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Fungi Associated with Herbaceous Plants in Coastal Northern California

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This thesis, written under the direction of the candidate's thesis advisor and approved by the program chair, has been presented to and accepted by the Biological Sciences Program, at Dominican University of California, in partial fulfillment of the requirements for the degree of Master of Science in Biological Sciences.

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Fungi Associated with Herbaceous Plants in Coastal Northern California

by

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A culminating thesis submitted to the faculty of Dominican University of California

in partial fulfillment of the requirements for the degree

Master of Science in Biological Sciences

Dominican University of California

San Rafael, CA

May 2021

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ABSTRACT

The presence of fungal species associated with herbaceous plants was monitored in coastal Marin County, California, USA. The research involved a combination of field sampling surveys and data collection using a stratified random design, pathogen identification through microbiological and molecular analysis, and descriptive analysis and ordination of results. A total of two years of repeated sampling (four times a year) was organized to allow for the detection of seasonal differences in pathogen presence on aerial plant parts. The objective of this study was to identify microbial species present on herbaceous plants using ITS1 sequence analysis. Throughout March of 2018 to March of 2020, a total of 843 samples were collected from five sample transect locations in coastal Marin County. From those 843 samples, thirty-four representative fungal and fungus-like isolates were grown into pure cultures; 16 isolates were identified at the species level, and 10 isolates on genus or family level, mostly belonging to the Ascomycota. At least one of the species identified in this study (*Phytophthora ramorum*, Oomycota) has not been found to be associated with the host plant it was isolated from (*Marah fabacea*) thus far. No significant effect of seasonality, geographic location and soil type on microbial biodiversity and abundance was detected.

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INTRODUCTION

Pathogens are associated with nearly every plant species linked in origin to a cosmopolitan range of habitats, producing damaging effects to agricultural and natural plant communities by creating change within the biodiversity of a healthy ecosystem (Alexander, 2010). Plant disease caused by pathogens can negatively affect biodiversity and impact the ecosystem by decreasing the food source of insects like bees, which are an important part of and are reliant on a healthy ecosystem. A native decline in natural plant communities can set the tone for ecological extinction where a species can still exist in limited numbers, but not fulfill its ecological role (for example as a reliable food source for certain animals). Therefore, the affected species has no or only limited ecological benefits to contribute.

Forest invasions by microbes, such as plant fungi, have received minor attention in literature focused on invasion biology (Wingfield et al., 2017). Additionally, little research focus has been directed towards plant diseases among herbaceous species in natural plant communities because economic interests in agriculture have set the majority of tone for past studies (Barber et al., 2020). Wingfield et al. (2017), adds that the lack of microbial species descriptions in literature provides a possible explanation as rationale for low levels of attention towards studies related to invasion biology within natural communities.

Many plant fungi are parasites and pathogenic to plants but not all fungi are pathogens. For example, saprobic fungi, are “the decomposers” of dead organic matter while mutualistic fungi provide benefits to living plants, in that mycorrhizal fungi (associated with the rhizosphere) improve plant uptake of water and nutrients (Moore et al., 2020). Plant pathogenic fungi have the

potential to alter biodiversity within a plant community by affecting an associated plant species' ability to compete for water, nutrients, and sunlight.

Aerial plant parts and surfaces (the Phyllosphere) are inhabited by diverse and competitive microbiomes (Leveau, 2019). Extensive microbial interactions exist on aerial plant parts where the surfaces provide nutrients and shelter for microorganisms (Farré-Armengol et al., 2016; Kirschner, 2018). Some plant pathogens are very host-specific and grow on only one plant species or on very few closely related plants (e.g., members of the *Erysiphales*, which cause powdery mildew), whereas other microbes can infect a wide variety of plant species and induce very different symptoms, such as *Phytophthora ramorum* (Panstruga & Kuhn, 2019).

In the United States, the study of native woody plant species such as tree and shrub species have largely been the focus concerning plant pathogens in natural ecosystems. A relatively recent example of disease research in natural communities in North America is Sudden Oak Death (SOD). *P. ramorum*, the causal agent of SOD, was discovered in the mid-1990's to have caused death in oak tree species in Coastal California and Southern Oregon. *P. ramorum* is a non-native pathogen of questionable origin (Grünwald et al., 2012). However, recent research indicates that the pathogen is native to tropical forests in Vietnam (Jung et al., 2020). Grünwald et al. (2012) write that the first detection of *P. ramorum* in the US has been traced to an ornamental nursery in Scotts Valley, CA, where appropriate plant disease management techniques are thought to have been neglected. Plant nurseries can harbor numerous plant pathogens, and plant trade of infected material is a major pathway for the long-distance spread of diseases (Frankel et al., 2018). SOD research has been helpful with identifying its symptoms and the detection of host plants. SOD is known to infect multiple unrelated host species, which contribute to the spread of the disease (Alexander, 2010). The growth of *P. ramorum* on these

foliar host species has increased SOD's ability to spread and decimate native tree populations in Coastal California and Southern Oregon, especially coast live oaks (*Quercus agrifolia*) and tanoaks (*Notholithocarpus densiflorus*). SOD affects oaks and other native woody species thriving in Marin County. Previous research conducted at National Ornamentals Research Site at Dominican University of California (NORS-DUC) in Marin County found significant seasonal differences in pathogen detection and abundance after several years of field study. Two new *Phytophthora* species were also discovered for the first time in the U.S. during this research (Pastalka et al., 2017). Another example of a non-native pathogen of forest trees in Coastal California is *Fusarium circinatum*, causal agent of Pine Pitch Canker, which affects Monterey Pines (*Pinus radiata*) in San Francisco and Bishop Pines (*P. muricata*) in Point Reyes, Marin Co. (Schweigkofler et al., 2004).

This research project was carried out on public land managed by the Golden Gate National Recreation Area (GGNRA) and the National Park Service (NPS) in southern coastal Marin County, California. GGNRA is an interesting example of a mixed-used, part-urban/part-rural, recreational area that displays significant micro-climatic, topographic, and environmental diversity. The diverse topographic setting of the San Francisco Bay Area has established a unique array of "local scale climates" and biodiversity within plant communities (Steers, 2016). GGNRA experiences a Mediterranean climate characterized by warm, dry summers and cool, wet winters. Coastal low clouds and fog are common during the late night and early morning hours. Average annual precipitation is approximately 960mm (38 inches), with most rain occurring November through March (United States Department of Interior, National Park Service, 2016). Natural terrestrial upland plant communities of the GGNRA support a diverse mix of land cover types including coastal scrub and grasslands, amongst others (National Park

Service, 2019). Coastal scrub is defined as a widespread cover type comprised of shrublands dominated by drought-deciduous shrubs (National Vegetation Classification System, 2020). Often referred to as “soft chaparral”, coastal scrub typically resides along the California coast and inner foothill areas in elevations up to 1500m (4900 ft) (Faber-Langendoen & Messick, 2014). The U.S. National Vegetation Classification (2020) describes the coastal scrub cover type within GGNRA as a setting that generally occurs within a seasonally wet climate as inland landforms are influenced by the Pacific Ocean, where “the inland distribution follows the corridors of marine influences of coastal fog or cool marine air where it is pushed inland by prevailing winds” (Ford & Hayes, 2007). In GGNRA, these areas are distinct and dominated by dense populations of *Baccharis pilularis* (coyote brush). Native herbaceous species, such as, soaproot (*Chlorogalum pomeridianum*), Indian thistle (*Cirsium brevistylum*), California poppy (*Eschscholzia californica*), California strawberry (*Fragaria vesca*), and catchweed bedstraw (*Galium aparine*) are found in understory areas of less dense coyote brush canopy cover within coastal scrub and amongst ground cover located along north-facing and south-facing slopes within grassland areas. Coastal scrub is known to intersperse with adjacent grassland areas within GGNRA (Pawley & Lay, 2013). Adjacent grasslands are characterized by the prevalence of native, cool-season bunchgrasses at elevations ranging from 10m (30 feet) to 1200 m (3500 feet) (National Vegetation Classification System, 2020).

This research was designed to assess the hypothesis that plant pathogens are present among herbaceous plant communities within the Golden Gate National Recreation Area (GGNRA), Marin County, California. To accomplish this goal the following objectives were met (1) Plant tissue collection to identify the presence of pathogens; (2) Pathogenic species identification through microbiological and molecular analysis; (3) Identify possible effects of

geographic variation on the presence of pathogens on herbaceous vegetation (4) Identify possible effects of seasonal variation on the presence of pathogens on herbaceous vegetation; (5) Determine through qualitative (descriptive) analysis if pathogens identified, ordinate in relationship to various geographic variables.

MATERIALS AND METHODS

Field Sampling

Review of Existing Site-Specific Environmental Data

A total of five transects were established at random within the GGNRA, two in the Marin Headlands and three in Tennessee Valley. Part 1 of the Supplemental Information which accompanies this paper provides figures in relation to study location information. Part 1, Figure 1 is a location map displaying the transect locations within Marin County, California. Part 1, Figure 2 is a USGS map displaying transect locations within Marin County, California.

Each transect consists of nine sample point locations arranged within a 2-axis cross design (Supplemental Information, Part 1, Figure 3 – Figure 7). Each sample point location within a transect was arranged 25 meters apart, with each axis totaling 100 meters in length (Supplemental Information, Part 1, Figure 8). The five transect locations account for a total of forty-five sample point locations. Sample Point locations were monitored using a portable 1-meter x 1-meter quadrat constructed of PVC pipe material laid onto the ground to assess herbaceous plant species identification and indicators of disease symptoms. Plant species within the 1-meter x 1-meter quadrat area were documented and aerial plant tissues displaying indicators of disease symptoms were collected and stored at the lab for further examination.

Transect and Sample Point locations were memorialized using a handheld Trimble Geo XH GPS device (Trimble, Inc., Sunnyvale, CA 94085). A sample transect was geolocated primarily by the latitude and longitude which correlates to its most central Sample Point (for example, Sample Point 1,0) (Supplemental Information, Part 1, Table 1). The eight sample points (for example, 1,1 – 1,8) associated with a transect were also located according to a correlating

latitude and longitude (Supplemental Information, Part 1, Table 2). Representative photos respective to when field sampling occurred can be found in Part 3 of the Supplemental Information. Table 1 provides an overview of the sample periods' seasonal relation and dates field sampling occurred during the study.

Table 1 Sample Period Overview.

Sample Period No.	Sample Period Name	Date of Sampling During Sample Period
SP1	Spring 2018	3-6-2018, 3-28-2018
SP2	Summer 2018	6-20-2018, 6-22-2018
SP3	Fall 2018	9-24-2018
SP4	Winter 2018/2019	12-29-2018, 1-4-2019
SP5	Spring 2019	6-14-2019, 6-18-2019
SP6	Summer 2019	9-6-2019, 9-9-2019
SP7	Fall 2019	11-21-2019, 11-22-2019
SP8	Winter 2019/2020	2-18-2020, 2-24-2020

Climate

Local precipitation and temperature data were taken from the California Irrigation Management Information System (CIMIS) to provide an accurate detailed collection of climatological data for the study. Weather data was taken from the weather station Point San Pedro (station IDs: (CIMIS #157, 2020) located at Latitude: 37 deg 59 min N and Longitude: 122 deg 28 min W. The weather station is located approximately 28.3 km (17.6 miles) from the research area of interest and sits at 1.5 m (5 ft) in elevation. The station has annually accounted for local precipitation and temperature from October 2002 to present (18 years).

Soils

Soil data related to the transects and sample points were obtained from U.S. Department of Agriculture, Natural Resources Conservation Service (NRCS). Data reports describing the soils setting respective to transect and sample point location coordinates were generated using NRCS' Web Soil Survey tool (United States Department of Agriculture, Natural Resources Conservation Service, 2019). Soils reports relating to each transect are in Part 2 of the Supplemental Information.

Vegetation Communities

Vegetation community "lifeform" classes within coastal scrub habitat were analyzed using the Marin County Draft Lifeform Map, version 12/1/2019 (Golden Gate National Parks Conservancy & Tukman Geospatial, 2019). The map describes a higher resolution of vegetation lifeform classes and acreage (when compared to coastal scrub) relevant to transect and sample point locations. The soil type and plant community cover were calculated using an approximately 4-mile x 6-mile boundary area which encompasses all transect locations (Figure 3).

Plant Species Identification

All herbaceous plant species within the 1-meter x 1-meter quadrat were identified visually and documented at the time of sampling. The data sheet was labeled for identification purposes with the date, sample period, transect and sample point relative to the when and where a field study was performed. Furthermore, the data sheet describes all herbaceous plant species found to be present within each sample point location at the time field sampling occurred. A determination of whether herbaceous plant species are flowering was also noted on the data sheet. Plant species unidentified visually at the time field sampling occurred were identified

morphologically via Calflora (www.calflora.org) and the Jepson Manual, Second Edition (University of California Press, Berkeley, CA, 2012).

All identified herbaceous plant species were logged onto a Microsoft Excel database (Supplemental Information, Part 2). Representative photos for herbaceous plant species identification were taken at each sample point during each sample period (Supplemental Information, Part 3).

Visual Indicators of Plant Disease

Sample Point locations were monitored visually for plants displaying disease symptoms. Indicators of disease include necrosis, leaf spots, and color variations. Symptomatic plant tissue (mainly leaves) was photographed, documented on a field data sheet, and then sampled using a sharp pair of shears. Plant tissue samples were placed individually in labeled Ziploc bags and transported on ice to the NORS-DUC laboratory for storage in dry refrigeration at 5° C until further analysis. Representative photographs of symptomatic plant tissues can be found in the results section and in Supplemental Information, Part 3.

Field sampling of aerial plant tissues among the five transects was conducted over a two-year period beginning March 2018 and ending March 2020.

Microscopic Analysis

Tissue Culture

Individual plant tissue samples were surface sterilized by being rinsed in deionized water for 30 seconds; then rinsed in 70 percent ethanol for an additional 30 seconds; and finally, the sample tissue was rinsed again with deionized water for a period of 1 minute. After completion of sterilization, the plant tissue was ready for the isolation of microbes growing within the plant.

A sterilized scalpel was used to cut twenty 5x5 mm sub-samples along the edge of where symptomatic areas visibly meet healthy leaf tissue, an area where microbial growth normally is highest.

The twenty tissue sub-samples were placed onto four plates (five sub-sample tissue cuttings per plate) containing two non-specific media to allow growth of a wide variety of microbial species, especially fungi. Two plates held BBL™ Corn Meal Agar (CMA) (Ref. 211132, Becton, Dickson and Company, Sparks, MD 21152 USA | 38800 Le Pont de Claix, France) and two plates held Potato Dextrose Agar (PDA) (Catalog No. 786341, Carolina Biological Supply Co., www.carolina.com). The four plates were incubated at 20° C. Incubated cultures were assessed for growths for a period of 15-21 days then moved to a 5° C refrigerator when the colonies were starting to grow close to one another (Figure 10). See Supplemental Information, Part 3 for representative photos of tissue cultures on potato dextrose agar and corn meal agar.

Culture Purification

For the purification of microbial isolates, representative samples were selected based on similar growth characteristics occurring within each sample period for the two-year study. One representative of every morphologically distinct microbial growth form was identified. If several growths looked visually identical, they were assumed to be the same microbe, and one of them was identified. Sample plugs were transferred onto new plates containing PDA + Antibiotics (0.25g Sodium Ampicillin (A9518-5G, Sigma Life Science, Sigma-Aldrich, Co. 3050 Spruce Street, St. Louis, MO 63103 USA) and 0.01g Rifampicin (R3501-1G, Sigma Life Science, Sigma-Aldrich, Co. 3050 Spruce Street, St. Louis, MO 63103 USA) to obtain clean cultures without contaminations. Incubation at 20° C occurred for a period of 15-21 days where purified

(isolated) growth was assessed and photographed (Supplemental Information, Part 3). Figure 9 provides a representative example of pure culture grown on PDA + AB media. Representative photos of purified cultures on PDA + AB can be found in Supplemental Information, Part 3.

Microscopy

Microscopic analysis was performed on purified microbial cultures growing on PDA+ AB medium using a light microscope Leica DM1000 LED fitted with a 5 MP HD Microscope Camera Leica MC170 HD. This identification method was performed to visually identify uniquely characteristic hyphal, sexual, and asexual features, such as the presence of conidia. Additionally, identifications were made by comparing colony growth patterns, colony colors and microscopic morphological characteristics with species descriptions in literature.

Molecular Detection Using ITS Sequence Analysis

DNA Extraction

Mycelium from pure cultures grown on PDA + AB plates were ground with a mortar and pestle after adding small amounts of liquid nitrogen. The resulting powder was transferred to a cryotube and stored at -80° C until DNA extraction.

DNA extraction was carried out according to manufacturer's specifications with slight modifications using the QIAGEN DNeasy® Plant Mini kit (50) (Cat. No. 69104 Qiagen, Hilden, Germany). Final elutions were produced with of 40 µL AE Buffer and centrifuged for two minutes. The samples were then analyzed via a Thermo Fisher Scientific NanoDrop™ 2000/2000c Micro Volume Spectrophotometer to obtain an accurate measurement of nucleic acid concentrations. The DNA samples were stored at -30° C until PCR amplification was initiated.

PCR Amplification of the ITS Region

PCR amplification was carried out using a T100 Thermal Cycler (Bio-Rad Laboratories, Inc.). PCR primers ITS1 (5' TCCGTAGGTGAACCTGCGG3') and ITS2 (5'GCTGCGTTCTTCATCGATGC3') were utilized to amplify all sample isolates at the internal transcribed spacer region (ITS) (White et al., 1990). ITS1 and ITS2 primers were chosen to minimize sequencing errors. 1 µL of 10µM ITS1 primer, 1 µL of 10µM ITS2 primer, 25 µL Taq 2X Master Mix (New England BioLabs., Ipswich, MA), 21 µL of nuclease free H₂O, and 2µL of DNA template (Sample 1 – Sample 34) were combined into a final 50 µL sample volume for amplification runs. Amplification runs consisted of an initial 3-minute denaturation at 95° C; followed by 34, three-step cycles, each consisting of a 30-second denaturization step at 95° C, a 30-second annealing step at 54° C, and a 1-minute extension step at 72° C; and a 10-minute final extension at 72° C. A High Pure PCR Product Purification Kit (Cat. No. agar11732668001 Sigma Aldrich St. Louis, MO) was used to purify the PCR products according to the manufacturer's instructions with a modification of using 225 µL binding buffer and 20 µL of Elution Buffer. 1.5% agarose gels stained with Ethidium Bromide were run to verify DNA presence and qualitative values after cleanup. Bands were visually inspected using an Invitrogen iBrightCL1000 (Thermo Scientific, Waltham, WA) and a 100 bp ladder (Figure 1).

Figure 1 Gel image of samples with 100 bp DNA ladder.

DNA Sequencing

DNA sequencing was carried out at the DNA sequencing facility at University of California-Berkeley in Berkeley, California (<https://ucberkeleydnasequencing.com>). Both forward and reverse strands of the PCR fragments were sequenced. Submission criteria for PCR product samples were 20-40 ng/1000bp PCR product + 8 pmol primer in a total volume of 13 μ L.

Microbial Species Identification

Sequences were analyzed visually utilizing BioEdit software version 7.2.5. (Hall & Hall, 1999). Steps were taken to eliminate any unknown gaps, trim sequences lengthwise, create a reverse compliment, and align the forward sequence and reverse compliment to compile a consensus sequence for species identification. The inquiry into microbial species identification was carried out by utilizing the Basic Local Alignment Search Tool (BLAST) application on the website of the NCBI, National Center for Biotechnology Information (Altschul et al., 1990; National Center for Biotechnology Information, 2020). A phylogenetic analysis of aligned

sequences was carried out using MEGA X (Kumar et al., 2018). A phylogenetic tree (Figure 10). was constructed with the Neighbor-Joining Tree method and bootstrapping with 1000 replications (Saitou & Nei, 1987; Tamura et al., 2004).

Statistical Analysis

Descriptive statistics based on averages and percentages through representation of tables and figures was utilized to assess species richness in relation to the soil type and plant community cover of the transect areas across all sample periods (Table 2). The soil type and plant community cover percentages were calculated using an approximately 4-mile x 6-mile boundary area which encompasses all transect locations (Figure 3). Table 3 shows the average values of soil qualities for locations where microbial species are absent and locations where microbial species are present. The physical characteristics of soils and their potential effects on seasonal microbial diversity were analyzed. The analysis is based on slope percentage, saturated hydraulic conductivity (Ksat), drainage class, and runoff class (all physical parameters) of soils to determine significant differences between an absence of microbial species and the species presence of microbes (Table 3).

Table 2 Comparison of percent preference based on soil type on plant community cover across all sampling periods. Values are the means of n locations.

	Spring 2018		Summer 2018		Fall 2018		Winter 2018/2019		Spring 2019		Summer 2019		Fall 2019		Winter 2019/2020	
	Microbes	Microbes	Microbes	Microbes	Microbes	Microbes	Microbes	Microbes	Microbes	Microbes	Microbes	Microbes	Microbes	Microbes	Microbes	Microbes
	Absent (n=40)	Present (n=5)	Absent (n=39)	Present (n=6)	Absent (n=41)	Present (n=4)	Absent (n=40)	Present (n=5)	Absent (n=41)	Present (n=4)	Absent (n=42)	Present (n=3)	Absent (n=42)	Present (n=3)	Absent (n=43)	Present (n=2)
Soil Type %																
CRONKHITE-BARNABE COMPLEX 30 TO 50 PERCENT SLOPES %	0.3	0	0.3	0	0.3	0	0.3	0	0.3	0	0.3	0	0	3.5	0.3	0
CRONKHITE-BARNABE COMPLEX 50 TO 75 PERCENT SLOPES %	3	0	2.7	2.6	2.2	7.7	2.6	3.1	2.2	7.7	2.9	0	2.9	0	2.8	0
HUMAQUEPTS SEEPED %	0.1	0.2	0.1	0.2	0.1	0	0.1	0	0.1	0	0.1	0	0.1	0	0.04	0.5
HYDRAQUENTS SALINE %	0.01	0.03	0.01	0	0.01	0.03	0.01	0.03	0.01	0.03	0.01	0	0.01	0	0.01	0
RODEO CLAY LOAM 2 TO 15 PERCENT SLOPES %	0.72	1	0.7	1	1	1.2	0.9	0	0.82	0	0.8	0	0.8	0	0.8	0
TAMALPAIS-BARNABE VARIANT VERY GRAVELLY LOAMS 30 TO 50 PERCENT SLOPES %	4	4.5	4.7	0	4.4	0	3.4	9.1	4.4	0	3.2	15.1	4.3	0	4.2	0
TAMALPAIS-BARNABE VARIANT VERY GRAVELLY LOAMS 50 TO 75 PERCENT SLOPES %	2.6	4.2	2.7	3.5	3.1	0	3.13	0	2.5	5.2	3	0	3	0	2.4	10.4
SIRDRAK SAND 15 TO 50 PERCENT SLOPES %	0.34	0	0.3	0.5	0.3	0	0.3	0.3	0.33	0	0.3	0.5	0.3	1	0.3	0
Plant Community %																
Shrub %	40.6	14.2	39.9	23.5	36.1	53	37.1	42.4	37.9	35.3	37	47.1	38.7	23.5	39.4	0
Non-native Forest & Woodland %	0.4	0	0.4	0	0.4	0	0.4	0	0.24	1.23	0.4	0	0.23	1.64	0.4	0
Upland Forb & Grass %	2.9	6.7	3	5.6	3.5	2.1	3.3	3.4	3.5	2.1	3.4	2.8	3.4	2.8	3.1	8.3

Table 3 The physical characteristics of soils at sampling locations where plant-dwelling microbes were absent, or present. Values are the means of n locations.

	Spring 2018		Summer 2018		Fall 2018		Winter 2018/2019		Spring 2019		Summer 2019		Fall 2019		Winter 2019/2020	
	Microbes	Microbes	Microbes	Microbes	Microbes	Microbes	Microbes	Microbes	Microbes	Microbes	Microbes	Microbes	Microbes	Microbes	Microbes	Microbes
	Absent (n=40)	Present (n=5)	Absent (n=39)	Present (n=6)	Absent (n=41)	Present (n=4)	Absent (n=40)	Present (n=5)	Absent (n=41)	Present (n=4)	Absent (n=42)	Present (n=3)	Absent (n=42)	Present (n=3)	Absent (n=43)	Present (n=2)
Soil (Physical Characteristics)																
Slopes	37.6	22.9	36.4	33.5	36.2	33.6	36.1	35.2	34.9	47.1	35.9	37.5	35.5	42.5	36.2	32.5
Ksat	3.5	4	3.6	3.3	3.5	4.2	3.6	3.3	3.5	4	3.6	3	3.6	2.2	3.5	4.3
Drainage Class	3.8	5	3.9	3.8	3.8	5.3	4	3.8	3.9	4.5	4	2.7	4	2.7	3.9	4.5
Runoff Class	5.3	5.8	5.4	5	5.3	5.8	5.4	5.2	5.3	5.8	5.4	5	5.4	4	5.3	6

RESULTS

Field Sampling

The following provides the results of field sampling. The sections below describe plant communities and soils underlying sample locations, herbaceous plant species present, indicators of disease symptoms found, culture characteristics, and molecular identification of pathogenic microbes.

Climate

Figure 2 displays the seasonal precipitation and temperature averages for Marin County during the two-year study. Winter 2018-2019 averaged 5.13 mm of daily precipitation; a higher accumulation compared to Winter 2019-2020 (0.99 mm). Spring 2018 -Winter 2019 (Year 1) had a precipitation total of 517 mm and Spring 2019- Winter 2019 (Year 2) resulted with 364 mm of rainfall.

Figure 2 Average Seasonal Precipitation and Temperature for Marin County, CA for both sampling years (2018-2019).

Plant Communities and Soil Associations

Table 4 provides an overview of plant communities and soils associations in relation to study area. Three plant communities, nine soil associations, and five landform types are present across the transect and sample point locations. The most common plant community type was shrub. Shrub plant community was most found present across all transects. Multiple soil types exist in all transects but Transect 1. Slopes across all transects ranged from 0% - 75%. Most areas consisted of slopes of 30% or more. The average Ksat value, or ease with which pores of a saturated soil transmit water, was moderately high. Soils across the transects were mostly considered to be moderately well drained to somewhat excessively well drained. Most soils were considered to have a very high runoff classification. Hills, a landform type, was also present across all transects. The setting of soils associations for each transect and sample point location can be viewed in Supplemental Information, Figures 8-12. The setting of soils for each transect and sample point location can be viewed in Supplemental Information, Figures 13-17.

Table 4 Soils and Plant Communities for all transect and sample point locations in the five transects.

Transect	Sample Point	Plant Community	Soils Association	Landform	Slope	Ksat	Drainage Class	Runoff Class
1	0	Upland Forb & Grass	SIRDRAK SAND 15 TO 50 % SLOPES	Dunes	15 - 50 %	High to very high	Somewhat excessively drained	low
1	1	Non-native Forest & Woodland	SIRDRAK SAND 15 TO 50 % SLOPES	Dunes	15 - 50 %	High to very high	Somewhat excessively drained	low
1	2	Shrub	SIRDRAK SAND 15 TO 50 % SLOPES	Dunes	15 - 50 %	High to very high	Somewhat excessively drained	low
1	3	Shrub	SIRDRAK SAND 15 TO 50 % SLOPES	Dunes	15 - 50 %	High to very high	Somewhat excessively drained	low
1	4	Upland Forb & Grass	SIRDRAK SAND 15 TO 50 % SLOPES	Dunes	15 - 50 %	High to very high	Somewhat excessively drained	low
1	5	Shrub	SIRDRAK SAND 15 TO 50 % SLOPES	Dunes	15 - 50 %	High to very high	Somewhat excessively drained	low
1	6	Shrub	SIRDRAK SAND 15 TO 50 % SLOPES	Dunes	15 - 50 %	High to very high	Somewhat excessively drained	low
1	7	Upland Forb & Grass	SIRDRAK SAND 15 TO 50 % SLOPES	Dunes	15 - 50 %	High to very high	Somewhat excessively drained	low
1	8	Shrub	SIRDRAK SAND 15 TO 50 % SLOPES	Dunes	15 - 50 %	High to very high	Somewhat excessively drained	low
2	0	Upland Forb & Grass	TAMALPAIS-BARNABE VARIANT VERY GRAVELLY LOAMS 30 TO 50 % SLOPES	Hills	30 - 50 %	Moderately high	Well drained	very high
2	1	Upland Forb & Grass	TAMALPAIS-BARNABE VARIANT VERY GRAVELLY LOAMS 30 TO 50 % SLOPES	Hills	30 - 50 %	Moderately high	Well drained	very high
2	2	Upland Forb & Grass	TAMALPAIS-BARNABE VARIANT VERY GRAVELLY LOAMS 30 TO 50 % SLOPES	Hills	30 - 50 %	Moderately high	Well drained	very high
2	3	Upland Forb & Grass	HUMAQUEPTS SEEPED	Drainageways	0 - 5 %	Moderately low to moderately high	Poorly drained	very high
2	4	Upland Forb & Grass	HUMAQUEPTS SEEPED	Drainageways	0 - 5 %	Moderately low to moderately high	Poorly drained	very high
2	5	Upland Forb & Grass	TAMALPAIS-BARNABE VARIANT VERY GRAVELLY LOAMS 30 TO 50 % SLOPES	Hills	30 - 50 %	Moderately high	Well drained	very high
2	6	Shrub	HUMAQUEPTS SEEPED	Drainageways	0 - 5 %	Moderately low to moderately high	Poorly drained	very high
2	7	Shrub	TAMALPAIS-BARNABE VARIANT VERY GRAVELLY LOAMS 30 TO 50 % SLOPES	Hills	30 - 50 %	Moderately high	Well drained	very high
2	8	Shrub	TAMALPAIS-BARNABE VARIANT VERY GRAVELLY LOAMS 30 TO 50 % SLOPES	Hills	30 - 50 %	Moderately high	Well drained	very high
3	0	Shrub	HYDRAQUENTS SALINE	Tidal flats	0 - 2 %	Low to moderately high	Very poorly drained	high
3	1	Shrub	TAMALPAIS-BARNABE VARIANT VERY GRAVELLY LOAMS 50 TO 75 % SLOPES	Hills	50 - 75%	Moderately high	Well drained	very high
3	2	Upland Forb & Grass	TAMALPAIS-BARNABE VARIANT VERY GRAVELLY LOAMS 50 TO 75 % SLOPES	Hills	50 - 75%	Moderately high	Well drained	very high
3	3	Upland Forb & Grass	TAMALPAIS-BARNABE VARIANT VERY GRAVELLY LOAMS 50 TO 75 % SLOPES	Hills	50 - 75%	Moderately high	Well drained	very high
3	4	Upland Forb & Grass	TAMALPAIS-BARNABE VARIANT VERY GRAVELLY LOAMS 50 TO 75 % SLOPES	Hills	50 - 75%	Moderately high	Well drained	very high
3	5	Shrub	HYDRAQUENTS SALINE	Tidal flats	0 - 2 %	Low to moderately high	Very poorly drained	high
3	6	Shrub	HYDRAQUENTS SALINE	Tidal flats	0 - 2 %	Low to moderately high	Very poorly drained	high
3	7	Shrub	TAMALPAIS-BARNABE VARIANT VERY GRAVELLY LOAMS 50 TO 75 % SLOPES	Hills	50 - 75%	Moderately high	Well drained	very high
3	8	Shrub	TAMALPAIS-BARNABE VARIANT VERY GRAVELLY LOAMS 50 TO 75 % SLOPES	Hills	50 - 75%	Moderately high	Well drained	very high
4	0	Upland Forb & Grass	RODEO CLAY LOAM 2 TO 15 % SLOPES	Basin floors, interior valleys	2 - 15 %	Moderately low to moderately high	Poorly drained	very high
4	1	Upland Forb & Grass	TAMALPAIS-BARNABE VARIANT VERY GRAVELLY LOAMS 30 TO 50 % SLOPES	Hills	30 - 50 %	Moderately high	Well drained	very high
4	2	Upland Forb & Grass	RODEO CLAY LOAM 2 TO 15 % SLOPES	Basin floors, interior valleys	2 - 15 %	Moderately low to moderately high	Poorly drained	very high
4	3	Shrub	RODEO CLAY LOAM 2 TO 15 % SLOPES	Basin floors, interior valleys	2 - 15 %	Moderately low to moderately high	Poorly drained	very high
4	4	Shrub	TAMALPAIS-BARNABE VARIANT VERY GRAVELLY LOAMS 30 TO 50 % SLOPES	Hills	30 - 50 %	Moderately high	Well drained	very high
4	5	UplandForb & Grass	RODEO CLAY LOAM 2 TO 15 % SLOPES	Basin floors, interior valleys	2 - 15 %	Moderately low to moderately high	Poorly drained	very high
4	6	Non-native Forest & Woodland	RODEO CLAY LOAM 2 TO 15 % SLOPES	Basin floors, interior valleys	2 - 15 %	Moderately low to moderately high	Poorly drained	very high
4	7	Upland Forb & Grass	RODEO CLAY LOAM 2 TO 15 % SLOPES	Basin floors, interior valleys	2 - 15 %	Moderately low to moderately high	Poorly drained	very high
4	8	Upland Forb & Grass	RODEO CLAY LOAM 2 TO 15 % SLOPES	Basin floors, interior valleys	2 - 15 %	Moderately low to moderately high	Poorly drained	very high
5	0	Shrub	CRONKHITE-BARNABE COMPLEX 50 TO 75 %SLOPES	Hills	50 - 75%	Moderately low to moderately high	Moderately well drained	very high
5	1	Shrub	CRONKHITE-BARNABE COMPLEX 50 TO 75 %SLOPES	Hills	50 - 75%	Moderately low to moderately high	Moderately well drained	very high
5	2	Shrub	CRONKHITE-BARNABE COMPLEX 50 TO 75 %SLOPES	Hills	50 - 75%	Moderately low to moderately high	Moderately well drained	very high
5	3	Shrub	CRONKHITE-BARNABE COMPLEX 50 TO 75 %SLOPES	Hills	50 - 75%	Moderately low to moderately high	Moderately well drained	very high
5	4	Shrub	CRONKHITE-BARNABE COMPLEX 30 TO 50 % SLOPES	Hills	30 - 50 %	Moderately high to high	Moderately well drained	very high
5	5	Shrub	CRONKHITE-BARNABE COMPLEX 50 TO 75 % SLOPES	Hills	50 - 75%	Moderately low to moderately high	Moderately well drained	very high
5	6	Non-native Forest & Woodland	CRONKHITE-BARNABE COMPLEX 50 TO 75 % SLOPES	Hills	50 - 75%	Moderately low to moderately high	Moderately well drained	very high
5	7	Shrub	CRONKHITE-BARNABE COMPLEX 50 TO 75 % SLOPES	Hills	50 - 75%	Moderately low to moderately high	Moderately well drained	very high
5	8	Shrub	CRONKHITE-BARNABE COMPLEX 50 TO 75 % SLOPES	Hills	50 - 75%	Moderately low to moderately high	Moderately well drained	very high

Figure 3 Soil association and plant community cover 7926-acre boundary area (~ 4-mi x 6-mi) which encompasses all transect locations.

Herbaceous Plant Species Identification

A total of 61 herbaceous plant species were identified within the 5 transects sampled. Of these 61 species, 27 (44%) were annuals, 40 (66%) were perennials, 24 (39%) were natives, 37 (61%) were non-natives, 44 were dicots, and 18 were monocots (Table 5, Figure 4, and Supplemental Information, Part 3). Information tables displaying the species identified per

sample point respective to transect locations and sample periods are provided in Supplemental Information, Part 2.

Figure 5 shows seasonal variation of plant species richness within the five transects. Plant species identification counts resulted 39 individual species each for Spring 2018 and Summer 2018, the highest species counts during the study. Fall 2018 and Winter 2018-2019 had 29 and 30 plant species counts, respectively. During Spring 2019, thirty-eight plant species were documented; Summer 2019 and Fall 2019 each resulted with 28; and 31 documented during Winter 2019-2020. Figure 6 provides examples of monitoring (with 1m² quadrat) of sample point locations at time of field survey.

Table 5 Ecological parameters of plant species identified during the study.

Plant Species		Life Cycle		Geographic Origin		Flowering Plant Class	
Scientific Name	Common Name	Annual	Perennial	Native	Non-Native	Dicot	Monocot
<i>Achillea millefolium</i>	Common yarrow		•	•		•	
<i>Agoseris apargioides</i> var. <i>apargioides</i>	Coast dandelion		•	•		•	
<i>Aira caryophylla</i> L.	Silver hairgrass	•			•		•
<i>Anagallis arvensis</i>	Scarlet Pimpernel	•			•	•	
<i>Avena fatua</i> L.	Wild oat	•			•		•
<i>Brassica nigra</i>	Black mustard	•			•	•	
<i>Briza maxima</i>	Rattlesnake grass	•			•		•
<i>Briza minor</i> L.	Little quaking grass	•			•		•
<i>Bromus diandrus</i>	Ripgut brome	•			•		•
<i>Bromus hordeaceus</i>	Soft brome, Soft chess	•			•		•
<i>Carduus pycnocephalus</i> L.	Italian thistle	•			•	•	
<i>Carpobrotus chilensis</i>	Sea fig					•	
<i>Chlorogalum pomeridianum</i>	Soap Root		•	•			•
<i>Cirsium brevistylum</i>	Indian Thistle		•	•		•	
<i>Cirsium vulgare</i>	Bull thistle		•		•	•	
<i>Claytonia perfoliata</i>	Miner's Lettuce	•		•		•	
<i>Conium maculatum</i> L.	Poison hemlock		•		•	•	
<i>Convolvulus arvensis</i>	Field bindweed		•		•	•	
<i>Daucus pusillus</i>	American wild carrot	•		•		•	
<i>Dichondra donelliana</i>	California ponysfoot		•	•		•	
<i>Dudleya farinosa</i>	Bluff Lettuce		•	•		•	
<i>Elymus glaucus</i>	Blue wildrye		•	•			•
<i>Erigeron glaucus</i>	Seaside Daisy		•	•		•	
<i>Erodium botrys</i>	Longbeak stork's bill	•			•	•	
<i>Eschscholzia californica</i>	California poppy	•	•	•		•	
<i>Festuca arundinacea</i>	Tall Fescue		•		•		•
<i>Festuca perennis</i>	Italian rye grass	•	•		•		•
<i>Foeniculum vulgare</i> Mill.	Sweet fennel		•		•	•	
<i>Fragaria vesca</i> L.	California strawberry		•	•		•	
<i>Galium aparine</i>	Catchweed Bedstraw	•		•		•	
<i>Geranium dissectum</i>	Cut-leaved Crane's-Bill	•			•	•	
<i>Geranium molle</i> L.	Crane's bill geranium	•	•		•	•	
<i>Helminthotheca echinoides</i>	Bristly ox-tongue	•	•		•	•	
<i>Heracleum maximum</i>	Common cowparsnip		•	•		•	
<i>Heterotheca sessiliflora</i>	Sessileflower False Goldenaster	•		•		•	
<i>Hirschfeldia incana</i>	Short podded mustard		•		•	•	
<i>Holcus lanatus</i>	Yorkshire Fog		•		•		•
<i>Hordeum murinum</i> L. ssp. <i>leporinum</i>	Foxtail barley	•			•		•
<i>Hypochaeris radicata</i>	Common Cat's-Ear		•		•	•	
<i>Juncus effusus</i> L. ssp. <i>pacificus</i>	Pacific rush		•	•			•
<i>Linum lewisii</i>	Lewis Flax		•	•		•	
<i>Lupinus bicolor</i>	Annual lupine, Bicolored lupine	•	•	•		•	
<i>Lysimachia arvensis</i>	Scarlet pimpernel	•			•	•	
<i>Marah fabacea</i>	California man-root		•	•		•	
<i>Melica torreyana</i>	Torrey's melica		•	•			•
<i>Mentha pulegium</i>	Pennyroyal		•		•	•	
<i>Narcissus pseudonarcissus</i>	Daffodil	•			•		•
<i>Oxalis pes-caprae</i> L.	Bermuda buttercup		•		•	•	
<i>Phalaris aquatica</i>	Harding grass		•		•		•
<i>Plantago coronopus</i>	Buck's-horn Plantain	•			•	•	
<i>Plantago lanceolata</i>	Ribwort Plantain		•		•	•	
<i>Pseudognaphalium californicum</i>	California cudweed	•	•	•		•	
<i>Rumex acetosella</i>	Sheep's Sorrel		•		•	•	
<i>Rumex crispus</i> L.	Curly dock		•		•	•	
<i>Sedum spathulifolium</i>	Broad-leaved Stonecrop		•	•		•	
<i>Spergula arvensis</i>	Corn spurrey	•			•	•	
<i>Taraxia ovata</i>	Sun Cup		•	•		•	
<i>Trifolium subterraneum</i>	Subterranean Clover	•			•	•	
<i>Vicia sativa</i>	Common Vetch	•			•	•	
<i>Vinca major</i>	Greater Periwinkle		•		•	•	
<i>Zantedeschia aethiopica</i>	Callalily		•		•		•

Figure 4 Relative abundance of Life cycle (annual vs. perennial), geographic origin (native vs. non-native), and flowering plant class observation (dicot vs. monocot) of plants identified during field studies.

Figure 5 Seasonal variation of plant species richness on the five transects during the sample periods.

Figure 6 Example sample point location at time of field survey. (a) 1m² quadrat, summer 2019 (SP6), Transect 1, Sample Point 6; (b) 1m² quadrat, winter 2019-2020 (SP8), Transect 2, Sample Point 3.

Indicators of Disease Symptoms

A total of 14 herbaceous plant species showed disease symptoms (Table 6), 7 native herbaceous plant species: common yarrow (*Achillea millefolium*), common soaproot (*Chlorogalum pomeridianum*), Indian thistle (*Cirsium brevistylum*), California poppy (*Eschscholzia californica*), California strawberry (*Fragaria vesca* L.), catchweed bedstraw (*Galium aparine*) and California man-root (*Marah fabacea*) and 7 non-native species: Italian thistle (*Carduus pycnocephalus* L.), field bindweed (*Convolvulus arvensis*), cut-leaved crane's-bill (*Geranium dissectum*), hairy cat's-ear (*Hypochaeris radicata*), ribwort (*Plantago lanceolata*), sheep's sorrel (*Rumex acetosella*), greater periwinkle (*Vinca major*). In Spring 2018, 6 different plant species were found showing symptoms on leaves including leaf spots, necrotic tips of leaves, and color variations of tissues. *Plantago lanceolata* was revealed to display symptoms during 7 of 8 seasons sampled. Leaf spots (85%) were the most common indicators among tissues observed through all seasons. *Achillea millefolium*, *Carduus pycnocephalus*,

Chlorogalum pomeridianum, *Eschscholzia californica*, *Fragaria vesca* L., *Galium aparine*, *Hypochaeris radicata*, *Marah fabacea*, *Plantago lanceolata*, *Rumex acetosella*, and *Vinca major* were all found to host known plant pathogens. Only 23% of species identified during the study showed symptoms on aerial plant parts during the study (Figure 7). See Supplemental Information, Part 3 for photos related to symptomatic plant tissues used for purified cultures. Figure 8 represents plant tissues displaying symptoms during field surveys

Table 6 Plant species sampled showing symptoms on leaves (necrosis, leaf spots, etc.) and associated microorganisms.

Sample Isolate	Sample Period	Season	Transect	Sample Point	Plant Species	Life Cycle	Geographic Origin	Flowering Plant Class	Tissue Symptoms	Associated Microorganism	Sequence Identity [%]	NCBI Accession number of best hit
1	SP1	Spring 2018	2	0	<i>Hypochaeris radicata</i>	Perennial	Non-Native	dicot	Leaf spots, lesion, necrosis at tips	<i>Rhizoctonia solani</i>	98.18	MT852563.1
2	SP1	Spring 2018	2	3	<i>Chlorogalum pomeridianum</i>	Perennial	Native	monocot	Necrosis at tips, white spots along leaf tissue	<i>Alternaria alternata</i>	99.30	MF422130.1
3	SP1	Spring 2018	2	3	<i>Eschscholzia californica</i>	Perennial/Annual	Native	dicot	chlorosis, necrosis at tips	<i>Plectosphaerella oligotrophica</i>	99.04	MT447499.1
4	SP1	Spring 2018	3	0	<i>Convolvulus arvensis</i>	Perennial	Non-Native	dicot	leaf spots	<i>Uncultured fungus clone 4248_815</i>	99.61	MT236804.1
5	SP1	Spring 2018	3	3	<i>Geranium dissectum</i>	Annual	Non-Native	dicot	chorosis, leaf spots	<i>Uncultured fungus clone 4248_815</i>	99.55	MT236804.1
6	SP1	Spring 2018	4	5	<i>Galium aparine</i>	Annual	Native	dicot	leaf spots	<i>Cladosporium bruhnei</i>	100.00	MG659641.1
7	SP2	Summer 2018	1	3	<i>Carduus pycnocephalus</i>	Annual	Non-Native	dicot	leaf spots	<i>Aspergillus fumigatus</i>	100.00	MT635279.1
8	SP2	Summer 2018	1	7	<i>Vinca major</i>	Perennial	Non-Native	dicot	chlorotic lesions, black powdery conidia or necrosis	<i>Alternaria infectoria</i>	99.31	MT635276.1
9	SP2	Summer 2018	2	4	<i>Rumex acetosella</i>	Perennial	Non-Native	dicot	leaf spots	<i>Didymella subherbarum</i>	97.37	KR534651.1
10	SP2	Summer 2018	3	2	<i>Rumex acetosella</i>	Perennial	Non-Native	dicot	leaf spots	<i>Pyrenophora sp.</i>	99.68	MT548668.1
11	SP2	Summer 2018	4	7	<i>Rumex acetosella</i>	Perennial	Non-Native	dicot	leaf spots, lesions	<i>Alternaria infectoria</i>	100.00	MT635276.1
12	SP2	Summer 2018	5	7	<i>Plantago lanceolata</i>	Perennial	Non-Native	dicot	necrosis at tips, leaf spots	<i>Alternaria infectoria</i>	99.27	MT561399.1
13	SP3	Fall 2018	3	6	<i>Convolvulus arvensis</i>	Perennial	Non-Native	dicot	leaf spots	<i>Alternaria sp. (KX343167)</i>	99.62	KX343167.1
14	SP3	Fall 2018	4	2	<i>Rumex acetosella</i>	Perennial	Non-Native	dicot	leaf spots	<i>Stemphylium sp.</i>	100.00	MT556676.1
15	SP3	Fall 2018	5	0	<i>Convolvulus arvensis</i>	Perennial	Non-Native	dicot	leaf spots, spots on stems	<i>Alternaria eureka</i>	100.00	MH861937.1
16	SP3	Fall 2018	5	7	<i>Plantago lanceolata</i>	Perennial	Non-Native	dicot	necrosis at tips, black blotch or staining	<i>Cladosporium cladosporioides</i>	100.00	MT609901.1
17	SP4	Winter 2018	1	3	<i>Achillea millefolium</i>	Perennial	Native	dicot	chlorotic spots	<i>Fusarium pseudograminearum</i>	100.00	MW341496.1
18	SP4	Winter 2018	2	0	<i>Hypochaeris radicata</i>	Perennial	Non-Native	dicot	leaf spots	<i>Fusarium sp.</i>	99.57	MN944544.1
19	SP4	Winter 2018	3	6	<i>Rumex acetosella</i>	Perennial	Non-Native	dicot	necrotic tips, leaf spots	<i>Fusarium pseudograminearum</i>	100.00	MW341496.1
20	SP4	Winter 2018	4	1	<i>Hypochaeris radicata</i>	Perennial	Non-Native	dicot	leaf spots, black blotch or staining	<i>Plectosphaerella cucumerina</i>	99.54	MH800332.1
21	SP4	Winter 2018	5	7	<i>Plantago lanceolata</i>	Perennial	Non-Native	dicot	chlorotic spots, black staining of entire leaf	<i>Glomium pusillum</i>	100.00	MT635300.1
22	SP5	Spring 2019	3	2	<i>Plantago lanceolata</i>	Perennial	Non-Native	dicot	leaf spots	<i>uncultured Boeremia</i>	99.06	MN065762.1
23	SP5	Spring 2019	3	6	<i>Rumex acetosella</i>	Perennial	Non-Native	dicot	leaf spots, necrosis at tips	<i>Alternaria sp. (MN105546)</i>	100.00	MN105546.1
24	SP5	Spring 2019	5	3	<i>Plantago lanceolata</i>	Perennial	Non-Native	dicot	leaf spots	<i>Stemphylium beticola</i>	99.61	MN401360.1
25	SP5	Spring 2019	5	6	<i>Plantago lanceolata</i>	Perennial	Non-Native	dicot	leaf spots, necrosis at tips	<i>Uncultured fungus clone 4248_749</i>	98.15	MT236706.1
26	SP6	Summer 2019	1	6	<i>Plantago lanceolata</i>	Perennial	Non-Native	dicot	necrosis at leaf tips, leaf spot	<i>Uncultured fungus clone 4248_815</i>	99.20	MT236804.1
27	SP6	Summer 2019	1	6	<i>Cirsium brevistylum</i>	Perennial	Native	dicot	necrosis at leaf tips	<i>Uncultured fungus clone 4248_815</i>	99.71	MT236804.1
28	SP6	Summer 2019	2	1	<i>Hypochaeris radicata</i>	Perennial	Non-Native	dicot	necrosis at leaf tips, leaf spot	<i>Uncultured fungus clone 2_7</i>	100.00	MF155979.1
29	SP6	Summer 2019	2	7	<i>Hypochaeris radicata</i>	Perennial	Non-Native	dicot	necrosis at leaf tips, leaf spot	<i>Aspergillus fumigatus</i>	99.61	MK450249.1
30	SP7	Fall 2019	1	1	<i>Marah fabacea</i>	Perennial	Native	dicot	necrosis at leaf tips, black specks	<i>Phytophthora ramorum</i>	99.26	MT248340.1
31	SP7	Fall 2019	1	4	<i>Fragaria vesca</i>	Perennial	Native	dicot	leaf spot	<i>Cladosporium cladosporioides</i>	99.58	LC325159.1
32	SP7	Fall 2019	5	4	<i>Plantago lanceolata</i>	Perennial	Non-Native	dicot	leaf spots	<i>Epicoccum nigrum</i>	99.51	MT573480.1
33	SP8	Winter 2019	2	3	<i>Plantago lanceolata</i>	Perennial	Non-Native	dicot	necrosis at leaf tips, leaf spot	<i>Uncultured fungus clone Unisequence #69-3488_0180</i>	99.57	GQ524966.1
34	SP8	Winter 2019	3	2	<i>Plantago lanceolata</i>	Perennial	Non-Native	dicot	chlorotic leaf spots	<i>Stemphylium eturmiunum</i>	100.00	MN401375.1

Figure 7 *Percentage of identified plant species showing symptoms.*

Figure 8 Plant tissues displaying symptoms during field surveys. (a) Necrotic leaf tips associated with *Phytophthora ramorum* on *Marah fabacea* (Sample 30); (b) Leaf spots associated with *Rhizoctonia solani* on *Hypochaeris radicata* (Sample 1); (c) Black powdery conidia or necrotic leaf tips associated with *Alternaria infectoria* on *Vinca major* (Sample 8); (d) Leaf spots and necrotic leaf tips associated with *Fusarium pseudograminearum* on *Rumex acetocella* (Sample 19). Photos: G. Huffman

Tissue Culture and Culture Purification

A total of 843 individual tissue sample cultures were prepared during the study. Each tissue samples showed microbial growth after incubation (100%). Typically, 3-4 morphologically distinct microbial cultures grew on each tissue sample. The microbial cultures

were grouped together according to morphological similarities, such as colony color and growth, within the media plates for each of the eight sample periods. Within each sample period, cultures with similar growth characteristics were selected for purification. In total, 34 cultures were selected for purification. An example of an environmental sample which then was purified is shown in Figure 9 (c) and (d). Figure 10 provides example images of conidia produced by conidiophores.

Figure 9 Example Tissue culture and culture purification plates. (a) and (b) Tissue culture plates with multiple morphologically distinct isolates incubated on potato dextrose agar and corn meal agar at 20 °C for 21 days; (c) and (d) Purified cultures incubated on potato dextrose agar with antibiotics at 20 °C for 21 days.

Molecular Detection Using ITS Sequence Analysis

Table 6 provides the molecular identification of the 34 plant associated microorganisms using DNA sequencing and BLAST search analysis. Molecular identification revealed a total of 16 known microbial species and 10 unknown species were present among the plant communities sampled.

The known fungal samples showed identity of 97% or more to *Alternaria alternata*, *Alternaria eureka*, *Alternaria infectoria*, *Alternaria sp. (KX343167)*, *Alternaria sp. (MN105546)*, *Aspergillus fumigatus*, *Cladosporium bruhnei*, *Cladosporium cladosporioides*, *Didymella subherbarum*, *Epicoccum nigrum*, *Fusarium pseudograminearum*, *Fusarium sp.*, *Glonium pusillum*, *Plectosphaerella cucumerina*, *Plectosphaerella oligotrophica*, *Pyrenophora sp.*, *Rhizoctonia solani*, *Stemphylium beticola*, *Stemphylium eturmiunum*, *Stemphylium sp.*, and *uncultured Boeremia* (all of them true fungi or Eumycota), and *Phytophthora ramorum* (an oomycete).

The fungal species with uncertain taxonomic identification ('unknown') are Uncultured fungus clone 2_7, Uncultured fungus clone 4248_749, Uncultured fungus clone 4248_815, and Uncultured fungus clone Unisequence #69-3488_0180.

The BLAST inquiry suggests that Sample 1, with a 98.18% sequence identity, is related to a *Rhizoctonia solani* sample isolated during a microbiome study of the Castillo coffee variety (*Coffea arabica*) in Colombia (MT852563.1).

Sample 2 (99.30% sequence identity) is related to an *Alternaria alternata* isolate collected from *Beta vulgaris* in the USA (MF422130.1).

Sample 3 (99.04%) is related to a *Plectosphaerella oligotrophica* strain GFRS31 previously collected from *Lycium barbarum* in China (MT447499.1).

Sample 4 (99.61%), Sample 5 (99.55%), Sample 26 (99.20%), and Sample 27 (99.71%) are all related to an uncultured fungus clone, "4248_815" isolated from irrigation water from the pond in Lithuania (MT236804.1) (Marčiulynas et. al, 2020) doi:10.3390/f11040459. BLAST lists the closest known related species as *Ectophoma multistrata* with 99% query coverage and a 93% sequence identity.

Sample 6 (100.00%) is related to a *Cladosporium bruhnei* isolate collected from grass in Zimbabwe (MG659641.1).

Sample 7 (100%) is related to an *Aspergillus fumigatus* isolate collected from lake water in Poland (MT635279.1).

Samples 8 (99.31%) and 11 (100.00%) are both related to an *Alternaria infectoria* isolate collected from lake water in Poland (MT635276.1).

Sample 9 (99.30%) is related to a *Didymella subherbarum* isolate collected from *Persicaria punctata* in Costa Rica (KR534651.1).

Sample 10 (99.68%) is related to an unclassified *Pyrenophora sp.* previously collected from *Danthonia californica* in Washington State, USA (MT548668.1).

Sample 12 (99.27%) is related to an *Alternaria infectoria* isolated from *Fagus sylvatica* in Germany (MT561399.1).

Sample 13 (99.62%) is related to an *Alternaria sp.* previously collected from *Quercus ilex* in Spain (KX343167.1).

Sample 14 (100%) is related to an unclassified *Stemphylium sp.* previously collected, in Catalonia, Spain (MT556676.1).

Sample 15 (100.00%) is related to an *Alternaria eureka* from a culture collection in Australia (MH861937.1).

Sample 16 (100.00%) is related to a *Cladosporium cladosporioides* sample isolated from a composting pile in Spain (MT609901.1).

Sample 17 (100.00%) and Sample 19 (100.00%) both are related to a *Fusarium pseudograminearum* sample isolated from a wheat plant in China (MW341496.1).

Sample 18 (99.57%) is related to an unclassified *Fusarium sp.* collected from *Thymus mongolicus* in China (MN944544.1).

Sample 20 (99.54%) is related to an *Plectosphaerella cucumerina* isolated from *Spirobolus bungii* (MH800332.1).

Sample 21 (100%) is related to an *Glonium pusillum* isolated from lake water in Poland (MT635300.1).

Sample 22 (99.06%) is related to an uncultured *Boeremia* from *Solanum tuberosum* in Estonia (MN065762.1). NCBI GenBank describes this uncultured *Boeremia* as an environmental sample collected on 2017-08-02, no publications featuring the environmental sample exist at the time of this writing.

Sample 23 (100%) is related to an unclassified *Alternaria sp.* previously collected from *Fagus sylvatica* in Denmark (MN105546.1).

Sample 24 (100.00%) is related to a *Stemphylium beticola* from a culture collection in Australia (MN401360.1).

Sample 25 (100%) is related to an Uncultured fungus clone “4248_749” previously collected from irrigation water in Lithuania (MT236706.1) (Marčiulynas et. al, 2020). BLAST data indicates the closest known genus as a *Cladosporium sp.* isolate with 97% query cover and a 96.90% identity.

Sample 28 (100%) is related to Uncultured fungus clone “2_7” previously collected from spices in Lithuania (MF155979.1). BLAST lists the closest known related genus as *Cladosporium sp.* with 100% query coverage and a 99.82% identity. Uncultured fungus clone 2_7 is listed only as an environmental sample, no publications featuring the environmental sample exist at the time of this writing.

Sample 29 (99.61%) is related to an *Aspergillus fumigatus* strain (F03YD) isolated from deep-sea sediment of the Pacific Ocean in Poland (MK450249.1).

Sample 30 (99.30%) is related to a *Phytophthora ramorum* isolate collected from a *Arctostaphylos morroensis* in Santa Cruz County, California, USA (MT248340.1).

Sample 31 (99.58%) is related to a *Cladosporium cladosporioides* sample isolated from linen wrapping of mummies in Egypt (LC325159.1).

Sample 32 (99.51%) is related to an *Epicoccum nigrum* isolate from *Rubus idaeus* in Poland (MT573480.1).

Sample 33 (99.57) is related to Uncultured fungus clone Unisequence #69-3488_0180 which has been collected from the phyllosphere of *Quercus macrocarpa* (GQ524966.1). BLAST lists the closest known related species as *Alternaria longipes* with 100% query coverage and a 99.64% identity. Uncultured fungus clone Unisequence #69-3488_0180 is listed only as an environmental sample collected May-September 2008, no publications featuring the environmental sample exist at the time of this writing.

Sample 34 (100.00%) is related to a *Stemphylium eturmiunum* isolate collected from *Lens culinaris* in Australia (MN401375.1).

Figure 10 Colony morphology. Conidiophore and conidia of *Alternaria alternata* and *Cladosporium cladosporioides*. (a) Colony morphology of *A. alternata*; (b) Conidiophore of *A. alternata*; (c) Conidium of *A. alternata*; (d) Colony morphology of *C. cladosporioides*; (e) Conidiophore of *C. cladosporioides*; and (f) Conidia of *C. cladosporioides*. Photos: G. Huffman

The phylogenetic analysis of the 34 samples indicates a significant homology between the 16 identified species where most of the tree nodes have a bootstrap support of at least 98% (Figure 11). The significance in homology displays genetic similarities among the species identified in the study in comparison to NCBI GenBank sequences. The evolutionary history was inferred using the Neighbor-Joining method (Saitou & Nei, 1987). The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed (Felsenstein, 1985). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (1985). The evolutionary distances were computed using the Maximum Composite Likelihood method

(Tamura et al., 2004) and are in the units of the number of base substitutions per site. This analysis involved 63 nucleotide sequences. All positions with less than 95% site coverage were eliminated, i.e., fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position (partial deletion option). There was a total of 140 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar et al., 2018).

Figure 11 Evolutionary relationships of microbial isolates based on ITS1 sequences. The best BLAST-match for each environmental sample was included in the analysis.

As shown in Table 7, most microbial strains isolated from herbaceous plants belong to the taxonomic group *Ascomycota* (20 out of 26). These include *Alternaria alternata*, *Alternaria eureka*, *Alternaria infectoria*, *Alternaria sp.* (KX343167), *Alternaria sp.* (MN105546), *Aspergillus fumigatus*, *Cladosporium bruhnei*, *Cladosporium cladosporioides*, *Didymella subherbarum*, *Epicoccum nigrum*, *Fusarium pseudograminearum*, *Fusarium sp.*, *Glonium pusillum*, *Plectosphaerella cucumerina*, *Plectosphaerella oligotrophica*, *Pyrenophora sp.*, *Stemphylium beticola*, *Stemphylium eturmiunum*, *Stemphylium sp.*, and uncultured *Boeremia*. Of the six remaining microbes, *Rhizoctonia solani* belongs to taxonomic group *Basidiomycota*. Both *Ascomycota* and *Basidiomycota* belong to the so-called ‘true fungi’, Kingdom *Fungi*. *Phytophthora ramorum* belongs to taxonomic group *Oomycota* (also known as ‘water molds’), Kingdom *Stramenopila*. Four microorganisms were found to be closely related to “uncultured fungi” or unclassified fungi having no official taxonomic position. Despite the lack of an official classification, the taxonomic position of the isolates can be defined. As such, the official species matching the highest percent identity nearest the “uncultured fungi” are all within the *Ascomycota*. BLAST indicates Uncultured fungus clone 2_7 and Uncultured fungus clone 4248_749, both probably belonging to the family *Cladosporiaceae*; Uncultured fungus clone 4248_815 probably belonging to *Didymellaceae*; and Uncultured fungus clone Unisequence #69-3488_0180 likely belonging to *Pleosporaceae*.

The relative abundance of taxonomic groups is shown in Figure 12: 77% of the microorganisms belong to the *Ascomycota*, 4% belong to *Basidiomycota*, 4% of microorganisms to the *Oomycota*, and 15% of microorganisms are of unknown taxonomic grouping.

Table 7 Taxonomic classification of microbial strains isolated from herbaceous plants (Dot: belongs to taxonomic group; Blank: does not belong to taxonomic group).

Microorganisms Found	Taxonomic Group			
	Ascomycota	Basidiomycota	Oomycota	Uncultured fungus
<i>Alternaria alternata</i>	•			
<i>Alternaria eureka</i>	•			
<i>Alternaria infectoria</i>	•			
<i>Alternaria sp. (KX343167)</i>	•			
<i>Alternaria sp. (MN105546)</i>	•			
<i>Aspergillus fumigatus</i>	•			
<i>Cladosporium bruhnei</i>	•			
<i>Cladosporium cladosporioides</i>	•			
<i>Didymella subherbarum</i>	•			
<i>Epicoccum nigrum</i>	•			
<i>Fusarium pseudograminearum</i>	•			
<i>Fusarium sp.</i>	•			
<i>Glonium pusillum</i>	•			
<i>Phytophthora ramorum</i>			•	
<i>Plectosphaerella cucumerina</i>	•			
<i>Plectosphaerella oligotrophica</i>	•			
<i>Pyrenophora sp.</i>	•			
<i>Rhizoctonia solani</i>		•		
<i>Stemphylium beticola</i>	•			
<i>Stemphylium eturmiunum</i>	•			
<i>Stemphylium sp.</i>	•			
<i>uncultured Boeremia</i>	•			
<i>Uncultured fungus clone 2_7</i>				•
<i>Uncultured fungus clone 4248_749</i>				•
<i>Uncultured fungus clone 4248_815</i>				•
<i>Uncultured fungus clone Unisequence #69-3488_0180</i>				•

Figure 12 Percent of taxonomic grouping of the isolates. *Taxonomic classification of isolated microbial strains.*

Most (96%) microbial strains were isolated from perennial plants with 85% isolated from non-native plants, and 96% isolated from dicots (Table 8, Figure 13). *P. lanceolata* displayed the highest host-microbe diversity with a total of 10 different microbial species isolated from tissues collected across all transect sample points (Figure 14).

Table 8 Ecological Parameters of the Host Plants of the Microbial Species Identified During the Study.

	Life Cycle			Geographic Origin			Flowering Plant Class	
	Annual	Perennial	Both Annual and Perennial	Native Host	Non-Native Host	Both Native and Non-Native	Monocot	Dicot
<i>Alternaria alternata</i>		•		•			•	
<i>Alternaria eureka</i>		•			•			•
<i>Alternaria infectoria</i>		•			•			•
<i>Alternaria sp. (KX343167)</i>		•			•			•
<i>Alternaria sp. (MN105546)</i>		•			•			•
<i>Aspergillus fumigatus</i>	•	•	•		•			•
<i>Cladosporium bruhnei</i>	•			•				•
<i>Cladosporium cladosporioides</i>		•		•	•	•		•
<i>Didymella subherbarum</i>		•			•			•
<i>Epicoecum nigrum</i>		•			•			•
<i>Fusarium pseudograminearum</i>		•		•	•	•		•
<i>Fusarium sp.</i>		•			•			•
<i>Glomium pusillum</i>		•			•			•
<i>Phytophthora ramorum</i>		•		•				•
<i>Plectosphaerella cucumerina</i>		•			•			•
<i>Plectosphaerella oligotrophica</i>		•		•				•
<i>Pyrenophora sp.</i>		•			•			•
<i>Rhizoctonia solani</i>		•			•			•
<i>Stemphylium beticola</i>		•			•			•
<i>Stemphylium eturmiunum</i>		•			•			•
<i>Stemphylium sp.</i>		•			•			•
uncultured <i>Boeremia</i>		•			•			•
Uncultured fungus clone 2_7		•			•			•
Uncultured fungus clone 4248_749		•			•			•
Uncultured fungus clone 4248_815	•	•	•	•	•	•		•
Uncultured fungus clone Unisequence#69-3488_0180		•			•			•

Figure 13 Host plant preference. (a) microbial strains associated with annual plants (green), perennial plants (red) or both (blue); (b) microbial strains associated with native host plants (green), non-native host plants (red) or both (blue); (c) microbial strains associated with monocot plants (green), dicot host plants (red) or both (blue).

Figure 14 Number of Microbial Species Isolated from Host Plants Each Season from All Transects.

A. fumigatus appeared in successive summers, and *C. cladosporioides*, appeared in successive falls (Table 9). The only other microbe to appear twice or more was Uncultured fungus clone 4248_815, which was present during successive springs and successive summers 2019. No strain was isolated in more than 2 seasons.

Figure 15 reveals the seasonal variation of microbial species on the five transects: 5 microbial species were identified by six sample isolations during Spring 2018 (18%); 4 microbial species were identified by six sample isolations during Summer 2018 (18%); 5 microbial species were identified by five sample isolations during Fall 2018; 4 microbial species were identified by five sample isolations during Winter 2018-2019; 3 microbial species were identified by three sample isolations during Spring 2019 (9%); 3 microbial species were identified by four sample isolations during Summer 2019; 3 microbial species were identified by three sample isolations

during Fall 2019; and 2 microbial species were identified by two sample isolations during Winter 2019-2020.

Table 9. Seasonal distribution of microbial diversity. Dot: the microbial species was identified during that season; Blank: microbial species was not identified during that season. Results are presented for both sampling years separately.

	<i>Alternaria alternata</i>	<i>Alternaria eureka</i>	<i>Alternaria infectoria</i>	<i>Alternaria</i> sp. (KX343167)	<i>Alternaria</i> sp. (MN105546)	<i>Aspergillus fumigatus</i>	<i>Cladosporium bruhnei</i>	<i>Cladosporium cladosporioides</i>	<i>Didymella subherbarum</i>	<i>Epicoecum nigrum</i>	<i>Fusarium pseudograminearum</i>	<i>Fusarium</i> sp.	<i>Glomus pusillum</i>	<i>Phytophthora ramorum</i>	<i>Plectosphaerella cucumerina</i>	<i>Plectosphaerella oligotrophica</i>	<i>Pyrenophora</i> sp.	<i>Rhizoctonia solani</i>	<i>Stemphylium beticola</i>	<i>Stemphylium eturmiunum</i>	<i>Stemphylium</i> sp.	uncultured <i>Boeremia</i>	Uncultured fungus clone 2_7	Uncultured fungus clone 4248_749	Uncultured fungus clone 4248_815	Uncultured fungus clone Unisquence # 69-3488_0180
Spring 2018	•						•									•		•								•
Summer 2018			•			•			•								•									
Fall 2018		•		•	•			•													•					
Winter 2018-2019											•	•	•		•											
Spring 2019																			•			•		•		
Summer 2019						•																	•		•	
Fall 2019								•		•				•												
Winter 2019-2020																				•						•

Figure 15 Seasonal variation of microbial species identified on the five transects (2018 and 2019).

DISCUSSION

It is necessary to understand plant pathogens and how they interact with host plants in the environment. This research provides a necessary first step to aid later research aimed at understanding pathogen effects on the biodiversity of natural herbaceous plant communities and understanding biological and ecological constraints that may be used to develop management tools related to disease management. The health of herbaceous plant communities within natural ecosystems deserves more attention than it has previously received. This research is significant because it directs the much-needed attention towards the relationship between natural herbaceous plant communities and plant pathogens. The general purpose of this research is to save native biodiversity and prevent non-native infections by detecting growth patterns and identifying plant pathogens within herbaceous plant communities in the GGNRA. This study provides further qualitative information that pathogens occur within natural communities. This research was designed to assess the hypothesis that plant pathogens are present among herbaceous plant communities within the Golden Gate National Recreation Area (GGNRA), Marin County, California. To accomplish this goal the following objectives were met (1) Plant tissue collection to identify the presence of pathogens; (2) Pathogenic species identification through microbiological and molecular analysis; (3) Identify possible effects of geographic variation on the presence of pathogens on herbaceous vegetation (4) Identify possible effects of seasonal variation on the presence of pathogens on herbaceous vegetation; (5) Determine through qualitative (descriptive) analysis if pathogens identified, ordinate in relationship to various geographic variables.

There is a significant scientific body of knowledge regarding the presence of plant pathogens and their effects on agricultural and landscape plants. Less is known about plant

pathogens associated with natural plant communities. During the study, I focused on a range of sample sites with characteristics based on coastal scrub habitat in coastal California. Transect locations were randomly chosen based on factors including, vegetation type, orientation of topography, and accessibility.

Based on the descriptive species diversity statistics on soil type and plant community cover across all sampling periods, there appear to be differences in diversity based on the soil and plant community types. However, further analyses are needed based on the findings to determine any statistical significance. A comparison based on physical parameters of soils to determine significant differences between species absent and species present was performed. Eight soil types related to respective transects were examined and no significant correlation between all physical soil characteristics and microbial species was present. A focus on a specific herbaceous plant genera or species could possibly reveal a relationship between environmental factors affecting plant host and microbial presence in this study or similar studies focused on plant-microbe interactions in nature. Perhaps under the right soil conditions where physical parameters such as drainage and runoff classifications allow for the optimal amount of moisture required in sustaining a microbe rich rhizosphere thus allow forb communities within a biodiverse setting to remain among known pathogens.

The higher percentage of non-native (61%) identifications among the transects could be due to a decline in native species in the area. It is possible that the greater numbers of non-native species are indicative of their ability to adapt to site conditions and outcompete native species. The high native species richness has declined and the abundance of non-native species within coastal California is now significant. An anthropogenic influence has contributed to the decline in native vegetation in GGNRA, resulting in a shift to European Mediterranean regional annual

species (Pawley & Lay, 2013). Many non-native tree species like eucalyptus, Monterey cypress, Monterey pine, and herbaceous species were introduced into the area nearly 150 years ago as anthropogenic influence became more prevalent, significantly changing the native environment (Seabloom et al., 2006). GGNRA is located within a globally significant region of biodiversity, the California Floristic Province (Myers et al., 2000; Steers, 2016). Conserving biodiversity amongst the world's representative examples of native concentrations and endemics is considered an ecologically important priority (Olson & Dinerstein, 2002).

Perennials by their very nature occupy the same space each year reducing the amount of available area for annuals. It is possible that perennials capture nutrients more efficiently, given their long-lasting life cycle. Their level of exposure to amounts of infection is greater. Similarly, this study found a greater number of dicots identified. It is possible that the greater number of dicots is related to a perennial life cycle.

Symptoms identified included leaf spots, necrotic leaf tips, and color variations of tissues. Only 23% of species identified during the study showed symptoms during the study. Leaf spots (85%) were the most common indicators among tissues observed through all seasons. A total of 14 herbaceous plant species showed disease symptoms, 7 native herbaceous plant species and 7 non-native species. Most (96%) microbial strains were isolated from perennial plants; with 85% isolated from non-native plants; and 96% isolated from dicots. All but two of the non-native species to display symptoms were perennials. The majority (74%) of isolates were cultured from perennial non-native tissues. This result suggests a potential preference for perennial non-native species. The preference possibly is related to senescence and the ability to resist pathogens as the plant ages during the perennial life cycle. Research does shed some light on the difficulties in determining pathogen resistance among perennials in a natural setting. It is possible that

perennials are more susceptible to the presence of pathogens due to age and genotype (Susi & Laine, 2015). Research (Whipps et al., 2008) suggests that a plant's genotype is potentially a greater determinate in development of microbial communities within the phyllosphere. More research into performance of perennial herbaceous species resistance among pathogenic fungi is needed to better understand this relationship. In this research, the perennial *P. lanceolata* was revealed to display symptoms during 7 of the 8 seasons sampled. Additionally, *P. lanceolata* displayed the highest host-microbe diversity with a total of 10 different microbial species isolated from tissues collected across all transect sample points. Perhaps a focus on a specific plant like the non-native *P. lanceolata* will further help develop a better understanding of host plant interaction.

Future studies into taxonomic groupings could further assist with determining successful treatment methods within areas like GGNRA. The observed dominance of *Ascomycota* among the plant-associated microorganisms could be due to its scale of species diversity, as *Ascomycota* is an extremely large taxonomic group that it is not surprising a greater number of representatives from this group that have environmental characteristics compatible for growth. Environmental conditions in the study area are possibly more favorable to the life cycles of *Ascomycetes* in contrast to two examples of *Basidiomycota* and *Oomycota* observed in the study. Focused work on the identification of the four species with unknown taxonomic groupings would aid in better understanding of species relationship to plant biodiversity in natural ecosystems. Additionally, most isolates (*Alternaria sp.*, *Aspergillus sp.*, *Cladosporium sp.*, *Epicoccum sp.*, *Fusarium sp.*, and *Stemphylium sp.*) were categorized as hyphomycetes, where many species are among the most ecologically and economically important fungal plant pathogens. These fungal species are unique in that conidia are produced on open conidiophores,

and not in closed fruiting bodies (pycnidia). Several of this fungal species are commonly known as ‘fungi imperfecti’ because no sexual stage is known. However, neither “fungi imperfecti” nor “fungi perfecti” (for which both asexual and sexual stages are known) are valid taxonomic units. The Marin Headlands area within GGNRA experiences high winds and heavy rainfall events during winters. Additionally, the coastal fog belt allows for moisture conditions to remain at a constant during sunrise and sundown. It is possible that open conidiophores provide a more suitable method for transmission in an open environment. A particular stage of senescence may provide vulnerabilities in a plants surface structure to allow for inoculation to occur. Senescence within the herbaceous species observed becomes readily identifiable by the beginning of summer (June).

Achillea millefolium, *Carduus pycnocephalus*, *Chlorogalum pomeridianum*, *Eschscholzia californica*, *Fragaria vesca* L., *Galium aparine*, *Hypochaeris radicata*, *Marah fabacea*, *Plantago lanceolata*, *Rumex acetosella*, and *Vinca major* were all found to host plant pathogens known for their effects on other plant species along a cosmopolitan range outside the GGNRA.

The host-pathogen relationships are summarized as follows:

Table 9 Summary of Host-Pathogen Relationship.

Plant Species	Associated Microorganism
<i>Achillea millefolium</i>	<i>Fusarium pseudograminearum</i>
<i>Carduus pycnocephalus</i>	<i>Aspergillus fumigatus</i>
<i>Chlorogalum pomeridianum</i>	<i>Alternaria alternata</i>
<i>Cirsium brevistylum</i>	Uncultured fungus clone 4248_815
<i>Convolvulus arvensis</i>	<i>Alternaria eureka</i> ; <i>Alternaria</i> sp. (KX343167); Uncultured fungus clone 4248_815
<i>Eschscholzia californica</i>	<i>Plectosphaerella oligotrophica</i>
<i>Fragaria vesca</i>	<i>Cladosporium cladosporioides</i>
<i>Galium aparine</i>	<i>Cladosporium bruhnei</i>
<i>Geranium dissectum</i>	Uncultured fungus clone 4248_815
<i>Hypochaeris radicata</i>	<i>Aspergillus fumigatus</i> ; <i>Fusarium</i> sp.; <i>Plectosphaerella cucumerina</i> ; Uncultured fungus clone 2_7; <i>Rhizoctonia solani</i>
<i>Marah fabacea</i>	<i>Phytophthora ramorum</i>
<i>Plantago lanceolata</i>	<i>Alternaria infectoria</i> ; <i>Cladosporium cladosporioides</i> ; <i>Epicoccum nigrum</i> ; <i>Glonium pusillum</i> ; <i>Stemphylium beticola</i> ; <i>Stemphylium eturmiunum</i> ; uncultured <i>Boeremia</i> ; Uncultured fungus clone 4248_749; Uncultured fungus clone 4248_815; Uncultured fungus clone Unisequence #69-3488_0180
<i>Rumex acetosella</i>	<i>Alternaria infectoria</i> ; <i>Alternaria</i> sp. (MN105546); <i>Didymella subherbarum</i> ; <i>Fusarium pseudograminearum</i> ; <i>Pyrenophora</i> sp.; <i>Stemphylium</i> sp.
<i>Vinca major</i>	<i>Alternaria infectoria</i>

Alternaria is a genus comprising common saprobic, endophytic, and pathogenic organisms that are found globally in warm, humid climates. They cause leaf spot symptoms which typically occur in the late summer months across a wide host range of plants (Woudenberg et al., 2013). Both *A. alternata* and *A. infectoria* are known to be pathogenic on two *Brassica* species (*B. napus* and *B. juncea*) harvested for Canola production in Australia (Al-Lami et al., 2019). Recent molecular analysis using the ITS region found that *A. alternata* and *A. eureka* are associated with apple core rot in South Africa (Basson et al., 2019). *A. alternata* was first reported as the causal agent of leaf spot on *Gossypium* spp. (cotton) in southern New

Mexico. (Zhu et al., 2019) *A. alternata* is associated with post-harvest rot of blueberries in California (Wang, F. et al., 2021). A study showed exposure to *A. alternata* was the lead sensitizer of child asthma sufferers (38%) in the United States (Salo et al., 2006). Molecular analysis revealed *A. infectoria* species group to be the cause of *Alternaria* leaf spot in *Triticum aestivum* (winter wheat) in the United States (Fulcher et al., 2017). To my knowledge, *A. eureka* was never found in the U.S.; it has been associated with apples in South Africa as mentioned above, and with native and introduced plant species in Australia (*Anigozanthus* sp., *Triglochin procera*, *Medicago rugosa*), and New Zealand (*Geniostoma* sp., *Leptinella dioica*) (Woudenberg et al., 2013). To my knowledge *A. alternata* has never been associated with necrotic tips or leaf spot symptoms occurring on *C. pomeridianum* in California or the United States. To my knowledge *A. infectoria* has never been associated with leaf symptoms occurring on *R. acetosella* (Sheep's Sorrel) or *V. major* (Greater periwinkle) in California or the United States. It should be noted that *Alternaria* spp. are associated with *Rumex crispus* (curled dock) in Mississippi (Roy et al., 1994).

Aspergillus spp. are among the most widely distributed micro-organisms on earth. Most *Aspergillus* species are not considered to cause plant disease, yet some species are associated with various disorders in plant species and products of economic interest (Perrone & Gallo, 2017). *Aspergilli* are named as the primary producing agents for aflatoxins, which create toxicities harmful to humans and animals, amongst agriculturally produced foods around the globe (Sarma et al., 2017). *A. fumigatus* produces a highly cytotoxic allergen called Asp f1 and can cause diseases in immunocompromised hosts (Madan et al., 1997). *A. fumigatus* is considered a common opportunistic soil born plant pathogen (Fang & Latgé, 2018). The presence of *A. fumigatus* has recently been associated with bananas, the fourth most

economically important crop in today's agriculture (Ali et al., 2021). To my knowledge *A. fumigatus* has never been associated with leaf spot symptoms occurring on *C. pycnocephalus* in California or *H. radicata* (Common cat's-ear) in California or the United States.

Cladosporium bruhnei is widely distributed among both living and decaying plant material of multiple genera and families found in temperate regions (Farr & Rossman, 2021). In California, *C. cladosporioides* has been linked to postharvest disease (brown spot) of late season table grape (*Vitis vinifera*) in the California central valley (Swett et al., 2016). Leaf spot symptoms were observed on *F. vesca* (California strawberry) and tissues were collected in Fall 2019. Culture isolates resulted in *C. cladosporioides*, which has been linked to blossom blight of strawberry in California (Gubler et al., 1999). To my knowledge *C. bruhnei* has never been associated with leaf symptoms occurring on annual native species *G. aparine* (Catchweed bedstraw) in California or the United States. To my knowledge *C. cladosporioides* has never been associated with leaf symptoms occurring on *P. lanceolata* (Ribwort) in California or the United States.

Didymella subherbarum is closely related to *D. corylicola*, a fungus newly associated with hazelnut fruit symptoms in Italy (Scarpari et al., 2020). To my knowledge *D. subherbarum* has never been associated with leaf symptoms occurring on *R. acetosella* (Sheep's Sorrel) in California or the United States.

Epicoccum nigrum is an endophyte and considered a plant pathogen with a cosmopolitan range of distribution. It is a saprophytic fungus thriving on dead or dying plant tissues where pustules containing sporodochia and conidia are evident (Schol-Schwarz, 1959). The species relies on a diverse host range of economically important crops in the US (Farr & Rossman, 2021). *E. nigrum* is associated with respiratory fungal allergies in humans (Kurup et al., 2000).

Culture morphology of *E. nigrum* displayed suede-like to downy, with a strong yellow to orange-brown diffusible pigment on potato dextrose agar after 15-21 days at 20° C. Numerous black sporodochia are typically visible but were not found in this study. To my knowledge *E. nigrum* has never been associated with leaf symptoms occurring on *P. lanceolata* (Ribwort) in California or the United States.

Fusarium spp. are filamentous fungi, many of its species are among the most important fungal plant pathogens. In the United States, *F. pseudograminearum* is prevalent in warm, dry areas within the Pacific Northwest United States where its presence is linked to chronic disease among cereal crops (Backhouse & Burgess, 2002; Cook, 1980; Smiley et al., 2005; Yin et al., 2020). The species was observed on shoot tissue sample during a survey of fungal endophyte communities inhabiting native switchgrass plants from the tallgrass prairie of northern Oklahoma (Ghimire et al., 2010). The three *Fusarium* samples displayed a red-shaded thallus on potato dextrose agar after incubation for 15-21 days at 20° C. Cottony aerial mycelia were present amongst the samples and sickle-shaped macroconidia were observed using a light microscope. A *F. circunatum* was detected on the seeds of *H. radicata* (Hernandez-Escribano et al., 2018). The study suggests a vertical transmission of *Fusarium sp.* to be possible, potentially validating the reasoning of presence and observation of leaf symptoms of *H. radicata* in this study. To my knowledge *F. pseudograminearum* has never been associated with leaf symptoms occurring on the native species *A. millefolium* (Common yarrow) in California or the non-native *R. acetosella* (Sheep's Sorrel) in California or the United States.

Farr and Rossman (2021) indicate that reports of *Glonium pusillum* distribution are scattered. The species was associated with *Yushania niitakayamensis* (Hayata) in Taiwan

(Sivanesan & Hsieh, 1989) To my knowledge *G. pusillum* has never been associated with leaf symptoms occurring on *P. lanceolata* (Ribwort) in California or the United States.

Phytophthoras is a genus belonging to the *Oomycetes* with many species, some of them very aggressive pathogens of native plants and agricultural crops. Several *Phytophthora* species which have emerged in the U.S. in recent years, among them *P. ramorum* which causes Sudden Oak Death (SOD) on native oak and tan oak populations on the west coast in North America (Grünwald et al., 2012), and now threatens rare habitats and conservation plantings (Frankel et al., 2020) by the inadvertent introduction of the species to restoration areas in California (Frankel et al., 2018). Molecular and phylogenetic analysis has recently concluded that *P. ramorum* is now linked in origin to East Asia (Jung et al., 2021). While *P. ramorum* has mainly been associated with its effect on tree species, the first report of leaf blight on *Vinca minor* (Periwinkle) caused by *P. ramorum* has occurred recently in Washington State in the USA (Elliott et al., 2021). *P. ramorum* is widespread in Marin County on several host plants, not only coast live oaks and tanoaks, but also foliar hosts like California bay laurel. *P. ramorum* was recently also detected on several manzanita species (Genus *Arctostaphylos*), which are a dominant plant group in the chaparral ecosystem in Coastal California. An annual citizen science program, called SOD Blitz, monitors the spread of *P. ramorum* in forested areas, which are traditionally the habitats most affected by SOD. *P. ramorum* is known to spread during the rainy season and needs water on the plant surface for the infection process. The detection of *P. ramorum* on an herbaceous plant in the coastal grasslands could be a hint that the pathogen is expanding its range and could maybe in future become a threat to native plants in those ecosystems. To my knowledge *P. ramorum* has never been associated with leaf symptoms occurring on the native species *Marah fabacea* (Manroot) in California or the United States.

Plectosphaerella is a widely distributed genus of filamentous fungi that are well-known as plant pathogens of various plants (Wang, Q. et al., 2019). *P. cucumerina* has been identified as the causal pathogen of diseases, leading to large losses of crops of economic importance (Carrieri et al., 2014; Garibaldi et al., 2013; Li et al., 2017; Xu et al., 2014). *P. oligotrophica* previously only has been isolated from soil (Giraldo & Crous, 2019; Liu, T. et al., 2013) and was collected during spring 2018 on a south-facing, sloped area within coastal scrub habitat. Yellowing of leaf commonly associated with *P. oligotrophica* (Raimondo & Carlucci, 2018) and necrosis at the tips were visually observed on *Eschscholzia californica* (California poppy), a dicot and perennial native herb. To my knowledge *P. cucumerina* has never been associated with leaf symptoms occurring on the introduced species *H. radicata* (Common cat's-ear) or the annual/perennial native species *E. californica* in California or the United States.

Some *Pyrenophora* species are known to infect wheat plants in Argentina (Perelló et al., 2019). To my knowledge no *Pyrenophora* sp. has never been associated with leaf symptoms occurring on *R. acetosella* (Sheep's Sorrel) in California or the United States.

Rhizoctonia solani is a common plant pathogenic fungus known to cause various plant diseases amongst a cosmopolitan herbaceous host range (Sneh et al., 1991). The fungus is usually found on herbaceous hosts in warm, wet climates. Outbreaks occur during summer and observable symptoms typically appear in late summer (Cubeta & Vilgalys, 1997) *R. solani* has been associated with melon root rot in California (Aegerter et al., 2000), and with stem canker on Buckwheat in Maine (Zhang et al., 2016). Most recently, the pathogen was found to be associated with root and stem rot of *Stevia rebaudiana* (sweetleaf) in Delaware and Maryland (Kessler & Koehler, 2020). To my knowledge *R. solani* has never been associated with leaf symptoms occurring on *H. radicata* (Common cat's-ear) in California or the United States.

Stemphylium spp. are saprophytic fungi which are associated globally among decaying crop vegetation and with the soil. *S. eturmiunim* was reported to cause postharvest rot of garlic sprouts in China (Fu et al., 2019). *S. eturmiunim* was discovered to be associated with black or “sooty” head mold of cereals in Iran (Poursafar et al., 2016). *S. beticola* and *S. eturmiunim* are newly associated with legume crops in Australia (Vaghefi et al., 2020). Pathogenicity of *S. beticola* is found to be severe on lupines worldwide and mild among lentil crops in Australia (2020). *S. beticola* is also associated with leaf spot of spinach in Italy (Farr & Rossman, 2021; Gilardi et al., 2018), and the United States (Liu, B. et al., 2020; Spawton et al., 2020; Woudenberg et al., 2017). *S. eturmiunim* is a pathogen of fava bean in Iran (Poursafar et al., 2016). To my knowledge *S. beticola* has never been associated with leaf symptoms occurring on *P. lanceolata* (Ribwort) in California or the United States. To my knowledge *S. eturmiunim* has never been associated with leaf symptoms occurring on *P. lanceolata* (Ribwort) in California or the United States. To my knowledge any *Stemphylium sp.* has never been associated with leaf symptoms occurring on *R. acetosella* (Sheep's Sorrel) in California or the United States.

Based on the results of the identification of the microbes listed above, the lack of determined associations with plant species is likely based on the lack of research into herbaceous species in the United States and California, which historically received little priority. Both PDA and CMA media proved to be effective in culturing sample tissues during the study. However, not all tissue cultures were selected for microbial isolation. A total of 34 samples were selected based on representing an abundance of culture characteristics and purified into isolated cultures using PDA + AB. Morphological identification was sometimes difficult. Out of 34 microbial strains just 11 produced conidia in pure culture. In some cases, conidia are produced under specific environmental conditions. Therefore, I could just identify the spore type of vary few

fungi, making morphological identification difficult. DNA sequence data proved to be valuable for identification of microbial strains in contrast to morphological identification. Also, often the conidia or other morphological structures are very similar within one genus (or between closely related genera) therefore molecular identification using ITS sequences proved to be a very efficient and helpful method.

Based on the results of this study, there was no clear seasonal relationship of the various microbial species to pathogen infection. *A. fumigatus* appeared in successive summers, and *C. cladosporioides* was isolated in successive falls. This may indicate a relationship of infection to dryer conditions when plant growth is less active. The only other microbe to appear twice, or more was Uncultured fungus clone 4248_815, which was present during successive springs and summers. This may indicate an ability to infect a host during both active and less active growth periods. No strain was isolated in more than 2 seasons. The rainy season in northern California typically occurs between October and April, however over the past several years the rainy season has shifted to November through April (Duginski, 2021). Most of the rainfall in Marin County occurs December through February (*Climate and average monthly weather in San Francisco (California)*. 2021). It should be noted that seasonal data (CIMIS #157, 2020) for the months of January and March from the year prior to the study (2017) resulted in an above normal rainfall amount. Based on the moist conditions occurring the year before the start of this study, it is possible the above normal rainfall resulted in the growth of more biomass and enabled higher microbial species counts. Whereby, there were more pathogens (22) found in 2018 than in 2019 (11). The higher rainfall amount in the year before this study may support the theory that increased biomass equates to a high microbial presence. In northern California, the onset of the growing season for vegetation typically occurs in January for native and non-native species,

following an initial heavy rain (> 100mm / 24hrs). More research is needed to develop a better understanding of how strong winds, heavy rainfall and associated storm water runoff, and fog may affect pathogen spread in the GGNRA.

This research, while focusing on microbial presence among a broad range of plant species in the natural environment, provides only limited insight regarding their effects on biodiversity within this ecosystem on the northern California coast. This coastal ecosystem, with its temperate climatic setting and history of intermittent human activities, influences significant species biodiversity but is also an example for the introduction of invasive species into our natural habitat. Future studies could focus on the native herbaceous species where research into host specificity and pathogenicity could be looked at in greater detail. The microbial associations with host plant species found during this study raises questions as to how they came into their relationships. *P. ramorum* and its association with *M. fabacea* (Manroot), as the dispersal method of how *P. ramorum* transmits from tree to herbaceous species, and vice versa, could perhaps be of significance. Microbiota among a plant's leaf tissues could utilize numerous methods to crossover from an epiphytic to an endophytic lifestyle (Chaudhry et al., 2021). It is possible that *P. ramorum* seized on an opportunity to infect *M. fabacea* through stomal openings. A direct focus on *P. lanceolata* and its host association with multiple pathogens across coastal California study areas can potentially provide further knowledge of non-native plant host characteristics within a natural setting.

Furthermore, many restoration projects have been implemented in recent years to restore the GGNRA using native coastal California plant species. Introducing new native and non-native plants poses the risk of introducing also new plant pathogens that are likely to affect a previously disturbed and less resilient area (Frankel et al., 2020; Jung et al., 2020). Data from a long-term

study on the presence of *Phytophthora* species in European nurseries indicate plantings during habitat restoration efforts have introduced plant pathogens in natural communities (Jung et al., 2016). The National Park Service has a mandate to protect natural resources. Sampling for plant pathogens is essential to gain a better understanding for how to focus research leading to the development of science-based, practicable adaptive management strategies to satisfy this requirement. This research project should provide resource managers with valuable information on the presence of plant pathogens within herbaceous species in the GGNRA and help to reduce the negative impact of plant diseases on the native vegetation.

REFERENCES

- Aegerter, B. J., Gordon, T. R., & Davis, R. M. (2000). Occurrence and Pathogenicity of Fungi Associated with Melon Root Rot and Vine Decline in California. *Plant Disease*, 84(3), 224-230. 10.1094/PDIS.2000.84.3.224
- Al-Lami, H. F. D., You, M. P., & Barbetti, M. J. (2019). Incidence, pathogenicity and diversity of *Alternaria* spp. associated with alternaria leaf spot of canola (*Brassica napus*) in Australia. *Plant Pathology*, 68(3), 492-503. <https://doi.org/10.1111/ppa.12955>
- Alexander, H. M. (2010). Disease in Natural Plant Populations, Communities, and Ecosystems: Insights into Ecological and Evolutionary Processes. *Plant Disease*, 94(5), 492-503. 10.1094/PDIS-94-5-0492
- Ali, F., Akhtar, N., Shafique, S., & Shafique, S. (2021). Isolation and identification of *Aspergilli* causing Banana fruit rot. Ptolemy Scientific Research Press. 10.30538/psrp-ojc2021.0019
- Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, 215(3), 403-410. 10.1016/S0022-2836(05)80360-2
- Backhouse, D., & Burgess, L. W. (2002). Climatic analysis of the distribution of *Fusarium graminearum*, *F. pseudograminearum* and *F. culmorum* on cereals in Australia - ProQuest. *Australian Plant Pathology*, 31(4), 321-327. <https://search.proquest.com/openview/dcf91cf32ea37d7c534c61aa3bcee6d5/1?pq-origsite=gscholar&cbl=105331>
- Baldwin, B. G., Goldman, D. H., Keil, D. J., Patterson, R., & Rosatti, T. J. (2012). *The Jepson Manual: Vascular Plants of California* (Second Edition ed.). University of California Press.

- Bankevich, A., Nurk, S., Antipov, D., Gurevich, A. A., Dvorkin, M., Kulikov, A. S., Lesin, V. M., Nikolenko, S. I., Pham, S., & Prjibelski, A. D. (2012). SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *Journal of Computational Biology*, 19(5), 455-477.
- Barber, A., Riedel, J., Sae-Ong, T., Kang, K., Brabetz, W., Panagiotou, G., Deising, H., & Kurzai, O. (2020). Effects of Agricultural Fungicide Use on *Aspergillus fumigatus* Abundance, Antifungal Susceptibility, and Population Structure. *mBio*, 11(10.1128/mBio.02213-20)
- Basson, E., Lennox, C., & Meitz-Hopkins, J. (2019). Morphological and molecular identification of fungi associated with South African apple core rot. *European Journal of Plant Pathology*, 153(3), 849-868. 10.1007/s10658-018-1601-x
- Bay Area Open Space Council. (2011). *The Conservation Lands Network: San Francisco Bay Area Upland Habitat Goals Project Report*. Berkeley, CA:
- Calflora. (2020). *The Calflora Database*. The Calflora Database [a non-profit organization]. <https://www.calflora.org>
- Carrieri, R., Pizzolongo, G., Carotenuto, G., Tarantino, P., & Lahoz, E. (2014). First report of necrotic leaf spot caused by *Plectosphaerella cucumerina* on lamb's lettuce in southern Italy. *Plant Disease*, 98(7), 998.
- Chaudhry, V., Runge, P., Sengupta, P., Doehlemann, G., Parker, J. E., & Kemen, E. (2021). Shaping the leaf microbiota: plant–microbe–microbe interactions. *Journal of Experimental Botany*, 72(1), 36-56. 10.1093/jxb/eraa417
- CIMIS #157. (2020). *Climatological Data*. California Irrigation Management System. <https://cimis.water.ca.gov/>

- Climate and average monthly weather in San Francisco (California)*. (2021). World Weather & Climate Information. <https://weather-and-climate.com:80/average-monthly-Rainfall-Temperature-Sunshine,San-Francisco,United-States-of-America>
- Cook, R. J. (1968). Fusarium root and foot rot of cereals in Pacific Northwest. *Phytopathology*, 58 (2), 127
- Cook, R. J. (1980). Fusarium foot rot of wheat and its control in the Pacific Northwest. *Plant Disease*, 64(12), 1061-1066.
- Cubeta, M. A., & Vilgalys, R. (1997). Population Biology of the *Rhizoctonia solani* Complex. *Phytopathology*, 87(4), 480-484. 10.1094/PHYTO.1997.87.4.480
- Duginski, P. (2021). California's rainy season is starting about a month later than it did in the 1960s, researchers say. *Los Angeles Times* <https://www.latimes.com/california/story/2021-02-13/californias-rainy-season-is-starting-about-a-month-later-than-it-did-in-the-1960s-researchers-say>
- Elliott, M., Rollins, L., Bourret, T., & Chastagner, G. (2021). First report of leaf blight caused by *Phytophthora ramorum* on periwinkle (*Vinca minor*) in Washington State, USA. *Plant Disease*, 10.1094/PDIS-08-20-1721-PDN
- Faber-Langendoen, D., & Messick, J. (2014). D327 *Adenostoma fasciculatum* - *Artemisia californica* - *Nassella pulchra* Scrub & Grassland Division. U.S. Geological Survey. <https://www1.usgs.gov/csas/nvcs/nvcsGetUnitDetails?elementGlobalId=957919>
- Fang, W., & Latgé, J. (2018). Microbe Profile: *Aspergillus fumigatus*: a saprotrophic and opportunistic fungal pathogen. *Microbiology*, 164(8), 1009-1011. 10.1099/mic.0.000651

- Farré-Armengol, G., Filella, I., Llusia, J., & Peñuelas, J. (2016). Bidirectional Interaction between Phyllospheric Microbiotas and Plant Volatile Emissions. *Trends in Plant Science*, 21(10), 854-860. 10.1016/j.tplants.2016.06.005
- Felsenstein, J. (1985). Confidence Limits on Phylogenies: An Approach Using the Bootstrap. *Evolution; International Journal of Organic Evolution*, 39(4), 783-791. 10.1111/j.1558-5646.1985.tb00420.x
- Ford, L. D., & Hayes, G. F. (2007). Chapter 7: Northern coastal scrub and coastal prairie. In M. G. Barbour, T. Keeler-Wolf & A. A. Schoenherr (Eds.), *Terrestrial vegetation of California* (pp. 180-207). University of California Press.
- Frankel, S. J., Alexander, J. M., Benner, D., & Shor, A. (2018). Coordinated response to inadvertent introduction of pathogens to California restoration areas. *California Agriculture (Berkeley, Calif.)*, 72(4), 205-207. 10.3733/ca.2018a0035
- Frankel, S. J., Alexander, J., Benner, D., Hillman, J., & Shor, A. (2020). Phytophthora pathogens threaten rare habitats and conservation plantings. *The International Journal of Botanic Garden Horticulture*. 18, 53–65.
- Fu, L., Jin, Y., Zhang, G. F., Qu, J. L., Fan, K., & Wu, Y. Y. (2019). First Report of Stemphylium eturmiunum Causing Postharvest Rot of Garlic Sprout in China. *Plant Disease*, 103(5), 1041. 10.1094/PDIS-09-18-1553-PDN
- Fulcher, M. R., Cummings, J. A., & Bergstrom, G. C. (2017). First Report of an Alternaria Leaf Spot of Wheat in the U.S.A. *Plant Disease*, 101(7), 1326-1326. 10.1094/PDIS-10-16-1541-PDN

- Garibaldi, A., Gilardi, G., Ortu, G., & Gullino, M. L. (2013). First report of a new leaf spot caused by *Plectosphaerella cucumerina* on field grown endive (*Cichorium endivia*) in Italy. *Plant Disease*, 97(6), 848.
- Ghimire, S., Charlton, N. D., Bell, J., Krishnamurthy, Y. L., & Craven, K. (2010). Biodiversity of fungal endophyte communities inhabiting switchgrass (*Panicum virgatum* L.) growing in the native tallgrass prairie of northern Oklahoma. *Fungal Diversity*, , 10.
10.1007/s13225-010-0085-6
- Gilardi, G., Franco-Ortega, S., Gullino, M. L., & Garibaldi, A. (2018). First Report of Leaf Spot of Spinach Caused by *Stemphylium beticola* in Italy. *Plant Disease*, 102(10), 2036-2036.
10.1094/PDIS-02-18-0265-PDN
- Giraldo, A., & Crous, P. W. (2019). Inside plectosphaerellaceae. *Studies in Mycology*, 92, 227-286.
- Golden Gate National Parks Conservancy, & Tukman Geospatial, L. (2019). *Marin County Draft Lifeform Map* (Marin Veg Map Datasheet – Marin County Lifeform ed.). Golden Gate National Parks Conservancy.
- Grünwald, N. J., Garbelotto, M., Goss, E. M., Heungens, K., & Prospero, S. (2012). Emergence of the sudden oak death pathogen *Phytophthora ramorum*. *Trends in Microbiology*, 20(3), 131-138. 10.1016/j.tim.2011.12.006
- Gubler, W. D., Feliciano, A. J., Bordas, A. C., Civerolo, E. C., Melvin, J. A., & Welch, N. C. (1999). First Report of Blossom Blight of Strawberry Caused by *Xanthomonas fragariae* and *Cladosporium cladosporioides* in California. *Plant Disease*, 83(4), 400.
10.1094/PDIS.1999.83.4.400A

- Hall, T. A., & Hall, T. A. (1999). BIOEDIT: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/ NT.10.14601/Phytopathol_Mediterr-14998u1.29
- Hernandez-Escribano, L., Iturrutxa, E., Elvira-Recuenco, M., Berbegal, M., Campos, J. A., Renobales, G., García, I., & Raposo, R. (2018). Herbaceous plants in the understory of a pitch canker-affected *Pinus radiata* plantation are endophytically infected with *Fusarium circinatum*. *Fungal Ecology*, 32, 65-71. 10.1016/j.funeco.2017.12.001
- Jung, T., Orlikowski, L., Henricot, B., Abad-Campos, P., Aday, A. G., Aguín Casal, O., Bakonyi, J., Cacciola, S. O., Cech, T., Chavarriaga, D., Corcobado, T., Cravador, A., Decourcelle, T., Denton, G., Diamandis, S., Doğmuş-Lehtijärvi, H. T., Franceschini, A., Ginetti, B., Green, S., . . . Pérez-Sierra, A. (2016). Widespread *Phytophthora* infestations in European nurseries put forest, semi-natural, and horticultural ecosystems at high risk of *Phytophthora* diseases. *Forest Pathology* 46(2), 134-163. 10.1111/efp.12239
- Jung, T., Jung, M. H., Webber, J. F., Kageyama, K., Hieno, A., Masuya, H., Uematsu, S., Pérez-Sierra, A., Harris, A. R., & Forster, J. (2021). The Destructive Tree Pathogen *Phytophthora ramorum* Originates from the Laurosilva Forests of East Asia. *Journal of Fungi*, 7(3), 226.
- Jung, T., Scanu, B., Brasier, C., Webber, J., Milenković, I., Corcobado, T., Tomsofsky, M., Panek, M., Bakonyi, J., Maia, C., Bačová, A., Raco, M., Rees, H., Pérez-Sierra, A., & Horta Jung, M. (2020). A Survey in Natural Forest Ecosystems of Vietnam Reveals High Diversity of both New and Described *Phytophthora* Taxa including *P. ramorum*. *Forests*, 11, 93. 10.3390/f11010093

- Kessler, A. C., & Koehler, A. M. (2020). First Report of *Rhizoctonia solani* AG 4 Causing Root and Stem Rot of Stevia in Delaware and Maryland. *Plant Disease*, 104(11), 3076-3076. 10.1094/PDIS-01-20-0214-PDN
- Kirschner, R. (2018). Fungi on the leaf – a contribution towards a review of phyllosphere microbiology from the mycological perspective. Biosystematics and Ecology Series|Biodiversity and Ecology of Fungi, Lichens, and Mosses| Biosystematics and Ecology Series 34. Retrieved from https://explore.openaire.eu/search/other?orpid=od_____386::792147ec2682162f49389e18934fa6a7
- Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Molecular Biology and Evolution*, 35(6), 1547-1549. 10.1093/molbev/msy096
- Kurup, V. P., Shen, H., & Banerjee, B. (2000). Respiratory fungal allergy. *Microbes and Infection*, 2(9), 1101-1110. 10.1016/S1286-4579(00)01264-8
- Leveau, J. H. (2019). A brief from the leaf: latest research to inform our understanding of the phyllosphere microbiome. *Current Opinion in Microbiology*, 49, 41-49. 10.1016/j.mib.2019.10.002
- Li, P., Chai, A., Shi, Y., Xie, X., & Li, B. (2017). First report of root rot caused by *Plectosphaerella cucumerina* on cabbage in China. *Mycobiology*, 45(2), 110-113.
- Liu, B., Stein, L., Cochran, K., du Toit, L. J., Feng, C., Dhillon, B., & Correll, J. C. (2020). Characterization of Leaf Spot Pathogens from Several Spinach Production Areas in the United States. *Plant Disease*, 104(7), 1994-2004. 10.1094/PDIS-11-19-2450-RE

- Liu, T., Hu, D., Liu, F., & Cai, L. (2013). Polyphasic characterization of *Plectosphaerella oligotrophica*, a new oligotrophic species from China. *Mycoscience*, 54(5), 387-393.
- Madan, T., Arora, N., & Sarma, P. U. (1997). Identification and evaluation of a major cytotoxin of *A. fumigatus*. *Molecular and Cellular Biochemistry*, 167(1-2), 89-97.
10.1023/a:1006823706119
- Mark, W. R., Hawksworth, F. G., & Oshima, N. (2011). Resin disease: a new disease of lodgepole pine dwarf mistletoe. *Canadian Journal of Forest Research*, 10.1139/x76-055
- Moore, D., Ahmadjian, V., & Alexopoulos, C. J. (2020). Fungus. *Encyclopedia Britannica*, <https://www.britannica.com/science/fungus>
- Moore, D., Robson, G. D., & Trinici, A. P. J. (2020). *21st Century Guidebook to Fungi* (2nd ed.). Cambridge University Press.
- Myers, N., Mittermeier, R., Mittermeier, C., Fonseca, G., & Kent, J. (2000). Biodiversity hotspot for conservation priorities. *Nature*, 403, 853-8. 10.1038/35002501
- National Center for Biotechnology Information. (2020). *Basic Local Alignment Search Tool (BLAST)*. National Center for Biotechnology Information. <https://blast.ncbi.nlm.nih.gov/Blast.cgi>
- National Park Service. (2019). *Natural Resource Condition Assessment Golden Gate National Recreation Area*. (). Fort Collins, Colorado:
- National Vegetation Classification System. (2020). *Coastal Scrub*. National Vegetation Classification System. <http://usnvc.org/>
- Olson, D. M., & Dinerstein, E. (2002). The Global 200: Priority Ecoregions for Global Conservation. *Annals of the Missouri Botanical Garden*, 89(2), 199-224.
10.2307/3298564

- Panstruga, R., & Kuhn, H. (2019). Mutual interplay between phytopathogenic powdery mildew fungi and other microorganisms. *Molecular Plant Pathology*, 20(4), 463-470.
10.1111/mpp.12771
- Pastalka, T., Rooney-Latham, S., Kosta, K., Suslow, K., Huffman, V., Ghosh, S., & Schweigkofler, W. (2017). Monitoring Using a Sentinel Plant System Reveals Very Limited Aerial Spread of *Phytophthora ramorum* From Infected Ornamental Plants in a Quarantine Research Nursery. *Plant Health Progress*, 18(1), 9-16. 10.1094/PHP-RS-16-0050
- Pawley, A., & Lay, M. (2013). *Coastal watershed assessment for Golden Gate National Recreation Area and Point Reyes National Seashore*. Fort Collins, Colorado: National Park Service.
- Peever, T. L., Ibañez, A., Akimitsu, K., & Timmer, L. W. (2002). Worldwide Phylogeography of the Citrus Brown Spot Pathogen, *Alternaria alternata*. *Phytopathology*®, 92(7), 794-802.
10.1094/PHYTO.2002.92.7.794
- Perelló, A. E., Couretot, L., Curti, A., Uranga, J. P., & Consolo, V. F. (2019). First report of spot lesion of wheat caused by *Pyrenophora teres* f. sp. *maculata* observed in Argentina. *Crop Protection*, 122, 19-22. 10.1016/j.cropro.2019.03.023
- Perrone, G., & Gallo, A. (2017). *Aspergillus* Species and Their Associated Mycotoxins. In A. Moretti, & A. Susca (Eds.), *Mycotoxigenic Fungi: Methods and Protocols* (pp. 33-49). Springer New York. 10.1007/978-1-4939-6707-0_3
- Pollack, A. (2016). *Preserving Biodiversity for a Climate Change Future: A Resilience Assessment of Three Bay Area Species--Adenostoma fasciculatum (Chamise)*,

- Arctostaphylos canescens* (Hoary Manzanita), and *Arctostaphylos virgata* (Marin Manzanita) <https://repository.usfca.edu/capstone/352>
- Poursafar, A., Ghosta, Y., & Javan-Nikkhah, M. (2016). A taxonomic study on *Stemphylium* species associated with black (sooty) head mold of wheat and barley in Iran. *Mycologia Iranica*, 3(2), 99-109. 10.22043/mi.2017.26183
- Raimondo, M. L., & Carlucci, A. (2018). Characterization and pathogenicity assessment of *Plectosphaerella* species associated with stunting disease on tomato and pepper crops in Italy. *Plant Pathology*, 67(3), 626-641. <https://doi.org/10.1111/ppa.12766>
- Rodriguez, R. J., Jr, J. F. W., Arnold, A. E., & Redman, R. S. (2009). Fungal endophytes: diversity and functional roles. *New Phytologist*, 182(2), 314-330. <https://doi.org/10.1111/j.1469-8137.2009.02773.x>
- Roy, K., Miller, W., & Mclean, K. (1994). Survey of Pathogenic Genera of Fungi on Foliage of Weeds in Mississippi. *Canadian Journal of Plant Pathology*, 10.1080/07060669409500784
- Saitou, N., & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, 4(4), 406-425. 10.1093/oxfordjournals.molbev.a040454
- Salo, P. M., Arbes, S. J., Sever, M., Jaramillo, R., Cohn, R. D., London, S. J., & Zeldin, D. C. (2006). Exposure to *Alternaria alternata* in US homes is associated with asthma symptoms. *Journal of Allergy and Clinical Immunology*, 118(4), 892-898. 10.1016/j.jaci.2006.07.037
- Sarma, U. P., Bhetaria, P. J., Devi, P., & Varma, A. (2017). Aflatoxins: implications on health. *Indian Journal of Clinical Biochemistry*, 32(2), 124-133.

- Scarpari, M., Vitale, S., Giambattista, G., Luongo, L., De Gregorio, T., Schreiber, G., Petrucci, M., Belisario, A., & Voglmayr, H. (2020). *Didymella corylicola* sp. nov., a new fungus associated with hazelnut fruit development in Italy. *Mycological Progress*, 19, 317-328. 10.1007/s11557-020-01562-y
- Schol-Schwarz, M. B. (1959). The genus *Epicoccum* Link. *Transactions of the British Mycological Society*, 42(2), 149-IN3. 10.1016/S0007-1536(59)80024-3
- Schweigkofler, W., O'Donnell, K., & Garbelotto, M. (2004). Detection and Quantification of Airborne Conidia of *Fusarium circinatum*, the Causal Agent of Pine Pitch Canker, from Two California Sites by Using a Real-Time PCR Approach Combined with a Simple Spore Trapping Method. *Applied and Environmental Microbiology*, 70, 3512-20. 10.1128/AEM.70.6.3512-3520.2004
- Seabloom, E. W., Williams, J. W., Slayback, D., Stoms, D. M., Viers, J. H., & Dobson, A. P. (2006). Human Impacts, Plant Invasion, and Imperiled Plant Species in California. *Ecological Applications*, 16(4), 1338-1350. [https://doi.org/10.1890/1051-0761\(2006\)016\[1338:HIPIAI\]2.0.CO;2](https://doi.org/10.1890/1051-0761(2006)016[1338:HIPIAI]2.0.CO;2)
- Sivanesan, A., & Hsieh, W. H. (1989). New species and new records of ascomycetes from Taiwan. *Mycological Research*, 93(3), 340-351. 10.1016/S0953-7562(89)80161-3
- Smiley, R. W., Gourlie, J. A., Easley, S. A., & Patterson, L. (2005). Pathogenicity of fungi associated with the wheat crown rot complex in Oregon and Washington. *Plant Disease*, 89(9), 949-957.
- Smiley, R. W., Gourlie, J. A., Easley, S. A., Patterson, L., & Whittaker, R. G. (2005). Crop damage estimates for crown rot of wheat and barley in the Pacific Northwest. *Plant Disease*, 89(6), 595-604.

- Smiley, R. W., & Patterson, L. (1996). Pathogenic fungi associated with Fusarium foot rot of winter wheat in the semiarid Pacific Northwest. *Plant Disease*, 80(8), 944-949.
- Sneh, B., Burpee, L., & Ogoshi, A. (1991). *Identification of Rhizoctonia species*. APS press.
- Spawton, K. A., McGrath, M., & du Toit, L. J. (2020). First Report of Stemphylium Leaf Spot of Spinach (*Spinacia oleracea*) Caused by Stemphylium beticola in New York State. *Plant Disease*, 104(11), 3068-3068. 10.1094/PDIS-02-20-0343-PDN
- Steers, R. (2016). *Plant community monitoring protocol for the San Francisco Bay Area Network of National Parks: Standard operating procedures version 1.0*. (). Fort Collins, Colorado: National Park Service. <https://irma.nps.gov/DataStore/Reference/Profile/2233013>
- Susi, H., & Laine, A. (2015). The effectiveness and costs of pathogen resistance strategies in a perennial plant. *Journal of Ecology*, 103(2), 303-315. <https://doi.org/10.1111/1365-2745.12373>
- Swett, C. L., Bourret, T., & Gubler, W. D. (2016). Characterizing the Brown Spot Pathosystem in Late-Harvest Table Grapes (*Vitis vinifera* L.) in the California Central Valley. *Plant Disease*, 100(11), 2204-2210. 10.1094/PDIS-11-15-1343-RE
- Tamura, K., Nei, M., & Kumar, S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences*, 101(30), 11030-11035.
- U.S. Department of Agriculture, Natural Resources Conservation Service. *National soil survey handbook, title 430-VI*. https://www.nrcs.usda.gov/wps/portal/nrcs/detail/soils/scientists/?cid=nrcs142p2_054242

- UC Berkeley DNA Sequencing. *Welcome to UC Berkeley DNA sequencing facility*. UC Berkeley DNA Sequencing Facility. <https://ucberkeleydnasequencing.com/home>
- United States Department of Agriculture, Natural Resources Conservation Service. (2019). *Web Soil Survey*. <https://websoilsurvey.sc.egov.usda.gov/App/HomePage.htm>
- United States Department of Interior, National Park Service. (2016). *Weather - Golden Gate National Recreation Area*. National Park Service. <https://www.nps.gov/goga/planyourvisit/weather.htm>
- Vaghefi, N., Thompson, S. M., Kimber, R., Thomas, G. J., Kant, P., Barbetti, M. J., & Leur, Joop A. G. van. (2020). Multi-locus phylogeny and pathogenicity of *Stemphylium* species associated with legumes in Australia. *Mycological Progress*, 10.1007/s11557-020-01566-8
- Wang, F., Saito, S., Michailides, T. J., & Xiao, C. (2021). Postharvest use of natamycin to control *Alternaria* rot on blueberry fruit caused by *Alternaria alternata* and *A. arborescens*. *Postharvest Biology and Technology*, 172, 111383. 10.1016/j.postharvbio.2020.111383
- Wang, Q., Li, H., Xia, Z., Hou, Y., & Liu, W. (2019). Characterization of the complete mitochondrial genome of *Plectosphaerella* sp. (Glomerellales: Hypocreomycetidae). *Mitochondrial DNA Part B*, 4(1), 1770-1771. 10.1080/23802359.2019.1610097
- Whipps, J. M., Hand, P., Pink, D., & Bending, G. D. (2008). Phyllosphere microbiology with special reference to diversity and plant genotype. *Journal of Applied Microbiology*, 105(6), 1744-1755. 10.1111/j.1365-2672.2008.03906.x

- White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. A. Innis, D. H. Gelfand, J. J. Sninsky & T. J. White (Eds.), *PCR Protocols* (pp. 315-322). Academic Press.
- Wingfield, M. J., Slippers, B., Wingfield, B. D., & Barnes, I. (2017). The unified framework for biological invasions: a forest fungal pathogen perspective. *Biological Invasions*, *19*(11), 3201-3214. 10.1007/s10530-017-1450-0
- Woudenberg, J. H. C., Hanse, B., van Leeuwen, G. C. M., Groenewald, J. Z., & Crous, P. W. (2017). Stemphylium revisited. *Studies in Mycology*, *87*, 77-103. 10.1016/j.simyco.2017.06.001
- Xu, J., Xu, X., Cao, Y., & Zhang, W. (2014). First report of greenhouse tomato wilt caused by *Plectosphaerella cucumerina* in China. *Plant Disease*, *98*(1), 158.
- Yin, C., McLaughlin, K., Paulitz, T. C., Kroese, D. R., & Hagerty, C. H. (2020). Population Dynamics of Wheat Root Pathogens Under Different Tillage Systems in Northeast Oregon. *Plant Disease*, *104*(10), 2649-2657. 10.1094/PDIS-03-19-0621-RE
- Zhang, X. Y., Zhang, X. M., Jiang, H. H., & Hao, J. J. (2016). First Report of *Rhizoctonia solani* AG-5 Causing Stem Canker on Buckwheat in Maine. *Plant Disease*, *100*(6), 1241-1241. 10.1094/PDIS-09-15-1060-PDN
- Zhu, Y., Lujan, P., Dura, S., Steiner, R., Zhang, J., & Sanogo, S. (2019). Etiology of *Alternaria* Leaf Spot of Cotton in Southern New Mexico. *Plant Disease*, *103*(7), 1595-1604. 10.1094/PDIS-08-18-1350-RE