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HUMBOLDT STATE UNIVERSITY

INRSEP/CNRS RESEARCH SYMPOSIUM

28 SEPTEMBER 2018 - 12-4 PM

POSTER SESSION 12-2 PM
ORAL PRESENTATIONS 2-4 PM

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Thank you for coming!
POSTER SESSION

POSTER # PRESENTER DEPARTMENT
1. Nicholas Vega Anguiano, Rangeland & Wildland Science
2. Fernando Flores, Environmental Resources Engineering
3. Colleen Smith, Forestry & Wildland Resources
4. Gabre Goff, Forestry & Wildland Resources
5. Dylan Neely, Wildlife Management & Conservation
6. Nathaniel Mcguigan, Biological Sciences
7. Katherine Stoneycypher, Environmental Science & Management
8. Kezia Rasmusson, Oceanography
9. Carolyn Westrick, Oceanography
10. Katherine Panebianco, Oceanography
11. Jacob Evans, Oceanography
12. Jackson Stillman, Physics & Astronomy
13. Joseph Davies, Geology
14. Delfina Navarro-Estrada, Chemistry
15. Octavio Acosta, Botany
16. Amanda Agosto Ramos, Cellular Molecular Biology
17. Tara Caso, Cellular Molecular Biology
18. Dixie Blumenshine, Cellular Molecular Biology
19. Maribel Perez Espinal Cellular Molecular Biology
20. Vannia Peña, Microbiology
21. Miranda Rodriguez and Amanda Pope, Microbiology
22. Kristian Bowman and Christopher Keyser, Microbiology
23. Emma Jones, Psychology
24. Yared Haile, Biological Sciences
25. Daniel Raemer, BS, Oceanography
26. Dana Christensen, BS, Geology
27. Kevin Soland, BS, Forestry
28. Kyle Anthoney, BS, Cellular Molecular Biology
29. James Lamping et.al, GSP 326, Forestry (Soils)

ORAL PRESENTATIONS

TIME PRESENTER DEPARTMENT (ABSTRACT NUMBER)
2:00 - 2:15 Dylan Neely, Wildlife Management & Conservation (5)
2:20 - 2:35 Maximilian Cox, Biological Sciences (31)
2:40 - 2:55 Amanda Agosto Ramos, Cellular Molecular Biology (16)
2:55 - 3:05 ---BREAK---
3:05 - 3:20 Nicholas Vega Anguiano, Rangeland & Wildland Sci (1)
3:25 - 3:40 Tara Caso, Cellular Molecular Biology (17)
3:45 - 4:00 Haley Nisson, Cellular Molecular Biology (19)
1. NICHOLAS VEGA ANGUIANO, RANGELAND & WILDLAND SCIENCE

Mentor: Dr. Susan Marshall, Lonyx Landry,

Investigating N2O Fluxes Of Fertilized Agricultural Fields in Differing Landscape Positions Utilizing Soil Textural Analysis

Landscape position influence Nitrous oxide fluxes in fertilized agricultural fields through many properties including soil texture. As a result, soil and hydrological physical process contribute to emissions of N2O-a greenhouse gas-which has a radiating potential of almost 300 times that of Carbon Dioxide. Soil texture plays a pivotal role in how much N20 is emitted. This is partially attributed to the proportions of sand, silt and clay. Past research shows there are higher fluxes in depressions in comparison to summits. In addition, it is thought that a higher clay content will lead to increased fluxes in soil due to clay’s high water holding capacity which is attributed to clays large number of micropores. Composite soil samples were collected from 5 different fertilized agricultural fields in summits and depressions using a 20 cm soil core, and then dried for 48 hours at 105 degrees celsius. Samples where subjected to the Hydrometer Analysis to analyze soil texture and proportion composition of sand, silt and clay. Statistical analysis was run before hand to identify high and low fluxes of sites to identify potential sampling locations with agricultural plots. A higher average flux is equivalent to a lower landscape position, whereas a lower flux is congruent to a higher landscape position. Current data shows that there is a higher N20 flux correlation to sand and silt instead of what was previously attributed to, which is clay. The reason being is that textures that are median grained - silt, which is larger than clay but smaller than sand- dictate the ratios of sand and clay. Thus percent differences in silt influence N20 flux. We had 13 sites located in the KBS LTER in Corn and Switchgrass systems. In a majority of the fields we found a significant positive correlation between fine-sized soil particles (silt and clay) and N2O fluxes when rainfall is consistent
2. FERNANDO FLORES, ENVIRONMENTAL RESOURCES ENGINEERING

Mentor: Dr. Sintana Vergara

Characterizing Greenhouse Gas Emissions and Operations from Small-Scale Composting Systems

The recent rapid impact of anthropogenic activity on climate change effects on our environment has brought scientists from around the world together to come up with strategies to reduce anthropogenic greenhouse gas emissions (IPCC 2018). Greenhouse gases (GHGs) of concern are carbon dioxide, methane, nitrous oxide and other gases (EPA 2018). A literature review was conducted to assess greenhouse gas emissions from small-scale composting systems. We also reviewed the methods to sample and analyze gases, and the environmental and waste material parameters that influence the production of GHG emission. Thus, the emission factors for CO2, an biogenic CO2 production, range from 0.126 to 0.423 kg/kg of wet waste. We reported emissions factors for CH4 which had higher variability, ranging over four orders of magnitude. N2O emissions factors varied over an order of magnitude. These results play a role in comparing small-scale and large-scale composting with the production of GHGs emissions. Other implications of the composting system included temperature and oxygen were correlated with GHG emissions. These inform practices for small-scale composting operations to improve operation and efficiency.
3. COLLEEN SMITH, FORESTRY & WILDLAND RESOURCES

Mentor: Dr. Susan Marshall

An Evaluation of Intensive Management Effects on Soil Microbial Biomass and Functional Diversity

The effects of forest plantation management on soil microbial biomass and functional diversity can often be significant, but little is known about changes over time. We conducted a study to observe the long-term effects of intensive management used in the Southeastern Tree Research and Education Site (SETRES). This well-characterized site is a loblolly pine (Pinus taeda) stand composed of 4 treatments: control, irrigated, fertilized, and both irrigated and fertilized. Microbial biomass and functional diversity were first measured at this site throughout the early-mid 1990’s where a significant increase in microbial biomass C and N was observed in the fertilized plots while irrigation had no effect. The use of irrigation in this study was subsequently terminated in 2010. Microbial biomass was quantified with the chloroform fumigation extraction technique for total C and N. We used Biolog EcoPlates™ and FF microplates™ to quantify both bacterial and fungal microbial functional diversity, respectively. By reevaluating the effects of these management strategies exactly 20 years later we can assess the long-term effects of fertilization and irrigation in a predominately sandy soil. Carbon source utilization was similar across treatments indicating that fertilization and irrigation did not have long-term impacts on functional diversity. In contrast to the prior study at SETRES, microbial biomass did not differ among treatments. Understanding the soil biological impacts of intensive management in plantation forestry may help us understand long-term nutrient cycling potential. The SETRES site is scheduled for harvest, and future research projects will evaluate the changes to the microbial community.
4. GABE GOFF, FORESTRY & WILDLAND RESOURCES

Mentor: Dr. Lucy Kerhoulas

Physiological Response of Quercus garryana to conifer encroachment in Northern California

Although previous research has investigated oak woodland responses to conifer encroachment, few studies have evaluated these responses at the physiological level. The objective of our study was to understand the physiological effects of conifer encroachment on Oregon white oaks (Quercus garryana) in open, moderately encroached, and heavily encroached woodlands of northwestern California. During the 2017 growing season, we measured predawn and midday water potential (Ψ) using a pressure chamber and stomatal conductance (gs, positively correlated with photosynthetic rate) using a leaf porometer. We found that Ψ was lowest (most stressed) in open stands, identifying water availability as the most limiting factor to oak productivity in these high-light environments. We also found that gs was highest in moderately encroached stands early in July but lowest in these stands late in the growing season, again suggesting that water availability can limit oak productivity in moderately encroached stands, particularly when winter water is exhausted. Lastly, we found a positive relationship between gs and 🍃 (reduced gas exchange under high water stress) in open stands and a negative relationship between gs and 🍃 (increased gas exchange under high water stress) in heavily encroached stands. This last finding indicates higher stomatal regulation in open stands, likely due to xeric acclimation, and lower stomatal regulation in heavily encroached stands, likely due to mesic acclimation. Our results suggest that despite increasing competition for water, a moderate level of conifer encroachment may increase water availability to oaks by reducing evaporative losses of soil water.
Using eDNA to determine the effectiveness of brook trout removal from high alpine lakes, California

Exotic species invasions are ranked as the second most destructive factor affecting biodiversity behind habitat degradation. In the western United States it is estimated that 95% of historically fishless lakes in alpine areas have been intentionally stocked with trout, often as a means of increasing sport fishing opportunities. In the Trinity Alps wilderness of northern California the Echo Lake basin was once stocked with brook trout (Salvelinus fontinalis) but has been identified as a high priority removal site for conservation. Ongoing sight surveys and electrofishing surveys throughout the basin have suggested the removal was 100% successful. In this study we used eDNA methods to determine whether or not the removal was successful. A total of eight sites were surveyed, including three fishless sites, two fish sites, and three removal sites. A minimum of three water samples were collected from each site, and three qPCR replicates were run for each sample. Our results were consistent with expectations, as we detected brook trout at all of the “fish” sites, and did not detect brook trout at “fishless” sites. The eDNA approach did not detect brook trout at the removal site, suggesting the Echo Lake brook trout removal effort was successful. This study validates the use of environmental DNA methods to sample for species in remote wilderness settings and illustrates the effectiveness of the approach for evaluation of exotic species removal efforts.
Implementing a 6 Month Weight Loss Program to address Dietary Health Related Issues Among Indigenous Peoples' in Northern California

What can we do to reduce dietary related health issues among indigenous communities in Northern California? Numerous studies conducted among Indigenous communities in Northern California found that dietary related health illnesses were all connected to a disruption in ancestral food systems. So, what are the solutions?

The Blue Lake Rancheria Tribal Office: Health and Wellness Program observed the effects of the health epidemic among members of the Native community. To address this health issues, the Blue Lake Rancheria has proposed the Six Month Weight Loss Program, an intervention program focusing primarily on Native elders who were chosen on a voluntary basis. The program primarily addresses diet and exercise while addressing other risk factors such as drug and alcohol abuse, tobacco use and socioeconomic conditions.

Due to the high rates of obesity and other dietary related health issues, the need to raise awareness and creating an intervention program is essential for the health of indigenous communities in Northern California. While the results of this implementation are still being processed we expect the majority of our participants to lose weight and expect a reduction in dietary health-related illnesses among tribal members.
7. Katherine Stonecypher, Environmental Sci & Management

Mentor: Sabra Steinberg, MS

Assessing Socio-Environmental Factors for a Childhood Leukemia Cluster in Cleburne County, Alabama

The communities of Fruithurst and Muscadine in rural Alabama host a combined population of no more than 1600 residents. However, cancer prevalence in these communities is very high. Beginning in 2013, four children living within the Fruithurst-Muscadine census block were diagnosed with cancer within four years. From 2016 to 2017, four adults residing in the same area were also diagnosed. The types of cancer diagnosed included Acute Lymphoblastic Leukemia, Chronic Lymphoblastic Leukemia, Chronic Myeloid Leukemia, and Lymphoma.

The homes of those diagnosed correspond spatially to a geologic formation known as the Heflin Phyllite, a type of metamorphic rock which is part of the regional Piedmont province. Similar metamorphic phyllites within the Piedmont province have been shown to host minerals enriched in uranium and could produce large concentrations of radon (222Rn). The link between lung cancer and radon gas is well established, but recent research suggests that radon in drinking water or in the air may contribute to a higher rate of childhood leukemia. Preliminary tests of well water in Fruithurst revealed concentrations of 222Rn exceeding the EPA proposed level of 4,000 pCi/L in four samples of patient wells. Although the Fruithurst city well was decommissioned in the 1990s due to concerns about radon, many citizens are still on private wells that could facilitate exposure to contamination. Elevated levels of trace elements (arsenic, chromium, lead and nickel) were also found in soils contaminated by industrial sources.

We used a combination of community-based research in the form of household surveys and geospatial analysis to identify incidences of cancer and associated risk factors such as smoking habit, occupational hazards, drinking water source, and family history of cancer. Survey results revealed a statistically significant correlation between well water and cancer diagnosis. Potential cancer clusters were identified using spatial analysis in ArcGIS and used as sampling locations for well water. Trace elements and radon level of the water samples will be analyzed using ICP-MS and RAD7 Radon Detector. These data will be used to identify households at risk of exposure and help obtain access to clean, safe sources of drinking water for the communities of Fruithurst and Muscadine.
8. KEZIA RASMUSSEN, OCEANOGRAPHY

Mentor: Dr. Kamila Larripa

Analyzing Taxonomic Diversity of the Human Gut Microbiome Using R

This research aims to quantify and illustrate microbiome diversity in the human gut. The microbiome describes interacting and ever-changing bacterial communities residing throughout the human body (as well as in other organisms), which contribute to and reflect our states of health. The process of analysis requires obtaining DNA, sample processing, data analysis, and representation using computer software. The sample was processed by uBiome, a company that analyzes various microbiome-related samples for the public and makes available the raw data. To analyze the raw data, an R package Metacoder, designed for illustrating hierarchical taxonomic diversity, was used for further data exploration and plot generation. Around 260 bacteria taxa were recovered (from phylum to species) and around 200,000 total counts of bacteria were recovered in the raw data. Four “very elusive” or low count bacteria taxa were found in this sample according to uBiome’s analysis. In addition, various bacteria with associated duties such as breaking down gluten and lactose, or managing metabolism were observed in this data set. Future research includes analyzing samples throughout changing diets and changing environments. An overall increase in data within this field is essential as the value, effect, and mechanisms of microbiomes have much to be understood.
9. CAROLYN WESTRICK, OCEANOGRAPHY

Mentor: Dr. Christine J. Cass

Benthic Microplastic Distribution In Humboldt Bay, Northern California: A Comparative Study Of Intertidal And Subtidal Sediments Based On Proximity From The Shore

Plastic production and use has increased steadily over the last century primarily because of plastic’s resistance to corrosion and low production costs. Plastics enter the marine environment from non-point sources such as rivers, wind, and fishing activities, and point-sources like sewage treatment plants, dumping and landfills. We focus on microplastics (MPs), specifically those within the size range of 0.335 to 5 mm, because of the uptake potential by detrital and filter feeding organisms. Quantifying the amount of MPs in the marine environment is crucial because bioaccumulation of plastics in marine life can affect humans who consume marine organisms. This study compares the concentration of MPs in sediments between the intertidal and subtidal environments of Humboldt Bay, California. We hypothesized that the intertidal samples would have higher MP concentrations due to their proximity to shore and increased anthropogenic activity. Sediment samples were collected using hand corers in the intertidal region and a Smith McIntyre grab in the subtidal region. Organic material in the samples was oxidized using 30% hydrogen peroxide, then a density differentiation technique was used to separate plastics for further microscope identification. Preliminary results show that about 95% of microplastics found in sediment are microfibers and the remaining 5% MPs are degraded hard plastic. To date, we have found more MPs in the subtidal region than the intertidal region, with 980 MP particles recovered in the subtidal region and 340 MP particles recovered in the intertidal region. This study confirms the presence of microplastics within Humboldt Bay, and defines their distribution with proximity to shoreline. These results can be used by the community to update recycling practices, wastewater treatment procedures to mitigate microfibers, and raise awareness about marine life ingestion of plastics.
10. KATHERINE PANEBIANCO, OCEANOGRAPHY

Mentor: Dr. Christine J. Cass

Comparison of microplastics in the benthic sediments of the northern, central, and southern regions of Humboldt Bay, Northern California

Plastics are durable, persistent, and have become deeply intertwined into the global economy. This research focuses on microplastics (MPs) that fall within the size range of 0.335 to 5.00 mm. Microplastics can bioaccumulate in marine organisms when consumed and eventually end up in human food sources. This size range of MPs found in the benthic environment are especially susceptible to consumption by the benthos. This study documents microplastic counts in the subtidal sediments of Humboldt Bay (HB) in northern California. This area supports a human population of about 47,000 people, and has a history of heavy industrial activities associated with lumber, shipping, and fishing. We hypothesized that the highest concentration of MPs would occur in the central portion of HB (Entrance Bay), where there is intensive use by humans, pollution point sources and a circulation pattern that concentrates flow towards Entrance Bay. Samples were collected from the northern (North Bay), central (Entrance Bay), and southern (South Bay) areas of HB using a Smith McIntyre grab sampler from the R/V Coral Sea. Organic material in subsamples was oxidized using 30% hydrogen peroxide, then isolated using a density separation technique and counted via optical microscopy. Preliminary counts of the MPs found in the subtidal region of HB are highest in Entrance Bay with 473 particles, lower in North Bay with 289 particles, and lowest in South Bay with 218 particles. Approximately 80% of these MPs were fibers, and the remaining 20% were degraded plastics. This information can be used to better understand the distribution of MPs within the soft-bottom, benthic ecosystem of HB, and to inform local communities and agencies as to the likely sources of these pollutants.
11. JACOB EVANS, OCEANOGRAPHY

Mentor: Dr. Christine J. Cass

Distribution of microplastics at the surface and within the water column in Humboldt Bay, Northern California

Plastic is a commonly used, man-made material that is highly durable, easy to produce, and used widely throughout society. The persistence of plastics results in their introduction to the oceans via river runoff from urban and industrial areas, intentional dumping, and fishing practices. This study investigates microplastics (MPs) in the size range of 0.335 to 5 mm. MPs directly affect marine ecosystems, as they are mistaken for food by marine organisms and are then transferred to humans when we consume seafood. MP concentration in the water column can vary due to river input, tidal flux, and source proximity. We hypothesized that the highest concentration of MPs within Humboldt Bay (HB) in northern California would be found in the harbor entrance (Entrance Bay), which is adjacent to a sewage treatment plant and near a solid waste transfer station. We surveyed MP concentrations within the water column and surface layer in the three sub-basins of HB. Surface and water column samples were obtained using 0.335-mm mesh neuston and ring nets, respectively. Organic material in the samples was removed via oxidation with 30% hydrogen peroxide. Density separation techniques were then used to separate plastics for microscope analysis. Preliminary results do not support our hypothesis, as air-sea interface MP concentrations are highest in North Bay (6.25x10^-5 ± 4.03x10^-5 plastic particles per liter (ppL)), followed by South Bay (3.48x10^-5 ± 1.04x10^-5 ppL), and lowest within Entrance Bay (2.23x10^-5 ± 0.87x10^-5 ppL). Within the water column, the highest average concentration of MPs was found in South Bay (5.81x10^-5 ± 11.89x10^-5 ppL), with lower concentrations in Entrance Bay (1.46x10^-5 ± 0.84x10^-5 ppL) and North Bay (1.21x10^-5 ± 0.87x10^-5 ppL). This study can help the public understand the quantity of plastic contained within HB, where it is most concentrated, and possible mitigation practices.
12. JACkSON STILLMAN, PHYSICS

Mentor: Dr. C.D. Hoyle

Novel Tests of Gravity Below Fifty Microns

Theories attempting to unify the Standard Model and General Relativity often include features that violate the Weak Equivalence Principle and gravitational Inverse-Square Law. Motivated by these considerations, undergraduates and faculty at Humboldt State University are operating an experiment to probe gravitational interactions below the 50-micron length scale. The experiment employs a torsion pendulum whose twist is measured as an attractor mass is oscillated nearby. The size and distance dependence of the torque variation provides a means to determine the existence of deviations from expected behavior at untested scales.

13. JOSEPH DAVIES, GEOLOGY

Mentor: Dr. Brandon Browne

Comparison of Cr-Spinel from Deer Mtn. Quarry and the Trinity Ophiolite, California.

Comparison of Cr-Spinel from Deer Mtn. Quarry and the Trinity Ophiolite, California. High magnesian andesite with olivine hosting spinel analyzed with the SEM EDS function compared with spinel within a dunite from the Trinity Ophiolite. Poster aims to show evidence for presence of Trinity Ophiolite as basement rock under Mt. Shasta, Siskiyou county, California.
14. DELFINA NAVARRO-ESTRADA, CHEMISTRY

Mentor: Dr. Matthew Hurst

Nutrient Analysis of Cenotes in the Yucatan Peninsula

Groundwater samples from three cenotes were collected and the levels of nitrogen and phosphorous species at different depths were analyzed with a spectrophotometer in order to determine their cycle, behavior and possible nutrient pollution from human activities. Other parameters including dissolved oxygen, temperature, pH, total dissolved solids, oxidation reduction potential and ion chemistry were analyzed with a multi-probe electrode system to provide explanations for the speciation of the nutrients and possible shifts from equilibrium. The groundwater in the Yucatan Peninsula is highly susceptible of nutrient pollution due to the high tourism and population in the area. The recreational activities taking place in the cenotes bring millions of tourists every year, which then promotes population growth and therefore introduces nutrients to the water.
15. OCTAVIO ACOSTA, BOTANY

Mentor: Dr. Cristian Martinez

Effect of Habitat Types on Detritivore and Fungivore Arthropod Communities in Southern Costa Rica

The importance of restoring historically logged forests has emerged as an area of interest in restoration ecology. Traditional aspects of ecological restoration focus on the floral community due to their ability to shape the physical environment of the forest. However, the arthropod community, especially detritivores and fungivores, must also be considered when assessing ecological restoration as they are an integral part of the brown food web which influences nutrient cycling. We ask the question of whether different restoration habitat types effect the community of arthropod detritivores and fungivores in leaf litter in order to assess the impacts restoration practices. We predicted finding a higher abundance of species in secondary forest, and a difference in communities in all three sites. Our study was conducted at Las Cruces Biological Station in southern Costa Rica. We assessed restoration at three different forest types (Secondary 10-25 years n=2, Primary selectively logged n=2, and Un-logged primary n=2) by analyzing arthropod detritivore communities. We collected 0.5m2 leaf litter samples and placed them in Berlese funnels to extract arthropod communities. Communities of detritivores in each site were then compared to study how site categories influence both the detritivore abundance and community structures. Our results suggest that site categories of restoration have an impact on detritivore communities. Additionally, we found fungivores to drive differences in community composition of arthropod communities at Las Cruces. Findings from this study can bring further knowledge to create more encompassing reforestation efforts in southern Costa Rica.
16. AMANDA AGOSTO RAMOS, CELLULAR MOLECULAR BIOLOGY

Mentor: Dr. Henrik Schneller

Characterizing Synthetic Transcriptional Regulators for Plant Engineering

Engineering of microorganisms has dominated the field of synthetic biology, a result of the detailed understanding of microbial systems and the availability of complete genome and proteome data. However, a carbon substrate must be provided, often in the form of plant-derived sugars. Plants fix carbon through photosynthesis using light and water, yielding the capacity to produce and utilize their own feedstock. This trait makes plants optimal systems for the generation of specialized compounds and therapeutic proteins. We are interested in creating tools that will facilitate the use of plants as bio-factories to produce high-value compounds, with the goal of exceeding the output potential of microorganisms. This work is also important because the metabolic engineering of plants will produce customized and optimized agricultural and biofuel crops. We started by generating a library of synthetic transcriptional regulators based on well characterized yeast elements for use in plant systems. To test our system, we simultaneously infiltrate various activator or repressor elements with a corresponding promoter sequence into Nicotiana benthamiana. The promoter drives the expression of green fluorescent protein (GFP) that we measure using a spectrophotometer. This allows us to quickly analyze the transcriptional output attained when certain components are used in tandem. So far, we have achieved an expression range of 0.5 to 50 times that of the normalized expression pattern of the plant. The library generated herein will offer a powerful tool for designing complex genetic circuits in plants, with specific and predictable outcomes.
Investigating seed composition over development in soybean fast neutron lines altered in central carbon metabolism

High oil (and protein) content of seeds makes Soybean (Glycine max [L.] Merr.) a valuable crop for biofuel and feedstock industries. The process of seed maturation leading up to the accumulation of these oil reserves, however, is nonlinear and results in a decline in lipids towards later stages of development that correspond to seed desiccation. Soybean seeds are also notorious for over accumulation of the non-digestible carbohydrates raffinose and stachyose which affect the quality of feedstock. Our data suggests that the timing of this accumulation corresponds to the decline in lipid content. We reasoned that a reallocation of carbon sources from maternal tissue or an inherent metabolic control directing carbon from lipid towards carbohydrates may lead to this decline. To that end, a select set of fast neutron (FN) mutagenized soybean seeds likely carrying deletions in regions of genome encoding central carbon metabolic genes were investigated for lipid and carbohydrate profiles over the course of seed development. A subset of these lines with high lipid and lower carbohydrate content especially raffinose and stachyose were suggested for future breeding efforts that could lead to desirable improvements of seed quality.
For the past 5 years the biological sciences have become enamored by the utilization of CRISPR/Cas9 gene editing tools. This system, originally found in bacteria as a viral defense mechanism, has been repurposed to create precise mutations and easy insertion of foreign DNA. An ever-expanding array of CRISPR technologies are currently available, permitting development of combined systems for simultaneous transcriptional activation, repression, and mRNA knockdown of multiple genes. Enzymatically dead Cas9 (dCas9) fused with either transcriptional activator VP64 or repressor KRAB, as well as RNA nuclease Cas13a, can be used to manage expression of multiple genes of interest. Our long-term goal is to use CRISPR-based tools for functional genetic screens and disease modeling. We hypothesize that the simultaneous use of dCas9-VP64/KRAB and Cas13a will support manipulation of two or more genes at the same or different loci, enabling us to carry out our goals in a variety of projects. Bicistronic plasmids containing cassettes of dCas9-VP64 or -KRAB and Cas13a, as well as other necessary components were constructed using HiFi assembly. They were screened and stably edited into HEK293t cells at the AAVS1 safe harbor locus. Their expression is under control of a Tet-On 3G inducible system. On-going studies use these tools to target multiple transcript variants of the Alzheimer's disease-associated MAPT gene, while regulating signaling and/or degradative pathways. EGFP, as a component of the integrated coding DNA sequence, acts as a marker of integration and inducible expression. dCas9-VP64/KRAB and Cas13a are co-expressed in the presence of doxycycline as indicated by GFP fluorescence. Due to differences in gRNAs between nucleases, they are currently expected to function efficiently in a single system, as test gRNAs are still being introduced to cells. This system will ease the construction of our intended disease models and allow study of protein-protein or protein-gene interactions within those models, as well as potential drug discovery. Gene repression and mRNA knockdown could be used in conjunction to ensure complete silencing in other single-gene models. Our bicistronic approach to CRISPR-based gene regulation could support efforts to investigate diseases studied in our lab, including Niemann-Pick diseases, Huntington’s disease, Alzheimer’s disease, and more.
19. MARIBEL PEREZ ESPINAL and HALEY NISSON, CELLULAR MOLECULAR BIOLOGY

Mentor: Dr. John Steele

Utilizing Cas13a genomic editing system for scientific inquiry

Generating a plasmid to contain a gRNA to work in tandem with another Cas13a-containing plasmid to selectively target specific exons, and therefore, splice variants. This tool has many applications, examples of which will be explored.

20. VANNIA PEÑA, MICROBIOLOGY

Mentor: Dr. John Steele

Progress toward gene regulation in Humulus lupulus L. (hops).

Primary metabolic compounds synthesized in higher plants follow a complex interconnected web of pathways, such that the regulation of enzymes within these pathways can affect down stream reactions. In order to study the enzymatic pathways of carotenoid production in Humulus lupulus L. (hops), plants were established in vitro by micropropagation of nodal explants. The young shoots of the Cascade variety were cultured on solid agar medium composed of a modified MS11 (Murashigee and Skoog, 1962) medium with plant growth regulators indole-3-acetic acid (IAA), 6-benzylaminopurine (BAP), and α-naphthalene acetic acid (NAA)14. Successful in vitro cultures were selected to study regulation of enzyme phytoene desaturase (PDS). Using mesophilic DNA (27.1 ng/μL) of Cascade hops and primers, we were able to show that the enzyme phytoene desaturase 3 in Arabidopsis thaliana (AtPDS3, ac: AT4G14210.1) shares some homology to hops phytoene desaturase (HIPDS, gnl|BL_ORD_ID|16589). Polymerase Chain Reaction (PCR) allows us to target and amplify the conserved exon 13 of the HIPDS scaffolding gene.
21. MIRANDA RODRIGUEZ and AMANDA POPE, MICROBIOLOGY

Mentor: Dr. John W. Steele

AAVS1 HiFi Assembly and Optimization of Different Plasmids for CRISPR/Cas9 Platform

The strive to find an effective therapy for neurodegenerative diseases such as Huntington's Disease and Alzheimer's have recently been obtainable due to the discovery of CRISPRs and the Cas9 systems. Four distinct Cas9 plasmids were constructed to assist in genome editing: Cas9-D10A, dCas9-VP64, dCas9-KRAB, and dCas9. To increase the versatility of these tools, an Adeno-associated Virus Integration site (AAVS1) plasmid was created using Hi-Fi assembly. The CRISPR tools were successfully integrated into the AAVS1 plasmid and later transfected into HEK293T by coupling it with the pLizzard Plasmid containing the indicator green fluorescent protein (GFP.) Green cells show a glimpse of the potential of the tool as it will be used for various different genes therapies.

22. KRISTIAN BOWMAN and CHRISTOPHER KEYSER, MICROBIOLOGY

Mentor: Dr. Jenny Cappuccio

Introduction of Serratia Marcescens chiA gene into E. coli for chitinase production

N-acetylglucosamine chains that comprise chitin. Chitin is found in the exskelton of arthropods such as insects and crustaceans. Successful cloning of the gene into a variety host bacteria strains can prove useful to agricultural and bioenergy fields. Isolated chitinase protein can be employed to combat phytopathogenic fungi and insects or to harvest biofuel feedstock from waste materials. Here we transformed a chemically competent strain of E. coli using the pET28a(+) expression vector containing a chiA gene insert derived from S. marcescens. With transformed E. coli, we can in the future express the pET28a(+) vector and extract the protein ChiA for enzyme activity analysis.
Development of AAVS1-Integratable Cassettes for Cell-Type Specific Expression of Fluorescent Proteins and CRISPR Tools in Human Neural Cell Types

Tauopathies are a class of neurodegenerative disease that are associated with the toxic cytosolic accumulation of the protein tau in neurons, astrocytes, and/or oligodendrocytes. There are fourteen described tauopathies that include primary (e.g. corticobasal degeneration and progressive supranuclear palsy) and secondary (e.g. Alzheimer’s disease, Niemann-Pick disease type C1) tauopathies. Each disease is distinct in the region of the brain, cell type, and predominant isoform(s) of the tau protein that accumulates. Our lab’s goal is to understand how the autophagy pathway is utilized by these cell types to manage excess tau protein burden. However, in order to understand how each of these cell types regulates this pathway, we first needed to develop tools that will allow us to visualize these cell types and target genes in a cell-type specific manner. The goal of this project was to develop a suite of cassettes that act as cell-type specific reporters and can be used for facile cloning and expression of CRISPR gene editing or regulatory tools in the desired cell type. In order to accomplish this goal, we developed a series of novel cassettes that: (i) can be integrated into the human AAVS1 locus by CRISPR gene editing and confer puromycin resistance; (ii) express an EGFP reporter driven by a cell-type specific promoter; (iii) contain a cleavable linker and unique cloning site for facile integration of CRISPR tools for genetic screens. We made unique cassettes with cell-type specific promoters to drive neuron-specific expression (SYN1, TUBA1A, or PRNP promoters), astrocyte-specific expression (GFAP promoter), oligodendrocyte-specific expression (CNP promoter), or expression in all mammalian cell types (EEF1A1). We successfully cloned, assembled, and screened plasmids for stable integration into the AAVS1 locus, then co-transfected plasmids along with dual nickase CRISPRs into HEK293t cells to confirm integration, puromycin resistance. As expected, we observed large amounts of EGFP expression in HEK293t cells which integrated EEF1A1-promoter driven constructs, but not other cell-type specific constructs. Future studies will utilize these cassettes by integrating them into human induced pluripotent stem cell-derived neural progenitor cells use for genetic screens in our 3-D neural sphere cultures and disease models.
A Role for β-catenin in Synaptic Scaling

Intrinsic to learning and development is the brain’s ability to refine and remodel synaptic networks. Synaptic scaling homeostatically regulates neuronal activity by proportionally modulating the number of postsynaptic AMPA receptors at the synapse, thus compensating for sustained perturbations in activity. Found vital to this process of synaptic scaling is Shank3, a scaffolding protein found in the postsynaptic density of excitatory neurons. Mutations in Shank3 are linked with disruptions to the development of neural networks and are strongly associated with cases of autism spectrum disorders and schizophrenia. Looking to ascertain the mechanisms disrupted by mutations to Shank3 we hypothesize an integral determinant is the nuclear localization of β-catenin. We knocked down Shank3 in cultured rat pyramidal neurons and quantified nuclear levels of β-catenin in control and TTX treated conditions. Lithium treatment rescues synaptic scaling in Shank3 knockdown neurons. Shank3 knockdown neurons were treated with lithium to determine if lithium influences β-catenin localization in the nucleus under conditions of reduced Shank3 levels. Regulation of synapse development is highlighted as causal to the deficiencies experienced in many psychiatric conditions. Our ability to study the rescuing effects of lithium on Shank3 will allow a greater understanding as to the optimizations that take place during key developmental phases; additionally generating new avenues conducive to the production of treatments for those suffering from or predisposed to neural network deficiencies.
25. DANIEL RAEMER, OCEANOGRAPHY

Mentor: Dr. Christine J. Cass

Quantification and comparison of microplastic contents in wild mussels and maricultured oysters from Humboldt Bay, California using enzymatic digestion methods

This research evaluates the microplastic contents of commercially maricultured Pacific oysters (Crassostrea gigas) and wild mussels (Mytilus edulis) from Humboldt Bay (HB), California. Ten bivalves were collected from each of three different locations in HB: North Bay, Entrance Bay, and South Bay. Oysters were purchased directly from a commercial oyster farmer who cultivates them in the North Bay. Bivalves were digested with proteolytic enzyme complexes, vacuum filtered, and microplastics were quantified by microscopic examination of filters. All samples contained microplastics, with plastic fibers being the most abundant items. Significantly different concentrations of microplastic particles (plastic particles/g tissue wet mass) were found between mussels collected from different locations and from the cultured oysters (p = 0.000). Mussels from North Bay contained the highest average concentration of microplastics (6.29 ± 1.73 items/g) and were significantly different from all other groups. Microplastic concentrations in Entrance Bay (3.39 ± 1.19 items/g) and South Bay (2.29 ± 1.75 items/g) mussels were intermediate and not significantly different from each other (p = 0.452). Oysters contained the lowest microplastic concentrations (0.72 ± 0.34 items/g) and were significantly different from North Bay (p = 0.000) and Entrance Bay (p = 0.001) mussels, but not from the South Bay mussels (p = 0.104). Half of the samples from each location were digested using the enzyme complex Corolase 7089 and the other half were digested with Corolase 8000, both supplied by AB Enzymes Inc. All samples treated with Corolase 8000 digested completely, whereas some of the samples treated with Corolase 7089 did not achieve complete digestion, suggesting that Corolase 8000 is more efficient for this purpose. No significant difference in microplastic recovery was found between the two treatments (p=0.253)


26. DANA CHRISTENSEN, GEOLOGY

Mentor: Dr. Melanie Michalak

Taxonomy of a Late Pleistocene Bryozoan from Megwil Point, Trinidad, CA using SEM

Paleoenvironment of northwestern California in Humboldt County may be determined using the classification of marine animals known as Bryozoans. They are widely distributed in all types of environments across the earth and express differences in their morphologies based on locality and climate. A Pleistocene fossil Bryozoan was extracted from a fossil bed located at Megwil Point in Trinidad, CA to undergo analysis using a Scanning Electron Microscope (SEM). The sample was glued to a sample mount and gold coated twice with two weeks between each coating. The secondary electron (SE) feature of the SEM was utilized for morphological analysis. Taxonomic classification was attempted using the morphological features remaining on the fossil Bryozoan. Due to lack of expertise with regard to fossil Bryozoan anatomy and the vast literature on the hundreds of possible species of Bryozoa to choose from, a full taxonomic classification was unable to be made beyond the Order Cheilostomata. It is not impossible, however, to make a more precise classification in the future.
27. KEVIN SOLAND, FORESTRY

Mentor: Dr. Lucy Kerhoulas

Efficacy of Forest Restoration

In 1968 and 1978, much of the land that Redwood National Park inherited was former industrial timberland that had been clear-cut and aerial seeding. Today, these acquired second-growth forests support a high density of even-aged trees with low tree vigor, stagnant development, and low redwood (Sequoia sempervirens, SESE) composition. Large-scale restoration efforts are underway to thin these forests and accelerate the development of late seral conditions. Given the widespread scale of these efforts, it is important to monitor long-term forest responses to treatments and evaluate the efficacy of various treatments. Our study investigated growth, as measured by basal area increment (BAI), under suppressed conditions for SESE, Douglas-fir (Pseudotsuga menziesii, PSME), and tanoak (Notholithocarpus densiflorus, NODE) in Redwood National Park. We also quantified spring (May) and fall (October) physiological rates, as measured by predawn water potential (ΨPD), midday water potential (ΨMD), and stomatal conductance (gs), in SESE and NODE. Finally, we evaluated SESE and NODE physiological responses to variable density thinning basal area (BA) reduction treatments (0, 25, 40, 55, and 75%) one month after thinning using post-/pre-treatment ratios. Our major findings and their implications follow: 1) Average annual BAI was low, realizing approximately one third the growth rates observed in unsuppressed redwood forests, and was significantly lower in the understory hardwood NODE compared to the two overstory softwoods. This finding confirms that these second-growth forests are suppressed and in need of restorative thinning. 2) Between spring and fall, NODE ΨPD decreased and gs increased, indicating anisohydric stomatal regulation. In SESE, ΨMD and gs decreased between spring and fall. Physiology between the two species was generally comparable, the only significant difference being that fall gs was higher in NODE than SESE. These quantifications of spring and fall physiology in suppressed SESE and NODE provide useful baseline information for long-term monitoring of forest responses to thinning. 3) For NODE and SESE, post/pre ΨPD was lowest in the 40% BA reduction treatment, indicating increased water stress. For SESE, post/pre ΨMD and post/pre gs were significantly higher in the 25% and 40% BA reduction treatments, respectively, compared to other treatments. These post/pre findings suggest that moderate thinning intensities (25-40% BA reduction treatments) foster the greatest vigor in residual trees. Overall, our measurements demonstrate that forests respond quickly to thinning treatments and that this release from competition can be physiologically detected rather immediately. This ability to detect an immediate response to thinning using physiology provides land managers with a complementary approach to traditional growth-based evaluations.
Mentor: Dr. John Steele

Characterization and Gene Expression Profiling of Human Induced Pluripotent Stem Cell-Derived Neural Sphere Cultures versus Monolayer Neural Cultures

Landscape position influence Nitrous oxide fluxes in fertilized With the addition of appropriate signaling factors, induced pluripotent stem cells (iPSC) have the ability to differentiate into various types of cells including brain tissue. Aggregates of differentiated neural cells can assemble and self-organize into brain organoids allowing the formation of neural circuits and distinct cellular interactions. Traditionally, cells are adhered to a surface and differentiated as separate monolayers of either astrocytes or neuronal cells, or as co-cultures in which conditions tend to favor one cell type but not the other. Compared with 2-D monolayers of neural cells, 3-D “neural sphere” cultures last up to 2 years and can consist of more types of cells which mimic key features of developing brains. Because some neurodegenerative diseases affect more than one type of brain cell, 3-D cultures would more accurately represent in vivo development and disease characteristics. We hypothesize that 3-D neural sphere lines are more accurate representations of in vivo connectome development with more healthy, stable, viable cells that represent the cell types of a developing forebrain (e.g. astrocytes, oligodendrocytes, neurons).

Human iPSCs from control or tauopathy disease models were differentiated and purified as neural progenitor cells. Neural progenitor cells (NPCs) were differentiated by FGF withdrawal for 28 days in either 2-D traditional adherent format or as 3-D neural spheres. Samples were collected every 4 days throughout differentiation, RNA extracted and purified, cDNA synthesized, and analyzed for expression of a panel of 38 genes. These genes were chosen based on prior publications for differential expression patterns throughout differentiation in NPCs, neurons, astrocytes, and oligodendrocytes. Primers were designed to amplify gene products from cDNA using the Harvard Primer Bank database. Primers have been screened for multiple gene isoforms and non-specific binding where inefficient/ineffective/inadequate sets were excluded or replaced. Our on-going work will identify the timing and pattern of expression this panel of genes in 2-D vs 3-D cultures of healthy control and tauopathy model lines. Future studies will employ plasmid reporters containing cell-type specific gene promoters to confirm gene expression and the presence of specific cell types in 3-D cultures. Furthermore, these cell-type-specific promoter tools can be used to drive expression of CRISPR-based tools to support our genetic screen programs in specific cell types.
29. James Lamping, Brian Murphy, Jeremy McFarland, Zachery Porteous, Colleen Smith, Sierra Monroe, Jeffrey Kennedy, Samuel MacAdam, Simon Bueche, Robert Becker, Travis Massey, Charles Sandhu, Kristy DeYoung, Samuel Wood, Lucy Corro, Michael Mc Dermott, Christine Emerson, Aaron Christiansen, Melanie Stevenson, Shayne Magstadt, Casey Thompson, Claire Thomas, Greg Stairs, John Soliz, Buddhika Madurapperuma, John Dellysse, Melissa Collin, Sean Fleming, FORESTRY (SOILS) GSP 326

Mentor: Dr. Buddhika Madurapperuma

UAV Photogrammetry for Surveying Dune Habitats: A Review of Research Needs of the Friends of the Dunes Land Trust

Humboldt Coastal Nature Center is managed as a Land Trust by the Friends of the Dunes (FOD). Local and international visitors are attracted to the dune and beach ecosystem and often unknowingly create social trails that diverge from FOD trails and boundaries. This social trails are vulnerable to endangered herbaceous communities due to trampling dune mat assemblage and also risk for habitat loss while increasing fragmentation of natural habitats. This study examines the habitat characteristics of dunes in terms of social trails, native plants, land-use classes and dune movement. As a project for intermediate remote sensing class, four groups of six students each carried out a detailed survey on the above topics of the dune ecosystem using high-resolution UAV imagery. A DJI Mavic Pro small Unmanned Aerial Vehicle (sUAV) was used to fly a 22.5 acre plot of the dunes managed by FOD at a height of about 30 m. The 427 images from this flight were used to create an orthomosaic image from Structure from motion (SfM) in Agisoft PhotoScan with 1.5 cm resolution. Ground control points were set up using real-time kinematic (RTK) GPS. Group 1 showcased the micro-topographical variation of the dune habitat and digital elevation models to pinpoint dune movements. Group 2 identified social trails, trails made by visitors walking off official trails. 970 meters of official trails and 1701 meters of social trails from 16 points of entry were identified. Social trails can create issues for conservation and may contribute to the spread of invasive species. Group 3 identified non-native species in the area using maximum likelihood classification methods aided by ground truth images taken in the study area to improve the classification accuracy. A pseudo-supervised classification was made to identify different types of vegetation and thus invasive species in the FOD area. Group 4 developed land-use/cover map of three classes such as, sand, vegetation, and shadow using supervised classification. With the high resolution orthoimagery, an overall classification accuracy of 80.79% was calculated for three land-use/cover types. Sand was classified with an accuracy of 76.19%, shaded areas were classified with an accuracy of 92.85%, and vegetation was classified with an accuracy of 73.33%. In conclusion, we can use these findings as baseline information to adapt management techniques to better fit the region of interest and the mission of the Friends of the Dunes Land Trust.
Identifying Bus Stop Locations: Isolating Trash Flow From Entering Streams and Parks

Municipal Solid Waste (MSW) has contributed to the global and local impact of the environment. MSW if not correctly disposed of will eventually accumulate in creeks and parks within cities or towns. Locations such as bus stop sites are likely to increase the spread of MSW into creeks and parks due to the frequent interaction of human activity at bus stop sites. The objective of this study observes bus stop sites of the Arcata Red and Gold bus routes utilizing GIS techniques such as buffering, intersecting, and clipping to analyze sites that are vulnerable to the spread of MSW based on proximity to parks, creeks, and creek lengths. Buffer zones are determined from the data sets of the Arcata bus routes, bus stop sites, city outline, creeks, and parks superimposed and clipped together onto a series of maps created from ESRI ArcMap 10.5.1. Map results feature bus stop sites with 100-meter buffers regions as Hot Zones, 200-meter buffer regions as Warm Zones, and 300-meter buffer regions as Mild zones a. Overall buffering analysis indicates Warm Zones and Mild Zones contain greater creek coverage along with continuous overlap with neighboring parks. Data suggests numerous bus stop sites within the Arcata Red and Gold bus routes would benefit from recycling and trash receptacles in order to minimize MSW from collecting in streams and parks.
The Effects of Light on Bioproduction from Engineered, Solar-Powered Microbial Consortia

The growing need for bioindustrial products (i.e., biofuels, plastics) requires a greater amount of carbohydrate inputs to meet consumer demands. Mass collection of carbohydrates from agricultural feedstocks in turn results in less viable land for food production, creating conflict of interest between food and chemical commodity markets. Alternatively, photosynthetic microbes (i.e., cyanobacteria or algae) may grow on non-arable lands and without potable water, potentially allowing for bulk sugar production without competing for agricultural resources. Our laboratory has engineered a strain of S. elongatus PCC 7942 that produces large quantities of sucrose by expressing two heterologous genes: sucrose-proton symporter (cscB) and sucrose phosphate synthase (sps). The engineered S. elongatus produces sucrose up to 80% of total biomass under laboratory light intensities, which could theoretically outperform the best plant-based carbohydrate feedstocks (e.g. sugarcane) by several fold. We explore the use of these cyanobacteria to create flexible co-cultures whereby the sugars secreted support the growth and bioproduction of useful metabolites, including the bioplastic polyhydroxybutyrate (PHB) from Halomonas bolievensis co-cultures. Preliminary studies demonstrate promising results for large-scale application, however experiments have so far been restricted to laboratory growth conditions. Herein, we assess the feasibility of large scale productivity using bioreactors that simulate more realistic light conditions for scaled production, including day-light cycles and variable light intensity. We report the performance of this system under a range of light conditions that are more representative of outdoor environments, which will help to determine the viability of this approach for largescale applications and biochemical production.