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TERRESTRIAL BIOME AND SEASONAL EFFECTS ON THE ABUNDANCE OF PLANT GROWTH-PROMOTING BACTERIA IN THE LOWER ATMOSPHERE

by
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To the Graduate Faculty:

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List of Abbreviations

AF	Aspen Forest
BSM	Basal Salts Medium
CFU	Colony Forming Units
DNA	Deoxyribonucleic Acid
HDMTA	Hexadecyltriethyl Ammonium Bromide
LBT	Luria Bertani-Tryptophan
LDD	Long Distance Dispersal
N	Nitrogen
NMDS	Non-Metric Multidimensional Scaling
O-CAS	Overlay-Chrome Azurol S
OTU	Operational Taxonomic Unit
P	Phosphate
P-solubilizer	Phosphate-solubilizer
PCR	Polymerase Chain Reaction
PGPB	Plant Growth-Promoting Bacteria
PGPM	Plant Growth-Promoting Microorganisms
PKA	Pikovskaya agar
RM-ANOVA	Repeated Measures-Analysis of Variance
rRNA	ribosomal Ribonucleic Acid
SASP	Small-Acid-Soluble Proteins
SS	Sagebrush Steppe
S	Suburban
S-producers	Siderophore-producers
TCP	Tri-Calcium Phosphate
TSA	Tryptic Soy Agar

Terrestrial Biome and Seasonal Effects on the Abundance of Plant Growth-Promoting Bacteria in the Lower Atmosphere

Abstract—Idaho State University—2016

I examined the ability of airborne microbes collected above three different terrestrial biomes in and around Pocatello, ID, USA, to promote plant growth using a model fern species as an indicator organism. Results demonstrated that plant growth-promoting microbes (PGPM) may be common members of the airborne microbiota over these biomes. This finding led to a year-long survey at these same sites to quantify the abundance of three types of PGPB within the lower atmosphere during all four seasons. Results indicated that the three types of PGPB were consistently present within the lower atmosphere at all biomes throughout the course of a year and that season correlated with PGPB abundance and shifts in the overall composition of airborne bacteria, while terrestrial biome did not correlate with either. This data sheds light on how season and terrestrial biomes affect microbial composition of the atmosphere, and specifically, the abundance of airborne PGPB.

Objectives of this Study

Given the scanty knowledge regarding the presence and abundance of PGPB in the atmosphere and how it is influenced by underlying terrestrial biome and season, this study addressed the following objectives:

Objective one was to determine if plant growth-promoting microbes are present in the lower atmosphere above three different terrestrial biomes by examining their ability to enhance the growth of a model fern gametophyte species (Chapter 2). **Objective two** was to determine how abundant three specific types of PGPB (IAA producers, siderophore producers, P-solubilizers) are in the lower atmosphere over three different terrestrial biomes during each of the four seasons (Chapter 3). **Objective three** was to determine how the overall airborne bacterial composition varied across season and biomes (using DNA-based techniques) and how this co-varied with the abundance of PGPB (Chapter 3). **Objective four** was to identify PGPB cultivated from air samples and gain insights into what fraction of the total airborne bacteria is comprised by PGPB (Chapter 3).

Chapter 1: Introduction

The atmosphere as a frontier for microbiological exploration

Aerobiology has been an active area of research for over the past 200 years, with efforts focused on modeling the dispersal of pathogenic microbes, determining the viability and metabolic activities of airborne microbes, and surveying the overall composition and biogeographical distributions of microbes in the atmosphere (14, 15, 60). Research over the past few decades has demonstrated that there are metabolically active microbes in the atmosphere that potentially influence concentrations of chemical compounds (e.g., nitrates, sulfates, chloride, phosphorus, iron), and also play roles in cloud formation via ice-nucleation (25, 23). Although conditions in the troposphere (lower 10-15 km of the atmosphere) vary widely (e.g., temperature range: -50° to 15 °C) and challenge microbial survival (e.g., limited nutrient availability, high levels of ultraviolet (UV) radiation), the troposphere harbors a microbiome with a diversity rivaling that of surface environments (78). Microbes survive these harsh conditions through several adaptations. For example, some microbes form spores or go into spore dormancy, during which DNA is stabilized with small-acid-soluble proteins (SASP); this is a primary mechanism for enduring high levels of UV radiation and repairing damage (78). Some bacteria generate high levels of protective pigments (i.e., carotenoids or violacein) to prevent DNA damage (78). Additionally, some microbes will reduce cell size and coat thickness, develop ultramicrocells, or even engage in cannibalism to survive harsh environmental conditions (70).

Bacteria are abundant in the atmosphere, with counts (viable and non-viable) ranging from 10^4 to 10^8 cells m^{-3} (54, 9). Bacterial fluxes from plant surfaces and soils to the atmosphere are considered the greatest contributors to the airborne microbiome, and are greatly influenced by meteorological factors (e.g., wind, precipitation) and human activities (e.g., sewage plant emissions, urbanization, agriculture; 54). An estimated 40-1800 billion grams of bacteria are emitted from surface environments into the atmosphere each year (60), and their distribution in the atmosphere obtains biogeographical patterns that mirror the distribution of the underlying biomes (15). It has been demonstrated that the microbial composition in surface environments is non-random and that genetic variation among species is related to their geographical separation (20, 65, 24); taxa-area relationships for microbes exist in which the number of taxa observed increases as more area is sampled, showing that microbes assume biogeographical distribution patterns in surface environments (46). Given that surface environments are the primary sources of microbes to the atmosphere and that the biogeography of microbes in the atmosphere mirrors the distribution of these environments, it is fair to hypothesize that as surface environments are variably impacted by anthropogenic activities, the distribution and composition of microbes in the atmosphere will be altered in parallel.

The dispersal of specific microbes through the atmosphere has garnered attention, particularly with respect to pathogenic bacteria and fungi, which cause human and economically important crop diseases (51). Occurrences of long-distance dispersal (LDD) through the atmosphere have been tracked and recorded in locations across the globe. For example, chestnut blight (*Cryphonectria parasitica*) in North America, which was introduced from East Asia, spread rapidly via wind over North American forests,

devastating chestnut populations. Another example is *ascochyta* blight of lentil, chickpea and pea crops in the northern Great Plains caused by *Ascochyta rabiei*, *Ascochyta lentis* and *Ascochyta pisi*. These pathogens' ascospores (sexual spores) can travel via wind up to a distance of five miles, facilitating the destruction of these crops across the Plains (58, 51). The origin of the pathogenic *Ascochyta* species is unknown, but they infect the above named crops worldwide and have likely traveled via LDD of infected seeds (42).

An area of research that has not garnered much attention is the dispersal of microbes to new habitats, where they can have positive effects on local ecology and ecosystem development. Although microscopic and, thus, a largely ignored part of the earliest stages of succession, airborne microbes are among the first colonists of severely disturbed terrestrial habitats (e.g., volcanic deposits, burned landscapes) and can influence the trajectory of ecosystem development. Microbial and plant communities are dependent on each other; for example, after the eruption of Mount St. Helens in 1980, lupines were among the first plants to recolonize the barren landscapes (26). As volcanic deposits lack endogenous sources of nitrogen and lupines are legumes, nitrogen-fixing bacteria were necessary to facilitate this recolonization and likely arrived via wind from nearby regions.

The above serve as examples of how atmospheric transport of microorganisms from one region to another can link their ecosystem health even if they are not contiguous. As landscapes are rapidly being changed by anthropogenic activities, the source populations of microbes that are emitted to the atmosphere are likely undergoing change as well. The United Nations (2008) predicted that the urban population will double by 2050, causing urban environments to expand and replace many natural

habitats. This growth of urban areas could have varied effects on airborne microbial assemblages and, furthermore, serious ecological consequences (e.g., increased pathogenic bacteria). By 2050, it is estimated that we will require a 70-100% increase in agriculture yields to feed the world's population (73). This will lead to an increased use of pesticides, which negatively affect the environment and are economically costly (73). Biopesticides (e.g., fungicides, insecticides, herbicides), made of live microbes, currently make up ca. 2.5% of the chemical pesticide industry; an increase in the use of microbial approaches could potentially augment the global market of chemical pesticides (73), but also change the composition and distribution of microbes in terrestrial biomes and the atmosphere.

One group of microbes that is likely abundant in the atmosphere and transported to new habitats, where they affect ecological interactions, is plant growth-promoting bacteria (PGPB). PGPB are abundant in terrestrial environments that are in constant contact with and serve as an inoculum source to the overlying atmosphere. Although recent studies suggest that airborne bacteria follow biogeographical distributions that are influenced by the underlying terrestrial biomes, the dynamics of bacterial fluxes between different terrestrial biomes and the atmosphere are still poorly understood (84), especially with respect to specific functional groups, such as PGPB, and what implications such fluxes might have on ecosystem ecology.

Plant growth-promoting bacteria (PGPB)

Much research has been conducted on plant-microbe interactions of the rhizosphere, demonstrating that microbes positively influence plant growth by synthesizing plant growth-promoting substances or by assisting plants with nutrient

acquisition from the surrounding environment. Direct mechanisms by which bacteria promote plant growth include: nitrogen fixation, phosphate solubilization, iron sequestration, indoleacetic acid (IAA)-production, and ethylene production (35). Indirect mechanisms by which bacteria promote plant growth include: antibiotic production, enzyme production, siderophore production and competition against pathogenic and nonpathogenic microbes colonizing surrounding soil as well as signaling the induced systematic response (ISR; 35). This study examined the abundance of three groups of culturable PGPB in the lower atmosphere: phosphate-solubilizers (P-solubilizers), indoleacetic acid-producers (IAA-producers) and siderophore-producers (S-producers). These three groups of PGPB will be described below.

Phosphate-solubilizers

Phosphorus (P) is a macronutrient necessary for plant metabolism, and without it, plant development and reproduction rates decrease (57). P is commonly bound in rock reserves and organic matter, or immobilized in soil from fertilizer application. For plants to acquire P from their surroundings, P must first be released into a form of low molecular weight organic phosphorus or in the form of soluble ions (PO_4^{-3}, HPO_4^{-2}, $H_2PO_4^-$; 74). P-solubilizing bacteria can increase the availability of P to plants by secreting a variety of organic acids (e.g., glycolic, oxalic, malonic, succinic, acetic, lactic, glutaric, isovaleric, isobutyric acid) into the soil matrix (74, 7, 5). The secretion of those organic acids lowers the pH of the surrounding environment and aids in releasing inorganic phosphate from rock and soil reserves (36). The mineralization of organic phosphate is catalyzed by phosphatases secreted by bacteria, which hydrolyze phosphoric esters (35). Bacterial genera that include P-solubilizing bacteria are *Pseudomonas*,

Bacillus, *Rhizobium*, *Burkholderi*a, *Achromobacter*, *Agrobacterium*, *Microccocus*, *Aereobacter*, *Flavobacterium*, and *Erwina* (74).

Siderophore-producers

PGPB can promote plant growth by producing siderophores. Siderophores are small (<100 Da), high affinity metal-chelating (i.e., iron) molecules that microbes synthesize and secrete into the environment. Siderophore biosynthesis in bacteria is induced by iron deficiency (75, 68). By reacquiring iron-chelated siderophores from their surroundings, microbes can obtain iron required for their biosynthetic and metabolic processes (75). Plants can also partially satisfy their own iron needs by taking up iron-chelated siderophores from the environment. Plants utilize iron for development and enzyme and chlorophyll production; iron deficiency in plant tissue can result in chlorosis and dramatically alter root-shoot ratios, halting new growth and limiting their ability to reach water and other nutrients for survival (61). In heavy metal contaminated environments, siderophores assist plants in counter-acting the toxic effects of high concentrations of heavy metals by binding to the heavy metal ions and immobilizing them in the soil to avoid their uptake through plant roots (75, 27). Microbial siderophores are classified by their iron coordination sites and chemical nature into the following three categories: 1) catecholates (produced exclusively by bacteria), 2) hydroxyamates (produced by both bacteria and fungi) and 3) α-carboxylate (produced by few bacteria but mainly by the fungal phylum, Zygomycota; 68, 83, 41, 28). Common bacterial genera known to include siderophore-producers include *Bacillus*, *Rhizobium*, *Pseudomonas*, *Staphylococcus*, and *Enterobacter*.

Indoleacetic acid-producers

Indoleacetic acid (IAA), also known as auxin, is a well-studied plant growth-regulating hormone (phytohormone), produced by both plants and bacteria (34). Plants vary in their sensitivity and responses to IAA concentrations, which can positively or negatively affect growth; however, despite the potential of adverse effects of IAA on plant growth, which has been attributed to endogenous production or production by neighboring plants, the effect of IAA produced by bacteria is generally at low concentrations and has only been documented to be involved promoting plant growth (12, 34, 35). Therefore, this thesis classifies IAA-producers as PGPB. Known effects of IAA on plant activities include, but are not limited to: plant cell differentiation, root development, xylem growth rate, light responses, photosynthesis rates, pigment formation and stress resistance via ethylene production (35). Tryptophan, a metabolic precursor to IAA, is secreted by plant roots and can then be taken up by PGPB, which convert it into IAA. Bacterial secretions of IAA can stimulate plant activities described above (35). Members of the bacterial genera *Rhizobium*, *Pseudomonas*, *Agrobacterium*, and *Enterococcus* are among the most commonly known and well-studied IAA producers within the rhizosphere.

Chapter 2: Airborne microbes from three different terrestrial biomes promote plant growth

Introduction

Recent studies suggest that airborne microbes obtain biogeographical distributions that are influenced by the microbes being emitted from the underlying terrestrial biomes (84). Therefore, these distributions are also likely influenced by both natural (e.g., volcanic eruptions) and anthropogenic activities (e.g., agriculture, urbanization) that alters terrestrial landscapes. Nonetheless, the dynamics of microbial fluxes between different terrestrial biomes and the atmosphere are still poorly understood, especially with regards to specific functional groups, such as plant growth-promoting microbes (PGPM). PGPM have the potential to impact ecological processes in the new habitats they reach via atmospheric transport; this could be especially important when they are among the first colonizers on disturbed terrestrial habitats (e.g., burned landscapes, volcanic deposits) where they can shape the earliest stages of ecosystem succession (37, 77, 32).

For plants to survive germination, they must procure nutrients and protect themselves from phytopathogens. When seedlings are in nutrient-poor conditions, characteristic of disturbed habitats, their likelihood of survival is decreased. Plant-microbe interactions have been well studied in the past few decades, and microorganisms found within the rhizosphere and phyllosphere have demonstrated their abilities to promote-plant growth even under these stressful conditions (6, 19). PGPM can stimulate root growth, mitigate plant stress or increase nutrient availability for plants (56) via indirect or direct mechanisms. Direct mechanisms of promoting plant growth, include controlling levels of plant hormones and assisting in acquisition of nutrients (e.g.,

phosphorus, iron, nitrogen), while indirect mechanisms include sustaining plant growth and development by inhibiting phytopathogens (e.g., siderophore production, antibiotic production, induced systemic resistance; 35). The potential importance of atmospheric dispersal of PGPM in ecosystem development is exemplified by ecosystem recovery after the eruption of Mount St. Helens in 1980, where lupines were among the first plants to recolonize the barren landscapes (26); as volcanic deposits lack endogenous sources of nitrogen and lupines are legumes, nitrogen-fixing bacteria were necessary to facilitate this recolonization and likely arrived via wind from neighboring regions.

For the first time, I directly assessed the ability of airborne microbial assemblages collected above three terrestrial biomes in and around Pocatello, ID, U.S.A (aspen forest, sagebrush steppe, suburban area; **Figure 1**) to promote plant growth through experiments utilizing a model fern gametophyte species (*Ceratopteris richardii*) as an indicator organism. Growth assays were conducted on four different agar medium types with varied nitrogen (N) and phosphorus (P) concentrations and stoichiometries designed to mimic conditions that might be encountered in nature. I hypothesized that gametophytes would grow larger in the presence of airborne microorganisms than in their absence, but N and P concentration and stoichiometry would moderate the magnitude of the effect.

Materials and methods

Experimental design and air sample collection

Using a SAS SUPER 180 (Bioscience International, Rockville, MD), air samples were collected 3 m above three sub-sites (~15 m apart) within each of three terrestrial biomes (sagebrush steppe, aspen forest, suburban) during a 7.5 hr period (9:00 AM- 3:30 PM) on 17 September 2014 (**Figure 2**). At each site, 180 L volumes of air were sampled onto agar plates

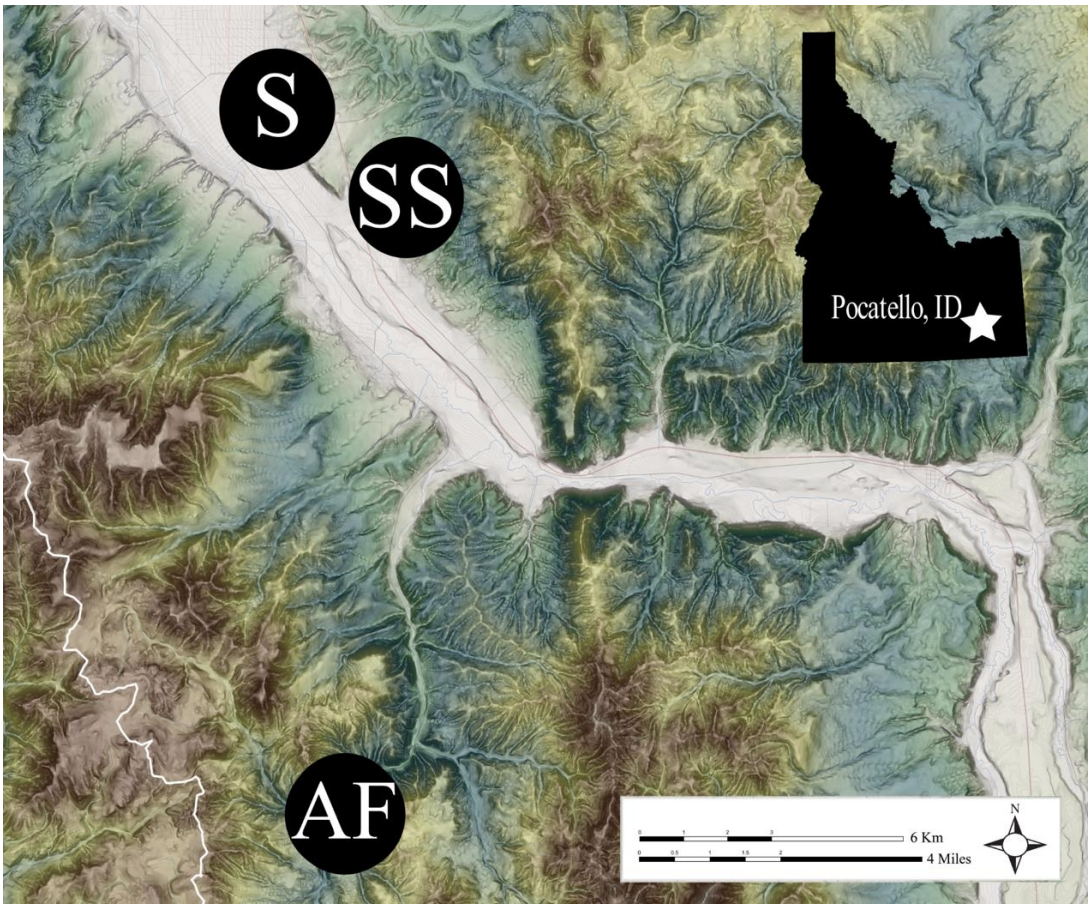

Figure 1. Map of air sampling locations. Air sampling locations in and near Pocatello, Idaho, U.S.A. Site labels: (AF) Mink Creek Trailhead in Caribou National Forest (aspen-forest site; 42°43'22.68" N, 112°25'13.5" W); (SS) Barton Road Ecological Research Site (sagebrush-steppe site; 42°51'7.15" N, 112°24'4.85" W); (S) Holt Arena parking lot on the Idaho State University campus (suburban site; 42°52'17.08" N, 112°25'39.58" W). Inset displays Pocatello's location within Idaho.

containing one of four different variations of basal salts medium (BSM; 17) with N:P stoichiometries of 1:1 or 16:1, where [P]=7.65 µM or 15.2 µM. At each terrestrial biome, air samples were collected onto a total of 36 plates, with triplicate plates of each of the four medium types being sampled onto at each of the three sub-sites.

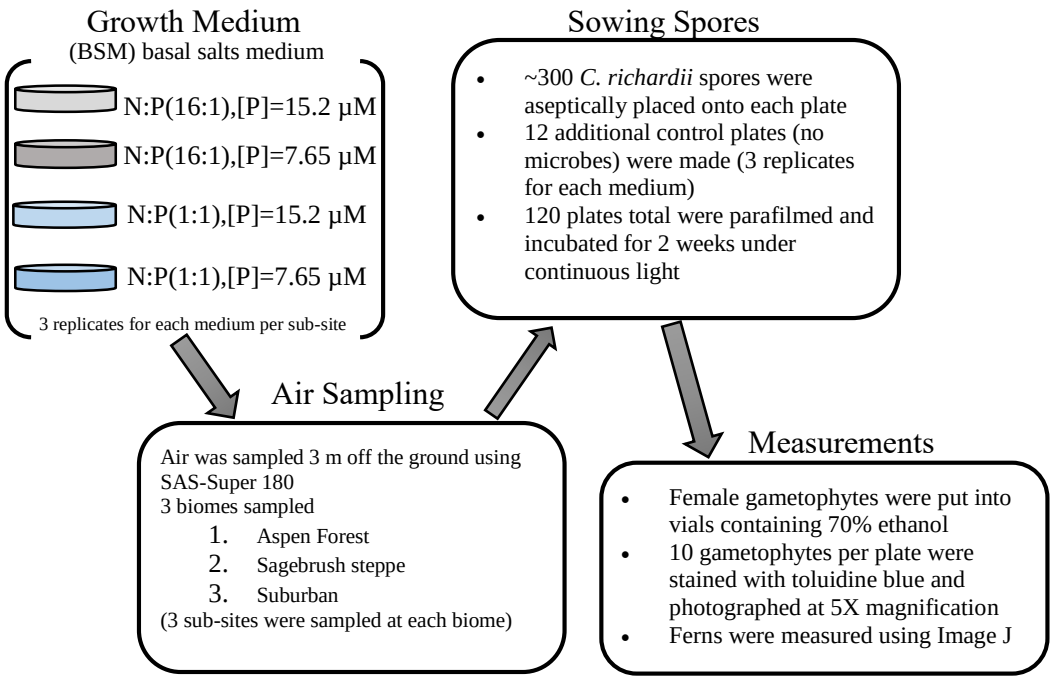

Figure 2. Experimental design for Chapter 2. Experimental design displaying characteristics of growth medium, air sampling, spore sowing, and measurements.

Sowing spores and plant growth conditions

In order to determine whether the microbes collected from air affected plant growth, ca. 300 *Ceratopteris richardii* (Carolina Biological, Burlington, NC) wild type spores were sowed onto each of the plates containing airborne microbes; spores were also sowed onto 12 control plates (three per medium type), devoid of airborne microbes. Following the 12-day growth period under the constant light of a 40 watt cool-white fluorescent tube bulb (44), representative *C. richardii* hermaphrodite gametophytes (n =

10) were selected at random from each of the 120 agar plates and preserved in 70% ethanol.

Fern gametophyte area

Fern gametophyte area was utilized as a metric of plant growth in each of the experimental treatments and the controls. Preserved gametophytes were stained with 0.01% (w/v) toluidine blue and photographed at 5X magnification using a Zeiss Primo Star Light microscope equipped with an AxioCam ERc5s HD digital camera (Carl Zeiss Meditec, Inc., Dublin, California, U.S.A.). Gametophyte area was determined by tracing the perimeter of each gametophyte using the program ImageJ version 1.49 (Bethesda, Maryland, U.S.A; 72) as previously described (45).

Statistical Analyses

Statistical analyses was carried out using the program R (version 3.2.2, R Development Core Team, 2011). Data were determined not to be normally distributed (Shapiro test) and were heteroscedastic (Fligner-Kileen test). A Welch's ANOVA (α=0.05), which does assume normality or homoscedasticity, followed by a Bonferroni correction for multiple comparisons, was used to determine: i) whether significant differences in gametophyte area existed among the four different medium types when grown in the presence of microbes collected from aspen forest, sagebrush steppe or suburban sites or in the absence of microbes (sterile controls) and ii) whether significant difference in gametophyte area existed when plants grown in the presence of microbes or in the absence of microbes (each medium type and airborne microbe sampling location considered separately).

Results

C. richardii gametophytes grew significantly larger in the presence of airborne microbes

On all four growth medium types, *C. richardii* grew significantly larger in the presence of airborne microbes than on sterile controls, irrespective of air-sampling location (**Figure 3**). For each of the four growth medium types, results of statistical comparisons between gametophyte area on plates with microbes and on sterile controls were as follows: N:P(1:1), [P]=15.2 µM (aspen forest (*P*-value=6.5 X 10^{-12}), sagebrush steppe (*P* -value=3.2 X 10^{-16}) and suburban (*P*-value=1.9 X 10^{-13}); N:P(1:1), [P]=7.65 µM (aspen forest (*P*-value=3.8 X 10^{-9}), sagebrush steppe (*P*-value= 2.3 X 10^{-11}), and suburban (*P*-value= 2.5 X 10^{-16}); N:P(16:1), [P]=15.2 µM (aspen forest (*P* -value=2.8 X 10^{-8}), sagebrush steppe (*P*-value= 1.0 X 10^{-14}), and suburban (*P*-value= 1.7 X 10^{-13}); N:P(16:1), [P]=7.65 µM (aspen forest (*P*-value= 1.7 X 10^{-6}), sagebrush steppe (*P*-value= 8.4 X 10^{-14}), and suburban (*P*-value=2.4 X 10^{-9}).

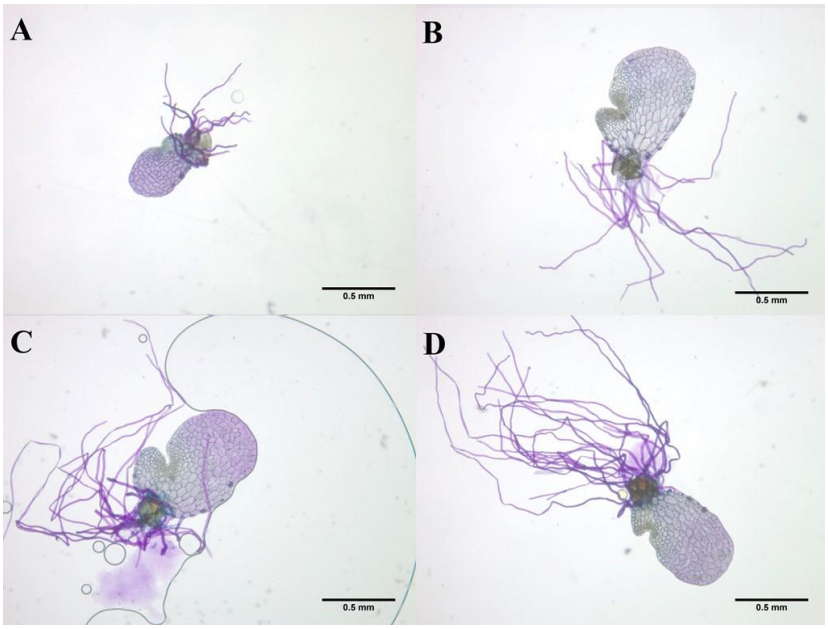

Figure 3. Images of *Ceratopteris richardii*. Gametophytes growing on N:P(1:1), [P]=15.2 µM that were representative of the average gametophyte area when grown in (A) the absence of microbes (controls) or in the presence of airborne microbes collected from (B) sagebrush steppe, (C) suburban site or (D) aspen forest. Gametophytes stained with toluidine blue. Images were taken at 5X magnification; scale bar = 0.5 mm.

Nutrient concentration and stoichiometry moderated microbial impacts on *C. richardii* gametophyte growth

Growth medium significantly impacted the gametophyte area on sterile control plates ($F=8.5354_{3,\ 62.471}$, *P*-value=7.806×10^{-5}), or when grown in the presence of microbes collected from air in aspen forest ($F=12.2631_{3,\ 182.374}$, *P*-value=2.403×10^{-7}), sagebrush steppe ($F=38.5545_{\ 3,\ 191.457}$, *P*-value< 2.2×10^{-16}), or the suburban site ($F=5.9749_{\ 3,\ 194.945}$, *P*-value= 6.455×10^{-4}). Macronutrient concentration impacted *C. richardii* growth when grown in the presence of microbes collected from aspen forest, sagebrush steppe and suburban sites; ferns always grew larger in the presence of microbes on media containing 15.2 µM P than on media containing 7.65 µM P (all *P*-values ≤ 0.0563). N:P ratio only affected *C. richardii* growth when grown with microbes

collected in the aspen forest ([P]=15.2 µM, *P*-value=0.0035) or on sterile control plates ([P]=15.2 µM, *P*-value=0.0002, **Figure 4**).

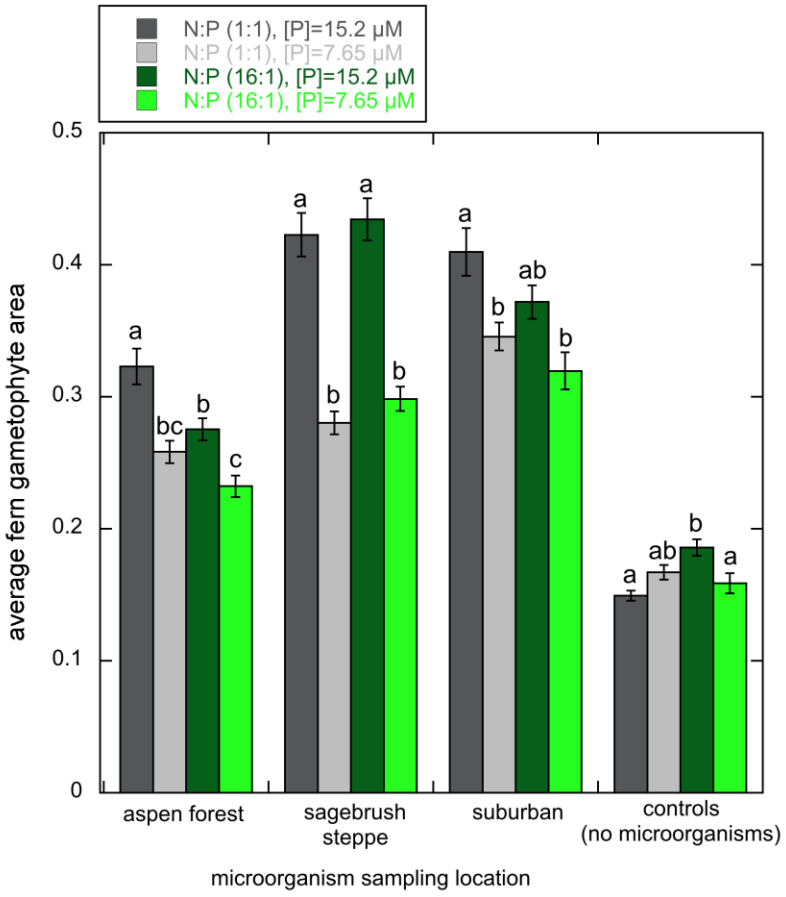

Figure 4. Bar plot of *Ceratopteris richardii* gametophyte growth. *C. richardii* growth (average area in mm^2), on four different growth medium types (N:P (1:1), [P]=15.2 µM; N:P(1:1), [P]=7.65 µM; N:P(16:1), [P]=15.2 µM; N:P(16:1), [P]=7.65 µM) in the presence of airborne microbes collected from three different terrestrial environments in Pocatello, ID, U.S.A. or on sterile controls. Small letters denote statistically significant differences in *C. richardii* growth among medium types inoculated with airborne microbes from each sampling location and within sterile controls (*P*-value < 0.05) as determined by a Welch's ANOVA followed by a Bonferroni correction for multiple comparisons (n = 90 for each bar with the exception of N:P(1:1), [P]=15.2 µM inoculated with airborne microbes from aspen forest (n = 88)).

Discussion

This study demonstrated that PGPM were present in the lower atmosphere above three different terrestrial biomes, which leads us to further postulate that PGPM may be ubiquitous in the lower atmosphere. The magnitude of the effect of airborne microorganisms on plant growth was moderated by N and P concentration and stoichiometry, but nonetheless suggests that atmospheric transport of PGPM to new terrestrial habitats could influence plant growth.

Experiments described here utilized the fern *C. richardii* to assess the ability of airborne microorganisms to promote plant growth. *C. richardii* is used to model non-seed plants (44). Such plants are important colonizers during the early successional stages of barren lands (43). This may be partly due to plant spores dispersing over long-distances, crossing oceans and reaching oceanic islands (79, 8). Therefore, *C. richardii* may have similar biology to many early-colonizing plants in disturbed terrestrial habitats for which inoculation by PGPM from the atmosphere may be quite important in catalyzing early stages of ecosystem succession.

The selection of *C. richardii* as a model plant species led to using basal salts medium (BSM), which is typically used to grow this fern in the laboratory (17), but the N and P concentrations and N:P ratios were altered. The N:P ratio for standard BSM is 0.9:1 and the P concentration is 3.8 mM, which is orders of magnitude above that typically found in soil (21). Güsewell (2004) found that N:P ratios of terrestrial plants in short term fertilization experiments were between 10 and 20. Therefore, a growth medium with an N:P ratio of 0.9:1 might impose N limitation for ferns, especially when nutrient concentrations are lowered to more closely approximate those found in nature

and in the event of competition with microbes for nutrients as happens in nature (48). Thus, our medium types were designed to bring N and P concentrations closer to those found in nature and impose N:P stoichiometries (1:1 and 16:1) that might limit N (common in soils) or provide adequate N to reduce microbe-plant competition for nutrients, permitting observation of the true potential for microorganisms to promote plant growth.

On each of the four growth medium types, *C. richardii* grew significantly larger in the presence of airborne microorganisms than on sterile controls, irrespective of where the airborne microbes were collected (**Figure 3, Figure 4**). Macronutrient concentration and stoichiometry moderated *C. richardii* growth in the presence and absence of microbes (**Figure 4**). Nutrient concentration impacted *C. richardii* growth when grown in the presence of microbes collected from any of the sites; on average, ferns always grew larger on media containing 15.2 µM P than on media containing 7.65 µM P, irrespective of N:P stoichiometry (all *P*-values $\leq$ 0.0563). Higher concentrations of macronutrients may have alleviated some competition between ferns and microbes allowing for larger fern growth. Plant-microbe competition was also evident in how stoichiometry impacted gametophyte area in two cases. In the presence of airborne microorganisms from the aspen forest, gametophyte area was significantly larger on media with an N:P stoichiometry of 1:1 than with an N:P stoichiometry of 16:1 ([P]=15.2 µM; *P*-value=0.0035), but on sterile controls ([P]=15.2 µM), gametophyte area was significantly larger when N:P stoichiometry was 16:1 than when N:P stoichiometry was 1:1 (*P*-value=0.0002). In the presence of microbes, the higher N:P ratio may have promoted faster use of N and growth by microbes, slightly limiting plant growth, than in cases

when the N:P stoichiometry was 1:1. Collectively, these results indicate that the impact of PGPM will be dependent on macronutrient concentration and stoichiometry in the environment.

This is the first experiment that demonstrates that mixed microbial assemblages sampled directly from air have the ability to promote plant growth and PGPM may be common components of airborne microbial assemblages. Because I utilized the same growth media at all three terrestrial biomes, this may have selected for the same microbial taxa in the air above each biome, regardless of differences in microbial abundance and species that may have existed above each biome. On the other hand, culture-based studies usually give insights to the ubiquitous species within the atmosphere and help map out their transport ranges (84). Future studies should determine the abundance and the identity of PGPM above each biome as well as the specific mechanisms by which they are promoting plant growth. Additionally, I only examined air samples from one time point and future studies should aim to determine whether or not there are seasonal trends in the abundance of PGPM in the atmosphere. This is a trend that one might posit would exist given that soils and plant surfaces are projected to contribute substantially to the microbial load of the atmosphere (55) and plant coverage changes seasonally in the cold temperate climate of Southeast Idaho. Although much research remains to be done, this study highlights the potential of atmospheric transport of PGPM from one location to another as a mechanism that links the health of terrestrial ecosystems even if they are not contiguous.

Chapter 3: The effect of season and terrestrial biome on the abundance of plant growth-promoting bacteria in the lower atmosphere

Introduction

Aerobiology has been an active area of research for the past 200 years, with efforts focused on modeling the dispersal of pathogenic microbes, determining the viability and metabolic activities of airborne microbes, and surveying the overall composition and biogeographical distributions of microorganisms in the atmosphere (14, 15, 23). Research over the past few decades has demonstrated that there are metabolically active microbes in the atmosphere that potentially influence concentrations of chemical compounds (e.g., nitrates, sulfates, chloride, phosphorus, iron), and also play a role in cloud formation via ice-nucleation (25, 23). Although conditions in the troposphere (lower 10-15 km of the atmosphere) can be harsh (e.g., high levels of ultraviolet radiation, low nutrient availability) and highly variable (e.g., temperature range: -50 to 15 °C), the troposphere harbors a microbiome with a diversity rivaling that of surface environments (78).

Bacterial fluxes from plant surfaces and soils to the atmosphere are considered the greatest contributors to the airborne microbiome, which contains 10^4 to 10^8 bacterial cells m^{-3} (54, 9), and are greatly influenced by meteorological factors (e.g., wind and precipitations) and human activities (e.g., sewage plant emissions; 55). An estimated 40-1800 billion grams of bacteria are emitted from surface environments into the atmosphere each year (15). This bacterial abundance within the atmosphere is supplied by the diverse underlying terrestrial and aquatic environments, which are variably distributed across the surface of the Earth. The distribution of microbes in the troposphere obtains

biogeographical patterns that mirror the distribution of the underlying biomes (14). Long-term microbial biogeography studies have shown that soil microbial diversity is influenced by plant diversity (85), environmental temperatures, precipitation patterns and plant productivity (38). As terrestrial environments contribute substantially to the airborne microbiome, understanding the composition and distribution of microbes in surface environments and how they are impacted by anthropogenic activities may allow us to predict the microbial composition and distribution in the atmosphere.

The dispersal of specific microbes through the atmosphere has garnered attention, particularly with respect to pathogenic bacteria and fungi, which cause human and economically important crop diseases (13, 31). Conversely, the atmospheric transport of microbes to new habitats, where they can have positive effects on ecosystem development and productivity, has not been considered extensively. Although microscopic and, thus, a largely ignored part of the earliest stages of succession, airborne microbes are among the first colonists of severely disturbed terrestrial habitats (e.g. volcanic deposits, burned landscapes) and can influence the trajectory of ecosystem succession (29, 64, 66). In this regard, one especially important functional group of microbes is plant growth-promoting bacteria (PGPB). PGPB have demonstrated their ability to positively influence plant growth through both direct and indirect mechanisms by synthesizing substances that enhance plant growth (e.g., indoleacetic acid, ethylene; 35), or assisting in nutrient acquisition from the surrounding environment (e.g., nitrogen fixation, iron sequestration; 35).

I hypothesized that PGPB are ubiquitous in the lower atmosphere, but their abundance may vary as a function of season and underlying terrestrial habitat, which also

may control the overall composition of airborne bacteria. I examined the abundance of three groups of culturable PGPB in the lower atmosphere (phosphate-solubilizers, indoleacetic acid-producers, and siderophore-producers) over three terrestrial biomes (aspen-forest, sagebrush-steppe, and suburban) during four seasons. Concurrently, I examined the total composition of airborne bacteria using cultivation-independent methods. I provide insights into how different terrestrial biomes, with varied land-uses, may affect the composition of airborne bacteria, and the abundance of PGPB, which potentially can be transported to new habitats where they can affect ecosystem development.

Materials and methods

Air sampling

Air samples were collected at three biomes (sites) in or around Pocatello, ID, U.S.A, in autumn (2014), winter (2015), spring (2015) and summer (2015) with a SAS SUPER 180 (Bioscience International, Rockville, MD). The SAS SUPER 180 impacts air samples (180 L min^{-1}) onto 100 mm perti dishes containing agar medium. The aspen forest site was at the Mink Creek trailhead in the Caribou National Forest (42°43'22.68" N, 112°25'13.5" W), the sagebrush-steppe site was within the Barton Road Ecological Research Area (42°51'7.15" N, 112°24'4.85" W), and the suburban site was in the parking lot of Holt Arena on the Idaho State University campus (42°52'17.08" N, 112°25'39.58" W; **Figure 1**). During each season, each site was sampled 3 times (ca. 1-

week between samplings; **Figure 5**). At each site, air was sampled with the SAS

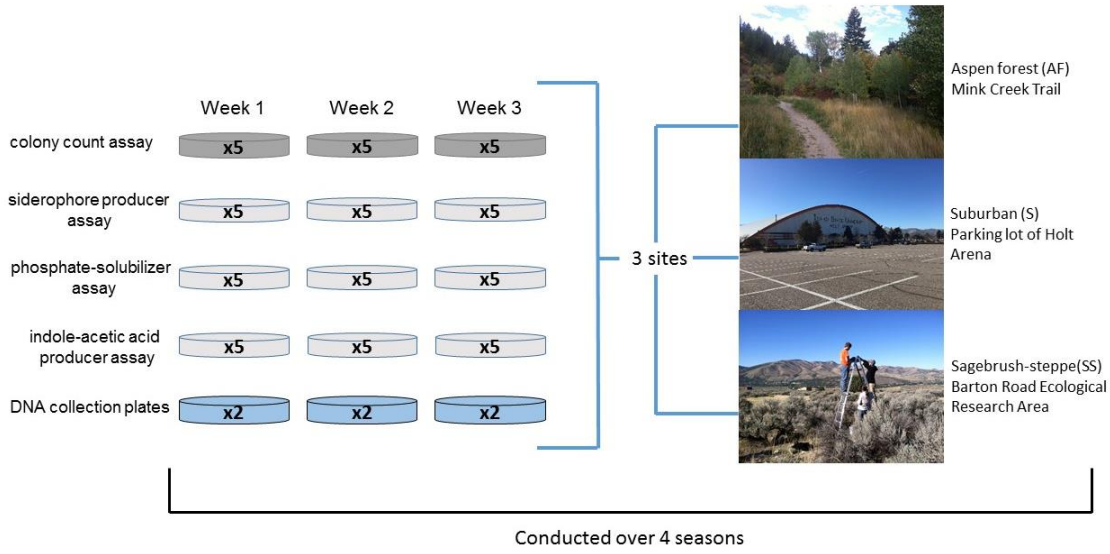

Figure 5. Experimental design for Chapter 3. Experimental design for airborne microbial collections from autumn 2014 to summer 2015 in and around Pocatello, ID, USA.

SUPER 180 facing northeast on the top of a ladder (three m above the ground), at the same time of day (in the morning) at each site to facilitate comparative analyses. Prior to sampling at each site, meteorological data was collected (average wind speed, dew point, barometric pressure, temperature, and relative humidity; **Appendix Table 1**) with a Kestrel 3500 (Kestrel Meters, Birmingham, MI). Three different agar mediums, tryptic soy agar, (TSA), Pikovskaya agar (PKA), and Luria Bertani-Tryptophan (LBT), were used to quantify the abundance of the three different groups of PGPB in air samples: siderophore-producers (S-producers), phosphate-solubilizers (P-solubilizers), and indole-acetic acid-producers (IAA-producers), respectively. TSA also was used to quantify the total abundance of culturable mesophilic bacteria collected in air samples. Air samples (180 L per plate) were collected onto five PKA plates, five LBT plates, and 10 TSA plates in random order at each site when sampling was conducted; this resulted in a total

of 60 plates per season, per site (**Figure 5**). At the midpoint of each sampling period at each site, air samples (1800 L per plate) were collected onto two agar plates, in succession, that were overlaid with autoclaved 0.2 µm polycarbonate membranes (EMD Millipore, Darmstadt, Germany). These membranes were removed from plates immediately after sampling (no microbes cultivated) and stored at -20 °C until DNA extraction was performed for bacterial 16S rRNA gene amplification and sequencing to determine the total bacterial composition of the air sampled at the sites. At the laboratory, plates for cultivating and quantifying PGPB were sealed with Parafilm, inverted and stored for one week at room temperature. Total bacterial and PGPB counts were performed seven days after air sampling.

Plant growth-promoting bacteria assay conditions

All agar medium were autoclaved on a liquid cycle for a sterilization time of 20 minutes at 121 °C, and then amended with cycloheximide (50 mg mL^{-1}; Sigma-Aldrich) to inhibit the growth of fungi. TSA (15 g L^{-1}; Sigma-Aldrich) was used for total colony counts because that general growth medium is suitable for growing a diversity of mesophilic bacteria. TSA also was used to cultivate S-producers. The overlay-chrome azurol S (O-CAS) was completed to detect and quantify the abundance of S-producers cultivated from air samples on TSA (68). Total bacterial counts were recorded from each TSA plate 7 days after sampling, prior to performing the O-CAS assay, which was conducted by overlaying the original growth medium with agar containing blue dye solution (60.5 mg chrome azurol S (CAS), 72.9 mg hexadecyltriethyl ammonium bromide (HDMTA) and 1 mM $FeCl_3$ $6H_2O$ in 10 mL of 10 mM HCl; 1, 62). The overlay medium contained piperazine-1,4-bis(2-ethanesulfonic acid (PIPES, 30.24 g L^{-1}, Sigma-

Aldrich) and agarose (0.9%). Overlay medium and blue-dye mixture were autoclaved separately, combined, and 20 mL was pipetted onto each plate for the O-CAS assay. After 15 min, but no more than 1 hr, color change was observed in the overlaid medium surrounding colonies that were producing siderophores. A change from blue to purple represented catecholate production, and a color change from blue to orange represented hydroxamate production by bacteria colonies. *Bacillus cereus*, which produces hydroxamate, was used as a positive control. Overlay medium also was pipetted onto a sterile TSA plate to assure that color changes could be attributed to siderophore production by the bacteria and not the medium.

IAA-producer assays were completed on LBT plates (15 g L^{-1} Luria-Bertani agar (Acros), 5 mM tryptophan (Sigma-Aldrich)). After four days of incubation, sterile 0.22 µm nitrocellulose membranes (ThermoScientific, Waltham, MA) were placed on top of colonies growing on the LBT plates allowing them to grow into the membranes. On day seven of incubation, the membranes were removed, placed into glass dishes with the biomass facing upwards, and plate counts were taken again. Membranes were then saturated with Salkowski reagent (1.2% $FeCl_3$ in 37% sulfuric acid) and incubated for 1 hr as previously described (12). IAA production was indicated by a red halo around bacterial colonies and counts were recorded (12).

The P-solubilizing bacteria assays were performed seven days after air sampling. PKA (31.3 g L^{-1} Pikovskaya Medium, Sigma-Aldrich) plates were observed for zones of clearing in the media around colonies, indicating that the colony was solubilizing phosphate from the insoluble tri-calcium phosphate (TCP) in the medium, which makes the medium cloudy (69). Total colony counts were recorded for each plate, as well as,

the number of colonies with zones of clearing. *Bacillus cereus*, a known P-solubilizer, was used as a positive control for this assay and a sterile plate was used as a negative control.

Bacterial isolate selection and identification

Over the course of each season, S-producing, P-solubilizing, and IAA-producing isolates representing diverse colony morphologies were selected and purified from each assay at each site. A total of 37 isolates were collected over the sampling year, 12 from autumn, 7 from winter, 9 from spring, and 11 from summer (**Appendix C**). Isolates were streaked onto TSA media and purified. Isolates were then transferred to tryptic soy broth (TSB, 15 g L^{-1}; Sigma-Aldrich) to propagate biomass for DNA extraction and cryopreservation in 25% glycerol at -80 °C. DNA was extracted from selected bacterial isolates with the MoBio UltraClean Microbial DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA), following the protocol of the manufacturer and stored at -20 °C.

To taxonomically classify the 37 PGPB cultures, 16S rRNA gene fragments were PCR amplified in triplicate from each of the DNA extracts and prepared for Sanger sequencing at the Molecular Research Core Facility (MRCF; Idaho State University, Pocatello, ID, U.S.A.). PCR amplification was performed with primers 27F and 1492R (52) in 20 µL reactions; each reaction contained 0.02 units μL^{-1} of Phusion Polymerase (New England Biolabs, Ipswich, MA), 1X Phusion HF Buffer (New England Biolabs, Ipswich, MA), 10 mM dNTPs, 0.4 mM of each primer, and 3% dimethyl sulfoxide. Thermalcycling was performed in an Eppendorf Mastercycler proS (Eppendorf, Westbury, NY) with the following program: denaturation at 98 °C for 30 s followed by 30 cycles of 98 °C for 15 s, 64 °C for 15 s, 72 °C for 30 s, with a final extension of 72 °C

for 5 min. PCR products were pooled from triplicate reactions to determine successful amplification by gel electrophoresis (1% agarose gel in tris acetate EDTA buffer) and visualization with ethidium bromide staining. Pooled products were purified with the Qiagen MinElute PCR Cleanup Kit (Qiagen, Valencia, CA). Quantified DNA products were finally sequenced with an Applied Biosystems 3130XL Genetic Analyzer (Life Technologies Laboratory, Grand Island, NY). Bidirectional reads were assembled with Sequencher 4.7 (Gene Codes Corporation, Ann Arbor, MI) and a nucleotide BLAST (3, 4) analysis was conducted to classify each isolate taxonomically.

DNA extractions from air samples

DNA was extracted from polycarbonate membranes impacted with air samples by aseptically cutting the membranes into two pieces and placing each half one into a separate bead-beating tube from the FastDNA soil extraction kit (MP Biomedicals, Solon, OH). DNA extraction was performed using the FastDNA soil extraction kit following the protocol of the manufacturer, with the exception that homogenization was performed for 70 s in step 4. Upon DNA elution (25 µL), duplicate extractions were combined and the DNA was purified with steps 16 to 22 of the MoBio PowerSoil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA). An autoclaved membrane was used as a control for extractions to ensure that DNA was extracted from the air sampled onto the membranes and not introduced via microbial contamination during the extraction process. Purified DNA extracts were stored at -20 °C.

16S rRNA gene amplification, sequencing and analysis

Bacterial 16S rRNA gene fragments were polymerase chain reaction (PCR)-amplified in triplicate with 515F and 806R barcoded primers (16) from each of the 36 DNA extracts from air samples. Each 25 µL reaction contained 0.4 µM of each primer and 2.5X HotMasterMix solution (5 PRIME, Inc., Gaithersburg, MD). PCR was performed in an Eppendorf Mastercycler proS (Eppendorf, Westbury, NY) with the following thermalcycling program: initial denaturation at 94 °C for 3 min followed by 32 cycles of 94 °C for 3 min, 50 °C for 1 min, 72 °C for 1:45 min, and a final extension of 72 °C for 10 min. Triplicate PCR products were pooled to determine successful amplification by gel electrophoresis (1% agarose gel in tris acetate EDTA buffer) and visualization with ethidium bromide staining. Pooled products were purified with Qiagen MinElute PCR Cleanup Kit (Qiagen, Valencia, CA). Sequencing of each barcoded 16S rRNA gene PCR product was conducted with the Illumina MiSeq (Illumina, San Diego, CA) at the MRCF.

The barcoded sequence libraries were trimmed, quality examined and analyzed with the software package "MOTHUR" version 1.34.3 (76). Contigs were made using forward and reverse fastq files and sequences that were ≤239 or ≥260 were eliminated from the libraries. Sequences that contained ambiguous bases, >2 mismatches to primers, >1 mismatch to a barcode or homopolymers (>7 bases) also were removed. Chimeras were detected and removed with the UCHIME algorithm (47). Sequences were aligned against the SILVA (80) bacterial 16S rRNA gene reference alignment. Operational taxonomic units (OTU's) were clustered at ≥ 97% similarity, using the average neighbor algorithm.

The 36-libraries were normalized to contain the same number of sequences (n = 40,115) prior to all comparative analyses among sampling sites and seasons. OTU-based rarefaction curves for each sample and evenness were calculated using 1,000 sampling iterations in the MOTHUR software package. All sequences were taxonomically classified using the Ribosomal Database Project (RDP) Classifier (82) within MOTHUR with the confidence thresholds set at ≥ 80%.

Statistical analysis

Repeated measure ANOVAs (RM-ANOVA; 2) were computed within the R environment (version 3.2.3, R Core Team 2015), relying on the Anova function from the package car (30), to determine if culturable colony counts for each PGPB assay differed depending on weeks, biomes, and seasons over the course of the year. Random factors (biome:weeks, season:weeks, and weeks) were adjusted for sphericity using the Greenhouse-Geisser epsilon correction (39).

A distance matrix of the 36-16S rRNA gene sequence libraries was generated based on the Bray-Curtis distance metric, which takes the relative abundance of sequences in each OTU into account (11). The matrix was used to generate non-metric multidimensional scaling (NMDS) ordinations to determine if airborne microbial composition clustered by sampling site and season. These analyses relied on the 'vegan' package (67) within the R environment (version 3.2.2, R Development Core Team 2011). Permutational tests were used to determine if the communities corresponding to treatments within the ordination were identical.

Accession numbers

Sequences for all 16S rRNA gene sequence libraries were deposited into the MG-RAST database (http://metagenomics.anl.gov) under the following accession numbers: 4683553.3, 4683560.3, 4683574.3, 4683570.3, 4683562.3, 4683588.3, 4683577.3, 4683568.3, 4683559.3, 4683583.3, 4683554.3, 4683565.3, 4683567.3, 4683580.3, 4683564.3, 4683557.3, 4683572.3, 4683585.3, 4683569.3, 4683584.3, 4683558.3, 4683582.3, 4683575.3, 4683587.3, 4683571.3, 4683556.3, 4683573.3, 4683563.3, 4683555.3, 4683579.3, 4683576.3, 4683561.3, 4683578.3, 4683586.3, 4683581.3, 4683566.3.

Results

Total bacterial and PGPB abundance

Across all sampling campaigns, total bacterial counts on TSA ranged from 0 to 88 colony-forming units (CFU) per air sample (180 L). PGPB counts and total CFU counts on TSA were affected by season, biome, weeks, and the interaction of season and weeks ($P < 0.01$, **Table 1**, **Figure 6**). Air samples cultured from all biomes contained P-solubilizers, S-producers, and IAA-producers and the abundance of those PGPB did not differ among biomes ($P > 0.05$ in all instances, **Table 1**). Overall, autumn had the highest abundance of P-solubilizers and S-producers, while summer had the highest abundance of IAA-producers (**Table 2**).

Table 1. RM-ANOVA results for year-long colony counts of PGPB. Biome:season, biome, and season are factors that have fixed effects; biome:weeks, season:weeks, and weeks, are random factors adjusted for sphericity using the Greenhouse-Geisser epsilon corrections. Statistical significance is denoted as follows: 0.001 '***' 0.01 '**' 0.05 '*'.

Factor	IAA-producers		P-solubilizers	
	Test statistic	*P*-value	Test statistic	*P*-value
biome:season	$F_{6, 48} = 0.389$	8.82E-01	$F_{6, 6} = 2.80$	2.02E-02**
biome	$F_{2, 48} = 0.0887$	9.15E-01	$F_{2, 48} = 1.70$	1.94E-01
season	$F_{3, 48} = 8.25$	1.58E-04***	$F_{3, 48} = 11.2$	1.35E-05***
biome:weeks	$F_{4.85, 96.85} = 1.07$	3.69E-01	$F_{4.71, 96.71} = 2.80$	4.91E-02*
season:weeks	$F_{6.95, 96.85} = 5.20$	3.16E-04***	$F_{6.71, 96.71} = 5.18$	8.27E-03***
weeks	$F_{2.85, 96.85} = 3.34$	4.81E-02**	$F_{2.71, 96.71} = 8.97$	1.34E-03**
	S- producers		TSA Plate Counts	
	Test statistic	*P*-value	Test statistic	*P*-value
biome:season	$F_{6, 6} = 1.33$	3.57E-01	$F_{2.97, 96.97} = 34.4$	7.21E-12***
biome	$F_{2, 48} = 0.365$	6.96E-01	$F_{3, 48} = 65.5$	2.20E-16***
season	$F_{3, 48} = 4.35$	8.61E-03***	$F_{4.97, 96.97} = 8.58$	6.57E-06***
biome:weeks	$F_{4.38, 96.83} = 0.320$	8.31E-01	$F_{6.97, 96.97} = 17.1$	3.51E-13***
season:weeks	$F_{6.83, 96.83} = 6.12$	7.49E-05***	$F_{6, 4.09} = 4.09$	3.51E-13***
weeks	$F_{2.83, 96.83} = 7.43$	2.06E-03***	$F_{2, 48} = 20.1$	4.66E-07***

Table 2. Average percentage of P-solubilizers, S-producers, and IAA-producers ± the SD in 180 L of sampled air above three terrestrial biomes (Pocatello, ID, U.S.A) over four seasons from autumn 2014 to summer 2015.

	Suburban			
Capability	autumn	Winter	spring	summer
P-solubilizers	22.7 ± 20.00	4.3 ± 5.00	2.8 ± 3.52	18.0 ± 26.80
S-producers	24.8 ± 26.96	10.6 ± 10.00	9.8 ± 3.23	7.7 ± 11.09
IAA-producers	0 ± 1.78	0 ± 0	0.2 ± 0.76	2.0 ± 4.09
	Sagebrush steppe			
	autumn	Winter	spring	summer
P-solubilizers	14.5 ± 13.29	3.2 ± 5.23	9.9 ± 11.58	7.0 ± 7.90
S-producers	33.5 ± 17.65	13.2 ± 17.68	8.5 ± 1.72	9.1 ± 11.44
IAA-producers	2.2 ± 8.61	1.0 ± 3.69	0.4 ± 0.91	8.0 ± 20.76
	Aspen forest			
	autumn	winter	spring	summer
P-solubilizers	17.5 ± 22.60	11.5 ± 18.83	5.4 ± 9.09	13.4 ± 7.00
S-producers	44.0 ± 33.33	19.1 ± 19.50	11.2 ± 15.93	16.7 ± 26.28
IAA-producers	0.7 ± 2.87	0 ± 0	0 ± 0	4.9 ± 11.38

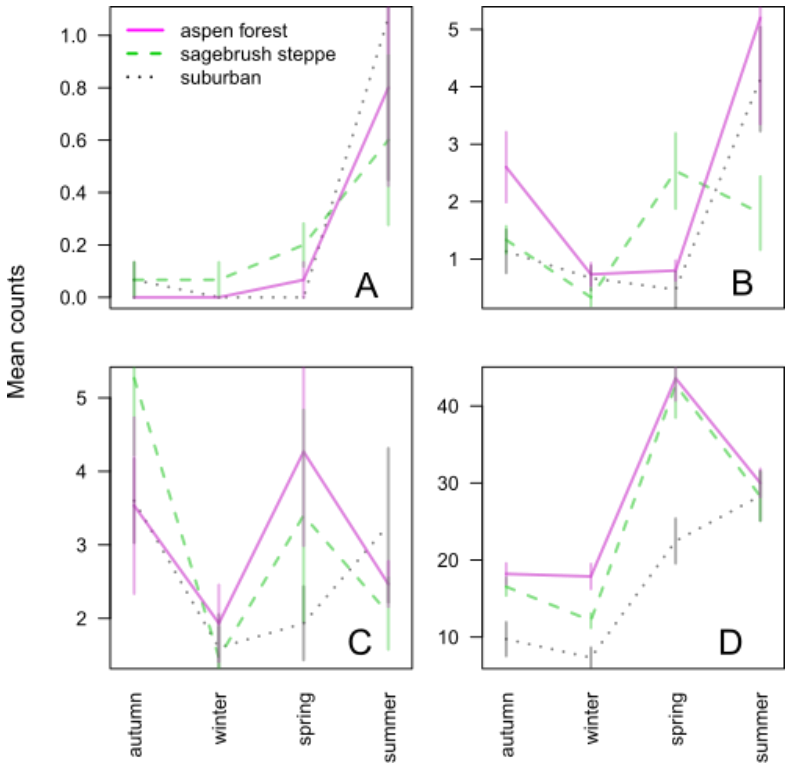

Figure 6. Biome and season interaction plots for PGPB abundance. Interaction plots of site and season for the abundance of surveyed PGPB and colony counts in the lower atmosphere in and around Pocatello, Idaho, U.S.A. (A) indole-acetic acid-producing bacteria abundance, (B) phosphate-solubilizing bacteria abundance, (C) siderophore-producing bacteria abundance, (D) total bacterial counts on TSA media. Note the differences in scale on the y-axis. Error bars represent standard errors (n = 3).

The percent of bacterial colonies cultivated on LBT that were IAA producers ranged from 0 to 7.97% (**Table 2**). The abundance of IAA-producers correlated with the following factors: season ($F_{3, 48}$ = 8.25, P <0.0001), weeks sampled during the season ($F_{2.85, 96.85}$ = 3.34, P <0.01), interaction of biome and weeks ($F_{4.85, 96.85}$ =1.07, P <0.01), and interaction of season and weeks ($F_{6.95, 96.85}$ = 5.20, P < 0.0001). The interaction of biome and season had no observed significant difference (P > 0.05) on the abundance of IAA-producers (**Figure 6a**).

P-solubilizers comprised 2.8 to 22.7% of the bacterial colonies cultivated on PKA (**Table 2**). The abundance of P-solubilizers correlated with the following factors: season ($F_{3, 48}$= 11.2, P < 0.0001), weeks sampled during the season ($F_{6.71, 96.71}$ = 5.18, P < 0.0001), interaction of biome and weeks ($F_{4.71, 96.71}$ = 2.80, P < 0.01), and interaction of biome and season ($F_{6, 6}$ = 2.80, P < 0.01; **Figure 6b**).

S-producers comprised 7.69 to 43.9% of the bacterial colonies cultivated on TSA (**Table 2**). The abundance of S-producers correlated with two factors: season ($F_{3, 48}$ = 4.35, P <0.001), and weeks sampled during the season ($F_{2.83, 96.83}$ = 7.43, P <0.001). S-producer abundance was not related to the interaction of biome and season ($F_{6, 6}$ = 1.33, P > 0.05; **Figure 6c**), the interaction of biomes and weeks, or the interaction of weeks and season (P > 0.05 in both instances).

Bacterial composition of air samples

A two dimensional NMDS plot (stress = 0.1470) based on the OTU composition of the 36-16S rRNA gene sequence libraries from air samples revealed clustering by season, but not by biome (**Figure 7**). Air samples collected during autumn from all sites clustered together with the exception of one biome. Permutational analyses indicated that community composition was correlated with season (P < 0.001, R^2=0.5568). Ellipses on the ordination plot delineate 95% confidence regions for the true cluster centroid (**Figure 7**). Six phyla were present over the year at all biome: Acidobacteria, Actinobacteria, Bacteroidetes, Cyanobacteria, Firmicutes, and Proteobacteria (**Figure 8**). The relative abundance of those phyla shifted across seasons. Proteobacteria composed the largest percentage of the sequence libraries from the aspen forest site in autumn (42%) and summer (37%), whereas Firmicutes composed the largest percent in the winter (35%) and

Actinobacteria composed the largest percent in the spring (31%). At the sagebrush-steppe site, Proteobacteria dominated the libraries from autumn (68%), spring (30%), and summer (47%); during winter, Firmicutes (33%) composed the largest percent of phyla. Additionally, Proteobacteria composed the largest percentage of the autumn (63%), winter (38%), and summer (55%) sequence libraries from the suburban site, but during spring, Actinobacteria (29%) dominated the libraries.

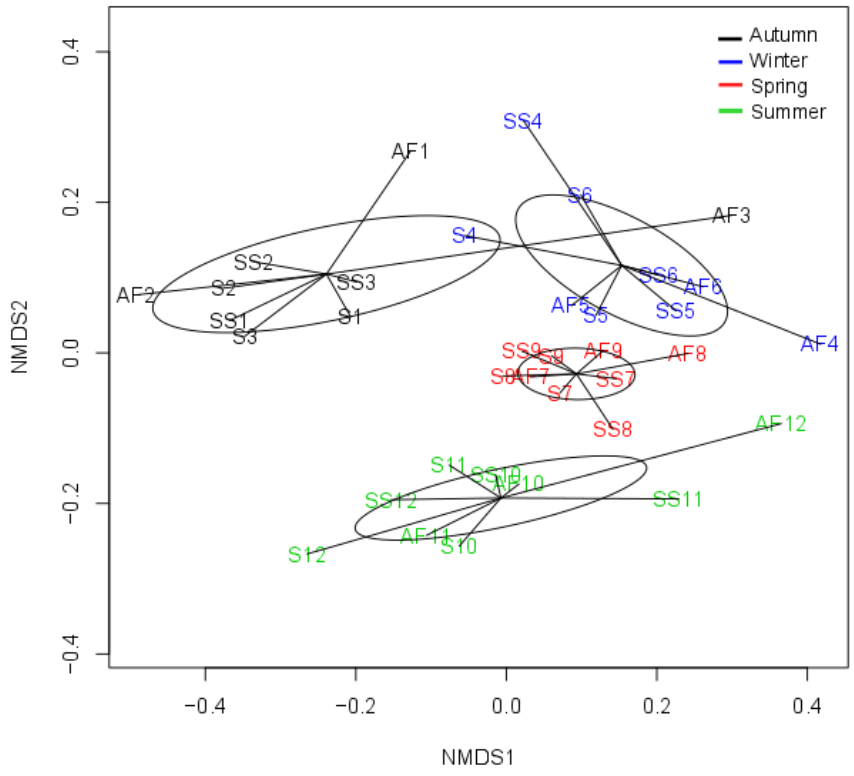

Figure 7. NMDS plot of airborne communities. NMDS plot of bacterial OTU composition of the 36-16S rRNA gene sequence libraries generated from air samples, based on the Bray-Curtis dissimilarity measure. Letters denote the location where the air sample was collected: AF=aspen forest, SS=sagebrush steppe, S=suburban; numbers indicate the week the air sample was collected: 1-3=autumn, 4-6=winter, 7-9=spring and 10-12=summer. Ellipses on the ordination plot delineate 95% confidence regions for the true cluster centroid and the lines connect libraries generated from air samples collected during the same season.

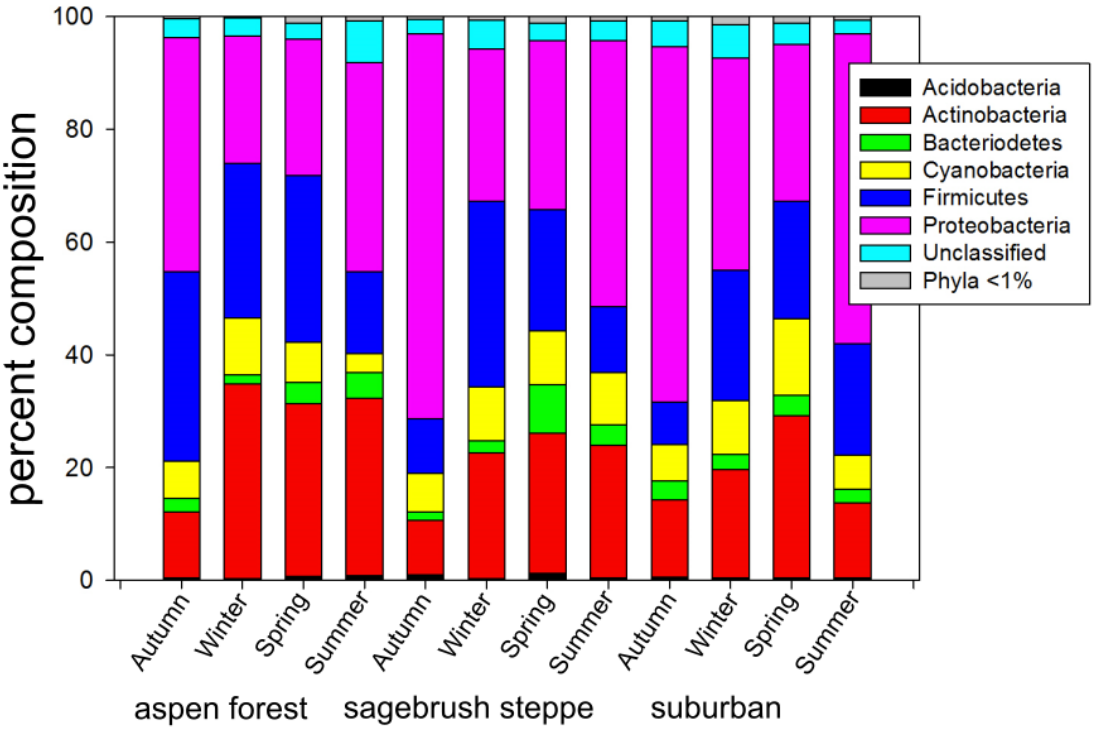

Figure 8. Dominant bacterial phyla identified libraries. Dominant bacterial phyla identified in 16S rRNA gene sequence libraries generated from air samples collected above three terrestrial biomes in the areas around Pocatello, ID, U.S.A, during four seasons (autumn 2014 to summer 2015). Each bar represents the percent taxonomic composition (n = 40,115 sequences) from each biome. Phyla that composed <1% of the libraries were Clamydiae, Chloroflexi, Deinococcus-Thermus, Fibrobacteres, Fusobacteria, Gemmatimonadetes, Nitrospirae, Plantomycetes, Spirochaetes, Verrucomicrobia, Candidate division BRC1, Candidate division 0D1, Candidate division OP10, Candidate division SR1, Candidate division TG-1, Candidate division TM6, Candidate division TM7, and Candidate divisionWS3.

Richness across all 36-sequence libraries ranged from 204 to 840 OTU's (**Appendix Figure 1**). Species richness in the sequence libraries from all three biomes shifted across seasons (**Appendix Figure 1**). The aspen forest site had the greatest richness in the summer, and lowest in the autumn. The sagebrush steppe site had the greatest richness in winter and the lowest in autumn. The suburban site had the greatest richness in autumn and lowest in summer (**Appendix A**). Rarefaction curves for each of the 36-libraries are in **Appendix B**.

Plant growth-promoting bacteria isolates

The 39 isolated PGPB represented 13 families: Pseudomonadaceae, Staphylococcaceae, Enterobacteriaceae, Bacillaceae, Micrococcaceae, Flavobacteriaceae, Streptomycetaceae, Microbacteriaceae, Coxiellaceae, Moraxellaceae, Paenibacillaceae, Brevibacteriaceae, and Nocardiopsaceae (**Appendix C**). The most abundant family represented in the isolate collections from all three sites was Bacillaceae (9-17%), followed by Micrococcaceae (8-17%). The P-solubilizing isolates were dominated by Microbacteriaceae, while the S-producers were dominated by Bacillaceae, and the IAA producers were dominated by Micrococcaceae. Family-based analysis of the 36 gene libraries indicated that families of the isolated PGPB represented 28.3% to 61.3% of sequences in each of the 36-sequence libraries derived from air samples (**Figure 9**). At all sites, the lowest relative abundance of those families was observed in the autumn libraries, but abundance increased in winter and then declined over spring and summer (**Figure 9**). On average, air collected above the aspen forest site had the highest amount of sequences from the PGPB-containing families that were represented in the isolate collection, whereas air collected above the suburban site had the lowest.

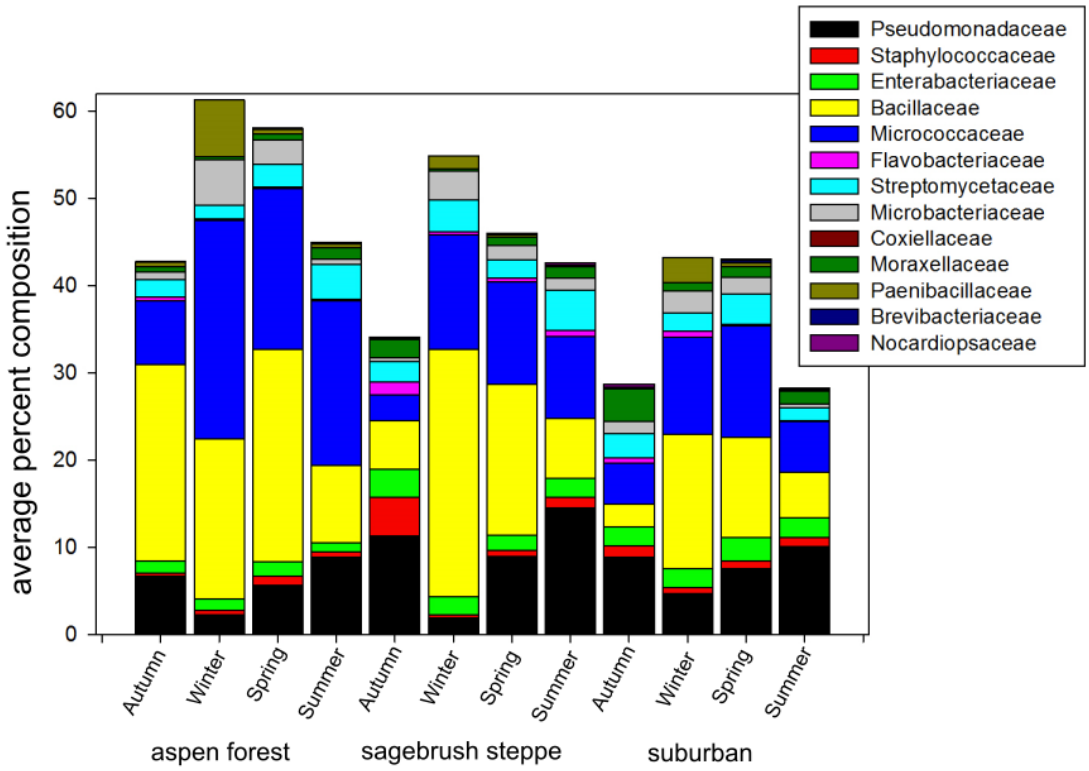

Figure 9. Bar plot of PGPB-containing bacterial families. Average percent composition of PGPB-containing bacterial families represented in 16S rRNA gene sequence libraries generated from air samples collected above three terrestrial biomes in and around Pocatello, ID, U.S.A., over four seasons (autumn 2014 to summer 2015). PGPB-containing families were determined by identification of the 39 PGPB cultures isolated from the PGPB assays conducted on air samples (Appendix Table 2).

Discussion

I used cultivation-based methods to explore how abundant three types of PGPB are within the lower atmosphere over three terrestrial biomes, and how their abundance may shift in conjunction with the overall composition of airborne bacteria across seasons using DNA-based methods. Our results indicated that although PGPB abundance in the lower atmosphere was affected primarily by season, PGPB were present during all seasons above the three sampling biomes (aspen forest, sagebrush steppe, and suburban).

Air sampling site, alone, had no effect on PGPB abundance, which was mirrored by the lack of obvious compositional differences among 16S rRNA gene libraries from the three biomes (**Figure 7, Figure 8**). Womack et al. (2010) hypothesized that biodiversity of atmospheric microbes exhibited biogeographical patterns; however, few studies aim to understand the spatial and temporal scales in which this pattern occurs. In this study, simultaneous sampling of sites was not possible, but sites were sampled successively, and at the same time of day for each effort. This allowed for comparison across sampling times, but may have masked site differences in our datasets, especially because there are known diurnal variations in bacterial fluxes from surface environments to the lower atmosphere (53). Future studies that simultaneously examine the bacterial communities over larger spatial scales could provide further insights into whether the geographical closeness of our sites contributed to the similarities in bacterial composition and PGPB abundance observed.

PGPB were present above all three sites during all seasons, but their abundance was significantly affected by season (**Table 1, Figure 6**). All three types of PGPB were most abundant in autumn and were least abundant in spring (**Table 2**). CFU counts on TSA also displayed seasonal trends, but those counts did not always correlate with the trends observed for each of the three types of PGPB. According to CFU counts on TSA media, autumn and winter had the fewest CFUs, whereas spring and summer had the most. These findings are similar, in part, with previous reports of airborne bacterial concentrations being greatest during autumn and summer and least during winter and spring (49, 10). Additionally, our results indicated that air samples collected during summer contained the greatest abundance of IAA-producers, whereas P-solubilizes and

S-producers were most abundant during autumn (**Table 2**). These variations in total CFU counts and PGPB abundance may be partly attributed to culture bias, but also to our lack of understanding in how underlying terrestrial biomes contribute to the abundance of specific PGPB relative to overall bacterial abundance, which may not be directly correlated in all seasons as the underlying vegetation goes through growth and senescence.

Although biome alone did not correlate with the abundance of airborne PGPB in the lower atmosphere, the interaction between site and season did have a significant effect on the abundance of P-solubilizers (**Figure 6**). Abundance of P-solubilizers at the sagebrush steppe site is greatest during spring and lower during summer, while the opposite occurred at the other two sites. At all three sites the same trends in abundance of IAA- producers were observed over the sampling year. Seasonal effects on the abundance of IAA-producers were observed; abundance increased significantly from spring to summer. Abundance of S-producers fluctuated with the interaction of biome and season, but was not significant. Trends in our surveyed PGPB abundance revealed autumn and summer had the greatest amount of IAA-producers, S-producers, and P-solubilizers, which paralleled previously noted trends in airborne bacterial concentrations being greatest during those two seasons (55). Because soil is nearly always covered by plant material, I hypothesize that seasonal variation in PGPB abundance in the lower atmosphere may be associated with plant cover. Additionally, each biome was dominated by different plant species, which are likely to harbor varying abundances and species of epiphytic bacteria that also may contribute to variations in PGPB abundance in air across seasons (**Table 2, Figure 7**).

PGPB isolated in culture were from 13 bacterial families, and these families were represented by 28 to 61% of the16S rRNA gene sequences in the libraries generated during this study (**Figure 9**). These percentages may not reflect the actual percentage of PGPB in the atmosphere above the biomes because not every member of those families is likely to be capable of promoting plant growth. Additionally, this study surveyed for only three PGPB capabilities (phosphate solubilization, siderophore production, indole-acetic acid production) because of time and assay-expense limitations and more PGPB containing families may have been identified if a survey had included additional PGPB capabilities (e.g., cyanide production, nitrogen-fixation, ACC deaminase production). Despite these limitations, our results demonstrate that the PGPB isolates were from families that comprised relatively large fractions of the sequence libraries and that PGPB-containing families are consistently present above all three biomes during all seasons.

PGPB have been well-studied to understand specific plant-microbe interactions, to develop techniques for their isolation and identification, and to quantify excreted plant growth-promoting substances (35, 12, 22); however, no dedicated survey has been conducted to determine the abundance of bacteria capable of promoting plant-growth within the rhizosphere or phyllosphere. Considering that soil and plants are primary sources of microbes into the atmosphere, I constructed my hypothesis with the knowledge that PGPB have been isolated from soil and plant-surfaces and are likely commonly transported through the atmosphere. Indeed, several of the PGPB that were isolated from air are representative of PGPB that commonly occur in association with plants and soils. As the atmosphere is a harsh environment with rapidly changing conditions, it is not surprising that a spore-forming genus, *Bacillus,* was the most

common genus isolated from our PGPB assays. Over half of our isolates had adaptations for surviving harsh atmospheric conditions, including spore formation, pigmentation, pleomorphic, or ability to produce endospores.

Several isolates, specifically *Bacillus* spp., are strains of bacteria that have been previously isolated from the phyllosphere, rhizosphere, or soil and are known for plant growth promotion. *Bacillus subtilis,* has demonstrated the ability to produce antibiotics and induced systemic resistance (50). A *Bacillus pumilus* strain was isolated, and this species has been studied for its ability to produce fungal cell-wall degrading enzymes (63). Moreover, Mengoni et al. (2003) observed that culturable communities from endophytic aerobic bacteria from stem and root tissues of two elm trees (*Ulmus laevis* and a hybrid elm clone "Lobel" [(*U. glabra* Huds. × *U. wallichiana* Planch.) × *U. hollandica* Wredei self.]) were dominated by the genera *Bacillus*, *Curtobacterium, Enterobacter, Pseudomonas, Stenotrophomonas, Enterobacter,* and *Staphylococcus*. Most of those genera also were isolated from our PGPB assays (**Appendix D**). Furthermore, Mocali et al. (2003) reported that *Bacillus* and *Curtobacterium* species were more abundant in the warmer months, which is consistent with our survey as well (**Appendix D**). To understand the fluxes of these isolated airborne PGPB from terrestrial biomes, a more extensive study would need to be conducted to examine the abundance of these taxa on plants in relation to their abundance in the overlying atmosphere.

At the three sampling biomes in and around the Pocatello area I demonstrated that PGPB are present in the overlying atmosphere during all seasons. Season had a more significant effect on PGPB abundance than underlying terrestrial biome, and paralleled fluctuations in percent composition of airborne phylum and families as determined by

16S rRNA gene sequencing. These findings provide insights into the total bacterial diversity of the overlying atmosphere as well as land-atmosphere fluxes of PGPB, specifically. PGPB that are actively present and viable within the lower atmosphere have the potential to contribute to ecosystem development as they reach new habitats, particularly disturbed landscapes (e.g., volcanic deposits, burned land) in early successional stages. As the human population grows and the expansion of urbanization into untouched habitats continues, microbial communities, both terrestrial and airborne, will be altered, perhaps affecting ecosystem development in neighboring ecosystems. This is the first study to look at PGPB presence within the lower atmosphere and by continuing to study microbial fluxes between terrestrial biomes the atmosphere, we can better understand how localized conditions and alterations of one surface environment can have influences at a larger scale.

Chapter 4: Conclusions

As wind sweeps across terrestrial biomes, microbes within them are emitted into the atmosphere, making terrestrial biomes the primary sources of microbes to the lower atmosphere. Thus, episodic alteration of terrestrial biomes by human activities (e.g., urbanization, agriculture) and natural disasters (e.g., volcanic eruptions, fire), which change the composition and distribution of microbes in terrestrial biomes, are likely to also change the microbial composition of the overlying atmosphere. This, in turn, has the potential to change the microbial taxa reaching new habitats via atmospheric transport. With regards to the atmospheric transport of specific microbial functional groups, pathogenic microbes have garnered much attention, but little is known about land-atmosphere fluxes of other groups of microbes, especially those that may have positive impacts on terrestrial ecosystem ecology. Airborne microbes are the primary colonizers of land devoid of vegetation (e.g., recent volcanic deposits) and are known to include plant growth-promoting bacteria (PGPB) that facilitate ecosystem succession via plant growth-promoting activities (e.g., increasing nutrient availability, secreting phytohormones, induced systematic resistance (ISR) to disease). Therefore, changes in the microbial composition of the atmosphere introduced by anthropogenic modification of underlying landscapes could impact ecosystem health and development. Although several lines of preliminary data indicate that PGPB may be ubiquitous in the atmosphere, this study surveyed the impact of different types of terrestrial habitats and seasons on the abundance of PGPB in the lower atmosphere.

The experiment in **Chapter 2** was conducted to determine if plant growth-promoting microbes were present in the lower atmosphere above three different terrestrial

biomes (aspen forest, sagebrush steppe, suburban) by examining their ability to enhance the growth of a model fern species. I found that across all air-sampling campaigns, *C. richardii* gametophytes grew significantly larger in the presence of airborne microbes, than on the sterile controls plates. Additionally, our results indicated that the impact of PGPM on plant growth is dependent on macronutrient concentration and stoichiometry in the environment. From this study (**Chapter 2)**, I was able to determine that PGPM were present in the lower atmosphere and that they enhanced plant growth (**Figure 4**). However, this study did not provide information about the specific PGPM present in the lower atmosphere or their abundance. This precipitated the year-long study reported in **Chapter 3**.

The study in **Chapter 3** was designed to test two hypotheses. First, I hypothesized that three specific types of PGPB (IAA-producers, S-producers, and P-solubilizers) were abundant in the lower atmosphere over three different terrestrial biomes during each of the four seasons. Second, I hypothesized that the overall airborne bacterial composition varied across season and biomes, and that this bacterial composition variation, co-varied with PGPB abundance. Study findings indicated that the three types of PGPB were present in the overlying atmosphere and that their abundance was correlated to changes in season, but not vary with terrestrial biome type (**Table 2, Figure 6, Figure 7**). I found that overall airborne bacterial composition varied significantly by season, but again biome had no effect (**Figure 8**). I had hypothesized that both biome and seasonality would have a significant effect of PGPB abundance and airborne bacterial composition, based on the findings in the literature that microbes have a biogeography that is reflected in the atmosphere (84, 14, 15). Although, vegetation and land-use within each biome was

distinct, their spatial closeness could be contributing to this limited effect of terrestrial biome. To fully determine the effect of terrestrial biome, the sampling locations and biome types should be sampled at greater distances from each other, in more prominent centers of each biome in the future. Additionally, I identified 37 PGPB that were cultivated from air samples. I isolated and identified these PGPB to gain insights into what fraction of the total airborne bacterial composition is comprised of PGPB. Some of the isolates identified have been studied previously and have been reported as plant growth-promoters (50, 63). I found that, from the 13 families identified, they comprised a significant portion of the airborne community composition (**Figure 9**). Although, not every member of each of the bacterial families is a plant growth-promoter, this data indicates that I was not isolating rare bacteria from the atmosphere, but instead commonly found taxa. This finding bolsters the potential significance of atmospheric transport in bringing PGPB to terrestrial habitats where they can play a role in ecosystem development.

The knowledge of microbial succession in ecology is evolving, and we now distinguish that microbial communities colonize barren land substrates (e.g., volcanic deposits, burned land) long before pioneer plants begin to grow (18). With this shift to studying the sequences of microbial successions on landscapes, understanding the interactions of terrestrial environments with atmospheric becomes more critical. We know that water, soil, and vegetation are the primary sources of microbes in the atmosphere (55), but we do not know how ecosystem health affects the airborne community that has potential to disperse and interact with distant ecosystems potentially controlling trajectory or succession.

This is the first survey of three types of PGPB in the lower atmosphere. I found that they are present and common players in the airborne community composition, above three different terrestrial biomes, and that seasonality was correlated to the shifts in abundance of those PGPB, as well as the composition of airborne communities. This thesis is just the tip of the iceberg at understanding the potential "fertility of the atmosphere" and our ability to monitor ecosystem health by examining the airborne microbial "signatures" above them. More in depth surveys of more types of PGPM need to be completed, along with more intensive sampling campaigns in which diurnal fluctuations of microorganisms are accounted for while simultaneously sampling at distant sites so that air samples are matched in time.

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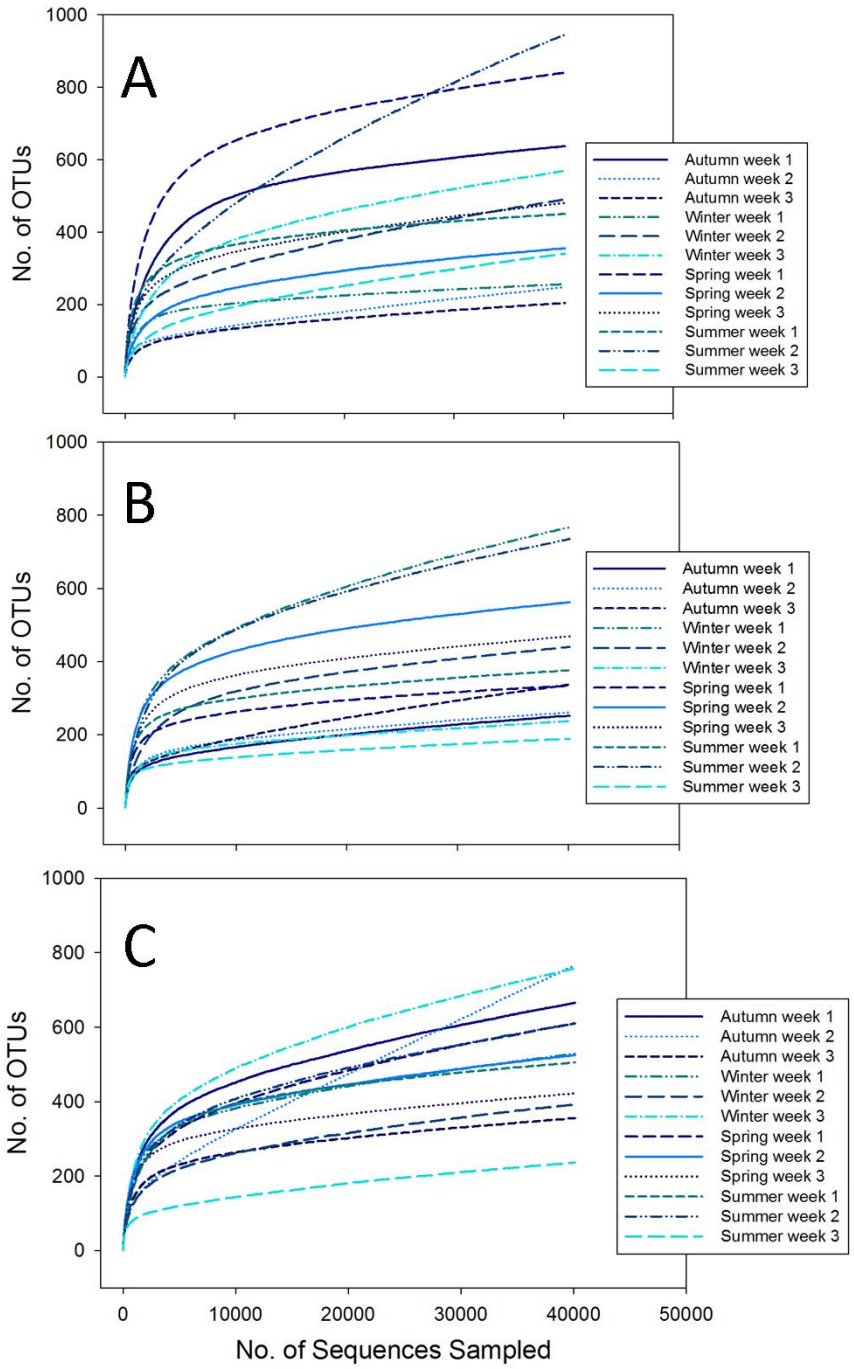
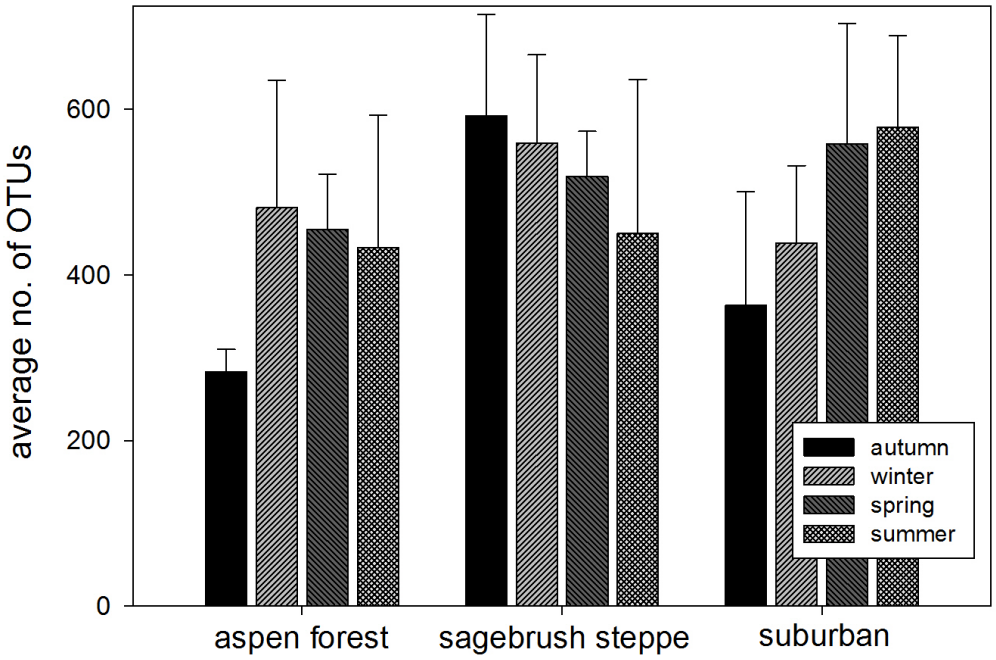
Appendix A



Appendix A. OTU-based rarefaction curves for the 36- 16S rRNA gene libraries generated from air samples. (A) suburban site, (B) sagebrush steppe site, (C) aspen forest site.

Appendix B



Appendix B. Normalized OTU-based richness of 16S rRNA sequence libraries generated from air samples collected above three terrestrial biomes in and around Pocatello, ID, U.S.A, during four seasons (autumn 2014 to summer 2015). Error bars represent standard errors (n = 3).

Appendix C

Appendix C. Meteorlogical data descriptions of sites collected over the course of the year.

Date	Land-use Type	Avg. Wind Speed	Temp. (°C)	Relative Humidit	Dewpoin t Temp. (°C)	Barometr ic	Start Time	End Time
05NOV2014	Aspen forest	0	8.00	54.1	2.3	25.2	0856	0945
05NOV2014	Sagebrush-steppe	1.2	11.2	52.7	1.9	25.56	1017	1104
05NOV2014	Suburban	2.5	13.2	47.1	1.9	25.8	1127	1213
12NOV2014	Aspen forest	0	-7.30	18.4	-23.4	24.96	0840	0926
12NOV2014	Sagebrush-steppe	1.5	-12.3	48.0	-21	25.36	0955	1042
12NOV2014	Suburban	2.0	-10.3.	30.0	-19.8	25.63	1055	1142
19NOV2014	Aspen forest	1.0	2.30	46.4	-7.5	24.92	0835	0920
19NOV2014	Sagebrush-steppe	1.1	0.70	48.7	-9.0	25.31	0955	1038
19NOV2014	Suburban	2.1	-1.10	54.6	-9.0	25.57	1055	1137
26JAN2015	Aspen forest	0	2.90	75.5	-1.1	25.12	0835	0935
26JAN2015	Sagebrush-steppe	0.6	3.90	78.1	0.1	25.48	1001	1047
26JAN2015	Suburban	2.8	6.00	71.0	2.0	25.72	1105	1210
02FEB2015	Aspen forest	0.7	9.70	54.0	0.9	24.92	0835	0928
02FEB2015	Sagebrush-steppe	2.3	4.90	75.3	0.9	25.29	0956	1055
02FEB2015	Suburban	1.3	6.70	69.3	1.4	25.54	1105	1157
12FEB2015	Aspen forest	0	10.2	36.2	-4.7	25.12	0825	0919
12FEB2015	Sagebrush-steppe	2.0	9.00	38.3	-4.1	25.49	0958	1056
12FEB2015	Suburban	2.8	11.7	34.4	-3.5	25.73	1115	1212
13APR2015	Aspen forest	0.6	5.80	52.1	-6.2	24.96	0830	0921
13APR2015	Sagebrush-steppe	2.3	9.80	39.0	-4.5	25.32	0958	1041
13APR2015	Suburban	0.7	15.4	24.0	-5.3	25.54	1058	1145
20APR2015	Aspen forest	0	14.3	14.2	-4.4	24.78	0820	0905
20APR2015	Sagebrush-steppe	2.2	10.8	49.4	-2.3	25.15	0935	1020
20ARP2015	Suburban	2.2	12.6	27.4	-3.2	25.38	1034	1120
27APR2015	Aspen forest	0	7.40	49.0	-4.4	25.02	0825	0909
27APR2015	Sagebrush-steppe	3.0	6.90	52.5	-1.2	25.43	0935	1018
27APR2015	Suburban	3.6	8.80	52.4	-0.4	25.68	1033	1133
24JUN2015	Aspen forest	0	20.6	47.2	9.1	24.90	0845	0940

Appendix C continued.

Date	Land-use Type	Avg. Wind Speed (mph)	Temp. (°C)	Relative Humidity (%)	Dewpoint Temp. (°C)	Barometric Pressure	Start Time (hours)	End Time (hours)
24JUN2015	Sagebrush-steppe	4.6	25.4	21.7	1.6	25.26	1020	1105
24JUN2015	Suburban	3.5	28.7	18.4	1.5	25.46	1120	1215
30JUN2015	Aspen forest	0.7	21.0	58.2	12.5	24.92	0758	0837
30JUN2015	Sagebrush-steppe	1.1	24.1	48.7	12.5	25.28	0911	0950
30JUN2015	Suburban	1.3	24.6	55.0	14.9	25.50	1005	1046
09JUL2015	Aspen forest	0	20.3	62.0	12.4	24.79	0815	0900
09JUL2015	Sagebrush-steppe	2.8	19.4	54.9	10.3	25.16	0933	1020
09JUL2015	Suburban	2.2	27.6	54.6	11.7	25.38	1031	1115

Appendix D.

Appendix D. Detailed table of isolates for which 16S rRNA genes have been sequenced and the nearest BLAST hits recorded.

Isolate	Nearest Blast hit	% Similarity	Season Isolated	Capability	Site
A1	*Pseudomonas frederiksbergensis* strain IHB B 765 16S ribosomal RNA gene, partial sequence	99	Fall	PS	Aspen forest
A3	*Staphylococcus succinus* strain IARI-J-24 16S ribosomal RNA gene, partial sequence	99	Fall	PS	Aspen forest
A5	*Enterobacter sp.* VTAN51 16S ribosomal RNA gene, partial sequence	99	Fall	PS	Sagebrush
A8	*Pseudomonas frederiksbergensis* strain IHB 765 16S ribosomal RNA gene, partial sequence	99	Fall	PS	Suburban
A10	*Pantoea ananatis* strain BSP9 16S ribosomal RNA gene, partial sequence	99	Fall	IAA	Aspen forest
A11	*Bacillus simplex* strain FJAT-10590 16S ribosomal RNA gene, partial sequence	99	Fall	IAA	Sagebrush
A12	*Kocuria sp*. Ats1 16S ribosomal RNA gene, partial sequence	100	Fall	IAA	Suburban
A14	*Arthrobacter sp.* AB70 16S ribosomal RNA gene, partial sequence	100	Fall	SP	Sagebrush
A15	*Bacillus atrophaeus* strain NXUSASNFB001 16S ribosomal RNA gene, partial sequence	99	Fall	SP	Aspen forest
A16	*Bacillus anthracis* IHB B 7021 16S ribosomal RNA gene, partial sequence	100	Fall	SP	Aspen forest
A17	*Bacillus sp.* 3HH-8 16S ribosomal RNA gene, partial sequence	100	Fall	SP	Suburban
A18	*[Brevibacterium] frigoritolerans* strain OL-3 16S ribosomal RNA gene, partial sequence	99	Fall	SP	Suburban
B3	*Flavobacterium sp.* AB 16S ribosomal RNA gene, partial sequence	98	Winter	SP	Aspen forest
B4	*Staphylococcus succinus* strain 57A (C3P2) 16S ribosomal RNA gene, partial sequence	100	Winter	SP	Sagebrush
B5	*Streptomyces candidus* strain PM425C 16S ribosomal RNA gene, partial sequence	99	Winter	SP	Suburban
B7	*Curtobacterium flaccumfaciens* pv. Flaccumfaciens strain x-2 16S ribosomal RNA gene, partial sequence	100	Winter	PS	Sagebrush
B9	*Steptomyces candidus* strain PM$25C 16S ribosomal RNA gene, partial sequence	100	Winter	SP	Aspen forest
B10	*Streptomyces candidus* strain PM425C 16S ribosomal RNA gene, partial sequence	99	Winter	SP	Suburban
B12	*Bacillus subtilis* strain ML 101B 16S ribosomal RNA gene, partial sequence	100	Winter	SP	Sagebrush
C4	*Rickettsiella sp.* 5- 186-2 16S ribosomal RNA gene, partial sequence	99	Spring	SP	Aspen forest
C6	*Bacillus thuringiensis* strain HS18-1, complete genome	99	Spring	SP	Suburban
C7	*Arthrobacter sp.* 18/4 16S ribosomal RNA gene, partial sequence	99	Spring	IAA	Suburban
C8	*Acinetobacter sp.* 4077 16S ribosomal RNA gene, partial sequence	99	Spring	IAA	Sagebrush
C12	*Curtobacterium flaccumfaciens* strain XA2-10 16S ribosomal RNA gene, partial sequence	99	Spring	PS	Sagebrush
C13	*Pseudomonas poae* RE*1-1-14 strain RE*1-1-14 16S ribosomal RNA gene, partial sequence	99	Spring	PS	Suburban
C18	*Bacillus sp*. KDJ060505 16S ribosomal RNA gene, partial sequence	99	Spring	SP	Sagebrush
C19	*Bacillus sp.* CMJ1-21 16S ribosomal RNA gene, partial sequence	99	Spring	SP	Suburban
D1	*Curtobacterium flaccumfaciens* pv. Flaccumfaciens strain x-2 16s ribosomal RNA gene, partial sequence	99	Summer	PS	Aspen forest
D7	*Curtobacterium sp.* Hz10 16S ribosomal RNA gene, partial sequence	99	Summer	PS	Sagebrush
D8	*Curtobacterium sp.* SaPS3 16S ribosomal RNA gene, partial sequence	99	Summer	IAA	Sagebrush
D9	Arthrobacter sp. TM4 1 16S ribosomal RNA gene, partial sequence	99	Summer	IAA	Sagebrush
D11	*Bacillus pumilus* partial 16S rRNA gene, strain CHRPB37	99	Summer	PS	Suburban
D13	*Paenibacilus sp.* THK-CF25 16S ribosomal RNA gene, partial sequence	99	Summer	SP	Suburban
D14	*Brevibacterium sp.* 210 46 16S ribosomal RNA gene, partial sequence	100	Summer	SP	Aspen forest
D15	*Exiguobacterium mexicanum* strain KBN-1-41 16S ribosomal RNA gene, partial sequence	99	Summer	SP	Aspen forest
D16	*Bacillus safensis* strain BS37 16S ribosomal RNA gene, partial sequence	100	Summer	IAA	Suburban
D17	*Nocardiopsis sp.* BNT5310 16S ribosomal RNA gene, partial sequence	99	Summer	IAA	Suburban