Combining environmental DNA analysis and soil stability metrics to assess the impact of pollinator habitat on USACE levees

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Well documented global decline of pollinators





of bumble bee species in decline

Rusty patched bumble bee (*Bombus affinis*) first bee species to be listed as endangered in continental U.S.



of butterflies in the U.S. at risk of extinction

The iconic monarch butterfly has experienced 80-97% declines in overwintering populations both east and west of the Rockies



27 species of non-native lady beetle have been established in the U.S. since the mid-1980's, threatening native species

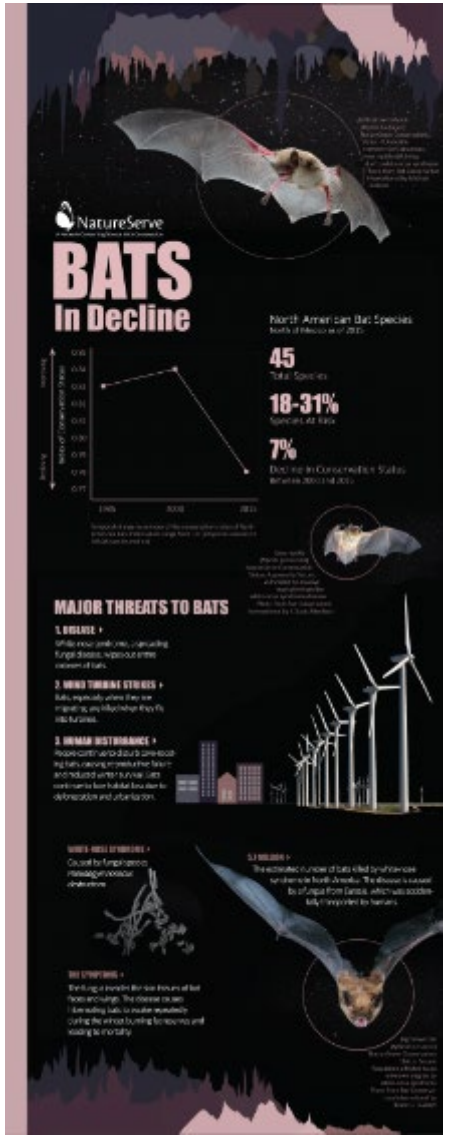
Once common across the U.S., the nine-spotted lady beetle is now locally extinct from many states where it was once plentiful.



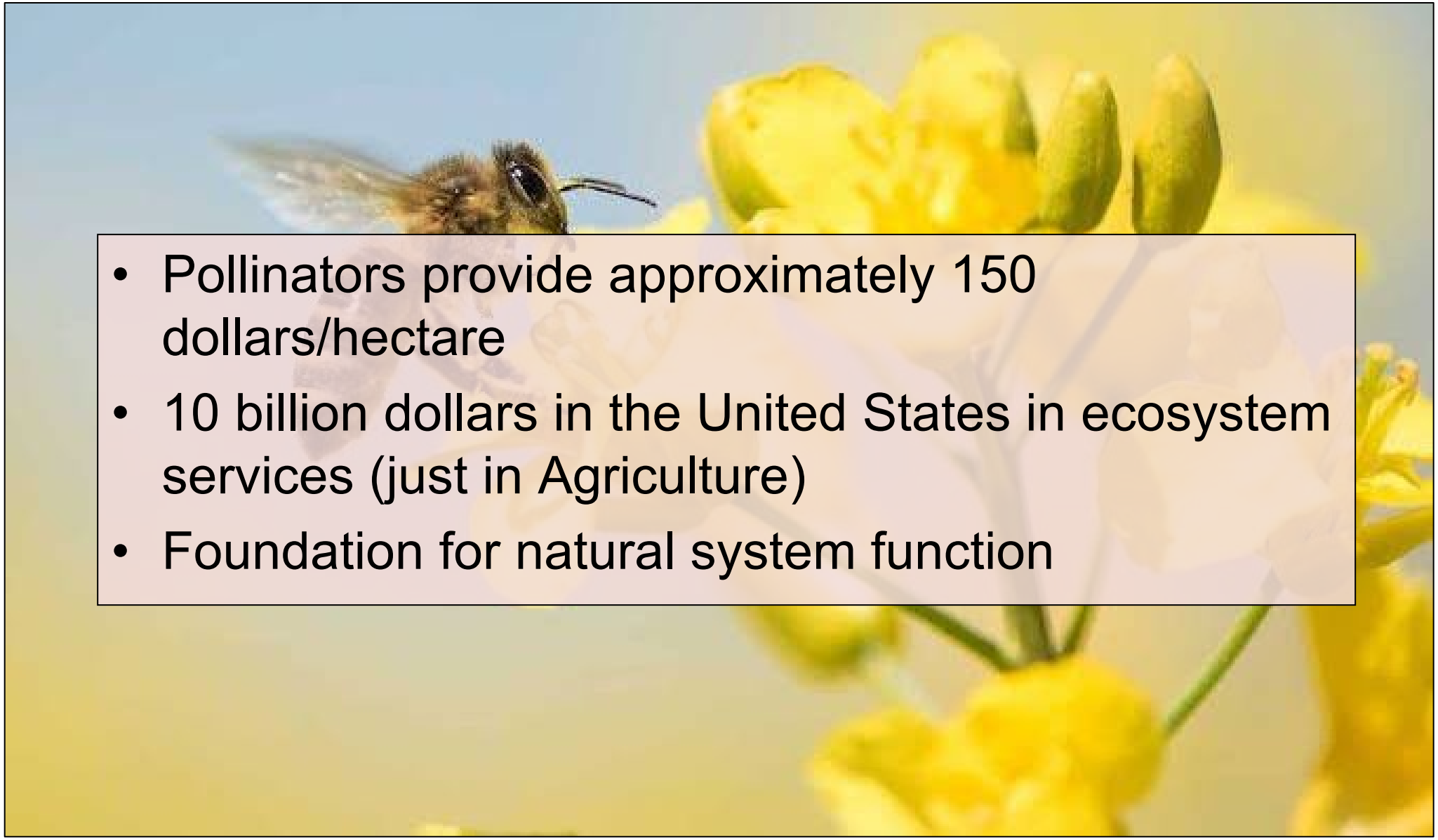
50% of leafcutter bees

27% of mason bee species

www.xerces.org



Pollinators provide distinct economic benefits



- Pollinators provide approximately 150 dollars/hectare
- 10 billion dollars in the United States in ecosystem services (just in Agriculture)
- Foundation for natural system function

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USACE manages approximately 30,000 miles of levees

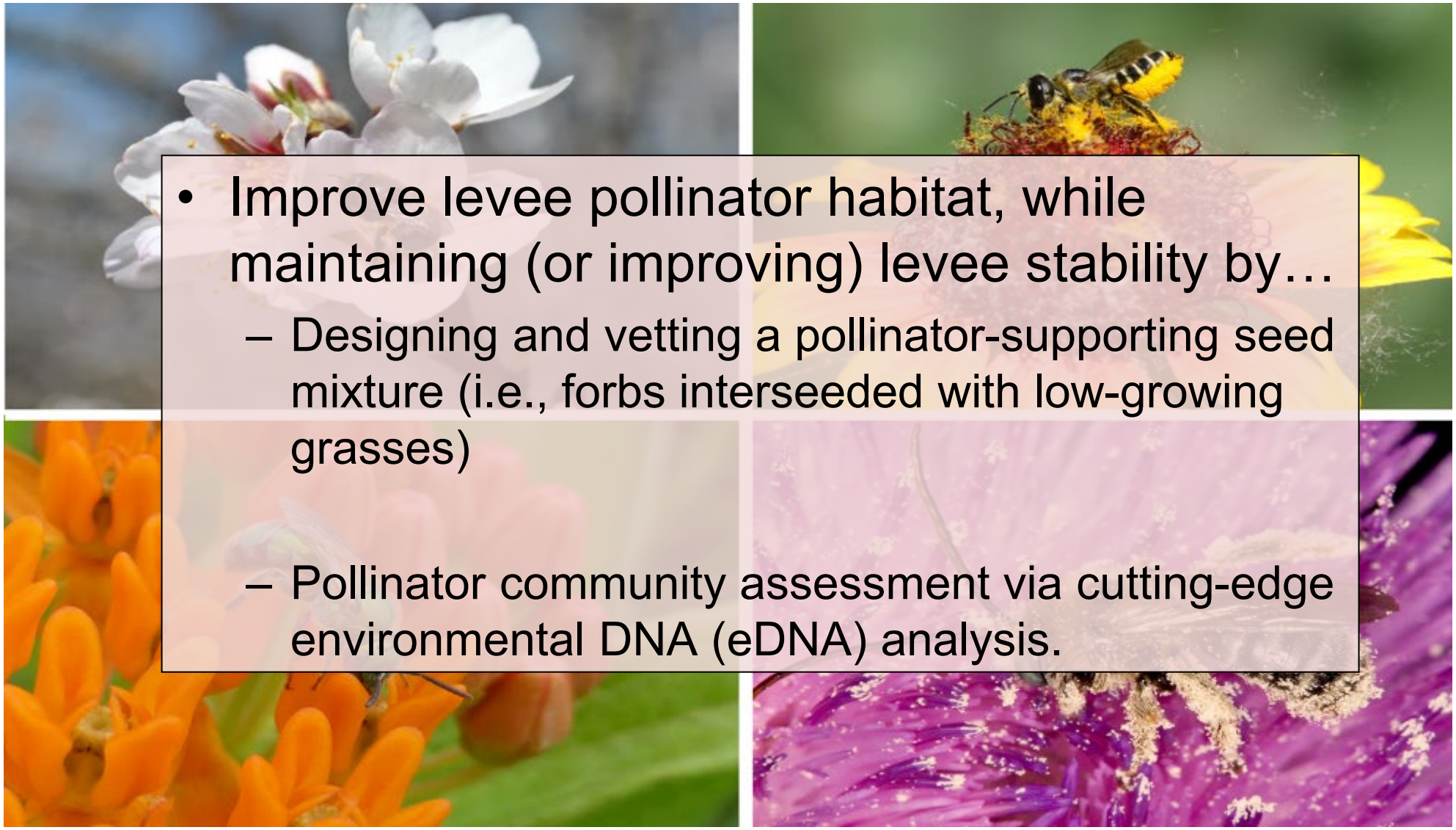


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Overall project goal

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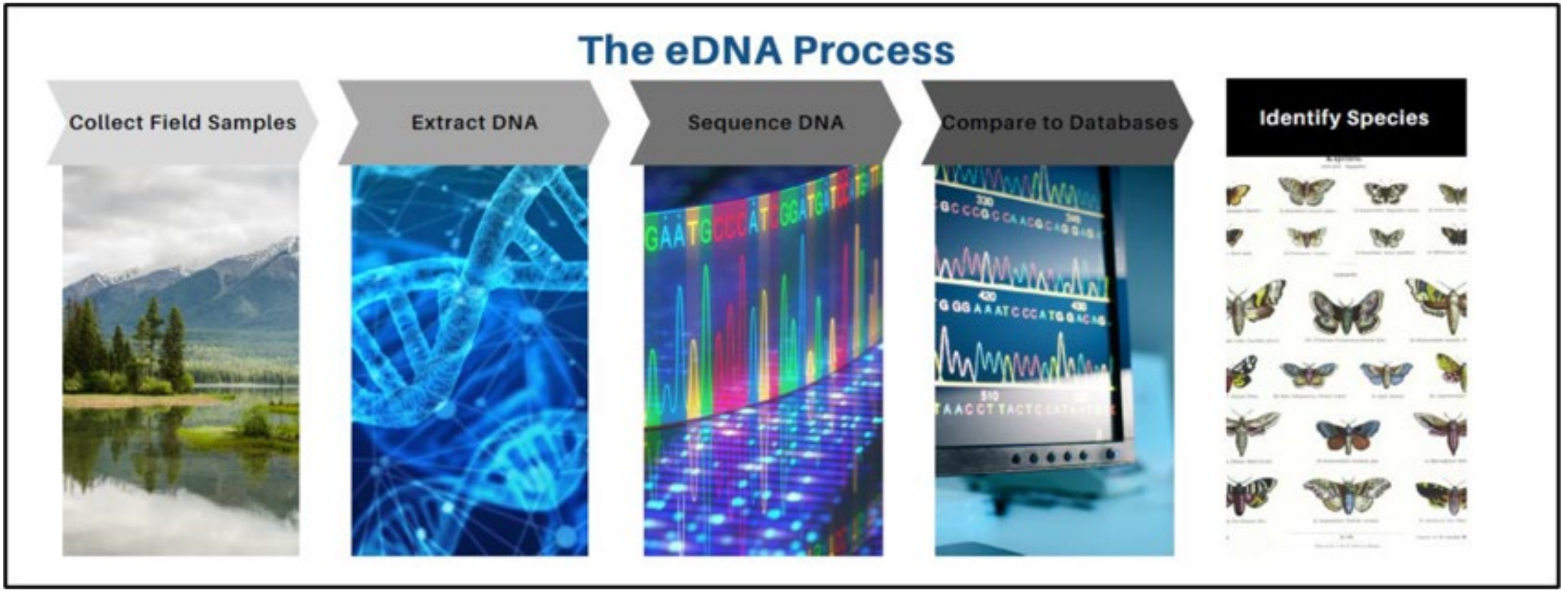
- Improve levee pollinator habitat, while maintaining (or improving) levee stability by…
  - Designing and vetting a pollinator-supporting seed mixture (i.e., forbs interseeded with low-growing grasses)
  - Pollinator community assessment via cutting-edge environmental DNA (eDNA) analysis.

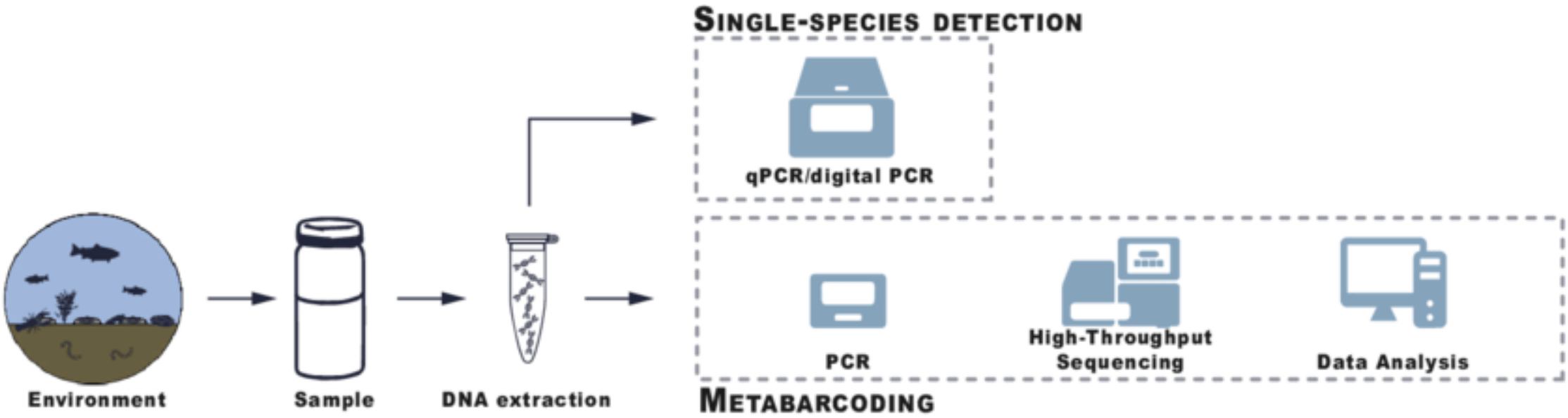
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eDNA analysis

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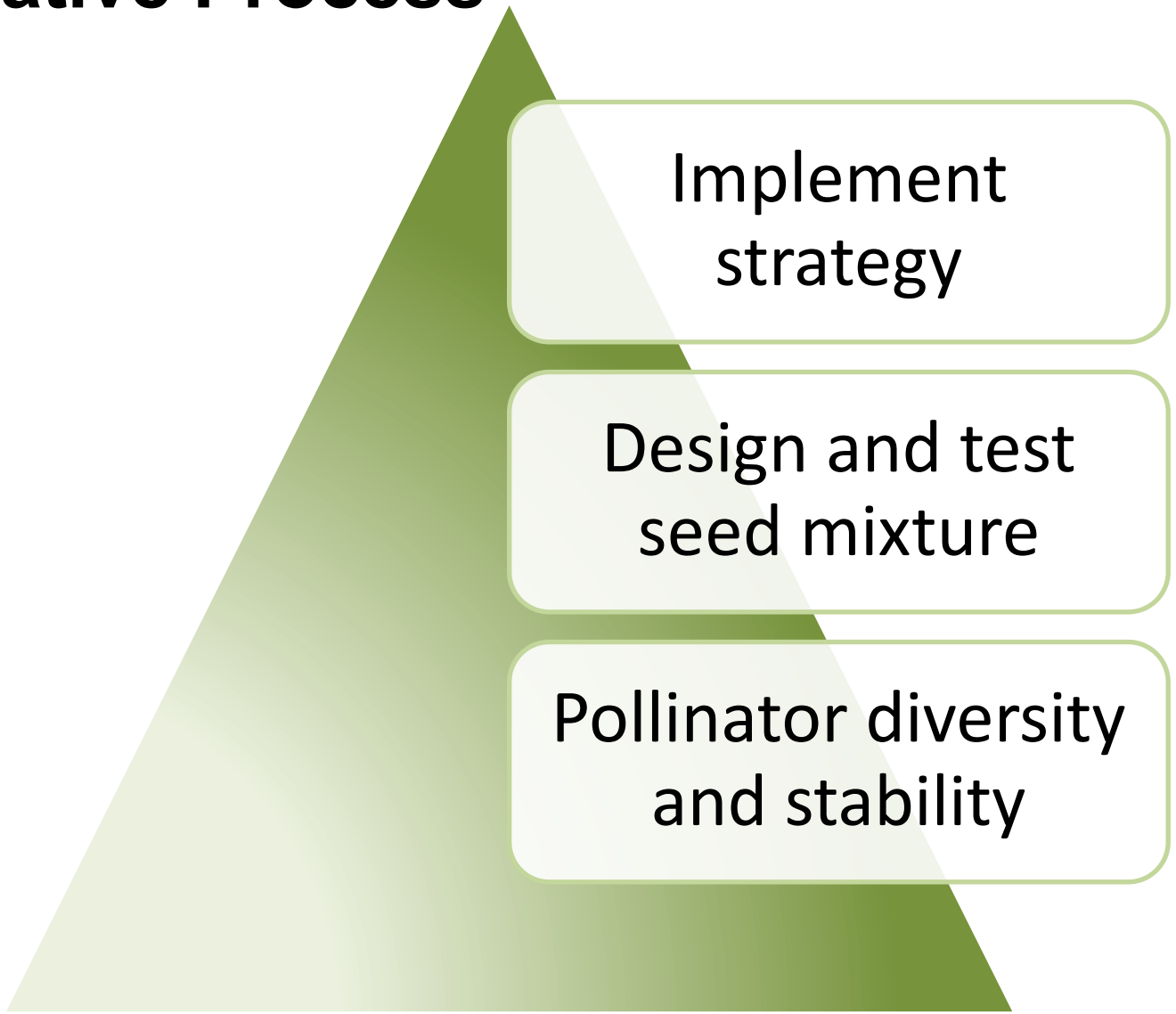

SINGLE-SPECIES DETECTION
qPCR/digital PCR
PCR
High-Throughput Sequencing
Data Analysis
METABARCODING
Environment
Sample
DNA extraction

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Iterative Process



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Texas levee analysis

- Common levee manager concern: Does pollinator presence impact levee performance?

- Leverage work from Franz Lichtner and Simone Whitecloud (CRREL)
  - Assess stability and plant communities along the Texas coast



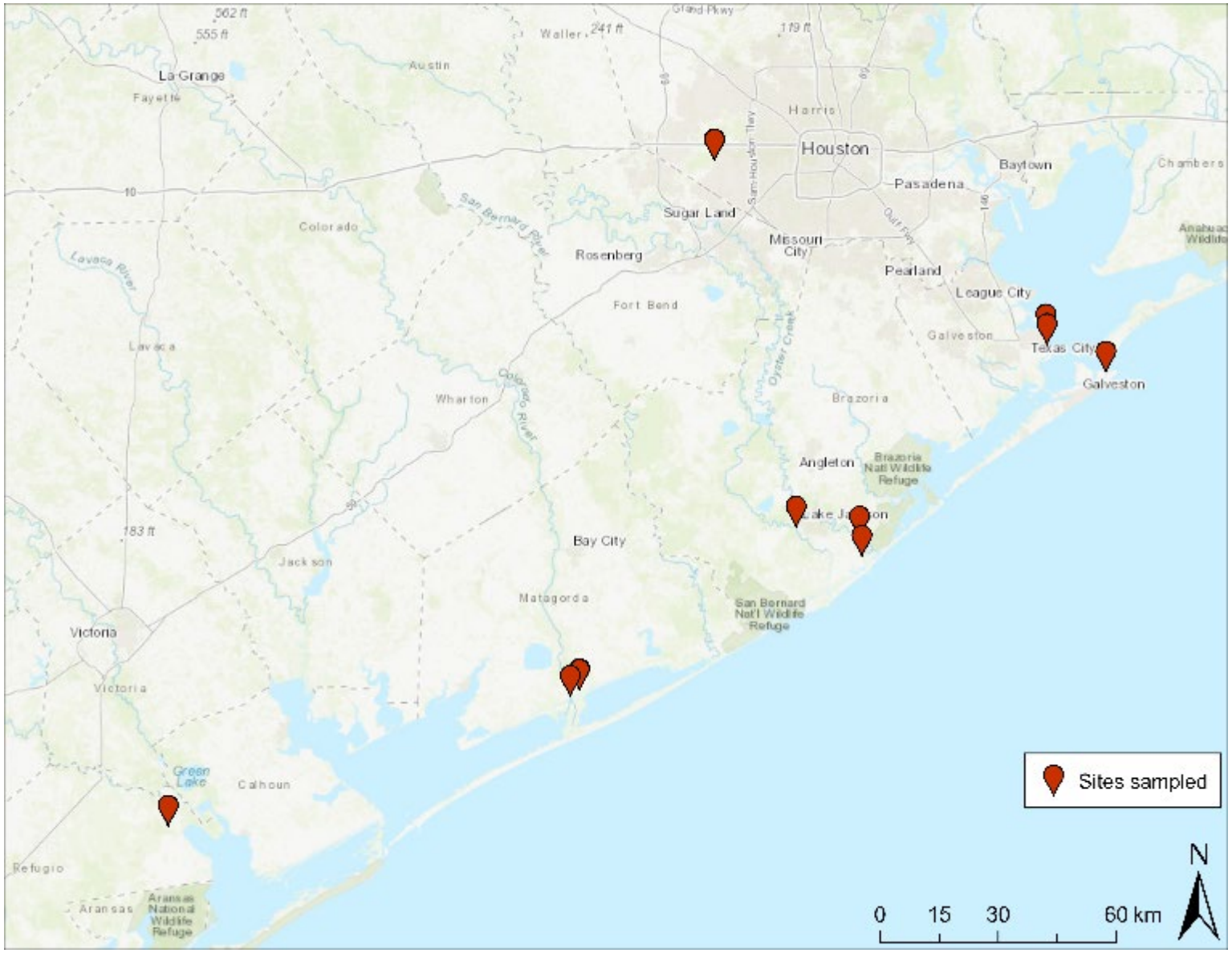
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Texas levee analysis

Pollinator diversity and stability

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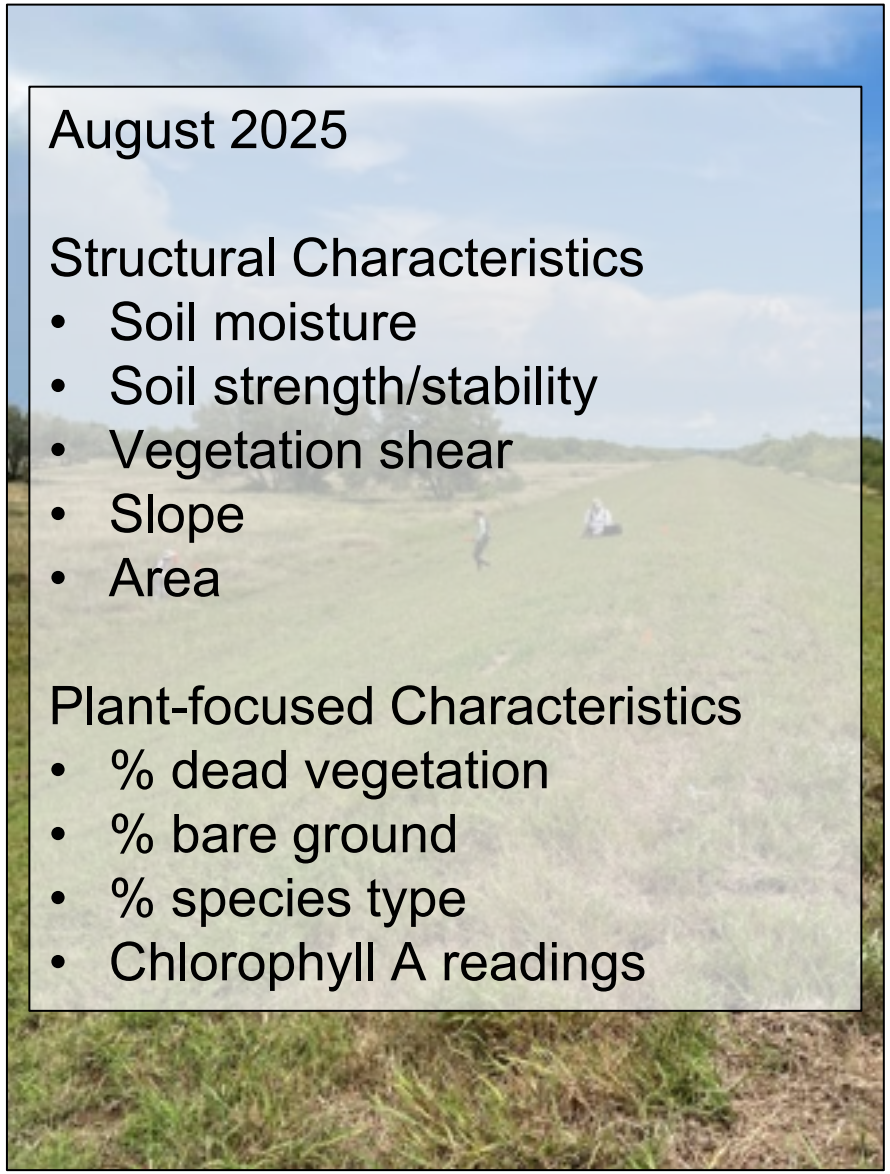
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Texas levee analysis

Pollinator diversity and stability

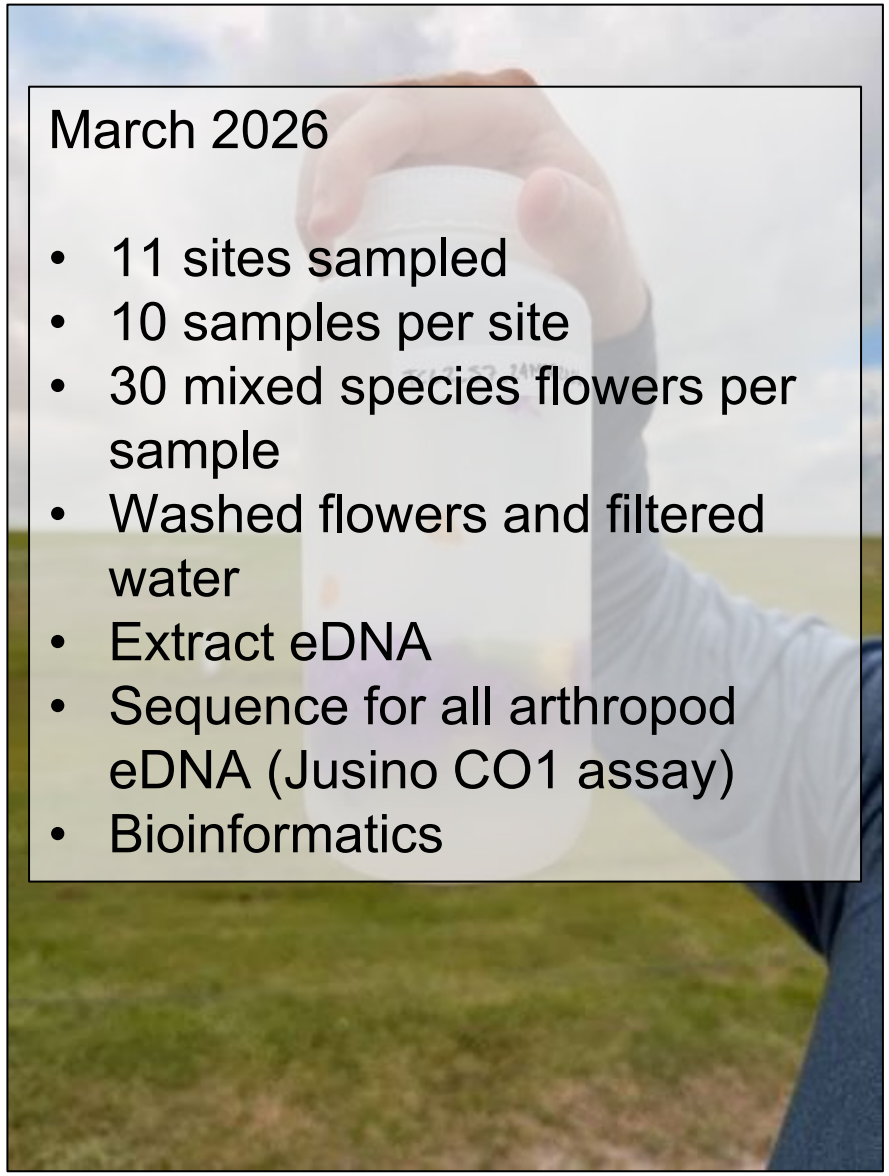
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August 2025

Structural Characteristics
- Soil moisture
- Soil strength/stability
- Vegetation shear
- Slope
- Area

Plant-focused Characteristics
- % dead vegetation
- % bare ground
- % species type
- Chlorophyll A readings



March 2026

- 11 sites sampled
- 10 samples per site
- 30 mixed species flowers per sample
- Washed flowers and filtered water
- Extract eDNA
- Sequence for all arthropod eDNA (Jusino CO1 assay)
- Bioinformatics

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Pollinator diversity and stability

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Texas levee analysis: eDNA results

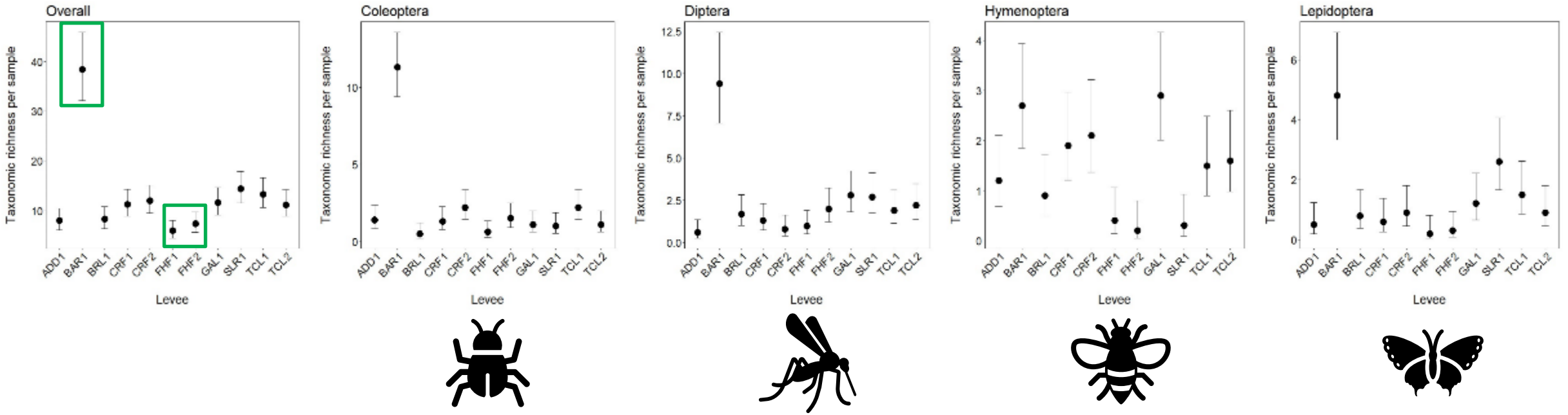
- 110 total samples
- 284,010,817 reads
- 215 species, 294 genera, and 149 families
- Detected pollinators, pests, predators, and parasites



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Texas levee analysis: eDNA results GLM



- Significant differences in arthropod biodiversity across sites

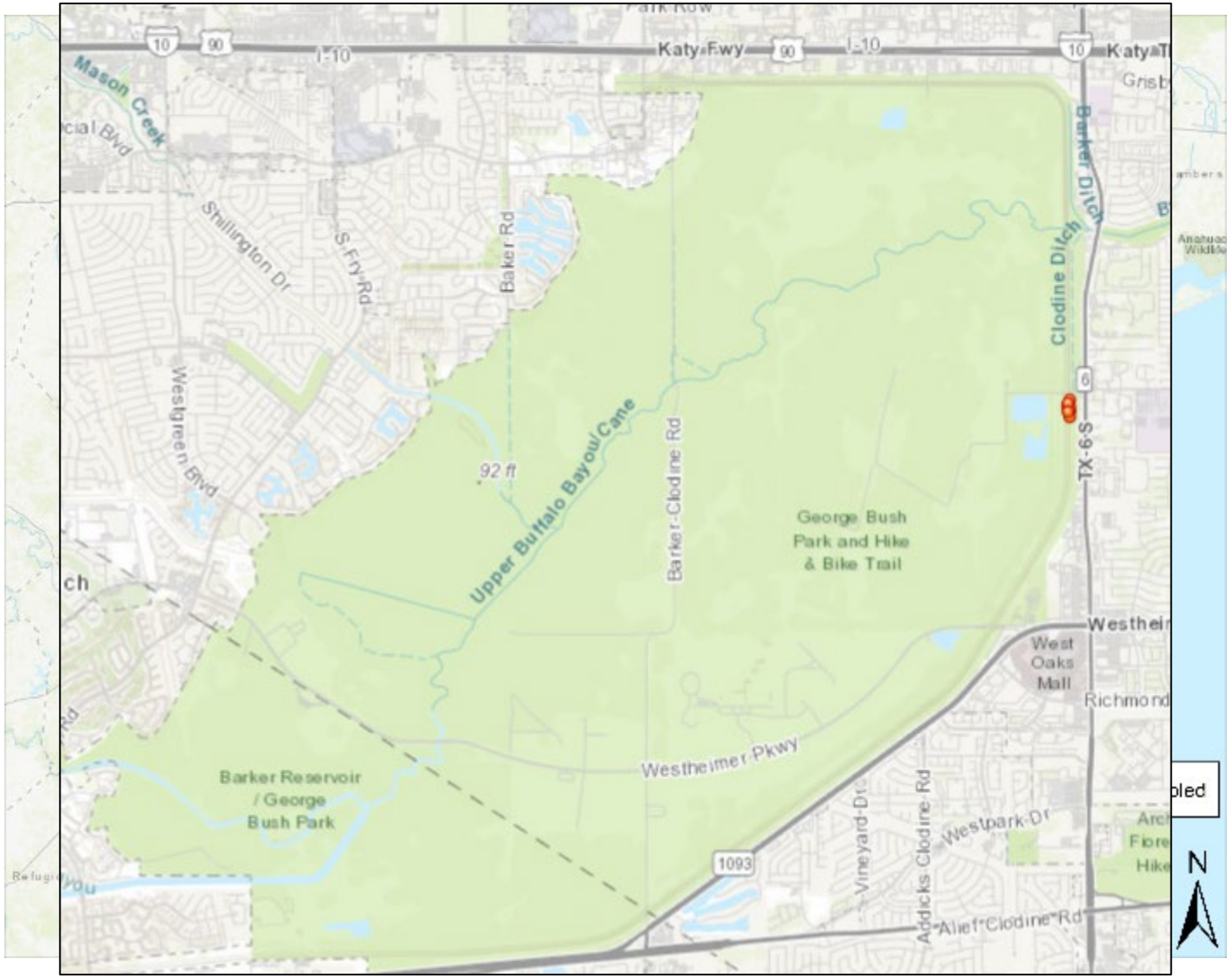
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Texas levee analysis: BAR1

Pollinator diversity and stability

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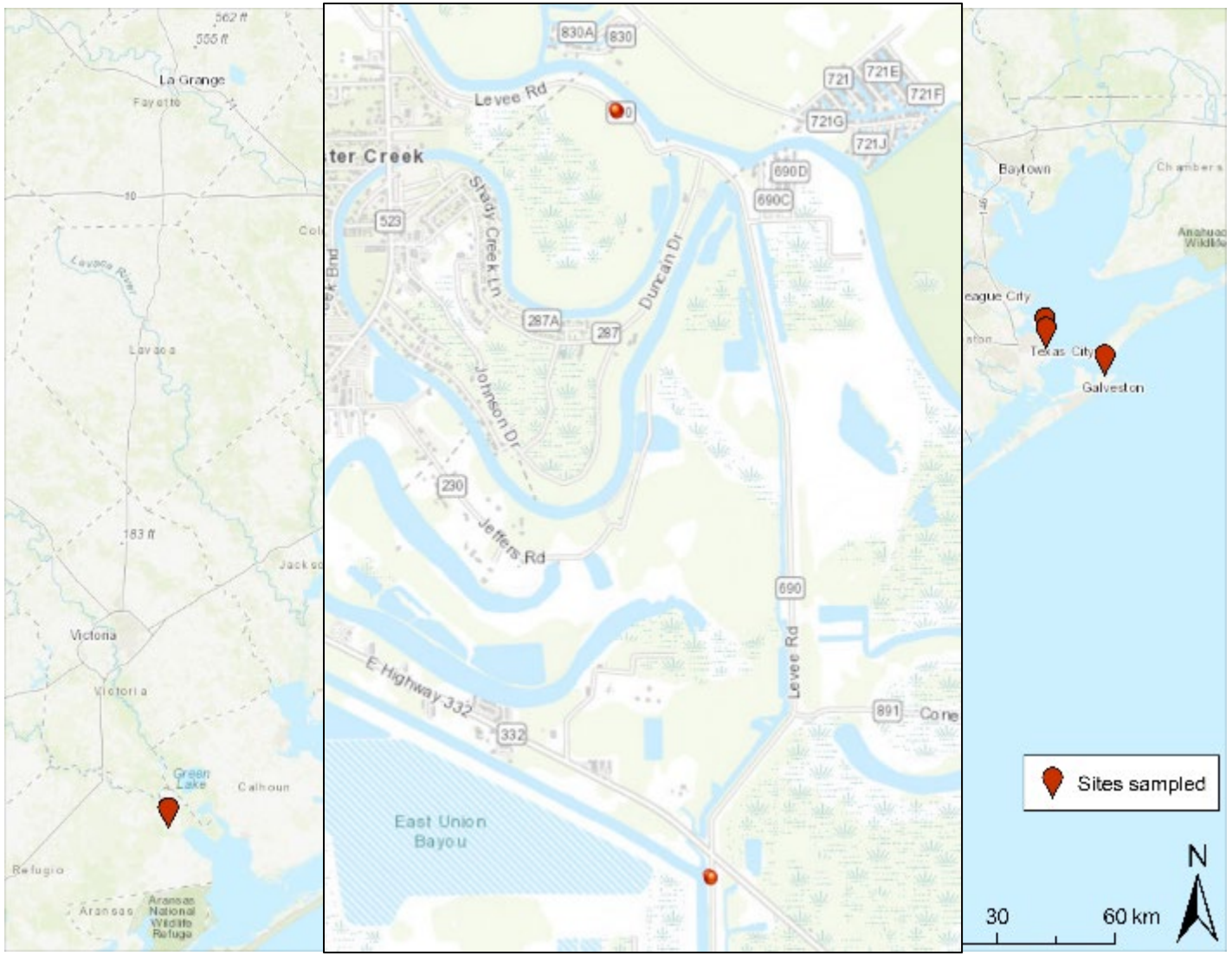
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Texas levee analysis: FHF1 and FHF2

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Texas levee analysis: Pollinators

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Texas levee analysis: Levee-specific pests

Atherigona reversura



Texas levee analysis: Levee-specific pests

Neoscapteriscus vicinus



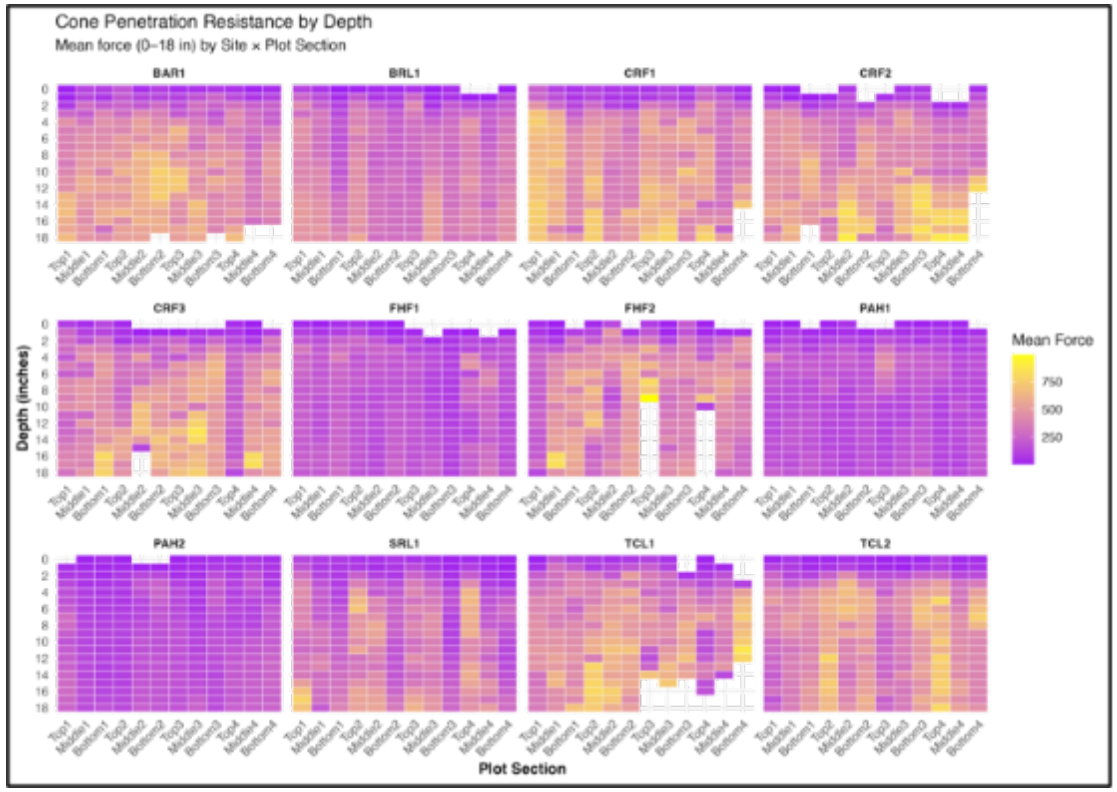
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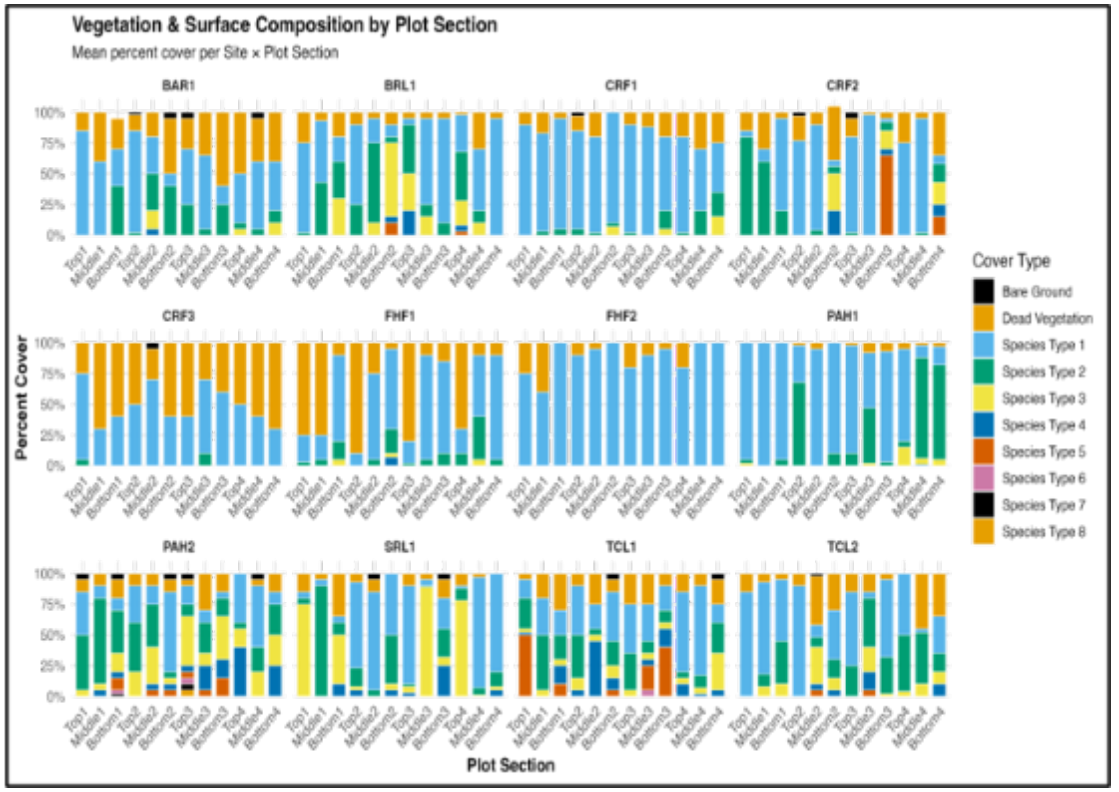
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Texas levee analysis: Principal component analysis

Pollinator diversity and stability

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Texas levee analysis: Principal component analysis

Structural Characteristics
- Soil moisture
- Soil strength/stability
- Vegetation shear
- Slope
- Area

Plant-focused Characteristics
- % dead vegetation
- % bare ground
- % species type
- Chlorophyll A readings

PCA

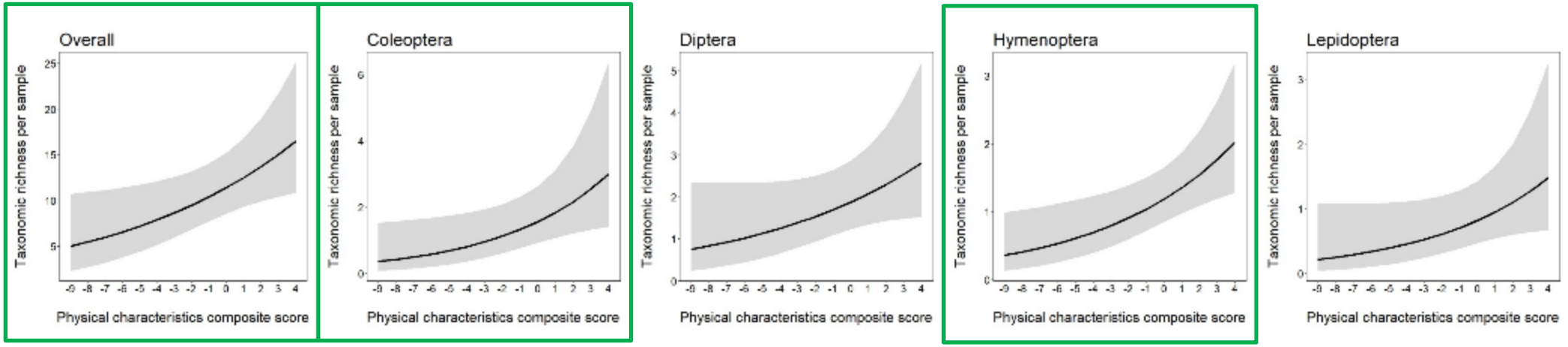
Structural characteristics principal component
- 56.10% variation
- Driven by soil strength and moisture levels

Plant-focused characteristic principal component
- 36.19% variation
- Driven by chlorophyll content and % species type

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Texas levee analysis: Structure characteristics GLMM



- Structural score positively predicts arthropod detection (compact and dry soils) overall and for Coleoptera and Hymenoptera

Texas levee analysis: Plant focused characteristics

- Plant-focused measurements did not significantly predict arthropod biodiversity
  - Taken during different seasons
  - Lacked data on specific flower diversity
- Assessing in next phase of project

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Texas levee analysis: Conclusions

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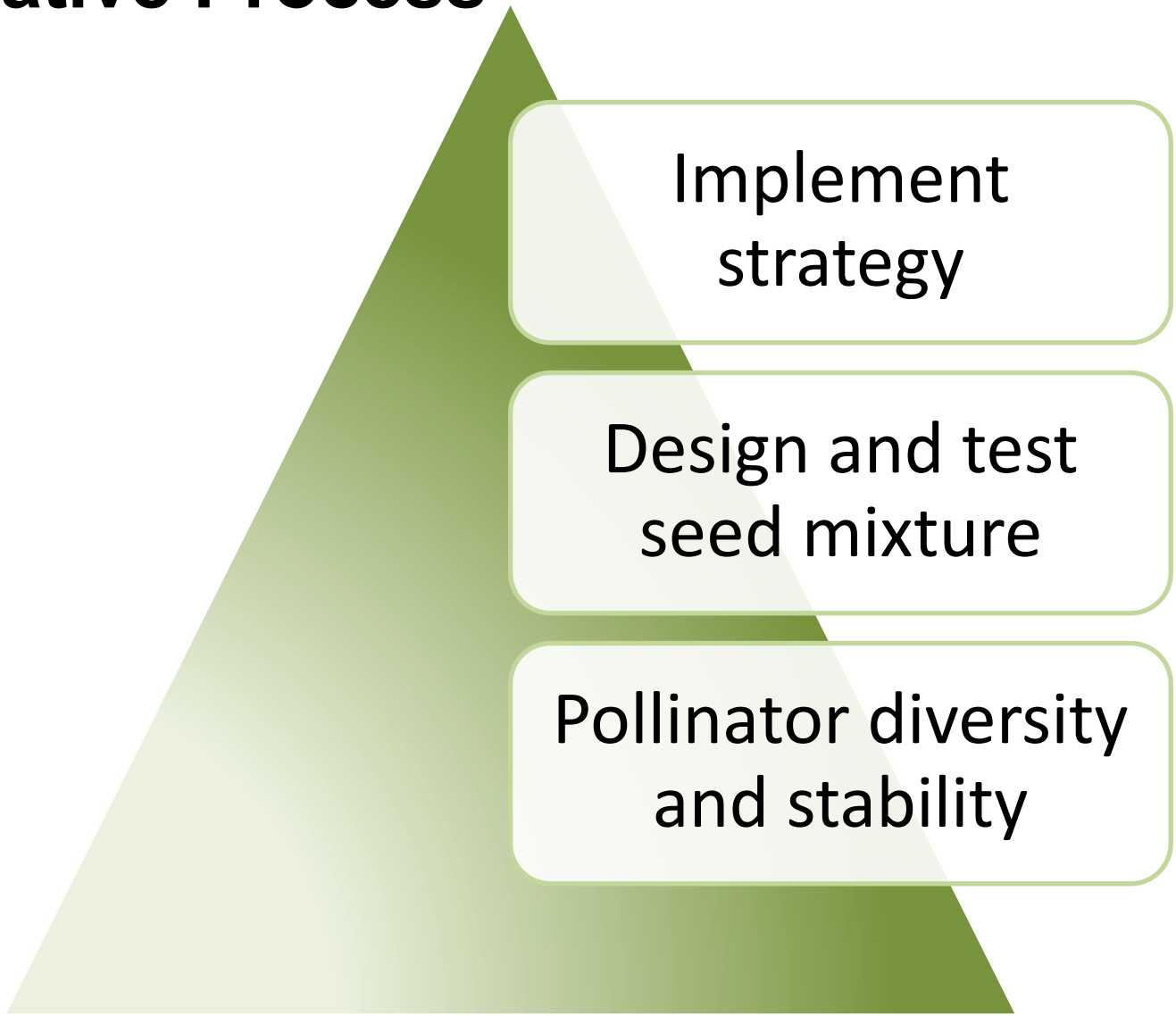
- Detect an extensive community of arthropods
- Broadly, floral resources on stable levees can improve arthropod biodiversity
- Flowering diversity did not relate to levee performance
  - Will be assessed with seed mixture experiment where we will sample specific flowers that we planted on levees
- Future steps
  - Contend with small sample size

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Iterative Process



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Pollinator seed mixture development and testing

Design and test seed mixture

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- Phase 1: Evaluate viability of pollinator-facilitating seed mixtures
  - Task 1: Develop and test pollinator-facilitating seed mixture
  - Task 2: Natural levee demonstration at USACE Rock Island District
- Phase 2: Assess pollinator biodiversity before and after establishment
  - Task 3: eDNA to assess current pollinator biodiversity on levees
  - Task 4: eDNA to assess pollinator biodiversity on planted levee





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Pollinator seed mixture development and testing

Design and test seed mixture

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Pollinator seed mixture development and testing

Design and test seed mixture

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- Modified Stantec Seed Mixtures…
  - Wet-to-Mesic prairie
  - Wet-to-Mesic prairie and buffalo grass
  - Midwest mesic pollinator mix
  - Midwest mesic pollinator mix and buffalo grass
  - Low profile prairie mix
  - Mesic-to-dry prairie
  - Mesic-to-dry prairie and buffalo grass



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Pollinator seed mixture development and testing

Design and test seed mixture

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Wet to Mesic Prairie	Mesic to dry Prairie	Midwest Mesic Pollinator + Buffalo Grass	Low-Profile Prairie	Wet-to-Mesic Prairie + Buffalo Grass	Midwest Mesic Pollinator	Mesic to dry Prairie + Buffalo Grass
Mesic to dry Prairie + Buffalo Grass	Wet to Mesic Prairie	Low-Profile Prairie	Wet-to-Mesic Prairie + Buffalo Grass	Mesic to dry Prairie	Midwest Mesic Pollinator + Buffalo Grass	Midwest Mesic Pollinator
Midwest Mesic Pollinator	Wet to Mesic Prairie	Mesic to dry Prairie	Wet-to-Mesic Prairie + Buffalo Grass	Midwest Mesic Pollinator + Buffalo Grass	Low-Profile Prairie	Mesic to dry Prairie + Buffalo Grass

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Pollinator seed mixture development and testing

Design and test seed mixture

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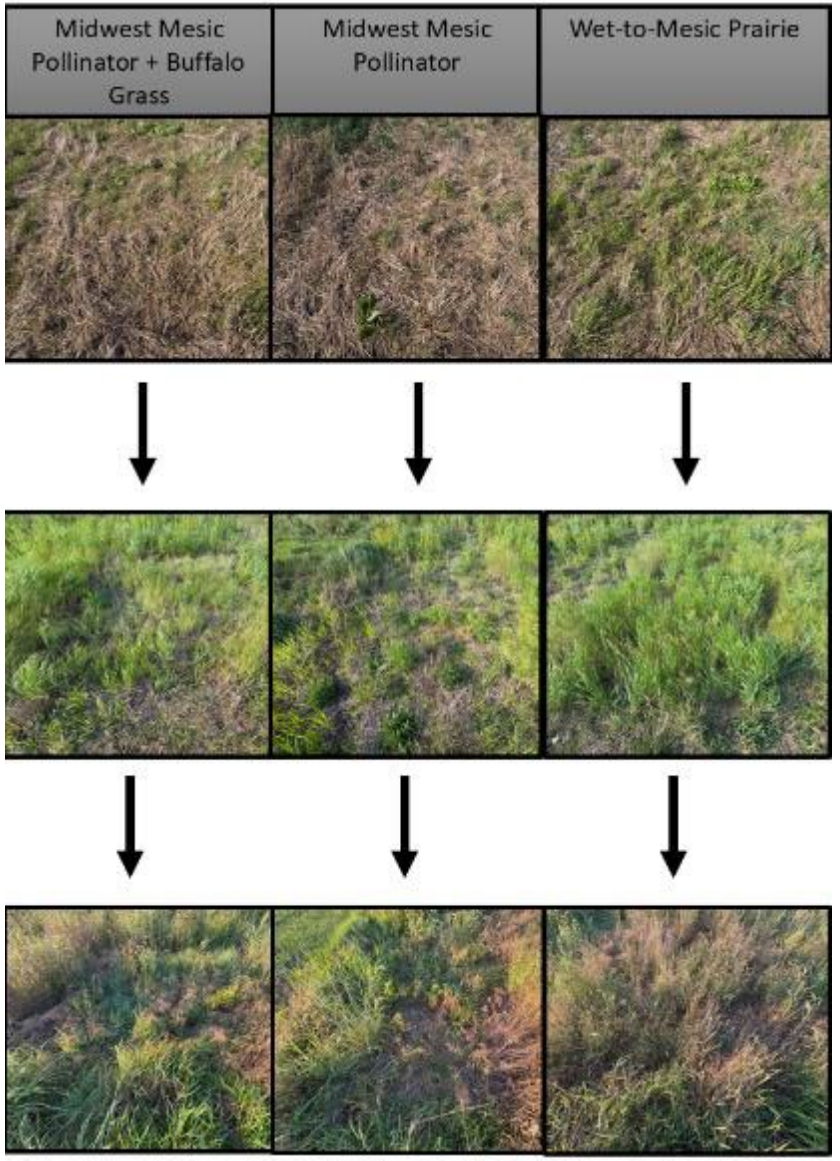
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Pollinator seed mixture development and testing

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- Seed May 2025
- Monitor November 2025 through July 2026



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Pollinator seed mixture development and testing

Design and test seed mixture

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	Midwest Mesic Pollinator	Mesic-to-Dry Prairie + Buffalo Grass	Low Profile Prairie	Midwest Mesic Pollinator + Buffalo Grass	Wet-to-Mesic Prairie + Buffalo Grass	Mesic-to-Dry Prairie	Wet-to-Mesic Prairie
Total Change in tons/acre	**6.7**	**-2.0**	**-2.4**	**-0.8**	**-11.9**	**-30.9**	**-3.3**

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Design and test seed mixture

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			RELATIVE COVER (%)					
Year	Mix	Bare Gound (%)	Total Vegetative Cover (%)	COVER CROP	WEEDS	SEEDED POLLINATORS	SEEDED GRASSES	UNKNOWNS
2025	Low Profile Prairie	80.6	19.4	57.3	54.5	28.6	25.9	11.7
2026	Low Profile Prairie	57.8	43.3	7.7	45.7	32.4	39.3	0.0
2025	Mesic to Dry	72.8	25.0	46.7	31.2	31.3	53.3	6.7
2026	Mesic to Dry	36.1	39.5	8.4	52.9	51.4	57.7	0.0
2025	Mesic to Dry + Buffalograss	67.2	20.6	67.6	43.4	13.6	13.6	2.8
2026	Mesic to Dry + Buffalograss	33.3	38.9	23.6	87.5	43.4	30.3	0.0
2025	Midwest Mesic Pollinator	64.9	24.4	77.2	34.1	16.0	11.5	4.6
2026	Midwest Mesic Pollinator	36.1	31.1	21.4	69.7	52.2	51.9	0.0
2025	Midwest Mesic Pollinator + Buffal	80.0	17.2	51.6	61.3	32.3	38.7	6.4
2026	Midwest Mesic Pollinator + Buffal	33.3	38.3	17.4	45.2	54.3	43.2	0.0
2025	Wet to Mesic	58.3	36.7	44.0	30.3	18.2	18.3	4.6
2026	Wet to Mesic	27.2	50.6	11.6	13.6	55.7	33.1	0.0
2025	Wet to Mesic + Buffalograss	84.4	15.6	78.6	46.3	18.0	39.8	14.6
2026	Wet to Mesic + Buffalograss	45.5	31.1	26.8	21.6	34.2	55.6	0.0

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Pollinator seed mixture development and testing

Design and test seed mixture

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Highest ranked seed mixtures

- **Midwest Mesic Pollinator + Buffalo Grass**
- Midwest Mesic Pollinator
- Wet-to-Mesic Prairie

Adjusted final seeding mixture based on species that grew well



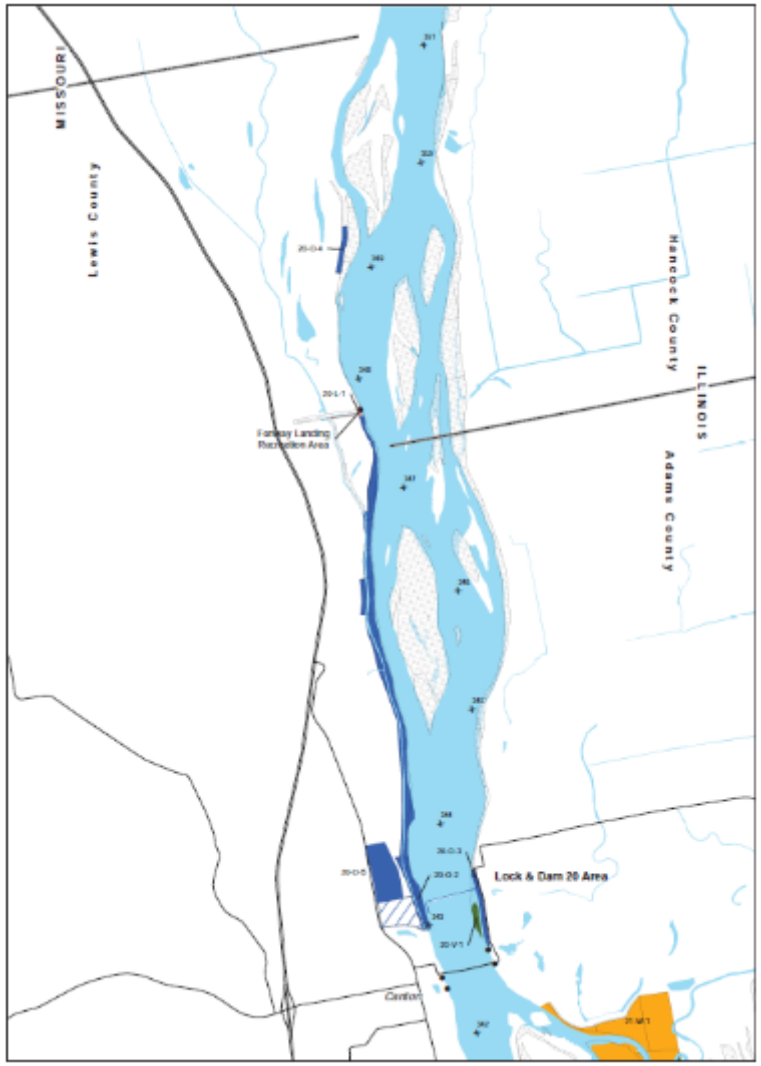
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Pollinator seed mixture development and testing: Next steps

Design and test seed mixture

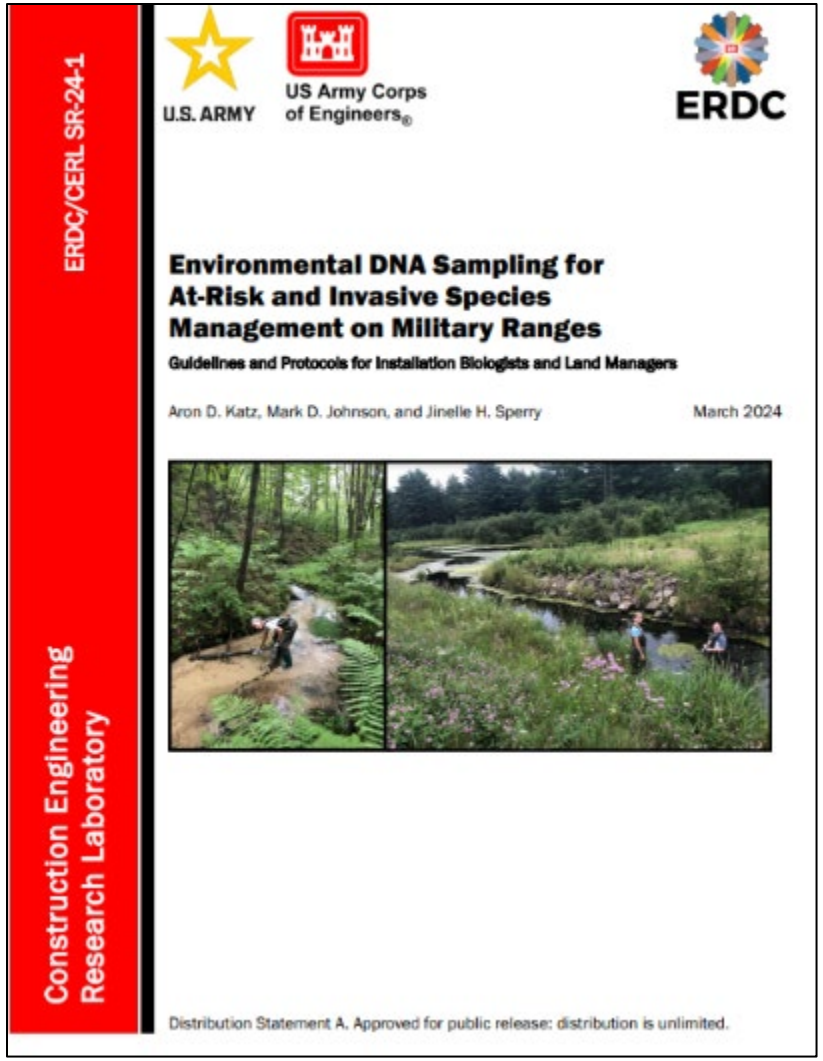
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Implementing Strategy



- One seed mixture not applicable to entire country
- Generate region-specific guidelines for seed mixture development
- Extensive standard operating procedures for eDNA
- Use lessons learned to illustrate benefits and challenges



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Implementing Strategy: Endangered species example



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- Reviewed distribution all potential listed species in the area
- Rusty patch bumblebee and monarch butterfly
- Reviewed foraging and habitat requirements for both species
- Offered a risk analysis for potential local habitat sites





Co-authors and Collaborators

Co-PIs
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- Jinelle Sperry
- Dan Gambill
- Aron Katz
- Sasha Tetzlaff
- Patrick Wolff
- Dylan O'Hearn

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- Catherine Dana
- Lauren Urie
- Bianca Saftoiu
- Sonia Tomcyzk

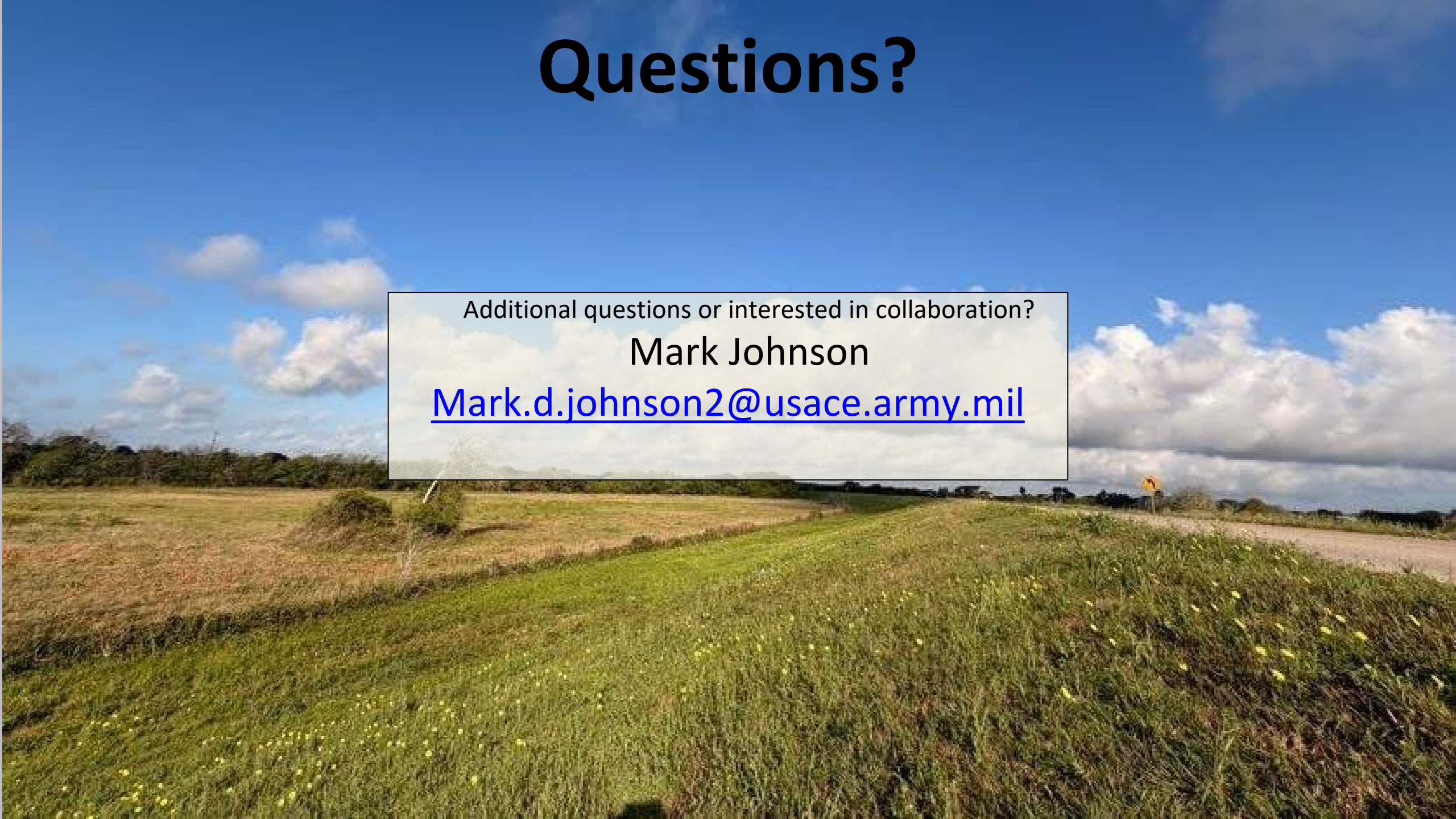
CRREL
- Franz Lichtner
- Tim Cary
- Alice Goldstein-Plesser
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- Simone Whitecloud

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Levee Safety Center
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- Daniel Rose
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- Allison Borkowski

Questions?



Additional questions or interested in collaboration?

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