10th International Conference on Serpentine Ecology, Eketerinburg, Russia (June 21-30)

Abstracts Accepted

Presenter/s in **BOLD**; Cal Poly Students Underlined

1) Devlin, M.C., Stephens, D., Negoita, L., and N. Rajakaruna. 2020. Impacts of Multiple Nutrient Element Enrichment on Native and Alien Plant Species in California's Serpentine Grasslands. Oral presentation at the 10th International Conference on Serpentine Ecology, Ekaterinburg, Russia. June 21-30.

Abstract: Nitrogen and phosphorus additions are linked to increased invasion of non-native plants onto serpentine soils. However, effects of combined N+P enrichment have not been thoroughly investigated. We grew two non-native annuals and two native annuals in serpentine soil to test competitive interactions under different nutrient addition treatments. Treatments included nitrogen (N), phosphorous (P), and N+P additions based on realistic current and future deposition rates. We ran five replicates of each treatment. After twelve weeks we measured seed mass, floret production, above ground biomass, and below ground biomass of each plant as indicators of competitive success. We used a mixed-model approach and tested for significant interactions between nativity and soil nutrient additions using likelihood-ratio model comparisons. We found that 1) N+P addition significantly increased seed mass in non-native species compared to natives ($\chi 2$ (1)=14.42, $P < 0.001$), 2) N+P addition significantly increased above ground biomass in non-native species compared to natives ($\chi 2$ (1)=7.33, $P < 0.01$), and 3) P addition significantly increased seed mass in non-native species compared to natives ($\chi 2$ (1)=5.04, $P < 0.05$). Phosphorous addition also showed a marginally significant interaction effect with inflorescence number ($\chi 2$ (1)=4.03, $P = 0.045$). Our results show that concurrent N and P deposition may competitively advantage non-native grasses over native grasses and forbs of serpentine substrates.

2) Fryday, A. M., Medeiros, I. D., **Siebert, S. J.**, Pope, N., and **N. Rajakaruna**. 2020. Lichen species discovery from ultramafic rocks in Mpumalanga, South Africa —problems and

consequences. Poster presentation at the 10th International Conference on Serpentine Ecology, Ekaterinburg, Russia. June 21-30.

Abstract: We explore the difficulties in finding the correct names for lichens collected from a region where the lichen biota has not been investigated for approximately 100 years and give examples of our successes and failures. There are many names available for South African lichens, but these were mostly proposed around the end of the 19th century by lichenologists who never visited the country – working instead with, usually meagre specimens that were sent to them by local botanists. In most cases the protologues of these names are inadequate to identify the species even to their current genus, and the species themselves are usually known only from the type collection that is held in a European herbarium and has not been studied since! The advantages and problems of using morphological or molecular OTUs are discussed. We describe the process that led us to recognize the new genus *Burrowsia* and the new species *B. cataractarum* and *Scoliciosporum fabisporum*, as well as the ongoing struggle to name several other distinctive, but apparently un-named taxa – including the 6–7 morphologically distinct entities of *Trapelia* that we observed although only three species have been reported from South Africa. The lack of basic taxonomic information significantly impacted our ability to carry out a meaningful ecological investigation of the lichen biota of the region and also has serious negative implications for the management and conservation of what is, undoubtedly, a rich and unique resource.

3) **Mulroy, M.**, Dart, J., Fryday, A.M., and N. Rajakaruna. 2020. An investigation of lichen biotas of ultramafic and sandstone outcrops along a maritime gradient in Central California. Poster presentation at the 10th International Conference on Serpentine Ecology, Ekaterinburg, Russia. June 21-30.

Abstract: Lichen community composition is largely a product of abiotic factors – in particular, climate (e.g., fog) and substrate properties (e.g., rock chemistry). Globally, ultramafic substrates often harbor distinct lichen assemblages, although few lichen species are ultramafic endemics. In North America, ultramafic lichen communities have received little attention, with only five studies to date. It is also unclear how other abiotic factors, such as maritime influence, interact with substrate to determine community composition. We plan to compare lichen biotas of ultramafic and sandstone outcrops along a large-scale (~65 km) maritime gradient. We will

sample outcrops in three zones (coastal, intermediate, and inland) along the gradient using qualitative and quantitative methods, as well as analyze substrate elemental chemistry and rock surface features. We hypothesize that 1) maritime influence will cause lichen communities to be more species-rich and diverse closer to the coast, and 2) ultramafic and sandstone communities will be more similar in the coastal zone than the interior due to maritime influences overriding substrate-level effects. This study will improve our understanding of the interactive effects of substrate properties and maritime influence on lichen community composition and add to our knowledge of regional species distributions. Here we present our preliminary floristic results and discuss the role substrate and climatic factors play in lichen community assembly.

4) **Mulroy, M.**, Dart, J., Fryday, A.M., and N. Rajakaruna. 2020. A review of the state of knowledge of lichens of ultramafic substrates in North America. Oral presentation at the 10th International Conference on Serpentine Ecology, Ekaterinburg, Russia. June 21-30.

Abstract: Lichens of ultramafic substrates have been a topic of study worldwide. However, the majority of published ultramafic lichen research comes from Europe, with only five published studies from North America. The North American research includes one quantitative investigation of lichens employing a quadrat sampling methodology, and four studies that compiled species inventories. Of the four inventory studies conducted, two included quantitative analyses of substrate chemistry that complemented the lichen species inventories, and three compared lichen communities of adjacent ultramafic and non-ultramafic substrates. A review of the results of these studies shows that ultramafic rocks support species assemblages that are distinct from adjacent non-ultramafic substrates, which is consistent with results from research on ultramafic lichens in Europe. However, the extent to which ultramafic lichen communities are unique to these substrates is unclear, and reports of lichens endemic to ultramafic substrates in North America are unconfirmed. The relative importance of ultramafic substrate chemistry compared to other substrate properties, such as surface texture and water retention capacity, also remains uncertain. Here we review the state of knowledge on lichens of ultramafic substrates of North America, focusing on emerging patterns from existing published studies as well as highlighting the knowledge gaps that currently exist.

5) **Stephens, D.**, K. Nelson, L. Negoita, and N. Rajakaruna. Evolutionary Ecology of Two Rare *Chorizanthe* (Polygonaceae) Species from central California, U.S.A. Poster presentation at the 10th International Conference on Serpentine Ecology, Ekaterinburg, Russia. June 21-30.

Abstract: *Chorizanthe aphanantha*, a recently discovered serpentine endemic species from central California, is found in sympatry with its congener, *C. breweri*. We analyzed the chemical and physical features of rhizospheric soils of 5 allopatric and 5 sympatric populations. A T-test found no significant differences in soil features between allopatric and sympatric sites. A principle component analysis was more revealing of the variability between allopatric and sympatric sites, showing overall poorer quality of soils where *C. breweri* is found, and higher quality among *C. aphanantha* sites. A competition study in the greenhouse with field-collected soils are currently underway to determine if the species are locally adapted to and are better competitors on their home soil. Each treatment includes 3 of each taxon in a tray of both soil types, replicated 4 times. We will measure the number of flowers, seed mass, and above-ground and below-ground biomass to determine fitness. We are also investigating mechanisms that might be responsible for the potential reproductive isolation between the taxa. *C. breweri* flowers for a longer period (March-July) than *C. aphanantha* (March-June), with *C. aphanantha* typically flowering first. Given there is overlap in flowering times we are examining potential mechanisms of reproductive isolation, including differences between species in chromosome numbers and pollinator guilds.

6) **Walsh, P.**, A. Ferrero, T. Cataldo, C. Knight, and N. Rajakaruna. Work in Progress: Competitive inhibition of Ni uptake by other divalent cations in California hyperaccumulator *Streptanthus polygaloides* (Brassicaceae). Poster presentation at the 10th International Conference on Serpentine Ecology, Ekaterinburg, Russia. June 21-30.

Abstract: The physiological and evolutionary mechanisms by which certain serpentine-associated species hyperaccumulate Ni is not truly understood. It is unclear whether Ni uptake proceeds through a newly evolved Ni-specific pathway or pre-existing transport pathways for Fe, Ca and other divalent cations. In this experiment we used Atomic Absorption Spectroscopy to examine tissue Ni concentrations of *Streptanthus polygaloides*, a California serpentine endemichyperaccumulator, under various concentrations of divalent metals. Three treatments of

modified Hoagland solution containing elevated Fe and Ni, elevated Ca and Ni or elevated Ni only, were applied to Ni starved *S. polygaloides* seedlings to test for competitive inhibition of Ni uptake systems by other divalent metals. We hypothesize that the low concentration of Ca in serpentine soils led plants to evolve highly sensitive, non-specific Ca uptake systems, which import Ni into the plant when soil Ni is high. Thus we expect lower Ni concentration in Ca+Ni treated plants than in Fe+Ni or Ni only treatments as a result of competitive inhibition by Ca ions. An even distribution of Ni concentrations across treatments, indicating no competitive inhibition, could point to the presence of a novel Ni specific uptake pathway. This study may provide insight into the process facilitating Ni absorption in *S. polygaloides* and the evolutionary mechanism through which this fascinating serpentine adaptation evolved.

7) **Rajakaruna, N.** Life on the Rocks: What plants on harsh substrate 'islands' can teach us about diversity, ecology, evolution, and conservation and restoration practices. Oral presentation at the 10th International Conference on Serpentine Ecology, Ekaterinburg, Russia. June 21-30.

Abstract: Rock outcrops are often characterized by unique plant communities with a high rate of rarity and endemism. Such plant communities offer exceptional opportunities for exploring ecological theory and provide model organisms for investigating the factors and mechanisms driving adaptive evolution, including the genetic bases for and architecture of traits conferring adaptation and reproductive isolation. Additionally, they offer opportunities to explore cross-adaptation, which is when a trait that evolved for tolerance to one harsh substrate becomes effective as an adaptation for another, allowing species to show cross-tolerance to multiple harsh substrates characterized by a suite of common stressors, including habitat bareness, drought, pH, ionic strength, and specific ions. Plants of harsh substrates offer unique challenges for conservation and restoration and are especially prone to stressors associated with climate change. Much of the research on harsh substrate-plant relations to date has focused on plants of ultramafic and other metal-enriched rock outcrops or saline soils, however, recent research on plants of gypsum and other calcareous substrates, solfatara fields, banded iron formation inselbergs, guano-derived soils, as well as soils that are enriched with anthropogenic inputs of major and trace nutrients and toxic metals, can help reexamine the plant-harsh soil relationship, including commonalities and differences across distinct plant-soil type associations.

8) Fernando, D. R., H. A. S. Weerasinghe, D. S. A. Wijesundera, G. W. A. R. Fernando, A. E. Fernando, M. C.M. Iqbal, C. H. Miranda, J. M. Gosse, Y. A. S. Samithri, and **N. Rajakaruna**. Plant-Soil Relations of Ultramafic Outcrops in Sri Lanka: A Reassessment. Poster presentation at the 10th International Conference on Serpentine Ecology, Ekaterinburg, Russia. June 21-30.

Despite the occurrence of ultramafic outcrops throughout South Asia, little is known about their geoecology, including their floristics and soil-plant elemental relations. We report 139 plant taxa (46 families) across five disjunct ultramafic outcrops of Sri Lanka. Our findings are based on extensive fieldwork conducted in 2017 and previous literature. The most species-rich families were the Fabaceae (17 spp), Euphorbiaceae (15 spp), and Asteraceae (11 spp). None of the taxa studied were found on all sites, while 85% were only found at one site. None of the taxa are considered to be serpentine endemic, and it is unclear if they are genetically distinct. We did not discover any new metal hyperaccumulators nor were we able to confirm Ni hyperaccumulation in taxa previously reported as such from Sri Lanka, including *Hybanthus enneaspermus* and *Evolvulus alsinoides*, or in *Rinorea bengalensis*, a known Ni-hyperaccumulating species from Southeast Asia. Soils on the Ussangoda, Indikolapalessa, Ginigalpalessa, and Yudagannawa sites were found to have the low Ca:Mg quotients and high heavy-metal content typical of serpentinites. Rupaha, a previously botanically unexplored site, also with low soil Ca:Mg quotients, had a comparatively far lower soil heavy-metal content, suggesting that the serpentinites at this site may have metamorphosed later than at the other sites. These soil results were further confirmed by elemental analyses of rock samples via x-ray fluorescence (XRF).

9) **Pena, A. J.**, O'Dell, R., Rajakaruna, N. 2020. Investigating the evolutionary mechanisms driving speciation in the *Layia glandulosa* - *L. discoidea* [Asteraceae] complex. Oral Presentation at the 10th International Conference on Serpentine Ecology, Ekaterinburg, Russia. June 21 - 30.

Abstract: *Layia discoidea*, a narrowly-distributed serpentine endemic, and its sandstone-associated progenitor, *L. glandulosa*, are both self-incompatible annuals native to California. The two, however, are interfertile and produce viable hybrids in a controlled setting, although no hybrids can be found in nature. The drastic contrast of the parental soils inhabited by each species has been suggested to play a role in partial or complete reduction of gene flow between

the congeners. It is not known if the two species can grow on each other's soil or if reduced hybrid fitness in parental soils may serve as a mechanism of post-zygotic isolation. Additionally, it is not known if other pre-zygotic mechanisms, such as distinct flower morphologies selecting for different pollinator guilds and differences in phenology reducing potential for gene flow, are also playing a role in their divergence. Using seed and soil collected from several populations within both species' range, we are conducting a reciprocal transplant study in a greenhouse to examine how individuals from both species perform in their native soil compared to the soil of their congener. Additionally, an ongoing breeding study will generate F1 seed that will be used in an in-field transplant study that will take place in the only location the two species co-occur. We hope our studies will confirm if post-zygotic isolation, through reduced hybrid fitness in parental soils, is responsible for the divergence of these species.

10) **Ferrero, A.** and N. Rajakaruna. Work in Progress: Isolation and characterization of Nickel-tolerant bacteria from disturbed and undisturbed serpentine outcrops at Irish Hills Open Space Preserve in San Luis Obispo, CA, U.S.A. Poster presentation at the 10th International Conference on Serpentine Ecology, Ekaterinburg, Russia. June 21-30.

Abstract: Serpentine-associated microbes have been observed to affect the growth and rate of metal accumulation in plants, and certain metal-sequestering bacteria have gained significant attention due to their potential use in bioremediation practices. While some studies show that microbes in disturbed settings differ significantly from those in undisturbed settings and other experiments describe trends in metal-tolerant species composition on serpentine soil, no study has yet elucidated differences in heavy metal-tolerant microbes isolated from disturbed vs. undisturbed serpentine habitats. In our study, we will focus on Ni-tolerant bacteria, as Ni is often found in high concentrations in serpentine soil and is an excellent target for bioremediation efforts due to the recent developments in biotechnologies utilizing other Ni-tolerant microbes and plants. We will collect biocrust samples from both undisturbed and disturbed serpentine soils at the Irish Hills Open Space Preserve, home to abandoned chromite mines and adjacent, undisturbed serpentine chaparral. We will use the samples for plate-count assays on media supplemented with $NiSO_4$ which will select for Ni-tolerant bacteria. After multiple rounds of dilution plating on media with increasingly higher Ni concentrations (i.e., 1mM, 5mM, 10mM),

some of the most Ni-tolerant colonies will be selected for further isolation and examination. Isolates will subsequently be identified via genetic sequencing of bacterial 16S ribosomal RNA.

11) **Raposo, A**., L. Negoita, C. Howington, A. Pena, A. Ferrero, P. Walsh, D. Stephens and N. Rajakaruna. The influence of fire and fire retardant (*Phos-chek*®) on plant diversity and non-native species abundance in California's serpentine chaparral. Oral Presentation at the 10th International Conference on Serpentine Ecology, Ekaterinburg, Russia. June 21 - 30.

Abstract: Fire and harsh soils play key roles in maintaining plant communities. However, the effects of fire-retardant on plant diversity on serpentine soils are unclear. We investigated how fire and a nutrient-rich fire retardant, *Phos-chek* (*PC*), influence diversity and the relative abundance of native and non-native species on serpentine soils. Our study area consisted of serpentine chaparral which burned in 2017 and was treated with *PC*. Treatments consisted of burnt serpentine, unburnt serpentine treated with *PC*, and unburnt serpentine as the control. Soil chemistry and plant species cover were assessed yearly for two years beginning 6 months after the fire. We then tested the effect of *PC* and fire on diversity and non-native abundance using generalized linear mixed-effect models, tukey pairwise comparisons, and the likelihood-ratio test. We found no significant effect of burning or *PC* application on diversity of species. However, non-native species abundance was significantly different among treatments directly following the fire, with complete elimination of non-native abundance in the burned treatment, and an increase in non-native abundance in the *PC* treatment. In the subsequent year, however, non-native abundance returned to levels found in the unburnt treatment, increasing on burnt serpentine and decreasing on unburnt serpentine treated with *PC*. This study helps better predict and plan for shifts in species distributions in response to fire and *PC* in serpentine chaparral.

12) **Dawley, R.**, D. Stephens, and N. Rajakaruna. Work in Progress: The Use of California Native Plants in Chromite Mine Restoration. Poster presentation at the 10th International Conference on Serpentine Ecology, Ekaterinburg, Russia. June 21-30.

Abstract: Deposits of chromite in mine tailings can prove hazardous to native ecology. We will test the ability of native, serpentine tolerant plants to grow on compost-amended chromite mine tailing soil collected from an abandoned chromite mine at the Irish Hills Open Space Preserve in San Luis Obispo, California. *Acmispon wrangelianus*, *Lupinus hirsutissimus*, *L. truncatus* (all Fabaceae), *Eschscholzia californic*a (Papaveraceae), *Festuca californica*, *F. microstachys* (both Poaceae), *Plantago erecta* (Plantaginaceae), and *Streptanthus glandulosus* (Brassicaceae) will be grown as a seed mix on mine soil and mine soil amended with local, organic compost. The plants will be grown outside the California Polytechnic State University greenhouse complex to simulate the climatic conditions at the chromite mine. The treatments will consist of 50:50 mine soil to compost, 50:25 mine soil to compost, and unamended mine soil (control). These treatments will be organized in a randomized complete block design and replicated five times. We will measure shoot and root biomass, root/shoot ratios, and total reproductive output to evaluate the effects of the treatments on native plant growth and fitness. The results of this study will provide baseline data for potential restoration of chromite mines in the region.

13) **Siebert, S. J**. and N. Rajakaruna. An overview of ultramafic geoecology in sub-Saharan Africa and the way forward. Oral Presentation at the 10th International Conference on Serpentine Ecology, Ekaterinburg, Russia. June 21 - 30.

Abstract: Elevated metal content, high Mg:Ca ratio and nutrient deficiencies of southern Africa's ultramafic rock types and associated soils have shaped unique plant communities over millennia. Typically, the vegetation is stunted and sparse, but provides refuges for plants that thrive under the serpentine influence. Consequently, ultramafic habitats have received attention from serpentine ecologists across Africa, particularly in Zimbabwe and South Africa. The aim of this study was to collate the research of the last 60 years into a detailed overview of ultramafic geo-ecology of sub-Saharan Africa which began in 1959 when a paper was published on the nickel and chromium toxicity of soils in Rhodesia (Zimbabwe). Since

then, 36% of published research has been concerned with species diversity, 34% with heavy metal accumulation and 30% with ecology. The species diversity topics vary, with alpha taxonomy (new species descriptions of edaphic endemics) and species diversity patterns accounting for most of the literature. The heavy metal-plant interface is dominated by metal hyperaccumulation and tolerance capabilities of selected species and phytoremediation. Ecology topics mainly explore plant-soil interactions and conservation of rare edaphic specialists. This review proposes future research to include soil algae and lichens, evolutionary processes of edaphic specialists, effects of climate change on edaphic endemics, and interactions between humans and the serpentine environment.

14) **Siebert. S**., F. Siebert, S. Adhikari, R. Boneschans, N. Rajakaruna. Foliar elemental profiles of forbs on metal-rich pyroxenite-derived soils of Sekhukhuneland, South Africa. Poster presentation at the 10th International Conference on Serpentine Ecology, Ekaterinburg, Russia. June 21-30.

Abstract: Foliar elemental profiles of most plants generally reflect site-specific soil chemistry. The aim of this study was to elucidate such patterns for the ultramafic flora of the Rustenburg Layered Suite in Sekhukhuneland. This region is ideal for studying plant elemental profiles because it has soil rich in heavy metals. Plots were placed at different topographical positions of across hills of exposed pyroxenite, namely crest, midslope, footslope and valley. The most dominant tree, shrub, dwarf shrub, forb and grass species were identified from frequency data for he topographic positions. Leaf tissue was analysed for five individuals per plant species per topographic position. Soil samples were taken from underneath each sampled individual and bulked for analyses per species. Foliar elemental profiles of 30 species in 14 families were constructed. In general, phytotoxic elements such as Cr, Mn and and Ni were excluded by plants. A limited number of species accumulated non-essential Al and the essential micro-nutrient Fe. In terms of forbs, the Asterids accumulated the most Fe (>1000 mg/kg), and overall the Eurosids were the better excluders of metals. In contrast, the Poales showed a better affinity for accumulation of metals and had the highest tissue levels of Al, Mn and Ni (100, 40 and 20 mg/kg, respectively). This study confirms the absence of a hyperaccumulator flora for Sekhukhuneland.

15) Venter, A., **S. Siebert**, A. Levanets, **N. Rajakaruna**, S. Barnard. Microbial diversity of biological soil crusts on serpentine soils in Mpumalanga, South Africa. Poster presentation at the 10th International Conference on Serpentine Ecology, Ekaterinburg, Russia. June 21-30.

Abstract: The unique geochemistry of serpentine soils generates habitats that are biologically unique and are known to harbor high levels of plant endemism. Biological soil crusts (BSC) are also found in these environments and are formed by a consortium of organisms such as bacteria, cyanobacteria, algae, fungi, lichens and bryophytes. These cryptogammic species show low levels of endemism but are found worldwide often in extreme habitats such as arid environments, rock faces and mine tailings. Therefore, the BSC community composition of serpentine soils in Mpumalanga was investigated by using integrative taxonomy, which combines morphological and DNA barcoding data. Diverse communities with 22 bacterial, 32 cyanobacterial, 27 algal and 26 fungal genera were found in nine serpentine sites. The most common bacterial genera were Caldithrix, Conexibacter and Chthoniobacter, which were found on all sites, except Kaapsehoop. The cyanobacterium Phormidium was found on all the sites, while the algal genera Bracteacoccus, Chlorella and Nanochloropsis were found on 89% of sites. Phoma, from the Dothideomycetes, was the most common fungus and found on 67% of sites. None of the recorded fungi and bacteria were unique (or endemic) to serpentine soils, but Hormotilopsis gelatinosa, which was found on the Kaapsehoop site, is a rare green alga that so far has been described from only seven localities in the world.