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Research article

Characterization of the fecal microbiota of Pampa Rocha pigs, a genetic resource endemic to eastern Uruguay

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ABSTRACT

Pampa Rocha (PR) is a breed of pig that emerged in eastern Uruguay during the 18th century. They represent an important resource for non-intensive production using purebred or crossbred animals. However, productive activities have been oriented towards intensive production using commercial breeds, abandoning, except by some academic and educational institutions, the promotion of this creole breed. Thus, a population of few animals is still maintained, which could be in danger of disappearing. This work focuses on the fecal microbiota of these animals, which is related to the animal genetic background but also to their grazing capacity and resistance to weather. The structure and diversity of bacterial communities in the intestines of four PR adult females and of other breeds, including crosses, reared under non-grazing conditions, were analyzed and compared. Results obtained indicate that PR fecal microbiota is clearly different from those of other animals analyzed. Some sequences, corresponding to particular groups apparently related to the consumption of fiber, were strongly associated with PR pigs.

1. Introduction

Worldwide, meat from pigs (*Sus scrofa domestica*) is consumed at high levels comparable to that of beef, chicken and lamb [1]. Diverse breeds of pig are raised in different farm systems around the world. However, swine production has several problems that are enhanced by incorporation of intensive farming methods in several countries. Under such systems, pigs remain confined to a small space during their entire life. Such confinement results in a higher risk of infectious diseases and may not comply with good practices for animal welfare [2]. Of particular significance is the use of antibiotics for pig production under confinement. These therapeutic agents are often supplied for disease treatment, but are also used for prophylaxis or even as growth promoters [3]. This practice is cause for concern in spread of antibiotic resistance, an arising global public health issue [4].

Alternative livestock production practices could contribute to diminish antibiotic use by providing better sanitary conditions for animals [5]. However, some systems of alternative production require a larger area for breeding; generally a smaller number of

Abbreviations: PR, Pampa Rocha; UCB, undefined crossbred; UB, unknown breed; LWxL, Large White x Landrace; Yorkshire and LxLWxDxSHR, Landrace x Large White x Duroc x Synthetic Hybrid Boar.

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animals are managed. In addition, several pig breeds are not well suited for such alternative practices [6], since they have been selected to optimize some parameters to detriment of others that would increase their survival outdoors.

Pampa Rocha (PR) originated as a result of crossings between breeds of pigs introduced by Portuguese and Spanish colonizers. Some of those first pigs roamed free in wild areas, particularly in eastern Uruguay, evolving into a distinct breed. PR has shown great grazing adaptability and resistance to inclement weather [7]. In addition, their black coats provide them with protection against UV radiation. These traits make them fit candidates for outdoor production systems. In addition, crossbreeding of these animals, with Duroc or Large White, results in superior weight gain [8].

Intestinal microbiota plays an important role during digestive processes. Composition of the microbial community in each zone of an animal's digestive tract can affect its ability to absorb nutrients, resulting in differences in feed conversion ratios [9,10]. Besides these obvious roles, the gut microbiota has other vital functions including the modulation of the immune system [11], and involvement in the gut-brain axis [12]. Moreover, community assemblages, rather than functioning as a collection of individual species, may be considered as real entities, with distinct abilities arising from the interaction of their members [13]. Microbiome compositions often show distinctive marker taxa that can be characteristic of each breed, growing conditions, health status or even considered as an individual fingerprint [10,14].

In this study, we describe and analyze the fecal microbiome of PR pigs. We hypothesized that the fecal microbiota of these pigs could contain certain singularities and even indicator species that could be useful genetic resources. We used fecal samples from four adult female pigs and sequenced part of their rRNA 16S genes, which were amplified using total DNA as template. We compared the community structure obtained from these samples, with that derived from fecal microbiomes of different breeds of pigs, obtained from a public database. In order to minimize unintended biases, we limited the comparison to data obtained from female pigs using the same sequencing technology and limited the comparisons to the same rRNA 16S region.

We consider it of great interest to develop a comparative study of bacterial fecal microbiota in PR pigs, which form an endemic breed in Uruguay. It is noteworthy that this breed is at risk given the limited protection policies for wild breeds implemented by local political authorities.

2. Materials and methods

2.1. Sample collection and DNA extraction

Fecal samples were obtained from four adult female Pampa Rocha pigs. Samples were obtained from animal's rectum, using a DNA Genotek kit, prototype P-164. Sample collection tubes were immediately stored at −20 °C until further processing. Fecal DNA was extracted from the samples using the Quick-DNA Fecal/Soil Microbe Miniprep Kit from Zymo Research, following manufacturer's instructions. Pigs were grown at the Unidad de Producción de Cerdos at the Facultad de Agronomía (UdelaR) (www.upc.edu.uy) in an open-air growing system. They had permanent access to grasslands cultivated with chicory (*Cichorium intybus*), red and white clover (*Trifolium pratense* and *T. repens*, respectively). Once a day, during the morning, they were offered a grain-based balanced commercial ration that included a vitamin-mineral nucleus, in amounts according to their physiological status. The rations used did not contain any antibiotic complement. Sanitary management was based on the supply of an antiparasitic (Ivermectin) every six months. Rations were

Table 1
Data of the sequences obtained from the SRA and used to compare with our results. Sequences belonging to four individuals were retrieved from each experiment included in the analysis.

BioProject	Run Accession numbers	Breed	Pigs age	average of million bases	Project description	Study site	Treatment	Reference
PRJNA622643	SRR11470352 SRR11470359 SRR11470382 SRR11470395	Large white x Landrace	18 days	18.7	Faecal microbiota transplantation and faecal microbiota analysis of piglets	Adelaide, Australia	Control	[34]
PRJNA634125	SRR11819538 SRR11819539 SRR11819541 SRR11819543	Landrace x Large White hybrid sows sired with Duroc x synthetic hybrid boar	12 weeks	63.8	Tail biting	Montreal, Canada	Control	[35]
PRJNA479505	SRR7505951 SRR7506120 SRR7506181 SRR7506410	Undefined crossbred	Post weaned (8 weeks)	30.3	pigs challenged with Salmonella and administered chlortetracycline	Iowa, USA	Non-inoculated control	[36]
PRJEB9053	ERR845286 ERR845288 ERR845287 ERR845289	Unknown	no data	35.1	Diet drivers of gut microbiota	Montreal, Canada	no data	Unpublished
PRJNA382998	SRR5450489 SRR5450490 SRR5450507 SRR5450520	Yorkshire cross	9 weeks	32.3	pigs challenged with Salmonella	Iowa, USA	Salmonella inoculated at 8 weeks of age	[37]

supplied in feeding trays and water was supplied at will with an automatic system. All reproductive cycle stages occurred at open-air (mating, pregnancy, farrowing, nursery and finishing), or on natural fields in individual or collective pen shelters. Sows were subject to a continuous farrowing system, with an average of two deliveries per year per sow. At the time of sampling, the pigs were 40 months old and weighed, on average 160 kg.

2.2. High-throughput sequencing

The extracted DNA was sent to the NGS service of Macrogen (Republic of Korea). Amplicons for a portion of the 16S rRNA gene were obtained using the 341F and 806R primers, which cover the V3 and V4 variable regions of the gene. Library was constructed with the Herculase II Fusion DNA Polymerase Nextera XT Index Kit V2 (Illumina, Inc.). Sequencing was performed on an Illumina MiSeq™ platform based on Sequencing by Synthesis technology. Pair-end reads of 301 bases in length were obtained. All fastq files obtained were deposited in the Sequence Read Archive (SRA) of the NCBI under the BioProject ID PRJNA870492.

2.3. Data collection

Microbiome data from other pig breed were obtained from the SRA of the NCBI (https://trace.ncbi.nlm.nih.gov/Traces/sra/). Accession numbers of the projects to be included were obtained from database using the term "*Sus scrofa*" as taxonomic query. The results were filtered by "platform" to include only Illumina, by "source" to include only DNA samples, by "strategy" to consider only amplicon obtained data, and by "library layout" to include only paired end data. The filters were used to obtain those data that were the most comparable to our sequences. With this same objective, only sequences from fecal samples of female pigs were included in this study. For those projects that included treatments such as fecal microbiota transplantation, pathogen challenge, or antibiotic supply, only control groups were considered. Four replicates were considered for analysis to match the number of biological replicates of our data. Then, replicates with the highest total sequences were selected (Table 1).

2.4. Bioinformatic processing

Sequences processing and analysis were performed with the software QIIME 2 version 2021.8 [15]. All sequences were trimmed at the 515F and 806R primer positions using the *cutadapt* command. Although in some studies, including ours, the sequences were obtained using the 341F primer, we cut all sequences at the 515F primer position to homogenize the data.

Each dataset of sequences were denoised and paired using *dada2* v1.18. Before pairing, forward and reverse reads were truncated to 280 nucleotides in length. To avoid sample-size-originated bias [16], before performing the diversity analysis, the number of reads per sample was rarified to the lowest obtained for a sample which was 11,764.

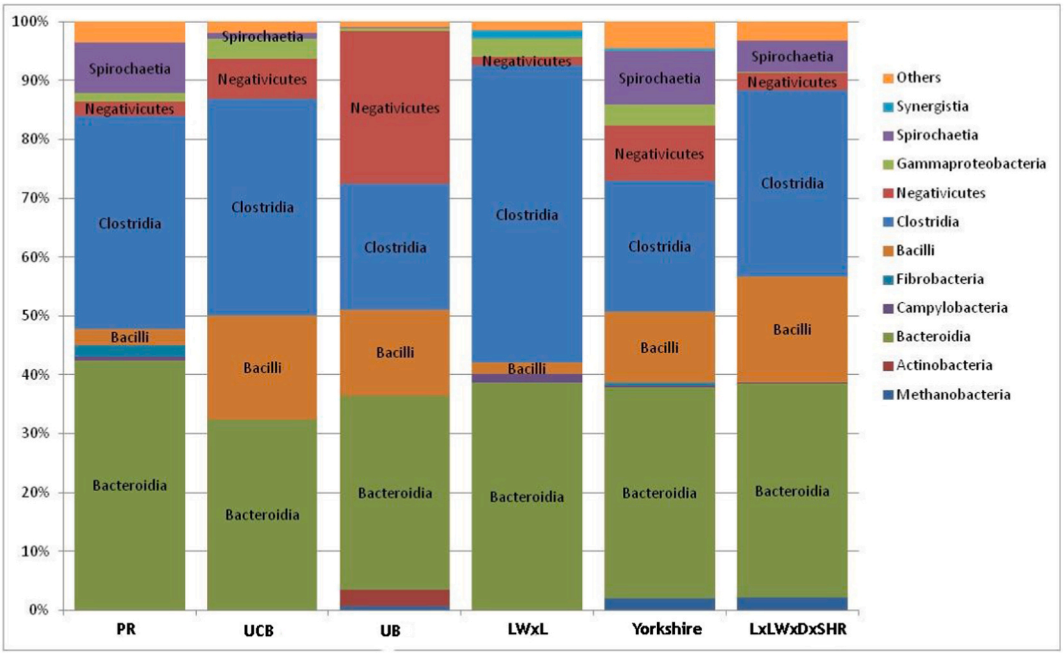

Fig. 1. Taxonomic composition and relative abundance of the fecal microbiota of different pigs analyzed at the Class level. Only the most abundant taxa are labeled in the chart. PR: Pampa Rocha; UCB: undefined crossbred; UB: unknown breed; LWxL: Large White x Landrace; Yorkshire and LxLWxDxSHR: Landrace x Large White x Duroc x Synthetic Hybrid Boar.

The taxonomy classifier was built using the Silva database version 138 with a 99% identity threshold. The classifier was trained with the Naive-Bayes algorithm with the following specific non-default parameters: -chunk-size 1000, –p-feat-ext –*n*-features 1024. These parameters were used in order to run the script in a computer with 16 GB of RAM. Taxonomy classification was performed with sklearn. Frequency tables of the OTUs observed in each samples were built for each taxonomic level ranging from class to genus. The relative abundance of each OTU at each taxonomic level analyzed was used to calculate Shannon's diversity index and Pielou's evenness index.

2.5. Statistical analysis

Fecal microbiome population structures were analyzed with non-metric multidimensional scaling (NMDS). Significance of the observed clustering of groups in the bidimensional representation of the NMDS was tested with the Multiple Response Permutation Procedure (MRPP). The R package *indicspecies* [17] was used to identify OTUs associated to the fecal microbiomes of the different pigs analyzed.

The significant differences of the diversity indexes between pigs at each taxonomic levels were evaluates evaluated by ANOVA and Tukey's HSD. Normal distribution of the data and homogeneity of variances were confirmed by Shapiro-Wilk's and Levene's tests, respectively.

All analysis were performed with the software R [18].

3. Results

3.1. Taxonomic composition of pigs fecal microbiomes

At class level, populations were mainly dominated by Bacteroidia and Clostridia, while Bacilli and Negativicutes were abundant in some pigs (Fig. 1). In PR, Bacteroidia accounted for 42.4% of the total community, while 36.2% belonged to Clostridia. Bacteroidales was the most abundant order in all pigs, which accounted for 42.3% of the total community in PR. Other abundant orders were Oscillospirales (19.4% in PR) and Lachnospirales (Fig. 2). Lactobacillales was an abundant order in some samples but not particularly abundant in PR (0.38%) and in Large white x Landrace (LwxL) (0.82%).

In total, 40 different classes of Bacteria were observed in the samples; 26 of which were present in at least one PR sample. Finally, the average number of classes present in PR samples was 21.25 (Table 2).

Thirty-seven families had abundances of over 1% of the population in at least one pig dataset. In PR samples, the most abundant families were Prevotellaceae (15.6%), Lachnospiraceae (12.4%), p-251-o5 (9.9%) and Oscillospiraceae (9.0%). With the exception of p-251-o5, the most abundant families in PR were also present in a considerable percentage in the other microbiomes (Fig. 3). Prevotellaceae was also the most abundant family in all other datasets except from LWxL, in which the most abundant family was Lachnospiraceae. In total, 125 families were observed in at least one pig dataset, 88 of them were present in at least one PR fecal sample. The average number of families present in PR samples was 67 (Table 2).

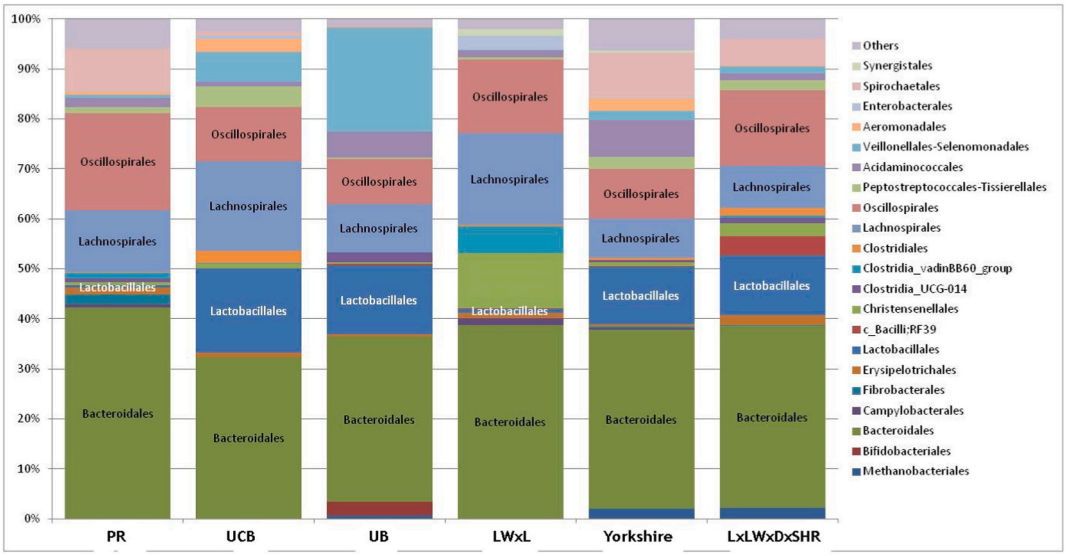

Fig. 2. Taxonomic composition and relative abundance of the fecal microbiota of different pigs analyzed at the Order level. Only the most abundant taxa are labeled in the chart. PR: Pampa Rocha; UCB: undefined crossbred; UB: unknown breed; LWxL: Large White x Landrace; Yorkshire and LxLWxDxSHR: Landrace x Large White x Duroc x Synthetic Hybrid Boar.

Table 2
Average number of observed OTUs (N), Shannon's diversity index (H') and Pielou's evenness index (J') at each taxonomic level for each pig breed. Numbers followed by the same letter indicate no significant differences between breeds for each parameter (Tukey's HSD, $p < 0.05$).

	Pampa Rocha[a]	Undefined crossbred[a]	Unknown breed[a]	LWxL[a]	LxLWxDxSHR[a]	Yorkshire[a]
Class (2.88, 0.088, 0.04532)[2]	21.25 ± 2.17 AB 1.461 ± 0.067 B 0.481 ± 0.009AB	14.75 ± 0.48C 1.416 ± 0.043 B 0.527 ± 0.018 A	12.50 ± 2.06C 1.404 ± 0.010 B 0.575 ± 0.046 A	16.25 ± 0.25 BC 1.139 ± 0.040C 0.408 ± 0.013 B	25.50 ± 0.65 A 1.558 ± 0.042 B 0.482 ± 0.016 AB	27.50 ± 1.66 A 1.873 ± 0.042 A 0.566 ± 0.012 A
Order (4.819, 0.123, 0.03437)[2]	41.75 ± 3.71 A 1.943 ± 0.083 BC 0.522 ± 0.013 B	25.5 ± 1.190 B 1.966 ± 0.052 BC 0.607 ± 0.011 A	23.25 ± 3.47 B 1.789 ± 0.060C 0.578 ± 0.025 AB	27.25 ± 0.48 B 1.850 ± 0.064C 0.560 ± 0.017 AB	43.00 ± 1.08 A 2.193 ± 0.062 AB 0.584 ± 0.019 AB	46.25 ± 2.50 A 2.281 ± 0.043 A 0.596 ± 0.012 AB
Family (7.105, 0.151, 0.03345)[2]	66.50 ± 4.87 A 2.937 ± 0.117 A 0.700 ± 0.016 A	38.50 ± 2.06 B 2.327 ± 0.076 CD 0.638 ± 0.015 AB	37.00 ± 5.05 B 2.126 ± 0.073 D 0.595 ± 0.023 B	42.00 ± 0.63 B 2.588 ± 0.052 BC 0.691 ± 0.016 A	67.00 ± 2.52 A 2.961 ± 0.075 A 0.705 ± 0.020 A	72.00 ± 3.94 A 2.750 ± 0.033 AB 0.644 ± 0.005 AB
Genus (15.76, 0.157, 0.02478)[2]	147.75 ± 8.10 A 3.650 ± 0.064 A 0.731 ± 0.007 A	90.00 ± 6.01 B 3.228 ± 0.073 B 0.719 ± 0.014 A	93.75 ± 12.02 B 2.788 ± 0.109C 0.618 ± 0.016 B	80.00 ± 3.49 B 3.037 ± 0.088 BC 0.693 ± 0.015 A	155.75 ± 3.40 A 3.754 ± 0.043 A 0.744 ± 0.011 A	166.75 ± 10.14 A 3.773 ± 0.079 A 0.738 ± 0.008 A

2 Residual standard errors for N, H' and J', respectively, are shown in parenthesis.
[a] Mean ± SE.

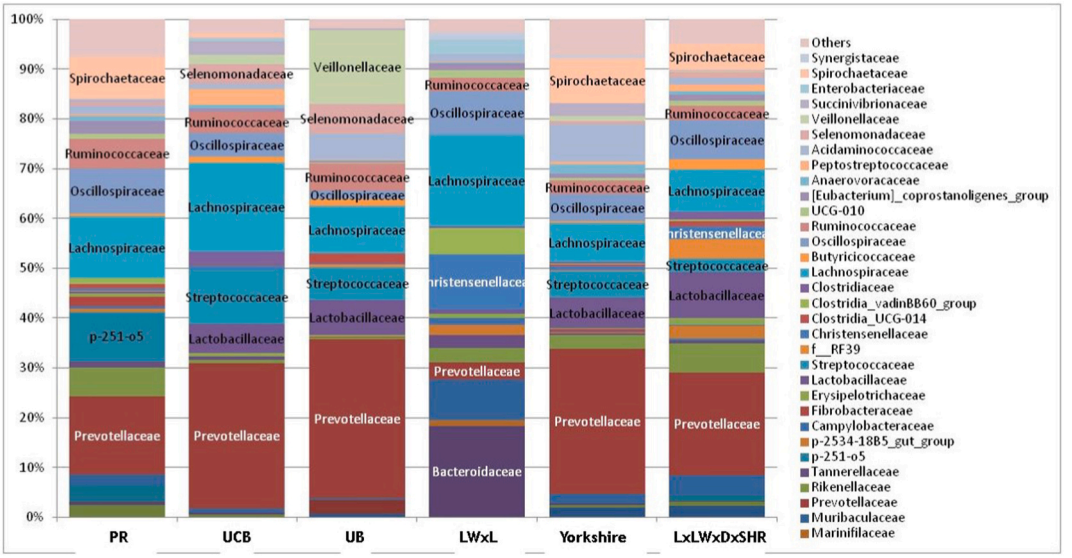

Fig. 3. Taxonomic composition and relative abundance of the fecal microbiota of different pigs analyzed at the Family level. Only the most abundant taxa are labeled in the chart. PR: Pampa Rocha; UCB: undefined crossbred; UB: unknown breed; LWxL: Large White x Landrace; Yorkshire and LxLWxDxSHR: Landrace x Large White x Duroc x Synthetic Hybrid Boar.

3.2. Diversity of fecal microbiomes and comparison of population structures of different pigs

A total of 323 genera were observed in at least one sample, of which 202 were present in at least one PR sample. The average number of observed genera in PR samples was 147.75 (Table 2), which was significantly higher than the number observed in samples from the undefined crossbred (UCB), the unknown breed (UB) and LWxL. Samples from Yorkshire and Landrace x Large White hybrid sows sired with Duroc x synthetic hybrid boar (LxLWxDxSHR) also presented a high number of genera (155.75 and 166.75 on average, respectively), which is consistent with their corresponding diversity indexes (Table 2).

In relation to diversity parameters at the family and genus levels, PR samples, together with Yorkshire and LxLWxDxSHR, harbored the most diverse communities. However, at the highest taxonomic levels studied (Class and Order), only Yorkshire consistently had the highest index values (Table 2). Samples from UCB, UB and LWxL showed the lowest index values at all taxonomic levels, with the exception of evenness index which often did not show significant differences between datasets in some taxonomic levels.

The structure of fecal microbiomes of each pig datasetanalyzed were markedly distinct at every taxonomic level examined. MRPP

results show that PR populations had an observed delta lower than expected delta at all taxonomic levels (Table 3). This indicates that differences observed within PR samples were lower than those observed between our PR samples and samples from other pigs. Populations of UB samples were the only ones with deltas higher than expected deltas at all taxonomic levels. This is consistent with NMDS scatterplots, which show the four UB samples forming a more dispersed cluster than those formed with samples of other datasets (Fig. 4).

Two families were significantly associated with PR fecal microbiomes, Paludibacteraceae and p-251-o5, both from the Bacteroidales order (Table 4). Significantly, the family p-251-o5, which is an uncultured family of Bacteroidales, has an average of 9.88% of the total population in PR samples and is present in all 4 samples of PR analyzed. We could not identify an order particularly associated with PR samples. However, Methanobacteriales, especially the family Methanobacteraceae, was significantly absent only in PR samples.

At the genus level, *Paraeggerthella*, *Papillibacter, Cellulosilyticum* and an unidentified genus of the *Ruminococcaceae* family were found by indicspecies analysis as OTUs significantly associated with PR fecal microbiomes (Table 4).

4. Discussion

Intestinal microbiota plays an important role in animal growth and health status. Its composition is affected by diverse factors, which can be intrinsic such as genetic background, or of environmental origin, like diet or sanitary conditions. The markedly distinct fecal microbiota of each dataset analyzed can be the result of a combination of these factors. However, in order to minimize the impact of confounding variables, we selected only samples obtained from adult females, and only those that served as controls in experiments with antibiotic treatments or pathogenic challenges, for example.

Differences in fecal microbiota have been observed in several previous studies comparing the microbiomes of different pig breeds [10]. This also has been observed in breeds of other animals, including ruminants such as cows [19] and non-ruminants like dogs [20] and pigs [10]. Although this result is expected, we also found one dataset among those analyzed, with a within-group variation greater than between-group variation. This suggests that the structure of fecal microbial communities in each breed is not always characteristic and distinct compared with others.

Community compositions may differ, but differences in diversity indexes are not so expected, as two different communities could have similar diversity index scores. Microbiome diversity plays a major role in the well-being of animals [14]. Larger animals can be considered as ecosystems, in which case the microbiome diversity gives resilience to the system in the same way that it is conferred to ecosystems in other environments. Even though the microbial composition at different gut locations may differ significantly [9], fecal microbiome has been used as a proxy for overall gut microbiome, mainly for practical reasons [21]. It is plausible that community's parameters such as diversity and evenness are to some degree correlated throughout the gut, and therefore useful for comparison purposes. A relatively high diversity in fecal samples could be assumed to correlate with the diversity that can be found in different gut locations, especially in the lower intestinal tract, and therefore to indicate a more resilient and healthy system compared to one in which lower fecal diversity values are obtained.

The p-251-o5 group is a whole family that consists of sequences from uncultured bacteria, that belongs to Bacteroidales order. The uncultured nature of the group determines that it is troublesome to predict or infer functionality of its members, even more so than when working with sequences derived from cultured bacteria. However, the studies and samples where this group has been significantly abundant could give a clue about its role. The p-251-o5 family has been among the most abundant families in the fecal microbiome of horses [22,23] and the rumen of cows and buffalos [24]. Moreover, a study that compared the fecal microbiome of 18 carnivores and 13 herbivores species from a zoo found that p-251-o5 was associated to animal of the Perissodacyla order, specially to Grévy's zebra (*Equus grevyi*) a congener of horses [25]. The significant presence of this group in the fecal microbiome of PR samples, could be an indication of a more fiber-oriented diet in these pigs.

The other family significantly associated with the PR samples was Paludibacteraceae, which consists of a single genus, *Paludibacter*. A non-identified member of this family has been considered as the OTU that better predicted immunity response to Influenza A vaccine in pigs, being significantly more abundant in the high-responsive group [26]. This could be indicative of a certain role in immunomodulation. Paludibacteraceae has also been identified to be more abundant in pigs under a low-protein diet supplemented with branched-chain amino acids [27]. This diet seems to increase the protein metabolism and to promote the fatty acid synthesis pathways. This also suggests, as well as with p-251-05, a correlation of this family of bacteria with a fiber oriented diet with a lower protein intake.

About the genus-level-OTUs identified by the indicspecies analysis as strongly associated to the PR fecal microbiomes, few studies

Table 3
Observed delta values from the MRPP analysis comparing the population structure of the fecal microbiome of different pig breeds at different taxonomic levels. Observed delta values lower than the expected delta are marked with an asterisk. Significance of delta was 0.001 for all taxonomic levels. Chance-corrected within-group agreement (A) was 0.3046, 0.2805, 0.3505 and 0.3571, for Class, Order, Family and Genus, respectively.

	Pampa Rocha	Undefined crossbred	Unknown breed	LWxL	LxLWxDxSHR	Yorkshire	Global	Expected delta
Class	**11.08***	**24.72***	34.44	**15.16***	**14.03***	**12.68***	18.69*	26.87
Order	**11.09***	**21.09***	32.09	**6.06***	**12.59***	**12.68***	17.74*	24.65
Family	**15.15***	**23.71***	31.22	**19.23***	**14.77***	**12.83***	19.49*	30
Genus	**11.65***	**22.55***	29.97	**20.17***	**14***	**11.69***	18.34*	28.52

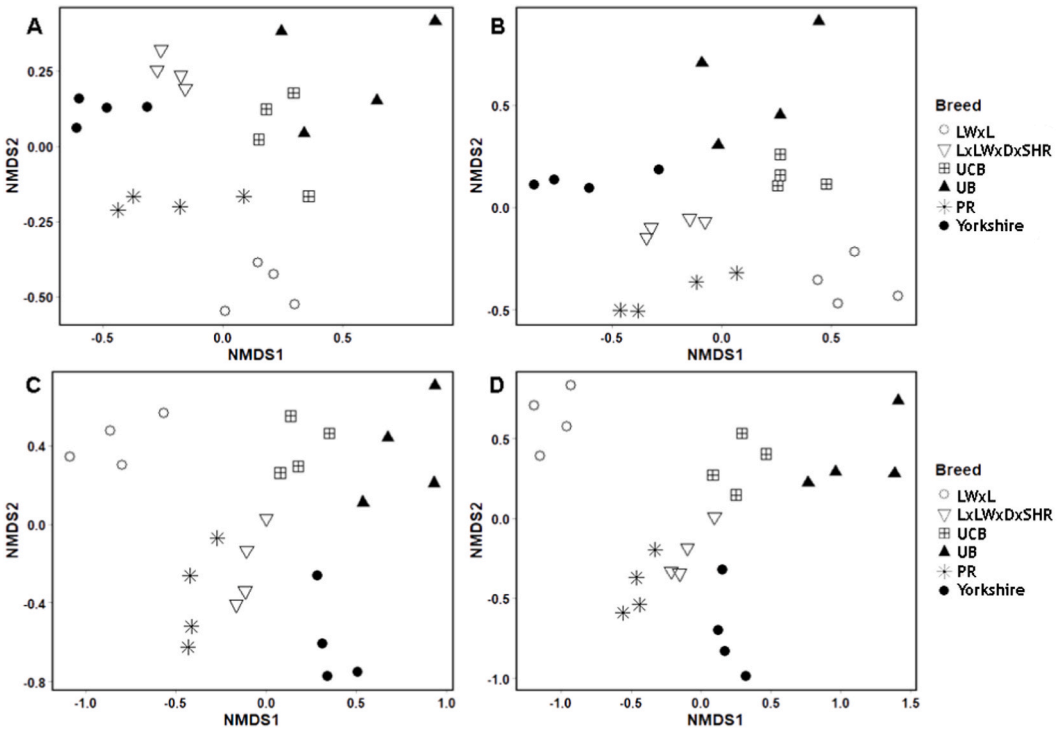

Fig. 4. Scatterplots of the NMDS analysis at different taxonomic levels showing the similarities or differences between the fecal microbiota of each individual analyzed. A: analysis at the Class level; B: analysis at the order level; C: analysis at the family level; D: analysis at the genus level. LWxL: Large White x Landrace; LxLWxDxSHR: Landrace x Large White x Duroc x Synthetic Hybrid Boar; UCB: undefined crossbred; UB: unknown breed; UB: unknown breed; PR: Pampa Rocha and Yorkshire.

Table 4
List of OTUs at different taxonomic levels identified by the indicspecies analysis as significantly associated to the PR microbiome either because of its presence or absence specifically in PR samples. The A value indicates to which degree the taxon occurs only in the breed analyzed (or is absent from it). The B value indicates to which degree the taxon occurs always in the breed analyzed (or is always absent from it). The stat value is the average between A and B.

	A	B	stat	p
Presence in PR				
Family				
p-251-o5	0.9621	1.000	0.981	0.001
Paludibacteraceae	0.9845	0.750	0.859	0.010
Genus				
Ruminococcaceae	0.7902	1.000	0.889	0.003
Paraeggerthella	0.9141	0.750	0.828	0.014
Papillibacter	0.8354	0.750	0.792	0.008
Cellulosilyticum	0.8545	0.750	0.801	0.026
Absence in PR				
Order				
Methanobacteriales	1.00	0.95	0.975	0.002
Family				
Methanobacteraceae	1.00	0.95	0.975	0.005

were found showing a potential role of these genera in digestive functions, particularly regarding fiber-rich diets.

Ruminococcaceae is a family composed of acid-sensitive fiber-degrading bacteria which has been shown to be associated with a better prognosis of pigs infected with PRRSV or PCV2 viruses [28]. At the same time, this better prognosis was associated also with a decrease of Methanobacteriaceae family which was particularly absent in PR fecal microbiomes. Bacteria of the genus *Papillibacter* have been described as unable to use carbohydrates, organic acids or alcohols as energy source; however they degrade aromatic compounds [29], particularly cinnamate, which is present in cinnamon. Cows subject to ruminal-acidosis-inducing diets have been found to have significantly less abundant *Papillibacter* populations in the rumen compared to the control group [30]. Bacteria of the genus *Cellulosilyticum*, as suggested by its name, have the capacity to degrade cellobiose anaerobically but are unable to ferment glucose. The genus has a clear relationship with fiber degrading capacities and the first isolated member was originally obtained from

the rumen of domestic Yaks (*Bos grunniens*) [31].

Our data strongly suggests that diet constitutes one of the most influential factors determining fecal microbiome differences, a fact supported by previous researches [27,32]. Although genetic differences of pig breeds must impact the microbiome composition, other assays where all factors but breed are equivalent should be performed to unambiguously determine the extent of such influence. However, the suitability of certain breeds to be fed and grown under determined conditions could also depend on the genetic background. Thus, the question of whether the differences observed arise as a consequence of dietary differences or due to genetic background remains open.

The distinctiveness of the fecal microbiome of the PR pigs, particularly due to the taxa significantly associated to PR pigs, can hold promise as a useful resource. This community can be used, for example, as microbiome donors in fecal transplantation either for industry or research purposes [33].

This is the first description of a microbiome belonging to PR pigs. We sampled four adult female PR pigs from a single farm and compared the microbiomes obtained with data from public databases. This strategy resulted in a series of limitations that should be considered. The extent to which breed and other uncontrolled factors such as growing systems or diet have shaped fecal microbiomes would remain unknown so far. Although it was restricted to fecal samples, it is important to note that the communities evaluated were significantly different from those present in the fecal samples of other pigs, moreover some OTUs were associated with the microbiome of PR pigs. This highlights the importance of evaluating microbiomes together with the animal genetic background when assessing the importance of the conservation of genetic resources.

Author contribution statement

Gastón Azziz: Conceived and designed the experiments; analyzed and interpreted the data; contributed reagents, materials, analysis tools or data; wrote the paper.

Matías Giménez: Conceived and designed the experiments; analyzed and interpreted the data; wrote the paper.

Nandy Espino: Performed the experiments; wrote the paper. Cecilia Carballo: Performed the experiments; wrote the paper.

Nelson Barloco: Conceived and designed the experiments; performed the experiments Silvia Batista: Conceived and designed the experiments; analyzed and interpreted the data; wrote the paper.

Data availability statement

Data associated with this study has been deposited at Sequence Read Archive (SRA) of the NCBI under the accession number BioProject ID PRJNA870492.

Additional information

Supplementary content related to this article has been published online at [URL].

Ethics approval

The present research was carried out following the recommendations of the CNEA, Uruguay (www.cnea.gub.uy) according to the protocol sent to the Comité de Ética en el Uso de Animales (Ethics Committee on Animal Use, CHEA) of the Facultad de Agronomía #1619.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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