Diversity above and below ground:
the genetics of vernal pool plants and seed banks

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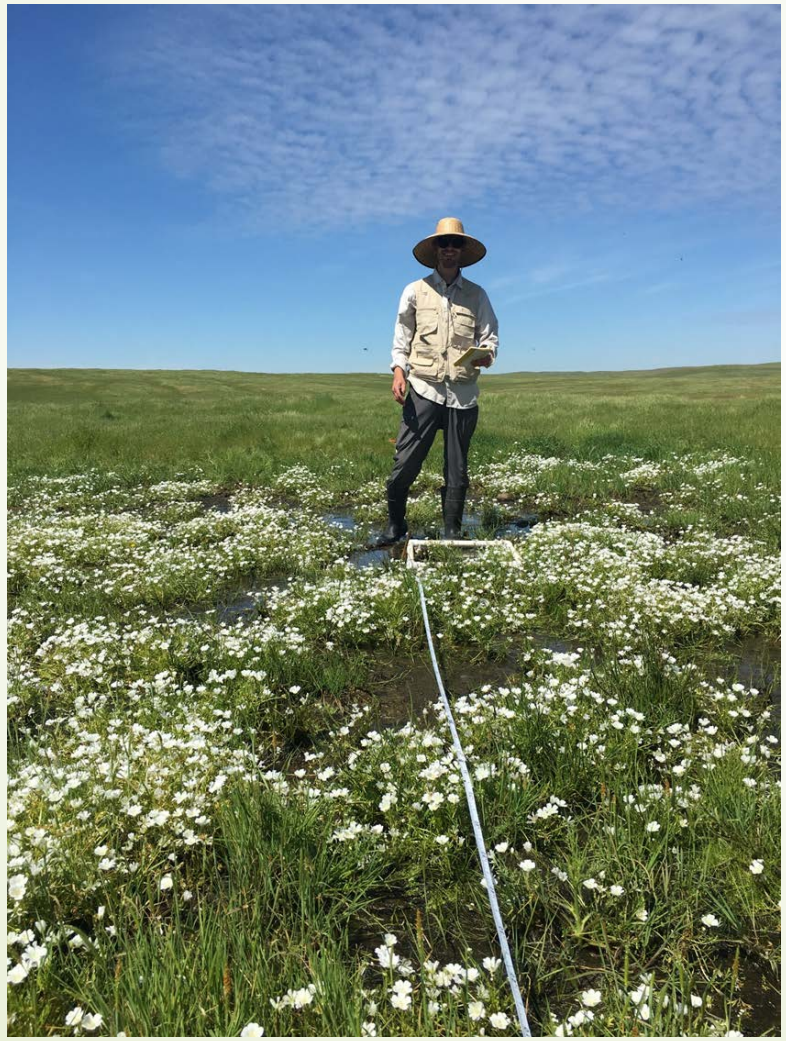
The problem:

- Understanding vernal pool (or any) plant communities is important...
- ...but hard!
  - time consuming
  - expertise
  - temporal variance
  - issues with ID'ing degraded/dried out samples
  - Can't ID below-ground species



Use eDNA and metabarcoding genomic techniques to:

- ID all plant species present
  - detect T&E species
- characterize plant spp genetically
- Reduce site visits
- Increase accuracy
- Increase understanding of VP communities
- Above/below ground ("seed bank") diversity
- Apply method to other soil systems

Neostapfia colusana "Colusa grass"
Fed: Endangered
CA: Threatened

Orcuttia inaequalis "San Joaquin Valley Orcutt Grass"
Fed 'Threatened
CA Endangered

Orcuttia pilosa "Hairy Orcutt Grass"
Fed Endangered
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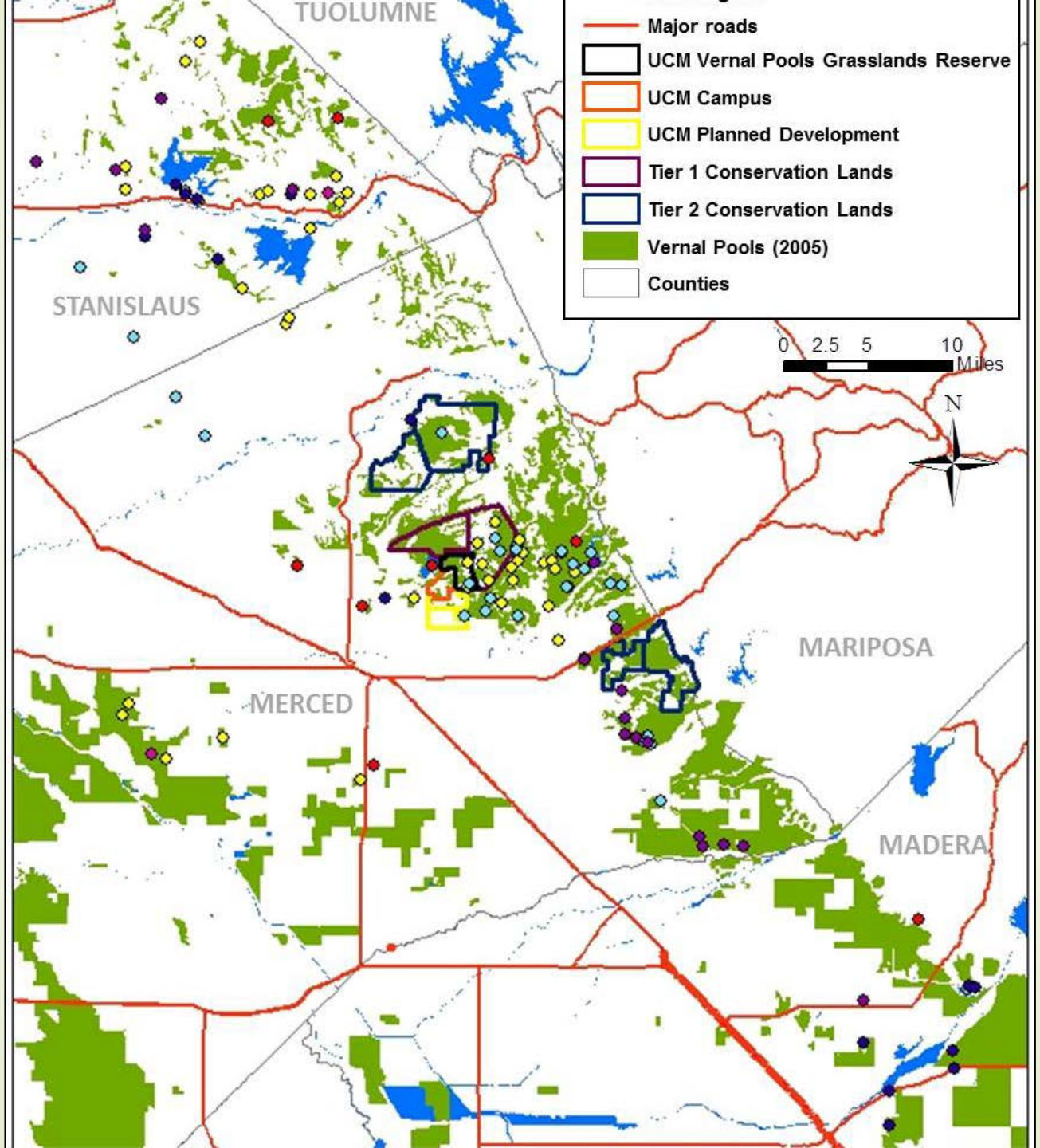
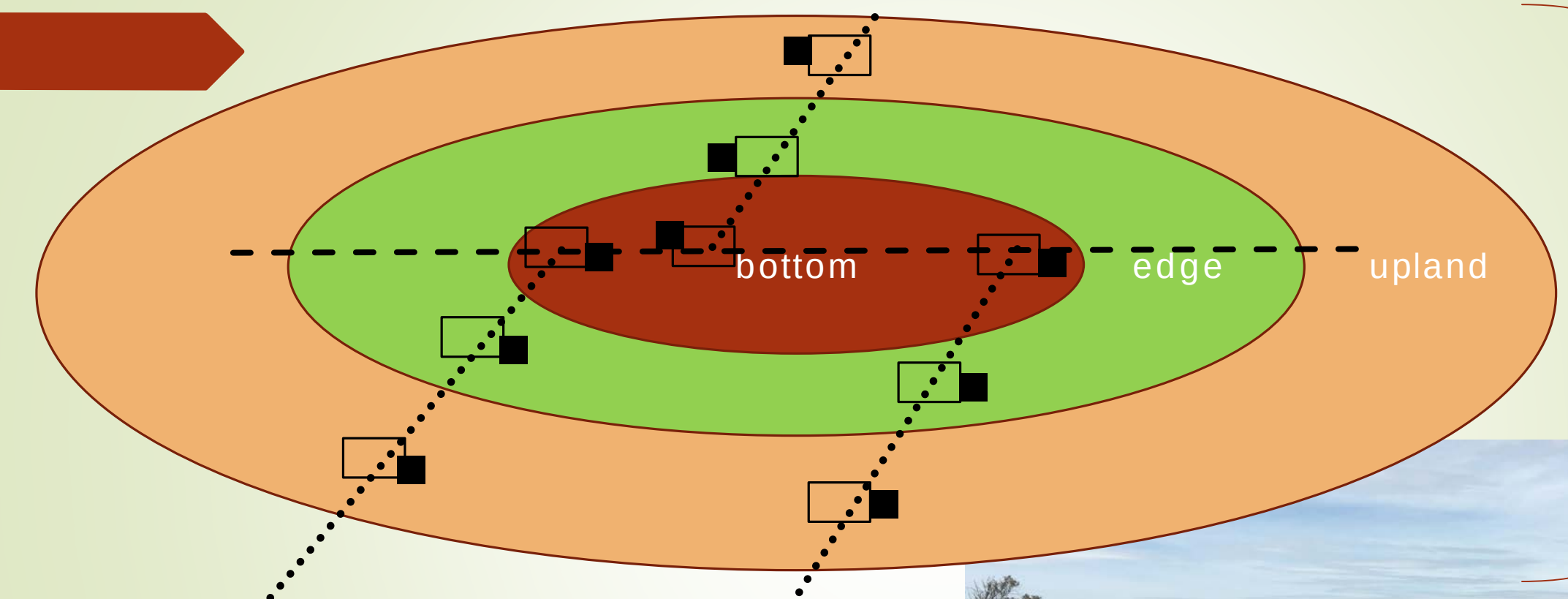

TUOLUMNE
STANISLAUS
MERCED
MARIPOSA
MADERA
Major roads
UCM Vernal Pools Grasslands Reserve
UCM Campus
UCM Planned Development
Tier 1 Conservation Lands
Tier 2 Conservation Lands
Vernal Pools (2005)
Counties
0 2.5 5 10
Miles
N

MVPGR veg. surveys and “seed bank” sampling



x36 pools

- Quadrat-transect surveys (above ground, % cover)
- Soil sampling (below ground. 4x4x2”)

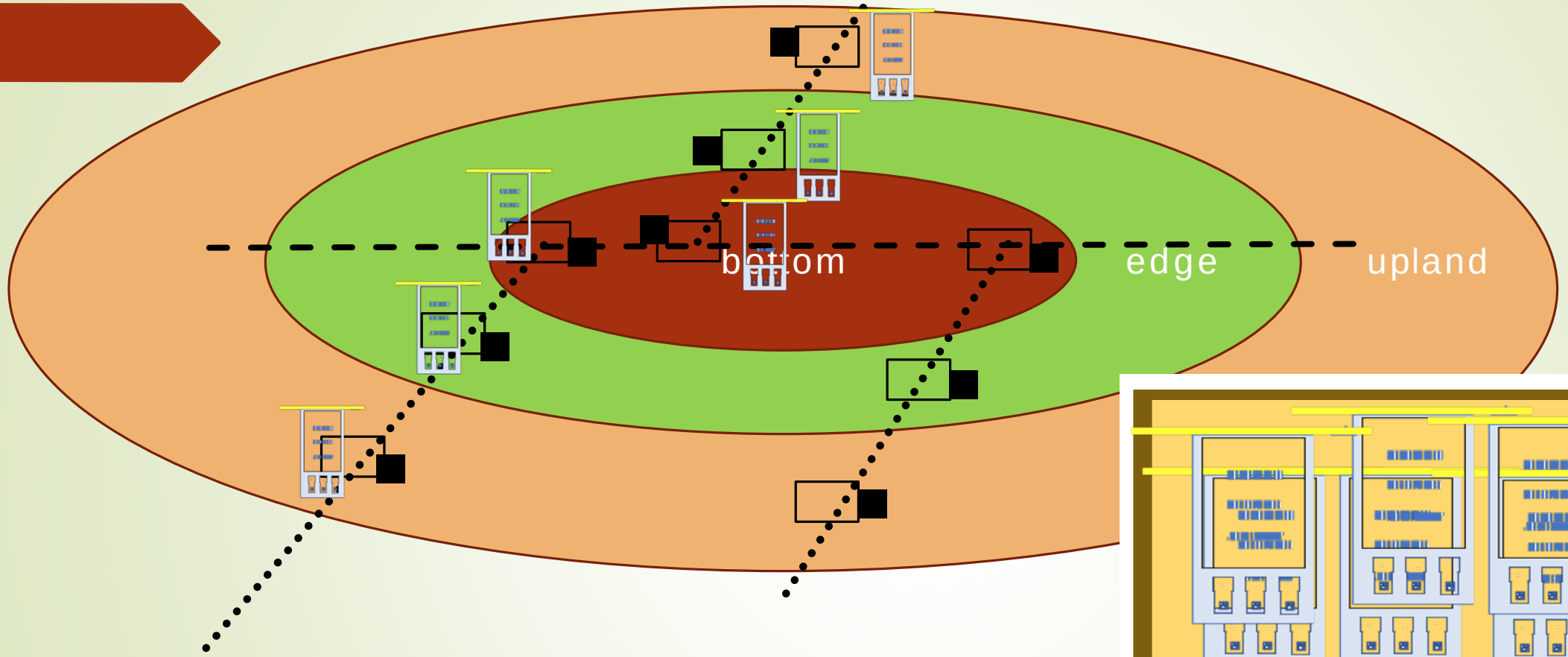
California environmental DNA

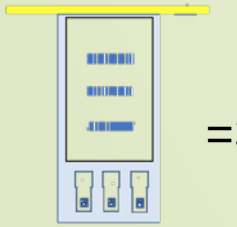
- establish a baseline of California's biodiversity
  - environmental DNA museum
  - Temporal replication
- Citizen science

UNIVERSITY OF CALIFORNIA

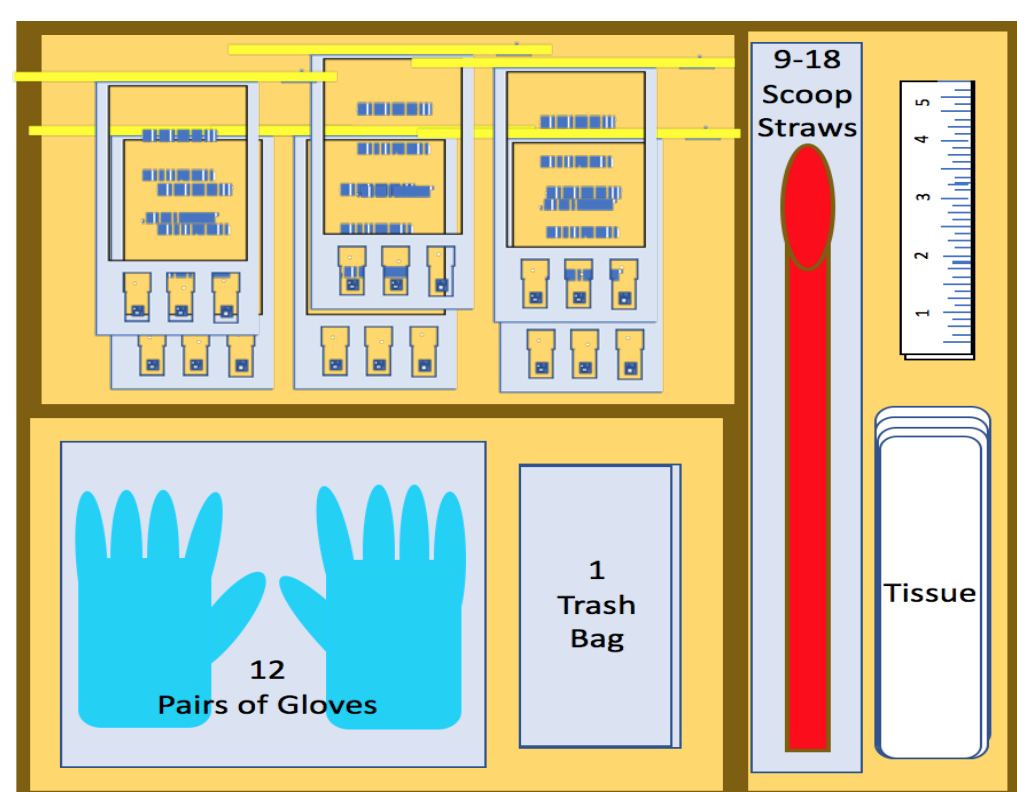
CALeDNA
Revolutionize Conservation in California

CaleDNA soil sampling (5 pools)





=3 tubes, each zone; 18 tubes total per pool



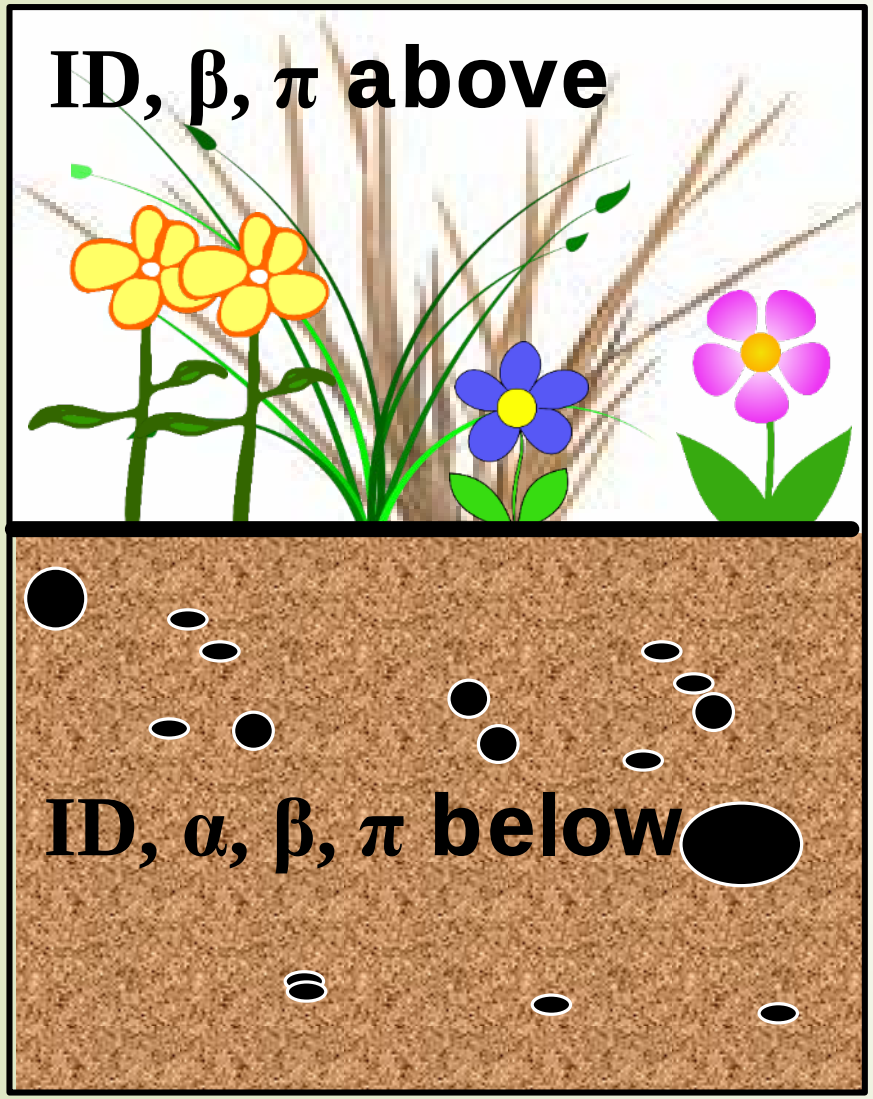
Pilot: Compare Soil Sample Processing

1. **Germinate in greenhouse → ID species**
2. **Sieve soil, extract, metabarcode → ID species***
3. **Grind soil, extract, metabarcode → ID species***
4. **Pool samples, extract, metabarcode → ID species***
5. **CaleDNA samples → ID species***

Compare

- *Metabarcode sequencing (Illumina miSeq)
  - COI, ITS2, rbcL and trnL
  - CaleDNA -- ITS1 universal primer and CO1 primer
- "known" seed bank samples

eDNA for VP soils

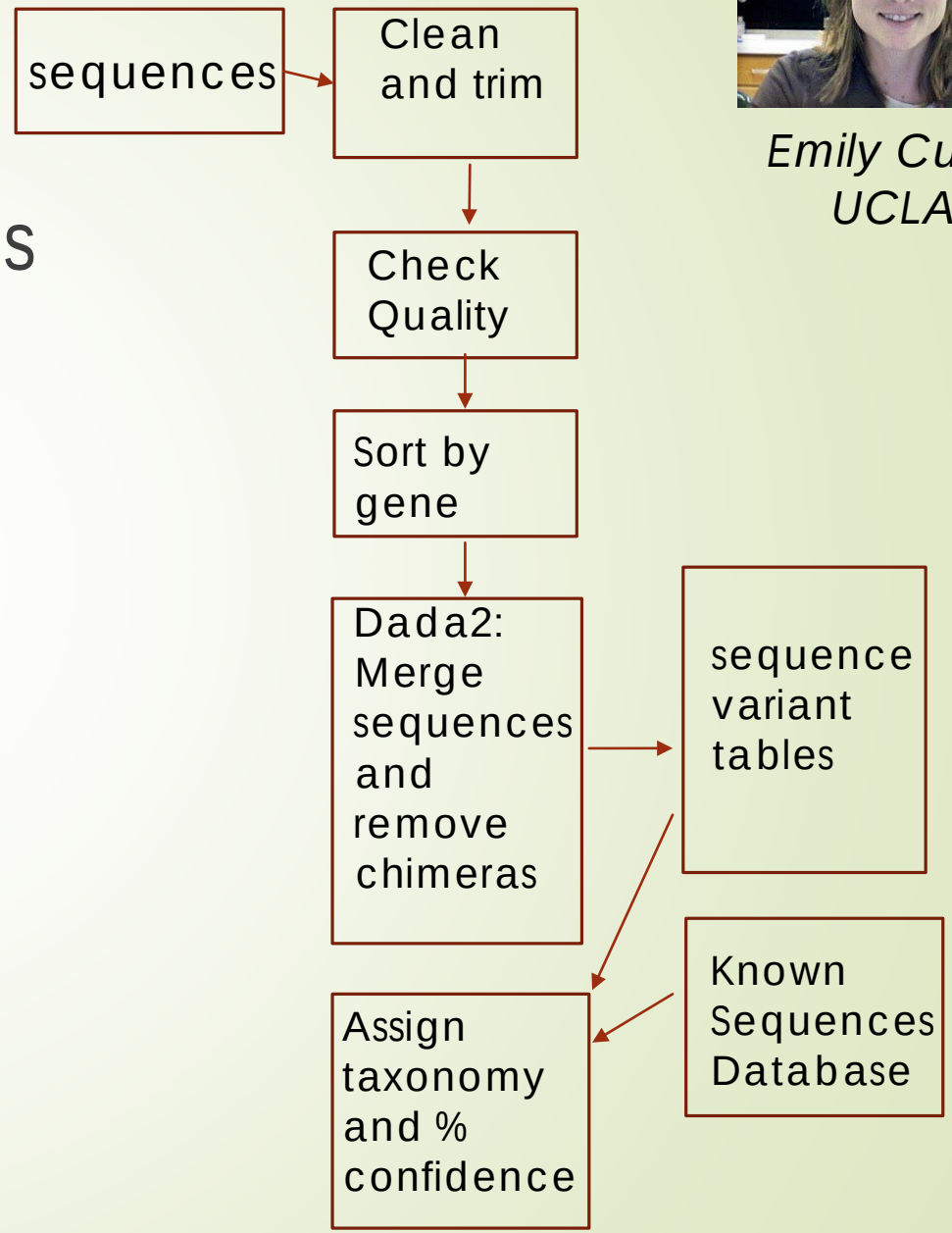


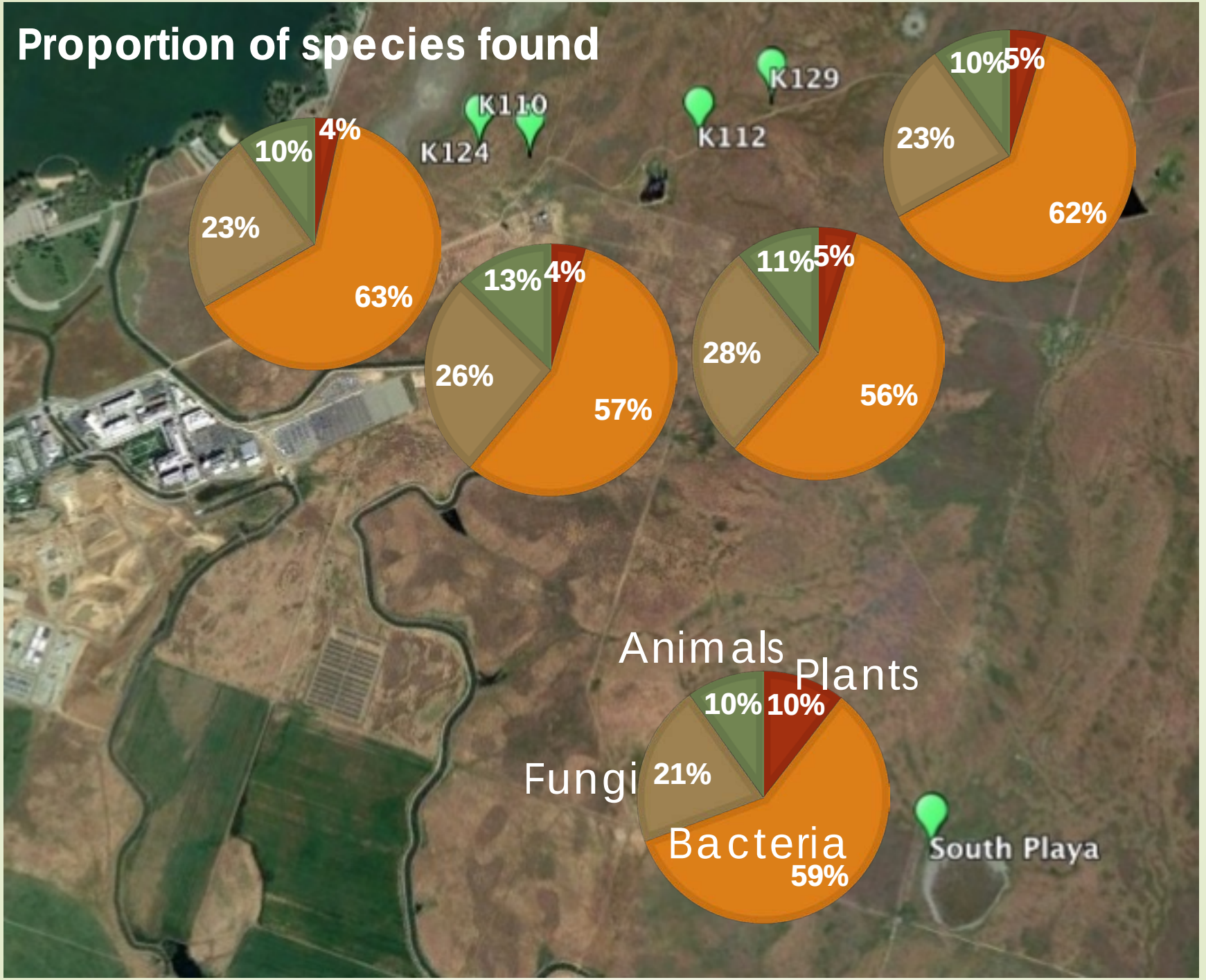
- Species ID (T&E) and detection rates (at known T&E plant sites)
- β (species) diversity
- π (genetic) diversity; pop. structure
- Environment:
  - hydrology (inundation, pool connectivity)
  - soil type
  - Climate
  - Spatial structure

Methods

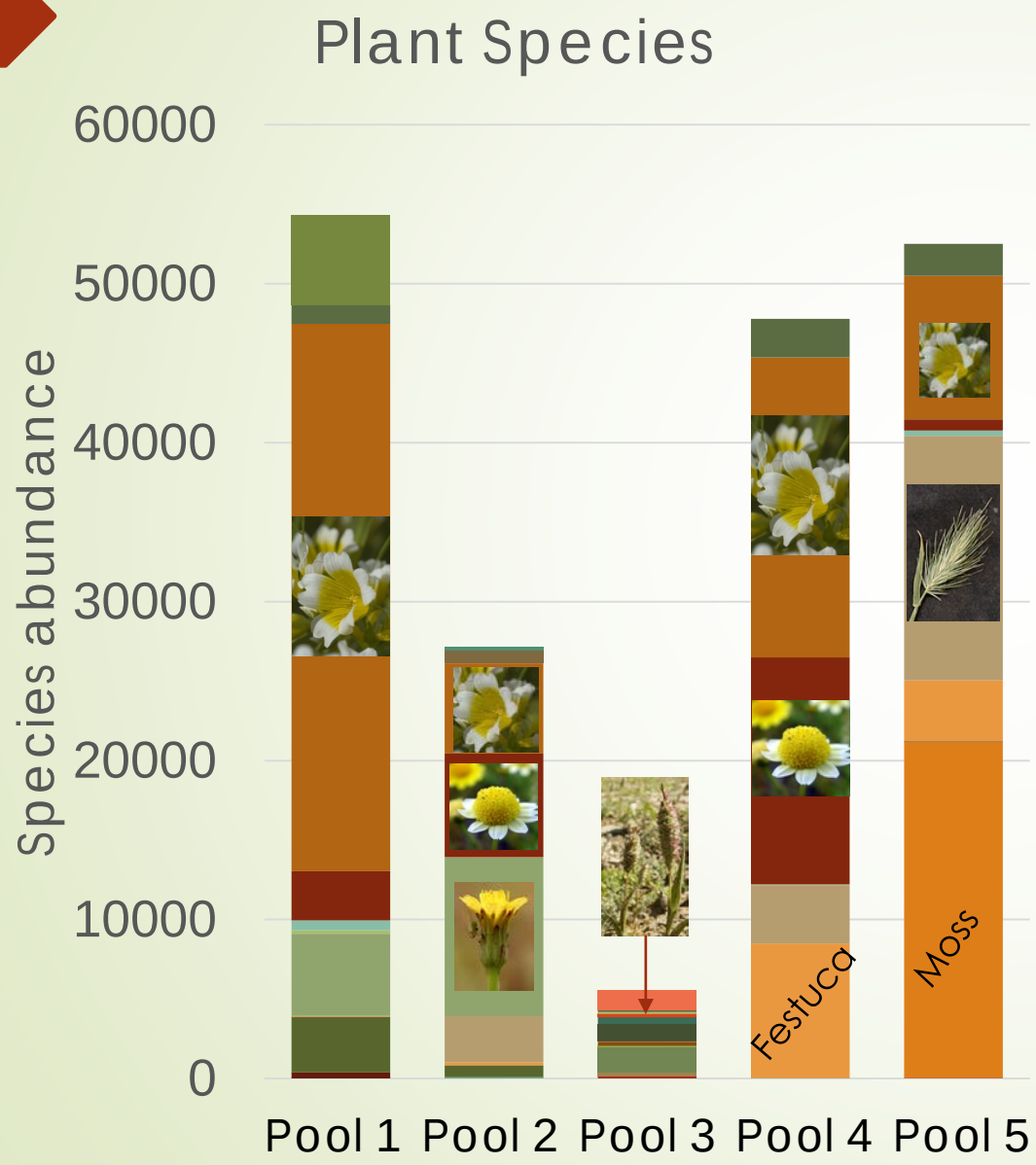
- DNA extracted from soil samples
- 5 genes:
  - 16S - prokaryotes
  - CO1- eukaryotes
  - 18S - eukaryotes
  - ITS1 - plants
  - ITS2 - fungi
- ANACAPA bioinformatics pipeline

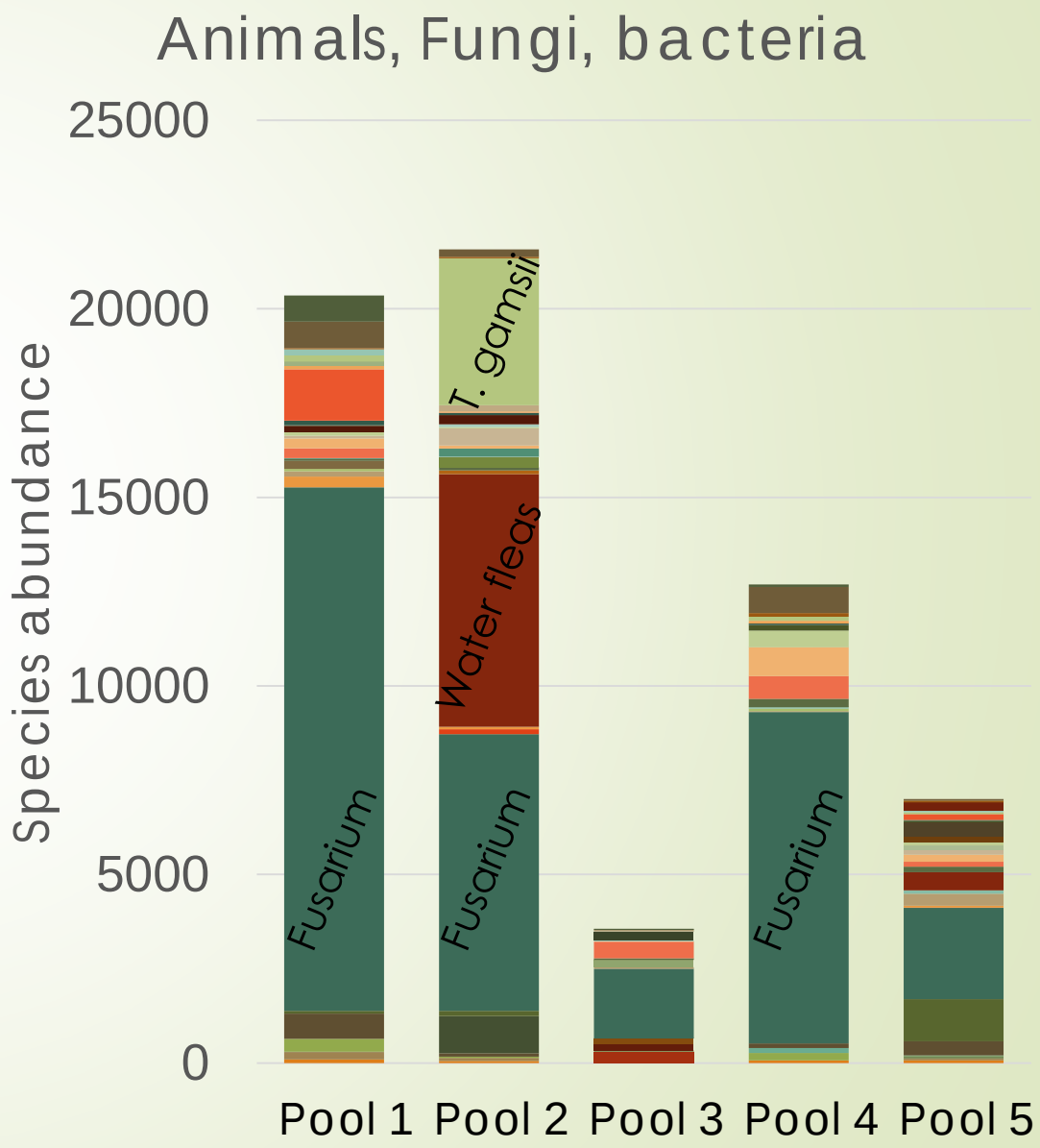
Emily Curd, UCLA




Proportion of species found
K110
K124
K129
K112
4%
10%
23%
63%
13%
4%
26%
57%
11%
5%
28%
56%
10%
5%
23%
62%
Animals
Plants
10%
10%
Fungi
21%
Bacteria
59%
South Playa

Species abundance and composition



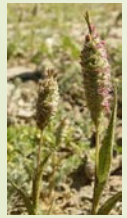


^ South Playa Pool, *N. colusana*

Plant species detected in soil samples using eDNA

- **Native and common vernal pool species**

  - *Lasthenia fremontii* - Frémont's goldfields
  - *Limnanthes douglasii* - *meadowfoam*
  - *Elatine californica* - CA waterwort
- **Endangered and rare species**

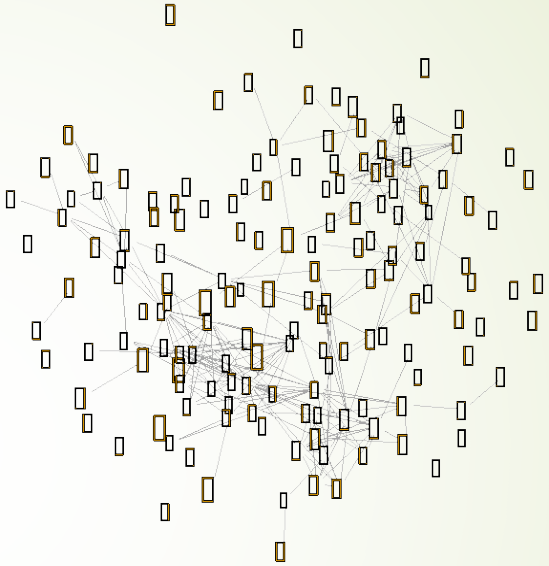


  - *Neostafia colusana*
- **Invasive non-native species**
  - ***Hordeum marinum, Festuca bromoides***

Community structure – pool connectivity

Bacteria

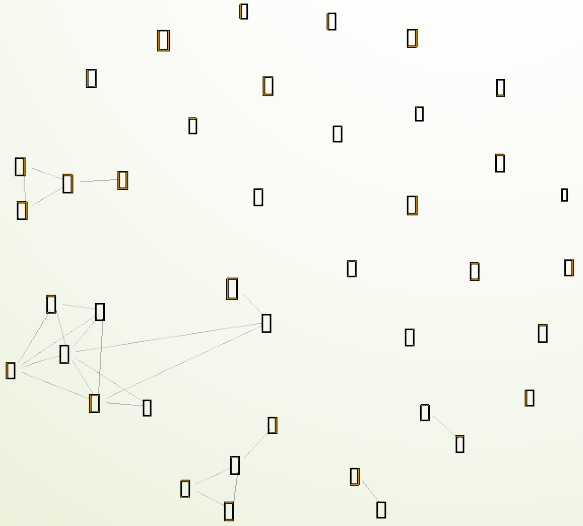
Fungi



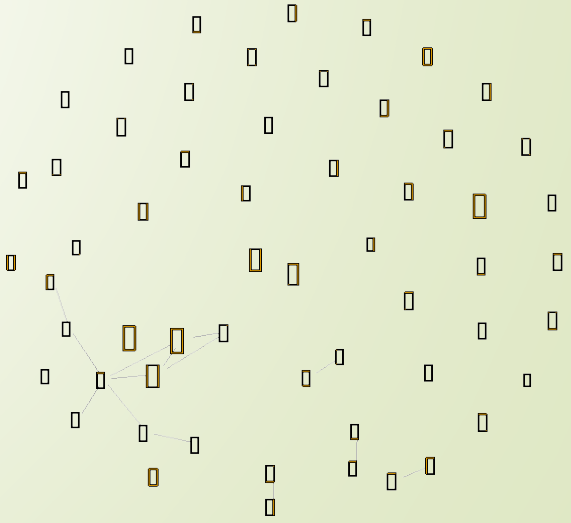
Species

Interaction

Plants and algae



Animals



Preliminary results: species diversity indices, 5 CaleDNA pool soils

β = the ratio between regional and local species diversity β = γ/α
α = mean species diversity in sites or habitats at a local scale
γ = total species diversity in a landscape γ = α * β

	Plant ITS				COI		
	bottom	**edge**	**upland**		**bottom**	**edge**	**upland**
β	2.5	2.2	1.8		2.3	2.5	2.2
α	13.8	12.6	11.6		20.9	18.2	14.5
γ	48.0	41.0	33.0		70.0	64.0	47.0

- Greater spp diversity in bottom, decreasing toward uplands
- Similar patterns for two different markers and classes of organisms

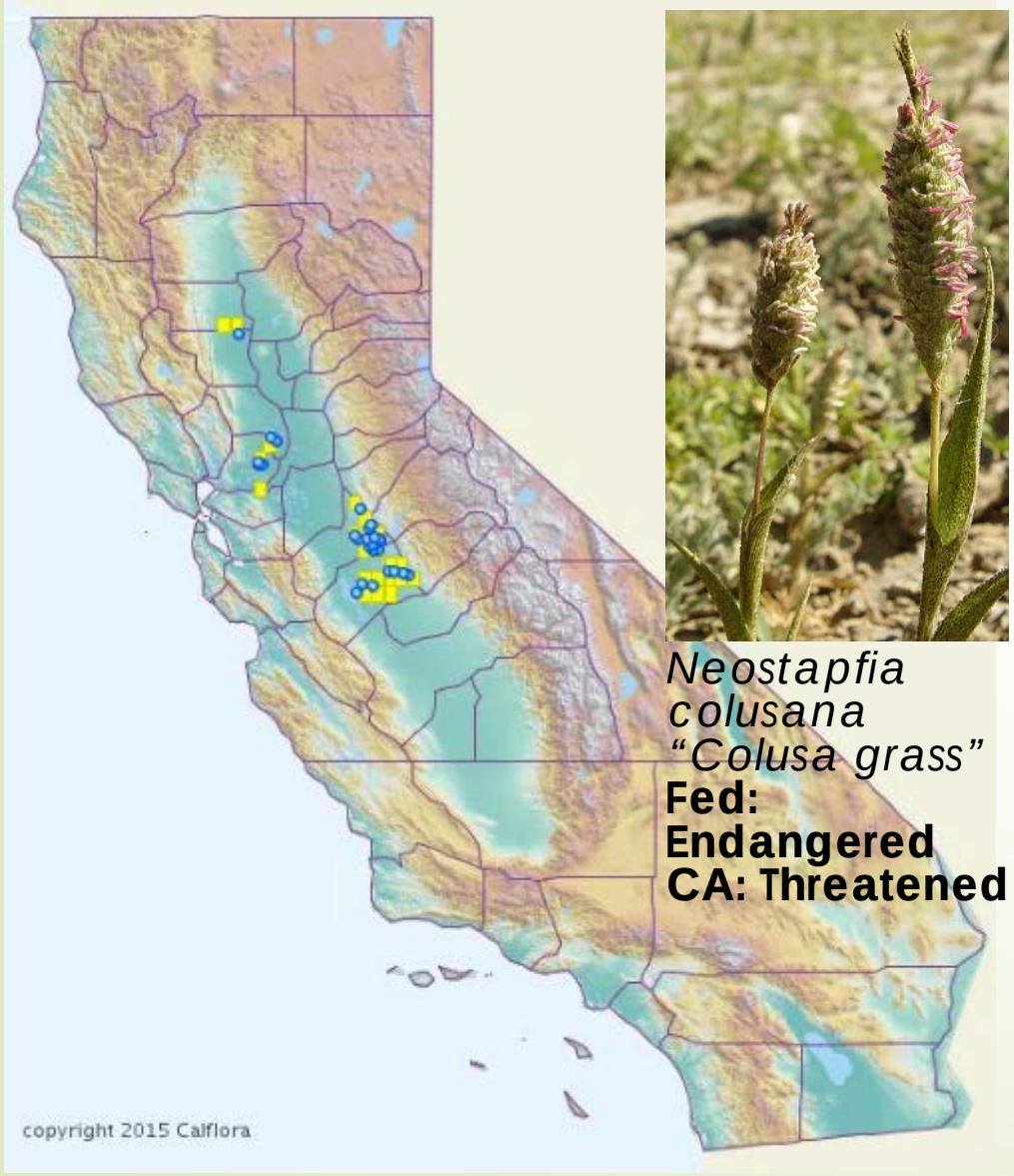
Limitations of eDNA for species detection

- Detection constrained by reference sequence database
  - *Plagiobothrys linifolius*
  - *Poa xenica*
- Sampling effort and area of the pool
- Duration of DNA in the environment
  - *N. colusana* detected before the growing season

Next steps

- Improving reference sequence database for vernal pool plants
- Analyze biodiversity above vs below ground
- Community assembly

Statewide Orcuttiae SNP marker development: *Neostapfia colusana* population genetic structure



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Orcuttia pilosa “Hairy Orcutt Grass”
Fed Endangered
CA Endangered

Tuctoria greenei “Greene's tuctoria”
Fed Endangered
CA Rare

Statewide Orcuttiae SNP marker development: Neostapfia colusana population genetic structure

- RADseq
  - SNP marker development for four Orcuttieae species
  - population genetic investigation of *N. colusana* rangewide population structure and diversity
  - How many “populations” exist in these isolated pools, how is diversity structured, and how much genetic diversity is left in this threatened species
- Genome Sequencing
  - sequenced *N. colusana* genome (UC Genomics Consortium)
  - 2.2GB
  - 87% completeness (core genes)
  - Next steps: Annotation

Acknowledgments

Funding

- USBR/USFWS CVPCP grant: “Genetic investigation of listed vernal pool plants and their communities in Merced County”
- UC Regents Catalyst grant; CaleDNA program and citizen scientists: “Conservation genomics of California vernal pool endemics”

Assistance

- Sexton Lab, undergraduate assistants
- Dawson Lab
- UC Conservation Genomics Consortium
  - Emily Curd, Rachel Meyer
  - Citizen scientists
- Carol Witham, Bob Holland, David Muths
- Monique Kolster, MVPGR

BACKGROUND

- USBR/USFWS CVPCP "Genetic investigation of listed vernal pool plants and their communities in Merced County"
  - improved protocols for soil plant species ID from soil samples
  - species diversity/richness estimates and environmental correlates
  - SNP marker development for Orcuttieae (four species)
  - population genetic investigation of a threatened vernal pool species (*Neostapfia colusana*)
  - Management implications
- UC Catalyst program – UC Conservation Genetics Consortium CaleDNA "Conservation genomics of California vernal pool endemics"
  - Genome sequencing for *N. colusana*
  - Soil eDNA sample collection and metabarcode species ID (plants + other)