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Validation of rumen sampling via stomach tubing to inoculate dual-effluent mini-fermenters

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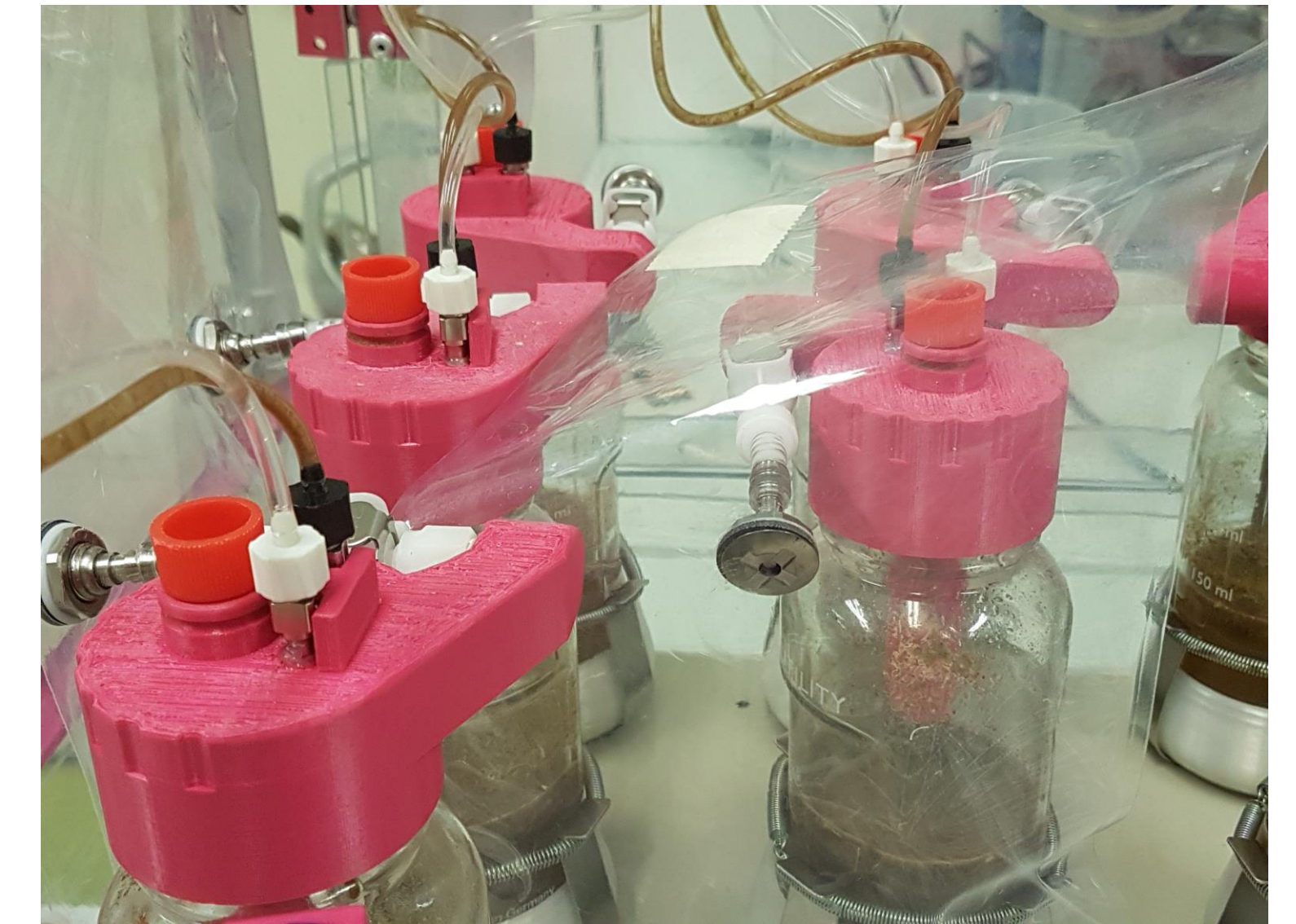
Validating alternatives to rumen cannulation to obtain rumen contents for inoculating *in vitro* devices is necessary due to societal demand for less invasive techniques in animal experimentation

Aim of the study

To compare, in dual-effluent mini-fermenters, stomach tubing (ST) and rumen cannulation (RC) for fermentation parameters
methanogenesis
microbiota composition

Materials and methods

- Donors of rumen inoculum : 2 rumen cannulated dry goats
- Dual-effluent fermenters : working volume 80 mL; liquid (7%/h) and solid (3%/h) turnover rates; artificial saliva at 7 mL/h
- Four 7-days periods with each condition tested in duplicate
- Diet (hay 0.6; barley 0.2; soybean meal 0.2) given twice a day at 8:30 and 15:30
- At the end of each period (days 6 and 7), all 8 fermenters sampled for
 - fermentation end-products production (CH₄ and SCFA production)
 - microbiota composition using 16S-rDNA sequencing of V4-V5 regions, FROGS pipeline (Escudie F, Auer L, Bernard M, Mariadassou M, Cauquil L, Vidal K, Maman S, Hernandez-Raquet G, Combes S, Pascal G. 2018. *Bioinformatics*,4, 1287–1294).



Results

Fermentation end-products

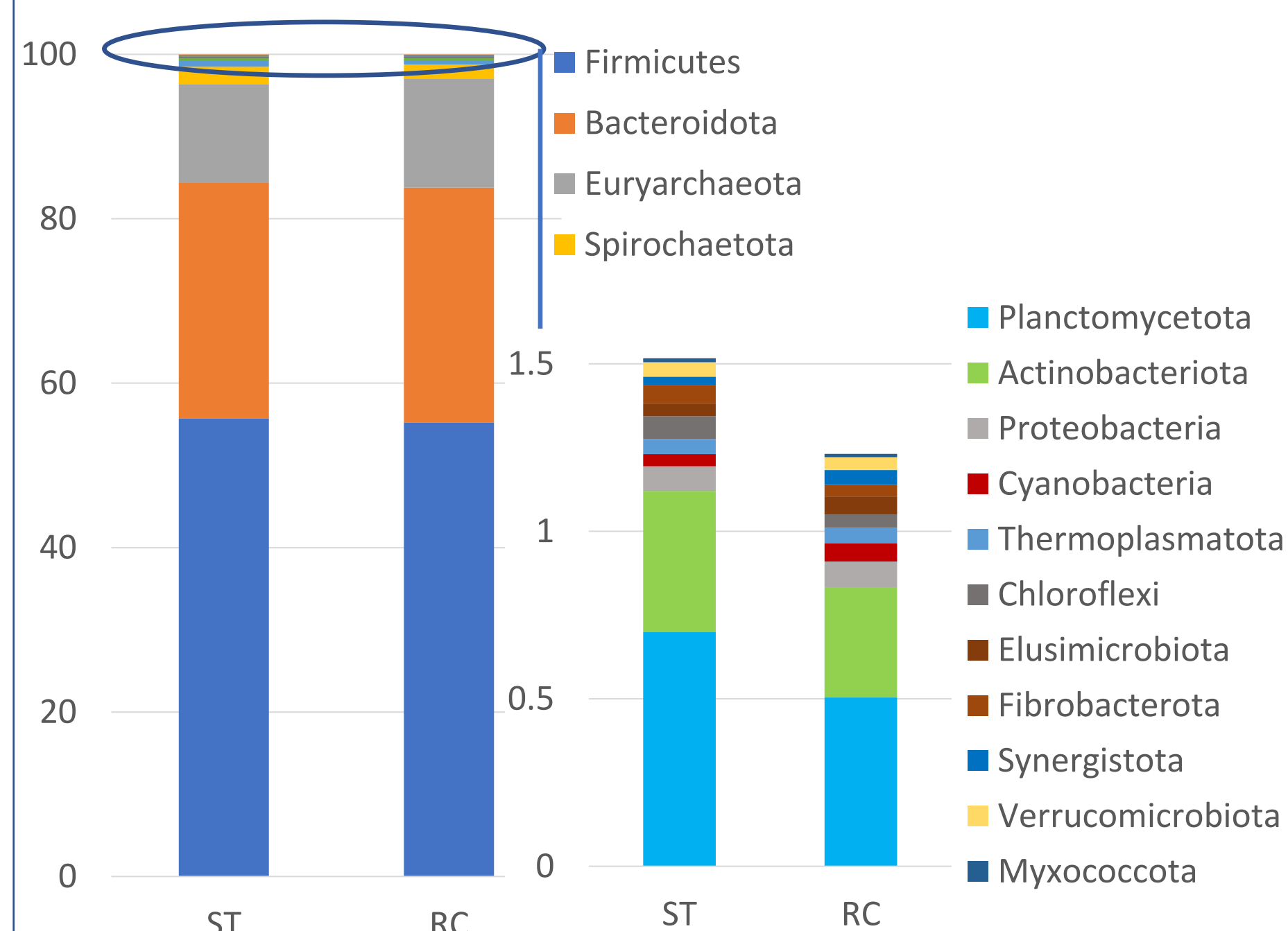
SCFA pattern in inocula, amounts of hexoses fermented (HF) and fermentation pattern in fermenters at days 6-7

	RC	ST	RMSE	P
SCFA in inocula				
Total (mM)	46.9	33.4	6.84	0.008
Acetate, C2 (%)	71.3	70.8	0.92	0.73
Propionate, C3 (%)	19.9	20.1	1.12	0.79
Butyrate, C4 (%)	8.7	9.1	1.39	0.99
HF (mmol/day)				
	4.52	4.54	0.333	0.57
Specific productions (mol/100mol HF)				
C2	115.7	116.7	3.41	0.45
C3	41.6	41.2	2.15	0.70
C4	21.4	21.1	1.88	0.64
CH ₄	55.5	55.5	0.98	0.98

The SCFA concentration of the rumen inocula was lower with ST than with RC because of saliva dilution but with similar proportions of individual SCFA

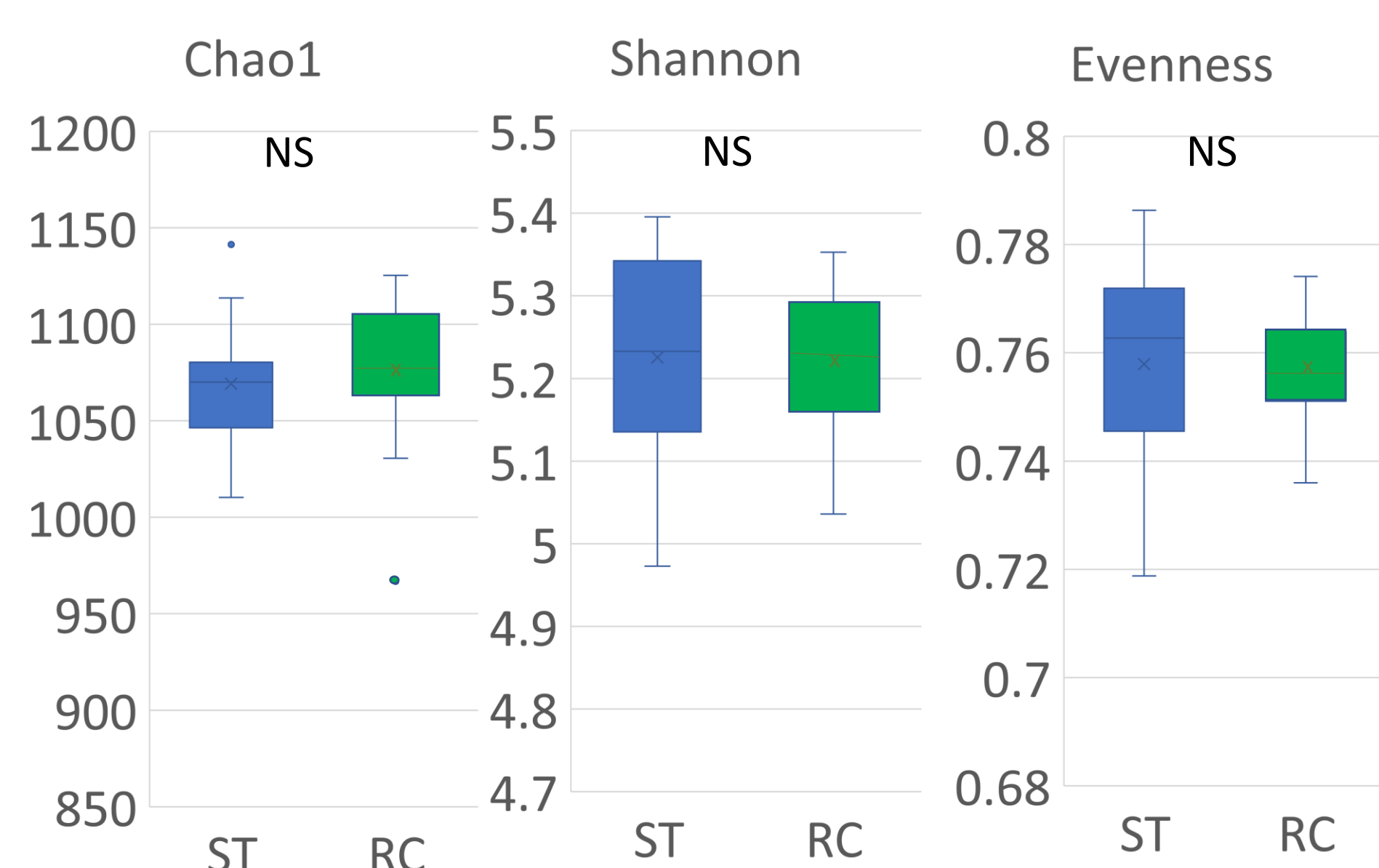
After 5 days of adaptation, fermentation parameters were not significantly different between ST and RC

Microbiota composition and diversity



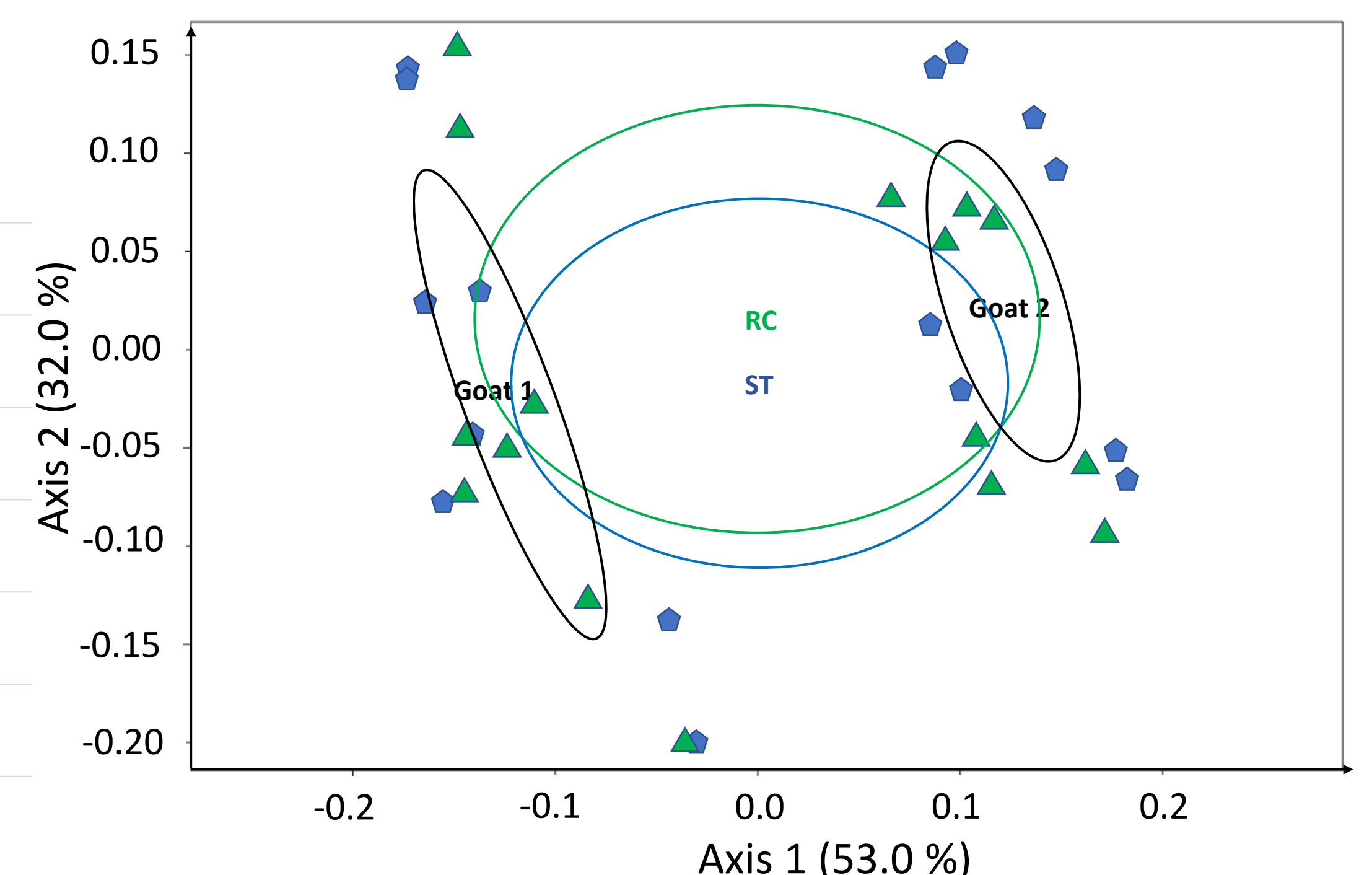
Composition (%) of fermenter microbiota at days 6-7 according to the inocula sampling at phyla level

The microbiota composition between ST and RC is similar at the phyla level



α-diversity indexes of fermenter microbiota at days 6-7 according to the inocula sampling

Alpha diversity of the fermenter microbiota not significantly different between ST and RC, with indexes closed to *in vivo* measurements



PCoA of inocula microbiota samples based on ASVs community dissimilarities (Bray Curtis) (● ST; ▲ RC)

Microbiota community structures similar between ST and RC with a clear clustering by goat

Conclusion

The activity and structure of microbiota in dual-effluent mini-fermenters inoculated with ST and RC were similar. Stomach tubing sampling is a good alternative to rumen cannulation