



# A cross-sectional observational study of the fecal microbiome composition in adult laying hens with and without access to an outdoor range

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## Introduction

Layers with access to outdoor ranges are exposed to additional environmental microorganisms, including the potential pathogens of wild birds. Alterations in fecal microbiome community parameters or the relative abundance of individual genera of outdoor-housed layers may serve as an indicator for risks of exposure to potential pathogens.

## Aim

To evaluate differences in the microbiomes of outdoor-housed vs indoor-housed layers across different farms under field conditions.

## Material & methods

Four indoor- and four outdoor-layer flocks were sampled once (Fig. 1). Ten cloacal swabs were taken per flock and analysed with 16S rRNA amplicon sequencing.

## Results

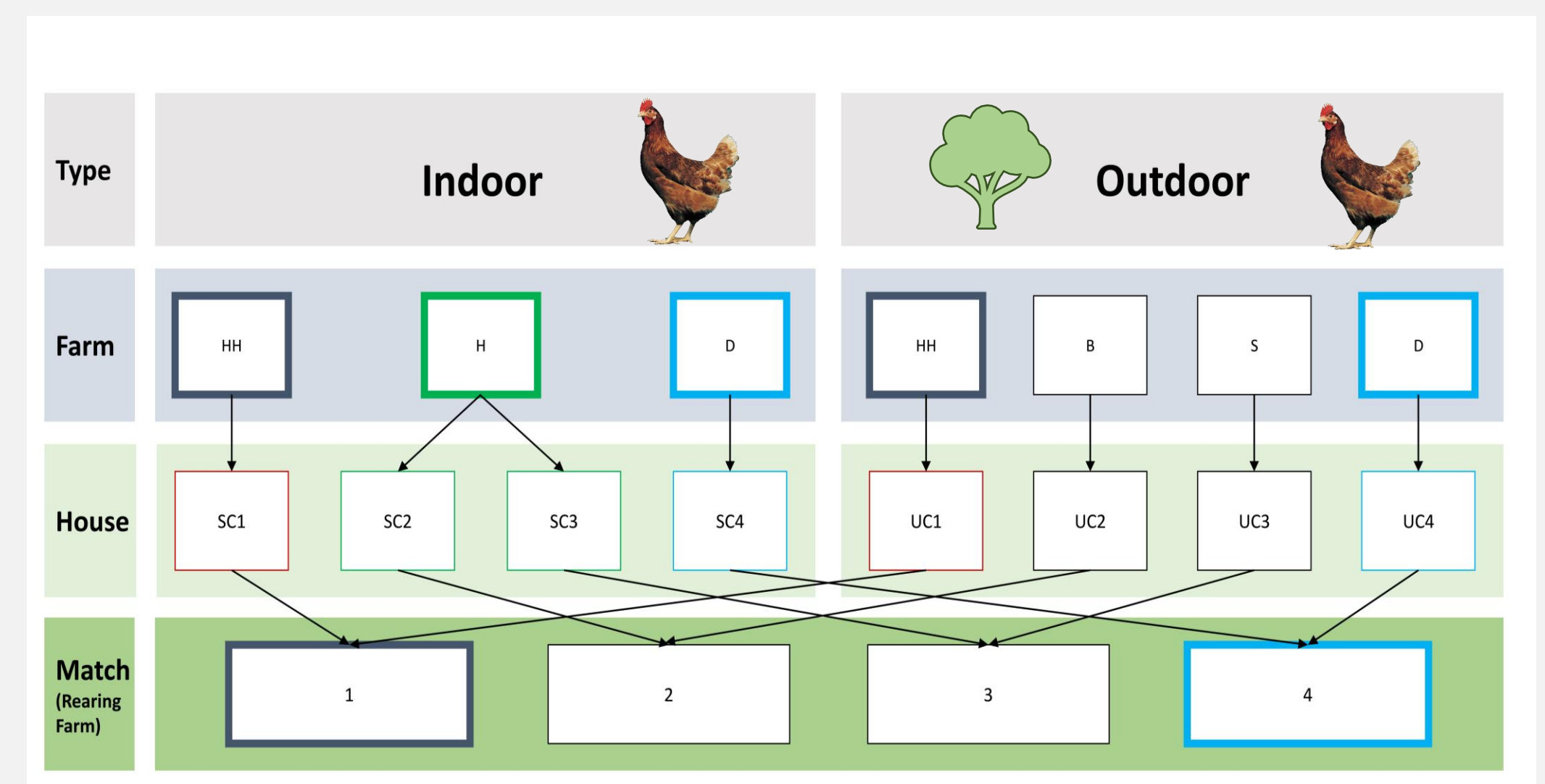
- Housing type (indoor vs outdoor), rearing farm, farm and house significantly contributed to the observed variation in microbiome composition (PERMANOVA  $p < 0.01$ ).
- Distance based redundancy analysis (dbRDA) on Bray-Curtis distances showed that a model with housing type and house explained 31.8% of the variation in community composition (Fig. 2).
- Variation partitioning showed that the combination of farm and rearing flock explained most variation (12.6%). Housing type explained only 0.2% of the variation (Fig. 3).
- Bray-Curtis distances between indoor-layers of indoor houses were significantly ( $p = 0.02$ ) higher than the distances between cloacal samples from layers of outdoor houses (Fig. 5).
- At the genus level, the abundances of five genera were different between housing types (Wilcoxon Rank Sum test  $p < 0.01$ ). None of these genera were consistently increased or decreased across houses within one housing type.
- Using a random forest classifier, one amplicon sequence variant (ASV) was detected (ASV499) that was only present in hens with an outdoor range ( $p < 0.001$ ). It was identified as *Dietzia maris*, a soil bacteria (Fig. 4).

## Conclusion

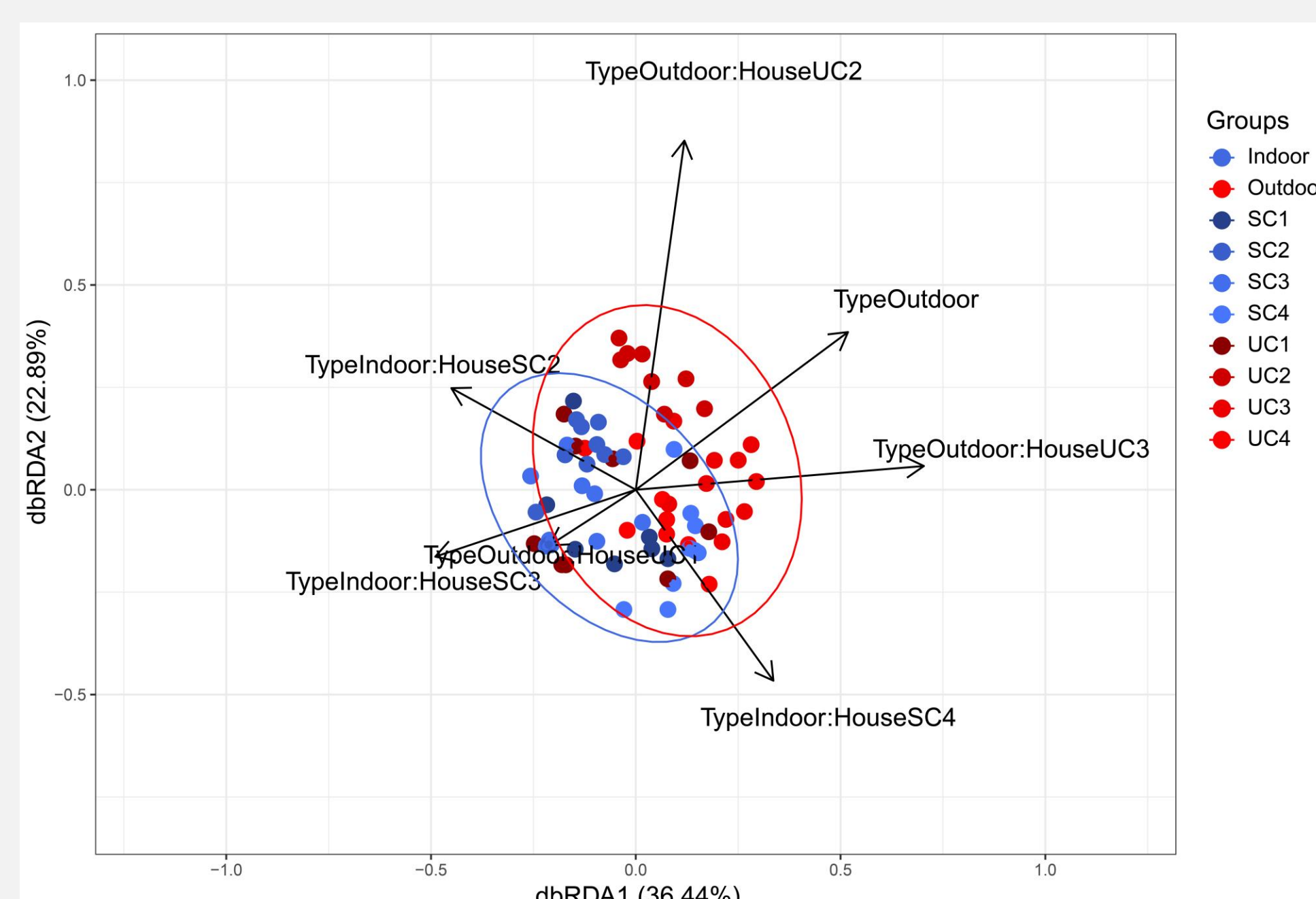
**Our study shows that the microbial composition of adult layers in field conditions is, to a limited extent, affected by the access to an outdoor range.**

**House, farm, and rearing flock play a greater role in determining their microbiomes.**

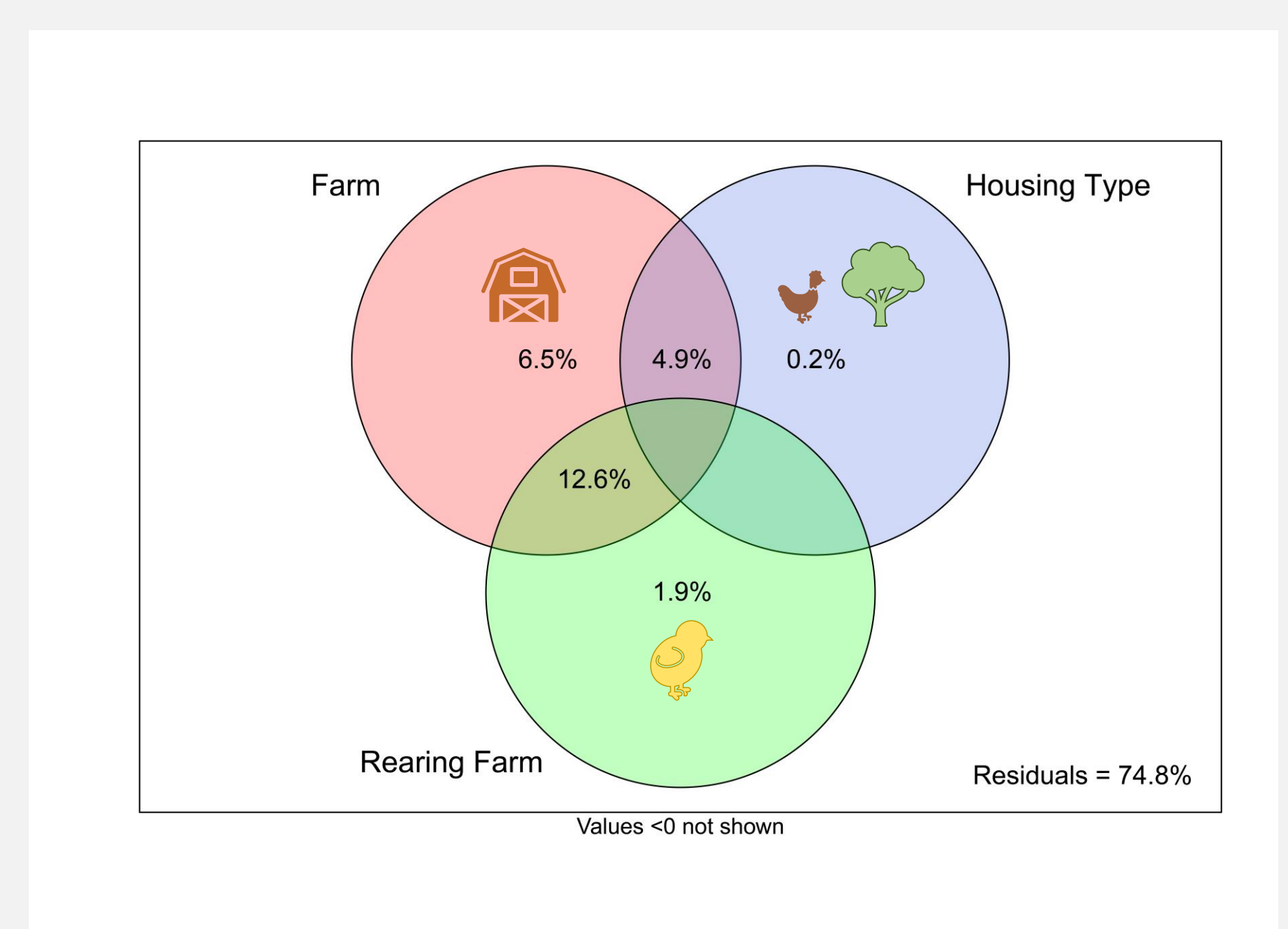
**Overall, measuring differences in fecal microbiota of layers as an indicator for the level of exposure to potential pathogens and biosecurity seems of little practical use.**



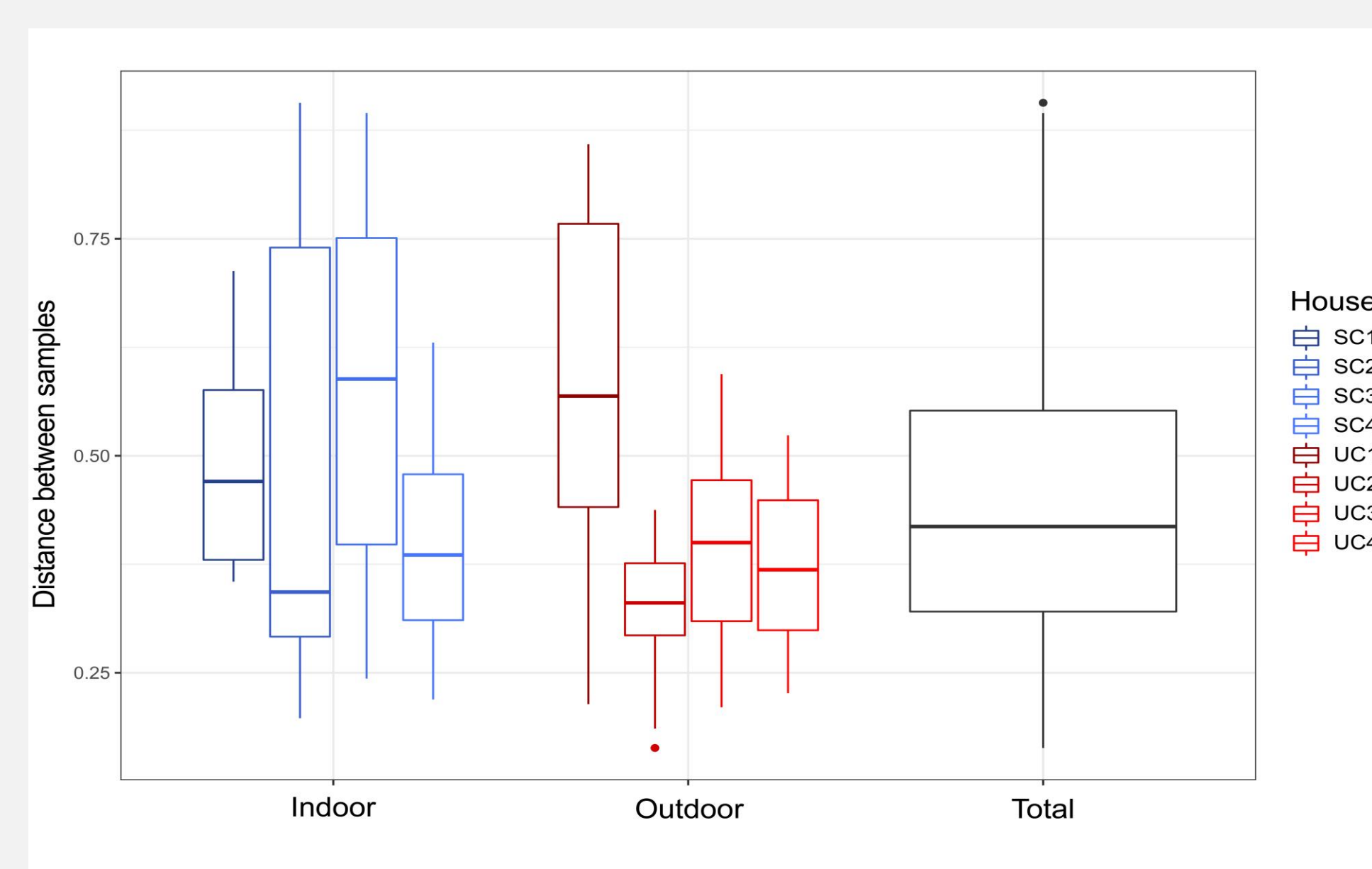
**Figure 1** Study designs of sampled flocks. Flocks were matched on rearing farm. Some houses were situated at the same farm. Flocks of rearing farm 1 and 2 went to houses situated on the same farm, HH and D. One farm housed 2 flocks (SC2 and SC3) that came from different rearing farms (2 and 3).



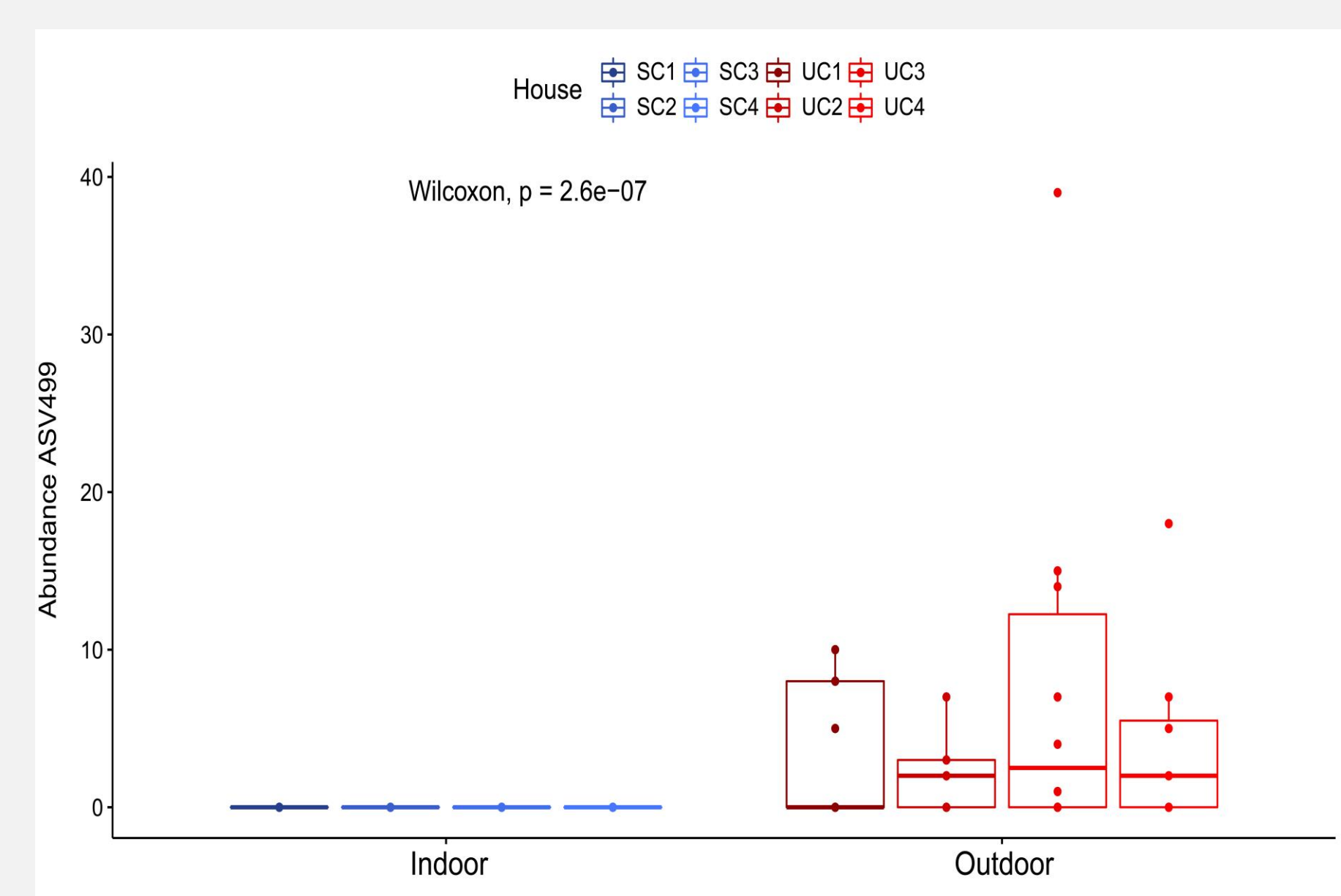
**Figure 2** dbRDA on Bray-Curtis distances. A model with housing type + HousingType:House was used.



**Figure 3** Venn diagram showing the explained variation of the microbiota composition per factor using Bray-Curtis distances.



**Figure 5** Bray-Curtis distances between the fecal microbiota of layers from the same house shown per housing type. Higher distances indicate higher dissimilarity.



**Figure 4** Absolute abundance of ASV499 per house and housing type.