6TH ANNUAL!

YOSEMITE SYMBIOSIS WORKSHOP
MAY 6-8, 2016

Yosemite Symbiosis Workshop
Sierra Nevada Research Institute
http://www.sachslab.com/symbiosis-2015.php
Co-organized by Joel Sachs and Carolin Frank

Friday May 6

Welcome Party *7:00pm* Landsneas House 2667 English Lane, Wawona CA (see attached map)

Saturday May 7

- Wawona Community Center (see attached map)

Lunch 12:00-1:00pm

1:00pm Introduction and welcome Joel Sachs

Session I ***Evolution and ecology of symbionts and their hosts***

1:15	Marisol Sanchez-Garcia	Increased diversification in two ectomycorrhizal clades of the suborder Tricholomatineae
1:30	Sydney Glassman	Life history and environmental drivers structuring host-associated assemblages of mycorrhizal and endophytic fungi
1:45	Eric Gordon	Largus than life: Ancient and persistent environmental symbiont reacquisition in bordered plant bugs (Pyrrhocoroidea: Largidae)

30 minute break for coffee and snacks

Session II ***Symbiosis and the alteration of hosts and their environments***

2:30	Sharon L. Doty	Plant-microbe symbiosis for improved removal of a broad range of carcinogenic pollutants
2:45	Amelia Wolf	Legumes are not hard wired for high nitrogen concentrations but incorporate more nitrogen in the presence of bacterial partners
3:00	Adriana Corrales Osorio	An ectomycorrhizal nitrogen economy facilitates monodominance in a neotropical forest
3:15	Pilar Martínez-Hidalgo	What are all those other bacteria in addition to rhizobia doing in the root nodule?

15 minute break for coffee and snacks

Session III ***Selection, cooperation and conflict in symbiosis services***

3:45	Laura Bogar	What stabilizes the ectomycorrhizal mutualism? An experimental test of partner choice by *Pinus muricata* in association with *Suillus brevipes*
4:00	Camille Wendlandt	Legume host effects on symbiotic benefit from complex soil microbial communities
4:15	David Hembry	Repeated transitions from mutualism to parasitism in leafflower-leafflower moth interactions
4:30	Andrew Siefert	Rhizobial mutualists mediate plant-soil feedbacks and plant-plant interactions of co-occurring, congeneric clovers
4:45	Megan Rúa	Variation in plant and fungal traits indicate mycorrhizal mediated selection in Pinus radiata

30 minute break for beer, wine and snacks

KEYNOTE LECTURE 5:30 – 6:30

Betsy Arnold

Mosaics, megabases, and matryoshki: a leaf-to-landscape perspective on the symbiotic renaissance

Dinner 6:30-8:00pm

Session IV **Poster Session - 8:00-9:00**

Sunday May 8

Breakfast 8:00-9:00am

Session V *Symbioses in a changing planet*

9:00	Dana Carper	The effect of climate change and site on the above- and belowground bacterial endophytic communities of subalpine conifer seedlings
9:15	Alyssa Carrell	Experimental warming effects on microbial communities of Sphagnum moss
9:30	Luis Cayetano	Symbiotic and plant associations of non-native Hawaiian hemipterans
9:45	Kimberly La Pierre	Unlocking the mechanisms behind legume invasions: Are rhizobial mutualists the key?
10:00	Emily Bellis	Evolutionary dynamics of Aiptasia-Symbiodinium symbioses in Caribbean Panama.
10:15	Joel Sachs	An experimental nitrogen deposition gradient increases mortality and eliminates symbiosis in a native legume

30 minute break for coffee and snacks

Session V *Mechanism and ecology of symbiosis*

11:00	A. Carolin Frank	Foliar nitrogen fixation in conifers – an ecological perspective
11:15	Katherine Muller	Physiological Mechanisms of Cooperation and Conflict in the Legume-Rhizobium Mutualism
11:30	Amanda M.V. Brown	Evolutionary genomics of rhizosphere endosymbionts: getting to the root of it
11:45	Phillip A. Cleves	Developing transgenic tools to study cnidarian-symbiodinium symbiosis in a sea-anemone model system
12:00	Peter Graystock	The importance of host phylogeny and geography on the microbiome, with implications for wild bee health and conservation

Lunch 12:15-1:30pm

Talks (in order of presentation)

1. Marisol Sanchez-Garcia, Brandon Matheny (University of Tennessee)

Increased diversification in two ectomycorrhizal clades of the suborder Tricholomatineae

Fungi are one of the most diverse groups of organisms, however little is known about the processes that shape such an outstanding taxonomic diversity. This study focuses on the evolution of ectomycorrhizal (ECM) fungi, symbiotic associates of many trees and shrubs, in the suborder Tricholomatineae of the Agaricales. Six clades of ECM fungi within the Tricholomatineae were identified. We used the binary state speciation and extinction (BiSSE) model and the Bayesian analysis of macroevolutionary mixtures (BAMM) program to test the hypothesis that the ECM habit represents an evolutionary key innovation that allowed the colonization of new niches followed by an increase in diversification rate. We detected two diversification rate increases in the genus *Tricholoma* and the Rhodopolioid clade of the genus *Entoloma*. However, no increases in diversification were detected in the four other ECM clades of the Tricholomatineae. We suggest that diversification of *Tricholoma* was not only due to the evolution of the ECM lifestyle, but to the expansion and dominance of its main hosts and ability to associate with a variety of hosts. Diversification in the Rhodopolioid clade could be due to the unique combination of spore morphology and ECM habit. The spore morphology may represent an exaptation that aided spore dispersal and colonization.

2. Sydney Glassman, Ian J. Wang, Kaitlin C. Lubetkin, Thomas D. Bruns (UC Berkeley)

Investigating life history and environmental drivers structuring host-associated assemblages of mycorrhizal and endophytic fungi

Fungi are an understudied group of microbes that play pivotal roles in all terrestrial ecosystems, yet the basic processes that structure their assemblages are poorly understood. Here a simple host-island setting in Yosemite National Park is used to test for the effect of dispersal distance, host type, and environment on community structure in two host-dependent guilds of fungi. Using cutting edge next generation sequencing technologies, we sampled the fungal community associated with both the roots and the leaves of 40 trees in YNP. Strong evidence of both dispersal limitation and host filtering was found for ectomycorrhizal fungi (EMF) in this subalpine tree island system composed of two Pinus congeners, and the same principles are being tested in foliar endophytic fungi (FEF). The comparison of the two functional guilds of symbiotic fungi, which has never been made in the same system, will shed light on the basic biology of these fungal symbionts, and has the potential to yield critical insights into the factors driving community assembly of microbial symbionts in general.

3. Eric Gordon, Quinn McFrederick, Christiane Weirauch (UC Riverside)

Largus than life: Ancient and persistent environmental symbiont reacquisition in bordered plant bugs (Pyrrhocoroidea: Largidae)

Obligate symbioses with bacteria are common in nature and symbiotic bacteria are typically vertically inherited through generations. Within Hemiptera, nearly all phytophagous taxa are thought to depend on bacteria for nutritional supplementation of their diet. However, identities and transmission routes of symbionts have not been investigated for all families, particularly for true bugs (Heteroptera). While Pyrrhocoridae have been relatively well studied with respect to bacterial symbioses, their closest relatives, Largidae, are only beginning to be explored using modern molecular methods. We conducted paired-end Illumina and Sanger sequencing of bacterial 16S amplicons of 30 pyrrhocoroid taxa, including 17 species of Largidae, in order to determine bacterial associates and similarity of associated microbial communities among this group. We also constructed a comprehensive phylogeny of this superfamily (4,800 bp; 5 loci; 56 ingroup + 9 outgroup taxa) to understand evolutionary patterns regarding bacterial associates. We found associations of the plant-beneficial-environmental clade Burkholderia within members of the family Largidae and marked differences between predatory and herbivorous members of the Pyrrhocoridae. Additionally, we undertook multiple lines of investigation (i.e., experimental rearing and phylogenetic and co-evolutionary analyses) to understand transmission routes of largid symbionts. We find evidence that suggests, that like other distantly-related Heteroptera, Largidae acquire bacterial symbionts from the environment every generation, a strategy that has seemingly persisted in some true bug lineages since the Cretaceous.

4. Sharon L. Doty, Zareen Khan, Jun Won Kang, Andrea Firrincieli, Robert Tournay, Dominic Sivitilli, Mitch Scott, John Freeman (University of Washington)

Plant-microbe symbiosis for improved removal of a broad range of carcinogenic pollutants

Phytoremediation is an effective technology for remediation of a wide range of environmental pollutants. However, when the pollutant is at phytotoxic concentrations, a modified technology is required. Endophytes are microorganisms that live within plants that can provide multiple benefits to the plant including increased nutrient acquisition and growth, and improved stress tolerance. In addition, some strains of endophytes have the capacity to degrade or otherwise reduce the toxicity of xenobiotics. We have isolated endophytes that degrade TCE, PAHs, and TNT, and addition of the strains to plants results in improved plant health on normally phytotoxic levels of these pollutants. The genomes of the TCE and PAH degrading strains have recently been sequenced, providing insight into mechanisms by which the strains can improve phytoremediation. Using fluorescent microscopy, we have demonstrated that multiple strains can co-colonize poplar, providing the potential to equip plants for sites polluted with different classes of chemicals. A field trial of the TCE-degrading strain with poplar trees is currently underway. In addition to the endophytes capable of degrading organic pollutants, we have recently isolated an arsenic-tolerant endophyte that reduces the phytotoxic effects of arsenic in inoculated Arabidopsis seedlings. Endophyte-assisted phytoremediation is an important technological advance enabling this green technology to be utilized on a broader spectrum of contaminated areas.

5. Amelia Wolf, Duncan Menge, Jennifer Funk (Columbia University)

Legumes are not hard wired for high nitrogen concentrations but incorporate more nitrogen in the presence of bacterial partners

Global climate change, agriculture, and pollution are changing the distribution and availability of essential plant nutrients, including nitrogen (N). Because of their key role in driving ecosystem processes such as carbon sequestration, understanding how symbiotic nitrogen fixing plants will be affected by changes in N availability is critical. In this study, we manipulated plant access to both microbial symbionts and soil N to address two questions: 1) Are N fixing plants hard wired to have high N concentrations across realistic gradients of N availability? and 2) Besides delivering fixed N, does the presence of the rhizobial symbionts affect plant nutrient uptake and allocation? We examined four herbaceous legumes species and subjected them to nine levels of N fertilization in a greenhouse; half of the individuals per species were inoculated with species-specific rhizobia, the other half remained uninoculated and therefore could not fix nitrogen. All four species we examined had greatly decreased foliar N concentrations in response to lower N availability, leading to highly variable N use efficiency (NUE) and N:P across the N availability gradient. Surprisingly, however, the flexibility in nutrient ratios exhibited by these species was much higher than has been described for other plant species. In addition, we found that access to symbiotic bacteria significantly increased N concentrations in the legumes, even after accounting for higher access to N from fixation. These results suggest that rather than being hard-wired for high N demand, legumes are highly plastic in their nutrient concentrations; high N in these legumes is due to the plant response to the symbiotic interaction as well as the fixed N produced by those partners.

6. Adriana Corrales Osorio, Scott Mangan, Benjamin Turner, James W. Dalling (University of Illinois)

An ectomycorrhizal nitrogen economy facilitates monodominance in a neotropical forest

Tropical forests are renowned for their high diversity, yet in many sites a single tree species accounts for the majority of the individuals in a stand. An explanation for these monodominant forests remains elusive, but may be linked to mycorrhizal symbioses. Here we tested three hypotheses by which ectomycorrhizas might facilitate the dominance of the tree, Oreomunnea mexicana. We tested whether access to ectomycorrhizal networks improved growth and survival of seedlings, evaluated whether ectomycorrhizal fungi promote seedling growth via positive plant–soil feedback, and measured whether Oreomunnea reduced inorganic nitrogen availability. We found no evidence that Oreomunnea benefits from ectomycorrhizal networks or plant–soil feedback. Instead, we found three-fold higher soil nitrate and ammonium concentrations outside than inside Oreomunnea-dominated forest and a correlation between soil nitrate and Oreomunnea abundance in plots. Ectomycorrhizal effects on nitrogen cycling might therefore provide an explanation for the monodominance of ectomycorrhizal tree species

7. Pilar Martínez-Hidalgo, Esteban Veliz, Pierrick Bru and Ann M. Hirsch (UC Los Angeles)

What are all those other bacteria in addition to rhizobia doing in the root nodule?

Nodules are plant organs generated mainly on the roots of leguminous plants, in cooperation with alpha- and beta-proteobacteria, which fix atmospheric nitrogen into ammonia to promote plant growth. It was initially thought that only symbiotic nitrogen-fixing bacteria could inhabit healthy N2-fixing nodules, but recent studies show that nodules are frequently populated by a broad and heterogeneous group of Gram-positive and Gram-negative bacteria. Numerous findings suggest that their presence in the nodule is not accidental, but that they may have an important ecological function.

Micromonospora is one of the commonly found inhabitants of Medicago sativa root nodules, and previous work indicates that it is an important Plant Growth Promoting Rhizobacterium (PGPR). After finding several Micromonospora strains in nodules in Spain and Australia, our work focused on the occurrence of Micromonospora, as well as other PGPR, in alfalfa nodules in the U.S.A.

In our study, we analyzed the microbiota present in nodules of Medicago sativa at different locations in California. We detected a diversity of microorganisms inhabiting the nodules of alfalfa. In addition to several strains of the genus Ensifer, we found strains from the following genera: Rhizobium, Ochrobactrum, Methylobacterium, Oceanobacillus, Bacillus, Paenibacillus, Streptomyces, Micromonospora, and others. All isolates were characterized for symbiotic traits in vitro, and based on the results, selected strains were used for in planta assays.

Our results demonstrate that Micromonospora is not commonly found in alfalfa nodules in California, but that the isolates that belonged to this genus were among the best plant growth promoters. Our results also point to the potential of engineering inoculants based on naturally occurring bacteria that promote plant growth. This strategy will help reduce the amount of fertilizer normally applied to agricultural crops, thereby reducing the amount of nitrogen runoff and pollution.

8. Laura Bogar, Kabir Peay (Stanford University)

What stabilizes the ectomycorrhizal mutualism? An experimental test of partner choice by *Pinus muricata* in association with *Suillus brevipes*

Ectomycorrhizal symbiosis can be thought of as a trading agreement between plants and fungi. In most cases, the market helps both partners acquire the goods and services they need. It remains unclear, however, what keeps this market stable. One possible mechanism would be preferential allocation of resources to the best available symbiotic partner. It is known that arbuscular mycorrhizal plants and fungi are capable of this kind of regulation, but the process has not been demonstrated in ectomycorrhizas. To test this hypothesis, we manipulated partner quality in a split-root experiment using Pinus muricata (Bishop Pine) seedlings and Suillus brevipes (Slippery Jack) fungi. Separate root chambers were planted with hyphal ingrowth bags containing either sand (low quality) or a mixture of sand and casein (high quality). After ten weeks, pines were labeled with 13CO2 and carbon allocation to each root compartment was measured using mass spectrometry. Although both root compartments contained the same fungal genotype, we expected that the fungus with access to protein would provide more nitrogen to the plant, and receive more carbon in exchange, than the fungus with only a sand bag. Our preliminary results suggest that the plants may, in fact, have provided slightly more carbon to the nitrogen-providing fungus. This pattern, however, was not entirely consistent from plant to plant, and variation in carbon allocation was high even when the split root systems had identical nitrogen treatments. Much more consistent was the effect of nitrogen addition on fungal colonization of the root system: adding casein to either chamber of the split root plants was sufficient to reduce ectomycorrhizal colonization, suggesting that the plants may have been inadvertently fertilized by the protein. We are currently replicating and expanding upon this experiment to determine the extent to which plant partner choice may determine the outcome of ectomycorrhizal symbiosis.

9. Camille Wendlandt, Kelsey Gano, Joel Sachs (UC Riverside)

Legume host effects on symbiotic benefit from complex soil microbial communities

The long-term stability of microbial symbioses partly depends on host control traits, which allow hosts to select cooperative lineages for symbiosis and/or adjust resource allocation to symbionts based on the benefit they provide. Host control traits are commonly studied using single or mixed inoculations of known partner genotypes, and some of these studies have uncovered intraspecific variation in host control traits. However, host control is rarely studied in a complex biotic environment. Here, we use a legume-rhizobium system to examine variation in the symbiotic quality of whole soil microbial communities and the role of host genotype in extracting benefit from such communities. We generated soil filtrates from six sites throughout California and inoculated them onto two sympatric inbred lines of Acmispon strigosus, as well as three “universal” host lines of A. strigosus or Lotus heermanii. In general, soil inocula from different sites produced consistent growth effects on universal hosts, but one universal host line (“UnH”) consistently achieved greater growth response to inoculation than the other two lines. Soil inocula differed greatly in their growth effects on sympatric hosts: lines from Yucca Valley and Griffith Park derived the least benefit (~400% growth response), whereas lines from Claremont derived the greatest (~870% benefit). These data suggest that host growth response to an inoculum depends more upon the host genotype than the source of the inoculum, with some host genotypes being better than others at extracting benefit from complex soil microbial communities.

10. David Hembry, Noah K. Whiteman, Katrina M. Dlugosch (University of Arizona)

Repeated transitions from mutualism to parasitism in leafflower-leafflower moth interactions

Although mutualism is commonly thought to be inherently unstable because of the opportunity for selection to favor antagonism by interacting partners, few macroevolutionary transitions from mutualism to either parasitism or free-living status have been reported. Leafflower moths (Lepidoptera: Gracillariidae: Epicephala) are distributed throughout tropical and warm temperate regions of the Old World, where most are specialized pollinating seed predators (in a manner analogous to fig wasps or yucca moths) of their host leafflower plants (Phyllanthaceae: Phyllanthus s. l.). Mutualistic leafflower moths actively pollinate their hosts' flowers, but oviposit into the flowers' ovaries, where the larvae consume a subset of the hosts' seeds. Multiple losses of pollination behavior—and therefore of mutualism—have been reported from the literature on this interaction in Asia. Here we report the discovery of non-pollinating leafflower moths associated with native species of leafflower plants (Phyllanthus spp.) in the United States (Oklahoma, Texas, and Florida). Through phylogenetic inference, we examine the phylogenetic relationships of mutualistic and parasitic leafflower moths and use this comparative framework to ask questions about the changes in the biology of these moths which have been associated with this repeated transition. Finally, we discuss the implications of these findings for studies of mutualism loss in other symbiotic interactions.

11. Andrew Siefert, Maren L. Friesen, Kenneth W. Zillig, Sharon Y. Strauss (UC Davis)

Rhizobial mutualists mediate plant-soil feedbacks and plant-plant interactions of co-occurring, congeneric clovers

Feedbacks between plants and soil microbes may influence plant community structure and coexistence by altering plant performance and species interactions. We examined the effects of diverse soil microbial communities, including rhizobial mutualists, on the performance and interactions of Trifolium (clover) species and asked whether these interactions inform patterns of species co-occurrence in natural communities. We conducted field surveys to determine rates of co-occurrence of three Trifolium species in coastal grasslands in northern California. In a greenhouse experiment, we examined effects of soil origin (home or away) and soil microbes (sterilized, small microbes, or combined small and large microbes) on plant performance, nodulation, and plant-plant interactions. In our greenhouse experiment, soil microbes had strong positive effects on plant growth and nodulation. Small microbes (including rhizobia) generated positive plant-soil feedbacks (higher growth in home vs. away soil) for one species, but plant-soil feedbacks were neutral for all species grown with complete soil microbial communities. Soil microbes relaxed plant competition and altered the relative strength of interactions with conspecific vs. heterospecific neighbors. Our results demonstrate that interactions between plants and rhizobial mutualists can strongly influence plant performance and interactions with conspecific and heterospecific neighbors, generating frequency-dependent feedbacks that potentially drive patterns of co-occurrence of congeneric species in natural communities.

12. Megan Rúa, Jason D. Hoeksema (National Institute for Mathematical and Biological Synthesis)

Variation in plant and fungal traits indicate mycorrhizal mediated selection in Pinus radiata

Coevolution describes evolutionary change in which two or more interacting species reciprocally drive each other's evolution, potentially driving trait diversification and ecological speciation. Yet, we still understand very little about how coevolution works in multi-species interactions or about the relative importance of biotic and abiotic sources of selection. Interactions among plant hosts and their microbes may provide an ecologically unique arena in which to examine the nature of selection in multispecies interactions. In particular, interactions between coniferous plants and their microbes provide a good system for experiments exploring the relative importance of biotic versus abiotic sources of selection, as conifers interact with a suite of belowground microorganisms including mutualistic ectomycorrhizal fungi (ECM), and these interactions vary along environmental gradients. Using populations of Monterey pine (Pinus radiata) from geographically separated sites along the west coast of California (USA) and Baja California (Mexico), we performed a common garden experiment at a site that contains native stands of Monterey pine on mainland California (Cambria) to investigate the relationship between sources of selection on pine traits, including candidate coevolving traits. In each garden, we planted seedling genotypes from three populations, as well as crosses to represent intermediate phenotypes/genotypes. We then measured seedling traits, ECM fungal traits, and soil ECM composition. We found direct linear selection on all traits, but the strength of selection differed for fungal and plant traits. The strength of selection was greatest for fungal traits (foraging strategy) while plant traits (relative growth rate, diameter, root:shoot, specific root length) were all undergoing moderate to no selection. Variation in these traits of the symbiosis indicate an important step in establishing evolution in response to geographic variation in climate. This work represents the first field-based, community-level approach towards investigating selection in mycorrhizal relationships.

13. Betsy Arnold (University of Arizona)

KEYNOTE LECTURE: *Mosaics, megabases, and matryoshki: a leaf-to-landscape perspective on the symbiotic renaissance*

We are fortunate to be living in one of the most exciting times in the history of symbiosis research: a true renaissance period in which ecological and evolutionary theory, organismal knowledge, and the dual powers of technology and computational biology are framing a new understanding of symbiotic interactions. From studies of diffuse coevolution and horizontal transmission to context-dependency, the -omics of interactions, and new evidence of nested symbioses, recent work across our field is rewriting the rules on how to think about 'the living together of unlike organisms.' In this presentation I will focus on one of earth's most prevalent symbioses -- the association of endophytic fungi with photosynthetic organisms -- to highlight (1) recent findings regarding ecological interactions at levels from leaves to landscapes; (2) new insights into mechanisms underlying plant-fungal associations from local to global scales; and (3) emergent questions, which together speak to the challenge, excitement, and promise that engage us across disciplines and organisms in the broader study of symbiosis.

14. Dana Carper, Alyssa Carrell, Lara Kueppers, Carolin Frank (UC Merced)

The effect of climate change and site on the above- and belowground bacterial endophytic communities of subalpine conifer seedlings

Climate warming is expected to drive uphill migration of subalpine forests, with seedling establishment presenting a critical bottleneck for migration and persistence. The plant microbiome plays a role in plant response to biotic and abiotic stress, yet is rarely examined in studies investigating plant response to climate change. Seedling endophyte communities may respond to environmental conditions, potentially aiding seedling establishment under a range of conditions. Alternatively, endophyte communities may be conserved across environmental conditions if the taxa perform critical metabolic or physiological functions required by seedlings. We used Illumina sequencing of the 16S rRNA gene to examine the root and shoot endophytic communities in 1-year old seedlings of limber pine establishing in common gardens subject to experimental climate manipulations across an elevation gradient The most abundant members of both root- and shoot communities were identical to strains with antifungal activity (e.g., Janthinobacterium and Massilia), perhaps suggesting a role in biotic stress protection. The root and shoot communities were significantly different in their diversity and taxonomic composition, and they responded differently to biotic and abiotic differences between forest and higher elevation sites, as well as to climate treatments. This potentially reflects differences in adaptation and colonization routes between root and shoot endophytes, and suggests that shoot and root communities could play distinct roles.

15. Alyssa Carrell, JE Kostka, M Kolton, J Glass, DJ Weston (Oak Ridge National Laboratory)

Experimental warming effects on microbial communities of Sphagnum moss

Bog ecosystems play an important role in carbon sequestration and other aspects of climate regulation. Bacteria in these ecosystems are responsible for several of these important ecosystem functions. The effects of climate change on bacterial communties in these ecosystems has not been well explored. Here we used experimental warming to evaluate changes in the composition and function of bacterial communities associated with Sphagnum in a bog ecosystem. Specifically we examined the 16S rRNA gene diversity as well as nitrogenous and methane monooxygenase genes to evaluate changes in nitrogen fixers and methanotrophs. We found variation in community composition and functional groups across warming treatments. We speculate these changes may help Sphagnum mitigate climate warming in these bog ecosystems.

16. Luis Cayetano (UC Berkeley)

Symbiotic and plant associations of non-native Hawaiian hemipterans

Facultative endosymbionts are well known in aphids and other members of the Hemiptera both for their ubiquity and the range of ecological benefits they confer to their hosts. Recent studies suggest that one of these benefits may include the ability to exploit novel host plants. In the current study, non-native hemipterans in Hawaii are investigated to determine the associations between the symbiotic composition of these insects and the plants that they exploit. The Hawaiian islands are famously inundated with alien species of animals and plant, but relatively little is known about the symbiotic interactions that prevail there. Knowledge of these interactions would shed light on the rapidity with which symbionts may facilitate the broadening of the range of hosts available to insects, the prevalence of horizontal transmission of symbiotic bacteria among newly arrived hosts, and how better to manage important pest organisms using biological control.

17. Kimberly La Pierre, Ellen L Simms, Stephanie S Porter (UC Berkeley)

Unlocking the mechanisms behind legume invasions: Are rhizobial mutualists the key?

Ecologists have a long history of examining the role of biotic interactions in determining invasion success. Currently, new molecular tools are driving a rapid understanding of the importance of feedbacks between plants and their associated microbial communities in determining species invasions. Invasive legumes present a particularly interesting case of plant-microbial feedbacks. An invasive legume can either associate with unfamiliar rhizobia in its invasive range or co-invade with rhizobia from its native range. Here, we examine legume-rhizobia specificity for three invasive legumes—French broom (Genista monspessulana), Spanish broom (Spartium junceum), and Scotch broom (Cytisus scoparius)—and several native legumes in the San Francisco Bay Area. Our results show that the communities of rhizobia associated with native vs invasive legumes in the Bay Area are quite distinct. Thus, it appears that the success of invasive legumes is due, in part, to co-invasion by their rhizobial symbionts, rather than an ability to widely utilize unfamiliar strains in their new range. Additionally, densities of rhizobia increase in areas invaded by these legumes compared to uninvaded areas. Interestingly, different invasive removal techniques (herbicide, mowing, hand-pulling) have vary in their effect on rhizobial density in the soil following removal. Overall, this research points to the importance of mutualisms in driving invasion success as well as restoration efforts.

18. Emily Bellis, Reid B. Edlund, Hazel K. Berrios, Dee R. Denver (Oregon State University)

Evolutionary dynamics of Aiptasia-Symbiodinium symbioses in Caribbean Panama.

Many marine invertebrates, including reef-building corals, sea anemones, jellyfish, and sponges, depend on mutualistic relationships with dinoflagellate algae of the genus Symbiodinium. The sea anemone Aiptasia is a burgeoning model system for studying cnidarian-dinoflagellate symbioses in the laboratory, however we still know very little about natural populations. We characterized populations of the sea anemone Aiptasia and its Symbiodinium from four sites in Panama that experience a range of thermal fluctuation regimes, based on long-term water temperature monitoring data recorded hourly for >15 years. We mapped anemone abundance and assessed within-anemone Symbiodinium density, diversity and mitotic index from 240 anemones from three sites in the Bocas del Toro Archipelago and one site near the Caribbean entrance to the Panama Canal. We are also performing restriction site-associated DNA sequencing to genetically characterize anemone host populations and search for candidate genetic loci under selection. By comparing natural populations of an anemone well-studied as a laboratory model for coral-algae symbioses, we hope to shed light on mechanisms through which cnidarians may respond to increasingly variable temperatures in the coming decades.

19. Joel L. Sachs, J. U. Regus, R. M. Bantay, K. A. Gano-Cohen״ A. C. Hollowell (UC Riverside)

An experimental nitrogen deposition gradient increases mortality and eliminates symbiosis in a native legume

Anthropogenic nitrogen deposition can provide legumes with a cheap source of nitrogen relative to symbiotic nitrogen fixation, leading to the potential breakdown of this critical symbiosis. Here, we tested the effects of nitrogen deposition on legume rhizobium interactions. We quantified deposition rates and soil nitrogen along a predicted deposition gradient in California. We exposed *Acmispon strigosus* seedlings to fertilization spanning nitrogen concentrations observed in the plant's California range. We tested inbred plant lines from pristine and nitrogen polluted sites and used rhizobial strains that varied in nitrogen fixation. We found that nitrogen deposition is a key predictor of nitrogen in soils. The growth benefits of rhizobial infection were dramatically reduced by even modest levels of mineral nitrogen, and all *Acmispon* lines failed to form root nodules at high nitrogen concentrations. Plant lines from high deposition sites showed no evidence of adaptation to increased nitrogen concentrations. Our dataset suggests that the benefits of rhizobial symbiosis have been eliminated in much of Southern California and that the tested plant populations have not adapted to increased nitrogen concentrations. If nitrogen deposition increases continue, plant host mortality and a total collapse of the symbiosis could result.

20. A. Carolin Frank, Andrew Moyes, Lara M Kueppers, Jennifer Pett-Ridge, Dana Carper, Dianne Quiroz, James Kupihea, Paola Saldierna (UC Merced)

Foliar nitrogen fixation in conifers – an ecological perspective

Mature temperate and boreal forest are nitrogen (N) limited, yet N budgets indicate unknown sources of N in these ecosystems. Symbiotic N-fixing plants are notably absent from coniferous forests, and sources to overcome N limitation are not well understood, but include epiphytic N_2 fixation in mosses, free-living $N_{2,}$ fixation in litter and soil, bedrock nitrogen where sedimentary rocks occur, and deposition of nitrogen pollution.

We found a consistent association between limber pine (*Pinus flexilis*) and specific bacteria, most notably potential N_2-fixing acetic acid bacteria (AAB), and Rhizobiales spp., prompting us to examine if native foliar endophytes may supply temperate and boreal forests with N. Using the acetylene reduction assay on surface sterilized foliar samples, we confirmed nitrogenase activity in the subalpine conifer *Pinus flexilis* (limber pine) growing at Niwot Ridge, Colorado, as well as more recently, in in bishop pine (*Pinus muricata*) and lodgepole pine (*Pinus contorta* ssp. *bolanderi* and ssp. *contorta*) growing along a gradient in soil age and associated variation in soil fertility at the “ecological staircase” in Mendocino, California.

Not surprisingly, N fixation rates of needle endophytes are much lower than those of nodulating N-fixers, and comparable to rates of free-living fixation in soils in temperate and boreal ecosystems. So far, we have found no evidence that local differences in soil N availability affected rates of foliar N fixation. Together, these results suggest that foliar endophytes represent a low-cost, evolutionarily stable N_2-fixing strategy for long-lived conifers that never fully alleviates N limitation in temperate and boreal ecosystems. These results open up the possibility that hidden symbiotic N fixers hide in other N-poor ecosystems as well.

21. Katherine Muller, Ford Denison (University of Minnesota)

Physiological Mechanisms of Cooperation and Conflict in the Legume-Rhizobium Mutualism

The mutualism between legume plants and nitrogen-fixing bacteria known as rhizobia involves a conflict between public and private goods. Nitrogen fixation by rhizobia in nodules is a public good that increases growth of the host plant, thereby increasing the supply of photosynthate available to all nodules. In addition to fixing nitrogen, rhizobia can divert plant-derived energy into private goods like polyhydroxybutyrate (PHB), a storage compound that aids survival and reproduction in individual rhizobia, but does not benefit the host. We know that nitrogen fixation and PHB accumulation compete directly for plant-derived energy. But we do not know to what extent this conflict between public and private goods contributes to variation in mutualistic benefit among rhizobia genotypes. During my dissertation work, I found genetic differences in PHB accumulation among rhizobia isolates from soybean nodules. However, experiments with a subset of strains did not find a clear relationship between PHB accumulation and mutualistic benefit. This result suggests other factors, besides allocation between public and private goods, contribute to variation in PHB accumulation and mutualistic benefit. One such factor may be the overall metabolic function inside nodules due to compatibility with a host genotype. I will present my results along with a conceptual framework that links genotype-by-genotype interactions due to compatibility with genetic differences in symbiont “cheating” (allocation to private over public goods) to explain variation in mutualism.

22. Amanda M.V. Brown, Dana K. Howe, Sulochana K. Wasala, Amy B. Peetz, Inga A. Zasada, Dee R. Denver

Evolutionary genomics of rhizosphere endosymbionts: getting to the root of it

The nutrient-rich and biotically complex rhizosphere poses a wealth of opportunities for species to interact. Most of the flow of life-producing carbon on land passes through soil microorganisms, yet the extent to which symbiosis plays a role in soils is unclear. While legume-rhizobia symbiosis, mycorrhizal fungi, and plant-growth promoting rhizobacteria have received attention, other symbioses have gone virtually unnoticed. For example, nematodes are major contributors to terrestrial carbon flow and root-parasitic species cost ~$100 billion in global annual agricultural damage, but their symbiotic partnerships are largely unknown. We conducted genome-sequencing surveys of six plant-parasitic nematodes and uncovered several bacterial associations. Fluorescence in situ hybridization and comparative functional genomic analyses demonstrated that three associated bacteria were endosymbionts with potentially large effects on their hosts. These included Xiphinematobacter (Verrucomicrobia) in the dagger nematode Xiphinema americanum, and Wolbachia (Alphaproteobacteria) and Cardinium (Bacteroidetes) in the root lesion nematode Pratylenchus penetrans. Comparative analyses pointed to a nutritional supplementation role for Xiphinematobacter, with enrichment for amino acid and vitamin synthesis. Phylogenomic analyses placed Wolbachia from P. penetrans as the earliest diverging member of this globally pandemic group, with the next branches comprising Wolbachia strains from sap-feeding insects. This result and genomic analyses suggest early Wolbachia may have been nutritional mutualists. Similar analyses for Cardinium, a widespread facultative symbiont in mites and wasps, suggest it arose early in nematodes where it may manipulate host reproduction in a similar manner to Wolbachia, through convergent evolution or lateral gene transfer. These results implicate diet specialization as a potential impetus fostering these partnerships. Together our study gets to the root of a previously underappreciated type of rhizosphere interaction, exposing a promising new area of symbiosis research.

23. **Phillip A. Cleves,** Benjamin M. Mason, Lauren L. Liddell, and John R. Pringle (Stanford University)

Developing transgenic tools to study cnidarian-symbiodinium symbiosis in a sea-anemone model system

Transcriptomic studies in cnidarians have identified many intriguing gene-expression correlations with the establishment and breakdown of symbiosis. Nonetheless, the molecular bases of these processes remain poorly understood, in part because of the lack of a tractable genetic system to functionally test candidate genes and pathways. The small anemone Aiptasia is symbiotic with Symbiodinium and has many experimental advantages over corals, including a high – but as yet unrealized – potential for the needed genetic studies. As a first step in developing transgenic methodologies, we are attempting to express fluorescent proteins from both capped-mRNA and Aiptasia-promoter expression constructs. If successful, these technologies should allow both expression of tagged proteins for localization studies and the overexpression of candidate genes to analyze gain-of-function phenotypes. To introduce the constructs, we are using microinjection of zygotes. In parallel, we are developing tools to knock down and knock out genes using morpholinos and CRISPR-Cas9 for analysis of loss-of-function phenotypes. If both gain-of-function and loss-of-function methods can be established, Aiptasia should become a uniquely powerful genetic model system for the study of cnidarian-Symbiodinium symbiosis.

24. **Peter Graystock,** Sandra M Rehan, Quinn S McFrederick (UC Riverside)

The importance of host phylogeny and geography on the microbiome, with implications for wild bee health and conservation

Abstract: Social corbiculate bees such as honey bees and bumble bees maintain a beneficial core microbiome which is absent in wild bees. Here we combine new and previously published data to compare the microbiomes of 10 wild bee species from the genera Augochlora, Megalopta, Halictus, Megachile, Osmia, Lithurgus and Ceratina. Our study will test for the existence of a wild bee core microbiome. We will additionally test for associations between the microbiome structure and host phylogeny, geography, and level of eusociality. We determine that microbiome composition is strongly correlated to host geography and provide evidence that wild bees may obtain much of their microbial community via environmental transmission from flowers. Despite the importance of environmental transmission, bee phylogeny was also correlated with the gut microbial community.The consequences of this enviro-phylo associated microbiota are discussed in relation to maintaining the health and avoiding dysbiosis of fragmented or introduced populations of wild bees.

POSTERS

1. Hailee Korotkin, K. –H. Chen, K. McFarland, F. Lutzoni, P.B. Matheny (University of Tennessee, Knoxville)

Fungal-bryophyte interactions: developing a model system using the fungus *Rickenella fibula*

Fungal-bryophyte interactions widely exist across the fungal tree of life, but in many cases have not been sufficiently studied. The mushroom-forming fungus, *Rickenella fibula* (Hymenochaetales, Basidiomycota)*,* is known to associate with the moss *Dicranum scoparium*, in addition to other mosses. *Rickenella fibula* has been observed fruiting on senescent stems of *D. scoparium*. However, the nature of the relationship has not been clearly established. Furthermore, it is unclear whether fungi in the Hymenochaetales evolved as lignin-degrading saprobes and switched to bryophyte associates or vice versa. Our objectives are to use a novel approach of combining *in vitro* experiments, genomics, transcriptomics, and phylogenetics to determine the trophic interactions between *R. fibula* and *D. scoparium,* and if *R. fibula* underwent a loss of the ability to decompose lignin. We hypothesize that *R. fibula* acts as a saprobe on senescent gametophyte tissue of *D. scoparium,* and that *R. fibula* has lost the ability to degrade lignin. Currently, *in vitro* experiments are being conducted and a full genome and transcriptomic data of *R. fibula* are being processed. Preliminary results from *in vitro*, genomic and transcriptomic analyses will be presented.

2. Su'ad Yoon, Joshua Harrison, Matthew Forister (University of Nevada, Reno)

Does the microbiome mediate novel host use, immune response, and performance in the specialist butterfly *Lycaeides melissa*?

The role of symbiotic microbes in mediating host use specificity has gained attention over recent years, however, this relationship has only been studied in a few systems. We investigated the role of the microbiome in the context of a host shift in the specialist butterfly Lycaeides melissa. L. melissa has colonized the exotic legume Medicago sativa in the past 200 years; L. melissa frequently utilizes M. sativa throughout its range despite the fact that L. melissa larvae suffer both reduced survival and decreased adult fecundity on this host, compared to the native host Astragalus canadensis. We hypothesized that L. melissa utilizing the exotic host M. sativa may experience trade-offs in the form of strengthened immune response or pathogen release. To test this, we reared L. melissa on the two hosts (exotic and native) and measured performance, immune response (phenoloxidase activity and melanization), and microbial density and richness. We found that microbial density (number of bacterial reads per milligram of host tissue) was significantly higher on the novel, exotic host M. sativa. In addition, standing phenoloxidase activity was also higher on M. sativa. Larval performance (final weight) was significantly lower on M. sativa, possibly suggesting higher investment in immune response or higher microbial load negatively affects growth and development. Interestingly, phenoloxidase activity and melanization (two measures of immune response), were affected by microbial factors in different ways. Microbial evenness had a positive effect on melanization, whereas phenoloxidase was positively affected by microbial richness and density. Taken together, these results suggest L. melissa experiences profound fitness effects as a result of increased microbial load on the exotic host M. sativa. Future research is needed to disentangle the exact mechanisms governing the interactions between host performance, immune response, and the microbiome.

3. Andrew Sher, Sharon Doty, Alisa Huffaker (UC San Diego)

Uncovering the Molecular Mechanisms of Salt Stress Tolerance Imparted by Endophytic Symbiosis

Many plants have been shown to house symbiotic microorganisms in the interstitial space between their cells, providing a nutrient-rich environment where these endophytic microbes can safely grow in exchange for a wide range of benefits. In harsh environments, interactions between plant and microbe become of increased importance. Plants such as those belonging to the Salicornia genus, commonly called pickleweed, are known halophytes and are capable of surviving the harsh salinity along tidal flats and estuaries. Despite their innate high salt tolerance, these plants may also depend on endophytes when facing the extremes of their environment. The endophytes from Salicornia plants growing despite complete submergence at high tide were isolated and identified using 16S rRNA gene amplification. Maize seedlings inoculated with these endophytes showed increased tolerance to salt stress. In order to elucidate the mechanisms of colonization and to better understand how salt tolerance is imparted, Arabidopsis plants have been inoculated with these endophytes. Using this model system allows for genetic manipulation of known defense pathways, as well as sodium transporters. We are currently evaluating whether Arabidopsis can be efficiently colonized by the endophyte and whether there is a conferred tolerance to high salinity as observed in maize. Current research is also focused on transcriptional and metabolic profiling of maize and Arabidopsis plants in response to endophyte association and upon exposure to high salt conditions.

4. Ellen Simms, Kimberly La Pierre, Peter Reich, and Sarah Hobbie (UC Berkeley)

Effects of the legume-rhizobia mutualism on biodiversity-ecosystem function relationships under global change

Ecological theory predicts that niche and relative fitness differences among plant species underlie variation in the biodiversity-productivity relationship. However, the mechanisms by which plant species differ are rarely experimentally quantified, leaving causal connections between plant diversity and productivity elusive. Plants in the legume family associate with beneficial soil bacteria called rhizobia, which fix atmospheric nitrogen (N) in exchange for carbon (C) from their plant hosts. As most plants obtain N from the soil, access to atmospheric N via this mutualism can differentiate legumes from non-legumes, thus influencing the productivity-diversity relationship. Increased atmospheric carbon dioxide (CO2) and soil N will likely influence the effect of legume species on the productivity-diversity relationship by altering the legume-rhizobia mutualism. We are experimentally examining the biotic processes that differentiate four legume species from each other and from non-legumes to understand how these differences cause variation in patterns of primary productivity within the BioCON experiment at Cedar Creek LTER. This research will inform management of ecosystem function under current and future global change scenarios, with important implications for human well-being.

5. Luis Marquez, G. von Maltzahn, R.B. Flavell, G.V. Toledo, S. Djonovic, D.M. Johnston, Y.A. Millet, J. Lyford, A. Jack (Symbiota Inc.)

Seed-origin endophytes provide environmental stress tolerance to agricultural plants

It is well established that microbial endophytes provide tolerance to extreme environmental stresses in wild plants. However, the application of those wild plant endophytes to large scale agriculture has proven challenging due to extreme variability in plant responses likely associated to a lack of co-adaptation. Natural selection should favor the incorporation in the seeds of beneficial endophytes, co-adapted to their host plants. Seed-origin endophytes that provide benefits throughout the life of the plant, including yield increase, would have a higher chance of being incorporated into the next generation of seeds. We isolated and extensively characterized 520 different OTUs of bacterial endophytes of seed-origin from 49 cultivars of corn, 5 cultivars of wheat and 23 cultivars of rice. Based on Symbiota derived hypotheses, we selected and tested 48 bacterial strains, representing 44 OTUs in germination assays in wheat and corn under salt and drought stress. All, but 2 of the strains tested improved weight, shoot or root length in the order of 5-15% compared to controls. Additionally, 8 out of 9 seed-origin endophyte improved corn growth in a two week sterile soil assay, in terms of biomass, root or shoot length in up to 27% under at least one condition (normal, drought or cold). A proteomic analysis of wheat inoculated with 3 seed-origin endophytes grown under heat stress and corn inoculated with one seed-origin endophyte grown under normal condition revealed three major pathways augmented or otherwise modified by the endophyte: growth promotion, resistance against oxidative stress and mechanisms involved in symbiosis enhancement. Finally, 11 seed-origin endophyte OTUs tested in non-irrigated field trials, at agriculturally relevant scale and using standard agricultural practices, increased yield relative to controls by up to 10% in wheat and 5% in corn. These results indicate that the inoculation of agricultural plants with seed-origin endophytes constitutes a viable technology to improve crop performance under environmental stress.

6. Mariana R Souza, Michael Dawson (UC Merced)

Jellyfish symbiosis and genome size variation

The study of genome size diversity is an ever-expanding field that is highly relevant in today's world of rapid DNA sequencing. Despite that, our knowledge on the distribution of and reasons for why certain lineages contain large genomes while others do not is still very poor. Three classes of Cnidarian present mutualistic interaction with *Symbiodinium*, a photosynthetic dynoflagellate, and the implications of the presence of symbiont on the systematics and genome of the species that host it is still unkown. To help clarify this uncertainty, the genome size of 54 species of the 18 families of Scyphozoa will be estimated by flow cytometry. Due to the almost obligate *Symbiodinium* presence in Scyphozoa that host it and due to the longevity of the association (about 240 million years), I hypothesize that some metabolic paths might have been lost in the host, resulting in reduction of genome size. The results have implication for the systematics of Scyphozoa as well as genome size evolution.

7. Jason Rothman, Pearl Le, Kaleigh Russell and Quinn McFrederick (UC Riverside)

The Effects of Metal Bioaccumulation on the Microbiomes of Pollinators

Significant effort has been invested into understanding the complex interactions that bees have with their environments. One aspect that was until recently overlooked is the detrimental effects of metal pollutants on bee health and abundance. Certain plant species growing in areas contaminated with metals can accumulate these pollutants in their pollen and nectar, which in turn negatively affects the insect pollinators that interact with those plants. We collected honey bee (*Apis mellifera*) workers and foragers from colonies fed with pollen patties containing cadmium, copper, selenium or lead. To characterize microbiomes, we extracted DNA from five pooled bee guts, amplified the hypervariable V5-V6 regions of the bacterial 16S rRNA gene through PCR, and sequenced the amplicons with the Illumina MiSeq. We then used Usearch 8 software to cluster the resulting read files into operational taxonomic units (OTUs) at both 97% and 99% sequence identity. To explore beta diversity, we used ordination and multivariate analyses. We were able to show shifts in the community composition of bee-associated bacteria due to metals exposure, which may in turn help elucidate measured effects that these contaminants have at the colony-level. These results suggest that metal contamination can affect the bee microbiome, which is thought to be critical to honey bee health. We intend to study these effects further in other social and solitary bee species.

8. **Hoang Q. Vuong**, Amanda Hale, Quinn S. McFrederick (UC Riverside)

Genomics of Flower and Bee Gut Associated Microbes

Lactobacillus is an extremely diverse genus of bacteria found in numerous environmental and host niches including the gut microbiome of mammals and insects alike. In wild bee guts, we have consistently found a bacterium that is closely related to, but distinct from *L. kunkeei.* Additionally, we detected this bacterium on flowers using 16S rDNA next-gen amplicon sequencing. These associations suggest pollinator to floral microbe transmission. However, with most bacterial species, high 16S rDNA similarity does not necessarily translate to genomic similarity. To confirm floral-bee microbe transmission and explore the intra-species diversity of this taxon, we cultured this bacterium from both flowers and their bee visitors. We sequenced ten isolates using the Illumina MiSeq platform and assembled the genomes for SNP counting and phylogenomic reconstruction. SNP counts between the two isolate pairs of *Lactobacillus* from bee guts the bee's corresponding flower were 20 and 284 SNP sites of 1,400,000-1,500,000 genomes bases, suggesting transmission of microbes during bee pollination. We used 436 orthologous amino acid sequences from *Lactobacillus* associated with bees and flowers in the phylogenomic analysis. All ten sequenced isolates fell into a clade that is reciprocally monophyletic to *Lactobacillus kunkeei.* Within this clade the isolates diverged into two well supported monophyletic groups, suggesting at least two new *Lactobacillus* species.

9. Joshua Harrison, G. W. Koch, T. L. Parchman, S. C. Sillett, Matthew L. Forister (University of Nevada, Reno)

A room with a view: vertical stratification of the foliar fungal community in the world's tallest trees

The canopies of the world's forests are largely unstudied reservoirs of fungal diversity. In particular, the patterns of foliar fungal endophyte diversity in forest canopies are poorly understood, which hampers the study of community assembly of these ecologically important fungi. Here we report on intracanopy variation in the fungal assemblage of the world's tallest trees, coast redwoods (Sequoia sempervirens). We took a culture-independent approach, using the Illumina MiSeq platform, to assay fungal diversity across the range of these giants at three heights within the canopy. Fungal community composition varied among trees, and we observed general distance decay in community similarity among trees sampled. Moreover, the fungal assemblage differed among canopy locations for each tree surveyed. While fungal colonization, as measured by the ratio of fungal reads to host reads, decreased with height in the canopy, we observed no such pattern for overall fungal richness. Interestingly, the turnover across trees in the fungal community increased with height in the canopy. In other words, the fungal assemblage in the treetops varied more among trees than did the fungal assemblage in lower branches. These results suggest that vertical stratification in the endophyte community may occur in other tall trees, and highlights the need for further mechanistic research aimed at understanding what drives the patterns we observed and how these fungi affect their hosts.

Map of Wawona and SNRI

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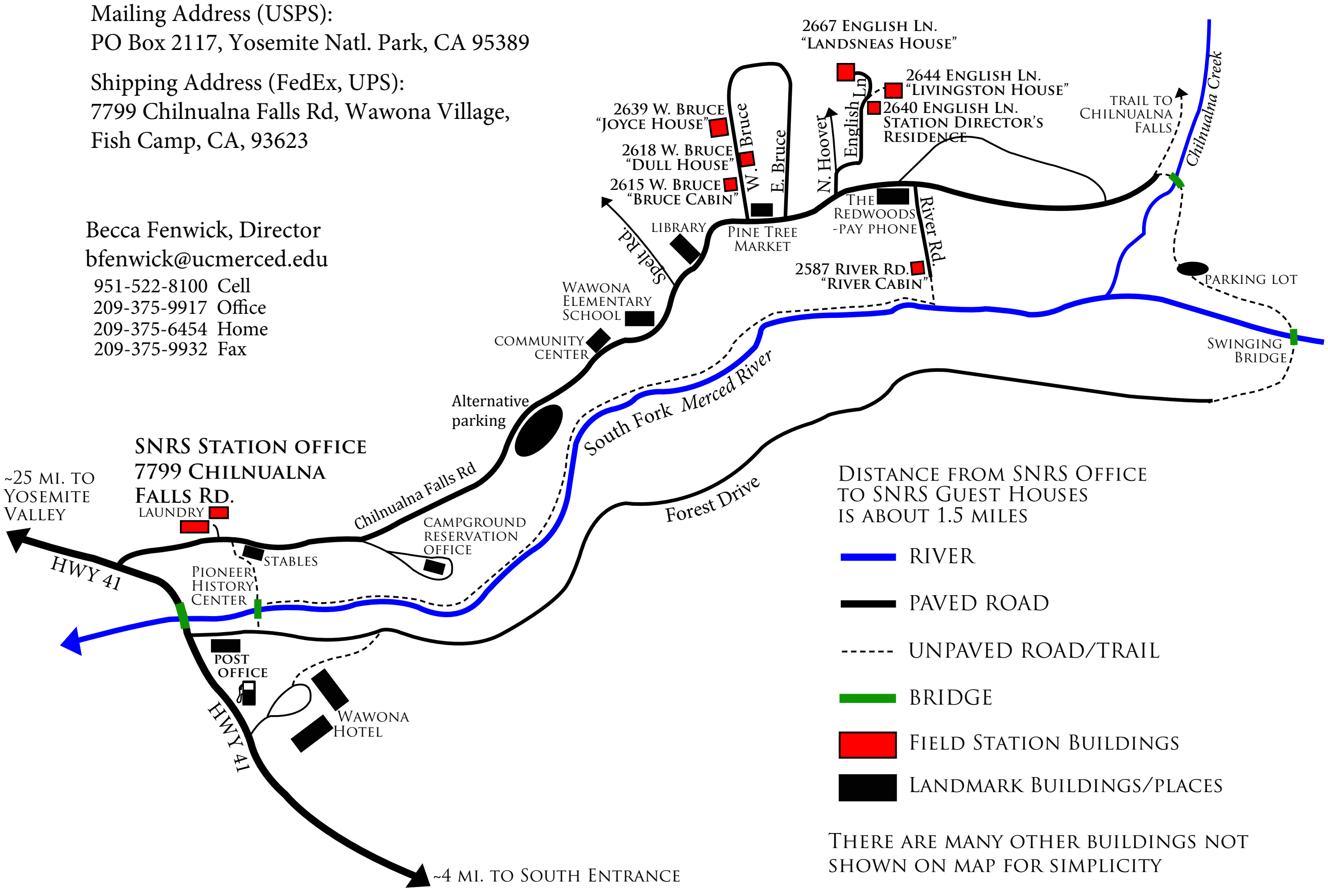

DISTANCE FROM SNRS OFFICE TO SNRS GUEST HOUSES IS ABOUT 1.5 MILES

🟦	RIVER
■	PAVED ROAD
- - - - -	UNPAVED ROAD/TRAIL
🟩	BRIDGE
🟥	FIELD STATION BUILDINGS
■	LANDMARK BUILDINGS/PLACES

THERE ARE MANY OTHER BUILDINGS NOT SHOWN ON MAP FOR SIMPLICITY