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RESEARCH ARTICLE

Domestication shapes the pig gut microbiome and immune traits from the scale of lineage to population

Sahana Kuthyar[1] | Jessica Diaz[1] | Fabiola Avalos-Villatoro[1] | Christian Maltecca[2] | Francesco Tiezzi[3] | Robert R. Dunn[4] | Aspen T. Reese[1,5]

[1]Division of Biological Sciences, University of California San Diego, La Jolla, California, USA

[2]Department of Animal Science, North Carolina State University, Raleigh, North Carolina, USA

[3]Department of Agriculture, Food, Environment and Forestry, University of Florence, Florence, Italy

[4]Department of Applied Ecology, North Carolina State University, Raleigh, North Carolina, USA

[5]Center for Microbiome Innovation, University of California San Diego, La Jolla, California, USA

Correspondence
Sahana Kuthyar, Division of Biological Sciences, University of California San Diego, La Jolla, CA, USA.
Email: skuthyar@ucsd.edu

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Abstract

Animal ecology and evolution have long been known to shape host physiology, but more recently, the gut microbiome has been identified as a mediator between animal ecology and evolution and health. The gut microbiome has been shown to differ between wild and domestic animals, but the role of these differences for domestic animal evolution remains unknown. Gut microbiome responses to new animal genotypes and local environmental change during domestication may promote specific host phenotypes that are adaptive (or not) to the domestic environment. Because the gut microbiome supports host immune function, understanding the effects of animal ecology and evolution on the gut microbiome and immune phenotypes is critical. We investigated how domestication affects the gut microbiome and host immune state in multiple pig populations across five domestication contexts representing domestication status and current living conditions: free-ranging wild, captive wild, free-ranging domestic, captive domestic in research or industrial settings. We observed that domestication context explained much of the variation in gut microbiome composition, pathogen abundances and immune markers, yet the main differences in the repertoire of metabolic genes found in the gut microbiome were between the wild and domestic genetic lineages. We also documented population-level effects within domestication contexts, demonstrating that fine scale environmental variation also shaped host and microbe features. Our findings highlight that understanding which gut microbiome and immune traits respond to host genetic lineage and/or scales of local ecology could inform targeted interventions that manipulate the gut microbiome to achieve beneficial health outcomes.

KEYWORDS
animal domestication, gut microbiome, immunity, pigs (*Sus scrofa*)

1 | INTRODUCTION

The gut microbiomes of domestic animals, like those of animals more generally, respond to both host evolution and ecology and, in turn, contribute to a wide range of host physiological processes (Donohoe et al., 2011; Garrett, 2020; McLaren & Callahan, 2020). During domestication, animals undergo artificial and natural selection for morphological and behavioural traits, which allow them to live near and with humans and cope with human settlements' dietary and ecological conditions (Wilkins et al., 2014). Apart from these recognizable changes in domesticated animals, however, are more inconspicuous but potentially equally consequential changes in their gut microbiomes (Alessandri et al., 2019; Metcalf et al., 2017; Reese et al., 2021). Such changes could be due to the effects of changing

host genetics or ascribed to differences in ecological conditions under which domestic animals are kept, whether concerning animal density, animal location (e.g. climate or habitat effects), medical interventions (such as antibiotic use) or other factors (McClure, 2013).

Domestic pigs are a particularly relevant and tractable model in which to investigate host and gut microbial responses to domestication because they are frequently studied (Chen et al., 2013; Groenen et al., 2012; Paudel et al., 2013), numerous and widespread, and raised in a wide range of conditions varying from free range, semi-natural conditions with little medical intervention to high density indoor husbandry with frequent interventions (including antibiotic use). The direct descendants of the wild progenitors of domestic pigs are still extant and, along with feral pigs, present in many parts of the world. The wild boar, *Sus scrofa*, first originated in Southeast Asia and diverged into European and Asian wild boar populations (Groenen, 2016; Groenen et al., 2012). Domestic pigs then originated from at least two domestication centres: western Asia in 8500 BC (Conolly et al., 2011; Ervynck et al., 2001) and China in 6500 BC (Cucchi et al., 2011; Jing & Flad, 2002), though recent genetic studies hint at additional regions (Larson et al., 2005). Most domestic pigs today come from a hybrid lineage that resulted when Chinese pigs were introduced to Europe in the 18th and 19th centuries (Giuffra et al., 2000). Domestic and wild pigs have been shown to harbour different gut microbiomes in terms of composition and functional potential (Chen et al., 2021; Correa-Fiz et al., 2019; Fenske et al., 2020), and within domestic pigs, different breeds can also have distinct microbial compositions (Bergamaschi et al., 2020; Xiao et al., 2016). These patterns, along with the evidence of partial microbiome heritability in pigs (Bergamaschi et al., 2020; Camarinha-Silva et al., 2017; Cheng et al., 2018; Crespo-Piazuelo et al., 2019), suggest that pig genetics exert some control over the gut microbiome.

Independent of host genotype, husbandry practices can cause differences in pig gut microbiomes (Frese et al., 2015; Looft et al., 2012) and lead to phenotypic changes. Dietary differences between wild and domestic pigs (Leus & Macdonald, 1997; Nafikov & Beitz, 2007; Schley & Roper, 2003; Ushida et al., 2016) as well as within domestic pigs (Mutua et al., 2012; Rozeboom et al., 2005) have been shown to alter microbial composition, diversity and metabolic gene composition (Huang et al., 2020). Additionally, antibiotic treatment can alter the composition and function of the pig gut microbiome (Allen et al., 2011; Looft et al., 2012; Looft et al., 2014). Such treatments differ between pigs living in domestic and wild settings and also vary among domestic settings (e.g. no antibiotics, therapeutic doses for illness or subtherapeutic use in feed). Variation in pig gut microbiomes may also arise from different patterns of contact with natural substrates and other animals as microbial transmission occurs through vertical inheritance during birth (Mach et al., 2015), pig interactions (Cadenas-Fernández et al., 2019; Kukielka et al., 2016) and contact with the physical environment (Mulder et al., 2011; Vo et al., 2017). Finally, living in high group densities as is common in industrial farm settings may increase opportunities for pig-to-pig transmission of pathogens or antibiotic-resistant strains and for horizontal gene transfer (Corn et al., 2009; Zhu et al., 2013). Pigs in these settings also have limited exposure to commensal microbes from the natural environment.

By changing gut microbial composition, domestication also likely alters host–microbe interactions. Domestication could result in increased host–microbe mutualism, promoting host adaptation. Because gut microbes evolve more rapidly compared to the host genome (Zilber-Rosenberg & Rosenberg, 2008) and their functions can be altered through acquisitions of novel strains or genes (e.g. Boto, 2014; Groussin et al., 2021; Maeusli et al., 2020), changes as a result of either host genotype or local ecology could contribute to the adaptation of pigs to domestication and domestic environments even before host evolution occurs (Rampelli et al., 2021). For example, the introduction of new diets could promote the selection of microbial genes that break down the new substrates (Kohl & Dearing, 2016; H. Li et al., 2018), allowing domestic pigs to digest those diets. Alternatively, the changes in gut microbiomes associated with domestication can lead to mismatches between the animal and their microbes with negative consequences for host, microbes or both host and microbes. Because domestic animal management is typically aimed at minimizing exposure to pathogens, this can minimize exposure to commensal microbes. Effects of altered microbial exposure can manifest in multiple ways, including but not limited to a decrease in microbial diversity, distinct microbial compositions and the loss or gain of specific microbes or functions (Cox et al., 2014; Kuthyar et al., 2022; Watson et al., 2022; Mulder et al., 2011).

Of the many functions the gut microbiome provides for the host, training and supporting immune function is of particular interest for domestic pigs. Domestic pigs are increasingly identified as sources and amplifiers of zoonotic disease (Holt et al., 2016; Morand et al., 2014) and commonly harbour antibiotic-resistant bacteria (Chen et al., 2021; Fenske et al., 2020). Importantly, high burdens of disease may lead to increased immune stimulation, resulting in reduced pig performance and increased economic costs (Cornelison et al., 2018; Huntley et al., 2018). Because immune function has been shown to be partly influenced by the gut microbiome (Hold & Hansen, 2019), investigating if gut microbiome changes associated with domestication also result in immune ramifications is vital. As immune traits can be highly plastic under different microbial exposures (Rampelli et al., 2021), altered microbial exposures experienced by domesticated animals may lead to reduced abilities to resist pathogens compared to their wild counterparts (Bisgaard et al., 2014; Clapperton et al., 2009). Of course, it could be that the domestic gut microbiome, as compared to the wild one, may better resist pathogens in contemporary agricultural settings where animals live in high density and are exposed to novel microbes.

Here, we investigated how domestication affects the gut microbiome and host immune state in wild and domestic pigs living across a variety of environments. To do so, we characterized gut microbial composition and potential microbial function from pigs living across multiple domestication contexts. We sampled multiple populations of free-ranging wild pigs (*Sus scrofa*), captive wild pigs of two species (*Potamochoerus porcus* (red river hogs) and *Sus cebifrons* (Visayan warty pigs)) and free-ranging and captive domestic pigs

(*Sus scrofa domesticus*). We also measured select immune features from a subset of these pigs and tested for associations with the gut microbiome. Differences in pathogen loads, while directly relevant to disease state, could reflect variation in exposure or immunity. By measuring immune traits, including pro- and anti-inflammatory cytokines and IgA, which are known to be stimulated by the microbiome (Peterson et al., 2007; Schirmer et al., 2016), it is possible to directly assess the investment in immunity made by pigs under differing conditions and harbouring differing gut microbiomes.

2 | METHODS

2.1 | Sample collection

While there is a global signal of domestication in mammalian gut microbiomes (Reese et al., 2021), domestic animals are raised under diverse husbandry techniques that will impact the gut microbiome distinctly. Previous studies have typically described microbial differences between domestic and wild pigs using only a single population of pigs in each domestication context (e.g. Fenske et al., 2020; Huang et al., 2020; Ushida et al., 2016; Wei et al., 2022 (but see exceptions Chen et al., 2021; Correa-Fiz et al., 2019)). This approach makes it difficult to dissociate the relative contributions of population-specific factors versus domestication to the pig gut microbiome. To detect a more robust signal of domestication effects, we collected 241 faecal samples from four different species of pigs (*S. scrofa*, *S. scrofa domesticus*, *S. cebifrons* and *P. porcus*) in multiple populations within each of the five domestication contexts (Table 1). Free-ranging wild populations were from Alabama (collected in 2017), Georgia (2016) and California (2021); these samples were collected in partnership with Auburn University, the University of Georgia and the USDA APHIS, respectively. Free-ranging domestic populations were from two farms in California (2021) and one farm in Vermont (2018); captive wild populations were from the Brookfield Zoo (2021), the Cincinnati Zoo (2021) and the San Diego Zoo (2021); captive domestic research populations were from University of California Davis (2021), California Polytechnic State University at San Luis Obispo (2021) and Premier BioSource, a research institution in California (2021); and captive domestic industrial populations were from one commercial farm in North Carolina (2021) and the North Carolina State University Swine Evaluation Station (2021).

Within each population, we collected samples from a minimum of 3 individuals and a maximum of 51 individuals. All individuals within a given population were of the same pig lineage (wild vs. domestic) and species (except for *P. porcus* and *S. cebifrons* at the San Diego Zoo) and experienced the same local ecology. We attempted to collect samples from both female and male pigs to mitigate sex differences, but for some wild collections, sex identification was not possible. Only adult pigs were sampled to limit the temporary effect of weaning on the gut microbiome (Guevarra et al., 2018).

Fresh faecal samples were collected for all individuals. For all populations except industrial ones, faecal samples were collected

TABLE 1 Sampling scheme for pig populations across domestication contexts.

Domestication context	Genetic lineage	Species	Number of populations	Population locations	Samples	Outside exposure	Antibiotic treatment
Free-ranging wild (feral)	Wild	*Sus scrofa*	3	California, Georgia, Alabama	Faecal and blood	Yes	No
Free-ranging domestic	Domestic	*Sus scrofa domesticus*	3	California (1), California (2), Vermont	Faecal	Yes	Yes and no
Captive wild	Wild	*Potamochoerus porcus*, *Sus cebifrons*	3	Cincinnati Zoo, Brookfield Zoo, San Diego Zoo	Faecal and blood	Yes and no	Yes
Captive domestic (research)	Domestic	*Sus scrofa domesticus*	3	University of California Davis (UC Davis), California Polytechnic State University at San Luis Obispo (Cal Poly), Premier BioSource	Faecal and blood	Yes and no	Yes
Captive domestic (industrial)	Domestic	*Sus scrofa domesticus*	2	North Carolina (1) and North Carolina (2)	Faecal and blood	No	Yes and no

from the ground within minutes to a few hours (<6) after defecation and placed in 2 mL tubes and stored on ice. For industrial populations, faecal samples were collected from the anus after rectal massage using an eight-inch nylon swab, then placed in sterile 5 mL tubes and stored on dry ice. All faecal samples were then moved to −80°C until shipment then stored at −80°C upon receipt at University of California San Diego. Across all populations, none of the individuals presented any clinical manifestations, such as diarrhoea, and stool consistency in faecal samples was normal.

We also collected blood samples from a subset of these individuals (see Table S1). Because blood samples were collected based on animal care guidance and equipment availability at each location, plasma samples were collected in some populations ($N = 39$ individuals across captive wild San Diego Zoo, free-ranging wild Alabama, industrial North Carolina (1)) whereas dried blood spots were collected in others ($N = 50$ individuals across free-ranging wild Alabama, captive wild San Diego Zoo, research UC Davis, research Premier BioSource populations). For plasma samples, 10 mL of blood was collected in tubes (Becton Dickinson Vacutainer Systems) by puncturing the jugular vein (0.8 mm × 32 mm needles, Eclipse, Becton Dickinson Vacutainer Systems). Samples were then centrifuged at 1500 × g at 4°C for 15 min (5811F, Eppendorf, Hamburg, Germany) and stored at −80°C until shipment. For dried blood spot samples, we followed the protocols in (McDade et al., 2012), with the exception that pig ears were punctured. Following shipment, blood samples were stored at −20°C at University of California San Diego. Collection of all faecal samples was non-invasive and thus did not need institutional approval. Blood sample collection was approved by the Institutional Animal Care and Use Committees (IACUC) for the free-ranging wild population at Auburn University (IACUC approval PRN 2017-3143 and PRN 2020-3779), the captive wild population at San Diego Zoo (IACUC approval 18-024 and 21-019) and the industrial population at North Carolina State University Swine Evaluation Station (IACUC approval 19–834). IACUC approval was not needed for blood sample collection for the research UC Davis population as animals were not transferred for this project. For samples collected from the industrial population at the commercial sow farm in North Carolina, USA, the Purdue University Animal Care and Use Committee approved all procedures involving live animals (Protocol #1912001990). Animal husbandry and use protocols were based on the Guide for the Care and Use of Agricultural Animals in Research and Teaching (Federation of Animal Science Societies, 2020).

2.2 | 16S rRNA gene sequencing and quantification

We extracted DNA from all faecal samples using the E.Z.N.A. Stool DNA Kit (Omega Bio-Tek; Norcross, GA) following manufacturer's instructions, except that we eluted DNA into 50μL of elution buffer. We amplified the target V4 region of the 16S rRNA gene using 515f/806r primers with Nextera overhangs and performed the reaction in KAPA HiFi HotStart ReadyMix (Roche). We then annealed index primers to the amplicon product, such that each sample was given a unique combination of primer barcodes through combinatorial Nextera i5 and i7 indexing. In addition to all biological samples, we also processed positive and negative DNA extraction and PCR controls. Indexed samples were then quantified, pooled in equimolar concentrations and cleaned with Kapa Pure Beads. Paired end 250 bp read sequencing was performed on a MiSeq500 at the University of California Davis DNA Technologies core. Samples were sequenced across three separate library runs, with different populations spread across the runs. We used the same positive control for all runs, and its sequencing output was consistent across runs.

All 16S rRNA gene sequencing processing was conducted using the Triton Shared Computing Cluster. Demultiplexed sequences were first trimmed with cutadapt (version 3.4 with Python 3.9.5). Using the dada2 R package (version 1.16.0 (Callahan et al., 2016)), sequences were quality filtered and dereplicated. After merging forward and reverse amplicon pairs, we assigned taxonomy according to the SILVA database version 138.1 (Quast et al., 2013) and removed contaminants from the extraction and PCR negative controls using the decontam R package (version 1.10.0; (Davis et al., 2018)). Amplicon sequence variants (ASVs) which mapped to chloroplasts, mitochondria, non-bacteria, and those not mapped at the phylum level, were also removed. The average number of reads was 36 757 (min = 1497, max = 123 328, standard deviation = 2609) with a total of 13 347 ASVs across biological samples. Sequences were rarefied with the phyloseq package (version 1.36.0 (McMurdie & Holmes, 2013)) to 12 000 reads for all statistical analyses. Raw reads are available through NCBI SRA (BioProject number PRJNA926635).

Total 16S rRNA gene copies were quantified in each sample on a Bio-Rad CFX-96 machine (Bio-Rad, Hercules, CA) and analysed using the CFX Maestro software. Extracted DNA was amplified using 515f/806r primers (515f: GTGCCAGCCGCGGTAA; 806r: GGACTACHVGGGTWTCTAAT) in Power SYBR Green PCR master mix (Applied Biosystems, Waltham, MA) as per the protocol in Reese et al. (2021). We calculated bacterial load (concentration of bacterial cells per gram of faeces), adjusting for weight of faecal material extracted, based on a standard curve created with the ZymoBIOMICS Microbial Community Standard (Zymo Research). An estimate of the absolute abundance of specific bacterial taxa was calculated by multiplying bacterial load with ASV relative abundance data.

2.3 | Metagenomic sequencing

To gain insight into the potential functions provided by the microbiome, we conducted metagenomic sequencing on 56 pigs from the larger faecal sample collection (Table S1). Metagenomic libraries were prepared with KAPA HyperPlus kits (Roche Diagnostics) and automated on EpMotion automated liquid handlers (Eppendorf). Libraries were prepared following manufacturer's instructions, except amplified libraries were pooled equi-volume and batch-cleaned with sparQ PureMag Beads (Quantabio). Two successive rounds of 0.8X

cleanings were performed, with each round including two ethanol washes. Sequencing was performed on the Illumina NovaSeq 6000 sequencing platform at the University of California San Diego Health Sciences Microbiome Core with paired-end 150 bp reads. Samples were sequenced across two library runs such that all samples from a given population were sequenced in the same run, but different populations were spread across all runs. We obtained approximately 393 GB of swine faecal metagenome data and achieved an average sequencing depth of 7.54 GB per sample.

All metagenomic read processing was conducted using the Triton Shared Computing Cluster (San Diego Supercomputer Center, 2022). Demultiplexed sequences were quality filtered using fastp (Chen et al., 2018), and reads that mapped to the pig genome (NCBI GCF_000003025_6_Sscrofa11_1_genomic.fa) were filtered out. Metagenomic reads were assembled and aligned to contigs using MegaHit (D. Li et al., 2015); unassembled reads were pooled and co-assembled using MegaHit again, and then merged with the previously assembled contigs. Prodigal software (Hyatt et al., 2010) was used to predict genes, and all complete genes were clustered at both protein and nucleotide levels at 95% using cd-hit (Fu et al., 2012). Dereplicated protein sequences were then aligned to the UniProt TrEMBL database, and taxonomic classification was assigned based on the lowest common ancestor using BASTA (Kahlke & Ralph, 2019). Raw reads are available at NCBI SRA (BioProject number PRJNA926638).

Dereplicated nucleotide sequences were annotated with the eggNOG 5.0 database (v2.1.7) using e-mapper (Huerta-Cepas et al., 2017) and the Virulence Factor Database (VFDB) using BLAST (Chen et al., 2005). The eggnog database annotated Kyoto Encyclopedia of Genes and Genomes (KEGG) orthologs, KEGG modules and carbohydrate-active enzyme (CAZy) genes. Salmon (Patro et al., 2017) and the *tximport* package (Soneson et al., 2015) in R were used to quantify and summarize gene level abundances of annotations.

2.4 | Pig immune phenotyping

We measured potential zoonotic pathogen burdens, faecal IgA concentration (an antibody marker of gut secretory immunity and barrier function (Brandtzaeg, 2013)), and concentrations of select circulating host cytokines (i.e. IL-6 [pro-inflammatory], IL-10 [anti-inflammatory] and TNF-α [pro-inflammatory]).

Abundances of potentially zoonotic pathogens were calculated by quantifying total 16S rRNA gene copies and combining that with 16S rRNA amplicon relative abundance data for relevant taxa. Bacterial pathogens were chosen initially based on a published list from the Center for Food Security and Public Health at Iowa State University (https://www.cfsph.iastate.edu/Assets/zoonotic-diseases-of-swine-table.pdf). Of the nine zoonotic bacterial pathogens included in the list, we observed six pathogens in our samples (not observed were *Yersinia enterocolitica*, *Leptospira* spp. and *Brucella suis*). Because the list from the Center for Food Security and Public Health did not include all known zoonotic bacterial pathogens typically found in pigs, we also quantified levels of *Mycoplasma* spp. (Fano et al., 2005), *Shigella* spp. (GuiBo et al., 2015) and *Listeria monocytogenes* (Kanuganti et al., 2002).

We measured faecal IgA concentrations from a subset of individuals ($N = 79$; Table S1) using the Bethyl Porcine Faecal IgA kit (Bethyl Laboratories). Faecal samples were first diluted 200-fold following (Seo et al., 2016), and samples were read in duplicate on a Cytation 5 machine at 450 nM. Concentrations were calculated based on the standard curve and average absorbance values. The detection limit for the faecal IgA assay was 0 ng/mL.

We measured IL-6, IL-10 and TNF-α concentrations using both dried blood spots and plasma from a subset of pigs ($N = 89$ individuals total; Table S1). Samples below the detection limits for each assay (IL-6, 20.5 pg/mL; IL-10, 29.7 pg/mL; TNF-α, 25.3 pg/mL) were not included in analyses. For dried blood spots, we followed the elution protocol in Grüner et al. (2015). For both types of blood, we quantified cytokine concentrations using the Millipore Sigma Porcine Cytokine/Chemokine multiplex analyte kit (Millipore Sigma) following manufacturer's instructions on a Luminex system. For a subset of individuals ($N = 18$ from captive wild San Diego Zoo and free-ranging wild Alabama), we collected both dried blood spots and plasma; using these samples, we first confirmed no significant differences between the two methods (paired T test, $p = 0.45$), so following results are reported together.

2.5 | Host SNP analysis

We genotyped a subset of pigs (48 individuals; Table S1) using the Porcine SNP60 Beadchip (Illumina) at the University of California San Diego IGM Genomics Center. These individuals were all also analysed for 16S rRNA gene and shotgun metagenomic sequencing. The data were analysed in GenomeStudio (Illumina); a quality control process was first performed where genotypes were filtered based on <95% call rate, and SNPs were clustered, with those with a call frequency of zero filtered out. After this filtering, the data set was composed of 59 319 SNPs. The PLINK plug-in (Purcell et al., 2007) was used to recode the data to vcf format, and the SNPRelate and gdsfmt (Zheng et al., 2012) packages in R were used to generate eigenvectors and eigenvalues to calculate a genetic covariance matrix based on principal component analysis (Figure S1).

2.6 | Statistical analysis

All statistical analyses were carried out in R (R Core Team (2020), version 3.3).

2.6.1 | Microbial composition

We first sought to understand how microbial composition and gene content differ across the scales of genetic lineage, domestication

contexts and population. For microbial composition, we calculated Bray-Curtis dissimilarities using phyloseq (version 1.36.0 (McMurdie & Holmes, 2013)) and visualized with Principal Coordinates Analysis (PCoA) ordinations. We employed multivariate Permanovas implemented with the adonis2 function in the vegan package (version 2.5–7 (Oksanen et al., 2009)) to test which host features were associated with differences in microbial community composition and functional potential. When analysing all pigs, the Permanova model based on Bray-Curtis distances included the following variables: domestication context, species or lineage, and population. When analysing domestic pigs, the Permanova model included outdoor access, antibiotic use and population. Using the *vst* and *plotPCA* functions in the DESeq2 package (Love et al., 2014), we generated eigenvectors and eigenvalues and plotted principal components analysis (PCA) ordinations of KEGG orthologs. We used the betadisper function in the vegan package to characterize dispersion within variables. Further, we performed Mantel tests comparing microbial compositional and KEGG functional distance matrices to determine if there were similar clustering patterns. For the subset of individuals with 16S rRNA gene sequencing, metagenomic and SNP data, we used Mantel tests to test for a correlation between pig genetic variation (based on SNP profiles) and variation in microbial community composition or functional potential.

2.6.2 | Microbial diversity and bacterial load

For both microbial diversity (Shannon and richness diversity metrics calculated using phyloseq) and bacterial load, we utilized generalized linear mixed effects models implemented with *lme4* within the *lmer* package (Boeck et al., 2011) to assess the impacts of domestication context, pig lineage and ecological factors such as exposure to outdoors and antibiotic, including these as fixed effects and population as a random effect. To test the hypothesis that microbial features could predict pig immune state, we utilized generalized linear mixed effects models for faecal IgA concentrations with PCoA Axis 1, PCoA Axis 2, microbial diversity and bacterial load as fixed effects and population as a random effect. The same model specifications were also used for each cytokine concentration as the dependent variable. We then ran type II ANOVAs on all linear mixed effects models to assess if predictor variables were significant. The distribution of residuals of all outcome variables was inspected using a normal quantile plot to determine normality. To better understand how domestication context groups differed from each other in terms of microbial diversity, bacterial load and gene abundances, we also utilized two-sample Wilcoxon rank sum tests with Benjamini-Hochberg (BH) corrections.

2.6.3 | Differential abundance of microbial taxa and genes

We tested for differential abundance of both microbial taxa and genes between wild and domestic pigs and among domestic pigs. We used analysis of composition of microbiomes with bias correction (ANCOM-BC) through the ANCOMBC package (version 1.2.2; (Lin & Peddada, 2020)) to test for differential relative abundance of microbial families, with a cutoff of log-fold change above 1 or below −1. To test for gene differential abundance, we utilized DESeq2with BH corrections for normalized gene counts of KEGG modules and orthologs and virulence factors (adjusted $p < 0.001$). We also tested for KEGG pathway enrichment using the enricher function in the clusterProfiler package (Yu et al., 2012) in the differentially abundant KEGG orthologs between wild and domestic pigs as well as across domestic pigs. We normalized gene counts to test for differences in overall CAZy genes and virulence factor abundances across domestication contexts.

2.6.4 | Immune trait analyses

To understand immune trait variation across domestication contexts, we used Kruskal–Wallis and two-sample Wilcoxon rank sum (with BH corrections) tests to examine differences in pathogen abundance, faecal IgA concentrations and cytokine concentrations. We ran Spearman's rank correlation analyses to test for any associations between faecal IgA concentrations, microbial diversity, bacterial load, total pathogen abundance and each cytokine concentration. We utilized nested ANOVAs to understand the effect of population within domestication contexts on faecal IgA concentrations. We also used the *envfit* function in the vegan package to calculate the association between immune features (faecal IgA, IL-6, IL-10 and TNF-α concentrations) and overall microbiome composition.

3 | RESULTS

3.1 | Gut microbial composition and diversity

Both host evolutionary history and local ecology shaped gut microbial composition in pigs (Figure 1a). Domestication context (PERMANOVA, F-statistic = 17.99, $R^2 = 0.24$, $\omega^2 = 0.27$, $p < 0.001$) explained the most variation in microbial composition, followed by population (F-statistic = 3.74, $R^2 = 0.156$, $\omega^2 = 0.17$, $p < 0.001$) and pig species (F-statistic = 2.72, $R^2 = 0.018$, $\omega^2 = 0.02$, $p < 0.001$). Because we had a small sample size for some pig species, we also ran the same model with pig lineage (domestic vs. wild) rather than pig species. The results were similar: domestication context (F-statistic = 19.57, $R^2 = 0.24$, $\omega^2 = 0.26$, $p < 0.001$) still explained the most variation in microbial composition, followed by population (F-statistic = 5.62, $R^2 = 0.157$, $\omega^2 = 0.17$, $p < 0.001$) and pig lineage (F-statistic = 2.36, $R^2 = 0.059$, $\omega^2 = 0.02$, $p < 0.001$). Within populations of domestic pigs, factors such as outside access and antibiotic use differed. This variation also shaped gut microbial composition, with categorical use of antibiotics (yes/no, F-statistic = 3.87, $R^2 = 0.03$, $\omega^2 = 0.15$, $p < 0.001$) and outside access (yes/no, F-statistic = 11.85, $R^2 = 0.09$, $\omega^2 = 0.06$, $p < 0.001$) both significant in our analyses once we had accounted for

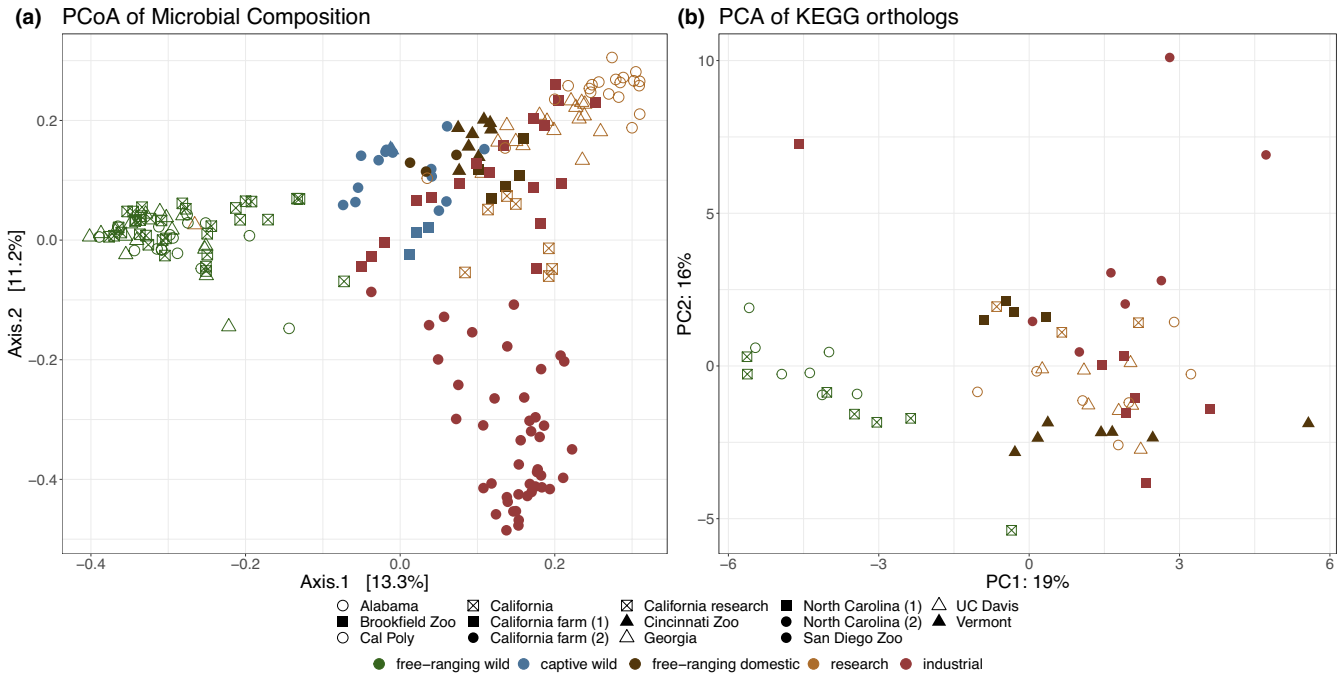

FIGURE 1 Ordination plots for microbial composition and functional potential across domestication contexts. Each domestication context is displayed by a unique colour, and populations are distinguished by shapes. Within a singular domestication context, each population is designated by a different shape, but shapes are repeated across contexts for different populations. Further, filled shapes represent samples from the domestic genetic lineage, whereas open shapes represent samples from the wild genetic lineage. (a) Principle coordinate analysis plot for microbial composition across domestication contexts based on Bray-Curtis dissimilarities for 16S rRNA gene sequencing ($N = 207$). (b) Principal component analysis plot for Kyoto Encyclopedia of Genes and Genomes (KEGG) orthologs across domestication contexts ($N = 56$). Captive wild pigs were not included for metagenomic analysis.

population. Pig lineage was not significant in explaining the extent of variation in microbial composition among pigs (beta-dispersion test, $p = 0.074$), but domestication context within domestic pigs was. Pigs from industrial contexts showed the most variation in microbial composition (beta-dispersion test, F-statistic = 12.84, $p < 0.001$). Microbial compositional dissimilarity and pig genetic dissimilarity based on SNP data were positively correlated (Mantel test, $r = 0.29$, $p < 0.001$), indicating that pigs which were more closely related had more similar microbiome community compositions.

Because population was a nested variable, we tested for associations between bacterial load or microbial diversity and ecological factors and genetic lineage when population was accounted for. Domestication context was a significant predictor of bacterial load ($p = 0.01$), but genetic lineage, outside exposure and antibiotic use were not ($p > 0.55$). Free-ranging wild pigs had lower bacterial load than pigs in other domestication contexts (two-sample Wilcoxon rank sum tests with BH correction, $p < 0.025$) (Figure S2). Domestication context (Figure S2) and species (Figure S3) were significant predictors of both Shannon diversity (domestication context, $p < 0.001$; species, $p = 0.01$) and richness (domestication context, $p < 0.001$; species, $p < 0.001$). Industrial pigs harboured microbiomes with the lowest Shannon diversity (two-sample Wilcoxon rank sum tests with BH correction, industrial versus all other contexts, $p < 0.001$) and richness ($p < 0.001$). Outside exposure ($p = 0.02$) and antibiotic use ($p = 0.002$) were also significantly associated with Shannon diversity. Across all domestication contexts, individuals with outside access harboured microbiomes with higher Shannon diversity than those without (two-sample Wilcoxon rank sum tests with BH correction, $p < 0.001$). Additionally, pigs with no antibiotic exposure harboured higher microbial diversity than pigs treated with antibiotics (two-sample Wilcoxon rank sum tests with BH correction, $p = 0.02$). We observed no correlation between bacterial load and Shannon diversity (Spearman's rank correlation, $p = 0.60$) or richness ($p = 0.12$).

Seventeen microbial taxonomic families were differentially abundant between wild and domestic pigs (ANCOM-BC, q-value<0.001, Figure 2a), and twenty microbial families were differentially abundant between industrial and free-ranging domestic pigs (ANCOM-BC, q-value<0.001; Figure 2b). We observed three microbial families (Akkermansiaceae, Bacillaceae and Acholeplasmataceae) that were higher in abundance in wild pigs compared to domestic pigs and in free-ranging domestic pigs compared to industrial pigs. Differential abundance analysis showed that Campylobacteraceae, Streptococcaceae and Clostridiaceae (families to which notable pathogens belong) were most abundant in domestic pigs compared to wild pigs. The microbes that were most differentially abundant in industrial pigs compared to free-ranging domestic pigs were also families that include potential pathogens (i.e. Coriobacteriaceae, Campylobacteraceae, Desulfovibrionaceae, Helicobacteraceae, Acidaminococceae, Porphyromonadaceae and Actinomycetaceae).

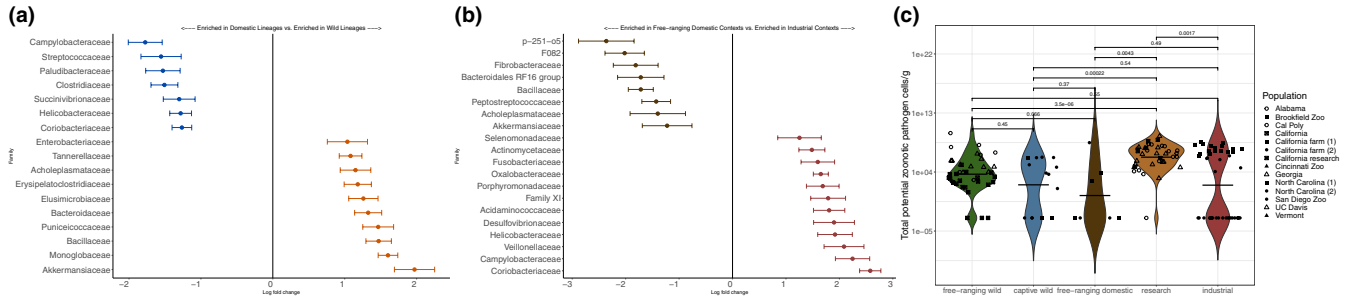

FIGURE 2 Microbial composition differs between genetically wild and domestic pigs as well as within domestic pigs. (a) Differential abundance analysis (ANCOM-BC) shows 17 microbial families that are enriched in either wild (positive log-fold change) or domestic (negative log-fold change) pigs. (b) Differential abundance analysis (ANCOM-BC) shows 20 microbial families that are enriched in either free-ranging domestic (positive log-fold change) or industrial (negative log-fold change) pigs. (c) Total potential zoonotic pathogen abundances (cells/g) across domestication contexts. Displayed p-values are from two-sample Wilcoxon rank sum tests with BH correction for comparisons between pairs of domestication context groups.

To test if this family level pattern reflected higher pathogen abundance in domestic pigs, we estimated the abundance of potential zoonotic pathogens by combining 16S rRNA amplicon and qPCR data. Potential pathogen abundances significantly differed between pig lineages (two-sample Wilcoxon rank sum tests with BH correction, $p=0.020$) and across domestication contexts (Kruskal–Wallis $\chi^2=9.41$, df$=4$, $p=0.050$), mainly driven by high pathogen abundances in research pigs (two-sample Wilcoxon rank sum tests with BH correction, $p<0.01$; Figure 2c). *Campylobacter jejuni*, a zoonotic pathogen of which pigs are a natural reservoir, was only found in industrial pigs. In contrast, very few free-ranging domestic pigs harboured any potential pathogens. Because ecological factors differ across populations within a singular domestication context, we also measured potential zoonotic pathogen abundance at the population scale. We observed differences in pathogen abundance between populations from the free-ranging wild domestication context (two-sample Wilcoxon rank sum tests with BH correction, Alabama vs. California, $p=0.004$; Alabama vs. Georgia, $p=0.408$; California vs. Georgia, $p<0.001$) and the industrial context ($p<0.001$), but not between populations from captive wild, free-ranging domestic and research contexts ($p>0.14$).

3.2 | Predicted functions of the pig gut microbiome

Gut microbial functional potential, as inferred from KEGG ortholog abundances ($N=56$ individuals out of 241), also varied significantly between domestication contexts (Figure 1b, PERMANOVA F-statistic$=5.44$, $R^2=0.21$, $\omega^2=0.19$, $p<0.001$) and populations (F-statistic$=2.86$, $R^2=0.18$, $\omega^2=0.14$, $p<0.001$). However, it did not vary with pig genetic lineage ($p=0.09$) and was uncorrelated with microbiome composition (Mantel test, $r=0.15$, $p=0.07$) or pig relatedness, as inferred from SNP data ($r=0.12$, $p=0.14$). Wild pigs were enriched with 32 pathways (enricher test, $p<0.05$; Figure S4A), whereas domestic pigs were enriched with 38 pathways, including starch and sucrose metabolic pathways as well as glycolysis pathways (enricher test, $p<0.05$; Figure S4B). Testing for differential abundance in specific gene sets (KEGG modules), we found that 356 KEGG modules were more abundant in domestic pigs. In contrast, only 104 modules were more abundant in wild pigs (Table S2). There were fewer differences in KEGG ortholog and module abundances between free-ranging domestic and industrial pigs (138 KEGG orthologs and 30 modules total) and between free-ranging domestic and research pigs (50 KEGG orthologs and 5 modules total) (Table S2). Focusing on specific functional groups, we found wild pigs had a significantly higher abundance of total carbohydrate metabolic enzyme (CAZy) genes compared to domestic pigs (two-sample Wilcoxon rank sum test with BH correction, $p<0.001$), and industrial pigs had the lowest abundance of CAZy genes of all pigs sampled (two-sample Wilcoxon rank sum tests with BH correction, $p<0.01$; Figure S5A). However, differential abundance analysis between wild and domestic pigs showed that domestic pigs had higher abundances of specific CAZy genes associated with chitin degradation and xylan- and glycogen-binding (DESeq2 adjusted $p<0.001$; Table S3).

We also investigated variation in genes encoding virulence factors, which enable pathogen colonization and are highly variable between microbial strains. Overall, the abundance of virulence factor genes varied significantly between domestication contexts (Kruskal–Wallis test $\chi^2=7.66$, df$=3$, $p=0.05$; S5B) and populations ($\chi^2=31.05$, df$=8$, $p<0.001$), but not between genetic lineages ($p=0.08$). In accordance with their low abundance of potential pathogens, free-ranging domestic pigs had the lowest abundances of virulence factor genes. However, across contexts, total potential pathogen abundance was not correlated with virulence factor gene abundance (Spearman's rank correlation, $p=0.94$).

3.3 | Pig immune state

We also examined whether domestication and its impacts on the gut microbiome were associated with variation in pig immune responses. Serum from pigs in three domestication contexts ($N=89$ individuals out of 241) revealed pro-inflammatory TNF-α concentrations

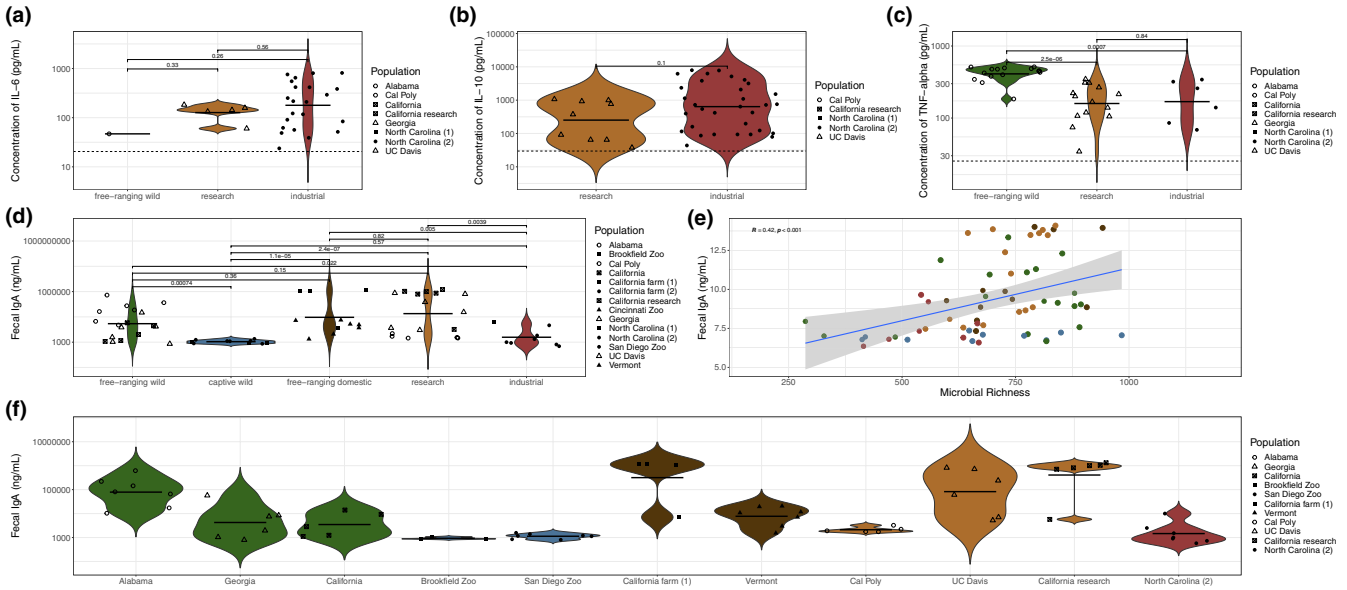

FIGURE 3 Pig immune measurements and correlations. Displayed *p*-values are from two-sample Wilcoxon rank sum tests with BH correction for comparisons between pairs of domestication context groups. (a) IL-6 concentrations (pg/mL) in free-ranging wild, research and industrial pigs. (b) IL-10 concentrations (pg/mL) in research and industrial pigs. (c) TNF-α concentrations (pg/mL) in free-ranging wild, research and industrial pigs. (d) Faecal IgA concentrations (ng/mL) across domestication contexts. (e) Positive correlation between microbial richness and faecal IgA concentrations. (f) Faecal IgA concentrations (ng/mL) across populations.

significantly differed across domestication contexts but pro-inflammatory IL-6 and anti-inflammatory IL-10 concentrations did not (Figure 3a–c; IL-6, Kruskal–Wallis $\chi^2=2.36$, df = 2, $p=0.31$; IL-10, Kruskal–Wallis $\chi^2=2.64$, df = 2, $p=0.10$; TNF-α, Kruskal–Wallis $\chi^2=20.58$, df = 2, $p<0.001$). Free-ranging wild pigs harboured higher TNF-α concentrations compared to industrial and research pigs (two-sample Wilcoxon rank sum tests with BH correction $p<0.001$). TNF-α concentrations were also significantly higher in pigs with outdoor access (two-sample Wilcoxon rank sum tests with BH correction $p<0.001$). We detected no significant relationships between any cytokine concentrations and potential pathogen abundances (Spearman's rank correlation, $p>0.18$). Based on the linear mixed effects model, PCoA Axis 1, PCoA Axis 2, microbial richness and bacterial load ($p>0.30$) were not significant estimators of cytokine concentrations. However, envfit analyses show variation in gut microbiome composition was significantly correlated with IL-10 concentration ($R^2=0.58$, $p=0.04$), but not IL-6 ($p=0.31$) or TNF-α ($p=0.78$) concentrations.

Faecal IgA concentrations also differed significantly across domestication contexts ($N=79$ individuals out of 241) (Kruskal–Wallis $\chi^2=26.52$, df = 4, $p<0.001$; Figure 3d), driven mainly by low concentrations in captive wild (two-sample Wilcoxon rank sum tests with BH correction, $p<0.003$) and industrial pigs ($p<0.04$). However, population within domestication contexts was also a significant driver of differences in faecal IgA concentrations (nested ANOVA, $p<0.001$; Figure 3f). We observed positive correlations between microbial richness and faecal IgA concentrations (Spearman's rank correlation, $\rho=0.422$, $p<0.001$, Figure 3e). Like TNF-α, faecal IgA concentrations were positively associated with outdoor access (two-sample Wilcoxon rank sum tests with BH correction $p=0.04$), but interestingly, TNF-α and faecal IgA concentrations were not correlated ($p=0.98$). Envfit analyses showed that overall microbial composition was not associated with faecal IgA concentrations ($p=0.92$). Further, linear mixed models demonstrated that bacterial load ($p=0.03$) and microbial richness ($p=0.018$) were significantly associated with faecal IgA concentrations but PCoA Axis 1 ($p=0.70$) and PCoA Axis 2 ($p=0.54$) were not. Finally, there was no significant relationship between faecal IgA concentrations and potential pathogen abundances ($p=0.11$).

4 | DISCUSSION

Sitting at the interface between the environment and host biology, the gut microbiome is increasingly considered a potential tool to improve both human and animal health. To succeed, however, any microbial manipulations must recognize the evolutionary and ecological drivers of gut microbiome variation and its resulting impact on host functioning. These dual forces are both prominent in the context of domestication, where the gut microbiome is thought to play a role in promoting host adaptation and health today (Hold & Hansen, 2019; Kolodny et al., 2020). Combining microbial sequencing and the characterization of pig immune features across a diverse range of domestication contexts, our study helps establish evolutionary and ecological drivers of pig gut microbiome composition and immune state. We observed a signal of pig lineage and diet in the types of metabolic genes present in the gut microbiome, whereas domestication context explained the most variation in gut microbiome taxonomic composition, pathogen load and immune markers. Population-level effects within domestication

contexts further accentuate the importance of local environmental and fine-scale genetic variation in shaping the gut microbiome and immune state. Altogether, our results provide a framework to inform future microbially-mediated health interventions to prevent or mitigate diseases in domestic animals by pointing to features more likely to respond to ecological manipulations such as diet or husbandry shifts.

4.1 | The potential role of the gut microbiome in domestic pig evolution

Selection and the resulting variation in host genomes are thought to be driving factors in shaping not only gut microbiome composition but also the functions the gut microbiome provides the host. In line with previous studies (Bergamaschi et al., 2020; Chen et al., 2021; Correa-Fiz et al., 2019; Xiao et al., 2016), we found pig genetic lineage was related to gut microbial composition and metabolic potential. Such ties could be due to the direct effects of genetic filtering of gut microbes or could reflect vertical inheritance of microbes by pigs during birth (Camarinha-Silva et al., 2017; Lu et al., 2018). Of course, these ties could also result from neutral filtering without any active selection (Burns et al., 2016; Venkataraman et al., 2015). If due to host selection or vertical transmission, however, these intertwined host–microbe relationships suggest that the retained microbes provide beneficial functions to the host in the domestic environment, such as assistance in digesting new diet components. These functions also could be artificially selected for during domestication because they provide functions valued by humans.

The pig gut microbiome encodes almost 800-fold more genes than does the pig host genome (Chen et al., 2021), including genes that assist with the digestion of a diverse variety of polysaccharides (Flint et al., 2012). We observed notable differences in the repertoire of microbial genes between wild and domestic pigs, perhaps in large part due to the need to adapt to distinct diets during domestication. In wild pig microbiomes, we observed indicators of diverse and fibre-rich diets: wild pigs had enriched pathways of amino acid biosynthesis, lipid metabolism and vitamin metabolism, as seen elsewhere (Chen et al., 2021). They also harboured higher abundances of CAZy genes—enzymes involved in the modification and degradation of carbohydrates—compared to domestic pigs. Because wild pigs eat a larger diversity of plants and insects than domestic pigs and have a higher cellulose content in their diets (Schley & Roper, 2003; Ushida et al., 2016), a higher diversity of CAZy genes may be associated with degrading a wide range of carbohydrates.

In contrast, diets for domestic pigs have been altered by humans for over 9000 years. Particularly for the last 200 years, these human-controlled diets have been characterized by their high starch content (Holman et al., 2022; Leus & Macdonald, 1997; Nafikov & Beitz, 2007; Ushida et al., 2016), much as is the case with industrialized humans (Sonnenburg & Sonnenburg, 2019). We found that domestic pig gut microbiomes were enriched in starch and sucrose metabolic pathways as well as glycolysis pathways. Domestic pig gut microbiomes also had increased abundances of glycoside hydrolases and CAZy genes related to starch degradation (GH13 and GH31) (Alessandri et al., 2019; Larson & Fuller, 2014; Møller & Svensson, 2016). Superficially, our results are reconcilable with a model wherein domestic pig gut microbiomes have expanded microbial metabolic capacities and adapted to a starch-rich diet, while losing some features associated with the wild diet. Such increased capacity for starch digestion has previously been associated with domestication in pigs and dogs (Axelsson et al., 2013; Wei et al., 2022) and is a major difference between human and chimpanzee metabolism (Perry et al., 2007). These adaptations would complement changes in the pig genome which promote digestion of domestic diets, changes which are likely under selection because pigs are selected to grow bigger more rapidly in commercial agriculture. However, an expanded repertoire of metabolic genes to digest domestic diets could come at the expense of other functions related to immunity (e.g. fighting off pathogens (Lochmiller & Deerenberg, 2000)). Whether these changes in the gut microbiome preceded host adaptation and thus promoted domestication (Reese et al., 2021) is not currently known for pigs. We also do not know how quickly changes in microbial metabolism come about or if they could be lost. For example, utilization of specific metabolic genes, such as those related to starch degradation, can be associated with either pig lineage or local ecology (as seen in mice and dogs (Reese et al., 2021)). Interestingly, there were only few differences in metabolic gene content among free-ranging domestic, research and industrial pigs. This result could either be due to host control reflecting the shared evolutionary history of domestic pigs or because all domestic pigs we sampled consume some amount of processed feeds containing the same carbohydrates (soy, corn and barley), necessitating the same types of microbial genes to break down their diets. Experimental work that manipulates diet in pigs from multiple domestication contexts could disentangle to what extent genetic and/or ecological factors shape microbial metabolism capabilities.

Indeed, while the effect of pig lineage on the gut microbiome was significant and in line with predictions from theory and the literature, for most microbiome features we investigated the lineage effect was weaker than the effects associated with domestication context and population. Pig lineage explained only 6% of the variation in gut microbiome composition overall and pig species only 2%. In contrast, the effect size of domestication context and population on microbial composition (24% and 16%, respectively) and function (21% and 18%, respectively) was akin to that of other major drivers of the gut microbiome (e.g. diet (Carmody et al., 2015), gut morphology (Sanders et al., 2015), age (Koenig et al., 2011)). Because the process of domestication encompasses changes in both pig traits and pig ecology, different microbial features could respond to evolutionary and/or ecological factors while manifesting with a domestication context signal. Population-specific factors reflecting local ecology or fine-scale genetic variation can further contribute to differences in microbial features. For example, we detected a large degree of

variation in gut microbiome composition between populations of industrial pigs, perhaps because the two farms utilize different husbandry practices. Additional variation in gut microbial composition and gene content not captured by our variables of interest may be attributed to factors that vary in geography, such as climate, diet and season.

4.2 | Domestication-related changes in pig gut microbiomes and pig health

Because the gut microbiome plays a crucial role in immune homeostasis, understanding which features respond to pig evolutionary history versus local ecology is fundamental to develop targeted interventions for pigs today. Pigs can suffer from a myriad of infectious diseases, including reproductive and respiratory syndrome, swine influenza and epidemic diarrhoea (Costa et al., 2014; Holtkamp, 2013; Messori et al., 2013). If domestication results in host–microbe mismatches that alter host immune functioning and reduce colonization resistance or resilience, this may play a role in increasing infectious disease burdens. Alternatively, the domestic gut microbiome may help protect against infectious disease in contemporary agricultural settings. Previous studies have shown higher abundances of pathogens in domestic animals compared to their wild progenitors (Craft et al., 2022; Kock & Caceres-Escobar, 2022; Reese et al., 2021). However, those studies typically did not consider variation among populations within domestic animal species or just examined pathogen prevalence within a singular domestication context (i.e. industrial; Toth et al., 2013). Because ecological factors can influence pathogen transmission (Haack et al., 2015; Kuthyar, Kowalewski et al., 2022; López-Pérez et al., 2021; Palmeirim et al., 2014), pathogen dynamics are likely to be influenced at both the scale of domestication context and population. Indeed, we found pigs from industrial and research contexts had much higher pathogen burdens compared to free-ranging domestic pigs. It is probable that the combined effects of stress and high density living conditions result in increased transmission and overall load of pathogens in pigs living in industrial and research contexts (Baer et al., 2013; Beura et al., 2016; Rosshart et al., 2017). Within industrial and free-ranging wild contexts, pathogen loads differed across populations. Different industrial pig farms have previously been found to have variable burdens of pathogens (de Oliveira Filho et al., 2018; Lovera et al., 2017; van Duijkeren et al., 2008), likely because local ecological factors distinct to each population (e.g. interactions with other animals (Miller et al., 2017), climate (Murray et al., 2006) and the physical environment (Faust et al., 2018)) shape what pathogens exist in the environment and how well they can colonize pigs (Adelman et al., 2010; Nédélec et al., 2016).

Ecological conditions under which domestic pigs live can also impact immunity more broadly. As seen in other work (Adelman et al., 2010; Wright et al., 2010), differences in local ecology, and thus differences in environmental stimuli, may predict cytokine patterns. For example, TNF-α, a pro-inflammatory cytokine that activates adaptive immunity (Kreikemeier et al., 2015; Pauli, 1995), has previously been associated with outdoor exposure (Wen et al., 2021). In our study, we see high TNF-α concentrations were associated with pigs with outdoor access (wild pigs), possibly because exposure to the natural environment stimulates TNF-α production. Whether this immune activation is acting directly on the host or through the gut microbiome (Kelly et al., 2007; MacGillivray & Kollmann, 2014; Mulder et al., 2011) is unknown. Faecal IgA concentrations were also specific to local ecology, both at the scale of domestication context and population. The positive associations between local environmental exposures, microbial diversity and faecal IgA concentrations potentially indicate that a diverse set of microbes stemming from multiple environmental sources is associated with the production of secretory IgA across pig populations (Bunker et al., 2015; Macpherson et al., 2000; Peterson et al., 2007).

Not all immune traits may be associated with domestication context, however. Our data suggest that microbial composition varies with IL-10 concentrations, largely reflecting high concentrations of IL-10 in industrial and research pigs. However, since we do not have cytokine values for free-ranging domestic pigs, we cannot say if high IL-10 concentrations or the associated microbiome composition are characteristic of all domestic pigs or just those in less natural settings. Additionally, because we were only able to collect cytokine data from a single population from each domestication context, we are unable to assess to what extent variation arises at the population or domestication context scale. Without experiments testing specific immune-microbiome interactions, the observational approach we took precludes direct connections from microbial and immune measurements to animal health. Altogether, creating strategies to improve pig health will require both a long term and short-term perspective on pig biology. Veterinary science needs to consider how common husbandry approaches influence microbiome composition, pathogen burdens and host inflammation to better assess ecological determinants of health. In sum, our results clearly showcase there is immense diversity in which variables respond at which scales. Understanding under which ecological and evolutionary contexts the domestic animal gut microbiome contributes to metabolism and defends against pathogens can inform gut microbial manipulations designed to improve animal performance and resilience.

AUTHOR CONTRIBUTIONS

Sahana Kuthyar: Conceptualization (equal); formal analysis (lead); investigation (equal); methodology (lead); project administration (equal); writing – original draft (lead); writing – review and editing (equal). **Jessica Diaz:** Methodology (supporting); writing – review and editing (supporting). **Fabiola Avalos-Villatoro:** Methodology (supporting). **Christian Maltecca:** Project administration (supporting); resources (supporting); writing – review and editing (supporting). **Francesco Tiezzi:** Methodology (supporting); writing – review and editing (supporting). **Robert Dunn:** Methodology (supporting); writing – review and editing (equal). **Aspen Reese:** Conceptualization

(equal); funding acquisition (lead); investigation (equal); project administration (equal); resources (lead); supervision (lead); writing – review and editing (equal).

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/jeb.14227.

DATA AVAILABILITY STATEMENT

All sequencing data are available in NCBI SRA (accession numbers PRJNA926635 for 16S and PRJNA926638 for metagenomics) with associated metadata files. Scripts for data processing, statistical analyses and visualization are available on Dryad (DOI: 10.5061/dryad.9zw3r22mb).

ORCID

Sahana Kuthyar https://orcid.org/0000-0001-5037-1692

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