

Biogeographical patterns of the rhizosphere microbiome of a native and invasive grass in the western United States

Plus other ongoing cheatgrass studies

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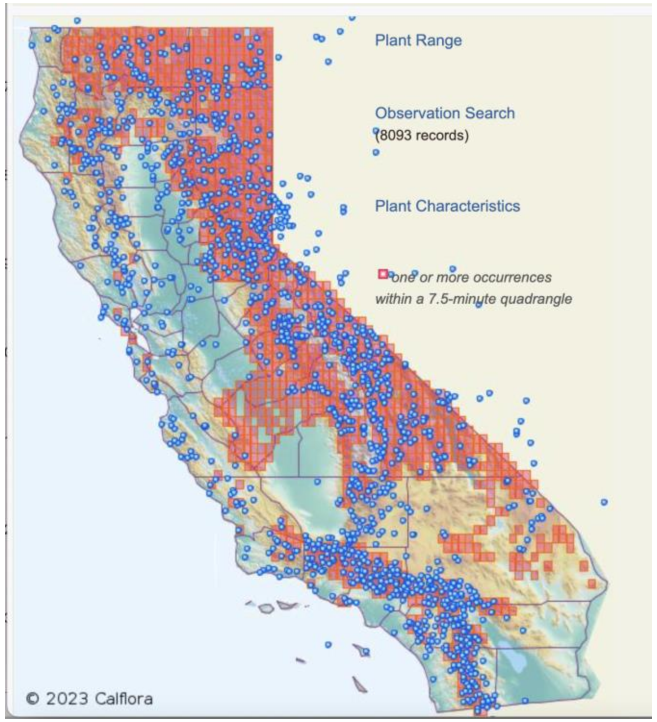
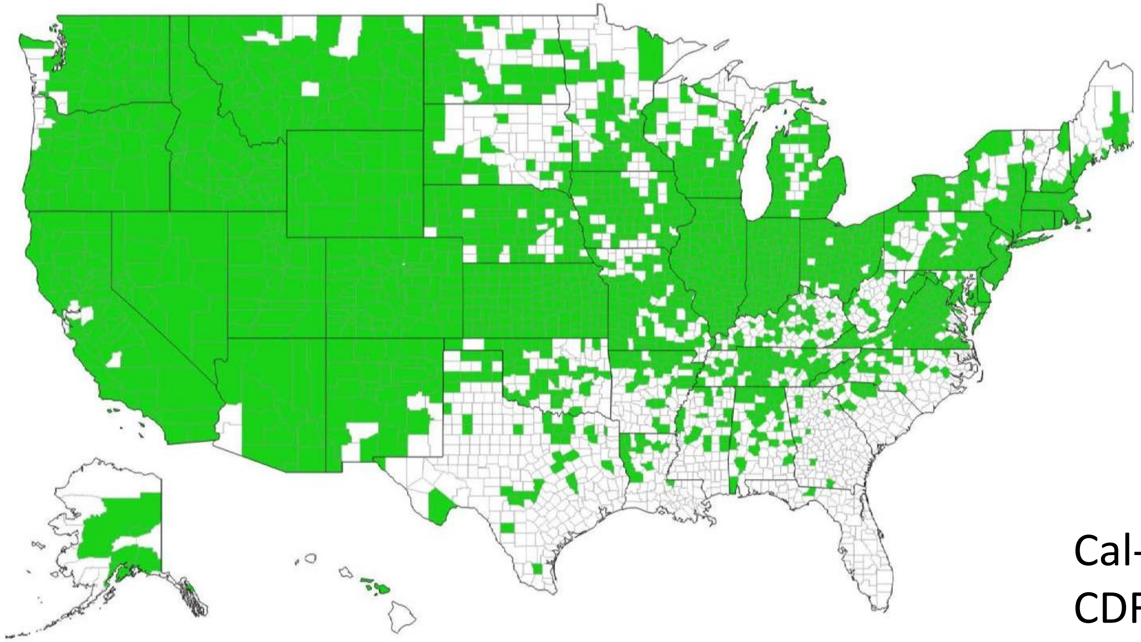
United States Department of Agriculture
Agricultural Research Service

Nemesis of the west: Cheatgrass



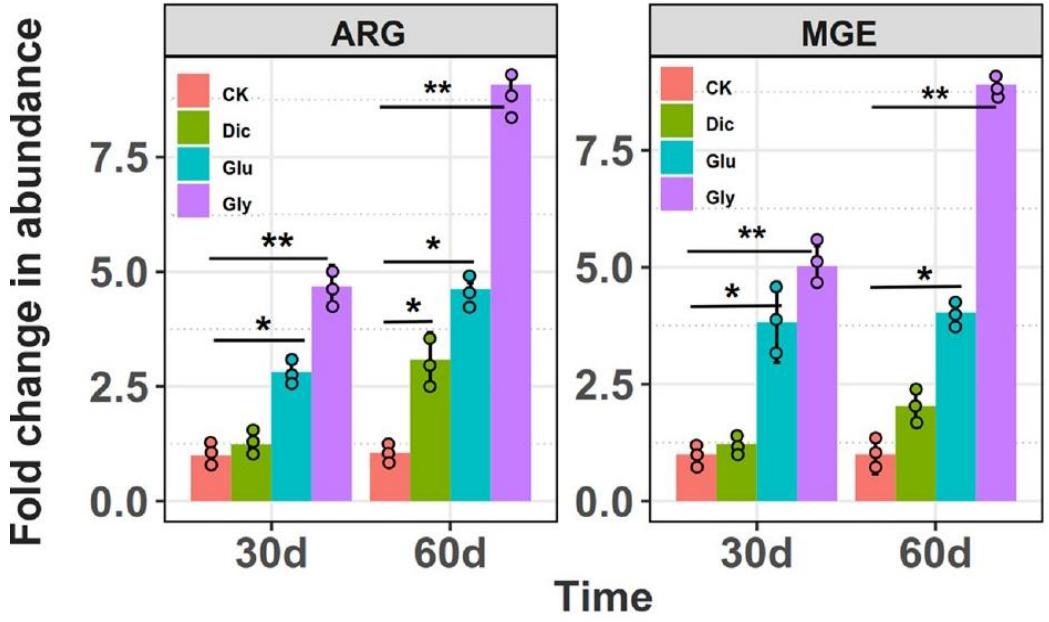
- Less desirable forage for livestock and wildlife
- Increase fire risk, intensity and frequency
- Displaces native plant species
- Altered soil nutrient levels and cycles

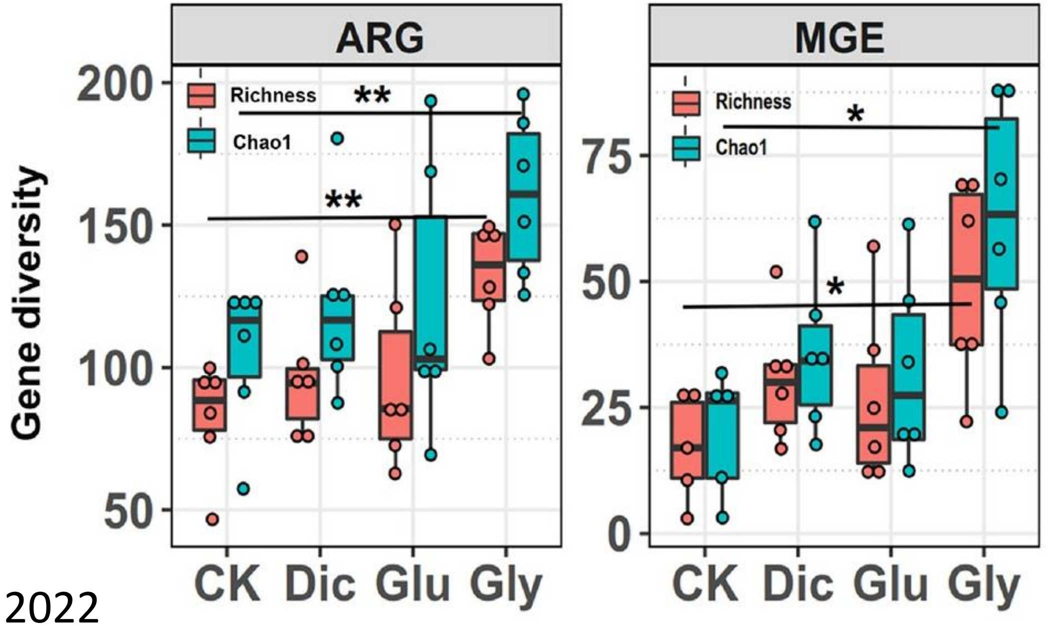


Cal-IPC rating = high
CDFA rating = C

Chemical and biological agents for control

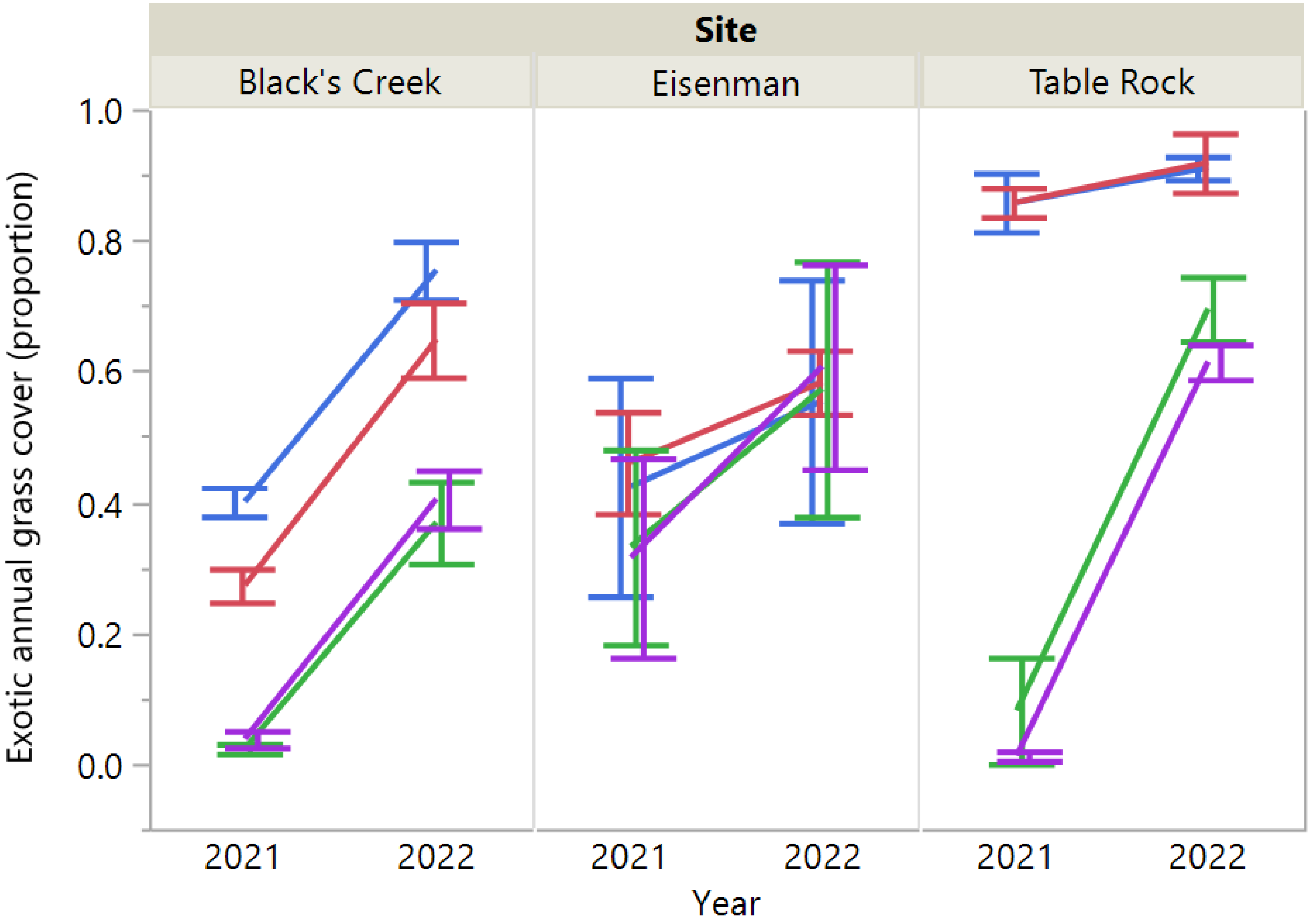
- Biocontrols like ACK55 (*Pseudomonas*) showed early promise, but ACK55 has had limited success in the field
- Chemical herbicides are widely used and effective (at least short term), but do they harm/alter the soil microbiome?
    - Increased antibiotic resistance found with broadly used herbicides





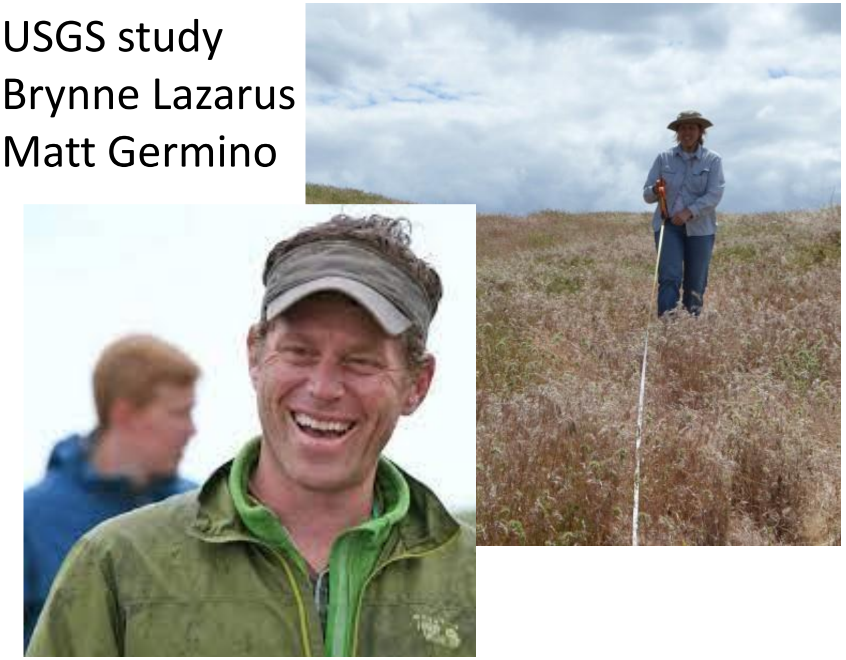
Liao et al. 2022

Chemical suppression of invasive annuals, but short-lived effects



Ordered_trt
1-Control
2-Battalion Pro
3-Imazapic
4-Battalion Pro + Imazapic

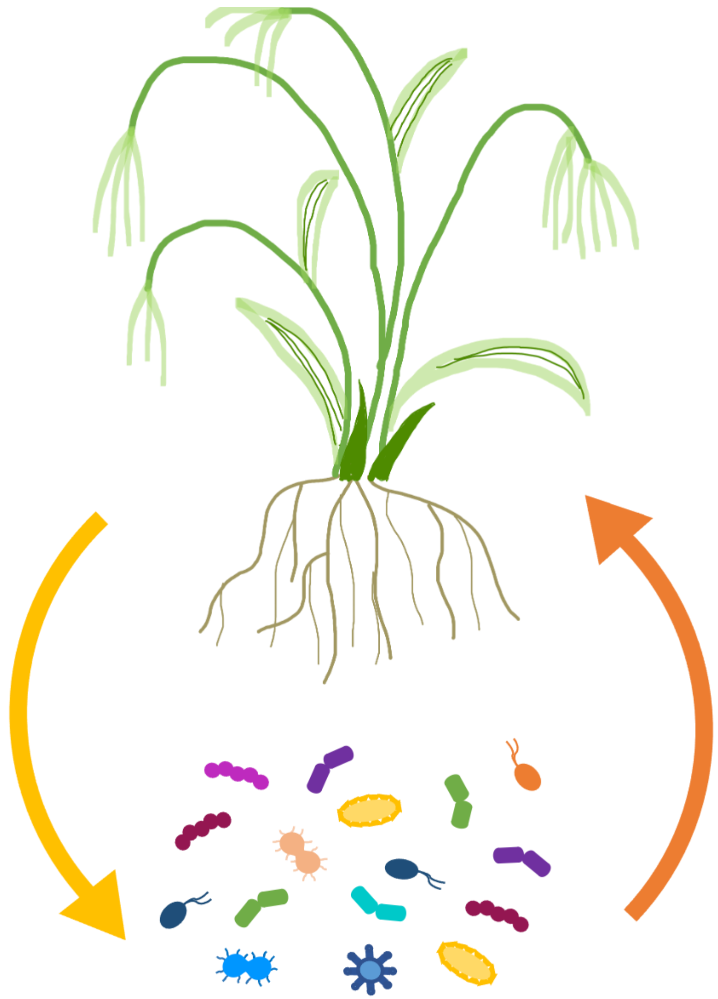
USGS study
Brynne Lazarus
Matt Germino



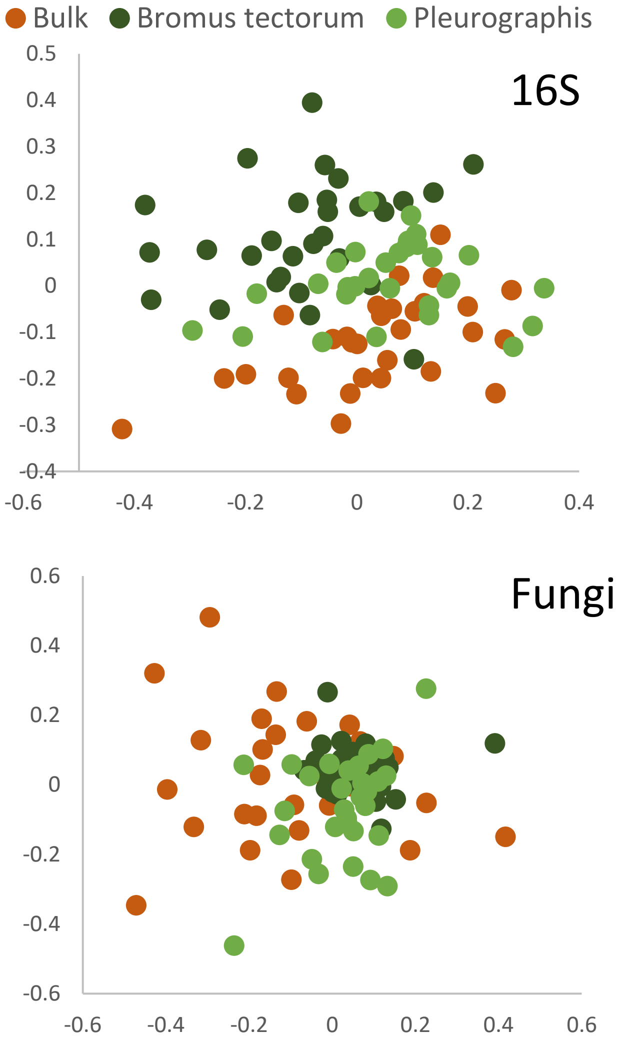
Funding from Idaho Transportation Department

Quest to identify better restoration tools

- Limited understanding of the fundamental ecology of many invasive species, such as cheatgrass
  - Soil alterations
    - Root exudate production
    - Allelopathy
  - Interactions with the microbiome
    - Rhizosphere
    - Seed endophytes
- How might this knowledge improve approaches to widespread control?

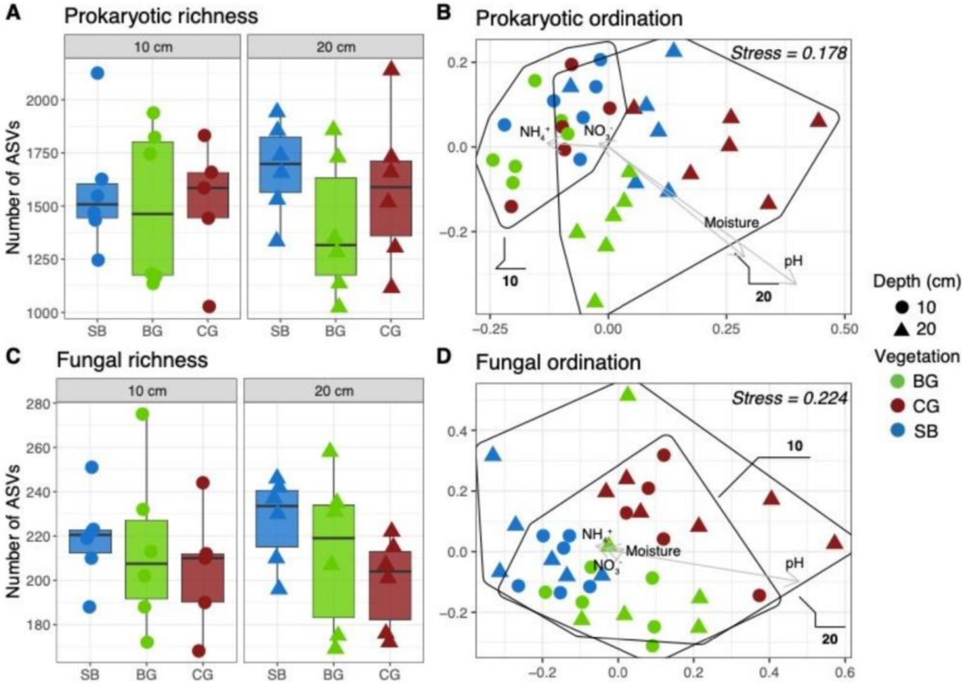


Results from other studies



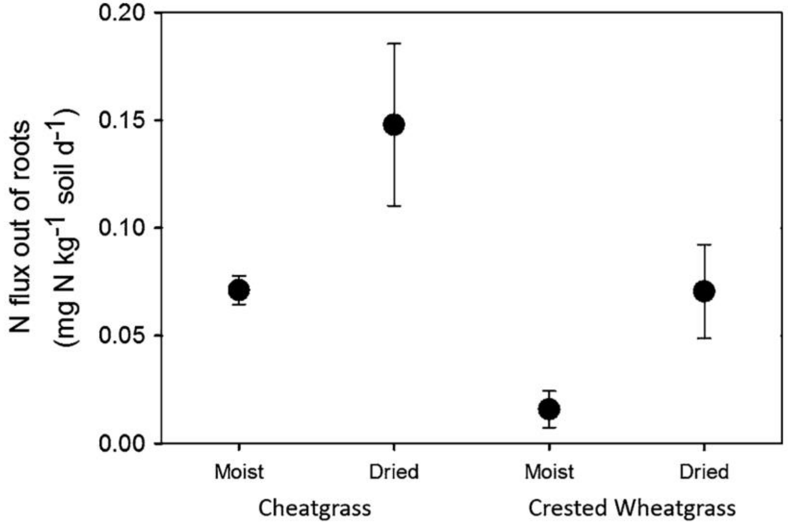
Albright et al. 2017

- Both studies done in Utah
- Multiple time points (Albright), two depths (Reitstetter)
- Significant shifts in community similarity, decreased fungal diversity



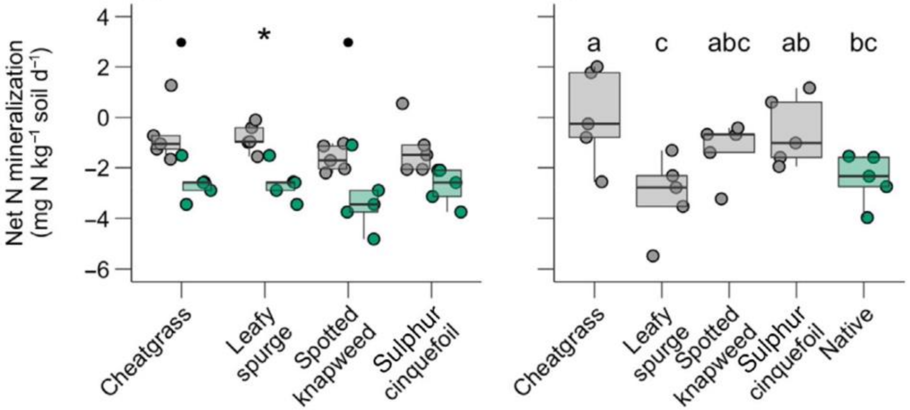
Reitstetter et al. 2022

Morris et al. 2016

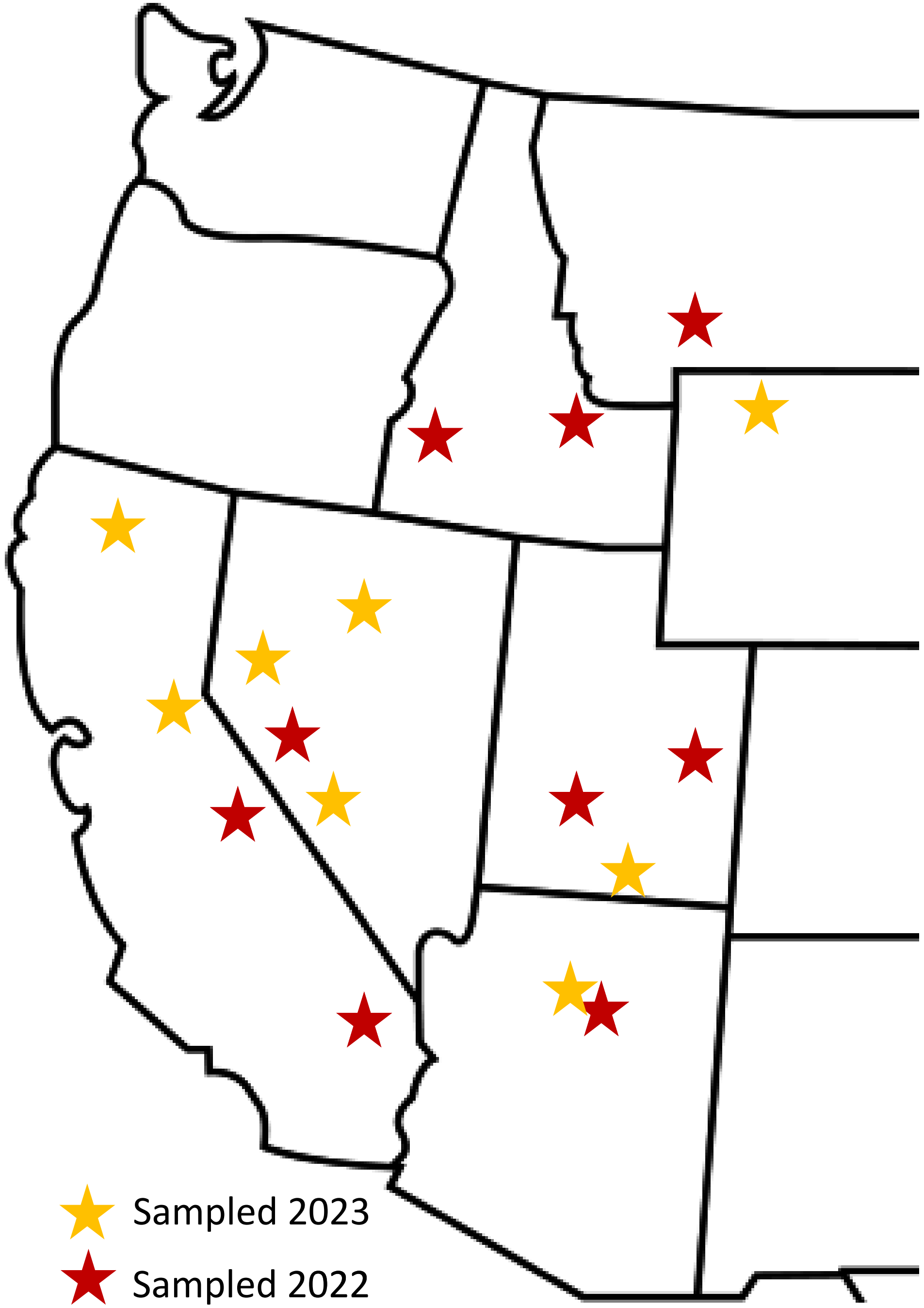


- Root exudate production (15N)
- Crested wheatgrass = non-native

Nasto et al. 2021



- Higher N mineralization in experimental cheatgrass plots



Spatial sampling: Cheatgrass and squirreltail

- Rhizosphere soil sampling
  - Microbiome: targeted and shotgun
  - Soil characteristics (chemistry, pH)
  - Culturing for potential biocontrol *Pseudomonas*
- Seed collection
  - Endophytes

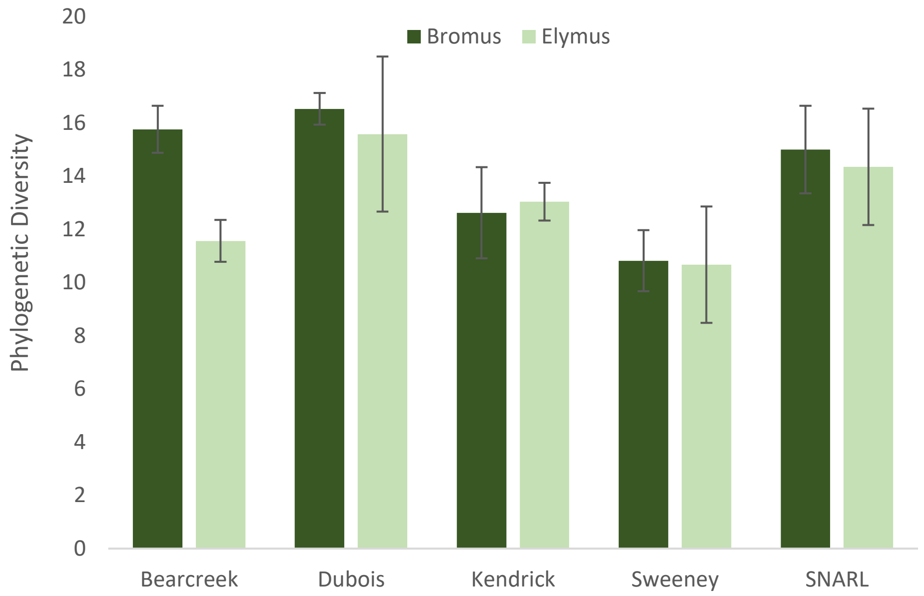
Sampling: soils and seeds

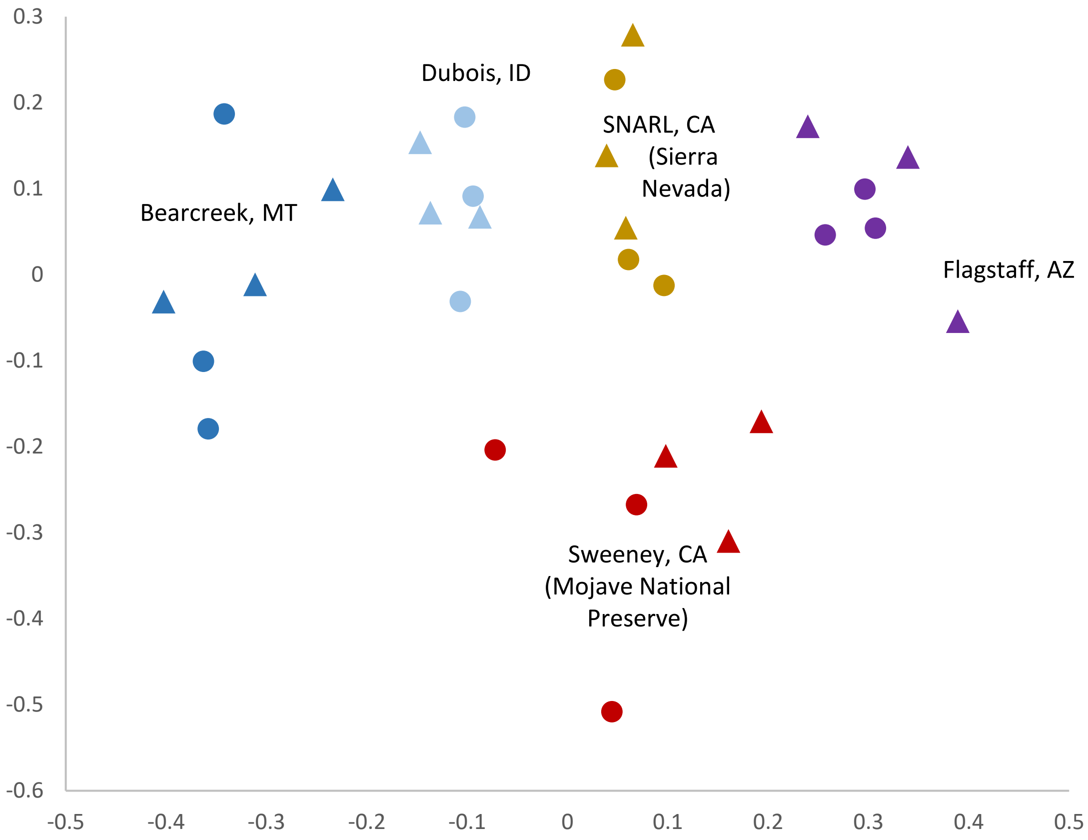
- Select paired cheatgrass and *Elymus* plants of similar size and developmental stage
- Collect soils at the base of the plant, targeting the root zone
- Stabilize DNA and RNA with chemical solution for amplicon, metaG
- Collect additional soils for pH, culturing and chemical analyses (ongoing)
- Collect seeds from cheatgrass and *Elymus*, plus any other mature native grasses

	Temperature		pH		Ecosystem
	Bromus	*Elymus*	*Bromus*	*Elymus*	
SNARL, CA	22.2	21.8	6.36	6.47	Sagebrush
Sweeney, CA	20.8	20.8	6.88	6.66	Pinyon-juniper
Flagstaff, AZ*	25.4	22.4	6.71	6.70	Ponderosa
Doney Park, AZ	22.0	21.0	6.46	6.40	Pinyon-juniper
Virginia City, NV	16.5	16.3	6.90	6.84	Sagebrush
Cedar City, UT	21.4	20.7	7.17	7.11	Sagebrush-oak
Moab, UT	35.9	35.7	9.15	8.91	Pinyon-juniper
Dubois, ID	20.4	19.4	7.19	7.16	Sagebrush
Boise, ID	16.3	15.8	7.35	7.04	Sagebrush
Bearcreek, MT	21.5	20.7	9.06	8.65	Sagebrush
Yreka, CA	21.0	20.4	8.07	8.01	Juniper
Susanville, CA	18.9	17.7	7.40	7.28	Sagebrush
Winnemucca, NV*	19.9	22.5	8.31	7.84	Sagebrush
Wellington, NV*	24.2	23.1	7.38	7.44	Pinyon-juniper
Tonopah, NV*	21.1	20	8.01	7.82	Rabbitbrush
Kanab, UT*	21.6	20.8	8.43	8.63	Pinyon-juniper
Emblem, WY*	23.9	23.4	8.49	7.9	Sagebrush
	21.9	**21.3**	**7.81**	**7.66**	

*Smaller scale sites

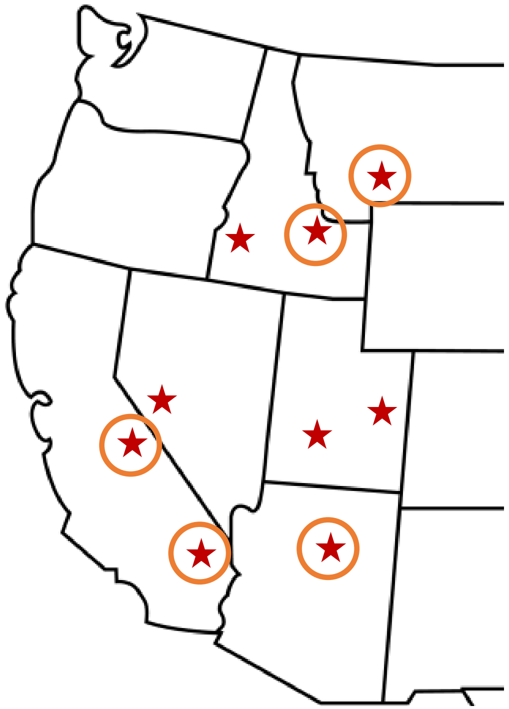
Rhizosphere microbiome: preliminary results for LSU





● *Elymus elymoides*

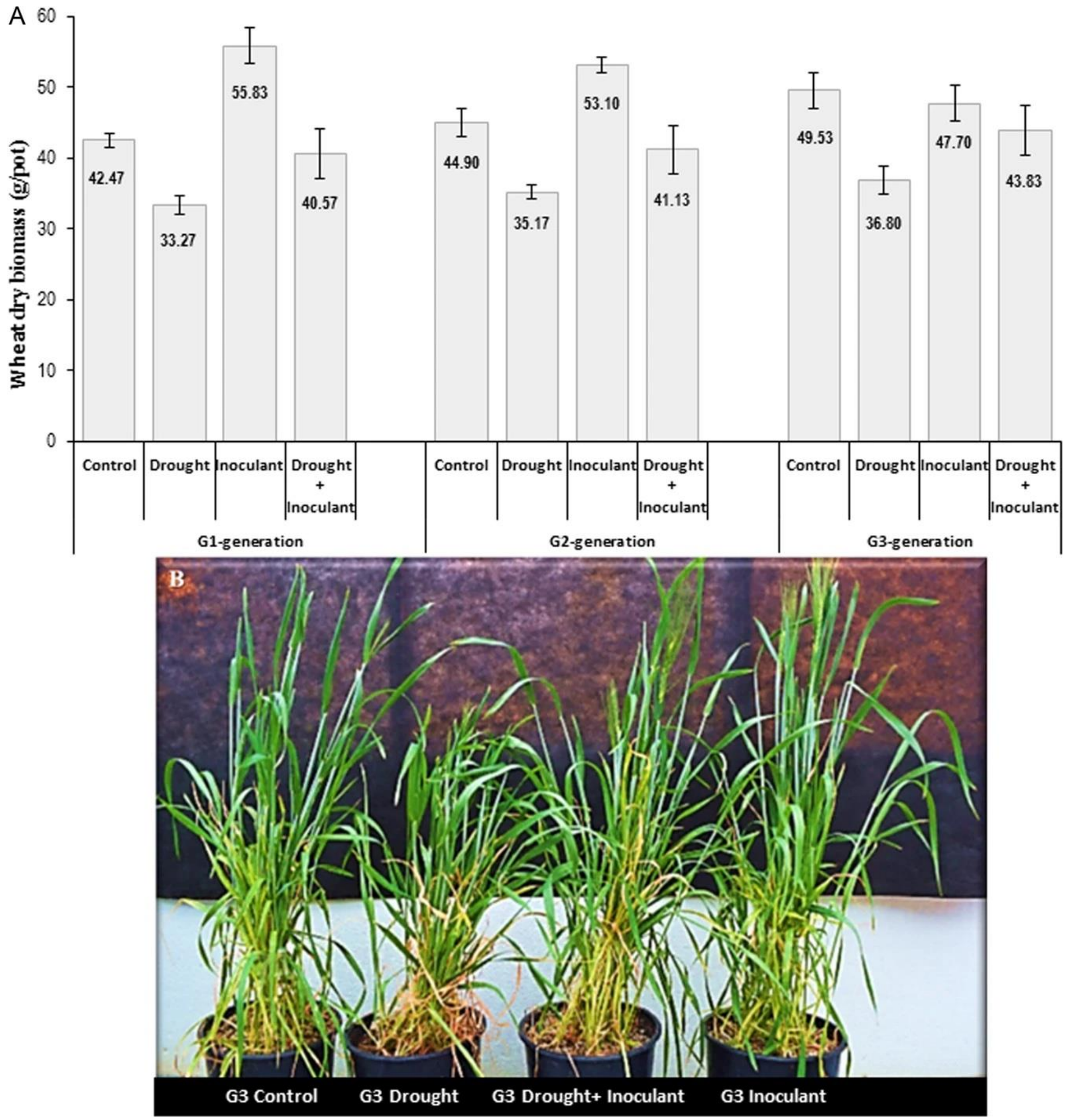
▲ Cheatgrass (*Bromus tectorum*)



- Site differences, no effect of plant species
- Low replication
- Additional sites currently on the MiSeq

Alternative targets for cheatgrass biocontrol

Penicillium seed endophytes in *Triticum* increased drought tolerance (Vujanovic et al. 2019)

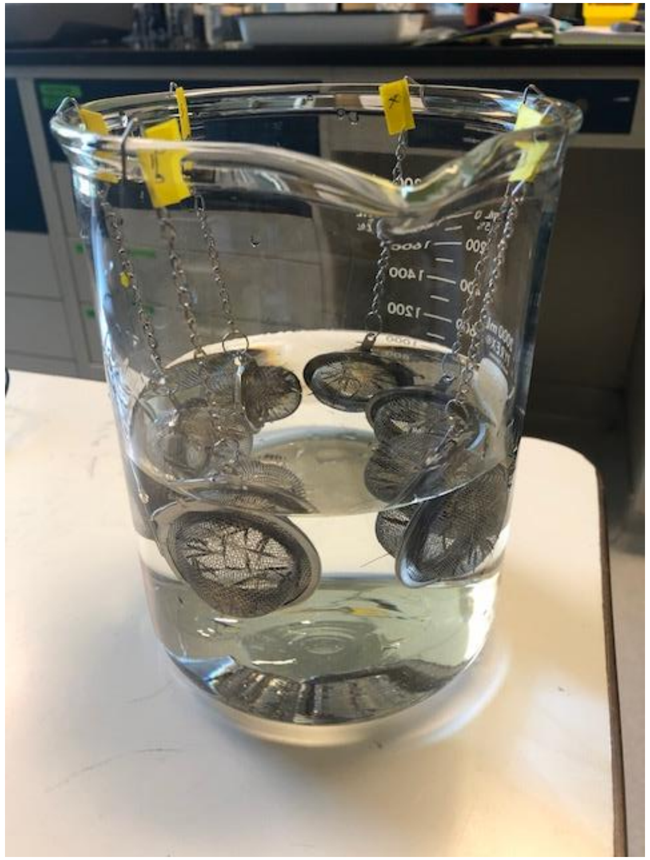


- Short-lived effects of herbicides suggest we need to target the seed bank for effective control
- Vertical transmission of endophytes in grasses, including *Bromus*
- No studies to date on the seed endophyte community of cheatgrass

Characterizing cheatgrass seed endophytes

Critical questions:

1. Do endophytes promote survival of cheatgrass?
2. Is there a common seed endophyte community across the range of cheatgrass?
3. Can we target the endophytes with biocontrol as a way for more effective, longer-term control of cheatgrass?

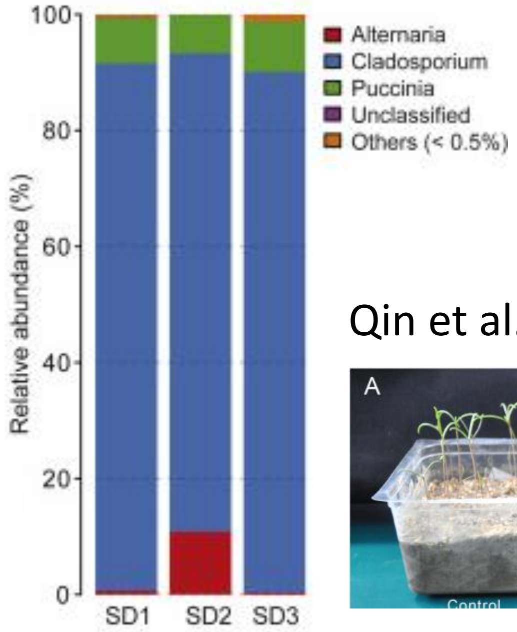


Preliminary seed analysis

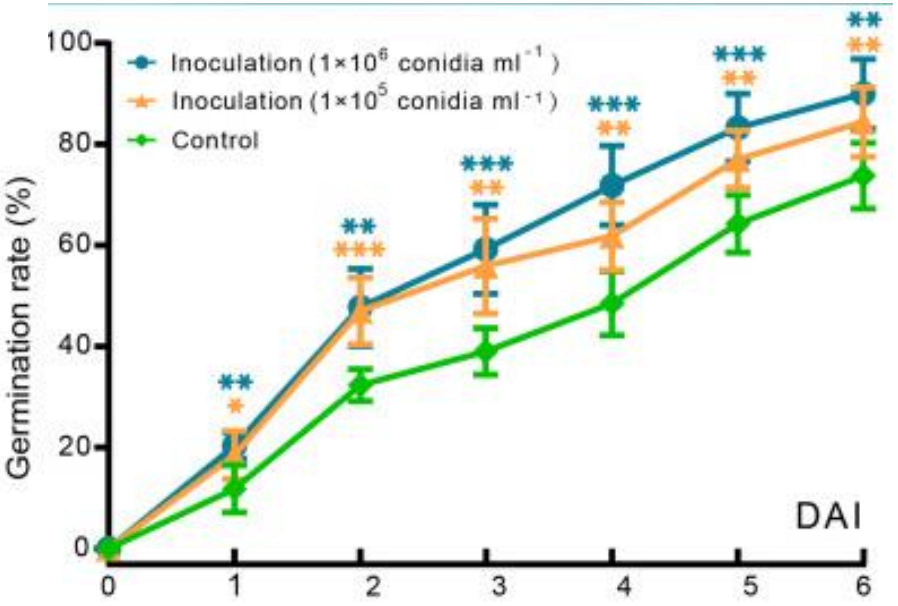
- Amplification of plant DNA during PCR reduced fungal sequences
- *Cladosporium* species identified from Boise seeds

Sandoval-Denis et al. 2015

- Ongoing culture and culture-independent analyses of cheatgrass and *Elymus* endophytes from 2022 and 2023

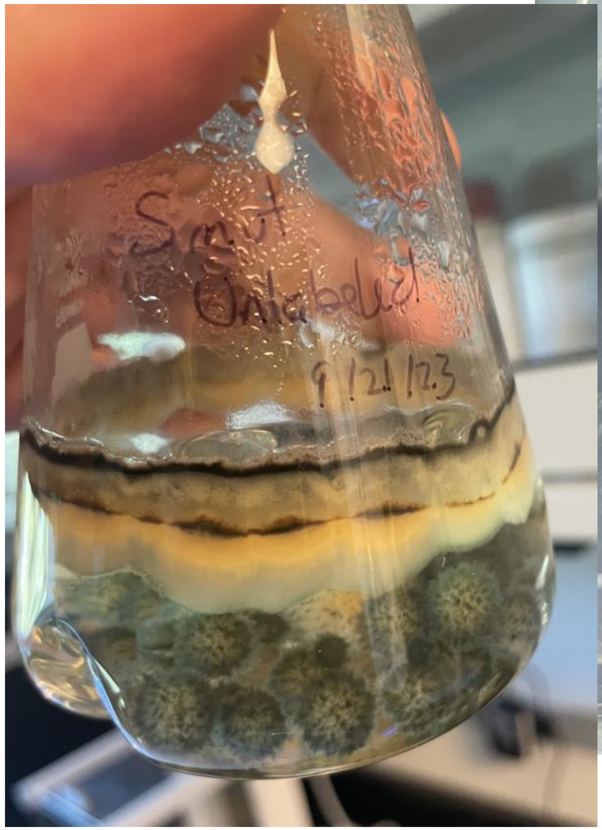


Qin et al. 2016

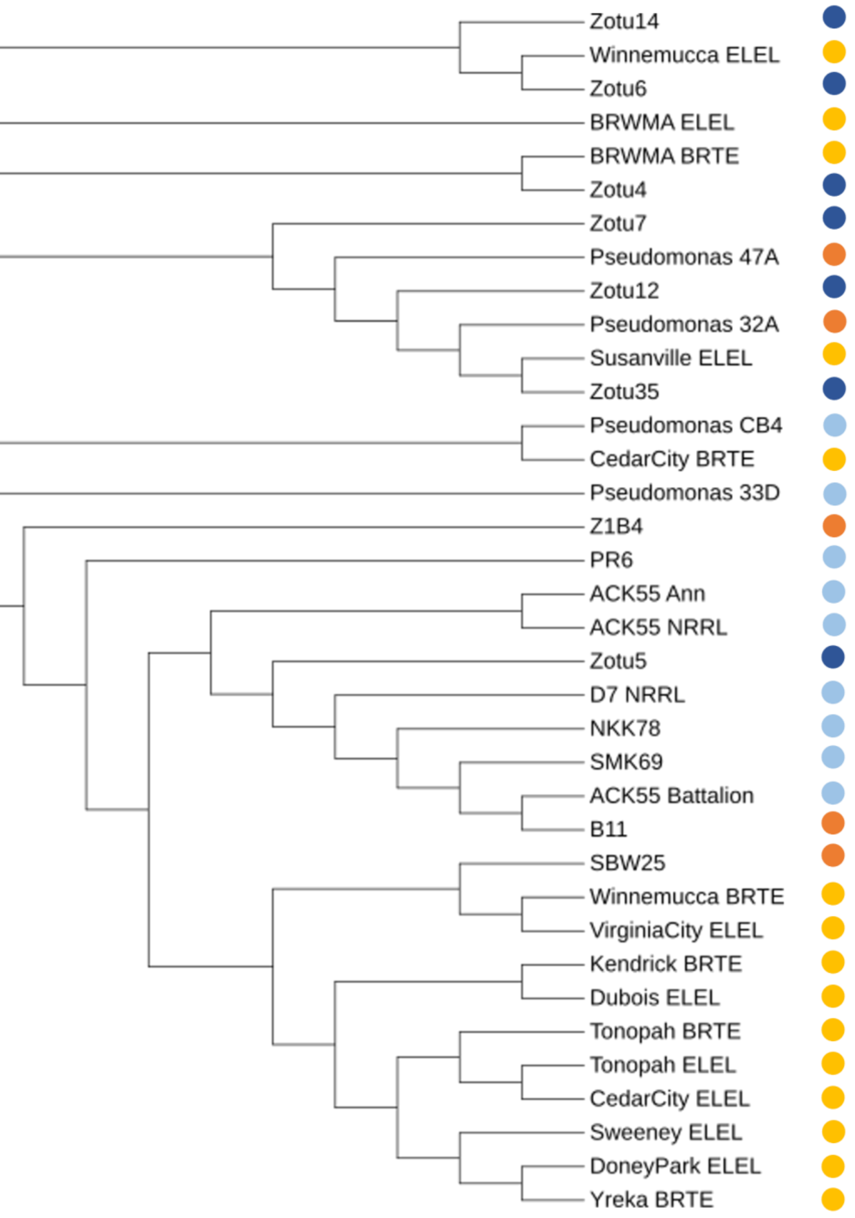


Naturally occurring pathogens: Smut and “Black fingers of death”

- Smut fungi and *Pyrenophora* kill cheatgrass seeds, but not at sufficient levels to control invasions
- Geographic distributions are not well known
- Genetic characterizations are patchy, particularly whole-genomes



Can we identify local *Pseudomonas* strains for control?



- Most bioherbicide and plant growth promoting strains cluster together
- Some related to CB-4 bioherbicide strain (crabgrass herbicide)
- 16S does not capture functional differences
- Genomic sequencing of known and new isolates forthcoming!


Questions?