Swansea University
Prifysgol Abertawe

# Microbiome-parasite relationships in lamb in response to pasture management

**Jack Jefferson**

**Submitted to Swansea University in fulfillment of the requirements for the Degree of *Masters of Research / MRes***

**Swansea University**

**December 2024**

# Declarations and Statements

**Declarations**

This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.

Signed..[redacted]..........................................................

Date.......25/6/25..............................................................................

This thesis is the result of my own investigations, except where otherwise stated. Other sources are acknowledged by footnotes giving explicit references. A bibliography is appended.

Signed...[redacted]..............................................................................

Date.......25/6/5..............................................................................

I hereby give consent for my thesis, if accepted, to be available for electronic sharing **after expiry of a bar on access approved by the Swansea University.**

Signed...[redacted]........................................................

Date...............25/6/25..............................................................................

The University's ethical procedures have been followed and, where appropriate, that ethical approval has been granted.

Signed.....[redacted]..............................................................

Date..........25/6/25..............................................................................

# Summary (Abstract)

Lambs and most ruminants with outdoor access are vulnerable to parasitic infections which significantly reduces feed intake, hinders growth, increases mortality rates. In farming environments, this negatively impacts carcass quality, wool growth, fertility, and milk yield. With resistance to anthelmintics growing among parasites, alternative studies of improving parasitic resistance in sheep must be explored. This study investigated the effects of pasture type, seasonal variation, and parasitic influences on the rumen microbiome composition and diversity in lambs. Twenty one lambs were divided into two dietary treatment groups: ryegrass-dominated pastures (R) and mixed sward pastures (S). Fecal samples were collected monthly over a three-month grazing period and analyzed using 16S rRNA gene sequencing to assess microbial diversity and community composition.

The results showed that alpha diversity, measured by Shannon and Chao1 indices, significantly increased over time in lambs grazing on ryegrass pastures, while no significant changes were observed for lambs on mixed sward pastures, though trends of increasing diversity were noted. Beta diversity analysis revealed distinct microbial community structures between treatments, with seasonal shifts in the relative abundance of key bacterial taxa, such as *Akkermansia*, *Oscillospiraceae*, and *Ruminococcus*. These changes were strongly influenced by pasture type and seasonal resource availability.

Parasitic interactions also played a role in shaping the microbiome, with low coccidia counts associated with reduced microbial diversity, while strongyle and *Nematodirus* egg counts showed no significant effects. These findings highlight the complex interplay between diet, seasonality, and parasitism in driving microbiome dynamics.

This study provides valuable insights into how pasture management practices and parasitic infections influence gastrointestinal microbiome diversity and composition. Future research should explore the functional implications of these microbial shifts on lamb health and productivity, incorporating long-term monitoring and performance metrics such as growth rates and immune function

# Statement of Expenditure

## Equipment or consumables

£1000 on laboratory consumables and sequencing costs

# Statement of Contributions

- Conceptualization: Konstans Wells, Tamsyn Webster, Claire Reigate, Jack Jefferson
- Data curation: Claire Reigate, Rothamsted Research
- Formal analysis: Jack Jefferson
- Funding acquisition: Konstans Wells, Tamsyn Webster, Claire Reigate, Jordana Rivero
- Investigation: Konstans Wells, Tamsyn Webster, Claire Reigate, Jack Jefferson
- Methodology: Konstans Wells, Tamsyn Webster, Claire Reigate, Jack Jefferson
- Project administration: Konstans Wells, Tamsyn Webster
- Resources: Konstans Wells, Tamsyn Webster, Claire Reigate, Jordana Rivero
- Software: Jack Jefferson, Konstans Wells, Tamsyn Webster
- Supervision: Konstans Wells, Tamsyn Webster
- Validation: Jack Jefferson
- Visualization: Jack Jefferson
- Writing – original draft: Jack Jefferson
- Writing – review & editing: Jack Jefferson, Konstans Wells, Tamsyn Webster

# Copy of Ethics Approval

**Microbiomes and parasites: how does type of pasture and drug treatment affect this important relationship in lamb?** **9119**

Project Tree

| Action Required on Form | Status | Review Reference | Date Modified |
|---|---|---|---|
| No | Approved | 3 2024 9119 8087 | 15/02/2024 20:18 |

# Copy of H&S and Risk Assessments

## Risk Assessment for Teaching, Administration and Research Activities

**Swansea University; College of Science**

**Name Jack Jefferson** ..........................................**Signature** [redacted]

**Supervisor* Konstans Wells/Tamsyn Webster Signature** [redacted] **Date 13/02/2024**

**Activity title** Microbiome-parasite relationships in lamb in response to pasture management and anthelmintic drugs

**Base location (room no.) Wallace 131a and Wallace 123** ............

(* the supervisor for all HEFCW funded academic and non-academic staff is the HOC)

**University Activity Serial # (enter Employee No. or STUREC No.** [redacted]...................................

**Start date of activity (cannot predate signature dates) 13/02/2024** ..................................................

**End date of activity (or 'on going') on going**......................................................................................

**Level of worker** (delete as applicable) PG ..........................................................................................

UG,PG, research assistant, technician, administration, academic staff, other (state)

| | |
|---|---|
| **Approval obtained for Gene Manipulation Safety Assessment by SU** ? | not applicable |
| **Licence(s) obtained under "Animals (Scientific Procedures) Act (1986)"** ? | not applicable |
| **Approval obtained for use of radioisotopes by COS** ? | not applicable |

### Record of specialist training undertaken

| Course | date |
|---|---|
| Lab 131a induction | 25/01/2024 |
| Lab 123 induction | 13/02/2024 |
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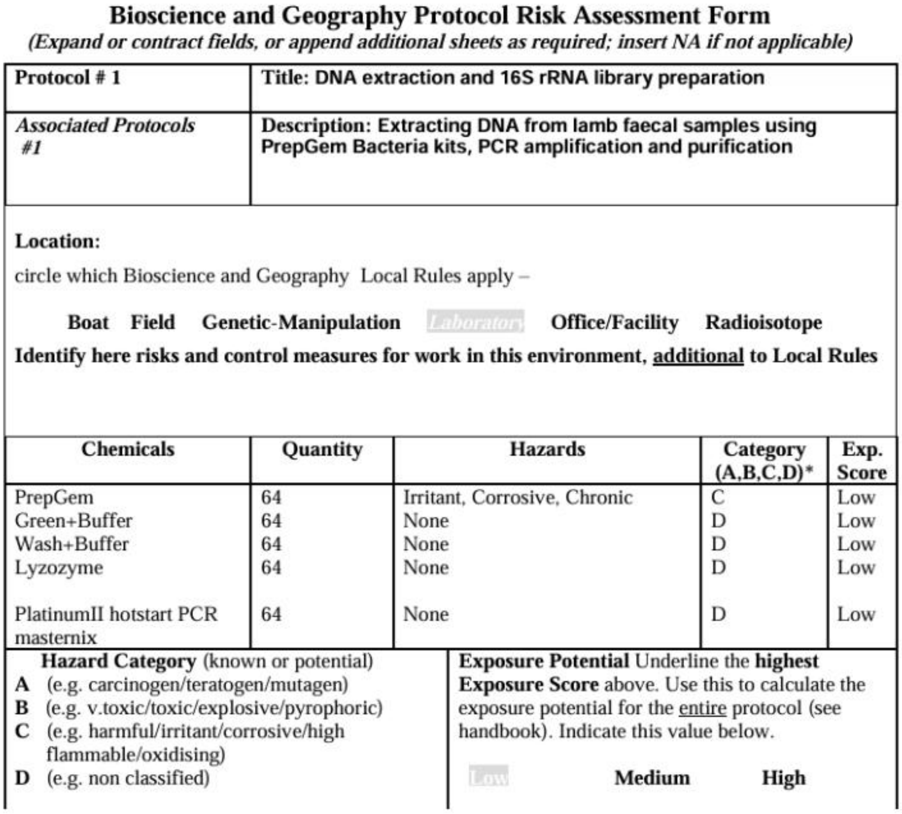


## Bioscience and Geography Protocol Risk Assessment Form

*(Expand or contract fields, or append additional sheets as required; insert NA if not applicable)*

| | |
|---|---|
| **Protocol # 1** | **Title: DNA extraction and 16S rRNA library preparation** |
| *Associated Protocols #1* | **Description: Extracting DNA from lamb faecal samples using PrepGem Bacteria kits, PCR amplification and purification** |

**Location:**

circle which Bioscience and Geography Local Rules apply –

**Boat** **Field** **Genetic-Manipulation** [✓ Laboratory] **Office/Facility** **Radioisotope**

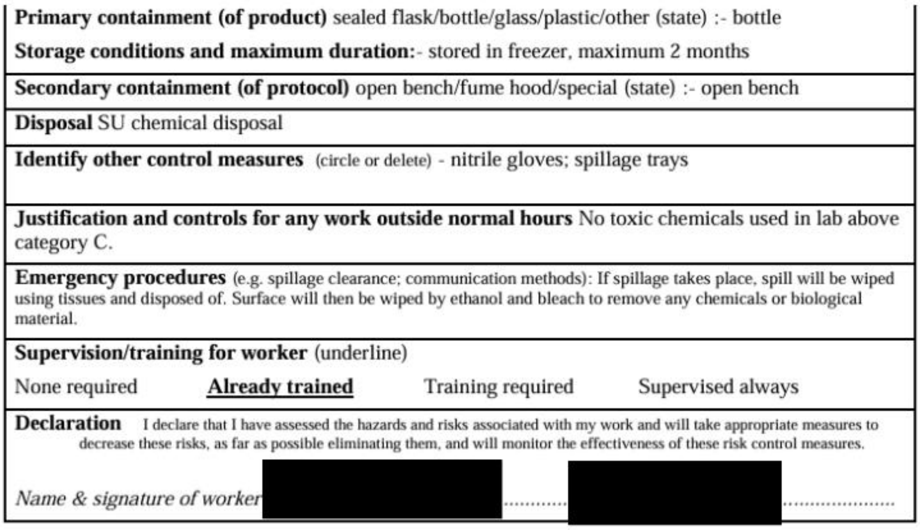
**Identify here risks and control measures for work in this environment, additional to Local Rules**

| Chemicals | Quantity | Hazards | Category (A,B,C,D)* | Exp. Score |
|---|---|---|---|---|
| PrepGem | 64 | Irritant, Corrosive, Chronic | C | Low |
| Green+Buffer | 64 | None | D | Low |
| Wash+Buffer | 64 | None | D | Low |
| Lyzozyme | 64 | None | D | Low |
| PlatinumII hotstart PCR masternix | 64 | None | D | Low |

**Hazard Category** (known or potential)
- **A** (e.g. carcinogen/teratogen/mutagen)
- **B** (e.g. v.toxic/toxic/explosive/pyrophoric)
- **C** (e.g. harmful/irritant/corrosive/high flammable/oxidising)
- **D** (e.g. non classified)

**Exposure Potential** Underline the **highest Exposure Score** above. Use this to calculate the exposure potential for the entire protocol (see handbook). Indicate this value below.

[✓ Low] **Medium** **High**



**Primary containment (of product)** sealed flask/bottle/glass/plastic/other (state) :- bottle

**Storage conditions and maximum duration:**- stored in freezer, maximum 2 months

**Secondary containment (of protocol)** open bench/fume hood/special (state) :- open bench

**Disposal** SU chemical disposal

**Identify other control measures** (circle or delete) - nitrile gloves; spillage trays

**Justification and controls for any work outside normal hours** No toxic chemicals used in lab above category C.

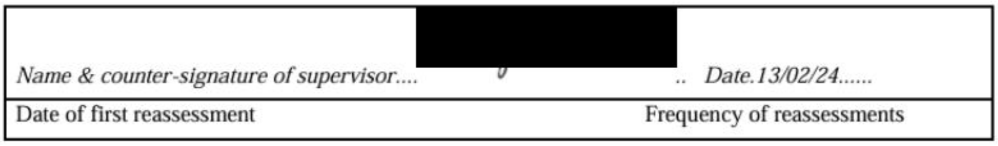
**Emergency procedures** (e.g. spillage clearance; communication methods): If spillage takes place, spill will be wiped using tissues and disposed of. Surface will then be wiped by ethanol and bleach to remove any chemicals or biological material.

**Supervision/training for worker** (underline)

None required [✓ Already trained] Training required Supervised always

**Declaration** I declare that I have assessed the hazards and risks associated with my work and will take appropriate measures to decrease these risks, as far as possible eliminating them, and will monitor the effectiveness of these risk control measures.

*Name & signature of worker*[redacted]............[redacted]......................



*Name & counter-signature of supervisor....*[redacted].. *Date.13/02/24......*

Date of first reassessment Frequency of reassessments

# Contents page

# Acknowledgements

- I want to thank my supervisors, Dr Konstans Wells and Dr Tamsyn Webster, for providing fantastic support guiding the creation and improvement of my work this year. I wouldn’t have accomplished this without them and have only gratitude for their helpfulness
- I also want to thank Claire Reigate for her help in this thesis and assistance in introducing me to the wider scientific community.
- Laboratory work was supported by the Ecological Continuity Trust
- Rothamsted field work: Dr Claire Reigate, Dr Jordana Rivero

## List of tables, illustrations, etc.

# Definitions and Abbreviations

1. ANOSIM (Analysis of Similarities): A statistical test used to evaluate differences in community composition between groups based on distance matrices.

2. ASV (Amplicon Sequence Variant): High-resolution sequences derived from DNA sequencing data, representing unique biological sequences without clustering into Operational Taxonomic Units (OTUs).

3. DNA (Deoxyribonucleic Acid): The molecular carrier of genetic information in living organisms, consisting of two strands forming a double helix structure.

4. rRNA (Ribosomal RNA): A type of RNA that forms the structural and functional components of ribosomes, playing a key role in protein synthesis.

5. FDR (False Discovery Rate): A statistical method used to control the expected proportion of incorrectly rejected null hypothesis in multiple hypothesis testing.

6. Gastrointestinal Microbiome (GM): The community of microorganisms, including bacteria, archaea, viruses, and fungi, residing in the gastrointestinal tract of an organism.

7. GI (Gastrointestinal): Pertaining to the stomach and intestines, part of the digestive system.

8. GLM (Generalized Linear Model): A flexible statistical model used to predict a response variable based on one or more predictor variables, accommodating different types of distributions.

9. NMDS (Non-Metric Multidimensional Scaling): An ordination method used to visualize differences or similarities in data, often applied to ecological datasets such as microbial community composition.

10. OTU (Operational Taxonomic Unit): A cluster of DNA sequences used to classify microorganisms based on sequence similarity, often at a specific percentage threshold.

11. PERMANOVA (Permutational Multivariate Analysis of Variance): A non-parametric statistical test used to assess the significance of differences in multivariate datasets, such as microbial community composition, based on a distance matrix.

12. PCR (Polymerase Chain Reaction): A laboratory technique used to amplify specific DNA sequences for analysis or further experimentation.

13. QIIME2 (Quantitative Insights Into Microbial Ecology 2): An open-source bioinformatics platform used for analyzing and interpreting microbial community data, including taxonomic and diversity analyses.

14. R: A programming language and environment for statistical computing and graphics, widely used for data analysis and visualization in scientific research.

15. SE (Standard Error): A measure of the variability of a sample mean, indicating the precision of the estimate.

16. SCFA (Short-Chain Fatty Acids): A group of fatty acids with fewer than six carbon atoms, produced by the fermentation of dietary fiber by gut microbiota, and associated with host metabolic and immune functions.

17. TAE (Tris-Acetate-EDTA Buffer Mix): A buffer solution commonly used in molecular biology for electrophoresis of nucleic acids such as DNA and RNA.

# Main Text

## Background

Lambs raised on pastures with a single grassland type are consistently exposed to the same environmental conditions, which can simplify feeding and management but also pose risks to their health. One significant concern is an increased exposure to helminth parasites, which can spread rapidly between grazing animals, who are vulnerable to reinfection in chain reactions (Kumar et al., 2013). Parasitic infections are acknowledged as one of the main obstacles to meeting future demands in the livestock industry (Mamun et al., 2020). For example, helminth infections can reduce animal productivity due to reduced weight gain and efficiency of food utilisation (Williams et al., 2021). Nematode infections are commonplace in pastures among fears of growing anthelmintic resistance (Waller, 2006). Furthermore, coccidia infections alone have negatively affected lamb growth and mortality, by damaging intestinal mucosa, leading to poor nutrient absorption which decreases weight gain (Charlier, 2020; Junkuszew et al., 2015).

Current strategies for managing production losses caused by gastrointestinal nematode infections primarily rely on commercial anthelmintic drugs (Corrêa et al., 2020). However, their efficacy has diminished due to the emergence of drug-resistant parasite strains. There is growing interest in understanding how variations in diet affect the microbial communities within the lambs' gastrointestinal tracts (Li et al., 2022). Perennial ryegrass (*Lolium perenne*) is a commonly used forage in pastoral systems throughout Europe and elsewhere, valued for its high nutritional quality and productivity (Santos, 2018). Meanwhile, multispecies sward pastures which incorporate other grasses, legumes, and herbs alongside ryegrass act as an alternative, offering different nutritional profiles and microbial community structures when compared to a ryegrass-only diet in dairy cows (Smith et al., 2020). Recent studies have explored the relationships between dietary variations and microbial diversity and abundance, which in turn has shown evidence to affect the lambs' growth, health, and overall performance (Grace et al., 2019).

The rumen microbiome, characterized by a diverse array of microorganisms, plays a critical role in meeting the dietary and metabolic requirements of ruminants (Corrêa et al., 2020). It is responsible for providing 70% of energy for the host animal from the breakdown of ingested plant material and the digestion of cellulose (Clemmons, 2019). Microbes are also responsible for the correct development of the intestines, as well as digestive and immune function (Mamun et al., 2020; Charlier, 2020). Research has demonstrated that the nutritional status of the host plays a crucial role in shaping the impact of nematode infections

(Miller and Horohov, 2005). Well-nourished animals are generally more capable of resisting the effects of infection compared to those on inadequate diets. While more research is necessary to fully learn its benefits, mixed swards may promote a more stable and resilient microbiome, helping ruminants maintain better digestive efficiency and overall health (Waller, 2006). Microbial communities are essential for the health and production performance of livestock (Li et al., 2022) and play a similarly vital role in other organisms. Diet is also known to impact parasitic infections in livestock and humans by modulating immune responses and altering the gut environment, which can either enhance or mitigate the effects of parasites (Clark et al., 2018; Hamer et al., 2019; Miller and Horohov, 2005).

Recent studies have explored novel methods to enhance feed efficiency and nutrient utilization, focusing on maintaining a healthy microbiome in lambs (Clemmons, 2019; Metcalf et al., 2017). For instance, the use of tannin-rich feed additives has been shown to reduce methane emissions and improve digestion in lambs that have been negatively affected by nematode infection (Corrêa et al., 2020). Mixed grazing systems, where lambs graze alongside other livestock species, have been demonstrated to disrupt parasite life cycles and reduce gastrointestinal nematode burdens, thereby improving overall health and growth performance (Waller, 2006). Additionally, the development of nutritionally optimized feed pellets, enriched with probiotics or specific nutrients, has proven effective in supporting gut health and enhancing feed conversion efficiency with reduced methane emissions (Petrič et al., 2022). Given the rising challenges associated with parasitic infections and the limitations of traditional anthelmintic approaches, exploring alternative and integrated methods is necessary. Different dietary and pasture management strategies, such as the use of mixed swards, alternative feed additives, and other nutritional interventions, need to be tried to identify effective and sustainable ways to support lamb health, reduce parasitic burdens, and promote beneficial microbial communities.

By comparing a ryegrass-only (R) pasture treatment with a mixed sward (S) pasture, this study aims to elucidate how different feeding strategies influence microbial populations. These insights can guide recommendations for optimising lamb diets to enhance their health and productivity. We hypothesise that lambs grazing on multispecies swards will exhibit distinct microbial profiles and lower parasitic loads compared to those on ryegrass-only pastures, with both temporal and environmental factors contributing to these differences.

## Methods

### 1. *Experimental Design and Sample Collection*

Twenty one individually marked lambs were held on 4 long-term grassland plots of the Rothamsted Research Farming Platform (latitude 51.8102433/ longitude -0.3762093) located in North Wyke, Devon, United Kingdom, in 2023. The composition of the mixed sward is specified in Table 1. Born in April, during the pre-experimental period (April - July) all lambs were given anthelmintic medication to remove any present helminths and other parasites to observe if the two pastures would have a difference in the rate of parasitic infections.

**Table 1.** Species and common names of mixed sward. Composed of 7 species of seed mix including 3 grasses, 2 legumes and 2 forbs.

| Common name | Latin name |
|---|---|
| Festulolium | *Festulolium cv* |
| Perennial ryegrass | *Lolium perenne* |
| Timothy | *Phleum pratense* |
| Red Clover | *Trifolium pratense* |
| White Clover | *Trifolium repens* |
| Chicory | *Cichorium intybus* |
| Ribwort Plantain | *Plantago lanceolata* |

The duration of the experiment was three months, where fresh faecal samples were collected non-invasively on 20/07/2023, 22/08/2023, and 13/09/2023. In this study, samples were classified into six treatments for analysis based around the combination of pasture type and sampling month: R1, R2, R3 and S1, S2, S3. Here, 'R' signifies the perennial ryegrass pasture, while 'S' identifies mixed sward pastures and each number signifies a different month (1: July, 2: August, 3: September). Faecal samples from droppings were only collected after the animals had moved at least 5 metres away to ensure they were not disturbed but no more than 10 minutes after defecation. Sample collections of the lambs were provided by the above-mentioned institution (collector: Dr C. Reigate). Samples stored in replicates of three using sterile containers and stored at -20°C in RNALater until further processing. In addition, some faecal material from the same droppings were then examined using FecPakG2 (Techion, Mosgiel, New Zealand) to detect the presence of parasites. Parasites were counted directly using the device, with larger parasites

(Nematodes/Strongyles) being counted directly and smaller parasites such as Cocci being divided into Low/Medium/High classifications.

**Table 2**: Raw parasite data averaged to the whole number based on treatment.

| **Treatment** | **Strongyle** | **Nematode** | **Tapeworm** | ***Strongyloides*** | **Cocci** | **Cocci Colour** | **Cocci Count** |
|---|---|---|---|---|---|---|---|
| R1 | 23 | 0 | 0 | 0 | 1 | Clear | Low |
| R2 | 62 | 9 | 0 | 0 | 1 | Clear | Medium |
| R3 | 52 | 9 | 0 | 0 | 1 | Mix | High |
| S1 | 49 | 5 | 0 | 0 | 1 | Mix | Medium |
| S2 | 171 | 23 | 0 | 0 | 1 | Clear | Medium |
| S3 | 87 | 9 | 0 | 0 | 1 | Clear | Medium |

2. DNA Extraction

Prior to DNA extraction, samples were thawed at room temperature. DNA was extracted from lamb faeces using the PREPGem Bacteria DNA extraction kit (MicroGem) following the manufacturer's instructions with these minor modifications: The replicants were first cleaned using a 10% dilution of bleach with water to remove any surface contaminants from the faeces, then the three replicants were homogenised together to ensure even distribution, with 0.2 ceramic beads, prior to DNA extraction.

3. 16S rRNA gene amplicon library preparation

A two-step PCR was performed to amplify the 16S rRNA V4 region from extracted DNA using 550F and 806R primers (Caporaso et al., 2011). Samples were diluted four times with water before they were used in the reaction mixture to avoid PCR inhibition. The first PCR reaction mixture contained 10 ul of Platinum II Taq mastermix (ThermoFisher), 0.4 ul each of the forward and reverse primers (10 uM), along with 7.2 ul of molecular grade water, and 2 ul of the extracted DNA. For the first PCR, amplification conditions consisted of an initial denaturation at 95°C for 3 minutes, followed by 28 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 30 seconds. A final extension step was performed at 72°C for 5 minutes. For the second step, barcoding, PCR reactions consisted of 1ul of each specific Illumina Nextera index, 2.5 ul of PCR product along with 7.5 ul molecular grade water and 10 ul platinum II taq. Amplification continued with an initial denaturation at 95°C for 3 minutes, with 8 cycles of denaturation at 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds. A final extension step was also performed at 72°C for 5 minutes. Negative controls (no template DNA) were included in each PCR run to detect contamination.

PCR products were examined using gel electrophoresis to confirm the presence of a single band of around 250-300 bp. Agarose gel at 2% dilution was prepared in a TAE buffer and stained with Gel Red. PCR products were mixed with loading dye and loaded onto the gel along with a DNA ladder Generuler 1 kb. Electrophoresis was conducted at 90V for 30 minutes until the dye front reached approximately 5 cm from the wells. The gel was visualised under UV light using a Chemidoc imaging system, and images were captured using Image Labs for analysis. PCR product bands were analysed using gel analysis software Image Labs by Bio-Rad. The size of PCR products was estimated by comparing their migration distance to the DNA ladder. Positive amplification was determined by the presence of a band of the expected size. Libraries were pooled based on band intensity, then purified using Ampure XP beads (Agencourt). The DNA was mixed with the magnetic beads at a ratio of 1:1.125 (DNA:beads) and incubated at room temperature for 5 minutes to allow binding of DNA to the beads. After incubation, the beads were washed twice with 80% ethanol and air-dried. The purified DNA was eluted in nuclease-free water and quantified using the Qubit 3.0 Fluorometer (Invitrogen) before final pooling and dilution to 4 nM for sequencing on an Illumina MiSeq platform at Swansea University.

### 4. Sequence analysis

Sequencing data was analysed using the bioinformatics platform QIIME2 version 2024.5 (Bolyen et al., 2019). A blend of the Linux based command line and graphical interface q2galaxy was used for the analysis. The following steps outline the methodology employed in this study. Raw sequencing data obtained from the Illumina MiSeq platform was imported into QIIME2. Pair-end demultiplexing was conducted to separate the sequence reads based on the sample-specific barcodes that were added in PCR steps. The quality of the demultiplexed sequences was then assessed using QIIME2 view with an aimed average quality score of 35 after trimming to ensure the reliability of downstream analyses by removing lower-quality data. Sequence data were subjected to quality filtering and denoising using the DADA2 algorithm, which also performed chimaera removal (Callahan, 2016). Truncation parameters were chosen, removing sequences longer than 230 base pairs for the forward reads and 160 for the reverse to ensure a high-quality score (Supplementary materials 1). Trimming of the first 19 base pairs was also carried out to remove primers. This process generated a feature table of amplicon sequence variants (ASVs) that depicted the number of ASV reads in each individual sample.

Taxonomic classification of ASVs was carried out using a pre-trained classifier based on the Silva 13_8 99% OTU database (Bokulich, 2018; Robeson et al., 2020). Filtering of the feature table was carried out in two steps to remove chloroplasts, mitochondria and unassigned bacteria that may have been present in the fecal samples. Sequences were aligned, then a phylogenetic tree was built using the `q2-phylogeny maft` plugin and ‘fasttree2’ (Katoh, 2002; Price et al. 2010). The resulting feature table, rooted tree, taxonomy and metadata files were then exported for downstream analysis in R version 4.4.1 (R Core Team, 2023).

### 5. *Statistical analysis*

Following the QIIME2 workflow, the generated ASV table and sequence taxonomies were analysed in R (R Core Team, 2023). After the filtering of low quality sequencing data, removing short sequences and chimaeras, a total of 653,097 sequencing reads were obtained from the 60 samples, with a mean of 10,885 sequencing reads for each sample. In addition, the total number of unique ASVs were also calculated. The raw amplicon sequence variant (ASV) table was processed to exclude non-bacterial sequences and filtered for taxa assigned at the domain and phylum levels.

To normalise the sequencing data across samples, a total of 100 subsamples of equal library size according to the minimum library size (10,866 reads) were randomly drawn for each individual sample to account for unequal sequencing efforts among individual samples. This ensures that variations in sequencing depth do not bias the comparisons of microbial diversity between samples. A rarefaction curve was plotted using the ‘iNEXT’ package in R to assess relative species richness across treatments, given the sampling effort, and to evaluate whether sampling was sufficient for capturing microbial diversity. Alpha diversity indices were iteratively calculated for each subsample, with Chao1 richness and Shannon diversity estimates derived using the ‘stats’ package in R. The summary statistics for Chao1 and Shannon diversity (mean, 95% confidence intervals based on the iterative computation) were then computed for each sample and subsequently linked to the sample metadata. Generalised linear models (GLMs) were then fitted to assess the effect of treatment (R1, R2, R3, S1, S2, S3) and additional parasitic covariates (egg counts for coccidia, strongyles and *Nematodirus*) on the Chao1 and Shannon indices using a Gamma family with the model displayed below.

*Mean Chao1/Shannon ~ treatment + parasite egg counts*

Plots displaying the distribution of Chao1 and Shannon diversity estimates across treatments were generated using violin plots, with overlaid point estimates using error bars and points representing the number of observed ASVs. Tukey post-hoc analysis was carried out comparing pairwise treatments using the results of the GLMs with the ‘emmeans’ package.

Beta diversity was measured using both Bray-Curtis dissimilarity and Weighted Unifrac distance using the ‘ordinate’ function using the ‘phyloseq’ package. The results were displayed using NMDS to display the separation of samples based on the pasture.

Taxonomic composition at the genus level was displayed by collapsing the ASV table to the genus levels using the ‘tax_glom’ function. Bar plots of relative abundance were generated to display the microbial composition in each sample group using the ‘ggplot2’ package. Genera with relative abundances below 2.5% were grouped into a single category named “Other” for clarity in the visualisations. Differential abundance analysis was performed using ANCOM-II (Analysis of Composition of Microbiomes) to identify genera with statistically significant differences across treatments (Lin et al., 2020). The method accounts for the compositional nature of microbiome data and adjusts for the different treatments, which were included as fixed effects in the model. ANCOM-II was applied with R1 as a baseline, using the `ancom-bc` R package, with taxa considered differentially abundant based on W-statistics and false discovery rate (FDR)-corrected p-values ($q < 0.05$).

Two separate PERMANOVAs were also carried out using the `adonis2` function from the ‘vegan’ package. We conducted this analysis using both weighted UniFrac and Bray-Curtis distance matrices to capture compositional differences across samples. One PERMANOVA model compared distances under the different treatments, and significance was evaluated by conducting 999 permutations. Meanwhile, the other PERMANOVA investigated individual factors (time/pasture/parasite metrics). Additionally, an ANOSIM was implemented using the `anosim` function from the ‘vegan’ package. The Bray-Curtis dissimilarity matrix was used with distances also compared between treatments as the predictor variable, and the distance measure as a response.

The diversity metrics and taxonomic composition analysis were performed using the R packages phyloseq, vegan, ggplot2, and dplyr, tidyverse, BiocManager, ANCOMBC, ComplexHeatmap, forcats, cowplot and DT (Gu, 2016; Gu, 2022; Lin, 2020; Lin, 2022; Morgan, 2024; McMurdie, 2013; Oksanen, 2024; Wickham, 2016; Wickham, 2019; Wickham, 2023; Wickham, 2023; Wilke, 2024; Xie, 2024).

## Results

### *1. Alpha Diversity Analysis*

A total of 6,853 unique bacterial ASVs were found in our study, with 22 (0.3%) being found in all examined individuals. To assess and compare microbial diversity across pasture types, rarefaction curves were used (Figure 1). The rarefaction curves approached a plateau approximately after a sample size of 100,000, indicating sufficient sequencing depth to capture the majority of microbial diversity within each treatment group. The S2, R2, S1 treatments visibly exhibit a marginally greater microbial diversity than R3, S3 and R1 treatments suggest a difference in richness between the two pastures.

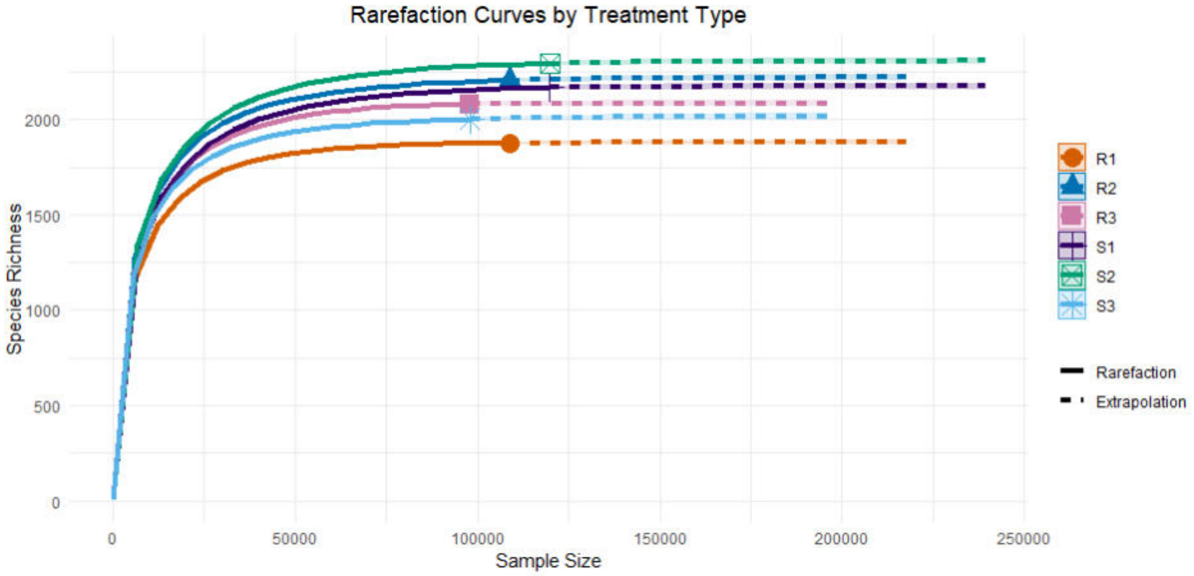


**Figure 1.** The rarefaction curve displays the observed and extrapolated microbial diversity (species richness) across samples from two pasture types, generated using the 'iNEXT' package in R. Solid lines represent the rarefaction curves based on sample richness, while dotted lines indicate extrapolated values, providing an estimate of potential diversity if sampling effort were increased.

Microbial diversity was assessed across the different treatment groups, and the results are presented in Figure 2. The GLM and post-hoc Tukey test for Shannon diversity showed significant differences between treatments, with R2 (Estimate = -0.01261, SE = 0.00399, p = 0.0325) and R3 (Estimate = -0.01458, SE = 0.00428, p = 0.0170) both displaying a significantly higher Shannon diversity in comparison to its baseline predictor, R1. No significant effects were observed for treatment S1 (Estimate = -0.00646, p = 0.6034). Treatment S2 (Estimate = -0.01219, SE = 0.00431, p = 0.0727) and treatment S3 (Estimate = -0.01090, SE = 0.00408, p = 0.1030) approached significance, suggesting a potential trend toward increased diversity. Coccidia counts showed that a low severity of infection was associated with a higher Shannon diversity (Estimate = -0.00601, SE = 0.00291, p = 0.04519 ). However, strongyle raw egg counts (Estimate = -2.28e-06, p = 0.809) and *Nematodirus* raw egg counts (Estimate = -1.90e-06, p = 0.9833) did not show significant associations with Shannon diversity. The model had a residual deviance of 0.0807 on 42 degrees of freedom.

The GLM for Chao1 richness similarly revealed significant effects for treatment R2 (Estimate = -0.0004395, SE = 0.000151, p = 0.00579) and R3 (Estimate = -0.000521, SE = 0.000159, p = 0.00213), both of which were associated with a decrease in Chao1 richness relative to the reference treatment R1. Treatment S1 and treatment S3 did not show significant effects compared to R1 (p = 0.12326 and p = 0.11655, respectively), while treatment S2 (Estimate = -0.0004394, SE = 0.000161, p = 0.00939) was significantly higher than R1. The coccidia count at low levels demonstrated a marginal association with reduced Chao1 diversity (Estimate = -1.74e-04, p = 0.094), while strongyle raw egg counts (p = 0.79985) and Nematodirus raw egg counts (p = 0.69023) were not significant predictors. The model's AIC was 679.79, with a residual deviance of 1.7302 on 42 degrees of freedom. Tukey post hoc analysis showed significant pairwise differences among treatments, with R1 vs. R3 (Estimate = 5.21e-04, p = 0.0243) and R1 vs. R2 (Estimate = 4.39e-04, p = 0.0601) indicating a consistent increase in richness for R2 and R3 treatments.

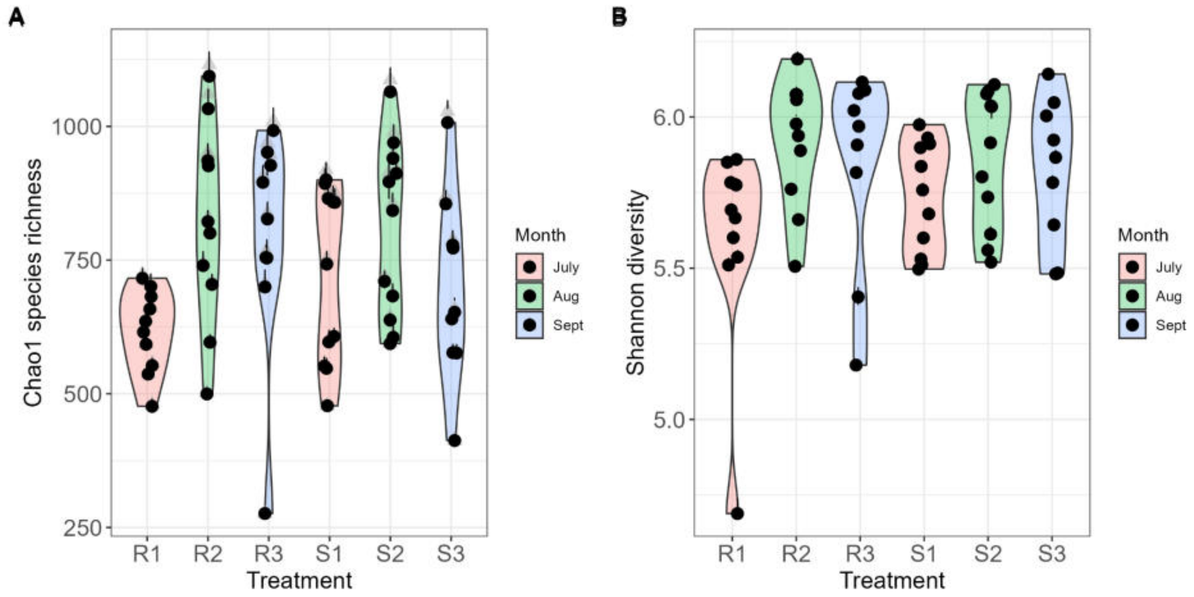


**Figure 2.** Violin plot displaying the relative per-capita Chao1 species richness (A) and Shannon diversity estimates (B) of each treatment group (given as a combination of pasture type and month of sampling). Black points represent means and error bars 95% confidence intervals resulting from an iterative model fit to rarefied data. The grey triangles represent the number of observed ASVs in each sample.

## 2. *Taxonomic Analysis*

The compositional bar plot (Figure 3) illustrates the relative abundance of bacterial genera in all samples obtained from the lambs in the opposing pastures. The plot includes the top genera by relative abundance, providing insights into the dominant microbial taxa in each pasture type.

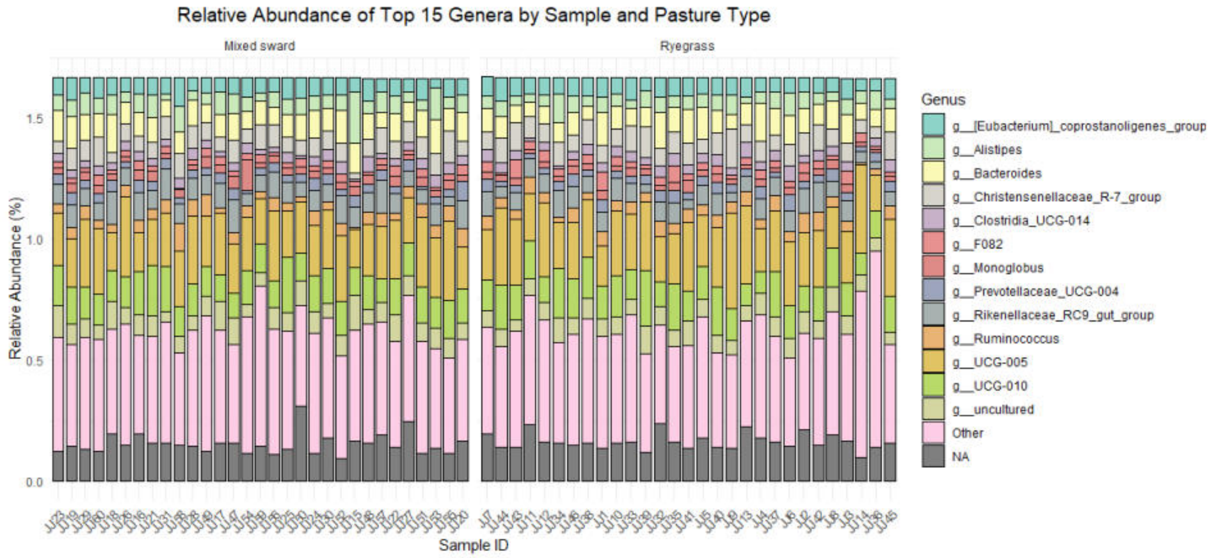


**Figure 3.** Compositional bar plot displaying the relative abundance of the most prevalent bacterial genera in gastrointestinal samples from lamb fed on mixed sward and perennial ryegrass pastures. The plot compares the microbial community composition between samples from the two pasture types, highlighting the dominant genera and their relative proportions.

The dominant eleven genera in both pasture types included *Alistipes*, *Bacteroides*, *Christensenellaceae*, *Rikenellaceae-RC9*, *Ruminococcus*, *Eubacterium*, *Monoglobus*, *Clostridia UCG-014*, *Prevotellaceae UCG-004*, *Oscillospiraceae-UCG-005* and *Oscillospirales UCG-010*. Using ANCOM (Analysis of Composition of Microbiomes), we identified several genera with significant differential abundance across different treatments ($p <= 0.05$). Uncultured taxa was significantly higher across all other treatments (R2, R3, S1, S2, S3) in comparison to R1. In the R1 group, *Akkermansia* and *WCHB1-41* were significantly enriched. The relative abundance of *Akkermansia* was more than double compared to S2, S3, and R2. *WCHB1-41* was highly abundant in R1, with minimal presence in other groups. In the R2 group, *Flexilinea* and *Mucispirillum* were notably enriched. *Mucispirillum* had a higher relative abundance than in any other group, while *Flexilinea* was considerably more abundant compared to S1 and S3. The relative abundance of *Akkermansia* was almost absent in R2. The R3 group showed significant enrichment of *Candidatus Saccharimonas* and *Aeriscardovia*. *Candidatus Saccharimonas* was more abundant in R3 compared to S2 and S1, while *Aeriscardovia* was more abundant than in all other groups. *S1* showed a significant increase in the relative abundance of the *Lachnospiraceae XPB1014 group*, which was approximately three times higher than in S3, R2, and R3. In S2, *Methylobacterium Methylorubrum*, *Oscillospiraceae*, and *Oribacterium* were enriched. *Methylobacterium Methylorubrum* had a higher relative abundance compared to R1 and R3. S3 showed increased abundance of *Bradymonadales*, *Negativibacillus*, and *Lachnospiraceae XPB1014 group*. *Bradymonadales* had the highest relative abundance in S3, surpassing R1 and R2.

A separate heat map illustrating the relative abundances of bacterial genera across treatments was generated to display the taxonomic composition of microbial communities by pasture type (Figure 4). Genera such as *Alistepes* were visibly more dominant in the mixed sward **(S)** pasture, whereas genera such as *Oscillospirales UCG-005*, *Oscillospiraceae-RC9*, *Christensenellacaea RC-7*, *Bacteroides* and *Coprostanolligenes* were prevalent in both pastures. Also, another differential abundance analysis was conducted to identify bacterial taxa significantly associated with each pasture type (Figure 5). This analysis revealed that specific genera were slightly enriched in each pasture type, suggesting weak environmental or treatment-related influences on microbial community structure. For example, taxa such as *Christensenellaceae-R-7*, *Oscillospiraceae* and *UCG-005* were significantly more abundant in the ryegrass **(R)** treatment, whereas *Alistepes* and *Rikennellacaea-RC9* visibly were more abundant in the mixed sward **(S)**.

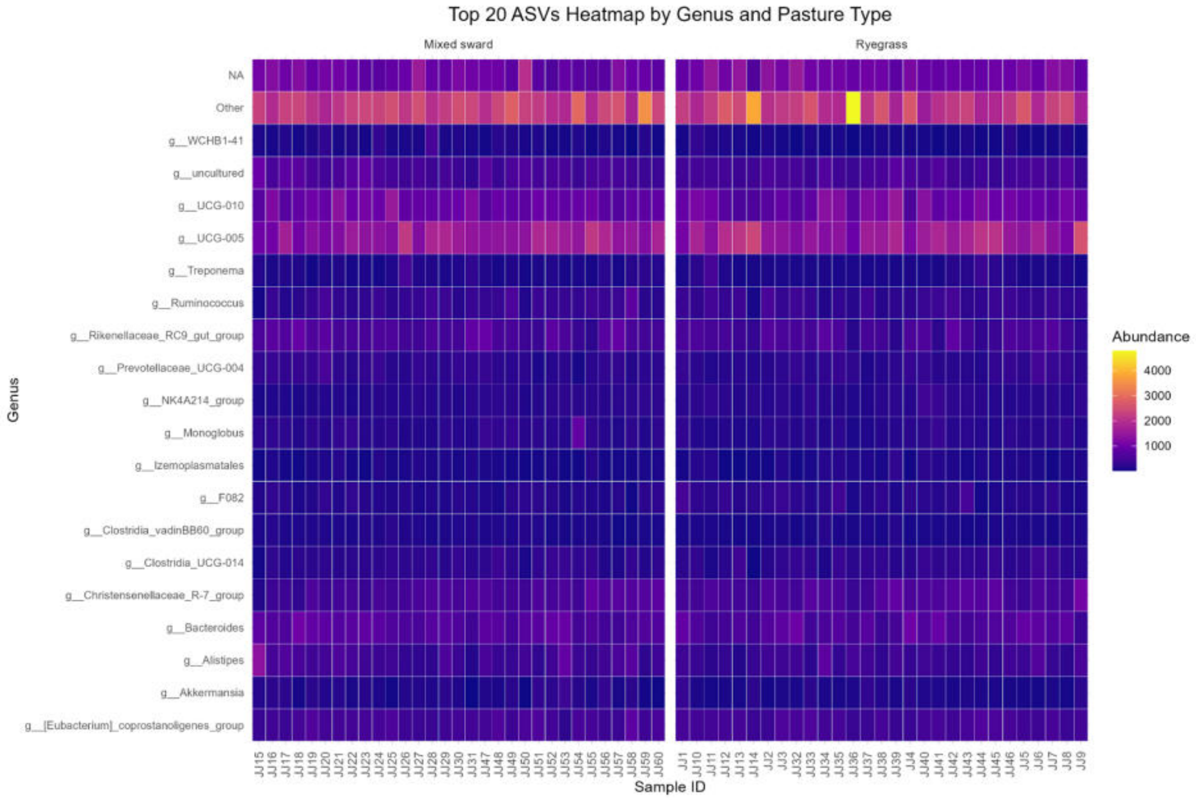


**Figure 4.** This heat map displays the relative abundance of the 15 most prevalent microbial genera across samples, separated by pasture type (Mixed Sward **(S)** and Ryegrass **(R)**). The plot was created using the 'ggplot2' package in R, with warmer colours indicating higher abundance levels of each genus within a sample. Rows represent microbial genera, and columns represent individual samples organised by treatment type.

### 3. *Beta Diversity*

A NMDS plot using UniFrac distances (Figure 5A) demonstrates strong similarities across the microbial communities. In contrast, the NMDS plot using Bray-Curtis distances (Figure 5B) shows much more variability among the microbial communities. The plot reveals a clear separation between the two pasture types, with the ryegrass samples predominantly clustering on the negative side of NMDS-2 and the mixed sward samples clustering on the positive side, indicating a distinct separation along this axis.

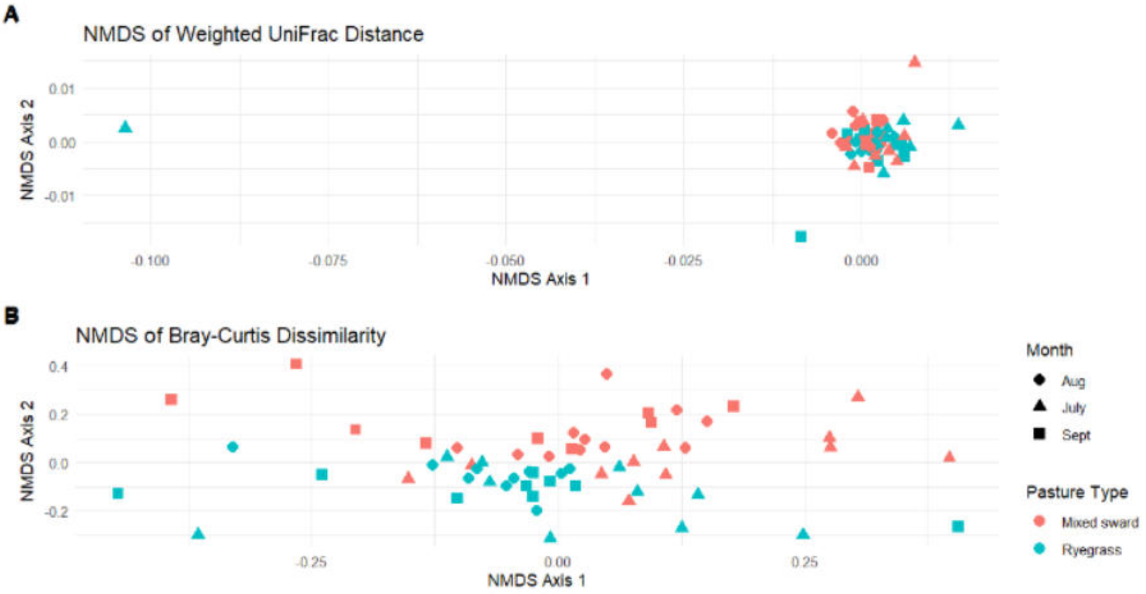


**Figure 5.** NMDS plot (Stress value 0.21) based on A. Weighted Unifrac Distance and B. based on Bray Curtis Dissimilarity (Stress value 0.12) showing clustering of samples by pasture. The different shapes describe the other months, and the red colour shows the ordination of the mixed sward (S) pasture while the blue colour shows the perennial ryegrass pasture (R).

The ANOSIM statistic using Bray Curtis dissimilarity revealed a statistically significant difference in microbiome composition across treatments (R = 0.2571, p = 0.001), suggesting that pasture diet influences the overall community structure of lamb microbiomes. Also, the ANOSIM indicated a significant variation in microbiome composition across different sampling dates (R = 0.1134, p = 0.001). This finding suggests that temporal factors, such as the sampling date and pasture type, influence the microbial community structure in lamb microbiomes. The results of the pairwise ANOSIM test based on treatment showed significant differences between several treatment groups, with adjusted p-values indicating robust separations. Specifically, GI microbial diversity in R3 significantly differed in R1, S2, S3, and S1, with adjusted p-values ranging from 0.0025 to 0.00375. R2 also showed significant differences in GI community composition compared to R1, S1, S2, and S3, with adjusted p-values between 0.0025 and 0.005. Similarly, R1 significantly differed from S3, S2, and S1 (p.adj = 0.0025 to 0.005). Comparisons between pasture types, such as S3 vs. S2 and S3 vs. S1, were also significant (p.adj = 0.036 and 0.0045, respectively), highlighting the distinctions in microbial composition between treatments.

The PERMANOVA results based on Bray Curtis distance and parasite metrics revealed significant effects on microbial community composition. The PERMANOVA investigating individual factors found pasture types ($R^2 = 0.0436$, $p = 0.001$), and sampling dates ($R^2 = 0.0556$, $p = 0.001$) both had highly significant impacts on the variation in community composition. The other PERMANOVA discovered that overall treatments were not significant ($R^2 = 0.346$, $p = 0.176$). These results indicate that the parasite metrics, particularly coccidia egg counts, explained the largest portion of variation.

## Discussion

1. Ryegrass pastures may cause a higher increase in gastrointestinal microbiome (GM) diversity of lambs over time

No significant differences in alpha diversity metrics were detected between the dietary treatments in our study, matching with prior findings in ruminants (McGovern et al., 2018; McLoughlin et al., 2020). Liu et al. (2023) similarly observed no effect of forage type on ruminal bacterial diversity in Ujimqin lambs, while Yang et al. (2018) reported comparable results for Hu lambs. Meanwhile, beta diversity analysis (ANOSIM and PERMANOVA, $p <= 0.05$) showed that diet significantly altered rumen microbiome composition. Such findings suggest that while alpha diversity metrics might remain stable, diet can still exert significant effects on the structure and function of microbial communities, as evidenced in other studies (Myer et al., 2015; Shabat et al., 2016). Auffret et al. (2017) found that dietary changes in cattle directly affect rumen microbial diversity, with implications for antimicrobial resistance and pathogenicity. Smith et al. (2020) showed that diverse sward types alter the relative abundance of rumen microbes in dairy cows, potentially enhancing resilience to parasitism. Additionally, a study by David et al. (2014) revealed how diet rapidly and reproducibly reshapes gut microbial communities, a finding echoed in comparative studies on sheep, where dietary practices rather than genetics were found to drive differences in GM composition. Mixed swards, consisting of multiple plant species with different growth rates, nutrient needs, and seasonal responses, often require complex strategies to maintain an optimal balance, productivity, and resilience (Barreta et al., 2023). Furthermore, while shorter management heights can increase nitrogen use efficiency and forage production, they have been shown to reduce species diversity. Higher nitrogen application rates, although beneficial for productivity, similarly decrease species diversity and increase uniformity in community traits, particularly during the primary growing season in the same study.

The GLMs exploring Shannon diversity showed that treatments R2 and R3 displayed significantly increased GM diversity in lambs compared to the baseline R1. However, none of the mixed sward treatments (S1, S2, S3) showed significant differences in alpha diversity from the baseline R1. Meanwhile, S2 and S3 approached significance, suggesting a possible trend of increased diversity with time in lambs feeding on the mixed sward pastures. This aligns with findings from Barrera et al. 2023, where on grazing systems where variability in phenology and growth rates of diverse sward types, particularly under variable conditions, has been noted to complicate management. However, this contrasts recent research of Williams et al. (2021), demonstrating that diets targeting GM composition can modulate intestinal immune responses, reduce inflammation, and potentially control helminth infections in livestock.  Although no changes in alpha diversity were observed over time in this study, the absence of significant variation does not necessarily negate the potential ecological relevance of subtle differences within specific treatments. In the ryegrass treatment, some microbial variation was detected, although this was not mirrored in alpha diversity metrics for the mixed sward treatments. This highlights the complex interactions between diet and microbiome diversity. Moreover, the time frame and resolution of sampling may play a role in these results. Studies reporting no temporal variation in alpha diversity often focus on comparable time windows, potentially limiting the detection of more nuanced dynamics.

Previous studies have highlighted the importance of plant diversity in shaping rumen microbiota, with diverse forage options fostering a more varied microbial community (Grace, 2019; Somasiri, 2014). However, more research would be necessary to understand how S and R treatments differentially impact lamb microbiomes and overall health over a longer period of time. Longitudinal studies examining microbial diversity and functional changes within the gut; alongside physiological measures of health such as weight, sex and age, would provide deeper insights into how these pasture types may shape lamb growth, immune function, and digestion.

## 2. Influence of Pasture Type on Microbial Community Composition

Compositional analysis revealed distinct microbial community compositions in the two pasture types. The primary phyla identified were Bacteroidetes and Firmicutes, aligning with findings from previous studies (Derakhshani et al., 2017; Liu et al., 2023). These phyla play key roles in the breakdown of cellulose and polysaccharides, supporting digestive processes in ruminants (Zhang et al., 2021). Additionally, the phylum Fibrobacteres is crucial for fibre and cellulose degradation, contributing essential nutrients for ruminant health (Chen et al., 2021). The abundance of these taxa align with previous studies showing seasonal impacts on microbial community dynamics in ruminants (Albakri, 2020; Golding, 2011).

Microbial communities play critical roles in modulating adaptive immunity and facilitating nutrient degradation, distribution, and absorption (Ellison et al., 2017). The microbial community in both pastures were dominated by several key genera, including: *Alistipes*, which is a recently discovered genus related to inflammation and cancer (Parker, 2020). *Bacteroides* was also present, known for its ability to metabolise polysaccharides and oligosaccharides, supplying nutrition and vitamins (Zafar, 2021; Wexler, 2007). *Christensenellaceae* is related to obesity prevention in humans and its presence as an anti-inflammatory (Waters, 2019). *Ruminococcus* is a prevalent gut microbe related to Crohn's disease in humans, produces an inflammatory polysaccharide and is linked to gut health by degrading plant material (Henke, 2019; Biddle, 2013; Ze, 2012). The family *Oscillospiraceae* is a prominent gut bacterial lineage consistently associated with various health biomarkers, particularly those linked to gut microbial richness and anti-inflammatory effects (Dai et al., 2023; Le Chatelier et al., 2013; Chen et al., 2020). Studies have highlighted *Oscillospiraceae UCG-005*, a genus within this family and the most abundant genera, for its positive correlations with short-chain fatty acid (SCFA) concentrations and its inverse relationship with inflammatory markers (Li et al., 2021; Xi et al., 2023; Zhang et al., 2022). Culturing this genus remains challenging (Yin, 2023), but these anti-inflammatory associations may underscore the significance of *Oscillospiraceae* in supporting host metabolic and immune health, warranting further investigation into its functional roles and potential as a therapeutic target for managing inflammation-related conditions. *Lachnospiraceae* is linked to obesity and colon cancer protection in humans, and the maintenance of gut health in mammals by breaking down foodstuff (Meehan, 2014; Biddle, 2013). *Rikenellaceae-RC9* was also among the most abundant genera. Although the function of *Rikenellaceae-RC9* remains unclear, studies in livestock suggest a close association with acetate and propionate (Holman & Gzyl, 2019; Liu et al., 2023). Notably, *Rikenellaceae-RC9* has been reported to have a negative correlation with propionic acid (Zhang et al., 2022), which is produced during the biohydrogenation of polyunsaturated fatty acids (PUFA) (Beam et al., 2000).

The ANCOM analysis revealed that relatively few bacterial genera exhibited statistically significant differences across treatments. In the R1 treatment, *Akkermansia* and *WCHB1-41* were significantly more abundant than other treatments. *Akkermansia*, involved with mucosal environments in humans and other mammals (Derrien et al., 2017), suggests that the microbial community in R1 may be favouring genera linked to the gut and mucosal systems. This could be due to the substrates present in the ryegrass pasture during July, or the availability of plant material that promotes the growth of these microbes. The enrichment of *WCHB1-41*, may be due to the presence of conditions that favour fermentative microbes in this pasture type during the early part of the season. *Flexilinea* and *Mucispirillum* were enriched in the R2 treatment. *Mucispirillum* is regarded for anti-inflammatory and mucus-degrading properties, suggesting that August may favour bacteria involved in breaking down or colonising mucosal layers (Herp et al., 2021). This suggests a higher availability of complex organic matter or specific microbial interactions in the ryegrass pasture during August. Similarly, *Flexilinea*, a bacterium associated with carbohydrate breakdown found in dairy cows (Mao et al., 2023), indicates that the ryegrass pasture during August may have a higher availability of plant-based polysaccharides, which may favour microbial fermentation processes. In the R3 treatment, the enrichment of pro-inflammatory *Candidatus Saccharimonas* and *Aeriscardovia* hints a shift towards microbes involved in the degradation of complex carbohydrates, such as plant polysaccharides (Li et al., 2023). The seasonal shift to September may result in changes in the composition of plant material available, supporting microbes that can degrade fibers and ferment carbohydrates. The increased abundance of these taxa in September suggests that ryegrass pasture may support a more complex set of microbial functions, such as fermentation of plant material.

Meanwhile, in the S1 treatment *Lachnospiraceae XPB1014 group* was significantly enriched. This genus, negatively associated with dietary starch, suggests that the S treatment in July provides an environment rich in plant fibers that favour microbial taxa capable of breaking down complex carbohydrates (Wu et al., 2024). The mixed sward has a different plant composition compared to ryegrass, providing an alternative set of resources for microbial fermentation. *Lachnospiraceae XPB1014 group* was also found to be abundant in the S2 and S3 groups, however its relative abundance varied. This suggests that it is able to thrive in different seasonal conditions with the S treatment. In the S2 treatment, the higher relative abundance of *Methylobacterium-Methylorubrum*, *Oscillospiraceae*, and *Oribacterium* suggests that the microbial community in August is more adapted to favour specific substrates such as methylated compounds (Green et al., 2018). Additionally, the enrichment of *Oribacterium*, positively correlated with concentrated amines and amino acids (Zeng et al., 2019), suggests that this genus may play a role in the fermentation of nitrogen-rich compounds in the S treatment. As a result, they may potentially contribute to the microbial breakdown of plant proteins and the production of nitrogenous metabolites. Furthermore, *Oscillospiraceae* and *Oribacterium* were also enriched in both S2 and S3. The seasonal increase in these taxa suggests that microbial communities in the S treatment become more active in breaking down methylated and nitrogenous metabolites in the plant material as the growing season progresses. This may result with specific microbial taxa becoming more prominent as environmental conditions and substrate availability change. In the S3 treatment, *Bradymonadales* and *Negativibacillus* were more abundant. The former is less explored but *Negativibacillus* is typically involved in the fermentation of plant polysaccharides (Zhang et al., 2021). Their higher abundance in September suggests that the mixed sward in this month supports microbes adapted to the breakdown of complex carbohydrates.

The observed shifts in microbial community structure across the different pasture types (ryegrass and mixed sward) and the seasonal variations in July, August, and September making up the treatments highlight the complex interplay between pasture type, seasonality, and microbial ecology. In particular, the abundance of specific taxa such as *Akkermansia*, *Mucispirillum*, *Candidatus Saccharimonas*, and *Lachnospiraceae XPB1014 group* reflects how microbial communities adapt to seasonal changes and the specific resources provided by different pasture types. However, it is important to consider the idea of functional diversity accounting for differences between the two pastures (Giger-Reverdin et al., 2023). Previous studies found that with an increase in dietary concentrate, Proteobacteria populations increased while reducing the proportions of Bacteroidetes and Firmicutes due to the bacteria having the same function in the rumen (Kibegwa et al., 2023). As this study didn't include physiological changes of the lamb between diets, there's no way of confirming the bacteria that account for the same functional similarities. Overall, these findings suggest that pasture management practices, in combination with seasonal shifts, could influence microbial diversity and function, potentially impacting soil and animal health in agricultural ecosystems

## 3. Parasitic Influences on Microbial Communities

The analysis revealed that GM beta diversity, as measured by both ANOSIM and PERMANOVA indices, varied significantly between ryegrass (R) and mixed sward (S) pasture. The GLM model showed that coccidia infection at low levels was associated with reduced Shannon diversity, while strongyle and *Nematodirus* egg counts were not significant predictors. Studies investigating how nematode parasites affect microbial community structure also find no changes in alpha diversity between groups (Mate et al., 2024). The relationship between low coccidia counts and alpha diversity metrics further highlights the potential role of parasite-related factors in shaping microbial community structure, although additional research is warranted to clarify these interactions. The significant changes iin coccidia count alpha diversity between the two pastures may reflect the differences in plant species richness, which could modulate host-parasite-microbiome interactions by influencing diet quality or microbial habitat within the gut. Previous studies have suggested that plant diversity can affect parasitic burdens and, as a result, gut microbial composition (Mamun, 2020; Williams, 2021). However, the life histories and transmission patterns of the parasites may influence their relationships with microbial diversity in distinct ways.

Coccidia, being intracellular parasites, have a more localized and host-specific impact on the gut environment, potentially altering microbial communities through immune modulation or competition for nutrients within the host (Junkuszew et al. 2015). The observed association between low counts and reduced Shannon diversity suggests that even low-level infections could influence the overall gut microbial community structure by affecting immune responses or altering nutrient availability in the gut. In contrast, strongyles and *Nematodirus*, which are nematodes with direct transmission through eggs in feces, may have a different ecological impact on the gut microbiome. Their transmission dynamics involve environmental contamination and may not directly interact with the microbial community in the same way as coccidia. These parasites often have life stages that live outside the host and may not exert immediate effects on the gut environment until maturation into adult worms in the mucosa of the small intestine (Miller and Horohov, 2005). Therefore, their relationship with microbial diversity could be more diffuse or dependent on factors such as production performance, infection intensity or host immune responses over time, rather than being immediately reflected in the microbial community composition.

Furthermore, differences in the dynamics of these parasites such as the environmental persistence of strongyle and *Nematodirus* eggs, versus the more acute and internalized infection cycles of coccidia; may shape how microbial communities respond over time, potentially influencing the long-term stability or resilience of the gut microbiome. As a result, parasite-induced shifts in microbial communities may not be uniform across different parasite types, underscoring the need for a more nuanced understanding of how parasitic infections and microbial diversity interact, particularly in the context of varying life histories and transmission modes of different parasites. Future studies should aim to disentangle these complex relationships by considering both parasitic load and ecological characteristics of each parasite species.

## 4. Implications for Livestock Management

The findings of this study have important implications for pasture management and livestock health. Mixed sward pastures appear to enhance microbial diversity, potentially contributing to improved gut health and resilience in lambs. These results suggest that diversifying pasture species may be a viable strategy for promoting microbial diversity and mitigating the negative effects of parasitic infections. Future research should explore the long-term effects of these management strategies on lamb health and productivity, as well as investigate the functional consequences of microbial shifts in relation to performance outcomes.

However, several limitations should be noted. Our study lacked a control group without anthelmintic treatment, limiting our ability to fully isolate the effects of pasture management from drug interventions. Additionally, the study's timeframe may not have been long enough to capture the full range of seasonal and environmental influences on microbial communities. We also did not collect data on lamb performance, such as weight gain or meat quality, which would provide valuable insights into the functional outcomes of microbial diversity.

Further studies should also address the limitations of this research by incorporating a control group with no anthelmintic treatment, extending the sampling period, and including performance metrics such as weight gain and meat quality to better understand the link between microbial diversity and lamb productivity.

## 5. Conclusion

This study examined how the rumen bacterial communities of lambs shifted over time under two distinct dietary treatments, providing key insights into the interplay between pasture management and gastrointestinal microbiota. While the overall richness and evenness of the bacterial communities remained relatively stable, significant differences emerged at the genus level across the monthly sampling periods. These findings underscore the critical role of dietary diversity, seasonal changes, and parasitic infections in shaping the gut microbiome. Moving forward, research should delve deeper into these complex interactions, aiming to develop targeted pasture management practices and refined parasite control strategies to improve lamb health and productivity.

# Appendices

1. Truncation parameters

| Forward | Reverse | % of inputs passed filter | % of inputs merged | % of inputs non-chimeric |
|---|---|---|---|---|
| 230 | 160 | 73.427 | 59.04116667 | 57.5845 |
| 236 | 162 | 71.68733333 | 45.362 | 28.7965 |
| 250 | 205 | 63.8835 | 52.91 | 51.8085 |
| 280 | 230 | 49.967 | 40.93183333 | 38.4685 |
| 290 | 230 | 48.08583333 | 30.39 | 27.34033333 |

The truncation parameters of 230 base pairs for the forward primer and 160 base pairs for the reverse primer were chosen based on the analysis of the sequence quality data. These were compared using q2view These parameters provided the highest percentage of reads passing filters (73.43%) and the greatest percentage of inputs merged (59.04%) compared to other parameter combinations. Additionally, the proportion of non-chimeric sequences (57.58%) was also higher for this combination, ensuring the quality and accuracy of the data. In contrast, longer truncation lengths resulted in significantly lower percentages of merged sequences and non-chimeric reads, making the 230f/160r combination the most optimal choice for maximising data quality and quantity.

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