2. Proposal body. **Do fungal connections increase the productivity of plants and/or biocrusts and enhance resource retention? A test of the fungal loop hypothesis.**

Introduction. Organisms can exchange resources such as water and nutrients, reducing resource loss. For example, fungi can transport water to plants[1] more rapidly than physical movement of water through the soil. The organisms have better access to needed resources and the resources remain under biological control.

In arid ecosystems, the dominant primary producers (plants and biocrusts) can be separated in space and active at different times, potentially accelerating resource losses from the system. Plants occur in a patchy distribution[2] and biocrust communities of cyanobacteria, mosses, lichens, and/or fungi occupy the surface interspaces. Many biocrust organisms fix atmospheric nitrogen[3], but it may be inaccessible to the plant roots. Temporal separation can occur because production is strongly controlled by soil moisture[4]. Biocrusts activate following rainfall events of many sizes[5], but plants require large events to increase moisture in the root zones[6].

Species interactions may couple the resource dynamics of primary producers that are disconnected in space and time. The **fungal loop hypothesis** proposes that fungi transport water and nutrients between plants and biocrusts, slowing resource loss[7] and increasing the performance of the producers. In a previous study, carbon and nitrogen were translocated rapidly between plants and biocrusts upto 1m apart[8]. Fungi are likely taking up and transporting resources because they can be active at lower soil moistures than plants[1], but this mechanism has not been experimentally tested.

In my experiment, I use mesh to inhibit physical connections of roots or fungi between biocrusts and plants. Additionally, I alter the water regime to provide large, rare pulses or small, frequent pulses. I hypothesize that when fungal connections are intact, nutrient translocation will increase producer performance and that nutrient content will be higher under either water regime.

All
Fungal and root connections intact; No mesh

Fungi
Root connections inhibited; 50µm mesh

None
Fungal and root connections inhibited; 0.45µm mesh

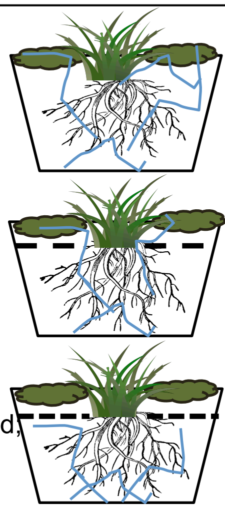

Fig. 1: Connection treatments. Blobs are biocrusts, zigzag lines are fungi, dotted lines are mesh.

Methods. I transplanted field-collected bunchgrasses (*Bouteloua gracilis*), soil, and biocrusts into pots in Aug. 2013. Pots were sunk into the ground to maintain root conditions but isolate them from neighbors.

To determine the role of fungi in resource transfer, hydrophilic mesh barriers were installed to inhibit connections between the biocrust and the rhizosphere (Fig. 1; 20 replicates). Controls had all connections intact. Mesh with 50µm pores (Small Parts, Fort Meade, FL) inhibit fine roots[10], but fungal connections remain intact. Mesh with 0.45µm pores (GE Healthcare, Pittsburg, PA) inhibit both roots and fungi[11].

To compare the effects of the same quantity of water delivered in different frequencies, I installed a watering system that provided well water to the pots. The watering regime was either **Large** infrequent events (400mL once per month, a 10mm event) or **Small** frequent events (100mL once per week, a 2.5mm event) from May to November (the frost-free months), patterns typical for this region (Western Regional Climate Center, 2014).

For performance, I calculate plant biomass from tiller number and diameter. I collect all inflorescences to calculate seed production. I will destructively harvest to determine root mass and root:shoot ratio. For biocrust performance, I extract chlorophyll *a*.

I will estimate nutrient content by sampling leaf and biocrust tissue for total N and C for 5 randomly selected pots in each treatment combination. **The GRAC funds will be used for N and C lab analysis in August 2015.** To detect plant available forms of N, PRS probes (Western

Ag, Saskatoon, Canada) will be installed under the mesh for one month[12] into ten replicates when plants become active after the monsoon rains in 2015.

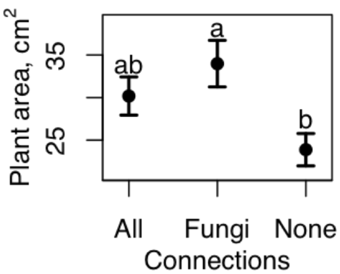

Fig. 2: Mean (± 1 *s.e.*) plant performance by connection treatment. Letters show results of Tukey HSD test, Connections treatment, P < 0.05.

Results. Initial results support the fungal loop hypothesis because the presence of fungal connections resulted in higher biocrust performance and the highest plant productivity. By Oct. 2014, biocrusts with hyphae inhibited had less chlorophyll than those with all connections intact ($F_{2,118}$=4.0, P=0.02). By July 2014, plants with hyphae inhibited were smaller than those with fungal connections intact ($F_{2,118}$ = 8.6, P < 0.01; Fig. 2).

PRS probes and total C:N analyses will determine if fungal connections improve nutrient retention, providing a potential mechanism for the improved performance of plants and biocrusts observed already. If fungi drive resource exchanges, biocrust or plant performance and nutrient content should not differ with all connections intact vs. only fungi intact, but should decline when fungal connections are inhibited. I will submit this work for publication by Spring, 2016.

Implications. This study is one chapter of my dissertation. The second chapter compares how soil fungi affect biocrust and plants when growing together or separately, testing for facilitation or competition in the interaction. The final chapter uses stable isotope labels to track water and nitrogen moving between plants and biocrusts with and without fungal connections.

By testing the fungal loop hypothesis, we will understand how the species interactions affects nutrient dynamics and can predict how arid land communities and nutrient cycling will shift with changing precipitation patterns[9]. Land managers can use this information for restoring degraded communities by accounting for the primary producers' interactions with the fungal community.

3. Literature Cited

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3. **Previous Use of GRAC Research Funds**
   I have never received GRAC research funds before.

4. **Budget**
   The entire experiment has been set up in 2013 (funded by the Harry Wayne Springfield Scholarship from the UNM Biology Department) so there is no additional cost for that.

Item	Description	Cost per unit	Total needed	Total cost	Funding source
Travel	Round trip, Corrales to La Puebla, NM to install probes and collect biocrust and grass samples.	$0.23 per mile, based on federal Standard Mileage Rates for moving expenses	180 miles	$41.40	Personal savings = $41.40
Lab Analysis	Soil sample analysis UNM Biology Annex Analytical Services using CE Instruments Model NC2100 for total C and N	$6.00	Mesh (3 levels) × water regime (2 levels) × 1 grass and 1 biocrust sample per pot × 5 replicates = 72	$432.00	**GRAC = $400.00.** Personal savings = $32.00.
Supplies and Lab Analysis	PRS probes. Western Ag. Innovations provides probe and analysis	$40.00	Mesh (3 levels) × water regime (2 levels) × 5 replicates = 30	$1,200.00	2015 Western Ag Innovations Research Award, proposal submitted Feb. 18 for $1,200.
Total	$1,673.40		**Total GRAC requested = $400.00, *pending*.** Total requested from other sources = $1,200.00, *pending*.		