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The Stool Microbiome in African Ruminants: A Comparative Metataxonomic Study Suggests Potential for Biogas Production

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Abstract: Lignocellulosic biomass is a promising substrate for anaerobic digestion (AD) in renewable energy generation but presents a significant challenge during the hydrolysis stage of conventional AD due to the recalcitrant nature of this biomass substrate. Rumen fluid is often employed as a bioaugmentation seed to enhance hydrolysis in the AD of lignocellulosic substrates due to its richness in hydrolytic bacteria. However, using rumen fluid to enhance AD processes presents substantial hurdles, including the procurement difficulties associated with rumen fluid and ethical concerns. In this study, the fecal microbiota of 10 African ruminant species from a large zoological park (Bioparc) in Valencia, Spain, were studied using 16S rRNA gene amplicon sequencing. In this study, the fecal microbiota of 10 African ruminant species from a large zoological park (Bioparc) in Valencia, Spain, were studied using 16S rRNA gene amplicon sequencing. The investigation revealed potential similarities between the fecal microbiota from the African ruminants' and cows' rumen fluids, as suggested by theoretical considerations. Although direct comparative analysis with cow rumen fluid was not performed in this study, the theoretical framework and existing literature hint at potential similarities. According to our results, the Impala, Blesbok, Dikdik and Bongo ruminant species stood out as having the greatest potential to be used in bioaugmentation strategies. Key genera such as *Fibrobacter*, *Methanobrevibacter*, and *Methanosphaera* in Impala samples suggested Impala rumen fluid's involvement in cellulose breakdown and methane production. Blesbok and Dikdik exhibited a high abundance of Bacillus and *Atopostipes*, potentially contributing to lignin degradation. The richness of *Prevotellaceae* and *Rikenellaceae* in the Bongo fecal samples is probably associated with structural carbohydrate degradation. Taken together, our results shed light on the microbial ecology of the gut contents of a whole set of Bovidae ruminants and contribute to the potential application of gut microbiota in AD.

Keywords: African ruminants; bioaugmentation; biogas production; microbial community; metataxonomic analysis

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1. Introduction

In response to the challenge of climate change, the EU has committed to reducing net greenhouse gas (GHG) emissions by at least 55% by 2030 and to attain carbon neutrality by 2050 [1]. As a result, there is an increasing demand for the exploration and adoption of renewable energy sources to gradually replace conventional fossil fuels. Anaerobic digestion (AD), a well-established biotechnology, not only generates renewable energy in the form of biogas through the breakdown of organic matter, but also contributes to the stabilization and minimization of organic waste, thereby mitigating negative environmental effects such

as GHG emissions [2]. AD offers a dual solution to critical environmental concerns: sustainable waste management and the generation of renewable energy [3,4]. The AD process typically consists of four sequential stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Each of these stages is carried out by a specific group of microorganisms: hydrolytic bacteria, acidogenic bacteria, acetogenic bacteria, and methanogenic archaea, respectively [5–7].

In practical applications, a diverse range of organic matter can serve as a substrate for AD, including agricultural residues, energy crops, and livestock waste. Among these organic materials, lignocellulosic biomass is recognized as the most abundant biomass on Earth, making it a promising source for generating renewable energy through AD, owing to its widespread availability [8]. The fundamental composition of lignocellulose primarily consists of cellulose (35–50%), hemicellulose (20–35%), and lignin (10–25%) [9]. Due to its insolubility in water and recalcitrant biomass structure, lignocellulose is resistant to biodegradation, making it a challenge to break down during the hydrolysis stage of conventional AD. Hence, the hydrolysis of lignocellulosic biomass often becomes the rate-limiting step during overall AD processes [8,10]. To enhance biogas production during the AD of lignocellulosic substrates, it is essential to employ effective approaches that improve the digestibility of these substrates and break down their recalcitrant biomass structures [11]. In this context, bioaugmentation has emerged as a promising strategy, as it involves the direct introduction of specific microorganisms that offer a range of enzymes for breaking down resilient compounds during the hydrolysis stage [12].

In recent years, there has been growing interest among researchers in using rumen fluid for bioaugmentation in AD, primarily because of its richness in hydrolytic bacteria [13]. Ruminants possess a specialized digestive system that depends on a symbiotic collaboration among the bacteria, archaea, protozoa, and fungi present in the rumen environment [14]. These microorganisms release hydrolytic enzymes and play a crucial role in digesting complex plant materials such as cellulose, hemicellulose, and lignin components [15,16]. Moreover, the addition of rumen as an inoculum in a bioaugmentation strategy to boost AD provides essential enzymes for the degradation of lignin and lignocellulosic substrates, along with the necessary methanogens for biomethane production [17]. Li et al. (2020) conducted a study comparing the rumen communities of 14 ruminant species, and they found that *Methanobrevibacter* spp. is the most widespread genus, consisting of strictly anaerobic archaea responsible for CH_4 production through CO_2 reduction with H_2 [18]. Singht et al. (2019) also remarked that many ruminants, especially those from the Bovidae family, such as buffalo and antelope, harbor substantial amounts of fibrolytic microorganisms [19].

Employing rumen fluids as bioaugmentation seeds in AD processes presents notable hurdles. The acquisition of rumen fluid for inoculation necessitates invasive techniques such as rumenocentesis and rumen cannulation [20]. This raises ethical concerns, as it requires accessing the digestive tracts of living animals [21]. Moreover, the controlled and consistent procurement of rumen samples can pose logistical challenges, particularly when conducted on a large scale or in specialized research environments [22]. In a recent study, Ozbayram et al. (2018) compared the composition of the microbial communities in cow rumen and manure, focusing on plant fiber-digesting microbes. The results showed that not only does the rumen microbiota have a higher potential for lignocellulosic biomass degradation, but ruminant manure also contains a microbiota similar to the rumen, highlighting its potential for use as bioaugmentation seeds. Therefore, fresh ruminant manure appears to be a promising source for bioaugmentation seeds due to their accessibility and similarities to rumen fluid microbiota [16].

Using ruminant manure as a source for bioaugmentation offers a promising approach to enhancing AD efficiency in biodigesters. However, research on the fecal microbiota in diverse ruminant species, especially on their potential as bioaugmentation agents, remains limited. This shift towards investigating fecal microbiota as potential bioaugmentation seeds not only addresses the challenges associated with rumen sampling but also facilitates more accessible and ethically sound practices in AD research and applications. Thus, this

study focused on shedding light on the fecal microbiota of ten African ruminant species from the Bovidae family. We posit a groundbreaking paradigm shift by asserting that the fecal microbiota in diverse ruminant species holds untapped and significant potential as agents for bioaugmentation purposes in AD, and we employed 16S rRNA gene amplicon sequencing (NGS) to compare biodiversity patterns, microbial community structure, and significant functional group identification.

2. Materials and Methods

2.1. Sample Description and Collection

Stool sampling was conducted in the zoological park (Bioparc) in Valencia, Spain (39°28′40.8″ N 0°24′28.8″ W). The selected ruminants and their dietary specifications are shown in Figure 1 and Table 1. Stools were collected in the morning from the floor of the areas where animals were housed overnight. For most species, each sample probably originates from a single animal, given their tendency to defecate at distinct locations. In this study, each sample was collected from different points, under the assumption that they each might come from a different animal. Falcon tubes containing 25 mL of anaerobic transfer media (composition per liter: 1.15 g Na_2HPO_4, 3 g NaCl, 0.20 g KCl, 0.20 g KH_2PO_4, 0.1 g $MgSO_4 \cdot 7H_2O$, 4 g Gellan gum, 4 mL 0.025% resazurin solution, 4 mL 25% cysteine hydrochloride solution, and 1 g sodium thioglycolate) were filled with stool up to a volume of 50 mL. Triplicates were taken for each species. Samples were immediately transported to the laboratory and homogenized in mortars.

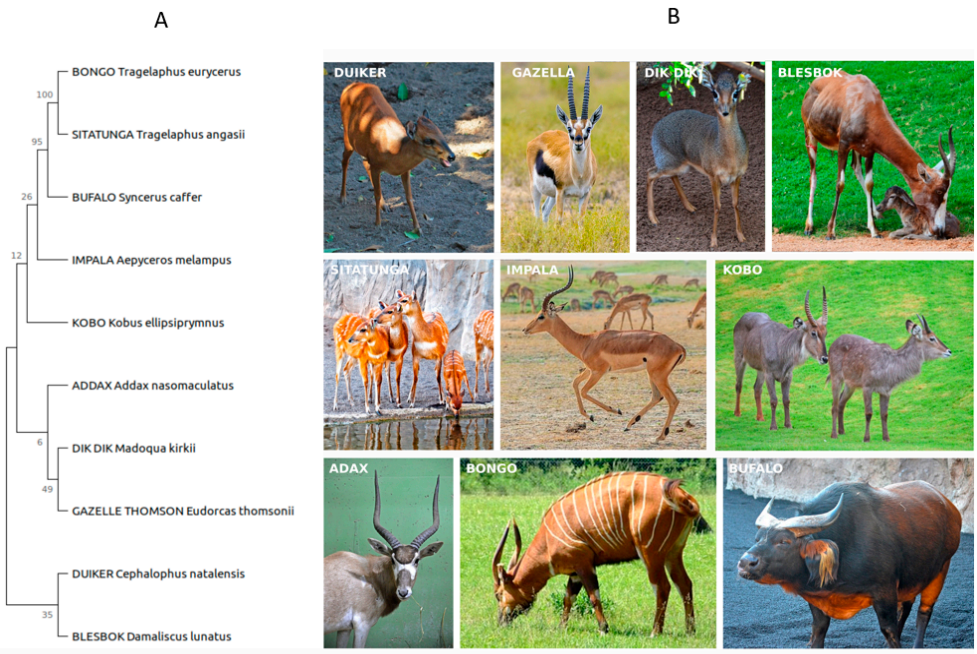

Figure 1. Selected ruminants from Bioparc whose stool samples were used in the present study. (**A**) Maximum likelihood phylogenetic tree arranged with mitochondrial cytochrome b gene alignment. Bootstrap support values for each branch are included in the tree [23]. (**B**) Ruminant species analyzed in this work.

Table 1. Dietary specifications of the animals.

No	Animal Type	Breakfast	Dinner
1	Sitatunga	Alfalfa; Feed * Vegetables	Alfalfa; Total pet food **
2	Adax	Hay; Alfalfa	Hay; Alfalfa; Total pet food

Table 1. *Cont.*

No	Animal Type	Breakfast	Dinner
3	Dikdik	Alfalfa; Feed	Alfalfa; Total pet food; Spinach
4	Buffalo	Hay	Hay; Alfalfa; Total pet food; Vegetables
5	Blesbok	Hay; Alfalfa; Feed	Hay; Alfalfa Total pet food
6	Gazella	Alfalfa; Feed	Hay; Alfalfa; Total pet food
7	Kobo	Hay; Alfalfa; Feed	Hay; Alfalfa; Total pet food
8	Duiker	Alfalfa; Feed	Hay; Alfalfa; Total pet food; Banana peel; Leafy vegetables
9	Bongo	Banana peel; Vegetables	Alfalfa; Total pet food; Vegetables
10	Impala	Hay; Alfalfa; Feed; Vegetables	Hay; Alfalfa; Total pet food

* Feed: Dry grass, flaked maize, carrots, corn, and oat grains supplemented with additional carrots. ** Total pet food: Meat byproducts, cereals, grain, vitamins, and minerals.

2.2. DNA Extraction and High-Throughput Sequencing

An aliquot of each sample was taken for DNA extraction using DNeasy PowerSoil Pro Kits® (Qiagen, Venlo, The Netherlands), according to the manufacturer's instructions. All processing steps were performed inside a Bactronez-2® anaerobic chamber (Bactron, Sheldon Manufacturing, Cornelius, OR, USA) using a mixture of gases (5% H_2, 10% CO_2, and 85% N_2). DNA extracted from the previous step was quantified using the Qubit High Sensitivity dsDNA quantification assays (Thermo Fisher Scientific, Waltham, MA, USA). These metagenomic DNA samples were used to amplify the hypervariable region V3-V4 of the 16S ribosomal RNA gene using the primers 341F (5′ CCTAYGGGRBGCASCAG 3′) and 806R (5′ GGACTACNNGGGTATCTAAT 3′) with the following PCR cycle: initial denaturation at 95 °C for 3 min; 30 cycles of amplification (30 s at 95 °C, 30 s at 55 °C, and 30 s at 72 °C); and 5 min of final extension at 72 °C [24]. The 16S rRNA amplicons were mixed with Illumina sequencing barcoded adaptors (Nextera XT index kit v2, FC-131-2001), and libraries were normalized and merged. The pools with indexed amplicons were loaded onto the MiSeq reagent cartridge v3 (MS-102-3003) and spiked with 10% PhiX control to improve the sequencing quality. Sequencing was conducted using paired-end 2 × 250 pb and 2 × 300 pb cycle runs on an Illumina MiSeq device.

2.3. Sequencing Data Analysis

The raw Illumina sequences were loaded into Qiime2 (v. 2021.2.0) [25]. To assess sequence quality, the plugin Demux was applied. Subsequently, the Qiime2-integrated DADA2 pipeline was used for sequence trimming and joining, as well as for removing chimeras and detecting amplicon sequence variants (ASVs) at a 97% similarity threshold. Taxonomic assignment for each sequence variant was carried out using the classify-Sklearn module from the feature-classifier plugin, with SILVA (v. 138) [26] serving as the reference database. The obtained results were further analyzed using the phyloseq R package (January 2022, phyloseq 1.36.0) [27].

2.4. Statistical Analysis

Differences in quantification of total bacteria between the 10 samples were assessed for significance using the ANOVA program in the statistical software PAST 4.11 [28]. Three alpha diversity indices were calculated: Chao1, which represents the species richness; Shannon index, providing information about richness and evenness; and Simpson index, measuring diversity while considering the number and abundance of species. For Chao1, Shannon, and Simpson indices, parametric ANOVA tests were employed after testing the normality of our data using the Shapiro–Wilk tests. These tests aimed to determine whether there were statistical differences among the groups of studied ruminants. Subsequently,

Tukey's pairwise post hoc tests were applied to identify specific groups that differed from each other. Significant differences were assumed when $p < 0.05$.

3. Results and Discussion

3.1. Microbial Community Diversity

In this study, the analysis of 16S rRNA gene sequences from fecal samples of 10 African ruminant animals generated a total of 2,761,783 quality sequences, with an average of 276,102 sequences per sample. The overall number of OTUs detected by the analysis was 18,327 based on a 97% nucleotide sequence identity between reads. The number of OTUs for each fecal animal sample was as follows: 762 (Sitatunga), 1598 (Adax), 643 (Dikdik), 1135 (Buffalo), 760 (Blesbok), 1629 (Gazella), 864 (Kobo), 1024 (Duiker), 779 (Bongo), and 1896 (Impala). Additionally, alpha (Shannon, Simpson, and Chao1) and beta (unifrac distance with PCoA) diversity indices were calculated for each sample to examine potential diversity differences in the fecal microbial communities among the ruminants, as shown in Figure 2. We observed that the Chao1, Shannon, and Simpson diversity indices were notably higher in the Adax sample. This suggests the presence of low-abundance species within the community and overall greater diversity compared to the other samples. In contrast, the Dikdik sample showed the highest abundance of species in the community but the lowest community diversity, a pattern similar to that of the Blesbok sample. Additionally, similarities were observed between the Impala and Bongo samples in terms of the abundance of species within the community.

In terms of the total Chao1 index, the statistical analysis indicated significant differences among the studied groups regarding the specific variations in microbial community diversity (ANOVA: $F = 4.776$; $p < 0.005$). Tukey's pairwise post hoc test showed that the Adax group exhibited significant dissimilarities in fecal microbiota composition compared with the Sitatunga and Dikdik groups, but not with the other groups. There were no significant differences among the remaining studied groups. In terms of the total Shannon index, the statistical analysis revealed significant differences among the studied groups (ANOVA: $F = 3.676$; $p < 0.005$). Tukey's pairwise post hoc test indicated that the Adax group showed significant dissimilarities compared with the Sitatunga group, but not with the other groups. No significant differences were observed among the rest of the studied groups. In terms of the total Simpson index, the statistical analysis indicated no significant differences among the studied groups.

Beta diversity, a crucial metric for comparing microbial communities, assesses the compositional differences between distinct samples. In this study, the unifrac distance, which considers the evolutionary information among microbial sequences within each sample, was employed. Principal coordinates analysis (PCoA) using unweighted unifrac distances was conducted to elucidate variations among the ruminant fecal samples, as illustrated in Figure 2D. The PCoA plot revealed that the Sitatunga, Duiker, and Buffalo samples exhibited closer microbial community similarities compared to the others, potentially attributed to their shared dietary habits (Table 1). Conversely, Impala exhibited lower similarity compared to the other samples, indicating a distinct microbial profile, possibly influenced by its unique dietary preferences and different environmental exposures among the diverse ruminant species in this study.

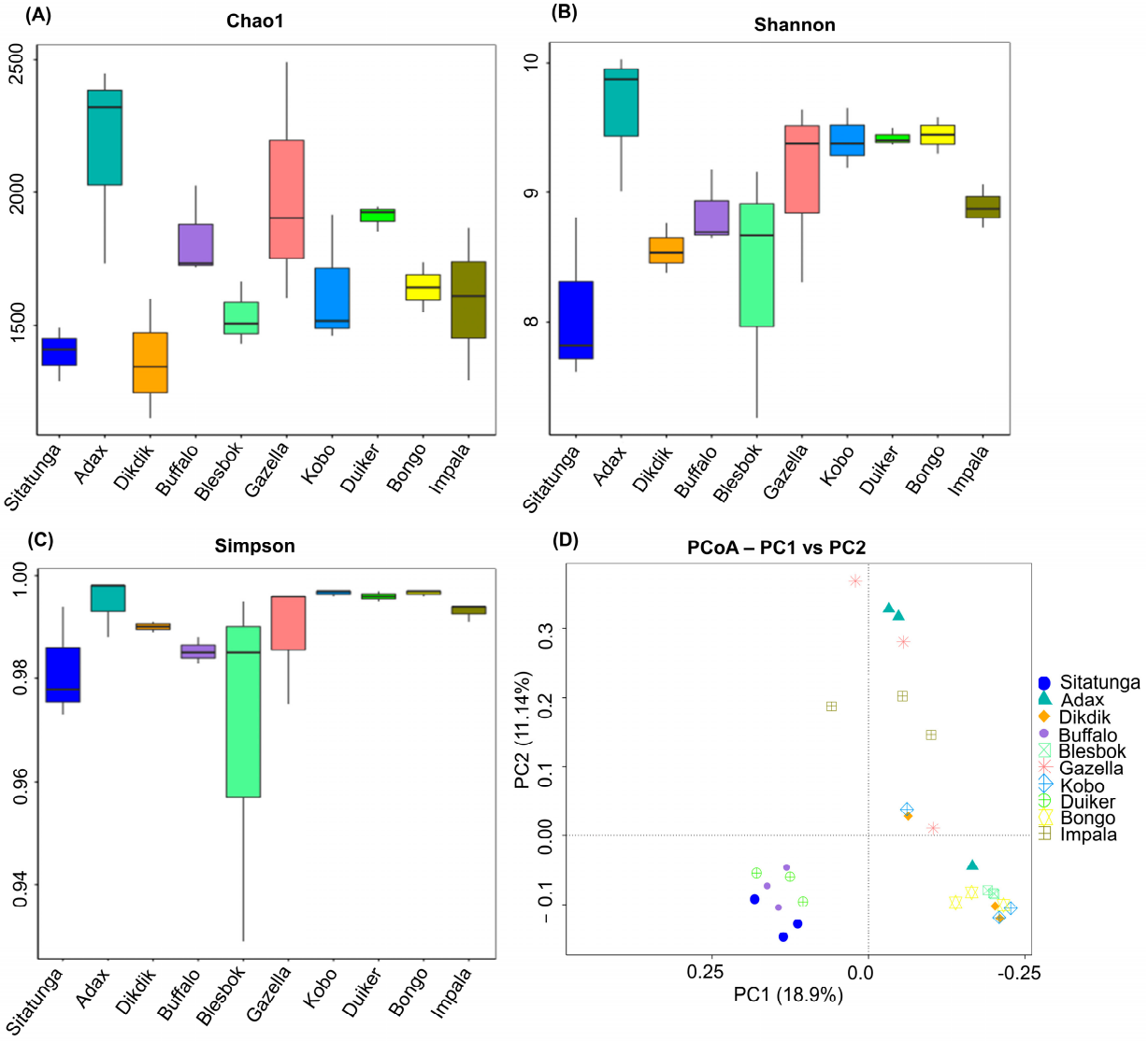

Figure 2. Alpha and beta diversity analysis of fecal microbiotas from the 10 species of ruminants. The boxplots represent Chao1 (**A**), Shannon (**B**), and Simpson (**C**) alpha diversity indices. The PCoA represents the (unweighted unifrac distances) beta diversity index (**D**).

In addition, a Venn diagram as well as a heatmap from the core representing the unique and common OTUs for the 10 ruminant fecal samples is depicted in Figure 3. Each circle in the diagram represents a specific ruminant species and lists the total number of OTUs for each sample. The core of the Venn diagram, indicated by the overlapping region among all samples, represents the shared OTUs present across all 10 ruminant species. The findings from the Venn diagram align with the taxonomic results and diversity patterns discussed in this study. The shared microbial community identified across all samples designates a dominance of certain bacterial phyla and genera, such as *Firmicutes* and *Bacteroidota* (Figure 3B), known for their roles in lignocellulose degradation and carbohydrate metabolism, which implies a conserved functional potential. This shared core microbiota likely contributes to the facilitation of biodegradation processes in the gut, indicating a collective adaptation to dietary and metabolic needs.

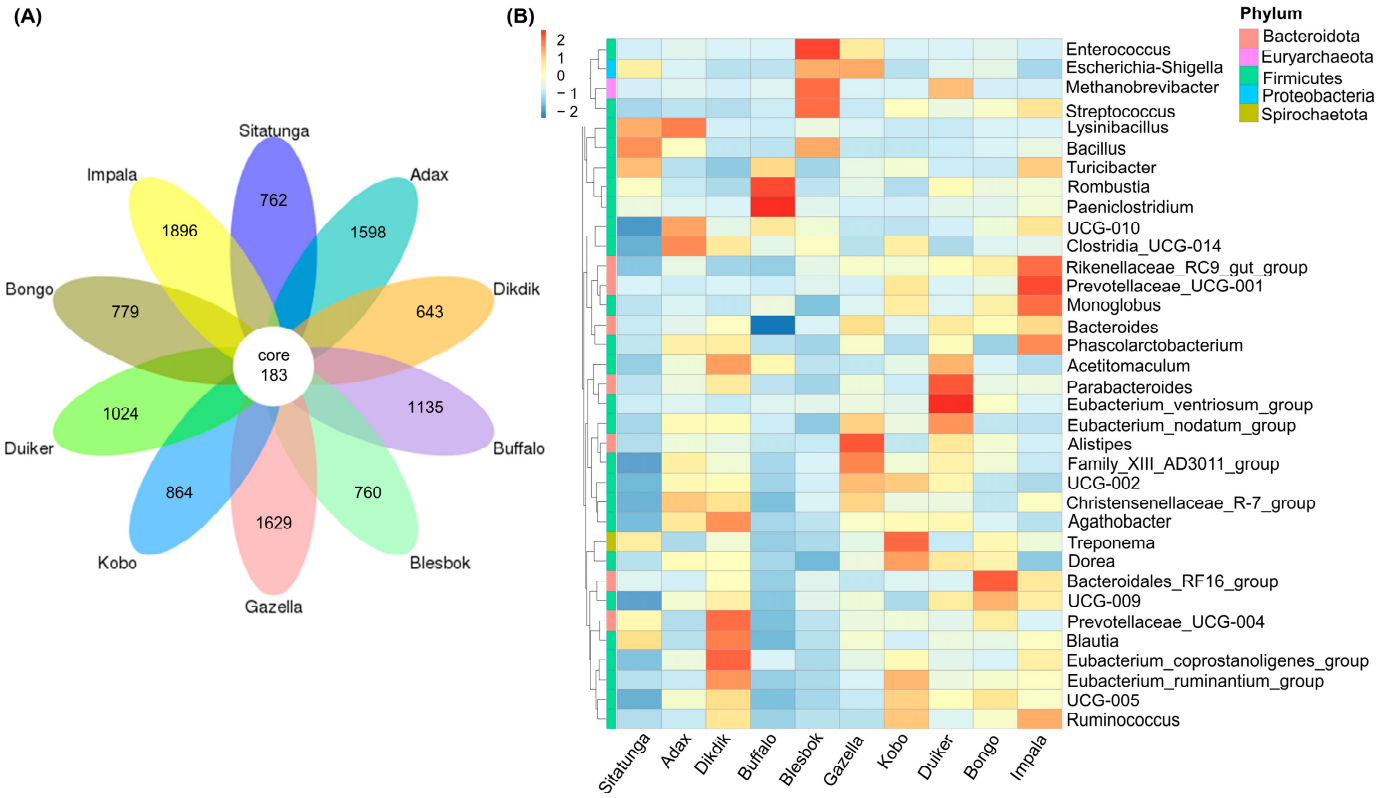

Figure 3. Flower diagram (**A**) and heatmap (**B**) representing the shared and unique taxa among the 10 ruminant stool samples analyzed. Each circle in the diagram represents a specific ruminant species with the total number of OTUs for each sample. The core of the Venn diagram, indicated by the overlapping region among all samples, represents the shared OTUs present across all 10 ruminant species.

3.2. Bacteria Community Structure

The fecal microbial community compositions of the 10 species of ruminants are visualized as a histogram graph and heatmap generated from the combined sequence dataset of the 10 animals (Figure 4). The histogram graphs (Figure 4A,B) illustrate the relative abundance of *bacterial* and *archaeal* phyla and genera. In total, 61 *bacterial* phyla were identified across all the ruminant fecal samples. Among the top 10 most abundant phyla, *Firmicutes* and *Bacteroidota* were the most abundant phyla in all samples, comprising ranges of 48.95% to 75.28% and 15.52% to 31.18% of the total sequences, respectively. *Proteobacteria* followed closely, ranging from 0.93% to 8.23% of the total sequences. The remaining sequences included *Spirochaetota* (0.49% to 4.28%), *Actinobacteria* (0.61% to 3.19%), *Verrucomicrobiota* (0.39% to 3.04%), *Desulfobacterota* (0.16% to 0.84%), *Fibrobacterota* (0.02% to 3.58%), and a number of low-abundance phyla. Upon analyzing each animal individually, it becomes evident that the Impala sample stands out, with *Proteobacteria* (8.23%), *Spirochaetota* (4.28%), and *Fibrobacterota* (3.58%) being the three most dominant phyla, surpassing the abundances observed in other ruminants. The Sitatunga samples exhibit a distinctive profile, with *Actinobacteriota* (3.15%) and *Proteobacteria* (5.62%) dominating. Blesbok also presents a relatively high abundance of *Proteobacteria* (3.42%), while Dikdik stands out with the highest relative abundance of *Firmicutes* (75.28%).

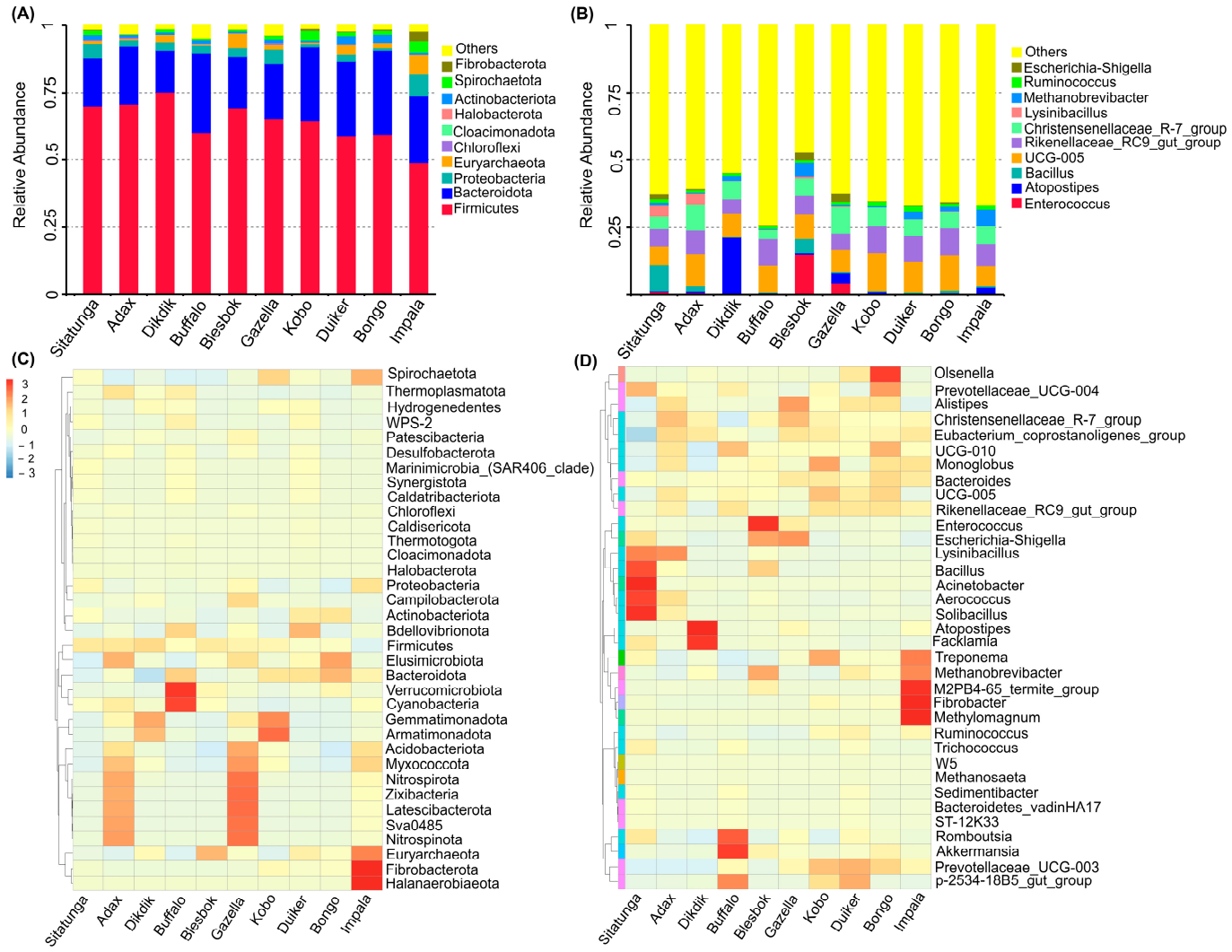

Figure 4. Relative abundance of phyla (**A**) and genera (**B**) in the 10 ruminant fecal samples; the heatmap of the top 35 most abundant phyla (**C**) and genera (**D**) in the 10 ruminant samples.

Firmicutes and *Bacteroidota*, dominant in all samples, are widely recognized components in the fecal matter and rumen of animals. Typically, in fecal matter, *Firmicutes* tend to be more abundant than *Bacteroidota* [15,29–35], which is consistent with our results. Members of the *Bacteroidota* phylum play various roles, including in the degradation of carbohydrates like complex plant cell walls and in the production of butyrate [36]. On the other hand, members of the *Firmicutes* phylum are crucial for the degradation of fiber and starch [37]. Additionally, members of *Fibrobacterota* and *Proteobacteria* are well-known constituents of both rumen fluid and manure, contributing to the digestion of plant fibers [38].

At the genus level (Figure 4B,D), the highest shared relative abundance among the sequences found in all samples was assigned to *UCG-005*, the *Rikenellaceae_RC9_gut_group*, and the *Christensenellaceae_R-7_group*. Although Gazella showed the highest abundance of the *Christensenellaceae_R-7_group*, reaching 9.62%, and Bongo stood out with the highest abundance of the *Rikenellaceae_RC9_gut_group*, reaching 10.06%, all of the samples revealed a relatively high abundance regarding these two groups when compared with results obtained in other studies on rumen samples. According to Wang (2023) and Liu (2022), who compared the rumen microbial communities between Yaks and Qaidam cattle, the relative abundance of the *Rikenellaceae_RC9_gut_group* and the *Christensenellaceae_R-7_group* in Yaks was revealed to be higher, reaching 10.6% and 8.0%, respectively [39]. On the other hand, Dong (2023) found a lower abundance of the *Rikenellaceae_RC9_gut_group*

(7.06%) and the *Christensenellaceae_R-7_group* (5.27%) when studying cow fecal flora [40]. In the same line of study, Mutungwazi (2022) compared four animal manures which are routinely used as inoculum in anaerobic digestion and revealed a lower abundance of the *Christensenellaceae_R-7_group* for pig (1.4%), horse (0.4%), chicken (0.2%), and cow (4.6%), and no significant presence of the *Rikenellaceae_RC9_gut_group* [17]. In fact, the *Rikenellaceae_RC9_gut_group* is known for its role in producing acetate and propionate as fermentation end products and is considered to be one of the main hydrolytic bacteria in the anaerobic process [41,42]. Also, the *Christensenellaceae_R-7_group*, as a member of *Firmicutes* phylum, plays a role in establishing magnetite-mediated direct electron transfer with methanogens during the methanogenic degradation of VFAs and promotes the hydrolytic acidification of refractory cellulose [36].

In addition to these two main groups, Impala exhibited a remarkable abundance of the genus *Fibrobacter* (3.57%), Bongo samples showed a high abundance of the family *Prevotellaceae* (4.65%), while Dikdik stood out with a very high abundance of *Atopostipes* (20.93%). The members of the *Prevotellaceae* family represent the most numerous bacteria that are cultivable from the rumen and hindgut of catt. This family plays an important role in breaking down proteins and carbohydrates. On the other hand, *Fibrobacter* has been suggested to be the main cellulose degrader in ruminant gut systems [43,44]. In addition, *Prevotellaceae* and *Fibrobacter* are usually the most dominant genera in ruminant fluid and are present in low abundance in fecal samples. According to results from previous studies on cow rumen fluid, *Prevotellaceae* and *Fibrobacter* can be found at frequencies of around 28% and 12%, respectively [30,31,37,45]. However, in ruminant fecal samples, *Prevotellaceae* and *Fibrobacter* are usually present in a rather low abundance, reaching between 0.4% and 1.4% [22,35,40,46]. Furthermore, with a similar function to *Fibrobacter*, members of *Atopostipes* are hydrolytic bacteria which promote the hydrolysis of cellulose and lignin contents to produce simple sugars [47,48]. On the other hand, Blesbok showed a significantly higher abundance of *Enterococcus* (14.98%) while the other animals showed less than 1%.

3.3. Archaeal Community

Along with the 61 *bacterial* phyla, 5 *archaeal* phyla were also identified across all the ruminant fecal samples. Figure 5 shows the relative abundance of the archaeal and *bacterial* phyla in each sample. Impala and Blesbok stood out, reaching 7.02% and 6.01% abundance of the phylum *Euryarchaeota*, followed by samples from Duiker and Dikdik, with 3.04% and 3.02% abundances, respectively. The rumen methanogenic communities for all samples analyzed were dominated by the genera *Methanobrevibacter* and *Methanosphaera* (order *Methanobacteriales*), which together accounted for 96% of all genera. Impala and Blesbok showed a relative abundance of 83% and 89% for *Methanobrevibacter* and 17% and 10% for *Methanosphaera*, respectively. However, according to O'Donnell (2017), after analyzing fecal samples from 10 different animals (ruminants, hindgut fermenter, and monogastric), they found around 0.01% of the reads were assigned to archaea, showing a lower relative abundance compared to our samples. On the other hand, results from previous studies showed *Methanobrevibacter* followed by *Methanosphaera* as the most abundant genera in the rumen community [22,49–53], which is in accordance with our results. The *Methanobrevibacter* has been described as a genus of characteristic hydrogenotrophic rumen methanogens [54]. In our results, *Methanobrevibacter* was also found to be the dominant genus among the archaeal community for all of our samples. Thus, the findings regarding the archaeal community align with the study's emphasis on understanding the fecal microbiota's potential role in anaerobic digestion (AD) for biogas production. The variation in the relative abundances of these methanogenic genera among different ruminant species, such as Impala and Blesbok, highlights the diversity in archaeal composition and suggests potential implications for methane production. These findings enrich the understanding of the functional roles of archaea in the gut microbiota of African ruminants, providing valuable insights into their ecological significance within the broader context of the study.

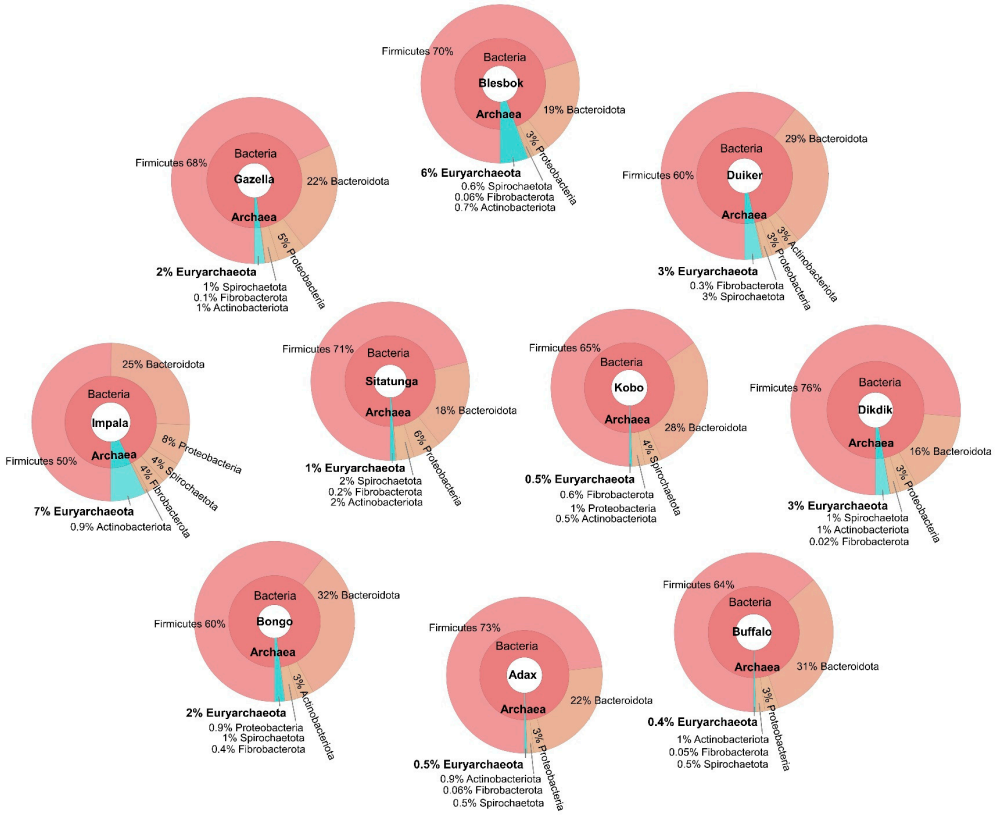

Figure 5. Krona chart showing the main bacterial phyla and highlighting the relative abundance of the archaeal communities of each stool sample.

3.4. Microbiome Profile and Bioaugmentation Potential

Among the African ruminant samples analyzed, Impala had the highest abundance in some important key bacteria and archaea. Members of *Proteobacteria*, *Fibrobacterota*, and *Euryarchaeota* stood out, with a higher relative abundance of the genera *Fibrobacter*, *Methanobrevibacter*, and *Methanosphaera*. Previous studies have highlighted that members of the genus *Fibrobater* (lignocellulolytic bacteria) are considered to be some of the most important cellulose-digesting anaerobic bacteria in the rumen, as well as some of the few isolated from the rumen capable of digesting crystalline cellulose [55,56], an important player in the context of biogas production. Furthermore, members of the *Methanobrevibacter* genus, which were overwhelmingly present in our samples, are hydrogenotrophic methanogens that use H_2 to reduce CO_2 for methane biosynthesis [57]. They also use formate as a carbon source for growth and energy metabolism [58] and are usually isolated from animal rumen [59,60]. On the other hand, *Methanosphaera*, unlike *Methanobrevibacter*, has the most restricted energy metabolism of all methanogenic archaea. It can only generate methane by reducing methanol with H_2 and depends on acetate as a carbon source [61].

Similar to Impala, the fecal samples from Blesbok had a high abundance of *Methanobrevibacter* and *Methanosphaera* compared to the other animals, but concerning the bacterial community, they also stood out for having a high relative abundance of *Bacillus spp*. According to studies carried out by Kim (1996) and Lv (2014), members of the *Bacillus* genus are robust to lignin degradation and function as biocatalysts for processing biomass from lignocellulosic waste, and they are also known to produce bacterial ligninases that can break certain Cα-oxidation and Cβ-Cβ bonds of the lignin structure [62,63]. Therefore, they are likely involved in the metabolic processing of the ruminant's food.

Finally, we can also highlight that the fecal samples from Bongo showed the highest relative abundance of the *Rikenellaceae_RC9_gut_group* genera and *Prevotellaceae* family. In addition, Dikdik had the highest abundance of *Atopostipes* compared to the other animals. In fact, previous studies show that members of the genus *Rikenellaceae_RC9_gut_group* (phy-

lum: *Bacteroidota*) are associated with the primary or secondary degradation of structural carbohydrates [64], but they also play vital roles in the colonization and decomposition of biomass composed mainly of pectin, cellulose, and hemicellulose [65,66]. Members of the *Prevotellaceae* family (genus: *Prevotella*) are fiber-degrading bacteria and are usually present in high abundance. In addition, the *Prevotella* genome is endowed with polysaccharide utilization loci (PUL), which are groups of genes that encode proteins specialized in processing complex carbohydrates [67,68]. It has been suggested that *Prevotella* species can act synergistically with other cellulolytic organisms and are involved in rumen fibrolytic activity [45,69]. Besides that, *Atopostipes* is an anaerobic hydrolytic bacterium which produces lactate, acetate, and formate (organic acids) by metabolizing glucose [70–72]. Sohail (2022) [48] and Duan (2021) [73] also showed that the genus *Atopostipes* was found in high abundance in the Yak rumen fluid, presenting a symbiotic relationship with the hydrogenotrophic methanogens and creating favorable conditions for *Methanobrevibacter*.

Taken together, the results of the present study reveal similarities in the bacteria and archaea, at the genus level, of the analyzed samples, which are surprisingly closer to rumen samples than to ruminant fecal samples. The taxonomic results presented in this study offer compelling insights into the potential of microbial communities within the fecal samples for biodegradation processes. The abundance of key bacteria such as *Fibrobacter*, *Bacillus*, *Prevotellaceae*, *Rikenellaceae_RC9_gut_group*, and *Atopostipes* identified across various ruminant species indicates a rich reservoir of microorganisms capable of breaking down complex organic compounds. Additionally, archaea (*Methanobrevibacter* and *Methanosphaera*) were found in high abundance, especially in the samples from Impala and Blesbok. The taxonomic diversity observed across the ruminant fecal samples suggests a robust enzymatic toolkit within these microbiomes, hinting at their ability to efficiently participate in biodegradation processes. These findings underscore the promise of harnessing these microbial communities for applications in AD and biogas production.

4. Conclusions

This study provides a comprehensive analysis of the fecal microbiota from 10 African ruminant species within the Bovidae family and hypothesizes their potential use for the bioaugmentation of AD, thus boosting biogas production. The analysis of microbial communities revealed that the Impala, Blesbok, Bongo, and Dikdik fecal samples shared similarities with rumen fluid microbiota, emphasizing their suitability as bioaugmentation seeds. The abundance of key bacterial genera such as *Fibrobacter*, *Methanobrevibacter*, and *Methanosphaera* in the Impala fecal samples suggested their potential significance in enhancing cellulose breakdown and methane production. Additionally, Blesbok exhibited a high abundance of *Bacillus*, known for lignin degradation, providing insights into its potential role in facilitating the breakdown of recalcitrant biomass. Furthermore, Bongo and Dikdik samples were underscored by the richness of bacteria like *Prevotellaceae*, *Rikenellaceae_RC9_gut_group*, and *Atopostipes*, known for their involvement in the degradation of structural carbohydrates. The analysis of community structures at the phylum and genus levels across the 10 fecal samples consistently identified *Firmicutes* and *Bacteroidota* as predominant phyla, with *UCG-005*, *Rikenellaceae_RC9_gut_group*, and *Christensenellaceae_R-7_group* as prevalent genera. The presence of these genera, along with *Fibrobacter*, *Bacillus*, *Prevotellaceae*, and *Atopostipes*, underscores their potential as bioaugmentation candidates for AD processes. The comparative assessment of fecal samples against previous rumen fluid studies highlighted the promising bioaugmentation potential of fecal microbiota, with similarities being found in microbial composition and functional roles, offering an alternative to rumen fluid. Our results may contribute to paving the way for future investigations and innovations in bioaugmentation strategies, ultimately fostering more accessible and ethically sound practices in AD research and applications.

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