# Biosphere 2 - Environmental Science Internship Program

Director: Mitchell Pavo-Zuckerman, PhD  Co-Director: Katerina Dontsova, PhD

UA's Biosphere 2 facility hosts this program for summer research experiences funded by the Mexican Ministry of Education and the United States Commission for Cultural and Educational Exchange (COMEXUS). By using a multidisciplinary approach (involving disciplines such as hydrology, geology, geochemistry, ecology, biology, physics, engineering, and atmospheric sciences) research teams focus on understanding how earth systems respond to environmental change. Open to freshmen, sophomores, juniors, and seniors.

# Ricardo Francisco Castrejon Martinez

B2 - Environmental Science Internship Program | PI: Dr. Katerina Dontsova

**National Autonomous University of Mexico** | Mexico City, Mexico

## FINE FRACTION PRODUCTION AND MINERALOGICAL CHANGES DURING BIOLOGICAL WEATHERING OF ROCK

Abstract: Soil is an open system through which various hydrological, biological and geochemical cycles operate. During the process of chemical weathering, the proportion of primary minerals decreases together with the particle size, and the presence of secondary minerals increases. There are few studies that analyze the relationship between microbiology and mineralogy in plant development, this knowledge is cornerstone for a better understanding of soil genesis processes and has direct application in some areas of study like edaphology and agriculture.

In this mesocosm study we evaluated the effect of biota, including bacteria, plants (Ponderosa pine and buffalo grass), and plants in symbiotic relationship with mycorrhizal fungi on weathering and formation of secondary phases. We extracted the fine fraction (FF), particles < 250 µm in size, from sand samples of four different types of rock (basalt, rhyolite, granite, schist) exposed to different biological treatments under controlled conditions. In this way we were able to connect the amount of FF with the different soil genesis factors. Later on we used the X-Ray Diffraction technique on the FF to find secondary minerals from the diffractograms and relate these with the biota.

We found that the amount of fine fraction is mainly related with the parent material. The second most important factor is the vegetation (pine and grass), with the samples where pines were grown having the largest weight of FF since their roots have a larger area of contact with the minerals. We were also able to notice a slight increment in FF weight on the cylinders where bacteria and mycorrhiza were present. XRD results indicated that extracted FF contained secondary minerals. This supports our initial hypothesis of the strong rock type and biota effect on incongruent weathering during soil formation.

# Rafael Chávez Garcia Silva

B2 - Environmental Science Internship Program | PI: Dr. Mitchell Pavao-Zuckerman

**Instituto Tecnológico y de Estudios Superiores de Occidente** | Tlaquepaque, Mexico

## LOW IMPACT DEVELOPMENT PERFORMANCE IN THE SOUTHWEST: MESQUITE RAIN BASINS THROUGHOUT THE MONSOON SEASON

Abstract: Traditional urban stormwater systems have negative environmental effects such as increased runoff and decreased water quality. Low impact development (LID) practices are a recommended alternative as a result of its potential to retain storm-water and pollutants; and also improve outdoor thermal comfort. Rain basins are structures where runoff water can be absorbed and stored, allowing rain events to have a greater impact on irrigation and transpiration of plantings in the basins. Research on different LID practices has increased in recent years, mainly on the East Coast and temperate regions. The ecological effect by different rain basin designs have not been quantified or thoroughly studied for arid regions. Our objective was to characterize the activity of mesquites with different soil treatments during the monsoon season in Oracle, AZ. We measured stomatal conductance, soil moisture and local normalized difference vegetation index (NDVI) at the infrastructures through the month of july in two different sites, one next to a road and one between one story residential buildings. The soil treatments were: gravel, gravel-compost layers and control canopies without soil modifications. Preliminary results show no significant stomatal conductance difference among the different treatments. Soil moisture was the lowest for the control design and for the basins next to the road. We suggest that these designs have an impact in water retention in the soil, especially when done in residential areas. Further research is needed on the effect this has on mitigating the heat urban island, a service that is most needed in urban aridlands.

# Xadani Simon Escobar Rito

B2 - Environmental Science Internship Program | PI: Dr. Peter Troch

**National Autonomous University of Mexico** | Mexico City, Mexico

## COMPUTING SENSIBLE HEAT FLUX FROM LEO

Abstract: To meet the challenge of predicting landscape-scale changes in Earth system behavior, the Biosphere 2 Landscape Evolution Observatory (LEO) has been constructed. The LEO consists of three identical, sloping, 333 m2 convergent landscapes inside an environmentally controlled facility. These engineered landscapes contain one meter deep ground consisting of a mixture of basaltic tephra and homogenous loam. These landscapes are being studied as replicas of "bare soil" for an initial period of three years. During this time investigations will focus on topics like energy cycles. The objective of this research was to determine how precisely sensible heat flux of LEO East hillslope can be calculated.

All of the data is from July 22nd to July 28th, every 15 minutes.

Net radiation Rn can be broken down into incoming shortwave Ris (emitted from the sun), outgoing shortwave Ros (reflected from land surfaces), incoming longwave Ril (emitted from constituent atmospheric gases), and outgoing longwave Rol (emitted from the land surface).

$$Rn=(Ris-Ros)+(Ril-Rol)$$

Each component was measured by CNR4 (installed on two vertical poles). Our results for net radiation (145.945 W/m2) and the albedo of the soil ($\alpha$=0.0948567) meet the theory that dark moist soil has a very low albedo.

On the other hand, from the heat flux plates (HFP) substratum heat flux was calculated (5.60183072W/m2) at the surface, considering soil temperature and water content. Using these results we could calculate sensible heat flux, which is 62.6901917W/m2 in average. Theory predicts certain behavior about sensible heat flux through the day, and our results match it.

## CHEMICAL DEFENSES IN THE GMB: THE IMPORTANCE OF SYMBIOTIC WOLBACHIA BACTERIA.

Abstract: The heteropteran Thasus neocalifornicus, commonly known as the Giant mesquite bug (GMB), is an insect found on mesquite trees (Prosopis veluntina: Fabaceae) feeding on seedpods and plant sap. Heteropterans often protect themselves from predators by releasing noxious or toxic aldehydes that are produced in the insects' abdominal glands, where wolbachia bacteria inhabits in symbiosis. Glands from adults and nymphs of the GMB were separated and analyzed using proteomic techniques to identify who is responsible in the symbiotic relationship for the manufacture of these chemicals. We specifically investigated whether aldehydes was a wolbachia or heteropteran alcohol dehydrogenase (ADH).

Adults and nymphs were collected off host plant, Arizonian mesquite trees (scientific name), from June 30th to August 4th, 2014 from mesquite trees at the Biosphere 2 (32°34'43.60"N 110°51'02.14"W).

By using two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) and a chemical reaction that detects ADH we achieved to isolate several potential enzymes, specifically the one that we were interested on has atomic mass of 25-35 KDa. To make sure of the accuracy of the enzyme isolation, the whole process was repeated at least six times.

The next step was to send all this enzyme samples to the BIO5 proteomics facilities for protein identification. Their analysis showed that one of the proteins that were more abundant in our sample corresponded to Nad-dependent glutamate dehydrogenase common in Wolbachia bacteria. Therefore, we conclude that the chemical compounds are not likely produced by the GMB but by Wolbachia.

# Edgardo Daniel Espinosa Ruiz

B2 - Environmental Science Internship Program | PI: Dr. Joost Van Haren

**Tecnologico de Monterrey Campus Queretaro** | Querétaro, Mexico

## THE EFFECT OF DROUGHT ON SAP FLOW RATE IN TROPICAL TREES OF THE BIOSPHERE 2 RAINFOREST

Abstract: Climate change is expected to bring warmer climates and drier conditions to the Amazon basin which effect is predicted to bring climatic feedback at a global scale. To have a better understanding of how tropical trees will respond to drought, this study aims to compare the relationship between sap flow velocity rates from a set of trees under controlled conditions inside biosphere 2 and their dependence to soil water from a wide range of depths during a drought. The hypothesis is to prove that although sap flow rates decreases with drought, the direct effect is delayed in the trees that obtain water from a deeper level.

Time-series sap flow and soil moisture data were collected, having readings taken every 15 minutes. The results were obtained by comparing both sap flow and soil moisture of varying depths during drought. Results and model analysis from experiment revealed moisture decline was higher in shallower levels. The top 25 cm layer of soil lost 8% of moisture within a month while 1 meter depth soil just had a 2% loss. Interestingly, Sap flow rate attenuation was present in just half of the trees analyzed. Considering this, it was stated there were trees that had a lower sensitivity to drought driven by a higher water potential found at the higher depth they took water in. Conclusions revealed deep roots exhibit a strategy to face drought periods, but address a necessity of deeper research to acknowledge how plants manage to satisfy their metabolic tasks against drought.

# Daniel Letza González Silva

B2 - Environmental Science Internship Program | PI: Dr. Joost Van Haren

**Universidad Nacional Autonoma de México** | Mexico City, Mexico

## ASK ME ABOUT ¨DIFFERENTIAL SUNBATHING¨! "EFFECTS OF SEASONAL VARIABILITY IN INCOMING RADIATION ON ECOSYSTEM-LEVEL WUE OF THE BIOSPHERE 2 TROPICAL RAINFOREST"

Abstract: Projected effects of climate change in the tropics will likely involve significant changes in the distribution of precipitation, rising levels of atmospheric CO2, and a warming atmosphere. Field-scale CO2-fertilization experiments showed that moisture input and temperature are important controls on terrestrial primary productivity, as well as on water-use efficiency (WUE). Although our theoretical understanding of the factors that affect WUE is relatively robust, results from experiments are not always incontrovertible because of limited capability to control environmental variables at the field scale. The Biosphere 2 Tropical Rainforest (B2-TRF) biome at the University of Arizona offers an excellent setup whereby environmental variables may be controlled and measured at high temporal resolution. However, although moisture input, temperature, and humidity can be controlled, incoming radiation due to seasonal effects cannot. This means that daylength is different in the winter from that in the summer. In this work, I compared the effects of temperature, humidity, and photosynthetically active radiation (PAR) on ecosystem-level WUE in the B2-TRF and a similarly wet rainforest in northeastern Puerto Rico (PR) for January and June. In PR, 86-96% of the variability in WUE can be explained by PAR for both months. In B2-TRF, 66% of the variability in WUE can be explained by PAR in June, while only 4% can be explained by PAR in January. These differences in PAR suggest that due consideration may need to be taken when conducting photosynthesis-related experiments in the winter at the B2-TRF.

# Saul Edmanuel López Saldaña

B2 - Environmental Science Internship Program | PI: Dr. Judith Becerra

**Universidad del Caribe** | Santo Domingo, Dominican Republic

## CHEMICAL DEFENSES IN THE GMB: THE IMPORTANCE OF SYMBIOTIC WOLBACHIA BACTERIA

Abstract: The heteropteran Thasus neocalifornicus, commonly known as the Giant mesquite bug (GMB), is an insect found on mesquite trees (Prosopis veluntina: Fabaceae) feeding on seedpods and plant sap. Heteropterans often protect themselves from predators by releasing noxious or toxic aldehydes that are produced in the insects' abdominal glands, where wolbachia bacteria inhabits in symbiosis. Glands from adults and nymphs of the GMB were separated and analyzed using proteomic techniques to identify who is responsible in the symbiotic relationship for the manufacture of these chemicals. We specifically investigated whether aldehydes was a wolbachia or heteropteran alcohol dehydrogenase (ADH).

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# Maria de Francisco Nava Morales

B2 - Environmental Science Internship Program | PI: Dr. Judith Becerra

**Instituto Tecnológico y de Estudios Superiores de Monterrey** | Monterrey, Mexico

## THE GENOME OF WOLBACHIA ENDOSYMBIONT OF THASUS NEOCALIFORNICUS

Abstract: Genome sequencing projects of endosymbiont intracellular bacteria are often challenging due to insufficient amount of DNA free of host contamination. As a result, analyses of these genomes often require enriching steps that facilitate de novo assemblies. The genome of Wolbachia endosymbiont of the Giant Mesquite Bug (Thasus neocalifornicus) poses a further challenge due to the absence of closely related reference genomes for both host and bacterium when attempting a mapping assembly. The objective of this study was to isolate specific NGS Illumina short reads to obtain a high quality Wolbachia genome assembly. The Illumina sequencing generated large amounts of sequence data, which are currently being assembled and analyzed. Overall quality was evaluated using FastQC to detect deviations from quality scores, adapter and k-mer content. Subsequent trimming with the Cutadapt package was used to eliminate adapter contamination. Also, several assembly steps were performed in Bowtie 2 with complete genomic and mitochondrial sequences from related Wolbachia hosts such as Cimex lectularius as references, along with other Wolbachia genomes already published. Open reading frames were identified followed by gene prediction with Glimmer 3. Finally, these sequences were submitted to the Kegg Automatic Annotation Server to identify the potential genes and reconstruction of metabolic pathways (still in progress). Preliminary results suggest that although there is a certain amount of host contamination, good coverage of the reference sequences was achieved. The described method should prove useful when dealing with NGS reads with mixed host and endosymbiont sequences, and of value to future genome sequencing projects.

# María Fatima Olmos Flores

B2 - Environmental Science Internship Program | PI: Dr. Joost Van Haren

**Universidad del Caribe** | Santo Domingo, Dominican Republic

## WATER USE EFFICIENCY (WUE) COMPARISON BETWEEN BIOSPHERE 2 TROPICAL RAINFOREST AND BRAZIL RAINFOREST

Abstract: Among others, water supply, light and vapor pressure deficit (VPD) are important factors affecting terrestrial productivity. Previous elevated [CO2] experiments at the field scale showed that water availability and temperature modulate photosynthesis and stomatal conductance. In a future climate scenario where the distribution of precipitation on land may be different from, and where temperature may become warmer than, today, the need to understand the controls on primary productivity in general, and water-use efficiency (WUE) in particular, has never been more urgent. While we understand the theoretical underpinnings of what controls WUE – from greenhouse and open-top chamber experiments – field-scale experiments usually do not yield unequivocal results. Having the ability to control environmental variables at a scale larger than a typical greenhouse, therefore, is necessary. The Biosphere 2 Tropical Rainforest (B2-TRF) biome provides an excellent setup whereby hypotheses related to WUE may be tested, not only because it resembles tropical ecosystems with similar environmental and vegetative characteristics but also because it provides an unprecedented ability to control a wide range of environmental variables at this scale. In this work, I seek to estimate ecosystem-level WUE of B2-TRF and a Brazilian rainforest by using an empirical relationship (WUE = 4.21 VPD-0.67). Results show that WUE is higher when atmospheric moisture demand is high (i.e. dry season) and vice versa at both sites particularly during the summer months. Differences in other factors, specifically daylength, at the two sites during the winter months may be able to explain the different WUE patterns observed between the two sites during this season.

# Maria Amparo Palacios Menéndez

B2 - Environmental Science Internship Program | PI: Dr. Katerina Dontsova

**Universidad del Caribe** | Santo Domingo, Dominican Republic

## CARBON AND NITROGEN VARIATION DURING INCIPIENT ROCK WEATHERING: IMPACT OF ROCK TYPE AND BIOTIC COMPOSITION

Abstract: The presence of carbon and nitrogen in soil is essential for life development as they provide energy to plants and microorganisms. Plants fix carbon in photosynthesis and transfer it to soil-root system. Nitrogen is mainly fixed by bacteria and is a limiting element for the entire ecosystem. While the contribution of ecosystem to C and N cycles in developed soils is relatively well understood, much less is known about their accumulation during early soil genesis.

Here we aim to understand how different rock substrates affect C and N distribution in porewater and plant using a highly controlled environment. 291 columns were loaded with 4 granular substrates (granite, schist, rhyolite and basalt) and 6 treatments: (control) biota-free, (B) bacteria, (GB) grass+B, (GBM) grass+B+mycorrhiza, (PB) pine+B, and (PBM) pine+B+mycorrhiza. Analysed were: water pH, electric conductivity, total organic carbon (TOC) and total nitrogen (TN) and plants TOC and TN.

Overall the pore water presented low nitrogen content, with basalt having the highest values and granite the lowest ones. Granite had also the lowest N variability in time, indicating it is the most N deficient. Conductivity and TOC slightly decreased in time reflecting increasing ecosystem demands and limited accumulation. TOC and TN uptake in plant was highest in Ponderosa pine, and higher for plants in rhyolite. Mycorrhiza decreased nitrogen concentration in both plants. TC in roots was higher than in shoot while N was evenly distribution. This means that root is a good C storage in nutrient poor systems. The results suggest that competition for N in plant-microbe interaction is constant but varies on rock type. This study allows a better understanding of the carbon and nitrogen cycle in early stages of life, and how these elements affect the plant growth.

# Estefanía C. Roldán Nicolau

B2 - Environmental Science Internship Program | PI: Dr. Jon Chorover

**Universidad Nacional Autónoma de México** | Mexico City, Mexico

## ROOT DEVELOPMENT AND NITROGEN ACQUISITION IN PONDEROSA PINE: SUBSTRATE AND MYCORRHIZA INFLUENCE.

Abstract: Notwithstanding their genetic disposition for growth, the structure of roots in plants can be modified by environmental factors and symbiotic relationships. Therefore an analysis of relationship between root architecture and its function is imperative for a better understanding of plant development under different conditions. Specific root length (SRL) provides a measurement of nutrient and water uptake among other relationships. This study is focused on nitrogen acquisition since plants are unable to extract enough of this element without the aid of proper developed root system and symbiotic microbiota. Ponderosa pine (Pinus ponderosa) was grown under controlled conditions inside 4x20 cm Plexiglas cylinders. Four granular rocks were used, basalt, granite, rhyolite and schist. Germinated seedlings with natural bacteria inoculum and bacteria-ectomycorrhiza were placed on each rock. Columns were extracted after 6 months, scanned and image analyzed using SmartRoot. There was no significant effect of mycorrhiza in terms of branching and root lengths. Pines in schist were more complex than in rhyolite. This was related to nitrogen availability indicating that lateral branching is propitiated when plants crave this element. Root development and its symbiosis with soil organisms has proved to be of great importance for nutrient uptake, specifically when the latter is scarce; this study provides a better understanding in this matter.

# Ana Paula Sosa Morfin

B2 - Environmental Science Internship Program | PI: Dr. Raphael D. Sagarin

**Instituto Tecnológico y de Estudios Superiores de Monterrey** | Monterrey, Mexico

## CULTURE-INDEPENDENT ANALYSIS OF THE MICROBIAL DIVERSITY IN THE BIOSPHERE2 OCEAN BIOME

Abstract: The study of microbial ecology is a lot of times limited by the inability to culture all organisms in a lab environment. The Biosphere2 Ocean provides an opportunity to test protocols for the evaluation of marine microbial organisms and the conditions they proliferate in. Microscopy and DNA analysis were utilized for the identification of microorganisms that exist in diverse conditions in the Biome. Biodiversity of water and sediment samples was analyzed and related to temperature, light and depth data. Samples were filtered for the quantification and identification of cyanobacteria under the microscope. From these samples, DNA was extracted for amplification and sequencing with domain-specific 16S primers for Archaea. Preliminary results for microscopy techniques showed cyanobacteria of the genera Oscillatoria, Schizothrix, Microcystis, and Cylindrospermopsis. Higher concentrations of colony forming units (CFU) in water were found in spots with a lower light exposure, increasing in a proportional matter with depth. The highest number of CFU in sediment samples was found in the shallower sampling points. DNA analysis results for the domain specific 16S primers presented nucleotide sequences that were aligned with Basic Local Alignment Search Tool (BLAST) and showed close matches to uncultured bacterioplankton from marine environments. Techniques like microscopy, PCR and amplicon sequencing allow culture-independent analysis of biodiversity. The optimization of these protocols and further testing of domain-specific primers is the next step to understanding the techniques necessary to study the biodiversity of the Biosphere2 Ocean Biome and any other marine environment.

# María Olga Vaquera Ibarra

B2 - Environmental Science Internship Program | PI: Dr. Katerina Dontsova

**Universidad de las Américas Puebla** | Cholula, Puebla, Mexico

## CHEMICAL WEATHERING AND CHEMICAL DENUDATION BY PLANT-MICROBE-MINERAL INTERACTION IN RHYOLITE, BASALT, GRANITE AND SCHIST

Abstract: Chemical weathering and chemical denudation are the essential parts of biogeochemical cycle on Earth. Chemical weathering involves reactions that transform primary mineral surfaces by constantly removing nutrients, which are further transferred into the ecosystem. Chemical denudation is a leaching by water of some elements obtained from chemical weathering. Microorganisms and plants are two of the ecosystem components that affect the amount of elements resulted from chemical weathering and denudation.

To observe the effect of plants and microorganisms on these two processes during initial stages of soil genesis, a mezocosm experiment was setup that included 4 rock substrates (rhyolite, basalt, granite and schist with a particle size between 250 to 500 µm) contained in 30×4cm columns. Each substrate was sown with a tree (Ponderosa pine) and grass species (Buffalo grass) with and without mycorrhizae. An un-inoculated control and bacteria treatment were also included. Columns were loaded in sealed sterile chambers fed by purified air and water. Concentrations of some of the most abundant soil ions/nutrients (F-, Cl-, NO3-, Br-, NO2-, SO42-, PO43-, Na+, Ca2+, Mg2+, K+, and total Si and P) were determined in porewater by ion-exchange chromatography and inductively coupled plasma mass spectrometry.

The results showed that the presence of plants increased the degree of chemical weathering compared to abiotic conditions, and decreased the degree of chemical denudation, in response to nutrient demand. Furthermore, a smaller fraction of denudation was observed in tree treatments as compared to grass. However, on mycorrhizal treatments, a much greater effect was noted. This enhances the importance of symbiotic association within the rhizosphere and allows a better comprehension of the microbial colonization influence in plant growth, which could help in feedbacks to soil fertility.

# Raziel Zavaleta Rodríguez

B2 - Environmental Science Internship Program | PI: Dr. Shirley A. Papuga

**Universidad de la Mixteca** | Huajuapan de León, Mexico

## AN AUTOMATED APPROACH TO ESTIMATING SNOW DEPTH FROM DAILY TIME-LAPSE DIGITAL IMAGES

Abstract: As part of the Santa Catalina Mountain Critical Zone Observatory (CZO), three time-lapse digital cameras were installed within the footprint of an eddy covariance tower that measures water and carbon exchange between a subalpine mixed conifer ecosystem and the atmosphere on Mount Bigelow. Since 2009, these cameras have been capturing hourly images as a means to monitor understory vegetation and snow depth to complement the tower measurements. Greenness indices have been shown to be an effective means to quantify vegetation dynamics using image analysis. However, using image analysis to quantify snow depth has proven more challenging. Snow depth stakes of 1,5 m with alternating 5 cm red and white segments were installed in the view of image frame for each of the cameras. Using this reference, images were analyzed visually, using segments uncovered by snow to estimate daily snow depth. While this method provided estimates of snow depth comparable to nearby snow depth sensors, the process was laborious and time consuming. Here, we present an image analysis method that automates this process and provides similar results. We argue that this automated image analysis for estimating snow depth is a valuable alternative to the traditional snow depth sensors. This is especially valuable in areas like the Santa Catalina Mountain Critical Zone Observatory (CZO) where snowpack is ephemeral and highly spatially variable, allowing for more cameras to be installed with the same budget to help capture this variability.

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