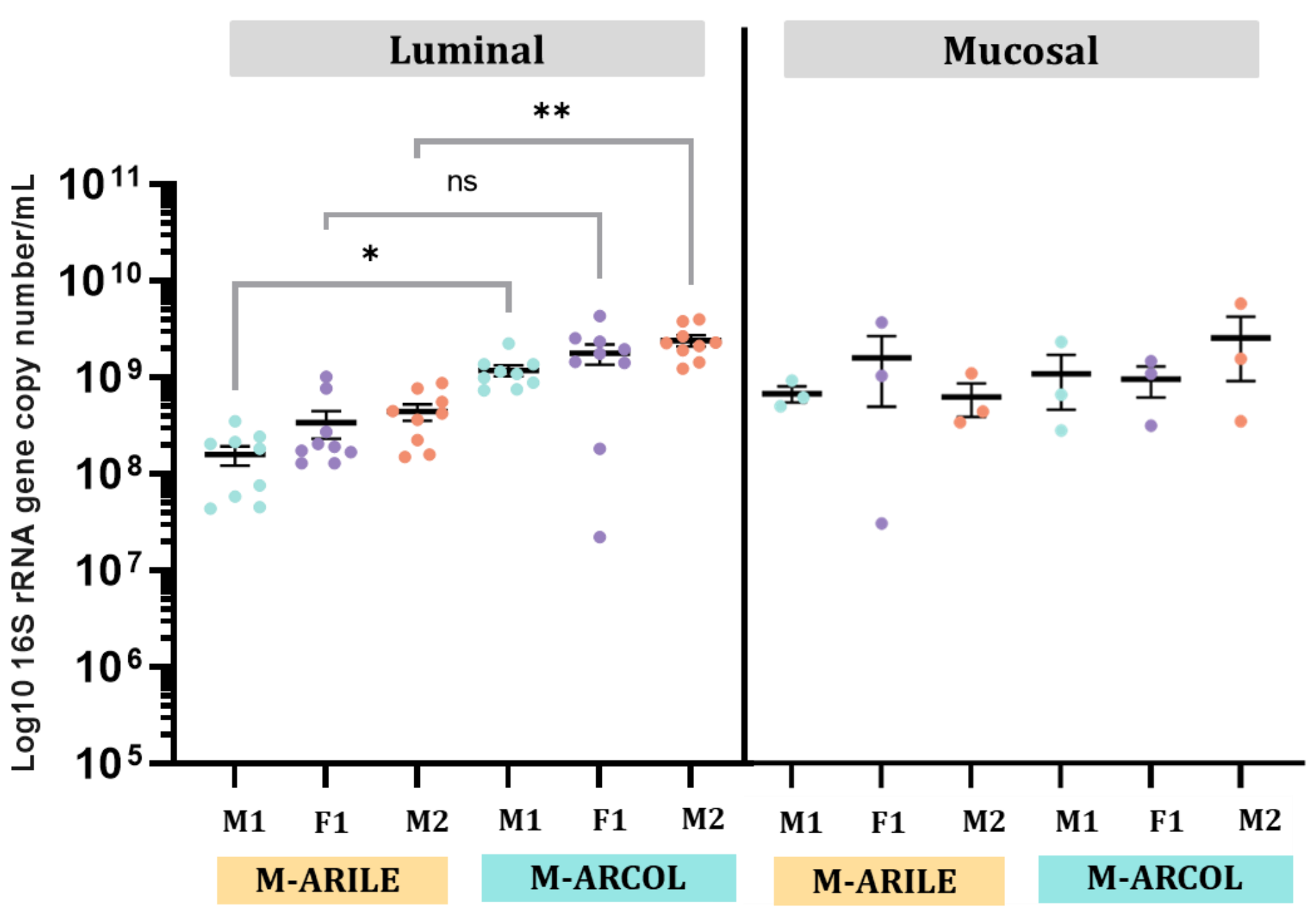
. SCFA composition stable at day 2 (D2) in both models, with variations between S1 & S2 in M-ARILE for all donors.

. In M-ARILE, SCFA proportions are donor dependant, but not in M-ARCOL.

(Inoc: inoculum)

RESULTS Microbiota composition

Total bacteria load

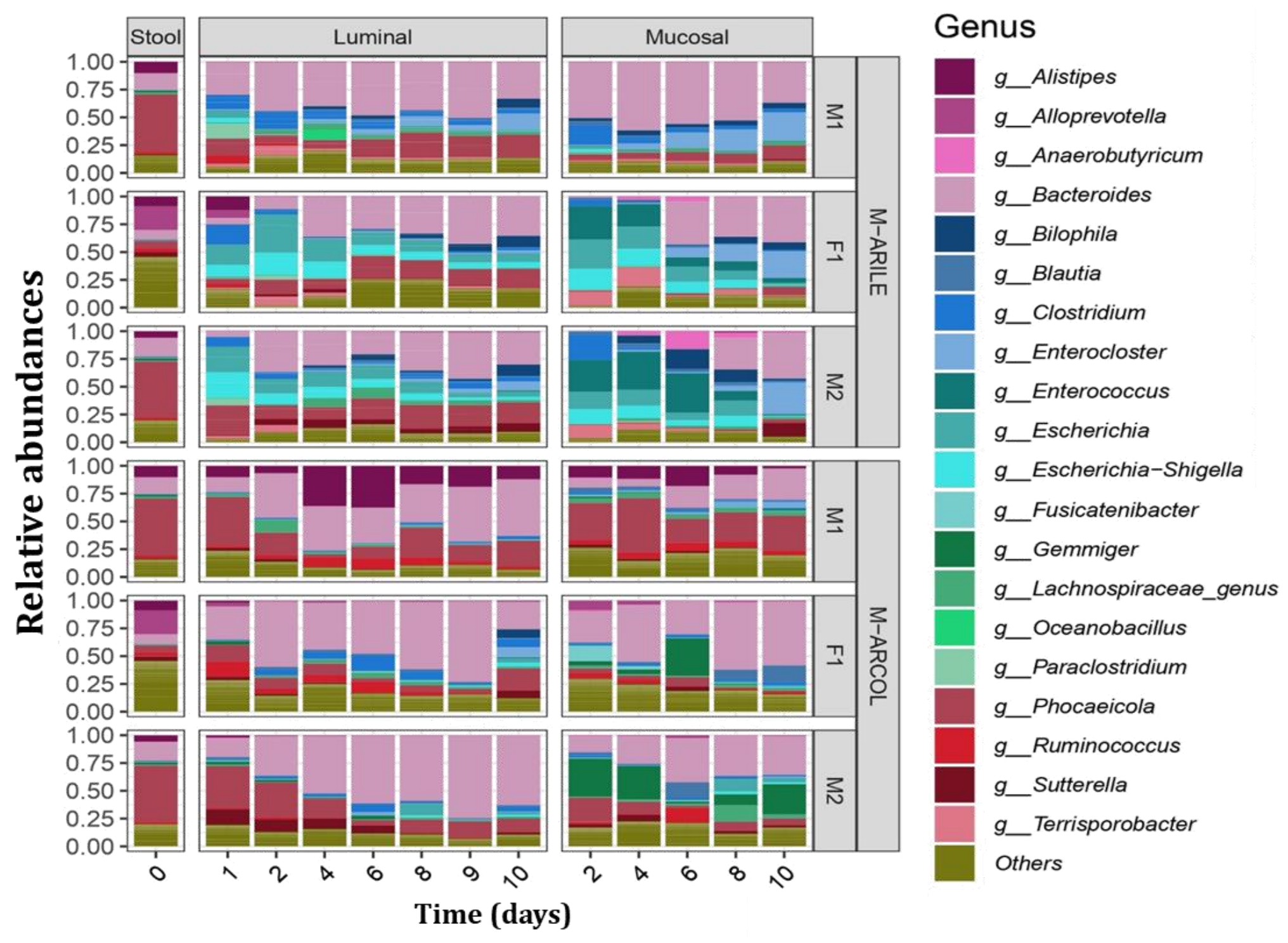


Kruskal-Wallis test on mean stabilized bacterial load (D6 to D10) ±SEM with Dunn's multiple comparisons test (n=3). ns: not significant; *p<0.05; **p<0.01

. Lower bacterial load in the luminal compartment from M-ARILE compared to M-ARCOL.

. In the mucosal part, no difference between the two models, whatever the donor.

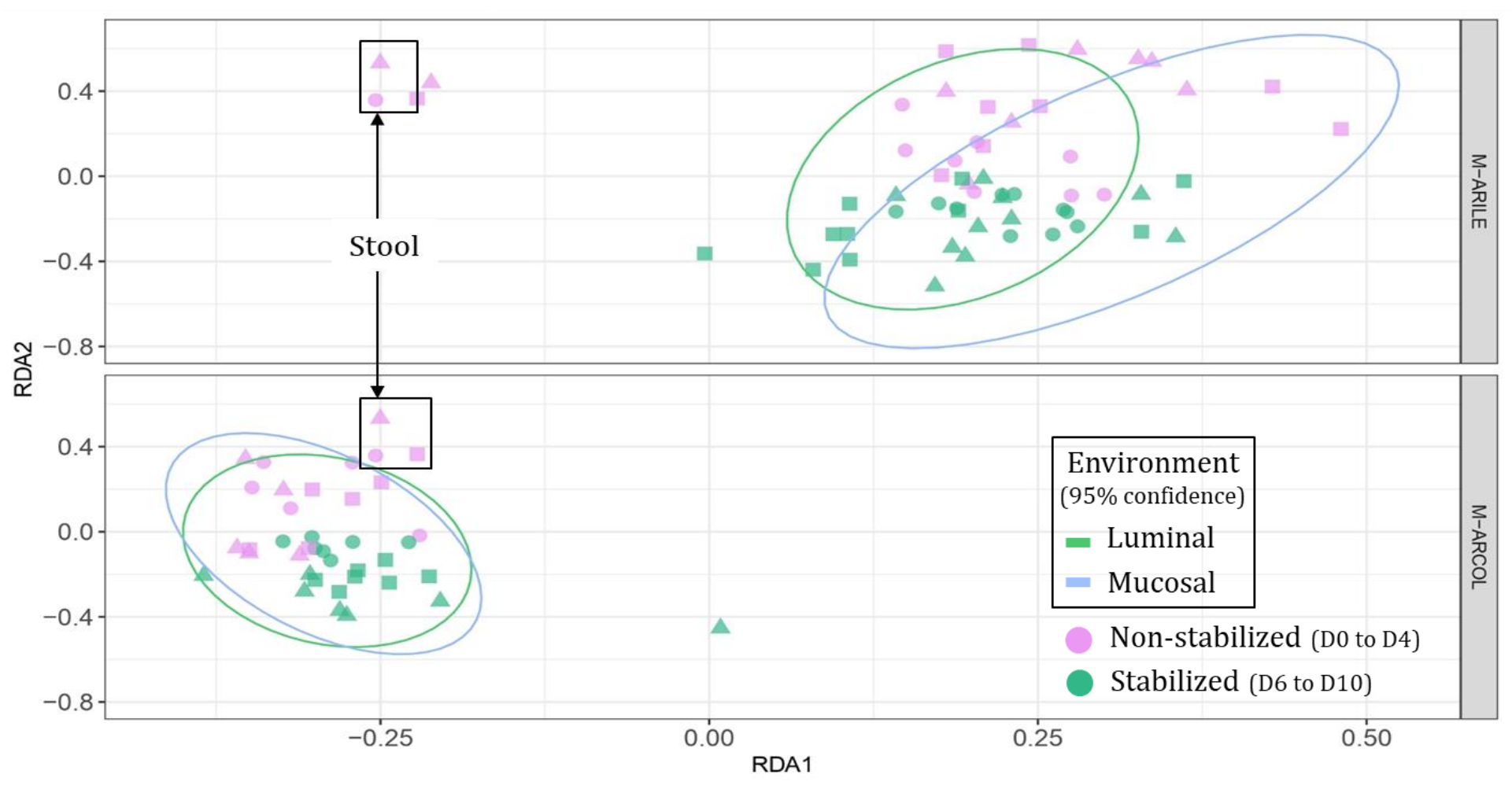
Taxonomy

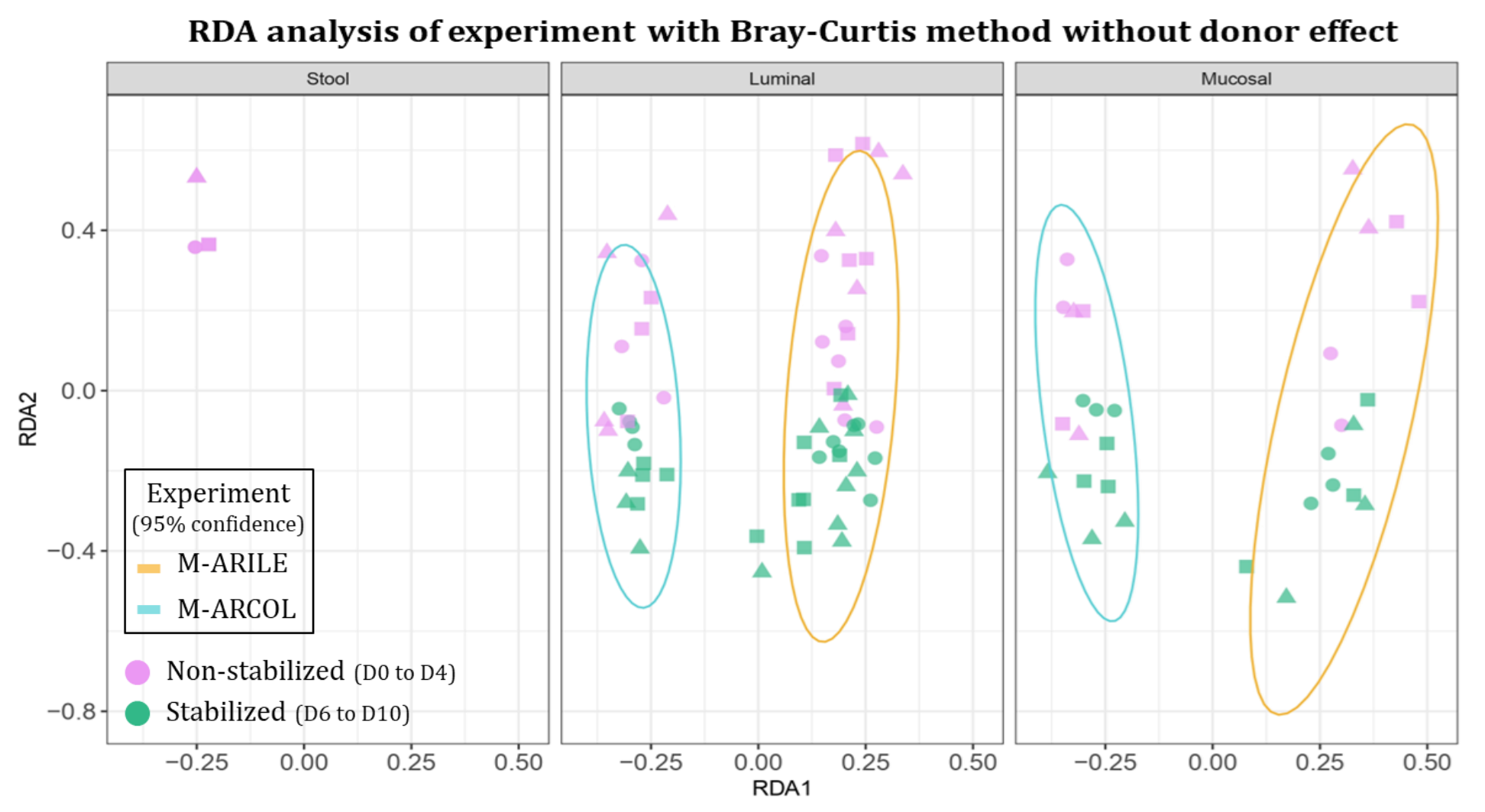


. Stabilization of bacterial profiles at day 6 in the luminal compartments of both models and, at day 8-10 in the mucosal phase.

. Higher abundances of *Clostridium*, *Escherichia* and *Enterococcus* genera in M-ARILE *versus* M-ARCOL.

β-diversity

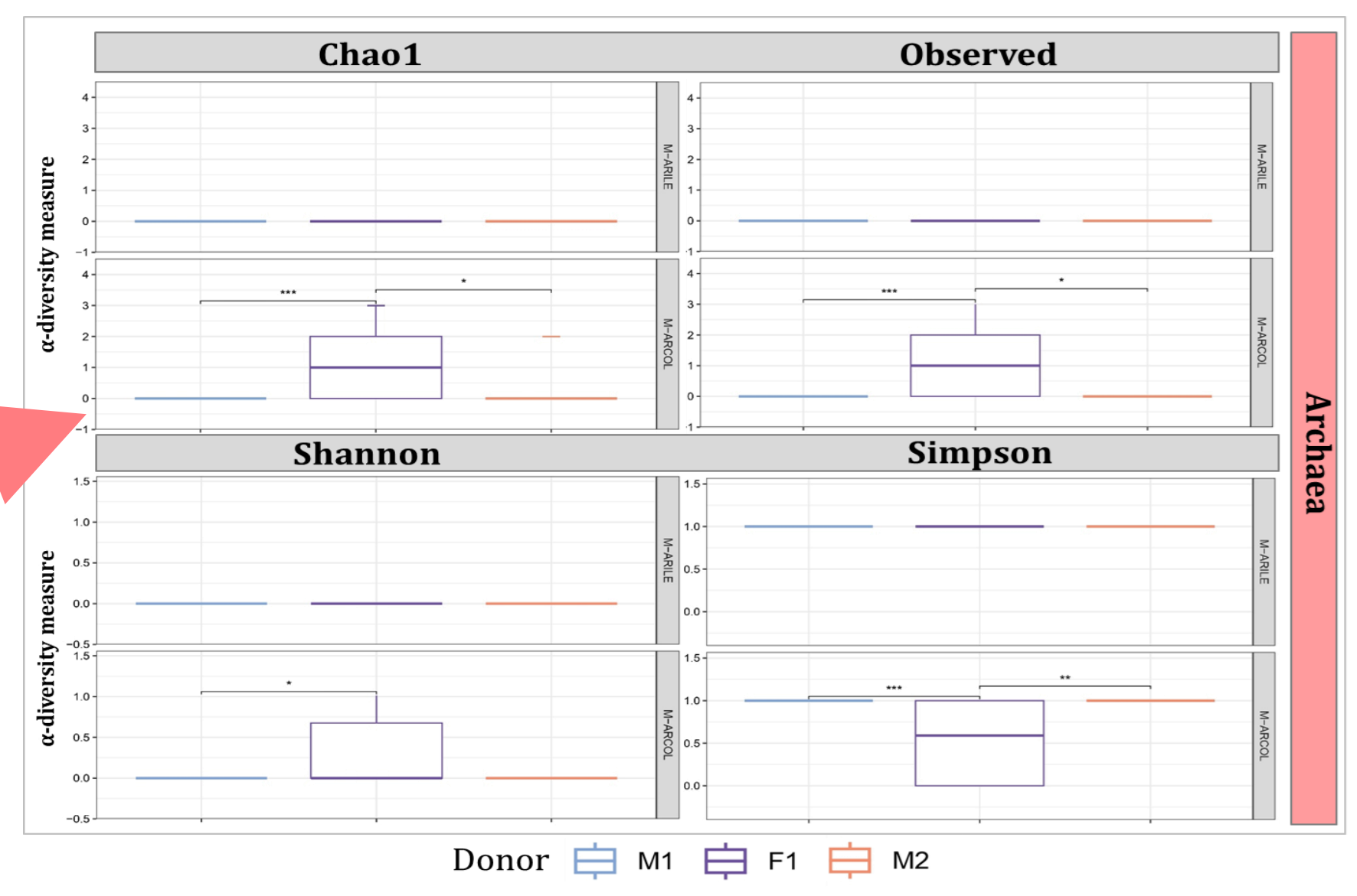

RDA analysis of environment with Bray-Curtis method without donor effect

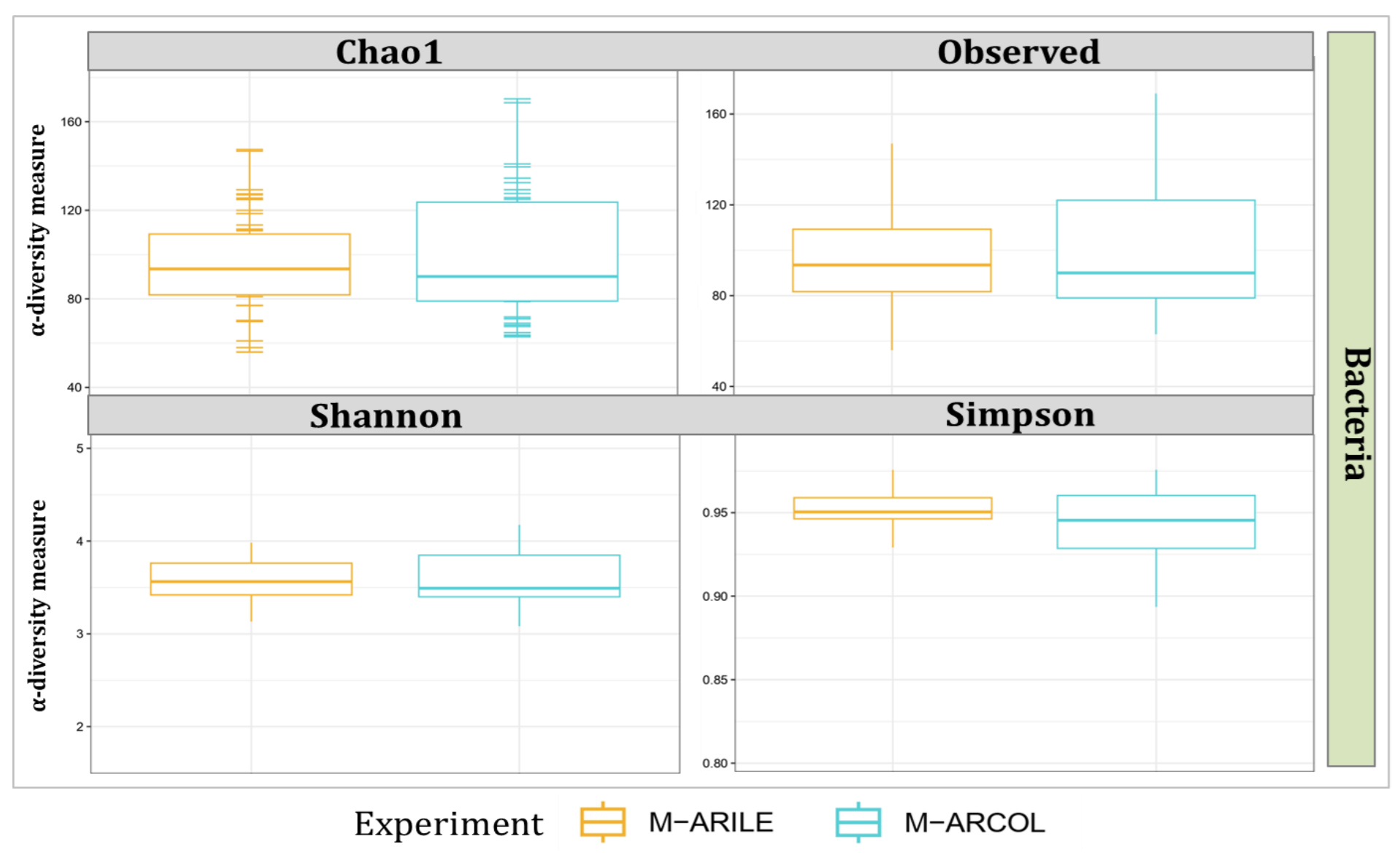

RDA analysis of experiment with Bray-Curtis method without donor effect

. Significant differences in lumen and mucus-associated microbiota, far more pronounced between the stool inoculum and the M-ARILE than the M-ARCOL.

. Differential clustering of ileal and colonic samples in both the luminal and the mucosal compartments.

α-diversity





. Consistency between metabarcoding analysis and methane production (only in M-ARCOL for donor F1).

. Similar α-diversity between M-ARILE and M-ARCOL (D6 to D10).

VALIDATION

Validation of M-ARILE regarding microbiota activity and composition

			M-ARILE *versus* M-ARCOL	***In vivo-in vitro* correlation**
Microbiota activity	SCFA production[I, II]	Total SCFA	↘	✓
		Acetate	↘	✓
		Propionate	↘	✓
		Butyrate	↘	✓
Microbiota load	Total bacteria[III, IV]	Luminal	↘	✓
		Mucosal	≈	✗
Microbiota composition[III, V] (Genera)	*Bacteroides*	Luminal & Mucosal	↘	✓
	Clostridium		↗	✓
	Escherichia		↗	✓
	Enterococcus		↗	✓
	Ruminococcus		↘	✓

[I]Cummings *et al.*, 1987; [II]Zoetendal *et al.*, 2012; [III]Delbaere *et al.*, 2022; [IV]Deschamps *et al.*, 2020; [V]Martinez-Guryn *et al.*, 2019

DISCUSSION

Most of the results in M-ARILE and M-ARCOL are in accordance with *in vivo* data in healthy human.

Metabolomic analysis are on going to better describe ileal and colonic *in vitro* samples

The M-ARILE model will provide a powerful platform for mechanistic studies on healthy human ileal microbiome and its interaction with nutrient, drug or enteric pathogen found in the small intestine.

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