Microbial Community Comparison of Nitrogen Fixation and Plant Growth between Invasive *Erodium cicutarium* and Native Species One-Year Postfire

221 B. Biome Street

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The Fire Regime

- Patterns of fire in an ecosystem
  - Frequency, intensity, size, and spread/structure
- CA has chaparral ecosystem - type of Mediterranean climate
  - Plants are “fire adapted”
    - Chamise, manzanita, laurel sumac, sagebrush
- A fire disturbance can cause a significant impact on the microbial community composition and beta diversity (Tas et al, 2014)

Image Credit: https://oregonexplorer.info/content/what-fire-regime

Invasive Species

- *Erodium Cicutarium* - Common stork's-bill
  - Native to Mediterranean Basin
  - Invasive in North America
  - Grow well after environmental disturbances (Howard, 1992)
- One year post-Skirball fire
- Soil microbial community
  - Composition + Function

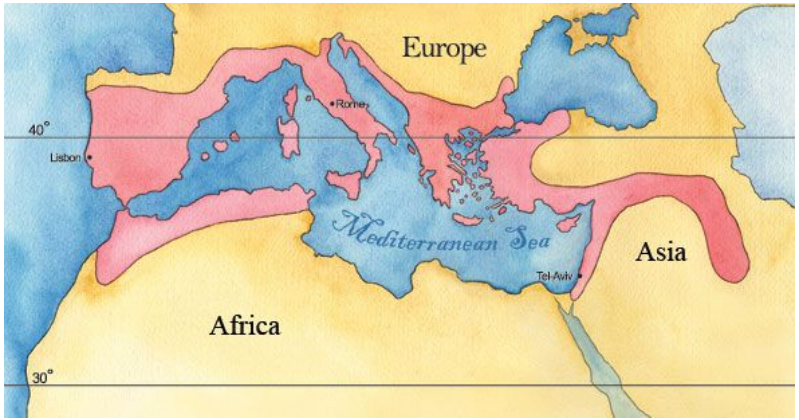


Image Credit: Harmeet Kaur, https://incomme.org/mediterranean-regions/mediterranean-basin-landscape.html

The Rhizosphere

- Area of the soil surrounding the plant root with microbes and fungi
- Maintains plant productivity in different environmental conditions (Mendes et. al, 2013)
- Plant growth promoting rhizobacteria (PGPR) are key players

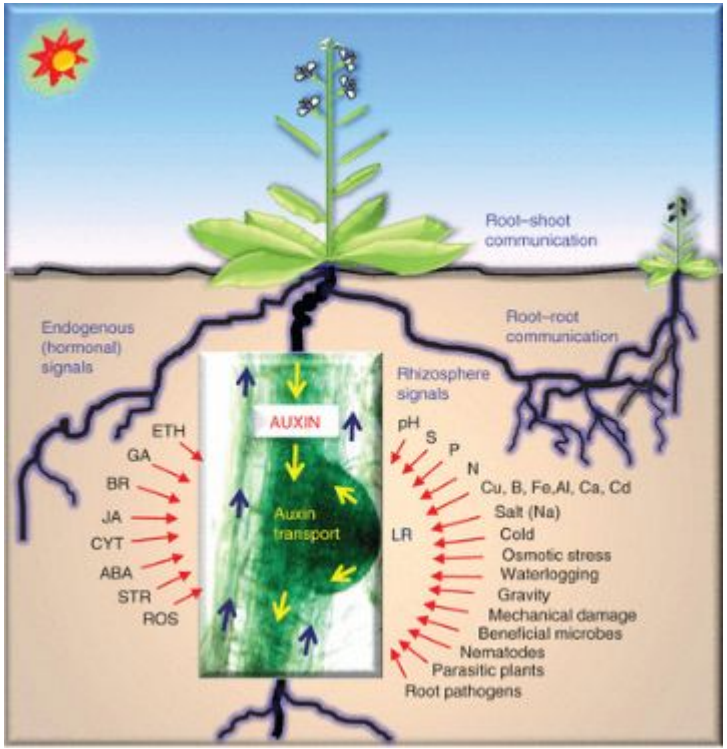


Image credit:
https://www.google.com/url?sa=i&source=images&cd=&ved=2ahUKEwjNkvqFjL3gAhUzFjQIHXsaBNwQjRx6BAgBEAU&url=https%3A%2F%2Fwww.sciencedirect.com%2Fscience%2Farticle%2Fpii%2FS0038071716301158&psig=AOvVaw2h-ha47xDuFPPqnlC81M_p&ust=1550297852381457

Plant Growth Promoting Rhizobacteria (PGPR)

- (PGPR) are microorganisms which support plant growth
    - Prevent or decrease a pathogenic organism's deleterious effects
    - Produce necessary compounds and support nutrient uptake (Penrose et al, 2002)
    - Examples
        - Nitrogen Fixation
        - Siderophore Production for iron uptake (Kloepper et al, 1980)
        - Phosphate solubilization (Rodriguez et al, 1999)
        - Phytohormone production (Cox et. al 2017)

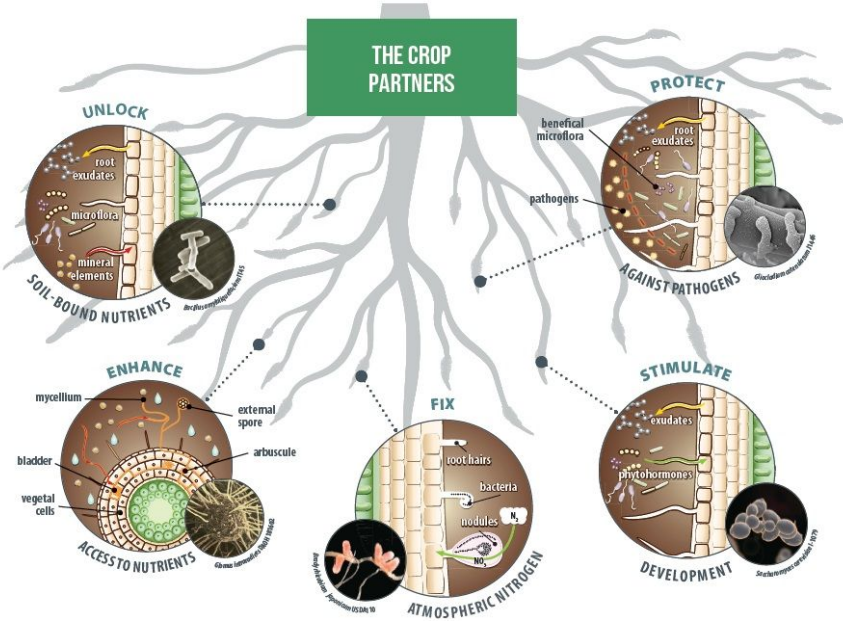


Image Credit:
http://www.lallemandplantcare.com/en/our-solutions/rhizosphere-inoculants/

What are we interested in?

- *Erodium cicutarium* continues to thrive in a nonnative environment post fire and rainfall

- How does the rhizosphere microbial community allow this plant to grow and recover in a foreign environment and after environmental stressors?

- Hypothesis: Will find microorganisms that fix nitrogen, produce siderophores, produce auxin, and solubilize phosphate

Image Credit: Harmeet Kaur, https://grist.org/food/the-secret-to-richer-carbon-capturing-soil-treat-your-microbes-well/

Why do we care?

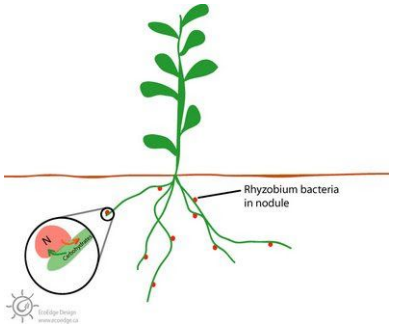
Microorganisms with Plant Growth Functions (PGPR) Can Potentially Be Used in:

- Agriculture and Horticultural Applications
- Promoting plant growth (Penrose et al, 2002)
- Biofertilizers or control agents for agriculture improvement
- Phytoremediation (Zhuang et al, 2007)
- Nitrogen production
    - Less cost and pollution (Souza, et al, 2015) fossil fuels running out (Bockman, 1997)

Image Credit:
http://www.lallemandplantcare.com/en/our-solutions/rhizosphere-inoculants/

Specific Experimental Goals

1. Rhizosphere of stork's-bill = capable of producing PGPR
2. Abundance of these bacteria has been altered in some way
    a. Affected microbial community and/or function
3. Surrounding microbial community composed of organisms that allow survival in non-native environments



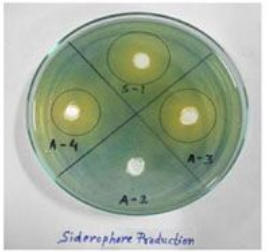
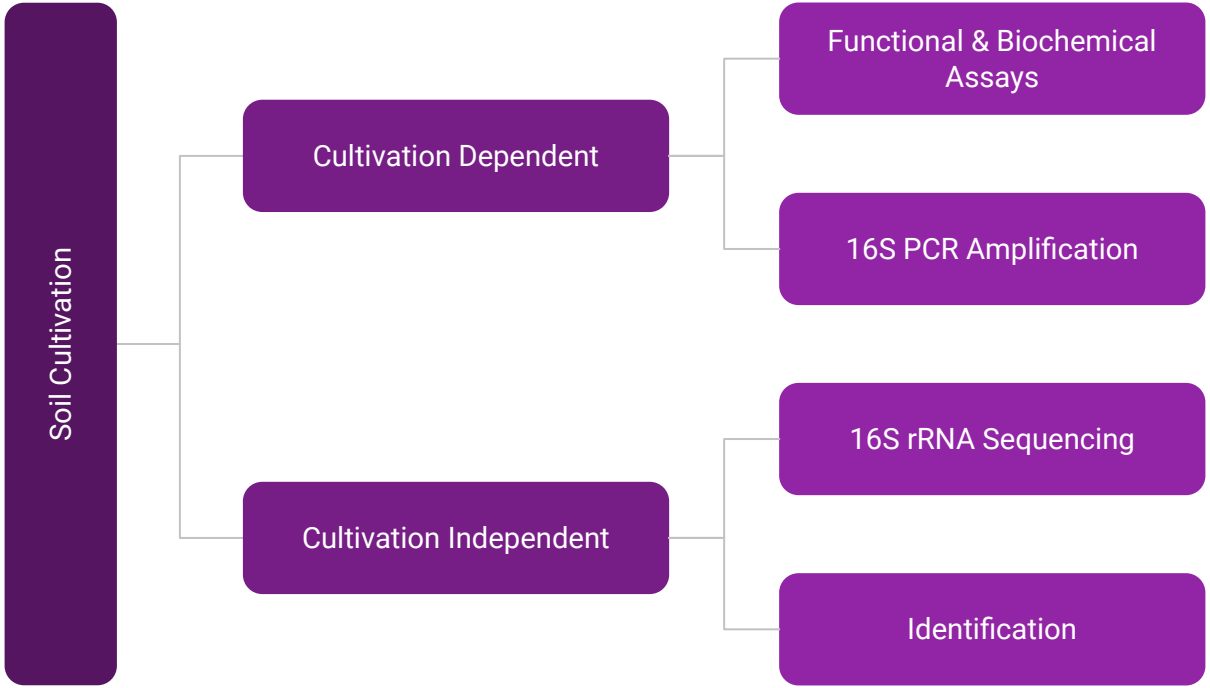


Fig. 9. Zone of development by siderophore producing isolates

Image Credit: http://callisto.ggsrv.com/imgsrv/FastFetch/UBER1/ZI-3AGB-2015-SEP00-IDSI-567-1
https://1.bp.blogspot.com/_uH8JDRwUtr0/SabyMchrTsI/AAAAAAAAAAc4/Ni6VHtfUWzY/s400/nitrogen-fixation.jpg
https://www.washingtonpost.com/news/post-nation/wp/2017/12/12/the-los-angeles-fire-that-destroyed-bel-air-homes-began-at-a-homeless-camp-officials-say/?noredirect=on&utm_term=.ec9f3c7ba573

Overall Experimental Approach



Hypothesis - Cultivation Dependent (CD)

Since fire can influence the microbial community and function due to changes in beta diversity in soil from before and after a fire (Tas et al, 2014); and PGPR microorganisms can help with stress tolerance, nutrient acquisition, and plant growth (Chaparro et al, 2012),

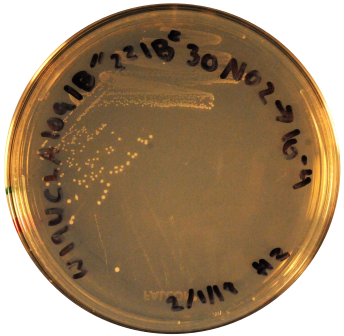
if microorganisms in the soil of the common stork's bill one-year postfire is compared to microorganisms in the soil of another plant directly after the Skirball Fire occurred,

then microorganisms found surrounding the common stork's bill will have *more functions* and *genes* that allow it to survive after an environmental stressor.

Cultivation Dependent Approach

Cultivation & Enrichment

- Collect soil samples
- Enrichment on R2A and N2-BAP media



Isolation

- Grow isolates on R2A and N2-BAP media until pure colonies obtained
- Streak Plate Method
- Liquid Cultivation
- Confirm purity through Gram stains

Functional + Biochemical Assays

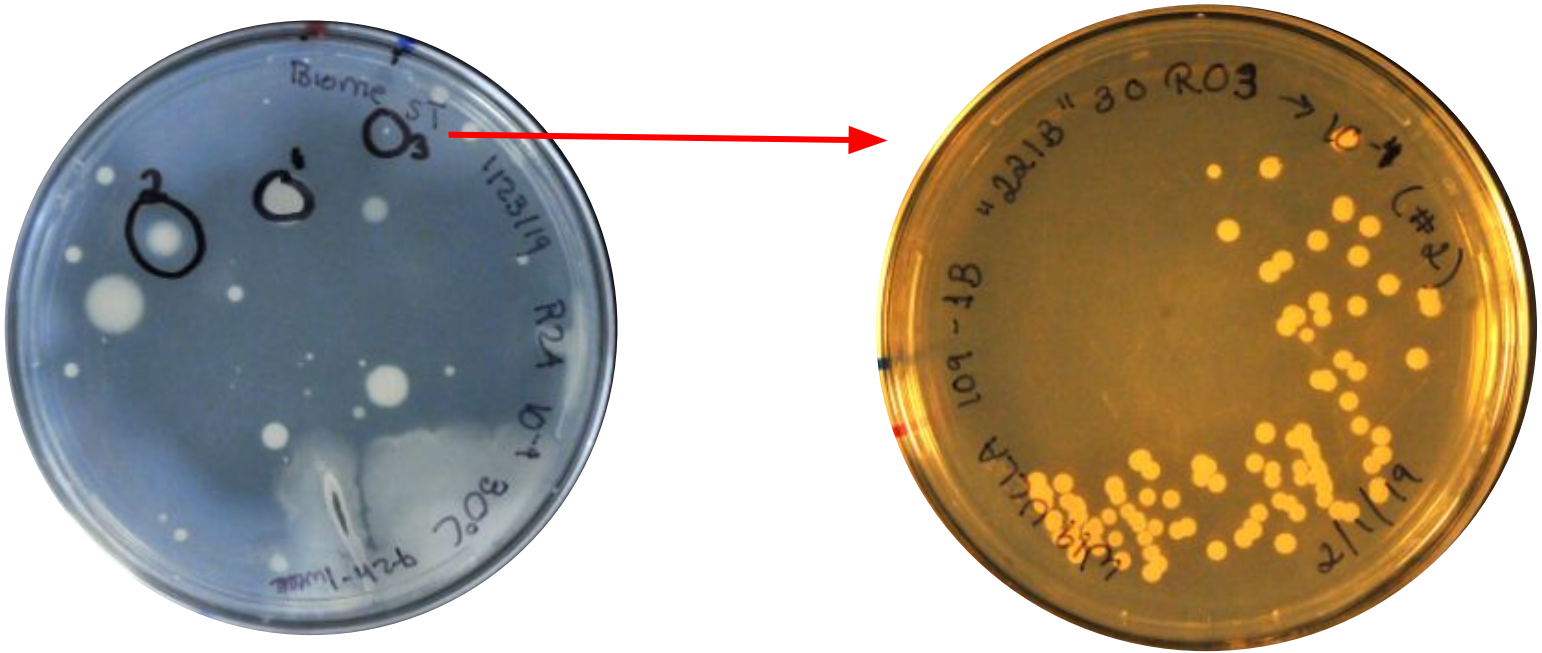
- Nitrogen-Fixation (DF + JMV)
- Phosphate Solubilization (PVK + PVK purple)
- Oxidation - Fermentation tests
- Siderophore Production
- SIM tests

Comparison

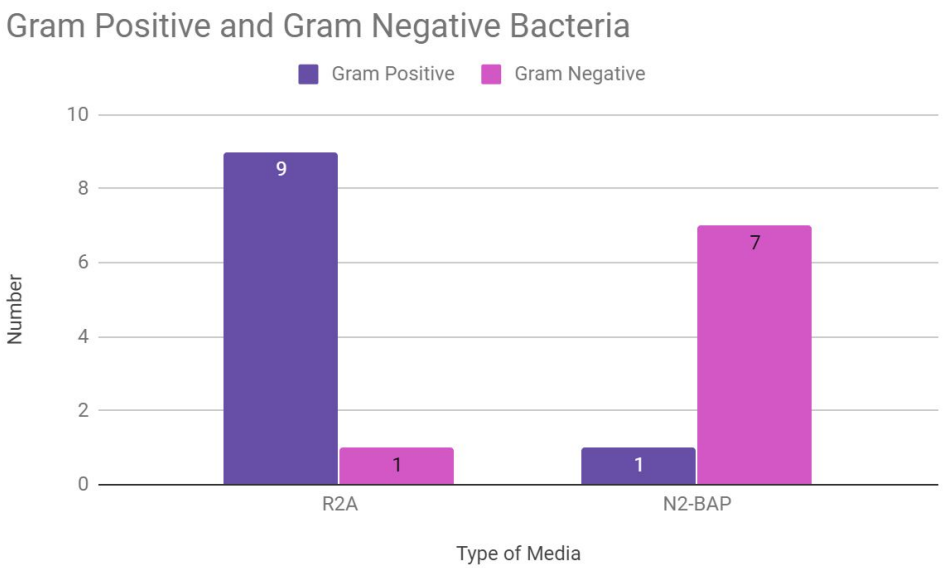
- Compare results (one-year postfire) to data collected directly after fire disturbance
- Native vs Invasive species microbial community

Enrichment & Isolation

Initial Isolation: W19UCLA1091B221B30R03

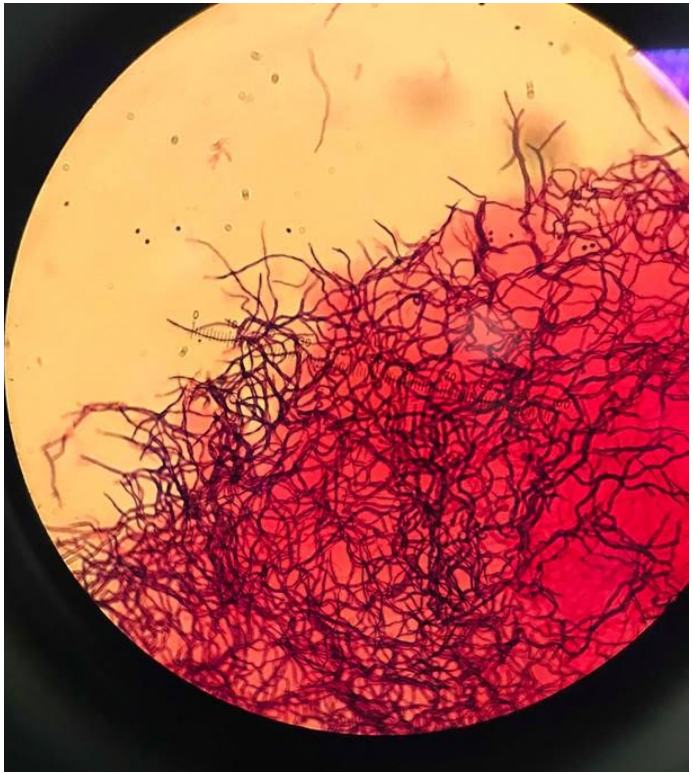


Gram Stains



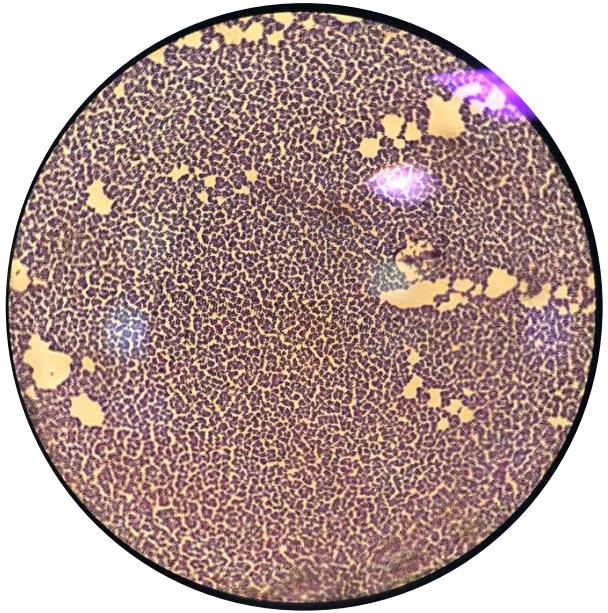
Majority of R2A isolates = Gram +

Majority of N2-BAP isolates = Gram -



W19UCLA109AL221BN13. Gram Positive. Filamentous morphology

Representative Gram Stains



W19UCLA109221BR16

Gram Positive

Spheres in clusters

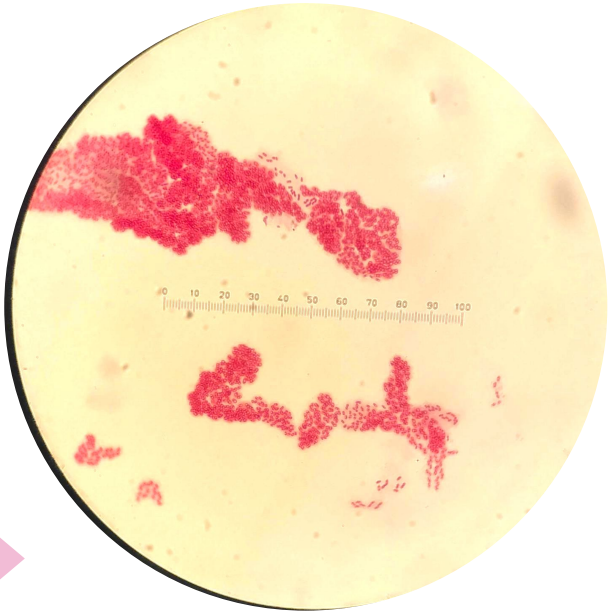
(staphylococci)

W19UCLA109221BN03

Gram Negative

Spheres in clusters

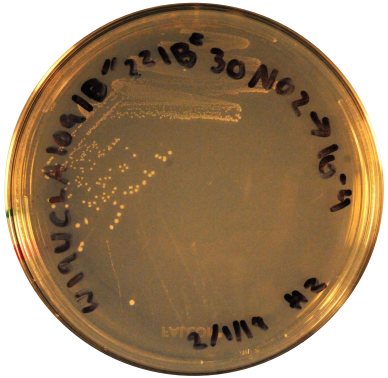
(staphylococci)

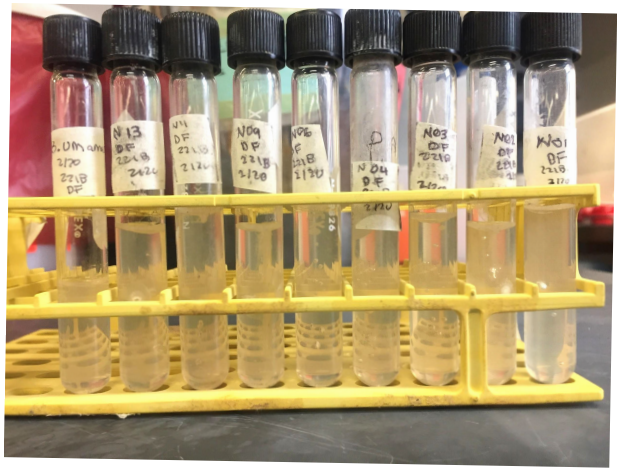


Nitrogen Fixing Bacteria: Using DF and JMV Media

- Dworkin & Foster (DF) & JMV Media
  - Nitrogen-free media
  - Investigate ability of isolates to convert atmospheric nitrogen into usable form
- Significance
  - Plants need nitrogen to grow
  - Invasive species may be comprised of nitrogen fixers that help adapt to environmental stressors

	N2-BAP	DF	JMV
Isolates Grown on Media	8/18	2.33/18*	5/18*





* Average among 3 trials

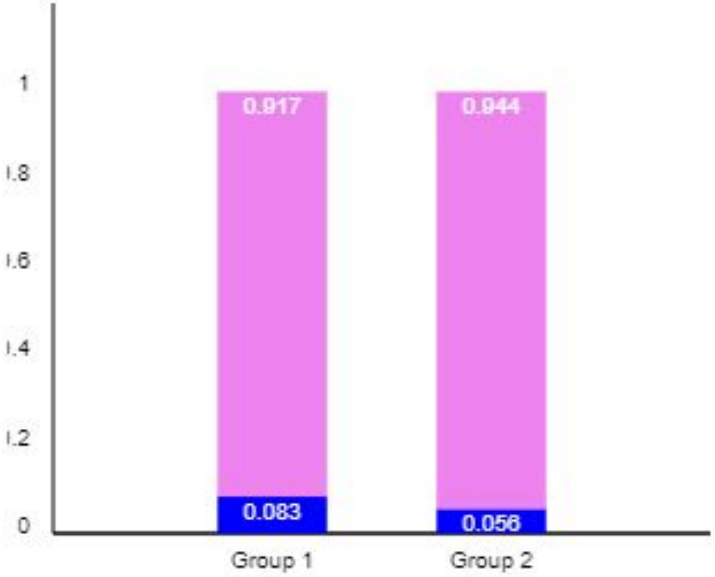
Dworkin & Foster Minimal Media

- Isolate N01 displayed consistent growth across all trials
- Variable incubation times
- Streak plate inoculation

Isolate	DF Trial 1	DF Trial 2	DF Trial 3
N01	growth	growth	growth
N02	growth	none	none
N03	growth	none	none
N04	none	none	none
N06	none	none	none
N09	growth	none	growth
N11	none	none	none
N13	none	none	none
B. unamae	growth	growth	growth

Team FIF (W18 Skirball) vs. Team 221B (W19)

- Team FIF analyzed *Pennisetum setaceum* (fountain grass) around stony soil
- Two-Proportion Theory Based Inference
  - Compared both proportions
  - z = 0.30
  - P-value = 0.6175
- Group 1: Team FIF
- Group 2: 221B Biome Street
- Blue: Isolate displayed growth on DF
- Pink: Isolate did not display on DF



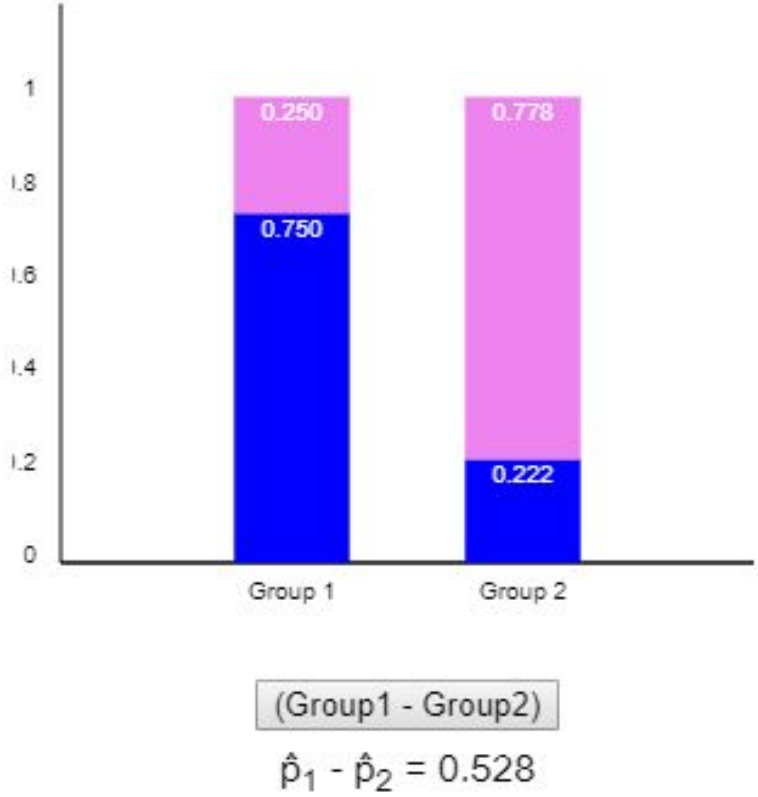
JMV Media

- 4/18 isolates grew on JMV media tubes consistently
- Will determine if isolates are *Burkholderia*
- Streak plate inoculation

Isolate	JMV Trial 1	JMV Trial 2	JMV Trial 3
N01	growth	growth	growth
N02	none	growth	growth
N03	none	growth	none
N04	none	none	growth
N06	growth	growth	growth
N09	growth	growth	growth
N11	none	growth	growth
N13	growth	growth	growth
B. unamae	growth	growth	growth

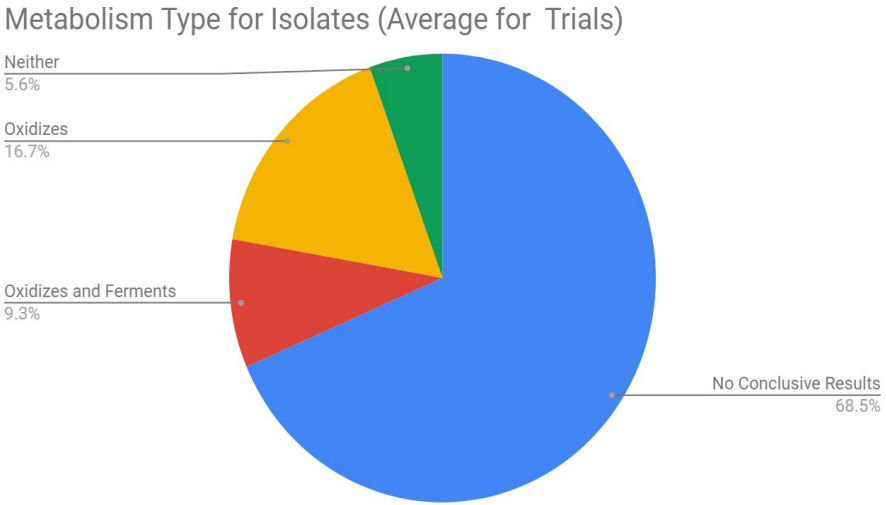
JMV Comparison - Team FIF (W18 Skirball)

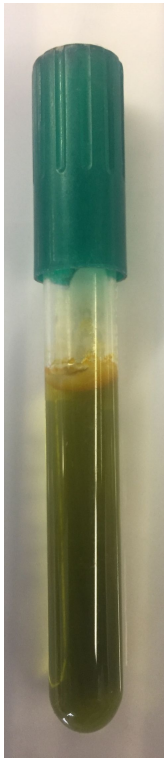
- Two-Proportion Theory Based Inference
  - Compared both proportions
  - z = 2.86
  - P-value = 0.9979
- Group 1: Team FIF
- Group 2: 221 B. Biome Street
- Blue: Isolate Can Grow on JMV
- Pink: Isolate Cannot Grow on JMV



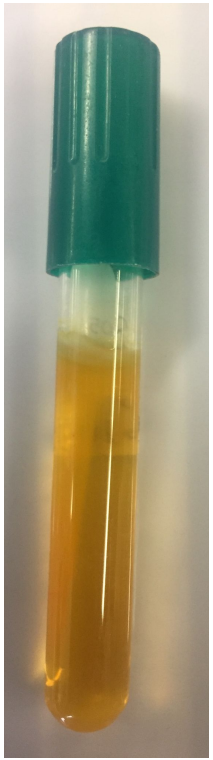
Oxidation vs Fermentation Metabolism

- Use OF Media with glucose
  - Color change = metabolic ability
- Mineral oil = anaerobic conditions
- No mineral oil = aerobic conditions





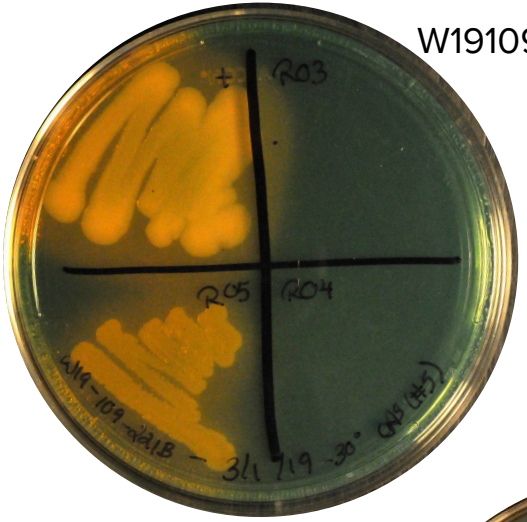
W19UCLA109AL221B30N06: Can oxidize glucose



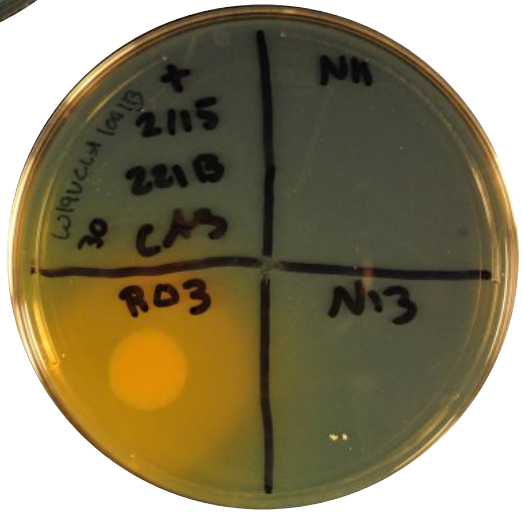
W19UCLA109AL221B30R05: Can oxidize and ferment glucose

Siderophore Production

- Plate isolates on CAS Media
  - Clearing around colony growth = siderophore production
- Trials 2-4 displayed no growth (including positive control)
- Trial 5 displayed siderophore production for 8/18 isolates
- Trials 1 and 6 each displayed clearings for 1 isolate



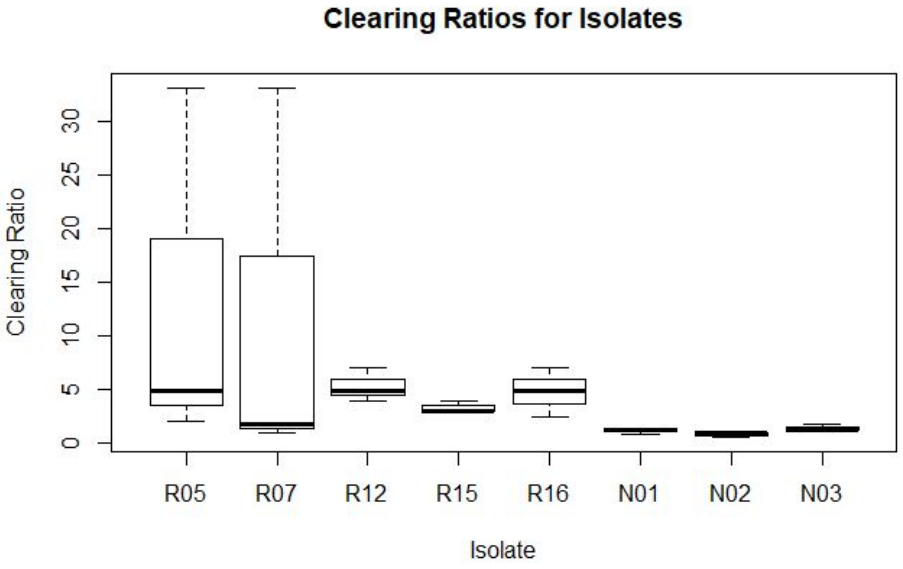
W19109AL221B30-Cas Trial #5

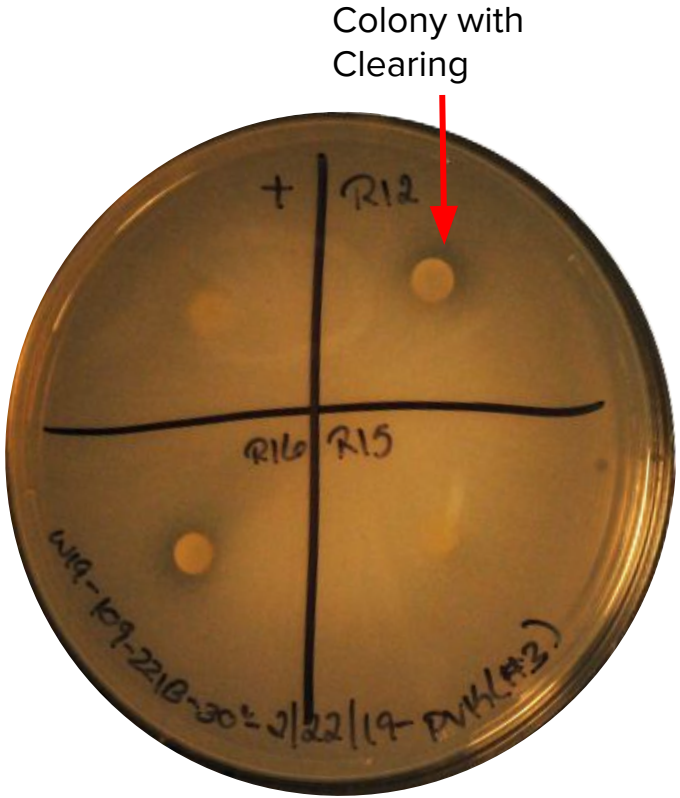


W19109AL221B30 - Cas Trial #1

Phosphate Solubilization: PVK Media

- Positive = clearing seen around colony
- 8/18 isolates were found to solubilize phosphate in 3 trials

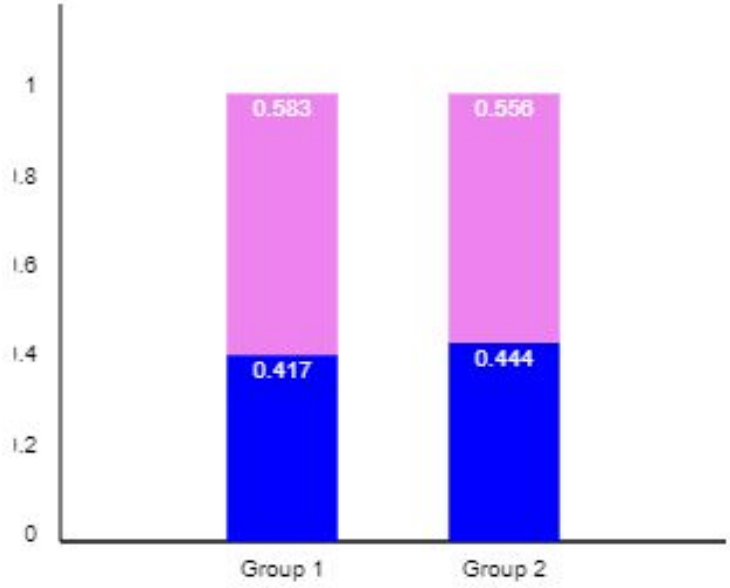




W19109221B30PVK - Trial 3

Compare to Team FIF (W18)

- Team FIF found 5/12 Isolates could solubilize phosphate
- Team 221B found 8/18 Isolates could solubilize phosphate
- 2 Proportion Test Using Theory Based Inference
  - z = -0.15
  - p=value =0.4402
- Not a Significant Difference ~ similar
- Group 1: Team FIF
- Group 2: 221 B. Biome Street
- Blue: Can Solubilize Phosphate
- Pink: Cannot solubilize phosphate



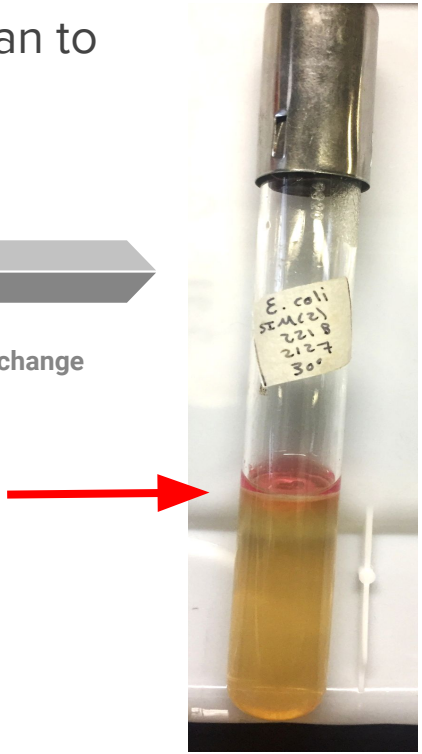
(Group1 - Group2)

$\hat{p}_1 - \hat{p}_2 = -0.028$

SIM Media for Auxin

- Sulfide Indole Motility (SIM) Media tests for catalysis of tryptophan to indole
- Indole-3-acetic acid is an auxin, a phytohormone

- *E. Coli* positive control changes to pink color
- Results: Negative for all trials
  - Demonstrated growth but no indole production



Hypothesis - Cultivation Independent (CI)

Since PGPR microorganisms help with stress tolerance, nutrient acquisition, and plant growth (Chaparro et al, 2012) and production of siderophores can make iron less available to native bacteria and fungi (Kloepper et al, 1980); since non-native species must adapt to a new environment,

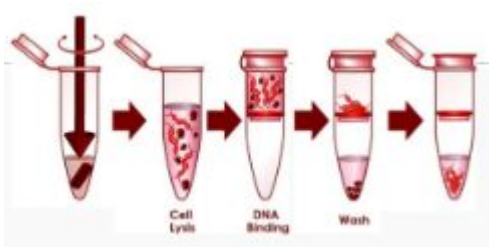
if they actively recruit plant growth promoting bacteria species to help them do this,

then we should expect to see *increased* N2 fixation and other PGP-related *functional properties* and *genes* in their rhizospheres.

Cultivation Independent Approach

Extract & Amplify → **Sequence** → **Identify & Compare**

- Extract genomic DNA
- Amplify genomic DNA from isolate (PCR)



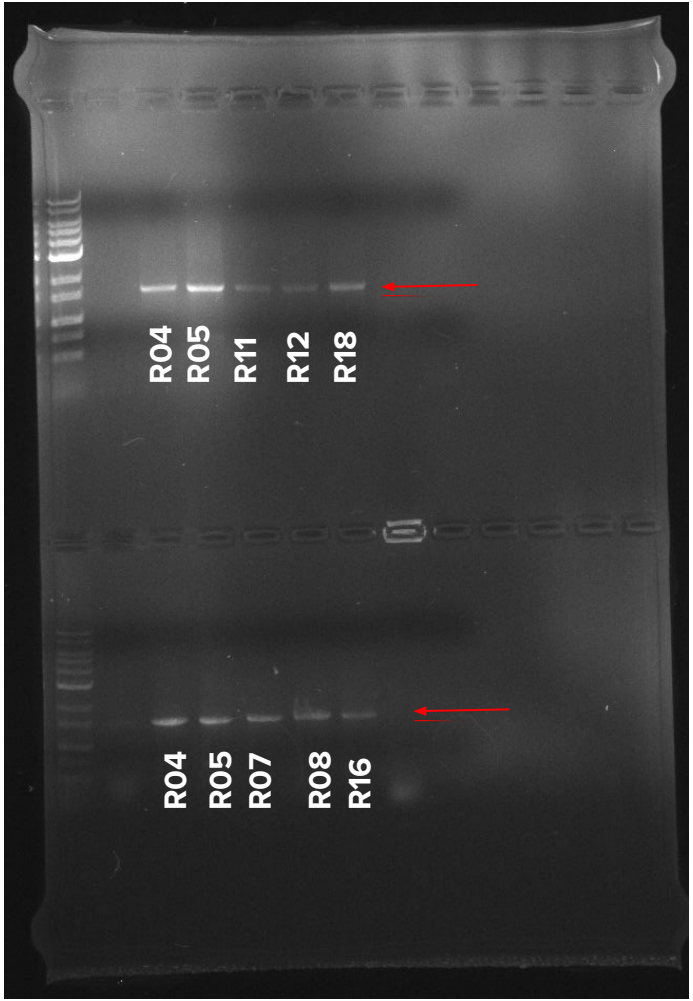
- Isolate & sequence the 16S rRNA

- Identify microorganisms present
- Phylogenetic trees
- Confirm functional genes (more or less coding regions?)

Image Credit:
https://image.slidesharecdn.com/mmpay2b5-161029122951/95/dna-extraction-and-quantityquality-check-9-638.jpg?cb=1478258459
https://www.thermofisher.com/blog/wp-content/uploads/sites/6/2017/05/shutterstock_430479463.jpg

16S PCR Amplification

- Top row DNA extracted using *Guanidine Thiocyanate* method
- Bottom row extracted using *Boiling Lysis*
- **8 total isolates** successfully amplified
- Isolated DNA will be sent for sequencing



Discussion

- Found presence of different plant growth promoting bacteria in cultivated isolates from soil collected 1 year post fire
  - Nitrogen fixation, phosphate solubilization, siderophore production all positive
  - No positive isolates tested for auxin production
- No significant difference in proportion of plant growth promoting factors tested in isolates from 1 month and 1 year post fire

Failure to Reject Null Hypothesis

However...

- Lack of statistical significance doesn't always mean no biological difference
- Further testing with larger sample size and more trials would be ideal

Biases & Limitations

- Past data to compare our results to was limited
  - Not able to control for species and conditions sampled from
- Isolates limited to 4 different enrichment media (R2A, RDM, N2-BAP, and ISP4)
  - Biased “significant” isolates
- Small sample size
  - Limited number of isolates
  - Only 3 trials per assay
- Assay Plates only tested for growth in specific conditions (laboratory setting, incubation time and temperature)

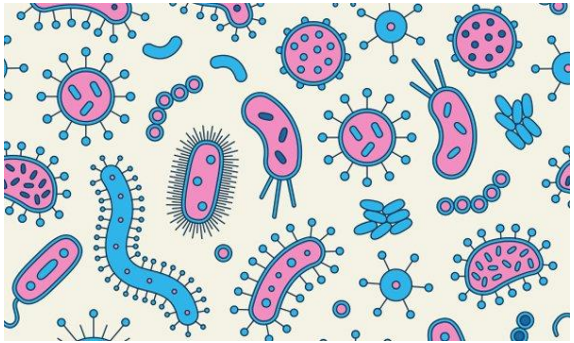


Image Credit: https://www.lifesciencetraininginstitute.com/wp-content/uploads/2017/03/course_microbiology.jpg
https://www.ase.org.uk/sites/default/files/Petri_Dish.jpg

Things to do in 109BL Next Quarter!

- Analyze isolate sequences to learn identity
- Learn more about microbes present in sample through metagenomic sequencing
    - Compare our metagenomic data to groups that collected from native plant rhizosphere
- Use sequence data to construct phylogenetic relationships

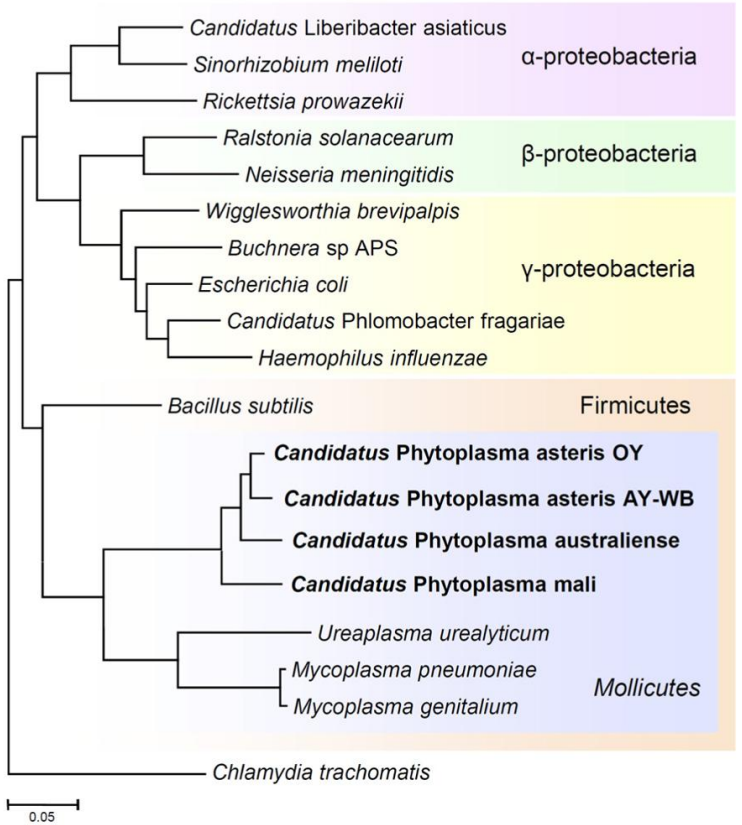


Image Credit: http://www.pnas.org/content/pnas/104/15/6394/F3.large.jpg

More Things to Consider

- Other environmental stressors effects on the microbial community
  - Snowstorms, tornados, earthquakes, etc
- Comparing microbiome of an invasive species in its invaded habitat vs. its own native habitat
- Negative effects of fire induced changes in the soil microbiome
  - ○Promote pathogenic bacteria?
  - ○Reduce plant defenses?

Image Credit: http://curlyzucchiniwellness.com/wp-content/uploads/2014/03/Dollarphotoclub_40209803.jpg
https://upload.wikimedia.org/wikipedia/commons/d/d1/Boreal_pine_forest_after_fire.JPG

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