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## Article

# Skin and Blood Microbial Signatures of Sedentary and Migratory Trout (*Salmo trutta*) of the Kerguelen Islands

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**Abstract:** Our understanding of how microbiome signatures are modulated in wild fish populations remains poorly developed and has, until now, mostly been inferred from studies in commercial and farmed fish populations. Here, for the first time, we have studied changes in the skin and blood microbiomes of the *Salmo trutta* population of the volcanic Kerguelen archipelago located at the northern limit of the Antarctic Ocean. The Kerguelen Islands present a natural framework of population expansion and reveal a likely situation representing further climate change in distribution areas. Our results showed that *S. trutta* of the Kerguelen Islands has a microbiome signature distinct from those of salmonids of the Northern Hemisphere. Our study also revealed that the skin and blood microbiomes differ between sedentary and migratory *S. trutta*. While 18 phyla were shared between both groups of trout, independent of the compartment, 6 phyla were unique to migratory trout. Further analyses showed that microbiome signatures undergo significant site-specific variations that correlate, in some cases, with the peculiarity of specific ecosystems. Our study also revealed the presence of potential pathogens at particular sites and the impact of abiotic factors on the microbiome, most notably due to the volcanic nature of the environment. This study contributes to a better understanding of the factors that modulate the microbiome signatures of migratory and sedentary fish populations. It will also help to better monitor the impacts of climate change on the colonization process in the sub-Antarctic region.



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**Keywords:** blood microbiome; skin microbiome; fish; *Salmo trutta*; migration; Kerguelen Islands; 16S rRNA

**Key Contribution:** This work highlighted the compartment-specific microbial signatures and the environmental influence on the microbiome signatures between migratory and sedentary trout of Kerguelen.

## 1. Introduction

The microbiota is now recognized as a key player in health and disease progression. Defining the microbiome signature, which includes nucleic acid originating from microorganisms, has thus become an essential component of the biomarker schematic in medicine and ecology. In clinical settings, for example, specific signatures will determine if a cancer patient will respond to particular therapies [1,2]. This progress has been greatly facilitated by the development of next-generation sequencing (NGS) technologies for analyzing 16S ribosomal RNA (rRNA) gene amplicons, which allows for rapid evaluation of microbial biodiversity while overcoming the limitations of cultivation-based methods. Despite these technical developments, our understanding of the microbiome in wild animals is only in its infancy. This is particularly true in fish populations, notably those inhabiting environments sensitive to pollution and climate change. Moreover, until now, studies of the fish microbiome have mostly focused on the gut microbiota and, to a lesser extent, on the

skin (mucosal microbiota). In fish, in particular, the skin mucus represents the first barrier defense against infectious stressors in the environment. The nonpathogenic skin microbial flora helps the immune system protect itself against pathogens [3,4]. Its composition depends on several factors, including host genetics and their surrounding environment. Captivity, pollution, and habitat transition are other factors that affect the microbiome signature of animals [5–7]. However, most microbiome studies in fish have been conducted in fish farms or experimental settings simulating freshwater-to-seawater transitions [8,9]. We still need to learn more about how the skin microbiome differs between migratory and sedentary wild fish populations. Considering the importance of the microbiome as a biomarker and the need to adopt less costly and invasive procedures, the concept of “circulating microbiome DNA” (cmDNA) has been developed as an alternative to studying the microbiome in clinical settings [10]. Since the middle of the 20th century, we have known that DNA fragments are present in the blood [11], but it is only in recent years that the advent of NGS methods has allowed us to better understand the origin of these circulating DNA fragments. We now know that blood contains a vast array of self and non-self DNA fragments originating from multiple tissues, including infected tissues, and that a significant percentage of the DNA within the blood originates from microorganisms [12–14]. Combined with the 16S rRNA approach, cmDNA analysis has thus become a central tool for studying an individual’s health status and measuring its ability to respond to treatment or environmental stressors [15–19]. Several recent studies have applied this concept to study the hemolymphatic microbiome of bivalves and its changes in response to environmental stressors [20–22]. The existence of a blood microbiome is a concept that is now widely accepted in humans and animals (including pigs, cows, goats, rodents, camels, and dogs [21,23–29]). A blood microbiome has also been reported in fish and discussed in reviews [5,30–32]. The existence of a circulating microbiome has also been reported in invertebrates. In many cases, this has been applied to facilitate the detection of pathogens. The Kerguelen Islands (also known as the Desolation Islands) are located in the Southern Ocean just north of the polar front. Long-term monitoring studies have shown that this archipelago, which comprises more than 300 islands that cover more than 7000 km<sup>2</sup> and has almost 3000 km of shoreline, is particularly sensitive to global warming [33,34]. Previously devoid of freshwater fish, the Kerguelen Islands were populated at multiple sites with various stocks of fish over three decades (1962–1993). Overall, 22 successful imports were conducted, with eggs collected from 8 salmonid species in the Northern Hemisphere [35]. *Salmo trutta* (*S. trutta*) is among the different species that successfully colonized the largest number of watersheds following the early occurrence of alternative anadromous (e.g., migratory) species to pursue its life cycle in seawater [36]. Given its remoteness and limited anthropogenic activities, Kerguelen’s salmonid populations thus provide a unique subpolar environment to study the impact of the environment and migratory activity on microbiome compartments and to better understand how climate change can impact these populations [37].

The objective of the present work was to define and compare the phylogenetic structure of the skin (mucus) and circulating (blood) microbiomes of migratory and sedentary *S. trutta* populations collected at different sites in the Kerguelen Islands. First, we assessed the overall composition of both the skin and blood microbiota in resident and migrating trout. We then proceeded to compare both skin and blood samples, as well as resident and migratory traits. We also analyzed site-specific variations to establish whether the local environment could trigger substantial contrasts in microbiota. Our study also revealed the presence of potential pathogens at certain sites and the impact of abiotic factors on the microbiome, most notably due to the volcanic nature of the environment. Finally, we further showed that FTA<sup>®</sup> Cards-based sampling is perfectly adapted for establishing mucosal and circulating 16S rRNA microbiome signatures, an interesting avenue for long-term monitoring programs in remote and sensitive polar environments.

## 2. Materials and Methods

### 2.1. Sample Collection

A total of 83 skin and blood microbiomes collected from 52 trout samples were analyzed (Table S1). Sedentary trout were captured by electrofishing in freshwater rivers, and migratory trout were captured by trolling (fly fishing). Immediately after capture, the fish were anesthetized, as described by Marandel et al. (2018) [38]. Blood was immediately withdrawn from the caudal vein using sterile nonheparinized syringes and spotted on Whatman 903™ Flinders Technology Associates filter paper (FTA® Cards) (Sigma–Aldrich, Oakville, ON, Canada). To avoid cross-contamination between samples, all samples were allowed to air dry and were stored individually in a plastic bag with a desiccant, as previously described [39]. Mucus samples were collected using a sterile scalpel blade by scraping along the fish's lateral line and were immediately spotted to cover an entire disc of the FTA cards. The same preservation method was applied as above. The care and use of field-sampled animals complied with the Government of France's animal welfare laws, guidelines, and policies (Comité d'Ethique), as approved by the Terres Australes et Antarctiques Françaises administration. All methods are reported using ARRIVE guidelines.

### 2.2. DNA Extraction, Preprocessing, and Sequencing

All procedures were conducted in a white room where pressure, temperature, and humidity were controlled to eliminate contaminants. Individual discs were cut from the FTA® Cards using a sterile 5.0 mm single round hole punch. Total DNA was isolated using the QIAamp DNA Investigator Kit (Qiagen, Hilden, Germany), according to the manufacturer's protocol. DNA was quantified in duplicate using a Quant-iT™ PicoGreen® dsDNA detection kit (Molecular Probes, Eugene OR, USA). Amplification of the 16S ribosomal RNA (rRNA) genes and 16S gene amplicon sequencing for all DNA samples were performed at the Centre d'Expertise et de Services Génome Québec (Montréal, QC, Canada) using the universal primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTAATCC-3') targeting the V3–V4 hypervariable regions [40]. Sequence libraries were prepared by Génome Québec with the TruSeq® DNA Library Prep Kit (Illumina, San Diego, CA, USA) and quantified using the KAPA Library Quantification Kit for Illumina platforms (Kapa Biosystems, MA, USA). Paired-end sequences were generated on a MiSeq platform PE300 (Illumina Corporation, San Diego, CA, USA) with the MiSeq Reagent Kit v3 600 cycles (Illumina, San Diego, CA, USA). The raw data files are publicly available on the NCBI Sequence Read Archive (PRJNA772886).

### 2.3. 16S rRNA Data Processing

Illumina sequence data (FASTQ files) were trimmed using Cutadapt (version 2.8, Uppsala, Sweden). The amplicon sequence variants (ASVs) were generated with the DADA2 pipeline (version 1.16.0, Stanford, CA, USA) [41] and subsequently analyzed within the R environment (R version 4.0.3, Vienna, Austria) [42]. Forward and reverse reads were then trimmed, filtered, and truncated with the filterAndTrim function. The error model (maxEE) was calculated for forward and reverse reads, and low-quality reads were removed. After denoising and merging, chimeric sequences (bimeras) were removed from the datasets. The minimum and maximum lengths were set at 400 bp and 428 bp, respectively. All reads had an average quality score of  $\geq 30$ . The RDP (Ribosomal Database Project) 16 classifier database was used for ASV taxonomy assignment [43]. The RDP contains 16S rRNA sequences available from the International Nucleotide Sequence Database Collaboration (INSDC) databases and can accurately classify bacterial 16S rRNA sequences [44,45]. Archaea and unclassified ASVs at the phylum level (representing 4.7% of the total ASVs) were removed. The phyloseq (version 1.34.0), microbiomeSeq (version 0.1), microbiomeMarker (version 1.3.3) and vegan (version 2.6.4) R packages were used to characterize the microbial communities [46–49].

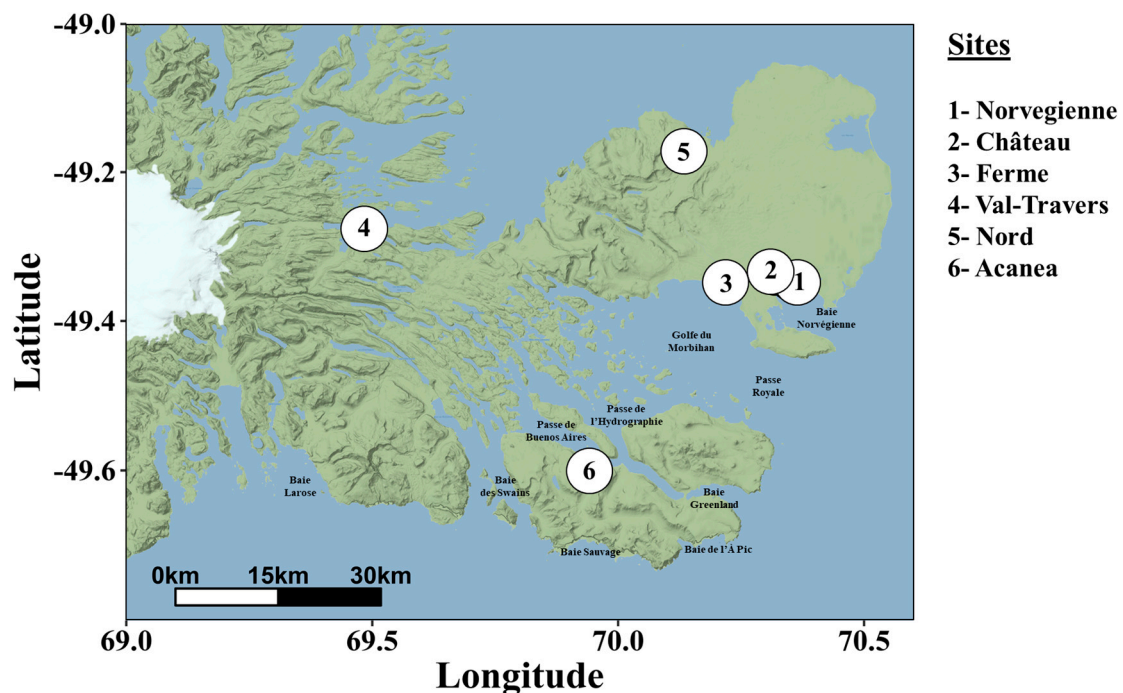
## 2.4. Statistical Analysis

The alpha diversity index, was calculated using the phyloseq R package [46]. The average values of each group were compared using Wilcoxon rank sum tests. Microbiota composition differences among groups were determined using multivariate analysis of variance with permutation (PERMANOVA) with 9999 permutations followed by pairwise permutation tests. We studied the functional content of the blood and skin microbiomes predicted from the KEGG database using the Piphillin tool (Piphillin server: <http://piphillin.secondgenome.com/> (accessed on 1 April 2021)) [50]. Heatmaps were generated based on the relative abundance and constructed with the 30 most abundant genera. Linear discriminant analysis (LDA) identified the effect size (LEfSe) that differentiates the samples among these taxa. The threshold on the logarithmic score of the LDA analysis was set to 3.0. Differences in the overall bacterial community composition among specimens were determined based on the UniFrac distance and visualized by principal coordinates analysis (PCoA). Permutation multivariate analysis of dispersion (PERMDISP) was also conducted with the betadisper function to test for the homogeneity of multivariate dispersions (i.e., deviations from centroids) among the specimens.

## 3. Results

### 3.1. General View of the Mucosal and Circulating Bacterial Microbiome

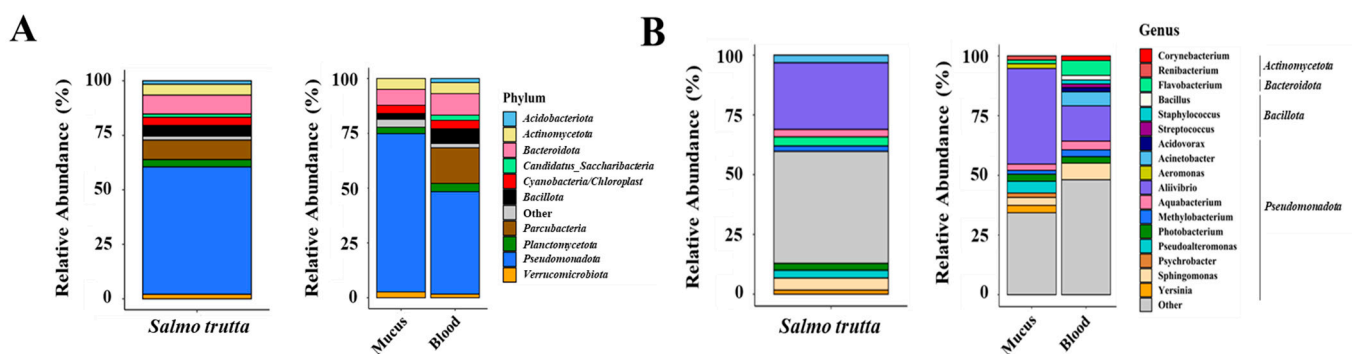
A total of 83 samples were collected from the blood ( $n = 45$ ) and skin mucus ( $n = 38$ ) of sedentary and migratory trout captured at different sites of the Kerguelen Islands (Figure 1). Each sample was immediately stored on-site on FTA cards (Table S1).



**Figure 1.** Map of the French subantarctic Kerguelen Islands showing the location of the rivers. Sedentary trout were collected from freshwater (FW), and migratory trout were collected from the mouth of the river (seawater (SW)).

For each sample, the cmDNA was determined by sequencing the V3-V4 hypervariable region of the 16S rRNA gene amplicons. A total of 1,976,043 paired-end sequences passed quality filtering ( $23,808 \pm 14,925$  per sample). Amplicon sequence variants (ASVs) were generated from 12,985 high-quality reads, and 89.2% of samples contained more than 10,000 reads. We first examined the overall skin and blood microbiomes at the phylum level, focusing on phyla representing at least 2% of all ASVs. Individual variations in

the circulating and mucosal microbiomes are shown in Figure S1. Our results showed that the microbiomes of *S. trutta* were dominated by *Pseudomonadota* (~45–75%) and *Bacteroidota* (~5–11%) and, to a lesser extent, *Parcubacteria* (~0.1–20%), *Bacillota* (~1–9%), *Actinomycetota* (~3–6%), and *Planctomycetota* (~2–5%) (Figure 2A). *Parcubacteria*, which has been described in the seawater of polar regions [51], was more abundant in the blood (17.1%) than in the mucus (0.3%) ( $p = 0.014$  (LefSE)). *Bacillota* and *Chloroflexota* were also significantly more abundant in the blood of the migratory trout compared to the sedentary population ( $p < 0.05$  linear discriminant analysis effect size (LefSE)). At the genus level, we found a dominance of genera under *Pseudomonadota*, the most abundant being the *Aliivibrio* genus, and to a lesser extent, bacterial DNA derived from *Sphingomonas*, *Pseudoalteromonas*, *Yersinia*, and *Aquabacterium*, among others (Figure 2B). Concerning differences between the skin and blood microbiomes, we found that the *Corynebacterium*, *Streptococcus*, *Acidovorax*, and *Acinetobacter* genera were found only in the blood, but not in the skin mucus. In contrast, *Psychrobacter*, *Yersinia*, and *Aeromonas* were only found in the circulating microbiome. Discriminatory genera between the blood and mucus are shown in Figure S2 (LDA score). *Aliivibrio* had the highest LDA score in the mucus, and *Flavobacterium* dominated the blood microbiome. Overall, these results indicate that the bacterial microbiome signature differs between the skin and blood microbiomes.



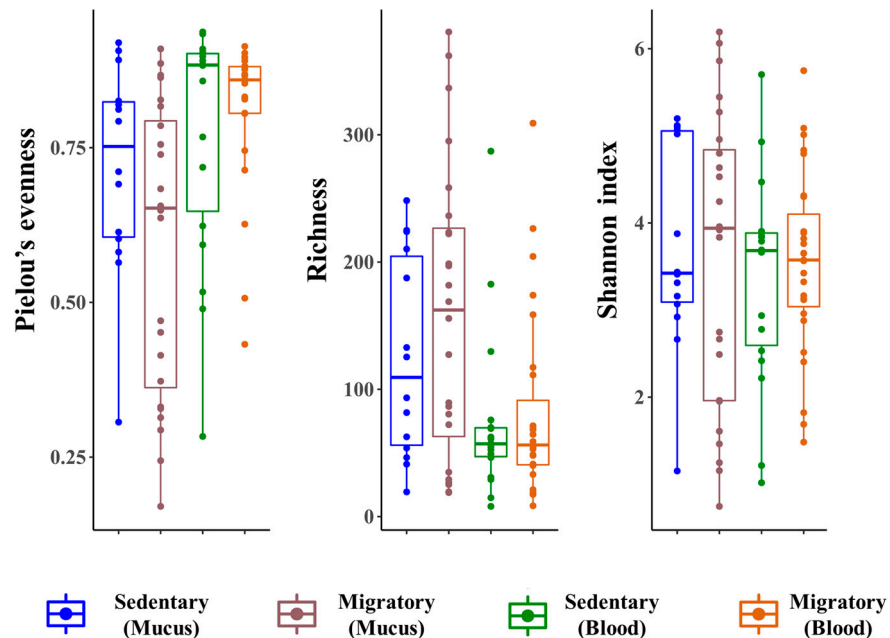
**Figure 2.** Phylum and genus level analysis of circulating and mucosal bacterial DNA. (A) Phylum and (B) genus levels were analyzed for all samples (left) and mucosal and circulating compartments (right) were analyzed in brown trout (*S. trutta*). Phylum and genus with a relative abundance of  $\leq 1.5\%$  are represented as “Other”.

### 3.2. Comparative Analysis between Sedentary and Migratory *S. trutta*

We next examined whether the skin and blood microbiome signatures differ between sedentary and migratory trout. We first compared the alpha diversity between migratory and sedentary populations using three diversity indices: richness, the Shannon index, and Pielou’s evenness. Globally, we found no significant differences in the alpha diversity between the two populations for either the skin or blood microbiome (Figure 3). Moreover, alpha diversity was also not dependent on sex differences (Figure S3). Further analyses, however, revealed significant differences at the phylum and genus levels in the composition of the microbiomes between the sedentary and migratory trout. The high abundance of rare ASVs in the blood of migratory trout was reflected by the Chao1 index (Figure 4A).

Focusing on unique and shared phyla, we found that 18 were shared between sedentary and migratory trout, independent of the compartment. A total of 7 phyla (*BRC1*, *Chlorobiota*, *Elusimicrobiota*, *Ignavibacteriota*, *Lentisphaerota*, *Poribacteria*, and *Mycoplasmata*) were unique to migratory trout (Figure 4B). Another 2 phyla (*Spirochaetota* and *SR1*) were unique to the mucus of both populations, and 1 phylum (*Chlorobiota*) was uniquely found in the blood, but not in the mucus. A clear dominance of unique bacteria in the mucosal microbiome of migratory trout was also found at the genus level (Figure 4C). Overall, migratory trout had more than six times the number of unique genera in their compartments compared to sedentary trout (233 versus 39). This result is consistent with a multivariate analysis (PERMANOVA) showing that the global composition of the microbiome was

significantly different between the two populations (UniFrac PERMANOVA,  $F(1, 82) = 1.44$ ,  $p = 0.02$ ). Principal coordinate analysis (PCoA) based on the unweighted UniFrac distance was carried out for both migratory and sedentary trout (Figure S4).



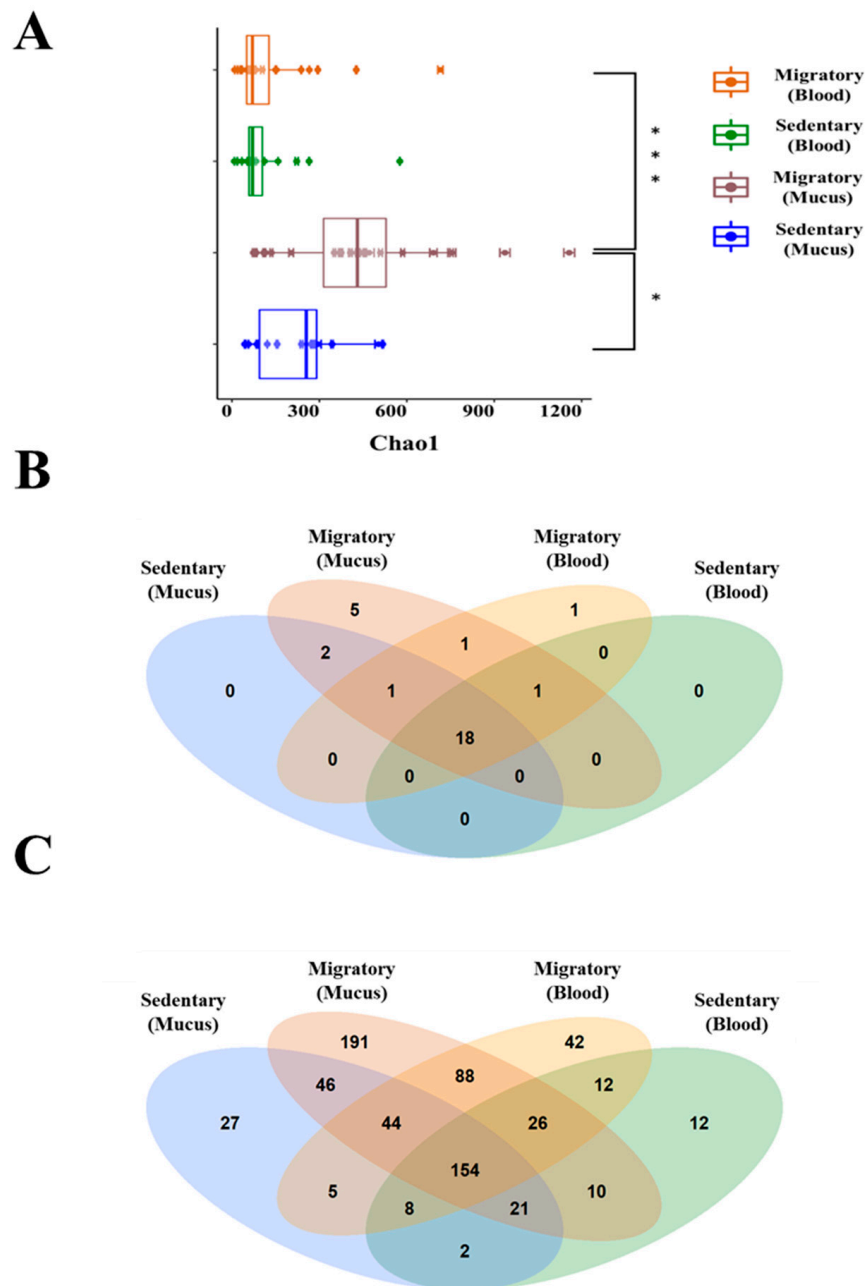
**Figure 3.** Alpha diversity analysis of both *S. trutta* phenotypes for circulating and mucosal microbiomes. Species evenness, observed richness and Shannon diversity indexes were calculated for each group. No significant differences between migratory and sedentary specimens for the same compartment were observed.

To further study the differences between migratory and sedentary trout, we examined the distribution of phyla and genera. At the phylum level, we found that the skin microbiome of sedentary trout contained significantly ( $p < 0.05$ ) higher DNA fragments of *Cyanobacteria/chloroplasts* and *Verrucomicrobiota* than that of migratory trout (Figure 5). In contrast, the latter had higher levels of *Bacillota* in both the skin and blood microbiomes. At the genus level, sedentary trout had a higher abundance of *Aquabacterium* ( $p < 0.05$ ), *Flavobacterium* ( $p < 0.01$ ), *Luteolibacteria* ( $p < 0.01$ ), and *Sphingomonas* ( $p < 0.05$ ) than the skin microbiome of migratory trout (Figure 6). In contrast, the skin microbiome of migratory trout had higher levels of *Aeromonas* ( $p < 0.05$ ) and *Yersinia* ( $p < 0.05$ ) (Figure 6). Other differences in the skin microbiome that approached conventional levels of significance ( $p < 0.10$ ) were also observed in the case of *Methylobacterium* and *Pseudoalteromonas*. Taken together, these results indicate that the skin microbiome signatures differed between migratory and sedentary trout.

### 3.3. Site-Specific Variations

A general overview of individual site-specific variations for phyla that constituted at least 1.5% of the total phylum is shown in Figure 7. Independent of the sampling site and the compartment, *Pseudomonadota* was the dominant phylum in the majority of samples, especially in the skin microbiome. This abundance of *Pseudomonadota* was almost absolute in samples collected at Rivière-du-Nord, independent of the population and the compartment. The presence of *Parcubacteria* was also omnipresent at all sites, except at Rivière-du-Nord. Focusing on the 30 most abundant genera at the genus level, we found that *Aliivibrio* was abundant in Rivière-du-Nord, in both sedentary and migratory trout, and in the skin microbiome of migratory trout in Val-Travers (Figure S5). Compared to other sites, at Rivière-du-Nord and other sites, the skin microbiome was particularly rich in psychrophilic genera in migratory trout (Figure S6). We also paid special attention

to bacterial genera associated with the degradation of hydrocarbons, such as polycyclic aromatic hydrocarbons (PAHs). We found that migratory trout sampled at Acaena were particularly rich in this regard compared to those at other sites (Figures S7 and S8) [52]. Migratory trout from this site also had skin and blood microbiome signatures that were abundant in potentially pathogenic genera compared to other migratory or sedentary trout (Figure S9). Taken together, these results showed that both skin and blood microbiome signatures are site-dependent and may reveal specific environmental conditions.



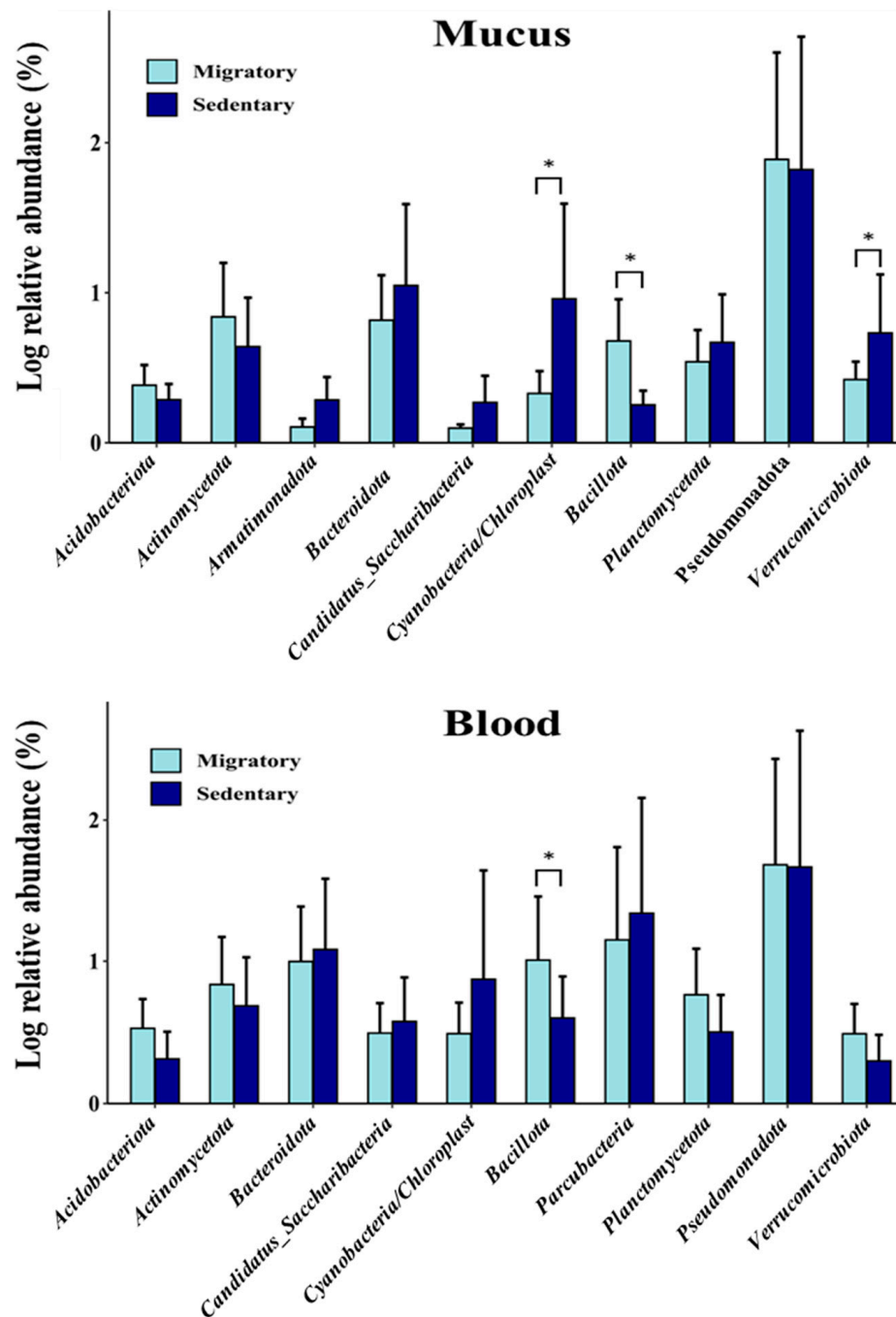
**Figure 4.** Comparisons between migratory and sedentary trout in both compartments. (A) The Chao1 index was calculated for each group. Venn diagrams showing the number of unique and shared (B) bacterial phyla and (C) genera in mucosal and circulating compartments in both sedentary and migratory brown trout (*S. trutta*). Note: \*  $0.01 < p \leq 0.05$ ; \*\*\*  $p \leq 0.001$ .

### 3.4. Functional Analysis of the CmDNA

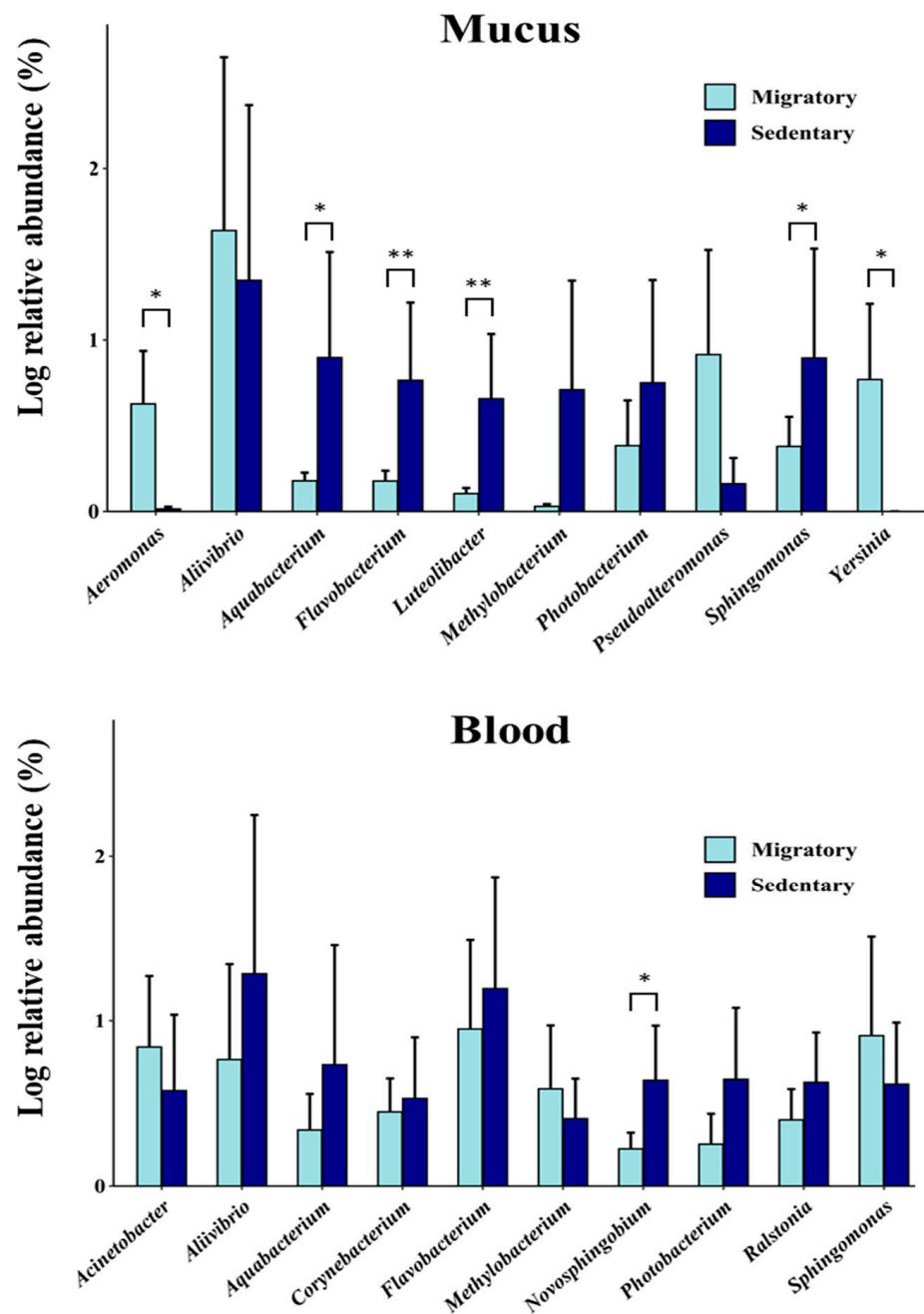
Predictive functional analysis from 16S rRNA profiling of cmDNA is routinely used to link the abundance of specific taxa with metabolic profiles [50,53,54]. To further explore



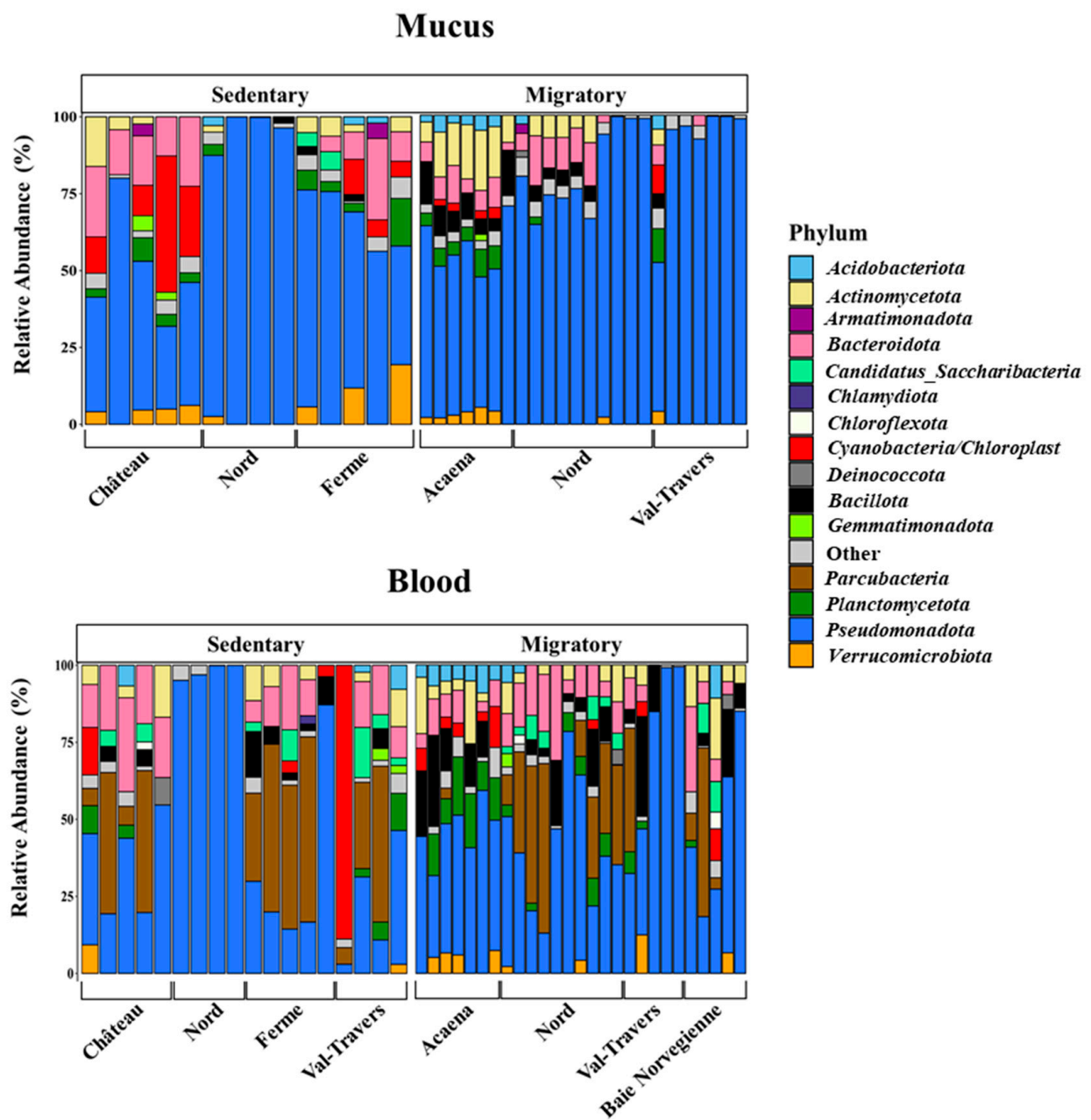
the distinctive traits between sedentary and migratory *S. trutta*, we studied the predicted metabolic pathways based on 16S rRNA. This analysis revealed two major differences. The first is the distinct difference in the metabolic profiles of migratory versus sedentary trout (Figure 8). We found a clear lipid metabolism shift consistent with previous studies in the salmonids [55]. A similar shift has recently been observed in lipid metabolism in the skin microbiome of *S. salar* during experimental seawater acclimatization [8]. Among the other potential differences between the sedentary and migratory microbial communities in terms of metabolism, we found a generally higher amino metabolism and biosynthesis of beta-lactams in the latter form, consistent with previous findings by Dehler et al. [8].



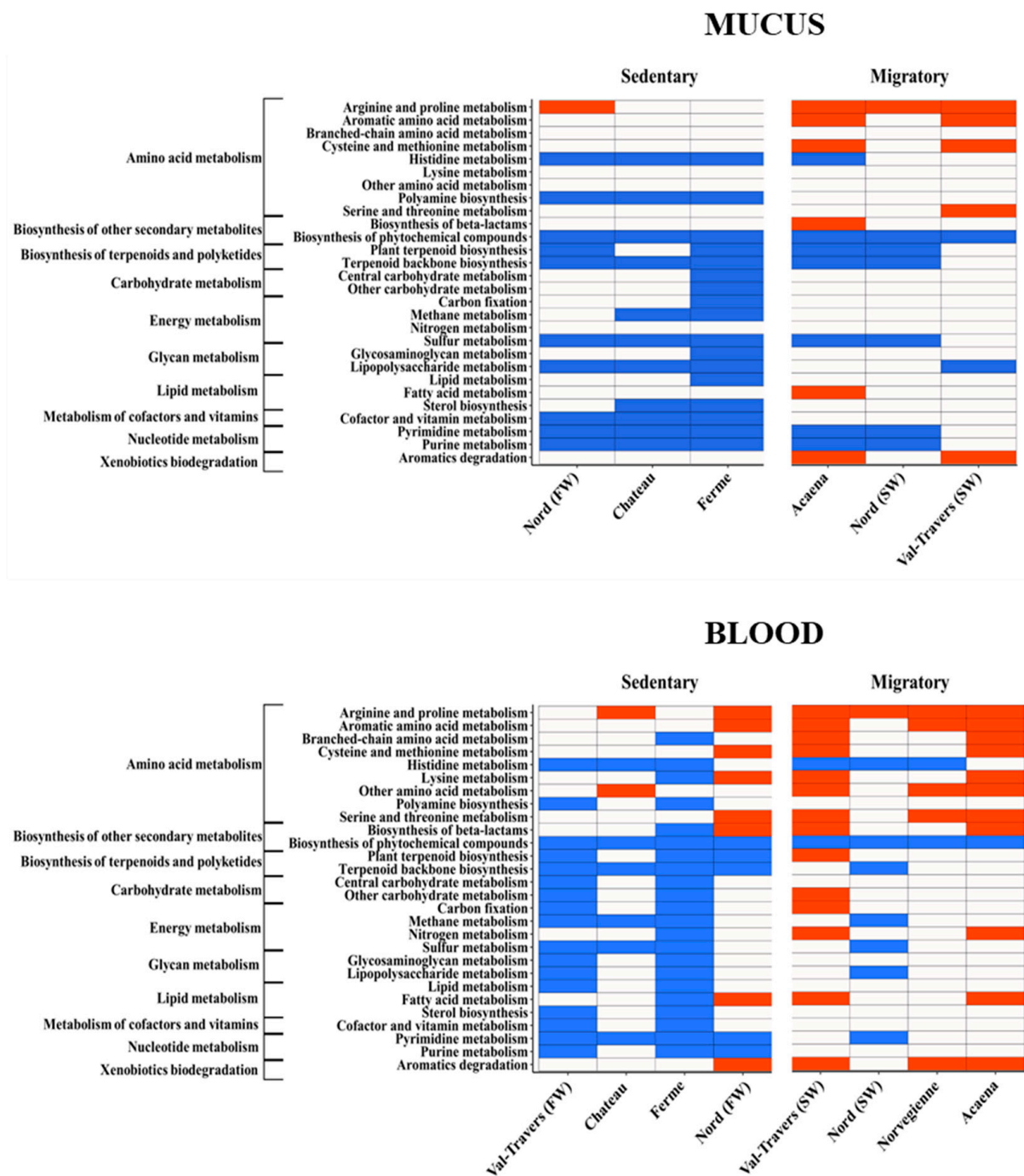
**Figure 5.** Relative abundance (%) of the top 10 bacterial phyla in circulating and mucosal microbiomes in *S. trutta*. Bar graph analysis displays the logarithm (base 10) of the relative abundance (mean  $\pm$  SE). White and black colors represent migratory and sedentary brown trout, respectively. Note: \*  $0.01 < p \leq 0.05$ .



**Figure 6.** Relative abundance (%) of the top 10 bacterial genera in circulating and mucosal microbiomes in *S. trutta*. Bar graph analysis displays the logarithm (base 10) of the relative abundance (mean  $\pm$  SE). White and black colors represent migratory and sedentary brown trout, respectively. Note: \*  $0.01 < p \leq 0.05$ ; \*\*  $0.001 < p \leq 0.01$ .



**Figure 7.** Inter-individual variability of circulating and mucosal microbiomes at the phylum level. Bar plots display the major phyla between sedentary (left) and migratory (right) brown trout (*S. trutta*) collected at different sites. Phyla with a relative abundance of  $\leq 1.5\%$  are represented as “Other”.



**Figure 8.** Heatmaps show the relative abundance (natural logarithm base) of metabolic pathways based on 16S rRNA data in migratory and resident trout collected from different sites.

#### 4. Discussion

In the present work, we compared the skin and blood microbiomes of the Kerguelen Islands' sedentary and migratory *S. trutta*. We have shown that the skin and blood microbiome signatures (1) differ at both the phylum and genus levels, (2) differ between migratory and sedentary trout, and (3) are site-dependent. This is the first study comparing the skin and blood microbiomes of wild sedentary and migratory salmonids and the first characterization of the circulating blood microbiome in a fish species. Finally, from a methodological perspective, our logistically simple and minimally invasive sampling platform offers an alternative approach for the long-term monitoring of fish populations in sensitive and remotely polar ecosystems.

Defining microbiome signatures is now recognized as a key step toward identifying factors shaping animal-associated microbiomes, including genetic (species-specific) and environmental factors. In teleost, in particular, the gut, mucosal, or blood microbiome is increasingly recognized as an important biomarker to detect disturbances in ecosystems or aquacultures. However, studies on the microbiome composition of wild fish populations remain relatively scarce and have, until now, mostly focused on the gut- and skin-associated microbiomes in fish farms. In the case of salmonids, Lokesh and Kiron (2016) have shown that the skin-associated microbiome of Atlantic salmon (*S. salar*) in the transition from freshwater to seawater in Norway was dominated by *Pseudomonadota*, *Bacteroidota*, and *Bacillota* [56]. Our data also revealed an abundance of *Pseudomonadota* and *Bacteroidota* in the skin-associated microbiome of both migratory and sedentary *S. trutta* of the Kerguelen Islands. This dominance was also found in the blood microbiome. In our study, however, the dominance of *Pseudomonadota* was not related to the abundance of the *Oleispira* genus, as reported for *S. salar* [8]. In fact, we did not find a dominance (or even a detection, for that matter) of *Oleispira* in the skin mucus (or in the blood) of migratory trout. Another difference from the study reported for *S. salar* is that we did not find a dominance of *Bacillota* in the skin-associated microbiome of sedentary trout [8]. Instead, we found that *Bacillota* were more abundant in migratory *S. trutta*. This was true for both mucosal and blood microbiomes. This increase in *Bacillota* in migratory *S. trutta*, which was driven by the presence of *Aerococcus*, *Bacillus*, *Hathewayia*, and *Clostridium\_sensu\_stricto* genera, which differed from the *Bacillota* found in the sedentary trout, which mainly included *Staphylococcus* and *Lactobacillus* genera. We also found an increased abundance of *Verrucomicrobiota* and *Cyanobacteria* in the skin mucus of sedentary trout. This shift was not found in the blood microbiome. Other bacteria previously found in the skin mucus of *S. salar*, such as *Thalassomonas*, *Psychromonas*, *Agarivorans*, *Pseudoalteromonas*, *Marinomonas*, *Arcobacter*, *Perluclidibaca*, and *Octadecabacter*, were also absent in *S. trutta*. However, a clear difference at the phylum level was noted by the abundance of *Actinomycetota* and *Parcubacteria* in the blood microbiome, but not in the mucus, of both migratory and sedentary trout. This signature is, in fact, similar to a recent metagenomic study showing that bacteria enriched in seawater in polar regions were mostly *Pseudomonadota*, *Actinomycetota*, *Bacteroidota*, and *Parcubacteria* [50], the latter being one of the most abundant phyla in intestinal fish microbiota [57,58]. Interestingly, we also found a similar signature in the hemolymphatic (blood-like) microbiome of mussel species (Figure S10) that inhabit the coastal marine ecosystems of the Kerguelen Islands [59]. These results suggest the existence of a possible “Kerguelen signature,” at least at the phylum level, driven by the environmental conditions of the Kerguelen Islands. Metagenomic profiling using the 16S rRNA microbiome signature is a cost-effective and rapid method to screen for candidate pathogens associated with infectious and noninfectious diseases in a given population [16,60–63]. Here, we paid particular attention to this aspect, given the history of salmonids in the Kerguelen Islands and their isolation from other salmonid populations. Our data revealed the presence of *Aliivibrio* and *Pseudomonas* within the skin mucus and blood microbiomes of all trout from the Kerguelen Islands. These genera include several pathogenic strains, such as *Aliivibrio salmonicida*, a common pathogen found in fish farms [64]. Our study also revealed the presence of *Renibacterium* in the skin microbiome of migratory trout at Acaena. This genus includes *Renibacterium salmoninarum*, the causative agent of bacterial kidney disease, a deadly disease affecting wild and cultured salmonids worldwide [65]. This pathogen was introduced in 1987 following the importation of Chinook salmon from the United States into the Armor basin. This was one of the reasons why the Aquasaumon Sea ranching project at Armor was abandoned [66]. There is a possibility that the bacteria have spread, since infected juveniles escaped from Armor in 1987. Moreover, Artic chars, kept in the Armor Hatchery, were released into the nearby Lac des Fougères in 1991 [35,67]. As of 2012, no signs of the disease have been observed [35]. We confirmed the presence of DNA derived from this bacterium by PCRs in mucosal and blood samples of brown trout collected in the Acaena river (Figure S11). Among the other sites that were distinguishable was

Rivière-du-Nord. The microbiome of either sedentary or migratory trout showed a unique signature. This was generally apparent in the blood microbiome of sedentary trout, which were dominated by two genera: *Aliivibrio* and *Photobacterium*. The dominance of *Aliivibrio* was also found in the skin microbiome. Whether such dominance of *Aliivibrio* reveals the presence of *Aliivibrio salmonicida*, a common pathogen known to cause cold-water vibriosis in salmonids [68], is certainly an issue that warrants further investigation. This pathogen, found mainly in estuaries, is usually found in high amounts in the blood of moribund fish. ASVs corresponding to *Aliivibrio salmonicida* were only found at Rivière-du-Nord, while all ASVs from other sites corresponded to *Aliivibrio logei*, a commonly found genus in the skin and gut microbiota [69]. Interestingly, the sedentary trout sampled at Rivière-du-Nord harbored an almost identical metabolic profile to that of migratory trout [70]. However, it is important to note that the 16S method does not allow for the identification of pathogens per se, but represents a rapid means to screen for potential pathogens and expression of virulence genes in subsequent analyses. Moreover, the presence of blood DNA fragments of bacterial pathogens does not necessarily reflect the onset of a disease. Rather, it provides a rapid, ethical, and sensitive means to signal their presence. The idea of a circulating microbiome is a concept increasingly studied in humans for identifying biomarkers in a clinical context. Despite all the precautions associated with the collection of samples, the fact remains that precautions are essential to minimize the impact of contaminants in the interpretation of the results [71–73]. These precautions are even more important when this concept is applied in environmental ecology, particularly in an inhospitable environment, such as the subantarctic islands such as those of Kerguelen. Despite the precautions we took during sampling, including proper aseptic cleaning methods, the use of sterile equipment, no template (water) controls, the use of FTA blank cards (and adjacent punches on the sampled cards), as well as the use of laboratories, materials, and equipment specifically dedicated to the preparation of DNA, it is necessary to remain critical in the interpretation of the results. This is particularly the case in studies that provide a snapshot of the microbiome, as in this case, and this is why it is important to carry out spatiotemporal analyzes and to carry out the validation of certain unexpected results, such as the presence of genera commonly associated with pathogenic species of interest, such as *Renibacterium salmoninarum*, which we found at specific sites in the Kerguelen Islands. Without a doubt, the use of logistically simple DNA sampling and preservation methods that minimize contamination in the field are useful tools for such future studies. Overall, our study may help better evaluate the impact of specific microbial structures on the fitness-related traits of specific populations, including their dispersal and reproductive abilities. Indeed, their presence in pathogenic strains would imply a higher energy expenditure on the immune system and a possible eco-evolutionary effect on MHC-related genes. For instance, MHC genes are highly homozygous in the Val Travers population, which is quite unusual compared to the data available in the literature. This could be partly related to inbreeding or the relaxation of selective pressures on pathogens [74].

Our data further revealed that another type of information that can be obtained from microbiome-based signatures is related to the physicochemical characteristics of the ecosystem. A clear example is the abundance of hydrocarbon degrader genera within the microbiomes of migratory trout sampled at Acaena. We hypothesize that their presence at the mouth of the Acaena River is possibly due to the nearby presence of multiple lignite deposits, also called “brown coal” at the Ravin du Charbon and the Ravin Jaune near the Acaena River [75].

To our knowledge, this is one of the rare studies of the blood microbiome of fish. Historically, most studies on the microbiome composition were carried out on the gut microbiome, with emphasis on their role in nutrition and related diseases, including inflammatory diseases. However, advances in sequencing NGS technologies have shown that plasma DNA fragments originate from multiple organs, providing an opportunity to obtain a systemic view of tissue damage. It is now possible to determine the exact tissue origin of plasma DNA fragments by examining footprints of consensus sequences

specific for transcription factors [76]. These findings have contributed to the emerging concept of the circulating microbiome and subsequent studies showing that the blood contains a rich source of microbial DNA to assess the host's health status [12,13,77]. As the blood microbiome originates from multiple tissues [78,79], the possibility of using a single drop of blood for monitoring a much broader microbial diversity within a host opens the door for easier monitoring and the understanding of factors that contribute to shaping the microbiome in aquatic species (as well as terrestrial species, for that matter), most notably in response to environmental stressors, including climate change.

Finally, we would like to briefly discuss the sampling approach used in our study. This study and our previous work on bivalves indicate that FTA<sup>®</sup> card-based sampling is perfectly adapted for establishing skin and blood (or hemolymphatic) 16S rRNA microbiome signatures. The efficacy of FTA<sup>®</sup> cards as a stable means to preserve DNA samples, even at room temperature, has been well documented [39]. Such a minimally invasive and ethical (nonlethal) sampling procedure is particularly well adapted for long-term monitoring programs in remote areas and for limiting the impact of large cohort studies on a given population inhabiting, for example, natural reserves (such as the Kerguelen Islands), for endangered species, or for storage and transport for fieldwork in areas where proper conditions for RNA preservation are challenging to achieve [80]. Sampling using FTA<sup>®</sup> cards is gaining momentum as it is compatible with basic nucleic acid-based detection methods. It has been used, for example, for molecular diagnosis, the detection of viruses, etc. [81,82]. It is particularly useful for safely transporting infectious material, which is rapidly inactivated upon binding the nucleic acid to the chemically modified paper [80]. This low-cost method is logistically simple (without the need to maintain a cold chain for sample integrity) and is ideally adapted for biobanking.

## 5. Conclusions

In conclusion, we have shown that migratory and sedentary trout of the Kerguelen Islands exhibit unique microbiome signatures compared to other salmonid populations reported in the Northern Hemisphere. It will be interesting to study the impacts of these microbial shifts on host physiology and how these signatures may change in response to climate change and the colonization process of salmonids in this unique ecosystem. This work also revealed the presence of potential pathogens at some sites and highlighted the impact of environmental factors on the microbiome. Finally, this study showed that FTA<sup>®</sup> Cards-based sampling is adapted for research in remote regions for establishing mucosal and circulating 16S rRNA microbiome signatures.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/fishes8040174/s1>, Figure S1: Individual variations in the circulating and mucosal microbiomes at the phylum level; Figure S2: Discriminant genera between the circulating and mucosal compartments; Figure S3: Alpha diversity analysis of mucosal and circulating microbiomes in (A) sedentary and (B) migratory brown trout (*S. trutta*); Figure S4: Principal Coordinates Analysis (PCoA) of bacterial DNA bacterial communities in both sedentary and migratory brown trout (*S. trutta*); Figure S5: Heatmaps showing relative abundance (%) of the top 30 bacterial genera of mucosal and circulating microbiomes between sedentary and migratory brown trout (*S. trutta*); Figure S6: Presence of psychrophilic bacterial genera isolated from Kerguelen sites and compared to Poli et al. (2017) identification of psychrophilic bacteria; Figure S7: Presence of Kerguelen bacterial genera commonly associated to oil spills (cited more than four times in different scientific papers) based on Hedao et al. (2018); Figure S8: Presence of Kerguelen bacterial genera known to degrade alkanes, phenols and phenanthrene which can be used as biomarkers for PAHs detection, based on Kuchi et al. (2021); Figure S9: Presence of potentially pathogenic bacterial genera found at different sites in Kerguelen; Figure S10: Bacterial cmDNA profiles in blue mussels (*Mytilus platensis*) and brown trout (*S. trutta*) collected at the mouth of the Norvegienne river; Figure S11: Amplified fragments shown on 1.5% agarose gel electrophoresis; Table S1: Characteristics of brown trout (*S. trutta*) collected at different sites at Kerguelen Islands.

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**Data Availability Statement:** The raw data files used in this study are publicly available on the NCBI Sequence Read Archive (PRJNA772886).

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