A new *in vitro* model of the human ileal ecosystem and its lumen and mucus-associated microbiome

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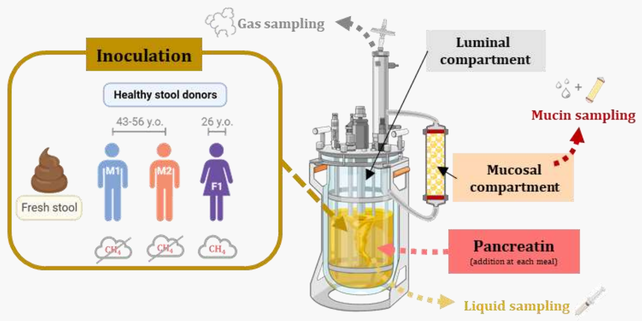
INTRODUCTION

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 has been largely understudied due to sampling invasiveness, especially in healthy volunteers. One alternative to *in vivo* assays is the use of ***in vitro* models simulating the human digestive tract**. However, up to now, there is no available *in vitro* system simulating the human ileal ecosystem and its associated microbiota, that has been fully developed and validated based on *in vivo* data.

Aim of this study: Develop and validate an *in vitro* dynamic model of the healthy human ileum microbiota, reproducing both **luminal and mucosal microenvironments** and the **kinetics of feeding** (alternance of fasted - fed states).

MATERIAL & METHODS

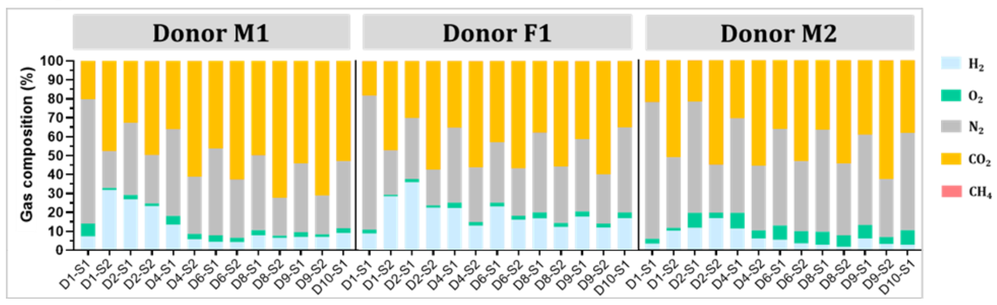
	M-ARILE	
	Rich (fed-state)	**Minimum** (fasted-state)
Nutritive Media		
• Carbohydrates	+++	**none**
• Lipids	+++	+
• Proteins	+++	+
• Bile acids	**[10mM]**	**[4mM]**
↳ BA I	+++	++
↳ BA II	++	+
pH	7.2	
Transit time	2h	
Stirring	Day 300rpm / Night 100rpm	
Fermentation	10 days continous fermentation	



RESULTS

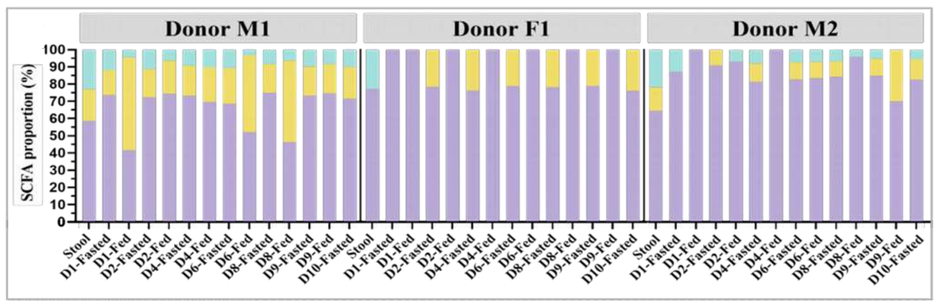
Microbiota activity

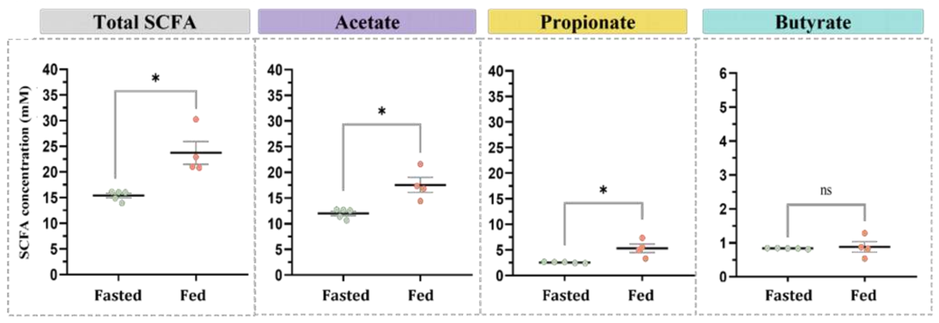
Gas composition



. Majority of CO_2 and H_2, low O_2 proportion and no CH_4 in the M-ARILE
. Significant higher proportion of CO_2 in fed (S2) than in fasted state (S1)

SCFA composition



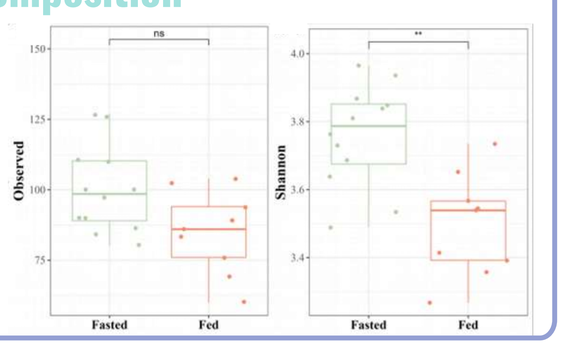


. SCFA proportions are donor-dependant, with an average acetate/propionate/butyrate ratio of 80/17/4 %, no butyrate for donor F1
. Total SCFA concentrations, as well as the three main SCFA, significantly higher in fed than in fasted state

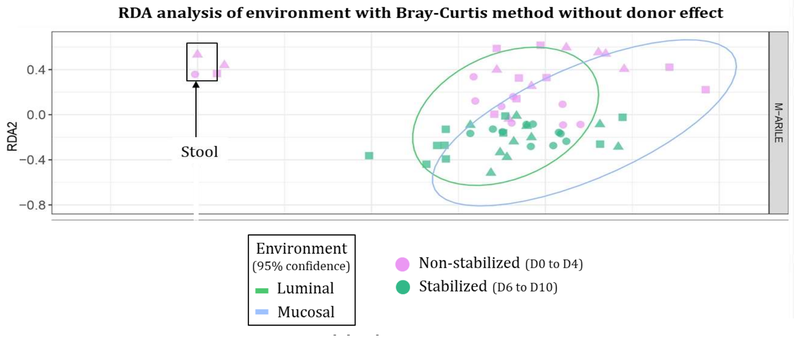
Microbiota composition

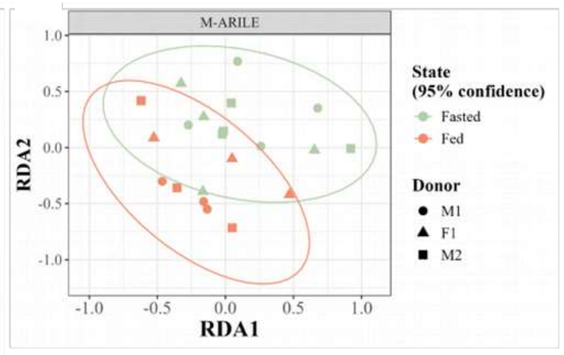
α-diversity

. Lower α-diversity in fed than in fasted state
Hypothesis: fast growing of dominant microbes, 'hiding' low abundant biomass



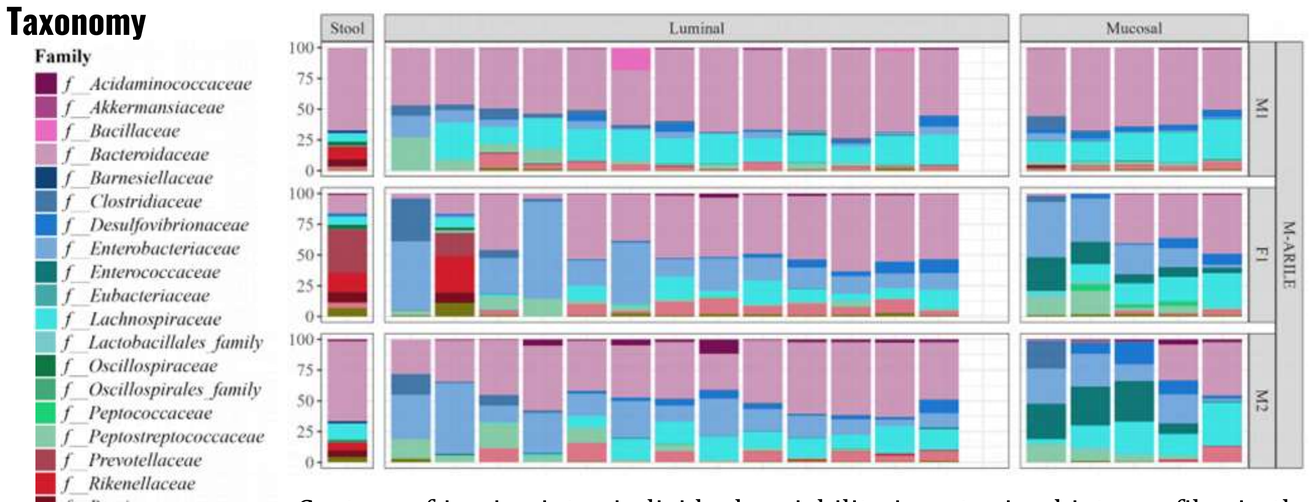
β-diversity



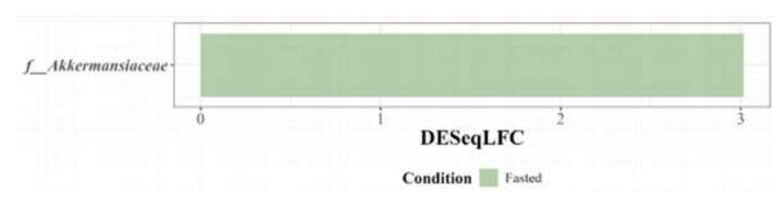


. Different clustering of lumen and mucus-associated microbiota in the M-ARILE
. β-diversity of the ileal microbiota driven by feeding status

Taxonomy



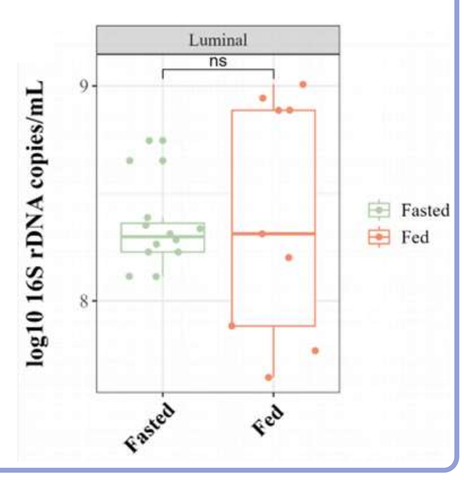
. Capture of in vivo inter-individual variability in gut microbiota profiles in the M-ARILE
. Presence of predominant bacteria colonizing the ileum *in vivo*: *Bifidobacteriaceae, Clostridiaceae, Enterococcaceae*



. Differential analysis: significant higher abundance of *Akkermansiaceae* in fasted than in fed state

Total bacteria load

. Bacterial load in the lumen similar to *in vivo* (10^8 copies/mL)
. No significant difference in the number of bacteria between fed and fasted states



VALIDATION

Comparison between *in vitro* data in the M-ARILE and *in vivo* data in healthy human ileum

			M-ARILE	*In vivo* (ileum)	*In vivo-in vitro* correlation
Microbiota activity	SCFA production[I, II] (mM)	Total SCFA	19.1	11.7-81.6	✓
		Acetate	14.5	7.9-64.6	✓
		Propionate	3.8	1.5-3.3	✓
		Butyrate	0.9	2.3-13.7	×
	Gas proportion[III, IV] (%)	CH_4	0	0	✓
		CO_2	50.3	6-17.5	×
		O_2	3.7	5-41	✓
		H_2	9.3	0.3-3.5	×
Microbiota load	Total bacteria[V]		10^8	10^7-10^8	✓
Microbiota composition[VI-XIII] (Family)	*Streptococcaceae*	Luminal & Mucosal	-	+	×
	Veillonellaceae		+	+	✓
	Clostridiaceae		+	+	✓
	Lactobacillaceae		-	+	×
	Enterobacteriaceae		+	+	✓
	Bacteroidaceae		+	+	✓
	Bifidobacteriaceae		+	+	✓

[I]Cummings *et al.*, 1987; [II]Zoetendal *et al.*, 2012; [III]Berean *et al.*, 2018; [IV]Kalantar-Zadeh *et al.*, 2018-2019; [V]Delbaere *et al.*, 2022; [VI]Booijink *et al.*, 2010; [VII]Fan *et al.*, 2020; [VIII]Kashiwagi *et al.*, 2020; [IX]Nagasue *et al.*, 2022; [X]Shalon *et al.*, 2023; [XI]Van Trijp *et al.*, 2024; [XII]Wang *et al.*, 2005; [XIII]Zilberstein *et al.*, 2007

DISCUSSION

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

The M-ARILE model can provide a powerful platform for mechanistic researches on personalized nutrition and medicine in the gut microbiome field by providing information on

- **the impact of ileal microbiota on food digestibility and micronutrients bio-accessibility**
- **the effect of drug on ileal microbes but also their potential for drug metabolism**
- **the interactions between food-borne pathogens and ileal microbial communities**

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