Diffuse symbioses: competition, coevolution, and pathogen suppression in the rhizosphere

Linda L. Kinkel
Department of Plant Pathology
University of Minnesota
1. Establish an international network of agricultural microbiome researchers to facilitate and advance coordinated synthetic, collaborative, and integrative scientific studies of agricultural plant and soil microbiomes.

2. Leverage existing national and international long-term agricultural research sites and projects to create an intercontinental-scale agricultural microbiome research platform.

3. Enhance development of foundational concepts and training in agricultural microbiomes research through a series of workshops targeted toward researchers in the field.

4. Enhance understanding of plant microbiomes and plant-microbiome-environment interactions using model plants and cropping systems, while establishing strategies for applying and translating model system data towards practical management of agricultural microbiomes.

To get involved, contact Linda Kinkel --- KINKEL@UMN.EDU
Post-doc position available.
Pathogen-suppressive populations

What factors mediate the capacities of antibiotic-producing *Streptomyces* populations to suppress plant pathogens in soil?

And how can we use this information to suppress diseases in cropping systems?
Naturally-occurring disease suppressive soil

30 years of potato monoculture:
no scab disease (*Streptomyces scabies*)

MANY *Streptomyces*

In vitro inhibition of diverse array of plant pathogens:
*S. scabies*, *Phytophthora*, *Pythium*, *Fusarium*, *Verticillium*,
*Aphanomyces*, *Rhizoctonia*, *Pratylenchus*, *Septoria*, *Sclerotinia*, ...
Streptomyces:

* Gram positive, filamentous saprophytes
* Ubiquitous in soils
* Extracellular enzymes
* Nutrient cycling
* Antibiotic producers, significant in human medicine
* Inhibit bacteria, fungi, and nematodes
* Plant growth-promoting hormones
Inoculative biocontrol: potato scab

(Liu et al., 1995)
Inoculative biocontrol: *Phytophthora* on alfalfa

(Xiao et al., 2002)
But why is the soil suppressive?

How did it become suppressive?
Suppressive vs. conducive soils
Suppressive vs. conducive soils

Density

- HIGHER *Streptomyces* population densities
Suppressive vs. conducive soils

Density

• HIGHER *Streptomyces* population *densities*

Antibiotic activity

• GREATER *Streptomyces inhibitory activities*
  *greater proportions of inhibitors*
  *greater mean inhibition zones*
Suppressive vs. conducive soils

Density
- HIGHER Streptomyces population densities

Antibiotic activity
- GREATER Streptomyces inhibitory activities
  * greater proportions of inhibitors
  * greater mean inhibition zones

Diversity
- HIGHER diversity in Streptomyces inhibitory phenotypes (Shannon-Wiener 2.56S vs. 1.85C)
More---Better---More Diverse.....

disease suppression

How (why) did the shift in antibiotic-producing populations occur?

*Density- and frequency-dependent selection?*
Long-term monoculture enriches saprophytic *Streptomyces* densities
Long-term monoculture enriches saprophytic *Streptomyces* densities

Density-dependent selection for antagonistic phenotypes

*Outcome: increased frequencies and intensities of inhibitory phenotypes*
Long-term monoculture enriches saprophytic *Streptomyces* densities

Density-dependent selection for antagonistic phenotypes
*Outcome: increased frequencies and intensities of inhibitory phenotypes*

Frequency-dependent selection for novel or rare antibiotics
*Outcome: increased diversity of antibiotic phenotypes*
Long-term monoculture enriches saprophytic *Streptomyces* densities

Density-dependent selection for antagonistic phenotypes
*Outcome: increased frequencies and intensities of inhibitory phenotypes*

Frequency-dependent selection for novel or rare antibiotics
*Outcome: increased diversity of antibiotic phenotypes*

**OUTCOME:** Disease suppressive *Streptomyces* community

*High densities and diversities of inhibitory phenotypes*
But what is the role of antibiotics in the community?

Antibiotics as weapons

Is there selection for antibiotic inhibitory phenotypes in local communities?

Local adaptation: Are sympatric isolates better at killing one another than allopatric isolates?
Inhibition is more intense among sympatric vs. allopatic *Streptomyces*.

Local selection for more intense inhibition of sympatric isolates (*antibiotic weapons*).

(Kinkel et al., 2014)
Inhibition zone and niche overlap were positively correlated among sympatric ($R^2 = 0.26; p<0.001$), but not allopatric ($R = 0.009; p=0.74$) Streptomyces.

Antibiotics as weapons against sympatric resource competitors
Selection for antibiotic inhibitory phenotypes is not independent of nutrient use.....

Niche differentiation as an alternative to inhibition?
Coevolutionary model for enrichment of antibiotic inhibitory phenotypes in the soil microbiome

Microbial community density, diversity, composition

Microbial species interactions

Evolutionary potential

Coevolutionary trajectory

Coevolutionary escalation arms race

Highly antagonistic community; high frequencies and intensities of antibiotic inhibition

Phylogenetic and phenotypic composition

Coevolutionary character displacement niche differentiation

Growth-efficient, niche-differentiated community

Two distinct coevolutionary trajectories....What drives these?
Coevolutionary model for enrichment of antibiotic inhibitory phenotypes in the soil microbiome

What drives these different trajectories?
3 specific hypotheses!
Are populations in plant monocultures more inhibitory than communities in polyculture plant communities?

Long-term field experiment: NSF-LTER Cedar Creek

2 C4 grasses: Andropogon gerardii  
Schizachyrum scoparium

2 legumes: Lespedeza capitata  
Lupinus perennis

Sampled the rhizosphere of each plant species in 1, 4, 8, and 16-species plots

Culturing and genomic approaches
Assaying pathogen inhibitory activity in soil

Streptomyces scabies  Fusarium oxysporum  Verticillium dahliae

Rhizoctonia solani  Fusarium graminearum
Rhizosphere microbial communities are consistently more inhibitory in low diversity vs. high diversity plant communities

(Bakker et al., 2013)
Nutrient use:

Are *Streptomyces* populations in plant monocultures less niche-differentiated than communities in polyculture plant communities?
Plant community richness and plant species influence nutrient use among soil *Streptomyces*.

Essarioui et al., 2017
Are polycultures more niche-differentiated?

Sympatric niche overlap among *Streptomyces* from monoculture is greater than among *Streptomyces* from polyculture plant communities.

Nutrient use:
- Biolog SF-P2

*n = 80 randomly-selected *Streptomyces*

(Essarioui, 2017)
Microbial species interactions drive functional characteristics

Monocultures: *Streptomyces* are more pathogen-inhibitory (coevolutionary escalation)

Polycultures: *Streptomyces* are more niche-differentiated (coevolutionary character displacement)
Plant community richness is correlated with the richness of soil carbon compounds.

**Pyrolysis gas chromatography mass spectrometry data:**
# carbon peaks in soil; Castle and Grandy, unpublished
1. Microbial interactions are critical to selection for distinct phenotypic or functional characteristics

2. Plants and plant communities (diversity) define the nutrient context for microbial interactions (what to eat)

What about other taxa......
**Streptomyces** inhibit sympatric more than allopatric *Fusarium*

![Bar chart showing mean proportion of inhibitors and mean size of inhibition zone for sympatric and allopatric interactions.](image)
Local selection: *Streptomyces* inhibit sympatric (but not allopatric) *Fusarium* isolates that pose a greater competitive challenge.

![Graph showing correlation between fusarium niche overlap and streptomyces inhibition]

- **Sympatric:** $R = 0.52^{**}$
- **Allopatric:** $R = 0.1$

- Sympatric interactions
- Allopatric interactions
Local selection: *Fusarium* inhibit sympatric (but not allopatric) *Streptomyces* isolates that pose a greater competitive challenge

![Graph showing the relationship between the total inhibition of *Streptomyces* and the niche overlap with *Fusarium*. The correlation coefficients are Sympatric: $R = 0.86^{***}$ and Allopatric: $R = 0.08$.](image-url)
Microbiomes are exquisitely adapted to local abiotic and abiotic environments.

Complex networks of interaction mold the functional capacity of the soil/rhizosphere microbiome.
Microbial interactions occur not between 2 individual populations, but within complex communities......

Networks of inhibitory interactions within individual communities
Complex networks of inhibitory and signaling interactions

***Unraveling the LAYERS of interactions***
Complex networks of inhibitory and signaling interactions

***Unraveling the LAYERS of interactions***
TRANSLATION to practice?
Managing soil microbiomes for disease suppression***

1. To manage microbiomes, manage species interactions.

2. Differentiate short-term (ecological) vs. long-term (evolutionary) goals and outcomes.

***
Targeting species interactions and coevolutionary dynamics suggests novel means of management.

---

**Evolutionary potential**
- Microbial community density, diversity, composition
- Microbial species interactions
- Polyculture

**Nutrients**
- Monoculture

**Coevolutionary trajectory**
- Coevolutionary escalation *arms race*
- Coevolutionary character displacement *niche differentiation*

**Outcomes**
- Highly antagonistic community; high frequencies and intensities of antibiotic inhibition
- Phylogenetic and phenotypic composition
- Growth-efficient, niche-differentiated community
<table>
<thead>
<tr>
<th>Management strategy</th>
<th>Short-term</th>
<th>Long-term</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutrient amendments (single vs. mixture)</td>
<td><em>Increase microbial population densities, diversities, activities</em></td>
<td><em>Select for inhibitory or niche-differentiated indigenous communities</em></td>
</tr>
</tbody>
</table>
Soil carbon amendments can alter microbial nutrient use preferences and potential for competitive interactions in soil communities

(Felice, unpublished)
<table>
<thead>
<tr>
<th>Management strategy</th>
<th>Short-term goal/outcome</th>
<th>Long-term goal/outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutrient amendments (single vs. mixture)</td>
<td><em>Increase microbial population densities</em></td>
<td><em>Select for inhibitory or niche-differentiated indigenous communities</em></td>
</tr>
<tr>
<td>Antagonistic inoculants</td>
<td><em>Increase antagonist densities</em></td>
<td><em>Select for resistant pathogens? Destabilize indigenous arms race?</em></td>
</tr>
<tr>
<td>Management strategy</td>
<td>Short-term goal/outcome</td>
<td>Long-term goal/outcome</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>----------------------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Nutrient amendments (single vs. mixture)</td>
<td><em>Increase microbial population densities</em></td>
<td><em>Select for inhibitory or niche-differentiated indigenous communities</em></td>
</tr>
<tr>
<td>Antagonistic inoculants</td>
<td><em>Increase antagonist densities</em></td>
<td><em>Select for resistant pathogens? Destabilize indigenous arms race?</em></td>
</tr>
<tr>
<td>Broad niche, growth efficient inoculants</td>
<td><em>Increase resource competition</em></td>
<td><em>Accelerate indigenous arms race? Destabilize indigenous niche differentiation?</em></td>
</tr>
<tr>
<td>Management strategy</td>
<td>Short-term goal/outcome</td>
<td>Long-term goal/outcome</td>
</tr>
<tr>
<td>-------------------------------------------------</td>
<td>------------------------------------------------</td>
<td>-------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Nutrient amendments (single vs. mixture)</td>
<td>Increase microbial population densities</td>
<td>Select for inhibitory or niche-differentiated indigenous communities</td>
</tr>
<tr>
<td>Antagonistic inoculants</td>
<td>Increase antagonist densities</td>
<td>Select for resistant pathogens? Destabilize indigenous arms race?</td>
</tr>
<tr>
<td>Broad niche, growth efficient inoculants</td>
<td>Increase resource competition</td>
<td>Accelerate indigenous arms race? Destabilize indigenous niche differentiation?</td>
</tr>
<tr>
<td>Signaling inoculants</td>
<td>Upregulate antibiotic production</td>
<td>Accelerate indigenous arms race?</td>
</tr>
</tbody>
</table>
Both incidence and severity of disease were smaller in the PRESENCE than in the absence of a SIGNALING STRAIN.

Field trial on potato

5 inoculant mixtures (3-5 inoculants), each inoculated WITH or WITHOUT a signaling strain.

Potato scab incidence (lesion number) and severity (% cover) were assessed at harvest.
<table>
<thead>
<tr>
<th>Management strategy</th>
<th>Short-term goal/outcome</th>
<th>Long-term goal/outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutrient amendments (single vs. mixture)</td>
<td>Increase microbial population densities</td>
<td>Select for inhibitory or niche-differentiated indigenous communities</td>
</tr>
<tr>
<td>Antagonistic inoculants</td>
<td>Increase antagonist densities</td>
<td>Select for resistant pathogens? Destabilize indigenous arms race?</td>
</tr>
<tr>
<td>Broad niche, growth efficient inoculants</td>
<td>Increase resource competition</td>
<td>Accelerate indigenous arms race? Destabilize indigenous niche differentiation?</td>
</tr>
<tr>
<td>Signaling inoculants</td>
<td>Upregulate antibiotic production</td>
<td>Accelerate indigenous arms race?</td>
</tr>
<tr>
<td>Synthetic communities to capture network properties</td>
<td>Increase population functions, multiple mechanisms of suppression</td>
<td>Harness power of coevolution</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Achieve stable colonization, coexistence, and function</td>
</tr>
</tbody>
</table>
Translating Science to Practice?

1. Managing the microbiome means managing species interactions.

2. Distinguish ecological vs. evolutionary dynamics of microbiomes.

3. Develop conceptual, predictive frameworks to advance hypothesis-testing, build infrastructure for translation to management.

4. Sequencing/’omics and culturing are symbiotic (mutualistic).

5. Deepened understanding of community structures and their relationships to function needed for effective management.
Acknowledgements

The Microbes

The People

The Money

Lindsey Hanson, Dr. Adil Essarioui, Dr. Sarah Castle, Dr. Zewei Song, Dr. Dan Schlatter, Dr. Matt Bakker, Laura Felice, Dr. Patricia Vaz-Jauri, Dr. JP Dundore-Arias, Miriam Gieske, ..........
Field trial, 2016: Amend fumigated and non-fumigated soils with microbial amendments, carbon amendments, or the combination.

**Effects on potato scab intensity**

<table>
<thead>
<tr>
<th>Chloropicrin fumigation</th>
<th>Vapam fumigation</th>
<th>No fumigation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent potato scab disease (%)</td>
<td>p=0.0024</td>
<td>p=0.0165</td>
</tr>
</tbody>
</table>

- **Carbon amendments** suppress disease and enhance microbial inoculants (but they can suppress potato yields)
What are the effects on YIELD?

But carbon amendments impose a yield cost
Soil carbon amendments can increase the inhibitory capacity among indigenous soil *Streptomyces*.

*(Schlatter et al., 2008)*
How do NPK amendments influence endophytic community composition and function (phenotypes)?

1) Does endophytic composition vary with NPK amendment?

2) Do microbial nutrient use phenotypes vary with NPK amendment?

3) Implications of NPK-associated shifts for microbial species interactions?

NSF Macrosystems
Borer, Seabloom, May

Hansen, Hanson, Johnson, Song, Spawn
Methods

- **Culture Collection**
  - Big Bluestem (*Andropogon gerardii*)
  - 12 leaves: 6 Control, 6 NPK-amended
  - Isolate collection – ~800 cultures
    - Random Sampling – 120 fungi, 120 bacteria

- **Assays**
  - DNA Extraction/Sanger Sequencing
  - BIOLOG SF-P2 carbon use profiles
  - Inhibitory phenotypes
  - Resistance phenotypes
NPK Influences Fungal Composition

Fungi

Bacteria

NPK

Control
Niche Width

(How many things they eat)
NPK amendments: altered nutrient use among endophytic fungi
Endophytes from NPK-amended plants vary in nutrient use as compared with endophytes from control plants
Phenotype and phylogeny are correlated among fungi. \( r^2 = 0.05 - 0.098; \ p = 0.001 \)
Sympatric competitive interactions?

Consequences for species interactions and selection?

Niche overlap

Greater Competition

Less Competition
SUMMARY: NPK amendments

- Altered fungal community composition
- Altered resource use profiles
- Bacterial relative competitiveness increases

Implications for microbial functional capacities and coevolutionary dynamics?

- Disease suppression
- Plant health
- Nutrient cycling