Can eDNA serve as a monitoring technique for pollinators on rights-of-way

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Today's Agenda

- Right-of-way biodiversity
- Rapid monitoring techniques
- Overview of 4 research projects
  - Pollinator eDNA in NY
  - Plant and pollinator eDNA in AZ
  - Native Plant / Pollinator Interactions
  - Airborne plant eDNA

Photos: A. Bennett


Photos: A. Bennett

Introduction

Background

- Utility lands managed with IVM provide biodiversity benefits to pollinators
- Utilities would like to monitor, measure, & track changes over time
- Field surveys are costly
- New rapid assessment methods are needed
- Is eDNA a possible solution?

LGE-KU Solar Site

EPRI Research Sites

Photos: A. Bennett

AEP – ROW

Introduction: Environmental DNA

What is eDNA:

- DNA shed by organisms into the environment
  - Can include microbes, plants, insects, and animals

Where you find eDNA:

- Water, air, soil
- Plants
- Carried on insects
- Carried on animals

Practical Application for ROWs:

- Rapid Biodiversity Monitoring
  - Pollinators visiting plants
- Rare or Invasive Species
  - Pollen from bees
  - Air samples
- Construction / IVM Impacts
  - Soil biota
  - Plant / pollinator community change
- Seed Mix Refinement
  - Pollen from bees
  - Understudied plant communities
  - Attractive pollinator plants

Project 1

NYPA ROWs

Project Collaborators:

- New York Power Authority
- Stantec

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Introduction

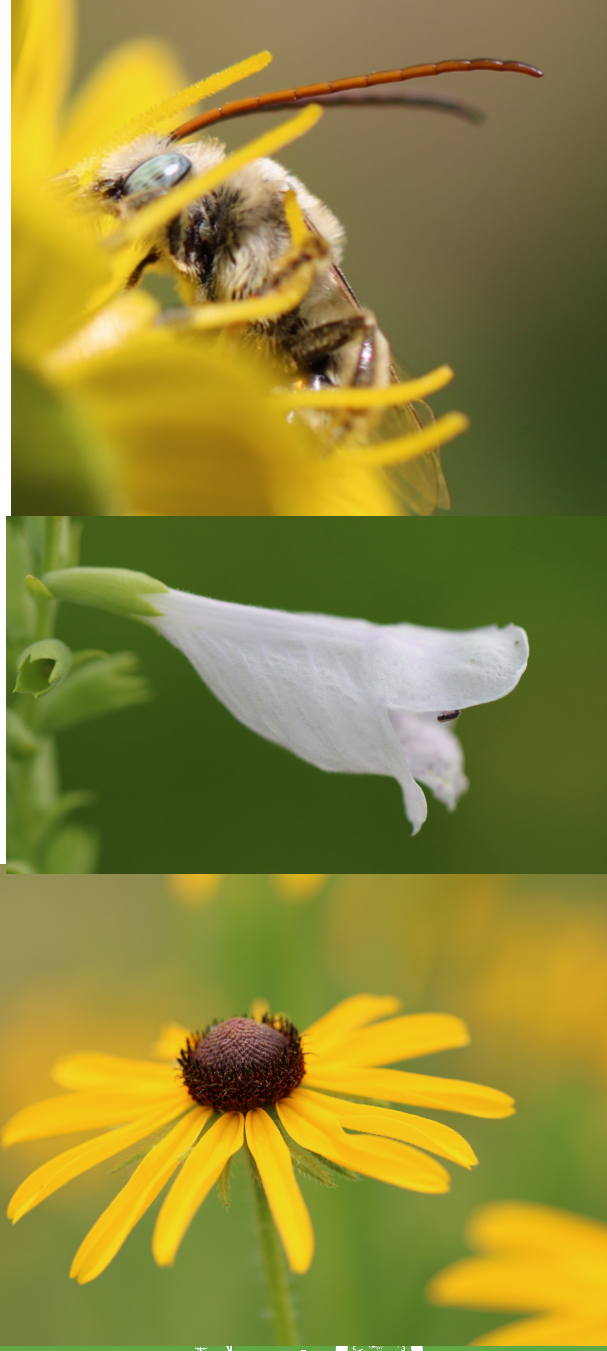
Study Questions:

1. Can eDNA assess pollinator communities along ROWs?
2. Does flower morphology impact detection of pollinators?
3. How does eDNA compare to field collections?

Leverage ROW Research:

- Ongoing research evaluating impact of construction mats on ROW vegetation and pollinators
- 3-year study, 2024 is final year of data collection

1. Do construction mats impact right-of-way vegetation and pollinator communities post disturbance?
2. Does proximity to areas disturbed by construction mats impact right-of-way vegetation?



Photos: A. Bennett

Methods

Methods Overview:

1. Field collected pollinators
   - ROW in New York State
   - Timed transects
   - Sampling method - Netting
   - Bees ID to species
2. Field collected flowers
   - Flower heads collected from 7 different species
   - 6 native and 1 non-native
   - 4 open and 3 tubular
3. Replicates collected
   - 10 replicates / flower species
   - 10 flower heads / replicate
4. DNA metabarcoding
   - Detects eDNA left by pollinators visiting flowers

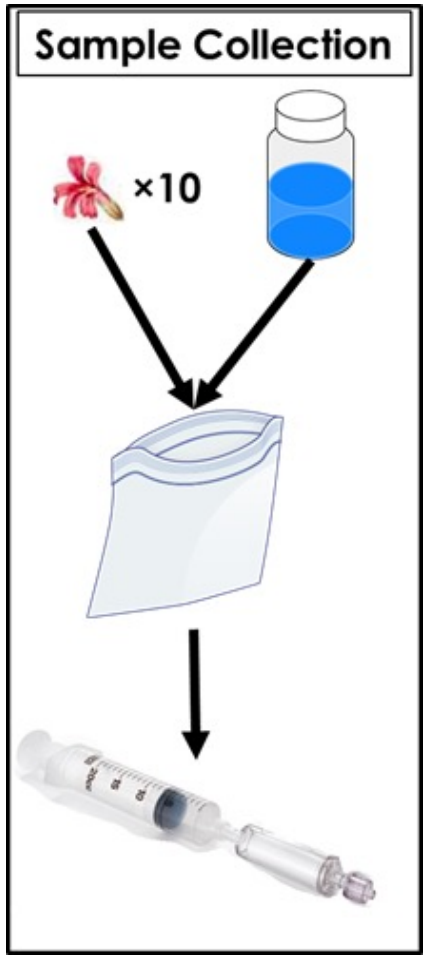
Photo: Lew Payne

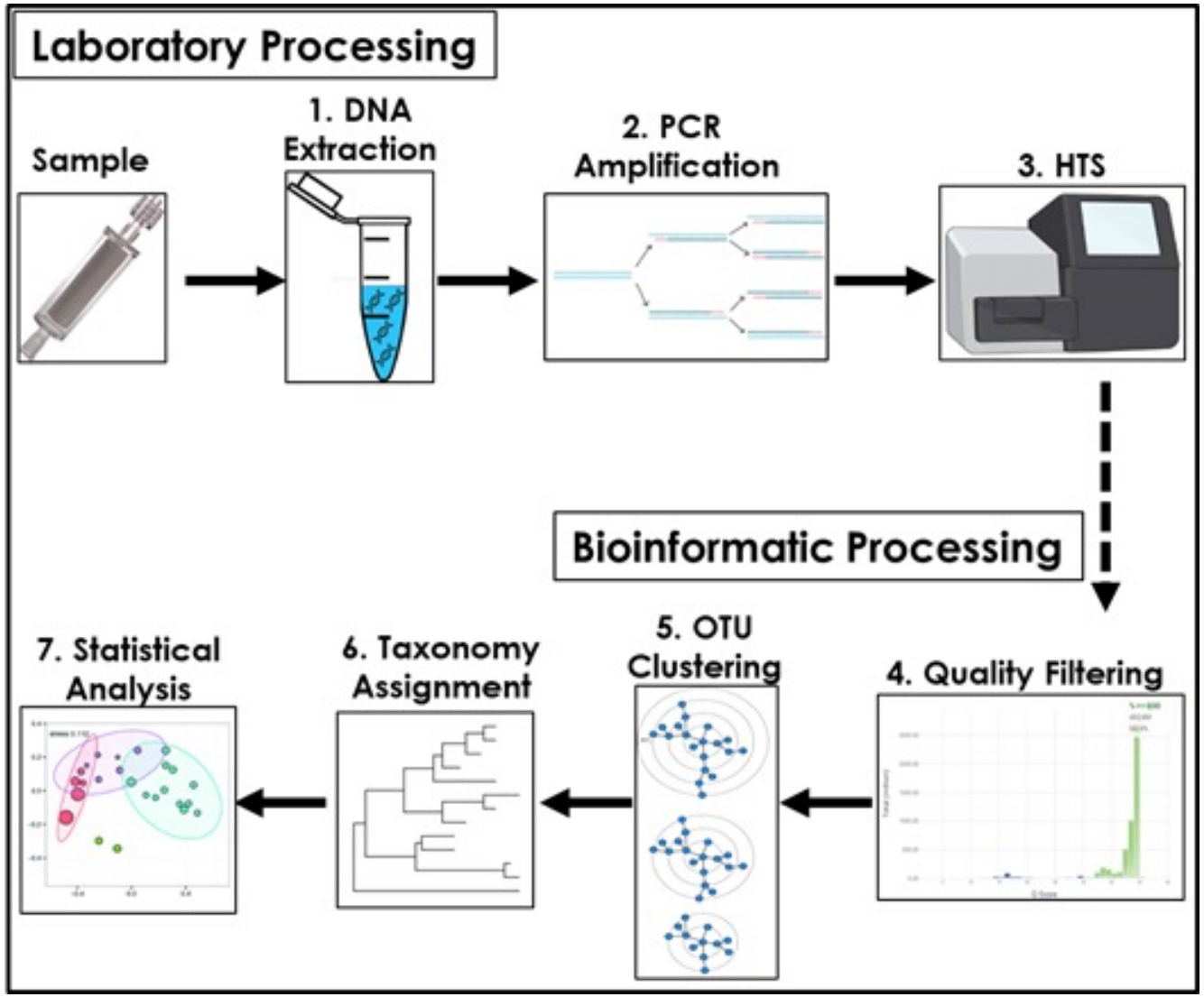
St. Lawrence Co

Lewis Co

Methods: eDNA Field & Lab

7 Flowers

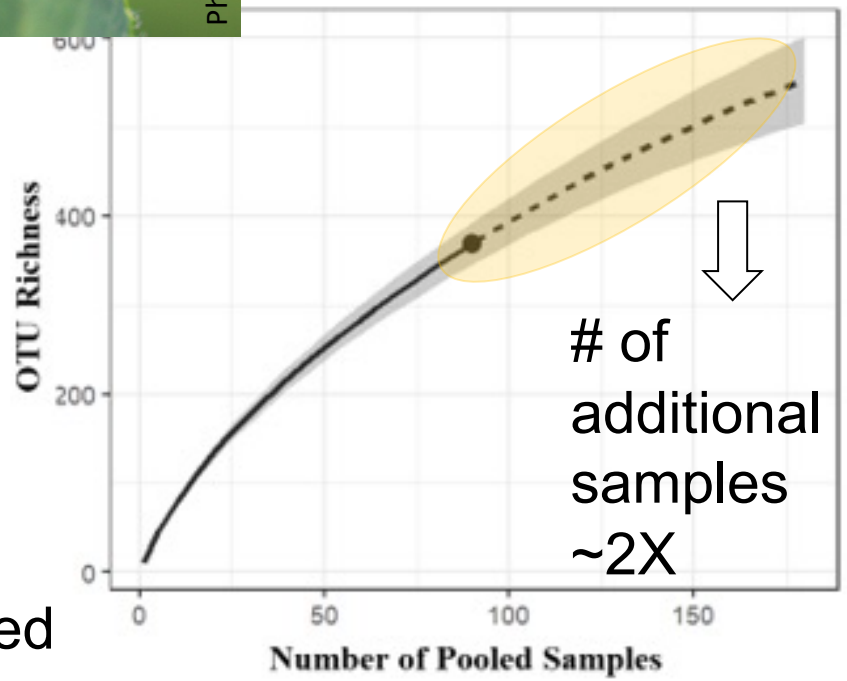




Results

Order	# of OTUs	# of Occurrences
Diptera	143	386
Coleoptera	46	115
Hemiptera	42	141
Hymenoptera	42	93
Lepidoptera	36	100
Orthoptera	17	38
Psocoptera	8	17
Ephemeroptera	4	4
Phasmatodea	4	5
Odonata	2	3
Thysanoptera	2	2
Blattodea	1	1
Mantodea	1	1
Mecoptera	1	1

Syrphid Fly



- High diversity of insect taxa detected
- Most detections were flies followed by beetles
- Richness curves estimated more sampling was needed

Results: Transects vs eDNA

Bees & Butterflies

➢ Bees

- Greater detection with netting
- Apidae highest followed by Andrenid (miner) & Halictid (sweat bees)
- Very low eDNA detections

➢ Butterflies

- Greater detection with netting
- Nymphalid (brush-footed) Pierid (whites / sulphurs), & Hesperid (skippers) most abundant leps not detected with eDNA
- Only 1 butterfly family detected with eDNA

Order	Family	Count with Ground	Occurrences with eDNA
Hymenoptera	Andrenidae	131	1
	Apidae	1672	24
	Chrysididae	1	0
	Cimbicidae	1	2
	Colletidae	30	0
	Crabronidae	26	0
	Halictidae	116	15
	Ichneumonidae	3	0
	Megachilidae	38	3
	Mellitidae	4	0
	Pompilidae	2	0
	Sphecidae	3	0
	Tenthredinidae	1	2
	Vespidae	8	3
Lepidoptera	Erebidae	17	4
	Geometridae	1	15
	Hesperiidae	98	0
	Lycaenidae	36	0
	Noctuidae	1	14
	Nymphalidae	231	0
	Papilionidae	41	1
	Pieridae	129	0

Photos: A. Bennett

Results

Ground counts for Hymenoptera by flower species

Red = Families undetected with eDNA; **Green** = Families detected with eDNA

Flower	Andrenidae	Apidae	Cimbicidae	Halictidae	Megachilidae	Tenthredinidae	Vespidae
Open Flowers							
Common Boneset	-	92	-	-	-	-	0
Swamp Candles	-	1	-	2	-	-	-
Black-eyed Susan	-	-	-	1	2	-	-
White Meadowsweet	10	41	1	2	2	1	1
Tubular Flowers							
Allegheny Monkeyflower	-	2	-	0	-	-	-
Bird Vetch	-	21	-	7	2	-	-
Blue Vervain	7	56	-	2	3	-	-


Photo: Roundstone native Seed

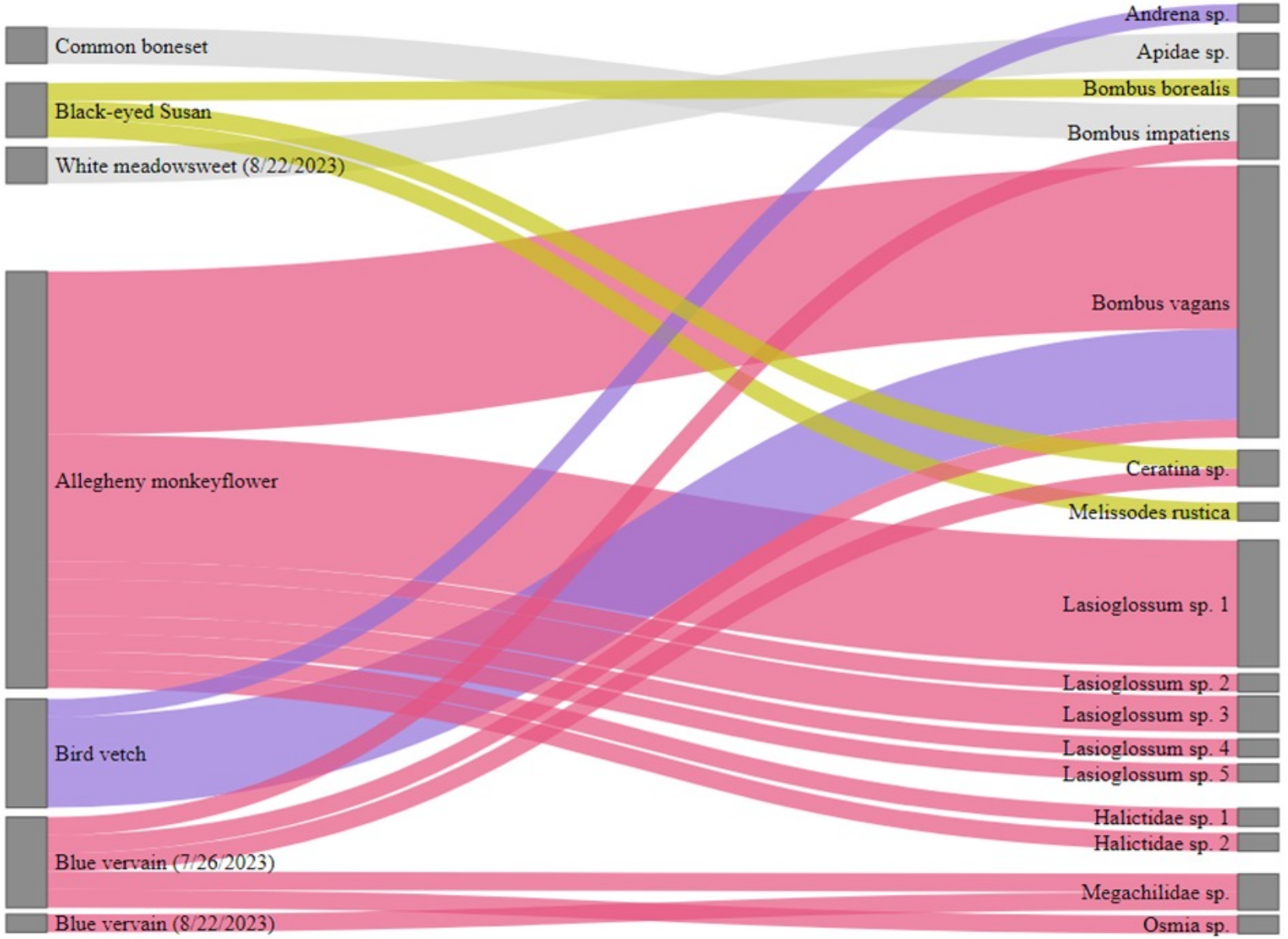

Photo: Truelove Seed


Photo: Prairie Moon Nursery

- High Apidae (bumble bee) eDNA detections
- Bees highly attracted to blue vervain and meadowsweet

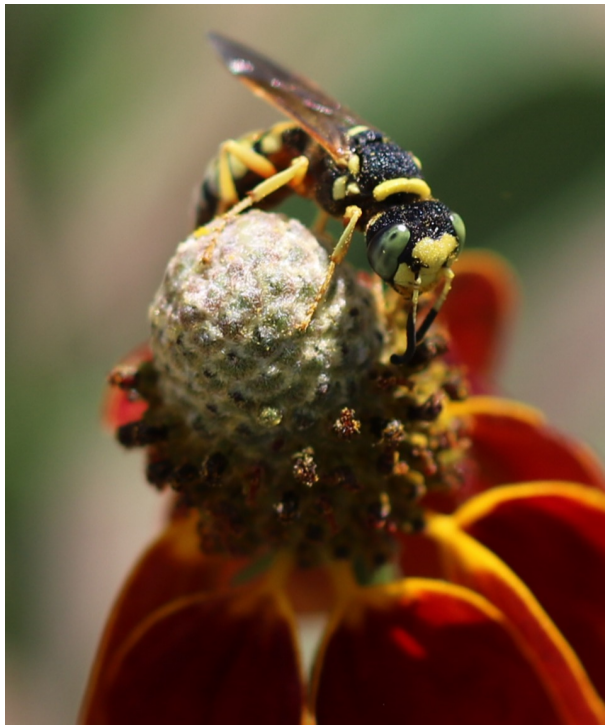
Results

- Relationship between bee groups and sampled flowers
- Bar thickness indicates number of DNA fragments detected for each flower
- Allegheny monkeyflower had more visits by *B. vagans* followed by *Lasioglossum* sp. 1
- Black-eyed Susan visited by
  - 3 bees but number of DNA fragments for each group was low
  - *Bombus borealis*, *Melissodes rustica*, *Ceratina* sp.



Key Findings

1. eDNA resulted in high detections of insect richness
   - Mostly non-pollinator groups; Hymenoptera ~10%
2. Species richness curves estimated more sampling
   - 2x more to increase richness by 50%
3. Insect richness differed by flower
   - Black-eyed Susan highest observed richness
   - Allegheny monkeyflower highest eDNA richness
4. eDNA bee detections
   - 4 bee families detected
   - *B. vagans* most common; Honey bees not detected
5. Flower morphology
   - 84% of bee detections were on tubular flowers
6. Aerial netting vs eDNA sampling
   - Overlap between methods was low
   - Halictid bees under-detected by eDNA



Photos: A. Bennett

Project 2

SRP ROWs

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Project Collaborators:

- Salt River Project
- Northern Arizona University

Project in Progress

- Determine the value of IVM practices to native plants & pollinators across three ecoregions in Arizona
- Compare pollinator abundance and richness on and off the ROW


Photo: K. Laushman

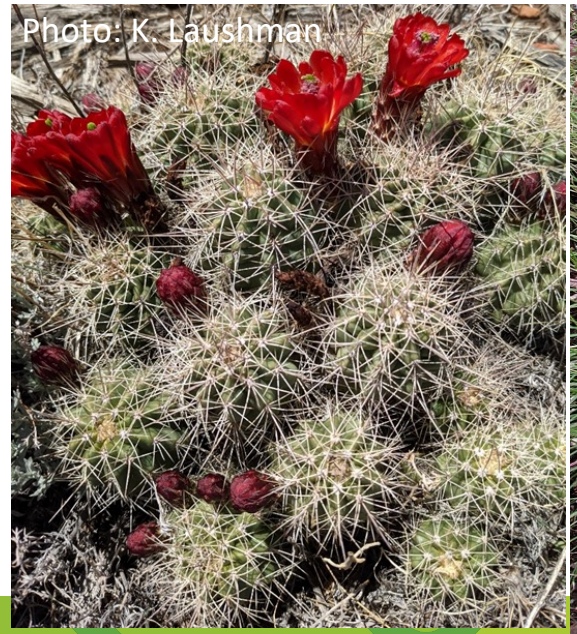


Photo: K. Laushman


Photo: J. Hayden


Photo: Pam Reschke


Photo: A. Bennett

Project in Progress


Sonoran Desert


Pinyon Juniper


Ponderosa Pine

2024

Results 2022: Ponderosa Pine

- Significantly higher bee **abundance** on ROW compared to off ROW
- Significantly higher bee **richness** on ROW compared to off ROW
- Differences in bees only found for Ponderosa Pine ecoregion

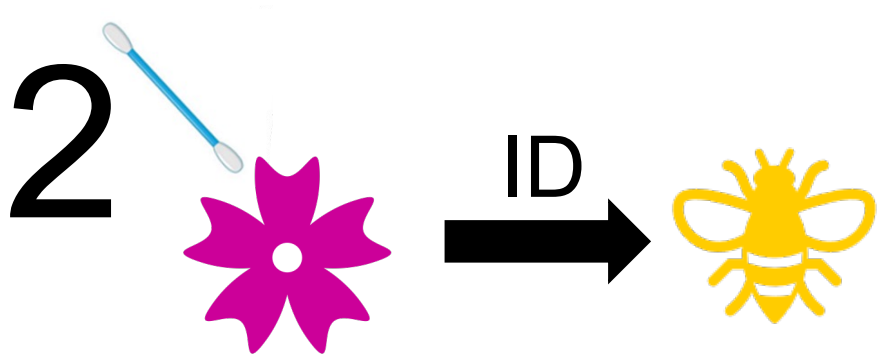
Common Bee Genera

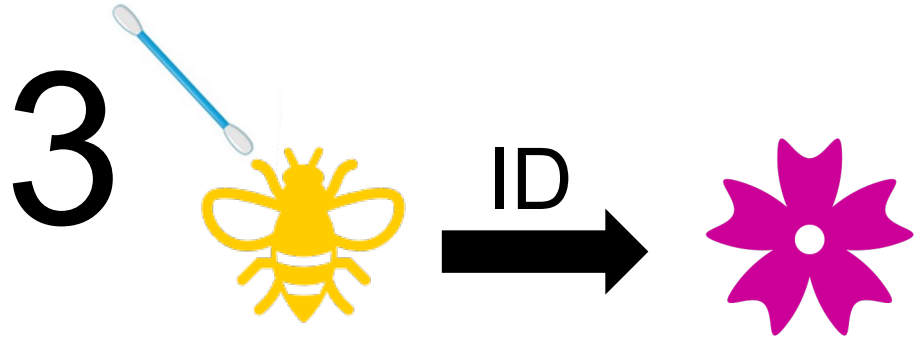
Off ROW		On ROW	
Genus	Individuals	Genus	Individuals
Agapostemon	0	Agapostemon	5
Apis	19	Apis	39
Centris	7	Centris	0
Hylaeus	5	Hylaeus	10
Lasioglossum	79	Lasioglossum	123
Melissodes	5	Melissodes	12

Photos: A. Bennett

Project Goals - eDNA

1. Compare pollinators collected by active sampling to data collected by eDNA sampling
2. Determine whether pollinator eDNA collected from flowers can detect differences in visitation
3. Evaluate whether eDNA collected from pollinators can identify flower species serving as foraging resources





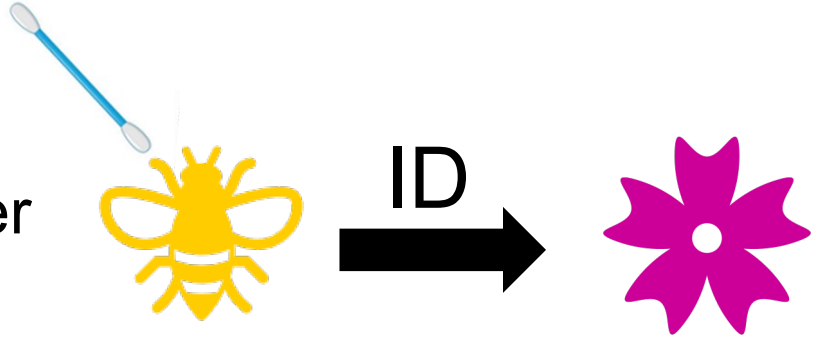
Methods

Study location: Ponderosa Pine
Sampled ROWs: 7

Ponderosa Pine: Elevation > 5000’

Pollinator Sampling

- Sampled 6 pollinator groups
  - 4 bees
  - 2 flies
- Netted ~7-30 bees / group
- Cooled pollinators on ice
- Swabbed bodies for eDNA
- Swabs placed in sterile vials
- Samples stored in -80º freezer
- DNA metabarcoding
  - Goal: Detect plant eDNA



Methods

Sampled Pollinator Groups

Photos: A. Bennett

Methods

Location: Ponderosa Pine
Sampled ROWs: 7

Plant Sampling

- Sampled 6 plants
  - 3 on ROW
  - 3 off ROW
  - Targeted 30 plants / spp.
- Flowers placed in sterile vials
- Samples stored in -80º freezer
- Samples stored in freezer
- DNA metabarcoding
  - Goal: Detect bee eDNA

False Pennyroyal

Macoun's rabbit-tobacco

Wright's Trefoil

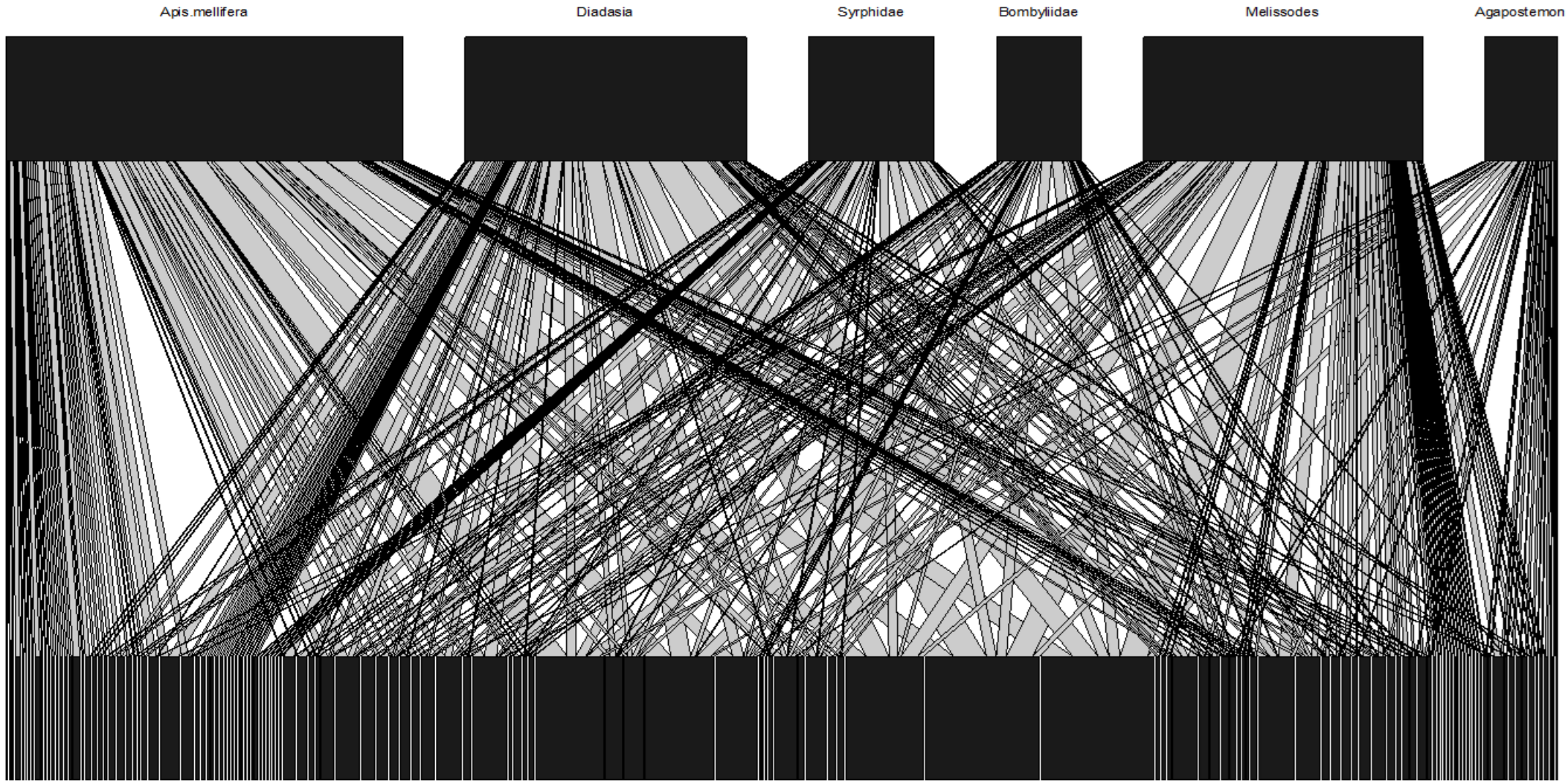
Pygmy Bluet

Fendler's Buckbrush

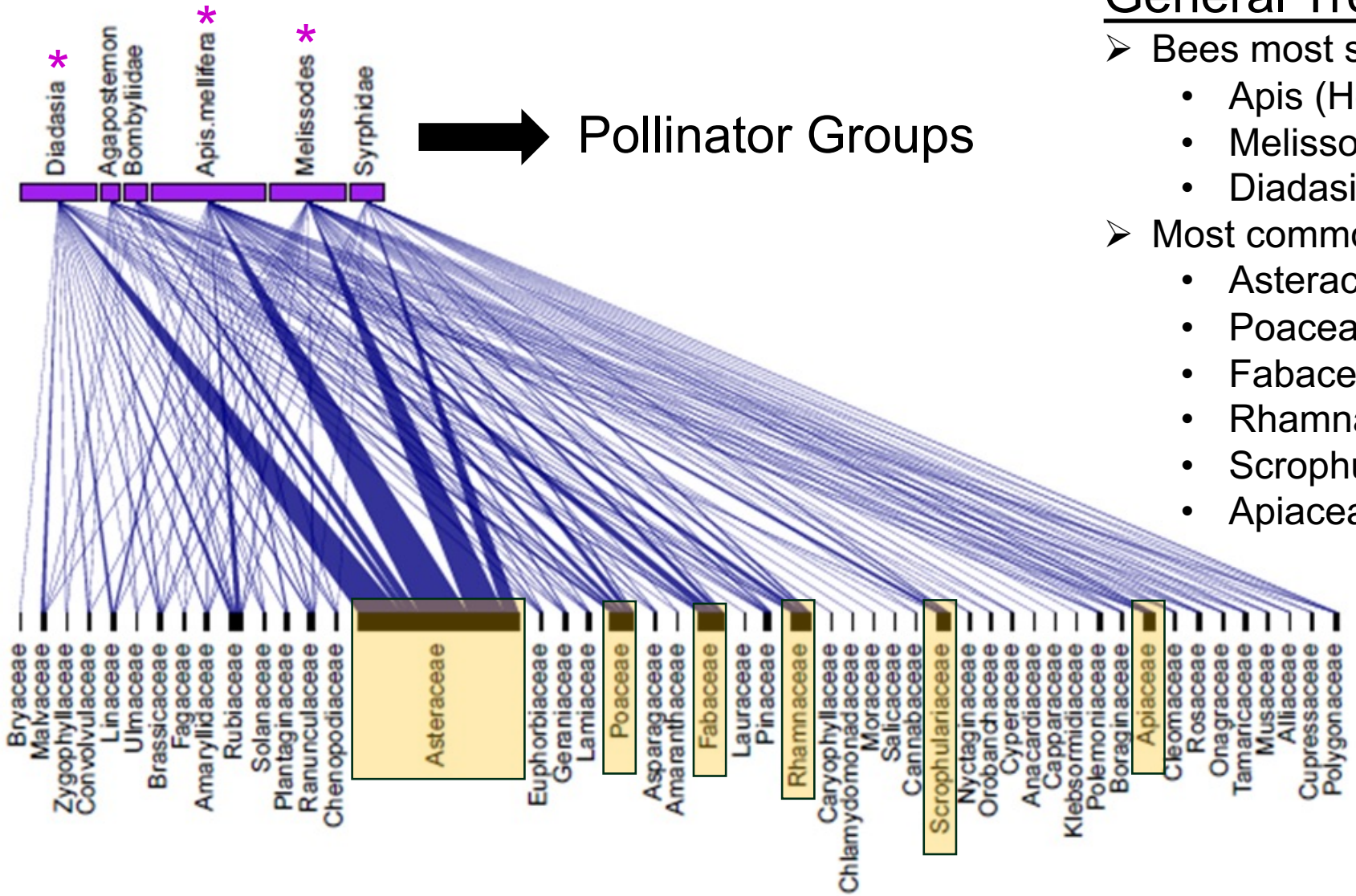
Bull Thistle

Results

Plant / Pollinator Network – All Taxon



Results



General Trends:

- Bees most sampled
  - Apis (Honey bees)
  - Melissodes (Long-horned bees)
  - Diadasia bees
- Most common plant families
  - Asteraceae (asters)
  - Poaceae (grass)
  - Fabaceae (pea)
  - Rhamnacea (buckthorn)
  - Scrophulariaceae (figwort)
  - Apiaceae (carrot)

Results

Plant Family	Agapostemon	Apis mellifera	Bombyliidae	Diadasia	Melissodes	Syrphidae	Total
Apiaceae	3	8	1	2	11	8	33
Asparagaceae		5		1	2		8
Asteraceae	25	161	33	142	120	50	539
Boraginaceae	1	3			1	1	6
Brassicaceae	5	2		5	3	1	17
Bryaceae				1			1
Cannabaceae			1		1		2
Capparaceae					1		1
Caryophyllaceae		2					2
Chenopodiaceae	2	2		2		1	7
Chlamydomonadaceae		1					1
Cleomaceae	1	1			2	1	5
Convolvulaceae	1			5	1		7
Cupressaceae		2				2	4
Cyperaceae		1			4		5
Euphorbiaceae		5	3	2	2		12
Fabaceae	5	38	5	9	19	4	83
Fagaceae		1		1	1		4
Geraniaceae		4	1	2	4		11
Klebsormidiaceae					1		1
Lamiaceae		1	4	1	2		10
Lauraceae			1				1
Linaceae		7		9	1		17
Malvaceae		1	1	11	1		14

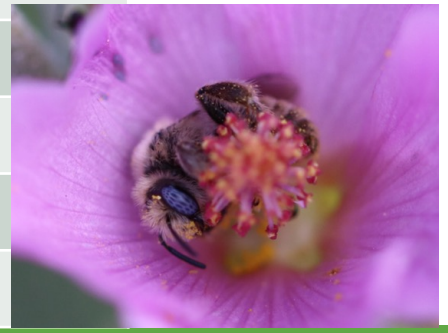
Plant Family	Agapostemon	Apis mellifera	Bombyliidae	Diadasia	Melissodes	Syrphidae	Total
Moraceae		1					1
Musaceae	1				1	1	3
Nyctaginaceae		1			1		2
Onagraceae		5			1	2	8
Orobanchaceae		1			1		2
Pinaceae	3	6	1	2	7	1	20
Plantaginaceae		6		2			8
Poaceae	4	21	10	15	22	5	77
Polemoniaceae		4	6			2	12
Polygonaceae	1	1	2		2	8	14
Ranunculaceae		5	3	6	5	1	20
Rhamnaceae	4	28	1	8	11	9	61
Rosaceae	1	4			5	2	12
Rubiaceae	4	19	3	13	3	2	44
Salicaceae		1					1
Scrophulariaceae		22		5	8	6	41
Solanaceae	1	2	1	1			5
Tamaricaceae		1		1	3	3	8
Ulmaceae	2			1	1		4
Zygophyllaceae	1			2			3
Total	**66**	**378**	**77**	**254**	**252**	**114**	**1159**

- Number of distinct DNA fragments / pollinator
  - 161 unique Asteraceae DNA fragments on Apis
  - Highest plant DNA fragments from: Aster, bean, grass, buckthorn, bedstraw, and figwort

Results

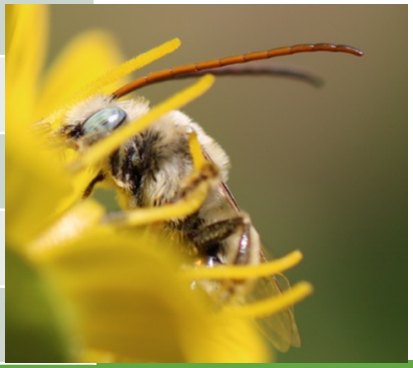
Diadasia - 1	Diadasia- 2
Asteraceae sp.	Achillea millefolium
Avena sp.	Achillea sp.
Calliandra sp.	Asteraceae sp.
Ceanothus sp.	Cirsium sp.
Cirsium sp.	Erigeron sp.
Cosmos bipinnatus	
Erigeron sp.	
Erodium sp.	
Fabaceae sp.	
Houstonia sp.	
Lepidium montanum	
Linum sp.	
Lotus sp.	
Penstemon sp.	
Tamarix sp.	
Xanthisma sp.	

- Large variability specimen to specimen
- 22 bees sampled
- 5-16 plant DNA fragments / bee were detected



Melissodes - 1	Melissodes - 2
Achillea millefolium	Achillea sp.
Apiaceae sp.	Asteraceae sp.
Asteraceae sp.	Cannabis sativa
Ceanothus sp.	Ceanothus sp.
Cirsium sp.	Cirsium sp.
Cyperaceae sp.	Erigeron sp.
Delphinium sp.	Melilotus sp.
Erigeron sp.	Musaceae sp.
Fagaceae sp.	Pinus sp.
Festuca arizonica	Schismus sp.
Helenium arizonicum	Verbascum thapsus
Helenium sp.	
Muhlenbergia sp.	
Pinaceae sp.	
Pinus sp.	
Poaceae sp.	
Tragopogon pratensis	
Verbascum sp.	
Verbascum thapsus	

- Large variability specimen to specimen
- 25 bees sampled
- 3-28 plant DNA fragments / bee were detected



Next Steps

1. Complete preliminary analyses of pollinator swabs
   - Informs plant eDNA on pollinators
2. Metabarcoding results from plants still needed
   - Will identify pollinators visiting plants
3. Data analyses of plant collected eDNA data needed
4. Compare field collected pollinators to eDNA data
   - Are results similar?
   - Are results different but complementary?
5. Evaluate plant and pollinator data for indicators of alignment
   - Do plant samples suggest common pollinator visitors
   - Do pollinator samples suggest preferred plants for foraging
   - Does data from both the plants and pollinators align

Photo: K. Laushman

Project 3

Native Plant and Pollinators

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Project Collaborators:

- University of Illinois
- Stantec

Background

- Builds upon previous eDNA studies
- Focus is on improving bee detections
  - Refining field methods
  - Evaluating different eDNA labs
  - Increasing sampling effort

<u>Study Objectives</u>

- Compare sampling methods
  - Active vs eDNA
- Assess richness & relative abundance across different flowers with eDNA
- Evaluate flower shape for detection differences: tubular *vs* open
- Compare costs across methods

Photos: A. Bennett

Methods

Study location:

- Stantec Nursery - Walkerton, IN

<u>Methods</u>

- Active pollinator sampling
  - Observations & vacuum
- Target 20 native flower species
- Collect flower heads from each species
  - 6 replicates / flower species
  - 5 flowers / replicated sample
- Flower heads into distilled water, shake
- Water then filtered; filter placed in CTAB
- Stored at room temperature until processed
- Metabarcoding used to ID pollinators (bees)

Data collection in progress……

Project 4

Airborne eDNA

Project Collaborator:
- University of Illinois

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Background

- Can airborne eDNA complement remote monitoring of pollinator habitat quality?
- Can airborne eDNA detect flowering native forb species?

Study Objectives

1. Compare the species of native flowering plants detected by ground vegetation surveys to airborne eDNA
2. Calculate the percentage of native flowering plants detected with airborne eDNA to ground vegetation surveys

Photo: A. Bennett

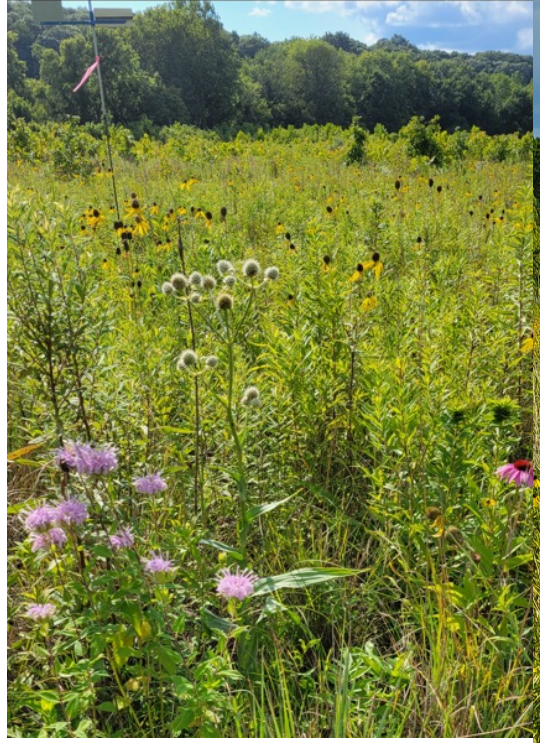
Methods

Study Location

- Central, Illinois
- 9 sites
  - Seeded to native seed mixes

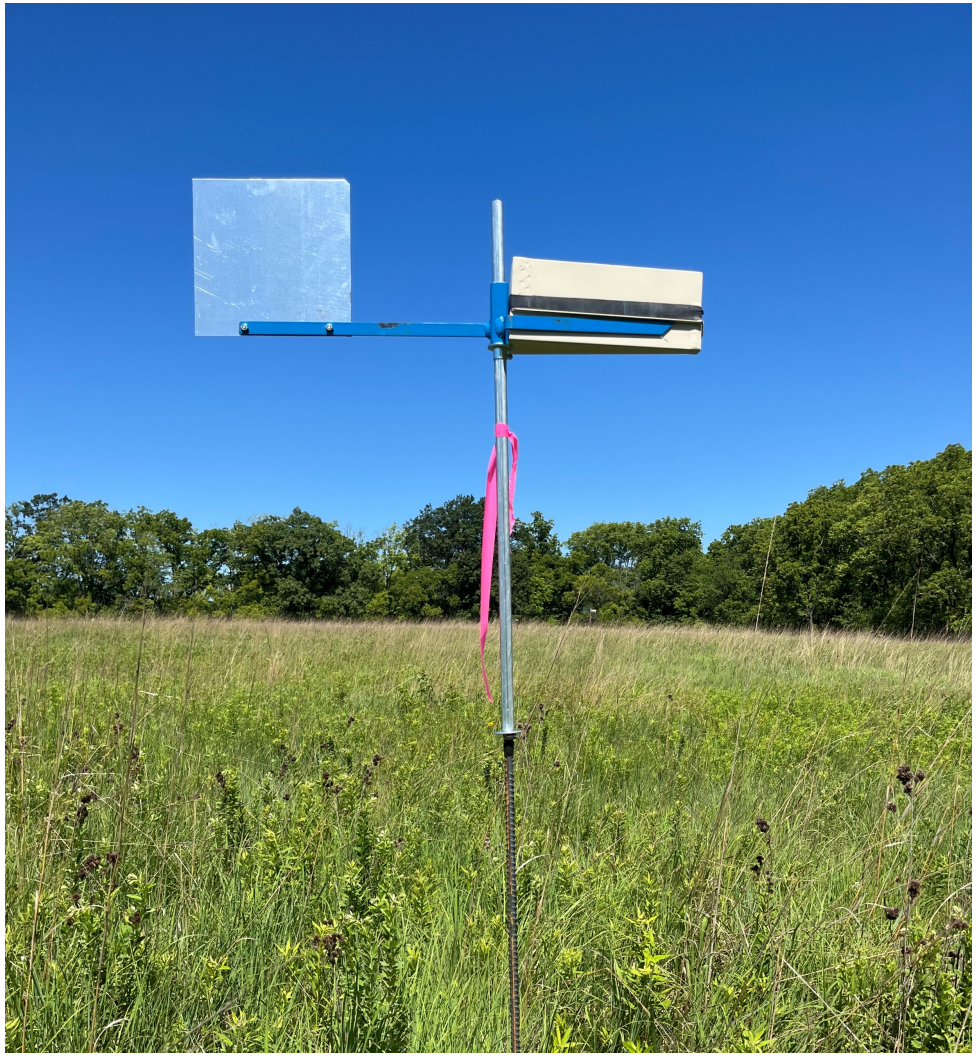
Vegetation Sampling

- Flowering plants are recorded using
  - 2 transects each 100m
  - 1 meander survey, 15 minutes
- 3m and 0.5m satellite imagery collected
  - Flower cover
  - Seasonal flower phenology
  - Lacks species level data



Airborne eDNA Sampling

Photos: A. Bennett



- 3 dust traps at all 9 sites

Photos: A. Bennett

Airborne eDNA sampling

- Traps checked twice a month + vegetation surveys performed
- Traps washed with distilled water in field
- Water + eDNA collected in sterile vials
- Samples filtered once back at lab
- Metabarcoding used to ID plant eDNA

Data collection in progress……

Summary

- eDNA monitoring technology is promising
  - Non-destructive sampling
  - Less time & labor intensive
  - Taxonomic experts not required
- Additional research needed to refine pollinator detections, specifically bees
- Pollinator eDNA is a developing field
  - Rapidly developing new lab methods
  - Refining field data collection methods
- Cost comparisons across techniques are needed
- Comparisons of eDNA methods are needed
  - *Example*: eDNA from flowers and bees
  - *Example*: value of airborne eDNA

Photos: A. Bennett

THANK YOU

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