Permit application for “Grassland arthropods across gradients: monitoring biodiversity and testing theory”

PI: Andrew Rominger

13) Describe project by specifically identifying timing, frequency, and how the project is expected to proceed:

We propose to collect taxonomic, genetic, and ecological data on arthropod communities at the Rio Mora National Wildlife Refuge (RMNWF). We will focus primarily on grassland arthropods as part of a larger science and monitoring project throughout the state of New Mexico. We are interested in both establishing a monitoring program for arthropods (of which summer 2018 will be the pilot season) and testing specific hypotheses (detailed in section 16) relating to how arthropod biodiversity responds to gradients in climate, land-use, and environmental heterogeneity as measured by plant and soil diversity. RMNWF is one of our more northerly sampling locations and represents natural land restored and/or rested from moderate agricultural use.

If granted permission to operate on RMNWF, we will establish our sampling plots (discussed in section 14) by 15 May 2018. Arthropod, plant, and soil sampling will proceed from then until 1 October 2018. Sampling will occur in 4 discrete campaigns (each 1 week long) during that period. Below we describe sampling the arthropods, plants, and soils. Figure 1 shows layout of sampling methods at each plot.

Arthropod sampling techniques during each of the 4 campaigns will consist of 4 methods:

1) Malaise trap: intercepts crawling and flying arthropods and collects them into a container of propylene glycol; 1 trap will be left out for 1 week
2) Pitfall trap: intercepts crawling arthropods and collects them into a container of propylene glycol; 4 traps will be left out for 1 week.
3) Berlese trap: one soil core is taken at the sampling plot and transported off site where it is placed in a funnel with a light source above, which forces soil arthropods move downward, fall out of the core, and to collect in a container of ethanol; the soil core is taken on the same day that the Malaise and pitfall traps are removed from the field. Soil cores of standard volume will be collected with an AMS soil auger.
4) Sweep and aerial netting: 2 researchers use nets to collect arthropods from the air and vegetation over a circular area of 15 m radius and during a concerted sampling duration of 7 minutes; netting occurs on the same day that Malaise and pitfall traps are removed.

Samples will be transported offsite and immediately processed. Specimens will be removed from the fluid into which they were collected and transferred to 95% ethanol to preserve their DNA. Vials containing specimens in ethanol will be labeled and databased using Darwin Core standards [1]. Specimens will be sorted into orders and a subset will be furthered keyed out to species. These fully keyed out specimens will be sequenced using a set of robust, phylogenetically informative arthropod primers [2] on an Illumina HiSeq. These voucher specimens will be deposited in the Museum of Southwestern Biology upon completion of the project. All other specimens will be sacrificed to destructive genetic sampling technique that provides sequence data and abundance estimates for all species at extremely low cost [2].

Plant sampling during each of the 4 campaigns will consist of recording the the identity of each plant found rooted within each 5 cm $\times$ 5 cm cell of a 2 m $\times$ 2 m grid. Plant sampling will take place on the same day Malaise and pitfall traps are deployed in the field. A tissue sample of each plant species at each sampling plot will be collected for DNA extraction and genetic analysis. Plant tissue samples will be stored offsite in 95% ethanol to preserve their DNA.

Soil sampling during each of the 4 campaigns will make use of the same soil core used in the Berlese trap. We will quantify 4 soil characteristics:

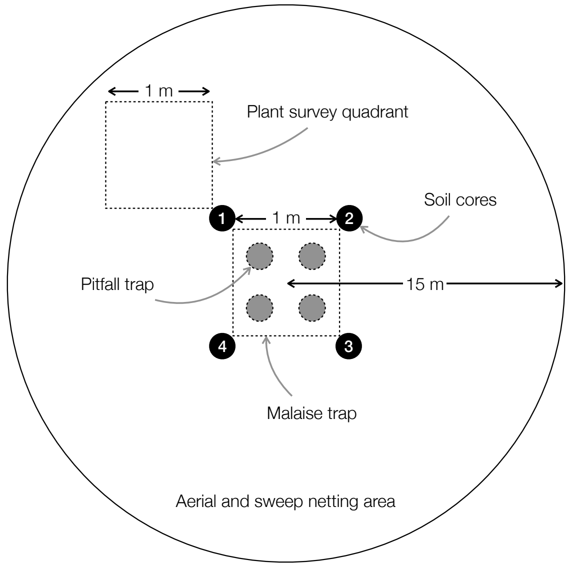

Figure 1: Aerial view of a sampling plot (not to scale). Numbers in soil cores indicate that only one soil core will be taken during each sampling campain, and the specific soil core taken will rotate clockwise around the edge of the Malaise trap. Plant survey quadrat will be randomly placed adjacent to the Malaise trap.

1) Soil moisture will be measured by weighing soil cores before placing them in the Berlese traps (which will partially dry them) and after fully drying them.
2) Soil bulk density will be used to evaluate porosity.
3) Soil texture will be measured using a hydrometer.
4) Soil chemical properties (total C, N, P) will be outsourced to Colorado State University.
5) Soil microbial community will be measured by sequencing a small sample (stored in 95% ethanol) of each soil core using standard universal microbial primers (16S and ITS) in an Illumina HiSeq.

14) Specifically identify location(s) and/or attach a map for the project:

We will establish 5 grassland and 2 woodland plots all at least 500 m distant from each other, and all within the polygon shown in Figure 2. These plots will be established by generating a random set of candidate sites and then rejecting candidate sites that are abnormal (e.g. coincide with prairie dog or harvester ant mounds) or are sensitive from a management perspective. The sensitivity of candidate sites will be discussed with Refuge staff. Candidate site rejection will proceed until 7 final plots have been selected.

15) Identify species or habitats being studied:

All arthropod species will be considered. We will focus primarily on grassland habitat, but include a small number of woodland plots for comparison (see Fig. 2).

16) Purpose/hypothesis:

We are interested in establishing long-term monitoring of arthropod diversity and populations in the Southwest. The purpose of this monitoring is two-fold: First we hope to provide useful information to conservation practitioners about arthropods and their associated plant and soil microbial communities; second we seek to use these data to test several specific hypotheses:

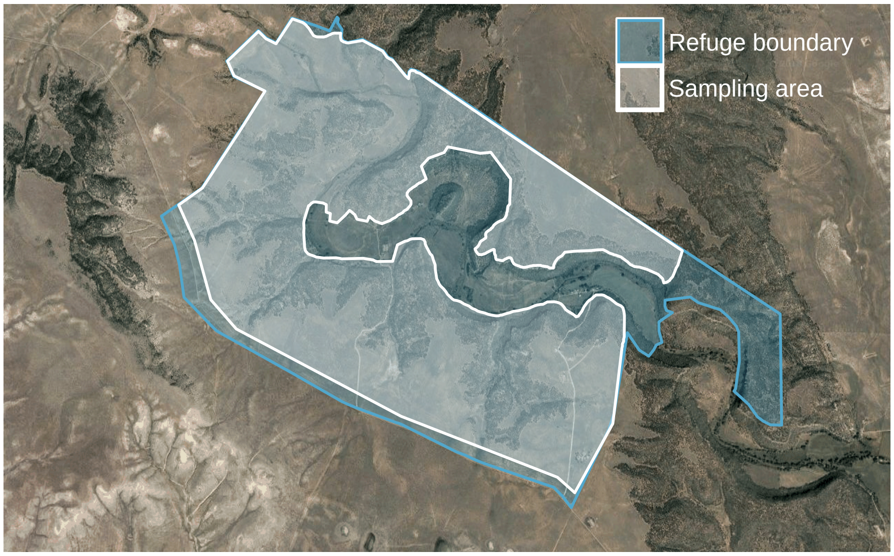

Figure 2: Map of the Refuge boundary and proposed sampling area within which a total of 7 plots will be established.

- **Climatic gradients**: Ecologists and evolutionary biologists have a long-standing interest in how diversity responds to broad climatic gradients such as those found across latitudes and elevations. Two central hypotheses in this field that we could like to address are whether such gradients in diversity are driven by historical factors (e.g. warm/more stable climates permit higher speciation rates and lower extinctions rates) or are driven by ecological factors (e.g. warm/more stable climates permit more primary productivity which facilitates greater coexistence of species). To address this debate we will sample grassland arthropods across latitudes and elevations in the state of New Mexico and evaluate whether changes in available resources (non-freezing days, moisture, biomass) or changes in evolutionary history better explain differences in diversity.
- **Gradients of environmental heterogeneity**: Another key hypothesis is that biodiversity is driven by diversity, or heterogeneity, of the environment. Here we will evaluate whether local and regional variation in plant communities and soil types predict arthropod diversity. We will integrate our local samples of plant and soil diversity with large-scale regional assessments (e.g. soil maps from the USDA's Natural Resources and Conservation Services).
- **Gradients of restoration**: The grasslands of the world have been vastly altered. In addition to understanding how biotic communities respond to natural gradients, we must also understand how land-use and our efforts to restore natural landscapes shape biodiversity. Here we hypothesize that the efficacy of restoration efforts will depend on whether communities are determined more by historical/regional factors or local/ecological factors (as tested in our previous two hypotheses). We also hypothesize that restoring the taxonomic composition of communities may depend more on these factors would restoring function—which could be largely independent of the deep evolutionary history of an area.

17) Expected benefits of research/monitoring:

Our expected benefits include documenting the response of arthropod, plant, and soil microbial communities to restoration efforts and changing regional climate. This information can be helpful in managing for these groups, which often suffer from a lack of information, particularly in the case of arthropods and microbes. We also anticipate benefits that can be generalized to other areas by embedding our results from RMNWF within our broader sampling of arthropod, plant, and microbial communities across the state, and using this larger dataset to test the hypotheses in section 16.

18) Briefly describe project history and context of research/monitoring project:

This is a new project and this year will be its pilot season. Our new work falls within the context of other arthropod research carried about by PI Rominger. The most relevant work (in terms of hypotheses tested and scale of sampling) was funded by the National Science Foundation and occurred across public lands in the state of Hawaii resulting in three publications thus far [2–4].

19) Briefly describe project's relationship to other research/monitoring projects either known of or conducted by the applicant:

We are aware of three highly relevant initiatives at RMNWF:

1) Brian Miller's project to monitor flying insects as part of a prey biomass quantification
2) David Lightfoot's project to document orthopteran abundance and diversity
3) Shantini Ramakrishnan and Luis Ramirez's grassland restoration project

We would be eager to work with Drs. Miller and Lightfoot to compare our sampling methods in order to access biases of the different techniques as well as sharing data to help augment their sampling. We would also be eager to establish arthropod monitoring plots in association with active restoration plots to understand how grassland restoration influences arthropod communities.

20) Identify the types of specimen collections to be taken (see specimen collection clause in the instruction section #20) or data to be collected during the proposed project:

Arthropod specimens will be collected from all plots using the methods described in section 13. Voucher arthropod specimens will be deposited in the Museum of Southwestern Biology. Plant tissue specimens will be taken and destructively sampled for DNA leaving no physical voucher. Microbial specimen sequences will be obtained through destructive sampling of small amounts of soil, again leaving no physical voucher. All sequence data will be archived and made publicly available through appropriate NCBI portals (e.g. https://www.ncbi.nlm.nih.gov/genbank/, https://www.ncbi.nlm.nih.gov/sra).

21) List other cooperators and institutions involved in the project:

The Juniper Hill Center (http://juniperhillcenter.org) will provide lab space for processing and storing specimens. Collaborators at the University of California Berkeley will lab space and materials for genetic analyses.

22) Generally identify the anticipated timeline for analysis, write-up and publication:

After each year's field sampling is completed, genetic data will be generated and analyzed within six months; a report of these results will be generated for documentation within our group and shared with the US Fish and Wildlife Service. We anticipate our first scientific manuscript to be submitted within two years.

23) For research involving animals, attach an Assurance of Animal Care Form or an approval from an Institutional Animal Care and Use Committee? Is a form or approval attached?

No form, not working with vertebrates.

License/Insurance/Certifications/Permits

24a) List and attach copies of any licenses you have for equipment operation (i.e., aviation or commercial boats), pesticide applications, transporters) or others if required:

None.

24b) List and attach copies of any insurance you have (i.e general liability, flight/grounding, contaminants, medical evacuation, or others if required:

Insurance Type	Carrier	Expiration Date
Health insurance for Andrew Rominger	United Healthcare	1 January 2019
Health insurance for Linden Schneider	United Healthcare	1 January 2019

24c) List and attach copies of any certifications you have, such as rat free, hull inspections, CPR/First Aid, or others if required:

None.

24d) List and attach copies of any other Federal, State, or Tribal permits if required:

None.

Logistics and Transportation

25a) Does project require personnel to stay overnight on the refuge?

No.

25b) If yes, how many? And list known personnel involved in overnight stay below:

NA

26) Specifically describe all major instrumentation/equipment/gear (i.e. use of drones) and materials used, if applicable or required:

27a) Provide details and schedule for the installation of instrumentation:

Malaise traps and pitfall traps are proposed to be installed by 15 May 2018. We will insure that sensitive areas are not damaged by installing this equipment.

27b) Provide details and schedule for the removal of instrumentation:

Equipment will be removed by 1 October 2018

27c) If instrumentation is permanent, describe need:

We request that a single rebar stake remain in place to mark the location of plots.

27d) If instrumentation requires a maintenance schedule, describe needs and schedule:

Maintenance is preformed when data are collected.

27e) Provide a data collection schedule:

We propose to complete our 4 sampling campaigns (each 1 week in duration) as follows:

- Campaign 1: middle May 2018
- Campaign 2: late June 2018
- Campaign 3: middle August 2018
- Campaign 4: late September 2018

During each campaign plots will only be visited 2 to 3 times: once for set-up, once for take-down, and a potential third time if not all data (particularly plant surveys) can be collected within two days.

28) Provide logistical arrangements for offsite transportation of samples:

Offsite transportation of samples will be by personal vehicle (detailed in section 29b-c)

29a) Provide detailed information on the logistics for onsite, intersite, and/or ship-to-shore transportation to or on the refuge, if required:

All transportation will be by personal vehicle (detailed in section 29b-c) on official Refuge roads, and by foot when travel off road is necessary.

29b) Provide descriptions, license plate and/or identification numbers of vehicles used for onsite transportation, if required:

Vehicle Type	Plate/I.D./Registration No.
Subaru Crosstrek 2013 (tan)	PFR309
Ford F150 2001 (white)	119TYT

29c) Provide descriptions, license plate and/or identification numbers of vehicles used for intersite transportation, if required:

Vehicle Type	Plate/I.D./Registration No.
Subaru Crosstrek 2013 (tan)	PFR309
Ford F150 2001 (white)	119TYT

29d) Provide descriptions, license plate and/or identification numbers of vehicles used for ship-to-shore transportation, if required

NA

30a) Is fuel cache needed?

No.

30b) Provide specific location(s) of fuel caches:

NA

31) Attach Safety Plan if required. Is Safety Plan attached?

Attached.

Work and Living Accommodations

32) Specifically describe onsite work and/or living accommodations, including spike camps:

Work will take place during daylight hours. Food and water as specified in the Safety Plan will be available during onsite work. Appropriate field gear will be used as specified in the Safety Plan.

33) Specifically describe on or offsite hazardous material storage or other on or offsite material storage space:

We will only use propylene glycol in the field, which is classified by the USDA as non-hazardous. We will use ethanol (flammable and hazardous) offsite only. Ethanol will be stored only in small quantities and kept in tightly sealed containers in a well ventilated space.

References

[1] Wieczorek, J., Bloom, D., Guralnick, R., Blum, S., Döring, M., Giovanni, R., Robertson, T. and Vieglais, D. (2012). Darwin core: An evolving community-developed biodiversity data standard. *PloS one* **7** e29715.

[2] Krehenwinkel, H., Wolf, M., Lim, J. Y., Rominger, A. J., Simison, W. B. and Gillespie, R. G. (2017). Estimating and mitigating amplification bias in qualitative and quantitative arthropod metabarcoding. *Sci. Rep.* **7** 17668.

[3] Rominger, A. J., Goodman, K. R., Lim, J. Y. and others. (2015). Community assembly on isolated islands: Macroecology meets evolution. *Glob. Ecol. Biogeogr.*

[4] Rominger, A. J., Overcast, I., Krehenwinkel, H., Gillespie, R. G., Harte, J. and Hickerson, M. J. (in review). Linking evolutionary and ecological theory illuminates non-equilibrium biodiversity. *Trends Ecol. Evol.*