

Research Article

Analysis of the Soil Microbiome of a Los Angeles Urban Farm

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This research aimed to address health and food security challenges affecting Los Angeles and beyond in our own backyard. One possible way to combat these challenges is through biotechnology. The purpose of this research was to characterize the arable soils at Los Angeles Pierce College and their potential uses. The methods employed were collection of soil physical and chemical properties, cultivation of soil microorganisms, and 16S rDNA sequencing with a citizen science platform. Statistical analysis in *R* revealed associations between field conditions and life in the soil. The testing of plant-based antimicrobial agents on antibiotic-resistant bacteria revealed that plant oils could be more efficacious for control than common antibiotics. We found evidence of plant growth-promoting, antibiotic-producing, and bioremediating bacteria in different fields with distinct microclimates based on the 16S rDNA taxonomy results and inferred functional profiles. The major findings included an abundance of *Streptomyces*, *Bacillus*, *Methylobacteria*, and others with desirable functions. Numerous *Streptomyces* isolates cultivated on selective media demonstrated the ability to reduce nitrate. These results will empower future trials and product development by identifying target soil microorganisms and the most viable fields.

1. Introduction

On a broad scale, it is urgent to address threats to public health and food security. These issues include increased food demand with a growing population, a limited number of arable acres, and threats to human health such as antibiotic-resistant bacteria and chemically contaminated land and aquifers [1–3]. One possible way to combat these challenges is through biotechnology. The Pierce Farm in Los Angeles has the potential to promote human health by harnessing natural biological pathways responsible for detoxification arising from the soil food web. In addition, the Farm can cycle nutrients from the landscape and provide a local source of produce to decrease the city's foodprint. The urban foodprint is the burden created by unsustainable consumption and waste generation [4].

Microorganisms can be unified with social and economic growth in a sustainable manner [5, 6]. For example, soil microorganisms can be biopharmed which perform the desired functions such as antibiotic production and

bioremediation. The first step is to mine genomic data for desirable functions [7, 8].

The purpose of this research is to characterize the arable soils at Los Angeles Pierce College and their potential uses. We are interested in the interaction between humans, plants, livestock, and soil microorganisms in urban fields which may help us respond to health challenges. Recently, soil microbiomes have received attention by researchers who searched for novel taxa and evaluated effects of environmental factors such as urbanization, fire, and different plant hosts [9–13]. Thus, soil physical and chemical properties data and 13 DNA samples have been collected during 2018 and 2019. The data include bacterial colony forming unit (CFU) counts, pH, EC (electrical conductivity), TDS (total dissolved solids), texture, N, P, and K concentrations, and organic matter.

We hypothesized that the amount of bacterial growth in the soil can be predicted by soil physical and chemical properties. What are the most important factors for

determining the number of colony forming units that may be cultivated from a field? A transformed linear statistical model was evaluated for hypothesis testing.

Field 19 at Pierce Farm is bordered by a newly constructed apartment development and can help us study the effect of urbanization on microbiomes. We hypothesized that there would be a higher proportion of antibiotic resistance genes in Field 19 due to population density [14].

Inferred functional profiles provided evidence to test this hypothesis.

We hypothesized that plant growth-promoting bacteria will be present in the Pierce College Arboretum with the ability to reduce nitrogen and complete the nitrogen cycle. To evaluate these claims, inferred functional profiles generated in Piphillin were considered.

Streptomyces were isolated and tested for nitrate reduction activity.

The effects of antibiotic-resistant bacteria are an important issue for human and livestock health and a high priority for the World Health Organization [15]. *Lavendula stoeches*, *Ocimum basilicum*, and *Eucalyptus pulverulenta* oils were tested using a standard microbiological assay to investigate effects on antibiotic-resistant bacteria isolated from several fields on the Pierce Farm. Our hypothesis was that the bacterial isolates would be better controlled by plant extracts than antibiotics. We hypothesized that Pierce Farm can also provide antibiotic-producing bacteria and possibly new antibiotics for biopharming. We looked to sequencing taxonomy results and inferred functional data to find out which fields may be most fruitful in this area.

We searched for endophytic bioremediating bacterial sequences in Field 21 within 16S rDNA sequencing results. We hypothesized that these bacteria can clean up soils contaminated by TCE (trichloroethylene) and hydrocarbons. Functional analysis data and taxonomy sequencing results were used to support or disclaim this hypothesis.

2. Materials and Methods

2.1. Description of Areas of Interest. Each region of the Pierce Farm tested had a distinct microclimate. A brief description of each field is given in Table 1.

2.2. Antibiotic-Resistant Bacteria. We tested *Lavendula stoeches*, *Ocimum basilicum*, and *Eucalyptus pulverulenta* oils to investigate effects on antibiotic-resistant bacteria. The plants are known to contain terpenes with antibacterial properties such as eucalyptol and beta-carophyllene [16]. The first step was isolation of antibiotic-resistant bacteria from the rhizosphere of the LAPC Farm. The sources of the antibiotic-resistant bacteria are summarized in Table 3. Penicillin (10 units)-resistant isolates and tetracycline (30 mcg)-resistant isolates were used for the plant extract assays.

Plant essential oils were procured by Soxhlet extraction which ran for three cycles. Standard microbiological assays outlined by Redfern et al. [17] were performed to determine

the efficacy of the oils in controlling bacterial growth. Bacterial isolates were cultured overnight in nutrient broth and pipetted onto nutrient agar plates. 20 microliters of the crude ethanolic extracts from dry plant biomass were pipetted onto paper discs atop the nutrient agar plates. A summary of the essential oil treatments is given in Table 2. The diameter of the area of inhibition (AOI) of bacterial growth was measured.

The standard errors were graphed about the mean AOIs in Excel, allowing visual inspection of the dataset for a significant difference between oil treatments [18]. In spring 2019, basil oil was tested alongside eucalyptus oil. This was to determine if there were any antibiotic-resistant isolates that reacted better to treatment with basil or lavender oil, in spite of the trend toward eucalyptus oil having the best performance.

2.3. Metagenomic Analysis. For the 16S rDNA sequences, ranacapa was used for diversity analysis [19]. Piphillin was used for inference of bacterial functions [20]. 16S ribosomal DNA sequences are the typical sequences used to identify bacteria.

Ranacapa diversity analysis, functional analysis with Piphillin, and STAMP were carried out according to the instructions in the Pipeline for Undergraduate Microbiome Analysis (PUMA) Manual [19].

STAMP was used for analysis and visualization of metagenomic profiles [21]. Soil classification data were collected using the sampling and testing methods published in the Introduction to Soil Science lab manual by St. Clair et al. [22].

The metagenomic soil DNA samples were collected according to the CalEDNA citizen science instructional videos and sent to UCLA for sequencing using Illumina technology. The total DNA was extracted using the PowerSoil DNA Extraction kit, followed by amplification using 16S metabarcoding primers and purification [23]. Quality control was carried out in QIIME.

The sampling locations at the Pierce Farm and the Arboretum, shown in Figure 1, were spread out in order to capture the most diverse picture of the soil microbiome.

3. Results and Discussion

3.1. Statistical Analysis of Soil Physical, Chemical, and Biological Properties Data. The results of an exploratory data analysis in the dlookr package for R 3.6.0 revealed that pH and moisture percentage were the only normally distributed values [24]. Most of the variables were skewed and exhibited a better distribution with a log transformation [25, 26]. Also, since the environmental data collected here had various units of measure, a transformation was needed [27, 28].

Results for multivariate regression of transformed data for CFU on plate D as a function of field, pH, organic matter content, N, P, K, and moisture were significant ($R^2 = 0.89$). This supports the initial hypothesis. However, none of the individual variables had a significant p value. It is possible that using the presence of antibiotic-producing bacteria as a

TABLE 1: Description of field characteristics and management practices at Pierce Farm.

Field name	Field characteristics	Type of sample(s) taken
Field 15	Horse manure dumping ground, planned future organic garden; overrun by squirrels	DNA, soil classification
Field 10	Goat grazing area near the hay barn	Soil classification
Field 28	Sudan grass, grazed followed by fallow	Soil classification
Marquis field	Compost in "C," chicken manure, animal bedding, spent grain; southeast of field "D" was former dairy; urban interface	DNA, soil classification
Arboretum	Urban tree park, urban, and residential interface	DNA, soil classification
Field 12	Irrigated plot in rye, alfalfa, oats, and fescue for sheep grazing	Soil classification
Field 21	Abandoned naturalist area, cow grazing area, pond and varied topography, calciferous parent material, alluvial sediment	DNA, soil classification
Field 19	Down grade from Field 21 and newly developed apartment towers; contains construction debris, drainage infrastructure	DNA, soil classification

TABLE 2: Summary of antimicrobial essential oil treatments.

Essential oil	Abbreviation	Amount tested	Term carried out
<i>Eucalyptus pulverulenta</i>	E	20 microliters	Fall 2018 Spring 2019
<i>Lavendula stoeches</i>	L	20 microliters	Fall 2018 Spring 2019
<i>Ocimum basilicum</i>	B	20 microliters	Spring 2019

TABLE 3: Description of antibiotic-resistant isolates from the Pierce Farm.

Isolate no.	Field	Dilution	Antibiotic resistance
1	Arboretum	10^{-3}	Tetracycline
2	Marquis C	10^{-3}	Penicillin
3	Marquis C	10^{-5}	Penicillin
4	Marquis D	10^{-4}	Penicillin
5	Field 28	10^{-3}	Penicillin
6	Field 15	10^{-3}	Tetracycline
7	Field 28	10^{-5}	Penicillin
8	Marquis D	10^{-3}	Penicillin

factor would help to improve the goodness of fit, if that data had been available.

Simple linear regression models showed that Field was significant for CFU D, organic matter, moisture, and P. Contrasts revealed no significant differences between fields for the moisture parameter. The two that were the most different were Marquis and Field 12 ($p = 0.19$). The results of Tukey's HSD on moisture did not show honest significant differences between groups. This determination is supported by Figure 3. It should be noted that 2018-2019 was an atypically heavy rainfall year.

The Arboretum showed a trend toward higher levels of organic matter by percentage, although there was a wide range of values for the areas sampled. The range was similar

to all fields except Field 12 which is cultivated and had significantly lower organic matter by percentage. Phosphorus levels were highest in Fields 15, 28, Arboretum, and Marquis, although not significantly higher. All fields had similar levels of phosphorus. The highest number of bacterial colonies grew on D plates (10^{-4} dilution) in Marquis field, which is significantly higher than those cultivated Field 12, and similar to other fields on the farm (Figure 4).

3.2. Principal Components Analysis (PCA) of Soil Classification Data. The PCA was run using the `prcomp()` function in R with imputed data from `dlookr` package and plotted using `ggplot2`. The principal component analysis showed some separation in the data by Term between Spring 2019 and Fall 2019. This is because in Spring 2019, samples tended to have lower values for TDS and P as a group, possibly owing to leaching related to the amount of rainfall received during fall and winter [29, 30].

As shown in Figure 5, however, there was no clear separation or clustering of samples by texture, term, or field which emphasizes similarity between samples overall. The variables that contributed the most to the PCA were the imputed values for the TDS and K concentrations in parts per million. Samples that separated out high on PC1 and positive on PC2 tended to have imputed missing values for K and lower values for the TDS experiment.

The highest sample on PC2 was a Spring 2019 sandy sample from Marquis C which had a low TDS (32 ppm) and low K-measured value (25 ppm). This makes sense due to the low cation exchange capacity of sand. In the imputed data set, pH was negatively correlated with N, and EC was positively correlated with P.

3.3. Correlation Matrix of Soil Classification Data. Figure 6 summarizes the strength of the correlation between the soil classification factors. The correlation matrix revealed moderate negative associations (0.6–0.8) between the CFU counts for plates C (10^{-3}) and D (10^{-4}) and percentage of silt by

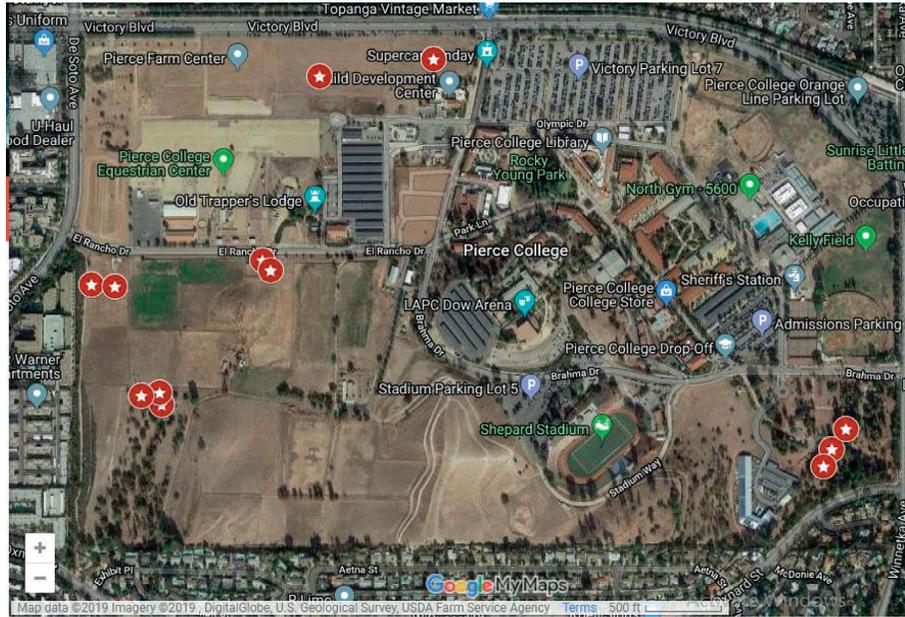


FIGURE 1: Soil sampling locations at Pierce College indicated with a star symbol. The metadata that were paired with the 16S DNA sequences came from an average of the data points for 2016–2018. The NPK was supplemented from 2018–2019 data. NPK results for Field 19 were averaged from 4 samples in Spring 2016–Fall 2016. The sampling location for Field 19 is shown in Figure 2. Table 4 shows selected metadata for the 16S isolates.



FIGURE 2: Sampling locations for Field 19 soil classification survey.

TABLE 4: Selected metadata for Pierce College 16S survey.

Sample ID	CalEDNA ID	Sample date	Field number	Latitude	Longitude	Plant common name	Texture by touch
S1	X16S461.A1.S50.L001	8/28/2018	19	34.18385	-118.588	White mustard	Sandy clay loam
S10	X16S_467.B1.S40.L001	8/28/2018	Marquis D	34.1878	-118.581	Dead brome	Sandy loam
S11	X16S_467.B2.S41.L001	8/28/2018	21	34.1055	-118.351	Dove bush	Sandy clay loam
S12	X16S_467.C1.S42.L001	8/28/2018	Marquis D	34.1878	-118.581	Brome	Sandy loam
S13	X16S_467.C2.S43.L001	8/28/2018	Arb	34.1051	-118.342	<i>Eucalyptus</i>	Clay
S2	X16S_461.A2.S51.L001	8/28/2018	21	34.1055	-118.351	Cottonwood	Sandy clay loam
S3	X16S_463.B1.S46.L001	8/28/2018	Arb	34.1051	-118.342	Sycamore	Clay
S4	X16S_463.B2.S47.L001	8/28/2018	19	34.111	-118.352	Dead brome	Silty clay loam
S5	X16S_463.C1.S48.L001	8/28/2018	Arb	34.1051	-118.342	Cactus	Clay
S6	X16S_463.C2.S49.L001	8/28/2018	15	34.18427	-118.585	Brome	Silty clay loam
S7	X16S_466.A1.S44.L001	8/28/2018	15	34.1833	-118.584	Brome	Silty clay
S8	X16S_466.A2.S45.L001	8/28/2018	Marquis C	34.1875	-118.583	Tumbleweed	Sandy loam
S9	X16S_467.A1.S38.L001	8/28/2018	21	34.18194	-118.587	Cattails	Sandy clay loam

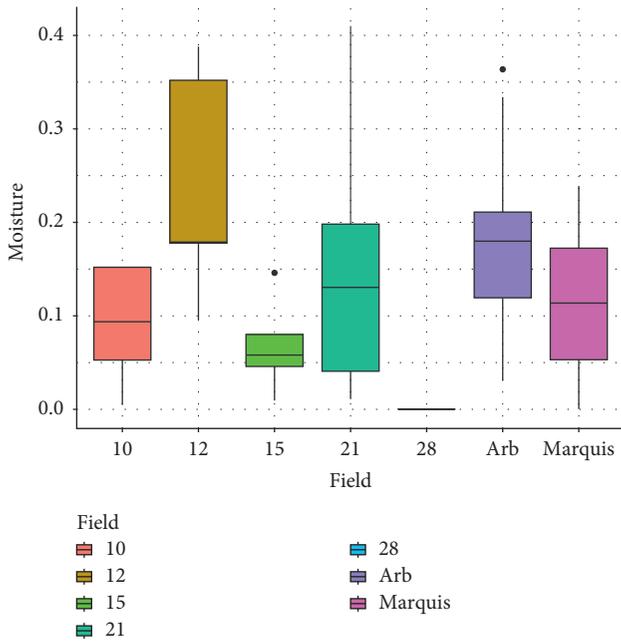


FIGURE 3: Comparison of soil moisture percentages by field.

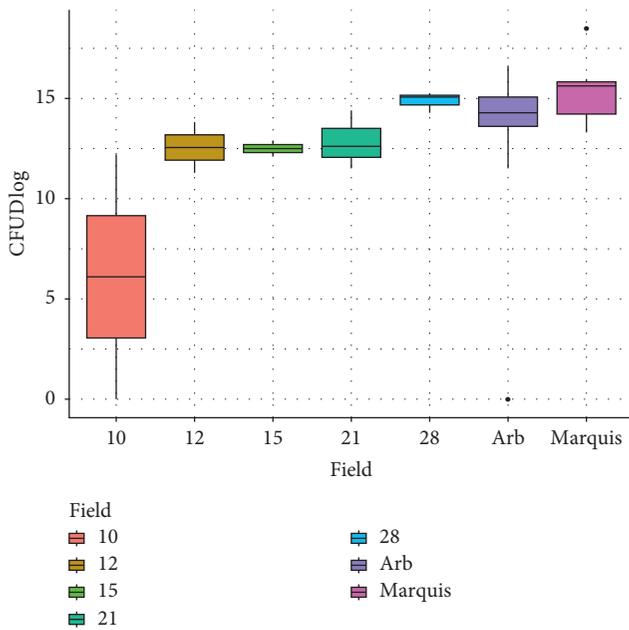


FIGURE 4: Comparison of log-transformed bacterial growth rates for 10^{-4} dilution by field.

hydrometer method; this was mirrored by moderate positive associations between the CFU counts on plates C and D with percentage of sand by the hydrometer method. This suggests the bacteria that were cultivated on these plates were mostly aerobes. In addition, there was a moderate positive association between the CFU counts on plate C with pH. There were moderate positive associations between P and TDS and slight negative associations between pH and both N and K.

EC and TDS showed a strong correlation as expected; there is a similar association between clay and sand percentages by hydrometer by nature of the relationship between the factors.

3.4. Antibiotic-Resistant Bacteria. During Fall 2018, essential oil trials on antibiotic-resistant bacteria, both eucalyptus and lavender oils, were effective in controlling the bacterial growth better than the antibiotics tested. The antibiotics tetracycline and penicillin sometimes had no effect, and the isolates displayed an AOI of 0. The mean of AOI for the eucalyptus treatment was 17.35 mm; the mean of AOI for the lavender treatment was 13.37 mm. A *t*-test confirmed that the bacteria were significantly more susceptible to the eucalyptus extract versus the lavender product ($p = 0.05$), as shown in Figure 7.

A visual inspection of the graphs of the standard errors about the mean for Spring 2019 trials revealed that basil and lavender were better at controlling some isolates, as shown in Figure 8.

Basil was the best at controlling antibiotic-resistant isolate 3 from Marquis C, and lavender was best at controlling isolate 7 from Field 28; the other isolates were better controlled by eucalyptus. Overall, this supported the hypothesis that plant extracts could control resistant bacteria better than common antibiotics.

3.5. Diversity Analysis. Next, the 16S rDNA sequencing results will be considered, beginning with diversity. The alpha diversity observed describes the number of taxa present, but does not describe the abundance [19]. It is interesting that sample S3 shows the lowest number of bacterial taxa in Figure 9, although the highest amount of plant diversity resides in the Arboretum.

3.6. Statistical Analysis of Inferred Metagenomic Profiles. When a principal coordinate analysis is carried out in STAMP and visualized with respect to the type of plant rhizosphere where soil was collected and metagenomic profiles, the samples that separate out the most are dead brome in Field 19 and cactus from the Arboretum. This separation is visible in Figure 10. The sycamore-associated sample from the Arboretum separates out PC1 vs. PC2 and on PC2 vs. PC3. This suggests that most of the variation in the functional hierarchy of the samples is coming from Field 19 and two different samples from the Arboretum.

The results from the STAMP analysis are statistically significant for comparisons between Field 21 and the Arboretum only due to the need for additional DNA samples from other fields. However, information from all of the samples can help to guide researchers in the future about where to sample to find bacteria with the indicated functions. In the L2 pathway, Field 19 did not have a trend toward a higher proportion of antimicrobial drug resistance. The Arboretum had a higher proportion of drug resistance antimicrobial genes in the L2 pathway, as shown in Figure 11. This did not support the hypothesis for Field 19 about drug resistance.

The sample from Field 19 in fact had a trend toward a lower proportion of human disease in L1 and bacterial disease pathway genes in L2 than the other fields studied. However, Field 19 had a trend toward the highest proportion

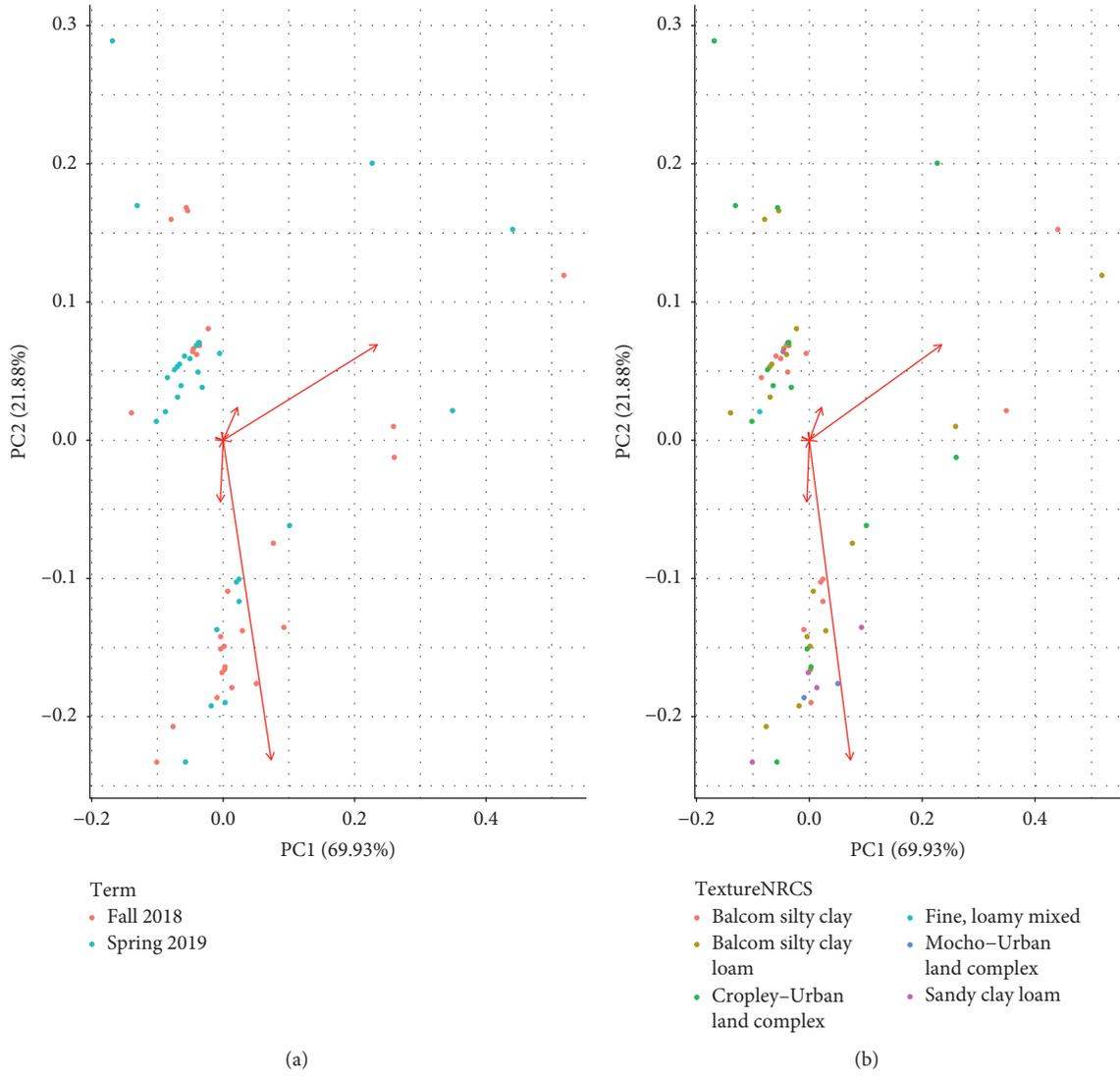
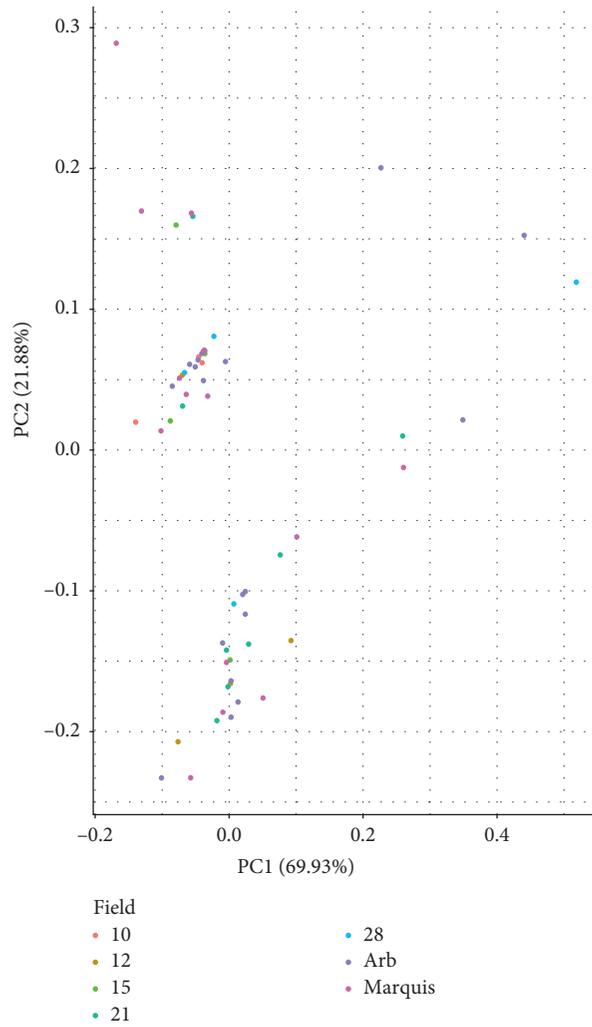


FIGURE 5: Continued.



(c)

FIGURE 5: Soil classification data PCA color-coded by season (a), NRCS texture (b), and field (c).

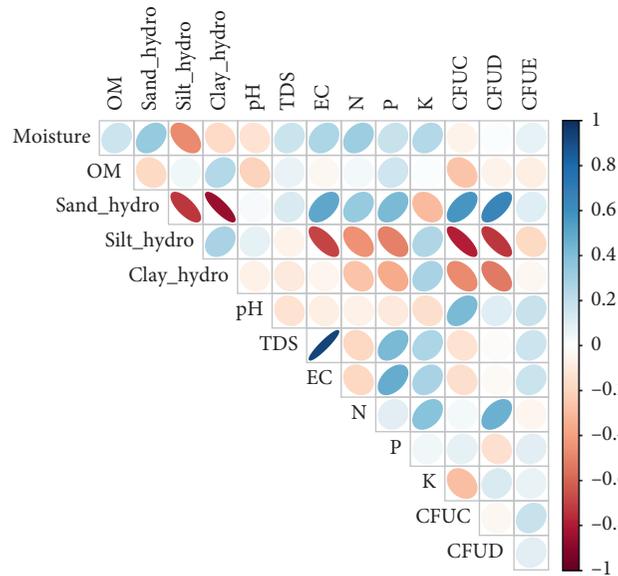


FIGURE 6: Correlation matrix of data from the soil classification survey.

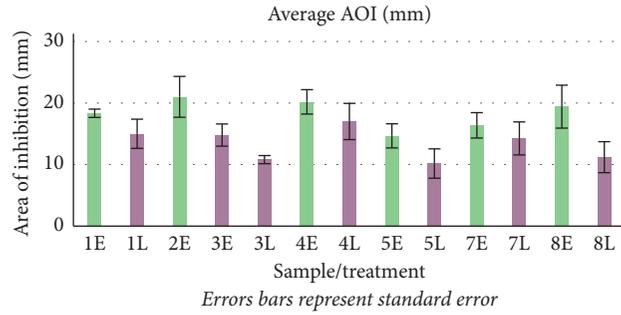


FIGURE 7: Graph of the effects of eucalyptus and lavender oils on bacterial growth during Fall 2018.

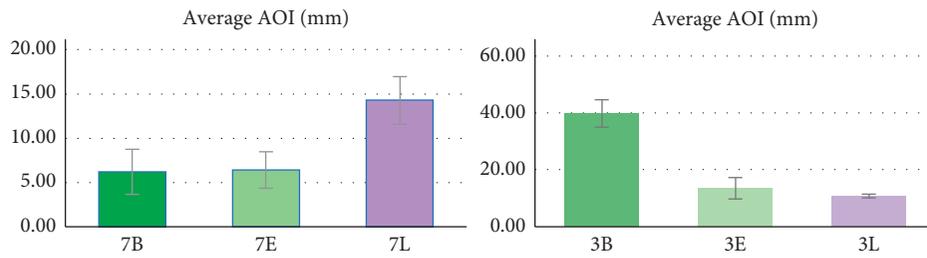


FIGURE 8: Results of repeated trials in Spring 2019 for treatment with lavender, basil, and eucalyptus oils.

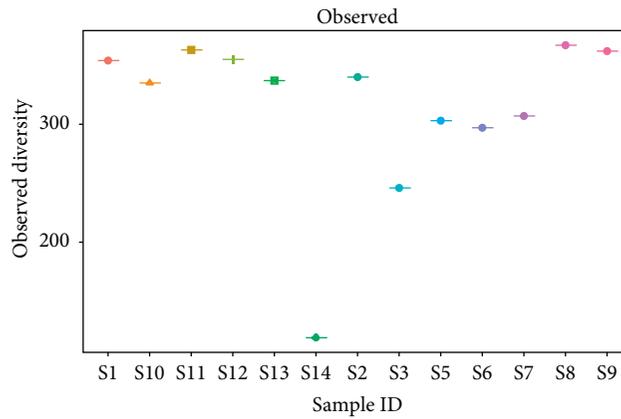


FIGURE 9: Results of alpha diversity analysis in ranacapa

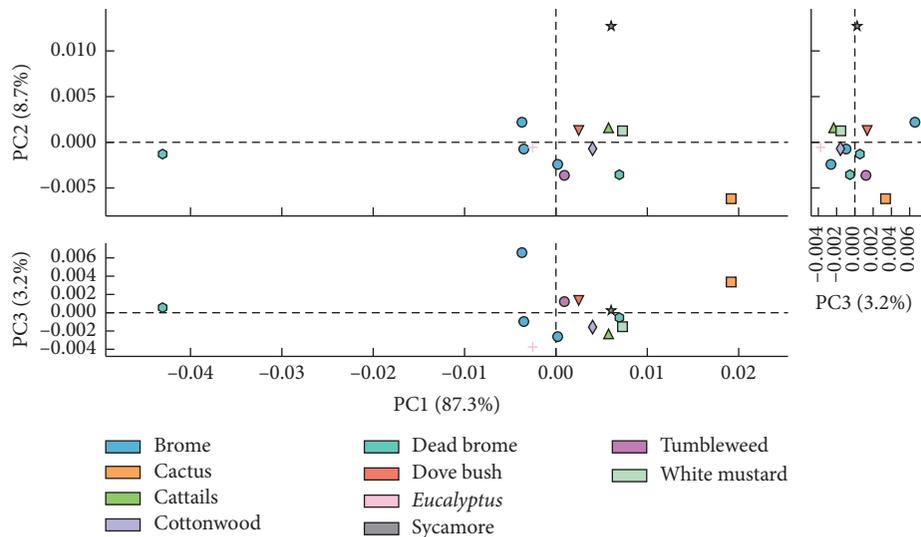


FIGURE 10: Principal coordinate analysis of functional profiles color-coded by plant rhizosphere.

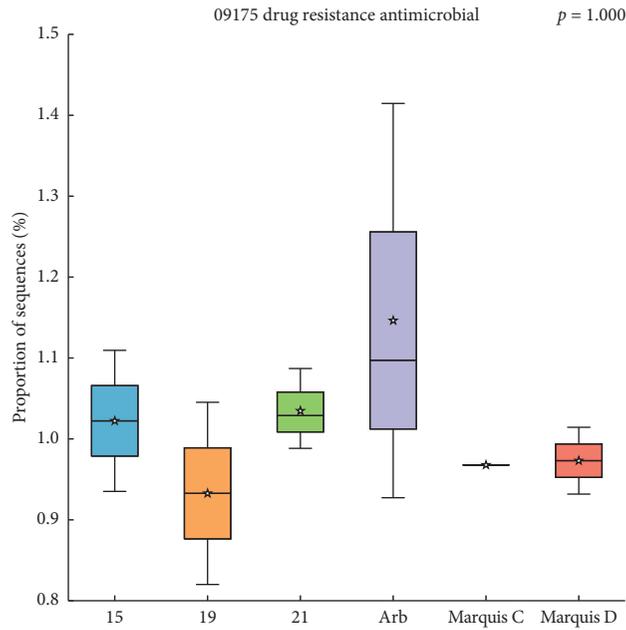


FIGURE 11: Comparison by field of proportion of sequences in the antimicrobial drug resistance pathway.

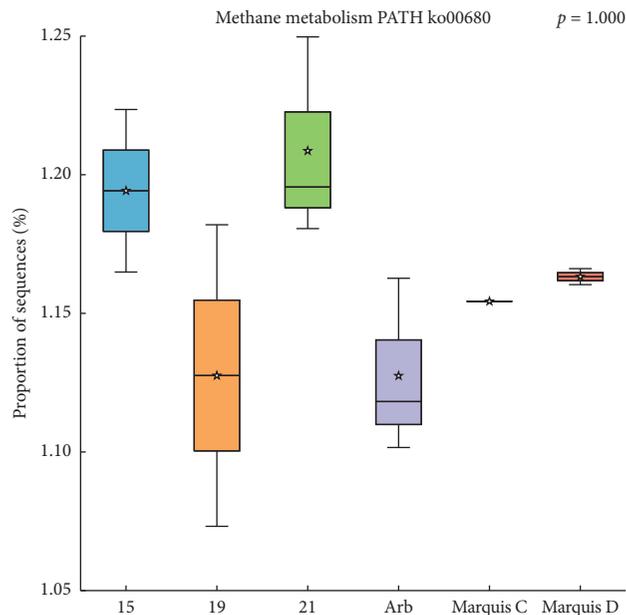


FIGURE 12: Comparison by field of proportion of sequences in the methane metabolism pathway.

of vancomycin-resistant genes in the L3 pathway which partially supports the hypothesis.

Several fields exhibited functional profiles that suggested the bacteria could be isolated and cultured to make a product. Neomycin, kanamycin, and gentamycin biosynthesis (L3) had a trend toward being the highest in terms of the proportion of sequences related to that function. In the L3 pathway, Field 19 had a high proportion of hits in potential bioremediation genes for chlorocyclohexane, chloroalkene, styrene, atrazine, and toluene degradation. Field 21 had lower numbers of reads

sequenced for this function. This partially supports our hypothesis and lets us know that in the future we should try to culture bacteria from Field 19 which is downgrade from Field 21.

Field 19 also had a high proportion of genes related to remediation of plastic byproducts, including BPA, styrene, and caprolactum degradation. There was a trend toward a high proportion of sequences attributed to penicillin production occurring in Field 19. In addition, the highest proportion of streptomycin and tetracycline production genes was attributed by Field 19.



FIGURE 13: Sampling location near California black poplar tree for Field 21 16S survey.

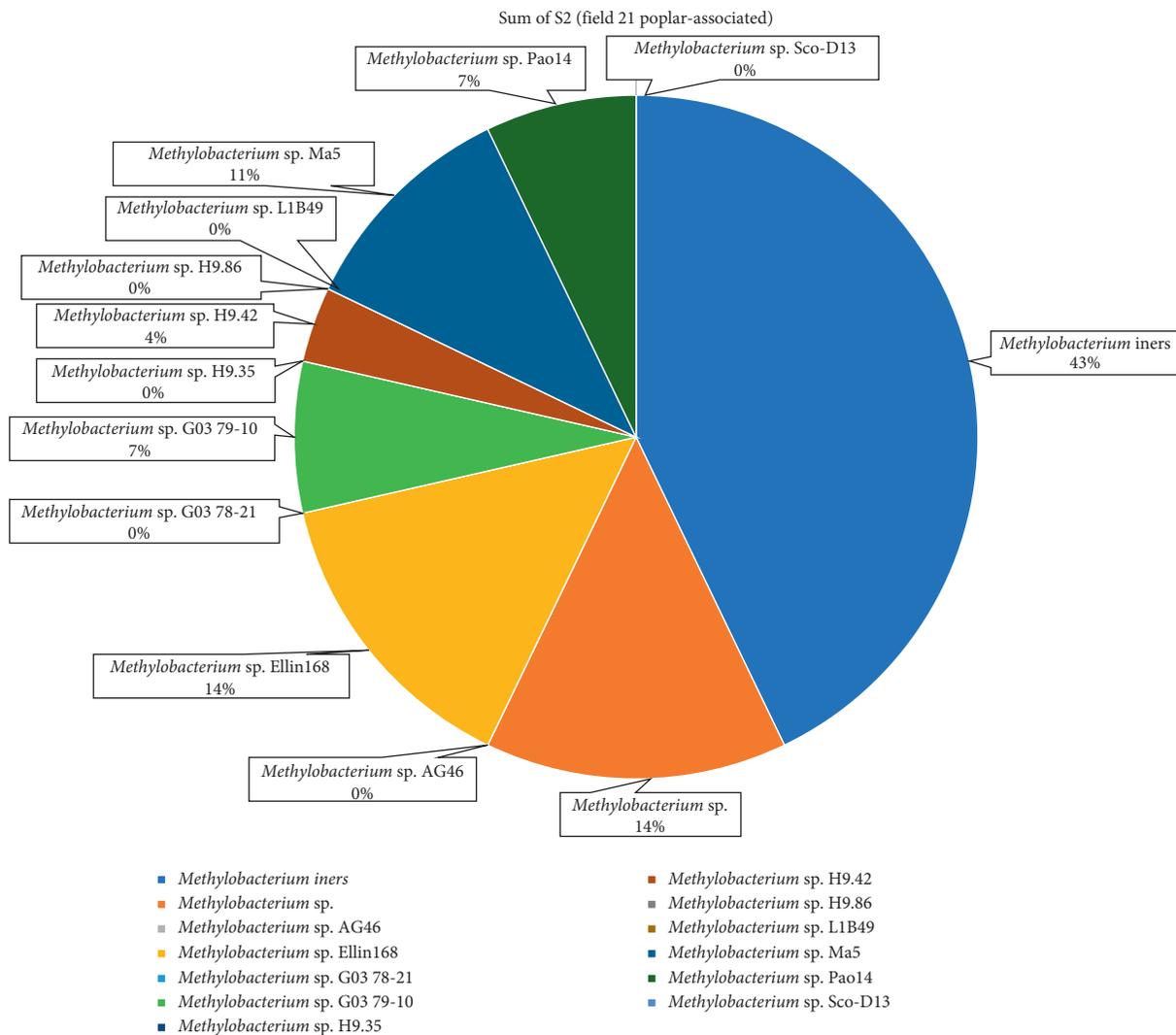


FIGURE 14: Sum of *Methylobacteria* sequences from Field 21.

Field 19 evidenced a high proportion of the functions related to terpene degradation, including geraniol, limonene, and pinene degradation. Taking samples from there in the future could help researchers explain why some bacteria are more tolerant of certain plant extracts than other extracts. Understanding the underlying genetic basis of antibiotic resistance can aid the development of treatments.

The Arboretum had a higher proportion of mineral absorption genes in the L3 pathway and a higher proportion of zeatin synthesis genes in the L3 pathway. Zeatin is a cytokine which promotes plant growth, bushy growth habit, and vegetative shooting [30]. Furthermore, the Arboretum had a higher proportion of hits in the plant-pathogen interaction pathway in L3.

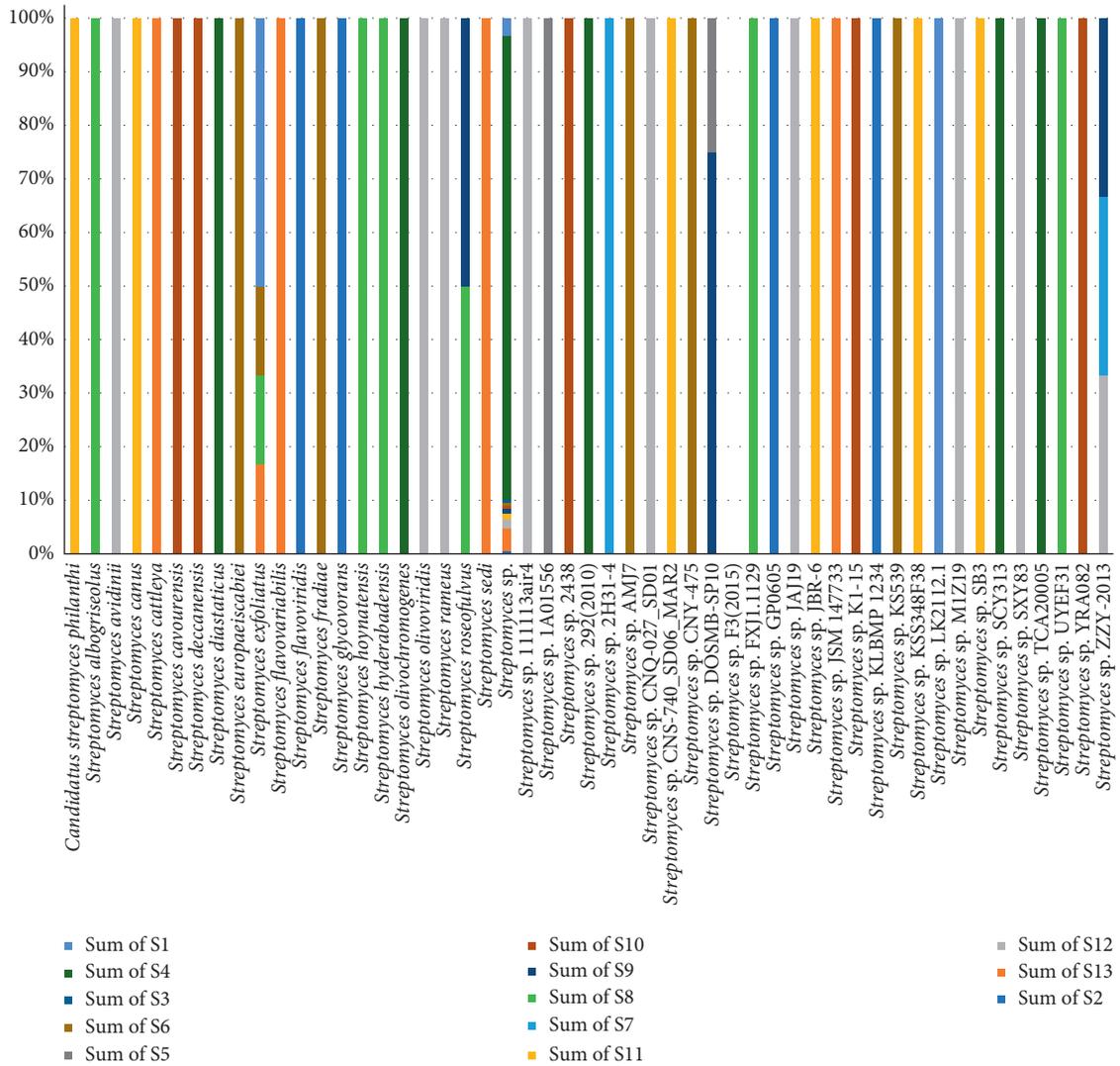


FIGURE 15: Relative abundance of *Streptomyces* sequence reads across the Pierce College Farm.

TABLE 5: Isolates of *Streptomyces* from Pierce Farm and nitrogen reductase activity.

Field	Isolate number	Dilution	Nitrate reductase
21	1St	10 ⁻⁴	Positive
21	2St	10 ⁻⁴	Positive
21	3St	10 ⁻⁵	Positive
Arboretum	4St	10 ⁻³	Positive
Arboretum	5St	10 ⁻⁴	Positive
Arboretum	6St	10 ⁻⁴	Positive
Arboretum	7St	10 ⁻⁵	Positive
Arboretum	8St	10 ⁻⁵	Positive
Arboretum	9St	10 ⁻⁵	Positive
Marquis A	10St	10 ⁻³	Positive
Marquis C	11St	10 ⁻⁴	Positive
Marquis C	12St	10 ⁻⁵	Positive
Marquis D	13St	10 ⁻³	Positive
Marquis D	14St	10 ⁻⁴	Negative
Marquis D	15St	10 ⁻⁵	Negative
Marquis D	16St	10 ⁻⁵	Positive

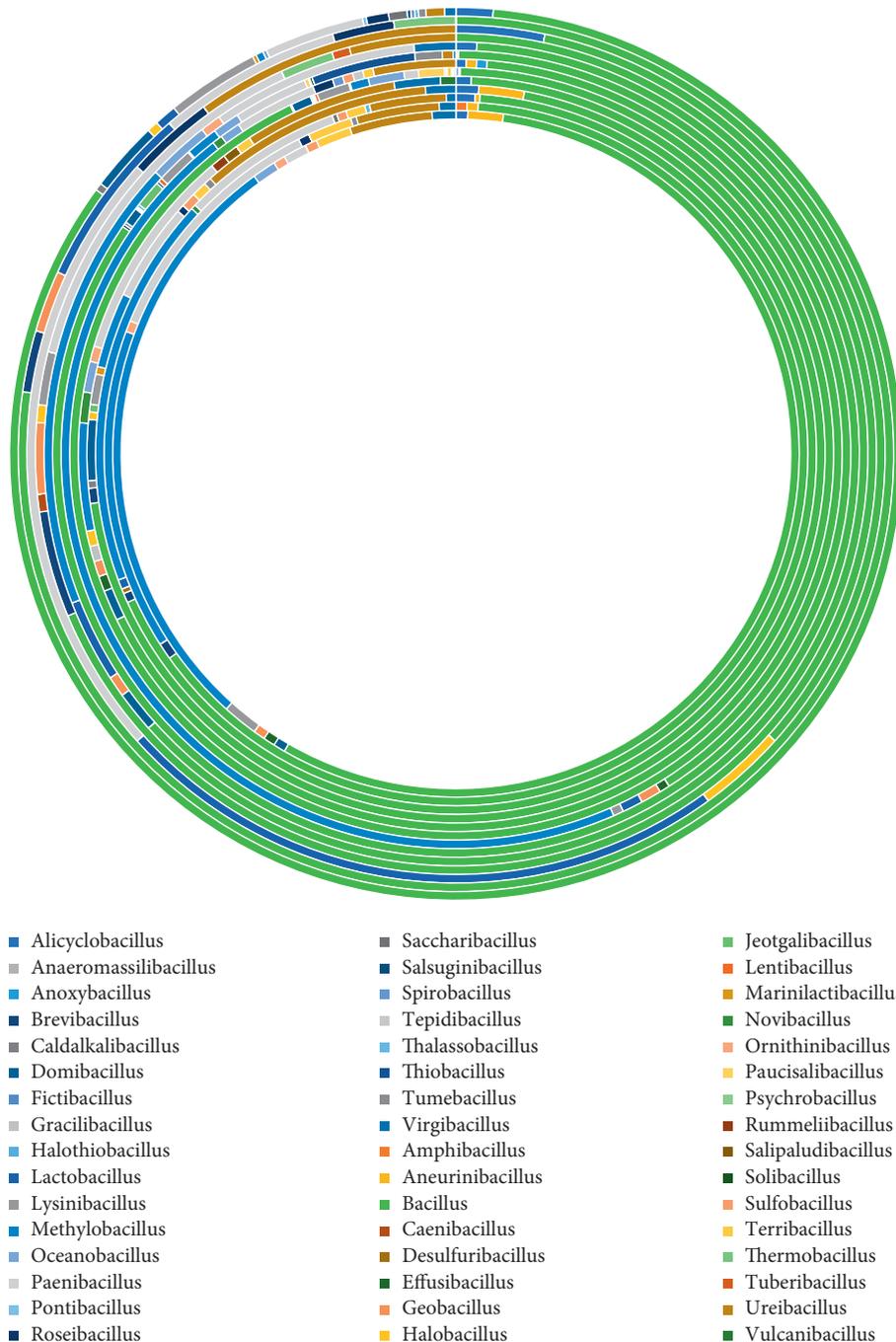


FIGURE 16: Relative abundance of *Bacillus*-related sequence reads in Pierce College fields.

The Arboretum also had a higher proportion of antibiotic resistance genes in L2 and the highest proportion of Staph infection pathway genes in L3. Furthermore, the Arboretum exhibited a trend toward having the highest proportion of sequences related to *E. coli* biofilm formation. This could be a source of genes for radiation cleanup. The Arboretum similarly had a high proportion of hits related to *Pseudomonas aeruginosa* biofilm formation which could be useful for plant growth-promoting compost teas.

Field 15 showed a trend toward a high proportion of sequences involved in polycyclic aromatic hydrocarbon

(PAH) degradation. PAHs in the urban environment are associated with carcinogenicity and have been shown to be remediated by microbes such as *Methylobacteria* sp. [31–33]. Field 15 exhibited a high proportion of sequences related to nitrogen metabolism as well. This makes sense due to the horse manure composting in the area.

Field 21 had a trend toward the highest proportion of hits in the L3 thermogenesis pathway, as well as the highest proportion of hits in the L3 methane metabolism pathway. The proportion of sequences for methane metabolism was significantly higher in the Arboretum than it was in Field 21

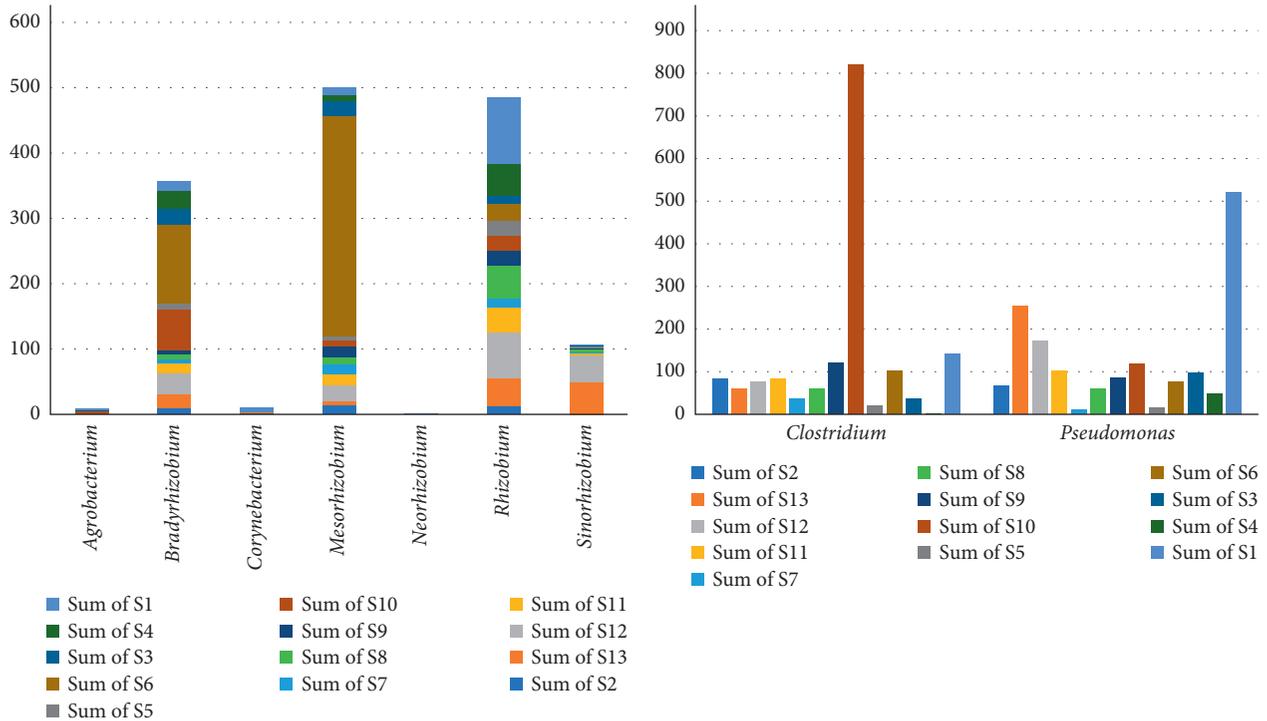


FIGURE 17: Relative abundance of zeatin-producers and *Clostridium* and *Pseudomonas* spp.

(Figure 12). Methanogenic bacteria can be associated with sulfate reduction as part of their metabolism [36]. Field 21 also had the highest proportion of plant hormone signal transduction pathway functions in L3. In L2, Field 21 had a trend toward a higher proportion of sequences related to environmental adaptation. In L3, Field 21, and Field 19 had similarly high proportions of dioxin degradation pathway hits. The highest proportion of siderophore production functions occurred at low moisture. This data point corresponded to Field 19.

Marquis C had the highest proportion of *Salmonella* infection genes in L3. This makes sense due to the presence of chicken waste and bedding in the compost. Marquis C and D ranked high in the proportion of sequences related to xylene degradation which could also be a source of genetic material for bioremediation.

3.7. *Relative Abundance of Methylobacteria Taxa.* Evidence of *Methylobacteria populii* growing in Field 21 was found at the location shown in Figure 13. This endophytic bacterium, which is usually symbiotic with poplar trees, was growing in the free soil near the plant rootzone of a California native mature poplar tree [35]. The sum of *Methylobacteria* sequences by species is shown in Figure 14. A possible application of these bacteria would be to provide inoculated California native black poplar trees for habitat restoration and bioremediation near lands contaminated by hydrocarbons [36]. For example, nearby Pierce College is the former nuclear engine testing site, the Santa Susana Field Laboratory. The Field Lab contains soils which are contaminated by TCE [37]. TCE is a potent carcinogen and a

risk to developing babies. The most effective method for TCE removal from water is aeration combined with activated carbon treatment [38]. A challenge with TCE degradation is that it resists oxidation (Ibid). Recently, Boeing has had the Santa Susana Field Lab Reclassified as an Open Space Preserve [39].

3.8. *Relative Abundance of Plant Growth Promoters and Antibiotic Producers.* We have found evidence of *Bacillus* and *Streptomyces* species which are known to produce antibiotics growing in the Pierce College Arboretum, Field 15, and the Nature Canyon (Field 21). It is possible that there are other bacteria or subspecies present that are capable of producing novel antibiotics. For example, recently, researchers in the EU and the US have discovered new antibiotics from soil. This is partially owing to the iChip, which is a new device for growing bacteria which were previously recalcitrant to common bacterial cultivation methods [40].

The distribution of reads for *Streptomyces* spp. spans all of the fields at Pierce College. This determination is supported by Figure 15. Each field has also provided isolated strains of putative *Streptomyces* (Table 5). These results support our hypothesis wherein we predicted the presence of plant growth-promoting bacteria on the farm.

The distribution of *Bacillus*-related genuses for all fields shows *Bacillus* was the most common of those queried, as illustrated by Figure 16. Each field has been confirmed to provide sequence reads and isolates for plant growth promotion and antibiotics, which supports our hypothesis. In addition, the natural presence of *Bacillus subtilis* in these fields may be an organic competitive advantage for

TABLE 6: Status of hypotheses at the conclusion of the 16S and soil classification surveys.

Hypothesis	Status	Supporting evidence	Limitations
Plant extracts can control resistant soil bacteria better than common antibiotics	Supported	High mean AOIs for eucalyptus, basil, and lavender oils	Lack quantifiable data for what constituents were responsible
Richness of bacterial CFUs at 10^{-4} dilution can be predicted by soil physical and chemical properties	Supported	High R^2 value for multivariate regression, significant univariate models	Presence of antibiotic-producing bacteria should be considered as a factor to improve the model
Hydrocarbon-remediating bacteria will be present in Field 21	Supported	<i>Methylobacteria</i> spp. Sequences were isolated from poplar root zone	Low number of reads overall for <i>Methylobacteria</i> spp.
Plant growth-promoting bacteria will be present in the Arboretum	Supported	Isolation of <i>Streptomyces</i> spp. and high proportion of reads for zeatin, mineral absorption, nitrogen metabolism, high relative abundance of <i>Rhizobia</i>	16S sequencing data are for the field at large rather than strain-specific
Field 19 will have the highest proportion of reads related to antibiotic-resistant genes	Partially supported	High proportion of vancomycin resistance and terpene degradation genes attributed to Field 19	Low amount of human disease and antimicrobial functions found in Field 19

production of commonly fumigated crops such as strawberries and carrots [41, 42], which are likely to grow well in the area.

Our 16S sequencing taxonomy data indicates the presence of plant growth-promoting bacteria such as the biopesticide *Bacillus subtilis* [43], nitrogen-fixing *Oxalobacteraceae* spp., and auxin-producing *Streptomyces* spp. on the Pierce College Farm. The *Oxalobacteraceae* spp. also help break down organic matter and mineralizing plant nutrients, and many *Streptomyces* spp. are capable of chelating iron and reducing nitrogen. Both are capable of solubilizing phosphate.

There was a high relative abundance of the archaea *Candidatus nitrocosmicus* which oxidizes ammonia and is part of the nitrogen cycle [44]. *Pseudomonas* spp. are known to enhance yield in red pepper and were also present in Pierce fields [45]. *Clostridium* spp. are sulfite-reducers, and compost tea containing *Clostridia* has been shown to increase greenhouse yield of peppers [46].

We isolated strains of putative *Streptomyces* spp. grown on selective media and tested them for nitrate reductase activity. The outcomes of the nitrate reductase test are summarized in Table 5. Most of the samples exhibited nitrogen reduction capabilities except for two isolates from Marquis D, putative *Streptomyces* isolates 14St and 15St.

The samples which had the highest number of reads from zeatin-producing bacterial genuses were S6 from Field 15 and S1 from Field 19. *Agrobacterium* sequences were not highly abundant in the samples. *Rhizobium* had the most hits from a variety of fields and was represented everywhere on the farm, including the Arboretum. The samples with the highest number of reads for *Clostridium* spp. were S10 from Marquis D. Figure 17 shows the relative abundance of these bacteria.

We should grow plants for beauty, food, and varietal trials using sustainable local inputs to offset the high cost associated with the high productivity of urban farms [47]. Currently, avocado and citrus orchards are proposed. Avocados, strawberries, clover, maintaining secluded preserves a sustainable urban food garden to feed hungry students, and

maintaining the urban tree park are popular ideas. Since several of the fields have not been cultivated in over five years, obtaining a USDA Organic certification is a viable option which would add value to the land.

The social benefit of bioremediation is the amount of revenue saved by not having to pay costs of remediation or medical bills for humans who experience ill effects from contamination.

For example, recently NASA announced that it is not feasible to clean up its portion of the Santa Susana Field Laboratory located near Pierce College because the cost exceeds \$500 million [48].

4. Conclusion

These results will empower future trials and product development by identifying target soil microorganisms and the most viable fields. Cultivation of these soil microorganisms along with crops can give our community a healthier and more sustainable future.

Terpene extracts from eucalyptus, basil, and lavender may have therapeutic value since our hypothesis was supported (Table 6). Use of plant material or extracts supplemented in animal feed may lead to decreased need for antibiotics. An extension of this research would be to test growing the plants which produce the phytoactive chemicals near livestock grazing areas; the crops may help to control antibiotic-resistant bacteria or at least keep them from spreading.

Future work to quantify the phytoactive compounds in the plant extracts is necessary. The possibility of synergistic effects of antibiotics and medicinal herbs as discussed by Budimir [49] should be investigated.

Different field conditions recorded such as field location, pH, organic matter content, N, P, K, and moisture were shown to be related to the amount of bacterial growth in the soil. It is clear that fertility is a collection of these properties within the top 6 inches of the plant rhizosphere. Further research should record the presence of antibiotic-producing

bacteria on agar plates as a factor in number of CFUs present in order to improve the statistical model.

Soil is a renewable resource over a long period of time. The land at Pierce College is classified as prime farmland (USDA NRCS). In the meantime, the value of the land for other enterprises increases over the years as buildable land becomes more scarce and this is one of a few remaining open spaces. Preserving this unique, biodiverse resource in the urban landscape is crucial for education and for productivity in its own right.

Preserving these plots of land for agriculture and education could yield tangible economic benefits from products such as food, antibiotics, and biofertilizers. Education also gives social benefits, as well as ornamental gardens. Imagine a world without beauty! The bacteria which can be isolated from the Pierce College urban farm can potentially aid in pollution remediation. Trees, turf, and other plants perform carbon fixation and have a cooling effect on the neighborhood. Further research should consider other soil microflora and macrofauna, such as the nematological composition of the fields.

Data Availability

Data from the 16S survey are available at <https://www.ncbi.nlm.nih.gov/sra/PRJNA556308>.

Disclosure

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. Daila Melendez is currently a horticulture BS student at Oregon State University, Corvallis, OR, USA 97331. Serina Reitz is currently a landscape architecture BS student at California State Polytechnic University, Pomona, CA, USA 91768. Destini Kananipour is currently a biology BA student at California State University, Northridge, CA, USA 91330.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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References

- [1] US Department of Agriculture, *Census of Agriculture Highlights*, US Department of Agriculture, Washington, DC, USA, 2014, https://www.nass.usda.gov/Publications/Highlights/2014/Highlights_Farms_and_Farmland.pdf.
- [2] Centers for Disease Control, *Antibiotic Resistance Threats in the United States*, Centers for Disease Control, Atlanta, GA, USA, 2013, <https://www.cdc.gov/drugresistance/biggest-threats.html>.
- [3] Department of Toxic Substances Control, *Santa Susana Field Laboratory Site Mitigation and Restoration Program*, DTSC, Sacramento, CA, USA, 2019, https://dtsc.ca.gov/sitecleanup/santa_susana_field_lab/.
- [4] B. Goldstein, M. Birkved, J. Birkved, and M. Hauschild, "Surveying the environmental footprint of urban food consumption," *Journal of Industrial Ecology*, vol. 21, no. 1, pp. 151–165, 2017.
- [5] A. A. Akinsemolu, "The role of microorganisms in achieving the sustainable development goals," *Journal of Cleaner Production*, vol. 182, p. 54, 2018.
- [6] G. M. King, "Urban microbiomes and urban ecology: how do microbes in the built environment affect human sustainability in cities?," *Journal of Microbiology*, vol. 52, p. 9, 2014, <https://link.springer.com/article/10.1007/s12275-014-4364-x>.
- [7] D. Prakash, P. Gabani, A. K. Chandel, Z. Ronen, and O. V. Singh, "Bioremediation: a genuine technology to remediate radionuclides from the environment," *Microbial Biotechnology*, vol. 6, no. 4, 2013.
- [8] D. B. B. Trivella and R. de Felicio, "The tripod for bacterial natural product discovery: genome mining, silent pathway induction, and mass spectrometry-based molecular networking," *mSystems*, vol. 3, no. 2, <https://msystems.asm.org/content/3/2/e00160-17>, 2018.
- [9] A. V. Lasa, A. J. Fernández-González, P. J. Villadas, N. Toro, and M. Fernández-López, "Metabarcoding reveals that rhizospheric microbiota of quercus pyrenaica is composed by a relatively small number of bacterial taxa highly abundant," *Scientific Reports*, vol. 9, no. 1, 2019.
- [10] B. Hillmann, G. A. Al-Ghalith, R. R. Shields-Cutler et al., "Evaluating the information content of shallow shotgun metagenomics," *mSystems*, vol. 3, no. 6, <https://msystems.asm.org/content/3/6/e00069-18>, 2018.
- [11] M. S. Huffman and M. D. Madritch, "Soil microbial response following wildfires in thermic oak-pine forests," *Biology and Fertility of Soils*, vol. 54, no. 8, pp. 985–997, 2018, <https://link.springer.com/article/10.1007%2Fs00374-018-1322-5>.
- [12] L. Flandroya, T. Poutahidis, G. Berg et al., "The impact of human activities and lifestyles on the interlinked microbiota and health of humans and of ecosystems," *Science of the Total Environment*, vol. 627, pp. 1018–1038, 2018.
- [13] K. Mise, K. Fujita, T. Kunito, K. Senoo, and S. Otsuka, "Phosphorus-mineralizing communities reflect nutrient-rich characteristics in Japanese arable andisols," *Microbes Environment*, vol. 33, no. 3, pp. 282–289, 2018, https://www.jstage.jst.go.jp/article/jsme2/33/3/33_ME18043/_pdf/-char/en.
- [14] N. Bruinsma, J. M. Hutchinson, A. E. van den Bogaard, H. Giamarellou, J. Degener, and E. E. Stobberingh, "Influence of population density on antibiotic resistance," *Journal of Antimicrobial Chemotherapy*, vol. 51, no. 2, pp. 385–390, 2003.
- [15] E. Tacconelli, E. Carrara, A. Savoldi et al., "Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis," *The Lancet Infectious Diseases*, vol. 18, no. 3, pp. 318–327, 2017.

- [16] NCBI PubChem, U.S. National library of National center for biotechnology information, 2018, <https://pubchem.ncbi.nlm.nih.gov>.
- [17] J. Redfern, M. Kinninmonth, D. Burdass, and J. Verran, "Using soxhlet ethanol extraction to produce and test plant material (essential oils) for their antimicrobial properties," *Journal of Microbiology & Biology Education*, vol. 15, no. 1, pp. 45-46, 2014, <http://www.asmscience.org/content/journal/jmbe/10.1128/jmbe.v15i1.656>.
- [18] E. H. Schultheis and M. K. Kjølvik, "Data nuggets," *The American Biology Teacher*, vol. 77, no. 1, pp. 19-29, 2015.
- [19] K. Mitchell, C. Dao, A. Freise, S. Mangul, and J. M. Parker, "PUMA: a tool for processing 16s rRNA taxonomy data for analysis and visualization," 2018, <https://www.biorxiv.org/content/biorxiv/early/2018/11/29/482380.full.pdf>.
- [20] S. Iwai, T. Weinmaier, B. L. Schmidt et al., "Piphillin: improved prediction of metagenomic content by direct inference from human microbiomes," *PLoS One*, vol. 11, no. 11, Article ID e0166104, 2016.
- [21] D. H. Parks, G. W. Tyson, P. Hugenholtz, and R. G. Beiko, "STAMP: statistical analysis of taxonomic and functional profiles," *Bioinformatics*, vol. 30, no. 21, pp. 3123-3124, 2014.
- [22] S. St Clair, M. Saraylou, and E. Maine, *Introduction to Soil Science Lab Manual v1*, Los Angeles Pierce College, Los Angeles, CA, USA, 2018.
- [23] R. S. Meyer, E. E. Curd, and T. Schweizer, "The California environmental DNA "CALeDNA" program," 2018.
- [24] C. Ford, *Understanding Q-Q Plots*, University of Virginia Library, Charlottesville, VA, USA, 2015, <https://data.library.virginia.edu/understanding-q-q-plots/>.
- [25] C. Ryu, "Dlookr package transformation information report," 2019, <https://cran.r-project.org/web/packages/dlookr/vignettes/transformation.html>.
- [26] N. Horsley, "Transformations," North Dakota State University Plant Science Department, Fargo, ND, USA, 2019, <https://www.ndsu.edu/faculty/horsley/Transfrm.pdf>.
- [27] N. C. Kenkel, "On selecting an appropriate multivariate analysis," *Canadian Journal of Plant Science*, vol. 86, no. 3, pp. 663-676, 2006.
- [28] D. Moriarty, "Advanced biometrics pac," California State Polytechnic University Pomona Biological Sciences Department, Pomona, CA, USA, 2013, <https://www.cpp.edu/~djmoriarty/advbiom/index.htm#adpac>.
- [29] M. al Kaisi, "Heavy rain, soil erosion, and nutrient losses," 2008, <https://crops.extension.iastate.edu/cropnews/2008/06/heavy-rain-soil-erosion-and-nutrient-losses>.
- [30] Hartmann and T. Hudson, *Hartmann & Kester's Plant Propagation: Principles and Practices*, Pearson, London, UK, 2018.
- [31] A. Cachada, E. Ferreira da Silva, A. C. Duarte R, and Pereira, "Risk assessment of urban soils contamination: the particular case of polycyclic aromatic hydrocarbons," *Science of the Total Environment*, vol. 551-552, pp. 271-284, 2016.
- [32] Lidstrom, Christoserdova, F. Eller, and W. Anesti, "Microbe wiki," 2010, <https://microbewiki.kenyon.edu/index.php/Methylobacterium>.
- [33] V. Ventrino, F. Sannino, A. Piccolo, V. Cafaro, R. Carotenuto, and O. Pepe, "Methylobacterium populi VP2: plant growth-promoting bacterium isolated from a highly polluted environment for polycyclic aromatic hydrocarbon (PAH) biodegradation," *The Scientific World Journal*, vol. 2014, p. 11, 2014.
- [34] A. Roy, P. Sar, J. Sarkar et al., "Petroleum hydrocarbon rich oil refinery sludge of north-east India harbours anaerobic, fermentative, sulfate-reducing, syntrophic and methanogenic microbial populations," *BMC Microbiology*, vol. 18, no. 1, 2018.
- [35] R. West, J. Malpas, C. Powell, M. Tollett, D. Anderson, and E. Dorrington, "California native poplar trees," 2019, <https://www.calflora.org/cgi-bin/specieslist.cgi?where-genus=Populus>.
- [36] B. V. Aken, C. M. Peres, S. Lafferty Doty, J. Moon Yoon, and J. L. Schnoor, "Methylobacterium populi sp. Nov., A novel aerobic, pink-pigmented, facultatively methylotrophic, methane-utilizing bacterium isolated from poplar trees (populus Deltoides×Nigra DN34)," *International Journal of Systematic and Evolutionary Microbiology*, vol. 54, no. 4, pp. 1191-1196, 2004.
- [37] US Environmental Protection Agency, *Site Inspection Report for Santa Susana Field Laboratory*, US EPA, Simi Valley, CA, USA, 2007, <https://19january2017snapshot.epa.gov/www3/region9/superfund/santasusana/SSFL-PASI-report-r2-complete.pdf>.
- [38] H. H. Russell, J. E. Matthews, and G. W. Sewell, "TCE," *Removal from Contaminated Soil and Ground Water*, United States EPA, Washington, DC, USA, 1992, <https://www.epa.gov/sites/production/files/2015-06/documents/tce.pdf>.
- [39] B. Boeing, "Secures historic santa susana site as open space habitat. Informative. Simi valley," 2017, <https://www.boeing.com/principles/environment/santa-susana/>.
- [40] B. Berdy, A. L. Spoering, L. L. Ling, and S. S. Epstein, "In situ cultivation of previously uncultivable microorganisms using the ichip," *Nature Protocols*, vol. 12, pp. 2232-2242, 2017.
- [41] Crop Life Foundation, "The importance of soil fumigation: califorgia strawberries," 2016, https://croplifefoundation.org/wp-content/uploads/2016/06/strawberries_fumigants.pdf.
- [42] Crop Life Foundation, "The importance of soil fumigation: califorgia carrots," 2016, https://croplifefoundation.org/wp-content/uploads/2016/06/carrots_fumigants.pdf.
- [43] University of California Agriculture and Natural Resources, *How to Manage Pests Bacillus Subtilis Pesticide Information*, University of California Agriculture and Natural Resources, Davis, CA, USA, 2019, <http://ipm.ucanr.edu/TOOLS/PNAI/pnaishow.php?id=9>.
- [44] L. E. Lehtovirta-Morley, J. Ross, L. Hink et al., "Isolation of 'Candidatus Nitrosocosmicus franklandus', a novel ureolytic soil archaeal ammonia oxidiser with tolerance to high ammonia concentration," *FEMS Microbiology Ecology*, vol. 92, no. 5, Article ID fiw057, 2016.
- [45] P. Chatterjee, S. Samaddar, R. Anandham et al., "Beneficial soil bacterium *Pseudomonas frederiksbergensis* OS261 augments salt tolerance and promotes red pepper plant growth," *Frontiers in Plant Science*, vol. 8, 2017.
- [46] M. Zaccardelli, C. Pane, D. Vilecco, A. Maria Palese, and G. Celano, "Compost tea spraying increases yield performance of pepper (*Capsicum annuum* L.) grown in greenhouse under organic farming system," *Italian Journal of Agronomy*, vol. 13, no. 3, pp. 229-234, 2018.
- [47] R. McDougall, K. Paul, and R. Rader, "Small-scale urban agriculture results in high yields but requires judicious management of inputs to achieve sustainability," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 116, no. 1, pp. 129-134, 2019.
- [48] S. Favot, "News: there's renewed anger in Chatsworth, Simi Valley, Over santa Susana Field Lab Clean-Up," 2018, <https://www.dailynews.com/2018/09/02/theres-renewed-anger-in-chatsworth-simi-valley-over-santa-susana-field-lab-clean-up/>.
- [49] I. S. Budimir, B. D. Kocić, V. M. Ćirić, O. G. Cvetković, and D. L. Miladinović, "An in vitro synergistic interaction of combinations of thymus glabrescens essential oil and its main constituents with chloramphenicol," *The Scientific World Journal*, vol. 2014, Article ID 826219, 12 pages, 2014.