

The influence of drought and warming on *Lupinus nipomensis*: Implications for survival, morphology, reproduction, and microbial communities

Peter Nguyen^{1,2}, Justin Luong^{3,4}, Rachel Meyer^{1,5}, Michael Loik³

¹Ecology and Evolutionary Biology Department, University of California, Santa Cruz ²Life and Environmental Sciences Department, University of California, Merced ³Environmental Studies Department, University of California, Santa Cruz ⁴Vernon and Mary Cheadle Center for Biodiversity and Ecological Restoration, University of California, Harder South, Santa Barbara ⁵Genomics Institute, University of California, Santa Cruz



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Background

- California is forecasted to have more droughts and heatwaves, which can hinder rare plant reintroduction efforts.
- Lupinus nipomensis* (Fabaceae) is an annual plant, located in southern San Luis Obispo County that is a California state and federally endangered species.
- It has variable interannual population sizes because of environmental factors.
- Microbial communities help regulate plant growth, nutrients, Water-use Efficiency, and reproduction, which can inform conservation management.



Figure 1. *Lupinus nipomensis* species in cage from Luong et al. (2019) microhabitat experiment

Methods

- We grew 80 *L. nipomensis* Jan-May 2022 under four treatments: control, drought, warming, and drought with warming.
- Withheld water until a threshold stomatal conductance of 0.05 mol m⁻² s⁻¹, then re-watered.
- Utilized a polycarbonate shelter with a heat lamp to increase temperature by 4°C.
- We collected eight soil samples grown with our experimental greenhouse plant.
- We collected soil samples from a field site (Luong et al. (2019)): wet-cool and dry-warm with or without *L. nipomensis* (+/-Ln).
- DNA was extracted using DNeasy PowerSoil Pro Kits and identified with metabarcoding PCR using 16S (bacteria and archaea), 18S (eukaryotes), fungal ITS (fungi), and CO1 (eukaryotes).
- DNA libraries were sequenced using Illumina MiSeq and the Anacapa Toolkit was used for identification.



Figure 2. *Lupinus nipomensis* greenhouse experiment



Figure 3. eDNA collection and metabarcoding PCR

Results

- Drought decreased total seed pods from 8.53 to 6.19 ($p = 0.034$), but led to higher reproductive efficiency (Seeds+Flowers), from 0.62 to 0.91 ($p < 0.001$).
- We detected **556** taxa in 16S, **377** taxa in 18S, **95** taxa in CO1 and **274** taxa in FITS.
- Using DESeq2, we found taxa that were overrepresented with higher abundance (Table 1,2).
- CO1 and FITS had different composition of wet-cool and dry-warm communities (Fig. 4).
- Greenhouse and field sites had different soil microbial richness and composition (Fig. 5).
- Drought reduced photosynthetic rates and stomatal conductance, thereby increasing Water-Use Efficiency (Fig. 6).

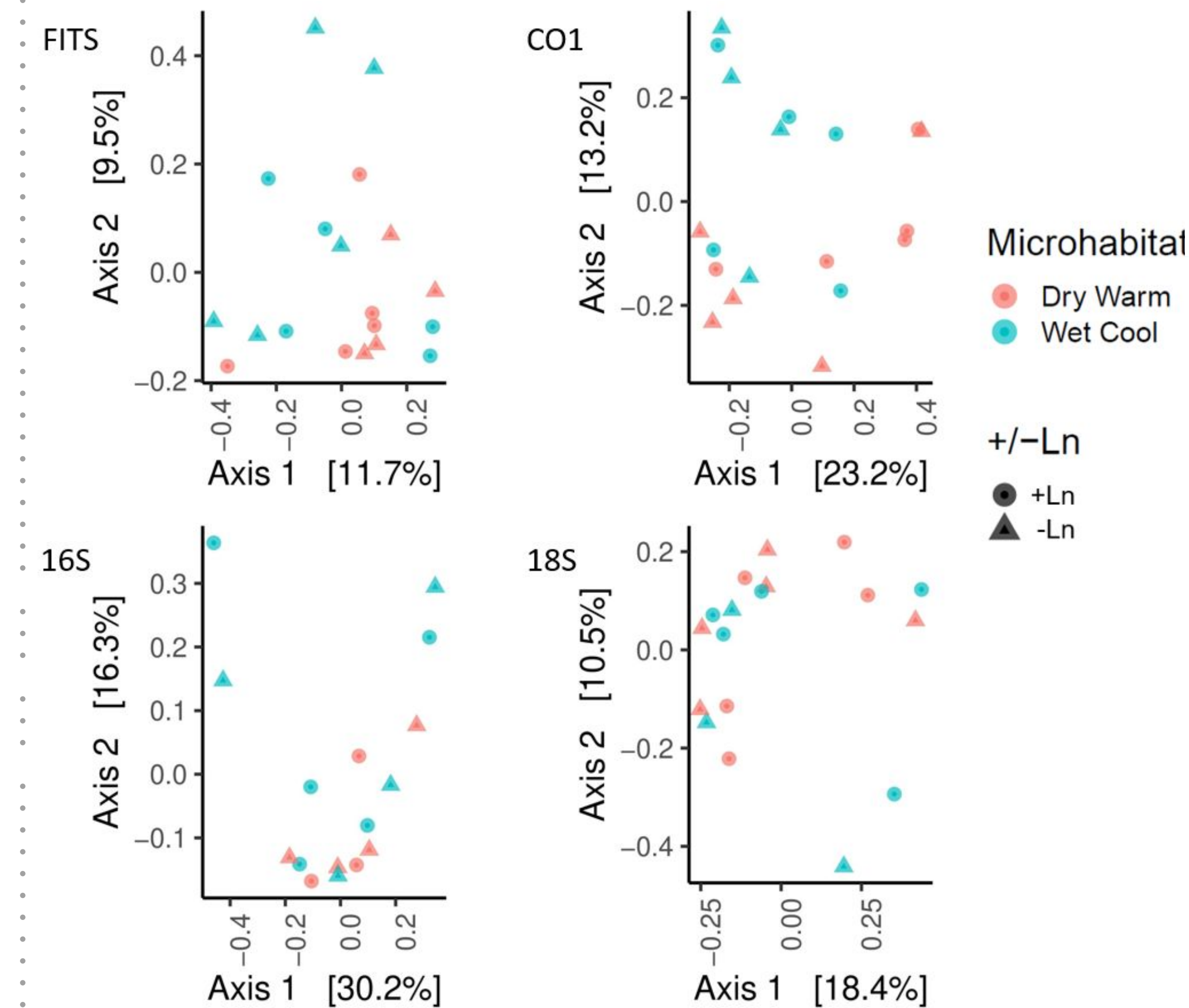


Figure 4. The principal coordinates analysis (PCoA) for FITS, 16S, 18S, and CO1 shows the relationship between samples based on their taxonomic composition.

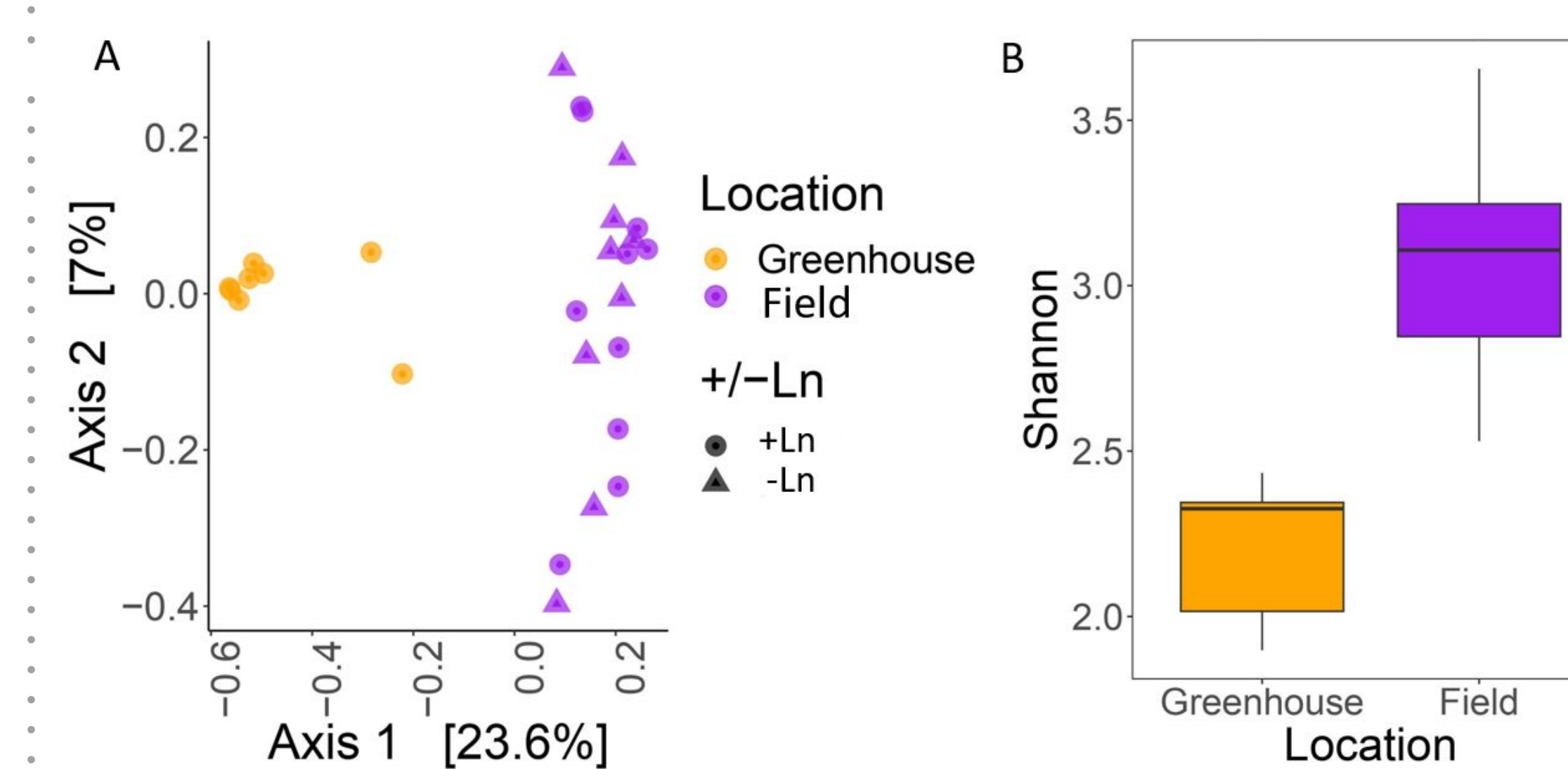


Figure 5. Visualization of beta diversity of FITS by using a Principal Coordinates Analysis (PCoA) plot of greenhouse and field samples (A). Comparison of alpha diversity in the greenhouse and BLEA soil samples using the Shannon diversity index (B).

Marker	Family	log2FoldChange	Adjusted p-value	Lower Classification
Overrepresented in wet-cool				
16S	Verrucomicrobia subdivision 3	1.33	0.11	NA
16S	Unknown Gammaproteobacteria (class)	0.97	0.04	NA
CO1	Aspergillaceae	5.89	0.11	<i>Penicillium sclerotiarum</i>
CO1	Erysiphaceae	5.34	0.14	<i>Blumeria graminis</i>
18S	Plasmodiophoridae	6.32	0.04	<i>Spongospora subterranea</i>
Overrepresented in dry-warm				
16S	Pseudonocardiaaceae	-2.04	0.04	NA
FITS	Dothideomycetes (Class)	-3.51	0.14	NA
Overrepresented in -Ln plots				
16S	Geodermatophilaceae	-2.63	0.04	Modestobacter (genus)
Overrepresented in +Ln plots				
FITS	Lasiosphaeriaceae	23.45	<0.001	<i>Cladarrhinum australe</i>
CO1	Glomerellaceae	24.16	<0.001	<i>Colletotrichum salicis</i>

Table 1. Taxa identified using DESeq2 for 16S, 18S, FITS, and CO1 taxa that were over or underrepresented across microhabitats and +/-Ln plots.

Marker	Family	log2FoldChange	Adjusted p-value	Lower Classification
Overrepresented in well-watered				
16S	Acidobacteriaceae	0.98	0.040	<i>bacterium Ellin6099</i>
16S	Gemmatimonadaceae	1.13	0.002	Gemmatimonas (genus)
16S	Nitrosomonadales (order)	2.63	0.002	NA
CO1	Helotiaceae	22.5	<0.001	<i>Articulospora tetracladia</i>
CO1	Hypocreales (order)	9.62	0.003	NA
FITS	Stachybotryaceae	24.2	<0.001	Stachybotrys (genus)
18S	Ascomycota	6.96	0.011	NA
Overrepresented in droughted				
16S	Burkholderiaceae	-4.06	0.048	NA
16S	Micropepsaceae	-1.85	<0.001	NA
16S	Oxalobacteraceae	-4.43	0.019	Massilia (genus)

Table 2. Taxa identified using DESeq2 for 16S, 18S, FITS, and CO1 taxa that were over- or underrepresented across well-watered and droughted greenhouse samples.

Conclusions

- Lupinus nipomensis* showed an increase in Water-Use Efficiency and reproduction in response to drought, exhibiting rapid acclimation.
- Soil microbial community structure of *L. nipomensis* varied across microhabitats; microclimate is key for microbial diversity.
- Lack of overlap in soil microbial communities between *in situ* and greenhouse soils shows that *L. nipomensis* occupies a broad microbial niche.
- Restoration using greenhouses to propagating rare plants may need to inoculate growth media with soil microbes from native habitats to ensure survival in the field.

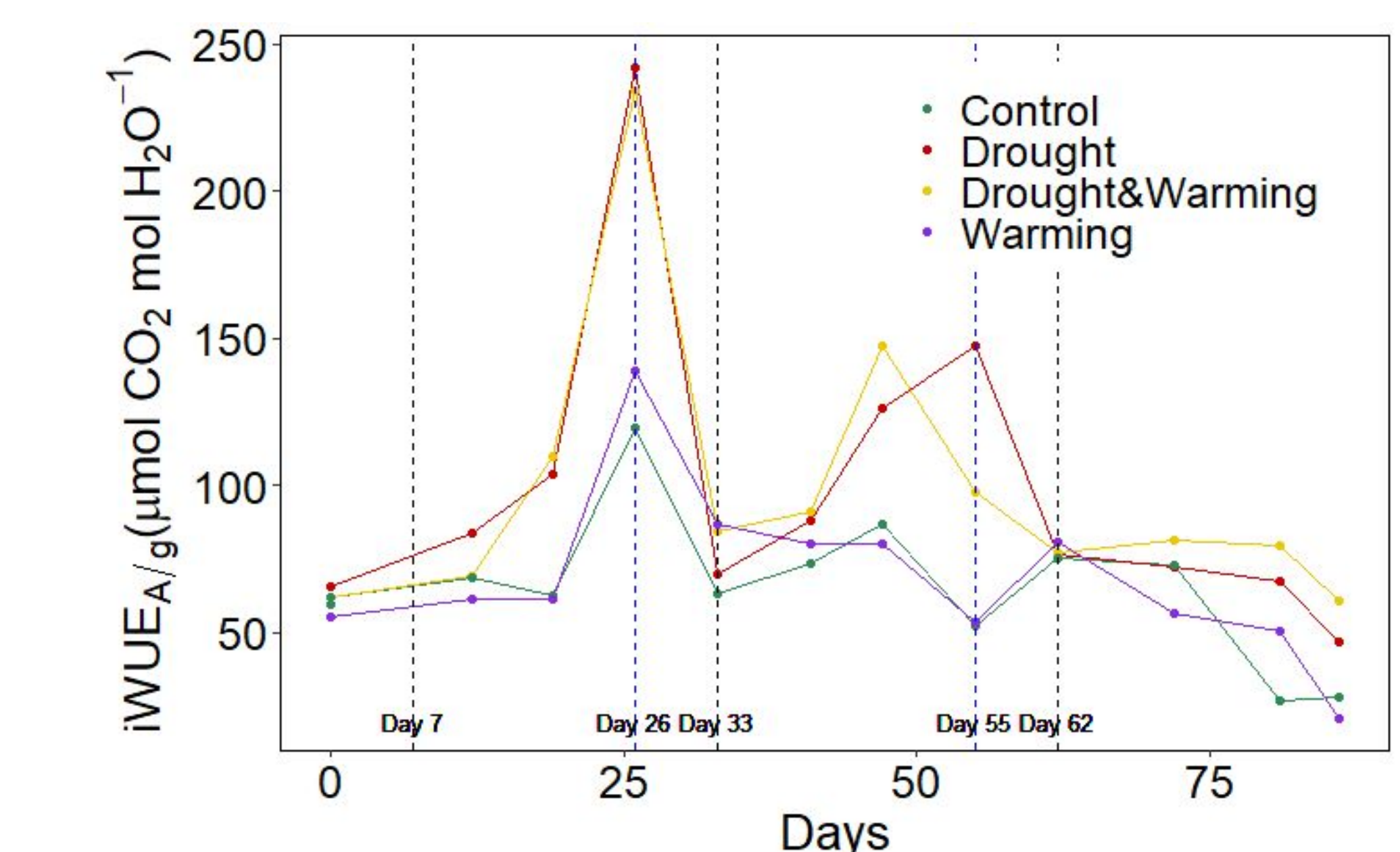


Figure 6. Water use efficiency of *Lupinus nipomensis* using GLM. The grey dotted lines represent the first, second, and third drought periods, while the blue dotted lines represent the first and second rewatering phases.

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Research Questions

- How does exposure to varying levels of drought and temperature stress influence the eco-physiological traits and reproductive success of *L. nipomensis*?
- In the field, are there soil microbial communities associated with drier or wetter microhabitats of *Lupinus nipomensis*?
- Do microbial communities of *L. nipomensis* differ between the greenhouse and the field?



pnguyen234@ucmerced.edu