

The gut microbiome and its role in regulating voluntary running behavior in male rats

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Introduction

Exercise has well-established physiological benefits, and a lack thereof often gives rise to health conditions, including metabolic syndrome, diabetes mellitus, and heart disease.

Exercise encompasses a spectrum of activities, but despite its beneficial impacts, its duration, intensity, and frequency may vary on an individual level.

Prior studies have established connections between our gut microbiome (GMB) and factors such as disease pathogenesis, recovery, and behavioral activities. Here, we further assess exercise variability in male rats and explore the relationship between voluntary wheel running and GMB composition.

Methods

Six- to eight-week-old male Sprague-Dawley rats were randomized into running (n=8) and sedentary groups (n=3). After an initial 7-day acclimation period during which no running occurred, all rats were housed individually. The running group had full access to a running wheel for an additional four weeks with living chambers equipped with a wheel.

Daily and cumulative distances were recorded daily, except for T+4 and T+5, which were rest days. Weight gain was recorded at the end of each running week. Fecal samples for sequencing were collected from each rat once per week, starting during the 7-day acclimation period, which resulted in five total samples. Seven-day average running behaviors identified two distinct groups of high (8,341 – 10,209 meters/week) and low (1,736 – 5,201 meters/week) runners.

Alpha and beta diversities of the GMB were conducted using QIIME2. We used generalized least square (GLS) mixed models on R to identify taxa that significantly correlated with the outcome.

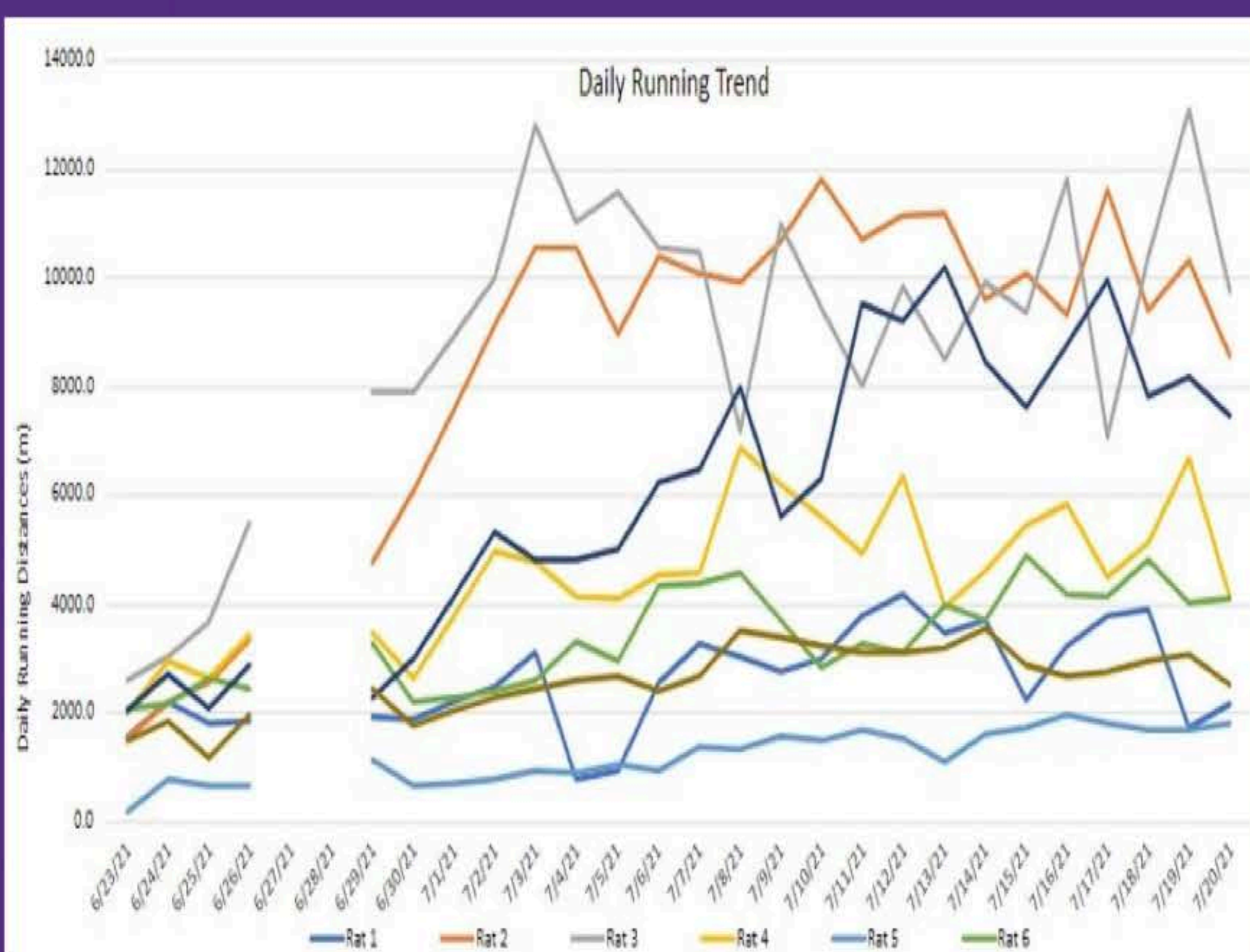


Figure 1. Daily running distance trend for rats over the experiment. First four days represent baseline (time=0)

Results

Alpha diversity using the Shannon metric was not significantly different between sedentary, low, and high runners ($H = 1.845$, p -value = 0.397). Beta diversity was significantly different between high and low runners (pseudo-F = 3.811, p -value = 0.006) and high runners and the sedentary group (pseudo-F = 4.677, p -value = 0.003) only.

We next determined whether the relative abundance of individual taxonomic features at baseline (pre-running) predicted the final 7-day average running distance. Only one taxonomic feature of the family Rikenellaceae was a near-significant predictor ($R^2 = 0.54$, p -value = 0.054), which suggests that **baseline GMB does not predict terminal running behavior**.

When assessing for taxonomic differences between run groups, an increased relative abundance of **Ruminococcaceae**, specifically ***R. bromii***, was noted in high vs low runners. When plotting the distribution between relative abundance and daily distance, Ruminococcaceae had a significant positive correlation ($R^2 = 0.33$, p -value < 0.001) with *R. bromii* having the strongest R^2 ($R^2 = 0.55$, p -value < 0.001) in high vs low runners.

A **significant positive** correlation between Ruminococcaceae abundance and daily running distance was observed in **high runners only** ($R^2 = 0.65$, p -value < 0.001). No significant correlation was seen in low runners ($R^2 = 0.14$, p -value = 0.070). There was a **significant positive** correlation between Ruminococcaceae and time in both **high** ($R^2 = 0.36$, p -value = 0.023) and **low** ($R^2 = 0.34$, p -value = 0.003) runners.

A **significant negative** correlation between Peptostreptococcaceae abundance and daily distance was observed in **high runners** (R^2 value: -0.47, p -value: 0.006) whereas low runners had no correlation. Peptostreptococcaceae had a **significant negative** correlation with time in the **high runners** ($R^2 = -0.42$, p -value = 0.012). A significant negative but weaker correlation was observed in **low runners** ($R^2 = -0.24$, p -value = 0.078). The sedentary group had a negative correlation that was not significant ($R^2 = -0.03$, p -value = 0.421).

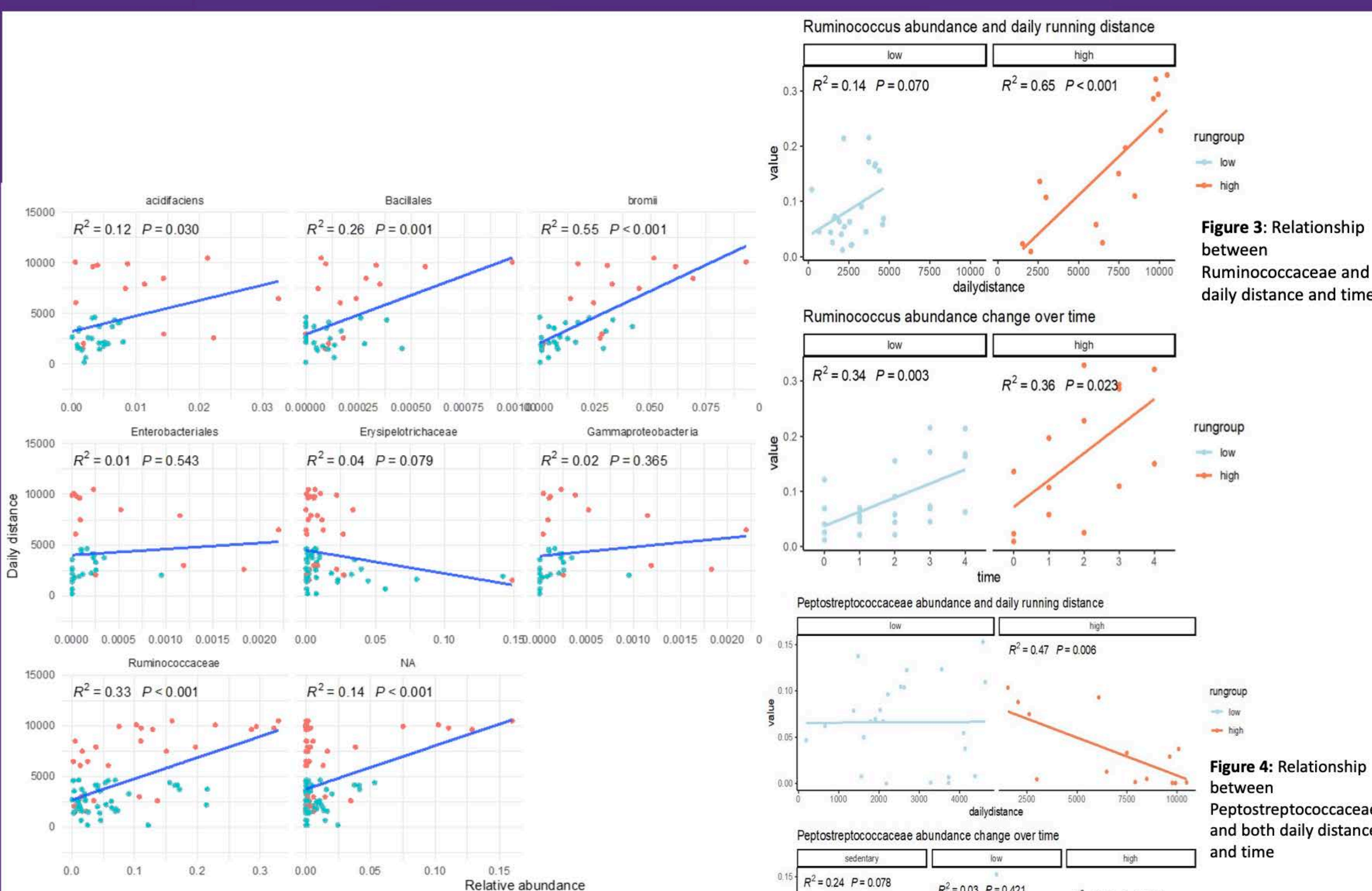


Figure 2: Relationship between abundance and daily distance of significantly different features by run group

Discussion

Voluntary running showed a marked influence on beta diversity. Our findings suggest differences in the composition and/or relative abundance of taxa in samples from the high and low run groups and the high run group and sedentary group. Existing literature describes associations between lower beta diversity and health complications such as hypertension, anxiety, and depression.

Further analysis demonstrated a stronger positive relationship between Ruminococcaceae abundance and daily running distance as well as time in the high run group compared to the low run group. This stronger association suggests that the abundance increases faster in high runners. It is important to note that “time” in our experiment also represents exposure to running for the running group. Ruminococcaceae converts starches into short-chain fatty acids (SCFAs), which are known to play a role in maintaining gut barrier integrity, immune/inflammatory responses, and metabolism.

A similar, yet inverse, finding was observed with Peptostreptococcaceae. The high run group had a stronger negative relationship with daily running distance and time, suggesting that the abundance decreases faster in high runners. The fact that the sedentary group also had a negative correlation with time, despite not being significant, demonstrates that this change in relative abundance is not due to running behavior alone. Interestingly, research has shown a beneficial role of Peptostreptococcaceae in the gut microbiome as this bacteria produces butyric acid, another SCFA that plays a role in increasing the absorption of nutrients and intestinal growth.

Further investigation is needed on the impact of these microbiome changes to determine their effect on the overall health of the host. The role of specific gut microbes on host health is a complex and active field of study. Our results show greater diversity of gut microbiota in rats with higher distance running behavior, suggesting an interaction between microbiome and exercise behavior. These findings are consistent with previous literature that gives evidence of a so-called gut-brain axis in which host microbiota influence host health and behavior.

Acknowledgements

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