1. Background

Since the advent of medicine and pesticides, microbial pathogens have been targeted with chemicals that have had the unintended and devastating effect of decimating cohabiting and often beneficial taxa. Over the last century this has led to the rise of drug-resistant strains and exacerbated the consequences these pathogens posed**[antibiotic/antifungal resistance]**. As the pharmaceutical industry ramps up their search for the next great compounds in this fight, microbiome research is beginning to produce successful alternatives to compound synthesis **[cite psl therapuetics]**. Currently accepted state-of-the-art microbiome therapies function by overcrowding pathogenic strains with a surplus of transplanted ‘good’ microbial (primarily bacterial) taxa sourced from healthy donors**[cite poop translate, vaginal microbiome papers, and plant ones]**. In plants, the foliar transplantation of fungal symbionts from related species has been shown to confer disease resistance to a population of the nearly extinct and fungal-treatment dependent plant species *P. kaalaensis****[p. kaalaensi paper]***. Pananma disease, caused by the fungus *F. oxysporum* and correlated to poor soils (those high fungal content and low bacterial diversity), has been shown to respond positively to the introduction of microbes from healthy soils **[Manipulating, Chao Xue]**. These findings show a promising alternative to chemical treatment, which perhaps can limit their devastating effects. To further expand the current armory against pathogens encroaching on the commercially important plants, better tools that can breach the gap in understanding the fundamental principles behind the establishment of microbiomes, their stability and dynamics, are necessary**[garrido]**.

2.1 Microbiomes

A microbiome is defined as the collective genomes of the totality of microorganisms interacting with and within a defined environment **[Microbiomes; Madupu]**. Simply put, microbiomes are collections of microorganisms that live and interact with one another and their specific habitat - ultimately forming complex ecosystems. These systems are not inconsequential and, in the case of some soil and ocean microbiomes have been implicated to contribute significantly to the respective nitrogen and oxygen cycles [**nitrogren/oxygen microbes**]. Microbiomes, however, are not limited to open environments where conditions are in constant flux such as those found in lakes and oceans, and are often described in conjunction with a host organism that is its environment [**closed vs open environents papers**]. Of particular interest in contemporary microbiome research are those associated with humans, crops,and the impact they have on the overall fitness of the host **[human gut project, soil-microbe]**. In humans, disturbed microbiomes have been linked to digestive disorders, cancers, Multiple Sclerosis and Alzheimer's among other afflictions **[review paper dysbiosis diseases]**. In plants, dysbioses of these microbiomes permit opportunistic pathogens to settle and disrupt their normal processes **[Deciphering microbe diversity, Dengbo, Zhou]**. As research in this area continues to expand, it promises to provide the scientific community with alternative methods of treating these and other diseases. Of particular interest to this Thesis are the microbiomes of plants and those of aquatic environments. **[norm, pace 1991 paper]**

2.1.1 The Rhizosphere and Phycosphere

Plant microbiomes are considered to be among the most diverse regions of earth’s ecosystems [**the good, the bad**]. Each of the plant’s organ harbors hosts its own unique microbiome, but the rhizosphere, defined at the root-soil interface, boasts the most diverse among them, as it is here where upwards of 20% of all photosynthetic products are released **[Plant microbiomes, Thomas Turner].** Rhizospheres are primarily comprised of soil-borne bacteria, fungal species, soil algae, and other microorganisms. Depending on the host species, a diverse but specific set of members at specific relative abundances is hosted **[psl spheres papers]**. Analogously, the phycosphere is the nutrient rich area surrounding phytoplankton, a diverse group of marine microorganisms. These autotrophs, like plants, convert sunlight to chemical energy and provide their immediate surroundings with chemical currency to exchange with the surrounding bacterial communities **[zooming in, Seymour]**. Both of these microbiomes are known to alter their immediate environment through modulating elements and compounds through their secretions [**zooming in, Seymour**]. The compounds exuded into the soil by plants are believed to initiate and support microbial colonization and stability. Similar mechanisms are presumably at play in the phycosphere [**regulation, Badri DV**]. Members of these communities in turn confer a certain level of defense on their host through the release of compounds into their respective spheres, which can adversely affect opportunistic pathogens **[Interplay, S.Haquard, Garrido]**. The similarity of the phycosphere to the rhizosphere makes it a great tool for continued exploration where the fundamental properties that drive colonization and stability can be gleaned and subsequently applied to the plant microbiome research.

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| Figure X. The physical similarities between the Rhizosphere and Phycosphere [Seymour, 2017] |

2.2 Metagenomics

The current state and ever-growing body of knowledge cementing the rhizosphere’s beneficial roles in plant health would of course not be possible without the advent of next-generation sequencing (NGS) technologies and techniques, especially Metagenomics and 16S profiling. This field of study and technique are defined as the study of genomes from environmental and community samples using shotgun whole-genome sequencing **[general Metagenomics review paper]**. Depending on the environment under study, there are detailed guidelines for sample collection and preparation, but beyond this, typical metagenomic pipelines are similar to most other NGS pipelines and consist of the following general steps: DNA extraction, library preparation, sequencing, assembly, annotation and analysis **[envr. Sampling methods papers]**. Metagenomic studies are not limited to determining ‘who’ is present in the sample; rather its main goal is to determine the functional genome composition in the sample **[Metagenomics review]**. These functional genomes, or metagenomes, allow investigators to extract probable biosynthetic pathways in that environment, which highlight possible metabolic inter-dependencies between present taxa. Determining these metabolic dependencies and probable biosynthetic pathways that exist in these communities provides a rich set of information from which fundamental insights may be obtained. The use of Metagenomic techniques in this Thesis, however, is limited 16S profiling in order to determine the presence and relative abundances of the known taxa in the synthetic communities used during in experimentation. **Explain a bit about 16S profiling**

2.2.1 Metagenomics Costs & Possible Solution

General Metagenomic studies document and contrast community compositions across space while others aim to document a specific community’s evolution across time during environmental change **[cite temporal and spatial studies].** While these environmental studies are important for myriad reasons, they only provide snapshots of the metagenomes and community composition during its collection. In order to glean more fundamental information, which can be subsequently exploited for plant therapeutic use, additional snapshots across shorter time intervals are required. While the price of NGS studies has gone down dramatically, studying microbiomes in the current paradigm is an expensive endeavor for most laboratories. In order to reduce cost and increase productivity, a high-throughput system and research method is currently under development in the Garrido-Oter lab at the Max Planck Institute for Plant Breeding Research (MPIPZ; Cologne, Germany). The system, currently in the prototyping phase, is meant to allow investigators to quickly iterate over hypotheses and experiments that require Metagenomics sequencing only sparingly. The system relies on the bridging of the rhizosphere and phycosphere, and the use of spectroscopic methods as a means of determining host and community fractions. In the current phase of the project, the group first aims to obtain a qualitative sense of the system’s composition and dynamics rather than quantitative information. However, as development progresses, the use of Metagenomics techniques should allow researchers to correlate the qualitative observations to quantitative metabolic information and ultimately help answer fundamental questions about the formation and dynamics of microbiomes.

2.3 Bridging the Microbiomes

The rhizosphere’s analogous aquatic counterpart, the phycosphere, has as its most appealing attribute to plant research a relative facility through which it lends itself for scientific study. Unlike rhizosphere samples, these already aqueous samples are quickly prepared for DNA extraction **[phycosphere methods paper]**. In addition, rhizosphere samples also require more involved DNA extraction steps including the meticulous selection of root-interface soil and filtration steps to separate microbes from it, resulting in time-costly overheads **[psl spheres]**. In order to benefit plant research, the rhizosphere needs to be transformed into a synthetic version of a phycosphere with reduced complexity. To achieve this, the system requires a candidate soil algae to serve as host and a collection of representative soil bacteria from which to engineer synthetic communities and present to the host. Lastly, these components must prove capable of behaving like one in a true phycosphere; in particular the members of the core root microbiota **[core microbiota papers]**, see **TableX.**

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| **TableX.** The ICL study shows significant overlap between Chlamy-enriched taxa and core root microbiota. |

2.3.1 Chlamydomonas and the ICL

*Chlamydomonas**rheinhardtii* (henceforth, Chlamy) has long been a model organism in biology and has been extensively used to understand chemo- and phototaxis as well as the mechanisms behind circadian rhythms **[cite chlamy research papers]**. Given its presence in soils and aquatic environments all over the world , the extensive body of research detailing its many features, as well as the amount of commercially available wild and genetically modified strains, it was an easy decision to use this organism as the host in the development of the synthetic phycosphere model **[cite more research and where to buy them].** More specifically, *C. rheinarhardtii* strain CC-1690 available from www.chlamymcollection.org was used for the system. To establish the viability of this system, Chlamy needed to show to differential colonization by soil bacteria than controls. This preliminary investigation was conducted over a two week period in which bacteria and Chlamy were co-inoculated in Erlenmeyer flasks and grown at specific conditions in a Percival chamber **[use pepe’s presentation as citation?]**. The control was similarly grown but supplemented with artificial root exudates. Samples were taken every two days and 16S profiling assays were performed to determine the relative abundances, which would indicate whether Chlamy was colonized deferentially from the control. The findings were positive and are highlighted in **FigureX.**

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The Indexed Chlamydomonas-associated bacterial Library (ICL) was specifically createdfor the purpose of engineering rationally designed synthetic communities in the use of the system under development **[does rgo paper exist?]**. Soil samples were collected from agricultural soil near the institute as they were known to harbor wild Chlamy. After their collection, they were washed and filtered for bacteria **[soil was protocol]**. Several filtrates were obtained and subsequently co-inoculated and regrown with the experimental Chlamy strain in Erlenmeyer flasks for three weeks using a tris-phosphate (TP) medium. After the growing period, the metagenomes of the resulting broths were sequenced and binned into 96% operational taxomic units (OTUs) **[cite Metagenomics paper]**. Serial dilutions (1:10000, 1:50000) from these broths were plated and resulted in over 3000 colony forming units. These recovered colonies were subsequently indexed and stored in special chambers at MPIPZ. Lastly, 200 of these colony forming strains had their genomes uniquely sequenced and were compared against the OTUs **[cite work]**. This final comparison ensured that the OTUs comprising the ICL and the uniquely sequenced strains represent members of the core root microbiota.

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| **FigureX.** After an 11 day growing period, metagenomic analysis represented in the PCoA plot shows that Chlamy ‘recruits’ a different subset than the artificial root exudates control. | |

2.4 Spectroscopy

It was obvious from the onset of the system’s development that the phycosphere would best be modeled through a photo bioreactor. It was evident that spectroscopic methods needed to be leveraged to probe the system. Spectroscopy is the general study of the interaction between light energy and matter with many sub-fields taking advantage of particular bandwidths of the electromagnetic spectrum. Matter can either absorb, transmit, or itself emit (fluoresce or phosphoresce) light of different wavelengths. These interactions can be measured thus allowing researchers to infer the matter’s intrinsic properties **[cit the spectrometry papers]**. The schematic in **FigureX** highlights a typical set-up. Light at a specific wavelength of radiation is passed through a sample in a vessel of known dimensions, and a detector measures the resulting light.

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| Input Light  SAMPLE  DETECTOR  Output Light |
| FigureX. Schematic of typical spectroscopic measurements |

2.4.1 Spectrophotometry & the Beer-Lambert Law

Spectrophotometry is a type of spectroscopy that measures the amount of light absorbed by matter. As light passes through a solution, the various particles which comprise the sample, including the media, become energized and thus subtract from the original intensity of the incident light **[chem textbook]**. The amount of light measured at the detector is the amount of light which was not absorbed by the system and is known as the transmitted light. The ratio between these two known quantities (transmitted vs. incident light) is known as transmittance and is highlighted in **FigureX** Equation 1**[cite chem textbook chapter on beers law].**

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| *Transmittance* | Equation 1 |

The Beer-Lambert law is based on the transmittance property and states that the absorbance of light by a substance is proportional to the concentration (*c*) of the sample and the path length (*l*) through which the light travels. This relationship is derived as follows:

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|  | Equation 2 |
|  | Equation 3 |
|  | Equation 4 |
|  | Equation 5 |
| |  |  | | --- | --- | |  | Equation 7 |   *Absorbance* | Equation 6 |

Equation 2 states that the infinitesimal decrease in light intensity with respect to path length is proportional to the product of the sample’s concentration and original intensity. A proportionality constant makes this relationship an equality, and is subsequently integrated in Equation 4. The integration results in the inverse log of the transmittance (i.e. absorbance), being equal to the product of the proportionality constant, the concentration, and the path length (Equation 5) **[cite source of derivation]**.

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| FigureX. Matter absorbs light as it passes through; the longer the path (L) the more light is absorbed. |

As noted in **FigureX,** the incident light used experimentally is usually monochromatic and is shone at the sample at a specific energy or wavelength (λ) of radiation. Compounds tend to absorb light at specific wavelengths, and any given compound may absorb light at multiple wavelengths. Monochromatic light absorption for a given compound can be represented as in Equation 7, where the proportionality constant becomes rewritten to symbolize the molar extinction coefficient . These coefficients are specific for any given compound at that particular wavelength and represent how well that compound absorbs the particular wavelength of radiation**[cite textbook]**. This equation allows researchers to create linear calibration curves and estimate the unknown concentration of a sample. This technique is further explained and employed in the methodology.

2.4.2 Fluorescence Spectroscopy

This type of spectroscopy is based on the observation that certain compounds emit visible light, i.e. fluoresce, when subjected to higher energy radiation, typically in the ultraviolet range. The emitted light’s intensity can be measured and is proportional to the concentration of substance in the system at low concentrations (< 0.1) of the sample as measured by optical density **[cite fluorescence papers]**. The derivation of the fluorescence linear equation utilizes Beer-Lambert’s Law at key steps. However, the determination of coefficients is much more complex for any given compound and involves knowing specific details about its physico-chemical properties. Since the project involves measuring concentrations of cells rather than compounds, derivation details are not provided; it suffices to know, however, that the end result is an equation similar to Equation 7 from which simple calibration curves can be produced [**cite second fluo paper**].

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|  | Equation 8 |