UNIVERSITY OF CALIFORNIA, MERCED

Eco-evolutionary Edaphic Effects on Meadowfoam, a Vernal Pool Plant, and eDNA Biomonitoring of Endemic Vernal Pool Plant Communities

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy in Environmental Systems

By

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2024

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2024

Dedication page

This dissertation is dedicated to the extraordinary individuals who have been my foundation, my inspiration, and my unwavering support through this incredible journey. I give special thanks to my advisor Jason and to my late co-advisor Marilyn. You opened a door and invited me into a world beyond anything I had ever imagined. Your positivity, wisdom and patience were a constant light and guiding force, not only helping me navigate the science and the inevitable uncertainties of the process, but the challenges of life along the way. I owe so much to your belief in me and your commitment to my success.

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Acknowledgements

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To Chris, Mo and Joy whose interest and tireless work ensured I had the opportunity to study the landscapes I love. To my committee, Rebecca, Nancy and Carolin - thank you for guidance and support when I needed it most. Your support made this journey possible.

To the army of undergraduates and volunteers who worked alongside me, shared in the challenges, and brought joy and energy to the research. I am especially grateful to Michael, Fidel, Andres, Kim, Yasmin, Amy, and so so many more -you inspired me daily with your curiosity and enthusiasm, and your contributions were invaluable to both my work and my growth as a scientist.

To my friends and colleagues—Monti, Lillie, Jackie, Brandon, Iolanda, Bailey, Joe, Lorenzo, and Dannise—thank you for your support, thoughtful feedback, and camaraderie. You made the challenges of this journey bearable and more meaningful. Your friendship created a sense of community and belonging that I will forever cherish and has helped me become a better person and scientist.

To my family, my mentors, my colleagues, friends and all those who have been part of this journey—thank you.

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EDUCATION

2015 - present: Ph.D., Environmental Systems, University of California, Merced, CA
The Evolution, Ecology, and Conservation Biology of Vernal Pool Plant Communities.

2015: B.S., Earth Systems Science, University of California, Merced, CA

PEER REVIEWED PUBLICATIONS AND MANUSCRIPTS IN PREPARATION

Ruiz-Ramos, D.V., Meyer, R., **Toews, D.J.**, Stephens, M., Kolster, M., and J.P. Sexton. 2023. Environmental DNA (eDNA) detects temporal and habitat effects on community composition and endangered species in ephemeral ecosystems: A case study in vernal pools. *Environmental DNA*, 00, 1– 17. https://doi.org/10.1002/edn3.360

Shay, J.E., Pennington, L.K., Montiel-Molina, J.A.M., **Toews, D.J.**, Hendrickson, B., and Sexton, J. 2021. Rules of plant species ranges: applications for conservation strategies. *Frontiers in Ecology and Evolution*, 664. https://doi.org/10.3389/fevo.2021.700962

Shay, J., Pennington, L., **Toews, D.J.,** Green, E., and J.P. Sexton. The leading edge matters too: fitness and the expression of adaptive climate variation are greatest at the high elevation, leading-edge of a species range. *Submitted.* Manuscript to *Ecology Letters.*

Toews, D.J., Pennington, L.K., Stephens, M., Ruiz-Ramos, D., Escalona, M., Toffelmeier, E., Shaffer, B.H., Meyer, R., J.P. Sexton. Reference genome of Greene's tuctoria, *Tuctoria greenei*, an endangered California vernal pool grass. *In prep.* Manuscript to *Journal of Heredity.*

Toews, D.J. & Pennington Reference genome of San Joaquin Orcutt grass, *Orcuttia inaequalis*, an endangered California vernal pool grass. *In prep.* Manuscript to *Journal of Heredity.*

Pennington, L.K., **Toews, D.J**., Stephens, M., Ruiz-Ramos, D., Escalona, M., Toffelmeier, E., Shaffer, B.H., Meyer, R., J.P. Sexton. Reference genome of Colusa grass, *Neostapfia colusana*, a threatened and endangered California vernal pool grass. *In prep.* Manuscript to *Journal of Heredity.*

TECHNICAL REPORTS

Swarth, C.W., Cronin, J., Araiza, D.N., **Toews, D.J.,** Nakamoto, B.J., Vega, M.C., Cook, K., Williams, E., and M. L. Fogel. Residual Dry Matter (RDM) Monitoring and Cattle Grazing in the Merced Vernal Pools and Grassland Reserve, University of California Natural Reserve System. University of California, Merced, California. January 2017.

TECHNICAL REPORTS *(cont'd)*

Toews, D.J., Swarth, C.W. Camera Survey to Detect the Endangered San Joaquin Kit Fox (*Vulpes macrotis mutica*) at the Merced Vernal Pools and Grassland Reserve, Merced, California. UC Merced Natural Reserve System. University of California, Merced. June 2015.

POSTERS AND TALKS FOR SYMPOSIA AND PROFESSIONAL MEETINGS

2024, talk	MVPGR Science Symposium and 10th Anniversary (Merced, CA). "*Varied fitness responses to edaphic gradients in Meadowfoam*," Principal investigators: **Daniel J. Toews**
2024, poster	Northern California Botany Symposium (Chico, CA). "*Reference Genomes for Three Vernal Pool Grass Species of the Orcutteae Tribe*," Principal investigators: **Daniel J. Toews,** Dr. Merly Escalona co-PI's: Dr. Lillie Pennington, Dr. Jason Sexton, Dr. Rachel Meyer. (2nd place award)
2023, talk	Freshwater Sciences 2023 (Brisbane, Australia). *"eDNA or bust: Comparing eDNA metabarcoding strategies and traditional plant survey methods in an endangered California ephemeral wetland ecosystem."* Principal investigator: **Daniel J. Toews**
2022, poster	California Native Plant Society Conference: Conservation Conference 2022 (San Jose, CA). "*Can environmental DNA detect rare plants? eDNA metabarcoding California vernal pool plant communities: A new tool for biodiversity assessments."* Principal investigator: **Daniel J. Toews.** Co-PI's: J. P. Sexton, D. Ruiz-Ramos, M. Stephens, R. Meyer
2019, talk	American Geophysical Union (San Francisco, CA). "*Differentiation of vernal pool communities assessed by eDNA: a community science experience*." Principal investigators: M. Dawson, and J. P. Sexton. Co-PI's: D. Ruiz-Ramos, **Daniel Toews**, M. Stephens, R. Meyer, E. Curd
2019, talk	California Native Plant Society: Conservation Symposium (Santa Cruz, CA). "*Insights from a CALeDNA case study: challenges and opportunities using eDNA to characterize vernal pool plant communities."* Principal investigators: **Daniel J. Toews**, and J. P. Sexton. Co-PI's: D. Ruiz-Ramos, D. Toews, M. Stephens
2019, poster	Northern California Botany Symposium (Chico, CA). "*Using an eDNA approach to quantify biodiversity in California vernal pool plant communities*," Principal investigators: **Daniel J. Toews,** co-PI's: Dr. Jason Sexton, Dr. Molly Stephens and Dr. Dannise Ruiz
2018, talk	UC Genomics Conservation Consortium Workshop: Modernizing ecosystem management in California (Monterey, CA). "*Detecting annual plant diversity in vernal pool soils with environmental DNA: challenges and opportunities*," Principal investigators: **Daniel J. Toews**, co-PI: Dr. Jason Sexton, Dr. Molly Stephens and Dr. Dannise Ruiz

POSTERS AND TALKS FOR SYMPOSIA AND PROFESSIONAL MEETINGS *(cont'd)*

2018, talk — 2018 Annual Meeting of the Ecological Society of America (New Orleans, LA). "*Adaptation and fitness variation is highest near the coldclimate limit of a species range*," Principal investigators: Jackie Shay and Dr. Jason Sexton, co-PI's: Lillie Pennington and Daniel J. Toews

2018, talk — AquAlliance Annual Conference: Vernal Pool Landscapes: Past, Present, and Future (Chico, CA). "*Soil effects on an endemic vernal pool annual plant, Limnanthes douglasii spp. rosea (Meadowfoam)*," Principal investigators: **Daniel J. Toews**

2018, talk — AquAlliance Annual Conference: Vernal Pool Landscapes: Past, Present, and Future (Chico, CA). "*Diversity above and below ground: the genetics of vernal pool plants and seed banks*." Principal investigators: M. Stephens and J.P. Sexton. Co-PI's: D. Ruiz Ramos, Daniel J. Toews

2017, poster — UC Merced Undergraduate Research Symposia *"Vernal Pool Plant Biodiversity among Merced Vernal Pools and Grassland Reserve,"* Principal investigators: Michael Spaeth, Molly Stephens, Jason Sexton, **Daniel J. Toews**

2016, poster — 42nd Southern California Botany Symposium (Pomona, CA). "*The role of soils in local adaptation in vernal pool plants, does soil type matter?*" Principal investigators: **Daniel J. Toews**

2015, poster — UC Merced Undergraduate Research Symposia (Merced, CA). "*Teasing Apart the Effects of Atmospheric Nitrogen Deposition from Grazing and Drought in Vernal Pool Wetlands and Adjacent Grassland,*" Principal investigator: Dr. Marilyn Fogel, co-PI's: Bobby Nakamoto, **Daniel J. Toews**, Christopher Swarth, David Ariaza, Dr. Christina Bradly

2014, talk — American Geophysical Union (San Francisco, CA). "*Teasing Apart the Effects of Atmospheric Nitrogen Deposition from Grazing and Drought in Vernal Pool Wetlands and Adjacent Grassland,*" Principal investigator: Dr. Marilyn Fogel, co-PI's: Bobby Nakamoto, **Daniel J. Toews,** Christopher Swarth, David Ariaza, Dr. Christina Bradly

RESEARCH AND PROFESSIONAL EXPERIENCE

2015 - present — **Graduate Student Researcher**; *School of Engineering, University of California, Merced; Supervisor: Jason Sexton:*

- Conducted molecular ecological (eDNA metabarcoding), traditional vegetation surveys and statewide sampling of threatened and endangered vernal pool plants and their communities; California Conservation Genetics Project (); Central Valley Project Improvement Act (U.S. Bureau of Reclamation/U.S. Fish & Wildlife Service).

2018 — **Consulting Biologist**; *LSA Consulting; Roseville, California*; *Supervisor: Kristin Nurmela:*

- Conducted residual dry matter (RDM) measurements used to assess rangeland health and rare vernal pool plant surveys as part of long-term monitoring efforts for rare plants on UCM conservation lands.

RESEARCH AND PROFESSIONAL EXPERIENCE (*cont'd)*

2017, 2018, 2021 **Staff Biologist**; *Vollmar Natural Lands Consulting, Berkeley, California*; *Supervisor: John Vollmar:*

- Conducted riparian and vernal pool plant monitoring at riparian and vernal pool restoration sites throughout the Central Valley, California.
- Duties included managing the planting, monitoring, and maintenance of native tree species in a riparian restoration site, in addition to vernal pool hydrological monitoring in natural, restored and created vernal pools.

2014 - 2015 **Intern**; *Merced Vernal Pools and Grassland Reserve, University of California, Merced; Supervisor: Christopher Swarth:*

- Coordinated science and educational programs as well as other activities for the MVPGR, including: the establishment of plant and animal databases, herbarium curation, research, campus and community outreach.
- Conducted and coordinated biological surveys for threatened and endangered species (California Tiger Salamander larval seine studies, aquatic invertebrate dip-net surveys, San Joaquin Kit Fox surveys, Tricolored Blackbird surveys, rare plant and botanical surveys).
- Conducted rangeland health assessments through residual dry matter (RDM) measurements.

2014 - 2015 **Research Assistant**; *School of Natural Sciences, University of California, Merced; Supervisor: Jason Sexton:*

- Conducted remote field sampling for genetic material used to investigate climate adaptation and population dynamics throughout the cutleaf monkeyflower's (*Mimulus laciniatus*) species range in the Sierra Nevada Mountains.

2011 - 2014 **Freelance consultant/broker and independent contractor**; *multiple locations*

- Provided professional broker, advice, and training services regarding logistics of metal recycling operations (e.g., the organization, purchase, logistics and sale of facilities, equipment and materials) to industrial recycling facilities, including: C&S Waste Solutions in Pahrump, NV, Ukiah, CA, and Lakeport, CA; TCS Metals LLC in Ukiah, CA and Lakeport, CA; and Atwater Iron & Metal, Atwater, CA.

2009 - 2011 **Operations Lead**; *Schnitzer Steel Industries Incorporated, Fresno, California; Supervisor: Suzanne Grigorieff:*

- Supervised crew members and operations of a high-volume non-ferrous metals department, including the pricing, shipping, receiving, and processing of materials.
- Managed and orchestrated equipment and facility repairs.

RESEARCH AND PROFESSIONAL EXPERIENCE (*cont'd)*

2008 - 2009 **Equipment operator/Buyer**; *Schnitzer Steel Industries Incorporated, Oakland, California; Supervisor: Ryan Gaurducci:*

- Conducted daily transactions from peddler and industrial accounts, including the shipping and receiving of domestic and non-domestic materials.
- Operated a verity heavy equipment in day-to-day operations of the non-ferrous department at Schnitzer Steel's port facility.

2006 - 2008 **Equipment operator/Buyer**; *Atwater Iron and Metal, Atwater, California; Supervisor: Jonathan Vann:*

- Operated a verity of light and heavy equipment in day-to-day operations, including the shipping, receiving and organizing of material.

TEACHING EXPERIENCE

Spring 2023, 2022, 2019	**Teaching Assistant.** Flora of California (BIO/ESS 133)
Fall 2022	**Teaching Assistant.** Ecosystems of California (ESS 050)
Fall 2019; Spring 2016	**Teaching Assistant.** Fundamentals of Ecology (BIO/ESS 148)
Fall 2018	**Teaching Assistant.** Fundamentals of Geology (ESS 020)
Fall 2017	**Teaching Assistant.** Introduction to Earth Systems Science (ESS 001)
Fall 2016	**Teaching Assistant.** Conservation Biology (BIO/ESS 149)

RESEARCH AWARDS AND GRANTS

2024 - Environmental Systems Summer Dissertation Fellowship ($8,000)

2023 - Environmental Systems Professional Development Fellowship ($1,000)

2022 - Society for Freshwater Science Graduate Student Conservation Research Award ($1,000) "Using eDNA to reveal plant-soil microbe relationships in California Vernal Pools."

2022 - Environmental Systems Graduate Bobcat Fellowship Award, School of Engineering, UC Merced ($7,000) "Multi-scale soil effects on vernal pool plant adaptation and biodiversity."

2022 - Environmental Systems Professional Development Fellowship ($1,000)

2022 - Environmental Systems Academic Year Fellowship ($450)

2020 - California Native Grassland Association, Grassland Research Awards for Student Scholarship ($500) "The role of soil heterogeneity on adaptation in an endemic vernal pool annual plant *Limnanthes douglasii ssp. rosea*."

2019 - California Native Grassland Association, Grassland Research Awards for Student Scholarship ($500) "Using eDNA metabarcoding sequencing to quantify biodiversity in California vernal pool plant communities."

2019 - Environmental Systems Graduate Student Professional Development Fellowship, UC Merced ($1,500)

RESEARCH AWARDS AND GRANTS *(cont'd)*

2018	-	Environmental Systems Graduate Bobcat Fellowship Award, School of Engineering, UC Merced ($7,500) "Metabarcode sequencing to identify soil seed bank species in California Vernal Pools."
2015, 2016	-	Environmental Systems Bobcat Travel Fellowship ($300)
2017	-	Environmental Systems Summer Bobcat Travel Fellowship ($1,600)
2016	-	Southern California Botanists travel award ($100)
2016	-	Climate Neutrality Curriculum Initiative Graduate Student Fellowship, UC Merced ($3,500) Principle Investigator: Anne Zanzucchi, co-PI: Tom Hothem
2016, 2017	-	Northern California Botanists travel award ($200)

INVITED LECTURES

2023	Flora of California: *"Flower morphology and Prominent California Plant Families"* (UC Merced)
2023	UC Merced Natural Reserve System Student Naturalist Training Program: *"Plant Ecology of California Vernal Pools"* (UC Merced).
2023	Evolution: *"Evolution of the Vernal pool Niche"* (UC Merced)
2023	Lecture. Terrestrial Ecosystems Ecology: *"Vernal pool and grassland ecology"* (UC Merced)
2022	Fundamentals of Ecology: *"Community Diversity and Ecology of UC Merced Vernal Pools and Grasslands Reserve"* (UC Merced)
2022	Flora of California: *"Plant Community Ecology and the Ecological Niche"* (UC Merced)
2022	Biodiversity and Conservation: *"The Ecology, Evolution and Biodiversity of Vernal Pool Plant Communities"* (UC Merced)
2022	Geomorphology and Surface Processes: *"Pedogenesis and Geomorphology of California Vernal Pools"* (UC Merced)
2021	Terrestrial Ecosystems Ecology: *"Patterns of Diversity and Adaptation in California Vernal Pools"* (UC Merced)
2016	Fundamentals of Ecology: "*Biodiversity*" (UC Merced).
2016	Conservation Biology: *"Grazing as adaptive management? A case study in vernal pools"* (UC Merced)
2015	Core 1: "*Vernal Pool Ecology*" (UC Merced, CA)

PROFESSIONAL DEVELOPMENT ACTIVITIES

2023	Bayesian Applications in Environmental and Ecological Studies (Freshwater Sciences 2023, Brisbane, Australia).
2022	UC Davis Genetic Variation Laboratory, eDNA methods and library preparation. (UC Davis, CA).
2019	University of California, Santa Cruz eDNA Summer Institute (UC Santa Cruz, CA).

PROFESSIONAL DEVELOPMENT ACTIVITIES *(cont'd)*

2018	University of California, Los Angeles CALeDNA Workshop (UC Los Angeles, CA).
2017-2018	Lab Safety Officer, Sexton Lab (UC Merced, CA).
2016	Isotopes-Past, Present, & Future (Carnegie Geophysical Laboratory, Washington D.C.)
2014	Jepson Vernal Pool Plant Workshop (UC Merced, CA).
2014	UC Merced Naturalist Training Program (UC Merced).

SELECTED SERVICE AND SYNERGISTIC ACTIVITIES

2023	Mentor for Student Undergraduate Research Fellowship, mentee Amy White (UC Merced).
2023	Lawrence Livermore National Laboratory: Intro to Soils and Plant Ecology on UCM's Vernal Pools and Grasslands Reserve (UC Merced).
2023	UC Berkeley Jepson Herbarium Vernal Pool Flora Workshop (UC Merced)
2023	California Native Plant Society: Plants of the Merced Vernal Pools and Grassland Reserve (UC Merced).
2022	Undergraduate Mentor. Swarth Fogel NRS Undergraduate Research Fellowship, mentee Mark Twomy (UC Merced)
2022	Vernal Pools Tour With The Chancellor (UC Merced)
2020	Ballico-Cressey School 2nd Grade Fieldtrip to the Merced Vernal Pools and Grasslands Reserve (UC Merced)
2019	Merced County Office of Education, Students Who Experience Engineering and Technology (SWEET) (UC Merced).
2019	Valley Land Alliance and Greater Merced Community Outreach – Vernal Pools 101 (Merced, CA).
2017	Merced County Supervisors at the Merced Vernal Pools and Grassland Reserve.
2017	Undergraduate Mentor. School of Natural Sciences, UC Merced; Student Success Internship Program, undergraduate mentee Michael Spaeth (UC Merced)
2016	UC Merced Regents tour of the Merced Vernal Pools and Grassland Reserve (UC Merced)
2014	Sacramento Splash, Merced Elementary School workshop (UC Merced).

Dissertation Abstract
Wetlands rank among the world's most imperiled ecosystems with the highest rates of habitat destruction, degradation, and loss of biodiversity. This is especially true for ephemeral vernal pool wetlands found in the Great Central Valley of California, USA, a region impacted by urbanization and high agricultural productivity. California vernal pools are small isolated ephemeral wetlands dominated by annual plant communities and represent centers of endemism and hotspots of native plant biodiversity. These taxonomically rich ecosystems are also highly threatened as most of the historical vernal pool habitat has been destroyed by agriculture and urban development and, consequently, host to numerous threatened and endangered plant species. Despite extensive conservation efforts, knowledge gaps remain about the ecological and evolutionary processes essential for maintaining these plant communities.

For my dissertation, I investigated the ecological dynamics and adaptive potential of the vernal pool annual plant *Limnanthes douglasii ssp. rosea* (meadowfoam), and assessed the utility of environmental DNA (eDNA) metabarcoding for monitoring rare and endemic vernal pool plant species. In the first chapter, I conducted a multivariate and ecological descriptive study to characterize habitat and morphological variation of meadowfoam plants occurring in different soil types associated with remaining vernal pool habitats in California. I found significant effects that environmental attributes and soil types strongly influence plant morphology, which could be essential for targeted conservation strategies. In the second chapter, I conducted a greenhouse experiment to explore plant-soil interactions and meadowfoam's adaptive potential. I found significant soil-driven effects on plant performance, attributed to differences in soil quality and adaptive potential of meadowfoam, highlighting the importance of preserving diverse soil habitats to maintain genetic diversity and species resilience. For my third chapter, I compared floristic surveys to plant DNA sequenced from vernal pool soil samples to assess the efficacy of eDNA as a biomonitoring tool of endemic and endangered vernal pool plant species. Here, I found eDNA effectively tracks a wide range of plant species, including rare and endemic vernal pool indicator species, with detection frequencies closely linked to plant abundance and ecological niche. This provides valuable insights for managers on the uses and limitations of eDNA as a monitoring tool in ephemeral ecosystems.

Collectively, these findings contribute valuable knowledge for the conservation and restoration of vernal pool ecosystems, informing management practices aimed at preserving these unique and threatened habitats. Importantly, this work supports local conservation efforts by prioritizing management strategies of particular vernal pools associated with high biodiversity or threatened species for conservation.

Chapter 1. Morphological Variation of An Endemic Vernal Pool Annual Plant Species Across Small-scale Edaphic and Environmental Gradients

ABSTRACT

Phenotypic variation is fundamental to the adaptation and persistence of species in diverse environments. Understanding the drivers of this variation is crucial for conserving biodiversity and managing ecosystems. This study investigates the influence of soil properties and habitat attributes on the morphological variation of meadowfoam (*Limnanthes douglasii* subsp. *rosea*), an annual wildflower endemic to California vernal pools. I measured plant abundance, size, and phenology of meadowfoam plants occurring in vernal pools across three distinct soil types in California's Central Valley to characterize morphological variation of meadowfoam across the sites and soil types. Through comprehensive soil analyses and field observations, I characterized the edaphic, topographical, and hydrological environments of each site. The goals of this study were to understand how soil properties and habitat attributes vary across soil types, assess morphological variation in meadowfoam, and identify specific environmental factors contributing to this variation. Results indicated significant variability in soil and habitat characteristics across sites, and significant soil type effects on plant growth and phenology, highlighting the importance of environmental heterogeneity in influencing plant morphology and abundance. The morphological variation observed in meadowfoam illustrates the adaptive potential of vernal pool plant species to their environments, which may be critical to their resilience under changing climatic and hydrological conditions. These findings enhance our understanding of the ecological mechanisms driving plant diversity and population dynamics in vernal pools, offering insights for the conservation and restoration of these unique ecosystems and their endemic species.

INTRODUCTION

Wetlands rank among the world's most imperiled ecosystems, experiencing high rates of habitat destruction, degradation, and biodiversity loss (Fluet-Chouinard et al., 2023). Vernal pool ephemeral wetlands, defined by their seasonal hydrological cycles, support diverse plant communities uniquely adapted to the extreme alternating wet and dry conditions characteristic of Mediterranean climates (Keeley & Zedler, 1998). Vernal pools, once a dominant ecosystem in the Great Central Valley of California, USA, support a rich flora and fauna, including many endemic species (Holland, 2009; Barbour et al., 2007). However, vernal pools in this region are heavily impacted by urbanization and high agricultural productivity, which has reduced vernal pool habitat by more than 90% of its historical extent (Holland, 2009; Witham, 2021). This dramatic habitat loss has placed numerous endemic species at risk of extinction, necessitating significant conservation and restoration efforts. (Shaffer et al., 2022; Vollmar, 2023; USFWS, 2005). Despite these efforts, many ecological and evolutionary dynamics that shape species composition and adaptation in vernal pools remain underexplored, particularly with regard to the role of soil heterogeneity in driving variation in plant species.

Phenotypic variation plays an essential role in the adaptation and survival of plant species, enabling them to persist and evolve in diverse and fluctuating environments. This variation, influenced by both genetic diversity and phenotypic plasticity, provides the raw material for natural selection and evolutionary change (Mitchell-Olds et al., 2007; Pigliucci, 2001; Bradshaw, 1965). In vernal pool ecosystems, environmental heterogeneity, including variation in soil properties and hydrology, exerts strong selective pressures on plant species, influencing both plant community composition and individual species adaptation strategies (Barbour et al., 2007; Rains et al., 2006; Keeler-Wolf, 1998). Although, previous studies have highlighted patterns of trait variation and adaptation across small-scale hydrological gradients in several vernal pool plant species (Linhart, 1974; Emery et al., 2009; Emery et al., 2011), the effects of soil heterogeneity on individual vernal pool plant taxa is underexplored. Vernal pools associated with distinct geomorphic surfaces exhibit unique physical, chemical, and hydrological properties, shaping plant species distribution patterns and resulting in different plant community associations that incompass specific edaphic specialists (Keeler-Wolf, 1998; Barbour et al., 2007; Buck-Diaz et al.,, 2012; Holland & Dains, 1990). Understanding how soil properties interact with hydrological regimes to shape species-level variation is essential to fully grasp the drivers of adaptive potential in vernal pool plant species and assess their resilience under shifting climatic and hydrological conditions.

Soil properties, such as texture (percent sand, silt, and clay), pH, electrical conductivity (EC), and cation exchange capacity (CEC), are crucial factors that determine water retention, nutrient availability, and plant development (Brady et al., 2008). Different soil types can influence water retention, root penetration, and nutrient uptake, thereby affecting plant growth and survival (Brady et al., 2008). Topographical features, such as maximum pool depth, total pool area, edge zone area, and slope have been correlated with geomorphic surfaces (Platenkamp, 1998; Smith & Verrill, 1998). These attributes contribute to spatial variation within and among vernal pools, creating microhabitats that have been found to influence species distributions (Buck-Diaz et al., 2012; Barbour et al., 2005).

Hydrology, including the duration of inundation and ponding depth, is another crucial factor driving species distributions and plant community dynamics in vernal pools (Barbour et al., 2005; Bliss & Zedler, 1997; Gosejohan et al., 2017). Plants in these habitats must be able to withstand periods of flooding followed by extreme drought conditions. The phenology of vernal pool plants, particularly their timing of germination, growth, and reproduction, is closely linked to hydrological regimes (Bliss & Zedler, 1997; personal obs). Linhart & Baker (1973) found significant variation of malic acid production in individuals of *Veronica perigrina* occupying center and periphery vernal pool zones, suggesting some adaptation to different flooding conditions between the zones. Similarly, Emery (2009) found differentiation in *Lasthenia fremontii*, a species occupying both pool bottom and transitional edge zones within individual pools, but did not detect local adaptation, despite differential phenology between zones and the potential for restricted

gene flow. Large variation in plant phenology, specifically the timing when cross pollination occurs between adjacent populations, can result in temporally segregated mating pools and differentiated subpopulations adapted to local environmental conditions (Peters & Weis, 2019).

Vernal pools are well-documented for their unique hydrological regimes and soil characteristics, and specialized plant and animal communities (Emery et al., 2009; Holland & Dains, 1990; Bauder, 2005; King et al.,1996). Previous research has highlighted the significant influence of soil type and hydrological conditions on the composition and diversity of vernal pool plant communities (Emery et al., 2009; Holland & Dains, 1990; Bauder, 2005). Furthermore, pools on different soil types and geomorphic surfaces show significant differences in hydrological characteristics, such as ponding duration, maximum water depth, and basin surface area (Alexander, 2007; Marty, 2005; Platenkamp, 1998). These environmental factors can drive local adaptation and phenotypic variation among plant populations, potentially leading to reproductive isolation and the formation of distinct populations and ecotypes (Silvertown et al., 1999; Levin, 2003). Despite this knowledge, many ecological and evolutionary dynamics within vernal pools are not fully understood. Thus, while significant effort has been made to conserve remaining vernal pool habitat, there is a dire need for information that can lead to better management and restoration of these systems.

In this chapter, I conducted a detailed floristic study of Meadowfoam (*Limnanthes douglasii* subsp. *rosea*), an annual vernal pool wildflower endemic to California vernal pools, to explore how environmental variation influences plant morphology. By examining meadowfoam abundance, maturity (used as a proxy for phenology), and morphological traits in relation to soil properties, hydrology, and topography, I sought to uncover patterns that could be important to ecological and evolutionary processes—such as local adaptation—driven by environmental heterogeneity. To test the effects of both categorical soil types and continuous environmental variables on morphological and phenological variation, I addressed the following questions over a single growing season: ***1) How do soil properties and habitat attributes vary across different soil types and individual vernal pools? 2) Do specific edaphic properties and habitat attributes contribute to morphological variation and abundance of meadowfoam?*** Ultimately, this research aims to use meadowfoam as a model to understand the ecological patterns driving morphological variation and adaptive potential in vernal pool plants across soil types and hydrological conditions. Understanding these conditions is essential for identifying adaptive strategies and potential reproductive isolation mechanisms in meadowfoam. More broadly, this work contributes to our knowledge of the ecological mechanisms underpinning plant diversity and adaptation in vernal pools, providing insights that can inform conservation practices and support biodiversity in these dynamic, imperiled habitats.

MATERIALS AND METHODS

Study area

The Merced Vernal Pools and Grassland Reserve (MVPGR) at UC Merced spans 2,553 ha of an intact vernal pool grassland ecosystem in eastern Merced County, California, managed by the University of California Natural Reserve System (ucnrs.org) (Fig. 1). It is located directly on the western periphery of the Sierra Nevada foothills and eastern edge of the California Central Valley. The MVPGR is primarily composed of stratified granitic alluvium terraces and volcanic mudflows deposited during the Pleistocene (12 to 2,600 ka) and Miocene (5-23 ma) epochs and includes remnants of an ancient alluvial fan (2-4 million years old) that represent some of the oldest and continuously exposed soils in North America (Marchand & Allwardt, 1981; Vollmar, 2002). The major substrata are the Riverbank, North Merced Gravels and Laguna formations that overlie older volcanic mudflow Merhten formations (Marchand & Allwardt, 1981; Vollmar, 2002). Representative vernal pool soil series found on the MVPGR include Reynor, Corning, Redding and Keyes gravely and/or clay loams (from oldest to youngest, respectively; Holland pers comm). The soils in the study area were initially extensively surveyed and mapped by Arkly (1962) and updated by the U.S. Department of Agriculture and Natural Resource Conservation Service (Beaudette & O'Geen, 2009). The MVPGR and adjacent rangelands encompass one of the largest contiguous networks of remaining grassland and vernal pool habitat in California and represent an important intact vernal pool landscape in the Great Valley vernal pool ecoregion that hosts many rare plant and animal species (Vollmar, 2002; Witham et al., 2021; USFWS, 2005).

Focal species

Limnanthes douglasii ssp. rosea (Benth.) C.T. Mason, commonly known as meadowfoam, is an annual dicot endemic to vernal pools and ephemeral swales in California (Kesseli & Jain, 1984). The species primarily occurs in the Central Valley and occupies shallower portions of pool basins and along vernal pool margins, germinating in late fall-early winter before pools begin to pond then flowering in the spring between March and May (Ornduff & Morin, The Jepson, 2012). Meadowfoam has an annual life cycle and is a common vernal pool plant wildflower that is broadly distributed across different soil types on the MVPGR (pers observation) (Fig. 1b and 1c). Meadowfoam is predominantly outcrossing, however, all *Limnanthes* species are self-compatible and capable of producing fertile nutlets (Mason, 1952; Kesseli & Jain, 1985). Kesseli and Jain (1984) found increased rates of homozygosity and inbreeding depression in populations that had both strictly outcrossing and hermaphroditic plants. High rates of habitat loss and fragmentation, as well as intermediate levels of gene flow in meadowfoam may be important components for population differentiation and adaptation to heterogeneous environments. Genetic exchange between *L. douglasii ssp. rosea* and *L. alba*, a closely related species, is known to occur through pollinator-mediated interactions (Runquist & Staton, 2013). These interactions also suggest that reproductive isolation and hybridization dynamics may shape species distributions and phenotypic variation in meadowfoam.

EDAPHIC AND MORPHOLOGICAL CHARACTERIZATION OF HABITATS

Soil properties

The soils examined here represent a variety of surficial alluvial deposits laid down along river and stream terraces from the Sierra Nevada and their foothills, as well as redeposited alluvium derived from local erosion and reworked volcanic mudflow deposits. Redding and Corning soils are primarily composed of highly weathered alluvium derived from igneous, metamorphic and sedimentary rock. Corning and Redding soils have similar soil characteristics, with clay contents between 35-60% and soil acidity between pH 5.2-5.7, respectively (USDA.gov). Keyes soils are derived from andesitic alluvium and associated with older volcanic mudflows of the Mehrten formation formed between 3-10 mya (reviewed in Vollmar, 2002). Although the parent material is quite old, the Keyes soils examined here are subject to local erosional forces and are much younger and less developed than Corning and Redding soil types. Soil characteristics of Keyes soil series range between sandy and sandy clay loam with clay content between 15-25% and an average soil pH of 6. I expect soils with different parent materials and age to differ in their chemical and physical properties and these differences might be important for growth and performance of vernal pool plants. For example, vernal pools occurring on older highly weathered soils might have a higher clay content and be less fertile than the younger soils developed from andesitic alluvium.

Soils were collected from nine semi-randomly selected vernal pools occurring across three soil types (Redding, Keyes and Corning) on the MVPGR. The three soil types were selected based on the following criteria: include vernal pools, are representative soil types of remaining vernal pool landscapes in California, and are habitat for *L. douglasii ssp. rosea*. Pool selection was accomplished using satellite imagery to create focus areas based on topography, suitable habitat for the species, and diversity of soil types. Soil type was determined using the Soil Survey Geographic data-bases (SSURGO) U.S. Department of Agriculture and Natural Resource Conservation Service Web Soil Survey (websoilsurvey.sc.egov.usda.gov).

Soils were collected in bulk at the upper edge in each of the nine study pools to a 10 cm depth and in the fall of 2016 before the rainy season. I defined the edge zone of each pool using several criteria in the field: the point of maximum ponding for that year determined during weekly field surveys over the 2016 winter, zonation of the target plant species (meadowfoam occurs along margins of pool edge) observed during peak flowering (Feb-Mar 2016), and presence of co-occurring edge species *Eryngium castrense* that is observable long after *Limnanthes* has senesced and is no longer present (personal obs). Soil samples were immediately transported to the lab, sieved to 2 mm and stored at room temperature until analyses were performed.

To characterize the edaphic environment of each soil type we measured the physical and chemical properties of soils from a subsample of each bulk collected soil sample of each site. I conducted particle size density analysis following the sedimentation method

adapted from Gee & Bauder (1986) to obtain the percent fraction of sand, silt and clay and determine soil texture. To characterize the chemical properties of soils I measured soil pH and electrical conductivity (EC) in a 1:1 soil and deionized water solution. I assessed soil fertility through cation exchange capacity (CEC) of base cations in soil solutions for two of the three soil types (Corning and Keyes). Base cations (Al, Ca, Mg, K and Na) were extracted following the BaCl2 Compulsive Exchange Method (Gillman & Sumpter, 1986) and exchangeable cations in the soil extracts were measured using a Perkin-Elmer 5300 DV Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) in the Environmental Analytical Lab at UC Merced. The CEC of each soil was determined by the summation method (Burt, 2011) and calculated as the sum of exchangeable base cations determined by ICP-OES.

Physical site attributes

The topography and physical attributes of each vernal pool were characterized using a 25 cm resolution digital elevation model (DEM) of the MVPGR generated from a light finding and range (LiDAR) dataset (Kalua et al., 2020). Each basin (i.e. vernal pool) was delineated and relief mapped by taking the difference of the smoothed raster DEM and the Wang & Liu (2006) hydrological model generated in SAGA tools QGIS (version 3.4) to fill the basins to maximum ponding depth. The simulation parameters of the model were determined as the best fit to known reference points collected at the bottom, edge and point of maximum ponding using a high resolution real-time kinematic (RTK) GPS unit over winter and spring of 2017. Surface area, maximum depth and average basin depth of each vernal pool was calculated from resulting basin polygons. The slope of the basin edge of each vernal pool was determined by examining elevation contours at 2.5cm increments from the bottom and top edge area. I used terrain profile tools to determine the bottom slope position and the known reference points of the upper edge zone to delineate the upper slope position. Mean slope was calculated of the edge polygon using topography tools in QGIS. Meadowfoam is an edge species and the amount of available niche space might be important for meadowfoam plants at each site. Thus, I calculated the edge to pool ratio by dividing the area of the edge zone by the total basin area.

Hydrology

Precipitation during the 2017 water year was the 4th highest recorded for Merced County, with rainfall reaching 19.26 inches compared to the annual average of 11.16 inches (records between 1895-2024) (ncei.noaa.gov). Differences in the hydrological regime of each of the nine vernal pools was monitored in weekly surveys of each site throughout the fall (2016), winter and spring (2017) months. Each site was visited before and after the onset of the first rains in the fall of 2016 and monitored after each rain event to record the initial date of ponding. I marked the deepest location of the pools with rebar after the onset of initial rains to determine the lowest point at the bottom of each pool. I monitored pools weekly after the date of initial ponding and until all pools were dry to determine the total inundation period of each pool (December 16, 2016 - May 9,

2017). Maximum ponding depth was determined as the maximum depth recorded at the location marked with rebar. Survey depth was considered to be the depth of ponding at the time floristic surveys for meadowfoam were conducted.

MORPHOLOGICAL CHARACTERIZATION OF SPECIES

Field surveys

I conducted an observational floristic study of meadowfoam plants in the field to characterize the abundance, phenology and growth form of meadowfoam plants in the nine study vernal pools occurring in different soil environments, Corning, Keyes, and Redding soil types. I monitored the presence and phenology of meadowfoam at each study vernal pool to determine the peak flowering time for floristic surveys over weekly time intervals during the Spring 2017 growing season. Floristic surveys of meadowfoam were conducted between April 1-3 and during the period of peak bloom. I measured plant abundance by counting the number of plants in each of 0.25 m^2 quadrats placed at 1-m intervals along three semi-randomly placed transects at each pool. I recorded the high edge, transition and bottom microhabitat position of each quadrat. Plant morphological characteristics for 30 individuals were measured at each site along the different hydrological gradients. These phenotypic traits were plant size (measured as the mean diameter of each plant), height, number of flowers and number of fruiting flowers. I used plant maturity (the ratio of fruiting to non-fruiting flowers) as an index of plant phenology to assess phenological differences between soil types.

Data analysis

I used principal component analysis (PCA), canonical discriminant analysis (CDA), multivariate analysis of variance (MANOVA) and generalized linear models to compare edaphic properties and morphological variation of meadowfoam plant traits within and among sites occurring across different soil types. I performed separate PCAs on soil continuous data (i.e., soil properties and habitat attributes) and categorical data of site and soil type, to characterize the edaphic and physical environments of the sites and soil types using the R package *FactoMineR* (Lê et al., 2024). Soil PCA data included the particle size fractions of % Clay, % Silt, % Sand, and chemical properties pH and EC for each site. The environmental PCA included hydrological parameters (inundation period, survey depth) and pool attributes (maximum basin depth, average basin depth and slope, pool area, edge area and edge:pool ratio) determined by LiDAR data (Kalua et al, 2020).

To test for significant correlations between site attributes and soil properties, I conducted pairwise correlations between soil properties and habitat characteristics using the GGally package in R (Schloerke et al., 2021). I conducted standard least squares linear models to test the effects of site and soil type on soil properties and habitat characteristics with soil type and site nested in soil type as fixed effects. Significant differences between the soil groups were determined through Tukey HSD post-hoc tests. An Analysis of variance (ANOVA) was performed on CEC with soil type as the dependent variable and pooled

t-test to examine differences between the paired Corning and Keyes soil types. Percent clay and EC were log-transformed to meet assumptions of parametric analyses. Correlation and linear model analyses were conducted in JMP (version 17, SAS).

To determine relative importance of the edaphic, hydrological and physical environment on phenotypic plant traits and phenology, I fit general linear models using principal component scores of the first two axes (PC1 & PC2) of either soil, environmental, or the interaction of soil and environmental PCs as fixed effects. Trait data were either log, square root or cube root transformed to meet assumptions of normality for parametric analysis. I performed discriminant analysis to explore if plants formed biologically relevant groupings by categorical soil types a priori, and used a MANOVA to test differences among observed groupings. Lastly, I constructed generalized linear models to determine the effects of categorical variables of site soil type on plant growth and phenology with soil type and site nested within soil type as fixed effects.

RESULTS

Edaphic and Environmental Analysis

Soil properties such as % clay, % sand, % silt, pH, EC, and CEC were significantly different across soil types and sites nested within soil types based on analysis of variance (**Table 1**). Both soil type and site effects significantly influenced all soil textures, percent clay, sand and silt, and EC ($p < 0.001$). Soil pH was also significantly affected by site ($p = 0.0169$) and soil type ($p < 0.0001$). A one-way ANOVA of CEC by soil type was also significant ($p = 0.008$). Tukey HSD pairwise comparisons revealed significant differences between soil types, whereas the least square mean of percent clay in Keyes soils was significantly lower compared to Corning and Redding soil types (**Figure 2**). Least square means from all pairwise comparisons for the other measured variables (percent sand and silt, pH, and EC) were significantly different from one another with no overlapping groups (Fig 2). A two-tailed t-test revealed Corning soils had a significantly higher CEC than Keyes soils. These results indicate strong variability in both soil type and specific site conditions on the measured soil properties.

Principal component analyses used to ordinate multivariate data effectively quantified the primary edaphic and environmental properties that distinguish sites among soil types and by hydrological and topographical habitat characteristics, to which meadowfoam plants could differentially respond. The PCA analyses identified the key combinations of soil and habitat characteristics that varied across nine sites and three soil types. I retained the first two principal axes of each PCA, which explained 81.4% of the variation in soils and 88.5% of the variation in environmental variables (**Figure 3**). In the soil PCA (**Figure 3a**), axis Soil PC1 explained 48.9% of total variation of soils. Soil texture explained >92% of variation in Soil PC1 and was driven by highly negative correlations of percent clay (47.2%) and highly positive correlations of percent sand (45.6%) and positive correlations of pH (7.15%) (**Figure 3b**). Principal component axis SoilPC2 explained 30.6% of total variation of soil and was primarily driven by positive correlations

with EC (56.5%) and highly negative correlations with pH (39.4%) (**Figure 3c**). Percent silt showed a significant positive correlation with percent clay and was removed from the analysis to exclude collinearity issues. Clustering of soil PC scores indicated that the edaphic properties substantially differed across soil types with large variation among sites within and between soil types. The soil PC1 values for vernal pools occurring on Redding and Corning soil types were more similar to one another, but distinct from sites on Keyes soils, whereas differences are primarily driven by differences in clay and sand content of the soil types with Keyes sites heavily influenced by sand, and Redding and Corning soils more influenced by clay. Soil PC2 values indicated that Keyes and Redding sites were more related to each other compared to Corning sites and the separation between these groups was primarily driven by soil acidity (pH) and salinity (EC). These patterns illustrate that the chemical and physical properties of soils are key components that differentiate the three soil types. In the environment PCA, (**Figure 4a**), PC1 (Env PC1) explained 57.9% of variation and was driven by positive correlations with hydrology, i.e. maximum ponding depth (26.2%), day inundated (26.4%), and average slope of the transition, or edge zone (23.8%), which combined contributed 76.4% to EnvPC1 (**Figure 4b**). Environment PC2 explained 30.6% of the variation and was primarily driven by positive correlations with topographical variables of basin surface area (44.9%), and maximum basin depth (31.4%), which totaled to 76.3% of Env PC2 (**Figure 4c**). The lack of clustering of sites by soil type along the environment PC1 axis illustrates similarities of habitat characteristics among pools across the three soil types, highlighting that differences of individual vernal pools are not driven by soil type. Large differences of sites along EnvPC1 suggests the variables included in the PCA do not fully capture the observed variation. Similarly, environmental PC2 reveals sites from different soil types are more similar than sites occurring on the same soil type. The variation in sites along EnvPC2 suggests sites to be separated by differences in hydrological conditions and topographical features.

Distribution and abundance across environmental gradients

Soil type strongly affected plant abundance; Redding and Corning soil types supported significantly higher plant abundance compared to Keyes soils. Generalized linear models of plant abundance revealed significant effects across soil types, sites and zones within sites. Both site and soil type effects on plant abundance were highly significant ($p<0.0001$ for both) (**Table 2**). A Tukey HSD post hoc test indicated significant variation across sites, including sites within the same soil type. Least square means from Tukey post hoc test indicated Corning and Redding soil types supported higher plant densities than Keyes soil types.

The analysis of plant abundance among pools and across hydrological gradients within pool zones showed significant effects for site ($p = 0.0013$), zone ($p = 0.0397$), and their interaction ($p = 0.0491$) (**Table 3**). Tukey post hoc tests indicated significant variation of plant abundance across sites, with sites from different soil types grouping. Significant zone effects indicate plant abundance to be correlated with hydrological zone. Least

squares plant abundance was significantly higher in the bottom zone compared to the transition zone, however there was high variability among pools.

Morphological Variation across soil types, sites and zones

Discriminant analysis of log transformed plant density, log plant area, log plant height, and square root transformed plant maturity showed significant groupings of plants for all nine sites by soil type (0.95%). I performed a stepwise variable selection analysis to keep statistically significant traits; log flowers were removed (**Figure 5**). Plants from Keyes soils separate along the Plant size axis, indicating differences of plant size where Keyes plants produced larger but not taller plants (**Figure 5)**. Plants in Redding and Corning soils were separated from plants in Keyes soils by plant maturity and height, with plants in Corning soils growing taller and plants from Redding further along in their developmental life cycle (**Figure 5**). A MANOVA on standardized trait data showed highly significant differences between and among subjects for all traits kept after the discriminant analysis ($p<0.0001$). Post hoc Tukey HSD test and least square means from the Manova revealed overlap between Corning and Keyes for plant height, whereas all other pairwise comparisons were different between soil groups and plant traits (**Figure 6**).

Standard least squares analysis of morphological traits reveals both site and soil type significantly influenced plant growth. For the number of flowers, site effects were significant ($p = 0.0128$), while soil type effects were not, indicating that flower production varies by site but not by soil type (**Table 4**). Tukey post hoc tests showed no significant differences of flowers among sites or soil types. On the other hand, plant maturity was highly influenced by both site ($p < 0.0001$) and soil type ($p < 0.0001$), with Tukey least square means showing site C3 had the highest maturity, and site K1 having the lowest maturity both significantly different from other sites (**Figure 7**). Plant maturity was significantly lower at sites of Keyes soil types and distinct from Corning and Redding soil types, which grouped together at the soil type level (Tukey HSD, $a = 0.05$) (**Figure 7**). Plant height also displayed significant variability across sites ($p < 0.0001$) and soil types ($p < 0.0001$), with site C3 having the tallest plants, distinct from other sites (**Figure 7**). Site C3 also had highest relative abundance, which might explain taller plants. However, site K2 had the highest plant density but supported some of the shortest plants. Tukey post hoc tests show large variation in plant height across soil types, with soil types all distinctly different (**Figure 8**). Plant size showed marginal variability across sites ($p = 0.0631$) and no significant effect of soil type (**Table 4**). These findings highlight the importance of site-specific conditions and variability in soil type in influencing meadowfoam growth and phenology.

The analysis of meadowfoam traits considering site, zone, and their interaction showed significant effects on plant traits. For the number of flowers, site effects were significant ($p = 0.0254$), whereas zone and interaction effects were not significant (**Table 5**).

Although square means showed differences in plant abundance, a Tukey test indicated no significant difference in flower production between the transition and bottom zones. Plant maturity showed highly significant effects of both site ($p < 0.0001$) and zone ($p < 0.0001$), with a significant interaction effect ($p = 0.0296$) (**Table 5**). The least square means showed that plants are significantly more mature in the transition zone compared to the bottom zone with high variability across sites. For plant height, site effects were significant ($p < 0.0001$), whereas zone effects and the interaction effects between site and zone were not (**Table 5**). These results highlight the significant role of site and zone in influencing meadowfoam traits, particularly for plant maturity and height.

Abundance vs.continuous environment data

A generalized linear model of plant abundance against the first two principal component axes of soil and environmental PCAs indicate that SoilPC1 and EnvPC2 significantly affect plant abundance (**Table 6**). SoilPC1 ($p = 0.0045$), had a strong influence on abundance, reflecting the importance of soil texture, which primarily loaded onto this axis. Topographical features such as basin surface area and maximum basin depth also significantly influences abundance, which is represented by EnvPC2 ($p = 0.0280$). There was a marginal interaction effect of SoilPC2*EnvPC2 ($p = 0.0691$) at alpha level of 0.05, which indicated a weak interaction effect of soil pH and EC by topographical features, i.e. basin size and depth.

Abundance vs. continuous environment data and soil type

The regression analysis of plant abundance against soil type and principal components reveals significant effects from both edaphic and environmental variables. For soil factors (**Table 7**), SoilPC1 ($p = 0.0044$), which primarily reflects soil texture and pH, significantly affects plant abundance. The interaction between soil type and SoilPC1 is also significant ($p = 0.0168$), indicating that the effect of soil properties on plant abundance varies depending on the soil type. For environmental factors (**Table 8**), EnvPC1 ($p < 0.0001$), which represents hydrological characteristics like maximum ponding depth and duration of inundation, significantly impacts plant abundance. The interaction between soil type and EnvPC1 is significant ($p < 0.0001$), suggesting that the effect of hydrological conditions on plant abundance is influenced by the soil type. However, EnvPC2, reflecting topographical features, does not seem to affect plant abundance by specific soil types.

Morphology vs. PCAs

The full factorial model analysis on plant traits with soil and environmental PCs as fixed effects revealed significant influences on several morphological traits (**Table 9**). For flowers, the interaction between SoilPC1 and EnvPC2 is significant ($p = 0.0170$), indicating that the number of flowers is affected by the combination of soil texture and topographical features. Plant maturity showed significant effects of SoilPC1 ($p = 0.0007$), SoilPC2 ($p = 0.0333$), EnvPC1 ($p = 0.0481$), and many significant interactions, highlighting the complex interconnections between soil properties and hydrological

conditions on phenological development. Height is significantly influenced by SoilPC1 ($p < 0.0001$), SoilPC2 ($p < 0.0001$), EnvPC1 ($p = 0.0002$), EnvPC2 ($p < 0.0001$), and their interactions, emphasizing the role of soil and environmental factors in determining plant height. Plant size, however, showed no significant effects from the individual PCs or their interactions.

Morphology vs. Soil PCA and soil type

The standard least squares analysis on plant traits with soil type and soil PCs as fixed effects revealed significant effects for several traits (**Table 10**). For the number of flowers, soil type ($p = 0.0049$), SoilPC1 ($p = 0.0302$), and SoilPC2 ($p = 0.0186$) are significant, indicating that soil texture and chemical properties influence flower production. Plant maturity is significantly affected by soil type ($p < 0.0001$), SoilPC1 ($p = 0.0331$), SoilPC2 ($p < 0.0001$), and their interactions, reflecting the role of soil conditions in plant phenology. Height also showed significant effects from soil type ($p = 0.0003$), SoilPC1 ($p = 0.0169$), and SoilPC2 ($p = 0.0390$), suggesting that variations in soil properties significantly impact plant height. Plant size, however, showed marginal significance for soil type ($p = 0.0798$) and SoilPC1 ($p = 0.0264$), but not for SoilPC2, indicating a weaker influence of soil properties on plant size.

Morphology vs. Environment PCA and soil type

The analysis of plant traits with soil type and environmental PCs as fixed effects revealed significant influences for several traits (**Table 11**). For the number of flowers, soil type ($p = 0.0262$) and EnvPC2 ($p = 0.0167$) are significant, indicating that topographical features significantly impact flower production. Plant maturity showed significant effects of EnvPC1 ($p = 0.0001$) and EnvPC2 ($p < 0.0001$), as well as their interactions with soil type, suggesting that hydrological conditions play a crucial role in phenological development. Height is significantly influenced by soil type ($p < 0.0001$), EnvPC1 ($p = 0.0209$), and their interactions, highlighting the importance of both soil and environmental conditions on plant height. Plant size showed significant effects of soil type ($p = 0.0061$) but not for the environmental PCs individually, suggesting that soil conditions are more influential on plant size compared to the measured environmental gradients.

DISCUSSION

Edaphic and Environmental Analysis

The analysis of soil composition represented by pH, CEC, EC, and percentages of sand, clay and silt, along with environmental variables such as topography (slope, basin depth, surface area) revealed significant variability of soil composition across all three soil types analyzed (Corning, Redding and Keyes), as well as individual vernal pools within soil type. These results underscored the substantial influence of soil type and site-specific conditions on soil texture, pH, electrical conductivity (EC), and cation exchange capacity (CEC). Although there was significant variation of sites within soil types, distinct

differences emerged among soil types, as is expected and aligns with the categorization of soil types.

The multivariate analysis illustrated distinct edaphic profiles of each soil type, whereas differences between soils were primarily driven by soil texture and soil chemistry. Keyes soils were characterized by higher sand content, whereas Redding and Corning soils had higher clay content, highlighting the age difference of the more weathered clay soils versus the younger, sandier Keyes soil type. The Redding and Corning soils examined in this study are predominantly found on higher alluvial terraces and have been subjected to more-or-less similar erosional conditions for millennia. On the other hand, Keyes soils at this site are found on lower alluvial terraces adjacent to a present-day stream and have likely been shaped by more recent geomorphic processes resulting in higher sand content than the older more weathered soils. Similarly, differences in EC and pH distinguished Corning soils from Keyes and Redding soils which had closer associations in ordination space. Keyes soils had significantly higher pH than the more acidic Redding and Corning soils. This is consistent with expectations that soils become increasingly acidic with age due to prolonged leaching of basic cations like calcium, magnesium, and potassium, and the accumulation of acidic cations such as hydrogen and aluminum (Brady et al., 2008; Rains et al., 2008). The large variation in EC measured in Corning soils also reflects variability across the landscape, which could result from differences in grazing pressure among pools; e.g., accumulation in salts from cattle defecation (Croel & Kneitel, 2011). Nonetheless, soils with higher clay content had higher average salinity and CEC values than the sandier Keyes soil type.

In contrast to soil factors, multivariate analysis of environmental factors did not show clear clustering by soil type, indicating habitat characteristics such as hydrology and topography exhibit significant variability within and across soil types. This lack of clustering emphasizes the complexity of multiple interacting environmental factors in shaping distinct vernal pool habitats, suggesting that soil type alone does not drive habitat differentiation, which has been well documented in this system (Platenkamp, 1998; Holland and Dains, 1990; Bauder, 2000). The significant influence of hydrological features (e.g., maximum ponding depth and days inundated) and topographical variables (e.g., basin surface area and depth) on environmental PC axes underscores the dynamic nature of these ephemeral wetlands and their susceptibility to variations in hydrological regimes (Rains et a., 2008; Hobson & Dahlgren, 1998; Keeley & Zedler, 1998; Barbour et al., 2005; Barbour, 2007; Platenkamp, 1998). Such variability in vernal pool soil and hydrological conditions play a crucial role in shaping plant communities and their distribution (Barbour et al., 2007; Rains et al., 2008).

Meadowfoam Distribution and Abundance

Soil type and specific site conditions of vernal pools (i.e., depth and surface area) are important determinants of meadowfoam community structure and dynamics. Vernal pools on Redding and Corning soils supported higher meadowfoam abundance

compared to Keyes soils, despite variability within sites of the same soil type. This finding suggests that although categorical soil type was a significant determinant of plant abundance, site-specific factors such as topography, localized soil properties and hydrological conditions also played a significant role. Deeper basins likely provide more stable hydrological conditions and reduced competition for water (Barbour et al., 2005; Keeley & Zeddler, 1998). Larger pool surface areas may support greater resource availability and reduce intraspecific competition, promoting higher plant abundance (Holland & Jain, 1981; Gerhardt & Collinge; 2003; Adler et al., 2018; Barbour, et al., 2005).

The significant effects of hydrological zones within pools on plant abundance further emphasize the importance of hydrology in vernal pool ecosystems. The higher abundance of meadowfoam in bottom zones demonstrates the effects hydrological gradients within pools can have on community assemblages (Buck-Diaz, 2012; Bliss & Zedler, 1997; Solomeshch et al., 2007). *Limnanthes* taxa are thought to be unable withstand extended periods of completely submerged conditions, occupying shallower positions in pool basins and in transitional pool-grassland edge zones (Barbour et al., 2005). *Limnanthes alba*, a closely related species in the genus, has been shown to occupy specific inundation gradients within the edge of vernal pools (Emery et al., 2009). In the present study, higher meadowfoam abundance found in the center and deeper pool basin zones in a high water year suggests that hydrology may not be as strong of an ecological driver for *L. douglasii subsp. rosea* compared to *L. alba*. Furthermore, Runquist & Stanton (2013) found that *L. douglasii subsp. rosea* experiences significant competitive pressure from *L. alba*, where pollinator-mediated interactions lead to competitive displacement between *L. alba* and *L. douglasii subsp. rosea* in sites where their geographic ranges overlap. Similarly, Runquist et al. (2016) found the two taxa rarely co-occurred in the same geographic complex. These interactions could force *L. douglasii subsp. rosea* to occupy pool bottoms as a strategy to avoid competition. This supports the idea that niche differentiation and competitive interactions, such as those mediated by pollinators (Runquist & Stanton, 2013), can influence the spatial distribution of *L. douglasii subsp. rosea* and *L. alba*.

Morphological Variation

Morphological variation in meadowfoam differed significantly across sites and soil types. Plants from Keyes soils were larger but not taller, while those from Redding and Corning soils were taller and more mature. These morphological differences suggest that soil properties, particularly texture and chemical composition, as determined by generalized linear models using PCA scores, can influence plant growth and phenological development. Plants grown in low pH and/or clay soils can have decreased root elongation, stunted growth and increased rates of development (Jones, 1983; Cottes et al., 2020). Meadowfoam plants measured in this study were taller, but more developed when occurring in acidic soils. Furthermore, a greenhouse experiment conducted on meadowfoam plants using soils and plants from the same sites as this study revealed

shorter root lengths in the more acidic clayey Corning and Redding soils compared to longer roots found in plants grown in Keyes soils (see Chapter 2).

Additionally, the significant site effects on traits such as the number of flowers and plant height suggest that localized environmental conditions, beyond soil type, substantially influence meadowfoam morphology. Variability in pool hydrological conditions (Linhart, 1974; Bauder, 2005), microbial interactions (Van Der Heijden et al., 2008; Wagner et al., 2014), and impacts from cattle grazing–e.g., nutrient input and trampling (Marty, 2005; Hendrickson, 2024)--could also contribute to site specific effects in plant morphology. Population dynamics such as density-dependence may also influence plant morphology and reproductive success (Runquist & Stanton, 2013). At the site with the tallest plants, higher relative abundance compared to other sites suggests that favorable site conditions can lead to increased plant productivity and reproductive output. Taller plants may also indicate intraspecific competition, where neighboring meadowfoam plants compete for light or other resources (Linhart; 1976; Adler et al., 2018). Conversely, however, the site with the highest plant density supported shorter plants, highlighting the potential for competitive interactions, site-specific stressors that limit growth, or trade-offs between reproduction and growth.

Ultimately, soil factors such as soil texture and chemistry, along with environmental variables - particularly pool hydrology - significantly influenced both plant abundance and morphological variation in meadowfoam. These findings emphasize the importance of soil properties and hydrological conditions in shaping demographic and ecological dynamics within vernal pool plant communities (Barbour et al., 2003; Bauder, 2005; Holland & Dains, 1990). The significant interactions between soil and environmental principal components highlight that the combined effects of these factors create a complex landscape of habitat suitability, influencing both plant abundance and trait expression, including phenological development -a trait important to gene flow in these patchy habitats. The ecological and evolutionary implications of these findings underscore the importance of abiotic factors in species distribution and adaptation across landscape and small-scale environmental heterogeneity, which is vital for conservation and management of biodiversity in threatened habitats (Hufford & Mazer, 2003; Linhart & Grant, 1996; Young et al., 1996).

CONCLUSION

This study provides the first evidence that morphological variation in a vernal pool species, meadowfoam, is partially driven by soil type, establishing a critical link between edaphic conditions and plant phenotype. This chapter highlights the importance of the landscape in shaping relationships between soil properties, environmental factors, and ecological dynamics of vernal pool plants. The significant variability in soil and habitat characteristics across different soil types and individual vernal pools underscores the need for a nuanced understanding of these factors in conserving and restoring vernal pool plant species and their habitats. The observed morphological variation in

meadowfoam further emphasizes the importance of considering both edaphic and environmental gradients in vernal pool conservation strategies at a regional scale. The observed morphological variation in meadowfoam, if genetically based, illustrates the adaptive potential of vernal pool species to their environments, which may be critical to their resilience under changing climatic and hydrological conditions. Future research should focus on genetic adaptive potential and the interactive effects of these factors and their evolutionary significance to develop more comprehensive conservation strategies for vernal pool ecosystems and their endemic plant species.

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Tables

Table 1. Analysis of variance and effect tests of soil type and pool (site) nested in soil type on physical (percent fractions of clay, sand and silt) and chemical (pH, EC, and CEC) properties of soils. Variance and effect tests conducted on % Clay & EC were tested on log transformed data.

Soil Attributes	*Source*	*df*	*SS*	*Mean Square*	*F*	*p*
% Clay (log transformed)	ANOVA	8	1.41	0.176	9.2672	<0.0001
	Error	9	0.05	0.005		
	Soil Type	2	0.81	-	74.6	<0.0001
	Pool	6	0.61	-	18.6438	0.0001
% Sand	ANOVA	8	1358.43	169.804	45147.25	<0.0001
	Error	9	0.04	0.003		
	Soil Type	2	755.78	-	100473.5	<0.0001
	Pool	6	602.65	-	26705.15	<0.0001
% Silt	ANOVA	8	581.48	72.69	125.88	<0.0001
	Error	9	5.20	0.577		
	Soil Type	2	288.52	-	249.83	<0.0001
	Pool	6	298.16	-	84.56	<0.0001
pH	ANOVA	8	2.04	0.255	13.5782	<0.0001
	Error	18	0.33	0.019		
	Soil Type	2	1.64	-	43.6582	<0.0001
	Pool	6	0.40	-	3.5515	0.0169
EC (log transformed)	ANOVA	8	3.91	0.488	260.5714	<0.0001
	Error	9	0.02	0.002		
	Soil Type	2	1.19	-	318.5791	<0.0001
	Pool	6	2.71	-	241.2355	<0.0001
CEC	ANOVA	1	5.93	5.935	24.147	0.008
	Error	4	0.98	0.246		

Table 2. Log-normal generalized linear regression analysis on abundance with site nested in soil type, and soil type as fixed effects.

Response	*Effect*	*DF*	*Wald Chi Square*	*P Value*
Abundance	Site	6	45.61613	**<.0001***
	Soil Type	2	25.776614	**<.0001***

Table 3. Generalized linear model with a log-normal distribution on plant abundance. Site, Zone and Site*Zone interaction is included as fixed effects in each model.

Response	*Effect*	*DF*	*Wald Chi Square*	*P Value*
Abundance	Site	8	25.399478	**0.0013***
	Zone	1	4.2293392	**0.0397***
	Site*Zone	8	15.560628	**0.0491***

Table 4. illustrates separate standard least squares linear models on plant traits. Soil type and site nested in soil type are included as fixed effects in each model.

Response	*Effect*	*DF*	*SS*	*F Ratio*	*P Value*
Flowers	Site	6	15.841671	2.761	**0.0128***
	Soil Type	2	3.562835	1.8629	**0.1573**
Maturity	Site	6	40..861606	9.0629	**<.0001***
	Soil Type	2	32.355530	21.529	**<.0001***
Height	Site	6	26.056036	5.9432	**<.0001***
	Soil Type	2	52.200392	35.7195	**<.0001***
Size	Site	6	11.795131	2.0225	0.0631
	Soil Type	2	3.487240	1.7939	0.1684

Table 5. Separate standard least squares linear models on plant traits with Site, Zone and Site*Zone interaction is included as fixed effects in each model.

Response	*Effect*	*DF*	*SS*	*F Ratio*	*P Value*
Flowers	Site	8	17.312549	2.2362	**0.0254***
	Zone	1	1.287568	1.3305	0.2498
	Site*Zone	8	4.388095	0.5668	0.8047
Maturity	Site	8	70.968391	14.5365	**<.0001***
	Zone	1	30.685596	50.2827	**<.0001***
	Site*Zone	8	10.636938	2.1788	**0.0296***
Height	Site	8	79.333034	13.5486	**<.0001***
	Zone	1	1.493265	2.0402	0.1544
	Site*Zone	8	4.664456	0.7966	0.6061
Size	Site	8	14.002666	1.7623	0.085
	Zone	1	0.019974	0.0201	0.8873
	Site*Zone	8	3.394529	0.4272	0.9042

Table 6. Log-normal generalized linear regression analysis on abundance with a full factorial model of the first two soil PCA axes and environmental PCA axes included as fixed effects. Interactions of the soil PCs with itself and environmental PCs and itself are orthogonal and not included.

Response	*Effect*	*DF*	*Wald Chi Square*	*P Value*
Abundance	SoilPC1	1	8.089195	**0.0045***
	EnvPC2	1	4.825914	**0.0280***
	SoilPC2*EnvPC2	1	3.304725	0.0691
	SoilPC1*EnvPC2	1	1.402464	0.2363
	EnvPC1	1	1.090243	0.2964
	SoilPC1*EnvPC1	1	0.732751	0.392
	SoilPC2	1	0.440997	0.5066
	SoilPC2*EnvPC1	1	0.00016	0.9899

Table 7. Generalized linear model with a log-normal distribution on plant abundance soil type and both soil PCs, and their interactions included as fixed effects in each model.

Response	*Effect*	*DF*	*Wald Chi Square*	*P Value*
Abundance	Soil type	2	8.712247	**0.0128***
	SoilPC1	1	8.095271	**0.0044***
	SoilPC2	1	1.364187	0.2428
	Soil type*SoilPC1	2	8.169024	**0.0168***
	Soil type*SoilPC2	2	0.118832	0.9423

Table 8. Generalized linear model with a log-normal distribution on plant abundance soil type and both env PCs, and their interactions included as fixed effects in each model.

Response	*Effect*	*DF*	*Wald Chi Square*	*P Value*
Abundance	Soil type	2	12.47027	**0.0020***
	EnvPC1	1	16.31329	**<.0001***
	EnvPC2	1	0.142603	0.7057
	Soil type*EnvPC1	2	21.11013	**<.0001***
	Soil type*EnvPC2	2	2.324342	0.3128

Table 9. Standard least squares linear regression analysis on plant traits with a full factorial model of the first two soil PCA axes and environmental PCA axes included as fixed effects. Interactions of the soil PCs with itself and environmental PCs and itself are orthogonal and not included.

Response	*Effect*	*DF*	*SS*	*F Ratio*	*P Value*
Flowers	SoilPC1	1	0.1309615	0.1369	0.7116
	SoilPC2	1	0.0643472	0.0673	0.7955
	EnvPC1	1	0.137614	0.1439	0.7047
	EnvPC2	1	0.2631026	0.2751	0.6004
	SoilPC1*EnvPC1	1	0.185441	0.1939	0.66
	SoilPC1*EnvPC2	1	5.5180572	5.7704	**0.0170***
	SoilPC2*EnvPC1	1	1.0436606	1.0914	0.2971
	SoilPC2*EnvPC2	1	1.1769564	1.2308	0.2683
Maturity	SoilPC1	1	8.762901	11.6614	**0.0007***
	SoilPC2	1	3.441612	4.58	**0.0333***
	EnvPC1	1	2.962923	3.943	**0.0481***
	EnvPC2	1	0.025712	0.0342	0.8534
	SoilPC1*EnvPC1	1	0.043539	0.0579	0.81
	SoilPC1*EnvPC2	1	23.710349	31.5531	**<.0001***
	SoilPC2*EnvPC1	1	13.607189	18.1081	**<.0001***
	SoilPC2*EnvPC2	1	6.903259	9.1867	**0.0027***
Height	SoilPC1	1	37.81892	51.7571	**<.0001***
	SoilPC2	1	17.638885	24.1397	**<.0001***
	EnvPC1	1	10.499535	14.3692	**0.0002***
	EnvPC2	1	15.05783	20.6074	**<.0001***
	SoilPC1*EnvPC1	1	12.711588	17.3965	**<.0001***
	SoilPC1*EnvPC2	1	12.596173	17.2385	**<.0001***
	SoilPC2*EnvPC1	1	1.489024	2.0378	0.1546
	SoilPC2*EnvPC2	1	27.506272	37.6437	**<.0001***
Size	SoilPC1	1	0.2489	0.6183	0.6183
	SoilPC2	1	3.5488	0.06	0.06
	EnvPC1	1	0.5529	0.4578	0.4578
	EnvPC2	1	0.048	0.8267	0.8267
	SoilPC1*EnvPC1	1	1.3995	0.2379	0.2379
	SoilPC1*EnvPC2	1	1.1374	0.2872	0.2872
	SoilPC2*EnvPC1	1	2.0126	0.1572	0.1572
	SoilPC2*EnvPC2	1	0.938	0.3337	0.3337

Table 10. Standard least squares results for Soil type, Soil PCs and interactions on plant traits.

Response	*Effect*	*DF*	*SS*	*F Ratio*	*P Value*
Flowers	Soil type	2	10.37086	**5.4225**	**0.0049***
	SoilPC1	1	4.539799	**4.7474**	**0.0302***
	SoilPC2	1	5.359365	5.6044	**0.0186***
	Soil*SoilPC1	2	8.648528	4.522	**0.0117***
	Soil*SoilPC2	2	0.720699	0.3768	0.6864
Maturity	Soil type	2	27.51439	18.3077	**<.0001***
	SoilPC1	1	3.449789	4.5909	**0.0331***
	SoilPC2	1	24.821	33.0311	**<.0001***
	Soil*SoilPC1	2	34.33882	22.8486	**<.0001***
	Soil*SoilPC2	2	5.812043	3.8673	**0.0221***
Height	Soil type	2	12.29871	8.4157	**0.0003***
	SoilPC1	1	4.220879	5.7765	**0.0169***
	SoilPC2	1	3.143974	4.3027	**0.0390***
	Soil*SoilPC1	2	8.208297	5.6167	**0.0041***
	Soil*SoilPC2	2	2.003259	1.3708	0.2557
Size	Soil type	2	4.964062	2.5536	0.0798
	SoilPC1	1	4.845523	4.9852	**0.0264***
	SoilPC2	1	0.06537	0.0673	0.7956
	Soil*SoilPC1	2	0.830128	0.427	0.6529
	Soil*SoilPC2	2	0.999783	0.5143	0.5985

Table 11. represents standard least squares results for Soil type, env PCs and interactions on plant traits.

Response	*Effect*	*DF*	*SS*	*F Ratio*	*P Value*
Flowers	Soil	2	7.0625322	**3.6927**	**0.0262***
	EnvPC1	1	0.0024867	**0.0026**	0.9594
	EnvPC2	1	5.5520244	5.8059	**0.0167***
	Soil*EnvPC1	2	4.8821437	2.5527	0.0798
	Soil*EnvPC2	2	5.0639641	2.6478	0.0727
Maturity	Soil	2	1.033047	0.6874	0.5038
	EnvPC1	1	11.660766	15.5178	**0.0001***
	EnvPC2	1	34.148812	45.4444	**<.0001***
	Soil*EnvPC1	2	28.832198	19.1846	**<.0001***
	Soil*EnvPC2	2	29.614933	19.7054	**<.0001***
Height	Soil	2	20.690449	14.158	**<.0001***
	EnvPC1	1	3.945639	5.3998	**0.0209***
	EnvPC2	1	0.389349	0.5328	0.4661
	Soil*EnvPC1	2	10.262135	7.0221	**0.0011***
	Soil*EnvPC2	2	8.91821	6.1025	**0.0026***
Size	Soil	2	10.101504	5.1964	**0.0061***
	EnvPC1	1	0.252887	0.2602	0.6104
	EnvPC2	1	0.480977	0.4948	0.4824
	Soil*EnvPC1	2	4.095446	2.1068	0.1237
	Soil*EnvPC2	2	6.183686	3.181	**0.0432***

Figures

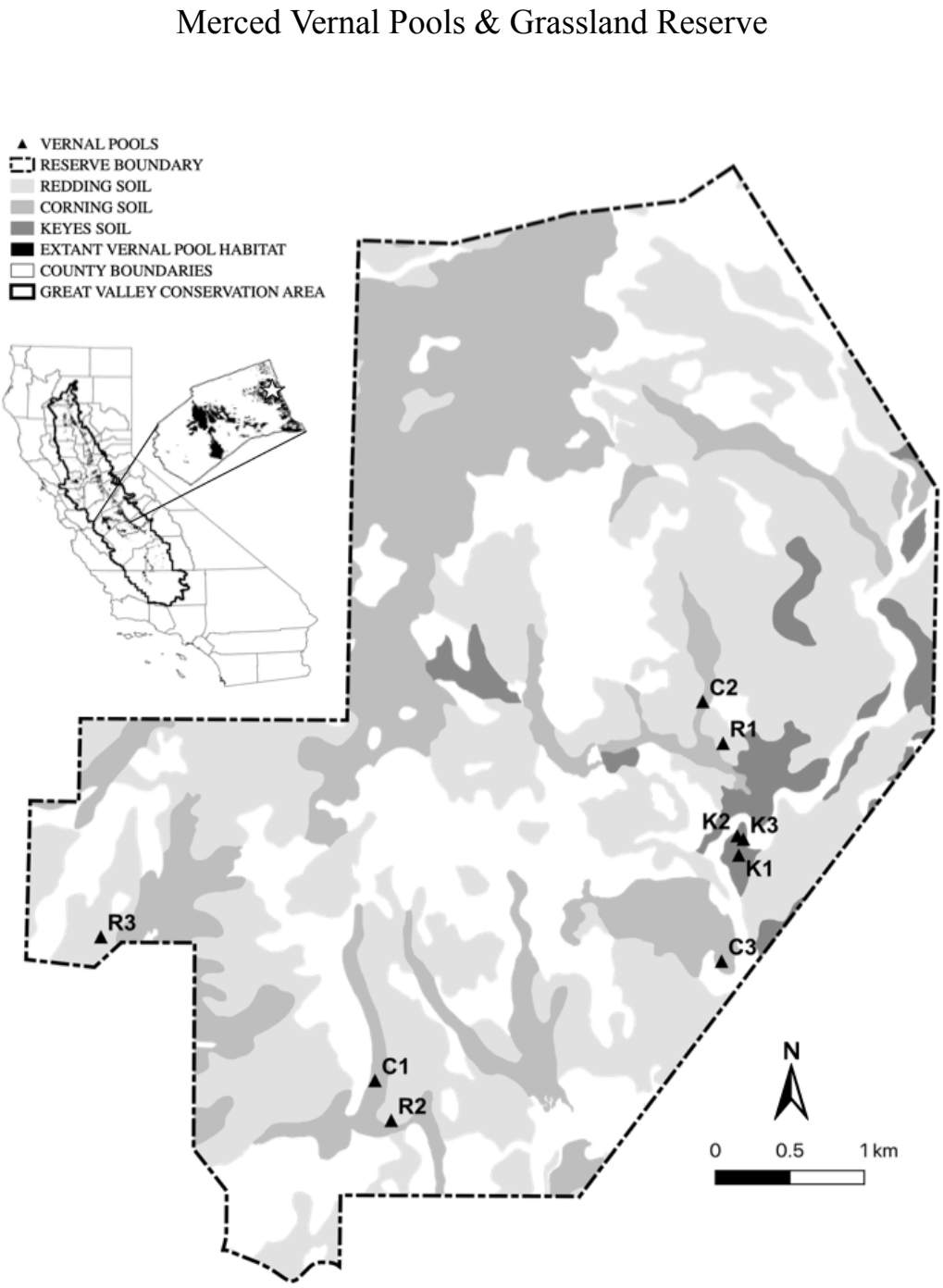

Figure 1. Map of the MVPGR, nine study vernal pools and distribution of three study soil types, Redding, Corning and Keyes.

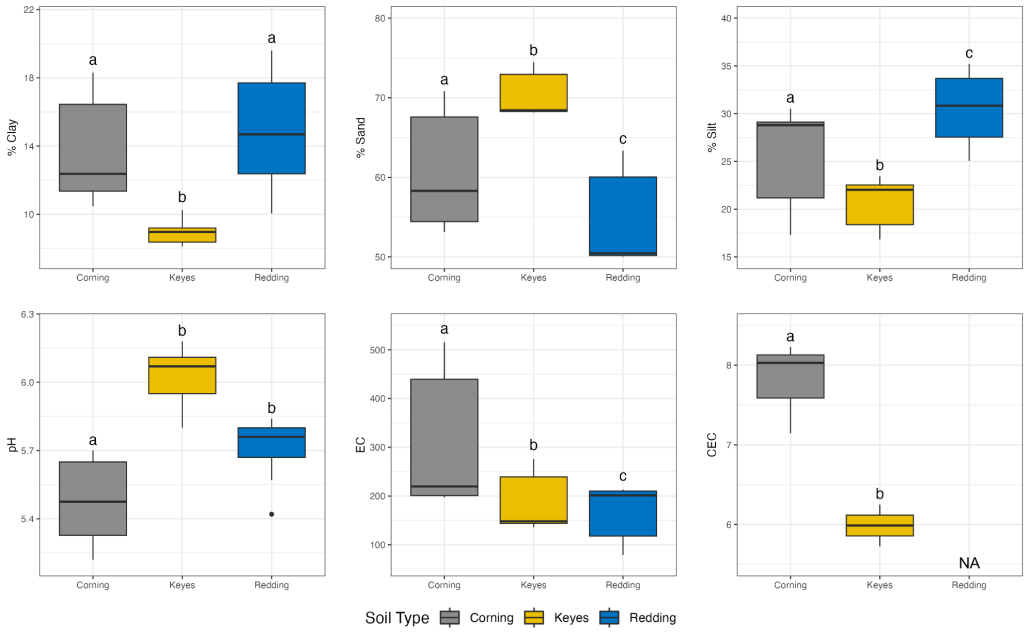

Figure 2: Box plots of untransformed physical and chemical properties of soils. Statistics represent linear regression models and letters represent Tukey post hoc tests, of which % Clay and EC are of log transformed data. Statistics for CEC are from a one-way ANOVA and pooled t-test and are not available (NA) for the Redding soil type.

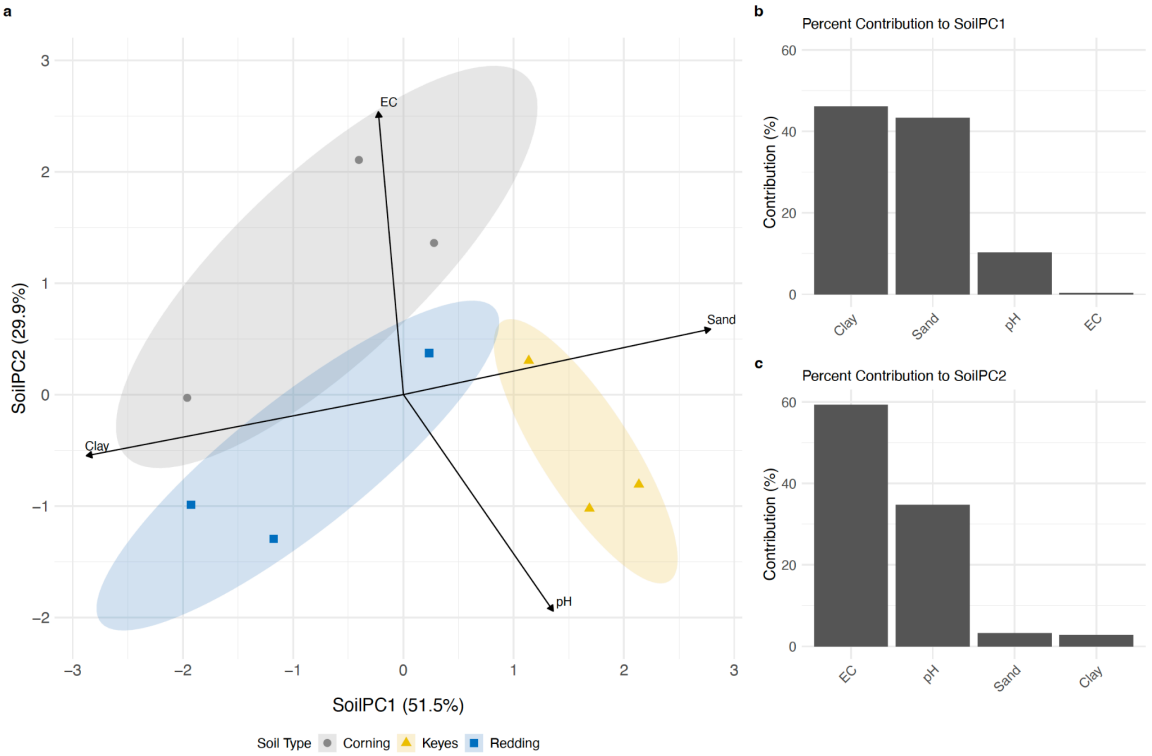

Figure 3. Biplot (a) of principal component analysis of the first two axes for soil variables, %Sand, %Silt, %Clay, pH and EC. Soil types are represented by different colors and shapes, and grouped by filled ellipsis. Barplots of percent contributions for PC1 (b) and PC2 (c) illustrate soil texture explains PC1 and chemical properties of pH and EC explain PC2.

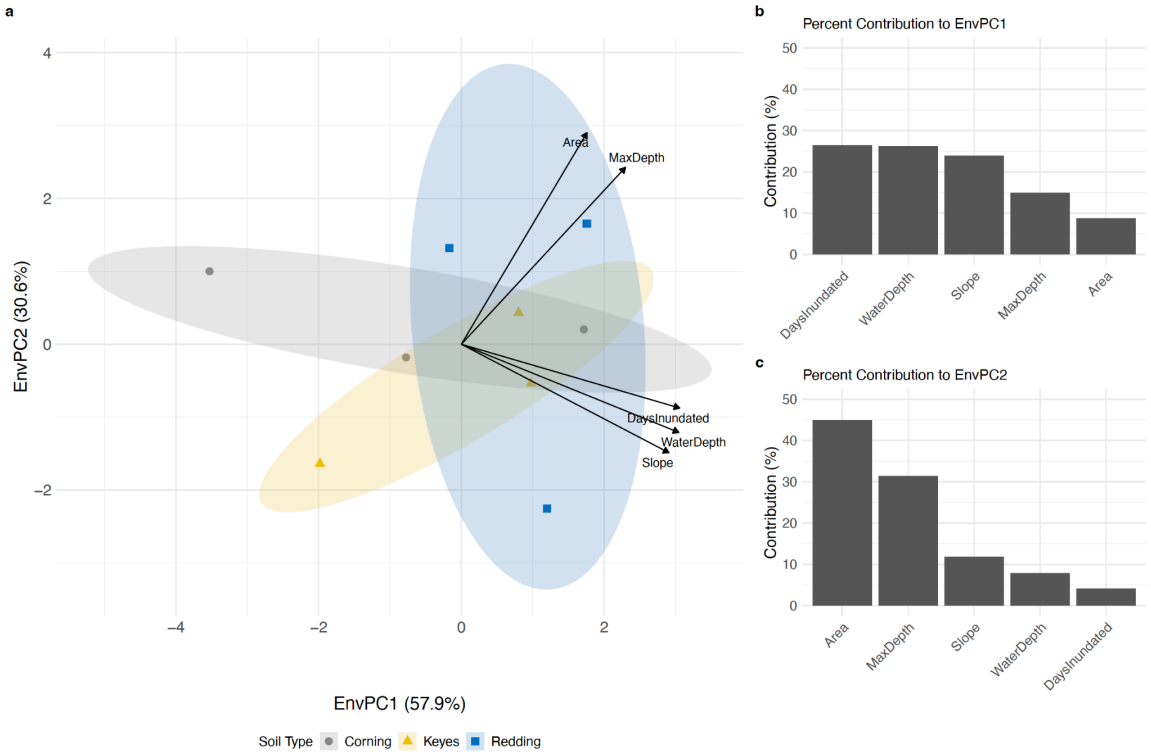

Figure 4. Biplot (a) of principal component analysis of the first two axes for topographical and hydrological variables, %Sand, %Silt, %Clay, pH and EC. Soil types are represented by different colors and shapes, and grouped by filled ellipsis. Barplots of percent contributions for PC1 (b) and PC2 (c) illustrate soil texture explains PC1 and chemical properties of pH and EC explain PC2.

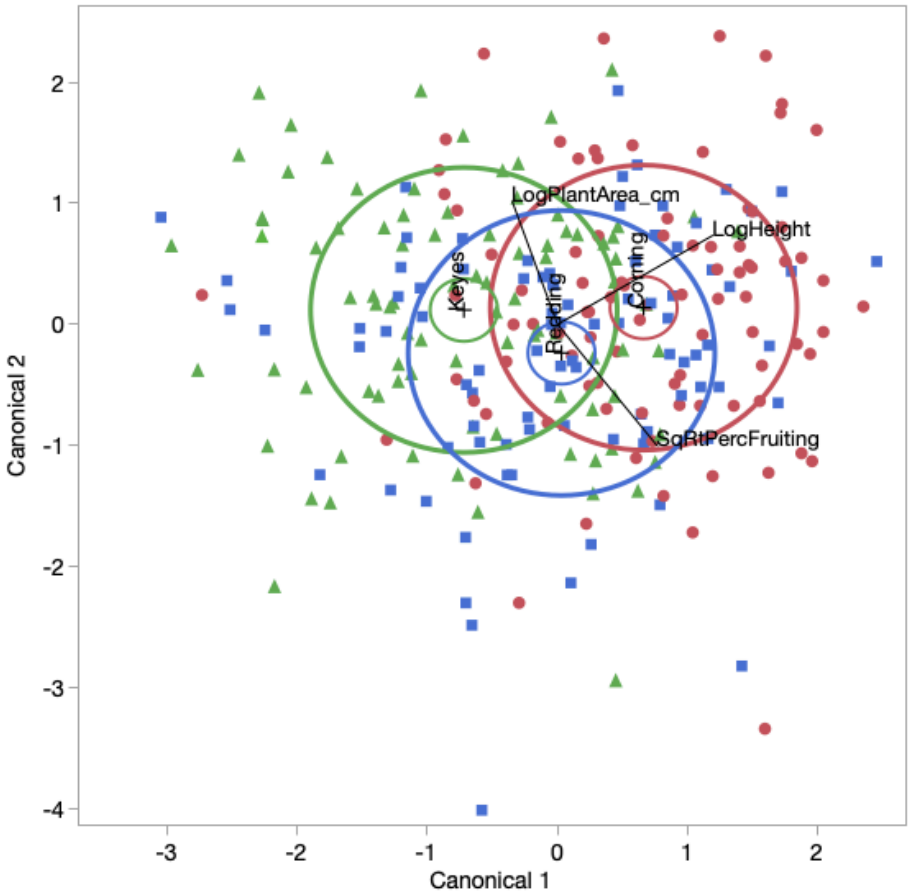

Figure 5. Canonical discriminant analysis (CDA) biplot for Log plant area, Log height, and square root transformed plant maturity by soil type for nine sites showing separation by soil type. I performed a stepwise variable selection analysis to keep statistically significant traits, Log Flowers was the only non significant trait and, thus was removed.

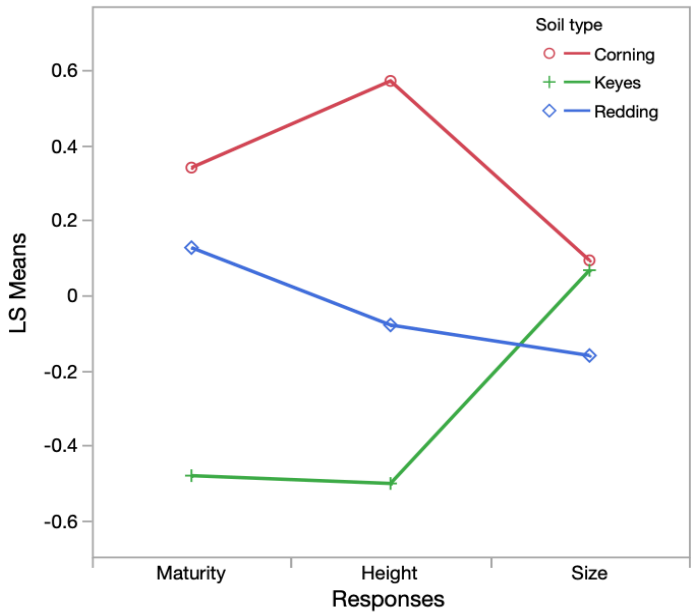

Figure 6. Least square means from MANOVA. Exact F and p-values for soil (27.58, p < 0.0001) and Site nested in soil type (6.94, p < 0.0001). Sphericity test, P > Chisq = 0.612. All within subject interactions are significant, p < 0.0001.

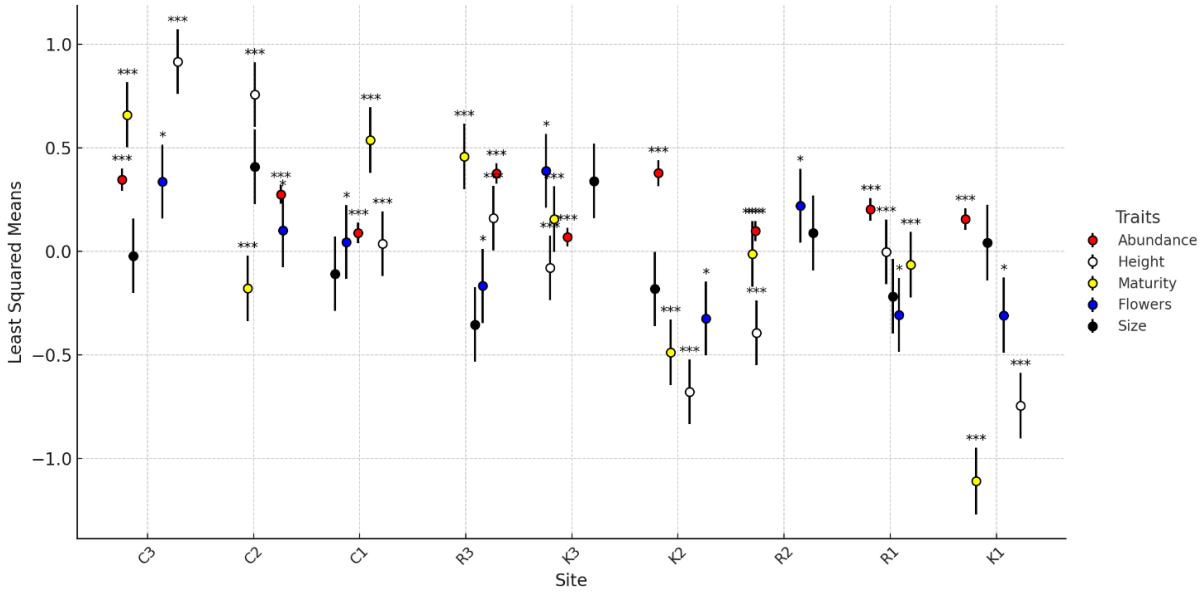

Figure 7. Least square means of meadowfoam abundance and plant morphological traits for each of the nine sites ordered from highest to lowest. C, K and R denote Corning, Keyes and Redding soil types, respectively. Asterisks indicate significant site effects.

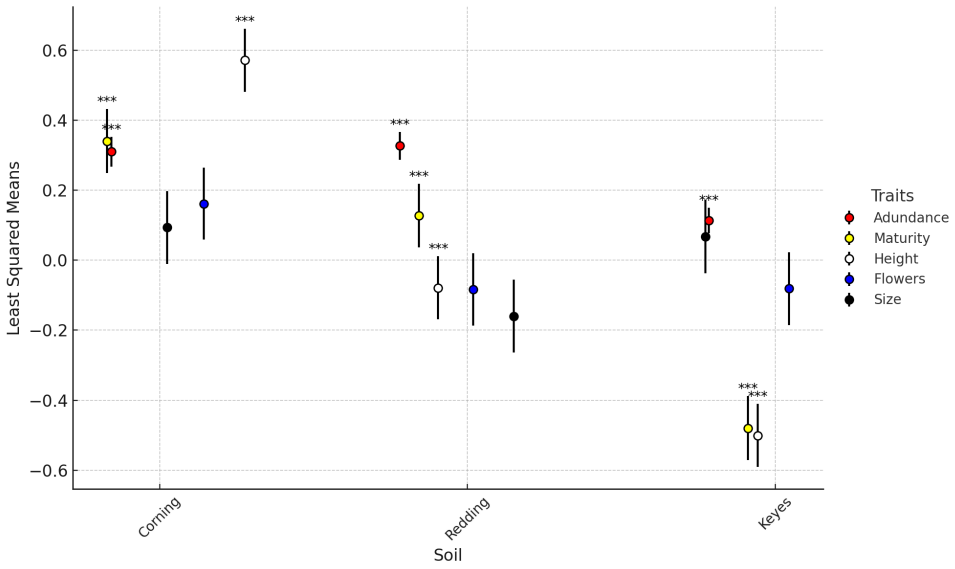

Figure 8. Least square means of meadowfoam abundance and plant morphological traits for soil type ordered highest to lowest. Asterisks indicate significant effects of soil type.

Chapter 2. Soil effects on phenotypic variation in a vernal pool annual plant, *Limnanthes douglasii ssp. rosea* (Meadowfoam)

ABSTRACT

The heterogeneity of soil environments found across vernal pool landscapes begs the question of whether plants are locally adapted and how this relates to conservation of vernal pool plant taxa. To examine the plant-soil relationship in *Limnanthes douglasii ssp. rosea* (meadowfoam), an endemic vernal pool annual plant, I employed a common garden experiment in a greenhouse and assessed plant fitness of plants grown among Corning, Keyes, and Redding soil types from nine populations across a large, intact vernal pool landscape, including three populations local to each soil type. Phenotypic trait measurements indicative of plant fitness (e.g. reproductive output, and plant size) of the reciprocally transplanted populations revealed statistically significant soil type effects. We found that on average, all plants from each soil type performed best in Keyes soils, a rare vernal pool soil type, and that plants grown in Redding soils, the most abundant vernal pool soil series, had the poorest performance. Additionally, plants originating from vernal pools on the Keyes soil type displayed higher fitness than all other plants when transplanted across Corning and Redding soil types. Aside from Keyes-derived plants, local populations rarely performed best in their home soils, however, Corning and Keyes variants were the top two performers in each soil treatment. Our findings indicate that fitness variation of Meadowfoam is significant across a landscape-scale continuum of high and poor-quality soils. This work illustrates that some genotypes of a vernal pool plant species distributed among small-scale soil gradients may be locally adapted to their native soils, whereas others exhibit phenotypic plasticity, allowing them to perform well across different soil types. Thus, managing and maintaining soil variation is a significant component of maximizing genetic variation in vernal pool ecosystems.

INTRODUCTION

Genetic variation among a species' populations represents its evolutionary potential and ability to adapt to rapidly changing environmental conditions (Rice & Emery, 2003). In plant populations, genetic diversity is maintained by dispersal of seeds and pollen; however, the sessile nature of plant species combined with habitat heterogeneity typically results in generations of selection by local environmental conditions, and the evolution of distinct genotypes (Galloway & Fenster, 2000; Linhart & Grant, 1996). As remaining areas of natural habitat become smaller and more fragmented, decreasing size and increasing spatial isolation of plant populations place them at risk of genetic erosion through genetic drift, inbreeding depression, reduced gene flow, and the extinction of local genotypes (Young et al., 1996). Thus, the preservation of natural plant communities and biodiversity depends on the conservation and restoration of species, their habitats, and the mechanisms responsible for structuring and maintaining genetic diversity within and among populations (Lande, 1988; Espeland et al, 2017; Wadgymar et al., 2022).

Since the classic common garden experiments of Turreson (1922), and Clausen, Keck, and Hiesey (reviewed in Núñez-Farfán & Schlichting, 2001) that demonstrated plants across spatial and environmental gradients can form morphologically distinct and locally adapted genotypes, numerous studies have shown that local adaptation plays a fundamental role in shaping genetic variation across plant populations (Linhart & Grant, 1996). Reciprocal transplant and common garden experiments can be used to study local adaptation and genetic variation of plant species in heterogeneous environments (Lechowicz, 1991; Savolainen et al., 2013; McNeily & Antonovics, 1968; Wright et al., 2006; Emery et al., 2011). These experiments directly test for genetic variation in plant populations by comparing fitness of plants from different habitats grown under the same environmental conditions. If locally adapted populations are expected to show higher fitness in their specific habitats or conditions (Kawecki & Ebert, 2004; Raabova et al., 2011). Of particular interest here, edaphic heterogeneity and differences in local, abiotic and biotic conditions of soils have been shown to result in differentiated soil ecotypes and locally adapted populations in plants (e.g. Smith et al., 2011; Lechowicz, 1991; Waser & Price, 1985; Wright et al., 2006; Raabová et al., 2011).

California vernal pools are small, island-like seasonal wetlands that support a unique array of plant species adapted to extreme seasonal cycles of flooding and drought conditions (Barbour et al., 2007). Although vernal pool grasslands once covered nearly half of California's Central Valley, destruction by agriculture and urban development have reduced vernal pool habitat to 5% or less of its historical distribution (Holland, 2009; Witham, 2021). Consequently, remaining vernal pool habitat is highly fragmented and sparsely distributed across complex geomorphic, edaphic and climatic conditions throughout California, and harbors many threatened and endangered status species (Holland, 2009; Barbour et al., 2007).

To help guide conservation and mitigation efforts, researchers developed classification schemes that characterize vernal pools by biogeographic region, geomorphic substrata and plant community assemblages (Holland, 2009; Barbour et al., 2003; Barbour et al., 2005; Buck-Diaz et al., 2012). However, few mitigation criteria exist that consider the limited geographic extent of distinct plant communities, localized population dynamics and unique ecological characteristics of species (Elam, 1998; Schlatter et al., 2016). Ultimately, small-scale edaphic heterogeneity results in vernal pool plant populations distributed over soil gradients that vary over scales of tens to hundreds of meters (Holland & Dains, 1990; Vollmar, 2002). Although patterns of trait variation and adaptation across small-scale hydrological gradients have been demonstrated in several vernal pool plant species (Linhart, 1974; Emery et al., 2009; Emery et al., 2011), it is unknown if vernal pool systems have this scale of soil adaptation. Furthermore, the soil-plant relationship among vernal pool plant taxa is relatively unexplored despite the dire need for conservation and information that can lead to better management of these systems.

To address whether vernal pool plant species may be adapted to vernal pools on specific soil types, I conducted a common garden experiment in a greenhouse using subpopulations (i.e., specific vernal pools) of meadowfoam, *Limnanthes douglasii ssp. rosea*, an endemic, vernal pool, annual plant species and asked the following questions: ***1) Are meadowfoam plants adapted to local soil conditions of distinct vernal pools? 2) Are meadowfoam plants adapted to distinct soil types within an intact vernal pool landscape?*** To answer these questions, I collected seeds and soils from nine vernal pools occurring on three common vernal pool soil types (Redding, Keyes and Corning) across the Merced Vernal Pools and Grassland Reserve (MVPGR), a large and intact heterogeneous vernal pool grassland landscape in Central California (Fig. 1). I germinated seeds and grew seedlings in soil blocks containing soils from each site/soil type combination in a full factorial design, to examine if plant growth and performance varies across (1) distinct vernal pools and (2) different pools but similar soil types.

MATERIALS AND METHODS

Study site

The MVPGR encompasses 2,553 hectares of an intact vernal pool and annual grassland matrix in eastern Merced County, California, and adjacent to the University of California, Merced (ucnrs.org) (Fig. 1). Located on the western edge of the Sierra Nevada foothills and the eastern border of the California Central Valley, the MVPGR experiences a Mediterranean climate, with an average annual rainfall of 12.1 inches from October through September, based on water year data from 1989 to 2017 (cdec.water.ca.gov). The landscape comprises stratified granitic alluvial terraces and volcanic mudflows deposited during the Pleistocene and Miocene epochs, with remnants of ancient alluvial fans (2-4 million years old), forming some of the oldest exposed soils in North America (Marchand & Allwardt, 1981; Vollmar, 2002). The major geological formations—Riverbank, North Merced Gravels, and Laguna—overlie older pyroclastic mudflows of the Mehrten formation (Marchand & Allwardt, 1981; Vollmar, 2002). Initially surveyed by Arkley (1962) and later updated by the USDA and NRCS (Beaudette & O'Geen, 2009), MVPGR soils encompass diverse surficial alluvial deposits, including those formed along river terraces from Sierra Nevada sources and redeposited volcanic alluvium. The dominant vernal pool soil types found on the reserve include Reynor clay (weathered Mehrten lahars), acidic Redding and Corning gravelly clay loams developed on high alluvial terraces, and Keyes sandy-clay loams associated with volcanic mudflows of the Mehrten formation (Toews unpublished data). Redding and Corning soils consist of highly weathered alluvium derived from igneous, metamorphic, and sedimentary rocks, with clay contents between 35-60%, soil acidity ranging from pH 5.2 to 5.7, and bulk densities between 1.10-1.35 and 1.45-1.60 g/cm^3, respectively. In contrast, Keyes soils are derived from reworked andesitic alluvium of the Mehrten formation lahars and exhibit lower clay content (15-25%), a slightly higher average pH (~6), and bulk density of 1.40-1.50 g/cm^3 (USDA.gov; see Chapter 1). Though the parent materials are ancient, recent erosional processes have led to comparatively younger, sandier Keyes soils than Corning and Redding soil types (reviewed in Vollmar, 2002). Together, these soils

represent a diverse mosaic of surficial alluvial deposits that are integral to the ecology of rare and endemic species within the Sierra Foothill Vernal Pool Ecoregion and the MVPGR's extensive vernal pool network (Vollmar, 2002; Witham et al., 2014; USFWS, 2005).

Study system

Limnanthes douglasii ssp*. rosea* (Benth.) C.T. Mason is an annual dicot endemic to vernal pools and ephemeral swells in California (Kesseli & Jain, 1984). The species primarily occurs in the Central Valley and grows along vernal pool margins, germinating in the winter months between November and February then flowering in the spring between March and May (Ornduff & Morin, The Jepson, 2012). *Limnanthes* taxa are thought to be vernal pool edge species and cannot withstand extended periods of completely submerged conditions (Solomeshch et al., 2007). *Limnanthes alba*, a closely related species in the genus, has been shown to be adapted to specific inundation gradients within the edge of vernal pools (Emery et al., 2009). *L. douglasii* ssp. *rosea* was selected for this study because it has an annual life cycle, it inhabits the edge zone along the inundation gradient in vernal pools, is a common vernal pool plant taxa and is abundant on the MVPGR (pers observation) (Fig. 1). *Limnanthes douglasii* ssp. *rosea* is a predominantly outcrossing species, however, all *Limnanthes* species are self-compatible and capable of producing fertile nutlets (Kesseli & Jain, 1985). Kesseli and Jain (1984) found increased rates of homozygosity and inbreeding depression in populations that had both strictly outcrossing and hermaphroditic plants. High rates of habitat loss and fragmentation, as well as intermediate levels of gene flow in *L. douglasii* ssp. *rosea* may be important components for population differentiation and adaptation to heterogeneous environments (Linhart & Grant, 1996).

Seed and soil sampling

Plant seeds and soils were collected from nine semi-randomly selected vernal pools occurring across three soil types (Redding, Keyes and Corning) on the MVPGR. The three soil types were selected based on the following criteria: harbor vernal pools, are representative soil types of remaining vernal pool landscapes in California, and are habitat for *L. douglasii* ssp*. rosea,* hereafter referred to as meadowfoam. Pool selection was accomplished using satellite imagery to create focus areas based on topography, suitable habitat for the species, and diversity of soil types. Soil type was determined using the Soil Survey Geographic data-bases (SSURGO) U.S. Department of Agriculture and Natural Resource Conservation Service Web Soil Survey (websoilsurvey.sc.egov.usda.gov).

Seeds were collected from 15-20 individuals with mature fruits at random sampling points spaced approximately every 2-3 meters throughout the pool. Seeds were collected in the spring 2016 at the end of the species growing season. Rapid desiccation of plants and dehiscence of seeds made it difficult to collect individuals later in the season and, thus, a range of 11-21 families were collected. In cases where family level

replication was not possible due to the comingaling of desiccated plants/seed heads, seeds were collected in bulk by selecting from multiple flower heads following methodology adapted from Emery et al. (2009). Whole plants and seeds were air dried in a greenhouse and then stored at room temperature in the lab until commencement of germination for the experiment.

Soils for the common garden experiment were collected in bulk at the upper edge of each study vernal pool to a 10 cm depth and in the fall of 2016 before the rainy season. I defined the edge zone of each pool using several criteria in the field: the point of maximum ponding for that year determined during weekly field surveys over the 2016 winter, zonation of the target plant species (meadowfoam occurs along margins of pool edge) observed during peak flowering (Feb-Mar 2016), and presence of co-occurring edge species *Eryngium castrense* (Coyote thistle) that is observable long after meadowfoam has senesced and is no longer present (personal obs). Soil samples were immediately transported to the lab, sieved to 2 mm and stored at room temperature until the experiment was initiated.

GREENHOUSE EXPERIMENT

Germination

Vernal pool plants are notoriously difficult to germinate and often have unique germination requirements to break dormancy; e.g. long imbibition, extreme temperatures, and complex microbial interactions (Keeley, 1988; Bliss & Zeddler, 1997; Collinge et al., 2003; personal experience). I conducted a series of pilot experiments to determine best germination conditions for meadowfoam. I found germinating seeds directly in native soils or potting mix resulted in low germination rates and had better germination success following the petri dish method reported by Toy and Willingham (1966). Toy and Willingham show 75% of meadowfoam seeds in petri dishes germinated in 6-12 days at 60°F (15°C), the reportedly best germination temperature for the species. The highest germination rates in my preliminary germination trials were based on Toy and Willingham (1966) methodology. Approximately 68% of seeds in petri dishes germinated over a three-week period with most seeds germinating within the first week, compared to 30% and <15% germination rate of seeds that were sown into potting mix (Sunshine No. 1) and native vernal pool soil, respectively.

Seeds that did not germinate in the petri dish method were found to be covered in a fungus or deformed and decaying. Further investigation found these seeds to be empty or infertile, presumably due to herbivory or lack of fertilization prior to experiment. Seed viability was determined prior to the greenhouse experiment by performing cut tests (Ooi & Whelan, 2005) and by assessing seed mass and deformities of randomly sampled seeds from each site x family combination. Seeds that were inflated, and/or small and deformed were typically non-viable and excluded from subsequent analysis and greenhouse experiment.

In February 2017, ~2,500 seeds representing 140 plant families and bulk collections were weighed then germinated to use in the common garden experiment in the greenhouse. All seeds from each population and or family were placed on moistened Whatman No. 1 filter paper in 3" petri dishes (30 seeds max per dish) and placed in a germination cabinet at 15°C for two weeks and until the bulk of seeds had germinated. Each dish was randomly assigned to a shelf position in the germination cabinet and rotated to different positions (1-5) on a weekly basis to account for microclimate variation in the germinating cabinet. A total of 1,582 seeds (64%) germinated over the course of two weeks, representing 952 seeds at family level and 630 from the bulk seed collection.

Maternal effects

To investigate differences of soil origin on seed mass and account for maternal effects for plants used in the experiment, I calculated the average family-level seed mass for each plant family. Family level seed mass was calculated by dividing the total seed weight by the number of seeds per family. Seeds of each family were weighed to the +-0.001 mg on a microbalance. Family-level information was not retained for plants grown from the bulk collected seed batch, however, I used the average site seed mass to include these individuals in statistical analysis (n=30).

Experimental design

To examine the effect of soil type on plant growth and performance, the common garden experiment was designed so that at least ~30 individuals from each of the nine vernal pools ("origin sites") would be planted within and between blocks ("destination sites") composed of the three different soil types. The final design included 33 individuals*3 sites*3 soil types*3 blocks/soil type for a total of 891 plants used in the experiment. Germinants were transferred from petri dishes into 66 ml Ray Leach Cone-tainers (Stuewe & Sons Inc., Tangent, OR, USA) containing vernal pool soil. The cone-tainer trays were placed into large tubs and filled with water (Fig 1). Extra seedlings were kept in trays of their native soil type and seedlings that died within the first two weeks after initial planting were replaced with members originating from the same soil type and/or site when possible. The plants in each block were bottom watered and the water level maintained just below the soil surface for the duration of the experiment. Seedlings of any species that emerged from the background seed bank of the soils were removed when found to reduce any confounding effects of competition. The experiment was kept in ambient conditions in a greenhouse at UC Merced until the cooling unit failed and internal temperatures reached 105°F, resulting in water dropping slightly below (1-2 cm) the soil surface and many plants dying. All blocks were expediently and carefully moved to a temperature-controlled greenhouse (80°F) at Merced Community College, Merced, CA. Plants were still in a vegetative stage and did not have reproductive structures when moved.

Plant growth and performance traits

I used survival, number of pedicels produced and biomass as measures of plant performance and as response variables in our models to test for soil adaptation. Survival and number of pedicels are directly related to reproductive success and evolutionary fitness, i.e. the ability to survive and reproduce. To assess the effect of soil type on plant growth, I measured phenotypic variation in final plant height, root length, size (biomass) and plant maturity (the ratio of fruiting to non-fruiting flowers) as a proxy for plant phenology non-zero data.

Data analysis

Normalization of data

Response variable data that were not normally distributed were either log transformed (log x+1), square-root or cube-root transformed to meet assumptions of parametric analyses and to improve homogeneity of variance and reduce heteroscedasticity. Normalization of data was confirmed using goodness of fit tests of distribution models using JMP statistical computing software (version 17, SAS).

Maternal seed mass

To determine the effects of site and soil type on maternal seed mass I constructed a standard least squares linear model with log-transformed maternal seed mass as the response variable. Thus, soil type, site nested within soil type, and their interactions were included as main effects in the final model. I evaluated and determined the best model performance using Akaike information criterion (AIC) scores of the untransformed and transformed models. I initially included block (i.e., destination site) as a random effect, however, this did not improve model fit and was excluded. I conducted multiple comparisons and post hoc tests (Tukey HSD) on least square means to detect significant differences of seed mass by soil origin.

Plant growth and performance

To determine the effect of the nine distinct vernal pool sites on plant performance, i.e. ‘site’ level effects, I performed separate linear or generalized regression models with maternal seed mass as a covariate, and seed origin and destination site (i.e., block) and interaction of origin and destination site as fixed effects. Survival was analyzed using a logistic regression model with a binomial data distribution and logit probability link specification (version 17, SAS). To determine the effect of the three soil types, models included seed mass as a covariate, and origin soil type, destination soil type,origin x destination soil type interaction, and block nested in soil type as main effects for all plant performance and plant growth models. Effect tests on the number of pedicels and plant biomass were conducted using generalized linear models with a log normal distribution and gamma distribution, respectively. Pedicels data were cube root transformed and biomass data were square root transformed prior to analysis and transformations were evaluated using AIC scores among models. The main effects and interactions on growth measures of final height and root length were tested using standard least squares linear

models on normally distributed cube-root transformed data. I performed generalized linear regressions on root:shoot ratio and plant maturity data with log normal and gamma model distributions and identity model probability link, respectively (JMPSAS). Significant differences between sites and soil types were determined with Tukey-HSD post hoc tests from multiple comparisons of the different factors.

RESULTS

The analysis at the site level (Table 1A) revealed highly significant effects of seed mass on all measured performance traits of meadowfoam, including survival ($p < 0.0001$), number of pedicels ($p < 0.0001$), and biomass ($p < 0.0001$). Similarly, maternal seed mass had a significant effect on performance traits at the soil type level: survival ($p = 0.0002$), pedicels and biomass ($p < 0.0001$, respectively). Analysis of growth traits were conducted on non-zero data. Significant effects of maternal seed mass were detected for growth traits of plant maturity ($p = 0.0195$), and with only near-significant effects on final plant height ($p = 0.0612$). Significant effects of maternal seed mass on other growth related traits of root length and root:shoot ratio were not detected.

1) Are meadowfoam plants adapted to local soil conditions of distinct vernal pools?

Survival: Analysis at the site level revealed significant effects on performance traits. (Table 1A). Overall, 48.7% of plants used in the experiment survived to produce reproductive structures or to the end of experiment (Figure 3a). Survival varied significantly across blocks, with 33% of origin sites performing best in their local soil conditions of their home vernal pool than at away vernal pool soil blocks, notably sites K3, K2 and C2 (Figure 3a & 3b). Alternatively, blocks C1 and R3 had the lowest survival in their home pool soils. A logistic regression on survival with all main effects included in the full model showed maternal seed mass to be the only significant predictor, despite the large variation in survival across sites and blocks. A reduced model with the interaction term removed showed significant effects for maternal seed mass ($p = 0.0002$), seed origin ($p = 0.031$), and destination site ($p < 0.0001$). Evaluation of AIC scores determined the reduced model to be a better fit and these results were retained. Tukey HSD pairwise comparisons of origin pairs revealed significant survival differences, where K2 had significantly higher survival odds compared to R3 and R2. All other pairwise comparisons of origin sites were not significant. Tukey HSD pairwise comparisons showed highly significant differences among most block pairs. Destination site K2 showed the highest least squares survival rate and destination K1 had the lowest least squares survival rate, both significantly different from most other blocks (Fig 3c). The strong block effect indicates that survivorship patterns were different across destination sites.

Pedicels: Significant effects were observed for seed origin ($p = 0.0319$), destination site ($p < 0.0001$), and their interaction ($p = 0.04$) (Table 1A). Plants originating from sites K3, K2, and C2 produced more pedicels in their home soils than compared to plants from

other sites and when grown at ‘away’ sites (Figure 4). Site K3 at home had the highest least square means and was significantly different from other pairs, while sites K2 and C2 had relatively high but not significantly different pedicel counts in their local home pool soils. Site R1 produced significantly more pedicels in K3 compared to pedicel counts at home. All other origin x destination comparisons were not significantly different from one another. Tukey HSD tests on the effect of origin on pedicel counts showed site K2 produced significantly more pedicels than other sites, with site R3 producing significantly lower pedicels than all other sites. Plants grown in destination sites K3 and K2 produced significantly more pedicels than other blocks. Plants grown in destination sites K1, R2 and C1 produced significantly fewer pedicles compared to other destination sites.

Biomass: A generalized regression on biomass with all main effects included in the full model showed the interaction effect of origin x destination to be non-significant and was removed from the model to improve model fit. The reduced regression model showed a significant positive relationship for maternal seed mass ($p < 0.0001$), seed origin ($p = 0.0024$), and destination site ($p < 0.0001$) (Table 1A). Biomass was highly variable across the experimental blocks (Figure 5). Origin sites K2 and K3 had the highest biomass, with Tukey HSD comparisons indicating significant differences between K2 and other origin sites. Alternatively, origin R3 produced plants with significantly lower biomass compared to other origin sites. Plant biomass was significantly higher in destination sites K2 and K3 with site K3 significantly different from other destination sites. As with survival and pedicel count, least square means for plant biomass in blocks K1 and R2 was significantly lower than compared to other blocks.

2) Are meadowfoam plants adapted to soil types within an intact vernal pool landscape?

Plant performance: At the soil type origin level (Table 1B), seed mass showed significant effects across all performance traits: survival ($p = 0.0002$), number of pedicels ($p < 0.0001$), and biomass ($p < 0.0001$). The effect of soil type destination had a strong impact on survival ($p < 0.0001$), number of pedicels ($p < 0.0001$), and biomass ($p < 0.0001$). Fixed effects for origin soil type and the interaction between origin soil and destination soil were not significant for any traits, suggesting that performance was more strongly affected by the destination soil and block. Block effects nested within destination soil were significant for all traits ($p <0.0001$), indicating variability within soil types and/or large block effects on plant growth. Survival, pedicel counts and biomass were highest in Keyes soils, regardless of the soil origin (Fig 6). Tukey HSD pairwise comparisons for pedicles indicated Keyes soil is significantly different from other soil pairs. Plants originating from seed of Keyes soil type produced more pedicles on average than any other soil type, but Tukey HSD post hoc tests showed only slight origin soil type effects between Redding and Keyes soils ($p=0.069$). Least square means on pedicel counts were highest in the Keyes soil treatment. Tukey post hoc tests showed significant differences of pedicels produced in Keyes soils compared to Corning ($p<0.0001$) and

Redding ($p<0.0001$). Corning and Redding pairwise comparisons were not significantly different.

Plant growth: Analysis of plant growth traits (Tables 2-5) further highlights the significant influence of soil type on meadowfoam. Final height was significantly affected by destination soil ($p < 0.0001$) and block nested within destination soil ($p = 0.0005$). Seed mass showed a marginal effect ($p = 0.0612$) and origin was not significant (Table 2). Tukey post hoc tests on plant height by soil types showed plants from all types had significantly higher least square means in Keyes and Corning soil, with plants growing taller in Keyes soils on average when compared to other groups (Figure 7). Root length was significantly influenced by destination soil ($p < 0.0001$) and block within destination soil ($p < 0.0001$) (Table 3). Root length was longest in plants grown in Keyes soils for all pairs, regardless of soil origin, and plants in Redding soils had the shortest root lengths (Figure 7). Tukey HSD post hoc tests on soil origin and destination soil revealed that root length differed significantly across all levels of soil treatments, whereas origin comparisons were not significantly different. For root-to-shoot ratio, significant effects were observed for origin soil ($p = 0.0447$), destination soil ($p < 0.0001$), and their interaction ($p = 0.0494$), whereas seed mass and block nested in soil type were not statistically significant (Table 4). Tukey HSD pairwise comparisons of origin and destination soils showed similar results to root length, where the root:shoot ratio was highest in plants grown in Keyes soils for all pairs and lowest in Redding soils (Figure 7). Plant maturity, measured as the ratio of fruiting to non-fruiting flowers, showed significant effects of seed mass ($p = 0.0195$), destination soil ($p = 0.0297$), and block within destination soil ($p < 0.0001$) (Table 5). Least square means of plant maturity were significantly higher in Keyes plants grown in sympatric soil conditions compared to other soil types. Corning plants had higher least squares for plant maturity when grown in sympatric soil types compared to plants of other soil origins, though Tukey pairwise comparisons show these are not significantly different when compared to other origin soil x destination soil pairs.

Maternal seed mass: Prior to analyses on performance and growth traits, I constructed a standard least squares linear model to investigate site and soil type effects on field -collected seed mass. Soil and site nested in soil type were included as fixed effects in the model with log transformed seed mass as the response variable. Both site and soil type significantly affected mean seed mass ($p <0.0001$). Tukey post hoc tests showed large variation in average seed mass among sites and soil types, with sites K2, K3, and C2 having the highest mean seed mass compared to other groups (Figure 2). Tukey HSD pairwise comparisons at the soil type level showed that mean seed mass differed significantly among the three soil types. Keyes soils produced significantly heavier seeds compared to Redding ($p <0.0001$) and Corning ($p <0.0010$) soils, and seeds from Redding soils produced significantly smaller seeds than both Keyes and Corning soil types (0.0126) (Figure 2).

DISCUSSION

Conservation of any particular species requires maximizing the preservation of genetic variation, especially for endangered species and ecosystems (Halbur et al, 2014; Ramp & Collinge, 2006). Thus, a major goal of habitat restoration is to establish populations that harbor high levels of genetic variation and enhance a given species' adaptive capacity to mitigate impacts of environmental change (Shay et al, 2021; Rice & Emery, 2003). Vernal pool plant species have demonstrated genetic variation and adaptation to environmental gradients across both broad and fine spatial scales (Ayres, 2015; Emery, 2009; Emery et al., 2009; Linhart, 1988; Sloop et al., 2011; Torres-Martínez & Emery, 2016; Torres-Martínez et al., 2019). It is unknown, however, if small-scale edaphic heterogeneity characteristic of vernal pool landscapes exhibits this scale of genetic diversity or adaptation despite the dire need for conservation in these systems.

I addressed this critical knowledge gap in vernal pool systems through a controlled greenhouse experiment to test the effects of soil type on plant growth and performance in meadowfoam. Here, I show that this highly outcrossing species has a differential response to soil conditions at both local site and broader soil type levels, highlighting the importance of soil heterogeneity and local genetic diversity on plant fitness in vernal pool systems. I found both an adaptive signal and evidence for plasticity in response to distinct soil conditions within vernal pools, with both maternal seed mass and soil type significantly influencing plant performance and growth traits in meadowfoam.

In all treatments, maternal seed mass significantly affected survival, pedicel production, and biomass at both the site and soil type levels, suggesting a transgenerational plasticity effect on plant performance (Herman & Sultan, 2011). That is, the maternal growth environment affected subsequent seedling performance, whereas larger seeds resulted in higher survival rates, greater reproductive output, and biomass. Initial field-collected seed mass showed significant variation across sites and soil types, with Keyes soils producing significantly heavier seeds compared to Redding and Corning soils. This variation in seed mass likely contributed to observed differences in plant performance and growth traits, supporting the hypothesis that maternal investment in seed size can enhance seedling establishment and subsequent growth (Galloway, 2005). Significant effects of soil type on seed mass and maternal seed mass on plant fitness suggest that the relationship between the seed's genetic background and the environment in which it grows is complex and context-dependent. This finding is particularly relevant in vernal pool ecosystems, where larger seeds may provide a competitive advantage in highly variable and often harsh environmental conditions (Venable & Brown, 1988)

Although maternal seed mass was a significant predictor of higher plant fitness, the effect of maternal seed mass on root length and the root-to-shoot ratio was not significant while site origin and soil destination type effects were significant. This suggests that the effects from the growing environment were stronger than the

transgenerational effects of seed mass. Furthermore, plants from some sites exhibited higher survival and pedicel production in their local soil conditions, signaling a positive response to their home vernal pool soil conditions. However, plants from other sites had lower survival rates in their home soils, suggesting that local soil conditions alone do not universally predict adaptive survival outcomes. It is important to note that a large die-off event, attributed to a greenhouse equipment failure, resulted in several plants in certain blocks dying. This could influence the statistical results and interpretation of the study in several important ways; e.g., reduction of statistical power, increased variance of response factors and confounding the effects of treatment and the die-off event. Nonetheless, statistically significant effects of site and soil type were observed.

Growth traits such as plant height and root length were significantly influenced by the destination soil, with plants growing taller and having longer roots in Keyes and Corning soils compared to Redding soils. The root-to-shoot ratio of plants was highest when grown in their same type compared to plants from other soil types, suggesting growth between above-ground and below-ground plant structures might be an adaptive trait to specific soil types, underscoring the critical role of soil type in plant performance. For instance, the higher root-to-shoot ratio in plants grown in their native soil type may reflect an optimization to cope with soil-specific moisture retention, nutrient availability, and structure (Schwinning & Ehleringer, 2001; Yamauchi et al, 2021). In vernal pool environments, where soils shift dramatically between saturated-anaerobic and dry-aerobic conditions, a high root-to-shoot ratio could be important to maximize water use, nutrient acquisition, and metal regulation during saturated conditions, and minimize water loss in dry periods (Hobson & Dahlgren, 1998). This might allow meadowfoam plants to modulate their growth patterns in order to balance the demands of root expansion for water and nutrient uptake with shoot growth to support photosynthesis and reproduction in an environment where timing and availability of resources are unpredictable and tightly coupled with soil characteristics (Bauder, 2005). For example, Rubio and Lavado (1999) found that two flood-tolerant grasses adapted their biomass allocation patterns under inundated conditions to reduce biomass and oxygen demand of the root system, leverage increased nutrient availability in waterlogged soil, and improve nutrient uptake efficiency.

Significant site destination and soil destination type effects on plant maturity suggest that soil variation affects phenology. I measured plant maturity as the ratio of fruiting to non-fruiting flowers, whereas a higher ratio of fruiting to non-fruiting flowers indicates a greater proportion of flowers have reached a more advanced stage in their reproductive cycle. Differences in plant maturity, i.e. flowering time, have been detected in other vernal pool plant species across landscape scales (Schiller et al., 2000) and between hydrological zones within pools (Linhart, 1988; Emery, 2009). Isolation by phenology can lead to isolated mating pools and barriers to gene flow that result in temporally segregated and genetically differentiated populations (Peters & Weis, 2019). Furthermore, several studies have attributed high genetic diversity within populations

and individual vernal pools to the heterogeneous and isolated nature of these habitats (Ayres et al., 2007; Gordon et al., 2012; Ramp et al., 2008). The differentiation in plant maturity observed in this study could be a response to localized environmental conditions, such as soil type and microhabitat variation, reinforcing the role of environmental heterogeneity in shaping genetic and phenotypic diversity within vernal pool plant species. This phenological differentiation may serve as an adaptive mechanism to optimize reproductive success under varying ecological conditions, further highlighting the ecological and evolutionary significance of maintaining diverse vernal pool habitats (De Jong, 2005).

The significant interactions between origin and destination site for the number of pedicels and root:shoot ratios suggests genotype-by-environment (GxE) interactions. These interactions indicate that the reproductive success of meadowfoam and allocation of resources for above or below ground plant growth is not solely dependent on genetic makeup or environmental conditions independently, but rather on the specific combination of both. Such GxE interactions are important to maintain genetic diversity within populations and can drive local adaptation (Kawecki & Ebert, 2004; Shay et al., 2021). In this experiment, plants from Keyes soils performed well across the experiment on average, but had highest fitness in their local soil conditions, suggesting a potential trade-off between plasticity and soil adaptation. The superior fitness of plants originating from, and grown into, Keyes soil supports theories of local adaptation, where populations are genetically fine-tuned to thrive in their native environments (Clausen, Keck, & Hiesey, 1940; Hereford, 2009). If this genetic differentiation of Keyes soil genotypes is generally adaptive, it will be important to understand why the Keyes genotypes do not take over in other pools, but there could be many reasons, e.g., the limited distribution and rarity of Keyes soils in this ecosystem, barriers to gene flow, and limited dispersal abilities.

Additionally, my results show that both origin and destination site and soil type profoundly affect plant performance across all measured traits. Specifically, Keyes soils consistently supported plants with higher survival rates, greater biomass, and more reproductive structures (pedicels) compared to plants when grown in either Redding or Corning soils. Keyes soils developed from redistributed andesitic alluvium derived from cemented volcanic mudflows of the Mehrten formation are younger, and potentially more fertile, than the older, more weathered soils found in high alluvial terraces, such as Redding and Corning clay loams of the North Merced Gravels formation (Marchand and Allwaldt, 1981). The superior performance of meadowfoam plants in Keyes soil may be attributed to more favorable physical and chemical soil properties, e.g., better water retention and nutrient availability, which are critical in the dynamic conditions of vernal pools. Similarly, Corning and Redding had crossing reaction norms in survival where each performed best in their local soil conditions, indicating potential adaptive differentiation between them, although this was not found to be statistically significant. Ultimately, these results suggest that while some genotypes may be locally adapted to

their home soils, others exhibit phenotypic plasticity, allowing them to perform well across different soil types, potentially leading to transgenerational genetic effects of seed mass. This is an important future direction of research.

Interestingly, Keyes soils are among the rarest soil types associated with California vernal pools, whereas Redding and Corning soils represent some of the most extensive soil types in these landscapes (Smith & Verrill, 1998). Grime (1977) theorized that plant species might exhibit different growth strategies based on soil resource availability, leading to adaptive traits that maximize performance in specific environments. The higher fitness of plants in the rarer Keyes soil could indicate ecological specialization, and aligns with the concept that rare or patchy soil types could harbor specific adaptations in plant species, e.g., adaptations to serpentine soils (Brady et al., 2005). Additionally, these findings highlight the adaptive significance of rare soils in supporting unique plant populations, suggesting that certain soil types may offer specialized conditions that foster plant success even across different environments (Brady et al., 2005). Such soils not only support niche specialization but may also enhance phenotypic plasticity and adaptability, which are critical for species persistence in fragmented and heterogeneous landscapes (Jump & Peñuelas, 2005).

Ultimately, the insights gained from this study have significant implications for the conservation of vernal pool ecosystems and their endemic plant species. Understanding the specific soil requirements and the role of seed mass in plant performance can inform restoration strategies (Elam, 1998; Wacker & Kelly, 2004). For instance, ensuring the availability of Keyes soil or soil amendments that mimic its properties, while maintaining a high degree of soil heterogeneity, could enhance the success of restoration efforts and promote adaptive genetic differentiation. Moreover, maintaining a diverse seed bank with a range of seed sizes can increase the resilience of plant populations to environmental variability (Rice & Emery, 2003; Wambugu et al., 2023).

CONCLUSION

This research provides new insights into the ecological and evolutionary dynamics of plant-soil interactions of vernal pool endemic plant species. The greenhouse experiment highlights the importance of soil type and maternal investment in seed mass as key determinants of plant fitness in *L. douglasii* ssp. *rosea*. These findings underscore the necessity of considering both genetic and environmental factors in conservation planning and restoration efforts. By addressing the ecological and evolutionary dynamics of vernal pool plants, this study contributes valuable knowledge for preserving these unique and threatened ecosystems.

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Tables

Table 1. (A) Site level analysis of fixed effects of seed mass, seed origin, destination (block), and interaction of seed origin and destination on performance traits, survival, number of pedicels, and biomass. **(B)** Soil type level effect tests of seed mass, origin soil type, destination soil type, soil type interaction, and block nested in soil type on performance traits, survival, number of pedicels, and biomass of the greenhouse experiment. Main effects and interactions for survival were tested using logistic regression models. Effect tests for pedicels and plant biomass were conducted using generalized regression models with a log normal distribution and gamma distribution, respectively. Pedicels data were cube root transformed and biomass data were square root transformed prior to analysis.

(A) Response variable at the site level	**Effect Test**	**DF**	**ChiX2**	***P value***
Survival	Seed mass	1	14.8738	**0.0001***
	Origin site	8	11.2459	0.1882
	Destination site	8	10.0267	0.2632
	Origin soil x Destination site	64	69.7962	0.2891
Pedicels	Seed mass	1	22.5357	**<.0001***
	Origin site	8	16.8366	**0.0319***
	Destination site	8	34.4133	**<.0001***
	Origin x Destination site	64	84.4463	**0.0444***
Biomass	Seed mass	1	19.4094	**<.0001***
	Origin site	8	11.9591	0.153
	Destination site	8	24.3500	**0.0020***
	Origin x Destination site	64	68.1782	0.3372
(B) Response variable at the soil level	**Effect Test**	**DF**	**ChiX2**	***P value***
Survival	Seed mass	1	6.9058	**0.0002**
	Origin soil type	2	136.7651	0.3918
	Destination soil type	2	20.7352	**<0.0001**
	Origin soil type x Destination soil type	4	13.6717	0.1410
	Block [Destination soil]	6	1.8739	**<0.0001**
Pedicels	Seed mass	1	24.2239	**<.0001***
	Origin soil type	2	0.4637	0.7931
	Destination soil type	2	36.9848	**<.0001***
	Origin soil type x Destination soil type	4	3.5636	0.4683
	Block [Destination soil]	6	186.5472	**<.0001***
Biomass	Seed mass	1	22.1414	**<.0001***
	Origin soil type	2	2.7357	0.2547
	Destination soil type	2	52.6986	**<.0001***
	Origin soil type x Destination soil type	4	5.6280	0.2287
	Block [Destination soil]	6	204.4468	**<.0001***

Table 2. Standard least squares model of Soil type-level analysis of square root final height.

Trait	Effect	DF	SS	F Ratio	Prob > F
	Seed mass	1	0.2720	3.6116	**0.0581**
	Origin soil	2	0.3438	2.2825	0.1033
Final height	Destination soil	3	3.9520	26.2341	**<.0001***
	Origin soil oil x Destination soil	4	0.4108	1.3636	0.2458
	Block [Destination soil]	6	1.8618	4.1198	**0.0005***

Table 3. Standard least squares model of cube-root root length.

Trait	Effect	DF	SS	F Ratio	Prob > F
	Seed mass	1	0.0136	0.0500	0.8233
	Origin soil	2	0.4316	0.7913	0.4542
Root length	Destination soil	3	9.1341	16.7450	**<.0001***
	Origin soil oil x Destination soil	4	1.0561	0.9681	0.4253
	Block [Destination soil]	6	9.4188	6.9067	**<.0001***

Table 4. Generalized regression with log normal dist. on cube root root:shoot.

Trait	Effect	DF	ChiX2	P > ChiX2
	Seed mass	1	0.4515	0.5016
	Origin soil	2	6.2157	**0.0447***
Root:Shoot	Destination soil	3	35.6235	**<.0001***
	Origin soil oil x Destination soil	4	9.5170	**0.0494***
	Block [Destination soil]	5	4.5131	0.4781

Table 5. Generalized regression with gamma plant maturity.

Trait	Effect	DF	ChiX2	P > ChiX2
	Seed mass	1	5.4542	**0.0195***
	Origin soil	2	3.9219	0.1407
Plant Maturity	Destination soil	3	7.0312	**0.0297***
	Origin soil oil x Destination soil	4	7.3296	0.1195
	Block [Destination soil]	5	28.3620	**<.0001***

Figures

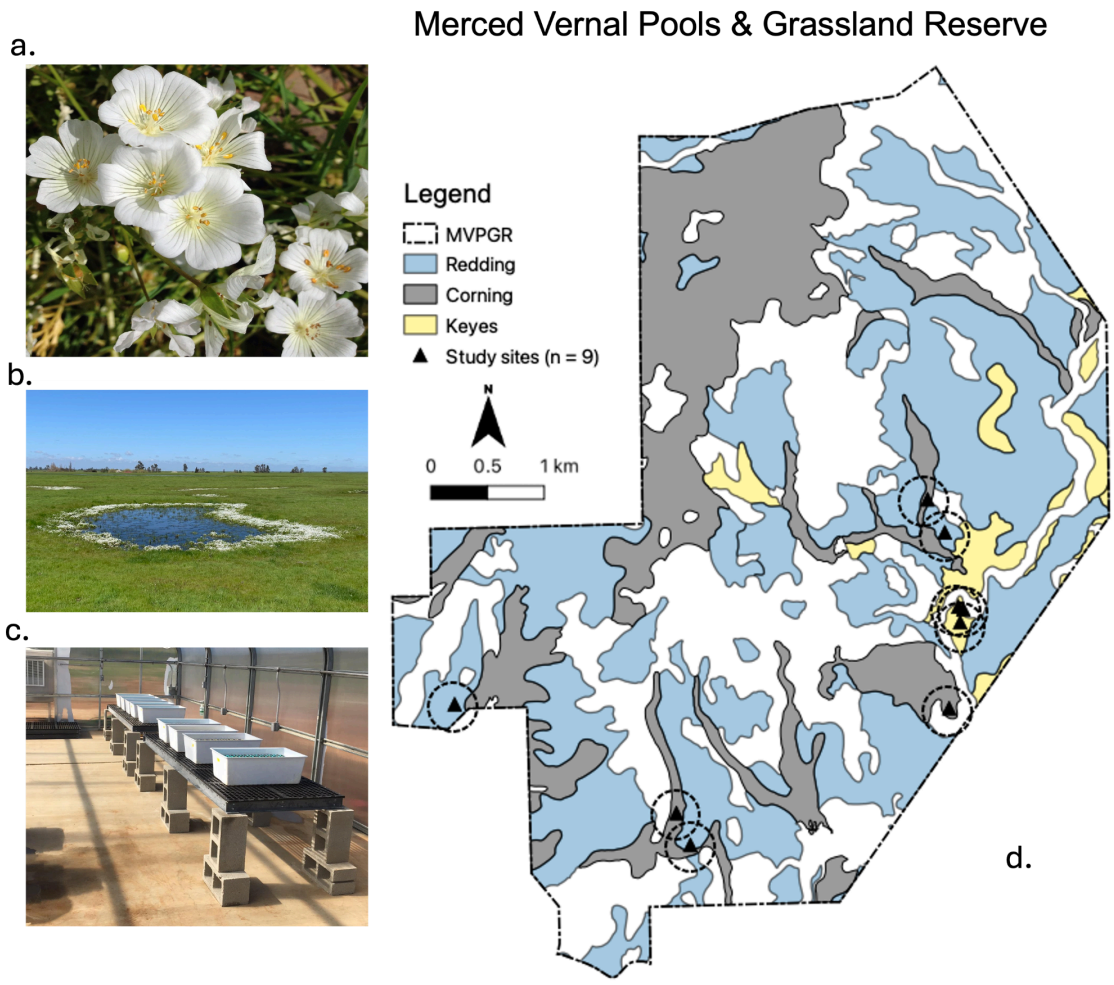

Figure 1. (a) Picture of focal species *Limnanthes douglasii subsp. rosea* (meadowfoam), **(b)** vernal pool with meadowfoam growing along pool margins, **(c)** nine experimental soil blocks in the greenhouse, and **(d)** map of the Merced Vernal Pools & Grassland Reserve with nine study sites across three soil types of Redding (blue), Corning (gray), and Keyes (yellow), in order from most to least abundant soil types.

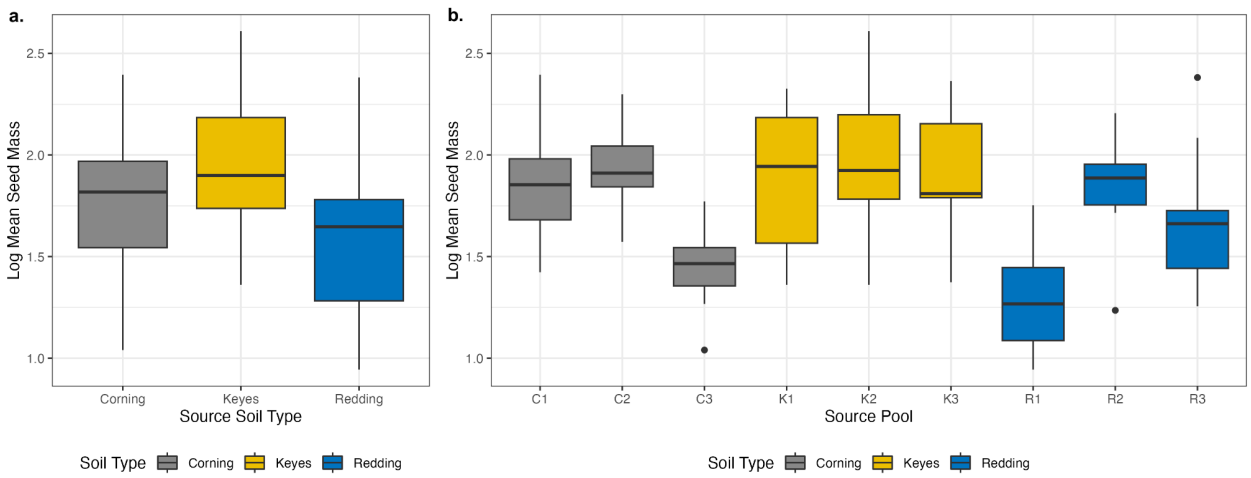

Figure 2. Box plots of mean log transformed maternal seed mass by **(a)** soil type ($p < 0.0001$) and **(b)** site ($p < 0.0001$).

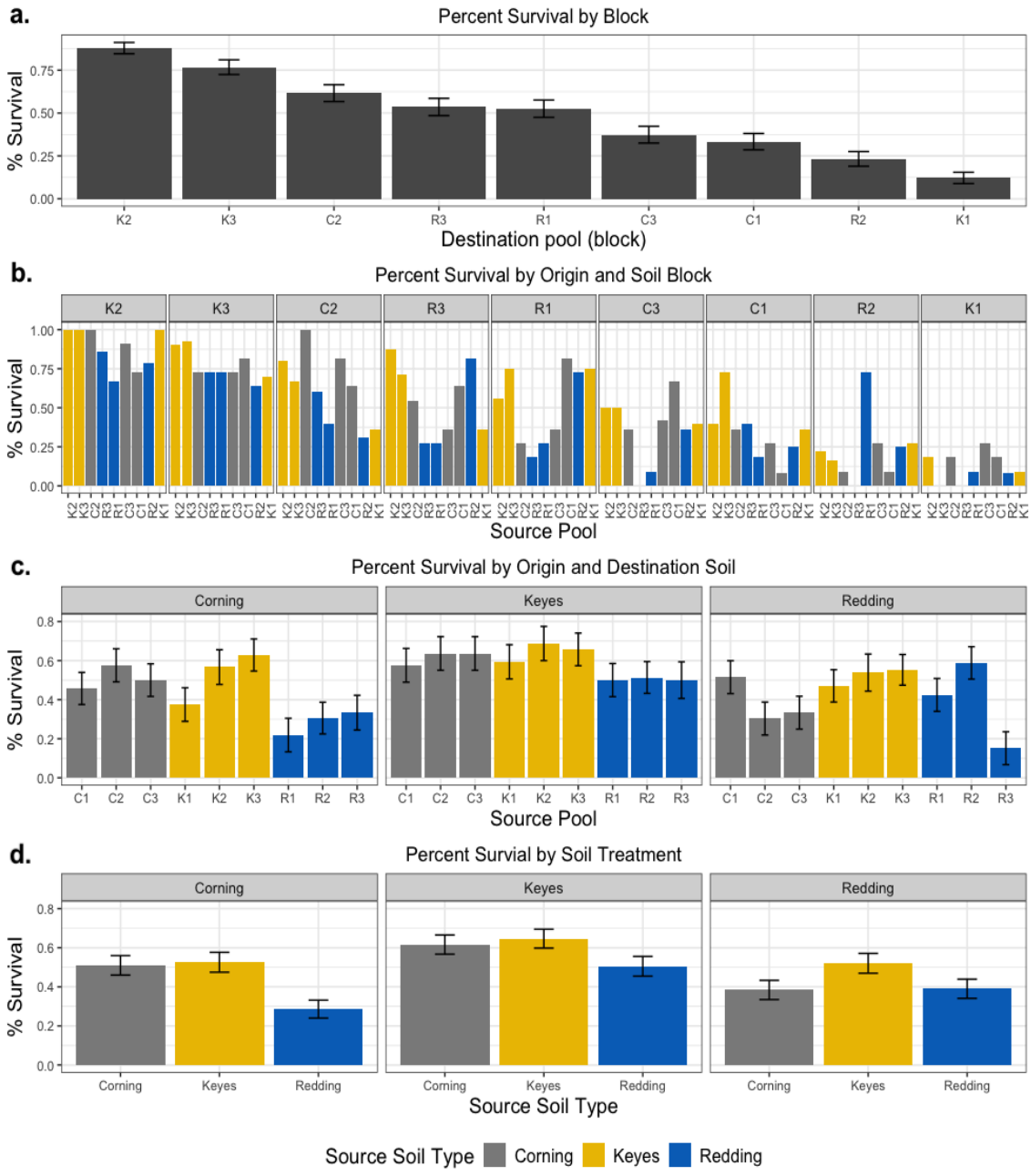

Figure 3: Percent survival by **(a)** block**, (b)** site vs. block, **(c)** site vs. soil destination and **(d)** soil origin vs. soil destination. Destination sites (b) and destination soil types **(c-d)** are indicated by the gray text box above bars. Source 'origin' sites and soil types are indicated by labels on the x-axis **(b-d)**.

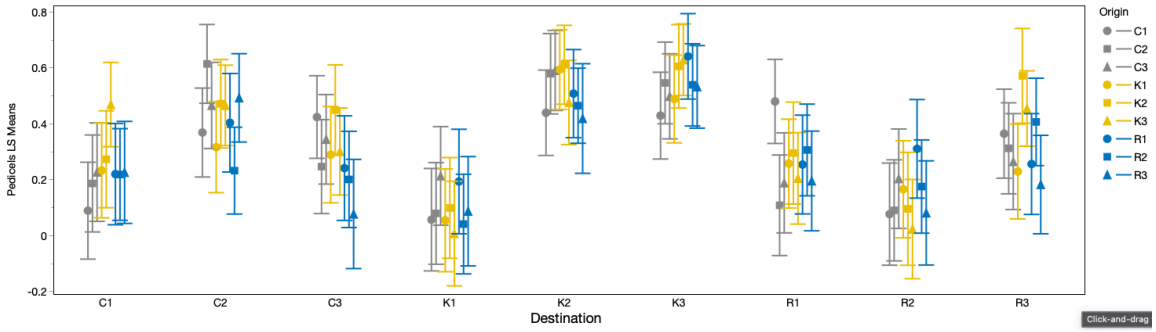

Figure 4. Pedicels least square (LS) means by site origin vs. destination block.

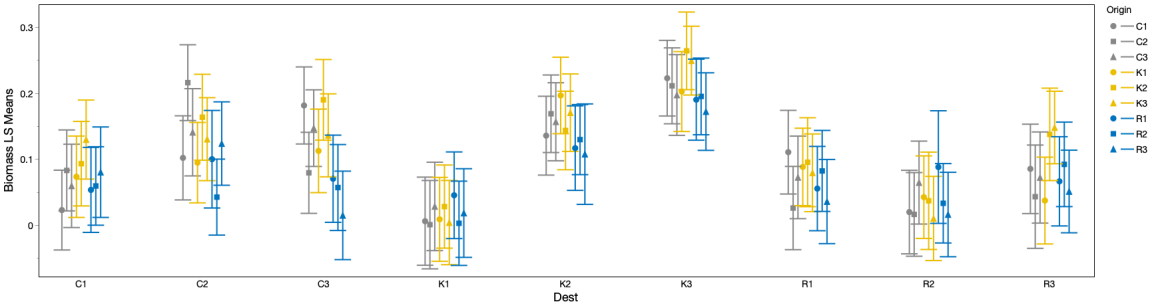

Figure 5. Biomass least square (LS) means by site origin vs. destination block.

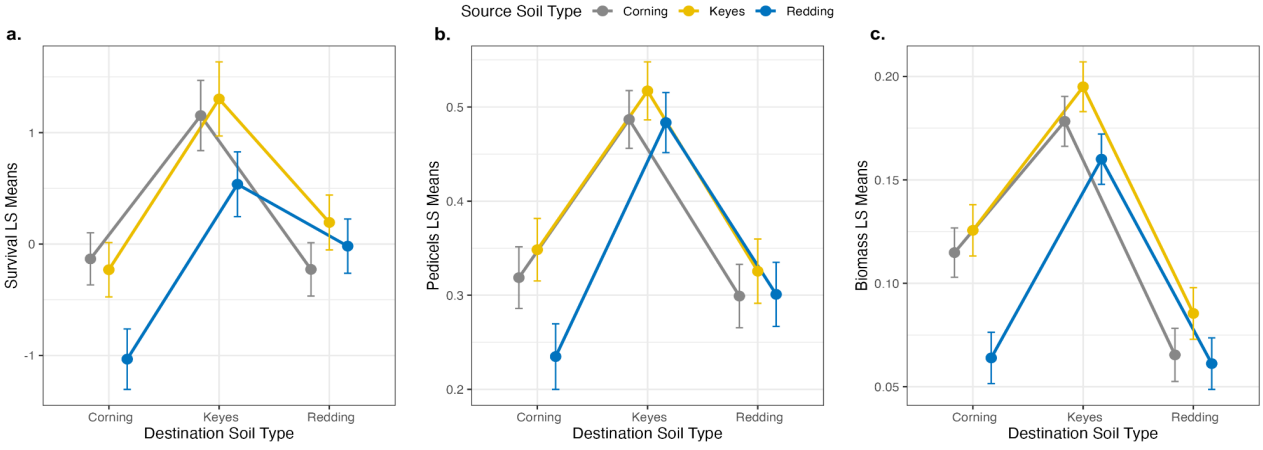

Figure 6: Performance traits least square (LS) means of (a) Survival, (b) pedicels and (c) biomass ordered by soil type destination on the x-axis.

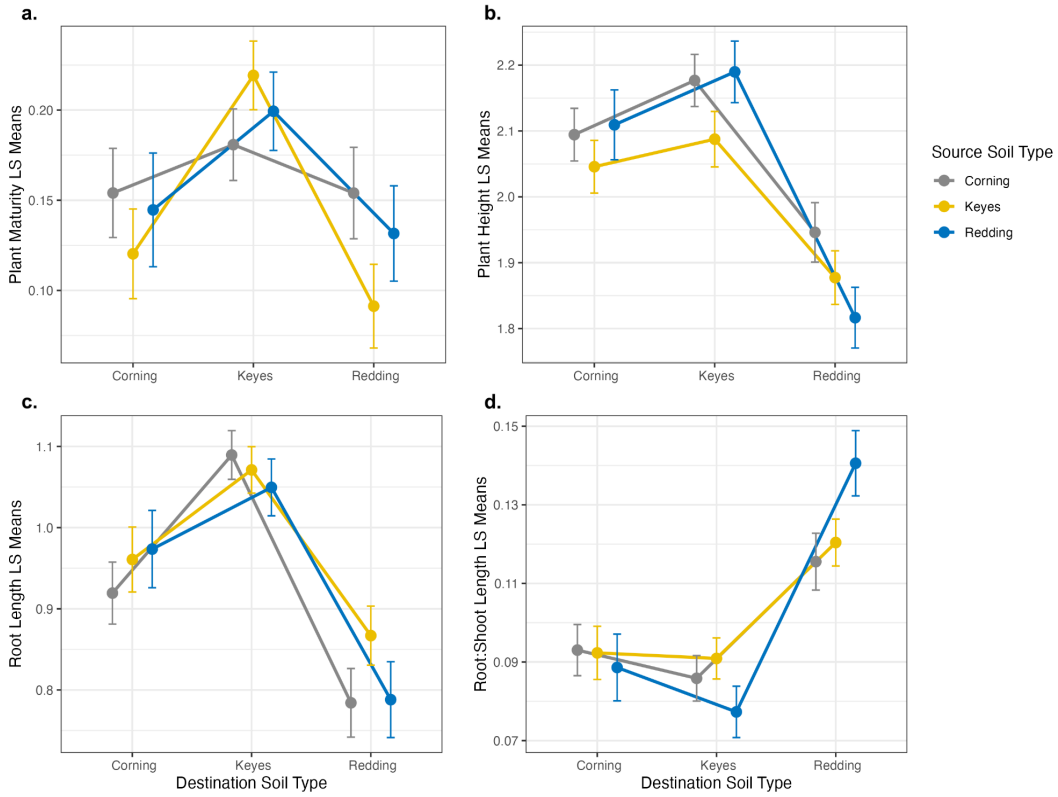

Figure 7: Least square (LS) means of plant phenology **(a)**, and phenotypic traits of plant height **(b)**, root length **(c)** and root:shoot ratio **(d)**.

Chapter 3. Evaluating the Utility of eDNA Metabarcoding for Biomonitoring Endemic and Rare Vernal Pool Plants

ABSTRACT

Environmental DNA (eDNA) metabarcoding is emerging as a powerful tool for biodiversity monitoring, and may prove to be useful in ecosystems that undergo regular, extreme environmental shifts, such as vernal pool wetlands. The ephemeral nature of these ecosystems, characterized by extreme annual cycles of wet and dry conditions, poses a significant challenge for managing and conserving vernal pool species. Interannual variations in rainfall can lead to the presence of species in some years and their absence in others, making it difficult to identify and track which species occur in which pools in a given year. Using eDNA to detect species' DNA in environmental samples, even when the species are not visually present, offers a potential solution. Despite its promise, there are still critical gaps in our understanding of eDNA's efficacy in detecting currently present but hidden species (e.g. seed banks). To evaluate the utility of eDNA for identifying and monitoring plant species in vernal pool ecosystems, I compared vegetation surveys with soil eDNA detections of plant species over a two-year period on the Merced Vernal Pools and Grassland Reserve, an important conservation area hosting numerous endemic and threatened species. The results demonstrate that eDNA metabarcoding is a powerful tool for monitoring plant biodiversity in vernal pools, successfully detecting a wide range of plant species, including endemic vernal pool indicator species and a rare species of conservation concern. Additionally, detection probabilities of plant taxa were significantly correlated with plant abundance, and eDNA effectively tracked focal taxa across sites and within their hydrological niche space within vernal pools. Overall, this research provides valuable insights for managers on the uses and limitations of eDNA as a monitoring tool in ephemeral ecosystems, where species detection and monitoring are inherently challenging.

INTRODUCTION

Most ecosystems on earth are impacted by anthropogenic global change and are experiencing an accelerated loss of biodiversity and unprecedented rate of species extinctions (Pereira et al., 2012). Terrestrial plants represent a major component of global biodiversity and are particularly threatened by rapid, human-driven environmental change, including pollution, climate change, biological invasions, and habitat destruction and fragmentation (Vellend et al., 2017). Changes to habitat connectivity, climate, and species interactions can have negative cascading effects that further impact biodiversity and ecosystem function (Balvanera et al., 2006). Understanding patterns and processes that govern plant species distributions are important for mitigating impacts of future environmental change (Pereira et al., 2012). However, quantifying biological diversity and uncovering the processes that structure terrestrial plant communities across environmental gradients remain central challenges for conservation biologists, ecologists and resource managers (Forest et al., 2007; Pereira et al., 2012; Pausas et al., 2001; Tscharntke et al., 2012).

Quantifying terrestrial plant diversity is typically accomplished using above-ground, visually-based species surveys and is complicated by several factors. Visually-based vegetation surveys can be costly, require ample time and labor, and botanical expertise. Additionally, a particular species presence may be missed due to spatial heterogeneity, temporal variation, or persistence over time in the soil seed bank (Faist et al., 2013; Barbour et al., 2003; Falahati-Anbaran et al., 2014). Recent advances in environmental DNA (eDNA) metabarcoding techniques enable detection of trace amounts of species DNA present in environmental samples and offer an efficient economical approach for biodiversity monitoring (Thomsen & Willerslev 2015; Taberlet et al., 2018). eDNA gives researchers the ability to detect species present as seeds, decomposed plants, or small seedlings, but otherwise missed by above-ground assessment methods (Kesanakurti et al.,2011; Hiiesalu et al., 2012; Yoccoz et al., 2012; Alsos et al., 2018). Combining vegetation survey data with eDNA metabarcoding assays represents a practical approach to study the spatial and temporal patterns of biodiversity within plant communities.

California's ephemeral vernal pool wetlands represent important hotspots of endemism and native plant diversity, yet are among the most endangered ecosystems in the state and lack protections around the world (Calhoun et al., 2017). The historical distribution of vernal pool ecosystems in California has been reduced by more than 90% over the last century due to habitat fragmentation, land conversion for agriculture, and urban development (Holland 2009). Although nearly a third of remaining vernal pool habitat is under some form of protection (e.g., conservation easements, state and federal wildlife refuges), thousands of acres of vernal pool habitat continue to be lost every year, with some of the largest recent losses in the state occurring in Merced County (Witham et al. 2014; Witham 2021). Consequently, remaining vernal pool habitat in California, and Merced County, host several state and federally listed threatened and endangered plant species that are of high conservation concern, including endemic grasses within the vernal pool grass tribe Orcuttieae (*Neostapfia colusana*, *Orcuttia* spp., and *Tuctoria* spp.; Crampton, 1959). Understanding how vernal pool plants will respond to future rapid environmental change is complicated by spatial and temporal variation in phenology, their limited dispersal abilities, and complex eco-evolutionary dynamics formed over generations of selection by local environmental conditions (Barbour et al., 2005; Bauder, 2000). Furthermore, quantifying biodiversity in vernal pools has typically relied on traditional observational approaches, which can be negatively impacted by cost, limited expertise and logistical challenges complicated by complex spatial heterogeneity and extreme temporal variation of the system; e.g., dormant seed (or cyst) banks, variability in germination, and invasibility (Bauder, 2005; Bliss & Zedler, 1997; Faist & Beals, 2018; Faist & Collinge, 2015).

To aid conservation and mitigation strategies, the United States Fish & Wildlife Service has adopted a Vernal Pool Recovery Plan (USFWS, 2005) and has invested significant

resources in predicting likely vernal pool habitat and surveying for species presence (Vollmar et al. 2013), including the use of eDNA to detect endemic and rare species. Environmental DNA techniques have been used to identify and monitor vertebrate and invertebrate taxa in aquatic vernal pool ecosystems (Kieran et al., 2021; Gold et al., 2020; Kieran et al., 2020; Montiel-Molina et al. 2021); however, its application to detect vernal pool plants in soils specifically is limited (Ruiz-Ramos et al. 2023). Nevertheless, important questions relevant to vernal pool plant species conservation and efficacy of eDNA to detect species remain. For example, while the ability of soil metabarcoding to inventory plant diversity and track patterns of environmental variation is promising (Ruiz-Ramos et al., 2022; Johnson et al., 2023), accurately identifying plants with high specificity across landscape scales is still challenging (Barnes et al., 2022; Banerjee et al., 2022).

To assess the utility of eDNA for identifying and monitoring plant species in vernal pool ecosystems, I conducted seasonal vegetation surveys of vernal pools over a two year period across the University of California Merced Vernal Pools and Grassland Reserve (MVPGR) (ucnrs.org), an important vernal pool conservation area. Subsequently, I collected soil samples for eDNA metabarcoding analysis to investigate the overlap in plant diversity between traditional taxonomy and eDNA approaches and the ability to track focal, endemic vernal pool plant taxa. Using results from eDNA in soil samples and traditional plant taxonomic surveys I asked, can DNA metabarcoding be applied to effectively detect and track endemic vernal pool plant species across a vernal pool landscape, and what is the relationship between species presence and detectability in eDNA? To accomplish this work, in collaboration with the Sexton Lab, I conducted a landscape-scale floristics study to investigate broadscale patterns of biodiversity across the landscape and to couple this with eDNA sampling of vernal pool soils. Moreover, I analyzed the abundance of several focal endemic vernal pool indicator species to specifically test the ability of eDNA metabarcoding to track individual, endemic vernal pool plants and rare species across a large heterogeneous landscape.

MATERIALS AND METHODS

Study location

Sampling of soils, traditional plant taxonomy and community-based vegetation surveys were conducted at 36 sites across the MVPGR, including 30 vernal pools and 6 perennial water bodies (stock ponds) with known endangered species occurrence records (CNDDB, pers.comm) (Figure 1). The MVPGR is located in the southern Sierra Nevada Foothills vernal pool ecoregion (Keeler-Wolf et al., 1998) and at the eastern periphery of the California Central Valley. Vernal pools at this site are of the northern hardpan and claypan vernal pool classification (Vollmar, 2002) and distributed across ancient granitic alluvium terraces and volcanic mudflows. The dominant vernal pool soil types found on the MVPGR include Reynor, Corning, Redding and Keyes gravely and/or clay loams (from oldest to youngest, respectively; Holland pers comm).

Community vegetation surveys

Plant community diversity surveys were conducted over spring 2017 and 2018 using 324 plots established using a transect and quadrat species sampling scheme. We targeted three plot locations for each of three hydrological zones (bottom, edge and upland) along three random transects following methods adapted from Marty (2005). At each vernal pool, the nine plant survey quadrat locations were marked with metal washers fixed to the ground and relocated using visual or metal-detector-assisted search for subsequent soil sampling and annual plant community diversity surveys. A total of ~648 37x70cm quadrats were surveyed and daubenmire cover class (% cover for each species) was recorded at the peak of pool community flowering period between April and May of 2017 and 2018.

I conducted a focused investigation of six endemic vernal pool indicator species using the community data to track individual plants across the MVPGR. These 'focal species' included, *Limnanthes douglasii* ssp. rosea (meadowfoam), *Eryngium castrense* (coyote thistle), *Lasthenia fremontii* (vernal pool goldfields), *Plagiobothrys stipitatus* (popcorn flower), *Downingia bicornuta* (calico flower), and *Trifolium variegatum* (white-tipped clover). These species have affinities to different hydrological conditions along elevational gradients, which are divided into bottom and transitional-edge zones within pools (Barbour et al., 2005). To test specific effects of microtopography on species detections within pools, I tracked species in their typical distributional zones within pools. Coyote thistle, white-tipped clover and meadowfoam are considered edge species and typically occupy transitional pool boundaries. Popcorn flowers, goldfields and calico flowers primarily occupy the longer-inundated pool bottom zones. Lastly, I conducted late season census surveys of *Neostapfia colusana* (Colusa grass) and *Orcuttia inaequalis* (San Joaquin Orcutt grass, Orcutt grass hereafter) (Crampton, 1959), two endangered plants prioritized for conservation management on the MVPGR, to compare targeted survey approaches to eDNA detection rates of these rare species. The two endangered Colusa and Orcutt grasses typically occupy larger and deeper claypan pools on heavier clay soils associated with the Mehrten formation (Vollmar, 2002), and flower in the late-spring and summer months (June-July), depending on the site and annual rainfall (Griggs 1981; Keeley & Zedler, 1998). Targeted sites for Colusa grass surveys were selected from known occurrence locations on the MVPGR (n = 6), and targeted sites for Orcutt grass included 1 known vernal pool location that overlaps with Colusa grass (Figure 1). I surveyed plants in peak season; i.e., after most plants had germinated and were in the flowering phase. I surveyed Colusa grass and Orcutt grass at known locations between June-July. Orcutt grass was also surveyed in May of both years based on my prior experience with this species and site. Abundance for the rare plants was obtained by counting the number of individuals detected by walking relevés at each site. I determined the peak flowering time for surveys over weekly site visits during the 2017 growing season (Feb-April).

Soil sampling

Soils were sampled under the USFWS Sacramento field office sub-permit A. Soil samples were collected directly to the left of each of the nine established quadrats. In 2017, we collected 432 (2mL) soil samples from a subset of sites representing 2 stock ponds and 14 vernal pools. In 2018, approximately 972 small samples were collected in triplicate from each marked sampling plot of all 36 sites. The samples were collected in triplicate and adjacent to each sampling plot and zone following sampling protocols developed by CALeDNA (ucedna.com; see Ruiz-Ramos et al. 2023). Briefly, sterile, stainless steel scoops were used to collect and place soil samples into 2mL cryotubes. Scoops, chisels and trowels were sterilized with 10% bleach for at least 10 minutes, rinsed with DI water and 70% ethanol in the field prior to the next sample. The small samples were transported to the lab and immediately transferred to an ultra-low freezer and stored at -80°C until DNA extraction.

DNA extraction, library preparation and sequencing

DNA extractions

We extracted DNA from approximately 468 samples representing each of the nine small, pooled triplicate soil samples collected from plots in 16 and 36 sites across 2017 and 2018, respectively. DNA extracts were pooled by zone within sites, and DNA metabarcoding libraries were constructed for 149 out of the 156 pooled sample extracts. Additionally, 17 extraction-negative and 4 pcr-negative controls (see below) were included for sequencing for a total of 170 samples. Soil samples were allowed to thaw on ice prior to sample processing and DNA extraction. All small, triplicate soil samples were pooled at the plot scale for each year following the CALeDNA protocol, resulting in 144 and 324 pooled small samples for 2017 and 2018, respectively. A homogenous 0.75 g sample of pooled soil was obtained by pooling a 0.25-g subsample from each biological triplicate and vortexing prior to extraction using Qiagen DNeasy Powersoil kit following the manufacturer's protocol. At least one extraction blank was included in every extraction round (up to 23 pooled samples + 1 blank per round) to track potential contamination during extraction procedures. All extractions were completed in an isolated DNA clean room. Sieves, spatulas and scoops were rinsed with water before being bathed in 10% bleach for 10 minutes and were rinsed with MilliQ water followed by a 70% ethanol rinse to reduce cross contamination between samples.

Library preparation and sequencing

Metabarcode libraries were prepared in a two-step, PCR-based approach. First, a metabarcoding PCR was performed to amplify two plant-specific gene regions followed by an indexing PCR to uniquely tag individual samples. Samples were amplified using the 450bp ITS2 region (ITSp3-F and ITSu4-R; Cheng et al., 2016) of plant nuclear ribosomal DNA, and the ~143bp P6 loop of the trnL (UAA) intron (trnLg-F and trnLh-R; Taberlet et al., 2007) of the chloroplast gene region. Illumina transposase primer adapter sequences were attached at the 5′ ends (Table 1). The ITS-p3/ITS-u4 primer pair has high universality and high specificity for land plants, 82.2% and 91.7%, respectively

(Cheng et al., 2016). The trnL chloroplast primer represents a shorter barcode and has been shown to have relatively great success amplifying and identifying taxa from highly degraded DNA in environmental samples (Valentini et al., 2009) but has lower specificity resulting in reduced coverage at higher taxonomic resolutions, e.g. at species level (Barnes et al., 2022; Espinosa Prieto et al., 2024).

Each barcode was amplified following a touchdown PCR in 15 µL triplicate PCR reactions using 1 µL of DNA template with 0.15 µL of each primer and 7.5 µL Qiagen Multiplex PCR Master Mix polymerase (Qiagen Inc., Valencia, CA, USA). Each sample was visualized on a 2% agarose gel to check amplification success of the targeted markers. Triplicate PCR products were pooled and cleaned using AMPure PCR purification beads (Beckman Coulter Life Sciences, Indianapolis, Indiana, USA) and quantified using a Qubit fluorometer. Prior to sample pooling, amplicons were extended with Illumina Sequencing Adapters (Nextera Transposase Adapters) compatible with the Nextera indexing kit (i.e., IDT® for Illumina Nextera DNA Unique Dual Indexes Set's A and B). Indexing PCRs were completed in 25µL reactions using 12.5 Kapa HiFi HotStart Ready mix, 1.25µL unique dual indexes and 11.25µL template-PCR water following CALeDNA's Indexing PCR protocol (ucedna.com). PCR conditions for both metabarcoding and indexing PCR's are listed in Table 4. Indexed PCR products were cleaned then visualized using the same protocol as the first PCR. The final cleaned and indexed PCR samples were pooled into two separate libraries, one for long fragment size libraries (ITS2) and one for the shorter trnL libraries. Each library was quality-checked on a bioanalyzer (Agilent Technologies) prior to sequencing. The combined long fragment libraries (ITS2) were sequenced using an Illumina MiSeq v3 600 cycle kit for 2×300 bp paired-end reads. The shorter trnL libraries were run on a MiSeq v2 300 cycle kit for 2×150 bp paired-end reads. Each MiSeq run was spiked with a PhiX Control v3 library as a standard quality control measure. Sequencing was conducted by the Genomics Core Facility, UC Davis, Davis, CA, USA. It is important to note that the ITS2 libraries here were pooled and sequenced with an additional set of sample libraries constructed using the rbcL barcode for another project in the Sexton Lab. Appropriate precautions were taken to mitigate risks of potential cross-contamination between libraries and reduce sequencing bias for different length amplicons. That is to say, I used unique dual indexes and normalization of libraries (pooling by equimolar ratios).

The trnL libraries generated a total of 13.4 million forward and reverse 150-bp reads; however, the majority of the reads were identified to Bryophyta and Chlorophyta and were not considered for the purpose of this analysis. Furthermore, only 20 Streptophyta taxa accounted for approximately 99.08% (340,540 reads) of the trnL dataset, versus 254 taxa and 3.4 million reads generated for the ITS2 dataset after filtering for Streptophyta. Additionally, taxa detected with trnL were above order level resolution and none of the focal taxa were detected in the trnL library. Thus, results reported in this study only represent the ITS2 libraries that had positive sequencing hits for taxa at or

below family level taxonomic resolution. The sequence data are backed up and stored in triplicate across three repositories: an external hard drive, Sexton Lab Box Cloud Storage at UC Merced, and the UC Merced Cluster.

Sequence processing and bioinformatics

Paired-end-read sequencing data were demultiplexed using cutadapt (Martin, 2011) and performed by the Bioinformatics Core, UC Davis, Davis, CA, USA. Sequence alignments and bioinformatic analysis were performed using the Anacapa Toolkit and CRUX reference databases to quality-filter, sort, and assign amplicon sequence variants (ASV's) and taxonomy (Curd et al., 2019). Reference libraries for each barcode region were constructed using a local species list of plants found on MVPGR, and running in silico PCR using Obitools and ecoPCR (Ficetola et al., 2010) against the GenBank nucleotide sequence database (www.ncbi.nlm.nih.gov) and generating a seed library of reads representing unique taxon identifiers. Amplicons were matched against the seed library reads by checking for the correct primer regions and trimming them using cutadapt (Martin, 2011). Bowtie2 (Langmead & Salzburg, 2012) and the Bayesian Lowest Common Ancestor (BLCA; Gao et al., 2017) algorithms were used to query and align ASV's to reference databases. Taxonomic classifications were made with confidence levels of at least a 70% Bayesian Confidence Cutoff (Curd et al., 2019). Plant taxonomic assignments of select sequences were checked using NCBI's BLASTn database (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Taxonomy tables were decontaminated using the 'prevalence' method in the decontam package in R, whereas the number of contaminate reads identified in negative controls were removed from the samples (Davis et al., 2018; R Development Core Team, 2008). Samples that had fewer than three reads per sample were removed and rarefied using ranacapa (Kandlikar et al., 2018) and phyloseq R packages (McMurdie & Holmes, 2013). Rarefaction depth was chosen after filtering for land plants (Streptophyta) and to 4,000 reads, the depth at which the species richness accumulation curve began to plateau (Figure S1). All focal species were found in NCBI's sequence reference database and were included in our locally generated reference library.

Statistical analysis

Species richness measures and alpha diversity analyses from community survey data were conducted using the R Vegan package (Oksanen et al., 2013). Wilcoxon signed-rank and paired t-tests were used to test differences between eDNA and vegetation survey-based measurements of Shannon diversity and species richness, respectively. I ran standard least-squares linear models to test for the main effects of sampling year, zone, site, and their interactions on observed alpha diversity determined by each method. Detection frequencies were determined for each species for both eDNA and plant surveys by the number of positive detections divided by the total number of detections as obtained by eDNA and plant surveys at that site and across both years. Separate generalized linear models with a lognormal distribution were conducted on eDNA relative read abundance and plant community survey abundance for the six focal

plant species with zone, site and year included as fixed effects. Separate logistic regressions were performed on eDNA and plant survey detection frequencies that included zone, site, and year as fixed effects. Interaction effects were not significant and excluded from all models to improve model fit. Abundance data were log-transformed prior to analysis. A Pearson's correlation was used to detect relationships between plant abundance and eDNA detection probabilities of the 35 overlapping plant species. A non-parametric Mann-Whitney U test was used to compare sample means between years and methods; i.e., eDNA-based and visually based detection frequencies of focal species. Linear models, correlations and statistical tests were conducted in JMP (version 17.0).

RESULTS

Metabarcode sequencing summaries

In this study, I sequenced a total of 149 sample extracts pooled by zone for each site in addition to 17 extraction negative controls and 8 PCR negatives for both the ITS2 and trnL fragment libraries. Sample extracts were missing for 6 pooled samples resulting in 102 sequenced samples for 35/36 sites in 2018. Decontamination of the ITS2 fragment libraries resulted in a total of 7.5 million forward and reverse 300-bp reads assigned to 1,258 unique taxa. After filtering for Streptophyta and exclusion of samples with fewer than three reads, 123 samples and 3.4 million reads were retained, resulting in 254 taxa at family-level taxonomic resolution. Approximately 80,000 reads were found in negative controls and removed from the dataset. Lastly, following standardization through rarefaction, the dataset was further filtered resulting in a total of 210 taxa across the 123 samples (Figure 2).

Community survey

There was concordance of 23 (58.9%) taxa at family level taxonomic resolution, 33 (27.3%) at genus level and 35 (16.7% at) species resolution detected between the eDNA and visual observation plant survey methods. Vegetation surveys identified 23, 33, and 35 unique taxa at family, genus and species levels, respectively. Unique taxa detected in eDNA assays included 11, 65, and 129 taxa at family, genus and species levels, respectively (Figure 3). We also found concordance between eDNA and plant surveys in terms of the most common genera detected across respective zones, e.g.the non-native grasses of *Festuca*, *Bromus*, and *Hordeum* (Poaceae), the native vernal pool genus *Plagiobothrys* (Boraginaceae) and *Isoetes* (quillworts) (Isoetaceae) (Figure 4). *Isoetes* was the most abundant genus detected in eDNA, and ranked 17th of the total 81 species detected in vegetation surveys. A Wilcoxon signed-rank test on Shannon diversity showed that differences in alpha diversity between the two methods was not significant across the study, indicating similar species evenness and distribution of plant diversity (Figure 5). A paired t-test indicated the number of species detected at the site level was higher for eDNA surveys on average and significantly different than visual surveys ($p = 0.005$) (Figure 6). The two sampling methods showed differences in species richness in linear models with zones having a strong effect on both eDNA and plant survey

methodologies ($p < 0.0001$). The Site effect on observed alpha diversity was highly significant for plant survey methodologies ($p = 0.0003$) and significant for eDNA-based measures ($p = 0.0177$) (Table 3). Mean observed alpha diversity was highest in the upland zones in eDNA-detected richness (Figure 7a), whereas visually observed diversity was highest in the transitional, edge zone (Figure 7b). The pattern between zones and years was consistent for each method.

Focal plant surveys vs. eDNA

A total of 6,866 reads, ranging between 150 to ~400 reads each, aligned to *Limnanthes douglasii ssp. rosea* (meadowfoam), *Plagiobothrys stipitatus* (popcorn flower), *Eryngium castrense* (coyote thistle) and *Lasthenia fremontii* (goldfields). *Downingia bicornuta* (calico flower) (7,134 reads) and *Trifolium variegatum* (white-tipped clover) (49,872 reads) had the highest read counts of the focal taxa. Trends in detection frequencies (i.e., the number of times species were recorded in each sample and site divided by the number of samples per site) in vegetation surveys were consistent between years and changed slightly depending on species and zones, whereas eDNA assays were less consistent between years and species (Figure 8). Coyote thistle, popcorn flower and calico flowers had the highest detection frequencies in focal plant surveys and across pool-edge zones in both years. However, coyote thistle and calico flower had low detection in eDNA assays. Calico flower had the second lowest average eDNA detection frequency across both years, despite having high relative sequencing read abundance in the samples where it was present. All focal species had much lower detection rates with eDNA than vegetation surveys for both years (Table 4). A Mann-Whitney U non-parametric test showed significant differences in detection frequencies between floristic survey and eDNA assay methodologies ($p < 0.0001$), but no difference between years (Table 5). Kruskal-Wallis non-parametric tests of the effect of zones on relative abundance of visual-based surveys of the six focal species were significant for all six species ($p<0.0001$) (Table 6). Similarly, significant zone effects were observed in eDNA-based observations and consistent with patterns detected in visual surveys (Figure 9). In particular, goldfields ($p = 0.0336$), white-tipped clover ($p = 0.0008$) and meadowfoam ($p = 0.0511$) exhibited significant zone effects in eDNA assays (Table 6).

Linear models on eDNA relative read abundance showed significant effects of both zone and site ($p < 0.0001$). Similarly, significant effects of site ($p = 0.0002$) and zone ($p < 0.0001$) were also found on focal plant survey abundance (Table 7). A logistic regression analysis on eDNA detection frequencies of the focal species demonstrated significant effects for zone ($p < 0.0001$) and site ($p < 0.0003$) (Table 8). Zone effects were also highly significant for detection frequencies of vegetation survey data ($p < 0.0001$), but site was not ($p = 0.19$). The year effect was not significant for either eDNA or plant survey methodologies.

A Pearson's correlation of plant survey abundance and positive eDNA detections of the 33 overlapping plant genera detected in both methods showed a slightly positive and

highly significant correlation ($r^2 = 0.30$, $p < 0.0001$) (Figure 10a), indicating that as aboveground plant abundance of a given species increases, the number of positive eDNA detections also increases. Likewise, relative plant survey abundance and relative abundance of eDNA sequencing reads for the overlapping genera showed a slightly positive and significant correlation ($r^2 = 0.27$, $p<0.0001$) (Figure 10b).

Rare plants

A total of 2,953 sequencing reads aligned to Colusa grass at above >90% Bayesian confidence level. A BLASTn search supported these species assignments to 100% identity scores. Orcutt grass was not detected in any of our eDNA samples, despite being detected at one site in field surveys. Colusa grass was detected in five samples from two different vernal pool sites and across two years. Colusa grass was detected with eDNA in only one site in 2017, and when plant abundance was >70% cover in visual surveys. We detected Colusa grass at two historically known locations (VP16 & VP14) during spring community floristic surveys in 2017. We also detected Orcutt grass co-occurring with Colusa grass in vernal pool VP14 during vegetation surveys in 2017, a known vernal pool location for Orcutt grass. Orcutt grass was also observed at this location in our 2018 field surveys. In 2018, we detected Colusa grass in eDNA samples at two locations (VP16 & VP05) where it was not detected in above-ground vegetation surveys. Vernal pool site VP05 represents a new observation record for Colusa grass occurring on the MVPGR, but this has not yet been confirmed with visual surveys.

Late-season vegetation surveys during 2017 field sampling for Colusa grass found the species present in all five of our targeted site survey locations, despite only being detected in 2 of 5 sites during spring surveys. Late season vegetation monitoring for 2018 resulted in a single detection for Colusa grass. Orcutt grass was detected in late season surveys and where it was present during spring community surveys. Overall, the total probability of detection of targeted vegetation surveys vs. eDNA for Colusa grass across the five sites over two consecutive years (2017 & 2018) was 70% and 20%, respectively. Orcutt grass was detected at the target site in both years surveyed. Lastly, we detected the rare vernal pool plant, succulent owl's clover, *Castilleja campestris* ssp. *succulenta*, in our spring 2017 plant community surveys, occuring in four of the 36 (11%) vernal pools surveyed. We did not detect it in any of our eDNA samples or in 2018 vegetation surveys.

Out of a total of 6,866 reads detected for Limanthaceae, 7 were aligned to *Limnanthes floccosa*, or woolly meadowfoam, with confidence scores of 100% to genus and 74% to species level taxonomic assignment. *Limnanthes floccosa* is a rare (CNPS 4.2B) species of conservation concern with limited distribution in northern California and southern Oregon (calflora.org). A BLASTn (nih.gov) search of these sequencing reads returned *Limnanthes* with hits of 100% sequence overlap having identity scores above 90%, and with most hits >95% identifying to *L. floccosa*. However, a BLASTn performed against *L. douglasii* ssp. *rosea* showed 99% sequence overlap and a 92% identity score. Given the

limited distribution and rarity of *L. floccosa*, and the high confidence scores of sequence alignment to *L. douglasii* ssp. *I* believe this detection most likely represents a miss identification, possibly due to the short sequence read length (248bp), compared to the >400 bp read lengths in the ITS2 reference database. All other *Limnanthes* sequences from ANACAPA results aligned to *L. douglasii* with confidence scores >80% at species level. A BLASTn search on the most abundant sequence identified them to the focal meadowfoam species, *L. douglasii* ssp. *rosea*, with 100% sequence overlap and 100% identity scores.

DISCUSSION

This study demonstrates the potential eDNA metabarcoding has as a monitoring tool for tracking plant species in highly fluctuating ecosystems such as vernal pools. Similar to previous research, these findings demonstrate the utility of eDNA in detecting a wide range of plant species, including many that were not identified through traditional floristic surveys (Ruiz-Ramos et al., 2023; Deiner et al., 2017; Taberlet et al., 2012). Nevertheless, the discrepancies between the two methodologies highlight the necessity of corroborating eDNA results with field observations (Barnes et al., 2022). eDNA metabarcoding had high concordance of the most abundant Streptophyta taxa with visual plant surveys, effectively tracked ecological patterns of focal species in their known habitat zones or niches, and was able to detect rare plant species of conservation concern.

The notable discrepancy between species detected by traditional floristic surveys and those identified through eDNA metabarcoding are consistent with findings from other vernal pool and plant metabarcoding studies (Ruiz-Ramos et al., 2023; Barnes et al., 2022). These findings are also consistent with studies that reported variations in community composition detected by different methods, e.g., pollen monitoring (Milla et al., 2022), remote sensing (Li et al., 2024), seed bank analysis (Faist & Collinge, 2015). For instance, eDNA detected 1,258 taxa before filtering and 254 taxa after filtering for Streptophyta in this study, whereas 129 taxa at the species level were unique to eDNA and 35 unique species were detected via vegetation surveys. This discrepancy emphasizes eDNA's sensitivity to a broader range of species, including taxa that might be seasonally dormant, cryptic or that might be overlooked in traditional surveys, such as algae, mossess, etc. (Yoccoz et al., 2012). Additionally, eDNA detected a large number of plant species DNA that do not occur in vernal pool systems, or are not known to be naturalized in the surrounding area, but are close relatives or occur in cultivated or otherwise human-influenced plantings (e.g., Pinaceae, walnuts, tropical plants such as Eryngium foetidum). These cases emphasize the risk of DNA movement (e.g., of pollen or other biological materials) into areas where species do not live, false positives (Piper et al., 2019; Zinger et al., 2019), contamination (Diener et al., 2017) or errors when aligning reference sequences (Taberlet, 2018).

Environmental filtering due to zonal variation is a strong determinant of vernal pool plant distribution patterns (Bauder, 2000; Emery et al., 2009; Collinge et al., 2013; Emery & La Rosa, 2019; Tittes et al., 2019). Here, I show significant zonation effects on biodiversity patterns in both eDNA and community survey methodologies, which aligns with findings from other vernal pool eDNA studies where habitat heterogeneity plays a crucial role in shaping biodiversity (Ruiz-Ramos et al., 2023; Montiel-Molina et al., 2021; Kneitel, 2016). For the six focal species analyzed here, I found significant effects of zone on eDNA detections, where the upland zone had significantly lower species detections compared to the pool or edge zones. Similarly, we successfully tracked species in their ecological niche space within pools. For example, detection frequency for calico and popcorn flowers were highest in the pool bottom zones where we expect them to occur, and coyote thistle and white-tipped clover had the highest detection rates in transitional edge zones. This study corroborates the hypothesis that environmental factors, particularly zonal variation, significantly influence patterns of plant diversity in vernal pools, and that eDNA can effectively track species in their ecological niche space owing to localized distribution of species DNA (Arrizabalaga-Escudero, 2018; Lopes et al., 2020; Kartzinel et al., 2015; Edwards et al., 2018).

The strong correlation between plant abundance and eDNA detection probabilities further supports the utility of eDNA in reflecting actual species presence and abundance in vernal pool ecosystems (Ruiz et al., 2023). This correlation supports the hypothesis that higher plant abundance increases the likelihood of DNA detection in environmental samples (Yoccoz et al., 2012). However, the lower detection probabilities for certain species in eDNA assays indicate that eDNA alone may not fully capture biodiversity patterns, necessitating its integration with traditional survey methods and multi-locus metabarcoding approaches (Deiner et al., 2017; Espinosa Prieto et al., 2023). Furthermore, lower detection probabilities of certain species in eDNA assays, despite their higher read counts, indicate potential biases in eDNA detection. Factors such as DNA degradation, primer specificity, and PCR efficiency might contribute to these discrepancies (Barnes et al., 2022; Deiner et al., 2017).

The observed temporal stability in eDNA detectability across years, despite significant zonal and site effects, suggests that eDNA can provide consistent biodiversity assessments over time. The non-significant effect of year in this study differs from findings reported by Ruiz-Ramos et al (2023), despite sampling overlap at 5 sites and across the same sampling years (2017 and 2018). Nevertheless, sample collection in this study occurred in late summer compared to sampling by Ruiz et al. (2023), which occurred in the spring when soils were wetter, highlighting potential seasonal bias. Thus, to fully understand the temporal dynamics of eDNA and its correlation with environmental changes, more extensive temporal sampling and longitudinal studies are needed (Ariza et al., 2023; Ruppert et al., 2019).

The detection of Colusa grass using eDNA, especially in locations where they were not observed during vegetation surveys, underscores the value of eDNA in monitoring rare vernal pool plants and uncovering hidden biodiversity (Boussarie et al., 2018). An obvious caveat to this finding also highlights a potential for false positive detections, inherent to eDNA metabarcoding. This finding is speculative and needs to be confirmed with visual surveys or seedbank investigations. Nonetheless, detecting rare plant species in this threatened landscape is particularly significant for conservation efforts, as it provides a method to monitor rare and endangered plant species across large landscapes and extreme seasonal ecosystems (Thomsen et al., 2012). The ability to detect species that are difficult to observe through traditional survey methods can greatly enhance the understanding and management of biodiversity in these sensitive habitats, whereas eDNA techniques can be especially useful in ephemeral vernal pools, where the presence of water is transient and species detection windows through traditional surveys are limited (Gold et al., 2020; Kieran et al, 2021; Carim et al., 2016). However, the absence of Orcutt grass and succulent owl's clover in eDNA samples, despite their presence in visual surveys, highlights the limitations and potential false negatives associated with eDNA methods (Diener et al., 2017; Carim et al., 2016). This discrepancy can be attributed to several factors, including the degradation of DNA in the environment, low concentrations of DNA from certain species, primer specificity or the inefficiency of the eDNA extraction and amplification processes (Barnes et al., 2022; Thomsen et al., 2012). These challenges indicate that although eDNA is a powerful tool for biodiversity monitoring, it should be used in conjunction with traditional survey methods to provide a comprehensive picture of species presence and abundance (Deiner et al., 2017). Integrating eDNA with conventional approaches can help mitigate the limitations of each method, improving the accuracy and reliability of biodiversity assessments in vernal pool ecosystems (Thomsen et al., 2012; Carim et al., 2016).

CONCLUSION

In this study, I assessed the utility of eDNA in identifying and monitoring endemic and rare plant species in vernal pool ecosystems. My results show that eDNA metabarcoding is a powerful tool for monitoring plant biodiversity in vernal pools, successfully detecting a wide range of plant species, including endemic vernal pool indicator species and a rare species of conservation concern. The high concordance between eDNA results and visual surveys for abundant species and tracking focal species in their ecological niches underscores its effectiveness in tracking both broad and fine-scale ecological patterns and species distributions. However, I also identified limitations of eDNA methods, such as false negatives and potential contamination leading to false positives. These findings suggest that while eDNA can detect plant species, it should be used alongside traditional survey methods to ensure a comprehensive and accurate assessment of biodiversity. Integrating both approaches can improve the reliability of biodiversity monitoring and conservation efforts in these sensitive habitats.

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Tables

Table 1. Expected amplicon length, primer pairs, adapter sequences, and primer reference for each barcode. Primers are italicized and adapters are not italicized.

Barcode region	Amplicon length	Primer	Sequence	Reference
trnL	10-143	trnLg-F	*5′*-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG *GGGCAATCCTGAGCCAA-3′*	Taberlet et al., 2007
		trnLh-R	*5′*-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG *CCATTGAGTCTCTGCACCTATC-3′*	Taberlet et al., 2007
ITS2	450	ITS2-F (ITS-p3)	*5′*-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG *YGACTCTCGGCAACGGATA-3′*	Cheng et al., 2016
		ITS2-R (ITS-u4)	*5′*-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG *RGTTTCTTTTCCTCCGCTTA-3′*	Cheng et al., 2016
5' F Illumina adapter sequence for Nextera			*5′*-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-*3′*	
5' R Illumina adapter sequence for Nextera			*5′*-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-*3′*	

Table 2. PCR conditions for each barcode: trnL conditions taken from CALeDNA. protocols and ITS2 adapted from Cheng et al., 2016.

Primer: trnL g/h		Reference: CALeDNA (adapted from Taberlet et al., 2007)		
Step		# cycles	Temp	Time
Activation		1	95°C	15 min
Touch Down	Denature	13	94°C	30 sec
	Anneal		70°C (2.0C/cycle)	30 sec
	Extend		72°C	60 sec
Amplification	Denature	35	94°C	30 sec
	Anneal		50°C	30 sec
	Extend		72°C	60 sec
Final extension		1	72°C	10 min
Hold			10°C	infinity

Primer: ITS2 (ITS-p3/ITS-u4)		Reference: Chen et al. 2016		
Step		# cycles	Temp	Time
Activation		1	95°C	15 min
Touch Down	Denature	13	94	30 sec
	Anneal		70 (-2.0 C /cycle)	30 sec
	Extend		72	60 sec
Amplification	Denature	40	94°C	30 sec
	Anneal		55°C	40 sec
	Extend		72°C	60 sec
Final extension		1	72°C	10 min
Hold			10°C	infinity

Table 3. Standard least-squares linear model on observed alpha diversity for survey and eDNA methodologies.

Method	Effect	Nparm	DF	*SS*	F Ratio	P-Value
Surveys	Site	28	28	1034.3447	2.6805	**0.0003***
	Zone	2	2	405.1655	14.6996	**<.0001***
	Year	1	1	9.1564	0.6644	0.4173
	Zone*Year	2	2	13.7503	0.4989	0.6090
eDNA	Site	28	28	2500.2431	1.8324	**0.0177***
	Zone	2	2	1980.3983	20.3198	**<.0001***
	Year	1	1	7.1866	0.1475	0.7019
	Zone*Year	2	2	79.4326	0.8150	0.4460

Table 4. Detection frequency summary statistics for focal species across years and methodologies, including the mean, standard deviation, minimum and maximum frequencies.

Year	Method	Mean	Std. Dev.	Min	Max
2017	Survey	0.94	0.08	0.81	1
2017	eDNA	0.18	0.16	0	0.43
2018	Survey	0.95	0.06	0.86	1
2018	eDNA	0.16	0.14	0	0.31

Table 5. Mann-Whitney U test comparisons of detection frequencies for both years, 2017 and 2018, and sampling methodologies.

Comparison	Statistic	P-Value
Year (2017 vs 2018)	72	1
Method (eDNA vs Survey)	0	**<0.0001**

Table 6. Kruskal-wallis tests on zone effects of relative abundance of eDNA and vegetation survey data for six focal species.

Method	Species	ChiSquare	P-Value
eDNA	Calico flower	4.189	0.1231
Survey	Calico flower	41.743	**< 0.0001**
eDNA	Coyote thistle	4.294	0.1168
Survey	Coyote thistle	87.078	**< 0.0001**
eDNA	Goldfields	6.615	**0.0366**
Survey	Goldfields	27.361	**< 0.0001**
eDNA	Meadowfoam	5.947	**0.0511**
Survey	Meadowfoam	29.678	**< 0.0001**
eDNA	Popcorn flower	1.047	0.5925
Survey	Popcorn flower	71.837	**< 0.0001**
eDNA	White-tipped clover	14.282	**0.0008**
Survey	White-tipped clover	40.003	**< 0.0001**

Table 7. Generalized linear models on relative eDNA sequencing read abundance and relative plant survey abundance with site, zone and year as fixed effects.

Method	Source	DF	ChiSquare	P-Value
eDNA	Site	28	64.0363	**0.0001***
eDNA	Zone	2	39.543	**<.0001***
eDNA	Year	1	0.795	0.3725
Survey	Site	28	62.492	**0.0002***
Survey	Zone	2	165.868	**<.0001***
Survey	Year	1	0.512	0.4743

Table 8. Logistic regressions on detection frequencies for both methods with site, zone and year as fixed effects.

Method	Source	DF	ChiSquare	P-Value
eDNA	Site	28	60.817159	**0.0003***
eDNA	Zone	2	27.215605	**<.0001***
eDNA	Year	1	0.3738589	0.5409
Survey	Site	28	34.226168	0.1935
Survey	Zone	2	241.96601	**<.0001***
Survey	Year	1	0.0240705	0.8767

Figures

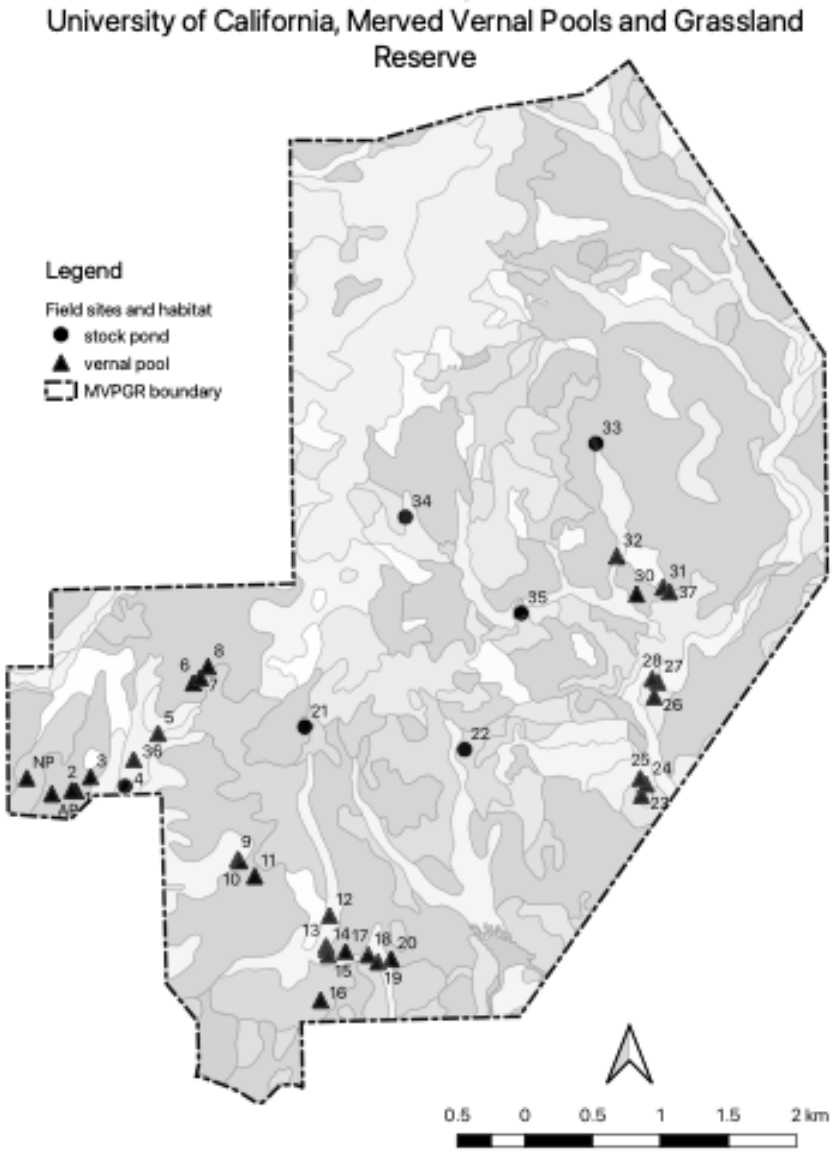

Figure 1. Map of study sites on the Merced Vernal Pools & Grassland Reserve.

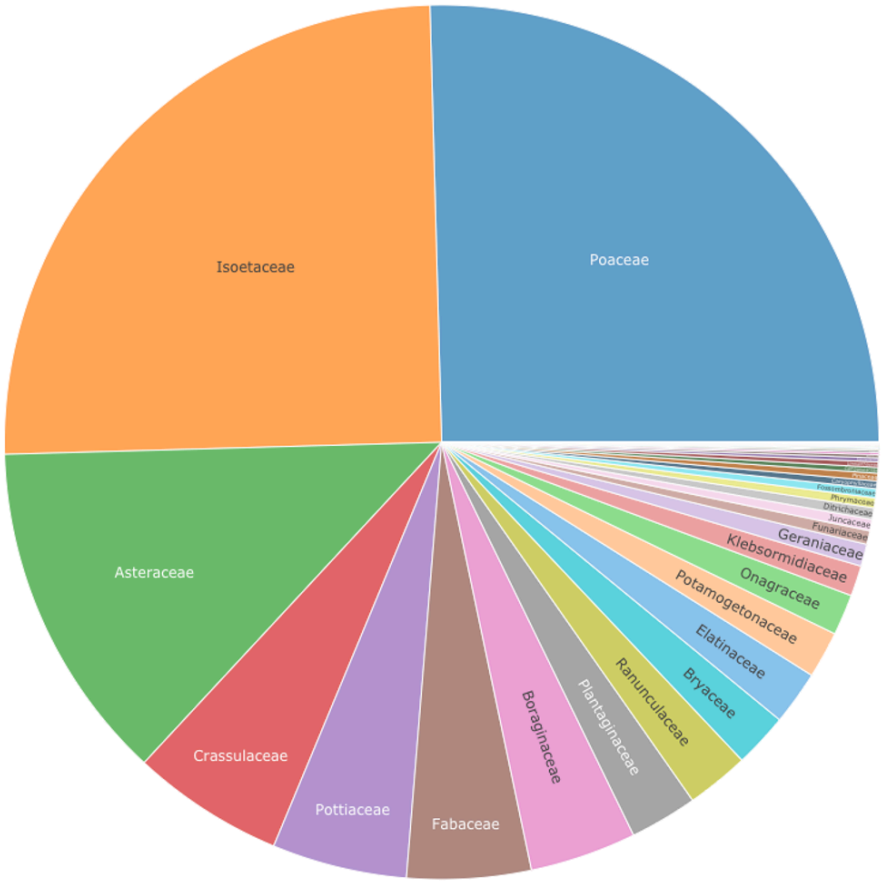

Figure 2. Sunburst plot representing 23 Streptophyta families detected through metabarcoding using ITS2.

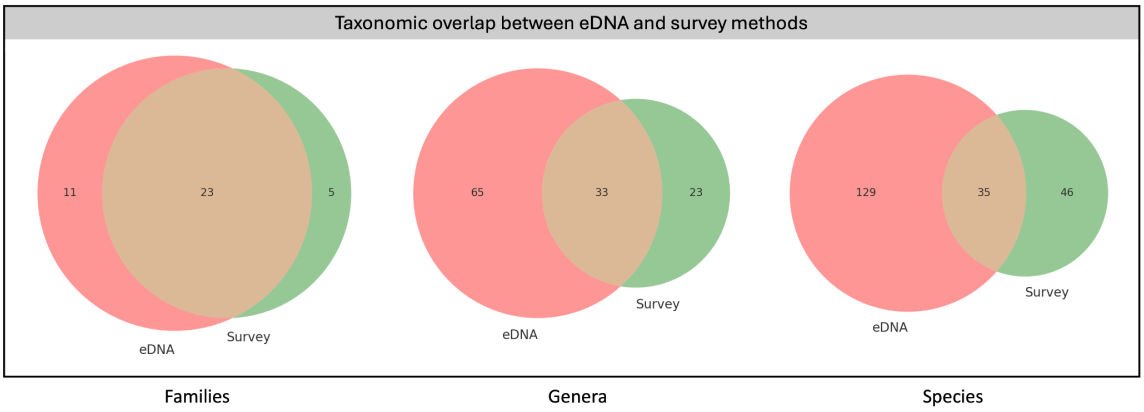

Figure 3. Venn Diagram representing total Streptophyta taxa for the eDNA dataset and community survey dataset for family, genus and species resolution.

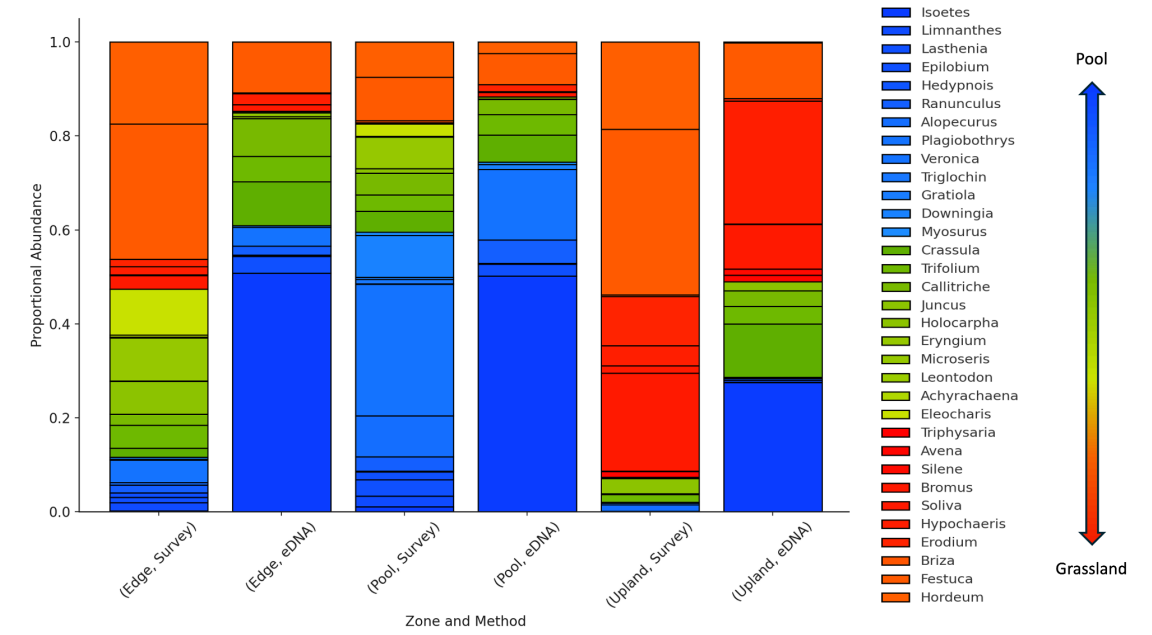

Figure 4. Genus-level relative abundance of zone habitats for the 33 overlapping genera between eDNA and plant survey methods across the study. Each bar represents plant composition sorted by detection frequency in zone: transitional (edge, green), pool bottom (pool, blue) and grassland (upland, red). The arrow shows the taxon's affinity for pool zone from pool bottom to upland habitats.

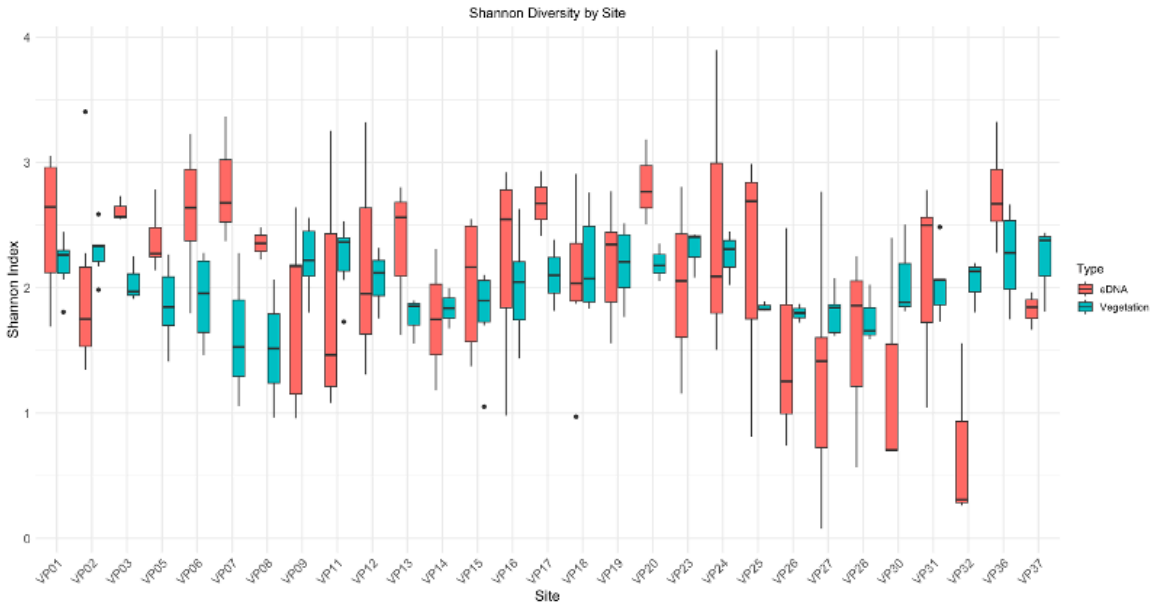

Figure 5. Box plot of shannon index representing both eDNA and community survey datasets across zones for each vernal pool site.

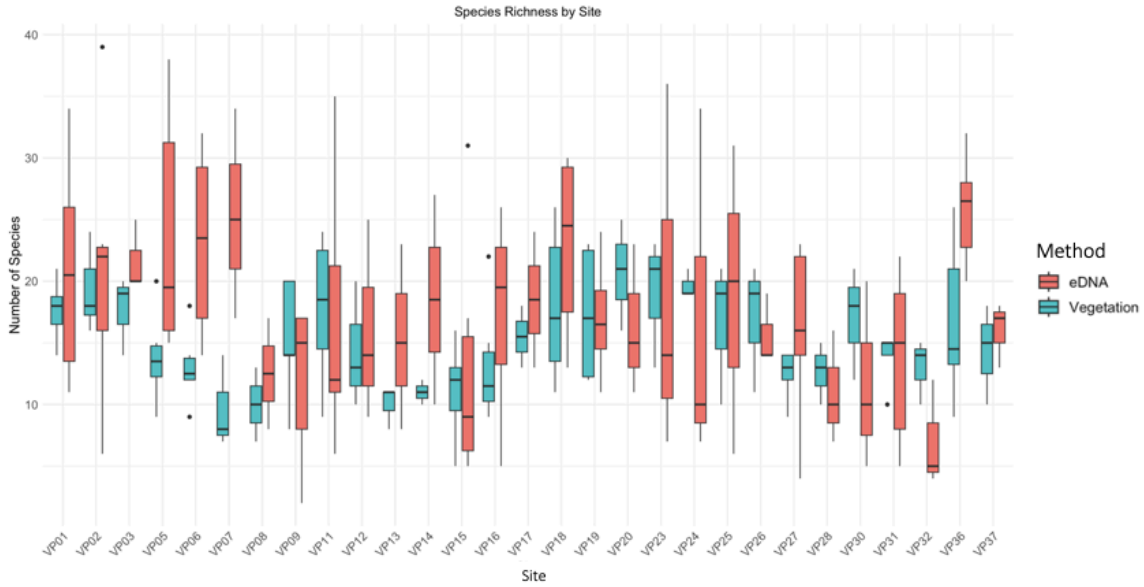

Figure 6. Boxplot of richness representing both eDNA and community survey datasets across zones for each vernal pool site.

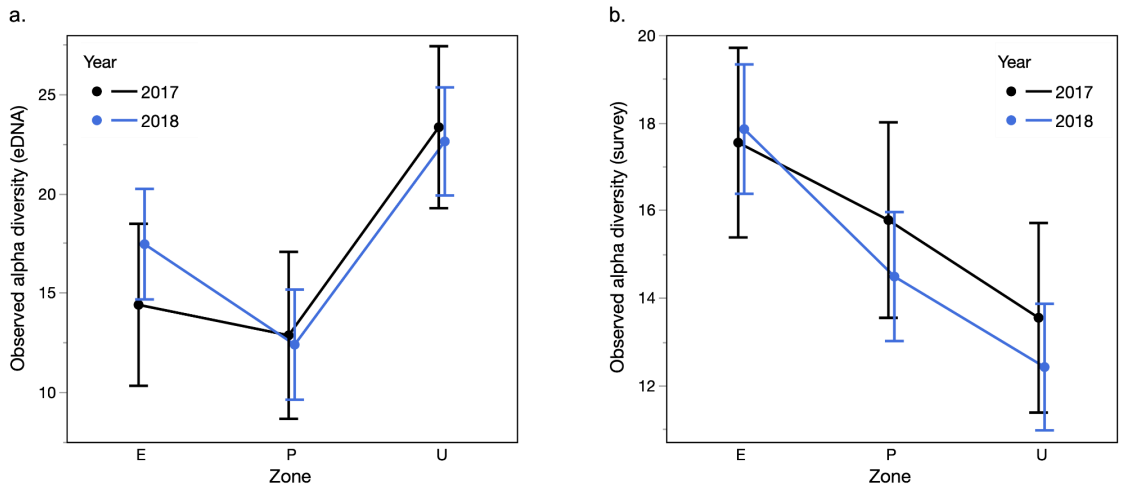

Figure 7. Least square means of species richness by zone and year detected in eDNA samples **(a)** and observed in visual vegetation surveys **(b)**.

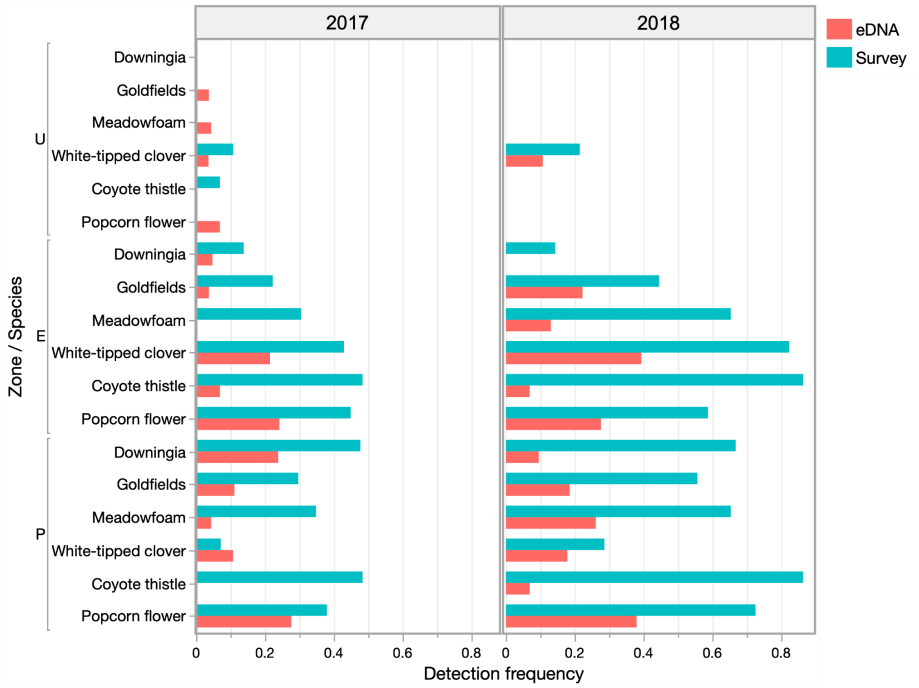

Figure 8. Bar graph of detection frequencies for the six focal taxa detected in eDNA and community surveys ranked by detection frequency for each year and zone (U, upland; E, edge; P, pool bottom).

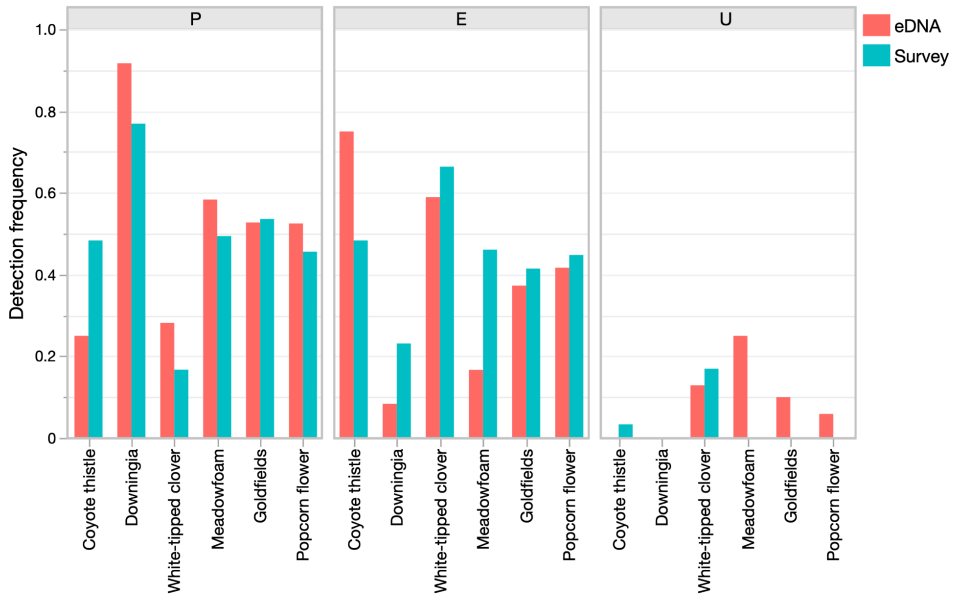

Figure 9. Bar graph of detection frequency for the six focal taxa in each pool (P), edge (E) and upland (U) zone ranked by detection frequency.

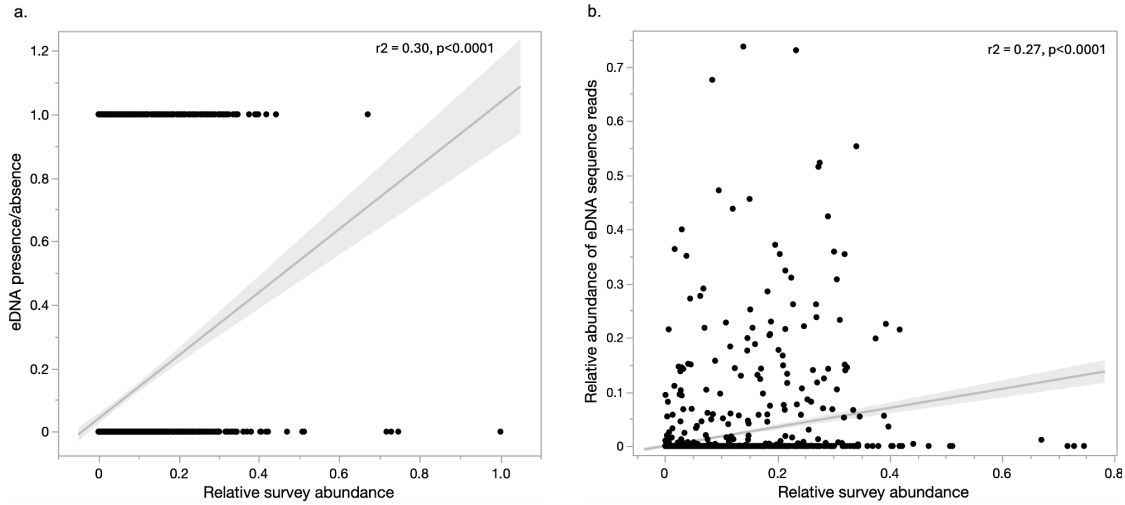

Figure 10. (a) Pearson's correlation of plant survey relative abundance and positive eDNA detections of the 33 overlapping plant genera detected in both methods. **(b)** Pearson's correlation of plant survey relative abundance and relative abundance of eDNA sequencing reads.

Supplemental information

Chapter 3.

Figure S1. Rarefaction curve of ITS2 samples.