

# Interactions Between Soil-, Plant-, and Host-Associated Microbiota in Laminitic and Control Horses



Veterinary Research  
Scholars Program  
University of Missouri

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## Background

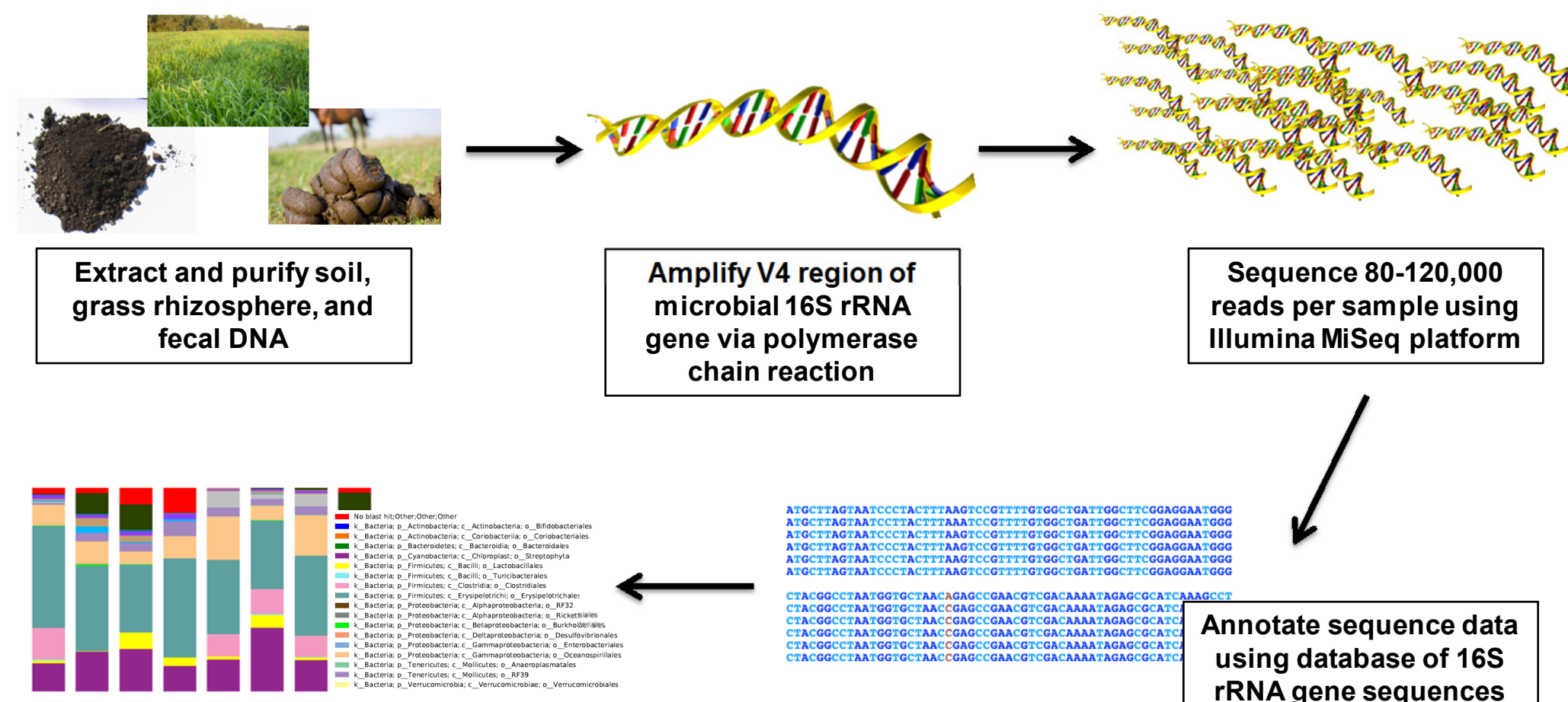
- Beyond 'time of year' and non-structural carbohydrate (NSC) content, risk factors for pasture-associated laminitis (PAL) in grazing horses are not well understood.
- PAL risk is likely attributable to host endocrinological factors (e.g., hyperinsulinemia) in response to increased NSC consumption.
- Other contributing factors such as interactions between the environmental and host gastrointestinal tract (GIT) microbiota have not been investigated.
- Using 16S rRNA amplicon sequencing, we evaluated the microbiota of soil and grass rhizosphere (root-associated microbiota) from pastures on which PAL-affected horses were grazing, and the fecal microbiota of PAL-affected and non-laminitic (control) horses grazing on the same pasture.
- We hypothesized that there would be differences in the soil- and grass-associated microbial populations of PAL-associated pastures, possibly affecting the GIT microbiota and predisposing to PAL.

## Sample Collection



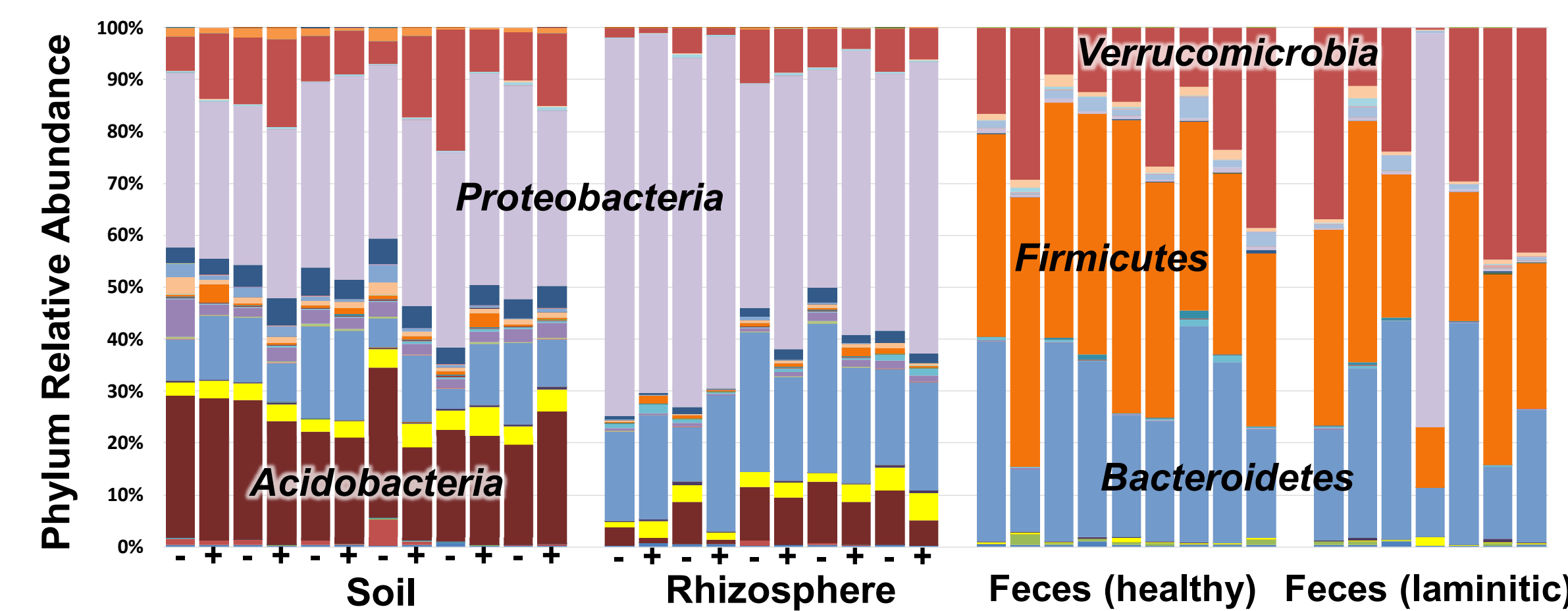
**Schematic depiction of sampling protocol.** Soil and grass samples were collected from both PAL-associated pastures and control (ungrazed) pastures. Fecal samples were collected from laminitic and control (unaffected) horses, grazing the same pasture.

## Sample Processing

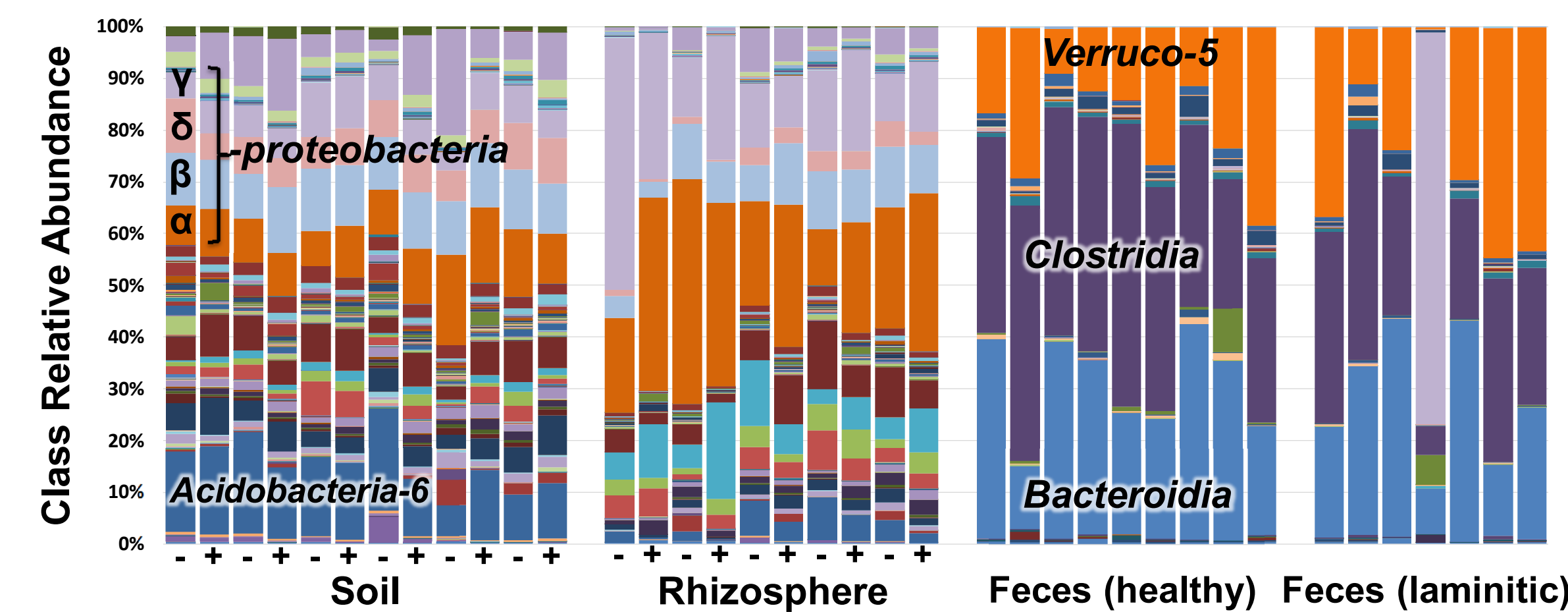


**Schematic of sample processing pipeline.** DNA was extracted and purified; V4 region of 16S rRNA gene was amplified, sequenced, and annotated.

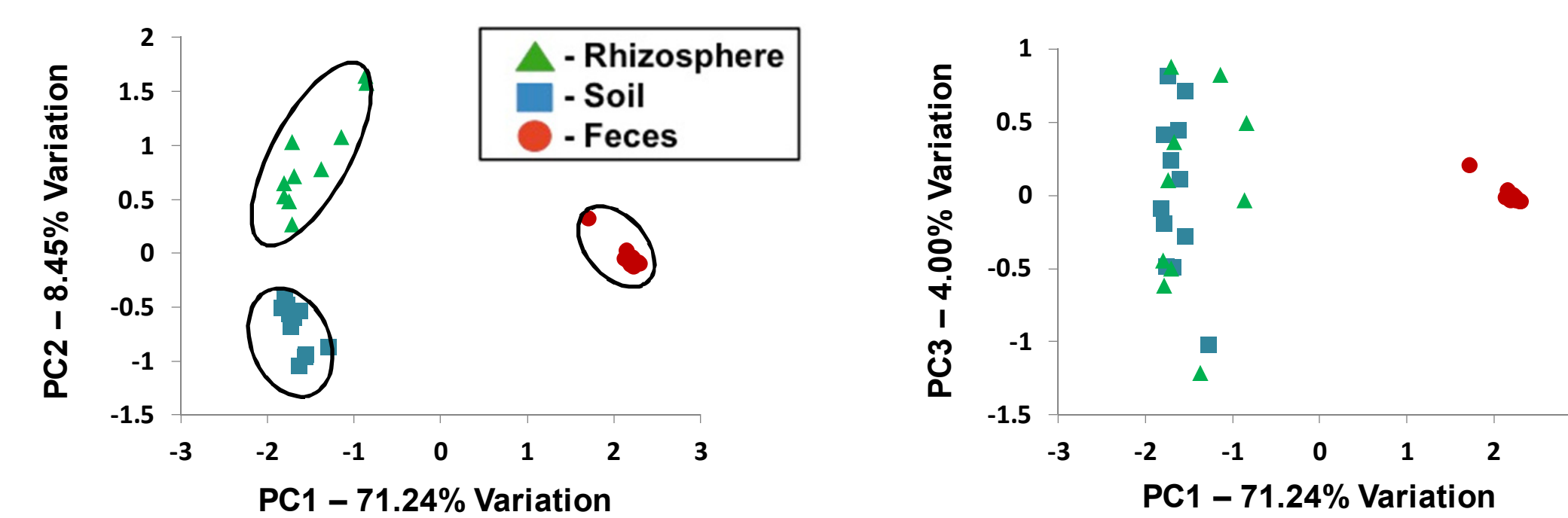
## Environmental and GIT Microbiota



**Figure 1:** Bar charts showing the relative abundance of soil-, rhizosphere-, and fecal microbiota at the level of phylum. Control (-) and PAL-associated (+) pasture samples are indicated.

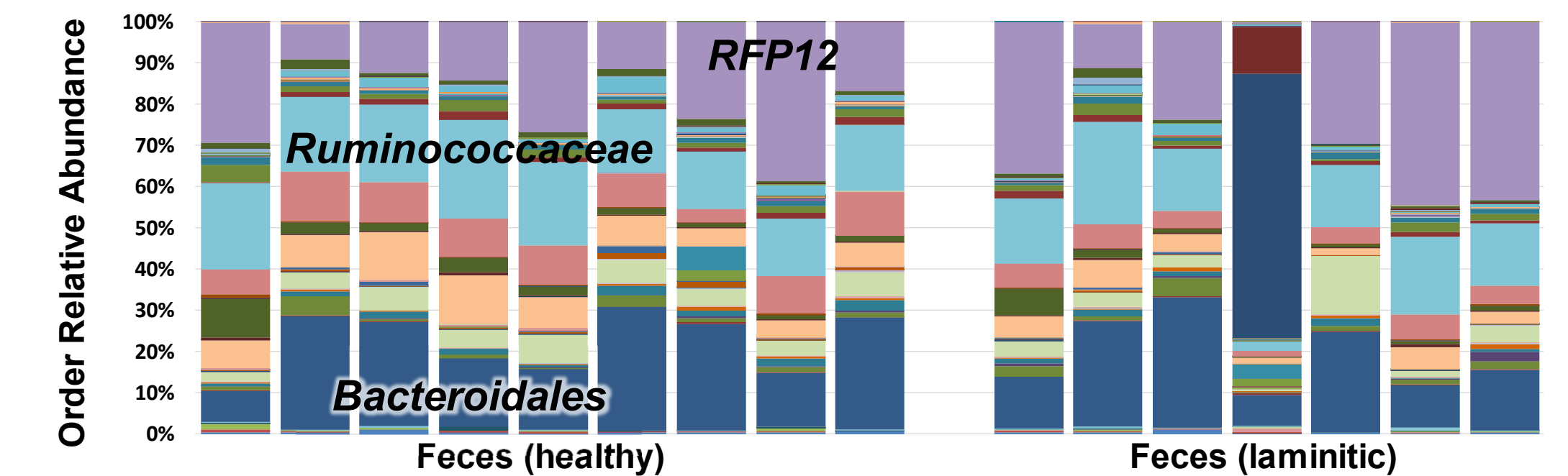


**Figure 2:** Bar charts showing the relative abundance of soil-, rhizosphere-, and fecal microbiota at the level of class. Control (-) and PAL-associated (+) pasture samples are indicated.

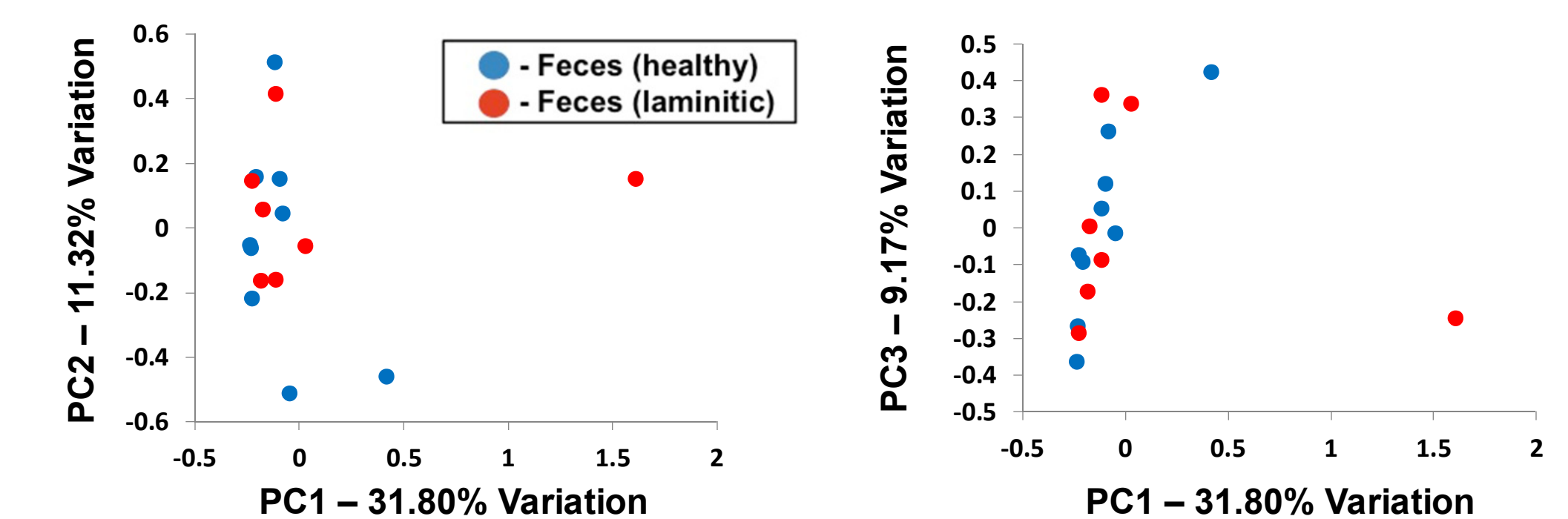


**Figure 3:** Principal component analysis of the samples shown in Figures 1 and 2, demonstrating distinct clustering of samples.

## GIT Microbiota in Laminitic Horses



**Figure 5:** Bar charts showing the relative abundance of fecal microbiota for unaffected and laminitic horses at the level of order.



**Figure 6:** Principal Component Analysis of the samples shown in Figure 5. No distinct clustering of samples is seen.

Family	p-value	Relative Δ In Laminitis
Lactobacillaceae	0.020	Decreased
Order Clostridiales, family unknown	0.0126	Decreased
Lachnospiraceae	0.0022	Decreased
Pasteurellaceae	0.00261	Decreased
Sphaerochaetaceae	0.0436	Decreased
Order RF39, family unknown	0.044	Decreased

**Figure 7:** Table of bacterial families detected at significantly different abundance in feces of laminitic and healthy horses (student's t-test).

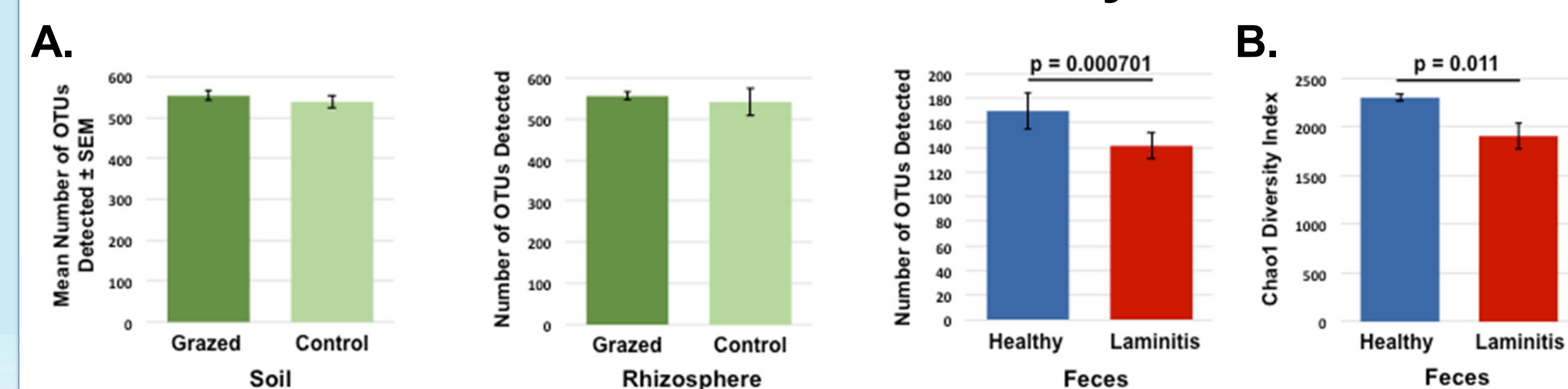
## Conclusions

- Soil and rhizosphere samples are extremely rich with only a minor subset of those microbiota being found in fecal samples; a small number of feces specific microbes were also detected.
- No differences in richness, diversity, or overall composition of environmental samples were detected (PAL-associated versus control pastures).
- A significant decrease in microbial richness and diversity was detected in feces of laminitic horses compared to healthy, unaffected horses.
- No global change is seen in microbiota of laminitic horses. Statistical testing found differences in abundance of select taxa, including putative probiotic families.

## Acknowledgements

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## Richness and Diversity



**Figure 4:** Bar charts showing richness (A) in grazed and control soil and rhizosphere samples, and feces of healthy and laminitic horses. Bar chart showing α-diversity (B) in feces of healthy and laminitic horses.