



PROCEEDINGS OF THE 12th WORLD RABBIT CONGRESS

Nantes (France) - November 3-5, 2021

ISSN 2308-1910

Session
BIOLOGY and PHYSIOLOGY

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How to cite this paper

Song B., Zhao J.P., Ren Z.J., 2021. The differences of cecal communities in rabbits and hares. Proceedings 12th World Rabbit Congress - November 3-5 2021 - Nantes, France, Communication BP-30, 3pp. + presentation

THE DIFFERENCES OF CECAL COMMUNITIES IN RABBITS AND HARES

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ABSTRACT

Rabbits and hares belong to the Leporidae family of the order Lagomorpha, though they are different in biology and feeding. Moreover, the cecal fermentation pattern of the hare is characterised by higher molar proportions of propionate and isobutyrate compared to those observed in rabbit cecum. But little is known about whether the species differences or healthy status differences contribute to their different cecal communities or not. The aim of this project is to estimate and compare the differences of cecal communities among healthy and diarrheal rex rabbits and hares by sequencing the V4 hypervariable region of the 16S rDNA using the Illumina MiSeq platform. Preliminary results showed that in the phylum level, Firmicutes dominated in the ceca of healthy and diarrheal rex rabbits, and Sphaerochaeta was the most dominant genus in hares; in the genera level, Christensenellaceae R-7 group and Ruminococcaceae unclassified genus were the most abundant genera in healthy rex rabbits, Escherichia-Shigella, Bacteroides, Bacteroidales S24-7 group norank and Ruminococcaceae unclassified genus were enriched in diarrheal rex rabbits, and Sphaerochaeta was the most dominant genus in hares. Our study demonstrated the features of cecal communities of healthy and diarrheal rex rabbits and hares, which have important implications for furthering the study of the cecal communities of Leporidae.

Key words: rabbit, hare, diarrhea, cecal communities, diversity

INTRODUCTION

Rabbits and hares are both herbage feeding small mammals sharing the general Leporidae characteristic of cecal fermentation of digesta. However, they have different digestive physiology and cecal fermentation characteristics (Mista et al. 2015). Previous study showed that wild and farm rabbits harbored clearly different bacterial and archaeal communities (Abecia et al. 2012). We thus hypothesize that species differences might also contribute to the differences in cecal microbial community between rabbits and hares.

In addition, the physiological status may be an important factor in determining the diversity of cecal communities among individuals of the same species. Some recent studies found that remarkable dysbiosis in cecal communities with a decrease in probiotics and an increase in some harmful microorganisms (Zhou et al. 2016) and digestive disorders are the main cause of morbidity and mortality in fattening rabbits and caused important economic losses in industrial rabbit farms.

Thus, the objective of this project is to study the difference of cecal microbiota of healthy and diarrheal rex rabbits and hares by sequencing the V4 hypervariable region of the 16S rDNA through the Illumina MiSeq platform.

MATERIALS AND METHODS

Animals and experimental design

Three healthy and three diarrheal growing rex rabbits *Oryctolagus cuniculus* (60 days old) from rabbitry in each of the four regions in China, including Changan region (CH 1-3, CD 1-3), Fengxiang county (FH 1-3, FD 1-3), Yangling region (YH 1-3, YD 1-3), and Zhashui county (ZH 1-3, ZD 1-3)

were collected. The syndromes of diarrhea in rabbits were cecal impaction, abdominal distension with gas and fluid accumulation in stomach and intestines, copious clear yellowish semi-fluid around the anus. Three healthy adult cape hares *Lepus capensis* were collected in Runzhen town (RH 1–3), Guanzhong Plain, China during field work. Experimental animals were placed in wooden cages in which they were transported to a laboratory. Animals were slaughtered by air embolism through ear vein immediately after they arrived at the laboratory, their caecum were excised and 2 g cecal contents were immediately sampled and stored at -20 °C for subsequent microbiological analyses.

Pyrosequencing and statistical analysis

Total DNA was extracted from cecum samples using a stool DNA kit (OMEGA Bio-Tek, Norcross, GA, USA) following the manufacturer's instructions. DNA concentrations were measured at 260 nm with a Nano Drop (3.0.1, ND-1000, Willington, USA) and purity was accessed by measuring the A260 / A280 ratio. Sequencing was performed at Shanghai Majorbio Bio-Pharm Technology Co., Ltd. Pyrosequencing was conducted on an Illumina MiSeq 2 × 250 platform. Barplots was generated using the R vegan package and family information for samples.

RESULTS AND DISCUSSION

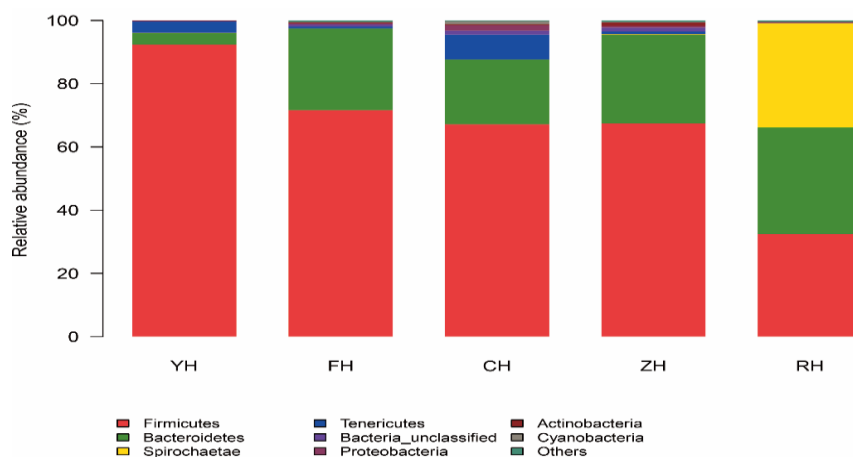


Figure 1: Relative proportion of predominant common phyla of healthy rex rabbits (YH, FH, CH and ZH) and hares (RH).

The relative abundance of Firmicutes and Tenericutes of healthy rex rabbits are more abundant than hares, whereas their relative abundance of Spirochaetae are lower than hares (Figure 1).

The most abundant genus of cecal community of CD was *Escherichia-Shigella/Enterobacteriaceae/Proteobacteria* which was more abundant than CH, and that of YD was more abundant than YH (Figure 2). The relative abundance of *Ruminococcaceae_unclassified* of FD decreased and *Bacteroides* increased comparing with FH. The relative abundance of *Ruminococcaceae_unclassified* and *Christensenellaceae_R-7_group* of ZD increased and *Bacteroides* decreased comparing with ZH.

The most dominant genus of cecal community of RH was *Sphaerochaeta/Spirochaetaceae/Spirochaete* which was higher than observed in healthy rex rabbits. It could be a result of the slightly different diets and digestive physiologies of these two species. First, hare is a facultative generalist herbivore that adapts its diet to the seasonal vegetation changes (Sokos, Andreadis, and Papageorgiou 2015). European hares selected diet for high energy content (crude fat and crude protein) and avoided crude fiber, so they preferred weeds/grasses and various crop types while avoiding cereals (Schai-Braun et al. 2015). In contrast, farm rex rabbits were fed with standard feed which contains more protein and less fiber. Moreover, the cecal microbiota composition can be changed by the high dietary protein (Hugenholtz 2015). Second, the cecal fermentation pattern of the hare was characterised by higher propionate and isobutyrate molar proportions compared with those observed in rabbit caecum, which indicates that the activity of the microbial populations colonising the hare and rabbit caecum was different.

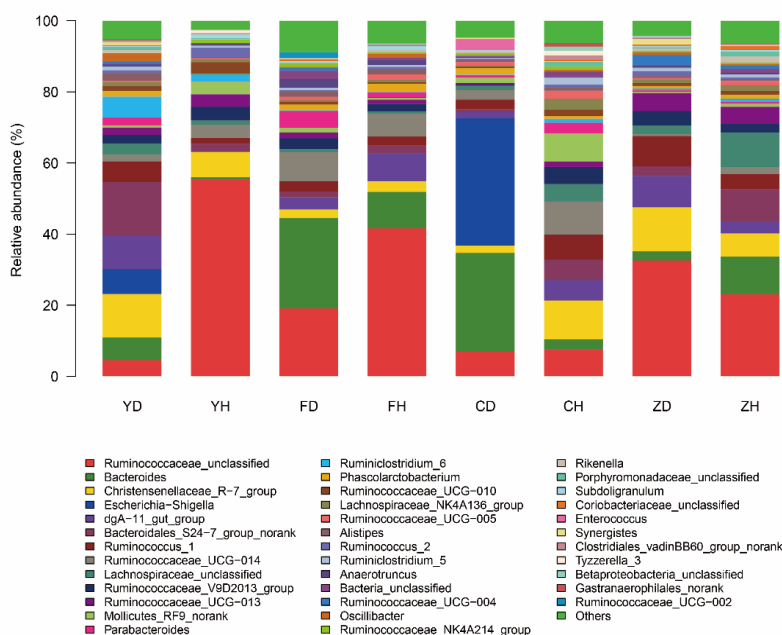


Figure 2: Relative proportion of predominant common genera of healthy (YH, FH, CH and ZH) and diarrheal (YD, FD, CD and ZD) rex rabbits.

The relative abundance of *Escherichia-Shigella* of CD was higher than CH, and the the relative abundance of *Escherichia-Shigella* of YD was also higher than YH, both of which only indicate that harmful microorganism quantity in the cecum of diarrheal rex rabbits has an increased trend. But we did not observe such differences between healthy and diarrheal rex rabbits in two other rabbitries. In conclusion, according to the changes of cecal communities of the diarrheal rex rabbits in each rabbitry, the relative abundance of pathogens of diarrheal rabbits were not significantly higher than healthy rex rabbits, the diarrhea is not caused by pathogens and doesn't belong to pathogenic diarrhea.

CONCLUSIONS

In summary, our study indicated that different diets and digestive physiologies caused the differences of cecal communities in rabbits and hares, and different healthy status contributes to the differences between healthy and diarrheal rabbits.

ACKNOWLEDGEMENTS

This work was supported by the Key Science and Technology Program of Shaanxi Province, China (2016 NY-108).

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The differences of cecal communities in rabbits and hares

Bing Song

15:30-16:30PM 03.11.2021

Sampling

Farm 1: D1 vs H1

Farm 2: D2 vs H2

Farm 3: D3 vs H3

Farm 4: D4 vs H4

Wild: RH

D: Diarrheal

H: Healthy

RH: Hares

Part I

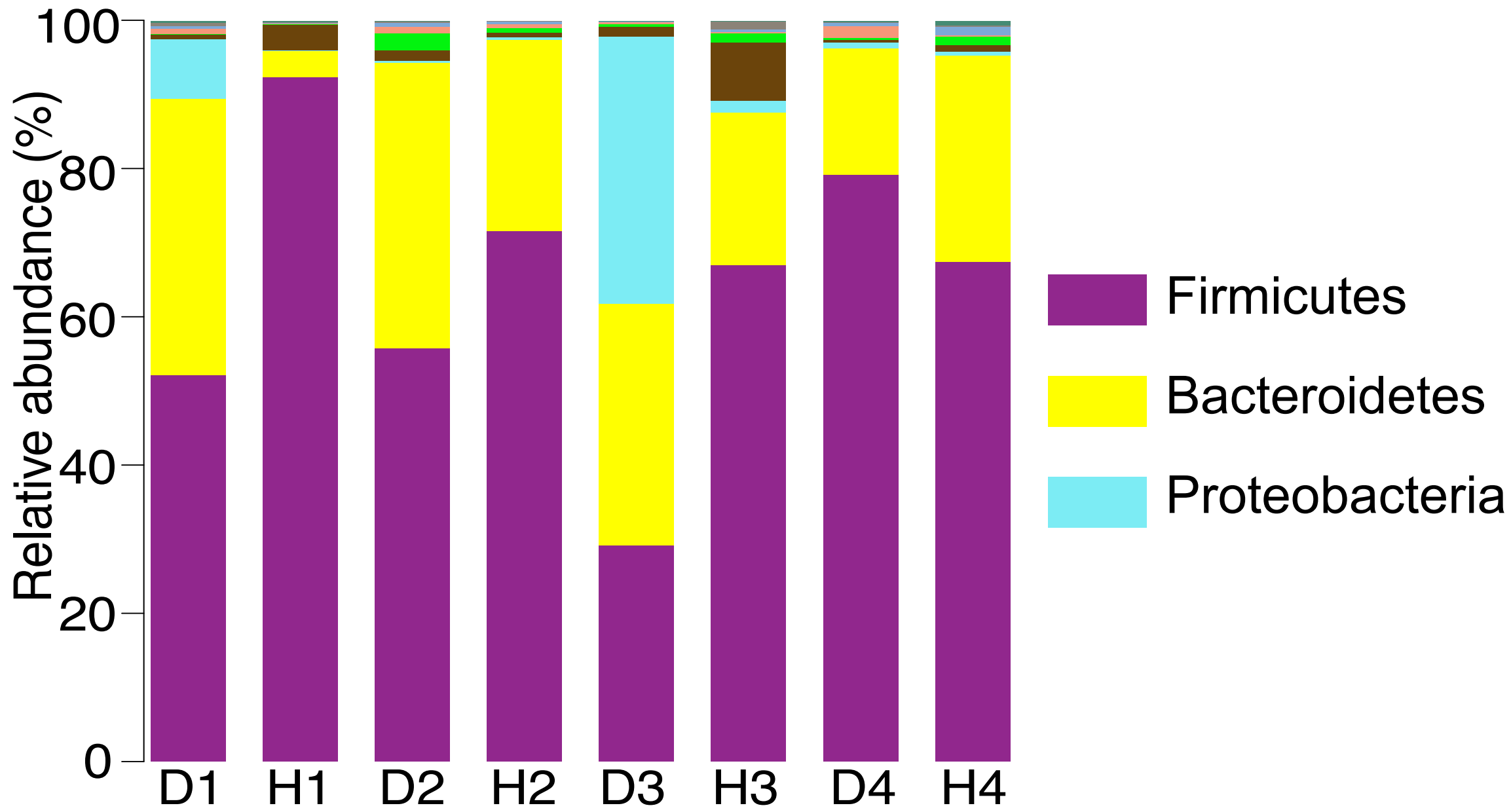
Diarrheal
VS
Healthy
Rabbits

Part II

Healthy
Rabbits
VS
Hares

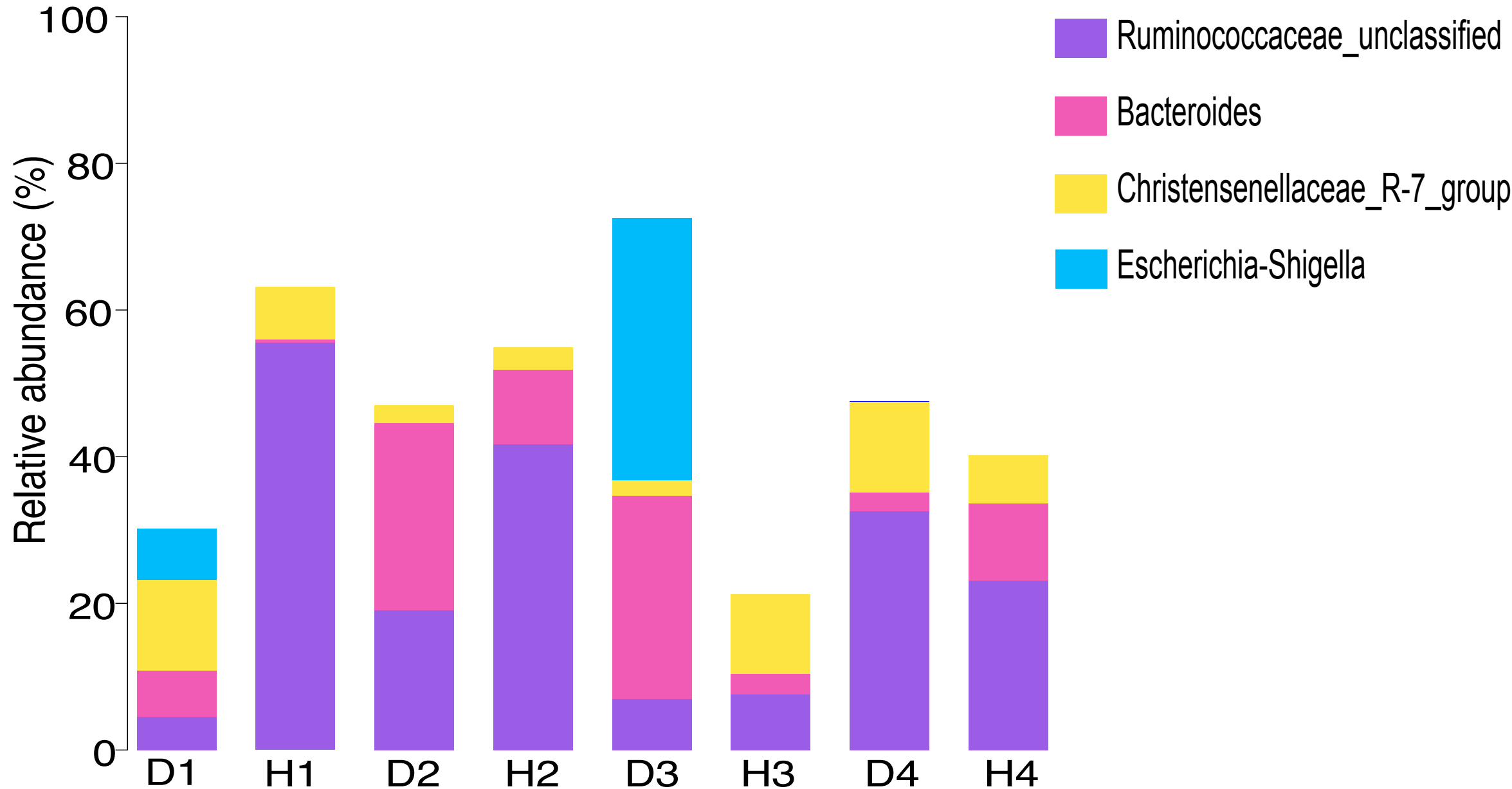


Dominant phyla in healthy and diarrheal rabbits



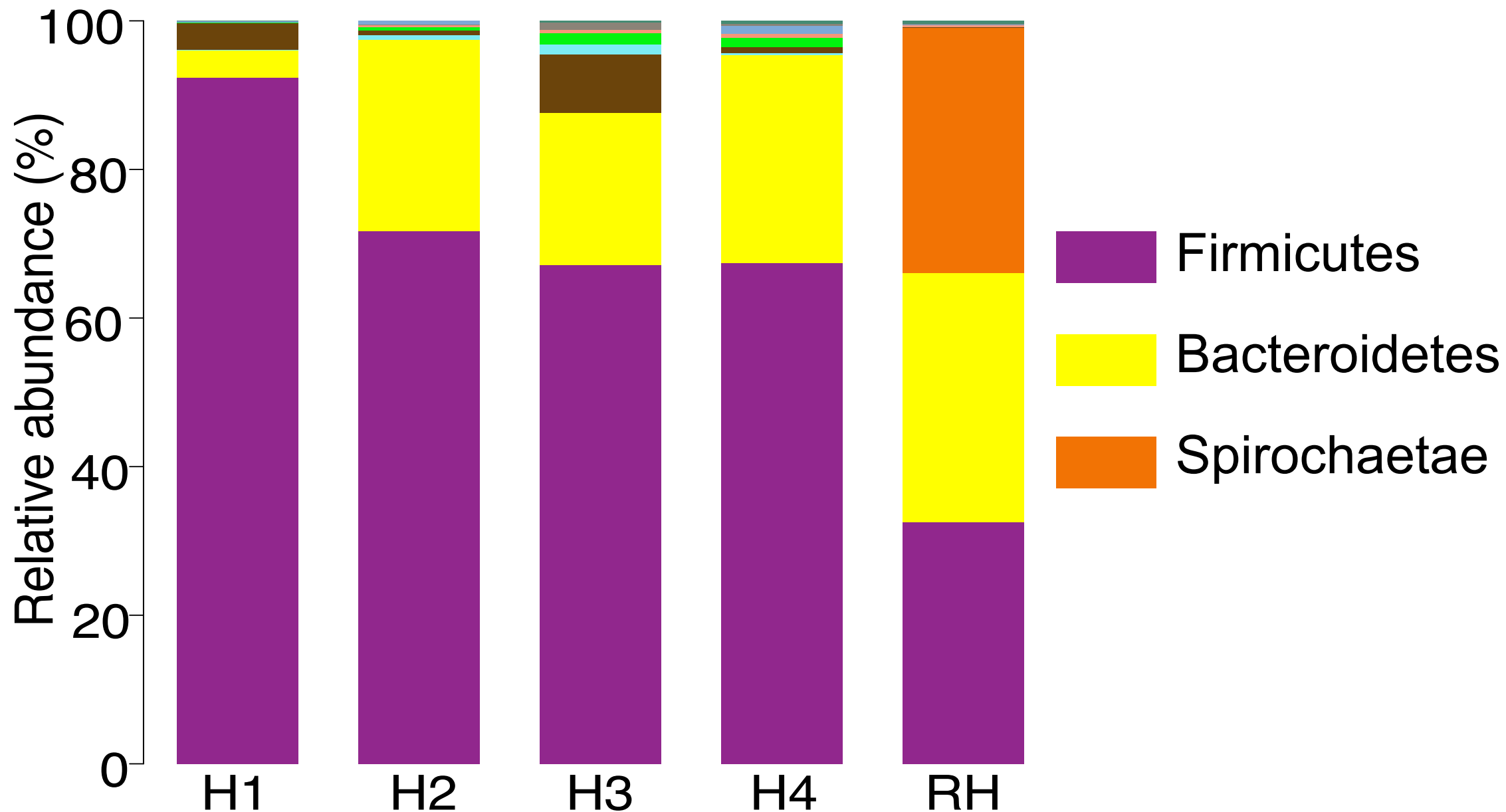


Dominant genuses in healthy and diarrheal rabbits



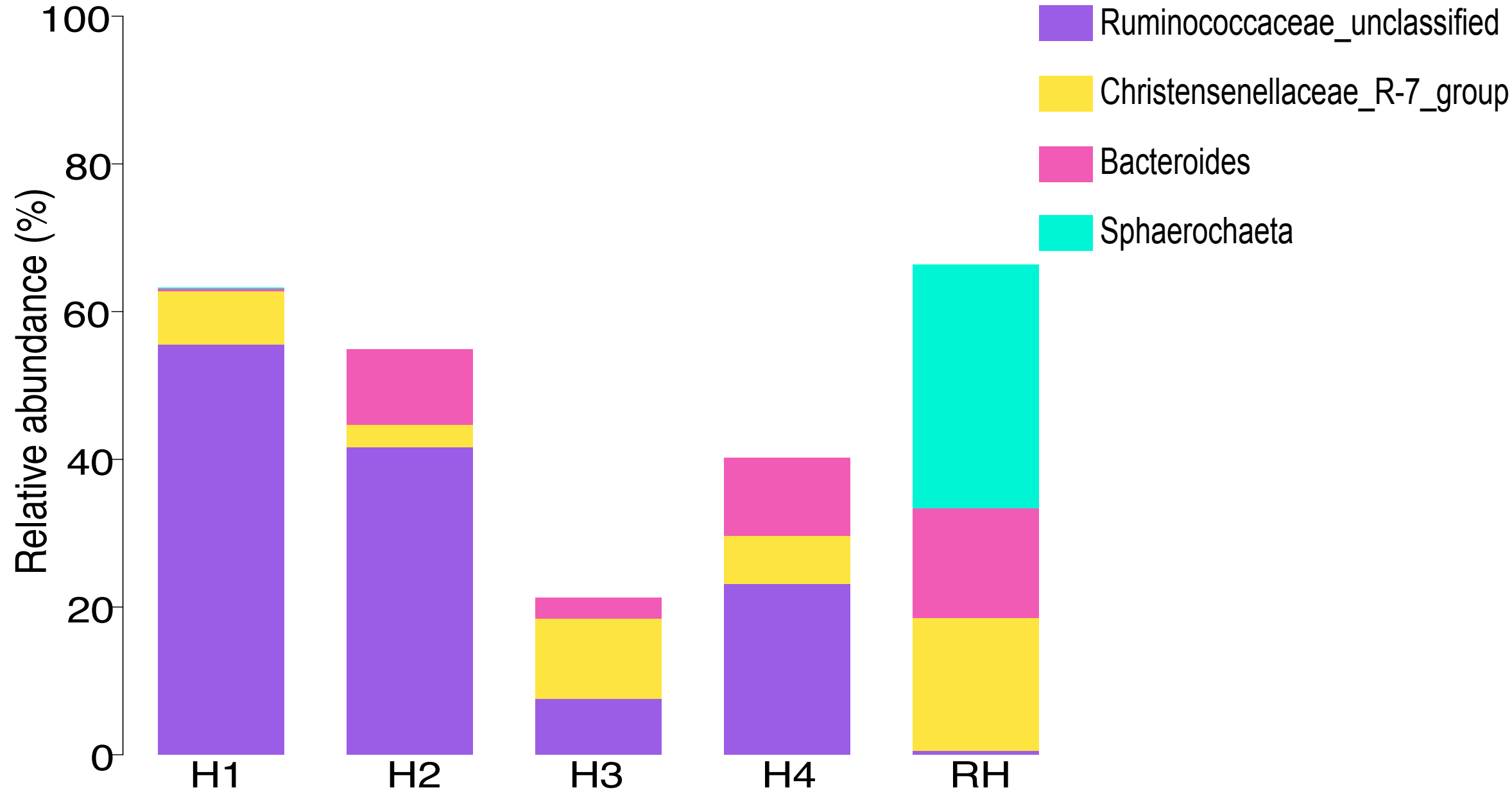


Dominant phyla in healthy rabbits and hares





Dominant genera in healthy rabbits and hares





Conclusions

Comparing with healthy rabbits,

- **A remarkable disorder** is caused by pathogenesis in diarrheal cecal microbiota.
- **Spirochaetae** dominates in hares' cecum due to their high fiber diet.