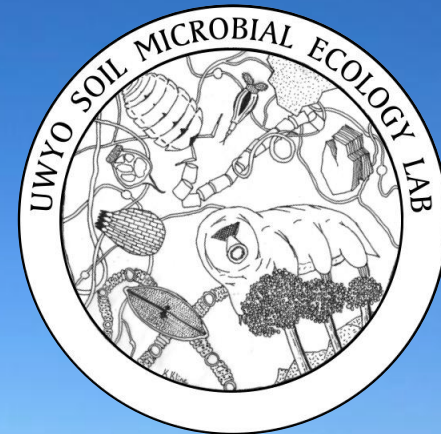




College of Agriculture, Life Sciences
and Natural Resources
Ecosystem Science
and Management



Assessing soil microbial community functional response to non-native *Bromus inermis* presence and restoration efforts in the Sagebrush Steppe of Grand Teton National Park

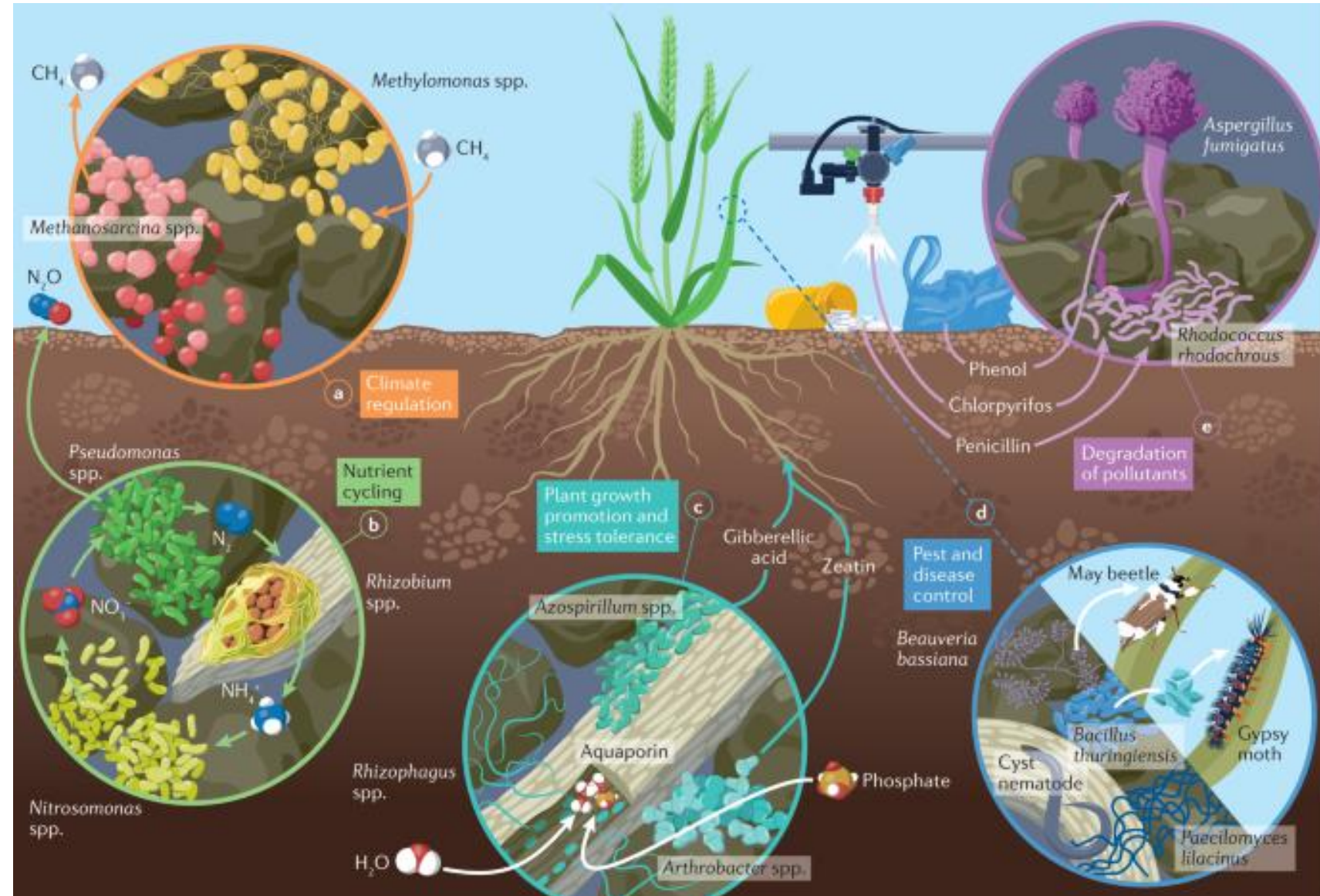
Dillon Romero, Linda van Diepen, Jennifer Bell

University of Wyoming, Department of Ecosystem Science and Management
Laramie, WY, USA

Why do we care about soil microbes?

(Hartmann and Six, 2022)

- Establish the conditions for plant growth
 - Nutrient cycling & availability
 - Soil Structure
- Associations with plants
 - Plant growth promotion (i.e., mycorrhizae)
- Succession

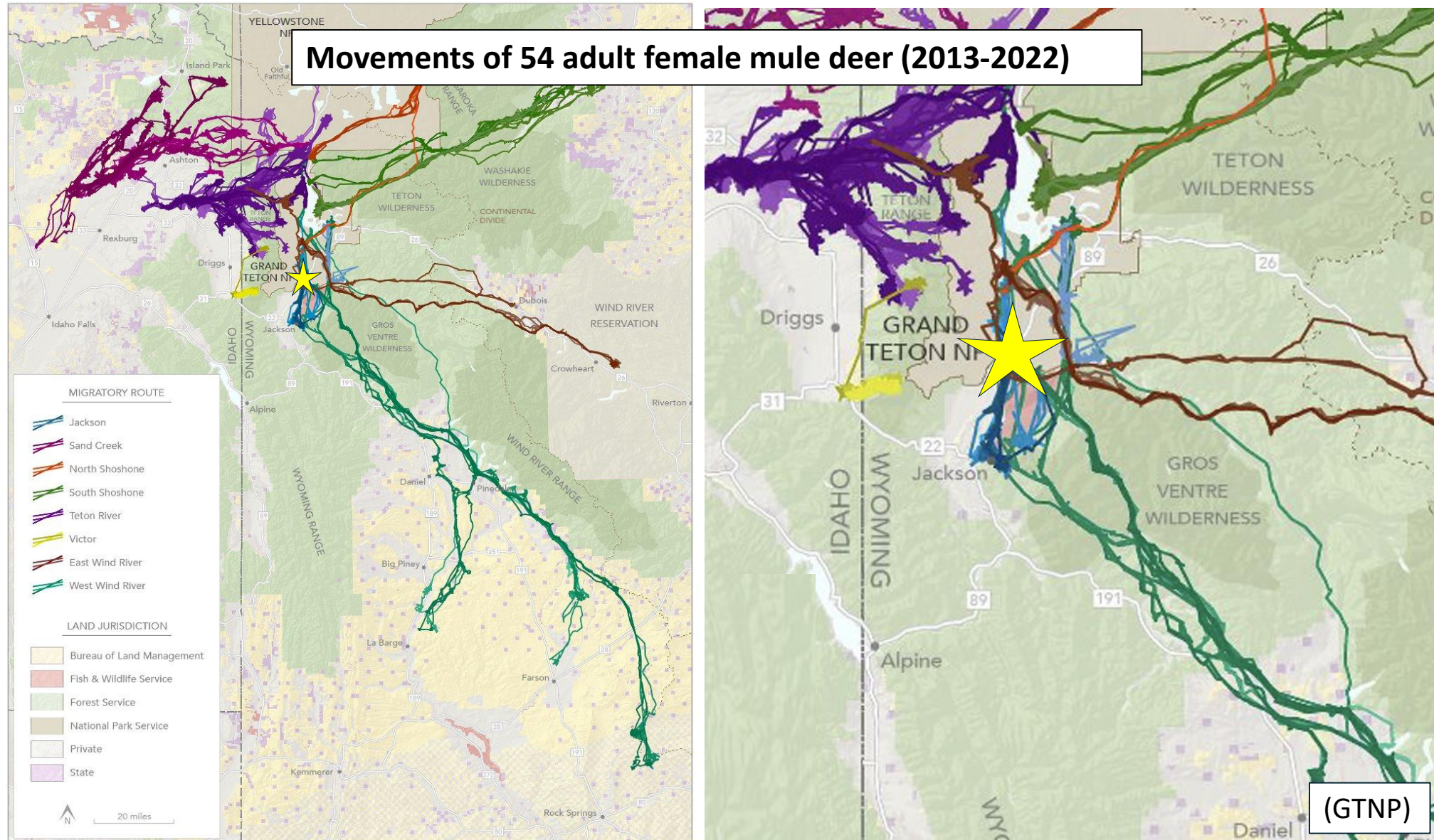




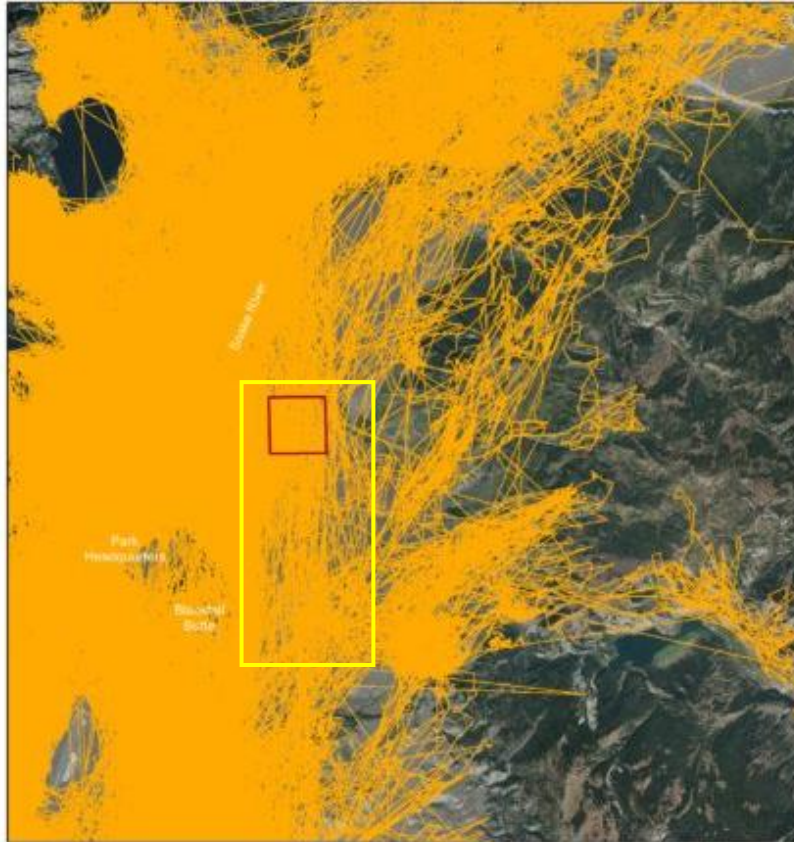
Sagebrush steppe ecosystem services: fauna



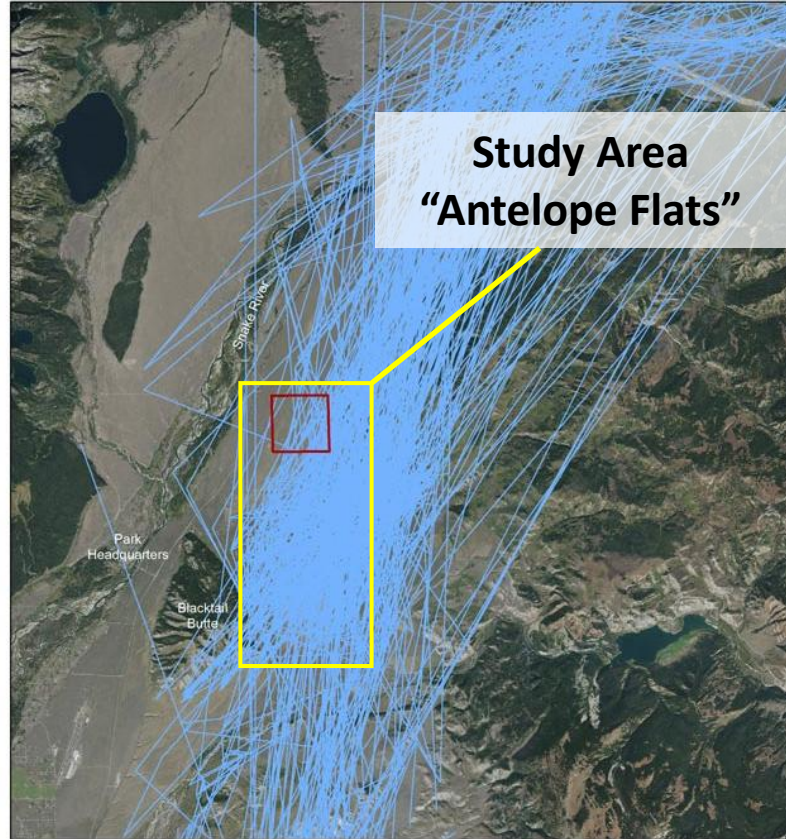
GTNP → critical bottleneck for ungulate migration



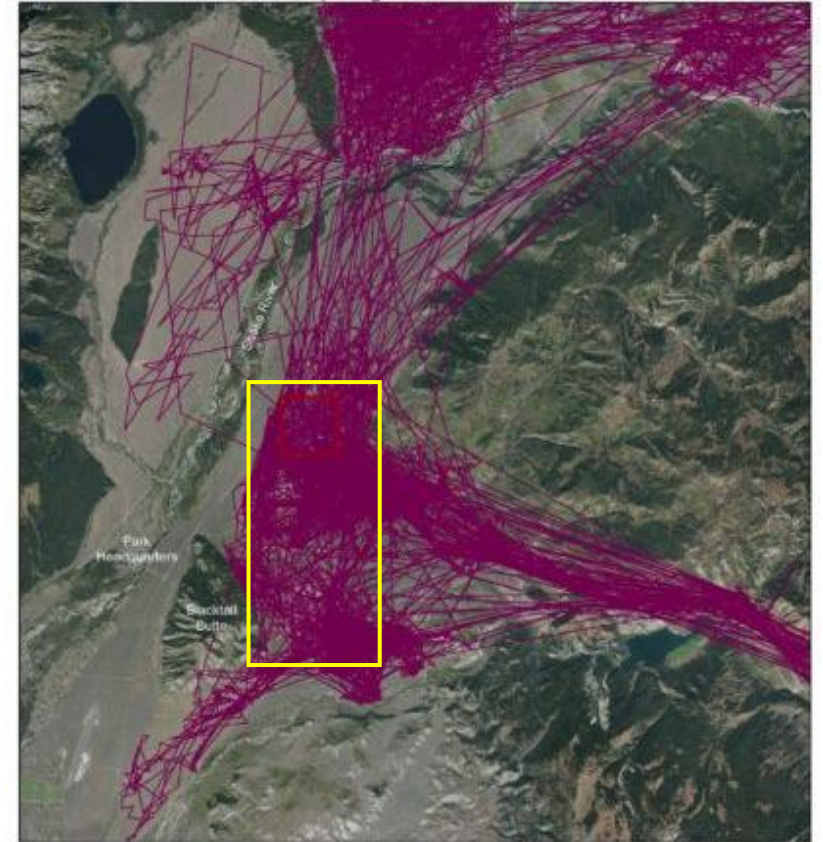
GTNP → critical bottleneck for ungulate migration



Movements of 111 individual elk (2006-2015)



Movements of 35 individual bison (1997-2007)



Movements of 21 individual pronghorn (1997-2007)

Haying in Antelope Flats

Settler activities

- Clear cut ~4,500ac native shrubland
- Plant *Bromus inermis* as forage for cattle
- Dig extensive ditch systems

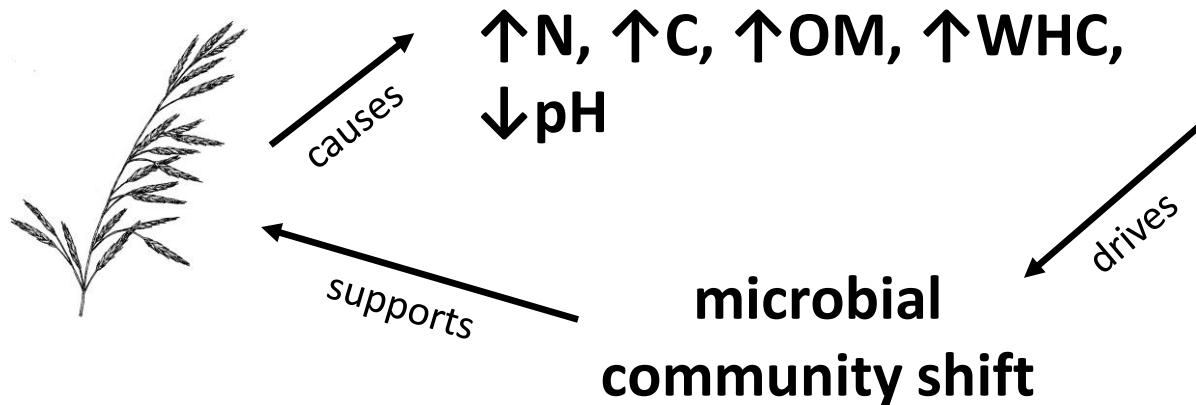


B. inermis and *thlaspi arvensis*

(NPS)

Smooth brome invasiveness

- Highly competitive → alters soil chemistry¹
- Changes in soil properties select for **taxonomically and functionally distinct microbial community**²
- Smooth brome is rarely studied in shrublands



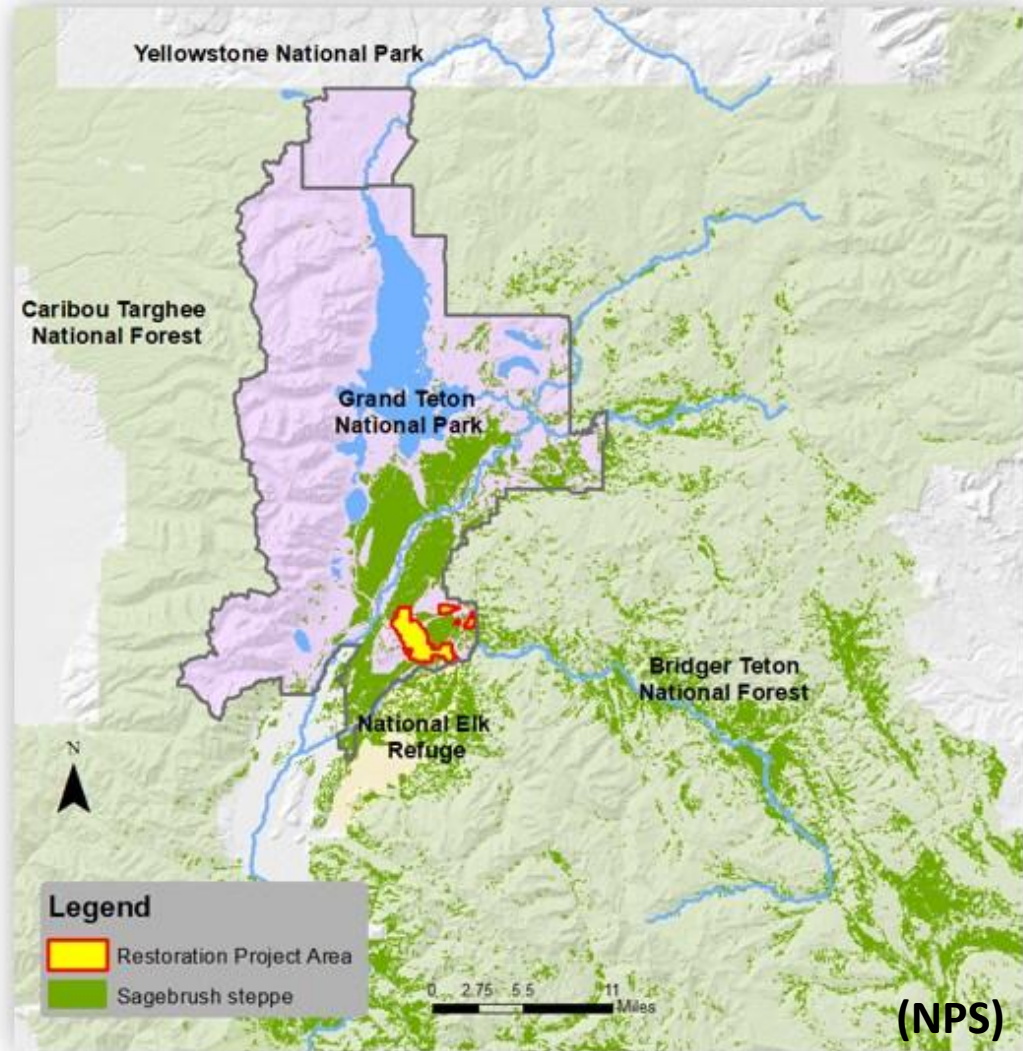
Reference – *A. tridentata* dominated shrubland



Non-native – *B. inermis* monoculture



GTNP restoration initiative



- Began in 2009
- Smooth brome removal – fire + herbicide
- Reseed with collected park seed

GTNP restoration protocol*

Year 1:

Early spring: **controlled burn** of smooth brome hayfield

Mid-late summer: **glyphosate** application

Year 2:

Mid-late summer: **glyphosate** application

Fall: **seed** with native seed mix

**Combination treatment most effective for smooth brome removal³ (Moeney, 2008).*





How are soils impacted by these practices?

Fire effects on soil

SOM

Shallow surface losses⁴:

- SOM combustion
- C/N/P/S volatilization⁵

pH

Ash deposition:

- Mg^{2+} , CaCO_3 rich ash
- Base cations buffer soil acidity⁶

Microbial Activity

Nutrient flush:

- Deposition of highly decomposable nutrients
- Increased moisture availability (↑ infiltration)
- Pulse of microbial activity and decomposition of SOM⁵

Glyphosate and “fallow”



Glyphosate and “fallow”

SOM

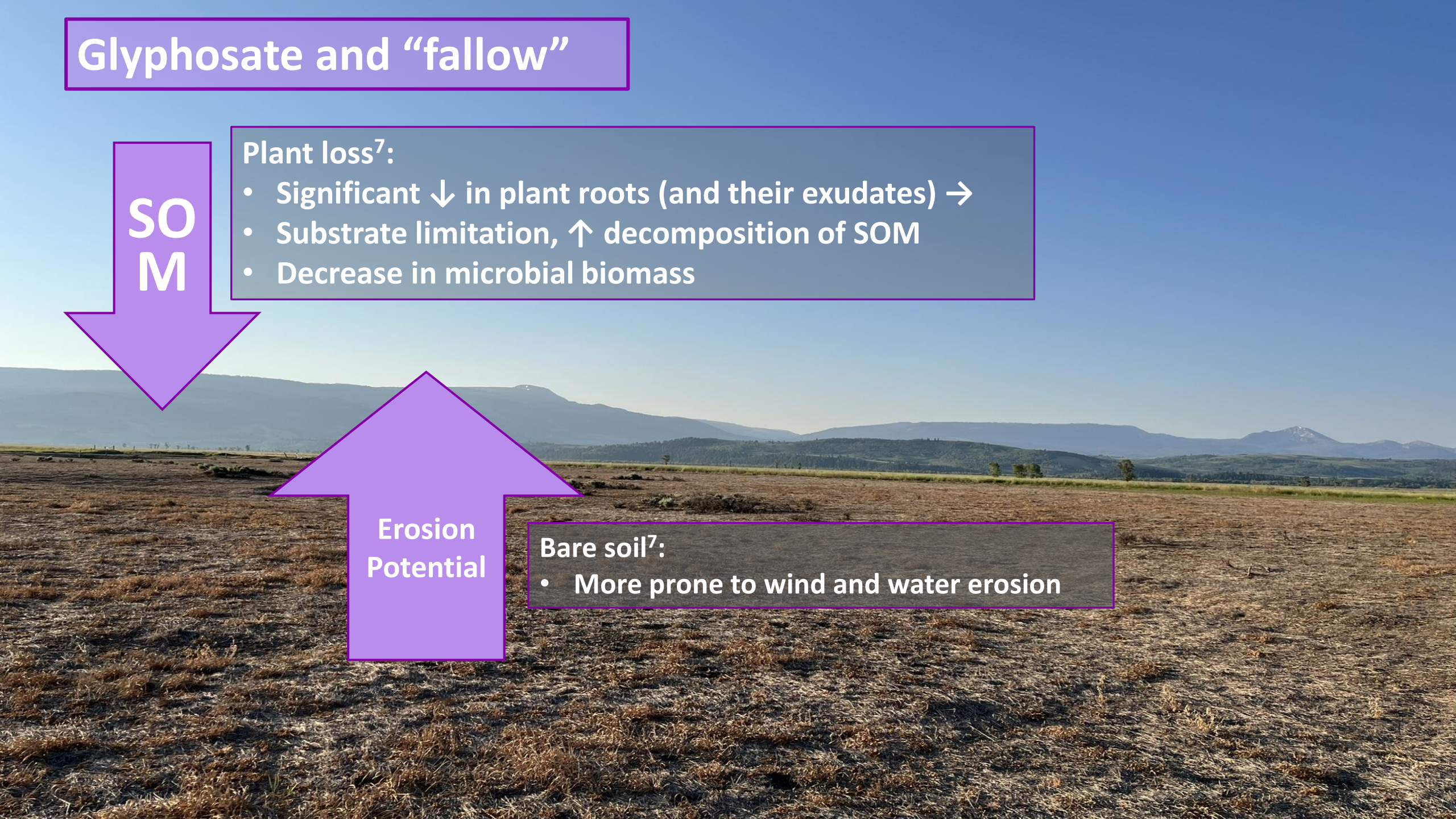
Plant loss⁷:

- Significant ↓ in plant roots (and their exudates) →
- Substrate limitation, ↑ decomposition of SOM
- Decrease in microbial biomass

Erosion
Potential

Bare soil⁷:

- More prone to wind and water erosion



Questions:

- 1) How does restoration impact soil chemical properties in Antelope Flats?
- 2) How are soil microbial communities responding to restoration in Antelope Flats?

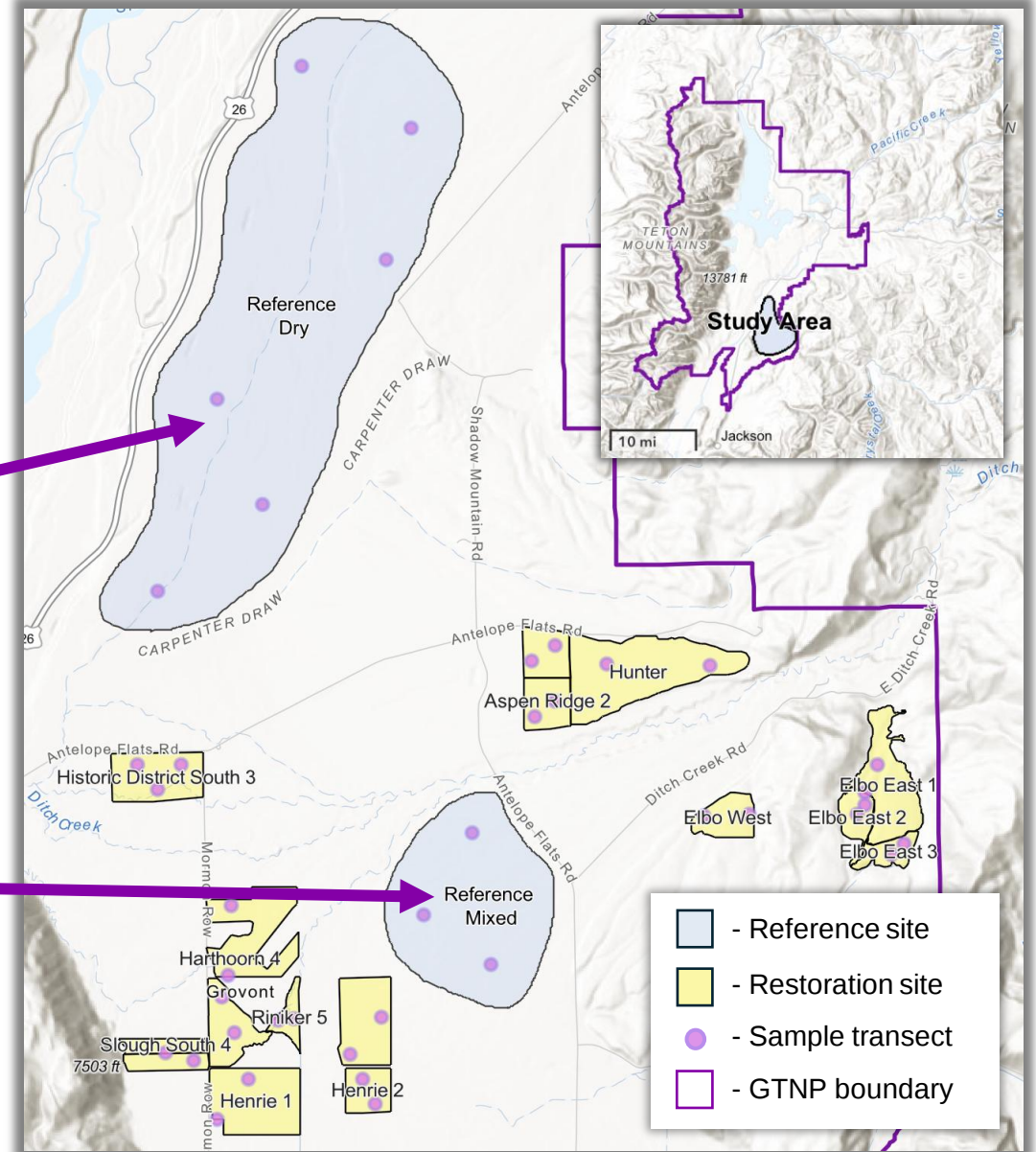
Study Area

- 2 reference sites
 - Dry – sagebrush dominated shrubland
 - Mixed – sagebrush/antelope bitterbrush

Reference Dry Shrubland

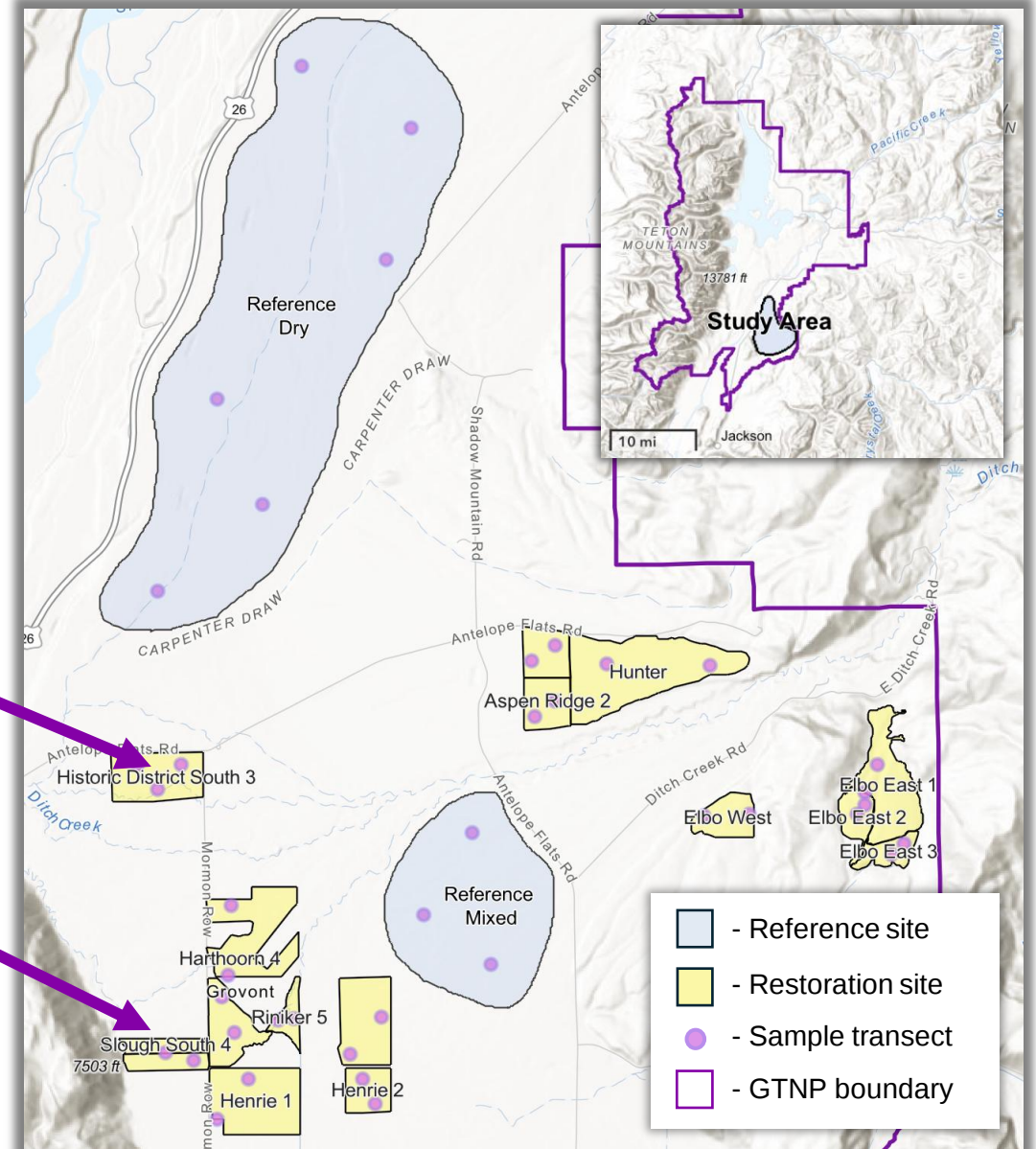


Reference Mixed Shrubland



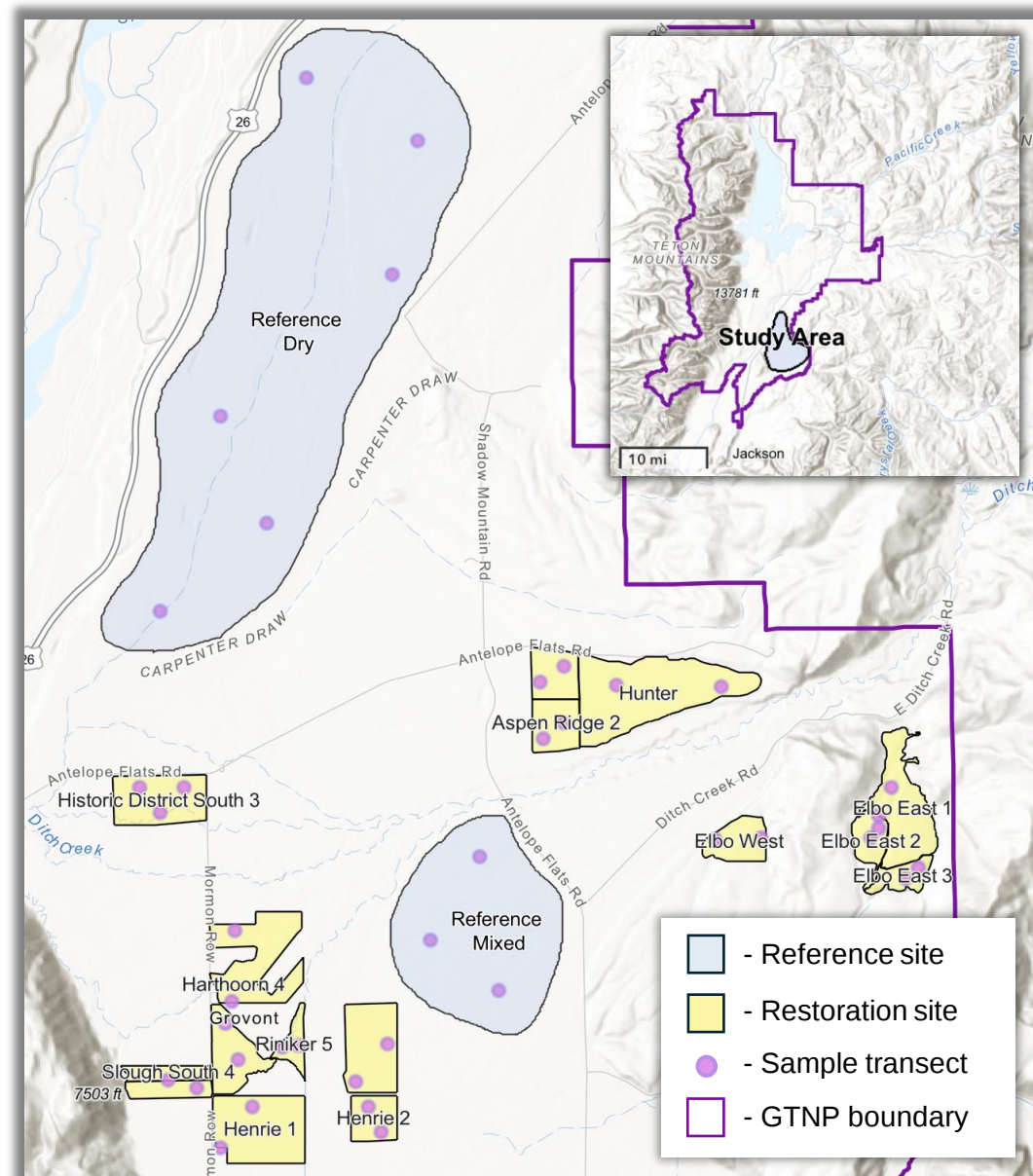
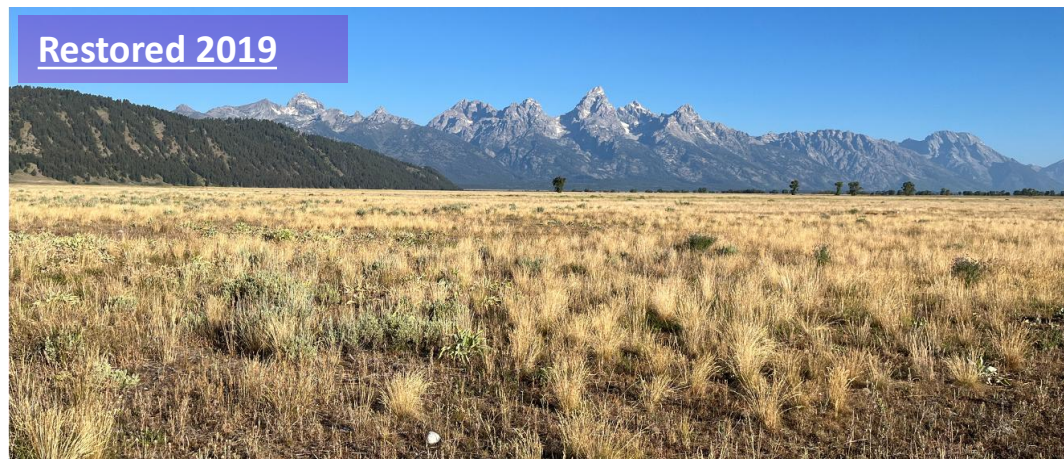
Study Area

- 1 unrestored site – smooth brome hayfield
- 1 prepped, unseeded site



Study Area

- 17 restoration sites (2009-2024)

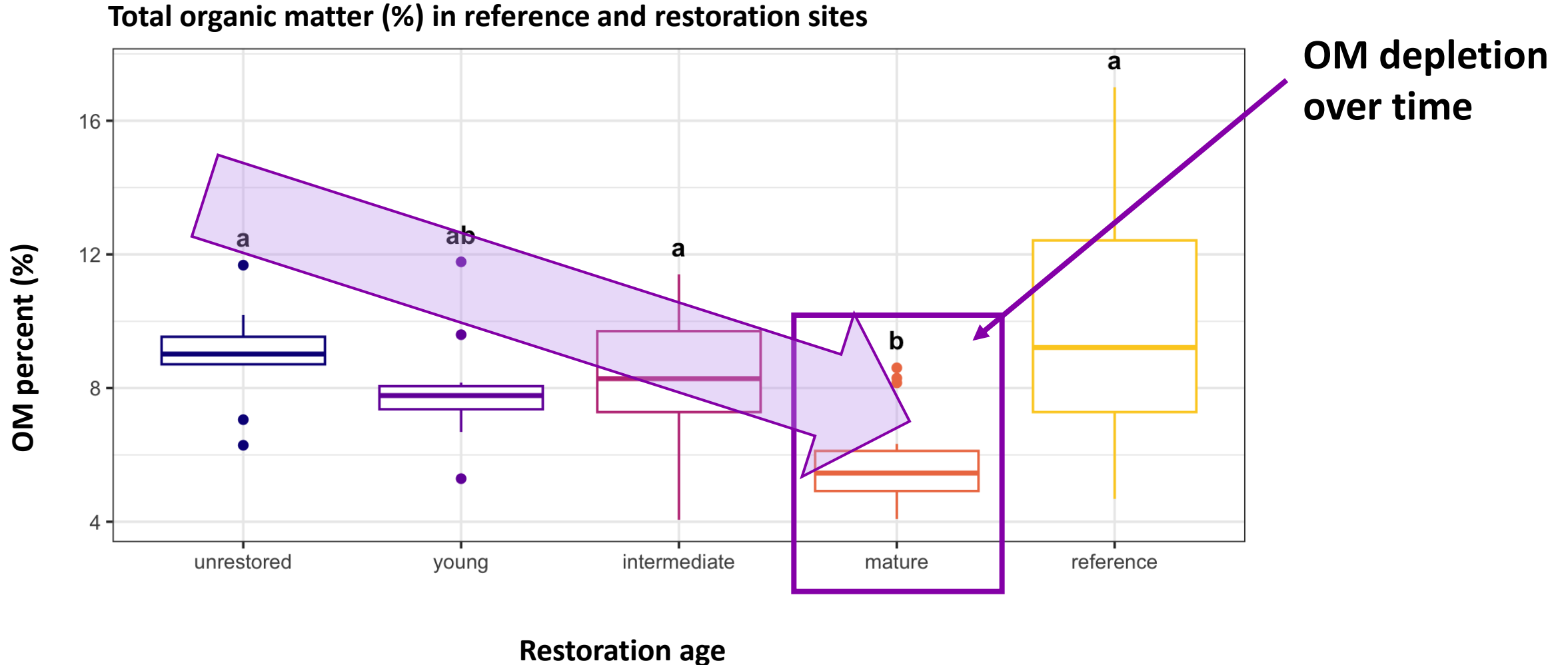


1) Assess changes in soil chemistry between sites

- C cycle –
 - Soil organic matter → “stable” C
 - Permanganate oxidizable carbon (POXC) → active C
- N cycle –
 - Acid citrate extractable (ACE) protein → organic, available N



1) Assess changes in soil chemistry between sites: **C-cycling**



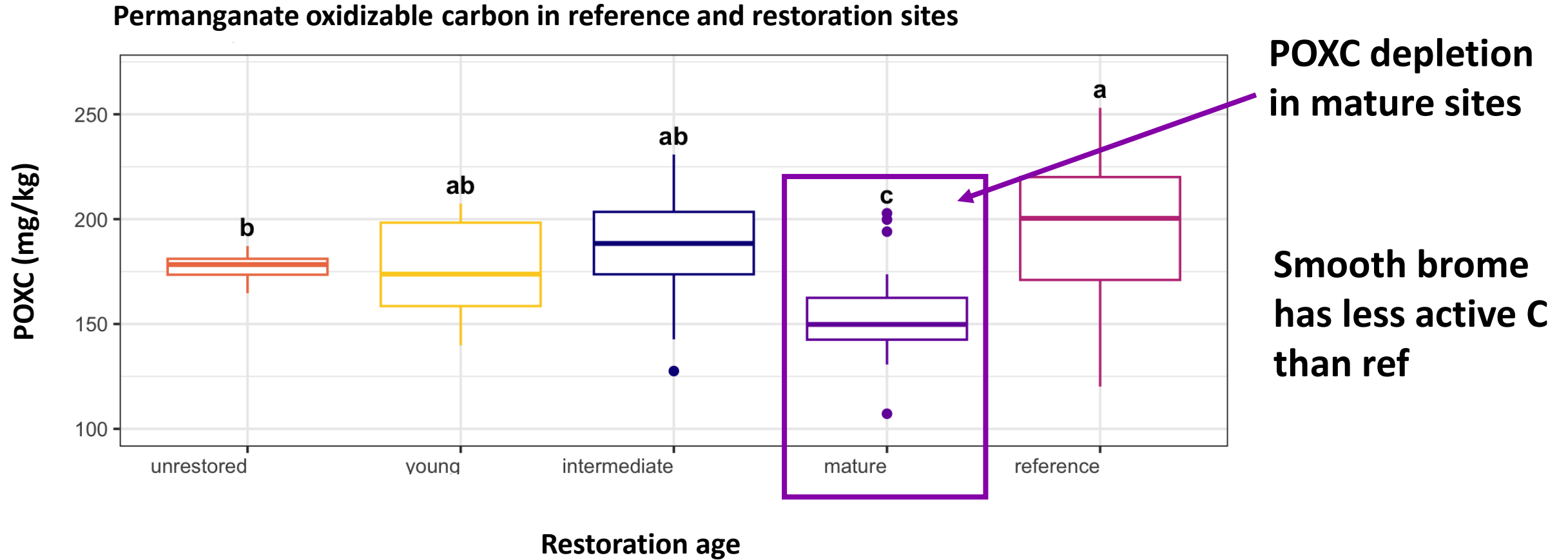
1) Assess changes in soil chemistry between sites: **C-cycling**



OM depletion over time

Smooth brome monoculture + shrub reference have ~ SOM

1) Assess changes in soil chemistry between sites: **C-cycling**



1) Assess changes in soil chemistry between sites: **N-cycling**



No observed effects

2) Assess microbial community response to restoration

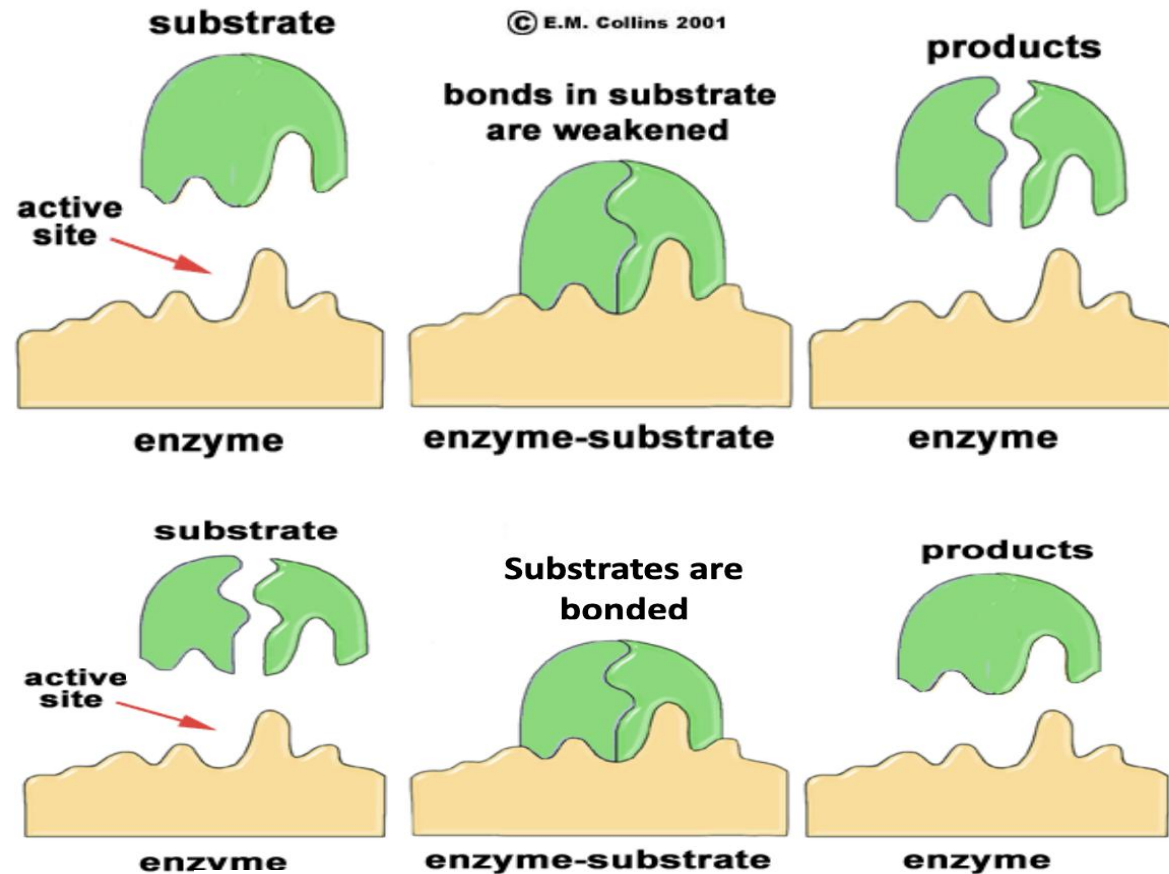
- Function – extracellular enzyme assay (EEA)
- Structure – DNA extraction

Structure → who's there?

Function → what are they doing?

2) Assess microbial community response to restoration

- Function – extracellular enzyme assay (EEA)
- Structure – DNA extraction



F _ : "Lock and Key" Enzyme Mechanisms
(Image from E.M. Collins 2001)

- Specific enzymes serve **specific functions**
- Critical for **organic matter degradation/nutrient cycling**
- Transformation of N/P/S to plant available forms
- Soil enzyme activities help us understand how microbes...
 - **function** in the soil
 - **respond** to environmental change

2) Assess microbial community response to restoration

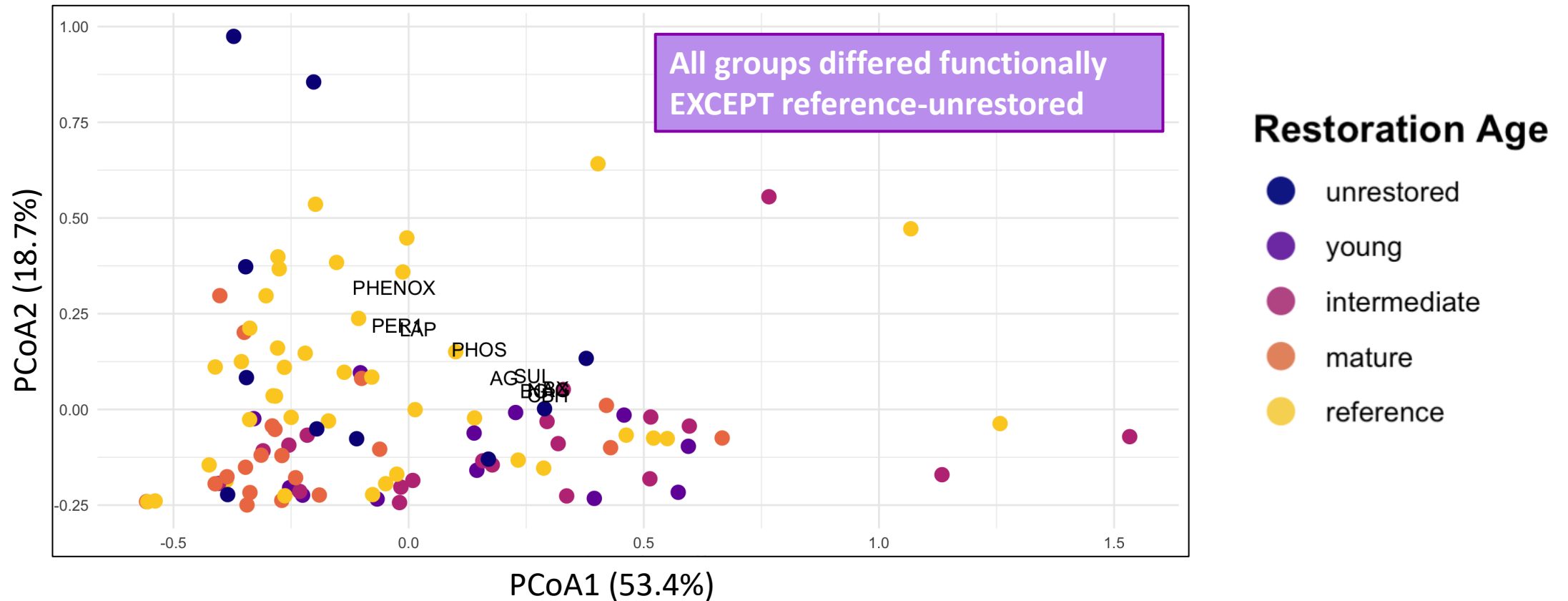
- Function – extracellular enzyme assay (EEA)
- Structure – DNA extraction



Enzyme	Nutrient cycle	Role
β -glucosidase (BG)	Carbon	Cellulose (simple)
Cellobiohydrolase (CBH)	Carbon	Cellulose (complex)
α -glucosidase (AG)	Carbon	Starch
N-acetyl- β -glucosaminidase (NAG)	Carbon	Chitin (insect/fungal cell walls)
β -xylosidase (BX)	Carbon	Hemicellulose
Leucine aminopeptidase (LAP)	Nitrogen	Polypeptides
Acid phosphatase (PHOS)	Phosphorus	Phosphate esters
Arylsulfatase (SUL)	Sulfur	Ester sulfates
Phenol oxidase (PHENOX)	Carbon	Organic matter
Peroxidase (PEROX)	Carbon	OM/lignin/humic substances

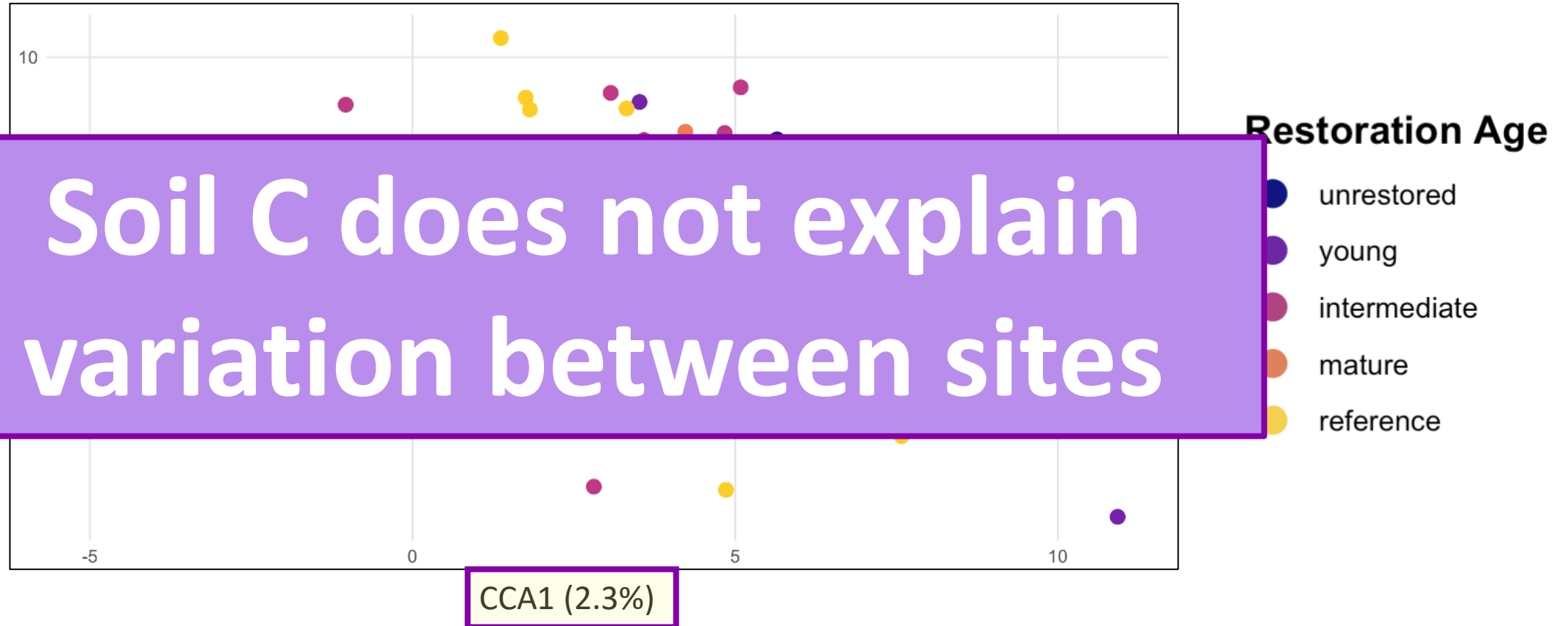
2) Assess microbial community response: function → Enzyme assay

Principle coordinates analysis of enzyme activities in reference and restoration sites (Euclidian distance)



2) Assess microbial community response: **function** → **Enzyme assay**

Canonical correlation analysis to determine effects of OM and POXC
on enzyme activities (Euclidian distance)



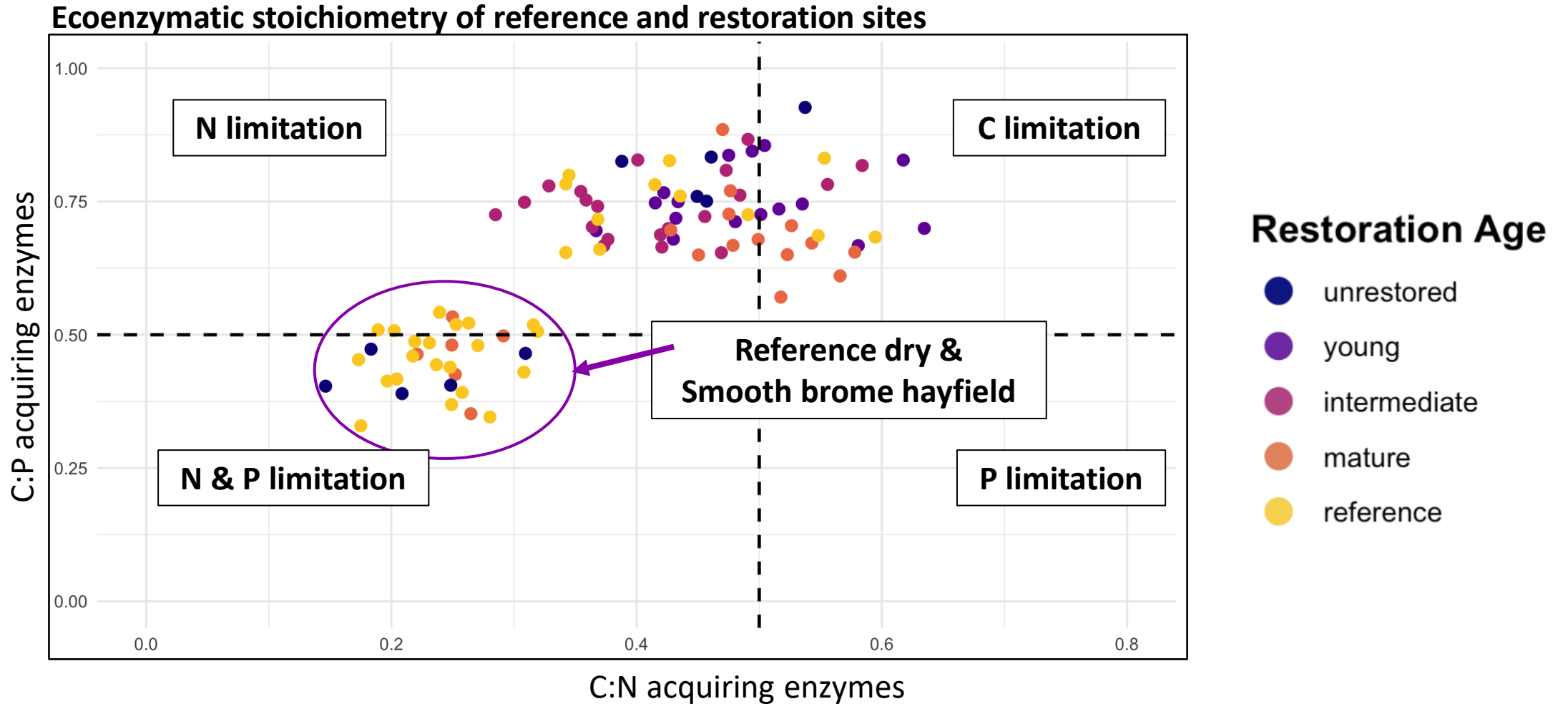
2) Assess microbial community response: **function → Enzyme assay**

Ecoenzymatic stoichiometry of reference and restoration sites

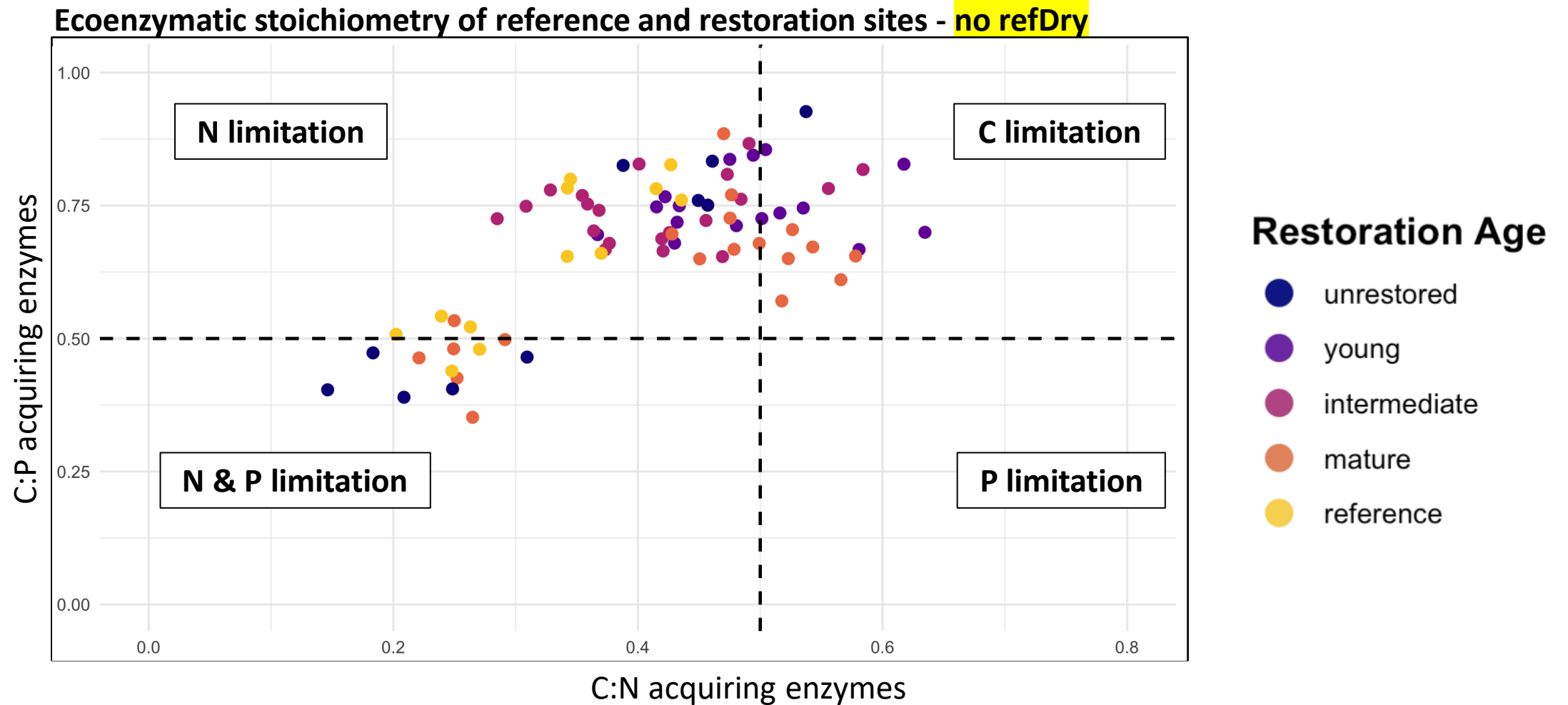
- Comparing the *relative* activities of C related enzymes to N and P related enzymes can help us understand **which nutrients might be limiting microbial metabolism** in the soil;
- Higher activity → greater metabolic requirement (i.e., **limitation**)
- Lower activity → lower metabolic requirement (i.e., **adequate supply**)

↑ C:N/P ~ ↑ C limitation

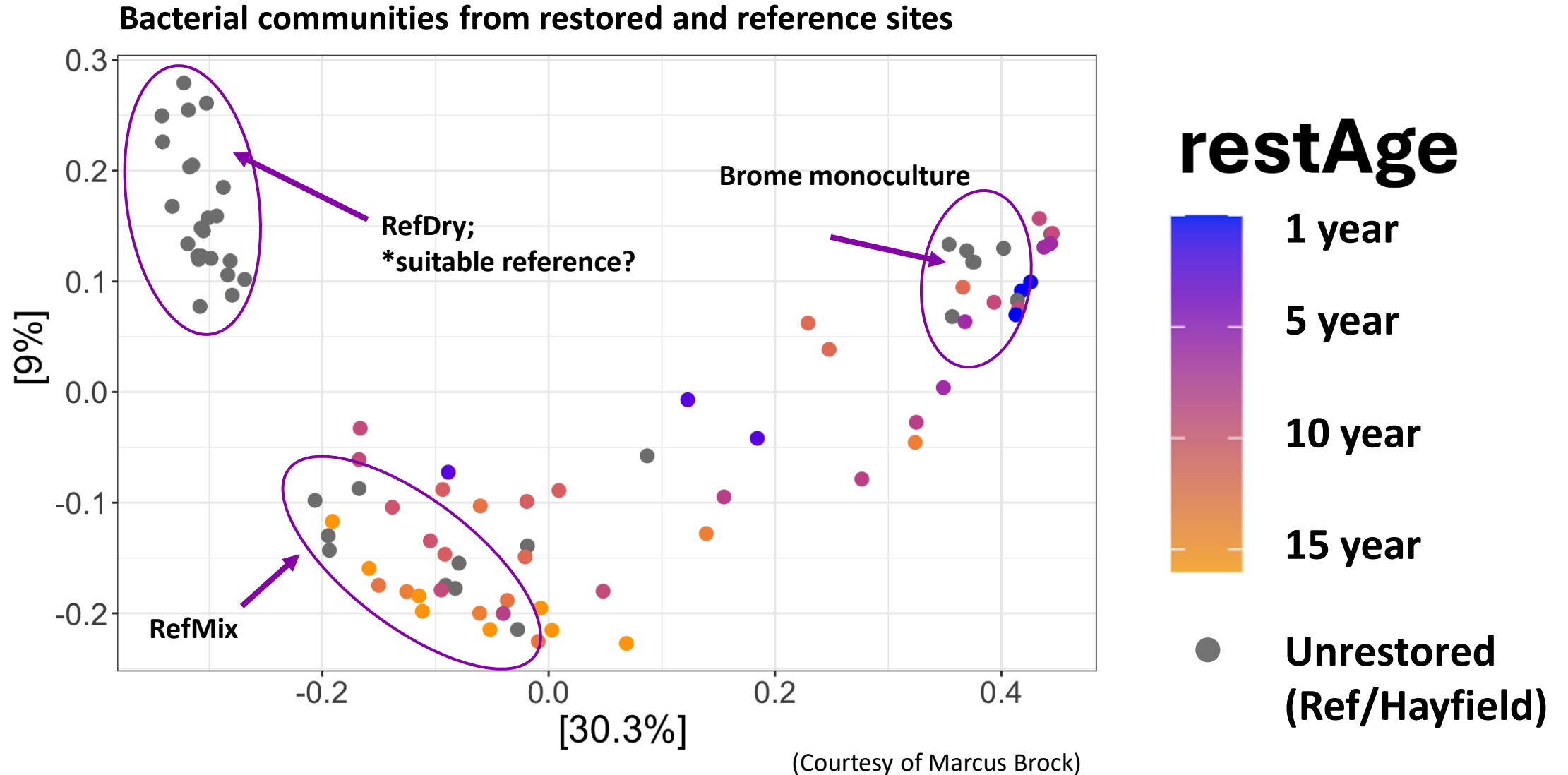
2) Assess microbial community response: **function** → **Enzyme assay**



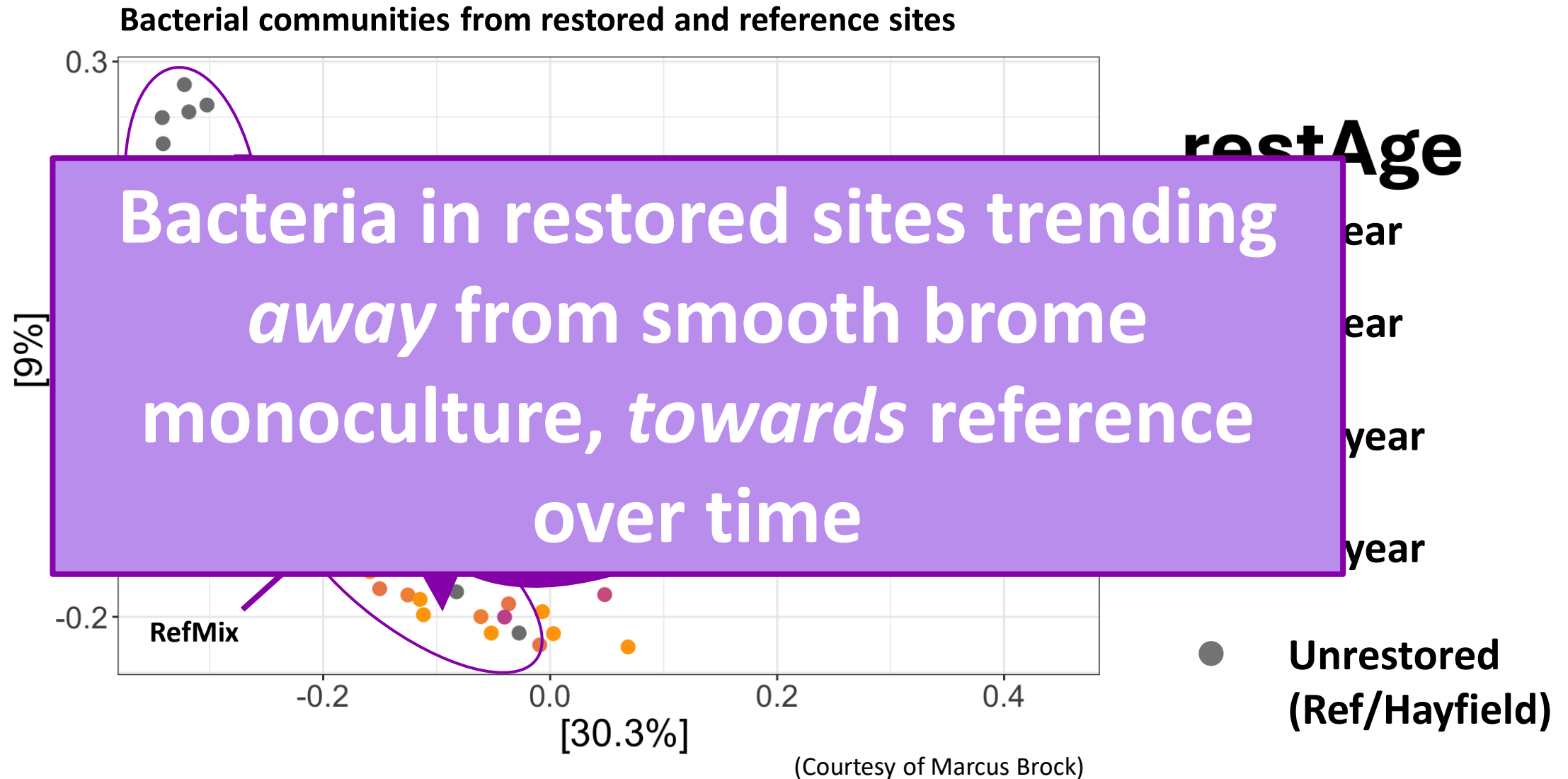
2) Assess microbial community response: function → Enzyme assay



2) Assess microbial community response: structure → DNA extraction



2) Assess microbial community response: structure → DNA extraction



Conclusions

1) Differences in plant community don't seem to be driving differences in soil chemistry

- Reference and smooth brome hayfields were equivalent in all soil chemistry metrics
- Soil C gradually declined over 15 years
- Likely due to **smooth brome removal protocol** and initial **productivity lag** when site is in active restoration

Conclusions

2) Form does not follow function

- The oldest restored sites have similar bacterial communities to the reference, but are still functionally distinct → function will likely recover as soil chemistry (especially C) recovers
- Smooth brome hayfields have a drastically different bacterial community, but equivalent function to reference sites

Conclusions

3) Soil recovery takes time

- Soil C is lagging behind plant community recovery → **long lived species and dry conditions** hinder biomass production and **slow OM accumulation**
- Decline in productivity and effects on soil are **unavoidable consequences** of effective smooth brome removal in this system

Conclusions

4) Soil microbiota *may* provide a valid means of assessing the validity of a reference site

- Dry reference site is clearly distinct in ecoenzymatic stoichiometry and bacterial community composition relative to reference, restored, and unrestored sites
- Indicative of **unrealistic restoration target?**

Next steps

- Differentiate into organic matter fractions
 - Particulate organic matter (POM)
 - Mineral associated organic matter (MAOM)
- Soil fungal communities
- ★ Continued collaboration with GTNP



Acknowledgements

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and Management



In loving memory of Dr. Linda van Diepen



References

- ¹Palit, R., & DeKeyser, E. S. (2022). Impacts and drivers of smooth brome (*Bromus inermis* Leyss.) invasion in native ecosystems. *Plants*, 11(10), 1340.
- ²Piper, C. L., Siciliano, S. D., Winsley, T., & Lamb, E. G. (2015). Smooth brome invasion increases rare soil bacterial species prevalence, bacterial species richness and evenness. *Journal of Ecology*, 103(2), 386-396
- ³Moeney, J. C. (2008). Control of smooth brome and restoration of native plant communities in the Kelly Hayfields of Grand Teton National Park (Doctoral dissertation, New Mexico State University).
- ⁴Binkley, D., Richter, D., David, M.B. and Caldwell, B. (1992), Soil Chemistry in a Loblolly/Longleaf Pine Forest with Interval Burning. *Ecological Applications*, 2: 157-164.
- ⁵DiCosty, R. J., & Callaham, M. A.. 2008. Fire effects on soils – overview. Encyclopedia collection — forest encyclopedia network.
- ⁶Woodmansee, R. G., & Wallach, L. S. (1981). Effects of fire regimes on biogeochemical cycles. *Ecological Bulletins*, 649-669.
- ⁷Nielsen, D. C., & Calderón, F. J. (2011). Fallow effects on soil. *Soil management: Building a stable base for agriculture*, 287-300.



Questions?



Supplemental

Reference Conundrum

Reference – coarse, < OM, shallow mineral lens



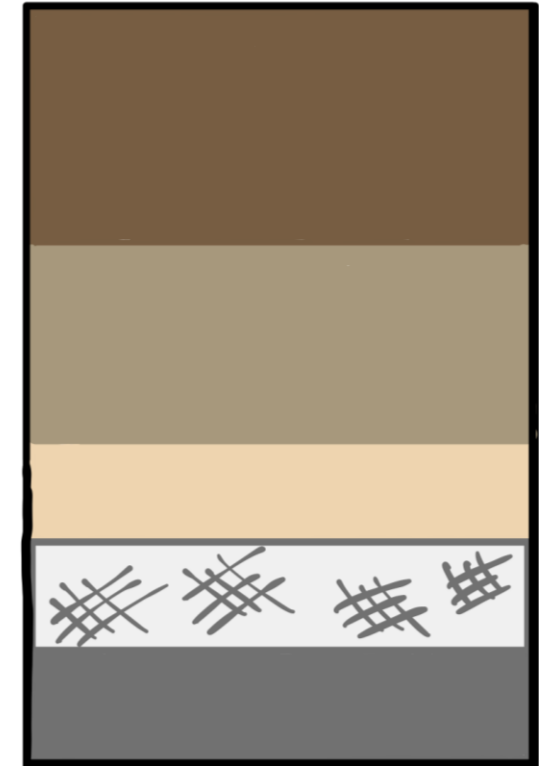
Non-native – intermediate texture, > OM, no mineral lens



refDry



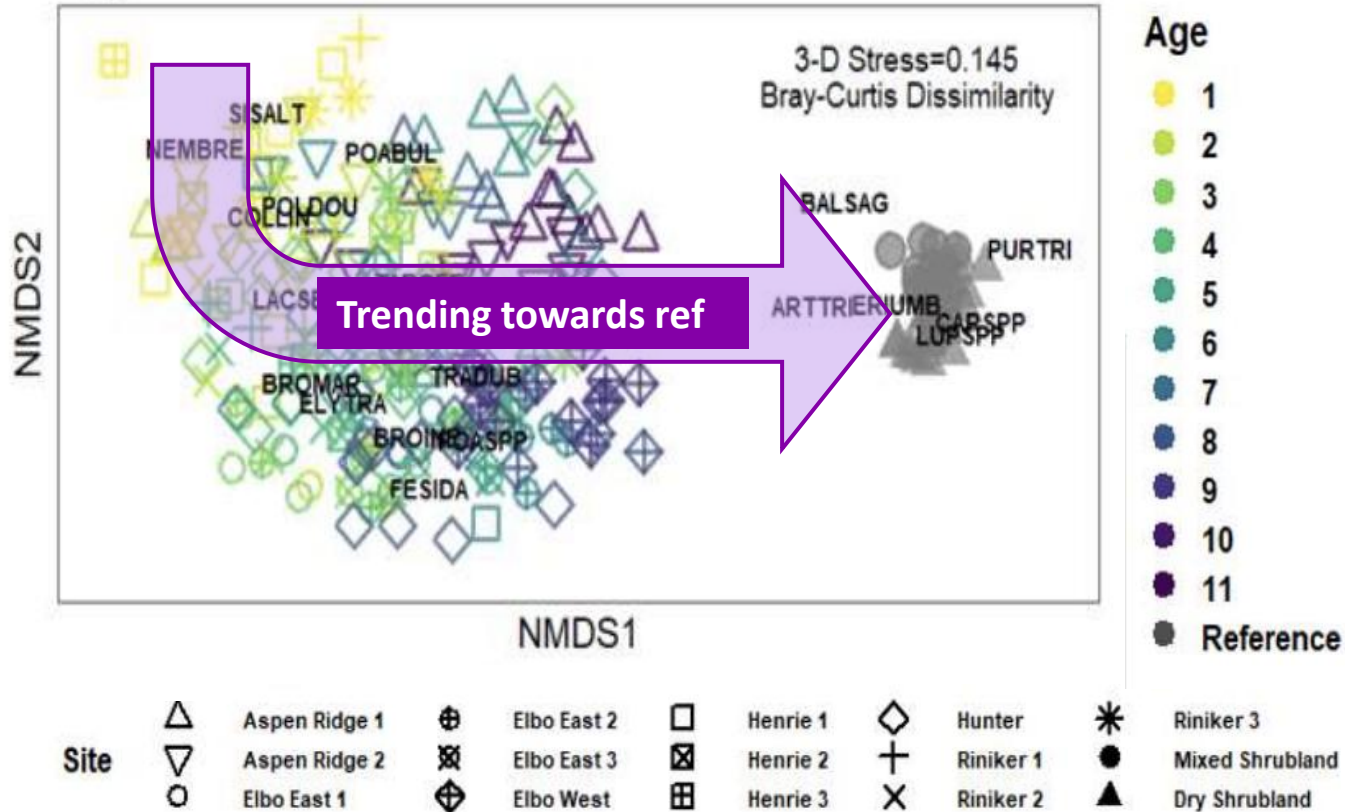
Non-native/restored/
refMix



Hypothesized refDry and NN/restored/refMix soil profiles based on sampling observations.

Mixed restoration outcomes in GTNP

Plant community composition from 11yrs monitoring



(Wessel, 2022)

Aspen Ridge (15 years rest.) – good shrub establishment

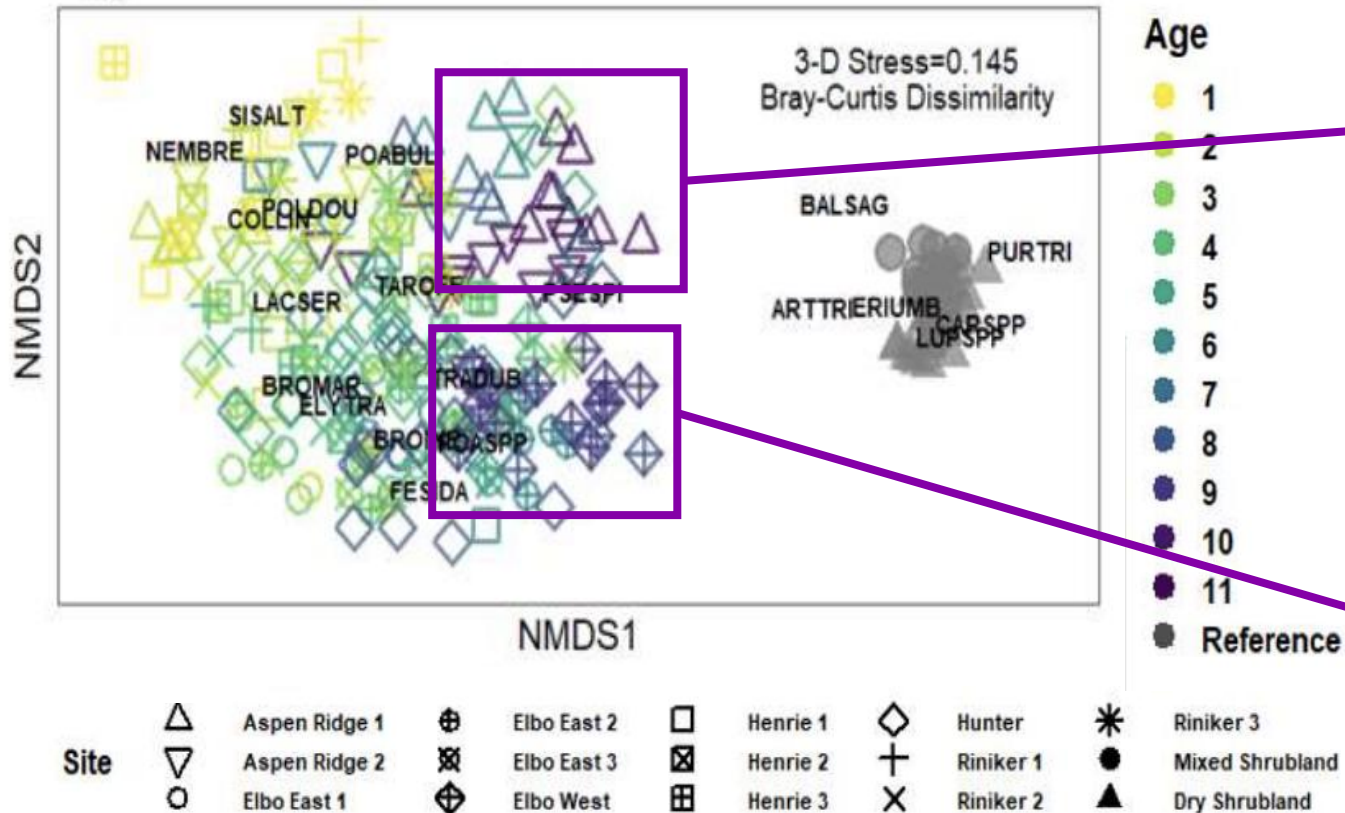


Elbo West (13 years rest.) – Poor shrub establishment, grass dominant



Mixed restoration outcomes in GTNP

Plant community composition from 11yrs monitoring



Aspen Ridge (15 years rest.) – good shrub establishment



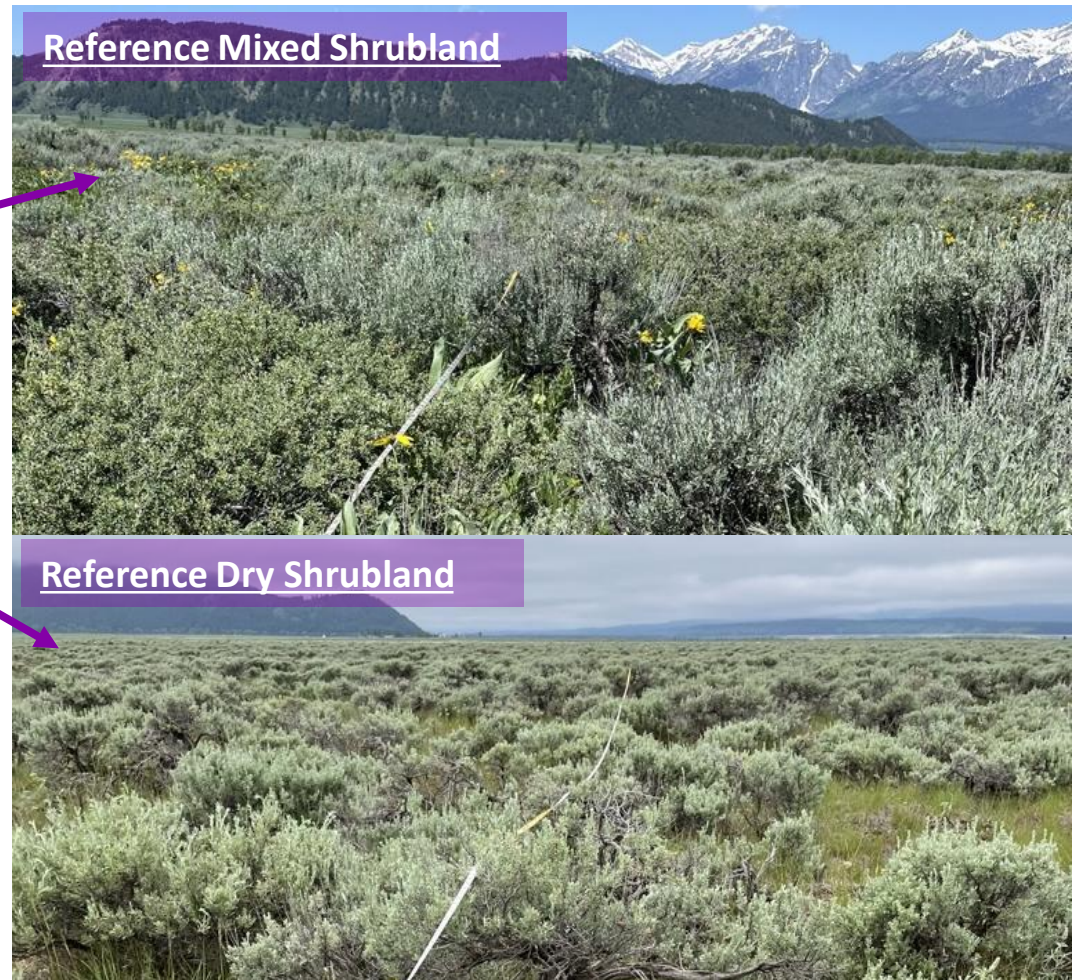
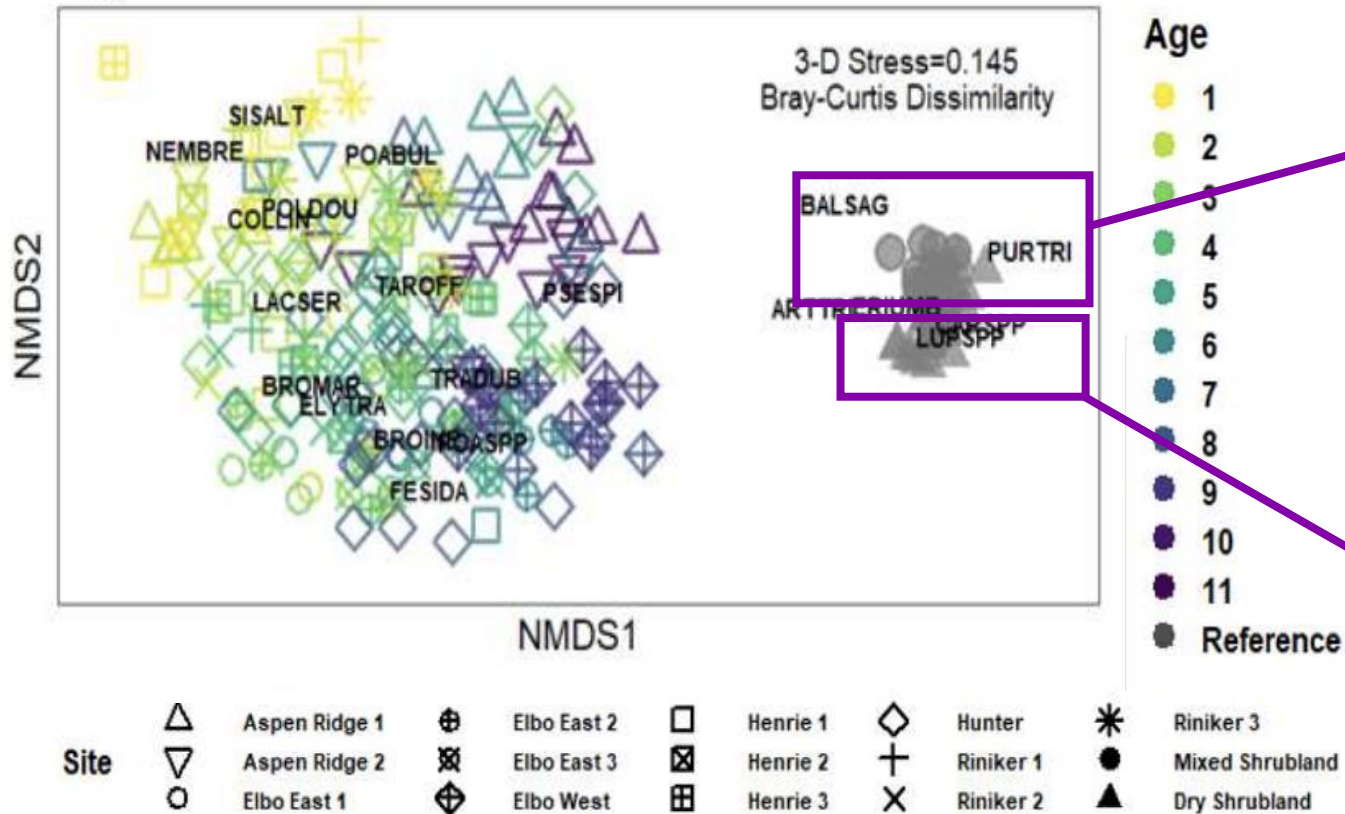
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