The Extraradical Hyphae (ERH) Microbiome

Based on Emmett et al. 2021

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Mutualistic fungi are important for many plants

- Arbuscular mycorrhizal fungi (AMFs) associate with **~72%** of land plants
- Legumes 🫘, cereals 🌾, tomatoes 🍅, and peppers 🌶️

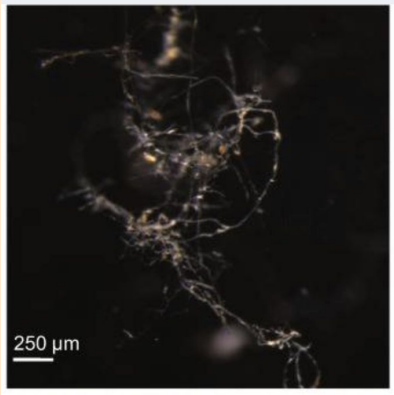
- Plant carbon compounds exchanged for fungal nitrogen and phosphorus
- Extraradical hyphae in the soil extend the reach of fungi

ERH-associated microbial communities contribute to these capabilities

- AMFs lack genes for phytases, secreted phosphatases, and lignases
- How do they solubilize these nutrients?

Microbial communities!

- Measured enzymatic activity in the hyphosphere
- Diversity of interactions



FISH of ERH bacteria (Emmett et al. 2021)

How does soil composition affect ERH communities?

Null hypothesis:
Soil composition has no effect on microbial richness or composition.

Alternative hypothesis 1:
Soils with a higher organic carbon content will have a greater microbial richness.

Alternative hypothesis 2:
Different soil types will have different ERH microbiome compositions (Bray-Curtis).

Experiment 1 of *Emmett et al. 2021* compared 3 soil types with ERH and a sand negative control

Table S1: Characteristics of source soils included in mesocosm experiments

Soil	pH	Al*	Ca	Cu	Fe	PK	Mg	Mn	Mo	Na	P	S	Zn	TC[a]	TN[b]
		mg/Kg	mg/Kg	mg/Kg	mg/Kg	mg/Kg	mg/Kg	mg/Kg	mg/Kg	mg/Kg	mg/Kg	mg/Kg	mg/Kg	%	%
Dryden	6.26	8.39	704.2	0.03	0.58	39.87	59.63	6.02	0.00	12.24	6.23	3.47	0.11	2.25	0.26
Florence	6.39	5.57	234.2	0.02	0.44	13.90	12.58	1.17	0.00	12.44	7.05	2.50	0.56	0.73	0.11
Pendleton	5.73	11.13	161.3	0.04	0.31	11.45	30.69	6.59	0.00	10.84	0.19	9.80	0.13	1.00	0.11

- Lansing: silty, highest carbon content
- Pendleton: more clay composition, and medium carbon
- Florence: sandiest soil, with lowest carbon
- Sand: uninoculated with fungi and inert (no nutrient content)

Interlude: coding a rounding function!

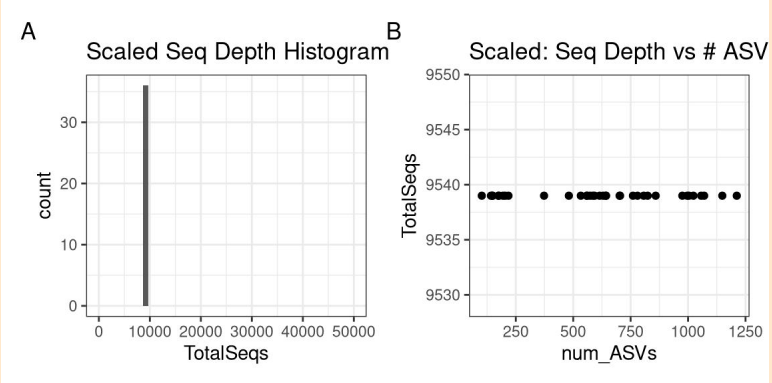
```
} else if (round == "exactround"){
  # exactround calculates the rounding threshold for each sample so that
  # all sample sums normalize exactly to the minimum sample sum
  transposed_otu <- t(otu_table(physeq.scale))
  for(sample in 1:nrow(transposed_otu)){
    sample_vec <- transposed_otu[sample,]
    asvs_to_round_up <- n - sum(floor(sample_vec))
    if (asvs_to_round_up == 0)
    {
      next
    }
    sample_sorted <- sort(sapply(sample_vec, function(x){x %% 1}))
    threshold <- sample_sorted[length(sample_sorted) - asvs_to_round_up]
    round_on_threshold_arr <- sample(which((sample_vec - floor(sample_vec)) == thres
    exactround <- Vectorize(function(x, ind){
      if ((x - floor(x)) > threshold)
        {ceiling(x)}
      else if ((x - floor(x)) < threshold)
        {floor(x)}
      else {if (ind %in% round_on_threshold_arr)
          {
          return(ceiling(x))}
        else {
          return(floor(x))}}})
    transposed_otu[sample,] <- exactround(sample_vec, array(1:length(sample_vec)))
  }
  otu_table(physeq.scale) <- t(transposed_otu)
}
```

"exactround" option when scaling samples

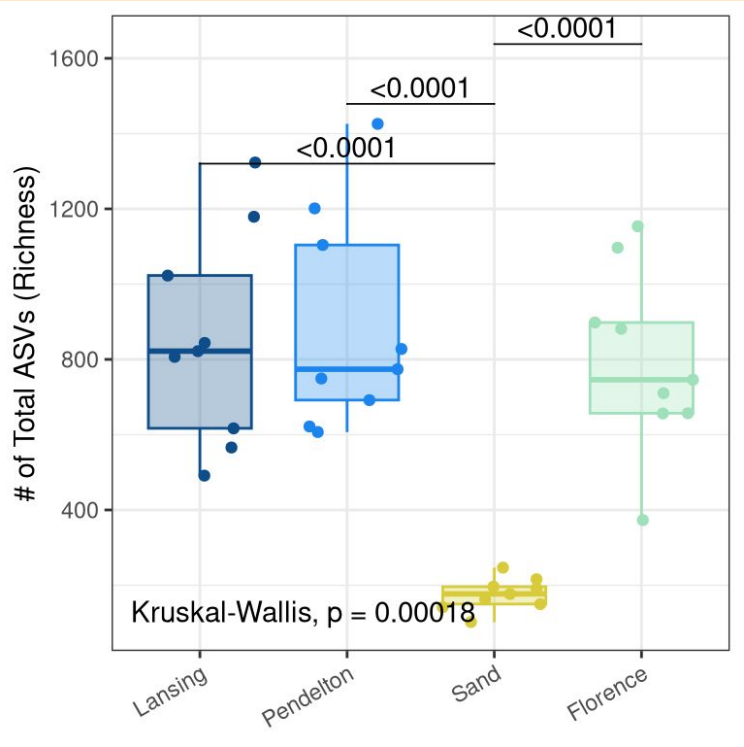
Same sequencing depth for all

Steps:

- Calculates rounding threshold for each
- Randomized selection for boundary cases



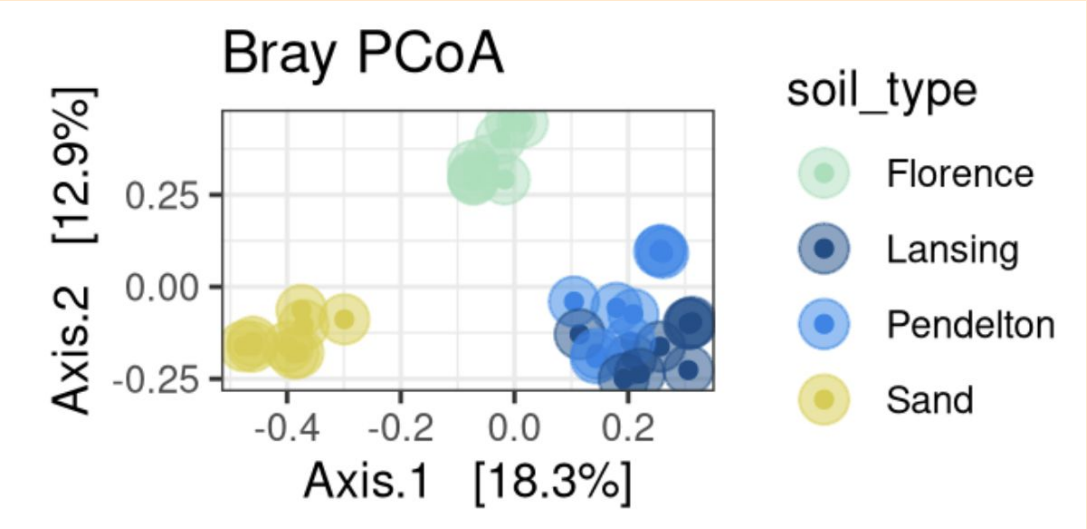
Soil type had no significant effect on richness



No significant difference between soils inoculated with ERH

All soils inoculated with ERH had significantly higher microbial richness than sand without ERH

Soil types have significant differences in the composition of abundant ASVs (Bray-curtis)




```
Response: Distances
           Df    Sum Sq    Mean Sq      F N.Perm Pr(>F)
Groups      3 0.003414 0.0011380 0.3706    999  0.779
Residuals  32 0.098275 0.0030711
```

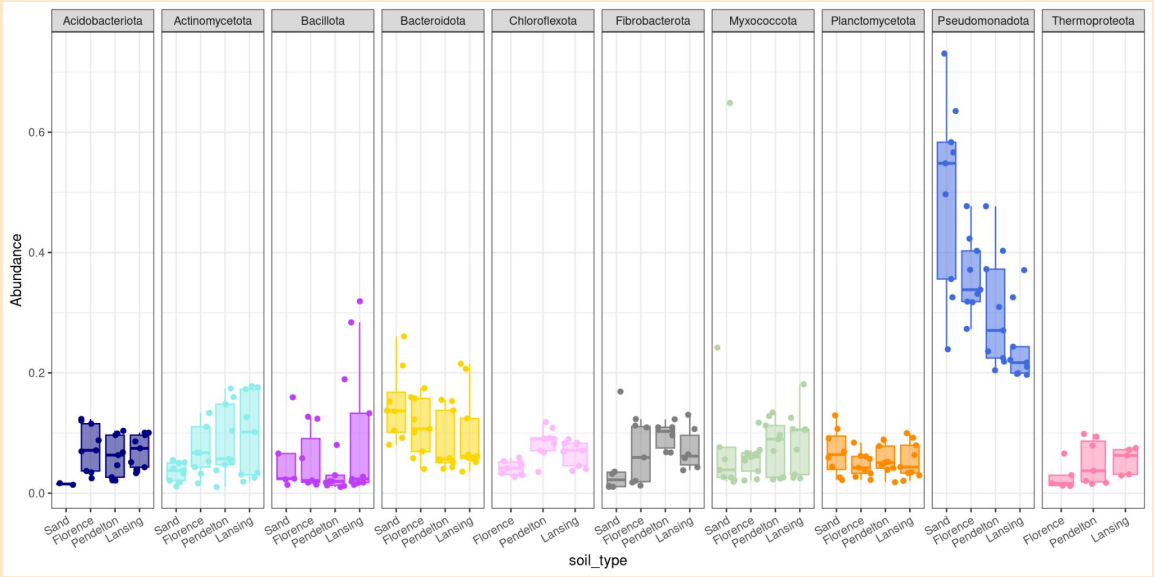
PERMANOVA

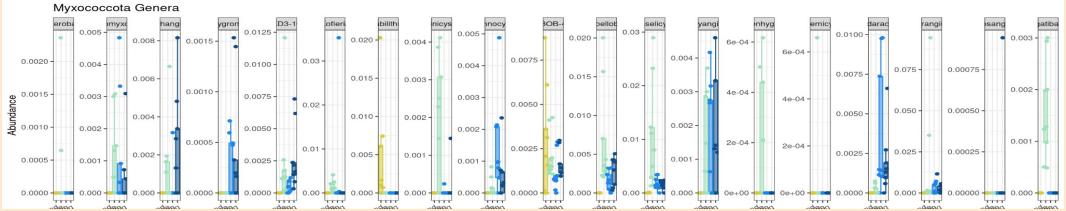
- $p < 0.001$
- $R^2 = 0.394$
- F-stat = 6.94
- Centroids are different among the groups

Post-hoc betadisper test ($p = 0.779 > 0.05$)

- Dispersion effect is not significant

Some phyla trend with soil composition while there is high variation among genera





Psuedomonadota:
- Generalists
- Possibly outcompeted

Thermoproteota:
- Ammonia-oxidizing archaea

Myxococcota:
- Predatory
- Phytase expression

Differences in ERH microbiota may inform engineered soil communities for agriculture

Soil ecological engineering (Bender et al. 2016)

- increased food security
- minimized environmental impact

Soil types had different ERH community compositions.

Tailoring ERH communities to specific conditions could help us design more sustainable soils!

Thank you!

:)