

Investigating the Distribution and Diversity of Bacterial ACC-d to Promote Plant Growth During Abiotic Stress

Jamell Dacon¹ and Patrick Kearns²

¹Department of Mathematics, Medgar Evers College CUNY, Brooklyn, NY 11225

²Department of Microbiology and Genetics, Michigan State University, East Lansing, MI 48825



Overview

- Introduction
- Hypothesis
- Research Question(s)
- Methods
- Results
- Conclusion

Introduction

- **What is abiotic stress?**

Abiotic stress is the negative effect of non-living elements on the living organisms in a specific environment.

Examples:

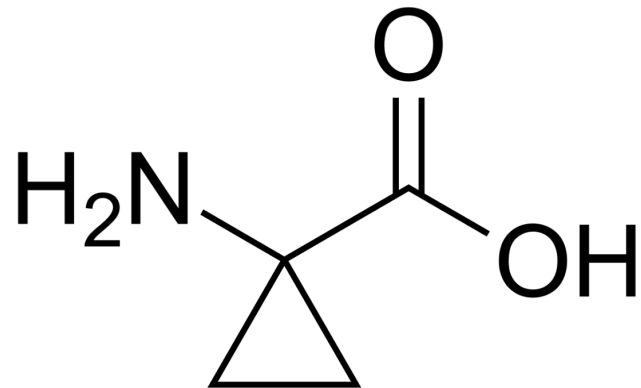
- Ultraviolet Radiation
- Temperature (High and Low)
- Drought
- Flooding
- High Salt Concentrations (Sea Blast)
- Organic/ Nonorganic Contaminants

What happens when a plant is stress?

When plants are stressed, they produce growth-inhibiting stress ethylene, which unfortunately limits their growth and development.

Focus: What is ACC-d?

The bacterial enzyme ACC-d (1-aminocyclopropane-1-carboxylate) deaminase promotes plant growth by lowering excessive plant ethylene levels during abiotic stress.



Hypothesis

Why do we care about ACC-d?

ACC-d is important for our agriculture, as a result of environmental factors such as global warming food security may be negatively affected.

Hypothesis: (A) ACC-d is spread across the phylogenetic tree of life where many organisms have it, not only those in close proximity to plants or have historical associations with plants.

(B) This enzyme will be overrepresented in microbes that have a strong affiliation with plants, then there is an evolved relationship.

Research Question(s)

- Q1: Geographical

What is the distribution of ACC deaminase in the environmental?

- Q2: Diversity

How related are the microbes that have ACC deaminase?

Objectives

- To understand the biogeographic distribution of ACC-d genes across different ecosystems and its diversity among microbial lineages.
- To compare the diversity of ACC-d genes between soil and plant communities, which are relevant to agricultural applications.

Methods

Mined the Joint Genome Institute Integrated Microbial Genomes (JGI IMG) database for ACC-d genes

- Retrieved ~157,000 ACC-d sequences, where nearly 150,000 were full length ACC-d sequences from ~3200 samples
- Collated metadata for each sample, exported in Excel
- Gathered ACC-d abundance and *rplB* abundance for each sample

QIIME

- Operational Taxonomic Unit (OTU) clustering at 85% sequence identity
- Calculate OTU richness
- Calculate alpha and beta diversity based on Bray-Curtis similarity

R

- Data visualization

Results

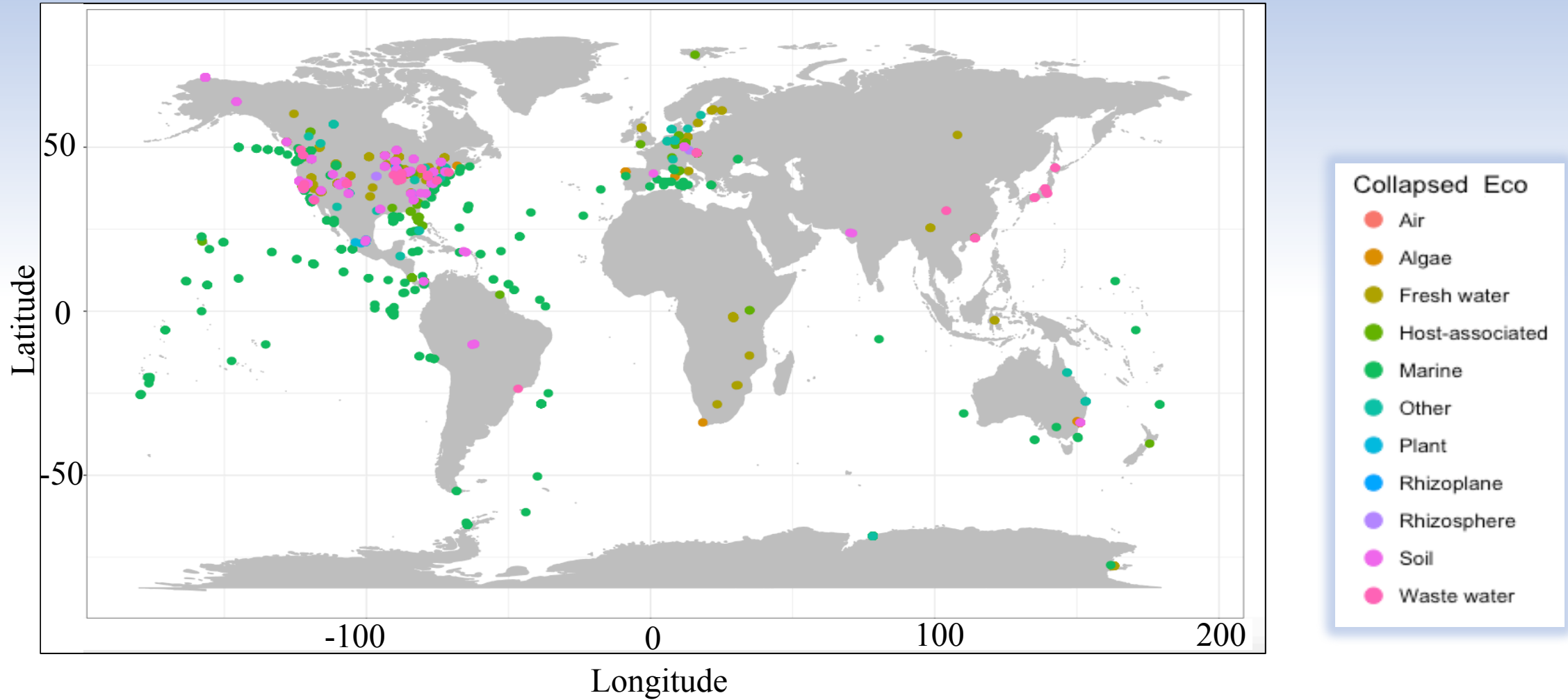


Figure 1. ACC-d Map demonstrates the distribution of ACC-d on a worldwide scale highlighting the areas of study after clustering.

Results

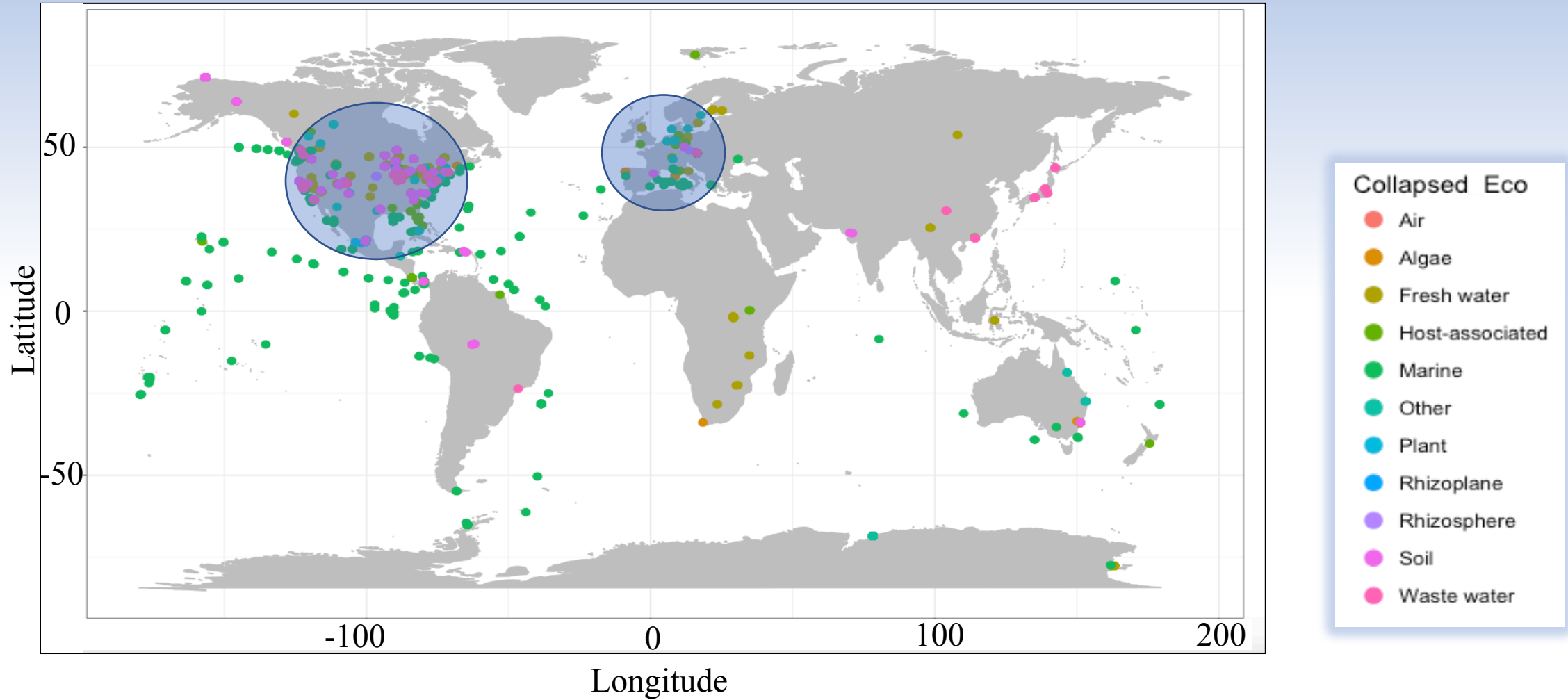


Figure 1. ACC-d Map demonstrates the distribution of ACC-d on a worldwide scale highlighting the areas of study after clustering.

Results

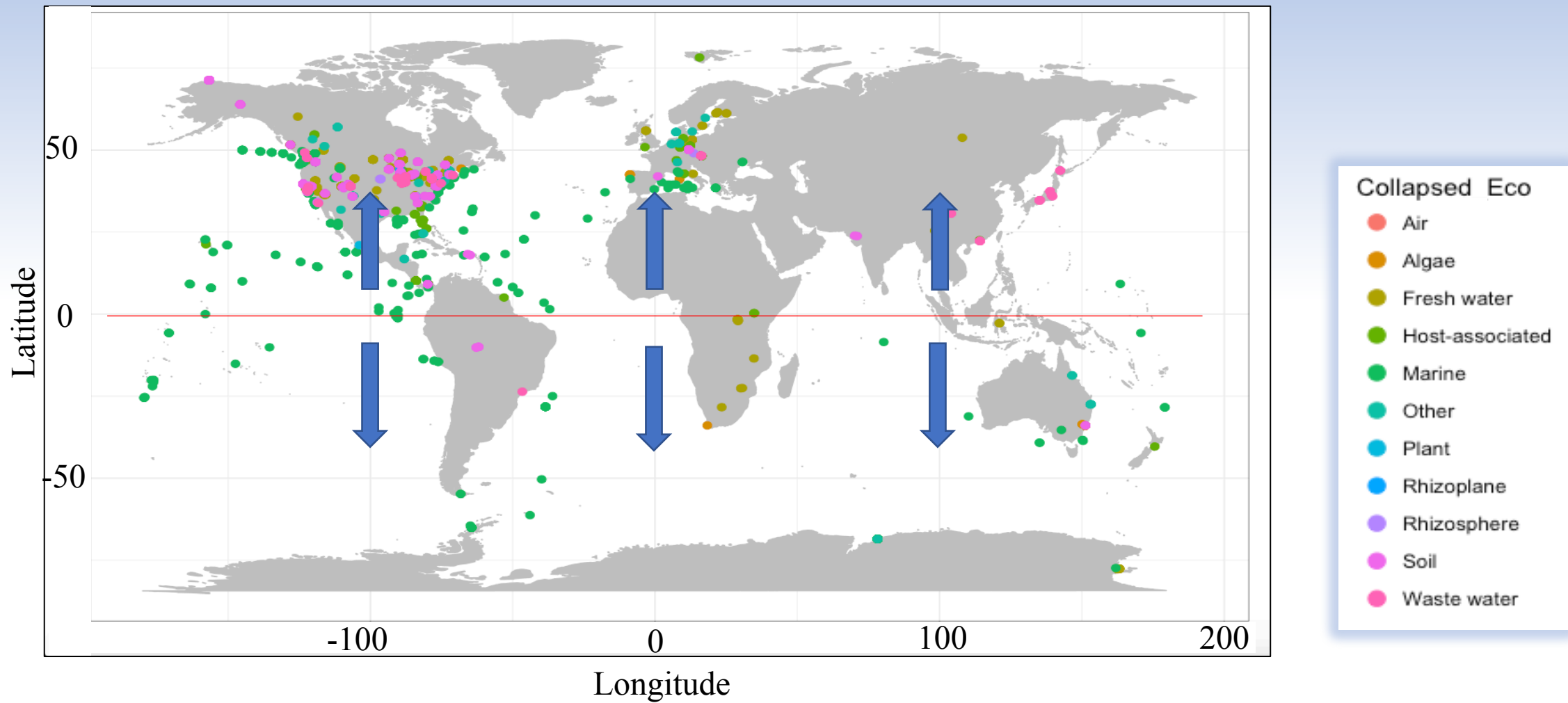


Figure 1. ACC-d Map demonstrates the distribution of ACC-d on a worldwide scale highlighting the areas of study after clustering.

Results

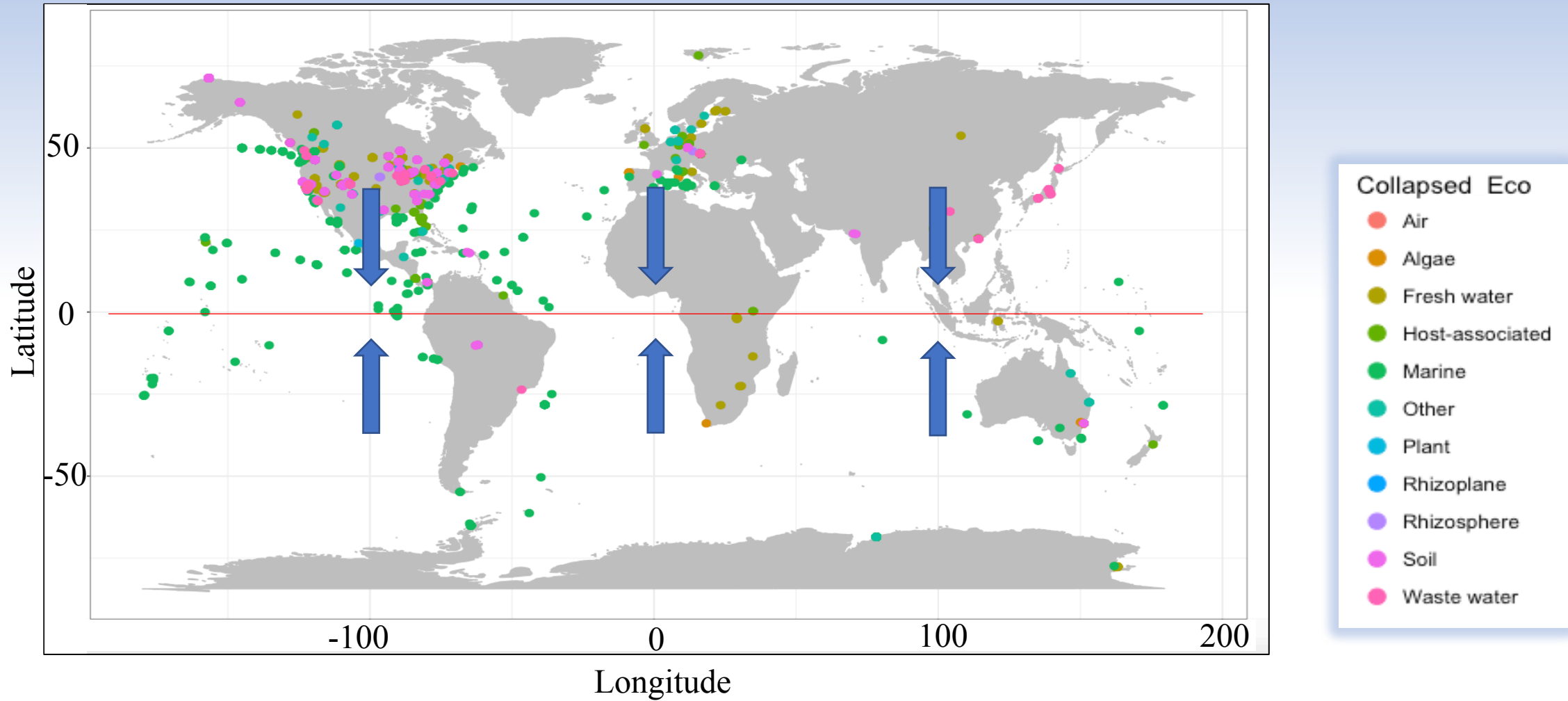


Figure 1. ACC-d Map demonstrates the distribution of ACC-d on a worldwide scale highlighting the areas of study after clustering.

Results

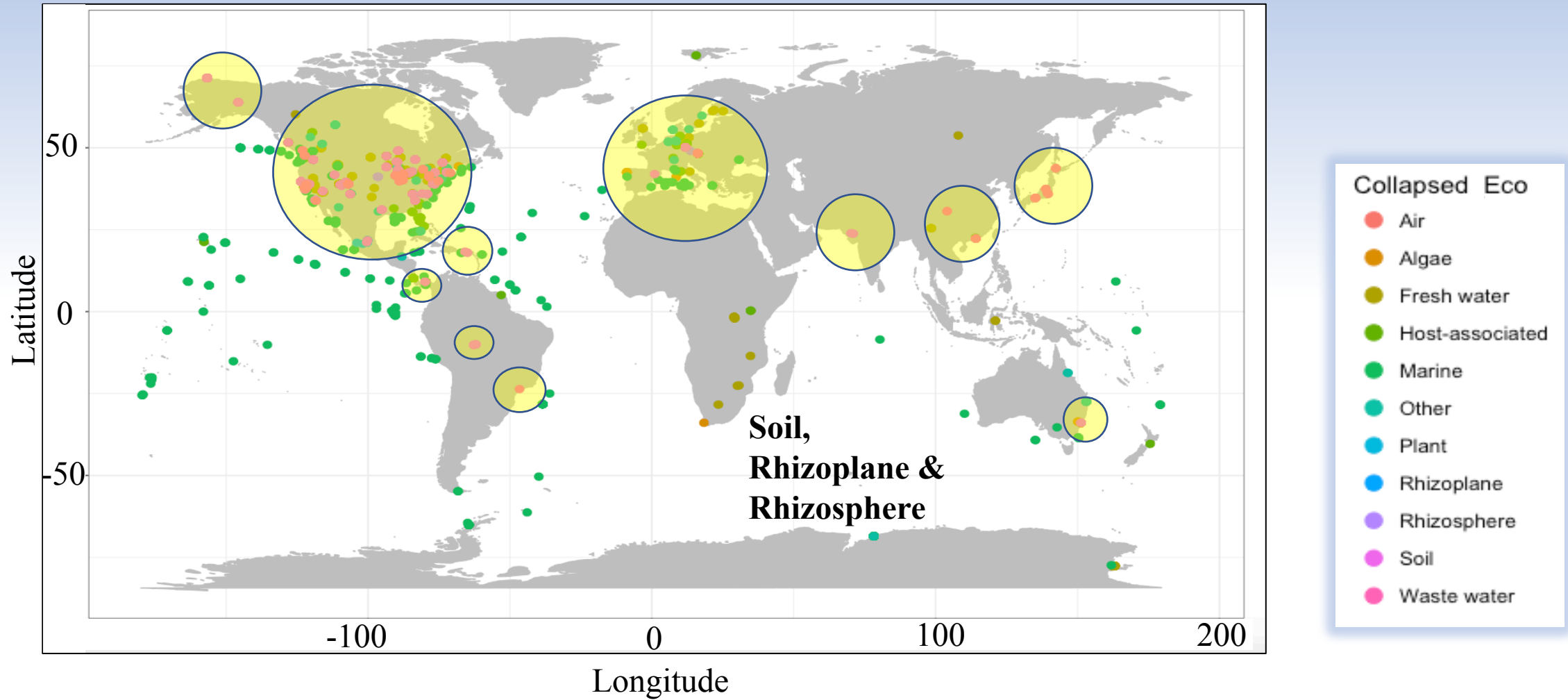


Figure 1. ACC-d Map demonstrates the distribution of ACC-d on a worldwide scale highlighting the areas of study after clustering.

Results

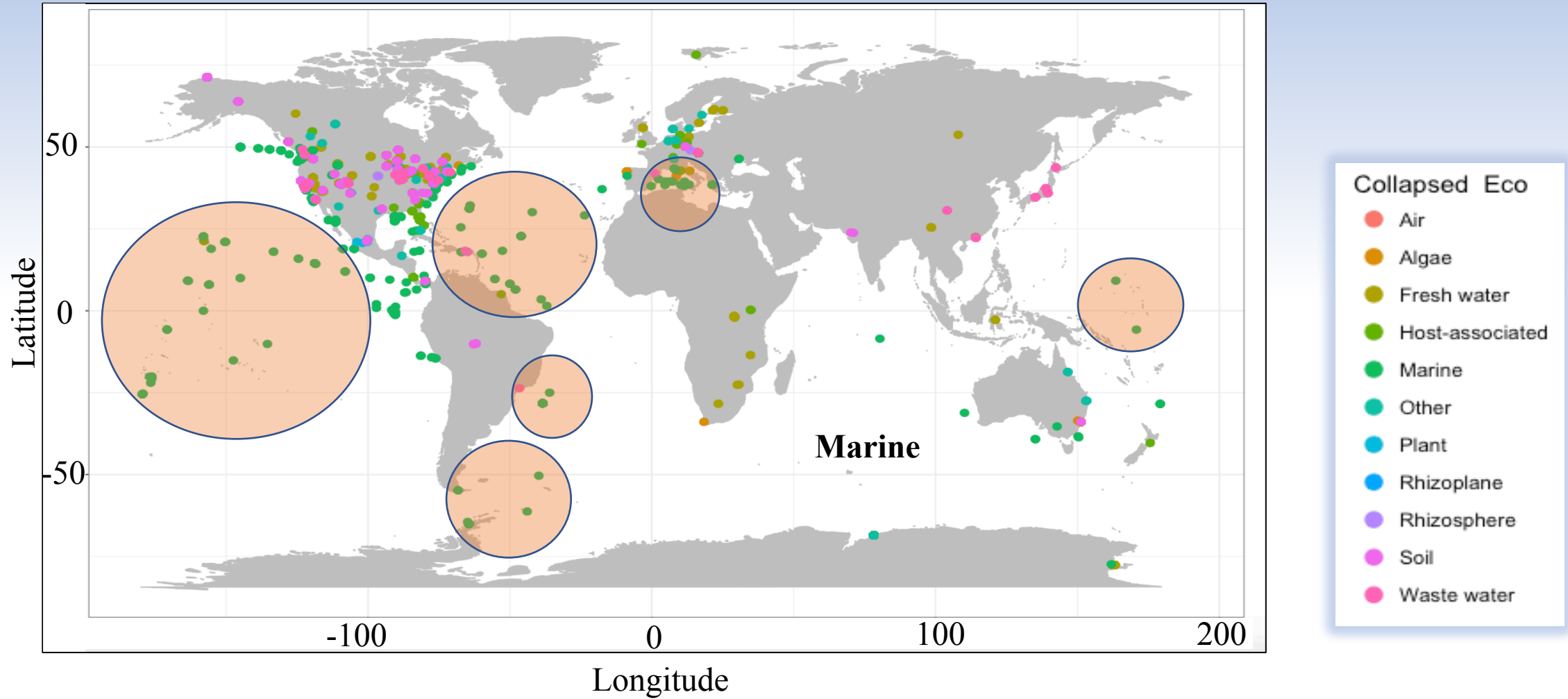


Figure 1. ACC-d Map demonstrates the distribution of ACC-d on a worldwide scale highlighting the areas of study after clustering.

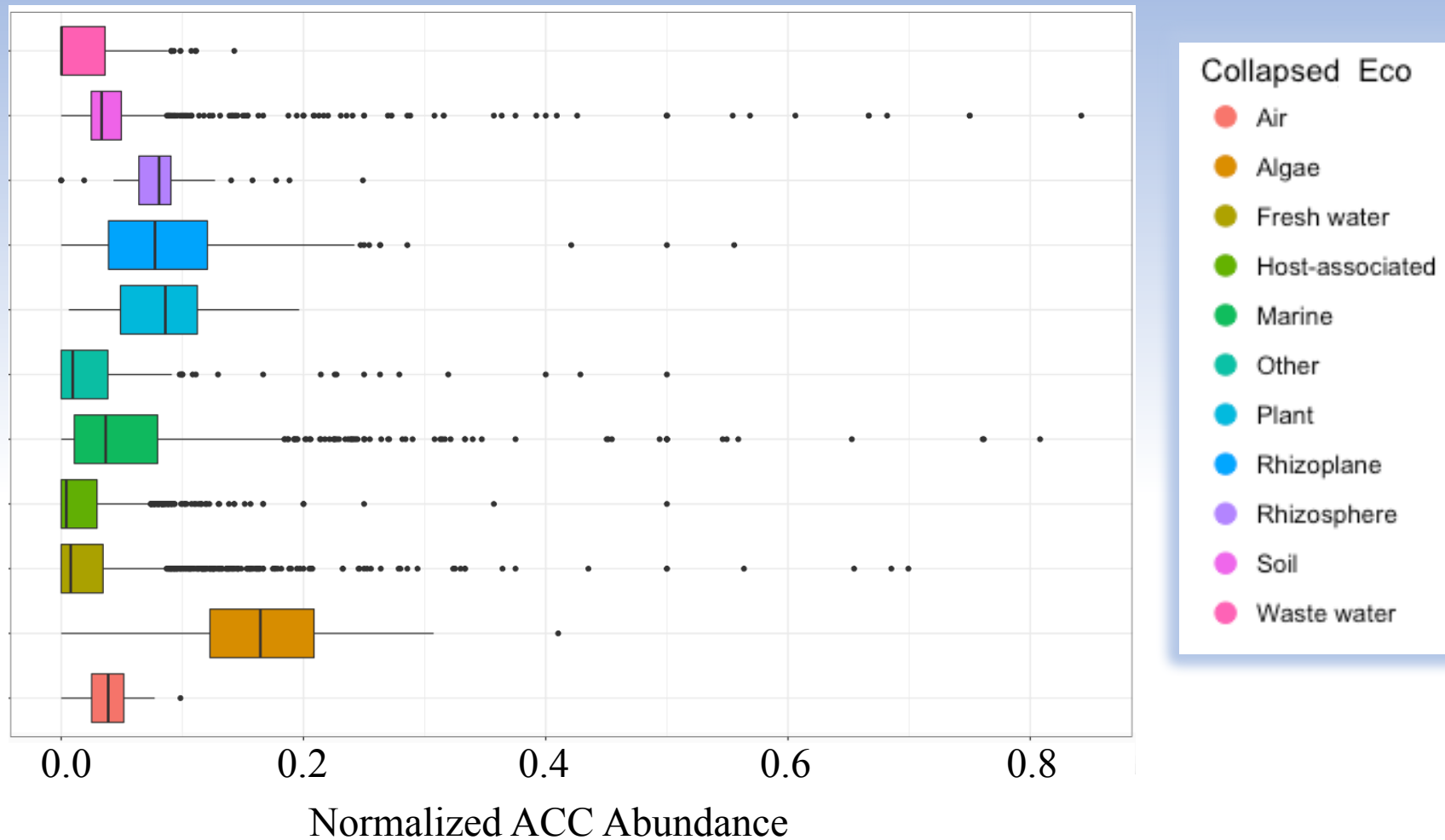


Figure 2. Normalized ACC-d Abundance box plot exhibits results of ACC-d with a corresponding *rplB* gene.

- The Normalized ACC Abundance graph depicts the relativized abundance of ACC-d in different ecosystems from all studies.
- The relationship between the abundance and the population displays the relative abundance of ACC-d in a specific ecosystem exist.
- The black line in the box is the media, the edges of the boxes are the 25th and 75th quartiles, and dots are outliers.

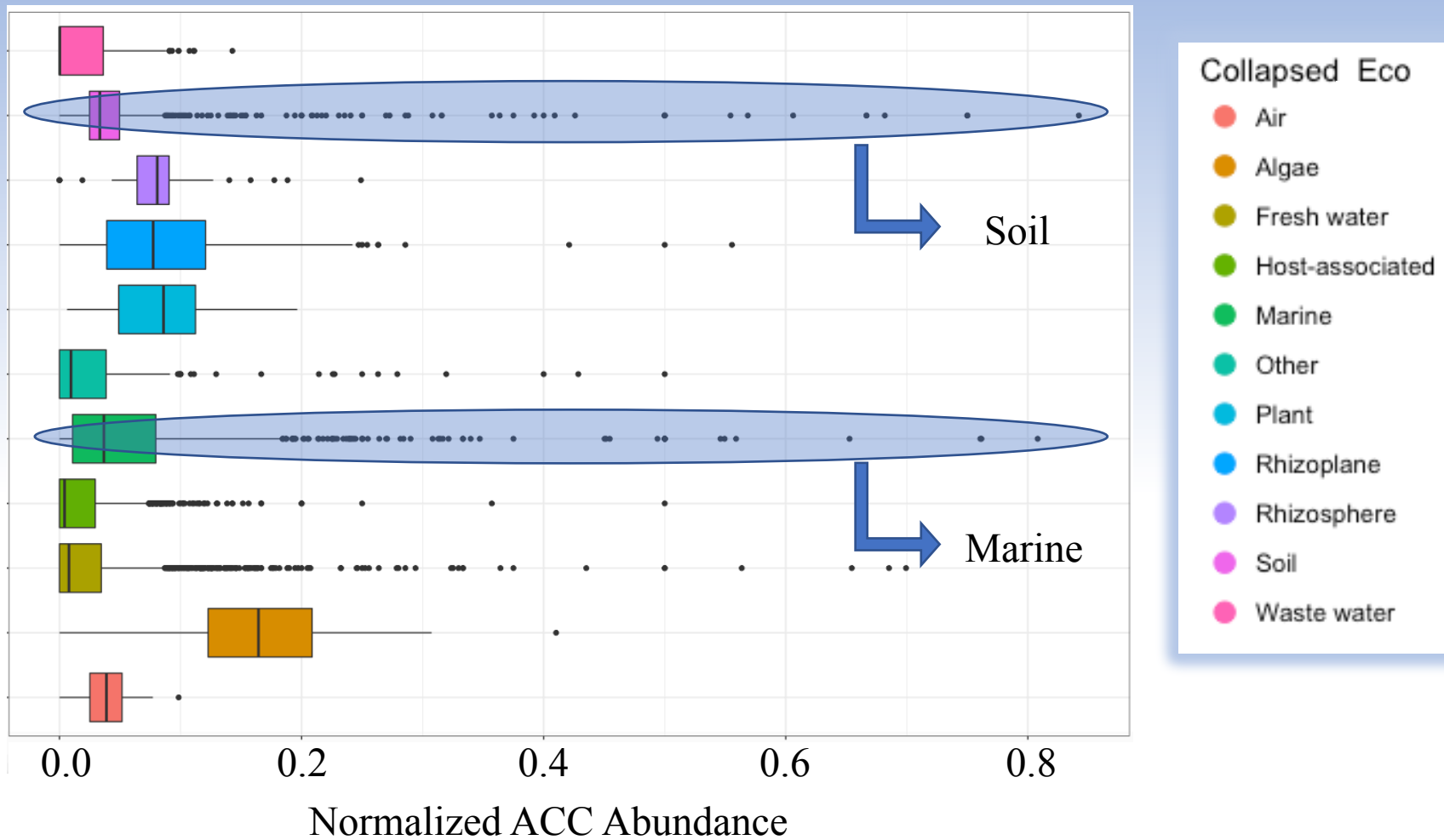


Figure 2. Normalized ACC-d Abundance box plot exhibits results of ACC-d with a corresponding *rplB* gene.

- The Normalized ACC Abundance graph depicts the relativized abundance of ACC-d in different ecosystems from all studies.
- The relationship between the abundance and the population displays the relative abundance of ACC-d in a specific ecosystem exist.
- The black line in the box is the media, the edges of the boxes are the 25th and 75th quartiles, and dots are outliers.

- Hypothesis: (A) ACC-d is spread across the phylogenetic tree of life where many organisms have it, not only those in close proximity to plants or have historical associations with plants.

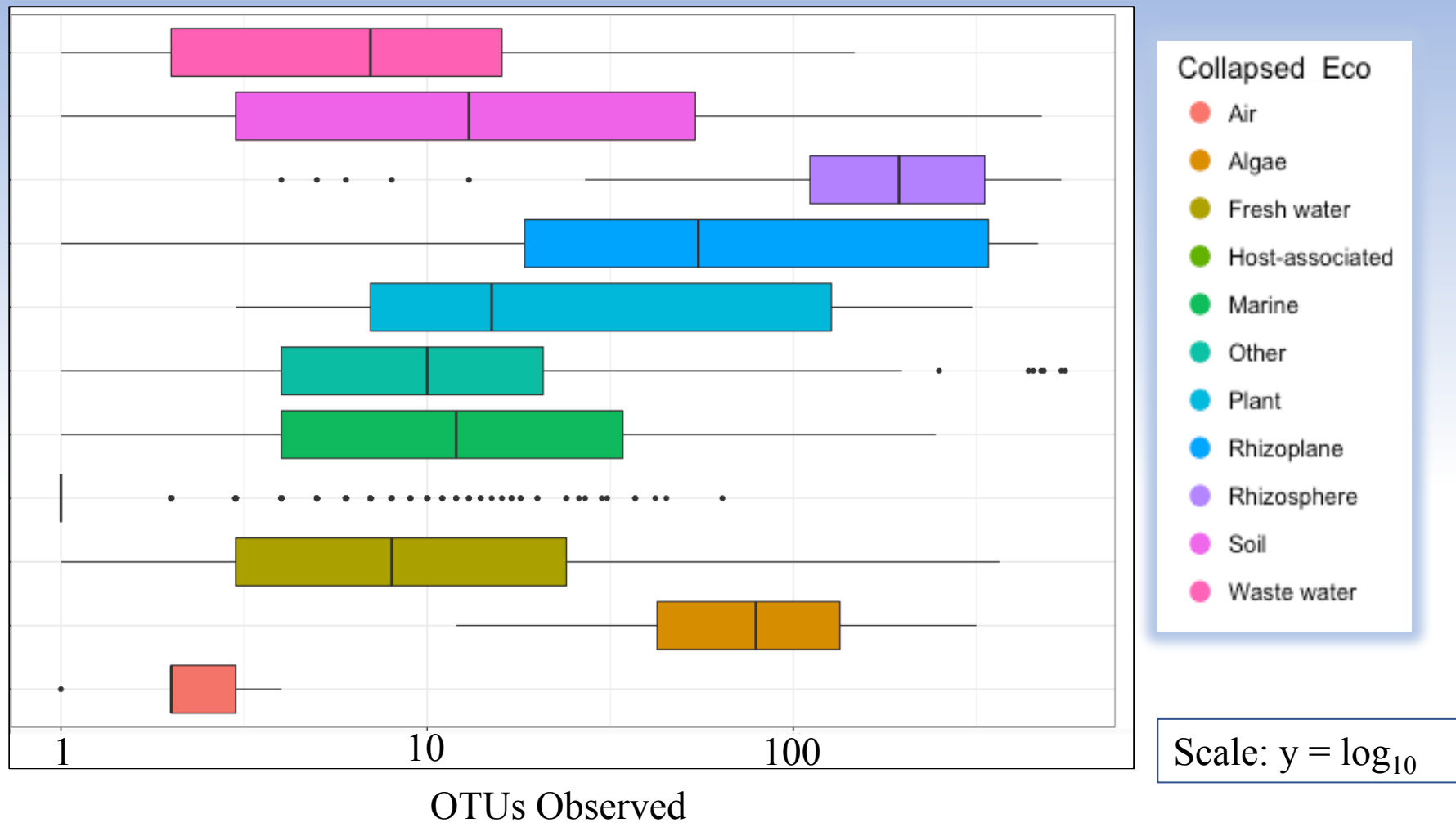


Figure 3. OTUs Observed box plot shows the results of entailing species richness referring to the number of species and species evenness within Alpha Diversity.

- Alpha diversity within the OTUs observed illuminates how many different species of ACC-d are present.
- The relationship between the OTUs observed and Shannon diversity shows how much of these ecosystems contain the bacterial gene ACC-d.
- The black line in the box is the media, the edges of the boxes are the 25th and 75th quartiles, and dots are outliers.

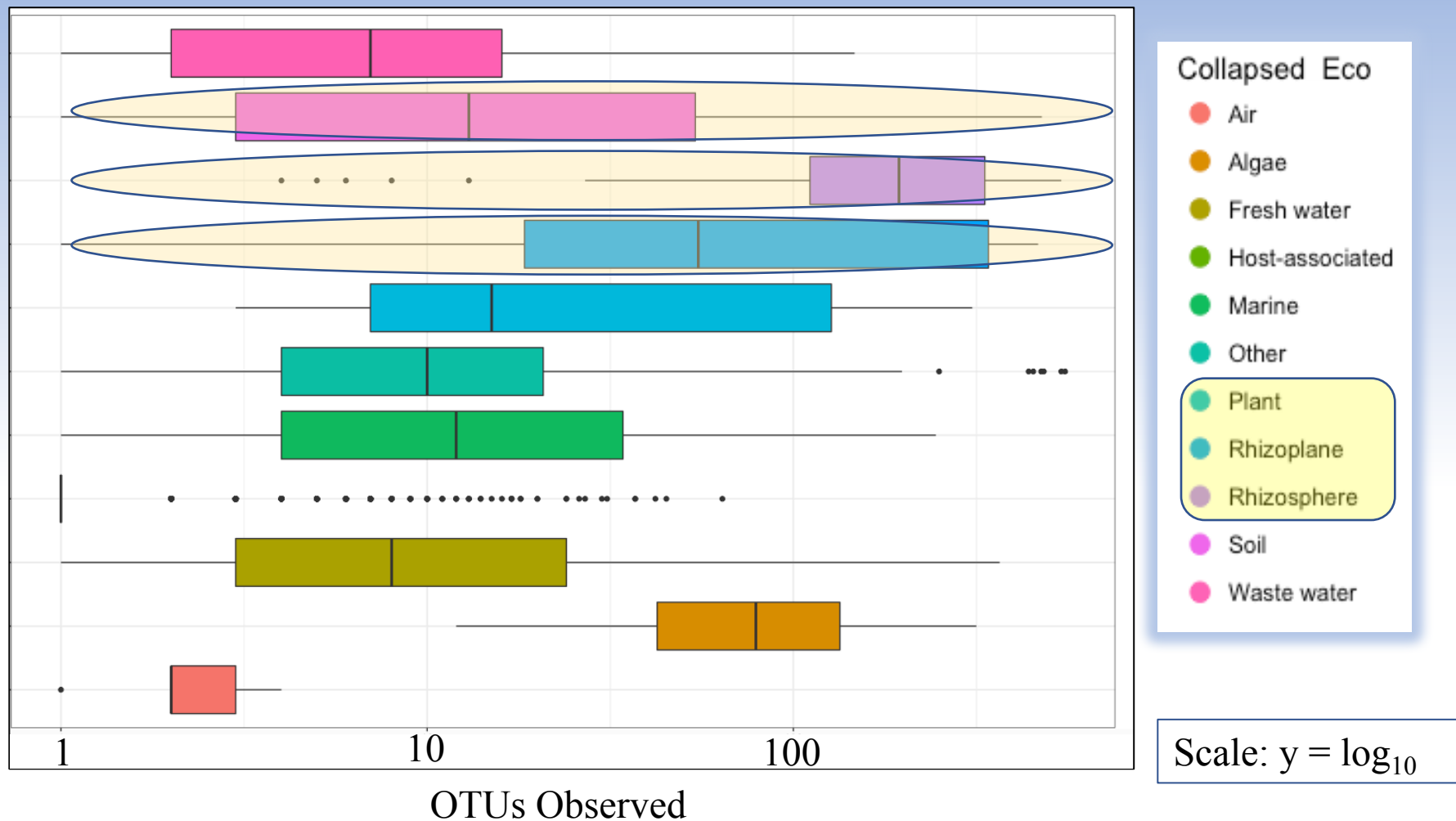


Figure 3. OTUs Observed box plot shows the results of entailing species richness referring to the number of species and species evenness within Alpha Diversity.

- Alpha diversity within the OTUs observed illuminates how many different species of ACC-d are present.
- The relationship between the OTUs observed and Shannon diversity shows how much of these ecosystems contain the bacterial gene ACC-d.
- The black line in the box is the media, the edges of the boxes are the 25th and 75th quartiles, and dots are outliers.

- Hypothesis: (B) This enzyme will be overrepresented in microbes that have a strong affiliation with plants, then there is an evolved relationship.

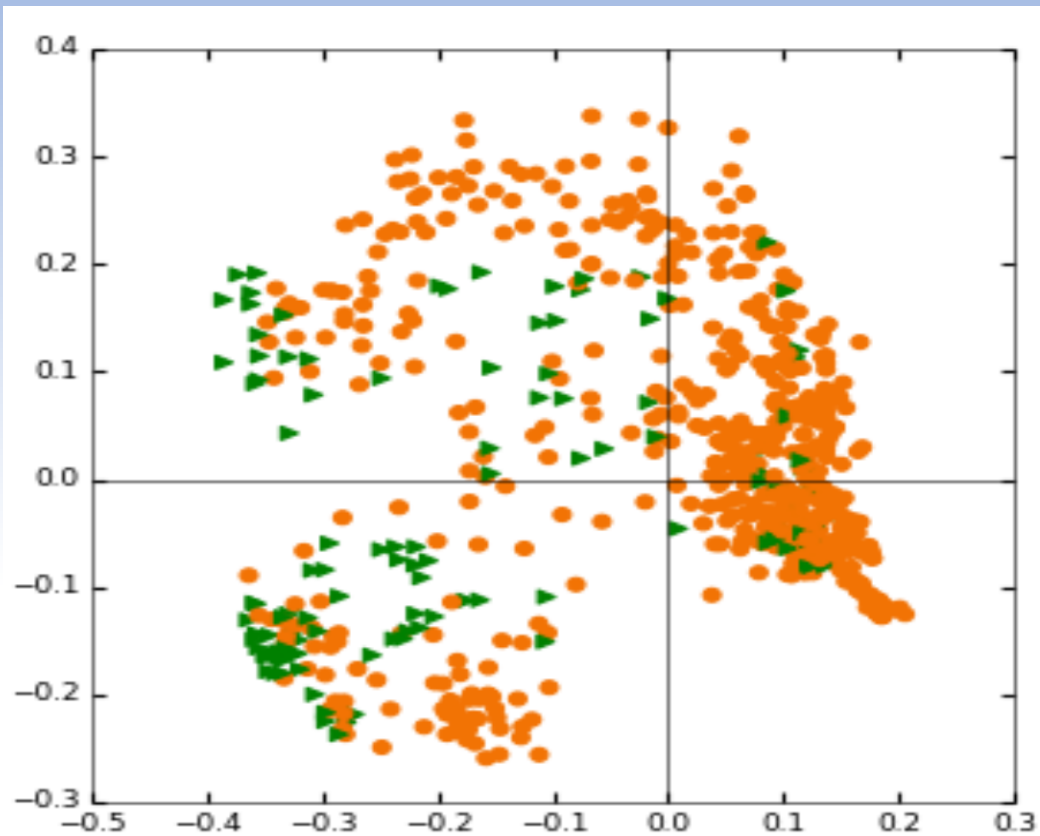


Figure 4. Principle coordinates analysis based on Bray-Curtis similarity. (PERMANOVA, $F=123.51$, $p<0.001$, $R^2=0.18$).

- Bray–Curtis dissimilarity is a mathematical equation to compute the dissimilarity between two different sites, based on composition via counts at each site.
- The Bray–Curtis dissimilarity is bounded between 0 and 1, where 0 means that the two sites have the same composition (they share all the species), and 1 means the two sites do not have the same composition (do not share any species).

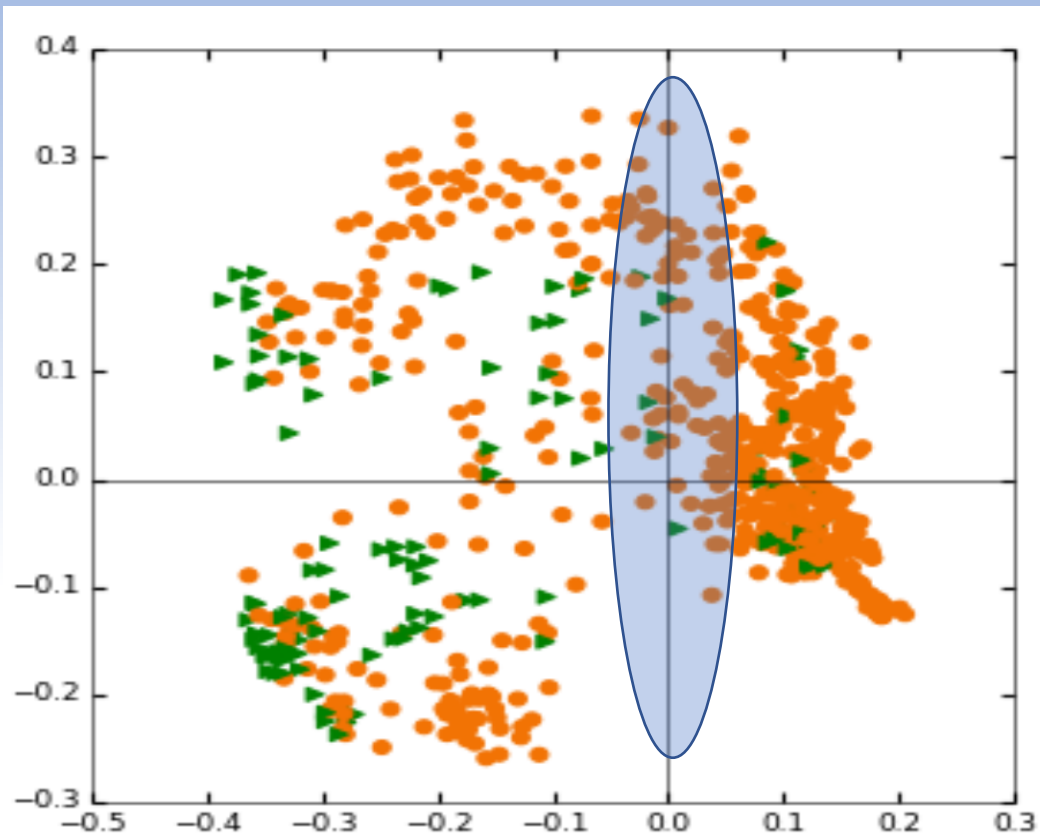


Figure 4. Principle coordinates analysis based on Bray-Curtis similarity. (PERMANOVA, $F=123.51$, $p<0.001$, $R^2=0.18$).



- Bray–Curtis dissimilarity is a mathematical equation to compute the dissimilarity between two different sites, based on composition via counts at each site.
- The Bray–Curtis dissimilarity is bounded between 0 and 1, where 0 means that the two sites have the same composition (they share all the species), and 1 means the two sites do not have the same composition (do not share any species).

Conclusion

- ACC-d is a widespread gene across many ecosystems (Fig. 1).
- Outside of soils, freshwater and marine environments are also hot spots for ACC-d abundance and diversity (Fig. 2).
- ACC-d is the most abundant and diverse in soil and plant-associated environments (soil, rhizosphere, rhizoplane, Fig. 3).
- Despite large geographical distances, soil and plant have different ACC-d diversity and representation (Fig. 4).

References

- Glick, B.R. *Microbiol Res* 169: 30-39 (2014).
- Caporaso, J. G. et al. *Nat Meth* 7: 335-336 (2010).
- Team, R. Core. *Vienna, Austria: R foundation for statistical computing* (2000).

Acknowledgements

- This work was supported by the BEACON Center for the study of Evolution in Action, the Summer Research Opportunities Program, the Michigan State Plant Resilience Institute, and the Advanced Computing Research Experience for Students REU program.
- Special Thanks:
 - Dr. Ashley Shade
 - Dr. Patrick Kearns
 - Steven Thomas
 - Dr. Judi Clarke Brown
 - Camille Archer

Questions?