

454 Pyrosequencing Reveals a Shift in the Fecal Microbial Community Composition of Genetically Identical Co-housed Pigs Fed Different Diets

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Abstract

The impact of diet on the microbial composition in the gastrointestinal tract (GIT) has been well documented. The GIT microbiome plays key roles in nutrition including modulation of nutrient uptake and utilization, promotion of GIT development and maturation, and extraction of energy from indigestible non-starch polysaccharides. However, quantifying the role of diet in shaping microbial composition in humans has been difficult. Pigs share a similar GIT anatomy and dietary composition with humans making them an attractive animal model. We used genetically identical co-housed pigs to assess the impact of diets on GIT microbial composition. For this study, the fecal microbiota of two genetically identical co-housed pigs were evaluated when fed diets containing either soybean hulls or wheat bran for 14-days. We found that microbial community composition clustered distinctly by diets with generally higher diversity in the wheat bran group than the soybean hull group. We also identified significant taxonomic differences in the bacterial composition between two different diets. This study allowed us to study the influence of diets on GIT microbiome composition independently of the confounding effects of host genotype and environment.

Introduction

Dietary intervention is considered to be one of the greatest potential methods to treat GIT-related diseases. In order to understand how dietary alterations affect the microbiome, it is important to be able to isolate the effect of the dietary intervention. This can be difficult given the numerous other factors such as host genetics, environment, and history. Identical twins studies have been used to control for host genetics, controlling past environmental exposures are still problematic or impossible. Although twins are regarded as one of the best models for studying non-genetic influences on the human GIT microbiome, cloned animal models offer an additional level of control. Swine make for a particularly attractive model in human medical studies. We utilize them here for the following reasons. First, pigs are omnivores and can be fed diets that mimic realistic human interventions. Secondly, the swine GIT is anatomically, metabolically and physiologically similar to human beings and already used in many medical studies. Thirdly, swine grow quickly and are especially susceptible to nutrient modulation. Fourthly, swine and human adults share similar intestinal microbial community composition.

Objectives

To evaluate the effects of different diets on fecal microbiome composition in genetically identical co-housed swine.

Materials and Methods

1. Make sure that diet is only variation (Fig. 1)

Two 18-month-old female healthy cloned adult swine created from Duroc gilt (2-14) using somatic cell nuclear transfer were used in an experimental design with four 14-d periods. The two swine had the same genotype and housed together since they were born. Two different diets were rotationally fed to pigs, one containing 20% soybean hulls and another containing 20% wheat bran. Swine were fed soybean hull diet before the experiment. In each experiment period, the two swine ate the same diet. Feces were collected at the last day of each period. Samples were stored at -20 °C until DNA extraction.

2. Characterize GIT microbiome composition through using 454-pyrosequencing of amplicons from 16S V3 region (Fig. 1)

Genomic DNA was extracted using the RBB+C method¹ with minor modifications including the addition of 1000 µl ASL buffer to the samples following the Qiagen DNA Stool Kit (Qiagen). The hypervariable V3 region of 16S rDNA was amplified in the PCR with specific primers for pyrosequencing analysis. After checked for size and purity, PCR products were subjected to pyrosequencing (454 GS FLX).

3. Obtain information about similarity, diversity and taxonomy of GIT microbiome (Fig. 1)

Similarity was analyzed using Compression-based Distance metric (CBD)² and Bray-Curtis dissimilarity. Simpson's diversity index was used to estimate bacterial diversity. Taxonomy was assigned using Ribosomal Database Project (RDP)³ classifier v2.5. Metastats⁴ was used to determine if two groups were significantly different from each other.

Materials and Methods

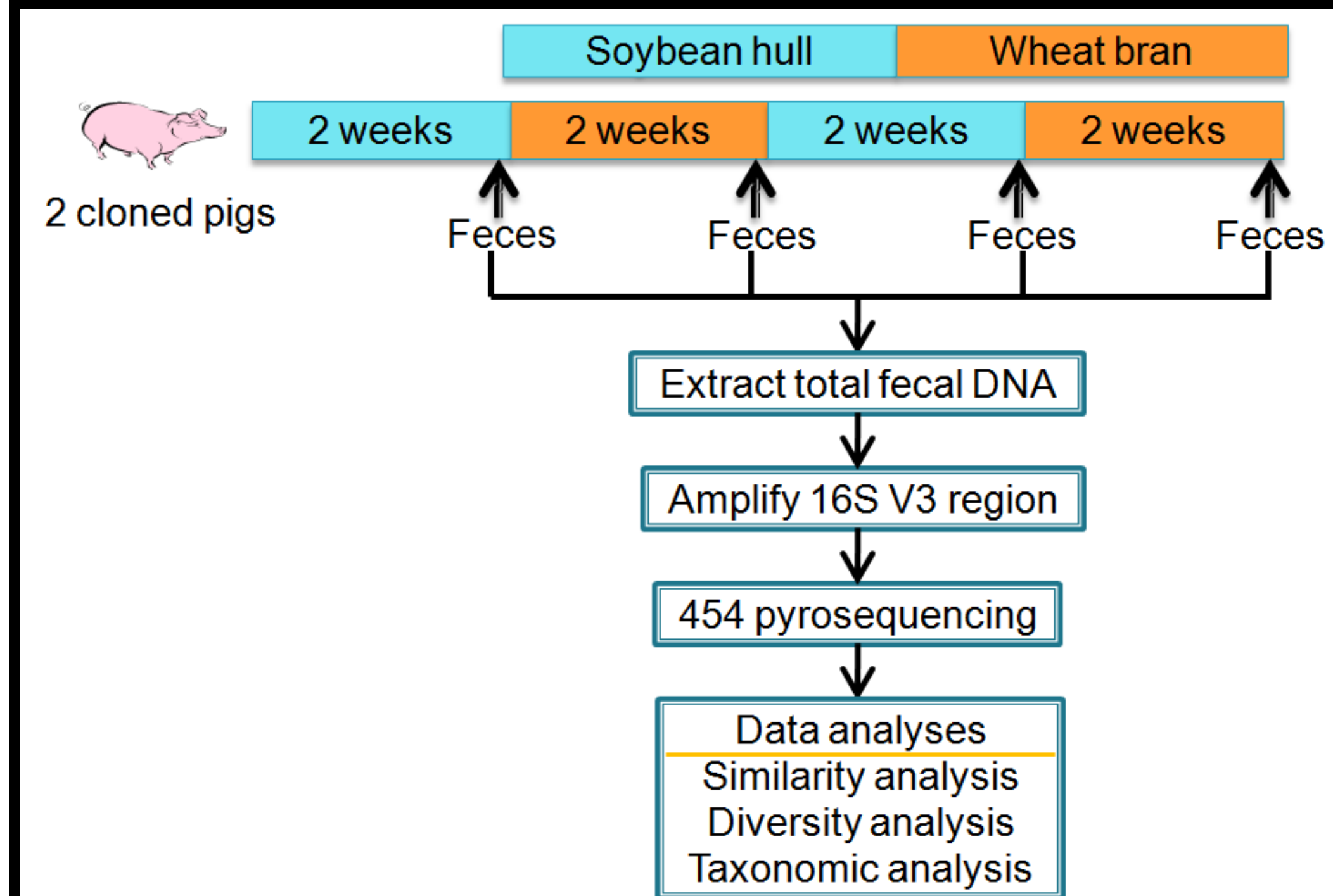


Figure 1. Experiment design. Upward black arrows represent fecal collection time points.

Results

1. Microbial Communities Clustered by Diet. Comparison of the individual bacterial community composition using CBD-based PCoA and Bray-Curtis dissimilarity-based NMDS (Fig. 2) exhibited a disparate clustering by diets. Cloned pigs responded similarly to diet change.

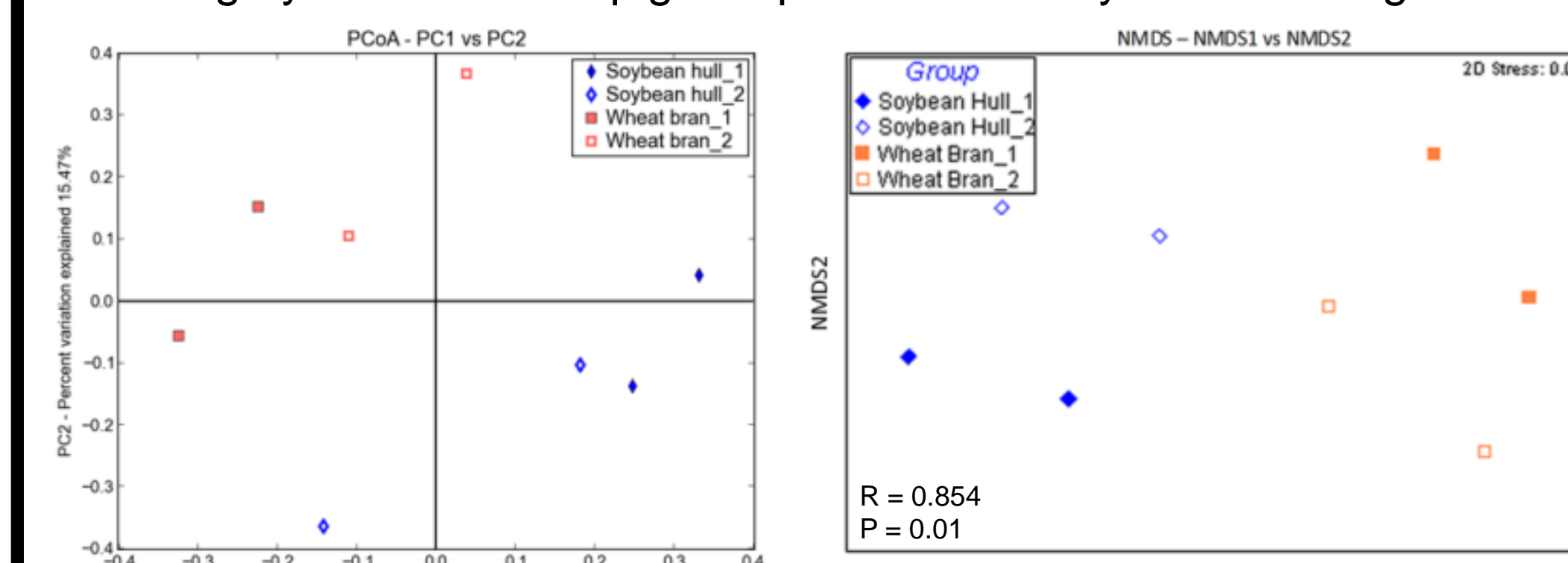


Figure 2. CBD-based PCoA (left) and Bray-Curtis dissimilarity-based NMDS (right) analysis showed clustering of microbiomes by diets.

2. Wheat Bran Results in Higher Microbial Diversity. Simpson's diversity index estimates were used to calculate diversity of microbial community samples. The difference in diversity between swine first fed soybean hull and wheat bran diet is bigger than that between swine secondly fed soybean hull and wheat bran diet (Fig. 3). When compared swine first fed soybean hull diet with other groups, the difference in diversity between swine first and secondly fed soybean hull diet is smallest, while the difference in diversity between swine first fed soybean hull and wheat bran diet is biggest, with the difference in diversity between pigs first fed soybean hull and secondly fed wheat bran diet being intermediate (Fig. 3). When compared swine first fed wheat bran diet with other groups, the difference in diversity between swine first and secondly fed wheat bran diet is smaller than that between swine first fed wheat bran and secondly fed soybean hull diet (Fig. 3).

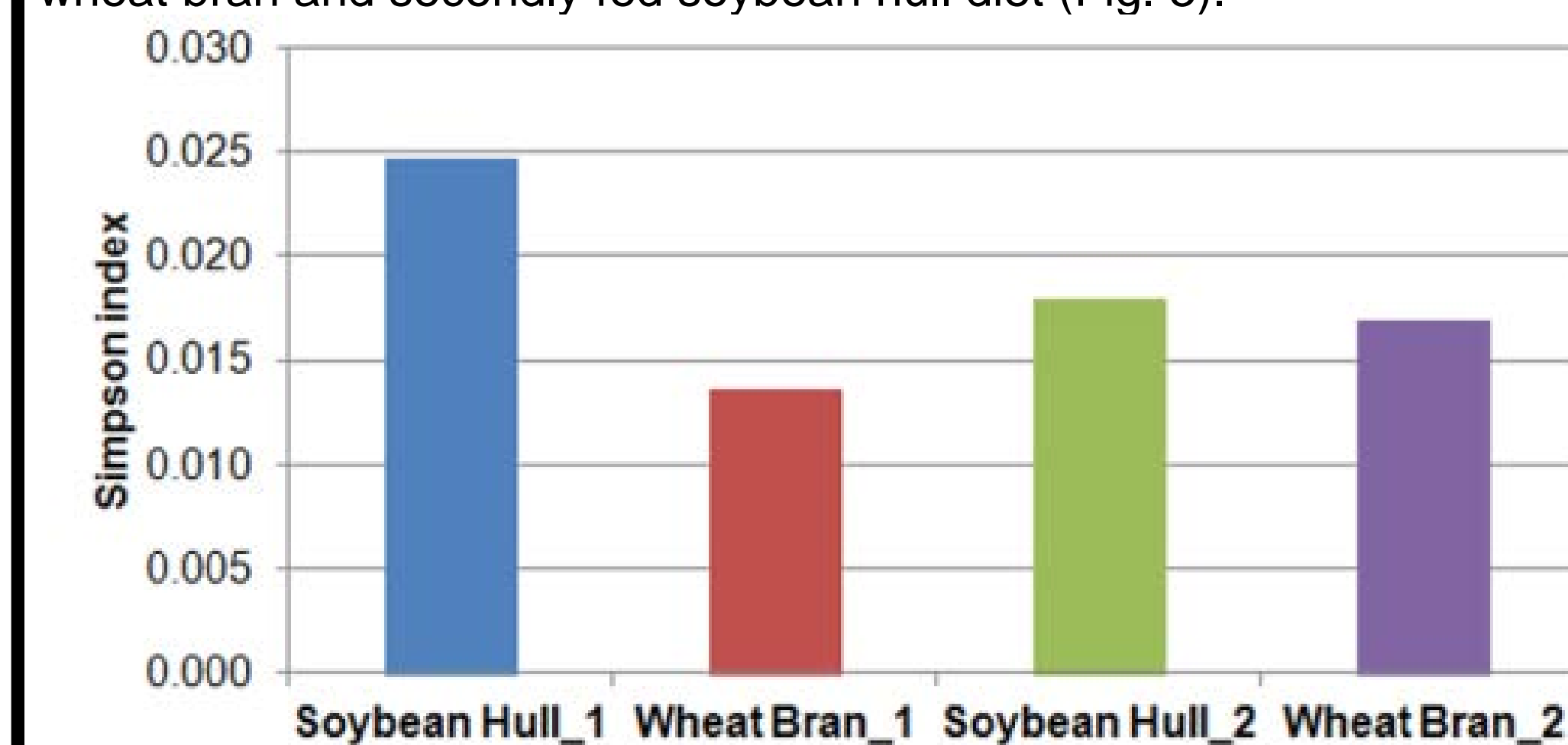


Figure 3. Diets affected the diversity of pigs' fecal microbiome. Average Simpson's diversity index in each period was shown.

Results

3. Diets Significantly Affected Bacterial Composition of Swine Fecal Microbiome at Operational Taxonomic Units (OTUs) Level. The OTU abundance differences between swine first fed soybean hull and wheat bran diet is bigger than that between swine secondly fed soybean hull and wheat bran diet (Fig. 4A). When compared swine first fed soybean hull diet with other groups, the OTU abundance differences between swine first and secondly fed soybean hull diet is smallest, while the OTU abundance differences between swine first fed soybean hull and wheat bran diet is biggest, with the OTU abundance differences between swine first fed soybean hull and secondly fed wheat bran diet being intermediate (Fig. 4B). When compared swine first fed wheat bran diet with other groups, the OTU abundance differences between swine first and secondly fed wheat bran diet is smaller than that between pigs first fed wheat bran and secondly fed soybean hull diet (Fig. 4C).

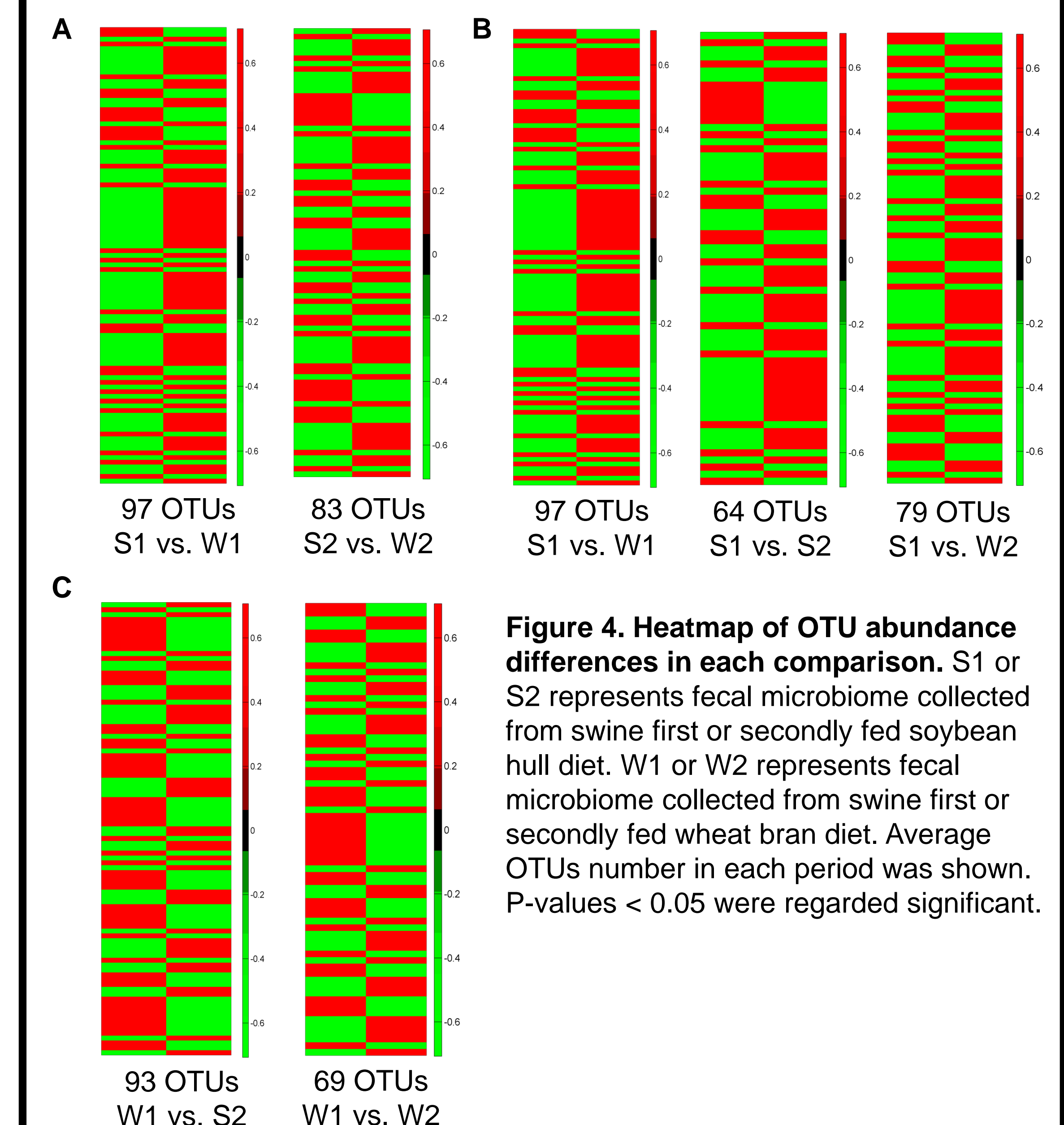


Figure 4. Heatmap of OTU abundance differences in each comparison. S1 or S2 represents fecal microbiome collected from swine first or secondly fed soybean hull diet. W1 or W2 represents fecal microbiome collected from swine first or secondly fed wheat bran diet. Average OTUs number in each period was shown. P-values < 0.05 were regarded significant.

Conclusions

Our results demonstrated that different diets affected fecal microbiome composition in isogenic swine. The influence of diet on GIT microbiome composition could be studied independently of the impact of host genotype and environment. The changes in gut microbiome composition induced by diet could be observed over short time scales. Clonal swine represent viable animal model for studying the impact of diet on gut microbiome composition.

Acknowledgements

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