Shipley-Skinner Reserve – Riverside County Endowment Proposal: Improving the Success of Plant Restorations by Comparing Soil Microbial Composition among Restored and Intact Native Systems

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**Background and Rationale**

Plant invasion by a non-native species can change various abiotic and biotic soil conditions, profoundly affecting the soil environment in ways that have been shown to inhibit the growth of native plants long after the removal of invasive grasses from an area (Cuddington, 2012; Hawkes et al., 2006; Jordan et al., 2007; Kourtev et al., 2010). Invasion can alter important nutrient cycles, prevent microbial mutualisms that provide the native plant with more water and mineral absorption, or inhibit natives through the introduction of novel plant pathogens (Jordan et al., 2007). Invasive plants directly alter the abiotic conditions of the soil through input of lower or higher quality litter, different timing of litter deposition, root exudates, as well as changes in pH, soil moisture and/or enzymatic activity caused by the change in plant composition (Dickens et al., 2012). Soil nitrogen is one common abiotic variable that is affected by invasive grass growth; this often results from altered nitrogen input of decomposing invasive grass biomass. This and other abiotic changes can lead to shifts in the soil microbial composition, which is why invasive plant species are commonly associated with bacterial communities that differ from native species. Such changes have been shown to inhibit the growth of native plants long after the removal of invasive grasses from an area (Hawkes et al. 2006, Jordan et al. 2007, Kourtev et al. 2010). Certain restoration techniques, such as soil inoculation, seek to reverse these soil microbial changes in an effort to create successful native plant establishment.

With this information in mind, I conducted a greenhouse and field experiment over the course of two years to determine whether microbial or abiotic legacy effects of invasive grasses inhibit the growth of natives and if these effects can be reversed. I grew 3 native plants and one invasive grass in either native intact soil or post-invasive grass soil (soil from which invasive grass has been removed) and determined that the soil type was correlated with native and invasive plant growth. I then grew the three native species in native soil and transplanted them into a 25 acre post-invasive grass site to determine if differences in native plant growth can be explained by host dependent changes in the microbial community, not just a change in the nutrient content of the two soils. I found that for one of the native species (*Artemisia californica*), plants that were inoculated with unsterilized native soil before planting had less mortality and larger growth than plants that were inoculated with sterilized native soil.

My goal now is to advance this study by determining what microbes actually associate with the native plant roots in a restored system with soil inoculum vs. a native system. Further information about the microorganisms that associate with these native plant species in restored vs. native sites may better inform our restoration methods, increase understanding of the role of microbes in restoration, and ultimately increase restoration success. This study will determine not only the microbial root associations of the native plants in restored vs. native sites, but also if soil restoration techniques in post-invasion sites yield native plant biomass that is comparable to plants grown among the intact soil of California sage brush stands.

**Research Objectives**

The following objectives are designed to understand if intact native sites vs. restored post-invasive sites using soil inoculum are comparable in plant growth success and microbial composition:

1. Determine if restoration strategies using native soil inoculum in post-invasive sites are as successful as native plants growing among intact California sage scrub. The primary method of comparing “success” will be plant height and viability.
2. Determine if the rhizosphere communities of the native plant species differ more between or within native and restored sites.
3. Determine if the non-plant associated microbes between the native and restored sites are different and if the bulk soil microbial composition is comparable to the root-associated microbial composition within sites.

**Research Plan**

In my previous research described earlier, I chose three native species(*Artemisia californica, Salvia leucophylla, and Baccharis pilularis*) that are most commonly used by the National Park Service for restorations. I did this to ensure that my findings would be useful in future restorations. I found that while *A. californica* grew better in intact native soil vs. post-invasive soil, the other two natives grew equally well in either soil type. I also demonstrated that *A. californica* and *B. pilularis* showed higher plant growth in the post-invasive site after native soil inoculation. Further findings about the underlying microbial associations of these plants and a comparison of restoration with inoculation vs. intact native stands would be valuable to this research. For these reasons, I have decided to use the same three native plants for this current project.

Approximately 3 months prior to the field study, all of the plants (n=120) will be grown in a UCR greenhouse in conetainers filled with sterilized standard potting mix combined with native soil. Half of the plants (n=60) will be transplanted from the conetainers into an intact stand of California sage scrub and the other 60 plants will be transplanted into a site where the grass has been cleared (post-invasive site). A total of 60 plants will be destructively sampled at the end of this experiment and the other 60 will remain intact as part of a small restoration. The chart below describes the amount of each plant species that will be transplanted into each site and the amount of plant species that will be destructively sampled vs. untouched in each site.

|  |  |
| --- | --- |
| **Plants in Intact Native Sage Scrub Site** | **Plants in Post-invasive grass site** |
| 10 *A. californica* for destructive sampling | 10 *A. californica* for destructive sampling |
| 10 *S. leucophylla* for destructive sampling | 10 *S. leucophylla* for destructive sampling |
| 10 *B. pilularis* for destructive sampling | 10 *B. pilularis* for destructive sampling |
| 10 *A. californica* to remain intact | 10 *A. californica* to remain intact |
| 10 *S. leucophylla* to remain intact | 10 *S. leucophylla* to remain intact |
| 10 *B. pilularis* to remain intact | 10 *B. pilularis* to remain intact |

Microbial DNA extraction and analysis of the root and soil samples will take place at the end of the field study. Primers targeting the V3 – V4 region of the 16S rRNA gene will be used to detect bacteria, while primers for the ITS2 region will be used to detect fungi. The methods for each objective are described in detail below:

1. *For objective 1*: in order to understand if plant growth in restorations using native soil inoculum is comparable to the growth of native plants growing in intact native sites, we will take plant height and mortality measurements once a week for 5 months for all plants (n = 120) in the post-invasive and native systems.
2. *For objective 2*: in order to understand if the rhizosphere communities of the native plant species differ more between or within natural and restored sites we will destructively sample a total of 60 native plants over 5 months. The microbial community of the collected rhizosphere soil and root samples will be analyzed. The chart below describes the amount of destructive sampling each month:

|  |  |  |
| --- | --- | --- |
| Month | Intact Native Sage Scrub site | Post-invasive grass site |
| 1 | 6 plants = 2 of each species | 6 plants = 2 of each species |
| 2 | 6 plants = 2 of each species | 6 plants = 2 of each species |
| 3 | 6 plants = 2 of each species | 6 plants = 2 of each species |
| 4 | 6 plants = 2 of each species | 6 plants = 2 of each species |
| 5 | 6 plants = 2 of each species | 6 plants = 2 of each species |

1. *For objective 3*: In order to understand if the non-plant associated microbes between the native and restored sites are different and if the non-plant associated microbes vs. root-associated microbial compositions are comparable within sites, I will collect 6 bulk soil cores per site each month (n = 60) to compare the microbial composition of the bulk soil and root-associated soil.

As stated above, some of the plants I will grow will be destructively sampled over the course of this field experiment, so I am leaving half of the plants intact as a restoration.

**Budget**

Brooke Pickett is a doctoral student in the Department of Evolution, Ecology, and Organismal Biology and UC Riverside. Three months of salary plus benefits is requested to support Brooke during this project ($6,614). Funds are requested for trips to and from each sampling location (0.5867/mile x 36 miles round trip from UCR to the Motte Reserve x 20 trips = $422 total). To extract DNA from 180 samples and prepare them for sequencing (6 rhizosphere soil samples per site x 5 time points = 30 x 2 sites = 60; 6 root samples per site x 5 time points = 30 x 2 sites = 60; 6 bulk soil samples per site x 5 time points = 30 x 2 sites = 60; 60 rhizosphere soil samples + 60 root samples + 60 bulk soil samples = 180 samples total), funds are requested for DNA extraction materials, DNA purification materials, polymerase chain reaction (PCR) master mix, barcoded primers, and DNA quantification materials. Altogether, these materials will total $50/sample ($50 x 180 samples = $ 9,000 total for sample preparation). In addition, funds are requested to sequence the samples on the Illumina MiSeq platform (paired end analysis, 300 base pair reads; $1,600). Other costs of consumables include molecular analysis, such as microcentrifuge tubes, pipette tips, and disposable gloves; $ 1,000 should cover this cost. The budget is outlined in the chart below:

|  |  |
| --- | --- |
| Three months salary plus benefits | $ 6,379 + $235 benefits = $6,614 |
| Travel to site | $ 422 |
| Sample extraction and sequence preparation | $ 9,000 |
| Illumina MiSeq run | $ 1,600 |
| Consumables | $ 1,000 |
| **Total** | **$ 18,636** |

**Literature Cited**

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